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Analysis of the Impact of Polyunsaturated Fatty Acids (PUFAs) on Placental Gene Expression

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Summary

There is an urgent need for effective strategies to manage the world-wide increasing prevalence of obesity, especially in children. A promising approach for primary prevention of obesity was hypothesized by Ailhaud and Guesnet in 2004 (Obes Rev 2004 5(1):21-26), suggesting that a decreased n-6/n-3 long-chain polyunsaturated fatty acid (LCPUFA) ratio in maternal nutrition during pregnancy and lactation programs lower offspring obesity risk. The INFAT (**I**mpact of **n**utritional **f**atty acids during pregnancy and lactation on early **a**dipose **t**issue development) study investigated this hypothesis in a randomized controlled human intervention trial conducted by Prof. Hauner (*Chair of Nutritional Medicine, TU München*).

In this context, the placenta provides a unique opportunity to assess the molecular impact of the reduced n-6/n-3 LCPUFA ratio in the maternal diet during pregnancy on an easily accessible human biopsy of mainly offspring-derived extraembryonic tissue, which consists in large parts of extraembryonic mesoderm and trophoblast cells. The placenta is also likely to play a key role in fetal / metabolic programming and thereof resulting sexual dimorphic outcomes. Sexual dimorphisms were commonly reported for placenta, adipose tissue distribution as well as for LCPUFA status and metabolism in adult humans. These observations together with the facts that LCPUFAs act predominantly by regulating gene expression and microRNAs are involved in the modulation of mRNA expression and placental physiology led to the main aim of this thesis: analysis of the impact of a reduced n-6/n-3 LCPUFA ratio in maternal nutrition during pregnancy on placental gene expression in a defined subpopulation of the INFAT study. Following this aim, at first, placental gene expression was investigated by transcriptomic analyses as well as RT-qPCR experiments and data were further analyzed with consideration of sex-specific effects. Subsequently, the influence of the n-3 LCPUFA intervention on the placental mRNA-microRNA expression network as well as its potential relations to biological processes was investigated using explorative miRNome profiling in combination with bioinformatics, and validated using RT-qPCR and Western blotting. Finally, observed changes in placental gene expression were assessed whether they are associated with metabolic changes or offspring obesity risk.

In this thesis, for placentas of the INFAT subpopulation, which reflect the excellent compliance of the whole INFAT population, it was demonstrated that a maternal n-3 LCPUFA supplementation during pregnancy impacts placental gene expression sex-specifically, with a more pronounced alteration in female offspring. Furthermore, it was found that the placental expression of *mechanistic target of rapamycin* (*MTOR*), a part of the nutrient sensor *mTOR complex 1 (MTORC1),* along with the *bona fide mTORC1* regulated amino acid transporters *L-type amino acid transporter 1 (LAT1)* and *taurine transporter (TAUT)* were regulated upon

the intervention. These data on alterations in nutrient sensing pathways and amino acid transport were in line with previous studies describing such alterations to be associated with fetal / metabolic programing of obesity risk. An interesting finding of this thesis was that *MTOR* and *LAT1* gene expression was up-regulated only in placentas of female offspring, whereas *LAT1* protein expression, along with methionine levels in umbilical cord plasma, which was likely to depend on the placental *LAT1* transporter, was down-regulated upon the intervention. Moreover, it was shown that *microRNA-99a* was sex-specifically up-regulated in female placentas upon the intervention which suggested that *microRNA-99a* is involved in the mRNA-microRNA expression network regulating placental amino acid transport by targeting *mTOR* and potentially *TAUT* and *LAT1.* For the placenta, only a few of the sex-specific expression changes were correlated with placental estradiol-17β / testosterone ratio or placental and umbilical cord plasma testosterone levels, which were found to be changed upon the n-3 LCPUFA intervention. This observation indicated that sex chromosomes (X, Y) contribute to a larger extent to sexual dimorphism in autosomal gene expression than sex steroids. With regard to programming fetal / metabolic programming of offspring obesity risk, it was shown that although placental *LAT1* gene expression was positively correlated with higher offspring weight at one year upon the n-3 LCPUFA intervention, there were no significant differences observed for offspring body weight at one year, neither in females nor all offspring of both whole INFAT population and the INFAT subpopulation. Potential counteracting mechanisms, as indicated by *proliferating cell nuclear antigen (PCNA)* / *cyclin-dependent kinase 6 (CDK6)* and *microRNA-99a* for offspring birth weight, could have contributed to the absence of differences upon n-3 LCPUFA supplementation during pregnancy. It is tempting to speculate whether a similar impact of the intervention could be exerted on the developing offspring adipose tissue, since expression of *mTOR, LAT1, TAUT, PCNA, CDK6* and *microRNA-99a* in adipocytes has been reported. Hence, since there have been no obvious differences in adipose tissue development observed upon the n-3 LCPUFA supplementation in the INFAT study so far, the INFAT follow-up to five years of age is necessary to assess whether this sex-specific impact of the maternal n-3 LCPUFA supplementation during pregnancy increases adipose tissue or weight more pronounced in female offspring later in life or if counteracting mechanisms as indicated by *microRNA-99a* prevent this.

Zusammenfassung

Es werden dringend effektive Strategien benötigt, um die weltweit steigende Adipositas-Prävalenz insbesondere bei Kindern zu bewältigen. Eine vielversprechende Hypothese für eine Primärprävention der Adipositas wurde 2004 von Ailhaud und Guesnet (Obes Rev 2004 5(1):21-26) aufgestellt, die vorschlugen, dass ein verringertes Omega-6 (n-6) zu Omega-3 (n-3) Verhältnis der langkettigen ungesättigten Fettsäuren (LCPUFAs) in der mütterlichen Ernährung während der Schwangerschaft und Stillzeit zur Programmierung eines geringeren Adipositasrisikos der Nachkommen führen könnte. Diese Hypothese wurde in einer randomisierten, kontrollierten humanen Interventionsstudie, genannt INFAT-Studie (**I**mpact of **n**utritional **f**atty acids during pregnancy and lactation on early **a**dipose **t**issue development) unter der Leitung von Prof. Hauner (Lehrstuhl für Ernährungsmedizin, TUM) untersucht.

In diesem Kontext stellt die Plazenta eine einzigartige Möglichkeit dar, um den molekularen Einfluss eines reduzierten n-6/n-3 LCPUFA-Verhältnisses in der mütterlichen Ernährung während der Schwangerschaft auf ein Gewebe der Nachkommen zu untersuchen, da die Plazenta relativ einfach zugänglich ist und zu einem großen Anteil aus extraembryonalem Mesoderm und Trophoblasten besteht. Des Weiteren spielt die Plazenta möglicherweise eine Schlüsselrolle in der fetalen/metabolischen Programmierung, die häufig assoziiert mit geschlechtsspezifischen Auswirkungen beschrieben wird. Ferner wurden geschlechtsspezifische Unterschiede für die Plazenta, Fettgewebsverteilung sowie dem LCPUFA-Status und -Metabolismus in erwachsenen Menschen berichtet. Diese Beobachtungen und das Wissen, dass LCPUFAs vorwiegend über die Regulation der Genexpression wirken und microRNAs an der Regulation der mRNA-Expression und Plazentaphysiology beteiligt sind, führten zu dem folgenden Hauptziel dieser Arbeit: die Analyse der Wirkung eines reduzierten n-6/n-3 LCPUFA-Verhältnisses in der mütterlichen Ernährung während der Schwangerschaft auf die plazentare Genexpression in einer definierten Subgruppe der INFAT-Studie. Dem Ziel dieser Arbeit folgend, wurde zunächst die plazentare Genexpression durch Transkriptomanalysen und RT-qPCR-Experimente ermittelt und unter Berücksichtigung von geschlechtsspezifischen Unterschieden analysiert. Anschließend wurden die Auswirkungen der n-3 LCPUFA Intervention auf das plazentare mRNA-microRNA Netzwerk und dessen potentieller Zusammenhang mit biologischen Vorgängen durch ein exploratives miRNome-Profil in Kombination mit bioinformatischen Auswertungen analysiert und mittels molekularbiologischer Methoden (RT-qPCR und Western blot) validiert. Abschließend wurden die Beziehungen zwischen Veränderungen der plazentaren Genexpression und Metabolitenkonzentrationen im Nabelschnurblutplasma mit dem Adipositasrisiko der Nachkommen ermittelt.

In dieser Arbeit wurde für die Plazenten der INFAT-Subgruppe, die auch die exzellente Einhaltung der Studienintervention der gesamten INFAT-Studienteilnehmerinnen widerspiegelt, gezeigt, dass die mütterliche n-3 LCPUFA-Supplementierung die plazentare Genexpression geschlechtsspezifisch beeinflusst und deutlichere Veränderungen in weiblichen Nachkommen auftreten. Des Weiteren wurde herausgefunden, dass durch die Intervention die plazentare Expression der Gene *mechanistic target of rapamycin (MTOR)*, [ein Teil des Nährstoff-Sensors *mTOR complex 1 (mTORC1)*] und auch seine *bona fide mTORC1*-regulierten Aminosäuretransportern *L-type amino acid transporter 1 (LAT1)* und *taurine transporter (TAUT)* reguliert werden. Diese Daten stimmen mit früheren Berichten überein, die ähnliche Veränderungen in Nährstoff-Sensor-Stoffwechselwegen und im Aminosäuretransport assoziiert mit einer fetalen/metabolischen Programmierung des Übergewichtsrisikos beschreiben. Interessanterweise wurde nur in Plazenten weiblicher Nachkommen die Genexpression von *MTOR* und *LAT1* durch die Intervention heraufreguliert, wohingegen die *LAT1*-Proteinexpression zusammen mit den Methioninspiegeln im Nabelschnurplasma, die möglicherweise vom plazentaren *LAT1*-Transport abhängen, herunterreguliert wurden. Ferner wurde die *microRNA-99a* durch die Intervention geschlechtsspezifisch in weiblichen Plazenten heraufreguliert. Die *microRNA-99a* könnte über eine Regulation von *MTOR* sowie möglicherweise auch *TAUT* und *LAT1* am mRNA-microRNA-Netzwerk beteiligt sein, das den plazentaren Aminosäuretransport reguliert. Die geschlechtsspezifischen Gen- und microRNA-Expressionsveränderungen sind jedoch nur geringfügig mit dem Estradiol-17β/Testosteron-Verhältnis oder dem Plazenta- sowie Nabelschnurplasma-Testosteronkonzentrationen korreliert, obwohl diese ebenso durch die Intervention verändert waren. Diese Beobachtung spricht dafür, dass Geschlechtschromosomen (X, Y) einen größeren Einfluss auf Sexualdimorphismen der plazentaren autosomale Genexpression ausüben als die Geschlechtshormone. In Bezug zur Programmierung des Adipositasrisikos der Nachkommen zeigte die plazentare *LAT1*-Genexpression eine positive Korrelation mit dem Körpergewicht zum ersten Lebensjahr, welches durch die Intervention aber weder Unterschiede in den Mädchen noch geschlechtsunabhängig in der gesamten INFAT-Population oder der INFAT-Subpopulation aufwies. Mögliche gegenregulatorische Mechanismen, wie sie für das Geburtsgewicht mit *proliferating cell nuclear antigen (PCNA) / cyclin-dependent kinase 6 (CDK6)* und *microRNA-99a* in dieser Arbeit angedeutet wurden, könnten zur Abwesenheit von Unterschieden durch die n-3 LCPUFA-Intervention während der Schwangerschaft beigetragen haben. In Analogie zu den Plazentadaten, könnten ähnliche Einflüsse der Intervention auch auf das sich entwickelnde Fettgewebe in den Nachkommen ausgeübt haben, da die Gene für *mTOR, LAT1, TAUT, PCNA, CDK6* und *microRNA-99a* auch in Adipozyten exprimiert sind. Obwohl in den Untersuchungen der INFAT-Studie bis jetzt keine deutlichen Einflüsse der

mütterlichen n-3 LCPUFA-Supplementierung auf das Fettgewebe oder Körpergewicht in Mädchen festgestellt wurden, sind Nachuntersuchung, wie zum Beispiel im INFAT-Follow-up bis zum fünften Lebensjahr notwendig, um herauszufinden, ob geschlechtsspezifische oder gegenregulatorische Mechanismen aufgrund der Intervention wie sie in dieser Arbeit beschrieben wurden, bezüglich des Fettgewebes oder Körpergewichts erst zu diesem späteren Zeitpunkt zur Ausprägung kommen.

1. Introduction

1.1. Fetal programming - a strategy to prevent the obesity epidemic?

1.1.1. The obesity epidemic

Obesity is characterized by an excessive fat accumulation in adipose tissue and increases the risk for several non-communicable diseases like cardiovascular disease, type 2 diabetes or some forms of cancer [1,2]. Body mass index (BMI) is the most widely used parameter for obesity diagnosis. A BMI above 25 and 30 kg/m2 classifies overweight and obesity respectively [1]. Worldwide over 200 million men and nearly 300 million women were obese in 2008 and more than 40 million children under the age of five were overweight in 2010 (http://www.who.int/mediacentre/factsheets/fs311/en/). In Germany, the obesity prevalence between 2004 and 2008 was 25% in adult men and 22% in adult women [3]. In German children starting school, the obesity prevalence (BMI > 97^{th} percentile of German reference values) ranged between 3% and 5% in 2008, but the increase in prevalence seems to be attenuated in the individual German states [4]. This attenuation of the increase in obesity prevalence in German children is in accordance with data from many other countries, including the US, Australia and the UK. In adults, the data for an increase in obesity prevalence are more heterogeneous, since leveling off and further increases are reported in different populations [5]. Despite this leveling off in several populations, the prevalence of obesity and overweight in children, adolescents and adults still resides at an epidemic level [1,5]. The individual and economic costs e.g. for treatment of obesity and its associated diseases, loss of productivity and health-care programs are an enormous economic burden, that will rise further [6]. Several strategies are applied to treat obesity, including dietary, life-style, exercise and pharmacological interventions as well as surgery. So far, the current dietary strategies do not exert the desired effect, due to the absence of sustainable weight reduction, while more severe weight reducing strategies, like medications and surgeries, can have serious complications [2]. Therefore, effective primary obesity prevention strategies are important, but still more research is necessary [7]. There is evidence from human and animal studies that already prenatal prevention could be a possible strategy to manage the obesity epidemic [8,9]

1.1.2. Fetal programming of obesity

The ‘thrifty phenotype hypothesis’ suggested by Hales and Barker was the first concept, that summarized the hints that a poor nutrition in fetal and early infant life increases susceptibility to later non-communicable diseases [10]. The following concepts of ‘metabolic programming’, which is also called ‘fetal, nutritional or metabolic programming’ [11,12] and DOHaD ‚developmental origins of health and disease’, all comprise that an early life event can have long-lasting consequences on health and disease risk [13]. These concepts have drawn the attention to pregnancy and lactation as critical windows for early prevention of obesity [14-17]. Fetal programming does not directly cause obesity, but may alter an individual’s sensitivity to an adipogenic environment, which then alters the risk for developing obesity in later life [16]. Both, *in utero* under-nutrition (e.g. intrauterine growth retardation) as well as *in utero* over-nutrition (e.g. high gestational weigh gain, maternal obesity or diabetes) increase offspring obesity risk, which seems to be responsible for the observed U or J-shaped association between birth weight and later obesity [16]. Epidemiological studies of the ‘Dutch famine’ / ‘hunger winter’ provided first evidence for a possible programming of obesity during pregnancy by *in utero* under-nutrition [18]. The obesity prevalence was lowest in 19 year old male offspring of mothers who experienced severe caloric restriction during their last trimester of pregnancy compared to male offspring with the same age without maternal caloric restriction during pregnancy. In contrast, obesity prevalence was increased in 19 year old male offspring of mothers exposed to severe caloric restriction during the first two trimesters of pregnancy [18]. Evidence for an association of *in utero* over-nutrition and obesity risk was provided by epidemiological studies in Pima Indians. It was shown that infants of diabetic mothers had a higher obesity prevalence from birth up to 19 years, compared to infants of pre-diabetic or non-diabetic mothers [19]. These and more studies in animals and humans provide evidence for a programming of obesity risk during pregnancy [9,20-22]. Experimental studies examining the underlying mechanisms for *in utero* programming of obesity risk were remarkable similar in their outcome for various maternal stimuli. Therefore, a common underlying mechanism was suggested for *in utero* under- and over-nutrition. Such a common mechanism could be an altered transfer of metabolic substrates between mother and fetus (e.g. glucose and amino acids). This alteration in the transfer of metabolic substrates can affect developmental structure and function of energy metabolism in fetal organs like pancreatic β-cells, hypothalamus, muscle and adipose tissue [15,16]. In addition, epigenetic mechanisms are discussed to mediate fetal / metabolic programming [20], which will be explained in detail (see **chapter 1.3.5**). However, the understanding of the underlying molecular mechanisms for programming obesity risk is still at the beginning [20].

1.1.3. Long chain polyunsaturated fatty acids (LCPUFAs) and fetal / metabolic programming of obesity risk

Based on *in vitro* and animal studies, Ailhaud and Guesnet suggested that lower obesity risk can be programmed by a decreased nutritional omega-6 / omega-3 long chain polyunsaturated fatty acid ratio (n-6/n-3 LCPUFA ratio) during pregnancy and lactation [8]. They showed that offspring of mice fed with a high-fat diet containing a low n-6/n-3 fatty acid ratio before mating and during pregnancy / lactation period, had lower body weight, from weaning until adulthood, compared to offspring of mice fed a high-fat diet containing a high n-6/n-3 fatty acid ratio [8,21]. Therefore, a decrease in the n-6/n-3 LCPUFA ratio during pregnancy in humans could represent a primary prevention strategy for childhood obesity [8]. Massiera *et al.* [21] proposed that the omega-6 (n-6) LCPUFA arachidonic acid (AA) via its metabolite prostacyclin activates the prostacyclin receptor and thereby promotes the transcriptional program for adipocyte differentiation and maturation (C/EBPβ/δ and PPARγ; Figure 1). Furthermore, they demonstrated that this stimulatory effect of AA on adipocyte differentiation and maturation was inhibited by the n-3 LCPUFAs eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). Therefore, they concluded that n-6 LCPUFAs are more potent in stimulating adipogenesis than n-3 LCPUFAs [21].

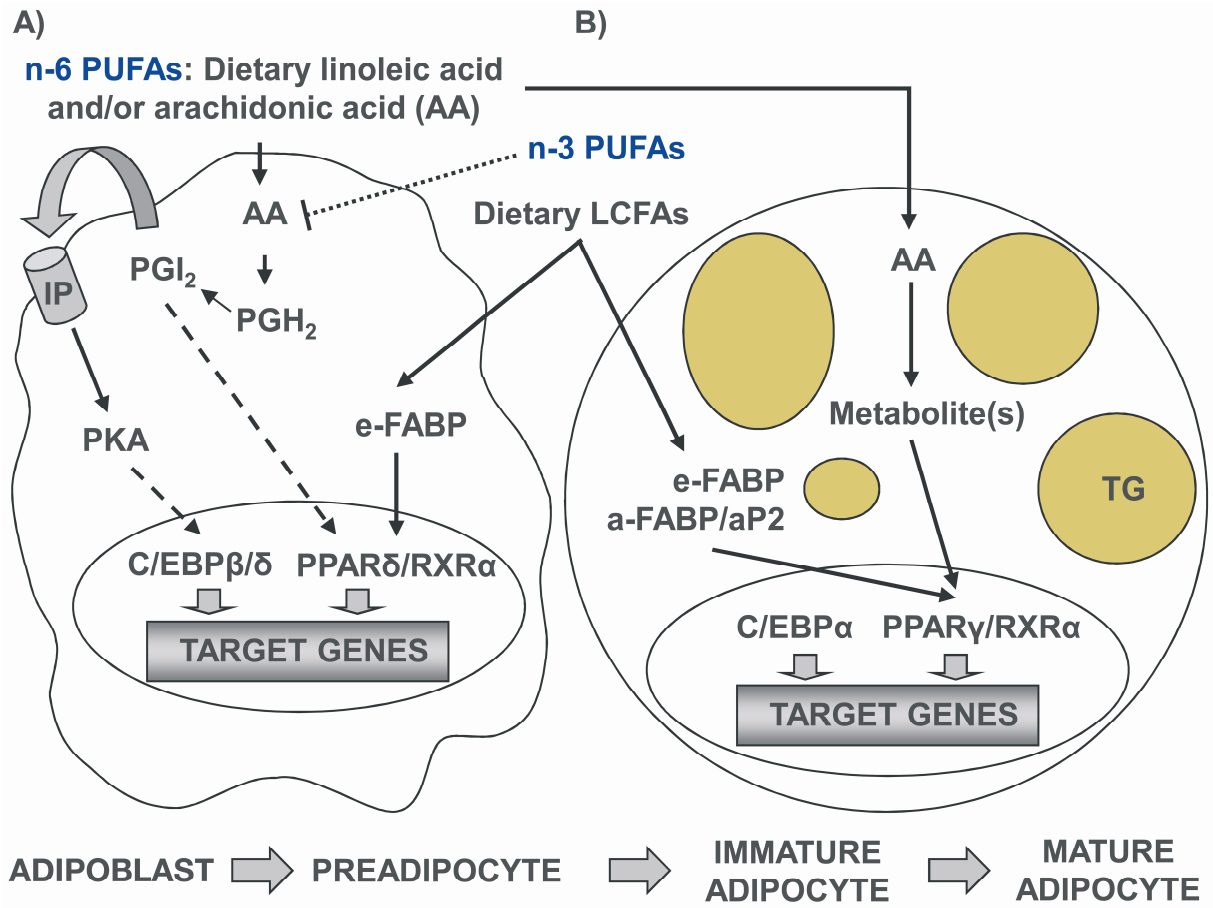

Figure 1: Involvement of dietary n-6 and n-3 polyunsaturated fatty acids in adipogenesis. *A) Dietary n-6 PUFAs like linoleic acid and AA are metabolized to prostaglandins in the preadipocyte. The derived prostacyclin (PGI_2) stimulates the prostacyclin receptor (IP), which via protein kinase A pathway (PKA) and a complex signaling cascade activates CCAAT / enhancer binding protein β (C/EBPβ) and C/EBPδ. Additionally, PGI_2 is assumed to bind also to PPARβ/δ. By this means, n-6 PUFAs enhance the differentiation process in the preadipocyte. N-3 PUFAs are less potent in stimulating adipocyte differentiation by interfering with the production of AA and / or PGI_2. B) In the immature adipocyte, metabolites of the AA can also impact differentiation / maturation as ligand of PPARγ. Other dietary long chain-fatty acids (LCFAs) can also influence PPARβ/δ and PPARγ as ligands. Epidermal (keratinocyte) fatty acid binding protein (e-FABP) in preadipocytes and also adipocyte fatty acid binding protein (a-FABP / aP2) in adipocytes are assumed to bind and transport LCFAs. TG, triglycerides. Modified from Massiera et al. [21]*

1.2. N-6 and n-3 long chain polyunsaturated fatty acids (LCPUFAs)

The n-6 and n-3 PUFAs, which were shown to impact adipogenesis, belong to the class of polyunsaturated fatty acids. Besides the classes of saturated and monounsaturated fatty acids, the polyunsaturated fatty acids (PUFAs) are characterized by two or more double bonds in their carbon chain skeleton [23]. The unsaturated fatty acids are classified into families according to the location of the double bond closest to the methyl end of the carbon chain skeleton. The n-6 PUFAs possess a double bond at the sixth carbon while the n-3 PUFAs possess the double bond at the third carbon counted from the methyl end. The position of the double bond is depicted by n or ω [23,24]. AA (20:4n-6) and linoleic acid (LA, 18:2n-6) belong to the n-6 PUFAs family, whereas α-linolenic acid (ALA, 18:3n-3), EPA (20:5n-3) and DHA (22:6n-3) belong to the n-3 PUFA family. Besides the n-6 and n-3 families, also other families exist like the n-9 and n-7 families. Within the family of PUFAs, a further classification is made, according to the chain length. Fatty acids consisting of a 20-24 carbon chain are also named long-chain PUFAs (LCPUFAs). Therefore, EPA and DHA belong to the n-3 LCPUFAs, whereas AA to the n-6 LCPUFAs [23].

1.2.1. Biosynthesis of n-6 and n-3 LCPUFAs

LCPUFAs and PUFAs from the n-6 and n-3 series are essential, because their endogenous biosynthesis is based on essential precursors. These precursors are dietary ALA (18:3n-3) for the n-3 family and LA (18:2n-6) for the n-6 family [24-26]. ALA and LA cannot be synthesized by humans and animals due to the lack of the *FAD8* (*Δ15-desaturase*) enzyme [24,26,27]. Moreover, the fatty acids from the n-6 family cannot be converted into the fatty acids of the n-3 family and vice versa [24,25].

The biosynthesis of n-6 and n-3 LCPUFAs from LA and ALA primarily occurs in the liver. It is catalyzed by *fatty acid desaturases 1 and 2* (*FADS1* / *Δ5-desaturase and FADS2* / *Δ6-desaturase*) and *elongases* (*ELOVL2* / *5*) which are responsible for a series of elongation and desaturation processes as depicted in **Figure 2A** [25,28]. Limited retro-conversion of DHA to EPA by peroxisomal β-oxidation is also possible [25]. However, the access of the different PUFA families to the enzymes for LCPUFA biosynthesis is not equal, because the rate liming enzyme *FADS2* shows a preference of n-3 compared to n-6 [24,25,27]. Despite this preference, there is a fairly inefficient conversion of ALA to DHA and EPA [29]. Therefore, a high intake of n-3 LCPUFA precursor ALA alone could not in any case be sufficient to cover the n-3 LCPUFA demand, especially for the increased demand in pregnancy and postnatal [25,29,30]. It can be necessary to include EPA and DHA direct in the diet. Predominant nutritional sources of n-3 LCPUFAs (EPA and DHA) are fish, other sea

food, or fish oil supplements, whereas n-6 LCPUFAs (AA) are predominantly obtained by the consumption of meat and meat products [25,31,32].

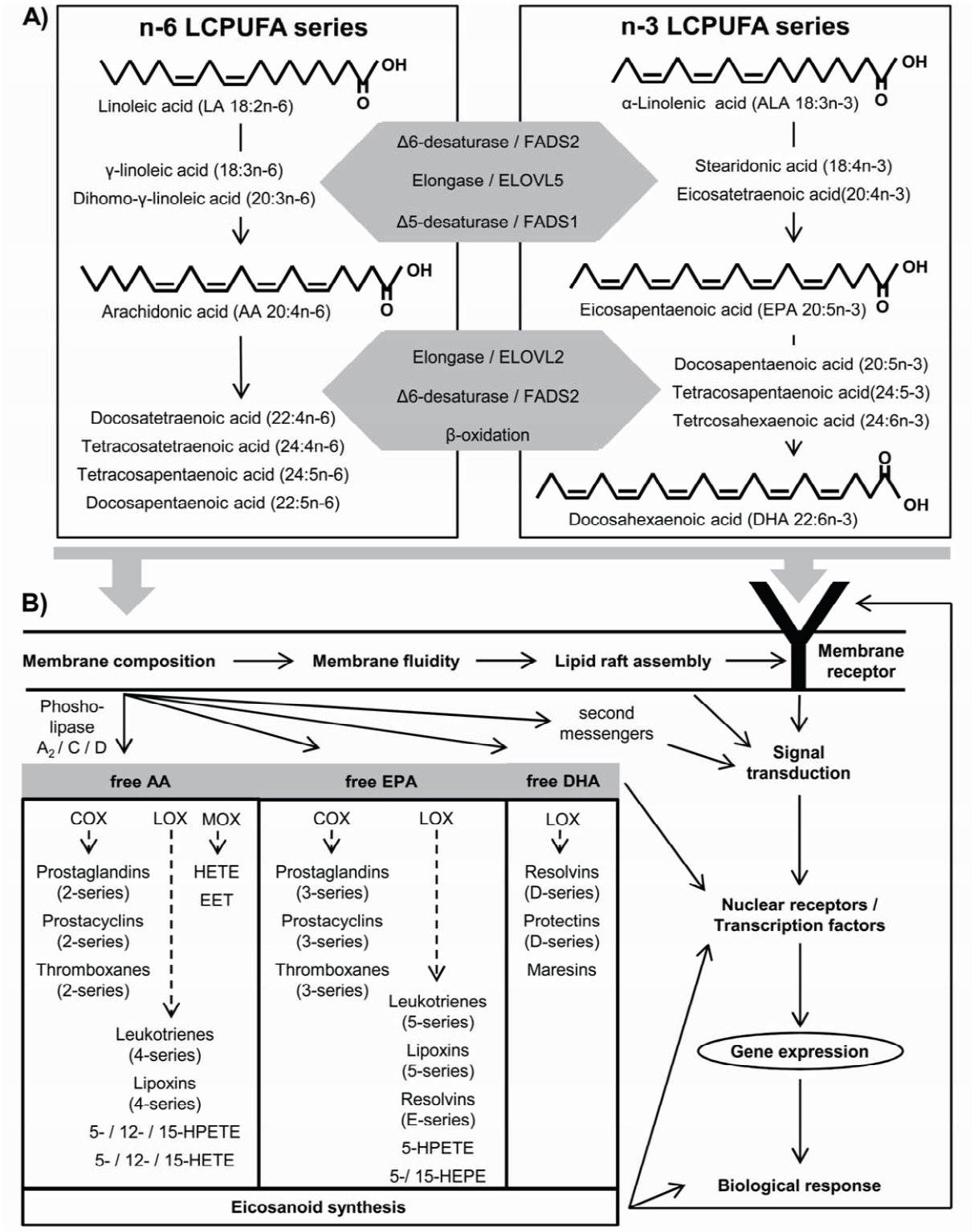

Figure 2: Schematic outline of n-6 and n-3 LCPUFA biosynthesis and the impact of n-6 and n-3 LCPUFAs and their derivatives on gene expression. *Summary based on [37,39,43,47]. Details are described in chapter 1.2. Cyclooxygenase 1 or 2 (COX); 5- / 12- / 15-lipoxy-genase (LOX); cytochrome P450 monooxygenase (MOX); Hydroxyeicosatetraenoic acid (HETE); hydroperoxyeicosatetraenoic acid (HPETE); Hydroxyeicosapentaenoic acid (HEPE); epoxyeicosatrienoic acid (EET);*

1.2.2. N-6 and n-3 LCPUFAs as precursors for eicosanoid synthesis

N-6 and n-3 LCPUFAs are the only precursors for eicosanoid synthesis, which are produced on demand (**Figure 2B** box: eicosanoid synthesis) [33]. The short-living eicosanoids are involved in a lot of different systems, like cardiovascular, reproductive, endocrine, immune systems and many more. Thereof, the n-6 and n-3 LCPUFAs and their eicosanoids are of great importance for many physiological functions. Several kinds of eicosanoids exist, like prostaglandins, prostacyclins, thromboxanes, leukotrienes and lipoxins, which are produced by a handful of enzymes. However, depending whether the eicosanoids are derived from the n-6 or the n-3 series, they exert different actions [33,34]. The metabolism of n-6 and n-3 LCPUFAs to eicosanoids will be explained in more detail in the following paragraphs.

Independent whether the LCPUFAs are derived from diet or endogenous synthesis, they first have to be activated to LCPUFA acyl-CoA-thioesters (LCPUFA-CoA) by *FA-CoA synthetases* for being metabolized [35]. Such activated LCPUFA-CoAs are usually incorporated into membrane phospholipids at the sn-2 position, whereas saturated or mono-unsaturated fatty acids are incorporated at the sn-1 position [36]. For eicosanoid synthesis, LCPUFAs, predominantly AA, but also EPA and DHA are released from membrane phospholipids by *phospholipase A_2 or phospholipase C* in combination with *diacylglycerol* (*DAG;* **Figure 2B**). AA is then converted to prostaglandins (PG), prostacyclins (PI) and thromboxanes (TX) of the 2-series by *type 1 and 2 cyclooxygenase* (*COX1* / *PTGS1* and *COX2* / *PTGS2*) or to leukotrienes (LT) of the 4-series, hydroperoxy-eicosatetraenoic acids (HPETE), hydroxyeicosatetraenoic acids (HETE) and lipoxins by 5-, 12-, or 15-lipoxygenases (LOX) [37]. Additionally, AA can also be converted to HETE, epoxyeicosatrienoic acid (EET) and hydroxyeicosatetraenoic acids (OH-AA) by cytochrome P450 monooxygenase pathway (MOX) [38]. Eicosanoids usually are short-living and regulate inflammation, platelet aggregation, vasoconstriction and immune functions by paracrine and autocrine processes [25,37,39].

In contrast, when available, n-3 fatty acids are preferentially incorporated into membrane phospholipids compared to n-6 fatty acids. Therefore, an increase in dietary n-3 LCPUFAs often leads to alterations in membrane fatty acid composition, with different sensitivity to incorporate these LCPUFAs in tissue or organelle membrane [40]. An increase in n-3 LCPUFAs in membrane phospholipids reduces the availability of AA for eicosanoid production. Furthermore, EPA can be converted to PG and TX of the 3-series, as well as LTs of the 5-series, lipoxins, HPETE and hydroxyeicosapentaenoic acid (HEPE) by *COX* and *LOX*. However, AA is a better substrate than EPA for *COX* and vice versa for *LOX* [37,41,42]. Moreover, EPA and DHA also can directly inhibit COX enzyme activity and eicosanoid metabolites from EPA are generally less potent than those produced from AA

[37]. In addition, the lipid mediators resolvins and protectins, derived only from n-3 LCPUFAs, were identified to possess anti-inflammatory and inflammation resolving effects [43]. Therefore, in humans consuming western diet, high in n-6 FAs, the high amount of AA-derived eicosanoids leads to a pro-inflammatory state. In contrast a consumption of fish or fish-oil rich in EPA and DHA can lead to a more physiologic state of inflammation by leading to a) the production of protective eicosanoids, b) eicosanoids with less potent inflammatory properties than AA-derived ones and c) a reduction in AA-derived eicosanoids [25,44]. These and yet undiscovered processes mediate the beneficial effects of n-3 fatty acids in ameliorating or decreasing the risk for several diseases like cardiovascular disease, type 2 diabetes and some forms of cancer. Additionally, a n-3 LCPUFA rich diet can also decrease disease risk factors by their hypolipidemic, antithrombotic, hypotensive, anti-inflammatory and insulin-sensitizing effects [25,26,45]. However, the effects of n-3 PUFAs for reducing obesity in humans remain to be clarified [8,46].

1.2.3. The impact of n-6 and n-3 LCPUFAs on gene expression

One important mechanism how the effects of n-6 LCPUFAs, n-3 LCPUFAs and their derivatives are mediated is by regulating gene expression [34]. LCPUFAs influence gene expression indirectly via changes in membrane composition, alterations in second messenger concentrations, and activation of membrane receptors as well as directly by regulating nuclear receptors (**Figure 2B**). The impact of LCPUFAs on gene expression was mainly studied in hepatocytes, *in vivo* and *in vitro*, but also in cells of adipose tissue, small intestine, pancreas, immune system and the neonatal mouse brain revealing tissue specific effects [48].

Indirect mechanisms to influence gene expression by LCPUFAs contain: A) alterations in membrane composition and fluidity leading to changes in cell signaling via disturbances of lipid rafts and alterations in membrane protein function and trafficking (**Figure 2B**) [39]. The underlying mechanism comprises that membranes consisting of phospholipids with incorporated LCPUFAs are more fluid than phospholipids containing saturated fatty acids [36]. B) Second messengers are released from LCPUFAs at the sn-2 position of membrane phosphatidylinositol or -choline by *phospholipase C / D or protein kinase C* (**Figure 2B**). The effects of the second messenger on cell signaling depends on the fatty acid type they are derived from [37]. C) Eicosanoids and LCPUFAs are able to activate G-protein coupled surface receptors (GPR) like eicosanoid receptors, GPR40 and GPR120, which results in activation of intracellular signaling (**Figure 2B**) [47]. All these signals are interrelated, but not exclusively. They integrate via cell signaling on transcription factors or nuclear receptors mediating their effects on gene expression (**Figure 2B**) [48].

Transcription factor activity is either regulated by this LCPUFA-mediated signaling or by direct activation with LCPUFAs as transcription factor ligands, but also nuclear abundance of transcription factors can be regulated (**Figure 2B**) [49]. The most accepted transcription factors activated directly by LCPUFAs or their derivatives are *peroxisome proliferator activated receptors* (*PPAR-α, -β/δ, -γ1* and *–γ2*)*, hepatic nuclear factor 4α (HNF-4α*), *retinoic acid X receptor* (*RXR*), and *liver X receptor α* (*LXRα*). Whereas, *sterol regulatory element binding protein 1* (*SREBP1*), *nuclear factor κ B* (*NFκB*), *carbohydrate regulatory element binding protein* (*ChREBP*), *max-like factor X* (*MLX*), *CCAAT / enhancer binding protein β* (*C/EBPβ*)*, and hypoxia-inducible factor 1* (*HIF1α*) are regulated by LCPUFAs or their derivatives independently of ligand binding [34,39,45,48].

Transcriptome analyses in animals and human cell cultures showed that an increase in n-3 PUFAs influences expression of genes involved in hepatic lipid metabolism (increase in lipid oxidation and decrease in lipogenesis), oxidative stress response, antioxidant capacity, prostaglandin synthesis, cell proliferation, cell growth, cell signaling and transduction [50].

1.2.4. The relevance of n-6 and n-3 LCPUFAs in pregnancy

The importance of n-6 and n-3 LCPUFAs is not only reflected by gene regulatory or immune modulatory functions, they also have relevant functions during pregnancy. N-6 and n-3 LCPUFAs are essential for fetal / neonatal growth and development, especially for visual and cognitive maturity [25,51], for offspring immune function [52,53] as well as for initiation of labor [54]. Additionally, positive effects for LCPUFAs are discussed for gestational diabetes (GDM) [55], preeclampsia [56] and psychiatric diseases [57]. Therefore, n-3 intervention studies during pregnancy were conducted to analyze potential improvements for maternal and fetal outcomes.

N-3 LCPUFA supplementation of pregnant women was suggested to improve maternal health. However, in humans, evidence is lacking for a decreased risk of GDM or preeclampsia upon maternal n-3 LCPUFA supplementation during pregnancy, although animal and epidemiological studies suggested a protective effect [56,58]. Furthermore, n-3 LCPUFA supplementation was also suggested for preventing maternal *post-partum* depression. However, further studies are necessary to investigate also the effects of EPA or DHA, separately [59].

In the fetus, AA and DHA are major components of cell membranes. Therefore, they are especially important for membranes in fetal nervous and visual systems, but also in all other developing organs [25]. An appropriate fetal supply with n-6 and n-3 LCPUFAs is therefore associated with fetal growth and maturation of numerous organ systems, fetal brain development and function, offspring visual function, learning, behavior and a more mature

neonatal sleep-state patterning [25,60]. However, the benefits of a short and long-term n-3 LCPUFA supplementation during pregnancy are not consistent for outcomes on psycho-motor, mental and visual acuity development [61]. In contrast, maternal n-3 LCPUFA supplementation in the second half of pregnancy was demonstrated to increase birth weight by around 50 g and birth length between 0.23 and 0.48 cm. This effect is mediated in large parts by an increase in gestational length of two to three days which is suggested to be associated with alterations in labor inducing prostaglandins [54,56,62]. Moreover, a lower relative risk for birth before the 34th, but not before the 37th week of gestation, was shown [56,62]. Additionally, data from intervention and epidemiological, suggested a protective role of n-3 LCPUFAs in pregnancy for sensitization to common food allergens and a reduced expression of allergic disease in the first year of life [63].

In summary, only a few intervention studies showed a beneficial impact of a maternal n-3 LCPUFA supplementation during a low-risk pregnancy on offspring or mother herself. To date, pregnant women are recommended to achieve a dietary intake of 200 mg DHA per day to avoid maternal and fetal deficiencies [64]. Despite hints from animal and epidemiological studies, analysis of the impact of a n-3 LCPUFA supplementation or a decreased n-6/n-3 LCPUFA ratio during pregnancy on offspring obesity risk as primary outcome has been neglected in humans, except for the INFAT study. The INFAT study was conducted at the *chair of nutritional medicine, Technische Univerität München, Germany*, by Prof. Hauner and started in the beginning of this thesis [65].

1.3. The human placenta

1.3.1. Development of the human placenta

During pregnancy, the human placenta represents the central interface between mother and fetus [66]. Placental development comprises several stages from pre-implantation stage over prelacunar and lacunar stage to the final villous stage [67]. In the pre-implantation stage the extraembryonic trophoblast cell lineage differentiates out of cells from the morula. These mononucleated trophoblasts constitute the outer layer of the developing blastocyst and surround the inner cell mass (embryoblast) and the blastocoel (blastocyst cavity). In the prelacunar stage, the attachment of the blastocyst to the uterine epithelium leads to differentiation of the attached trophoblasts in oligonucleated syncytiotrophoblasts (ST). The remaining trophoblast cells are then named cytotrophoblasts (CT). The cytotrophoblasts constantly fuse with the syncytiotrophoblast to maintain the syncytium. The syncytiotrophoblasts, exhibiting an invasive phenotype, penetrate the uterine epithelium for implantation of the blastocyst. In the lacunar stage, lacunae develop from fluid filled spaces within the syncytiotrophoblast and the remaining syncytiotrophoblast between the lacunae form the trabeculae, which will later develop in the placental villous trees [67]. Cells from the extraembryonic mesoderm and cytotrophoblasts build up the chorionic plate of the placenta and chorionic cytotrophoblasts migrate via the trabeculae to the maternal side (decidua / basal plate). There they turn into extravillous trophoblasts and connect the placenta with the maternal circulation by remodeling the maternal spiral arteries. In the final villous stage, primary villi, consisting only of trophoblastic structures, start to branch from the trabeculae into the lacunae (intervillous space). Cells from the extraembryonic mesoderm of the chorionic plate also migrate into the trabeculae and primary villi, converting them in secondary villi. These cells stop before they reach the maternal side and the trabeculae without a mesenchymal core are named trophoblastic cell columns. The secondary villi turn into tertiary villi when the cells of the mesodermal core start to differentiate in placental blood cells and vessels [67].

An important regulator of placental development is *PPARγ,* which belongs to the family of ligand activated receptors [68]. To this family belong *PPARα, PPARβ/δ, PPARγ1* and *PPARγ2. PPARγ1* is ubiquitously expressed in every tissue, but knock-out in mice showed severe placental defects at a time point when the placenta takes over embryonic nutrition (E10) [68]. *PPARγ1* is expressed in the human placenta from the 7th week of gestation onwards. In the first trimester, *PPARγ1* is primarily detected in villous cytotrophoblasts and invading extravillous trophoblasts, in the second trimester in columns of the anchoring villi and cytotrophoblasts and in the third trimester in villous syncytiotrophoblasts, as well as

villous and extravillous cytotrophoblasts [68]. Upon activation by small lipophilic ligands (e.g. prostaglandin 15-deoxy- 12,14 Prostaglandin J2, naturally occurring fatty acids and their derivatives - especially LCPUFAs and synthetic thiazolidinediones), *PPARγ* forms a heterodimer with *RXRa* to activate target genes. *PPARγ1* without ligand is able to repress gene expression in combination with promoter-bound co-repressor complexes [68]. Mouse studies showed that *PPARγ1* is essential for placental development, trophoblast invasion, differentiation of trophoblasts into the syncytium, the regulation of fat accumulation in trophoblasts and lipid uptake [68]. Moreover, it is also discussed to modulate the onset of parturition via and increase *PPARγ1* / COX_2 ratio in fetal membranes. In addition also *PPARβ/δ* was shown to have a pivotal role in placental development, but *PPARβ/δ* knock-out mice displayed less severe placental defects than *PPARγ1* [68].

1.3.2. Anatomy of human term placenta

The human term placenta, weighing on average 470 g, is of circular discoidal shape with a diameter of about 22 cm and an average thickness of 2.5 cm. It possesses villous and hemomonochorial properties. The placenta consists of a chorionic plate on the fetal surface, a microvillous fraction within the intervillous space in the middle and a basal plate (decidua) on the maternal surface (**Figure 3a**). Within the chorionic plate two arteries and one vein of the fetal umbilical cord (UC) branch into 16 to 24 pairs of large arteries and veins (chorionic vessels) to supply 60 to 70 main stem villi. Within the central region of the placenta these main stem villi branch into stem villi and further into smaller villi building up the villous tree with terminal villi floating in the intervillous space freely (**Figure 3b**). The intervillous space is filled with maternal blood supplied by endometrial spiral arteries, located in the maternal basal plate and is drained by endometrial veins. Stem villi connect the fetal chorionic plate with the maternal basal plate and stabilize the villous trees. After birth, lobes can be observed at the maternal surface of the decidua, corresponding to the placental septa, which separate the intervillous space within the placenta [67,69].

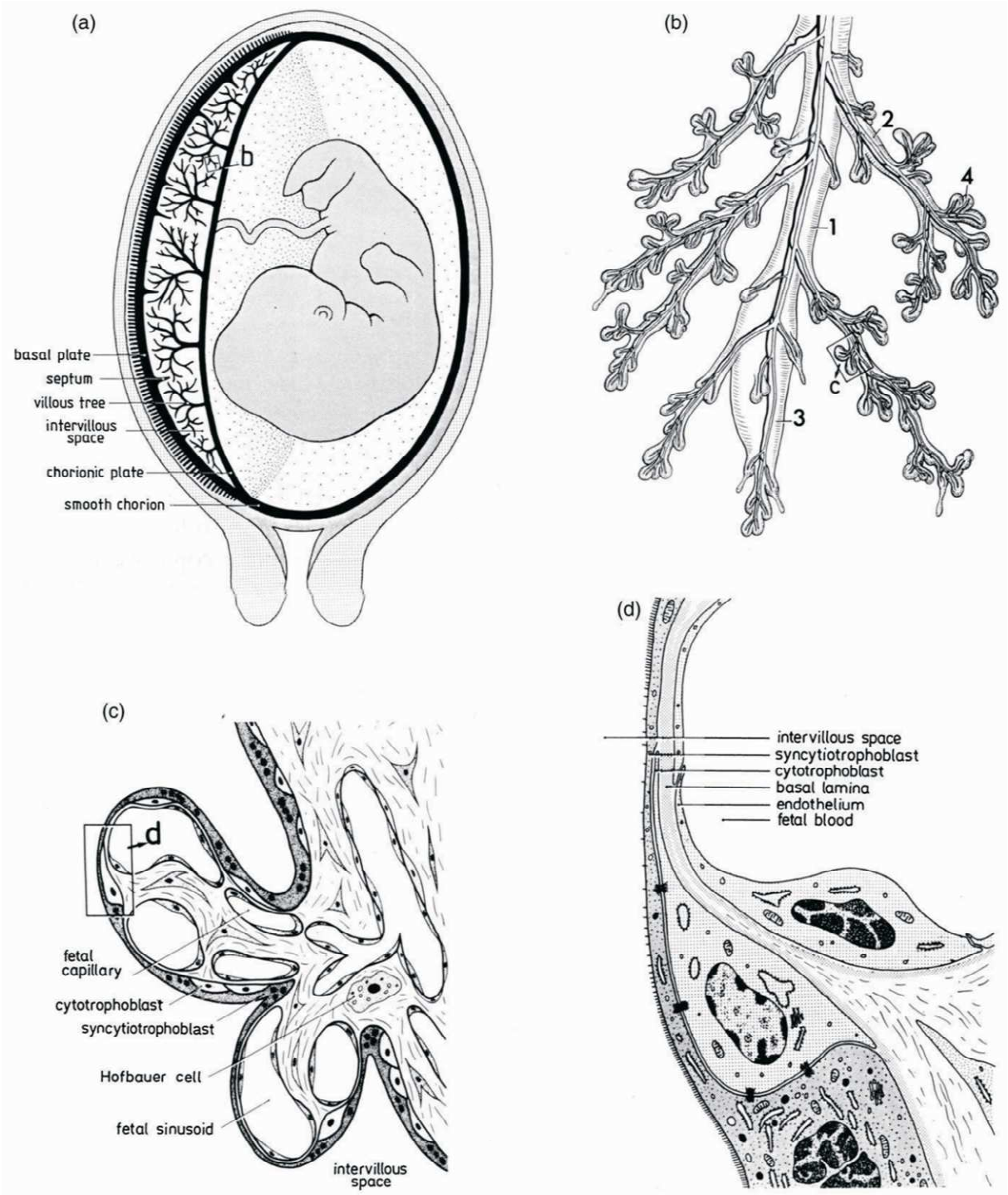

***Figure 3**: **Schematic view of placental structures depicted in increasing resolutions. (a)**The placenta and its branching villous trees are connected to the fetus via the umbilical cord. **(b)** The furthermost branching of a mature villous tree shows the different placental villous types: From the stem villous [1] branch off mature intermediate villi [2], which end in grape-like terminal villi [4]. Stem villi finish in immature villi [3]. In **(c)** a simplified picture of two terminal villi shows the location of placental cell types and the functional units of the placenta, the so called epithelial plates. The four typical layers of an epithelial plate are shown in **(d)**. Reprinted from figure 6.1 on page 56 in chapter 6 'Basic structure of the villous trees' from 'Pathology of the Human Placenta' (6th ed. 2012), with written permission kindly provided by Springer Science+Business Media (original copyright notice in **Appendix 11.22**) [78].*

The syncytiotrophoblast is considered the most important placental cell type, due to its functions in maternal-fetal exchange, placental metabolism as well as in the synthesis of placental hormones and growth factors. It is a multinucleated and polar layer enclosing every villous tree and the intervillous space facing parts of the chorionic and basal plate. Thereby it represents the main cell type in contact with maternal blood (**Figure 3c / d**). The syncytiotrophoblast is derived by fusion of the underlying monolayer of villous CTs (Langhans cells), providing their organelles, proteins and nucleic acids for maintenance of the syncytiotrophoblast. Throughout gestation, the number of villous CTs is reduced, leading to frequent direct contact between syncytiotrophoblast and the basement membrane in term placenta. Below the basement membrane the villous stroma (mesenchymal core) is located, containing fibroblasts, reticulum cells, Hofbauer cells (macrophages), mast and plasma B cells. Further placenta cells are assigned to the fetal vessels including endothelial cells, pericytes and smooth muscle cells. The decidua contains cell types of maternal as well as extraembryonic origin. The extravillous trophoblasts are derived from extra-embryonic tissue, whereas decidual stroma cells, natural killer cells, macrophages as well as other immune cells are derived from the mother [67,69].

1.3.3. Functions of the human placenta

The diverse functions of the placenta substitute fetal organs until their maturity. The main functions of the placenta are supply of the fetus with oxygen and nutrients, waste disposal, production of hormones and protection of the fetus, especially against xenobiotic molecules, infections, maternal disease and maternal immune response (reviewed by [66]). These tasks are mostly dependent on transport mechanisms, as for example certain xenobiotics are transported back from the placenta into maternal circulation [66], while IgG antibodies are transported to the fetal circulation by endocytosis [70].

Furthermore, the placenta produces a variety of hormones and most of them are released into the maternal circulation, but some are also released into the fetal circulation. These hormones mediate paracrine or autocrine effects on metabolism, fetal growth, maintenance of pregnancy, immune tolerance, parturition and other functions. Placental hormones, mainly produced by the syncytiotrophoblast layer, are estrogen, progesterone, eicosanoids, chorionic gonadotrophin, placental lactogen, placental growth hormone, insulin-like growth factor 1 and 2, leptin vasoactive autacoids, pregnancy associated proteins and many others [66,69].

From gestational week 10 to 12 onwards, the placenta plays a key role in fetal nutrient supply, since almost all nutrients have to cross the placental barrier. The fetus is provided with oxygen, water, carbohydrates, amino acids, lipids, vitamins, minerals and other nutrients

by simple diffusion, facilitated or active transport. Transport mechanisms are also involved in removing fetal waste products like carbon dioxide. The functional units of the placenta, responsible for nutrient transport from maternal to fetal circulation, are terminal villi containing the epithelial plates (**Figure 3d**). Within these epithelial plates the syncytiotrophoblast layer facing the maternal circulation and the highly branched sinusoids of the fetal capillary endothelium reside in close proximity (0.5 – 1.0 µm) to allow maternal-fetal exchange, but they still separate maternal and fetal circulation [66,69]. The two polarized membranes of the syncytiotrophoblast, the apical microvillous and the basolateral basal plasma membrane, regulate placental transfer, since endothelial cells of the fetal capillary endothelium contain permeable paracellular clefts [71,72]. The factors which influence placental exchange are blood flow in umbilical cord and placenta, concentration gradients, placental metabolism, as well as number and activity of transport proteins. Transport proteins in the syncytiotrophoblast barrier seem to be the primary regulating factor for the transfer of macronutrients like glucose, amino acids and fatty acids [71].

The transporters with the highest relevance for term placental glucose transfer are the facilitated glucose transporters *GLUT1 (SLC2A1)* and *GLUT3 (SLC2A3). GLUT1* is ubiquitous expressed throughout the term placenta, whereas the high affinity transporter *GLUT3* primarily expressed in the endothelial cells of feto-placental vessels and stroma cells at term [66,73]. Amino acids are transported to the fetal circulation by various families of selective active sodium-dependent and sodium-independent amino acid transporter. These families are either assigned to accumulative transporters (System A and X^-_{Ag}), exchangers (System y^+L, $b^{0,+}$, asc and several transporter of system L) and facilitated diffusion (System T, y^+ and several transporter of system L) or to monomeric or heterodimeric composition [74]. For uptake and translocation of fatty acids, the placenta expresses several *fatty acid transport proteins* [*FATP* (*SLC27A*) *1- 4* and *6*] and *fatty acid binding proteins* [*FABP1, 3 – 5* and *7*]. LCPUFAs are crucial for fetal development and their fetal and placental transformation from precursors is limited. Therefore, the fetus depends on the active placental transport from maternal circulation [75,76]. In placental transport, LCPUFAs, especially DHA, and essential fatty acids are preferred over non-essential fatty acids, which is called bio-magnification [76,77]. Although, there is already substantial knowledge about placental transport mechanisms, further investigations are necessary to obtain deeper insight in the detailed mechanism and regulation of placental transport [76].

1.3.4. Pathologies of the human placenta

There are manifold pathologies known in human singleton placentas, which can be roughly distinguished by disruption of maternal or fetal vascular supply, inflammation or invasion into the uterine wall [79,80]. The impact of these placental pathologies can range from unremarkable to fetal or maternal death [79,80]. For further details see [79,80]. Moreover, placental pathologies are also associated with maternal diseases during pregnancy (e.g. preeclampsia or diabetes) and adverse fetal outcomes (e.g. preterm delivery or intrauterine growth retardation).

The development of maternal preeclampsia is accompanied by abnormalities in placental implantation and disturbed remodeling of uterine spiral arteries, which causes placental ischemia. Therefore, such placentas display signs of infarctions, chronic abruption, increased perivillous fibrin, retroplacental hematoma and fetal thrombotic vasculopathy with different severeness. These placental pathologies can lead to hypertension, oedema and proteinuria on the maternal side and preterm delivery on the fetal side [79,81]. Also in pregnancies complicated by maternal diabetes, including hyperglycemia and macrosome offspring, alterations in the human term placenta were identified. These alterations were an increase in villous and capillary surface area and intervillous space, villous immaturity and increased villous membrane thickness, which impairs oxygen transport [79,82,83].

In preterm deliveries, chorioamnionitis, vascular obstructive disorders, acute and chronic marginal abruption as well as lymphoplasmatic deciduitis are found to be overrepresented in human term placentas [79]. In placentas of offspring with intrauterine growth retardation (IUGR) maternal and fetal vascular obstructive lesions, high grade villitis of unknown etiology, perivillous fibrinoid deposition as well as chronic abruption have been observed more frequently [79]. These examples demonstrate that the placenta plays a key role for optimal fetal development.

1.3.5. Epigenetic mechanisms in the placenta involved in fetal programming

Due to the importance of the placenta in fetal development, it is likely to play a key role in mediating fetal / metabolic programming [84]. The placenta adapts to environmental stimuli with alterations in nutrient transport and hormone production, thereby leading to fetal / metabolic programming of the offspring *in utero* (reviewed in [84]). It is frequently discussed that epigenetic mechanisms are involved in the adaption to environmental stimuli [85].

Epigenetic mechanisms are defined as gene expression changes occurring without alterations in the DNA sequence, which are mitotically or meiotically inherited [86,87]. These changes in gene expression are mediated by a close interaction between DNA methylation,

histone modifications and non-coding (nc) ribonucleic acid (RNA) including microRNAs [86]. The best characterized epigenetic mark is DNA methylation, where a methyl group derived from S-adenosylmethionine is covalently bound to DNA cytosine bases in CpG dinucleotides by *DNA (cytosine-5) methyltransferases* (*DNMT1, 3A* and *3B*) [88]. DNA methylation is critically involved in silencing of somatic genes, repetitive elements, X chromosome inactivation in females as well as in imprinting [86,88]. Imprinting is of particular importance in placental development, morphology and physiology, e.g. for nutrient transport [89,90]. In imprinted genes one of the two alleles is silenced depending on the maternal or paternal origin. According to Haig's 'kinship theory' or the 'genetic conflict theory', maternal expression of an imprinted gene limits growth of conceptus and paternal expression of an imprinted gene enhances feto-placental growth [89,90]. Around 140 imprinted genes are known in human and most of them are expressed in the feto-placental unit, especially in the placenta (see also **chapter 1.4.2**) [91,92]. Examples for two well-known imprinted genes in the placenta are *H19* und *IGF2. H19* is exclusively expressed from the maternal allele and *IGF2* from the paternal allele, thereby regulating placental growth and nutrient transfer [90]. Although methylation of CpG-dinucleotides in imprinting control regions is required for imprinted genes, histone modifications and non-coding RNAs (nc RNAs) are also involved in imprinting [93].

The group of ncRNAs includes macro ncRNAs, involved in regulation of imprinting, as well as short ncRNAs, which are further classified in short interfering (si)RNAs, short nucleolar (sno)RNAs and microRNAs [94]. MicroRNAs are made up of about 22 nucleotides in length and are involved in post-transcriptional regulation of about 30% of all protein-coding genes [94,95]. It has been shown that microRNAs are involved in almost every cellular process examined today and especially in signal transduction [96]. Usually, microRNAs repress target messenger RNA (mRNA) expression, but activation of mRNA targets by microRNAs has also been reported (**Figure 4**) [95,97,98]. It is suggested, that microRNAs are involved in fine-tuning of gene expression [98,99]. However, it was also reported that changes in even one microRNA can lead to diseases, for example different forms of cancer, since one microRNA can lead to expression changes in several target genes [99]. In addition, the mRNA-microRNA regulatory network seems to be of great importance in the placenta, as indicated by the high abundance of placenta-specific microRNAs and the necessity of an intact microRNA machinery for proper placental development [100,101]. In addition, the chromosome 19 microRNA cluster, which is the largest human microRNA cluster, is exclusively expressed in the placenta [102], further supporting the crucial role of microRNAs in placental tissue. A promising feature of microRNA is that in humans placenta-specific microRNAs can be detected in maternal blood during pregnancy, providing an option to use certain microRNAs as easily accessible biomarkers for alterations in the placenta [103].

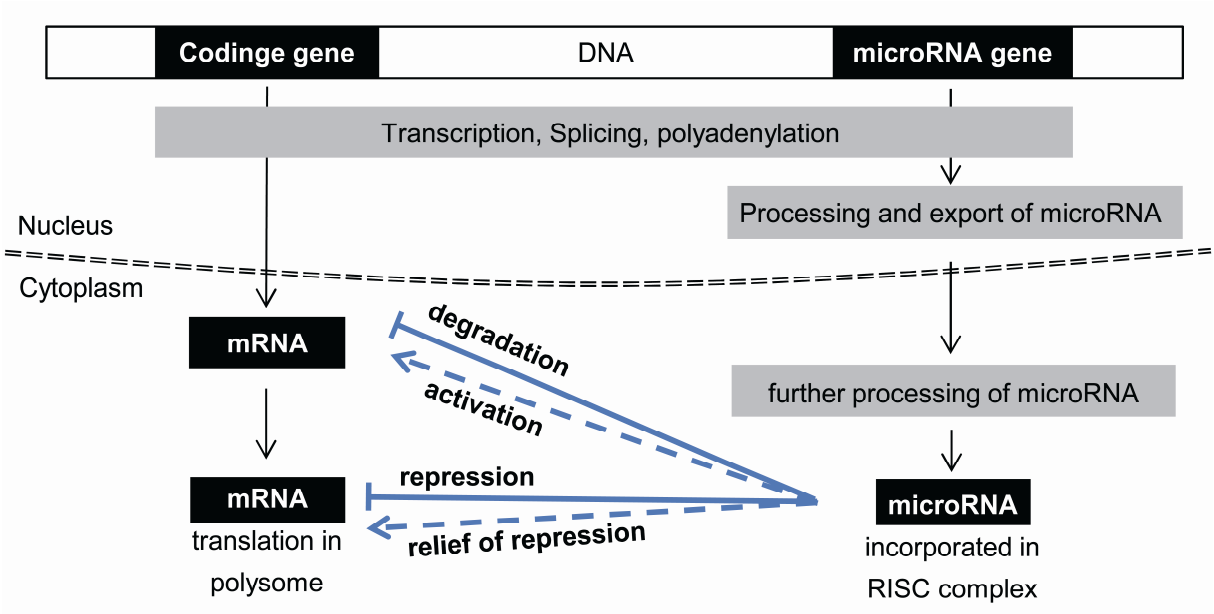

***Figure 4: Simplified scheme of the mRNA-microRNA regulatory network**. Based on Kosik [97] and Vasudevan et al. [98]*

Histone modifications are located on amino acids of the protruding amino-terminal tails of the nuclear histones. These histones form an octamer (2x H2A, H2B, H3 and H4) and together with the wrapping DNA, build up the nucleosome [104,105]. Selected amino acids [serine (Ser), lysine (Lys), arginine (Arg), threonine (Thr), glutamic acid (Glu) and proline (Pro)] of these tails can be dynamically modified by acetylation, methylation, phosphorylation, ubiquitylation, sumoylation, ADP-ribosylation, deimination and proline isomerization via enzyme systems of *histone methyltransferases* / *-demethylases, histone acetyltransferases* / *-deacetylases, kinases* and *ubiquitylases*, respectively [104]. These modifications mediate repression or activation of transcription by altering the interaction of the DNA with its histones leading to regions in the chromatin which are in a more repressive or permissive transcriptional state [104,105]. The signal from one histone modification and / or the pattern of histone modifications in one or several nucleosomes is referred to as 'histone code' [104-106]. Histone modifications are also involved in appropriate placental development and selective activation of placental-specific gene expression requires expression of histone-modifying enzymes [106,107].

1.4. Sexual dimorphism

1.4.1. Sexual dimorphism in placental physiology

The placenta is also described as a 'diary' for intrauterine life [79]. Due to its easy accessibility, the placenta represents a unique opportunity to study influences of fetal / metabolic programming acting during intrauterine life [79]. In addition, since the whole placenta, except for the decidua, is derived from extraembryonic trophoblast cells [67], containing the genetic make-up of the fetus, placental analyses possess the advantage to investigate influences exerted during pregnancy, without taking unethical fetal biopsies. However, for a long time, the placenta has been regarded as an asexual organ, since offspring sex was neglected in most of the placental analyses [108]. Now, evidence is rising that the placenta functions in a sex-specific manner. It was found that only in female human placentas the ratio of *interleukin 5* to *tumor necrosis factor α* mRNA ratio was significantly higher and *11β-HSD2* activity was significantly lower in asthmatic women using inhaled steroids than in controls [109]. Moreover, in the same study population and another study of diabetic pregnancies, morphologic analyses showed that the parameters of the human placental fetal capillaries were altered by diabetes or asthma in a sex-specific manner [110,111]. However, there is no data published comparing morphological parameters between male and female placentas in uncomplicated conditions. Further evidence for placental differences between sexes was provided by Sood *et al.* [112], who clearly showed in a transcriptome analysis that gene expression differed between male and female placentas.

1.4.2. Basic mechanisms mediating sexual dimorphism

Information on the basic mechanism mediating sexual dimorphism is scarce in humans and therefore, the herein described mechanisms are mostly based on observations from mouse or rodent models [113,114]. Before the 7^{th} – 8^{th} week of gestation in humans or embryonic day 10.5 in mice, male and females only differ in the sex chromosomes (XX vs. XY) [113,114]. Afterwards, the bipotential gonad starts to differentiate into testis or ovary, driven by either testosterone or estrogen production initiated by the presence or absence of *sex determining region on the Y chromosome (SRY)* transcription factor, respectively [114]. These steroid hormones are thought to provide the initiation of sexual differentiation in somatic tissue via activation of signal transduction, stimulation of pituitary growth hormone production or *sex steroid hormone receptors* (*SSHR*), all of them in turn regulating further transcription factors [113]. Some of the discovered down-stream transcription factors of the complex transcription network implicated in the establishment of sexual dimorphism in rodent

liver are the hormone-dependent *signal transducer and activator of transcription 5b (Stat5b), hepatocyte nuclear factor 4α (Hnf-4α)* and the hormone-independent *regulators of sex limitation 1 and 2 (Rsl1 and Rsl2)* [113,114]. Male predominant gene expression is mediated by *Stat5b* and *Hnf-4α*, whereas female predominant expression arises from *Rsl1* and *Rsl2* [114].

Not only sexual dimorphisms exist, which are mediated by sex steroids. Also the expression of genes from the sex chromosomes contributes to different gene expression between male and female somatic tissues, since only one X is present in males compared to two X in females. Therefore, in females, the maternal or the paternal X allele is randomly silenced to reduce X chromosome gene dosage and the active one is two-fold upregulated to reach the same levels as other autosomal genes [113]. However, about 15% of X-linked genes can escape this X-inactivation *in vitro*, especially in preimplantation embryos [115], which implicates a higher expression of these genes in female tissues. Genes located on the Y chromosome show, if they have no homologue on the X chromosome, male-specific expression [113]. Moreover, differences in the expression of paternal or maternal imprinted genes were observed between male and female blastocysts, indicating that also an early involvement of epigenetic mechanisms in sexual dimorphism [116].

A comprehensive analysis of gene expression differences between murine male and female organs revealed a large extent of sexual dimorphism with rather small fold changes between 1.2 and 2.0 [117]. They showed that liver, adipose, muscle and brain tissues had a sex-biased gene expression of 72%, 68%, 55.4% and 13.6% (> 1.2 fold change < 2.0) respectively, which demonstrated that the degree of sexual dimorphism is highly tissue specific. The genes displaying different expression between male and female organs were not only located on the sex chromosomes, but also on the autosomes. A possible explanation for this was that sex hormones, growth hormone or sex chromosomal transcription factors (e.g. *Sry*) activate mediators which further regulate sexual dimorphic expression in down-stream genes [117].

1.4.3. Sexual dimorphisms in the context of obesity, LCPUFAs and fetal programming

Differences between males and females are not only observed in the placenta, but also in fetal / metabolic programming of disease risk, obesity as well as LCPUFA status and metabolism [118-121]. Already at birth, the fat distribution is different between male and female newborns [121]. The skinfold ratio of (triceps + biceps) / (subscapular + suprailiac) is at birth 3% higher in female than in male newborns [121]. At puberty, the differences become more pronounced, which are maintained until the fifth decade of life, due to a tendency of females for increased peripheral fat accumulation compared to males. This is reflected in the

different fat distribution types, where men are generally considered to have an android distribution (upper body, trunk) and women a gynoid distribution (hip and thigh) [121].

Sex-specific differences have also been described in LCPUFA metabolism and status. Childs *et al.* [118] reviewed that plasma n-3 LCPUFA concentration, especially DHA, is higher in adult females than in adult males, which was associated with a higher synthesis rate of n-3 LCPUFAs from their precursor alpha-linolenic acid. The sex steroids estradiol, progesterone and testosterone were discussed to be involved in the higher synthesis rate of n-3 LCPUFAs in female adults [118]. However, in babies (2 - 46 days) or children (3 - 5 years) no significant differences in LCPUFAs could be observed [122]. Since the number of analyzed probands was limited, further studies are necessary to confirm these results [122]. Moreover, in human umbilical cord blood there are no reports for differences between male and female fetuses. In contrast to the absence of sex-specific differences in LCPUFA status between male and female newborns, babies and children, LCPUFA supplementation in pre-term children showed a sex-specific outcome. Ryan *et al.* found that supplementation of formula in pre-term children decreased weight gain, length gain and head circumference gain as well as had a lower fat-free mass in 6 month old boys, but not in 6 month old girls [123]. However, there are no reports addressing sex-specific effects of a LCPUFA supplementation during pregnancy. At the moment, sex-specific impact of LCPUFA supplementation during pregnancy for fetal programming cannot be excluded.

Sex specific outcome of fetal / metabolic programming was shown for small size at birth, which was associated with increased insulin resistance and hyperinsulinemia in young male adults only [119]. Moreover, animal studies support sex-specific programming even in a transgenerational manner. It was reported that female offspring of pregnant rats fed with lard had increased blood pressure in contrast to male offspring with unchanged blood pressure. In animal studies, even transgenerational sexual dimorphism was observed, when a maternal low protein diet modified growth and metabolism only in the progeny of female offspring (F2 generation) [119].

The field of human sexual dimorphism, although it is a well-known phenomenon, is still in its infancy. Therefore, further investigation is necessary, especially to further clarify the involved molecular mechanisms. Moreover, it is important to assess the impact of sex-specific differences for fetal / metabolic programming and when sex-specific programming begins.

2. Aim of the study

Observations from *in vitro* experiments demonstrated that n-6 LCPUFAs promote and n-3 LCPUFA counteract adipogenic growth and differentiation processes. Additionally, in animal studies, it was shown that a reduced n-6/n-3 LCPUFA ratio in the maternal diet during pregnancy and lactation reduces offspring obesity. This gave rise to the hypothesis that a dietary n-3 LCPUFA intervention could be used as a new primary prevention strategy against obesity, if in humans similar molecular mechanisms operate. To investigate this hypothesis, the INFAT (**I**mpact of **n**utritional **f**atty acids during pregnancy and lactation on early **a**dipose **t**issue development) study was conducted at the *chair of Nutritional Medicine* by Prof. Hauner, *Technische Universität München, Germany*, in a randomized, controlled, human intervention trial.

In this context, the placenta represents the unique opportunity to assess *in vivo* the impact of the reduced n-6/n-3 LCPUFA ratio in the maternal diet during pregnancy on an easy accessible human biopsy, which consists in large parts of extraembryonic mesoderm and trophoblast cells. Since the placenta plays a central role to ensure proper fetal development, it is discussed to be critically involved in mediating fetal / metabolic programming effects, which are investigated by the n-3 LCPUFA intervention. On the molecular level, LCPUFAs exert most of their effects by directly or indirectly influencing gene expression. Therefore, the analysis of the impact of the n-3/n-6 LCPUFA intervention on placental gene expression could allow drawing conclusion how the intervention molecularly impacts gene expression in other fetal tissues like in the developing adipose tissue. Furthermore, with the analysis of placental gene expression possible molecular and physiological mechanisms could be identified which are involved in the programming of offspring obesity risk. Since, evidence is rising that fetal / metabolic programming has often sex-specific outcomes and there are hints for differences in placental gene expression between male and female offspring, offspring sex will for the first time be considered in an analysis of human placentas upon a dietary intervention

The aim of this thesis was:

(1) to analyze the impact of a reduced n-6/n-3 LCPUFA ratio in maternal nutrition during pregnancy on placental gene expression with consideration of sex-specific differences.

(2) to investigate the impact of a maternal n-3 LCPUFA intervention during pregnancy on the placental mRNA and miRNA expression and mRNA-microRNA expression network as well as their relations to biological processes.

(3) to assess whether changes in placental gene expression are associated with metabolic changes or offspring obesity risk.

3. Subjects and Methods

All analyses in the context of this thesis were conducted in a subpopulation of the INFAT study, which was conducted at the *chair of Nutritional Medicine* (Prof. Hauner), *Technische Universität München, Germany.* The primary outcome of this open-label, randomized, controlled, prospective human intervention trial was infant fat distribution assessed by skin fold thickness (SFT) measurements at four body sites at three to five days, six weeks, four and twelve month post-partum. Secondary outcomes were the definition of possible risk factors for weight gain during early infancy and to obtain new data on the underlying mechanisms [65]. To explore the long-term effects of this reduction in maternal n-6/n-3 LCPUFA ratio during pregnancy, a follow-up until five years of age is currently in progress. The follow-up and the primary endpoint are part of other theses.

3.1. Subjects – the INFAT study population

The placentas used in this thesis were obtained from the INFAT study. The details of the whole INFAT study population and the results of the primary outcome are published in Hauner *et al.* [124]. In brief, 208 study participants were recruited from July 2006 until April 2009 in and around Munich by practice-based gynecologists, research assistants of the outpatient clinic of the Division of Obstetrics and Perinatal Medicine of the University Hospital Klinikum rechts der Isar, Technische Universität München (Munich, Germany), advertisements in local newspapers, pregnancy specific internet pages and the freely available journal 'Baby und Familie'. These recruited pregnant women were screened for inclusion and exclusion criteria. Inclusion criteria were: gestational age < 15th weeks of gestation; age between 18 and 43 years; BMI at conception between 18 and 30 kg/m^2, sufficient German language skills, and written informed consent. Inclusion criteria for follow-up of the newborns were gestational age at birth between 37th and 42nd weeks (full-term), appropriate size for gestational age, and an APGAR (Appearance, Pulse, Grimace, Activity, Respiration) score > 7 at 5 min postpartum. Pregnant women were excluded when: there was a high-risk pregnancy (multiple pregnancy, hepatitis B or C infection, or parity > 4), hypertension, chronic diseases such as diabetes or gastrointestinal disorders, psychiatric diseases, supplementation with n–3 FAs before randomization, alcohol abuse, hyperemesis gravidarum and smoking. Exclusion criteria for follow-up of the newborns were severe malformations or diseases, chromosomal anomaly and inborn metabolic diseases.

The 208 pregnant women were randomly assigned to the control group (CG) or the n-3 LCPUFA intervention group. In the following paragraphs, the n-6/n-3 LCPUFA intervention is named n-3 LCPUFA intervention or simply intervention. Both groups were advised for a

healthy diet during pregnancy according to the guidelines of the German Nutrition Society (DGE). The women in the intervention group were additionally advised to decrease their n-6/n-3 LCPUFA ratio to about 3:1. Therefore, the women in the n-3 LCPUFA intervention group (IG) ingested three fish oil capsules per day, which contained in total of 1020 mg DHA, 180 mg EPA and 9 mg vitamin E (Marinol D-40^{TM}, Lipid Nutrition, Loders Croklaan, Wormerveer, The Netherlands, **Appendix 11.1**). Concomitantly, the women in the intervention group should reduce their AA intake to 50 - 90 mg per day by consuming less meat, meat products and eggs, until the end of lactation. Capsule intake was assessed by capsule diaries and AA-balanced diet was assessed by seven-day nutritional records.

Maternal baseline data like pre-pregnancy weight, height and para-status were collected from the maternity card and further data like smoking status, education, and alcohol consumption were collected by questionnaires. Maternal blood samples were collected in the 14th - 15th (P_15) and 32nd week of gestation (P_32) after an overnight fast. Data for birth weight, gestational age, mode of delivery, sex of the child, and APGAR score were documented from the maternal obstetric record or the midwifes. At defined time points the skin fold thicknesses (SFT) and body fat mass, as measures for adipose tissue development of different adipose tissue depots of the children, were assessed by a Caliper (Holtain T/W Skinfold Caliper). In a subset of children whole body composition was analyzed by ultrasonography and magnet resonance imaging (MRI). Further anthropometric data of the children and their mothers, like weight, height and upper arm circumference were collected.

Placenta, umbilical cord and umbilical cord blood were collected at birth. Immediately after blood collection, venous umbilical cord and maternal blood samples were centrifuged at 2,000 x g for 10 min. Plasma layer and buffy coat were removed from the red blood cells (RBCs) and stored until analysis at -80°C. RBCs were washed three times with 0.9% saline solution. Residual RBCs were stored in aliquots at -80°C until analysis. For more detailed information on the rationale and design of the INFAT study see Hauner *et al.* [65].

The study protocol was approved by the ethical committee of the Technische Universität München (Faculty of Medicine; 1479/06/2006/2/21). Participant data was encoded by randomly assigned numbers and current data privacy law was considered for data management. The protocol of the International Conference on Harmonization Good Clinical Practice guidelines (ICH-GCP, valid from 1997/1/17), the last revision of the declaration of Helsinki (October 2008, Seoul, South Korea) and applicable local regulatory requirements and laws were applied for conducting the INFAT study. The study protocol was registered at clinicaltrials.gov with NCT00362089.

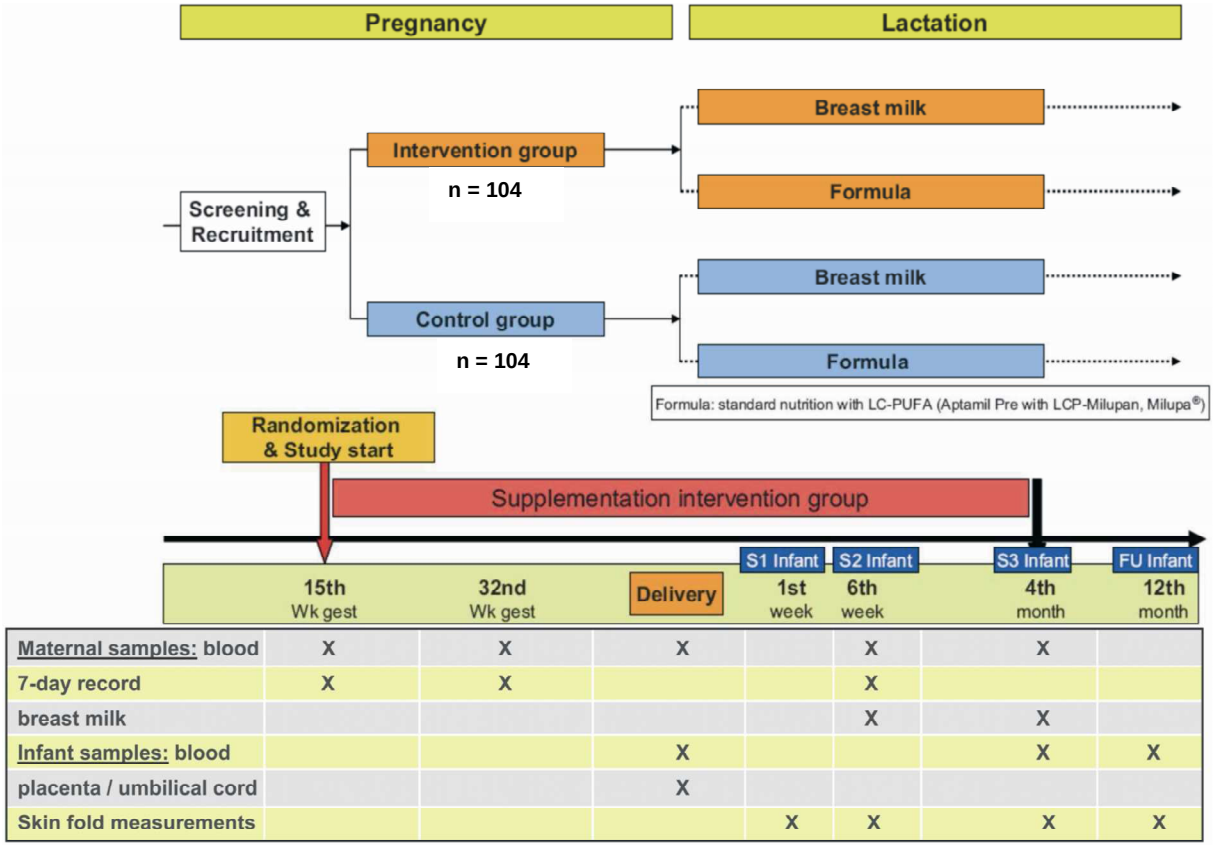

***Figure 5: Study design of the INFAT study**, depicting the two intervention arms during pregnancy and lactation and the collection scheme of data from seven-day (7d) records and biological samples (maternal blood (blood), infant blood, breast milk (milk), umbilical cord blood and placental tissue). Follow-up (FU); week of gestation (Wk gest);*

3.2. Sampling of human term placenta

Placentas were only included in placental analysis when the study participant gave informed consent. After either spontaneous delivery or caesarean section, placentas were kept on 4℃, also during sample preparation according to a standardized sampling protocol. Before the dissection, the placenta was examined (for completeness, infarcted or calcified areas and other abnormalities), the shape was documented by photographs and the weight was measured (**Figure 6A / B**). Six placental pieces were dissected from each of the four quadrants with 3 cm distance to the middle of the placenta by means of a positioning device, avoiding large vessels, calcifications or other obvious abnormalities (**Figure 6C**). To obtain enrichment in the chorionic villous fraction, the maternal basal plate and the fetal chorionic plate were removed by cutting away one third of the upper and one third of the lower part of the dissected placental piece (**Figure 6D**). Five pieces of the remaining villous fractions of about 1 cm^3 per quadrant were immediately snap-frozen in liquid nitrogen and subsequently stored at -80℃ until further processing. The remai ning piece per quadrant was subjected to formalin-fixation. To minimize influences that can obscure the effects of the n-3 LCPUFA intervention, several exclusion criteria were applied for placental analysis. Placentas from

mothers with pregnancies complicated by gestational diabetes as well as from mothers giving birth to newborns with a birth weight not appropriate for gestational age (below the 10th or above the 90th birth weight percentile). Furthermore, placentas where labor was initiated with cervical ripening agents were excluded because often prostaglandin gels with derivatives of n-6 LCPUFAs are used.

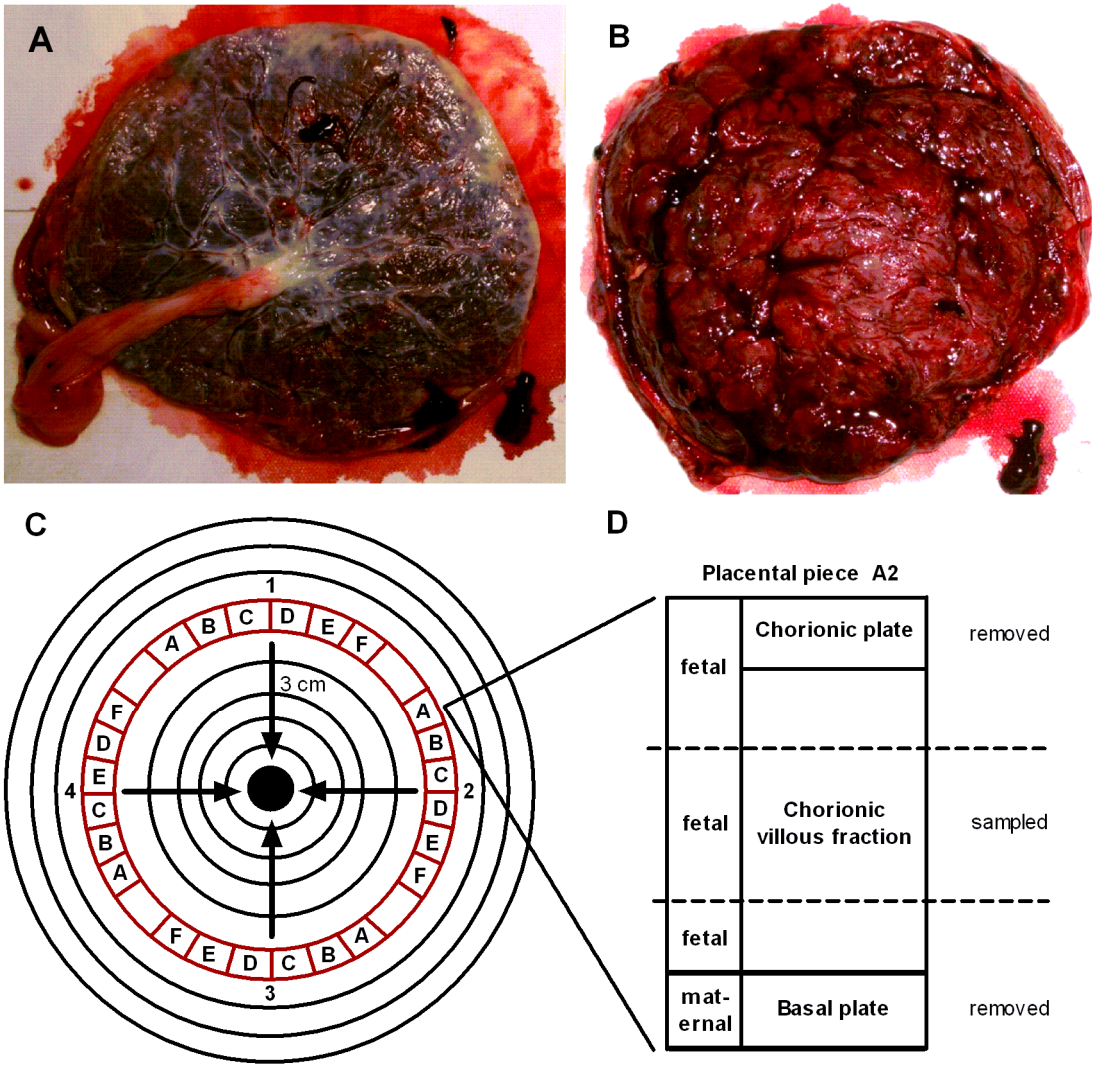

Figure 6: A) View on the fetal facing chorionic plate of a human term placenta after birth with umbilical cord. B) View on the maternal-facing basal plate of a human term placenta after birth. C) Schematic figure of the chorionic plate with umbilical cord insertion *(black circle) including the sampling plan of the six pieces (A - F) per quadrant (1 - 4). Samples A - E were snap-frozen in liquid nitrogen, whereas F samples were subjected to formalin-fixation.* ***D)*** *chorionic villous fraction was dissected by removing the chorionic and basal plate as described in chapter 3.2.*

3.3. Extraction of total RNA and total RNA containing small RNA

For gene expression analysis, total RNA with more than 200 nucleotides is usually extracted from biological material. However, microRNAs, which consist of about 22 nucleotides [95], are not obtained with these total RNA extraction methods. Therefore, another extraction method, which purifies already RNAs with more than 18 nucleotides, was necessary to obtain total RNA including small RNAs (microRNAs). For placental gene or microRNA expression analyses, total RNA (> 200 nucleotides) as well as total RNA including small RNAs (> 18 nucleotides) without further microRNA enrichment was extracted from placental tissue. Four different pieces per quadrant from the A-series of the placenta were completely homogenized by a Rotor-stator homogenizer in *TRIzol® Reagent* (Invitrogen, Darmstadt, Germany) in short intervals and pooled together in equal parts for both extractions. For isolation of total RNA or total RNA with small RNA, the *TRIzol®* method was combined with the *midi RNeasy Kit* (Qiagen, Hilden, Germany) or *mirPremier® microRNA isolation Kit* (Sigma-Aldrich, Taufkirchen, Germany) respectively. In principle, during homogenization of the placental tissue, the chaotrope components of the *TRIzol®* solution protect RNA from degradation by RNAses. After incubation of the homogenate with chloroform and subsequent phase separation, the RNA is located in the water phase, in contrast to desoxyribonucleic acid (DNA) and protein located to the interphase and the organic phase [125]. Mixing the water phase with 96% ethanol provides optimal conditions for exclusive binding of total RNA or total RNA including small RNA to the silica-based membranes. The instructions from the *TRIzol®* manual were followed until the separation of the watery and organic phase. Subsequently, the samples were processed according to the manufacturer's protocol of the *midi RNeasy Kit* or the *mirPremier® microRNA isolation Kit*.

NanoDrop™ 1000 Spectrophotometer (peqlab Biotechnologie GmbH, Erlangen, Germany) was used to quantify the eluted total RNA (with or without small RNA). Purity of the extracted RNA is indicated by the 260/280 ratio for the presence of protein or phenol as well as by the 260/230 ratio for the presence of phenol, also measured by the *NanoDrop™ 1000 Spectrophotometer*. The mean 260/280 ratio was 2.06 ± 0.10 [mean ± standard deviation (SD)] and 2.06 ± 0.04 for total RNA and total RNA containing small RNA respectively. The 260/230 ratio was 2.20 ± 0.21 and 2.04 ± 0.19 for total RNA and total RNA containing small RNA respectively. These ratios indicated good purity for the extracted RNAs used for further analyses. Furthermore, the integrity of the total RNA (with or without small RNA) was analyzed on the *Agilent Bioanalyzer 2100* with *Agilent RNA 6000 Nano Assay Kit (Agilent Technologies, Santa Clara*, *CA, US)*. Placenta samples with RNA integrity numbers (RIN) > 5 were considered appropriate for inclusion in further analyses [126].

3.4. DNA microarray analysis

DNA microarray analyses were conducted to analyze in a large scale 1.) the differential gene expression between male and female placentas as well as 2.) the impact of the n-3 LCPUFA intervention on placental gene expression by comparing the intervention group to the control group. The principle of DNA microarrays is based on reverse-transcription of mRNAs, expressed in the target tissue, to cDNAs, which are subsequently amplified to cRNAs labeled as biotin or fluorescent dye labeled cRNAs, depending on the microarray type. These labeled cRNAs hybridize to the complementary DNA probe sets for transcripts of genes spotted onto the DNA microarray. Upon binding of the cRNA to its respective probe set and subsequent washing steps, the intensity of the label can be read out and reflects the gene expression level, thereby enabling the identification of expression changes for each gene [127].

For human placental transcriptome analysis, extracted total RNAs from selected placentas were hybridized to *Affymetrix Custom Array - NuGO_Hs1a520180 array* (*NuGO array*), containing 17699 genes [128]. All steps and controls of the DNA microarray execution were performed according to the *Affymetrix's GeneChip Expression Analysis Technical manual*. Unless stated otherwise, all reagents, chips and equipment used were derived from *Affymetrix Inc.* (*Santa Clara, CA, USA*). Per placenta, 5 µg of total RNA was reverse transcribed to cDNA with *Gene Chip® Expression 3'-Amplification one cycle cDNA Synthesis Kit*. The cDNA was *in vitro* transcribed to biotin-labeled cRNA with the *Chip® Expression 3'-Amplification IVT labelling Kit.* An equal amount of the labeled fragmented cRNA was hybridized on the *NuGO* array, washed and stained. Intensity of the probe sets was read out on the *GeneChip® Scanner 3000*.

The DNA microarray experiment was controlled with the *MADMAX (Management and analysis Database for Multi-platform microArray eXperiments)* quality control procedure [128,129]. Various plots like probe-set intensity plots (before and after normalization), clustering blots (e.g. principal component analysis and correlation plots), *NuSE*, *RLE* plots and many others helped to decide whether the quality of the DNA microarrays is appropriate [129]. Statistical analysis for *NuGO* DNA microarray data was conducted with the *MADMAX* statistical analysis procedure. Intensity data of the DNA microarray analysis was normalized using the *gc Robust Multichip Average (slow)* algorithm. The probe sets were annotated to their corresponding genes by the Custom Chip Definition Files (CDF-files) version 13.0.0. No previous filtering for fluorescence intensity was applied to the datasets. In *MADMAX* fold changes (FCs) and p values (p) were calculated in moderated t-tests using the *R Bioconductor LIMMA* package [129-131]. For the factor n-3 LCPUFA intervention, fold changes and p values were calculated by pooling the data of male and female placentas. Furthermore, for the factor n-3 LCPUFA intervention a model including an offspring sex term

was applied to investigate the impact of the intervention in a larger sample size with consideration of offspring sex. The latter analysis was kindly provided by Dr. Philipp Pagel from the *chair of genome-oriented bioinformatics* (*Technische Universität München*, *Germany*). For the factor offspring sex, fold changes and p values were calculated separate for the control and the intervention group. For the factor n-3 LCPUFA intervention, fold changes and p values were calculated separate for male and female placentas. The applied significance criteria for the identification of regulated genes were fold changes ≥ +1.5 and $P < 0.05$ or fold changes ≤ -1.5 and $P < 0.05$.

3.5. Metabolic pathway analysis

Pathway analyses were conducted to identify whether the significantly regulated genes of the transcriptome datasets were enriched in particular biochemical metabolic pathways. Pathway analysis (also known as functional enrichment or knowledge base-driven pathway analysis) integrates the normalized DNA microarray data and their annotations to metabolic pathways or gene ontology functional classification [132,133]. This integration leads to a reduction in complexity of the analysis by grouping thousands of genes to several hundred pathways as well as to the identification of gene groups that function in the same pathway in order to identify regulated biological processes or to generate new hypothesis [133,134]. There are three different pathway analytic approaches: over-representation analysis (ORA) approaches, functional class sorting (FCS) approaches and pathway topology (PT)-based approaches [133]. The application PathVisio 2.0.9, used in this thesis, is based on the ORA approach [133,134]. ORA statistically assess the fraction of genes in a particular pathway identified in the set of genes displaying expression changes. For this purpose, an input gene list is created containing all genes from the transcriptome data above a certain threshold or criteria. For each pathway, the genes from the input list which are included in the pathway are counted. Likewise, the genes from a background list (e.g. all genes measured on the DNA microarray) which are included in the pathway are counted. With these counts, every pathway is statistically analyzed for over- or under-representation in the genes from the input list [133]. PathVisio uses the standardized difference score, short Z-score, for statistical analysis [134]. The pathways from Hsa-KEGG_20100914 and wikipathways_Homo_sapiens _Curation-AnalysisCollection_gpml (2010-11-04) were both downloaded from http://www.pathvisio.org/wiki/PathVisioDownload. The pathways derived from both resources have been curated carefully. The 159 pathways from the wikipathways_Homo_ sapiens_Curation-Analysis Collection and the 213 pathways from hsa-KEGG_20100914 were both selected, resulting in about 364 unique pathways for analysis. All these pathways were loaded into PathVisio 2.0.9, together with the gene database Hs_derby_20100601.bridge. Z-scores were calculated by subtracting the number of

expected genes from the number of significantly regulated genes (fold change ≥ +1.5 and p-value < 0.05 or fold change ≤ -1.5 and p-value < 0.05) in the respective pathway and dividing the difference by the SD of the significantly regulated genes [135]. A Z-score of 0 indicates no enrichment, a positive or negative Z-score (> 0.0 / < 0.0) indicates that this pathway contains significantly regulated genes from the transcriptome datasets in an over-representative respectively under-representative manner [134,135].

3.6. Gene expression analysis by RT-qPCR

For biological validation of selected genes, RT-qPCR is often described as the method of choice or gold standard [136]. RT-qPCR detects and quantifies minute amounts of specific nucleic acids sequences in real-time over a wide dynamic range [136-138]. To achieve this, three steps are necessary: 1) reverse transcription (RT) of mRNA into cDNA, 2) amplification of cDNA by polymerase chain reaction (PCR) and 3) detection and quantification of amplification products. These steps can be carried out for RT and PCR (including quantification) separately, by two subsequent steps, which is called two-step PCR, or together in a single-tube called one-step PCR. Advantages of a one-step PCR include less hands-on time and reduced potential for contamination. For real-time quantification the increase in the amplification products at each cycle in the PCR is monitored using reporter-dyes, which are either sequence specific probes or non-specific dyes. SYBR green non-specifically intercalates in the newly amplified double-stranded PCR products. The cycle at which the fluorescence first rises over a certain threshold (background) is defined as cycle of quantification (Cq). The Cq is inversely correlated with the amount of the target gene in the analyzed sample, whereas a higher amount of the target gene leads to earlier rise of the fluorescence over the background [136].

To assess gene expression differences in placental target genes from the transcriptome and pathway analysis, a one-step RT-qPCR with the non-specific reporter-dye SYBR green was conducted by using the *QuantiTect SYBR Green RT-PCR Kit* (*Qiagen, Hilden, Germany*) according to the manufacturer's protocol. Therefore, target specific primers were applied in the RT as well as in the qPCR, representing the most specific and sensitive method for converting RNA into complementary DNA (cDNA) [136]. If at least one intron was present in the target gene, intron-spanning primer were designed [136] or *Quantitect primer assays* were used (*Qiagen, Hilden, Germany*; see **chapter 10.1**). All primers were carefully tested for optimal annealing temperature, amplification efficiency and target specificity by melting curve analysis of the amplification products. The mean efficiency of the primer pairs and assays, analyzed from the amplification response curves, were 85.9 ± 4.1% (mean ± SD).

For RT-qPCR, 10 ng total RNA were used to generate amplification products in the range of 64 to 163 base pairs (bp). Cycling conditions included the following steps: 1 cycle at 50℃ for 30 min, 1 cycle at 95℃ for 15 min, 40 cycles at 95 ℃ for 15 sec / 60℃ (or as stated in **chapter 10.1**) for 30 sec / 72℃ for 30 sec, and a terminal melt ing curve. Cq values were determined by *Mastercycler ep realplex* with *CalqPlex based* on the *second derivative maximum algorithm* (*Eppendorf, Hamburg, Deutschland*). 'No-template' controls were included to assess contamination in each run and genomic contamination was assessed by '*no-RT*' controls for each gene and every sample. Cq values were measured in duplicate and normalized to the geometric mean of the four reference genes β-actin (ACTB), Polymerase (RNA) II (DNA-directed) polypeptide A (POLR2a), beta-2-microglobulin (B2M) and Topoisomerase (DNA) I (TOP1) [= Bestkeeper BK]. These genes showed no significant differences between the analyzed groups, and the repeated pair-wise correlation analysis in *BestKeeper® program (version1)* showed their stability which demonstrated their suitability as reference genes (**Appendix 11.16**) [139]. Relative gene expression levels were calculated by the $2^{-\Delta\Delta Cq}$ method ($Cq_{\text{mean BK}} - Cq_{\text{target gene}} = \Delta Cq \rightarrow 2^{\Delta Cq}$ for each placenta in the control and the n-3 LCPUFA intervention group separately; mean $2^{\Delta Cq\ (IG)}$ / mean $2^{\Delta Cq\ (CG)}$) [140]. The expression levels of the female placentas of the control group (CF) samples were assigned to an arbitrary value of 100% and all other analyzed groups were expressed relative to placentas of female offspring in the control group (CF). The checklist for the *Minimum information for publication of quantitative real-time PCR experiments (MIQE) guidelines* is provided in **Appendix 11.2** [137].

3.7. Explorative microRNA profiling

To analyze whether microRNAs were involved in the observed regulation of gene expression or pathways in the transcriptome analysis, a placental microRNA profile was assessed in female placental villous fractions. The explorative microRNA profiling was conducted at the Children's Research Center, Dr. von Hauner Children's Hospital, Ludwig-Maximilians-Universität München, Germany in collaboration with Prof. Dr. A.A. Roscher and Dr. K. Fleischmann. The detection of the microRNAs was RT-qPCR-based (as described above in **chapter 3.6**), including preloaded microRNA primers on *TaqMan® Array Human Micro RNA A+B Cards Set [141]*.

Total RNA containing small RNA of three female placentas assigned either to the control or the n-3 LCPUFA intervention group, were pooled together in equal parts for placental microRNA profiling. These female placentas of the INFAT study were obtained from spontaneous labor without the use of analgesics or anesthetics. All reagents and kits applied were obtained from *Applied Biosystems (Darmstadt, Germany)* and used according to the

manufacturer's protocol. In brief, reverse transcription of 350 ng total RNA containing small RNA (RIN > 5) was conducted with *TaqMan® MicroRNA Reverse Transcription Kit* using *Megaplex™ RT primer*. Further pre-amplification of cDNA was carried out with *TaqMan® PreAmp Primers* and *TaqMan® PreAmp Master Mix* for twelve cycles. Diluted, pre-amplified cDNA was analyzed with the *TaqMan® Array Human Micro RNA A+B Cards Set v3.0* and *TaqMan® Universal PCR Master Mix (No AmpErase® UNG)* on the *7900HT Fast Real-Time PCR System* to assess the profile of 754 microRNAs including endogenous and negative controls.

Data were read out by the *SDS (Sequence Detection System) Software v2.4.* Statistical calculations were conducted in R (version 2.11.1). Log fold changes were calculated as following: (Cq_{IG} – Cq_{CG}) and normalized with a cyclic *LOESS* procedure, separately for microRNAs analyzed on plate A and plate B [142,143]. For the calculation of putative differentially expressed microRNAs, an adaptive cut-off was determined by *polynomial quantile regression (quadratic model) [144],* to avoid a bias by increasing fold changes of microRNAs with higher Cq values. MicroRNAs with a log fold change below the 0.05 or above the 0.95 quantile of the cut-off were regarded as suitable targets for further validation.

3.8. Analysis of microRNA binding sites within mRNA sequences

To assess the impact of the n-3 LCPUFA intervention on the mRNA-microRNA network, it was analyzed whether microRNAs identified in the explorative approach could be involved in the gene expression changes observed upon the n-3 LCPUFA intervention. Therefore, the genes differentially expressed upon the intervention in the transcriptome analysis were assessed for binding sites of microRNAs identified in the explorative microRNA profiling. Therefore, the *Diana mirExTra* web server application was used, as a bioinformatics approach [145]. The complete list of significantly regulated genes from three DNA microarray datasets analyzed for the effect on n-3 LCPUFA intervention (IG vs. CG in female, male and pooled male and female placentas under consideration of sex) were used as input gene list. All genes not present in the input list served as background. A microRNA filter was used containing the microRNAs which were identified to be regulated by the intervention in female placentas in the explorative profiling. However, *hsa-miR-550*, hsa-miR-200a*, hsa-miR-30c-2*, hsa-miR-30c-1*, hsa-miR-497*, hsa-miR-130a** and *MammU6* were not listed in the application and were thus not included in the analysis. The option 'conservation between human and mouse' was not applied. MicroRNAs with overrepresented binding sites in the 3' UTR of significantly regulated genes were identified by comparing the microRNA target predictions scores calculated by *DIANA microT* of the input gene list to the gene list of all unchanged genes (background) by a one-sided *Wilcoxon rank test* [145].

Additionally, the target prediction algorithms *rna22* [146], *DIANA-microT-CDS v5.0* [147], *DIANA-microT 3.0* [148], *microRNA.org* [149], *miRDB* [150], *TARGETMINER* [151], *TARGETSCAN* [152], or *PICTAR* [153] were used to identify binding sites for microRNAs within specific microRNAs. Moreover, databases storing information on experimentally identified microRNA targets were applied like *miRecords, miRSel, miRWalk, StarBase* [154]. To analyze the sequence identity between microRNAs and potential target genes, a sequence alignment was conducted. The multiple sequence alignment tool *Clustal Omega* was used with default settings. *Clustal Omega* is an open access web-service provided by the *European Bioinformatics Institute o EMBL (EBI-EMBL)* [155].

3.9. microRNA expression analysis by RT-qPCR

For biological validation, candidate microRNAs, selected from the placental microRNA profiling and the bioinformatics analysis, were measured by a TaqMan RT-qPCR. The principle of a RT-qPCR was already described in **chapter 6.6**. The difference of TaqMan to the SYBR green based RT-qPCR is that the fluorescence detection of the amplification-product in a TaqMan-based approach depends on a sequence-specific probe. This probe is labeled with a fluorescent dye and a quencher, which absorbs the emission spectra of the fluorescent dye when they are located on the same probe. Upon extension of the target sequence, the probe is cleaved by the Taq-Polymerase, which separates the fluorescent dye from its quencher. By this separation, the fluorescence of the dye can be measured and is inversely correlated with the amount of the target gene [156].

For the biological validation of target microRNAs, a larger sample size of male and female placentas was used. Placenta samples corresponding to those used for the biological validation of mRNA expression were applied for microRNA validation. All reagents were purchased from *Applied Biosystems / Life technologies (Darmstadt, Germany)*. The selected microRNAs were analyzed with commercially available TaqMan® MicroRNA Assays in a two-step RT-qPCR according to the manufacturer's protocol [157]. In brief, 10 ng of total RNA including small RNAs were reversely transcribed with the *TaqMan® MicroRNA Reverse Transcription Kit* and analyzed by RT-qPCR with the *TaqMan® Universal PCR Master Mix, No AmpErase® UNG*. Thermal cycling conditions for reverse transcription included the following steps: 1 cycle at 16℃ for 30 min, 1 cycle at 42℃ for 30 min, and 1 cycle at 85℃ for 5 min. The thermal cycling conditions for the subsequent quantitative PCR were the following: 1 cycle at 95℃ for 10 min, 40 cycles at 95℃ for 15 sec / 55℃ (**chapter 10.1**) for 30 sec / 72℃ for 30 sec. Cq values were determined by *"Mastercycler ep realplex"* (*Eppendorf, Hamburg, Deutschland*) with *CalqPlex* analogous to the gene expression analysis (**chapter 3.6**). 'No-template' controls were included to assess contamination in each

run and genomic contamination was controlled by 'no-RT' controls for each gene and every sample (**Appendix 11.17**). Cq values were measured in duplicates and normalized to the geometric mean of three well controlled reference genes (*SNORD24 small nucleolar RNA, C/D box 24 (RNU24); RNU6-2 RNA, U6 small nuclear 2 (RNU6b), microRNA-26b* = Bestkeeper BK; **Appendix 11.17**). These microRNAs showed no significant differences between the analyzed groups. Repeated pair-wise correlation analysis in the BestKeeper® program (version1) showed their stability, which demonstrated their suitability as reference genes (**Appendix 11.17**) [139]. Relative gene expression levels were calculated by the $2^{-\Delta\Delta Cq}$ method, analogously to the gene expression (**chapter 3.6**) [140]. The expression levels of the female placentas of the control group (CF) samples were assigned an arbitrary value of 100% and all other analyzed groups were expressed relative to CF. The checklist for the *MIQE guidelines* for microRNA RT-qPCR is provided in **Appendix 11.3** [137].

3.10. Analysis of protein expression by Western blot analysis

The positively validated mRNA expression for human *L-type amino acid transporter 1 (LAT1)* was additionally validated on protein level by Western blot analysis according to a standard protocol [158]. Unless stated otherwise, all chemicals were purchased from *Sigma Aldrich Chemie (Taufkirchen, Germany)*. Ground placental tissue powder (50 – 100 mg) was homogenized in 5 µl/mg radioimmunoprecipitation assay buffer (RIPA: 150 mM NaCl, 50 mM Tris, 1 mM EDTA, 1% Nonidet-P40, 0.2 % SDS, 0.25 % sodium deoxycholate) supplemented with 1 % phosphatase inhibitor cocktail II and 1% phosphatase inhibitor cocktail 2 by a rotor-stator homogenizer. Protein concentration of the supernatant was measured using the *Pierce® BCA protein Assay Kit (Thermo Scientific, Rockford, IL, USA).*

A 10% sodium dodecyl sulphate (SDS)-polyacrylamide gel was used to separate per lane 50 µg of protein extract denatured in *Laemmli* buffer. A standard protein ladder (PageRuler®, Fermentas) and appropriated positive controls were included in all measurements. The separated protein was transferred onto a 0.45 µm nitrocellulose membrane by a semidry Transblot B44 (Biometra GmbH, Göttingen, Germany). Then, the membrane was blocked in 2% ECL Advance™ blocking reagent (*GE Healthcare, Munich, Germany*) dissolved in TBS (20 mM Tris, 140 mM NaCl, pH 7.6) for 1 h. Blocking and antibody incubations were always followed by serial washing steps. The membrane was incubated with the primary antibody against human *LAT1* – anti goat (SAB2501232) diluted 1:1000 in 2% ECL-TBS-Tween 20 (TBS-T) overnight at 4℃. For detection, the membrane was incubated with secondary donkey anti-goat conjugated with *IRDye 800CW* (*LI-COR Biosciences GmbH, Bad Homburg, Germany*) diluted 1:10,000 in 2% ECL-TBS-T for 1h at room temperature. The intensity signal was detected at 800 nm wavelength using the *Odyssey infrared imaging system* (*LI-*

COR Biosciences GmbH). For normalization, the scanned membrane was re-probed without stripping for Glyceraldehyde-3-phosphate dehydrogenase primary antibody (GAPDH AM4300, 1:4000, *Ambion / Life technologies, Darmstadt, Germany*, 4℃ overnight). Secondary goat anti-mouse antibody conjugated with *IRDye 680CW* (*LI-COR Biosciences GmbH*) was used to detect GAPDH intensity at 680 nm wavelength. The Integrated intensities after background correction (t*op / bottom)* were obtained by *Odyssey Application Software V3.0 (LI-COR Biosciences GmbH*). Specificity of *LAT1* bands was demonstrated by experiments with *LAT1* blocking peptide. The whole blots used for *LAT1* quantification are shown in **Appendix 11.18** along with obtained raw data and calculation of normalized values.

3.11. Amino acid analysis

Measurement of amino acid levels in placental tissue and umbilical cord plasma samples were conducted in collaboration with Professor H. Daniel and performed by M. Sailer and R. Scheundel at the *Chair of Nutritional Physiology*, *Technische Universität München, Germany.* Amino acid levels were analyzed as described previously [159], except that instead of the *iTRAQ™ Reagent Kit*, the *aTRAQ™ Reagent Kit 200 Assay (ABSciex, Foster City, USA*) was used in combination with liquid chromatography-tandem mass spectrometry (LC-MS/MS). In brief, ground placental villous tissues of four different locations per placenta (100 mg) were separately dissolved in 150 µl MeOH/H_2O (50:50 vol / vol) / mg. Equal parts of the four supernatants from one placenta were then pooled together. For further sample preparation, 40 µl of the pooled placenta supernatant or 40 µl of plasma were used according to the manufacturer's instructions [159]. Mass analysis was performed using the *3200QTRAP LC/MS/MS (Applied Biosystems, Foster City, USA*). For quantification, the *Analysist® 1.5 Software (Applied Biosystems, Foster City, USA*) was used. Placental amino acid concentrations were normalized to the whole tissue protein concentration, measured by Bradford assay (*Biorad Laboratories GmbH, München, Germany*).

3.12. Sex steroid analysis in placental tissue and umbilical cord plasma

Sex steroid analysis was conducted in collaboration with Prof. Dr. Dr. H.H.D. Meyer, PD. Dr. S.E. Ulbrich and performed by Waltraud Schmid at the *Chair of Physiology, Technische Universität München, Germany*. The description of these methods was kindly provided by PD. Dr. S.E. Ulbrich. Hormone concentrations were determined in umbilical cord plasma and in placental tissue samples using enzyme immunoassays according to Prakash *et al.* [160] (progesterone), Blottner *et al.* [161] [testosterone (T)] and Meyer *et al.* [162] [estradiol-17β (E2) and total estrogen]. The progesterone enzyme-linked immunoabsorbent assay (ELISA)

was modified concerning the primary antibody, which was kindly donated by Frank Weber, *Clinic for ruminants, Ludwig-Maximilian Universität München, Germany* (serum 1.8 immunized against progsterone-7α-carboxyethylthioether). Plasma aliquots from umbilical cord blood (50 µl) were extracted with 30% tertiary butylmethylether / 70% petroleum ether (v/v). After freezing overnight at -60°C, the super natants were decanted, dried, diluted in assay buffer, and subjected to the respective ELISA. To additionally determine the total amount of conjugated estradiol-17β and total estrogen, the water residue after extraction was hydrolyzed with *β-glucuronidase / arylsulfatase* (from *Helix pomatia*) (*Merck, Grafing, Germany*) at 37°C for 2h according to Meyer *et al.* [163] and then subjected to a second alcohol extraction as described above. The hormone concentration of placental tissue was determined following Blottner *et al.* [161]. Briefly, four placenta pieces from different sites per placenta were homogenized in liquid nitrogen and pooled in equal parts. A 10 mg aliquot of the pooled placental tissue was transferred into 400 µL of a 0.9% NaCl solution. The tissue emulsion was then subjected to the same extraction procedure as for the plasma determination and hydrolysis was performed likewise. The cross-reactivity of the testosterone ELISA with testosterone was 100%, with 5α-dihydrotestosterone 10%, with androstenedione 2%, with estradiol < 0.1% and with progesterone < 0.1%. The antibody used in the ELISA for the detection of estradiol-17β was raised against E2-6-carboxymethyloxim bovine serum albumin and showed minimal cross reactivity towards other estrogens (0.7% with estrone). For analysis of total estrogen, an antibody raised against estradiol-17β-hemisuccinate bovine serum albumin reacting with estradiol-17β (100%), estrone (100%) and estradiol-17α (70%) was used [162]. The lower detection limit for testosterone, progesterone, estradiol-17β and total estrogens was 0.05 ng/mL, 0.5 ng/mL, 20.0 pg/mL and 20.0 pg/mL plasma and 0.025 ng/g, 2.5 ng/g, 100 pg/g and 100 pg/g placenta, respectively. The plasma intra- and interassay coefficient of variations were < 10%.

3.13. Fatty acid analysis

Fatty acids analysis was performed at the *Laboratory of Lipid Research, Danone Research – Centre for Specialised Nutrition, Friedrichsdorf, Germany* as described in Hauner *et al.* [124,164]. In brief, the method according to Bligh and Dyer [165] was used to extract the plasma lipids with chloroform / methanol / water. The lipids of the extract were fractionated by high performance liquid chromatography (HPLC) in phospholipids, which were stored for later analysis, and neutral lipids [166]. Fatty acids of RBCs, placental tissue (pooled from four different sites) and plasma phospholipids were chemically converted from the non-volatile fatty acids into their corresponding volatile fatty acid methyl ester derivates (FAME) with acetyl chloride following the method of Lepage and Roy [167] with slight modifications.

Subsequently, FAMEs were analyzed using the *gas chromatograph Agilent 6890N (Agilent Technologies Inc., Santa Clara, CA, USA*), equipped with a *cold-on213 column injector, DB-23 column* (30 m, 0.25 mm, 0.25, *µ*m) from *Agilent J&W (Agilent Technologies Inc., Santa Clara, CA, USA)* and a flame ionization detector, as reported in detail by Beermann *et al.* (2005) [166]. *ChemStation* software was used for data acquisition and analysis. Fatty acids were identified by assignment of their peak retention time to those of pure standards (*Sigma Aldrich, Taufkirchen, Germany*). Their contents are presented in % fatty acids / total fatty acids (%wt). All analyses were carried out in duplicates.

3.14. Statistical analysis

Normal distributed variables of the data from subject characteristics, ΔCq values from mRNA and microRNA analysis, intensity ratios of Western blot analysis, amino acid, fatty acid and sex hormone levels were analyzed for differences between the study groups by two-way ANOVA for the factors n-3 LCPUFA intervention and offspring sex. Normal distributed data are presented as mean ± SD. Relative gene expression is presented as mean relative gene expression in % + SD corrected for error propagation. Normal distribution was assessed by Shapiro-Wilk test. Not normal distributed variables or variables that violated homoscedascity (Levene's test) were analyzed by two-way ANOVA on ranks and presented as median with interquartile range (IQR: 25^{th}-75^{th} percentile). Significant results were further analyzed by pairwise comparison with Holm-Sidak post-hoc test. For qualitative data cross-tables with Fisher exact test were used. All tests were carried out with Sigma Plot 11.0 (Systat Software GmbH, Erkrath Germany).

Usually, when a lot of statistical test are performed, corrections for multiple testing have to be applied. However, this would lead to very conservative results. Therefore, as proposed by Saville [168], two-sided P values < 0.05 are considered significant and all test results are presented, so that the reader can do an informal adjustment of the significance level.

For bivariate correlation analysis, non-parametric *Spearman-rho correlation coefficient* (Rs) and two-sided significance was calculated with *IBM SPSS Statistics 19 (IBM Deutschland GmbH, Ehningen, Germany*). P values < 0.05 were considered as significant. Correlation coefficients between 0.0 - 0.4, 0.4 – 0.7, and 0.7 – 1.0 were considered as weak, moderate and strong correlations respectively.

4. Results

4.1. Overview of applied experimental and analytical strategies

A scheme of the applied experimental and analytical strategies is shown in **Figure 7** and described in more detail as follows.

Selection of placentas and INFAT-subpopulation: At the beginning of this thesis the placentas for the control group (n = 20) and intervention group (n = 21) used for all experiments had to be carefully selected from the 208 pregnant women of the whole INFAT population (control group n = 104 and intervention group n = 104). The selection was conducted by well-defined selection criteria to exclude effects of birth mode, labor initiation and extreme birth weights (**chapter 3.2**) on gene expression. Afterwards, one has to assess whether the applied selection criteria led to differences between the groups of the INFAT subpopulation and the groups of the whole INFAT population with regard to maternal baseline parameters, maternal compliance, newborn birth parameters and offspring growth and fat distribution measurements up to one year of life. For the characterization, also the percentages of EPA, DHA, AA and the n-3 LCPUFA ratio were of interest to analyze in the placenta, umbilical cord blood RBCs and maternal blood from the 32nd week of gestation in order to investigate whether the applied intervention influenced the percentages of these fatty acids in maternal and fetal circulation as well as in placental tissue.

Placental expression analyses and bioinformatics: Since this thesis strongly focused on the sex-specific analysis of the mRNA-microRNA network, the next steps were to analyze placental expression of mRNA and microRNAs in a sex-specific manner. Therefore, profiles of placental mRNA and microRNA expression had to be generated by transcriptome and miRNome analysis. For the profiling approaches, the placentas were further selected to exclude possible effects of analgesics or anesthetics (see **chapter 4.3**). The data from the DNA microarrays for transcriptome analysis were investigated for differences in mRNA expression according to offspring sex and the n-3 LCPUFA intervention. Moreover, the DNA microarray data were analyzed for interactions between offspring sex and the intervention. The different datasets of the transcriptome analysis were subjected to metabolic pathway analysis to identify whether significantly regulated genes were overrepresented in distinct pathways. In parallel, an explorative miRNome analysis using only female placentas was conducted by low-density arrays to identify putative alterations in microRNA expression upon the intervention. In a bioinformatics approach, the data of the transcriptome and the explorative miRNome were combined for mRNA-microRNA network analysis by using *DIANA-mirExTra*. With this bioinformatics approach one can analyze whether significantly

regulated mRNAs of the DNA microarray analysis possess binding sites in their 3'UTR for the regulated microRNAs of the explorative miRNome analysis.

Biological validations: To confirm the results of the bioinformatics mRNA-microRNA network and the pathway analysis, target mRNA and microRNAs were selected for biological validation by RT-qPCR. For the significantly regulated target genes, associated metabolites can be measured to assess a possible impact of the placental gene expression changes on metabolism.

Correlations: Significantly regulated mRNAs and microRNAs were correlated with offspring weight and fat distribution measurements to assess whether the placental gene expression changes upon the intervention could be involved in programming of offspring obesity risk.

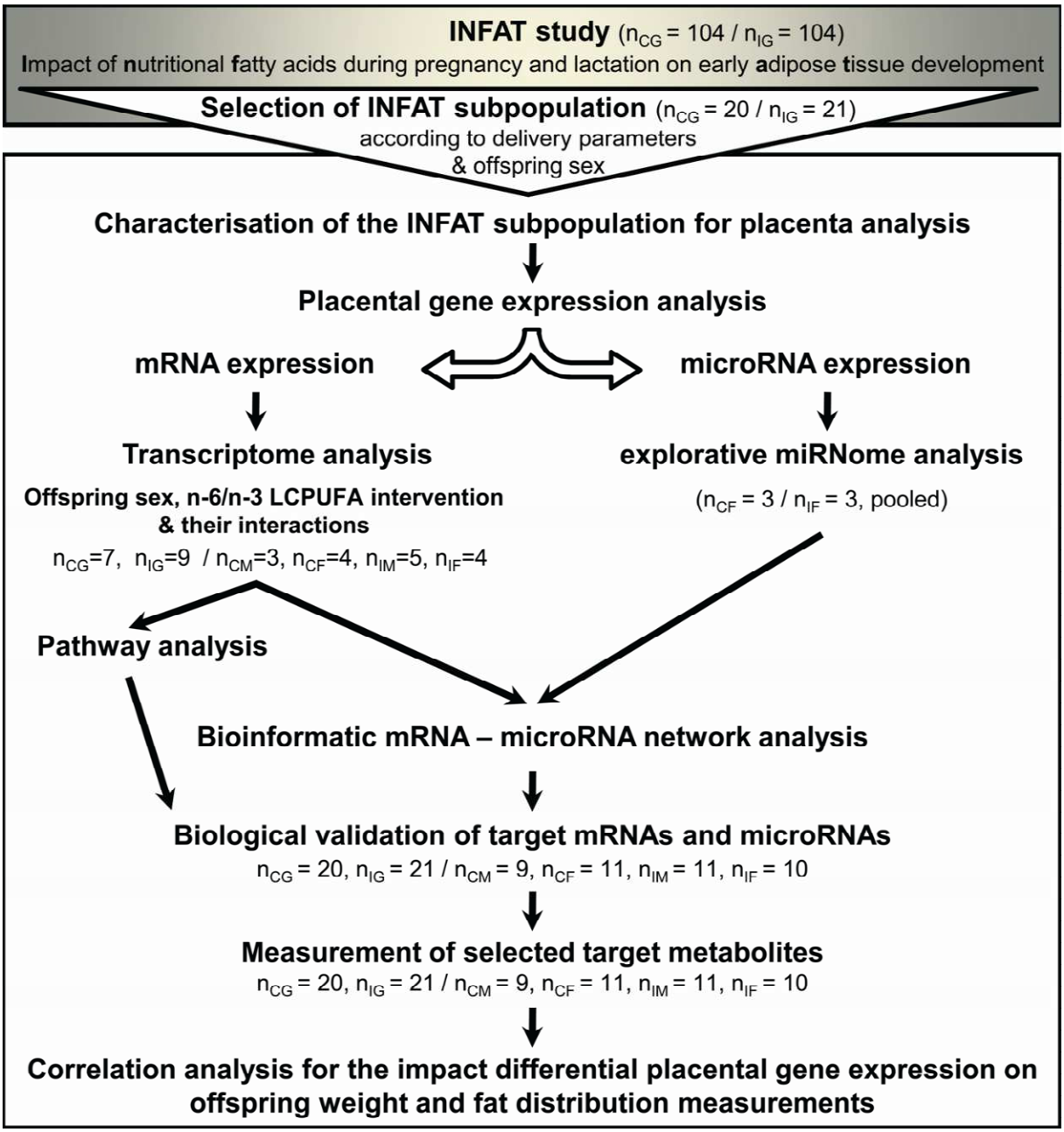

***Figure 7: Overview of applied experimental and analytical strategies**. CG, women / offspring / placentas of the control group; IG, women / offspring / placentas of the n-3 LCPUFA intervention group; CM, placentas of male offspring from the control group; CF, placentas of female offspring from the control group; IM, placentas of male offspring from the n-3 LCPUFA intervention group; IF, placentas of female offspring from the n-3 LCPUFA intervention group;*

4.2. Characterization of the INFAT subpopulation selected for molecular analysis of placental tissue

4.2.1. Analysis groups and statistics

For the characterization of the INFAT subpopulation, the pregnant women were separated in four analysis groups by n-3 LCPUFA intervention and offspring sex (CM = pregnant women of the control group with male offspring, CF = pregnant women of the control group with female offspring, IM = pregnant women of the intervention group with male offspring, IF = women of the intervention group with female offspring). Significance of the analyzed parameters was tested by a two-way ANOVA or a two-way ANOVA on ranks for non-normal distributed data. In two-way ANOVAs, a p value is calculated for the factor n-3 LCPUFA intervention, the factor offspring sex and an interaction of intervention with sex. A p value below 0.05 for the factor intervention or offspring sex indicated a significant effect of the intervention (P*) or offspring sex (P#). However, for a p-value below 0.05 of the interaction term (P*#), it cannot be anticipated *per se* which factor influences the other factor. Therefore, a significant interaction term can indicate 1.) a significant difference between the control and intervention group which is modified by offspring sex, or 2.) a significant difference between male and female placentas that is modified by the intervention. By means of further post-hoc tests, which are allowed to conduct upon a significant p-value in one of the three tests in the two-way ANOVA, it can be assessed how the analyzed parameter is influenced in the single groups (CM, CF, IM and IF) in more detail.

4.2.2. Maternal baseline characteristics

The analyzed subpopulation of the INFAT study was of Caucasian origin, well-educated and on average 32 years old, which was comparable to the whole INFAT population [124]. Maternal characteristics of the INFAT subpopulation are presented in **Table 1**. The maternal baseline parameters (**Table 1**) were not significantly different between the four analysis groups, except for height. The pregnant women in IF were on average about four cm taller than pregnant women in CF and IM (2.3%, $P_{IM\ vs.\ IF} = 0.020$ / 2.6%, $P_{IF\ vs.\ CF} = 0.032$). The published baseline characteristics for the whole INFAT study were not stratified for offspring sex [124]. However, the maternal baseline characteristics of the INFAT subpopulation analyzed for the effect of the n-3 LCPUFA intervention (P*), are in agreement with the data from the whole INFAT population [124].

Table 1: **Maternal baseline characteristics.**

		male offspring (M)			female offspring (F)				P*	P#	P*#
		n	mean ± SD / median (IQR)		n	mean ± SD / median (IQR)		CM vs. CF	IM vs. IF	IF vs. CF	IM vs. CM
Maternal age (years)	CG	9	33.6 ± 3.4		11	32.0 ± 4.3			0.446	0.257	0.948
	IG	11	32.6 ± 5.1		10	30.8 ± 5.1					
Primiparae‡	CG	9	44.4%	n: 4	11	45.5%	n: 5	Chi-Square test			
	IG	11	54.5%	n: 6	10	60.0%	n: 6	0.8800			
Smoking during pregnancy‡	CG	9	11.1%	n: 1	11	0.0%	n: 0	Chi-Square test			
	IG	11	0.0%	n: 0	10	10.0%	n: 1	0.4850			
Alcohol during pregnancy‡	CG	9	11.1%	n: 1	10	10.0%	n: 1	Chi-Square test			
	IG	11	0.0%	n: 0	10	20.0%	n: 2	0.504			
Attended ≥12 years at school‡	CG	9	88.9%	n: 8	11	63.6%	n: 7	Chi-Square test			
	IG	11	81.8%	n: 9	10	50.0%	n: 5	0.219			
Weight before pregnancy (kg)	CG	9	60.9 ± 8.1		11	63.6 ± 8.2			0.941	0.061	0.415
	IG	11	59.1 ± 6.4		10	65.7 ± 7.9					
Weight gain in pregnancy[1] (kg)	CG	9	16.1 ± 3.8		11	16.6 ± 6.5			0.641	0.506	0.715
	IG	11	14.9 ± 3.7		10	16.4 ± 3.5					
Height (cm)	CG	9	168.3 ± 4.5		11	166.5 ± 2.3			0.507	0.345	**0.020**
	IG	11	166.1 ± 4.4		10	170.4 ± 4.7		0.309	**0.020**	**0.032**	0.226
BMI before pregnancy (kg/m^2)†	CG	9	21.0	(19.2-24.0)	11	22.7	(20.9-25.5)		0.652	0.080	0.962
	IG	11	20.8	(20.1-22.3)	10	21.9	(20.7-24.0)				

Data are presented as n, mean ± SD or percentage for each of the four analysis groups (CM, CF, IM, IF). For quantitative variables the corresponding P values are calculated with two-way ANOVA (P, P#, P*#). † Not normal distributed variables or variables that violated homoscedascity were presented as median (interquartile range IQR = 25th-75th percentile). Their corresponding P values were calculated with two-way ANOVA on ranks (P*, P#, P*#). In case of a significant two-way ANOVA P value, post-hoc tests (grey shaded) were conducted with Holm-Sidak test to adjust the significance level (CM vs. CF, IM vs. IF, IF vs. CF and IM vs. CM). ‡ For qualitative variables the Chi-Square test was used. P values < 0.05 were considered as significant and marked bold; P* < 0.05 significant difference between the n-3 LCPUFA intervention and the control group, effect of the intervention, P# < 0.05 significant difference between male and female placentas, effect of offspring sex; P*# < 0.05 significant interaction between sex and the intervention; [1]last measured value at booking minus self-reported weight before pregnancy.*

4.2.3. Compliance of the pregnant women in the INFAT subpopulation

The compliance of fish-oil capsule intake, dietary intake of AA-balanced diet, and biomarkers, was assessed for the INFAT subpopulation. Fatty acid percentage of total fatty acids for EPA, DHA and AA as well as n-6/n-3 LCPUFA ratio in RBCs served as biomarker for the maternal n-3 LCPUFA intervention.

In the intervention group (IG = IM+IF), total intake of the fish oil capsules was greater than 90% in all analyzed women of the INFAT subpopulation (**Table 2**). This was in agreement with the fish-oil capsule intake rates reported for the whole INFAT population [124].

Table 2: Maternal nutritional compliance at 32nd week of gestation

		male offspring (M)			female offspring (F)				P*	P#	P*#
		n	mean ± SD / median (IQR)		n	mean ± SD / median (IQR)		CM vs. CF	IM vs. IF	IF vs. CF	IM vs. CM
Fish oil capsule intake											
90 - 95% intake fish oil capsules‡	CG	-	-	-	-	-	-	Fisher exact test			
	IG	11	45.5%	n: 5	10	30.0%	n: 3	0.659			
> 95% intake fish oil capsules‡	CG	-	-	-	-	-	-	Fisher exact test			
	IG	11	54.5%	n: 6	10	70.0%	n: 7	0.659			
Dietary intake											
Linoleic acid (g/d)	CG	9	10.0 ± 4.7		9	11.1 ± 3.8			0.347	0.250	0.795
	IG	11	8.4 ± 2.8		10	10.2 ± 4.4					
Alpha-Linolenic acid (g/d)	CG	9	1.3 ± 0.4		9	1.1 ± 0.2			0.965	0.118	0.632
	IG	11	1.3 ± 0.5		10	1.1 ± 0.3					
Eicosapentaenoic acid (mg/d)†	CG	9	18.0	(9.5-76.5)	9	42.0	(17.0-81.5)		0.727	0.907	0.214
	IG	11	55.0	(4.0-0.160.0)	10	26.0	(2.0-81.5)				
Docosahexaenoic acid (mg/d)†	CG	9	136.0	(63.0-209.0)	9	110.0	(70.0-187.5)		0.357	0.701	0.555
	IG	11	78.0	(52.0-317.0)	10	70.5	(54.3-136.5)				
Arachidonic acid (mg/d)†	CG	9	181.8	(123.5-252.0)	9	97.2	(91.3-187.5)		0.085	0.132	0.516
	IG	11	104.9	(84.9-243.3)	10	113.7	(49.9-153.4)				
n-6/n-3 PUFA ratio[1,†]	CG	9	6.0	(5.0-8.4)	9	8.2	(5.3-13.3)		**< 0.001**	0.055	0.410
	IG	11	2.9	(2.2-4.2)	10	4.2	(2.9-5.4)	0.436	**0.045**	**< 0.001**	**< 0.001**

Data are presented as n, mean ± SD or percentage for each of the four analysis groups (CM, CF, IM and IF). For quantitative variables the corresponding P values are calculated with two-way ANOVA (P, P#, P*#). †Not normal distributed variables or variables that violated homoscedascity were presented as median (interquartile range IQR = 25th-75th percentile). Their corresponding P values are calculated with two-way ANOVA on ranks (P*, P#, P*#). In case of a significant two-way ANOVA P value post-hoc tests (grey shaded) were conducted with Holm-Sidak test, to adjust the significance level (CM vs. CF, IM vs. IF, IF vs. CF and IM vs. CM). P values < 0.05 were considered as significant and marked bold; P* < 0.05 significant difference between the n-3 LCPUFA intervention and the control group, effect of the intervention; P# < 0.05 significant difference between male and female placenta, effect of offspring sex; P*# < 0.05 significant interaction between sex and the intervention; ‡For qualitative variables the Fisher's exact test was used. P values < 0.05 are marked bold. [1] n-6/n-3 LCPUFA ratio: [C18:2n-6 + C20:4n-6) / (C18:3n-3 + C20:5n-3 + C22:6n-3 + 1200mg n-3 LCPUFA supplement (for intervention group only)]*

The dietary intake characteristics of the INFAT subpopulation were in agreement with most of the dietary characteristics of the whole INFAT population, except for AA (mg/d). In the INFAT subpopulation, taken together the women who delivered male and female offspring, AA decreased in the same range (30.2 mg/d) as reported for the whole INFAT population (30.8 mg/g) [124]. However, there was only a trend for statistical significance in the subpopulation (P* = 0.085). Calculating the total LCPUFA intake (fish-oil supplementation and diet), the maternal dietary n-6/n-3 LCPUFA ratio was significantly lower by 97.0% in the intervention compared to the control group [CG = CM+CF: median (IQR): 6.5 (5.2-9.1) vs. IG = IM+IF: 3.3 (2.4 – 4.4), P* < 0.001]. Post-hoc analysis for the total dietary n-6/n-3 LCPUFA intake revealed a significant lower n-6/n-3 LCPUFA ratio in the intervention compared to the control group for pregnant women bearing offspring of both sexes. Moreover, the n-6/n-3 LCPUFA ratio was also significantly lower by 44.8% in pregnant women with male offspring compared to pregnant women with female offspring in the intervention group ($P_{IM\ vs.\ IF}$ = 0.045; **Table 2**).

The biomarker analysis at baseline (P_15) of EPA, AA, DHA and n-6/n-3 LCPUFA ratio in maternal RBCs displayed no significant differences between the four analysis groups in the INFAT subpopulation (CM, CF, IM and IF), except for AA (**Table 3**). During pregnancy in the 32nd week (P_32), all biomarkers were significantly different in maternal RBCs between the n-3 LCPUFA intervention and the control group of the INFAT subpopulation. However, RBC AA at baseline (P_15) were significantly 8% lower in IM compared CM (P*# = 0.022, $P_{CM\ vs.\ IM}$ = 0.009). In P_32, RBC AA levels further decreased in the IM to a difference between IM and CM of 20% ($P_{IM\ vs.\ CM}$ < 0.001). However, at P_32 the RBC AA levels in CF were as low as in IF ($P_{IF\ vs.\ CF}$ < 0.615). Upon the n-3 LCPUFA intervention (P_32), the significant differences in RBC DHA and EPA led to a significant lower maternal RBC n-6/n-3 LCPUFA ratio (P* < 0.001). Post-hoc analysis revealed that in mothers of male offspring the RBC n-6/n-3 LCPUFA ratio at P_32 was significantly lower by 43.1% upon the intervention [CM 2.32 (1.91-2.69), IM: 1.32 (1.07-1.99); $P_{IM\ vs.\ CM}$ = 0.001]. In mothers of female offspring the RBC n-6/n-3 LCPUFA ratio at P_32 was significantly 49.0% lower upon the intervention [CF: 2.86(2.20-4.64), IF: 1.46 (1.30-1.74); $P_{IF\ vs.\ CF}$ < 0.001]. In summary, the results from the biomarker analysis in maternal RBCs at P_32 and P_15, not stratified for sex, basically were in agreement with the results from the whole INFAT population, except that maternal RBC AA levels at P_15 and P_32 were in CF already as low as in the intervention group. However, these differences did not interfere with the equal lowering of the n-6/n-3 LCPUFA ratio upon the n-3 LCPUFA intervention of women bearing male or female offspring.

Table 3: Maternal LCPUFA biomarkers in RBCs at 15th and 32nd week of gestation

		male offspring (M)			female offspring (F)				P*	P#	P*#
		n	median	(IQR)	n	median	(IQR)	CM vs. CF	IM vs. IF	IF vs. CF	IM vs. CM
EPA RBC P_15 (20:5n3)	**CG**	9	0.43	(0.32-0.54)	11	0.47	(0.28-0.57)		0.847	0.553	0.274
	IG	10	0.50	(0.39-0.78)	10	0.38	(0.30-0.54)				
EPA RBC P_32 (20:5n3)	**CG**	9	0.44	(0.35-0.45)	10	0.27	(0.10-0.40)		**0.003**	0.193	0.473
	IG	11	0.72	(0.25-0.96)	10	0.67	(0.33-0.79)	0.165	0.665	**0.008**	0.089
DHA RBC P_15 (22:6n3)	**CG**	9	4.57	(4.25-4.87)	11	4.87	(3.76-5.59)		0.100	0.442	0.206
	IG	11	5.64	(5.10-6.33)	10	4.89	(4.19-5.32)				
DHA RBC P_32 (22:6n3)	**CG**	9	5.67	(4.83-6.15)	11	4.25	(0.59-5.49)		**0.004**	0.162	0.355
	IG	11	8.49	(2.19-9.75)	10	8.03	(3.26-9.08)	0.108	0.726	**0.007**	0.148
AA RBC P_15 (20:4n6)	**CG**	9	13.81	(13.06-14.52)	11	13.00	(11.56-13.48)		0.130	0.614	**0.022**
	IG	11	12.82	(12.15-13.11)	10	13.21	(12.23-14.15)	**0.050**	0.184	0.547	**0.009**
AA RBC P_32 (20:4n6)	**CG**	9	12.59	(11.91-12.86)	11	10.57	(2.72-12.34)		**< 0.001**	0.056	**0.005**
	IG	11	9.98	(4.26-10.76)	10	10.32	(5.08-11.33)	**0.001**	0.461	0.615	**< 0.001**
n6/n3 LCPUFA ratio RBC[1] P_15	**CG**	9	2.64	(2.33-3.04)	11	2.45	(2.26-3.13)		0.276	0.301	0.136
	IG	11	2.14	(1.84-2.67)	10	2.74	(2.33-2.93)				
n6/n3 LCPUFA ratio RBC[1] P_32	**CG**	9	2.32	(1.91-2.69)	11	2.86	(2.20-4.64)		**< 0.001**	0.160	0.650
	IG	11	1.32	(1.07-1.99)	10	1.46	(1.30-1.74)	0.195	0.488	**<0.001**	**0.001**

Data are presented as n, median (interquartile range: 25th-75th percentile) for each of the four analysis groups (CM, CF, IM, IF), because the variables were not normal distributed. Their corresponding P values are calculated with two-way ANOVA on ranks (P, P#, P*#). In case of a significant two-way ANOVA P value post-hoc tests (grey shaded) were conducted with Holm-Sidak test, to adjust the significance level (CM vs. CF, IM vs. IF, IF vs. CF and IM vs. CM). P values < 0.05 were considered as significant and marked bold; P* < 0.05 = significant difference between the n-3 LCPUFA intervention and the control, effect of the intervention; P# < 0.05 = significant difference between male and female placenta, effect of offspring sex; P*# < 0.05 = significant interaction between sex and n-3/n-6 LCPUFA intervention; Values for fatty acids are expressed as % fatty acid / total fatty acids (wt%). [1]n-6/n-3 LCPUFA ratio: (C20:2n-6 + C20:3n-6 + C20:4n-6 +C22:2n-6 + C22:4n-6 +C22:5n-6) / (C20:3n-3 + C20:4n-3 + C20:5n-3 + C22:3n-3 + C22:5n-3 + C22:6n-3)*

4.2.4. Offspring parameters in the INFAT subpopulation

Offspring birth, growth and fat distribution measurements were assessed in the INFAT study to investigate the impact of the n-3 LCPUFA intervention on offspring obesity risk. Selected birth, growth and adipose tissue parameters are presented for the time points at birth and one year in **Table 4**. No significant differences were found between the four analysis groups (CM, CF, IM and IF) in the analyzed birth parameters (pregnancy duration, APGAR-Score, umbilical cord pH), growth parameters (weight at birth and one year, birth weight percentiles, birth height, birth head circumference, ponderal index at birth, weight per length ratio at birth, birth weight to placental weight ratio) and fat distribution measurements [sum of four skin fold thicknesses (SFT), percentage fat distribution in the upper abdomen by subcutaneous / preperitoneal ratio in ultrasonography measurement (SC/PP)]. Moreover, placental tissue weight did not significantly differ between intervention and the control group or between male and female placentas (CM: 551 ± 94 g, CF: 552 ± 70 g, IM: 529 ± 100 g, IF: 537 ± 52 g, p values of two-way ANOVA > 0.05). A comprehensive list containing additional growth and fat distribution measurements and additional time points is provided in **Appendix 11.4**.

In contrast to the INFAT subpopulation, we previously reported for the whole INFAT study population that pregnancy duration was significantly prolonged in the n-3 LCPUFA intervention group compared to the control group [124]. Pregnancy duration was increased by 4.8 days (control: 275.1 ± 11.4d, intervention: 279.9 ± 8.5d, p = 0.001) in the whole INFAT study population (**Figure 8)**. To analyze whether this difference could be attributed to the selection criteria for the subpopulation, the same selection criteria were applied to the whole INFAT population. Therefore, the gestational ages of pregnancies with pre- and post-term born children, gestational diabetes, with birth weight < 10^{th} or > 90^{th} percentile, initiation of labor and primary sectios were excluded from the whole INFAT population. **Figure 8** shows that the exclusion of these parameters resulted in a non-significant mean difference of 1.3 days between control and intervention group (control: 279.1 ± 8.3d, intervention: 280.4 ± 5.7d, p = 0.378), which was in the range observed for the INFAT subpopulation (control: 281.2 ± 8.8d, intervention: 280.5 ± 6.4d, p = 0.762).

Furthermore, it was shown by Hauner *et al.* [124] that the significant differences for weight, weight-per-length ratio and ponderal index at birth disappeared when the data were adjusted for pregnancy duration and offspring sex. The identified absence of statistical significant differences for weight, weight per length ratio and ponderal index at birth in the INFAT subpopulation was in agreement with these data adjusted for pregnancy duration and offspring sex from the whole INFAT population, because in the subpopulation pregnancy duration was not statistically different and the effect of offspring sex was analyzed separately. The absence of differences upon the intervention in growth and fat distribution

measurements from birth up to one year was in line with the data adjusted for pregnancy duration and offspring sex from the whole INFAT study population.

Table 4: Offspring birth, weight and fat distribution characteristics

		male offspring (M)		female offspring (F)			P*	P#	P*#
		n	mean ± SD / median (IQR)	n	mean ± SD / median (IQR)	CM vs. CF	IM vs. IF	IF vs. CF	IM vs. CM
Pregnancy duration (d)	**CG**	9	279.4 ± 8.7	11	282.6 ± 4.8		0.811	0.649	0.309
	IG	11	281.1 ± 5.4	10	279.9 ± 7.5				
APGAR Score†	**CG**	9	10.0 (9.0-10.0)	11	10.0 (10.0-10.0)		0.632	0.784	0.369
	IG	11	10.0 (9.0-10.0)	10	10.0 (9.0-10.0)				
pH-Wert†	**CG**	9	7.3 (7.2-7.3)	9	7.3 (7.3-7.4)		0.870	0.545	0.246
	IG	11	7.3 (7.2-7.4)	10	7.3 (7.2-7.3)				
placental weight (g)	**CG**	9	551.0 ± 94.4	11	552.2 ± 70.0		0.469	0.859	0.896
	IG	11	529.0 ± 99.9	10	536.9 ± 51.8				
birth weight (g)	**CG**	9	3487.2 ± 280.8	11	3544.6 ± 298.0		0.993	0.922	0.577
	IG	11	3536.8 ± 167.8	10	3496.5 ± 338.0				
weight 1 year (g)	**CG**	8	9636.3 ± 1073.7	11	9058.2 ± 922.4		0.365	0.184	0.745
	IG	11	9839.1 ± 1259.4	10	9486.0 ± 1020.7				
birth weight percentile†	**CG**	9	48.0 (29.5-54.0)	11	54.0 (28.0-80.0)		0.857	0.210	0.900
	IG	11	41.0 (32.0-50.0)	10	57.5 (32.8-82.3)				
birth height (cm)	**CG**	9	52.2 ± 1.6	11	51.4 ± 1.4		0.495	0.469	0.350
	IG	11	52.1 ± 1.7	10	52.2 ± 1.8				
birth head circumference (cm)	**CG**	9	35.6 ± 1.1	11	35.0 ± 0.8		0.312	0.064	0.914
	IG	11	35.3 ± 0.9	10	34.7 ± 1.1				
birth ponderal index (kg/m^3)	**CG**	9	24.6 ± 3.4	11	26.2 ± 2.2		0.469	0.498	0.192
	IG	11	25.1 ± 2.0	10	24.6 ± 2.2				
birth weight / length (g/cm)	**CG**	9	66.9 ± 6.2	11	69.0 ± 5.3		0.749	0.711	0.330
	IG	11	67.9 ± 2.5	10	66.9 ± 5.6				
birth weight / placenta weight†	**CG**	9	6.4 (6.0-6.9)	11	6.5 (5.9-6.9)		0.540	0.713	0.610
	IG	11	6.9 (5.5-7.6)	10	6.6 (5.9-7.0)				
Sum of 4 SFT[1] 3-5d pp. (mm^2)	**CG**	8	14.5 ± 1.6	11	15.4 ± 2.5		0.140	0.111	0.756
	IG	11	15.3 ± 1.9	9	16.6 ± 1.8				
Sum of 4 SFT[1] 1 year (mm^2)†	**CG**	8	23.1 (19.4-25.6)	11	25.4 (21.0-26.9)		0.983	0.322	0.545
	IG	11	23.8 (21.8-25.0)	10	24.7 (19.7-27.7)				
Ratio $[SC/PP]^2$ 6 weeks	**CG**	8	3.2 ± 0.9	7	3.4 ± 1.0		0.157	0.892	0.663
	IG	9	2.7 ± 1.6	8	2.6 ± 1.2				
Ratio $[SC/PP]^2$ 1 year	**CG**	8	1.6 ± 1.1	10	1.8 ± 1.0		0.614	0.418	0.824
	IG	9	1.4 ± 0.5	10	1.7 ± 0.6				

Data are presented as n, mean ± SD for each of the four analysis groups (CM, CF, IM and IF). For quantitative variables the corresponding P values are calculated with two-way ANOVA (P, P#, P*#). †Not normal distributed variables or variables that violated homoscedascity were presented as median (interquartile range IQR = 25th-75th percentile). Their corresponding P values are calculated with two-way ANOVA on ranks (P*, P#, P*#). In case of a significant two-way ANOVA P value post-hoc tests (grey shaded) were conducted with Holm-Sidak test, to adjust the significance level (CM vs. CF, IM vs. IF, IF vs. CF and IM vs. CM). P values < 0.05 were considered as significant and marked bold; P* < 0.05 = significant difference between the n-3 LCPUFA intervention and the control, effect of the intervention, P# < 0.05 = significant difference between male and female placentas, effect of offspring sex; P*# < 0.05 = significant interaction between sex and the intervention. ; [1]sum of the four skinfold thickness (SFT) was calculated as: biceps + triceps + subscapular + suprailiac SFT, [2]the ratio of subcutaneous to preperitoneal (SC/PP) fat was calculated as [(subcutaneous-area sagittal + axial)/2]/preperitoneal-area sagittal [124].*

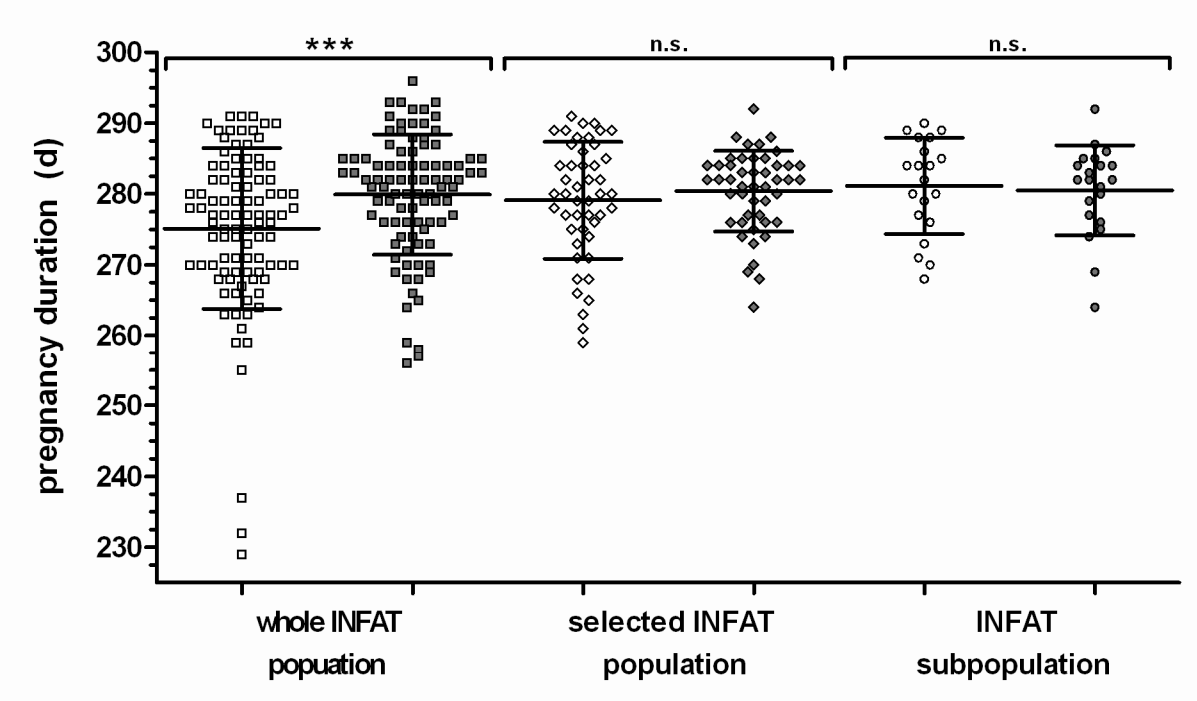

Figure 8: Differences for pregnancy duration in days (d) *between control and n-3 LCPUFA intervention group of the whole INFAT population (n_{CG} = 96 / n_{IG} = 92), the whole INFAT population with selection analogous to the INFAT subpopulation (exclusion of pregnancies with pre- and post-term born children, gestational diabetes, birth weight < 10th or > 90th percentile, initiation of labor and primary sections; (n_{CG} = 47 / n_{IG} = 47) and the INFAT subpopulation (n_{CG} = 20 / n_{IG} = 21). Data are presented as scatter blots with mean ± SD. The control group is depicted by white symbols and the n-3 LCPUFA intervention group by grey symbol. Statistical significance was calculated by unpaired t-test. P values < 0.05 were considered as significant; P*** < 0.001 = significant difference between the intervention and the control group, n.s. = no statistical significant difference between intervention and control group.*

4.2.5. LCPUFA analysis in offspring compartments and placental tissue

The relative percentage of LCPUFAs in lipid content of fetal compartments was analyzed to evaluate the impact of dietary changes upon the n-3 LCPUFA intervention on fetal tissues. The fetal part of the placenta and the umbilical cord RBC are derived from extraembryonic and embryonic mesoderm cells of the offspring. Therefore, EPA, DHA and AA levels as well as the n-6/n-3 LCPUFA ratio were measured in umbilical cord RBCs as well as in the placenta of the INFAT subpopulation (**Table 5**).

The placental phospholipid fraction contained significant higher EPA and DHA levels as well as a significant lower n-6/n-3 LCPUFA ratio in the n-3 LCPUFA intervention group than in the control group, independently of offspring sex (EPA: P* < 0.001, DHA: P* = 0.015, n-6/n-3 LCPUFA ratio: P* < 0.001). EPA levels in the phospholipid fraction significantly increased by 200% in male placentas upon the intervention ($P_{IM\ vs.\ CM}$ = 0.001). Similarly, in female placentas, EPA was significantly 200% higher in the intervention group compared to the control group ($P_{IF\ vs.\ CF}$ = 0.001). DHA levels in the phospholipid fraction were significantly elevated by 57.1% in male placentas in the intervention group compared to the control group ($P_{IM\ vs.\ CM}$ = 0.058). In female placentas an increase of 40.7% was observed for the DHA levels ($P_{IF\ vs.\ CF}$ = 0.107). Likewise, the n-6/n-3 LCPUFA ratio decreased by 39.8% in male placentas upon the intervention ($P_{IM\ vs.\ CM}$ < 0.001) and by 28.8% in female placentas ($P_{IF\ vs.\ CF}$ = 0.005). AA levels remained unchanged upon the intervention. A very similar pattern was observed for these biomarkers in the triglyceride (TG) fraction of the placenta and in total (phospholipids and triglyceride fraction) placenta lipids (**Appendix 11.5**).

In umbilical cord RBCs significant differences in EPA levels and the n-6/n-3 LCPUFA ratio were detected in the n-3 LCPUFA intervention group compared to the control group by two-way ANOVA (EPA: *P** = 0.002 / n-6/n-3 LCPUFA ratio: P* = 0.005). DHA levels and AA levels did not display significant differences between the analysis groups.

In agreement with the data from the whole INFAT study, significant higher umbilical cord RBC EPA levels and a lower umbilical cord RBC n-6/n-3 PUFA ratio upon the n-3 LCPUFA intervention, as well as unaltered AA levels were also observed in the INFAT subpopulation [124]. In contrast to the whole INFAT study population, the RBC DHA level, were not significantly elevated upon the intervention in the INFAT subpopulation. Since the placental fatty acid profile only was analyzed in the INFAT subpopulation, it was not possible to compare these data to the whole INFAT population.

Table 5: LCPUFA fatty acid percentages of placental phospholipid (PL) fraction, and umbilical cord RBCs.

% of total fatty acids (wt%)		male offspring (M)		female offspring (F)			P*	P#	P*#
		n	mean ± SD median (IQR)	n	mean ± SD median (IQR)	CM vs. CF	IM vs. IF	IF vs. CF	IM vs. CM
EPA placenta tissue PL (20:5n3)†	**CG**	8	0.10 (0.08-0.13)	11	0.09 (0.07-0.15)		**< 0.001**	0.778	0.818
	IG	11	0.30 (0.18-0.35)	10	0.27 (0.19-0.37)	0.725	0.970	**0.001**	**0.001**
DHA placenta tissue PL (22:6n3)	**CG**	9	2.66 ± 1.15	11	3.07 ± 1.17		**0.015**	0.617	0.802
	IG	11	4.18 ± 1.65	10	4.32 ± 2.55	0.601	0.858	0.107	0.058
AA placenta tissue PL (20:4n6)	**CG**	9	16.21 ± 4.94	11	17.12 ± 3.56		0.150	0.662	0.816
	IG	11	14.53 ± 3.77	10	14.81 ± 5.03				
n-6 /n-3 LCPUFA ratio placenta tissue PL[1]	**CG**	9	7.24 ± 2.07	11	6.49 ± 1.36		**< 0.001**	0.996	0.480
	IG	11	4.36 ± 1.32	10	4.62 ± 1.79	0.624	0.609	**0.005**	**< 0.001**
EPA UC RBCs (20:5n3)	**CG**	6	0.06 ± 0.04	8	0.07 ± 0.05		**0.002**	**0.046**	**0.017**
	IG	8	0.26 ± 0.12	7	0.10 ± 0.10	0.760	**0.002**	0.525	**< 0.001**
DHA UC RBCs† (22:6n3)	**CG**	8	1.03 (0.66-3.88)	9	2.25 (0.76-4.59)		0.492	0.376	0.131
	IG	9	4.36 (1.68-6.27)	8	1.03 (0.52-2.22)				
AA UC RBCs†(20:4n6)	**CG**	8	3.91 (2.88-11.08)	9	8.73 (3.49-13.70)		0.305	0.503	0.088
	IG	9	8.29 (4.58-11.35)	8	2.99 (2.41-5.06)				
n-6/n-3 LCPUFA ratio UC RBCs †,1	**CG**	8	4.90 (4.03-6.22)	9	5.39 (4.07-7.39)		**0.005**	0.106	0.291
	IG	9	2.62 (2.45-3.59)	8	3.88 (3.18-5.16)	0.680	0.062	0.172	**0.007**

Data are presented as n, mean ± SD for each of the four analysis groups (CM, CF, IM and IF). For quantitative variables the corresponding P values are calculated with two-way ANOVA (P, P#, P*#). †Not normal distributed variables or variables that violated homoscedascity were presented as median (interquartile range IQR = 25th-75th percentile). Their corresponding P values are calculated with two-way ANOVA on ranks (P*, P#, P*#). In case of a significant two-way ANOVA P value post-hoc tests (grey shaded) were conducted with Holm-Sidak test, to adjust the significance level (CM vs. CF, IM vs. IF, IF vs. CF and IM vs. CM). P values < 0.05 were considered as significant and marked bold; P* < 0.05 significant difference between the n-3 LCPUFA intervention and the control, effect of the intervention, P# < 0.05 significant difference between male and female placentas, effect of offspring sex; P*# < 0.05 significant interaction between sex and n-3/n-6 LCPUFA intervention; [1]n-6/n-3 LCPUFA ratio: (C20:2n-6 + C20:3n-6 + C20:4n-6 +C22:2n-6 + C22:4n-6 +C22:5n-6) / (C20:3n-3 + C20:4n-3 + C20:5n-3 + C22:3n-3 + C22:5n-3 + C22:6n-3).*

4.3. Transcriptome analysis of placental gene expression

To investigate the impact of a decreased n-3 LCPUFA intervention on the placental transcriptome, influences on gene expression from other factors than the n-3 LCPUFA intervention should be kept at a minimum. Influences of birth mode on placental gene expression were already described by Lee *et al.* [169]. In addition, there were hints abstracted from literature that analgesics or anesthetics administered during labor can also have an impact on placental gene expression [170-174]. Therefore, it was decided to consider these factors in the analysis of this thesis. The transcriptome analysis only included placentas from vaginal deliveries where no cervical ripening agents (e.g. prostaglandin gels), analgesics or anesthetics were administered during labor from full-term newborn appropriate for gestational age (AGA: 10^{th} – 90^{th} percentile) from a healthy gestation (e.g. no gestational diabetes). The 17 conducted DNA microarrays were further assessed for their quality. Since one DNA microarray failed in more than three quality criteria, the data of nine placentas from the n-3 LCPUFA intervention group were compared to seven placentas of the control group (in total n = 16). This placental selection is denoted in the following as: placental setup used in the transcriptome analysis (n_{CG} = 7 / n_{IG} = 9) without the use of anesthetics or analgesics during labor.

4.3.1. Transcriptome analysis for the impact of the n-3 LCPUFA intervention on placental gene expression

Of 17016 genes assessed by the DNA microarrays, in total, 22 genes showed significant differential expression between the n-3 LCPUFA intervention group and the control group in the placenta (fold change ≥ +1.5 and P < 0.05 or fold change ≤ -1.5 and P < 0.05; **Table 6**). Ten of the 22 genes were lower expressed and twelve were higher expressed in the intervention than in the control. These 22 significantly regulated genes were by far less genes that were expected to be regulated. Since sex-specific placental gene expression [112] and sex-specific differences in LCPUFA metabolism [175] were already reported, it was decided to investigate the DNA microarray data for gene expression changes upon the intervention in a sex-specific manner. The sex-distribution of the placentas analyzed by DNA microarray were three male (CM) and four female (CF) placentas in the control group as well as five male (IM) and four female placentas in the n-3 LCPUFA intervention group. Offspring sex was considered in the statistical analysis for the impact of the n-3 LCPUFA intervention by including a term for sex. Due to this consideration of offspring sex, 222 genes [61 (27.5%) were up-regulated and 161 (72.5%) were down-regulated] were observed to have significant differential expression upon the n-3 LCPUFA intervention (**Figure 10**). This list with 222 regulated genes contained 19 of the 22 genes identified without the consideration of offspring

sex, but *phosphoribosyl pyrophosphate amidotransferase* (*PPAT*), *tubulin polymerization-promoting protein family member 3* (*TPPP3*) and *tandem C2 domains, nuclear* (*TC2N*) were not contained in the list of 222 genes any more. A reason for this might be their borderline significance levels (raw p values 0.0371 - 0.0496). The lists for all significantly regulated genes between the n-3 LCPUFA intervention group and the control group for male and female placentas together with and without the consideration of offspring sex are provided on the compact disc attached to the thesis (**Appendix 11.6.1 and 11.6.2)**.

Table 6: 22 significantly regulated genes upon the n-3 LCPUFA intervention independent of offspring sex.

gene name	FC	raw p-value	Intensity normalized by gcRMA(slow) in unlogged scale		description
			Control	n-3 LCPUFA intervention	
CBR1	-1.74	0.04048	22 (16-39)	17 (14-19)	carbonyl reductase 1
LPGAT1	-1.65	0.00120	92 (85-101)	51 (47-75)	lysophosphatidylglycerol acyltransferase 1
PRR16	-1.62	0.00682	40 (33-43)	23 (17-26)	proline rich 16
LACTB2	-1.61	0.00172	26 (19-34)	17 (13-18)	lactamase, beta 2
CSTA	-1.61	0.02346	183 (123-226)	110 (90-121)	cystatin A (stefin A)
CENPK	-1.59	0.01885	37 (36-43)	24 (20-37)	centromere protein K
ANXA3	-1.56	0.00111	493 (438-629)	338 (304-374)	annexin A3
SFRP1	-1.56	0.01890	30 (24-39)	19 (18-27)	secreted frizzled-related protein 1
PPAT	-1.51	0.03710	18 (17-20)	11 (8-19)	phosphoribosyl pyrophosphate amidotransferase
PRSS23	-1.50	0.03012	177 (138-246)	125 (109-149)	protease, serine, 23
THOP1	1.58	0.00374	16 (12-19)	22 (21-25)	thimet oligopeptidase 1
CCDC69	1.62	0.04745	82 (46-108)	109 (102-119)	coiled-coil domain containing 69
TPPP3	1.66	0.04968	147 (130-189)	230 (177-354)	tubulin polymerization-promoting protein family member 3
FURIN	1.66	0.03882	213 (191-245)	290 (287-454)	furin (paired basic amino acid cleaving enzyme)
LOC100288618	1.67	0.00001	8 (7-8)	13 (12-15)	hypothetical protein LOC100288618
PAFAH2	1.70	0.00177	20 (20-25)	42 (29-42)	platelet-activating factor acetylhydrolase 2, 40kDa
PRRG4	1.80	0.02551	51 (39-61)	87 (63-105)	proline rich Gla (G-carboxyglutamic acid) 4 (transmembrane)
SH3GLB2	1.85	0.04398	110 (87-209)	224 (173-256)	SH3-domain GRB2-like endophilin B2
CX3CR1	1.87	0.01984	17 (15-37)	40 (32-51)	chemokine (C-X3-C motif) receptor 1
TC2N	2.00	0.04455	77 (64-126)	124 (101-192)	tandem C2 domains, nuclear
CORO6	2.10	0.01762	210 (139-279)	458 (283-667)	coronin 6
HINT3	3.03	0.01622	13 (10-15)	27 (16-97)	histidine triad nucleotide binding protein 3

Data are presented as median (interquartile range IQR = 25th-75th percentile). Control = placentas of female and male offspring in the control group (n_{CG} = 7), intervention = placentas of female and male offspring in the n-3 LCPUFA intervention group (n_{IG} = 9). The genes are sorted according to their fold change. FC, fold change;

4.3.2. Transcriptome analysis for the impact of offspring sex on placental gene expression

DNA microarray data were analyzed in more detail for sex-specific placental gene expression. It was investigated whether gene expression differences exist *per se* between male and female placentas independently of the n-3 LCPUFA intervention. Comparing gene expression data of male (n_{CM} = 3) versus female placentas (n_{CF} = 4) of the control group, significant expression differences were found for 399 genes (fold change ≥ +1.5 and P < 0.05 or ≤ -1.5 and P < 0.05). Thereof, 183 (45.9%) were higher expressed and 216 (54.1%) genes were lower expressed in male compared to female placentas (**Figure 9**). All significant differentially expressed genes between male and female placentas in the control group are listed on the compact disc attached to the thesis (**Appendix 11.6.3)**.

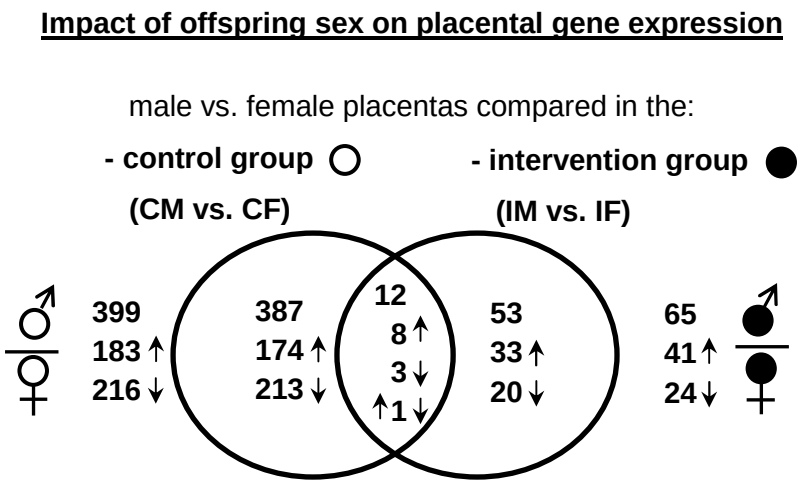

Figure 9: The Venn diagram compares the number and intersection of genes with significant differential expression *(fold change ≥ +1.5 and p-value < 0.05 or fold change ≤ -1.5 and p-value < 0.05)* ***between male and female placentas in the control group*** *(CM vs. CF, n_{CF} = 4 / n_{CM} = 3)* ***and in the n-3 LCPUFA intervention group*** *(IM vs. IF, n_{IF} = 4 / n_{IM} = 5). White symbols = control group, black symbols = intervention group, arrow up = higher expressed genes, arrow down = lower expressed genes in comparison to the respective control group (CF of IF).*

To confirm the gene expression differences between male and female placentas within the control group, the significantly regulated genes of this transcriptome dataset (CM vs. CF) were compared to a list of similar transcriptome analyses extracted from literature. Earlier reports of microarray data from human term placentas provided 56 significantly regulated genes between male and female offspring in control groups [112,176]. Thereof, ten genes could not be measured by the herein applied DNA microarrays, because there were no probe sets on the DNA microarray or the probe sets could not be assigned to genes by the chip definition file (CDF). Further ten genes showed no or very low expression levels [intensity < 20 (threshold for intensity above background) in 90% of the DNA microarrays]. Of the remaining 36 genes twelve (33.3%) showed significant differential expression between male and female placentas in the INFAT subpopulation (**Table 7**)**.**

Table 7: Twelve genes showing significant expression differences between male and female human term placentas, which were already reported

gene symbol	gene description	Chr.	Fold change CM vs. CF	P CM vs. CF
CD99L2	CD99 molecule-like 2	X	1.92	0.0461
EIF1AX	eukaryotic translation initiation factor 1A, X-linked	X	-1.67	0.0447
HDHD1A	haloacid dehalogenase-like hydrolase domain containing 1A	X	-1.81	0.0013
KDM6A	lysine (K)-specific demethylase 6A	X	-1.87	0.0071
DDX3Y	DEAD (Asp-Glu-Ala-Asp) box polypeptide 3, Y-linked	Y	Expressed above background only in male placentas (fluorescence intensity > 20)	
EIF1AY	eukaryotic translation initiation factor 1A, Y-linked	Y		
KDM5D	lysine (K)-specific demethylase 5D	Y		
RPS4Y1	ribosomal protein S4, Y-linked 1	Y		
USP9Y	ubiquitin specific peptidase 9, Y-linked	Y		
UTY	ubiquitously transcribed tetratricopeptide repeat gene, Y-linked	Y		
ZFY	zinc finger protein, Y-linked	Y		
LPL	lipoprotein lipase	8	5.60	2.4 E-05

The identification of sex-specific expression differences between male and female placentas independent of the n-3 LCPUFA intervention led to the question whether the intervention influences these sex-specific expression differences or not. In the comparison between male (n_{IM} = 5) and female (n_{IF} = 4) placentas from the n-3 LCPUFA intervention group, 65 genes were identified to exhibits sexual dimorphic expression (fold change ≥ +1.5 and $P < 0.05$ or fold change ≤ -1.5 and $P < 0.05$). Thereof, 41 (63.1%) genes were higher expressed and 24 (36.9%) genes were lower expressed in male than in female placentas (**Figure 9**). For the complete list of all genes with significant differential expression between male and female placentas in the n-3 LCPUFA intervention see the data provided on the compact disc attached to the thesis (**Appendix 11.6.4**).

A Venn diagram was used to assess whether sex-specific differences exist in the control and the n-3 LCPUFA intervention group or only in one of the two analysis groups. The intersection area of the Venn diagram (**Figure 9**) shows that twelve genes displayed significant differential expression between male and female placentas in both the control and the n-3 LCPUFA intervention. The eight genes *DDX3Y, RPS4Y1, USP9Y, ZFY, KDM5D, EIF1AY, UTY,* and *zinc finger protein 711* (*ZNF711*) were significantly higher and the three genes *KDM6A, Sjogren syndrome antigen B (SSB)* and *HDHD1A* were significantly lower expressed in male than in female placentas of both groups. One gene, *LIM and calponin homology domains 1* (*LIMCH1*), showed an inverse expression in the control group compared to the n-3 LCPUFA intervention group. *LIMCH1* was higher expressed in male than in female placentas of the control, but lower expressed in male compared to female placentas of the n-3 LCPUFA intervention.

Overall, fewer genes (65 genes) were found to have significant sexual dimorphic expression in placentas of the n-3 LCPUFA intervention compared to placentas of the control group (399 genes). Furthermore, 387 genes with sex-specific expression were uniquely observed in the control group and 53 genes were uniquely observed in the n-3 LCPUFA intervention group.

4.3.3. Transcriptome analysis for the impact of the n-3 LCPUFA intervention separately in male and female placentas

It was also investigated whether genes can be altered in their gene expression upon the n-3 LCPUFA intervention specifically in male or in female placentas. Therefore, the n-3 LCPUFA intervention group was compared to the control group in male (n_{CM} = 3, n_{IM} = 5) and female (n_{CF} = 4, n_{IF} = 4) placentas, separately (fold change ≥ +1.5 and $P < 0.05$ or fold change ≤ -1.5 and $P < 0.05$). In male placentas, 93 genes were differentially expressed between the n-3 LCPUFA intervention and the control [IM vs. CM: 34 (36.6%) genes were up-regulated and 59 (63.4%) were down-regulated]. In female placentas, 239 genes showed significant differential expression between the n-3 LCPUFA intervention group and the control group [IF vs. CF: 80 (33.5%) genes were up-regulated and 159 (66.5%) were down-regulated; **Figure 10**]. The entire lists of all significantly regulated genes between the n-3 LCPUFA intervention group and the control group for male and female placentas separately are provided in **Appendix 11.6.5 and 11.6.6** on the compact disc.

Here also, a Venn diagram was applied to compare the genes significantly regulated upon the n-3 LCPUFA intervention in all placentas considering offspring sex as well as in male and female placentas separately. The Venn diagram shows that only six genes were significantly regulated upon the n-3 LCPUFA intervention in both the male and the female dataset. All of these six genes were inversely regulated in male and female placentas. *UDP-N-acetyl-alpha-D-galactosamine:polypeptide N-acetylgalactosaminyltransferase 11 (GALNT11), CDC28 protein kinase regulatory subunit 2 (CKS2)* and *chromosome 8 open reading frame 59 (C8orf59)* were down-regulated upon the intervention in female placentas, but up-regulated in male placentas, whereas *LIMCH1, EPS8-like 2 (EPS8L2)* and *stimulated by retinoic acid 6 (STRA6)* were up-regulated upon the intervention in female placentas, but down-regulated in male placentas (**Figure 10**). No genes were enclosed in the intersection area between all three analyzed transcriptome datasets (IG compared to the CG in male and female placentas analyzed separately and together under consideration of offspring sex). However, 22 genes were enclosed in the intersection area between the dataset for male and female placentas together, considering offspring sex, and the dataset of male placentas. On the other side, 67 genes were assigned to the intersection area between the dataset for male and female placentas together, considering offspring sex, and the dataset of female placentas. Most of the genes were detected to possess significant differential expression between the n-3 LCPUFA intervention group and the control group uniquely in one dataset. In detail, 133 genes were only observed in male and female placentas analyzed together, under consideration of offspring sex, whereas 166 genes were found to be regulated only in female placentas and 65 genes only in male placentas.

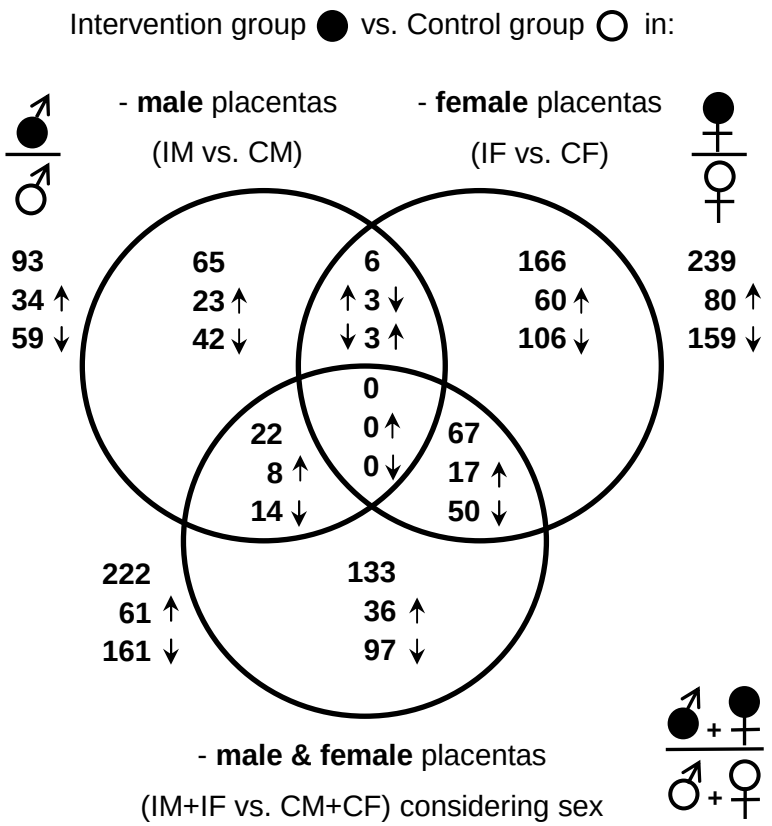

Figure 10: Venn diagram comparing the number and intersections of significantly regulated genes *(fold changes ≥ +1.5 and p-value < 0.05 or fold changes ≤ -1.5 and p-value < 0.05)* ***between the n-3 LCPUFA intervention group and the control group in male placentas*** *(IM vs. CM, n_{CM} = 3 / n_{IM} = 5),* ***female placentas*** *(IF vs. CF, n_{CF} = 4 / n_{IF} = 4)* ***and male and female placentas under consideration for sex*** *(IM+IF vs. CM+CF, n_{CG} = 7 / n_{IG} = 9). White symbols = control group, black symbols = intervention group, arrow up = higher expressed genes, arrow down = lower expressed genes in comparison to the respective control group (CF of IF).*

4.3.4. Transcriptome dataset investigation for known LCPUFA regulated genes

No transcriptome analyses have so far been reported investigating the impact of a maternal n-3 LCPUFA intervention on placental gene expression. However, reviews summarizing gene expression analysis of LCPUFAs supplementation, especially in white adipose tissue or liver and thereof cell models of mammalian origin, often reported changes in genes of lipid metabolism, *PPAR* targets and several other genes [28,45,46,48,49,177-179]. Therefore, it was assessed whether the n-3 LCPUFA intervention impacts the corresponding genes in the placenta. The genes extracted from literature were analyzed for significant regulations upon the n-3 LCPUFA intervention in the respective DNA microarray datasets (IM vs. CM, IF vs. CF, IM+IF vs. CM+CF under consideration of offspring sex).

For genes involved in lipid metabolism and regulated by LCPUFAs, 115 genes were extracted from literature [28,48,49,180,181]. Out of the 99 genes present on the DNA microarray, 46 genes were not expressed or expressed at very low levels in the analyzed placentas [fluorescence intensities below 20 (threshold for intensity above background) in 90% of the DNA microarrays]. However, from the remaining 54 genes only two genes - *lipoprotein lipase* (*LPL*) and *medium-chain acyl-CoA dehydrogenase* (*ACADM*) - were significantly regulated between the n-3 LCPUFA intervention group and the control group in male and female placentas, separately or together under consideration of offspring sex (**Appendix 11.7**). Moreover, none of the placental genes shown to be regulated in the placental cell line BeWo upon DHA or EPA treatment [180] or in the labyrinth zone of rat placenta upon maternal high n-3 LCPUFA diet during pregnancy [182] were found to be regulated in the placental transcriptome analysis of this thesis. The reasons for the absence of expression differences in lipid metabolism upon the n-3 LCPUFA intervention could be that DNA microarrays have lower sensitivity compared to RT-qPCR *[acyl-CoA synthetase long-chain family member 5 (ACSL5), fatty acid binding protein 3, muscle and heart (FABP3*), *arachidonate 15-lipoxygenase, type B (ALOX15B), arachidonate 5-lipoxygenase (ALOX5), PTGS1*], that there was a lower sample size [*perilipin 2 (PLIN2 / ADRP), lipin 1 (LPIN1*)] or a lower applied n-3 LCPUFA dose (*ACSL1/5, ADRP, FABP3, LPIN1, ALOX15B, ALOX5, PTGS1*) [180,182].

The gene list for genes in lipid metabolism regulated by LCPUFAs did also include the transcription factors *peroxisome proliferator-activated receptor PPARα, PPARβ/δ* and *PPARγ*. Their expression was also not significantly different upon the n-3 LCPUFA intervention or offspring sex in the transcriptome analysis (**Appendix 11.7**). It was reported in various tissues, that binding of LCPUFAs to the *PPARs* changes expression of *PPAR* target genes [34,39,45,48]. *PPARα, PPARβ/δ* and *PPARγ* gene and protein expression was shown to be predominant in syncytiotrophoblasts of human term placenta [183]. In the transcriptome

analysis of this thesis, *PPARβ/δ* and *PPARγ* showed expression above background (intensities above 20 in all analyzed DNA microarrays), however *PPARα* did not (intensities below 20 in all DNA microarrays). Fluorescence intensities of *PPARβ/δ* were higher compared to those of *PPARγ*. In contrast, Wang *et al.* [183] showed with a semi-quantitative PCR that *PPARβ/δ* and *PPARα* bands were weaker than *PPARγ* in human term placenta. The difference to the transcriptome data could depend on the analysis of whole placental tissue compared to villous tissue in this thesis. The importance of *PPARγ* and *PPARβ/δ* for placental development was shown by knock-outs of these genes in mice, which resulted in lethal placental defects [184]. Therefore, the transcriptome datasets were investigated for changes in *PPARβ/δ* and *PPARγ* target genes. For *PPARβ/δ,* 37 target genes identified in murine aorta tissue and for *PPARγ,* 69 target genes identified in human myofibroblasts were extracted from literature, which were present in the transcriptome datasets [185,186]. After excluding the genes not expressed or expressed at very low levels (intensities < 20 in 90% of the DNA microarrays), 20 and 32 genes remained for LCPUFA-regulated *PPARβ/δ* and *PPARγ* target genes respectively. For the 20 *PPARβ/δ* target genes, there was only one significant regulation observed for *Kruppel-like factor 11 (KLF11*) in the analyzed DNA microarray datasets. There was no significant regulation for *hydroxysteroid 11-beta dehydrogenase 1* (*HSD11B1*), which was demonstrated to be *PPARβ/δ* target genes in the human term placental trophoblasts [187,188]. Out of the 32 *PPARγ* target genes, *IGFBP1* and *LPL* were the only ones showing significant regulations in the n-3 LCPUFA intervention group compared to the control group (**Appendix 11.10**). Neither the experimentally validated *PPARγ* target genes *ADRP*, *SLC27A1* (*FATP1*) nor *SLC27A4* (*FATP4*) in primary human term placental trophoblasts showed significant regulations in the DNA microarray datasets upon the n-3 LCPUFA intervention (**Appendix 11.8**) [68]. This might be again a result of the lower sensitivity of DNA microarrays compared to RT-qPCR (*FATP1, FATP4*), the lower sample size (*FATP4, ADRP1*) or *in vitro* experiments with a single cell type compared to an *in vivo* dietary supplementation on placental tissue including several cell types (*FATP1, FATP4, HSD11B1, ADRP*).

Further genes reported to be altered upon LCPUFA treatment were retrieved from literature [45,46,48,49,177-179]. From different pathways and different tissues, 79 of these genes were present in the transcriptome datasets. Of these 79 genes, 39 showed no expression or very low expression (intensities below 20 in 90% of the DNA microarrays). Only the previously observed *LPL* and *ACADM* were exhibited significant differential expression between the n-3 LCPUFA intervention and the control of the remaining 40 genes (**Appendix 11.10**).

From these comparisons, *LPL* was selected for further biological validation due to several reasons. *LPL* is associated with lipid metabolism and is a *PPARγ* as well as a LCPUFA

regulated gene. However, only a few LCPUFA regulated genes reported from other organs seem to be regulated. In general, only very small effect sizes for regulations upon nutritional interventions in humans are observed [189]. However, only a few more PPARβ/δ genes were additionally identified to be regulated with a 1.3 fold change cut-off and p value < 0.05 (*biogenesis of lysosomal organelles complex-1, subunit 4, cappuccino, CNO / diaphanous homolog 1 (Drosophila), DIAPH1 / iron-sulfur cluster assembly 1 homolog (S. cerevisiae), ISCA1*; **Appendix 11.7-11.10**). Therefore, these comparisons showed that only very few reported LCPUFA-regulated genes being expected to change upon the n-3 LCPUFA intervention, seem to be actually regulated in the placenta.

4.3.5. Search for placental pathways to be regulated upon n-3 LCPUFA intervention by metabolic pathway analysis

To identify alternative pathways from the DNA microarray datasets for genes significantly regulated by the n-3 LCPUFA intervention or offspring sex, pathway analysis was conducted separately for the five datasets from the transcriptome analysis (**Appendix 11.11 and 11.12**). Pathways with a significantly overrepresented number of regulated genes in *PathVisio* (Z-score > 0.0), which included more than four significantly regulated genes in two of the five analyzed DNA microarray datasets were summarized in **Table 8**. The pathways were listed according to a) the number of datasets showing significantly regulated genes in an over-representative manner and b) the total number of genes with significant differential expression in the respective pathways. The five highest ranked pathways were oocyte meiosis, insulin signaling pathway, cell cycle (A) and (B), as well as Cytokine-cytokine receptor interaction. Furthermore, genes from the cell cycle pathways (A and B) were detected in 9 from 15 presented pathways.

Additionally, genes of the adipogenesis pathway were observed to be significantly overrepresented in the dataset of the intervention group compared to the control group and the dataset of male compared to female placentas in the control group (**Table 8**). This is of interest in the context of the INFAT study, investigating the impact of a n-3 LCPUFA intervention during pregnancy and lactation on adipose tissue development.

Table 8: Summary of pathways containing overrepresented differentially expressed genes from the DNA microarray datasets analyzed by offspring sex or the n-3 LCPUFA intervention

Pathway	m / t	Offspring sex		n-3 LCPUFA intervention			Significantly regulated genes contained in the pathways	
		CM vs. CF	IM vs. IF	IF vs. CF	IM vs. CM	IM+IF vs. CM+CF	analyzed for the effect of offspring sex	analyzed for effects of the n-3 LCPUFA intervention
Oocyte meiosis†	103 / 122	7	-	6	-	4	PRKX, PPP3CA, CALM1, 2xCALM2, CAMK2G, YWHAB	PRKACB, CDK1, **ANAPC4**, SMC3, **MAD2L1**, PPP3CA, PPP2R1A, **ANAPC4**, CCNB1, FBXO5
Insulin signaling pathway	126 / 147	7	-	5	5	-	SH2B2, TRIP10, GSK3B, FASN, 2xCALM1, 2xCALM2, PRKX,	PRKACB, SH2B2, RHOQ, PPP1R3D, PYGL, TRIP10, HK2, CALM1, IRS2, SH2B2
Cell cycle A	114 / 131	5	-	6	-	5	GSK3B, CDKN1A, YWHAB, CCNH, **CDK6**	**TGFB1**, SMC3, **MAD2L1**, **ANAPC4, CDK6**, CDK1, CDK4, **ANAPC4**, CCNB1, MCM3, DBF4
Cell cycle B	81 / 94	4	-	5	-	4	GSK3B, **CDK6**, CDKN1A, CCNH	**TGFB1, CDK6,** CDK1, **MAD2L1**, **HDAC5**, CDK4, CCNB1, DBF4, MCM3
Cytokine-cytokine receptor interaction†	228 / 267	-	4	5	4	-	CXCR7, CCR5, LEP, BMP2	IL8, FLT4, **TGFB1**, CSF3R, CCL13, CXCR7, TNFRSF21, PRL, NGFR
Adipogenesis†	118 / 131	11	-	5	-	-	LIFR, IL6ST, **DVL1**, FRZB, ID3, SREBF1, MBNL1, NRIP1, CDKN1A, **LPL,** LPIN1	**LPL, TGFB1**, MEF2C, BMP1, FRZB
B Cell Receptor Signaling Pathway†	144 / 159	9	-	6	-	-	RAP2A, PIK3AP1, SH2B2, ATP2B4, GSK3B, ACTR2, RASGRP3, **CDK6**, PPP3CA	PIK3AP1, SH2B2, PP3CA, **CDK6, HDAC5,** BCL6
Myometrial Relaxation and Contraction Pathways	146 / 161	9	-	-	5	-	GNB1, GUCY1A3, ACTB, CALM1, 2xCALM2, RGS5, YWHAB, CAMK2G	IGFBP1, PRKAR1B, CXCR7, RXFP1, GNG8
Wnt signaling pathway	139 / 162	9	-	5	-	-	**SFRP1**, GSK3B, PRKX, TBL1X, ROCK1, CAMK2G, PPP3CA, **DVL1**, FZD6	**SFRP1, LRP6**, PRKACB, TBL1X, PPP3CA
TGF-beta receptor Signaling Pathway†	134 / 152	6	-	6	-	-	ROCK1, **DVL1**, CAMK2G, CDKN1A, **CDK6**, YAP1	**TGFB1,** CDK1, **ANAPC4, CDK6,** MEF2C, ZEB1
Neurotrophin signaling pathway	115 / 136	8	-	-	4	-	SH2B2, GSK3B, CALM1, 2x CALM2, CAMK2G, MAGED1, YWHAB	IRS2, SH2B2, PLCG2, BAD
Melanogenesis	95 / 112	8	-	-	4	-	FZD6, PRKX, **DVL1**, GSK3B, PRKX, CALM1, CALM2, CAMK2G	**FZD7,** FZD3, WNT8A, CALM1
Salivary secretion	80 / 108	7	-	4	-	-	ATP1A4, KCNN4, PRKX, CALM1, 2x CALM2, GUCY1A3,	PRKACB, KCNN4, LYZ, ?
MicroRNAs in cardiomyocyte hypertrophy†	77 / 109	6	-	4	-	-	CALM1 (CALM2) PPP3CA, GSK3B, ROCK1, IL6ST, **DVL1**	**HDAC5**, PPP3CA, **TGFB1, LRP6**
Endochondral Ossification†	61 / 68	4	-	4	-	-	SERPINH1, FRZB, TIMP3, CALM1	FRZB, **TGFB1,** MEF2C, TIMP3

Number and names of significantly up- and down-regulated genes in each dataset. '–' denotes pathways which have no positive Z-scores or where less than four genes are significantly regulated in the pathway; †pathways including significantly overrepresented differentially expressed genes also detected in cell cycle pathways (A and B). Pathways and genes selected for biological validation are marked in bold; m, measured genes in the pathway; t, total genes contained in the pathway.

4.4. Explorative microRNA profiling for the influence of the n-3 LCPUFA intervention

DNA microarray analysis of placental gene expression showed significant changes upon the n-3 LCPUFA intervention. Since microRNAs are involved in the regulation of gene expression and are of great importance for placenta function (see **chapter 1.3.5**) [95,98,100-102,190,191], it was investigated whether the n-3 LCPUFA intervention has an impact on placental microRNA expression. An explorative approach was used to generate a profile of all known human microRNAs in the placenta. It was decided to analyze the placental miRNome also in a sex-specific manner, since sex-specific effects of the n-3 LCPUFA intervention on microRNA expression could not be excluded. More changes in microRNA expression were anticipated for female placentas, because more regulations in gene expression were observed upon n-3 LCPUFA intervention in the DNA microarray analysis. Therefore, to minimize costs and complexity, only the female miRNome was investigated in a pool of three female placentas for each group. The placentas for the pool of the control and intervention group were obtained by spontaneous birth without the use of analgesics or anesthetics analogous to the placental setup used for the transcriptome analysis.

The low-density array used contained 666 unique microRNAs. Thereof, 483 (69.5%) microRNAs showed amplification in female placentas of both, the control and the n-3 LCPUFA intervention group (**Appendix 11.13**, data provided on the compact disc attached to the thesis). 25 microRNAs were identified as differentially expressed between the n-3 LCPUFA intervention and the control pool ($< 5^{th}$ and $> 95^{th}$ quantile of Cq-based adaptive threshold). Of these 25 microRNAs, 12 (48.0%) were up-regulated and 13 (52.0%) were down-regulated compared to the control group. Additional 21 microRNAs exhibited Cq values either in the n-3 LCPUFA intervention group or in the control group. These microRNAs were therefore assumed to be switched-on or switched-off, respectively. Of these 21 microRNAs, 16 (76.2%) were switched on and five (23.8%) were switched-off upon the n-3 LCPUFA intervention. It was found that the technical duplicates of Cq values below 30 were more reliable compared to those above Cq 30. This observation was taken into account for the biological validation. In summary, the expression of in total 46 of 666 (6.9%) measured microRNAs was influenced by the n-3 LCPUFA intervention and were thus selected for further analyses (**Table 9**).

Table 9: Summary of the explorative microRNA profiling for the impact of the n-3 LCPUFA intervention in female placentas

microRNA ID	Norm. Cq CG	Norm. Cq IG	median Cq	FC IG vs. CG
Up regulated microRNAs				
hsa-miR-302b	39.63	35.22	37.42	21.22
hsa-miR-641	31.80	30.04	30.92	3.39
hsa-miR-550*	32.37	30.70	31.53	3.17
hsa-miR-668	26.79	25.14	25.96	3.14
hsa-miR-100	15.83	15.20	15.52	1.55
hsa-miR-99a	17.81	17.20	17.51	1.53
hsa-miR-139-5p	19.05	18.45	18.75	1.51
hsa-miR-30e*	16.49	15.96	16.22	1.45
hsa-miR-517b	17.33	16.83	17.08	1.41
hsa-miR-495	17.99	17.56	17.77	1.35
MammU6	13.79	13.40	13.59	1.31
hsa-miR-30d	16.78	16.41	16.60	1.29
Down regulated microRNAs				
hsa-miR-888	32.21	35.73	33.97	-11.45
hsa-miR-216a	29.77	32.69	31.23	-7.52
hsa-miR-375	23.70	26.46	25.08	-6.75
hsa-miR-581	31.38	33.56	32.47	-4.53
hsa-miR-586	30.90	33.07	31.98	-4.50
hsa-miR-923	19.86	21.34	20.60	-2.79
hsa-miR-130b	20.95	22.04	21.50	-2.12
hsa-miR-10b	20.83	21.87	21.35	-2.06
hsa-miR-223	14.51	15.05	14.78	-1.46
hsa-miR-320	16.65	17.13	16.89	-1.40
hsa-miR-21	14,93	15,38	15,15	-1,36
hsa-miR-522	15.36	15.76	15.56	-1.32
hsa-miR-451	14.96	15.34	15.15	-1.30

microRNA ID	Norm. Cq CG	Norm. Cq IG	median Cq	FC IG vs. CG
MicroRNAs switched-on				
hsa-miR-485-5p	Inf	27.64	NA	switched-on
hsa-miR-361-3p	Inf	29.00	NA	switched-on
hsa-miR-30c-2*	Inf	29.76	NA	switched-on
hsa-miR-219-5p	Inf	32.02	NA	switched-on
has-miR-155	Inf	32.11	NA	switched-on
hsa-miR-30c-1*	Inf	32.21	NA	switched-on
hsa-miR-208	Inf	32.26	NA	switched-on
hsa-miR-630	Inf	32.46	NA	switched-on
hsa-miR-219-1-3p	Inf	32.92	NA	switched-on
hsa-miR-497*	Inf	33.05	NA	switched-on
hsa-miR-130a*	Inf	33.22	NA	switched-on
hsa-miR-936	Inf	33.45	NA	switched-on
hsa-miR-591	Inf	33.68	NA	switched-on
hsa-miR-767-3p	Inf	33.81	NA	switched-on
hsa-miR-216b	Inf	34.22	NA	switched-on
hsa-miR-569	Inf	35.63	NA	switched-on
microRNAs switched-off				
hsa-miR-200a*	32.02	Inf	NA	switched-off
hsa-miR-649	33.57	Inf	NA	switched-off
hsa-miR-367	33.99	Inf	NA	switched-off
hsa-miR-302c*	34.84	Inf	NA	switched-off
hsa-miR-302a	36.37	Inf	NA	switched-off

MicroRNA profiling of n-3 LCPUFA intervention group compared to the control group in female placentas (IF vs. CF, pool of n = 3 in each analysis group). The normalized Cq (norm. Cq) after loess normalization are shown for each pool (CF and IF). Median Cq was calculated from normalized Cq values. Log RQ was calculated by (norm. Cq IF – norm. Cq CF). Fold changes (FC) were calculated by 2^{logRQ} or -2^{logRQ} (in case of negative logRQ). The high and low thresholds were calculated with quantile regression with a quadratic model. LogRQ values below the 5th and above the 95th percentile were considered to be regulated and are shown in this table. Furthermore, microRNAs with a detectable Cq value in one group and a Cq value below detection limit in the other group were also shown in this table (switched-on / switched-off). Inf, infinite = Cq value below detection limit; NA, not applicable = no median Cq could be calculated.

The chromosome 19 microRNA cluster (C19MC) represents the largest cluster of human microRNAs, which is exclusively expressed in the placenta and undifferentiated cells. The 46 microRNAs in this cluster were shown by reports of microRNA profilings to be among the most abundant microRNAs in human term primary trophoblast cells [192]. To evaluate the results of the explorative microRNA profiling in female placentas, the obtained expressed microRNAs were compared to the microRNAs of the C19MC cluster. Of 46 microRNAs reported to belong to the C19MC cluster [192], 42 (91.3%) microRNAs were also expressed

in the explorative profiling (**Appendix 11.14**). The median Cq values ranged between 11.8 and 30.3. Three microRNAs of the C19MC cluster could not be measured, because there were no primers on the low-density array. Only *miR-520d-3p*, belonging to the C19MC cluster, was not expressed in the microRNA profiling. It cannot be clarified whether this is due to failure of the primer, whether it was expressed in other placental cell types of the decidua or the fetal chorionic plate or is only expressed in male placentas. Two microRNAs of the C19MC cluster (*miR-517b* and *miR-522*) were differentially expressed between the n-3 LCPUFA intervention group and the control group in female placentas in the explorative profiling.

4.5. Combined analysis of data from transcriptome analysis with microRNA profiling data by Diana mirExTra

To investigate whether the microRNAs identified in the explorative miRNome analysis can interact with the genes significantly regulated upon the intervention, a combined strategy was applied by using *DIANA mirExTra*. Therefore, the significantly regulated genes from the transcriptome analysis (fold change ≥ +1.5 and $P < 0.05$ or fold change ≤ -1.5 and $P < 0.05$) were investigated for binding sites of regulated microRNAs from the explorative miRNome profiling (< 5th and > 95th quantile of Cq-based adaptive threshold). On the one hand, *DIANA mirExTra* provided a ranking of the regulated microRNAs in the input list, which possess binding sites in the 3'UTR of significantly regulated genes upon the n-3 LCPUFA intervention in an over-representative manner. On the other hand, it also provided a ranking of pathways which contain regulated genes of the transcriptome analysis upon the intervention with binding sites in their 3'UTR for the regulated microRNAs of the explorative miRNome analysis in an over-representative manner.

Of the 46 microRNAs identified upon the n-3 LCPUFA intervention in female placentas by the miRNome profiling, only 39 (84.8%) microRNAs could be included in the analysis of *DIANA mirExTra* (see chapter 3.8). Thereof, 23 (59.0%) regulated microRNAs showed significantly higher DIANA microT scores (P value < 0.05) in the significantly regulated genes of the transcriptome analysis (input gene list: all significantly regulated genes in the five analyzed transcriptome datasets) than in the background gene list (all other genes). Thus these microRNAs were assumed to have binding sites in the 3'UTR of several significantly regulated genes of the DNA microarray datasets for the n-3 LCPUFA intervention in an over-represented manner (**Table 10**). Since the Cq duplicates below a value of 30 were more reliable, it was decided to only select microRNAs for the biological validation with median Cq values below 30. Thirteen of the 23 (56.5%) microRNAs with significantly over-represented binding sites in the 3'UTR of significantly regulated genes displayed median Cq values below 30. The single significantly regulated genes of the transcriptome analysis, which possess binding sites for the same microRNAs of the explorative profiling, are depicted in **Appendix 11.15**.

Table 11 shows the pathways that include the regulated genes of the transcriptome datasets with binding sites for the different input microRNAs in an over-representative manner. The three pathways, where most genes possess binding sites in their 3'UTR for the different regulated microRNAs of the explorative profiling, were WNT signaling pathway (8 genes), insulin signal transduction (7 genes) and cell cycle (6 genes). These pathways were also observed in the summary of pathway analysis of the DNA microarray datasets.

Table 10: Summary of the results for microRNA binding sites in significantly regulated genes from transcriptome analysis and positively validated target genes by DIANA miR-ExTra

microRNA	Fold change	median Cq	P value	Genes over threshold
hsa-miR-888	-11.45	34.0	7.29 E-05	33
hsa-miR-375	**-6.77**	**25.1**	2.12 E-03	**4**
hsa-miR-586	-4.50	32.0	6.45 E-05	1
hsa-miR-130b	-2.13	21.5	4.66 E-02	22
hsa-miR-320	**-1.40**	**16.9**	**2.16 E-03**	**13**
hsa-miR-21	-1.36	15.2	9.12 E-06	11
hsa-miR-522	-1.32	15.6	8.72 E-04	10
hsa-miR-30d	**1.29**	**16.6**	5.38 E-03	**47**
hsa-miR-451	1.30	15.2	2.61 E-02	0
hsa-miR-495	1.35	17.8	1.22 E-12	63
hsa-miR-517b	1.41	17.1	5.16 E-04	43
hsa-miR-139-5p	1.51	18.8	7.22 E-03	13
hsa-miR-99a	**1.53**	**17.5**	1.00 E-02	**16**
hsa-miR-100	**1.55**	**15.5**	4.40 E-03	**15**
hsa-miR-668	3.14	26.0	1.73 E-04	6
hsa-miR-641	3.39	30.9	1.68 E-03	8
hsa-miR-302b	21.22	37.4	3.32 E-03	18
hsa-miR-367	Switched-off	†	7.80 E-05	23
hsa-miR-649	Switched-off	†	8.78 E-03	2
hsa-miR-302a	Switched-off	†	1.47 E-02	21
hsa-miR-569	Switched-on	†	1.57 E-03	2
hsa-miR-630	Switched-on	†	2.47 E-02	27
hsa-miR-155	Switched-on	†	4.65 E-02	10

P-value calculated by one-sided Wilcoxon rank sum test; microRNAs selected for further validation are marked in bold. †Median Cq below detection level.

Table 11: List of pathways which contain genes from the transcriptome analysis with binding sites for putatively regulated microRNAs in an over-representative manner

Pathway name	KEGG ID	Gene number	P value	Gene name
Streptomycin biosynthesis	hsa00521	3	2.57E-14	HK2, IMPA1, PGM1
Biosynthesis of steroids	hsa00100	3	1.10E-05	LSS, SC5DL, CYP51A1
Wnt signaling pathway	hsa04310	**8**	4.37E-05	**FZD7, LRP6**, TBL1X, **SFRP1**, PPP2R1A, **DKK1**, PPP3CA, PRKACB
Olfactory transduction	hsa04740	3	2.00E-04	CALM1,CALM2,CALM3, GNAL, PRKACB
Insulin signaling pathway	hsa04910	**7**	6.03E-04	PYGL, MKNK2, CALM1,CALM2,CALM3, RHOQ, TRIP10, PRKACB, PPP1R3D
Cell cycle	hsa04110	**6**	1.00E-03	DBF4, CCNB1, CDK4, **CDK6, MAD2L1**, MCM3
p53 signaling pathway	hsa04115	4	5.01E-03	CCNB1, CDK4, **CDK6**, APAF1
Bladder cancer	hsa05219	3	5.62E-03	DAPK1, CDK4, IL8
Regulation of actin cytoskeleton	hsa04810	1	0.011	FGF2
Nicotinate and nicotinamide metabolism	hsa00760	2	0.017	NUDT12, NT5C3
beta-Alanine metabolism	hsa00410	2	0.027	ACADM, HIBCH
Pentose phosphate pathway	hsa00030	2	0.033	PGM1, PGD
Hedgehog signaling pathway	hsa04340	3	0.037	GAS1, HHIP, PRKACB
Amino sugars metabolism	hsa00530	2	0.049	NAGK, HK2

4.6. Biological validation of selected target genes and microRNAs by RT-qPCR

For the biological validation of selected target genes and microRNAs, the sample size for RT-qPCR was increased to $n_{CM} = 9$, $n_{CF} = 11$, $n_{IM} = 11$ and $n_{IF} = 10$ (in total n = 41). This was achieved by measuring placentas from labors with and without the application of analgesics or anesthetics during labor. Still, all placentas for biological validation were obtained by vaginal deliveries, where no cervical ripening agents (e.g. prostaglandin gels) were administered during labor from full-term AGA newborn from a healthy gestation (e.g. no gestational diabetes). For the group CM, only seven placentas fulfilled these criteria. To meet the sample size requirement, two placentas obtained by secondary caesarean sections were included. Secondary sections were chosen, because they also were exposed to labor like placentas of spontaneous birth mode. This selection is in the following named: placental setup used for biological validation independent of the use of analgesics or anesthetics. P values < 0.05 were considered significant. The RT-qPCR data of all analyzed target genes measured in biological validation are summarized in **Appendix 11.16**.

4.6.1. Biological validation of selected target genes from metabolic pathway analysis on gene expression level

Since it was one aim of this thesis to investigate the mRNA-microRNA network, the focus was put on the cell cycle and WNT signaling pathways for biological validation, because several genes in these pathways were over-represented in the metabolic pathway analysis as well as in the combined analysis of microRNA profiling and transcriptome analysis. Significantly regulated genes playing a central role for the respective pathways or exhibiting binding sites for regulated microRNAs of the explorative miRNome profiling were selected. For cell cycle pathway, *cyclin-dependent kinase 6 (CDK6), cyclin-dependent kinase 1 (CDK1), transforming growth factor beta 1 (TGFB1), MAD2 mitotic arrest deficient-like 1 – yeast - (MAD2L1), anaphase promoting complex subunit 4 (ANAPC4)* and *histone deacetylase 5 (HDAC5)* were analyzed. Furthermore, *proliferating cell nuclear antigen (PCNA)*, which was just below the significance level in the DNA microarray analysis (IF vs. CF: fold change 1.4 p = 0.004), was added to the list of target genes for biological validation due to its common use as cell proliferation marker [193]. For WNT signaling pathway, *dickkopf 1 homolog - Xenopus laevis - (DKK1), secreted frizzled-related protein 1 (SFRP1), frizzled family receptor 7 (FZD7), low density lipoprotein receptor-related protein 6 (LRP6)* and *dishevelled, dsh homolog 1 - Drosophila - (DVL1)* were investigated in the biological validation. Furthermore, *LPL* was included for biological validation because it was found to be regulated (LCPUFA targets in **chapter 4.3.4**). In addition to *TGFB1*, the selection of *LPL*

for biological validation assessed also the significant overrepresented adipogenesis pathway. The significantly regulated genes between male and female placentas and / or control group and n-3 LCPUFA intervention group are presented in **Figure 11A / B**.

For the selected genes in the cell cycle pathway, *CDK6* and *PCNA* gene expression was significantly higher in the n-3 LCPUFA intervention group than in the control group as identified by the two-way ANOVA on ranks. The post-hoc tests showed that there were only significant differences between the n-3 LCPUFA intervention and the control in female placentas (**Figure 11A** IF vs. CF: *CDK6* 132% ± 49% vs. 100% ± 37%, $P_{IF\ vs.\ CF}$ = 0.046 / *PCNA* 134% ± 55% vs. 100% ± 17%, $P_{IF\ vs.\ CF}$ = 0.005). The two-way ANOVAs for *HDAC5* and *TGFB1* showed significant interactions between the n-3 LCPUFA intervention and offspring sex. With the post-hoc test for *HDAC5,* it was observed that its gene expression was significantly higher in male than in female placentas in the control, but not in the n-3 LCPUFA intervention (**Figure 11A** CM vs. CF: 135% ± 52% vs. 100% ± 41%, $P_{CM\ vs.\ CF}$ = 0.016). Post-hoc analysis for *TGFB1* revealed also a significant difference between male and female in the control group, but not in the n-3 LCPUFA intervention group (**Figure 11A** CM vs. CF: 160% ± 71% vs. 100% ± 46%, $P_{CM\ vs.\ CF}$ < 0.001). At the same time *TGFB1* gene expression was significantly higher by 32% in the n-3 LCPUFA intervention group than in the control group in female placentas, whereas *TGFB1* expression was significantly lower by 27.5% in the control compared to the n-3 LCPUFA intervention in male placentas (**Figure 11A** IF vs. CF: 132% ± 48% vs. 100% ± 46%, $P_{IF\ vs.\ CF}$ = 0.018 / IM vs. CM: 116% ± 50% vs. 160% ± 71%, $P_{IM\ vs.\ CM}$ = 0.016). *CDK1, MAD2L1* and *ANAPC4* were not significantly different expressed (**Figure 11A**).

Two-way ANOVAs for the selected genes in the WNT signaling pathway identified significant expression differences between the n-3 LCPUFA intervention groups as well as significant differences between male and female placentas. Post-hoc analysis revealed that *LRP6* gene expression was significantly lower in the n-3 LCPUFA intervention compared to the control in female placentas, but not in male placentas (**Figure 11B** IF vs. CF: 84% ± 30% vs. 100% ± 33%, $P_{IF\ vs.\ CF}$ = 0.049). For *DVL1,* post-hoc tests showed that gene expression was significantly higher in male than in female placentas in the control but not in the n-3 LCPUFA intervention (**Figure 11B** CM vs. CF: 122% ± 41% vs. 100% ± 41%, $P_{CM\ vs.\ CF}$ = 0.009). There were no significant differences observed for the placental expression of *DKK1, SFRP1* and *FZD7. LPL* gene expression was also not significant different between n-3 LCPUFA intervention and / or offspring sex.

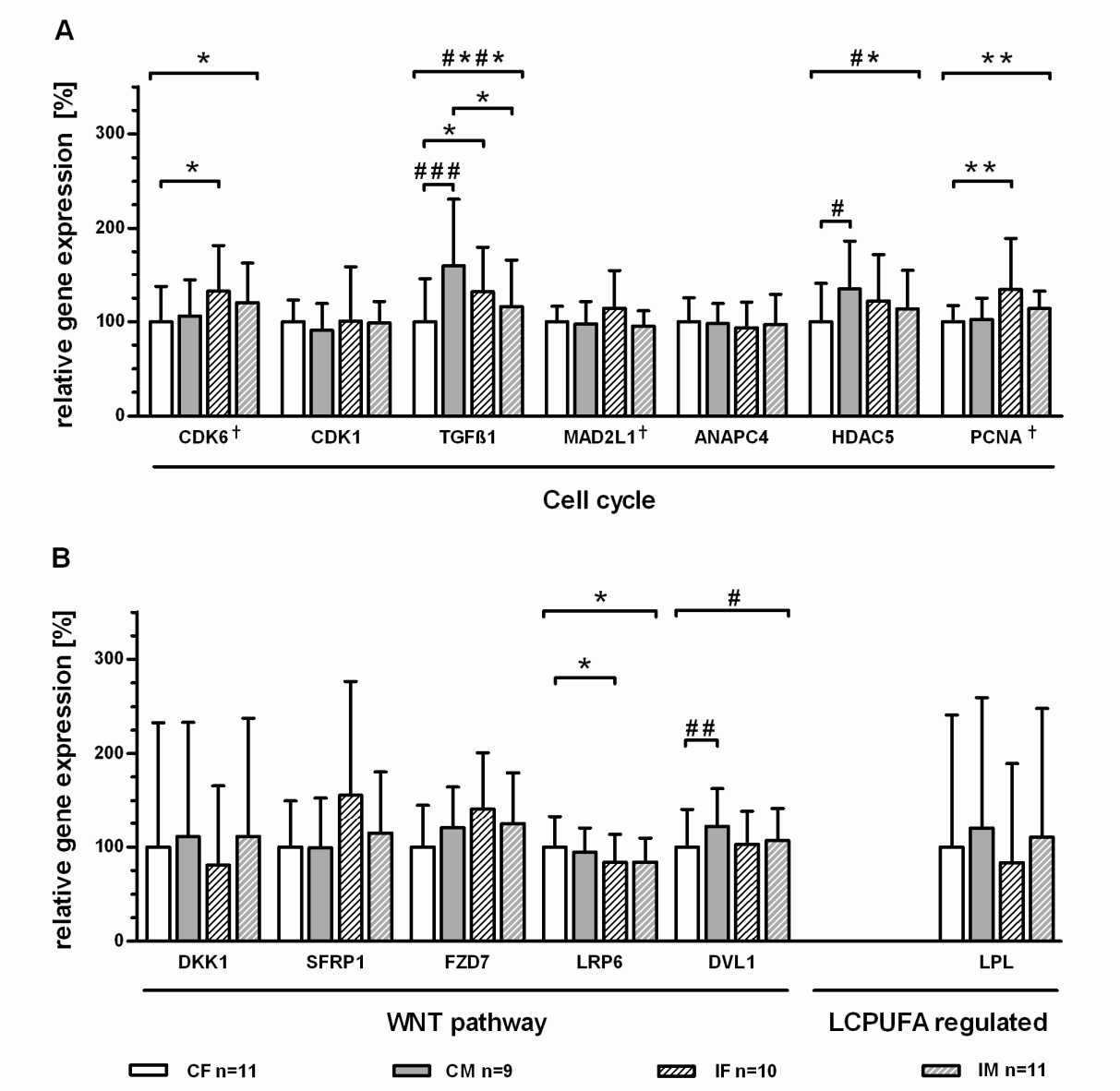

Figure 11: Results for the biological validation of selected placental target genes in cell cycle, WNT signaling pathway and LPL. *Quantitative real-time PCR (RT-qPCR) generated Cq values, which were subsequently normalized to the geometric mean of four reference genes (ACTB, POLR2a, B2M, TOP1). Relative gene expression levels were calculated by the $2^{-\Delta\Delta Ct}$ method [140]. The expression level of the CF samples was assigned an arbitrary value of 100% and all other analyzed groups (CM, IF and IM) were depicted relative to CF. Statistical significance was tested by two-way ANOVA from normal-distributed ΔCq values or by two-way ANOVA on ranks for †rank transformed ΔCq values. Significant results were further analyzed by pairwise comparison with Holm-Sidak post-hoc test. Data are presented as mean relative gene expression in % + SD. Significant effects for the factor offspring sex were marked with #, significant effects for the n-3 LCPUFA treatment with * and significant interactions with * #. # or * two-sided P < 0.05, # # or * * two-sided P < 0.01, # # # or * * * two-sided P < 0.001.*

4.6.2. Biological validation of *mTORC1* pathway and *mTORC1* target genes on gene expression level

It has been shown that the WNT signaling pathway is connected with *cell cycle* by the *mechanistic target of rapamycin* (*mTOR) complex 1 (mTORC1)* pathway [194,195]. Therefore, the transcriptome datasets were re-investigated for *mTORC1* components and *mTORC1* targets (reviewed by Laplante and Sabatini [195]). *MTOR* was expressed in the placenta (median intensity 30.5), but gene expression was not significantly differential upon the n-3 LCPUFA intervention in all five DNA microarray datasets (**Table 12**). Moreover, several components that are contained in both *mTOR complex1 and 2 (DEPTOR, MLST8, TELO2)* and unique components of *mTORC1 (RPTOR),* only showed very weak placental expression [intensity levels below 20 (background) in more than 90% of the 16 DNA microarrays]. The only significant difference was found for the *TELO2 interacting protein 1* (*TTI1*) gene*. TTI1* was 1.6 fold lower expressed in the intervention compared to the control in male and female placentas together under consideration of sex (P = 0.02) and in male placentas alone (P = 0.01). However, *TTI1 i*s not specific for *mTORC1*.

Table 12: Summary of DNA microarray data for the effect of n3 LCPUFA intervention on *mTOR complex* components

gene name	gene description	IM+IF vs. CM+CF		IM vs. CM		IF vs. CF	
		FC	p	FC	p	FC	p
mTOR complex							
MTOR	mechanistic target of rapamycin (serine/threonine kinase)	-1.07	> 0.05	-1.08	> 0.05	-1.03	> 0.05
DEPTOR[§]	DEP domain containing MTOR-interacting protein	-1.06	> 0.05	1.08	> 0.05	-1.20	> 0.05
MLST8[§]	MTOR associated protein, LST8 homolog (S. cerevisiae)	1.04	> 0.05	- 1.03	> 0.05	1.04	> 0.05
TTI1	**TELO2 interacting protein 1**	**-1.62**	**0.0215**	**-1.49**	**0.0111**	1.02	> 0.05
TELO2[§]	TEL2, telomere maintenance 2, homolog (S. cerevisiae)	-1.06	> 0.05	-1.14	> 0.05	-1.00	> 0.05
mTORC1-specific components							
RPTOR[§]	regulatory associated protein of MTOR, complex 1	-1.06	> 0.05	-1.05	> 0.05	1.01	> 0.05
AKT1S1	AKT1 substrate 1 (proline-rich)	1.53	> 0.05	1.17	> 0.05	1.25	> 0.05

[§] median intensity below 20 in 90% of the DNA microarray; FC, fold change; p = raw p value, CM, placentas of male offspring in the control group; CF, placentas of female offspring in the control group IM, placentas of male offspring in the n-3 LCPUFA intervention group; IF, placentas of female offspring in the n-3 LCPUFA intervention group.

Due to the important role of *mTORC1* in placental nutrient sensing, it was decided to biologically validate the most important *mTORC1* components. Therefore, gene expression of *MTOR* and *RPTOR*, the major components of *mTORC1* [195], was assessed in the larger sample size of the biological validation.

For *MTOR* gene expression, in the two-way ANOVA significant effects of the n-3 LCPUFA intervention (p* = 0.008) were observed (**Figure 12**). *MTOR* showed a significantly higher gene expression in the intervention group than in the control group in female, but not in male

placentas, in the post-hoc analysis (IF vs. CF: 136% ± 38% vs. 100% ± 13%, $P_{IF\ vs.\ CF}$ = 0.003). For *RPTOR* gene expression, only a significant effect of offspring sex was detected by two-way ANOVA (p# = 0.049; **Figure 12**). The post-hoc tests showed that *RPTOR* gene expression exhibited a sexual dimorphism, with significant higher expression in male than in female placentas of the control group, but not in the n-3 LCPUFA intervention group (CM vs. CF: 135% ± 37% vs. 100% ± 24%, $P_{CM\ vs.\ CF}$ = 0.007).

The effect of the n-3 LCPUFA intervention on *mTORC1* was further investigated. Roos *et al.* [196] reported a down-regulation of two amino acid transporters, *L-type amino acid transporter 1 (LAT1)* and *taurine transporter (TAUT / SLC6A6)*, by inhibition of *mTORC1* in human primary trophoblast cells. These two amino acid transporter were also found significantly differential expressed in the transcriptome analysis (*TAUT* / *SLC6A6*: IM+IF vs. CM+CF: FC -1.72, P = 0.031 / IM vs. CM: FC -1.84, P = 0.003 and *SLC7A5* / *LAT1*: IF vs. CF: FC 2.45, P = 0.021) (**Appendix 11.6.2, 11.6.5 and 11.6.6**, data shown on the compact disc). Therefore, *TAUT, LAT1*, and in addition *solute carrier family 3 (activators of dibasic and neutral amino acid transport) member 2 / CD98 heavy chain (SLC3A2 / CD98*), the second subunit for functional cell surface expression of *LAT1* [197], were investigated in the setup of the biological validation.

Two-way ANOVA and post-hoc tests showed that *TAUT* gene expression was significantly decreased in the n-3 LCPUFA intervention group compared to the control group, independently of offspring sex (IG vs. CG: 53% ± 25% vs. 100% ± 60%, P* < 0.001). For *LAT1*, in the two-way ANOVA a significant effect of the intervention and offspring sex as well as a significant interaction of both factors was observed. Post-hoc tests revealed that *LAT1* gene expression was sexual dimorphic, since it was significantly higher in male than in female placentas of the control group, but there was no sexual dimorphism upon the n-3 LCPUFA intervention (CM vs. CF: 180% ± 104% vs. 100% ± 60%, $P_{CM\ vs.\ CF}$ < 0.001). Furthermore, in female placentas the gene expression was significantly up-regulated in the n-3 LCPUFA intervention compared to the control (IF vs. CF: 170% ± 90% vs. 100% ± 60%, $P_{IF\ vs.\ CF}$ = 0.002; **Figure 12 / Appendix 11.16**). No significant differences were observed for *SLC3A2* gene expression.

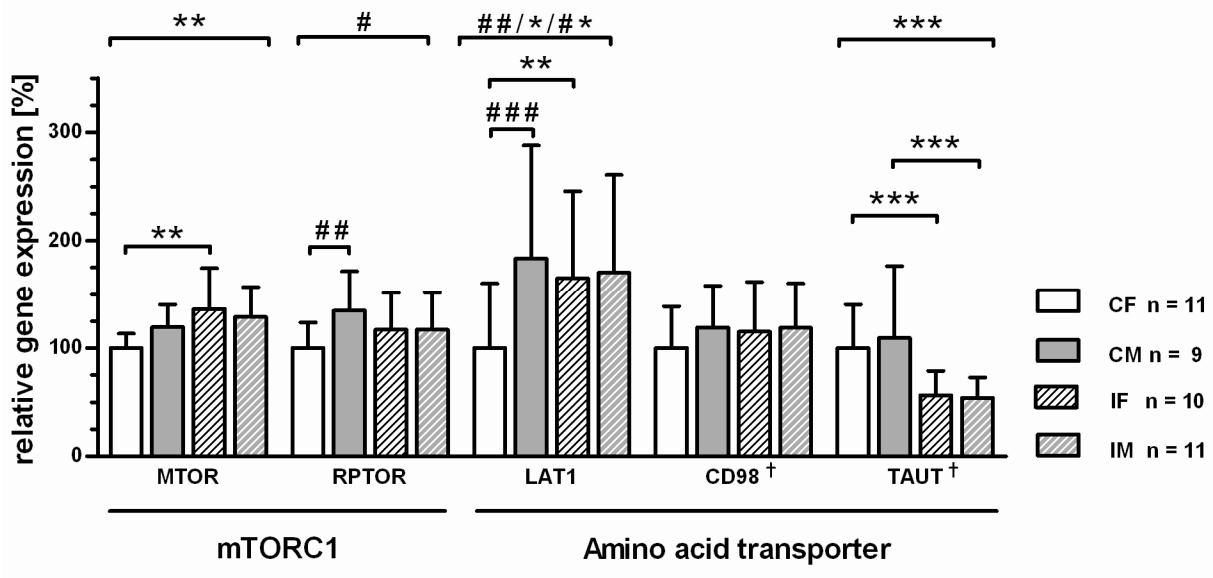

***Figure 12**: **Biological validation of mTORC1 components and amino acid transporter (mTORC1 targets) in the placenta.** Cq values measured by quantitative real-time PCR (RT-qPCR) were normalized to the geometric mean of four reference genes (ACTB, POLR2a, B2M, TOP1). Relative gene expression levels were calculated by the $2^{-\Delta\Delta Ct}$ method [140]. The expression level of the CF samples was assigned an arbitrary value of 100% and all other analyzed groups (CM, IF and IM) were depicted relative to CF. Statistical significance was tested by two-way ANOVA from normal-distributed ΔCq values or two-way ANOVA on ranks from †rank transformed ΔCq values. Significant results were further analyzed by pairwise comparison with Holm-Sidak post-hoc test. Data are presented as mean relative gene expression in % + SD. Significant effects for the factor offspring sex was marked with #, significant effects for the factor n-3 LCPUFA treatment with * and significant interactions with * #. # or * two-sided P < 0.05, # # or * * two-sided P < 0.01, # # # or * * * two-sided P < 0.001.*

4.6.3. Biological validation of selected microRNAs by RT-qPCR

All placental microRNAs selected for biological validation were found to be significantly regulated in the explorative miRNome profiling and identified by *DIANA mirExTra* to possess binding sites in the 3'UTR of significantly regulated genes of the transcriptome analysis. *MiR-375* was selected, because it displayed the highest fold change in the explorative miRNome profiling within the overrepresented microRNAs (-6.77) with a median Cq value below 30. *MiR-375* is known to promote adipocyte differentiation by over-expression of *miR-375* in murine *3T3-L1* cells [198]. Moreover, it is important in the development of human pancreatic islets, during which *miR-375* expression was steadily increased [199]. In addition, *miR-320* and *miR-30d* were chosen for biological validation, because binding sites for these microRNAs were identified in *CDK6* and *TAUT,* respectively, by *DIANA miR-ExTra*. It was reported that *miR-30d* and *miR-320* both were associated with processes in adipocytes. *MiR-30d* over-expression was shown to stimulate adipogenesis in human adipose tissue derived stem cells [200], whereas the expression of *miR-320* was increased in insulin-resistant murine adipocytes (3T3-L1 cells) and insulin-sensitivity was increased upon *miR-320* knock-down [201]. Furthermore, *miR-100* and *miR-99a*, also significantly regulated in the

explorative miRNome profiling, were biologically validated. There was evidence in various cancer and normal cell lines (prostate cancer cell models, c-Src-transformed cells, human embryonic kidney 293 cell) supporting a regulation of *MTOR* expression by demonstrating a binding of these microRNAs to its 3'UTR in luciferase experiments [194,202,203]. Together, these selected microRNAs could impact the gene expression of *CDK6, HDAC5, TAUT* and *MTOR* as indicated by *DIANA miR-ExTra* bioinformatics analysis (**Appendix 11.15**).

For biological validation of the microRNAs, the same set of placentas was used as for the biological validation of gene expression independent of the use of analgesics and anesthetics during labor (n = 41) (**see chapter 4.6**). Therefore, these microRNAs were also analyzed for possible sex-specific effects on microRNA expression. Placental *miR-99a* alone showed a different expression with a significant effect of the n-3 LCPUFA intervention (P* = 0.001) and a significant interaction of the intervention with offspring sex (p*# = 0.026) in the two-way ANOVA (**Figure 13**). The post-hoc analysis revealed that *miR-99a* exhibited a sexual dimorphism. It was significantly higher expressed in male than in female placentas of the control group (CM vs. CF: 142% ± 63% vs. 100% ± 63%, $P_{CM\ vs.\ CF} = 0.039$), but there was no difference between male and female placentas in the intervention group. Additionally, in female placentas alone, there was a significant increase in expression upon the intervention (IF vs. CF: 186% ± 70% vs. 100% ± 41%, $P_{IF\ vs.\ CF} < 0.001$). A summary for all analyzed target microRNA measurements by RT-qPCR is shown in **Appendix 11.17**.

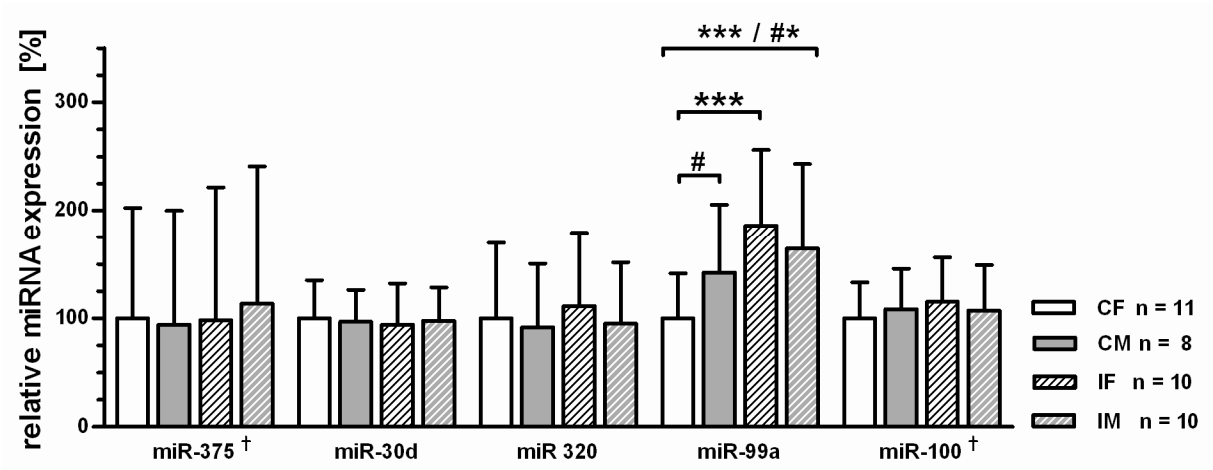

Figure 13: Biological validation of selected placental microRNAs from the explorative microRNA profiling. *Cq values of the quantitative real-time PCR (RT-qPCR) were normalized to the geometric mean of three reference RNAs (RNU24, RNU6b, miR-26b). Relative gene expression levels were calculated by the $2^{-\Delta\Delta Ct}$ method [140]. The expression level of the CF samples was assigned an arbitrary value of 100% and all other analyzed groups (CM, IF and IM) were depicted relative to CF. Statistical significance was calculated by two-way ANOVA from normal-distributed ΔCq values or by two-way ANOVA on ranks from †rank transformed ΔCq values. Significant results were tested analyzed by pairwise comparison with Holm-Sidak post-hoc test. Data are presented as mean relative microRNA expression [%] + SD. Significant effects for the factor offspring sex were marked with #, significant effects for the n-3 LCPUFA treatment with * and significant interactions with #*. # or * two-sided P < 0.05, ### or *** two-sided P < 0.001.*

4.6.4. Analysis for binding sites of *miR-99a* within *LAT1* and *TAUT* genes

MiR-99a was reported earlier to target *mTOR* protein expression and activity by functional analyses. However, there is nothing known whether *miR-99a* also acts more downstream of *mTOR* and can regulate *TAUT* and *LAT1* expression. The *DIANA-mirExTra* algorithm did also not predict that *miR-99a* can target the expression of these two amino acid transporters. Therefore, possible binding sites of *miR-99a* within *LAT1* and *TAUT* were assessed in their DNA sequence. First, computational predictions for microRNA targets were used. The computational prediction of microRNA targets are mostly and with different weight based on a) complementarity to the miRNA seed region, b) evolutionary conservation of the microRNA recognition element, c) free energy of the miRNA-mRNA heteroduplex, and d) mRNA sequence features outside the target site [204]. Another approach is the collection of experimental supported microRNA targets in large databases, e.g. tarbase [154].

A *miR-99a* binding site was predicted in the *TAUT* gene by the microRNA target prediction algorithm *rna22* [146] with two mismatches in the seed sequence. No prediction for a *miR-99a* binding site in *LAT1* 3'UTR or CDS was found by the prediction algorithms of *DIANA-microT-CDS*, *DIANA-microT, microRNA.org*, *miRDB*, *TARGETMINER, TARGETSCAN-vert*, or *PICTAR-vert*. Moreover, none of the databases storing information for experimentally identified microRNA targets (*miRecords, miRSel, miRWalk, StarBase*) [154] found *LAT1* and *TAUT* as targets for *miR-99a*. Therefore, a sequence alignment was conducted to obtain information on the complementarity between the *miR-99a* seed sequence and the *LAT1* mRNA sequence. The alignment of *LAT1* mRNA and *miR-99a* sequences matched in the seed sequence (nucleotide 2-8 of microRNA) with *LAT1* 3' UTR with two mismatches at nucleotide two and seven (5' – 3') of *miR-99a*.

4.6.5. Validation of *LAT1* protein expression by Western blot analysis

Gene expression of the reported placental *mTORC1* target gene *LAT1* was regulated in the same direction as *MTOR* gene expression upon the n-3 LCPUFA intervention. This observation was in line with literature, where it was shown that the *LAT1* transporter gene expression and activity is regulated by *mTORC1* in trophoblasts obtained from human term placenta [196]. To further assess the impact of the n-3 LCPUFA intervention on placental nutrient transport, *LAT1* protein expression was analyzed by Western blot. *LAT1* was measured in placental protein extracts analogous to the setup of the transcriptome analysis, with no analgesics or anesthetics applied during labor ($n_{CF} = 4$, $n_{CM} = 4$, $n_{IF} = 4$ and $n_{IM} = 5$, in total n = 17). The fluorescence scans of the complete membranes, as well as experiments for *LAT1* antibody specificity are provided in **Appendix 11.18**. A significant effect for the factor n-3 LCPUFA intervention (P* = 0.010) and for the factor offspring sex (P# = 0.004) on

relative *LAT1* protein expression was found by two-way ANOVA (**Figure 14**). Post-hoc analysis identified a significant lower relative *LAT1* protein expression in male compared to female placentas of the control (CM vs. CF: 71% ± 20% vs. 100% ± 31%, $P_{CM\ vs.\ CF} = 0.010$), but not in the n-3 LCPUFA intervention group (IM vs. IF: 58% ± 16% vs. 74% ± 19%, $P_{IM\ vs.\ IF} = 0.085$). Additionally, the post-hoc analysis found a down-regulation of *LAT1* protein expression upon the n-3 LCPUFA intervention in female placentas (IF vs. CF: 74% ± 19% vs. 100% ± 31%, $P_{IF\ vs.\ CF} = 0.014$), but not in male placentas (IM vs. CM: 58% ± 16% vs. 71% ± 20%, $P_{IM\ vs.\ CM} = 0.183$). Therefore, *LAT1* protein expression pattern was inverse compared to *LAT1* gene expression.

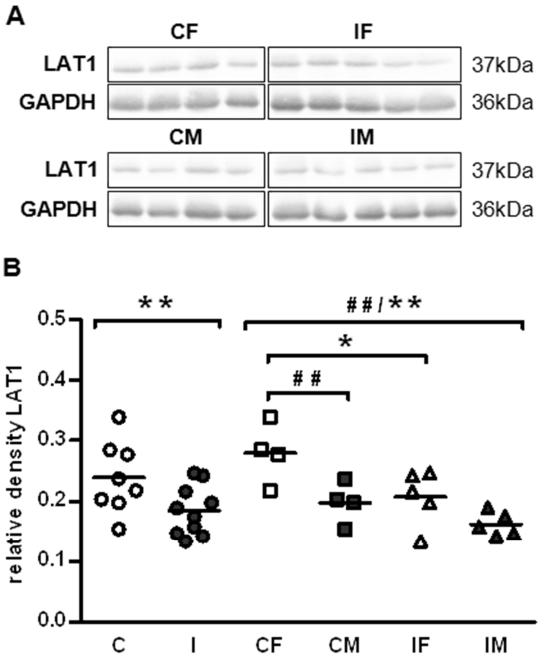

***Figure 14**: **Relative LAT1 protein expression in placental protein extracts.** LAT1 protein expression was analyzed by Western blot analysis from placentas obtained by spontaneous birth mode without anesthetics or analgesics. **A)** The Western blots show all LAT1 and GAPDH bands in the placenta separately for the n-3 LCPUFA intervention and offspring sex (CM, CF, IM and IF). **B)** Western blot data are summarized in a scatter blot for control group (CG) and n-3/n-6 LCPUFA intervention group (IG) together as well as separated for offspring sex, respectively (CF, CM, IF and IM). Band intensities of LAT1 were normalized to those of GAPDH. LAT1 relative density was plotted on the graph with the mean for the respective group. Significance was tested by two-way ANOVA with Holm-Sidak post-hoc tests. Significant effects for offspring sex differences are marked with #, significant effects for the n-3 LCPUFA intervention with * and significant interactions with *#. # or * two-sided $P < 0.05$, ## or ** two-sided $P \leq 0.01$.*

4.7. Analysis of amino acid levels in placenta and umbilical cord plasma

Since placental changes for the amino acid transporters *TAUT* and *LAT1* were observed upon n-3 LCPUFA intervention, the amino acid profiles of the placenta and the umbilical cord plasma were also assessed for alterations upon the n-3 LCPUFA intervention. Analogous to the placental setup of the biological validation with independent usage of anesthetics and analgesics during labor, 41 placentas (n_{CM} = 9, n_{CF} = 11, n_{IM} = 11 and n_{IF} = 10) and 34 matching umbilical cord plasma samples were measured (n_{CM} = 8, n_{CF} = 8, n_{IM} = 9 and n_{IF} = 9) for their amino acid levels. Unfortunately, too few plasma samples were available from the mother at birth and the amino acid levels in the maternal circulation at the 32nd week of gestation have not been measured until now.

There were no significant differences in placental amino acid levels between the n-3 LCPUFA intervention group and the control group or between male and female placentas (**Table 13**). However, in umbilical cord plasma significant differences upon the n-3 LCPUFA intervention and / or offspring sex were observed for taurine (Tau), glutamic acid (Glu), sarcosine (Sar), methionine (Met) and ß-aminoisobutyric acid (bAib) levels (**Table 14**). Significant interactions of the n-3 LCPUFA intervention and offspring sex for umbilical cord plasma taurine, glutamic acid, sarcosine and methionine levels were identified by two-way ANOVA. Umbilical cord plasma taurine levels were significant higher by 51.5% in male than in female children in the n-3 LCPUFA intervention group [**Figure 15A**: IM 300.88 (215.28-359.47) µmol/l vs. IF 198.60 (142.50-231.37) µmol/l, $P_{IM\ vs.\ IF}$ = 0.035], but not in the control group, which was shown by post-hoc analysis.

Post-hoc analysis of umbilical cord plasma methionine levels revealed a sexual dimorphism, because in the control group they were significantly lower by 72.1% in male compared to female placentas [**Figure 15C** CM 1.36 (1.11-4.45) µmol/l vs. CF 4.87 (3.36-6.57) µmol/l; $P_{CM\ vs.\ CF}$ = 0.049], but not in the n-3 LCPUFA intervention. Additionally, an impact of the intervention was found, as significant lower umbilical cord plasma methionine levels by 59.3% were observed in the intervention compared to the control in female placentas alone [**Figure 15C** IF 1.98 (1.14-3.86) µmol/l vs. CF 4.87 (3.36-6.57) µmol/l; $P_{IF\ vs.\ CF}$ = 0.037]. There were no significant differences in the levels for the branched chain amino acids leucine, isoleucine and valine in both, the placenta and the umbilical cord plasma (**Table 13 and 14**), which were shown to increase *mTORC1* activity in human muscle in the recovery period after exercise or whose transport is regulated by *mTORC1* in primary trophoblasts of human term placenta [196,205].

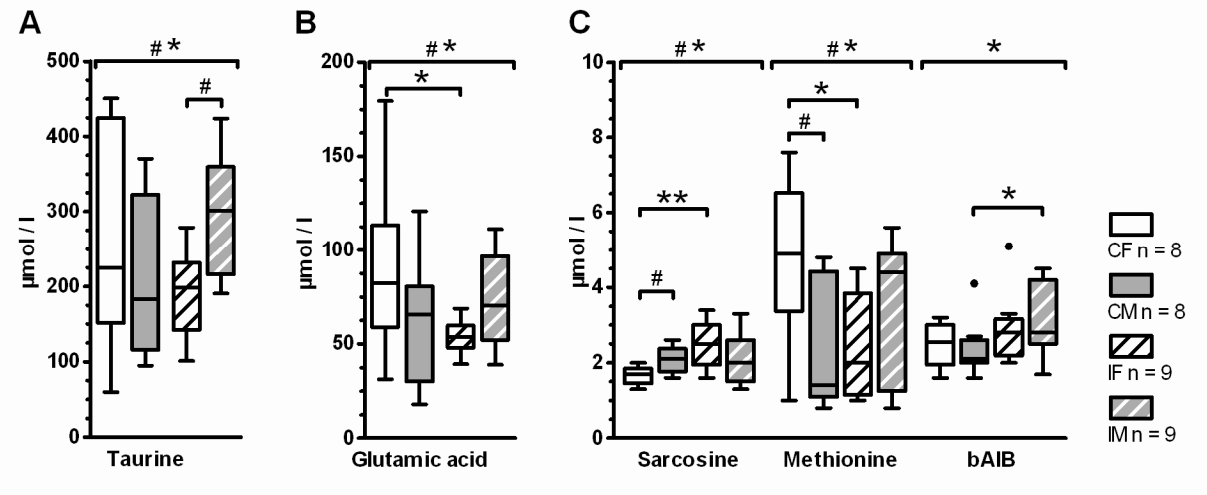

Figure 15: Amino acids levels in umbilical cord (UC) plasma with significant differences between n-3 LCPUFA intervention groups and / or offspring sex. *Amino acid levels for **A)** taurine, **B)** glutamic acid and **C)** sarcosine, methionine and ß-aminoisobutyric acid (bAIB) are presented as box-plots with median and interquartile range (IQR: 25th - 75th percentiles). Tuckey-Whiskers show values within 1.5 times the IQR, or stop at the lowest or highest value within. All data points outside 1.5 times the IQR are shown as individual dots (=outlier). Statistical significance was calculated by two-way ANOVA on ranks with Holm-Sidak post-hoc test. Significant effects for sex differences are marked with #, significant effects for the n-3 LCPUFA treatment with * and significant interactions with *#. # or * two-sided P < 0.05, ## or ** two-sided P < 0.01.*

Table 13: Amino acid levels in placental villous tissue

µmol/g protein	Control group		n-3 LCPUFA intervention group		Two-way ANOVA on ranks:
	female (n = 11) median (25^{th} - 75^{th})	male (n = 8) median (25^{th} - 75^{th})	female (n = 10) median (25^{th} - 75^{th})	male (n = 11) median (25^{th} - 75^{th})	
PEtN	31.89 (24.19-53.99)	32.84 (21.30-38.28)	37.20 (23.41-59.58)	30.66 (27.19-40.71)	P*, P# and P*# for all placental amino acid levels >0.05
Tau	163.25 (113.5-204.9)	132.37 (99.3-169.7)	138.63 (102.8-247.1)	114.71 (103.9-161.3)	
Asn	6.55 (5.82-8.04)	5.93 (4.55-7.48)	5.73 (4.22-11.57)	5.39 (4.48-6.98)	
Ser	12.79 (10.19-15.09)	9.68 (7.48-12.92)	10.06 (6.96-18.42)	8.31 (7.59-11.63)	
Hyp	1.45 (0.98-1.86)	1.36 (0.87-1.51)	1.32 (0.94-2.68)	1.24 (1.07-1.51)	
Gly	31.63 (23.72-45.02)	24.67 (19.08-38.61)	30.65 (20.22-50.59)	24.03 (22.24-30.84)	
Gln	31.79 (23.66-43.43)	28.26 (21.96-38.96)	30.21 (22.67-54.69)	28.21 (20.41-46.42)	
Asp	42.66 (31.91-52.51)	29.58 (22.52-38.22)	40.01 (24.93-64.91)	29.41 (25.29-60.35)	
EtN	28.41 (20.27-33.81)	22.03 (17.25-33.34)	25.25 (15.64-40.85)	24.98 (16.68-31.37)	
Cit	0.35 (0.25-0.39)	0.32 (0.26-0.52)	0.41 (0.24-0.75)	0.35 (0.25-0.50)	
Ala	31.38 (27.03-52.41)	31.22 (22.04-40.35)	26.40 (20.73-58.83)	31.84 (23.27-42.46)	
Thr	20.01 (14.25-23.57)	17.83 (10.49-23.28)	16.49 (12.88-27.12)	13.98 (12.11-20.98)	
Glu	90.24 (62.77-119.72)	68.75 (55.49-99.52)	84.23 (57.34-143.52)	67.76 (53.43-107.66)	
His	4.57 (3.79-6.76)	4.36 (3.10-6.35)	4.33 (2.80-9.66)	3.99 (3.19-5.59)	
X1MHis	0.10 (0.02-0.15)	0.07 (0.03-0.49)	0.06 (0.03-0.11)	0.02 (0.01-0.06)	
X3MHis	0.10 (0.08-0.13)	0.09 (0.06-0.13)	0.10 (0.06-0.18)	0.09 (0.08-0.12)	
GABA	1.09 (0.62-1.49)	0.82 (0.55-1.38)	0.91 (0.47-1.59)	0.81 (0.44-1.13)	
bAIB	1.23 (1.06-1.67)	1.15 (0.74-1.80)	1.30 (0.83-2.26)	1.06 (0.76-1.59)	
Abu	3.64 (2.33-4.78)	3.16 (2.01-4.19)	3.12 (2.03-4.54)	2.50 (1.89-3.56)	
Aad	0.66 (0.56-0.91)	0.54 (0.36-0.97)	0.67 (0.44-1.11)	0.63 (0.50-0.80)	
Pro	13.29 (9.71-17.27)	11.19 (8.65-16.50)	11.11 (7.96-23.46)	11.16 (8.79-14.13)	
Arg	5.46 (5.02-7.28)	4.49 (3.78-7.09)	5.35 (3.43-10.82)	5.23 (3.65-6.25)	
Orn	1.86 (1.46-2.27)	1.67 (1.31-2.65)	1.89 (1.26-2.74)	1.80 (1.24-2.29)	
Cys	1.19 (1.06-1.37)	1.13 (0.83-1.51)	1.11 (0.78-1.78)	1.19 (0.89-1.41)	
Asa	0.14 (0.09-0.41)	0.16 (0.09-0.29)	0.23 (0.11-0.87)	0.29 (0.11-0.44)	
Lys	11.61 (10.49-14.63)	9.62 (8.01-13.87)	12.67 (7.16-17.89)	10.73 (7.99-13.44)	
Val	9.73 (8.54-14.17)	8.76 (6.88-13.85)	9.94 (6.92-18.69)	9.60 (6.46-10.42)	
Met	0.20 (0.08-0.26)	0.21 (0.16-0.39)	0.30 (0.18-0.42)	0.22 (0.11-0.39)	
Tyr	4.03 (3.26-5.35)	2.99 (2.55-5.24)	4.22 (2.48-7.19)	3.49 (2.41-4.06)	
Ile	4.56 (4.14-5.58)	4.11 (3.03-5.67)	3.79 (2.92-8.30)	4.16 (2.74-4.92)	
Leu	9.41 (8.47-13.46)	8.70 (6.78-11.71)	8.19 (6.32-17.31)	8.33 (5.97-10.38)	
Phe	4.53 (4.08-6.70)	4.07 (3.24-5.53)	4.07 (3.06-8.84)	4.06 (3.08-4.98)	
Trp	1.27 (1.11-1.70)	1.15 (0.82-1.53)	1.30 (0.81-2.25)	1.09 (0.93-1.36)	

Amino acid levels are presented as median with Interquartile range (25^{th} – 75^{th} percentile) due to non-normal distribution. They were measured by iTRAQ™ method in 3200QTRAP LC/MS/MS. The placental setup was the same as in the biological validation (n = 41) with spontaneous birth mode and independent of the usage of analgesics or anesthetics during labor. The measured amino acid levels were normalized to the protein content of the sample. Significance was tested by two-way ANOVA with Holm-Sidak post-hoc tests.

Table 14: Amino acid levels in umbilical cord plasma

µmol/l	control group		n-3 LCPUFA intervention group		Two-way ANOVA on ranks			Holm-Sidak post-hoc			
	female (n_{CF} = 8)	male (n_{CM} = 8)	female (n_{IF} = 9)	male (n_{IM} = 9)	P*	P#	P*#	CM vs CF	IM vs IF	IF vs CF	IM vs CM
	median(25^{th} - 75^{th})	median(25^{th} - 75^{th})	median(25^{th} - 75^{th})	median(25^{th} - 75^{th})							
PEtN	14.14 (8.46-34.95)	11.82 (6.92-16.24)	11.21 (7.05-17.62)	20.60 (11.72-25.81)	>0.05	>0.05	>0.05				
Tau	224.42 (151.43-424.76)	183.71 (115.93-322.23)	198.60 (142.5-231.37)	300.88 (215.98-359.47)	>0.05	>0.05	**0.043**	0.416	**0.035**	0.269	0.072
Asn	56.78 (54.28-59.4)	52.52 (49.99-63.11)	55.20 (49.36-61.44)	53.00 (50.28-69.88)	>0.05	>0.05	>0.05				
Ser	151.04 (130.56-158.46)	129.02 (121.09-135.42)	132.10 (119.3-143.36)	130.05 (117.25-148.48)	>0.05	>0.05	>0.05				
Hyp	23.69 (19.14-25.41)	22.81 (19.46-25.91)	24.51 (21.06-27.48)	24.62 (21.65-27.37)	>0.05	>0.05	>0.05				
Gly	252.45 (216.22-258.76)	237.49 (213.41-256.89)	244.97 (235.62-279.57)	239.36 (211.78-247.31)	>0.05	>0.05	>0.05				
Gln	508.09 (399.42-533.62)	495.01 (448.59-560.63)	508.93 (470.95-562.10)	554.51 (459.14-592.07)	>0.05	>0.05	>0.05				
Asp	9.17 (4.97-19.75)	7.66 (5.48-11.95)	6.28 (4.47-11.16)	10.31 (4.97-18.04)	>0.05	>0.05	>0.05				
EtN	26.11 (24.87-29.89)	25.75 (22.42-28.20)	28.22 (19.99-31.01)	31.36 (27.13-38.10)	>0.05	>0.05	>0.05				
Cit	8.83 (8.28-13.48)	11.92 (9.44-15.76)	11.62 (10.19-13.53)	11.72 (9.09-13.43)	>0.05	>0.05	>0.05				
Sar	1.68 (1.44-1.83)	2.10 (1.78-2.37)	2.49 (1.94-2.97)	1.96 (1.49-2.58)	**0.045**	>0.05	**0.011**	**0.038**	0.115	**0.002**	0.674
Ala	485.03 (453.84-529.19)	449.10 (384.98-563.12)	448.10 (427.14-500.50)	509.98 (444.61-696.60)	>0.05	>0.05	>0.05				
Thr	318.48 (258.54-400.28)	305.61 (284.00-358.86)	294.29 (269.08-363.24)	317.96 (240.79-345.74)	>0.05	>0.05	>0.05				
Glu	82.19 (58.77-113.24)	65.56 (30.00-80.43)	53.71 (47.94-59.69)	70.28 (52.03-96.56)	>0.05	>0.05	**0.030**	0.181	0.072	**0.027**	0.379
His	110.44 (102.17-135.00)	113.84 (95.62-122.60)	107.03 (99.83-130.87)	117.73 (99.98-134.27)	>0.05	>0.05	>0.05				
X1MHis	11.83 (7.57-14.22)	7.73 (3.86-14.55)	7.71 (5.44-8.71)	8.82 (6.45-12.19)	>0.05	>0.05	>0.05				
X3MHis	3.22 (2.97-3.44)	3.19 (2.73-3.55)	2.89 (2.12-3.39)	3.69 (2.95-4.55)	>0.05	>0.05	>0.05				
GABA	0.46 (0.34-0.51)	0.48 (0.30-0.60)	0.47 (0.37-0.55)	0.46 (0.39-0.61)	>0.05	>0.05	>0.05				
bAIB	2.56 (1.97-3.02)	2.11 (1.97-2.57)	2.76 (2.21-3.16)	2.80 (2.52-4.21)	**0.043**	>0.05	>0.05	0.369	0.593	0.451	**0.034**
Abu	16.84 (12.89-29.58)	17.51 (13.94-21.44)	19.92 (16.33-22.54)	15.61 (11.96-19.87)	>0.05	>0.05	>0.05				
Aad	1.43 (0.92-1.71)	1.29 (1.07-1.77)	1.09 (1.01-1.33)	1.24 (1.15-1.48)	>0.05	>0.05	>0.05				
Car	0.71 (0.38-1.7)	0.94 (0.52-1.02)	0.89 (0.75-1.07)	0.83 (0.74-1.07)	>0.05	>0.05	>0.05				
Pro	182.04 (173.45-208.62)	194.39 (181.51-209.97)	206.21 (192.78-229.30)	205.13 (178.82-228.76)	>0.05	>0.05	>0.05				
Arg	49.27 (35.86-70.77)	70.73 (48.49-76.78)	60.45 (51.44-73.74)	52.90 (42.81-63.09)	>0.05	>0.05	>0.05				
Hyl	1.44 (0.91-1.81)	1.38 (1.11-1.82)	1.46 (1.34-1.48)	1.30 (1.20-1.66)	>0.05	>0.05	>0.05				
Orn	86.83 (71.09-105.64)	90.94 (78.65-109.47)	98.33 (82.18-106.32)	110.60 (89.84-123.19)	>0.05	>0.05	>0.05				
Cys	35.08 (22.36-36.58)	34.59 (30.02-37.91)	29.06 (15.60-36.75)	32.33 (20.79-42.51)	>0.05	>0.05	>0.05				
Lys	267.36 (243.60-327.12)	282.72 (264.48-333.12)	294.72 (264.48-343.20)	312.00 (276.00-350.40)	>0.05	>0.05	>0.05				
Val	209.07 (178.02-226.67)	210.62 (187.34-236.50)	229.77 (175.43-248.40)	207.00 (197.17-222.53)	>0.05	>0.05	>0.05				
Met	4.87 (3.36-6.57)	1.36 (1.11-4.45)	1.98 (1.14-3.86)	4.37 (1.27-4.92)	>0.05	>0.05	**0.029**	**0.049**	0.253	0.037	0.298
Tyr	62.09 (48.41-77.30)	59.49 (53.46-73.58)	76.13 (58.71-83.62)	60.42 (57.10-62.09)	>0.05	>0.05	>0.05				
Ile	60.95 (50.40-74.79)	65.88 (47.88-79.28)	63.26 (54.59-71.84)	61.72 (53.66-72.66)	>0.05	>0.05	>0.05				
Leu	112.22 (98.27-122.33)	109.69 (90.38-142.05)	113.23 (94.33-126.38)	105.14 (98.67-118.29)	>0.05	>0.05	>0.05				
Phe	73.73 (64.47-77.60)	70.49 (67.81-77.70)	74.17 (73.35-85.77)	76.30 (72.57-80.50)	>0.05	>0.05	>0.05				
Trp	71.75 (56.81-82.80)	65.96 (58.50-69.77)	66.49 (60.99-91.87)	69.96 (63.59-75.90)	>0.05	>0.05	>0.05				

Amino acid levels are presented as median with Inter-quartile range (25^{th} – 75^{th} percentile) due to non-normal distribution. They were measured by iTRAQ™ method in 3200QTRAP LC /MS/MS. 34 umbilical cord blood plasma matching to the placentas of the biological validation with spontaneous birth mode and independent of the usage of analgesics or anesthetics during labor. Significance was tested by two-way ANOVA with Holm-Sidak post-hoc tests. Significant two-sided p < 0.05 are marked in bold.

Significant effects for the n-3 LCPUFA treatment are shown with P, significant effects for offspring sex with P# and significant interactions with P*#.*

4.8. Sex steroid analysis in placenta and umbilical cord plasma

Sexual dimorphism in the expression of several mRNA, microRNAs, proteins and amino acids were observed upon the n-3 LCPUFA intervention in the placenta or umbilical cord plasma. In the transcriptome analysis, genes located on sex chromosomes (X and Y) were shown to exhibit sex-specific expression. However, in the transcriptome analysis and the biological validation also genes on autosomes displayed sex-specific expression. Sex differences in placental gene expression at term are suggested to arise not only from sex-chromosomes, but also from sex steroids [108,114]. Furthermore, during pregnancy the placenta is one of the key organs for sex steroid synthesis [206,207]. Therefore, placental and umbilical cord plasma sex steroid levels were investigated additionally. The concentrations of sex steroids free estradiol-17β (E2), total free estrogen and free progesterone were analyzed in the same placental setup used for biological validation independent of the use of analgesics or anesthetics during labor [n_{CM} = 9, n_{CF} = 11, n_{IM} = 11 and n_{IF} = 10 (in total n = 41)] and the matching umbilical cord plasma samples (n_{CM} = 8, n_{CF} = 8, n_{IM} = 9 and n_{IF} = 9). Additional, total levels of free testosterone, which is mainly produced in fetal testis, were also measured, since it is the main placental precursor for estrogen synthesis [207]. For estradiol-17β and estrogen, the conjugated metabolites (sulfatation and glucuronidation) were also analyzed, because they are the main form for transport in fetal plasma [206,207].

Except for testosterone levels, two-way ANOVA showed no significant effects for the analyzed sex steroids in placental tissue or umbilical cord plasma upon n-3 LCPUFA intervention or by offspring sex (**Appendix 11.19**). For testosterone levels, a significant effect of offspring sex was observed in the placental tissue as well as in the umbilical cord plasma (**Figure 16A / C**). Placental testosterone showed in the post-hoc analysis a sexual dimorphism in the n-3 LCPUFA intervention, with significant lower levels by 45.7% in male compared to female placentas, but no significant differences between male and female placentas in the control group [median (IQR): IM 10.7 (9.7 - 14.4) ng/g placenta vs. IF 19.7 (16.6 - 22.6) ng/g placenta; $P_{IM\ vs.\ IF}$ = 0.008; **Figure 16A**]. Sexual dimorphic testosterone levels were observed in umbilical cord plasma by the post-hoc analysis. Testosterone levels were significantly lower by 12.5% in female compared to male placentas independently of the n-3 LCPUFA intervention [CM: 1.3 (1.0 - 1.3) ng/g placenta, CF: 1.1 (0.8 - 1.3) ng/g placenta, IM: 1.2 (1.0 - 1.4) ng/g placenta, IF: 0.9 (0.8 - 1.1) ng/g placenta; CM+IM vs. CF+IF: P# = 0.049; **Figure 16C**].

Furthermore, the estradiol-17β / testosterone ratio was calculated, since this ratio is frequently used as activity index for the *aromatase* enzyme. *Aromatase* conducts the transformation of testosterone to estradiol-17β [208,209]. Two-way ANOVA for placental

estradiol-17β / testosterone ratio showed a significant effect of offspring sex and a significant interaction of offspring sex with the n-3 LCPUFA intervention (**Figure 16B**). A significant higher estradiol-17β / testosterone ratio by 171.4% in male compared to female placentas of the n-3 LCPUFA intervention [IM: 5.7 (3.6 - 6.3) vs. IF: 2.1 (1.7 - 3.7); $P_{IM\ vs.\ IF} = 0.002$], but not in the control was observed by the post hoc analysis. Additionally, estradiol-17β / testosterone ratio was significantly decreased by 49.9% upon the n-3 LCPUFA intervention in female placentas in the post-hoc analysis [IF: 2.10 (1.72-3.66) vs. CF: 4.19 (2.53-6.11); $P_{IF\ vs.\ CF} = 0.042$], but no significant differences were observed in male placentas. For umbilical cord plasma the two-way ANOVA found no significant differences in the estradiol-17β / testosterone ratio (**Figure 16D**).

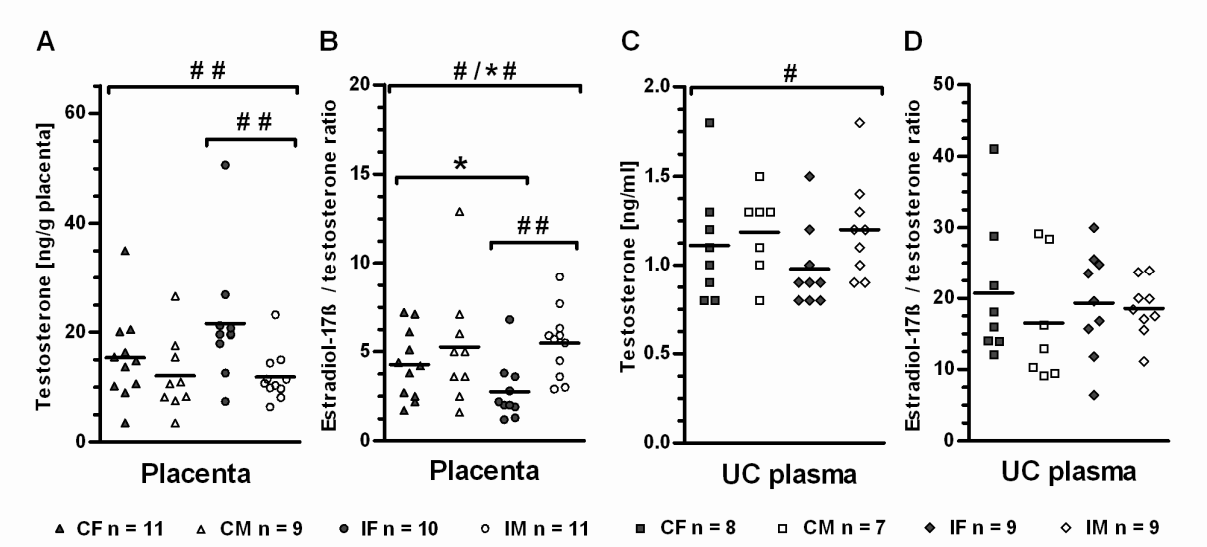

Figure 16: Free testosterone levels in placenta A) and umbilical cord plasma C) and free estradiol-17β / testosterone ratio in placenta B) and umbilical cord plasma D) *are presented as scatter plots with median. Statistical significance was calculated by two-way ANOVA on ranks with Holm-Sidak post-hoc test. Significant effects for sex differences are marked with #, significant effects for the n-3 LCPUFA treatment with * and significant interactions with *#. # or * P < 0.05, ## or ** P < 0.01.*

4.9. Correlation analysis of significantly regulated placental gene, microRNA and protein expression with selected parameters

4.9.1. Correlation analysis between selected placental gene and protein expression as well as related umbilical cord plasma amino acids

Very frequently, a certain pattern was observed in which placental gene or microRNA expression was significantly increased upon n-3 LCPUFA intervention only in female offspring, like for the placental gene expression of *MTOR, CDK6, PCNA, miR-99a* and *LAT1*. Therefore, it was of interest whether expression changes in these genes were related to each other. In addition, it was assessed whether the *mTORC1*-associated placental amino acid transporter *LAT1* and *TAUT* were associated with their related amino acids methionine and taurine in umbilical cord plasma.

Placental gene expression values of *mTOR* correlated significantly with the placental expression values of *CDK6, PCNA, miR-99a* and *LAT1* in a weak (Rs 0.0 – 0.4) to moderate (Rs 0.4 – 0.7) positive manner (**Table 15**). Furthermore, placental gene expression values of *CDK6* correlated significantly with those of *PCNA* in a moderate, positive manner. The gene expression values of *miR-99a* were significantly correlated with those of *LAT1 and TAUT* mRNA in a moderate manner, although with opposite directions of the correlation (**Table 15**). Moreover, it was found that the gene expression values of *TAUT* were significantly correlated with those of *MTOR* in a weak, inverse manner.

In addition, umbilical cord plasma methionine levels were significantly correlated with the gene expression values of *MTOR* in an inverse manner. However, umbilical cord plasma amino acids methionine and taurine were not correlated with their placental amino acid transporters *LAT1* and *TAUT*, respectively. Only a trend was observed for an inverse correlation of the gene expression values of *LAT1* with umbilical cord methionine levels in a weak manner.

Table 15: Correlation analysis between placental expression of selected genes as well as with related umbilical cord plasma amino acids

		ΔCq MTOR	ΔCq CDK6	ΔCq PCNA	ΔCq miR-99a	ΔCq LAT1	rel. density LAT1	UC MET	ΔCq TAUT	UC TAU
ΔCq MTOR	Rs	1.00	**0.60**	**0.57**	**0.35**	**0.46**	-0.40	**-0.43**	**-0.34**	-0.21
	P	.	**< 0.001**	**< 0.001**	**0.033**	**0.003**	0.101	**0.010**	**0.033**	0.233
	n	41	**38**	**41**	**38**	**41**	18	**34**	**40**	34
ΔCq CDK6	Rs	**0.60**	1.00	**0.55**	0.17	0.03	-0.23	-0.24	-0.19	-0.19
	P	**< 0.001**	.	**< 0.001**	0.317	0.844	0.387	0.190	0.269	0.318
	n	**38**	38	**38**	35	38	16	31	37	31
ΔCq PCNA	Rs	**0.57**	**0.55**	1.00	0.23	0.19	**-0.47**	-0.33	**-0.36**	0.06
	P	**< 0.001**	**< 0.001**	.	0.160	0.241	**0.049**	0.055	**0.022**	0.742
	n	**41**	**38**	41	38	41	**18**	34	**40**	34
ΔCq miR-99a	Rs	**0.35**	0.17	0.23	1.00	**0.43**	-0.39	-0.19	**-0.42**	-0.17
	P	**0.033**	0.317	0.160	.	**0.006**	0.131	0.296	**0.009**	0.366
	n	**38**	35	38	38	**38**	16	32	**37**	32
ΔCq LAT1	Rs	**0.46**	0.03	0.19	**0.43**	1.00	-0.09	-0.31	-0.11	0.20
	P	**0.003**	0.844	0.241	**0.006**	.	0.735	0.072	0.488	0.250
	n	**41**	38	41	**38**	41	18	34	40	34
rel. density LAT1	Rs	-0.40	-0.23	**-0.47**	-0.39	-0.09	1.00	0.42	**0.59**	0.32
	P	0.101	0.387	**0.049**	0.131	0.735	.	0.104	**0.010**	0.235
	n	18	16	**18**	16	18	18	16	**18**	16
UC MET	Rs	**-0.43**	-0.24	-0.33	-0.19	-0.31	0.42	1.00	0.04	0.10
	P	**0.010**	0.190	0.055	0.296	0.072	0.104	.	0.813	0.593
	n	**34**	31	34	32	34	16	34	33	34
ΔCq TAUT	Rs	**-0.34**	-0.19	**-0.36**	**-0.42**	-0.11	**0.59**	0.04	1.00	-0.01
	P	**0.033**	0.269	**0.022**	**0.009**	.0488	**0.010**	0.813	.	0.958
	n	**40**	37	**40**	**37**	40	**18**	33	40	33
UC TAU	Rs	-0.21	-0.19	0.06	-0.17	0.20	0.32	0.10	-0.01	1.00
	P	0.233	0.318	0.742	0.366	0.250	0.235	0.593	0.958	.
	n	34	31	34	32	34	16	34	33	34

Correlation coefficients between 0.0 - 0.4, 0.4 – 0.7, and 0.7 – 1.0 were considered as weak, moderate and strong correlations respectively. A negative value indicates an inverse correlation, whereas a positive value depicts a positive correlation. The correlation analysis was conducted independent of n-3 LCPUFA intervention status. P values < 0.05 were considered as significant correlations and are marked in bold. Rs, spearman-rho correlation coefficient; p, p-value for the respective correlation; UC MET, umbilical cord plasma methionine; UC TAU, umbilical cord plasma taurine; rel. density, relative density.

4.9.2. Correlation of significantly regulated parameters with n-6/n-3 LCPUFA ratio of biomarkers

It was further investigated whether the n-6/n-3 LCPUFA ratio is related to significant changes in placental parameters. Therefore, correlation analysis was performed for significantly regulated placental gene, protein and microRNA expression as well as umbilical cord amino acids and sex steroids with the n-6/n-3 LCPUFA ratios in umbilical cord RBCs, maternal RBCs at P_32 and in placental phospholipids.

Most of the significant correlations were obtained with the maternal RBC n-6/n-3 LCPUFA ratio (P_32) and the placental parameters. The gene expression values of *CDK6, PCNA, MTOR* and *miR-99a* were correlated with the n-6/n-3 LCPUFA ratio in maternal RBC in a moderate, inverse manner (**Table 16**). Normalised *LAT1* protein and the gene expression values of *TAUT* were significantly related to maternal n-6/n-3 LCPUFA ratio as well as

umbilical cord RBCs in a moderate, positive manner. Moreover, the gene expression values of *PCNA, LRP6* and *TAUT* showed significant correlations with placental n-6/n-3 LCPUFA ratio (**Table 16**). No significant correlations for any of the n-6/n-3 LCPUFA ratios were observed with gene expression values of *LAT1, DVL1, HDAC5* and *RPTOR* or the significantly regulated sex-steroid and amino acid parameters.

Table 16: Correlation of significantly regulated placental parameters with n-6/n-3 LCPUFA ratio in UC RBCs, placental phospholipids and maternal RBCs at P_32

	UC RBCs	Placenta PL	P_32 RBCs	UC RBCs	Placenta PL	P_32 RBCs	UC RBCs	Placenta PL	P_32 RBCs	UC RBCs	Placenta PL	P_32 RBCs
	CDK6			TGFB1			HDAC5			PCNA		
Rs	0.15	-0.12	**-0.46**	0.25	0.15	-0.08	0.04	0.03	-0.09	-0.30	**-0.33**	**-0.48**
p	0.416	0.491	**0.004**	0.156	0.351	0.621	0.827	0.85	0.559	0.09	**0.033**	**0.002**
n	31	38	**38**	34	41	41	34	41	41	34	**41**	**41**
	LRP6			DVL1			MTOR			RPTOR		
Rs	0.28	**0.31**	0.11	-0.14	0.07	-0.09	0.02	-0.20	**-0.37**	-0.15	-0.11	-0.12
p	0.113	**0.049**	0.486	0.445	0.644	0.576	0.924	0.216	**0.018**	0.401	0.487	0.438
n	34	**41**	41	34	41	41	34	41	**41**	34	41	41
	LAT1			TAUT			miR-99a			LAT1 protein		
Rs	-0.23	-0.07	-0.19	**0.44**	**0.62**	**0.54**	-0.08	-0.16	**-0.48**	**0.58**	0.28	**0.66**
p	0.198	0.679	0.23	**0.01**	**<0.001**	**<0.001**	0.663	0.332	**0.002**	**0.016**	0.265	**0.003**
n	34	41	41	**33**	**40**	**40**	31	38	**38**	**17**	18	**18**
	Placental T			Placental E2/T			UC plasma T			UC methionine		
Rs	0.08	-0.03	0.03	-0.10	-0.13	-0.06	-0.25	-0.24	-0.19	0.26	0.16	0.23
p	0.663	0.841	0.855	0.567	0.434	0.707	0.172	0.182	0.285	0.148	0.370	0.186
n	34	41	41	34	41	41	31	33	33	32	34	34
	UC taurine											
Rs	0.23	-0.10	0.01									
p	0.186	0.558	0.937									
n	34	34	34									

Correlation coefficients between 0.0 - 0.4, 0.4 – 0.7, and 0.7 – 1.0 were considered as weak, moderate and strong correlations respectively. A negative value indicates an inverse correlation, whereas a positive value depicts a positive correlation. The correlation analysis was conducted independent of n-3 LCPUFA intervention status. P values < 0.05 were considered as significant correlations and are marked in bold. Rs, spearman-rho correlation coefficient; p, p-value for the respective correlation; UC, umbilical cord; RBC, red blood cells; P_32, 32nd week of gestation.

4.9.3. Correlation of sex steroids with placental expression

Placental testosterone (Pl_T) levels and the estradiol-17β / testosterone ratio (Pl_E2/T) as well as testosterone levels in umbilical cord plasma (UC_T) were candidates for mediating the sex-specific effects of the n-3 LCPUFA intervention on placental gene, protein or microRNA expression changes. First of all, placental testosterone levels did not significantly correlate with umbilical cord plasma testosterone levels (Rs = -0.031, p = 0.865, n = 33). However, the estradiol-17β / testosterone ratio was significantly correlated between placental tissue and umbilical cord plasma in a positive, moderate manner (Rs = 0.424, p = 0.014, n = 33).

Of the 13 placental expression parameters assessed, only four expression values were significantly correlated with placental or umbilical cord plasma testosterone or placental estradiol-17ß / testosterone ratio (**Table 17**). The gene and protein expression values of *LAT1* were both significantly correlated with umbilical cord plasma testosterone in a moderate, inverse manner (**Table 17**). In addition, the gene expression values of *LRP6* and *DVL1*, from the WNT signaling pathway, were both significantly correlated with placental estradiol-17ß / testosterone ratio in a positive manner.

Table 17: Correlations of candidate sex steroid parameters with placental genes, microRNAs, or proteins showing validated sex-specific regulations

	ΔCq CDK6			**ΔCq PCNA**			**ΔCq HDAC5**			**ΔCq LRP6**		
	Pl_T	Pl_E2/T	UC_T	Pl_T	Pl_E2/T	UC_T	Pl_T	Pl_E2/T	UC_T	Pl_T	Pl_E2/T	UC_T
R	-0.06	0.19	0.08	-0.13	-0.01	-0.03	-0.09	0.18	-0.17	-0.28	**0.48**	0.13
p	0.734	0.254	0.658	0.422	0.969	0.864	0.591	0.268	0.339	0.080	**0.002**	0.476
n	38	38	31	41	41	33	41	41	33	41	**41**	33
	ΔCq DVL1			**ΔCq FZD7**			**ΔCq TGFB1**			**ΔCq MTOR**		
	Pl_T	Pl_E2/T	UC_T	Pl_T	Pl_E2/T	UC_T	Pl_T	Pl_E2/T	UC_T	Pl_T	Pl_E2/T	UC_T
R	-0.25	**0.32**	0.01	0.07	0.06	-0.21	-0.14	0.11	-0.15	0.01	0.05	0.00
p	0.115	**0.044**	0.955	0.688	0.728	0.233	0.395	0.490	0.422	0.931	0.766	0.991
n	41	**41**	33	41	41	33	41	41	33	41	41	33
	ΔCq RPTOR			**ΔCq TAUT**			**ΔCq LAT1**			**ΔCq miR-99a**		
	Pl_T	Pl_E2/T	UC_T	Pl_T	Pl_E2/T	UC_T	Pl_T	Pl_E2/T	UC_T	Pl_T	Pl_E2/T	UC_T
R	-0.06	-0.03	0.07	-0.08	0.03	-0.05	-0.02	0.12	**-0.48**	-0.07	0.09	-0.18
p	0.689	0.850	0.707	0.619	0.855	0.791	0.893	0.441	**0.005**	0.673	0.583	0.337
n	41	41	33	40	40	32	41	41	**33**	38	38	31
	Relative density LAT1											
	Pl_T	Pl_E2/T	UC_T									
R	0.39	-0.13	**-0.52**									
p	0.111	0.598	**0.045**									
n	18	18	**15**									

Correlation coefficients between 0.0 - 0.4, 0.4 – 0.7, and 0.7 – 1.0 were considered as weak, moderate and strong correlations respectively. A negative value indicates an inverse correlation, whereas a positive value depicts a positive correlation. The correlation analysis was conducted independent of n-3 LCPUFA intervention status. P values < 0.05 were considered as significant correlations and are marked in bold. Rs, spearman-rho correlation coefficient; p, p-value for the respective correlation; PL_T, placental testosterone; PL_E2/T, placental estradiol17ß / testosterone ratio; UC_T, umbilical cord plasma testosterone.

4.9.4. Correlation analysis of placental expression with offspring weight and fat distribution measurements

In order to identify associations of significantly regulated placental genes, microRNA or protein expression upon the n-3 LCPUFA intervention with factors for placental parameters, and factors indicating offspring adipose tissue distribution as well as obesity risk, correlation analysis was conducted. The assessed placental expression values were *LAT1, TAUT, LRP6, DVL1, PCNA, CDK6, HDAC5, TGFB1*, *MTOR* and *RPTOR* genes as well as *miR-99a* and normalized *LAT1* protein. Each of these expression values was correlated with placental weight, birth weight-to-placental weight ratio, birth weight, ponderal index at birth, weight at one year, sum of four skin fold thicknesses (SFT; 3-5 days and one year) as well as the ratio of subcutaneous to preperitoneal (SC/PP) fat at 6 weeks and one year. All parameters displaying significant correlations are presented in **Table 18.** The complete list for all analyzed correlations is shown in **Appendix 11.20**.

RPTOR was inversely correlated with placental weight in a weak manner. Moreover, a weak, positive correlation was identified for *MTOR* with birth weight-to-placental weight ratio (**Table 18**). Furthermore, the expression values of *PCNA, CDK6* and *miR-99* showed significant correlations with birth weight and birth-weight-to-placental weight ratio in a weak manner, but with different directions of the correlation (**Table 18**). A positive correlation was found for *PCNA* and *CDK6* with birth weight, whereas *miR-99a* was inversely correlated with birth weight. The most significant correlations were observed for weight at one year with *DVL1, HDAC5* and *LAT1* gene expression ($P < 0.01$; **Table 18**). Their gene expression values were correlated with weight at one year in a positive manner. Moreover, *LAT1* protein showed a positive correlation with SC/PP ratio at six weeks in a weak manner (**Table 18**). There were no significant correlations for any expression parameter with the SC/PP ratio at one year, with ponderal index, sum of four SFTs at 3-5 days and one year (**Appendix 11.20)**. Furthermore, the gene expression values of *TAUT, LRP6* and *TGFB1* did not correlate with any offspring weight and fat distribution measurements analyzed.

Table 18: Correlation of significantly regulated parameters with selected placental parameters, offspring weight and fat distribution measurements up to one year of life

	LAT1	TAUT	LRP6	DVL1	PCNA	CDK6	HDAC5	TGFB1	MTOR	RPTOR	miR-99a	LAT1 protein
	with placental weight											
Rs	-0.28	-0.03	0.13	-0.15	0.12	-0.09	-0.23	-0.30	-0.30	**-0.31**	-0.05	0.28
p	0.074	0.860	0.402	0.366	0.462	0.595	0.151	0.055	0.056	**0.045**	0.755	0.254
n	41	40	41	41	41	38	41	41	41	**41**	38	18
	with birth weight / placental weight ratio											
Rs	0.23	0.06	-0.12	0.16	0.08	0.26	0.28	0.18	**0.37**	0.25	-0.10	-0.29
p	0.149	0.705	0.471	0.325	0.626	0.111	0.079	0.250	**0.017**	0.117	0.565	0.250
n	41	40	41	41	41	38	41	41	**41**	41	38	18
	with birth weight											
Rs	-0.04	-0.01	0.06	-0.02	**0.31**	**0.33**	0.06	-0.15	0.10	-0.03	**-0.33**	0.25
p	0.805	0.957	0.719	0.918	**0.049**	**0.042**	0.717	0.339	0.523	0.834	**0.041**	0.309
n	41	40	41	41	**41**	**38**	41	41	41	41	**38**	18
	with weight / length ratio at birth											
Rs	-0.11	0.01	0.06	0.01	**0.32**	0.26	0.04	-0.19	0.03	-0.12	**-0.37**	0.24
p	0.498	0.947	0.721	0.931	**0.039**	0.111	0.825	0.232	0.876	0.443	**0.023**	0.341
n	41	40	41	41	**41**	38	41	41	41	41	**38**	18
	with Subcutaneous-to-preperitoneal fat mass ratio (SC/PP) 6 weeks											
Rs	-0.15	0.21	0.20	0.01	-0.08	0.06	0.01	-0.09	-0.07	-0.04	-0.05	**0.49**
p	0.428	0.253	0.273	0.961	0.669	0.758	0.971	0.619	0.717	0.820	0.802	**0.045**
n	32	31	32	32	32	29	32	32	32	32	30	**17**
	with weight at 1 year											
Rs	**0.38**	-0.08	0.03	**0.49**	0.15	0.22	**0.48**	0.20	0.28	0.21	0.15	-0.19
p	**0.014**	0.651	0.834	**0.001**	0.350	0.193	**0.002**	0.210	0.085	0.205	0.383	0.468
n	**40**	39	40	**40**	40	37	**40**	40	40	40	37	17

Correlation coefficients between 0.0 - 0.4, 0.4 – 0.7, and 0.7 – 1.0 were considered as weak, moderate and strong correlations respectively. A negative value indicates an inverse correlation, whereas a positive value depicts a positive correlation. The correlation analysis was conducted independent of n-3 LCPUFA intervention status. P values < 0.05 were considered as significant correlations and are marked in bold. Rs, spearman-rho correlation coefficient; p, p-value for the respective correlation;

5. Discussion

Strategies for primary obesity prevention could help to manage the increasing obesity prevalence [210]. Therefore, the INFAT study investigated the hypothesis, brought forward by Ailhaud and Guesnet [8], that a decreased n-6/n-3 LCPUFA ratio in maternal diet during pregnancy and lactation can program a reduced offspring obesity risk [124]. The placenta is assumed to play a key role mediating fetal / metabolic programming effects [84]. Moreover, considering that the placenta consists mainly of cells from extraembryonic mesoderm and trophoblasts representing offspring tissue [67], it provides a unique opportunity to explore the molecular impact of the n-3 LCPUFA intervention on a fetal tissue also with regard to offspring sex. In this thesis, it was an aim to investigate in placentas of the INFAT study whether the n-3 LCPUFA intervention can have an impact on placental gene expression, microRNAs and the mRNA-microRNA regulatory network with the consideration of sex-specific effects. A further objective was to assess whether molecular changes in placenta are associated with metabolic changes or obesity risk in offspring.

5.1. The n-3 LCPUFA intervention impacts biomarkers in maternal and newborn circulation as well as in placental tissue

The characterization of the INFAT subpopulation (n_{CG} = 20, n_{IG} = 21) showed its concordance with the whole INFAT population (n_{CG} = 104, n_{IG} = 104) for almost all analyzed parameters, despite the smaller sample size. Both populations demonstrated the excellent compliance and the thereof resulting decrease of the n-6/n-3 LCPUFA ratio in maternal and newborn circulation as well as in placental tissues upon the n-3 LCPUFA intervention during pregnancy [124,164]. In the measured placental tissue, unaltered AA levels in combination with increased levels of n-3 LCPUFAs EPA and DHA concomitantly decreased the n-6/n-3 LCPUFA ratio of the INFAT subpopulation. This decreased in the n-6/n-3 LCPUFA ratio was also observed in umbilical cord RBCs. These data are in accordance with published observations that a nutritional n-3 LCPUFA intervention during pregnancy is able to change placental fatty acid percentage of n-3 LCPUFAs EPA and DHA [211].

The most notable difference between the whole INFAT population and the subpopulation was the unchanged gestational duration in the subpopulation, compared to the 4.8 days prolonged gestational duration in the whole INFAT population. Surprisingly, the INFAT population and the INFAT subpopulation did not differ between gestational age in the intervention group, but in the control group. It could be shown that this difference is attributed to the selection criteria applied for the subpopulation. However, the absence of differences between pregnancy duration represents an opportunity to study the impact of the n-3

LCPUFA intervention on placental gene expression independent of a prolonged gestational duration, which is often reported for n-3 LCPUFA supplementation during pregnancy [124]. Differences in gene expression between different gestational ages were described already [212]. Therefore, a prolonged gestational duration might also exert intrinsic effects on placental gene expression and therefore, in this study only the effects of the n-3 LCPUFA supplementation were assessed.

5.2. The n-3 LCPUFA intervention impacts placental gene expression sex-specifically

Upon the maternal n-3 LCPUFA supplementation during pregnancy, transcriptome analysis identified only a few significant differences in placental gene expression by simply pooling male and female placentas (n_{CG} = 7, n_{IG} = 9, spontaneous birth mode without the use of anesthetics or analgesics during labor). However, consideration of offspring sex (n_{CM} = 3, n_{CF} = 4, n_{IM} = 5 and n_{IF} = 4) revealed a more detailed picture for the impact of the n-3 LCPUFA intervention on placental gene expression. The first major finding was the disappearance and to a lesser extent the appearance of sexual dimorphism in placental gene expression upon the intervention. The second one was that the n-3 LCPUFA intervention exerted a more pronounced impact on gene expression in placentas of female compared to male offspring.

5.2.1. Disappearance and appearance of sexual dimorphic gene expression in placenta upon the n-3 LCPUFA intervention

The observed data from the control group confirmed that sexual dimorphic gene expression exists in healthy term placentas, which was published earlier by Sood *et al.* [112] and Tsai *et al.* [176]. However, an impact on sexual dimorphisms of a dietary n-3 LCPUFA intervention on placental gene expression was not reported before. It can be suggested that these disappearance and appearance of sexual dimorphism in placental gene expression upon the intervention could depend on transcription factors mediating sexual dimorphisms, which are in addition responsive for regulation by n-3 LCPUFAs. One example for such a transcription factor is *HNF-4α*. *HNF-4α* was shown to be involved in the sexual dimorphic expression of liver *cytochrome P450 (CYP)* genes in mice [213]. The authors suggested that *HNF-4α* induces the expression of several male-specific *CYP* genes, whereas it down-regulates expression of predominant female *CYP* genes by suppressing the transcription factors *HNF-3β* and *HNF-6β* [213]. Interestingly, the HNF-4α transcription factor activity was shown to be suppressed by binding of LCPUFAs like C18:3n-3-CoA, EPA (C20:5n-3-CoA) and C22:6n-6-CoA [214]. However, *HNF-4α* seems to be absent in the placenta, because there are no reports on its expression in whole placental tissue and it was not detectable in primary

trophoblast or trophoblast cell lines [215]. Unfortunately, the probe set for HNF-4α was also not annotated in the DNA microarray analysis (**Appendix 11.7**). Hence, other transcription factors with a similar mechanism of action or other mechanisms leading to these differences in sexual dimorphic placental gene expression remain to be identified. These findings raise the question whether less sex-specific expressed placental genes have physiological consequences for the placenta or the offspring, which will be discussed later.

In this context, it is of interest that in male and female placentas almost no genes were regulated in the same direction upon the intervention. A similar observation was made by Rudkowska *et al.* [216] in peripheral blood mononuclear cells (PBMCs) upon a n-3 LCPUFA intervention. The authors reported in a transcriptome analysis that for 860 genes with significant different expression only nine were regulated in the same direction in adult men and women with a healthy status. They suggested that different genes are regulated in human male and female PBMCs upon a n-3 PUFA intervention [216]. For murine placenta, a recent transcriptome analysis of Gabory *et al*. [217] show that a small portion of genes (11 out of 178) displayed sexual dimorphic gene expression upon both a maternal control diet and a high-fat diet during pregnancy. The larger portion of murine placental genes were either sexually dimorphic upon control diet (86 genes) or upon high-fat diet (81 genes) [217]. A comparison of these data with the transcriptome analysis is difficult, since they are derived from human PBMCs or from a mouse model. However, despite the differences, these data support the finding, that only a small number of genes are regulated in the same direction in male and female placentas upon n-3 LCPUFA intervention. Furthermore, this could be a possible explanation for the fact that without considering placental sex, only 22 genes were identified to be significantly regulated upon the n-3 LCPUFA intervention.

5.2.2. More pronounced impact on placental gene expression in female offspring upon the n-3 LCPUFA intervention

The observation that in placentas of female offspring more genes were regulated upon the n-3 LCPUFA intervention (239 genes) than in male offspring (93 genes) was also a new finding of the transcriptome analysis. Rudkowska *et al.* [216] also published that in a transcriptome analysis of PBMCs more genes were significantly changed upon the n-3 PUFA intervention in men than in women. In contrast to this, a human placental transcriptome analysis revealed that more genes were significantly altered in female than in male offspring by maternal asthma [218]. In addition, murine placentas of female offspring showed about two-thirds more alteration in gene expression in response to maternal diet than placentas of male offspring [219]. In this case, the data of this thesis seem to be closer related to the data of other placental analysis, than to an n-3 PUFA intervention in adult PBMCs, since the data of placental transcriptome upon different stimuli analysis in human and mouse are in line with

the observation that more genes were significant differentially expressed in female placenta upon n-3 LCPUFA intervention than in male placentas. Clifton *et al.* [108] hypothesized that 'the minimal placental gene alterations of the male placenta may be a mechanism that allows the male fetus to continue growing in an adverse environment'. However, a maternal supplementation of n-3 LCPUFAs during pregnancy is suggested to have beneficial rather than adverse effects (see **chapter 1.5.4.**). Therefore, a generalization of the hypothesis of Clifton *et al.* [108] could be suggested towards: 'the minimal gene alterations of the male placenta may be a mechanism that allows the male fetus to maintain growth relatively independent of environmental stimuli'. Conversely, the considerable changes in gene expression of female placentas could allow the female fetus to adapt more comprehensive to environmental cues. However, the physiological relevance of the comprehensive adaption of female placental gene expression to their environment remains to be explored.

In this context, it is important to consider whether factors of the INFAT subpopulation could confound this sex-specific impact of the n-3 LCPUFA intervention. For the placental biomarkers, an increase in n-3 LCPUFAs (EPA and DHA) and the concomitant decrease in the n-6/n-3 LCPUFA ratio were observed, without any sexual dimorphism. Therefore, placental biomarkers would not explain the sex-specific differences of the intervention. However, in the maternal and newborn circulation differences were observed between male and female offspring of the intervention group. Upon the intervention, umbilical cord RBC EPA levels were significantly increased in male offspring only, but remained at the level of the control in female offspring. Similarly, in the maternal circulation during pregnancy (P_32), AA levels were only decreased in pregnant women with male offspring to the level of women pregnant with female offspring of control and the intervention group. In this case if at all, placental expression changes in male and not in female offspring would have been expected. That despite of these differences in male offspring and equal changes in placental biomarkers more pronounced placental expression differences were observed in female offspring strongly supports that the decrease in the n-6/n-3 LCPUFA ratio in maternal nutrition during pregnancy has a more pronounced impact on placental gene expression in female placentas.

Of course, the number of genes that showed sexual dimorphism or were regulated upon the n-3 LCPUFA intervention independent and dependent of offspring sex in the transcriptome analysis cannot be considered to be absolute. The number of regulated genes can vary by applying other significance criteria than fold change +1.5 and $p < 0.05$ or fold changes -1.5 and $p < 0.05$ or by measuring a larger sample size in the DNA microarrays. It has to be admitted that the sample size for DNA microarray analysis after stratification for sex was the relatively low (n = 3 - 5). However, the reason for this low sample size in the DNA microarray analysis was the very strict selection for placentas with a spontaneous birth mode without

reception of anesthetics or analgesics during delivery. In addition, the analyzed placentas represent a very specific and well-defined analysis sample and the thereof described observations in the transcriptome analysis are supported by published literature. Therefore, the transcriptome data in this thesis are the first to argue for a more comprehensive adaption of placental gene expression from female offspring to the n-3 LCPUFA intervention in maternal nutrition during pregnancy.

5.2.3. Genes of the cell cycle pathway are only increased in female placentas upon n-3 LCPUFA intervention

Several of these genes specifically regulated in female placentas were identified in the cell cycle and WNT pathways and confirmed in the biological validation in an increased placental sample size by applying the same strict selection criteria like spontaneous birth mode, but independent of the use of anesthetic and analgesics during labor [n_{CM} = 9, n_{CF} = 11, n_{IM} = 11 and n_{IF} = 10 (in total n = 41)]. The biological validation confirmed the general observations made in the placental transcriptome analysis. Predominant placental expression changes in female offspring upon the n-3 LCPUFA intervention (*CDK6, TGFB1, PCNA*, *LRP6*) as well as the more common disappearance of sexual dimorphism (*TGFB1, HDAC5, DVL1*) were also identified in the biological validation. Moreover, the obtained data suggested that the n-3 LCPUFA intervention is associated with an increasing gene expression in the cell cycle / cell proliferation pathway for female placentas. In contrast, in male placentas, the n-3 LCPUFA intervention might not change cell proliferation due to down-regulation in upstream regulators like *TGFB1* or an unresponsiveness of genes which are regulated in female placentas upon the intervention.

All the biologically validated genes (*CDK6, PCNA, HDAC5, TGFB1, DVL1* and *LRP6*) showed in the *Human Protein Atlas* signals for protein expression in the trophoblastic cells of the placenta by immunohistochemistry [220]. Klingler *et al.* [221] reported that a nutritional supplementation of DHA, EPA and folate together during pregnancy increased *PCNA* protein expression (Western blot and immunohistochemistry) exclusively in trophoblast cells of the human term placenta. DHA and EPA supplementation without folate increased *PCNA* expression without reaching significance. This non-significance of the increase in *PCNA* expression could be a result of the lower amounts of EPA (150 mg) and DHA (500 mg) in their supplementation regime, compared to the 180 mg EPA and 1200 mg DHA in the INFAT study supplementation or from different sex distribution, which they not stated [221]. In addition, Johansen *et al.* [222] showed *in vitro* that DHA, but not EPA stimulated cell proliferation of a human extravillous trophoblast cell line. However, this kind of cell type was due to the sample preparation not assessed in this thesis.

Cell proliferation was described to be of utmost importance for development and maintenance of the human placenta. The continuous syncytium is not able to proliferate and thus depends on the continuous proliferation of the underlying cytotrophoblasts, which then fuse with the syncytiotrophoblast [223,224]. The highest proliferation rate of cytotrophoblasts is observed in the first trimester of human pregnancy. Towards term, the proliferation rate decreases due to a declining number of cytotrophoblast cells, but a small extend of proliferation can still be detected at term [223]. This remaining proliferation activity was suggested to be necessary for the regeneration of syncytium, but seems not to have this big impact on placental growth as in early pregnancy [223]. Cell proliferation has to be tightly controlled by numerous factors like oxygen, *insulin-like growth factor, fibroblast-growth factor 4, placental growth factor* [224], to which also *CDK6* and *PCNA* belong [193,225]. *PCNA* is an important factor in DNA replication during S-phase of cell cycle, where it shows the highest expression levels and is also involved in DNA excision repair during G2-phase of cell cycle and in quiescent cells [193]. *CDK6* is a kinase, which activated upon mitotic stimuli via D-type cyclins promotes cell division by inactivating cell cycle inhibitors [225]. However, the physiological relevance of this observed increase in cell cycle / cell proliferation upon the n-3 LCPUFA intervention in placentas of female offspring is unclear. Klingler *et al.* stated that the consequence of increased placental cell proliferation is still unknown. It was reported that increased placental proliferation is rather associated with preeclampsia, anaemia or diabetes in mothers [221].

However, the time point when higher cell proliferation occurs might be decisive. An increased proliferation of cytotrophoblasts only a term would indicate a higher regenerative capacity and maintenance of the syncytium. When the observed higher proliferation at term also would be indicative for higher proliferation of cytotrophoblasts during gestation, a higher placental weight would be expected. Significant higher placental and fetal weight at day 22 (near term) was reported for a n-3 LCPUFA-enriched diet during pregnancy in nulliparous albino Wistar rats [226]. However, there was no significant difference in placental weight in the subpopulation or the whole population, which would have supported this. A possible impact of the gene expression changes in cell cycle only in female placentas on offspring will be discussed later.

5.3. Possible Interaction between *mTOR* gene and *microRNA-99a* expression in female placentas upon n-3 LCPUFA intervention

5.3.1. Indication that placental mRNA-microRNA network is influenced upon n-3 LCPUFA intervention

Remarkably, the cell cycle pathway was also identified by the combined analysis of miRNome (pool of n_{CF} = 3 and n_{IF} = 3) and transcriptome (n_{CM} = 3, n_{CF} = 4, n_{IM} = 5 and n_{IF} = 4) in placentas with spontaneous birth mode without the use of analgesics and anesthetics. This analysis revealed that genes in the cell cycle pathway could be targeted by microRNAs found to be regulated in the explorative profiling. Fu *et al.* [227] recently summarized for interactions between single microRNAs and target genes that microRNAs in human placental trophoblasts modulate cell proliferation either by inhibiting or enhancing trophoblast cell proliferation. No reports were found for an effect of n-3 LCPUFA or any other dietary intervention on interactions between gene and microRNA expression in a mRNA-microRNA network in any tissue. Therefore, the bioinformatics analysis strategy of combining miRNome data with transcriptome analysis in this thesis are the first indicating that a dietary n-3 LCPUFA intervention during pregnancy has an impact on the placental mRNA-microRNA network and suggest the changes in microRNAs are involved in the regulation of the differentially expressed placental genes.

5.3.2. Sex-specific impact of the n-3 LCPUFA intervention on placental *microRNA-99a* expression

In more detail, the data of this thesis hypothesized that miR-99a is involved in the mRNA-microRNA network in the placenta and the alterations upon the n-3 LCPUFA intervention. This microRNA was identified in the explorative miRNome profiling (pool of n_{CF} = 3 and n_{IF} = 3) and confirmed in the biological validation (n_{CM} = 9, n_{CF} = 11, n_{IM} = 11 and n_{IF} = 10, in total n = 41, with spontaneous birth mode but independent of anesthetics or analgesics). Interestingly, *miR-99a* displayed also a sexual dimorphism, with a significant increase in female placentas upon the n-3 LCPUFA intervention, but no significant difference in male placentas. Expression of *miR-99a* in human placental tissue was already demonstrated by RT-qPCR [228] as well as trophoblast-specific expression in humans by transcriptome analysis [229], but there is no literature about the *miR-99a* expression in the syncytiotrophoblast. *MiR-99a* was reported to be dysregulated in many cancer cell models as well as in several diseases [194,202,203,230,231]. Since down-regulation of *miR-99a* was predominantly observed in various cancer models, *miR-99a* was claimed as a tumor suppressor [202,203,230]. Evidence that a maternal diet during pregnancy in mice can influence microRNA expression in a long-lasting manner was provided by Zhang *et al.* [232]

by a miRNome analysis. In their mouse study, a maternal high-fat diet, fed from pre-conception until weaning, decreased 23 murine microRNAs in offspring livers at the age of 15 weeks [232]. Additionally, several other authors reported that a DHA treatment resulted in expression changes of microRNAs in human aortic endothelial, glioblastoma and breast cancer cell lines measured by RT-qPCR [233-235]. No comparable literature is available and thus again information had to be abstracted from data of other tissues and mouse models. Once more, the data of this thesis are the first to show sex-specific change of placental *miR-99a* expression in female placentas upon a maternal n-3 LCPUFA intervention during pregnancy. It could be hypothesized that *miR-99a* is involved in the mRNA-microRNA interactions as well as in the mediation of sex-specific effects upon the n-3 LCPUFA intervention.

5.3.3. Sex-specific impact of the n-3 LCPUFA intervention on placental *mTOR* expression

Reports exist that *miR-99a* can target *MTOR* and *RPTOR* of the *mTOR complex 1* (*mTORC1*), which is involved in nutrient sensing [194,195,202,203]. For various human cancer cell lines (like Human Embryonic Kidney 293- and Esophageal Squamous Cell Carcinoma cells), it was demonstrated by luciferase reporter assays that *miR-99a* directly suppresses *MTOR* mRNA by binding to its 3'UTR [194,202,203]. In addition, Oneyama *et al.* [202] showed that expression of *miR-99a* in c-Src transformed cells reduced not only *mTOR* protein expression, but also phosphorylation of *ribosomal protein kinase S6 (p70S6K)*, which represents a marker for *mTORC1* activation. Since *MTOR* gene expression was significantly increased in female placentas upon the n-3 LCPUFA intervention, it was speculated to be targeted by *miR-99a* in placenta.

MTOR protein was shown to be expressed in the human syncytiotrophoblast cells by immunohistochemistry [236]. *Mechanistic target of rapamycin (mTOR)* is a serine / threonine kinase that is found in two protein complexes named *mTORC1* and *mTORC2* [195]. *MTORC1* is one of the key sensors of nutrients [195,237]. In addition, the *mTORC1* pathway is anticipated to play a major role in the placental mediation of intrauterine programming [92,237]. In this context, there is emerging evidence that placental *mTOR* signaling determines fetal growth via regulation of nutrient transporters and that *mTOR* signaling links maternal nutrient availability with fetal growth [238]. *MTORC1* integrates a large number of upstream-regulators and senses cues from growth factors, stress, energy status, oxygen and amino acids. Amino acids, particularly *leucine (Leu)* and *arginine (Arg)*, are especially important for *mTORC1* activation, because their presence is necessary for any up-stream-signal to activate *mTORC1* [195]. Down-stream processes mediated by *mTORC1* include cell cycle progression, cell growth, lipid and protein synthesis, autophagy, energy metabolism

as well as lysosome biogenesis. The best characterized direct *mTORC1* targets are *eukaryotic translation initiation factor 4E-binding protein 1 (4E-BP1)* and *p70 ribosomal S6 kinase 1 (S6K1),* which both promote protein synthesis [195].

There are no reports on sex-specific analyses of *mTOR* expression as well as on the effects of n-3 LCPUFAs on *mTOR* gene expression, but several *in vivo* and *in vitro* studies found that n-3 LCPUFAs can change *mTORC1* activation (phosphorylation of *S6K1*). A n-3 LCPUFA supplementation in healthy humans did not activate *mTORC1* in muscle tissue under basal conditions. However, during a hyperinsulinaemic–hyperaminoacidaemic clamp *mTOR* (Ser 2448) phosphorylation and *S6K1 (Thr 389)* phosphorylation was increased only in the n-3 LCPUFA supplemented group [239,240]. In contrast, *in vitro* studies in various cancer cell lines showed a decreased *mTOR* activation upon n-3 LCPUFA stimulation [241-243]. The reported results for the effect of n-3 LCPUFA stimulation seem to be different according to health status or tissue.

Very recently, it was discussed that *mTORC1* can act as a lipid sensor [244]. Phosphatidic acid, which is a central metabolite in the synthesis of membrane lipids, is required for *mTORC1* stability and *mTORC1* is responsive to changes in phosphatidic acid levels [244]. Phosphatidic acid contains two fatty acid chains, which can be either derived from newly synthesized saturated fatty acids or dietary fatty acids. Supporting an impact of the n-3 LCPUFA intervention on mTOR is the fact, that phosphatidic acid, which interacts with *mTOR* to promote complex stability and activity, needs at least one of the two fatty acids unsaturated. However, at the moment there is nothing known whether there is a difference on *mTOR* stability or activity between n-3 or n-6 LCPUFAs in phosphatidic acid [244]. Therefore, it remains elusive how the n-3 LCPUFA intervention could be involved in the regulation of *mTOR*, but incorporation of more unsaturated fatty acids in phosphatidic acid could be a likely mechanism. For a better understanding of the impact of the n-3 LCPUFA intervention on placental nutrient sensing, further analyses which address *mTORC1* activation, possible mechanisms of action and sex-specific effects are necessary.

5.3.4. Possible regulation of placental *mTOR by miR-99a* in female placentas upon n-3 LCPUFA intervention

Another mechanism of action how MTOR gene expression could be regulated upon the n-3 LCPUFA intervention in female placentas is *miR-99a*. Placental *MTOR* and *miR-99a* expression both were significantly increased in female placentas upon the n-3 LCPUFA intervention in a very similar manner and were significantly correlated in a weak positive manner. The experiments described in **chapter 5.3.3**, which showed that *miR-99a* over-expression decreases *MTOR* gene expression, were based on *miR-99a* transfections or

knock-downs, with much higher reported concentration changes as the two-fold increase of *miR-99a* reported in this thesis [194,202,203]. Moreover, these experiments were also conducted in transformed cancer cells and not in healthy tissue. In addition, as reported in the introduction (**chapter 4.3.4**), microRNAs can not only decrease mRNA or protein expression, but also increase mRNA and protein expression [98,190]. Reports show that in murine primary peritoneal macrophages and the macrophage cell line RAW264.7, *IL-10* mRNA expression was increased in a dose-dependent manner by binding of *miR-4661* to the 3'UTR [245] and that IL-10 mRNA half-life was extended [245]. Other mechanisms proposed to be involved in the microRNA-dependent increase in mRNA expression are internal ribosome entry site (IRES), lack of or short poly-A tail or a 5' terminal oligopyrimidine tract (5' TOP) sequence [98]. However, there is no literature for activation of *MTOR* mRNA expression upon *miR-99a* binding.

The data obtained for *MTOR* and *miR-99a* in this thesis are observational and based on correlation analyses. On the one side, the data suggest that the two-fold up-regulation of *miR-99a* in healthy placental tissue could increase *MTOR* gene expression, but on the other hand *miR-99a* could prevent an otherwise much higher MTOR gene expression. However, further functional experiments in primary placental cell culture will be interesting to clarify the impact of *miR-99a* on placental *MTOR* expression upon the n-3 LCPUFA intervention, especially such experiments considering sex. From the reported data and the observations made in this thesis, it could be speculated that the regulation of *miR-99a* and its possible interaction with *MTOR* upon the intervention is a mechanism which is involved in the placental adaption of female offspring to the environmental stimuli of the dietary n-3 LCPUFA intervention during pregnancy in the maternal diet.

5.4. Impact of the n-3 LCPUFA intervention on placental amino acid transporter

Apart from the observed regulations in *MTOR* and *miR-99a* expression as well as the cell cycle / proliferation pathway, it was very surprising that there were no significant regulations observed in the biological process of lipid metabolism, *PPARγ* or *PPAβ/δ* target genes in the placenta, although such alterations were found in other organs upon stimulation with n-3 LCPUFA. The demonstrated higher incorporation of EPA and DHA in placental membranes would at least suggest changes in placental eicosanoids and their placental target gene expression [39]. Unfortunately, due to their short-living nature [33,34], placental eicosanoids were not assessed in this thesis. A possible reason why the n-3 LCPUFA intervention did not lead to placental gene expression changes could be that there was, apart from the incorporation in the phospholipids of the placental cell membranes, no increase in the concentration of free n-3 LCPUFAs in the placental cells upon the intervention. A reason for this could be an immediate transport through the placenta into the fetal circulation. This hypothesis is supported by Dunstan *et al.* [246], who stated the saturation of placental DHA transport occurs at 8.87% DHA of total fatty acids in maternal RBCs at gestational week 37 upon supplementation of 4 g DHA and EPA in an Australian population (n = 81). In the analyzed INFAT subpopulation upon a 1.2 g intervention with EPA and DHA, the median DHA% of total fatty acids in maternal RBCs at gestational week 32 was just below this concentration [IM: 8.49% (2.19-9.75), IF: 8.03 (3.26-9.08)]. However, it seems that this threshold for saturation of DHA transport can vary between different populations, since in three Tanzania tribes with high, low and intermediate habitual fresh water fish consumption the saturation of placental DHA transport was already found at 5.6% of total fatty acids in maternal RBCs (n = 73) [247]. Since the investigated Australian population in this report has a European genetic background, it could be likely that the threshold for the placental transport capacity of EPA and DHA lies in a similar range. Therefore, the amount of supplemented n-3 LCPUFAs in the INFAT study seems not saturate EPA and DHA transport, which could be responsible for the unaltered gene expression in lipid metabolism or *PPARγ* or *PPAβ/δ* target genes. In contrast to this, a surprising observation was that changes in placental gene expression of *LAT1* and *TAUT* amino acid transporter upon the n-3 LCPUFA intervention indicated alterations in amino acid transport.

5.4.1. The n-3 LCPUFA intervention alters placental *LAT1* gene and protein expression in different directions

LAT1 belongs to the family of *light chains of hetero(di)meric amino acid transporter* or also called *catalytic chains of hetero(di)meric amino acid transporter* [248-250]. Heterodimeric System L transporter are buildup of one catalytic light chain (*LAT1* or *LAT2*) and one heavy chain (*SLC3A2 / CD98)* [248-250], which is essential for *LAT1* surface localization [248]. The sodium-independent antiporter *LAT1* possesses 1:1 stoichiometry for exchanging essential substrate amino acids against other substrate amino acids via a concentration gradient. *LAT1* substrates are large neutral amino acids like phenylalanine (Phe), tyrosine (Tyr), leucine (Leu), isoleucine (Ile), histidine (His), tryptophan (Trp), valine (Val), methionine (Met) and glutamic acid (Glu), but also homocysteine [248-250]. The importance of *LAT1* for cell proliferation becomes apparent with its up-regulation in proliferating cells (activated human lymphocytes, rat hepatoma cells), its high expression in nearly all tested tumors and tumor cell lines as well as its up-regulation in regenerating liver. Its tissue distribution indicates an involvement of *LAT1* in amino acid transport of growing cells and across endothelial / epithelial secretory barriers, like the placenta [197]. In the placenta, *LAT1* is localized predominantly in the apical membrane of the syncytiotrophoblast layer for transporting essential amino acids from the mother to the fetus [251,252]

The measured gene expression changes of *LAT1* resembled the pattern of *MTOR, miR-99a, CDK6* and *PCNA*, which were only increased in female placentas upon the n-3 LCPUFA intervention. Therefore, the up-regulation of *LAT1* and the proliferation markers *CDK6* and *PCNA* in female placentas upon the intervention are in line with the reported association of *LAT1* in proliferating cells [197]. Surprisingly, in female placentas, *LAT1* gene (n_{CF} = 11, n_{IF} = 10) and protein (n_{CF} = 4, n_{IF} = 4) expression changes upon the n-3 LCPUFA intervention were inverse. Roos *et al.* also reported reduced mRNA level and transport of *LAT1* substrates upon *mTORC1* inhibition by rapamycin, although *LAT1* protein expression remained unaltered in cultured human primary trophoblasts [196]. This was later explained by less abundance of *LAT1* protein in the microvillous membranes [253]. A lot of such posttranslational steps are involved in the processes from mRNA to protein, which can obscure a correlation between mRNA and protein expression [254-256]. Studies analyzing a correlation between mRNA and protein expression in human tissues with combined transcriptomic and proteomic approaches showed that the overall correlations between gene and protein expression are often weak and exhibit huge variances [254,255]. The reported inverse correlations were hypothesized to be caused by negative feedback loops, delay between mRNA and protein accumulation or technical reasons [255,256]. However, the inverse direction of regulation might also depend on the placenta selection criteria for the different analyses. *LAT1* gene expression analysis was performed in placentas obtained by

spontaneous birth mode independent of the application of analgesics or anesthetics during labor, whereas *LAT1* protein expression was assessed in placentas obtained by spontaneous birth mode without the use of analgesics or anesthetics. The exact mechanisms are not yet understood and therefore further investigations are necessary to confirm the observed inverse correlations between *LAT1* mRNA and protein expression and their possible reasons.

Direct effects of LCPUFAs on *LAT1* expression or activity were not reported. Lager *et al.* [257] provided a hint that fatty acids could stimulate placental amino acid transport by demonstrating that oleic acid, a very abundant mono-unsaturated fatty acid, stimulated system A amino acid transport activity in trophoblast cells. An impact of the n-3 LCPUFA intervention on amino acid metabolism was further supported by the decrease in the amino acid levels of the *LAT1* substrate methionine in umbilical cord plasma.

5.4.2. The n-3 LCPUFA intervention sex-specifically alters umbilical cord plasma methionine levels

Methionine is an essential, neutral, sulfur-containing amino acid, which is rapidly transported from maternal to fetal circulation through the placenta [258]. During pregnancy, methionine balance is of great importance for offspring long-term health, as deficiency and excess of methionine in maternal nutrition leads to intrauterine growth retardation animal studies [259]. Methionine functions as a key amino acid for the initiation of protein synthesis [260]. In addition, in the one-carbon (C1) cycle, methionine represents an essential component, due to its involvement in producing S-adenosyl-methionine (SAM). In turn, SAM is important for the biosynthesis of phospholipids, DNA and protein methylation as well as chromatin function [259]. This central role of methionine in C1-metabolism seems to account via its effects on DNA synthesis and cell cycle / proliferation for this association with fetal growth [261].

Umbilical cord plasma methionine levels (n = 34, matching to the placental setup used in the biological validation independent of analgesics or anesthetics use during labor) were decreased upon the n-3 LCPUFA intervention in female offspring. This was an interesting observation, since methionine is transported by *LAT1* [248,249]. In addition, it was described that *LAT1* and to a smaller extent *y+LAT2* seem to be most important for uptake of *L*-methionine in primary term placental trophoblasts and BeWo cells, *in vitro* [262]. In contrast to this, placental methionine levels (n = 41, setup of the biological validation) were not significantly different upon the n-3 LCPUFA intervention. However, changes in human placental transport were reported to influence amino acid levels in umbilical cord plasma [71,263]. The decrease in umbilical cord plasma methionine levels upon the intervention

reduced female umbilical cord plasma methionine levels to the range of male newborns of both the control and intervention group.

Umbilical cord plasma methionine levels showed a non-significant positive, moderate correlation with *LAT1* protein expression, whereas it was inversely correlated with *LAT1* mRNA expression in a weak manner as a trend. However, methionine can also be transported by nine other placental amino acid transporters like *SLC38A1 (SNAT1), SLC7A9 ($b^{0,+}$ AT), SLC7A8 (LAT2), SLC43A1 (LAT3), SLC43A2 (LAT4), SLC7A7 (y^+LAT1), SLC7A6 (y^+LAT2), SLC38A1 (SNAT1)* and *SLC38A4 (SNAT4)* [74]. Since these transporters were not significantly altered upon the n-3 LCPUFA intervention in the transcriptome analysis (**Appendix 11.21**), the alterations in umbilical cord plasma methionine levels could depend on placental *LAT1* protein and / or gene expression changes upon the intervention.

This is the first study examining an impact of DHA or EPA supplementation during pregnancy on umbilical cord plasma amino acid levels. Unfortunately, amino acid levels in the maternal circulation have not been measured until now, so that it cannot be addressed whether the observed changes in umbilical cord plasma are associated with maternal methionine levels or fetal turn-over. Several *in vivo* studies showed that DHA or n-3 supplementation in infants, female piglets and chicken can alter methionine levels in plasma and tissues [264-266]. Kale *et al.* proposed a link between DHA and the C1 metabolism from data in never-medicated schizophrenia patients, as in a state of low DHA availability less methyl groups are required for the conversion of phosphatidylethanolamine-DHA to phosphatidylcholine-DHA and therefore more methyl groups are available for DNA and histone methylation [267]. Rees *et al.* reported that feeding pregnant rats a low protein diet with increasing methionine concentrations (0.2% - 0.5%) resulted in a lower offspring birth and body weight [268]. They suggested that a programming effect could be mediated, at least in part, by influencing DNA methylation with altered availability of methyl groups from C1-metabolism [261]. However, in the INFAT study there were no differences observed in birth weight or weight at one year. Therefore, it cannot be concluded whether this decrease of methionine levels in female placentas has consequences on body weight later in life or whether the female levels were decreased to a normal levels like in male offspring.

5.4.3. The n-3 LCPUFA intervention decreases placental *TAUT* gene expression and introduced a sex difference in umbilical cord plasma taurine levels

Another placental amino acid transporter - *TAUT* - (n = 41, biological validation independent of analgesics or anesthetics use during labor) and the umbilical cord plasma amino acid taurine (matched n = 34) were both regulated upon the n-3 LCPUFA intervention. The sulfur-containing, non-essential, non-proteinogenic amino acid taurine can be obtained from diet as

well as by endogenous synthesis from degradation of methionine by the transsulfuration pathway mainly. Important physiologic functions of taurine are bile salt formation, osmoregulation, impact on membrane structure and function, Ca^{2+} homeostasis, anti-oxidation, ion channel function as well as modulation of neurotransmission [269]. Placental taurine is the most abundant amino acid in the whole body and placental tissue, where taurine levels are 100 - 200 times higher than maternal levels. Placental *TAUT* is anticipated to be obligatory for fetal supply with taurine from the maternal circulation, since the fetus cannot synthesize it himself [270]. The importance of placental taurine transport for fetal development is underlined by a reduced taurine transport observed in fetal growth restriction [271-273]. *TAUT* belongs to the system ß family and transports taurine, hypotaurine, ß-alanine and to a lesser extent α-alanine and γ-amino-butyric acid (GABA) [274]. This Na^+/Cl^- /ß-amino acid symporter, with a stoichiometry 2:1:1, is located at the maternal facing syncytiotrophoblast of the human placenta [274,275]. *TAUT* was shown to be down-regulated by *mTORC1* inhibition with rapamycin in primary trophoblast cells [196]. A reduced *TAUT* activity and thereof reduced taurine levels in human cytotrophoblasts were described to impair syncytialization and increases the susceptibility for apoptosis [276].

Placental *TAUT* gene expression was down-regulated independently of offspring sex, whereas umbilical cord plasma taurine levels increased only in male offspring upon the n-3 LCPUFA intervention. Significant correlation between ΔCq values of *TAUT* and the umbilical cord plasma taurine levels were also absent. The different directions of regulation upon the intervention and the absence of correlation between *TAUT* gene expression and umbilical cord plasma taurine levels were unexpected. However, posttranslational modifications, like the phosphorylation of *TAUT* at Ser 322 [269], which alters the affinity of *TAUT* for taurine, could be involved. Additionally, taurine levels in the umbilical cord plasma depend not only on placental transport, but also on the fetal / newborn metabolism.

Only indirect hints from literature indicate that n-3 LCPUFAs could influence taurine levels. Li *et al.* [266] showed, that dietary supplementation with DHA in female neonatal piglets increased taurine in plasma as a trend ($p = 0.059$). Moreover, Hwang *et al.* [277] showed that EPA and DHA increased gene expression for cystathionase (CTH), the rate-limiting enzyme for the degradation of homocystein to taurine in HepG2 cells. Another possible mechanism for an impact of LCPUFAs on taurine levels was proposed in osmotic regulation. Osmotic exposure of Ehrlich ascites tumor cells leads to cytosolic phospholipase A2-mediated release of arachidonic acid from the nuclear membrane, which metabolized to the leukotrienes LTC4 and LTD4 stimulates a cysteine receptor CysLT1 triggered taurine release [269]. A decrease in the concentration of these leukotrienes could occur upon a n-3 LCPUFA intervention and might be responsible for decreased taurine release from certain cell types. However, this

direction does not fit with the increased taurine levels in umbilical cord plasma taurine in male offspring.

A supplementation of pregnant rats with taurine, leading to a doubling of taurine concentration in fetal circulation, had no impact on birth weight, but increased offspring body weight from one week of age onwards in females and from 4 weeks onwards in males. Furthermore, it was reported that abdominal (parametrial and preperitoneal) fat depots were increased in female offspring and epididymal fat depots in male offspring at 12 weeks upon taurine supplementation [278]. However, there are no comparable data for human pregnancy. The 1.6 fold increase of taurine levels in umbilical cord plasma upon the n-3 LCPUFA intervention observed in male offspring was nearly as high as in the described animal study. However, as mentioned earlier, neither significant differences in birth weight, nor in weight at one year were observed in the INFAT subpopulation of the whole INFAT population. However, it cannot be excluded that weight differences might occur later at life which is assessed in the conducted INFAT follow-up. Our data suggest that the n-3 LCPUFA intervention impacts placental taurine transport and taurine levels in newborn male circulation, but further sex-specific investigations are necessary to unravel whether this influences male offspring obesity risk.

5.4.4. Placental *MTOR* and *miR-99a* expression are correlated with placental *LAT1* and *TAUT* expression

Correlation analyses were performed to obtain information whether *miR-99a* is, in addition to MTOR, involved in the regulation of placental amino acid transport. Therefore, it was investigated whether the n-3 LCPUFA mediated changes in the placental transporters *LAT1* and *TAUT* as well as their associated amino acids were correlated with *MTOR* gene expression or *miR-99a* expression. In line with the report from Roos *et al.* [196], it was found in this thesis that *MTOR* gene expression was correlated with the gene expression of *LAT1* and *TAUT*. Surprisingly, *LAT1* gene expression was positively correlated and *TAUT* gene expression was inversely correlated with *MTOR* gene expression. Roos *et al.* [279] suggested that the increase in *TAUT* activity by glucose deprivation could be *mTORC1* independent. For n-3 LCPUFA intervention, the obtained correlation data suggest that upon n-3 LCPUFA intervention *MTOR* gene expression might be involved in the regulations of *LAT1* and *TAUT* gene expression, but influences them in different directions.

Interestingly, placental *miR-99a* expression was significantly correlated with the placental gene expression of *LAT1* and *TAUT*, with the same directions of the correlations as observed for *MTOR*. Therefore, *miR-99a* could also be involved in the regulation of *LAT1* and *TAUT* gene expression via *MTOR* or by directly regulating the expression of these two transporters.

There is no literature about *miR-99a* binding to the mRNA of *LAT1* or *TAUT* directly, but Filipowicz *et al.* [96] reported that microRNAs can target amino acid transporters, as shown for miR-122 repressing translationally cationic amino acid transporter 1 in human hepatoma cells by transfection experiments. Due to this possibility, it was bioinformatically assessed whether *LAT1* and *TAUT* possess a *miR-99a* binding site in their mRNA sequences. [154]. A *miR-99a* binding site was predicted for *TAUT* mRNA by the microRNA target prediction algorithm *rna22* [146] with two mismatches in the seed sequence. However, none of the applied prediction algorithms identified a *miR-99a* binding site within the *LAT1* mRNA. Unfortunately, until now the available prediction methods have only precision of about 50% and sensitivity of 12% [280]. However, collections of experimental supported microRNA targets in large databases did also not contain information on binding sites of *miR-99a* within *LAT1* or *TAUT* mRNA sequences. The conducted alignment of *LAT1* mRNA with *miR-99a* sequences revealed agreement in the seed sequence (nucleotide 2-8 of microRNA) with *LAT1* 3' UTR with two mismatches at nucleotide two and seven (5' – 3') of *miR-99a,* similar to the 2 mismatches in the prediction for *TAUT* as *miR-99a* target gene. The reason why this binding site was not identified could depend on the additional features used in the applied prediction algorithms, but it seems worth to perform a functional biological validation of *miR-99a* targeting *LAT1* or *TAUT.*

5.4.5. Placental amino acid transport and fetal / metabolic programming

Taken together, the data of this thesis argue for an impact of the n-3 LCPUFA intervention during pregnancy on amino acid metabolism via *MTOR* mediated alterations in the placental amino acid transporter *LAT* and *TAUT*, which are associated with changes in umbilical cord methionine and taurine levels. In neonatal rats, a higher abundance of hepatic proteins involved in amino acid metabolism and protein synthesis (*elongation factor 1γ, protein disulfide-isomerase A6, serine hydroxymethyltransferase and argino-succinate synthase*) was found, which the authors attributed to higher n-3 LCPUFA levels in maternal diet during pregnancy and lactation [281]. Moreover, in livers of neonatal rats, a maternal diet adequate for n-3 PUFAs increased serine levels and decreased glycine levels compared to a maternal diet deficient for n-3 PUFAs [282]. Innis and Novak [283] therefore proposed that n-3 fatty acids alter gene expression of key genes and proteins to shift macronutrient metabolism towards oxidation of fatty acids with sparing of amino acids for anabolic pathways of protein and peptide synthesis in liver. In addition, Gingras *et al.* [284] reported that in steers, the activation of *Akt-mTOR-S6K1* signaling pathway was associated with the increase of insulin-stimulated amino acid disposal by enteral infusion of menhaden oil, which is rich in n-3 LCPUFAs. These few reports point towards an association of n-3 LCPUFA interventions during pregnancy with amino acid metabolism and a possible regulation by *MTOR*.

Interestingly, it was proposed that an altered transfer of amino acids between mother and fetus is one of the underlying mechanisms for programming offspring obesity risk (see **1.1.2**) by influencing developmental structure and function of energy metabolism in fetal organs like pancreatic β-cells, hypothalamus, muscle and adipose tissue [15,16]. A key role was proposed for placental nutrient sensing pathways, including *MTOR,* to be involved in placental adaption to environmental signals and mediation of programming effects [92,237]. Therefore, the data of this thesis are in line with literature. However, there were no programming effects observed until one year of age in the INFAT study [124]. Nevertheless, these observed alterations in the placental amino acid transporter *LAT1* and *TAUT* mediated in part by *MTOR* could be involved in programming of offspring obesity risk at a later time point, which can only be assessed in future with data from the INFAT follow up.

5.5. Several placental gene expression changes are correlated with n-6/n-3 LCPUFA ratio of maternal and fetal biomarkers

To further support that the observed placental expression changes in this work are a result of the n-3 LCPUFA intervention, the significantly regulated parameters (gene, microRNA, protein expression and amino acid levels) were correlated with the n-6/n-3 LCPUFA ratio in maternal (RBCs 32nd week of gestation), placental and newborn (umbilical cord RBCs) biomarkers. Most of the placental parameters showing significant correlations with *MTOR* (*CDK6, PCNA, MTOR, TAUT, miR-99a* and *LAT1* protein) were correlated with the maternal n-6/n-3 LCPUFA ratio. In line with the data of this thesis, significant correlations of placental gene expression with maternal LCPUFAs were also identified by Larqué *et al.* [211]. They showed that the percentage of EPA, DHA, AA and DHA / AA ratio in maternal plasma phospholipids at birth were significantly correlated with gene expression of *FATP-1* and *4* in human placental tissue without decidua [211] similar to the placenta sampling in this thesis.

The sample size for maternal blood samples from birth was too small to be analyzed. Therefore, the n-6/n-3 LCPUFA ratio from the maternal RBCs at the 32nd week of gestation was used as indirect marker for maternal status at birth in the correlation analysis. Most of the significant correlations were observed for maternal RBCs. A reason for this close connection of the placental expression changes with maternal RBC can be that the genes correlating with maternal RBCs were all expressed in the trophoblast cells or the syncytio-trophoblast, which are close to or in direct contact with maternal blood. This suggests that the LCPUFAs which contribute to the decreased n-6/n-3 LCPUFA ratio in maternal blood are associated more closely with placental gene expression changes than in the membrane composition of the placenta or the umbilical cord blood. Unfortunately, free AA, EPA, DHA and n-6/n-3 LCPUFA ratio in the placental compartment were not assessed and umbilical cord plasma samples were too few available, to investigate whether these levels were

stronger correlated to placental gene expression than the maternal biomarkers. However, the correlations support that the observed placental expression changes in these genes and the *LAT1* protein are connected to the n-3 LCPUFA intervention.

5.6. Minor contribution of estradiol-17β / testosterone ratio on sex-specific placental gene expression

Possible biological factors which could be involved in the n-3 LCPUFA-mediated, observed sexual dimorphic gene expression are sex steroids. Of all analyzed sex steroids, placental estradiol-17β / testosterone ratio (n = 41, corresponding to the placental setup for biological validation independent of the use of analgesics or anesthetics) alone showed a sex-specific decrease upon the n-3 LCPUFA intervention in female offspring. Since placental estradiol-17β levels did not show any significant differences, it was suggested that changes in placental testosterone levels most likely contribute to the significant differences of the estradiol-17β / testosterone ratio. Measured placental testosterone and estradiol-17β levels were in the same range of those described earlier [285-287].

Estradiol-17β / testosterone ratio is often used as an indirect marker for *aromatase* activity, because *aromatase* is the key enzyme for the conversion of testosterone to estradiol-17β or androstenedione to estrone [208,209]. Placental *aromatase* deficiency in human fetuses causes increased testosterone levels in maternal and female fetal circulation [288]. Therefore, differential *aromatase* expression could be involved in the offspring sex and n-3 LCPUFA-based differences observed for placental testosterone levels and estradiol-17β / testosterone ratio. Sathishkumar *et al.* [289] reported that *aromatase* mRNA and protein expression were lower in female compared to male placentas in normal human pregnancies. Since lower aromatase protein expression would result in less production of estradiol-17β from testosterone, an increase in testosterone compared to estradiol-17β is anticipated. These data are in concordance with the observed lower estradiol-17β / testosterone ratio in female compared to male placentas.

No effect of a n-3 LCPUFA intervention during pregnancy on placental testosterone or aromatase expression is described. Reports on the impact of n-3 LCPUFA on circulating testosterone levels in humans and animals studies were contradictory [290-297] and most likely depend on differences in n-3 LCPUFA supplementation (concentration and duration), subject disease status or animal model. It was hypothesized that n-3 LCPUFAs influence testosterone production in adult pig testis via their impact on PGE_2 synthesis or their regulation of *PPARs*, which are involved in steroidogenesis [291]. This indicates that the n-3 LCPUFA intervention could decrease estradiol-17β / testosterone levels in female placentas upon n-3 LCPUFA intervention.

Placental function itself could be influenced by increased placental testosterone levels, since the human placenta expresses androgen receptor [298]. In the transcriptome analysis of this thesis, there were no significant differences found for placental androgen receptor expression upon n-3 LCPUFA intervention (IM+IF vs. CM+CF: FC 1.75, p = 0.136 / IM vs. CM: FC 1.42, p = 0.150 / IF vs. CF: FC 1.25, p = 0.388). Moreover, there is no literature available for an impact of placental testosterone levels on placenta function. Reports for increased maternal testosterone levels during pregnancy showed an association with low birth weight [299], mediated by a reduction of placental *amino acid transporter SNAT2 (SLC38a2)* [300]. However, no significant regulation was found for this transporter upon n-3 LCPUFA intervention (**Appendix 11.21**).

Clifton [108] stated that 'testosterone is the most obvious steroid that may control sex-specific differences observed in the human placenta [108]. However, our correlation analysis showed that in total only two out of 13 placental expression parameters with sex-specific regulations were significantly correlated with placental estradiol-17β / testosterone ratio or umbilical cord plasma testosterone, respectively. Bermejo-Alvarez *et al.* [301] demonstrated in bovine blastocysts that one third of the active expressed genes exhibit sexual dimorphism already before sex steroids are produced. They furthermore proposed that sex chromosomes impose an extensive transcriptional regulation upon autosomal genes without hormonal interaction [301]. In addition, Mao *et al.* [219] showed in mice that different maternal nutrition during pregnancy led to sexual dimorphism in the placental transcriptome at a time-point where the contribution of sex hormones seems unlikely. The data from other tissues or species had to be abstracted and are difficult to compare to the data in this thesis. However, they suggest that placental estradiol-17β / testosterone ratio might only contribute to a minor extent to sexual dimorphisms in gene expression dependent of the n-3 LCPUFA intervention.

5.7. Implications of placental expression changes upon n-3 LCPUFA intervention for offspring obesity risk

The analysis of placental gene expression and further placental parameters was conducted to investigate whether changes in the placenta upon the n-3 LCPUFA intervention during pregnancy have a physiological impact and are involved in programming offspring obesity risk. Correlation analyses assessed whether significant expression changes in the placenta are associated with selected placenta, weight and body composition measurements. The complex network of regulations upon the n-3 LCPUFA intervention and their correlations with weight and body composition measurements are summarized in **Figure 17**.

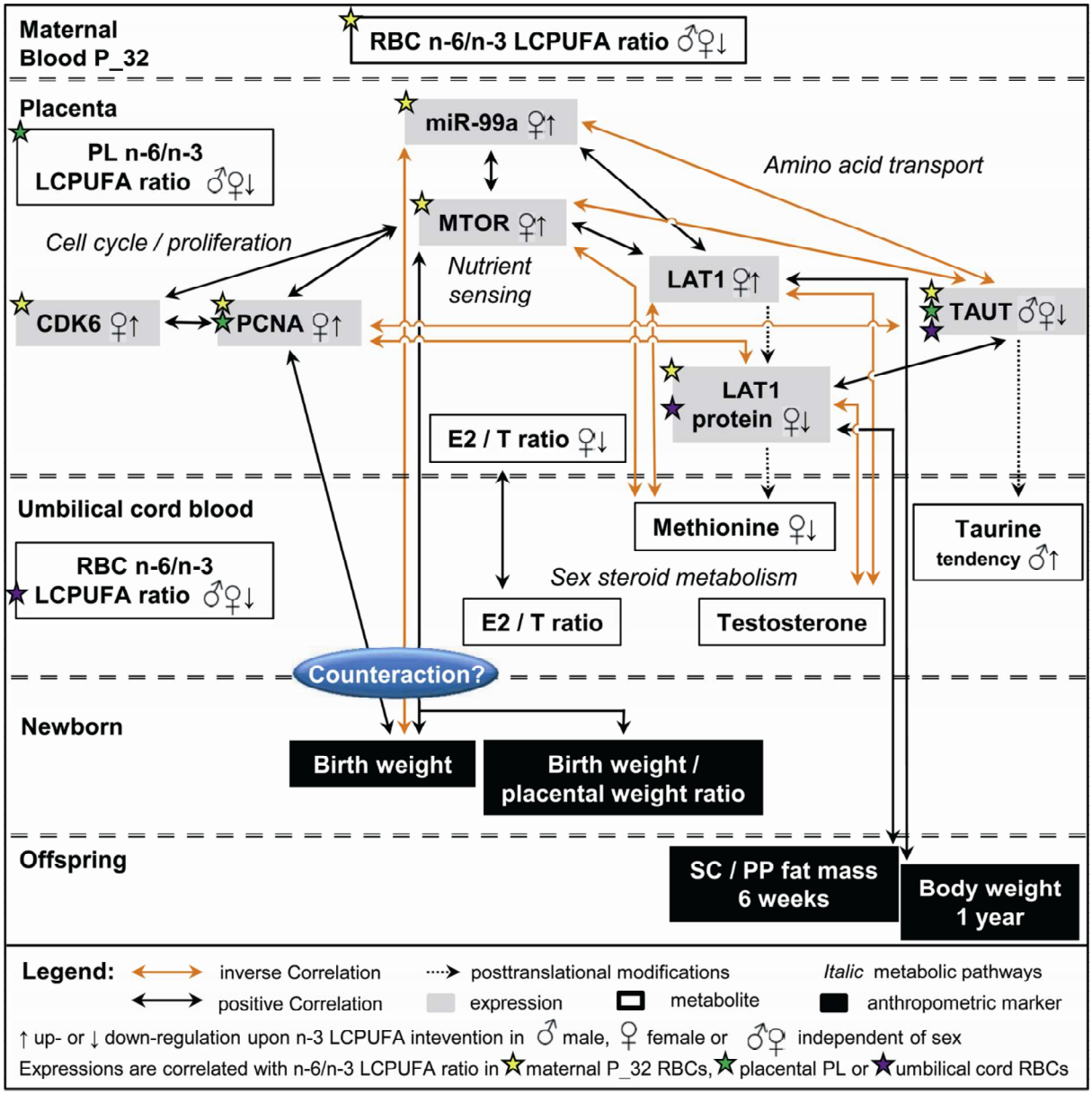

Figure 17: Summary of the complex network of regulations upon the n-3 LCPUFA intervention and their correlations with weight and body compositions measurements. *RBC, red blood cells; P_32, 32nd week of gestation; PL, phospholipid; E2 / T ratio, Estradiol-17β / Testosterone ratio; SC / PP, subcutaneous fat / preperitoneal fat ratio.*

5.7.1. Opposing correlations for offspring birth weight with placental expression of *PCNA* and *CDK6* compared to *miR-99a*

For birth weight, opponent correlations were observed between placental *miR-99a* and CDK6 / PCNA, where *miR-99a* expression was inversely and *PCNA* and *CDK6* expression positively correlated. Higher placental ΔCq values corresponding to higher gene expression of *PCNA* (mean ΔCq_{IG} = -2.10 / mean ΔCq_{CG} = -2.36) and *CDK6* (mean ΔCq_{IG} = -2.88 / mean ΔCq_{CG} = -3.18) upon the n-3 LCPUFA intervention were associated with higher offspring birth weight. There is no literature for an association of placental *CDK6* expression with birth weight. However, observations of Zadrozna *et al.* [302] support this, because they showed that in intrauterine growth retardation, which leads to lower birth weight, less placental cells of terminal villi express *PCNA* compared to control placentas. At the same time, they observed reduced cell proliferation in cytotrophoblast cells and a reduction in syncytiotrophoblast nuclei in terminal villi of placentas affected by intrauterine growth retardation [302]. These data together with the data from the thesis suggest that higher *PCNA* gene and protein expression in placental cells is associated with higher birth weight.

In contrast, higher *miR-99a* ΔCq values (mean ΔCq_{IG} = 0.92 / mean ΔCq_{CG} = 0.33), and thus higher placental expression, were associated with lower offspring birth weight. However, there is no literature about an association of *microRNA-99a* with birth weight. It was shown by Cui *et al.* [230] that overexpression of *miR-99a* induced a *mTORC1*-mediated G1 cell cycle arrest in renal cell carcinoma cells. Moreover, Turcatel *et al.* [303] demonstrated that inhibition of *miR-99a* decreased also *TGFB1* signaling in normal mouse mammary gland cells, which is associated with increased cell proliferation.

As mentioned earlier for the INFAT study, there were no significant differences observed for offspring birth weight between the n-3 LCPUFA intervention group and the control group. In the whole INFAT population offspring birth weight was significantly different between the intervention and the control group, however differences did not remain statistical different after adjustment for sex and gestational duration [124]. The interesting observation of opposite directions of correlations for offspring birth weight with *PCNA* and *CDK6* compared to *miR-99a* allows the speculation that *miR-99a* has a neutralizing or counter regulatory effect on offspring birth weight upon the n-3 LCPUFA intervention. This would further support that *miR-99a* prevents an excess in up-regulation of *MTOR.* This should be further investigated along with functional experiments to improve the knowledge about the function of *miR-99a* in placental tissue.

5.7.2. *Placental LAT1* expression is significantly correlated with several weight and body composition measurements

The most significant (positive) correlations for selected placental gene expression with weight and body composition measurements were observed for *LAT1, HDAC5* and *DVL1* with weight at one year. *LAT1* gene expression showed the strongest regulation upon the n-3 LCPUFA intervention, but only in female placentas. Independent of sex, higher ΔCq values of *LAT1* (mean ΔCq_{IG} = -1.42 / mean ΔCq_{CG} = -1.81) corresponding to higher gene expression were associated with higher offspring weight at one year. There are no data published for associations of placental *LAT1* expression or activity with offspring weight at birth or later time points. Moreover, placental *LAT1* protein expression, which was decreased upon n-3 LCPUFA intervention, showed a positive correlation to the subcutaneous to preperitoneal fat mass ratio at 6 weeks after birth (Rs = 0.49, p = 0.045, n = 17). Lower *LAT1* protein expression was therefore associated with lower subcutaneous to preperitoneal fat ratio indicating a relative increase in preperitoneal compared to subcutaneous fat mass.

Therefore, both the higher *LAT1* gene expression and the lower *LAT1* protein expression upon n-3 LCPUFA intervention were associated with higher weight at one year as well as with a relative higher preperitoneal fat mass at 6 weeks, respectively. The analyzed data suggest that an increase of placental *LAT1* gene expression and the decrease in *LAT1* protein are associated with higher weight parameters in female offspring. Despite many parameters that point towards higher weight especially in females (cell proliferation, umbilical cord plasma methionine levels, LAT1 expression), but also in males (taurine levels), there was no significant difference in female offspring upon n-3 LCPUFA intervention in weight at one year or in the subcutaneous to preperitoneal fat mass ratio at 6 weeks of age in the whole INFAT population or the INFAT-subpopulation. The correlation of *LAT1* mRNA was not observed with birth weight, but later with weight at one year. It could be speculated that there is an impact in later life. Therefore, a counteracting mechanism might be proposed for miR-99a which might delay or prevent this effect. Therefore, a correlation analysis with the follow-up data of the INFAT study up to five years will be interesting. However, the presented data are mainly of observational origin and from correlation analysis with clinical data. Therefore, they have to be interpreted very carefully. Nevertheless, these acquired data are very valuable to generate hypotheses for underlying molecular mechanisms acting upon a n-3 LCPUFA intervention during pregnancy. Further investigations are important to confirm whether the observed association of higher placental *LAT1* gene expression is associated with higher offspring weight.

5.7.3. Analogy of molecular changes in the placenta upon the n-3 LCPUFA to adipose tissue

This thesis revealed several molecular changes in the placenta and correlations with weight body composition measurements upon the n-3 LCPUFA intervention. However, the n-3 LCPUFA intervention does not solely act on the placenta. Since also RBCs in umbilical cord blood show a lower n-6/n-3 LCPUFA ratio, it is likely that also other fetal organs are influenced which can be involved in fetal / metabolic programming like for example the hypothalamus [304]. For the INFAT study with its primary aim to influence adipose tissue development, it is of special interest to address whether a decreased n-6/n-3 LCPUFA ratio could have a similar impact as found in placenta on the developing fetal adipose tissue.

It is not surprising that expression of *mTOR, PCNA, CDK6, LAT1* and *TAUT* was reported for adipocytes in other studies, since these genes have central cellular functions like cell proliferation, nutrient-sensing and amino acid transport, [305-309]. However, these expression data are mostly derived from murine models, like 3T3-L1 cells, as information on the expression of these genes is rare in human adipocytes or adipose tissue. Interestingly, also the expression of *miR-99a* is described in murine 3T3-L1 adipocytes and human subcutaneous primary adipocytes [310,311].

The functions of these genes in adipocytes are summarized as follows:
For *MTOR*, several authors showed that inhibition by rapamycin markedly reduced the gene expression of transcriptional adipogenesis regulator *PPARγ2* and other adipocyte marker genes (*C/EBPα, adipsin, aP2, LPL, FATP-1* and *SREBP-1c*) as well as the lipid uptake and storage in rat adipose tissue and during differentiation of 3T3-L1 adipocytes [305,312]. Recently, Yoon *et al.* reported, that the level of mTOR protein and / or activity influences the balance between adipogenesis-promoting and adipogenesis-suppressing functions and determines the net outcome of fat formation in the murine 3T3-L1 cell line [313]. Moreover, chronic treatment of rodents with rapamycin decreased their body weight gain and led to lower adipose tissue mass containing fewer adipocytes with a smaller diameter compared to control rodents [312].

For *PCNA* and *CDK6,* it is known that several cell cycle regulators are involved in clonal expansion of adipocytes, which is one of the two critical steps for expanding adipose tissue mass [307]. The importance of *PCNA* for adipose tissue proliferation was demonstrated by abolishing the phosphorylation site at tyrosine-114 of the *PCNA* protein. Mice with this modification on a high-fat diet showed less weight gain and fewer adipocytes compared to control mice [307]. In addition, the *cyclin D3 – CDK6* complex was reported to phosphorylate *PPARγ2*, thereby leading to transcriptional activation of *PPARγ2* target genes in *3T3-L1* adipocytes. A point-mutation in cyclin D3 resulted in smaller adipocytes and reduced gene

expression of adipocyte markers (*PPARγ, aP2* and *LPL*) as well as a 30% reduced weight gain upon a high-fat diet compared to wild-type mice [308].

Information on the function of *LAT1* and *TAUT* in adipocytes or adipose tissue is scarce. It was only reported that *LAT1* mRNA is higher expressed in subcutaneous white adipose tissue of ob/ob mice than in wild-type mice [306]. It was shown that a restriction of the *LAT1* substrate methionine in the diet of mice protects from visceral fat mass accretion compared to mice on a control diet [314]. For TAUT, there are no publications, but it was shown that abdominal (parametrial and preperitoneal) fat depots were increased in female offspring and epididymal fat depots in male offspring at 12 weeks upon taurine supplementation [278].

MiR-99a was reported to be 1.2-fold higher expressed in adipocytes from subcutaneous fat in obese women compared to non-obese women, where *miR-99a* expression was correlated with BMI [311]. Furthermore, in *3T3-L1* adipocytes *miR-99a* expression increased during differentiation. However, anti-sense inhibition of *miR-99a* did not exhibit an impact on adipocyte differentiation, which was measured by marker gene expression and lipid droplet accumulation [310]. The function of *miR-99a* in adipocytes is still unclear, but could be associated with its regulatory influence on *mTOR*.

Altogether, one could speculate that, if the same directions of gene expression regulation as described for the placenta are anticipated for adipocytes upon the n-3 LCPUFA intervention, the higher gene expression for *mTOR*, *PCNA, CDK6* and *LAT1* may lead to an increase in adipocyte size followed by weight gain. Moreover, since the above mentioned genes were only up-regulated in the placenta from female offspring upon the intervention, it would be interesting to assess whether sexual dimorphic expression of these genes occur also in adipocytes or adipose tissue upon n-3 LCPUFA intervention.

5.8. Identified placental regulations in the context of the INFAT study

The publications for the primary outcome and sub analyses of the INFAT study showed no evidence for an impact of the n-3 LCPUFA intervention during pregnancy and lactation on offspring adipose tissue development or body weight up to one year of life [124,164,315-318]. This negative finding on offspring body weight was supported by a Cochrane analysis [319] and a meta-analysis of studies, where body weight was only represented as a secondary outcome [320]. However, several investigations of the INFAT population, independent of the n-3 LCPUFA supplementation, taking advantage of the broad distribution of n-6/n-3 LCPUFA ratio, identified factors, which are associated with offspring body weight and fat mass like for example umbilical cord plasma insulin or leptin [315,316]. With this kind of analysis, it was also shown that higher levels for DHA, n-3 LCPUFA and n-6 LCPUFAs maternal RBC were associated with higher birth weight, birth length and lean body mass,

which are all markers of prenatal growth [164]. These observations are concordant with the identified regulations of placental gene expression associated with nutrient sensing, amino acid transport and cell cycle / proliferation upon the n-3 LCPUFA intervention. This indication for an effect of the LCPUFAs was found when the analysis is performed considering the entire INFAT population as one group. However, this effect disappears when the population is analyzed separately in n-3 LCPUFA intervention and control group.

Of special interest is that several analyses of the INFAT study [164,318] as well as the correlations of placental gene expression with offspring weight markers pointed towards a higher body weight upon the n-3 LCPUFA supplementation during pregnancy and lactation, rather than on the decrease in body weight, which was hypothesized based on corresponding animal studies. However, support for these observations in the INFAT study came from a study in mice, where a n-3 LCPUFA supplementation limited to the pregnancy period increased the percentage of body fat through accumulation of subcutaneous fat depots [321]. In accordance to the absence of placental regulations in *PPARγ* and genes of lipid metabolism reported in this thesis, Mühlhausler *et al.* did also not observe regulations of key genes in adipogenesis and lipogenesis upon n-3 LCPUFA supplementation in the murine adipose tissue [321]. Despite the valuable insights from the INFAT study and other studies, it is still not clear whether a n-3 LCPUFA supplementation during pregnancy and lactation can program offspring obesity risk [317]. To draw definitive conclusions, long-term follow-ups, like the one conducted for the INFAT study, are mandatory.

Sex-specific effects have to be considered for further experiments, since surprisingly the placental gene expression changes upon the n-3 LCPUFA intervention were more pronounced in female offspring. In the whole INFAT population also a more pronounced effect in female offspring was found for the association of higher umbilical cord blood insulin levels with higher weight gain from birth up to two years [315]. Considering the data from the placenta analysis, on the one hand, this more pronounced impact of the intervention can suggest that the intervention could have a more pronounced impact on female offspring later in life. On the other hand, possible counter-regulatory effects, especially in female offspring, like the one proposed for *miR-99a*, could be responsible for the absence of an impact of the intervention on female offspring obesity risk and await further investigation.

5.9. Concluding remarks

The INFAT subpopulation, reflecting the excellent compliance of the whole INFAT population, provided an unique opportunity to investigate the impact of a n-3 LCPUFA supplementation during pregnancy on placental gene expression and to analyze molecular mechanisms involved in programming offspring obesity risk. The data of this thesis demonstrated that the n-3 LCPUFA intervention impacts placental gene expression sex-specifically. Global transcriptome data and gene-specific biological validation showed that the intervention predominantly altered placental gene expression in female offspring. Moreover, this thesis revealed that placental amino acid transport was regulated upon the maternal n-3 LCPUFA supplementation during pregnancy, which other authors described to be associated with fetal / metabolic programing of obesity risk. Gene expression of *MTOR*, a part of the nutrient sensing pathway *mTORC1*, and the *bona fide mTORC1* regulated amino acid transporter *LAT1* and *TAUT* were regulated upon the n-3 LCPUFA intervention. Only in placentas of female offspring, *MTOR* and *LAT1* gene expression were up-regulated, whereas *LAT1* protein expression, along with methionine levels in umbilical cord plasma, which are likely to depend on the placental *LAT1* transporter, were down-regulated upon the intervention. Moreover, *microRNA-99a*, which could be involved in the mRNA-microRNA expression network regulating placental amino acid transport by targeting *MTOR* and potentially *TAUT* and *LAT1*, was identified to be sex-specifically up-regulated in female placentas upon the n-3 LCPUFA intervention. However, in the placenta only a few of the sex-specific expression changes were associated with placental estradiol-17β / testosterone ratio or placental and umbilical cord plasma testosterone levels, which were found to be altered upon the n-3 LCPUFA intervention. This observation favors the hypothesis that sex chromosomes (X, Y) could contribute to sex-specific autosomal gene expression to a larger extent than the observed changes in sex steroid levels. Finally, although placental *LAT1* gene expression correlated with higher offspring weight at one year, there were no significant differences for offspring weight at one year, either in females or all offspring, upon the n-3 LCPUFA intervention in the whole INFAT population or the analyzed subpopulation. Potential counteracting mechanism, as indicated for offspring birth weight by the expression for *PCNA* / *CDK6* and *miR-99a*, could contribute to the absence of differences by the n-3 LCPUFA intervention. Since there is evidence that *MTOR, LAT1, TAUT, PCNA, CDK6* and *miR-99a* are also expressed in adipocytes, the intervention could have a similar sex-specific impact on the developing offspring adipose tissue. Data of the INFAT follow-up until five years of age will contribute to assess whether this sex-specific impact of the maternal n-3 LCPUFA supplementation during pregnancy especially on the nutrient sensor *MTOR* and its associated amino acid transporter *LAT1* increase adipose tissue or weight more pronounced in female offspring or if counteracting mechanisms as indicated for *miR-99a* prevent this.

5.10. Future perspectives

Additional to the pathways and microRNAs investigated in this thesis, genes in significantly overrepresented pathways from pathway analysis or the combined transcriptome-microRNA-analysis (e.g. the insulin signaling pathway) are further promising targets. Moreover, the combined analysis could only be done for microRNA binding sites in the 3'UTR of significantly regulated genes, since no program was available that could analyze binding sites in the entire gene body. Therefore a re-analysis of the transcriptome and miRNome datasets when such a program is available could reveal additional regulated genes and pathways targeted by regulated microRNAs. Likewise, from the 46 regulated microRNAs in the explorative profiling only four were biologically validated until now. Therefore, some other microRNAs, like those switched on or off upon the n-3 LCPUFA intervention are interesting targets for further biological validation. It will be important to analyze amino acid levels in maternal circulation and placental LCPUFA eicosanoids to complement the already available data. In addition, although the observed gene expression changes in the biological validation were mostly supported to be expressed in placental trophoblast cells by literature, it will be important to confirm the cell type-specific localization of differentially regulated genes and *miR-99a* by in situ RNA-hybridization experiments and immunohistochemistry in the available paraffin-embedded tissues. Moreover, it will be interesting to analyze whether the observed expression changes of placental *miR-99a* could also be detected in maternal plasma, because placenta-specific microRNA were reported to circulate in maternal blood during gestation.

The results of this thesis also raise new hypothesis that await further investigation. Therefore, cell culture experiments are necessary to improve the understanding of the sex-specific impact on the n-3 LCPUFA intervention, for example by analysis of *mTORC1* activation or overexpression / knock-down of *miR-99a*. Moreover, alterations in the activity of the amino acid transporters *LAT1* and *TAUT* could also be assessed *in vitro*. In addition, it would also be possible to address the opposing directions of regulation between *LAT1* gene and protein expression. However, in order to investigate the sex-specific effects of the intervention in cell culture, it will be necessary to analyze primary cells from male and female placentas, since all commercially available placental cell lines are derived from male donors.

In case that such cell culture experiments support an important role for *miR-99a* in mediating n-3 LCPUFA intervention effects, a knock-out animal model, either with a complete or cell-type-specific knock-out (trophoblast, adipocyte), will contribute to understand the functions of this microRNA. In addition, such a *miR-99a* knock-out model under the same treatment regime during pregnancy as described by Massiera *et al.* [21] would provide valuable insights in the effect of the low n-6/n-3 LCPUFA ratio during pregnancy on obesity risk.

Especially important is the unique opportunity that is given by the conducted follow-up of the INFAT study. Since programming effects are reported to occur often in later life, it might be possible that during later time periods of the conducted follow-up studies the impact of alterations upon n-3 LCPUFA intervention on obesity risk of female offspring could be observed.

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7. Abbreviations

AA	Arachidonic acid
AAR	Amino acid response
ACADM	Medium-chain acyl-CoA dehydrogenase
ACTB	β-actin
a-FABP / aP2	Adipocyte fatty acid binding protein
AGA	appropriate for gestational age (10^{th} to 90^{th} birth weight percentile)
ALA	α-linolenic acid
ANAPC4	Anaphase promoting complex subunit 4
APGAR	Appearance, Pulse, Grimace, Activity, Respiration
Arg	Arginine
ASCL1, 3, 5	Acyl-CoA synthetase long-chain family member 1, 3, 5
bAIB	ß-aminoisobutyric acid
bp	base pair(s)
BK	Bestkeeper
BMI	Body mass index
BSA	Bovine serum albumin
B2M	Beta-2-microglobulin
CBP	CREB-binding protein
CDF-file	Custom Chip Definition file
CDK1	Cyclin-dependent kinase 1
CDK6	Cyclin-dependent kinase 6
cDNA	complementary DNA
CD99L2	CD99 molecule-like 2
C/EBPβ	CCAAT / enhancer binding protein β
C/EBPδ	CCAAT / enhancer binding protein δ
CF	Female offspring / placentas of the control group
CG	Control group
CM	Male offspring / placentas of the control group
C19MC	The chromosome 19 microRNA cluster
ChREBP	Carbohydrate regulatory element binding protein
CKS2	CDC28 protein kinase regulatory subunit 2
COX1 / PTGS1	Cyclooxygenase 1
COX2 / PTGS2	Cyclooxygenase 2
Cq	Cycle of quantification
CREB	cAMP-responsive element binding protein
CT	Cytotrophoblast
CV	Coefficient of variation
CYP	Cytochrome P450
C8orf59	Chromosome 8 open reading frame 59
DAG	Diacylglycerol
DDX3Y	DEAD (Asp-Glu-Ala-Asp) box polypeptide 3, Y-linked
DEPTOR	DEP domain containing MTOR-interacting protein
DHA	Docosahexaenoic acid
DKK1	Dickkopf 1 homolog (Xenopus laevis),
DNA	Desoxyribonucleic acid
DNMT 1 / 3a / 3b	DNA (cytosine-5) methyltransferase 1 / 3a / 3b
DOHaD	Developmental origins of health and disease
DVL1	Dishevelled, dsh homolog 1 (Drosophila)
EET	Epoxyeicosatrienoic acid
e-FABP / Mal1	Epidermal (keratinocyte) fatty acid binding protein
EIF1AX	Eukaryotic translation initiation factor 1A, X-linked

EIF1AY	Eukaryotic translation initiation factor 1A, Y-linked
ELISA	Enzyme-linked immunoabsorbent assay
ELOVL 2 / 5	Elongase 2 / 5
EPA	Eicosapentaenoic acid
EPS8L2	EPS8-like 2
ERK	Extracellular signal-regulated kinase
E2	Estradiol-17β
F	Female
FABP	Fatty acid binding protein
FA-CoA	Fatty acid acyl-CoA-thioesters
FADS1	Fatty acid desaturase 1 / Δ5-desaturase
FADS2	Fatty acid desaturase 2 / Δ6-desaturase
FAME	Fatty acid methyl ester derivates
FATP1-4 / 6	Fatty acid transporter 1-4 / 6
FC	Fold change
FCS	Functional class sorting approach
FGF-10	LIF and fibroblast growth factor
FGF-R	LIF and fibroblast growth factor receptor
FZD7	Frizzled family receptor 7
GABA	γ-amino-butyric acid
GALNT11	UDP-N-acetyl-alpha-D-galactosamine:polypeptide N-acetylgalactosaminyl-transferase 11
GAPDH	Glyceraldehyde-3-phosphate dehydrogenase
GDM	Gestational diabetes
GLU	Glutamic acid
GLUT 1 / 3	Glucose transporter 1 / 3
GPR	G-protein coupled surface receptor
HDAC5	Histone deacetylase 5
HDHD1A	Haloacid dehalogenase-like hydrolase domain containing 1A
HETE	Hydroxyeicosatetraenoic acids
HIF1α	Hypoxia-inducible factor 1
His	Histidine
HNF-4α	Hepatic nuclear factor 4α
HPETE	Hydroperoxy-eicosatetraenoic acids
HPLC	High performance liquid chromatography
HSD11B1	Hydroxysteroid (11-beta) dehydrogenase 1
IF	Female offspring / placentas of the n-3 LCPUFA intervention group
IG	n-3 LCPUFA intervention group
IM	Male offspring / placentas of the n-3 LCPUFA intervention group
IGFBP1	Insulin-like growth factor binding protein 1
IGF1R	Insulin-like growth factor 1 receptor
Ile	Isoleucine
INFAT	**I**mpact of **n**utritional **f**atty acids during pregnancy and lactation on early **a**dipose **t**issue development
IP	Prostacyclin receptor
IQR	Interquartil range: 25^{th}-75^{th} percentile
KDM5D	Lysine (K)-specific demethylase 5D
KDM6A	Lysine (K)-specific demethylase 6A
LA	Linoleic acid
LAT1	L-type amino acid transporter 1 (SLC7A5)
LC-MS/MS	Liquid chromatography-tandem mass spectrometry
LCPUFA	Long-chain polyunsaturated fatty acid
Leu	Leucine
LIF	Leukaemia inhibitory factor
LIF-R	Leukaemia inhibitory factor receptor
LIMCH1	LIM and calponin homology domains 1

LPIN1	Lipin 1
LOX	Lipoxygenase
LPL	Lipoprotein lipase
LRP6	Low density lipoprotein receptor-related protein 6
LT	Leukotrienes
LXRα	Liver X receptor α
M	Male
MADMAX	Management and analysis Database for Multi-platform microArray eXperiments
MAD2L1	MAD2 mitotic arrest deficient-like 1 (yeast)
Met	Methionine
MIQE	Minimum information for publication of quantitative real-time PCR experiments
MLST8	MTOR associated protein LST8 homolog (S. cerevisiae)
MLX	Max-like factor X
MOX	Cytochrome P450 mono-oxygenase pathway
MRI	Magnet resonance imaging
mRNA	Messenger RNA
mTOR	Mammalian target of rapamycin
mTORC1 / 2	mTOR complex 1 / 2
nc RNA	Non-coding RNA
NFκB	Nuclear factor κ B
NuGO	European Nutrigenomics Organisation
n-3 LCPUFA	Omega-3 LCPUFA
n-6 LCPUFA	Omega-6 LCPUFA
n-6/n-3 LCPUFA ratio	Omega-6 / omega-3 long chain polyunsaturated fatty acid ratio
ORA	Over-representation analysis approach
PBMCs	Peripheral blood mononuclear cells
PCNA	Proliferating cell nuclear antigen
P_15	14^{th} - 15^{th} week of gestation
P_32	32^{nd} week of gestation
Phe	Phenylalanine
PG	Prostaglandin
PGE_2	Prostaglandin E_2
PI	Prostacyclin
PL	Phospholipid
PLIN2 / ADRP	Perilipin 2
POLR2a	Polymerase (RNA) II (DNA-directed) polypeptid A
PPAR	Peroxisome proliferator activated receptor
PT	Pathway topology-based approach
PTGS1 / 2	Prostaglandin-endoperoxide synthase 1 / 2 (prostaglandin G/H synthase and cyclooxygenase)
RBC	Red blood cell
RIN	RNA integrity number
RIPA	Radioimmunoprecipitation assay buffer
RNA	Ribonucleic acid
RNU24	SNORD24 small nucleolar RNA, C/D box 24
RNU6b	RNU6-2 RNA, U6 small nuclear 2
RPS4Y1	Ribosomal protein S4, Y-linked 1
RPTOR	Regulatory associated protein of MTOR complex 1
Rs	Spearman-rho correlation coefficient
RT	Reverse transcription
RT-qPCR	Reverse transcription quantitative real-time polymerase chain reaction
RXR	Retinoic acid X receptor
SAM	S-adenosyl-methionine
Sar	Sarcosine

SC/PP	Subcutaneous / preperitoneal ratio in ultrasonography measurement
SFRP1	Secreted frizzled-related protein 1
SD	Standard deviation
SDS	Sequence Detection System
Ser	Serine
SFT	Skin fold thickness
siRNA	Short interfering RNA
SLC2A1 / 3	Solute carrier family 2, member 1 / 3
SLC3A2 / CD98	Solute carrier family 3 (activators of dibasic and neutral amino acid transport), member 2 / CD98 heavy chain
SLC6A6	Solute carrier family 6 (neurotransmitter transporter, taurine), member 6 (TAUT)
SLC7A5	Solute carrier family 7 (amino acid transporter light chain, L system), member 5 (LAT1)
SLC27A1 - 4 / 6	Solute carrier family 27, member 1-4 / 6
snoRNA	Short nucleolar RNA
SREBP1	Sterol regulatory element binding protein 1
SSB	Sjogren syndrome antigen B (autoantigen La)
ST	Syncytiotrophoblast
STRA6	Stimulated by retinoic acid 6
S6K1	p70 ribosomal S6 kinase 1
T	Testosterone
Tau	Taurine
TAUT	Taurine transporter (SLC6A6)
TELO2	TEL2, telomere maintenance 2 homolog (S. cerevisiae)
TG	Triglyceride
TGFB1	Transforming growth factor beta 1
Thr	Threonine
TOP1	Topoisomerase (DNA) I
Trp	Tryptophan
TTI1	Telo2-interacting protein
TX	Thromboxane
Tyr	Tyrosine
UC	Umbilical cord
USP9Y	Ubiquitin specific peptidase 9, Y-linked
UTY	Ubiquitously transcribed tetratricopeptide repeat gene, Y-linked
Val	Valine
ZFY	Zinc finger protein, Y-linked
ZNF711	Zinc finger protein 711
4E-BP1	Eukaryotic translation initiation factor 4E-binding protein 1
%wt	% fatty acids / total fatty acids

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10. Materials

10.1. Primers

Gene name	for / rev	Sequence (5´-3´)	Accession number	Primer location in exon	length (bp)	T (℃)
ACTB	for	CCTGGAGAAGAGCTACGAGCTG	NM_001101.3	3 - 4	108	60
	rev	GACTCCATGCCCAGGAAGGAAGG				
PolR2a	for	CTTGTGTGATACCATGACCTGTCGTG	NM_000937.4	25 - 26	115	60
	rev	GCACGTCCACCGTTTCCTCAAAGG				
hB2M	for	GGACTGGTCTTTCTATCTCTTGTAC	NM_004048.2	2 - 3	120	60
	rev	TCTCGATCCCACTTAACTATCTTGG				
TOP1	for	CTTCATCGACAAGCTTGCTCTG	NM_003286.2	14 - 15	98	60
	rev	TGATGTGCTCCACACGAAGTGA				
TGFB1	for	TGGTTGAGCCGTGGAGGGG	NM_005474.4	3 - 4	98	63
	rev	AACCCGTTGATGTCCACTTGCA				
HDAC5	for	CCT ACA GCA GAA GTT GAA CGT GG	NM_000660.4	20 - 21	139	60
	rev	GAA GTT CCC GTT GTC ATA GCG ATG				
DKK1	for	ACA ACT ACC AGC CGT ACC CGT	NM_012242.2	1 - 2	116	60
	rev	CTT CCT GCA GGC GAG ACA GAT				
LRP6	for	GGATCTCTTCCAGGAATGTCTCG	NM_002336.2	20 - 21	150	60
	rev	TGCAGGGAAGTAAGTGCCTTTGG				
DVL1	for	CAGCAGAGTGAAGGGAGCAAA	NM_004421.2	14 - 15	117	60
	rev	TGTGATCCGATTCACTGCCACT				
LPL	for	AAACCCATACCAATCAGGCCTTTG	NM_000237	7 - 8	93	63
	rev	TTGTGGAAACTTCAGGCAGAGTGA				
LAT1	for	CCGTGAACTGCTACAGCGTGA	NM_003486.5	2 - 3	121	60
	rev	ACATCACCCTTCCCGATCTGGA				
hTAUT	for	TGGAGGTGCGTTTCTCATACCG	NM_001134367.1	3 - 5	157	60
	rev	GGAGGCATAGCCGATACCAGA				

Gene name	Order number (commercial primer)†	Accession number	Primer location in exon	length (bp)	T (℃)
CDK6	QT00019985	NM_001259	5 - 7	82	55
CDK1	QT00042672	NM_001786	1- 3	133	55
MAD2L1	QT00094955	NM_002358	1 - 2	104	55
ANAPC4	QT00027153	NM_013367	3 - 4	70	55
PCNA	QT00024633	NM_002592	5 - 6	105	55
SFRP1	QT00031927	NM_003012	2 - 3	91	55
FZD7	QT01010919	NM_003507	only 1 Exon	64	55
mTOR	QT00056133	NM_004958	51 - 52	66	55
RPTOR	QT00023436	NM_020761	26 - 27	69	55
SLC3A2 (CD98)	QT00085897	NM_002394	3 - 4	126	55

microRNA	microRNA sequence	Accession number	Assay ID§	length (bp)	T (℃)
RNU6b	CGCAAGGATGACACGCAAATTCGTGAAGCGTTCC ATATTTTT	NR_002752	001093	n.s.	60
RNU24	ATTTGCTATCTGAGAGATGGTGATGACATTTTAAA CCACCAAGATCGCTGATGCA	NR_002447	001001	n.s.	60
hsa-mirR-26b	UUCAAGUAAUUCAGGAUAGGU	MI0000084	000407	n.s.	60
hsa-miR-99a	AACCCGUAGAUCCGAUCUUGUG	MI0000101	000435	n.s.	60
hsa-miR-100	AACCCGUAGAUCCGAACUUGUG	MI0000102	000437	n.s.	60
hsa-miR-375	UUUGUUCGUUCGGCUCGCGUGA	MI0000783	000564	n.s.	60
hsa-miR-30d	UGUAAACAUCCCCGACUGGAAG	MI0000255	000420	n.s.	60
hsa-miR-320	AAAAGCUGGGUUGAGAGGGCGA	MI0000542	002277	n.s.	60

commercial primer ordered from Qiagen (Hilden, Germany), n.s. = was not stated, § commercial primer ordered from Applied Biosystems, Darmstadt, Germany

10.2. Consumables

Item	Company	City	Country
Dewar flask	KGW-Isotherm	Karlsruhe	Germany
mortar	Morgan Technical Ceramics - Haldenwanger	Waldkraiburg	Germany
pestle	Morgan Technical Ceramics - Haldenwanger	Waldkraiburg	Germany
safety goggles	UVEX Winter Holding	Fürth	Germany
cotton gloves, Softline	Zefa-Laborservice GmbH	Harthausen	Germany
tube racks	Brand GmbH & Co. KG	Wertheim	Germany
plastic beakers	Vitlab®	Großostheim	Germany
water bottle	Vitlab®	Großostheim	Germany
aluminum foil	Sylvana, Penny-Markt GmbH	Köln	Germany
laboratory aluminum foil	Carl Roth GmbH & Co. KG	Karlsruhe	Germany
detergent "Baktolin"	Bode-Chemie GmbH	Hamburg	Germany
microtube PP, 1.5 ml	Paul Böttger OHG	Bodenmais	Germany
microtube PP, 2 ml	Diagonal GmbH & Co. KG	Münster	Germany
reaction tubes, 0.5 ml	Brand GmbH & Co. KG	Wertheim	Germany
reaction tubes, 0.2 ml	Zefa-Laborservice GmbH	Harthausen	Germany
reaction tubes, 2 ml (safe-lock)	Eppendorf AG	Hamburg	Germany
spatula	Carl Roth GmbH & Co. KG	Karlsruhe	Germany
waste bags	PAA Laboratories GmbH	Pasching	Austria
paper towels	Anton Schlecker	Ehingen	Germany
forceps	Fine Science Tools GmbH	Heidelberg	Germany
lab coat	CWS-boco GmbH	Dreieich	Germany
latex gloves, size "M"	Rösner-Mautby Meditrade GmbH	Kiefersfelden	Germany
purple nitrile exam gloves, size "M"	Kimberley-Clark Health Care	Zaventem	Belgium
tips, blue 1000 µl	Brand GmbH & Co. KG	Wertheim	Germany
tips, yellow, 100 µl	Brand GmbH & Co. KG	Wertheim	Germany
tips, white, 10 µl	Brand GmbH & Co. KG	Wertheim	Germany
Biosphere® filter tips (10, 100, 1000 µl)	Sarstedt AG & Co. KG	Nümbrecht	Germany
pipettes 2,5; 10; 100, 1000 µl	Eppendorf AG	Hamburg	Germany
tubes, 15 ml	Greiner Bio-One GmbH	Frickenhausen	Germany
tubes, 50 ml	Greiner Bio-One GmbH	Frickenhausen	Germany
pipetting aid	Gilson Intl. B.V.	Limburg	Germany
disposable pipettes 2; 5; 10; 25; 50 ml	Falcon™, BD Biosciences	Heidelberg	Germany
Corning® Costar® "stripette", disposable pipettes 2; 5; 10; 25; 50 ml	Corning® Inc., Sigma-Aldrich Chemie GmbH	München	Germany
multi-channel pipette 30-300 µl	Brand GmbH & Co. KG	Wertheim	Germany
Eppendorf Multipette® plus	Eppendorf AG	Hamburg	Germany
Eppendorf Combitips plus	Eppendorf AG	Hamburg	Germany
pipettes 10; 200, 1000 µl	Gilson Intl. B.V.	Limburg	Germany
96-well plate	Nunc / Thermo-Fisher Scientific	Langenselbold	Germany
Corning® Costar® reagent reservoirs	Corning® Inc., Sigma-Aldrich Chemie GmbH	München	Germany
Whatman paper	Carl Roth GmbH & Co. KG	Karlsruhe	Germany
plastic ware (beakers, cylinders, funnels)	Diagonal GmbH &Co KG	Münster	Germany
glassware (beakers, cylinders, funnels)	Diagonal GmbH &Co KG	Münster	Germany
glass plates, clips, rubber, chamber, combs	Biometra GmbH	Göttingen	Germany
OPTITRAN BA-S85 nitrocellulose membrane (0.45 µm)	Whatman GmbH	Dassel	Germany
scalpel	B. Braun Melsungen	Melsungen	Germany
Parafilm®	Pechiney Plastic Packaging	Chicago	USA
gel chamber, sled, comb	UniEquip GmbH	Planegg	Germany
twin.tec real-time PCR plates 96	Eppendorf AG	Hamburg	Germany
heat sealing film	Eppendorf AG	Hamburg	Germany
cool rack 96-wells	Eppendorf AG	Hamburg	Germany
cuvettes, polystyrene	Sarstedt AG & Co. KG	Nümbrecht	Germany
dissection scissors	Fine Science Tools GmbH	Heidelberg	Germany
forceps	Fine Science Tools GmbH	Heidelberg	Germany
Pasteur pipettes, glass	Zefa-Laborservice GmbH	Harthausen	Germany
SuperFrost® Plus slides	Menzel GmbH & Co. KG	Braunschweig	Germany
histosettes	Simport Scientific	Beloeil	Canada

microtube boxes	Zefa Laborservice		
Mini-PROTEAN®™TGX™ Precast gels	Bio-Rad Laboratories GmbH	München	Germany
paper towels	Anton Schlecker	Ehingen	Germany
boxes for freezing	Zefa-Laborservice GmbH	Harthausen	Germany

10.3. Machines

Item	Company	City	Country
7900HT Fast Real-Time PCR System	Applied Biosystems GmbH	Darmstadt	Germany
Agilent 2100 Bioanalyzer	Agilent Technologies GmbH	Böblingen	Germany
DNA/RNA UV-Cleaner UVC/T-M-AR	UniEquip GmbH	Planegg	Germany
Centrifuge 5415 R	Eppendorf AG	Hamburg	Germany
Centrifuge 5430	Eppendorf AG	Hamburg	Germany
Centrifuge 5810	Eppendorf AG	Hamburg	Germany
Centrifuge 5424	Eppendorf AG	Hamburg	Germany
realplex^4 Mastercycler epgradient S	Eppendorf AG	Hamburg	Germany
freezer -20 ℃ Liebherr premium	Liebherr GmbH	Biber ach	Germany
Freezer "ThermoForma"	Thermo Fisher Scientific	Schwerte	Germany
Freezer "ThermoScientific HeraFreeze"	Thermo Fisher Scientific	Schwerte	Germany
fridge 4 ℃ Liebherr	Liebherr GmbH	Biberach	Germany
GeneChip® Scanner 3000	Affymetrix Inc.	Santa Clara, CA	USA
heat sealer	Eppendorf AG	Hamburg	Germany
Heraeus Megafuge 1.0 R	Heraeus, Thermo Scientific	Waltham	USA
homogenizer ("Dispergierwerkzeug")	Miccra (ART Labortechnik)	Mühlheim	Germany
ice machine AF 100	Scotsman ice systems	Milan	Italy
incubator HeraCell 150	Thermo Fisher Scientific	Schwerte	Germany
microtome HM 355 S	Microm / Thermo Fisher Scientific	Walldorf	Germany
microwave	MDA		
Mini Centrifuge GMC-060	LMS Group	Tokyo	Japan
mini scale EW3000-2M	Kern GmbH	Balingen	Germany
mini scale PE360	Mettler-Toledo	Ingolstadt	Germany
ND-1000 Spectrophotometer	Peqlab biotechnology GmbH	Erlangen	Germany
Odyssey® Infrared Imaging System	LI-COR Biosciences GmbH	Bad Homburg	Germany
Thermocycler T3000	Biometra GmbH	Göttingen	Germany
S20-K SevenEasy™ pH meter	Mettler-Toledo	Ingolstadt	Germany
spectrophotometer infinite M2000	Tecan Austria GmbH	Gröding	Germany
spectrophotometer Ultrospec 3100pro	Amersham Biosciences / GE Healthcare	München	Germany
Power pack P25 T	Biometra GmbH	Göttingen	Germany
Electrophoresis power supply CONSORT E861	Consort	Turnhout	Belgium
printer	Intas Science Imaging Instruments GmbH	Göttingen	Germany
Scale, max 820 g	Sartorius AG	Göttingen	Germany
HeraSafe bench	Heraeus / Thermo Fisher Scientific	Waltham	USA
Thermomixer comfort	Eppendorf AG	Hamburg	Germany
timer	Oregon Scientific GmbH	Neu-Isenburg	Germany
UV-VIS gel electrophoresis detection system	Intas Science Imaging Instruments GmbH	Göttingen	Germany
Concentrator 5301	Eppendorf AG	Hamburg	Germany
VARIOSCAN	Thermo Electron / Thermo Fisher Scientific	Marietta	USA
Vortexer "Vortex Genie 2"	Bender-Hobein		
Vortexer 2x^3	Velp Scientifica		
Julabo SW 22	JULABO GmbH	Seelbach	Germany
Mikrom SB 80 water bath	Microm / Thermo Fisher Scientific	Walldorf	Germany

10.4. Chemicals

Item	Company	City	Country
1 kb DNA ladder	New England Biolabs GmbH	Frankfurt am Main	Germany
Agarose, pegGOLD universal	Peqlab biotechnology GmbH	Erlangen	Germany
Acetic acid (100 %)	Merck KGaA	Darmstadt	Germany
Acrylamide/ Bisacrylamide "Roti-Gelektrophorese" Gel 30	Carl Roth GmbH & Co. KG	Karlsruhe	Germany
ε-Amino-n-caproic acid	Sigma-Aldrich Chemie GmbH	Taufkirchen	Germany
Ammoniumpersulphate (APS)	Bio-Rad Laboratories GmbH	München	Germany
Water, purified	SG Water USA, LLC	Nashua	USA
Bradford protein assay dye reagent	Bio-Rad Laboratories GmbH	München	Germany
Boric acid	Merck KGaA	Darmstadt	Germany
Bromophenolblue	Merck KGaA	Darmstadt	Germany
Bovine serum albumine	Sigma-Aldrich Chemie GmbH	Taufkirchen	Germany
Calcium chloride ($CaCl_2$)	Merck KGaA	Darmstadt	Germany
Chloroform	Carl Roth GmbH & Co. KG	Karlsruhe	Germany
Coomassie Brilliant Blue G 250	SERVA Electrophoresis GmbH	Heidelberg	Germany
Sodium-deoxycholate	Sigma-Aldrich Chemie GmbH	Taufkirchen	Germany
Dimethylsulphoxide (DMSO)	Sigma-Aldrich Chemie GmbH	Taufkirchen	Germany
Dithiotreitol (DTT)	AppliChem GmbH	Darmstadt	Germany
dNTP mix (200 mM)	Qiagen GmbH	Hilden	Germany
Carbon dioxide (CO_2 solid; "dry ice")	TKD GmbH	Fraunberg-Tittenkofen	Germany
Titriplex® Ethylenediaminetetraacetic acid (EDTA)	Merck KGaA	Darmstadt	Germany
Ethanol	J.T. Baker, Mallinckrodt	Deventer	Netherlands
Ethanol, vergällt	CLN GmbH	Niederhummel	Germany
Ethidium bromide	Carl Roth GmbH & Co. KG	Karlsruhe	Germany
Formaldehyde, 37 %	Carl Roth GmbH & Co. KG	Karlsruhe	Germany
Gene Ruler, 50 bp ladder	Fermentas GmbH	St. Leon-Rot	Germany
Glycerol	Carl Roth GmbH & Co. KG	Karlsruhe	Germany
Glycine	Merck KGaA	Darmstadt	Germany
H_3PO_4 (phosphoric acid), 85 %	Merck KGaA	Darmstadt	Germany
Hematoxylin (Gill)	Carl Roth GmbH & Co. KG	Karlsruhe	Germany
Hydrochloric acid (HCl)	Carl Roth GmbH & Co. KG	Karlsruhe	Germany
Isopropanol	J.T. Baker, Mallinckrodt	Deventer	Netherlands
Nitrogen, liquid	Linde Gas	Unterschleißheim	Germany
Magnesium chloride ($MgCl_2$)	Merck KGaA	Darmstadt	Germany
Methanol	Merck KGaA	Darmstadt	Germany
ECL Advance™ blocking agent	GE Healthcare	München	Germany
Nonidet P-40	Sigma-Aldrich Chemie GmbH	Taufkirchen	Germany
Water, nuclease-free	Sigma-Aldrich Chemie GmbH	Taufkirchen	Germany
Oil Red-O	Sigma-Aldrich Chemie GmbH	Taufkirchen	Germany
Oligo-dT Primer	Promega Corporation	Mannheim	Germany
Orange G	Sigma-Aldrich Chemie GmbH	Taufkirchen	Germany
PageBlue™ Protein staining solution	Fermentas GmbH	St. Leon-Rot	Germany
Pageruler, prestained	Fermentas GmbH	St. Leon-Rot	Germany
Paraplast®	Carl Roth GmbH & Co. KG	Karlsruhe	Germany
PBS ready-made	Biochrom AG	Berlin	Germany
Phosphatase inhibitor	Sigma-Aldrich Chemie GmbH	Taufkirchen	Germany
BCA protein assay kit	Pierce / Thermo Fisher Scientific	Bonn	Germany
Ponceau S	Sigma-Aldrich Chemie GmbH	Taufkirchen	Germany
Oligonucleotides (PCR primer)	Metabion	Martinsried	Germany
Phosphatase Inhibitor cocktail II	Sigma-Aldrich Chemie GmbH	Taufkirchen	Germany
Protease Inhibitor cocktail	Sigma-Aldrich Chemie GmbH	Taufkirchen	Germany
Protease inhibitor	Sigma-Aldrich Chemie GmbH	Taufkirchen	Germany
Rnase inhibitor RNasin®	Promega Corporation	Mannheim	Germany
Rnase-Zap®	Sigma-Aldrich Chemie GmbH	Taufkirchen	Germany
Sodium dodecyl-sulphate (SDS)	Omnilab GmbH & Co. KG	Bremen	Germany
Sodium azide (NaN_3)	Merck KGaA	Darmstadt	Germany
Sodium chloride (NaCl)	Merck KGaA	Darmstadt	Germany
Sodium hydroxide (NaOH)	Merck KGaA	Darmstadt	Germany

Item	Company	City	Country
Sodium dihydrogen phosphate (NaH_2PO_4)	Merck KGaA	Darmstadt	Germany
Sodium sulphate (Na_2SO_4)	Merck KGaA	Darmstadt	Germany
Sodium hydrogen carbonate ($NaHCO_3$)	Merck KGaA	Darmstadt	Germany
Tetramethylethylenediamine (TEMED)	Merck KGaA	Darmstadt	Germany
TRIzol® Reagent	Invitrogen	Darmstadt	Germany
Tris base	AppliChem GmbH	Darmstadt	Germany
Triton® X-100	Sigma-Aldrich Chemie GmbH	Taufkirchen	Germany
Tween® 20	Sigma-Aldrich Chemie GmbH	Taufkirchen	Germany
Xylol	Carl Roth GmbH & Co. KG	Karlsruhe	Germany

10.5. Kits

Item	Company	City	Country
Agilent RNA 6000 Nano Kit	Agilent Technologies	Waldbronn	Germany
Chip® Expression 3'-Amplification IVT labelling Kit	Affymetrix Inc.	Santa Clara, CA	USA
Custom Array - NuGO_Hs1a520180 array (NuGO array)		Santa Clara, CA	USA
Gene Chip® Expression 3'-Amplification one cycle cDNA Synthesis Kit		Santa Clara, CA	USA
HotStarTaq® PCR Kit	Qiagen GmbH	Hilden	Germany
Megaplex™ RT primer	Applied Biosystems Deutschland GmbH	Darmstadt	Germany
miRNeasy® Mini Kit	Qiagen GmbH	Hilden	Germany
mRNeasy® Mini / Midi Kit		Hilden	Germany
Omniscript® reverse transcription kit		Hilden	Germany
QuantiTect® SYBR® Green PCR kit		Hilden	Germany
RNase-free DNase set		Hilden	Germany
TaqMan® Array Human Micro RNA A+B Cards Set v3.0	Applied Biosystems Deutschland GmbH	Darmstadt	Germany
TaqMan® MicroRNA Reverse Transcription Kit		Darmstadt	Germany
TaqMan® PreAmp Primers and TaqMan® PreAmp Master Mix		Darmstadt	Germany
TaqMan® Universal PCR Master Mix (No AmpErase® UNG)		Darmstadt	Germany

11. Appendix

11.1. Fatty acid analyses for the fish-oil capsules

Certificate of analysis - Marinol-D40 capsules 1000mg (13-06-2006)

Free fatty acid as oleic acid	0.19%
Manual peroxide value	1.2 meqO2/kg
C18:3n-3	0.3%
C18:4n-3	1.2%
C20:4n-3	0.4%
C20:5n-3	5.84%
C21:5n-3	0.4%
C22:5n-3	1.8%
C22:6n-3	40.23%
Total Omega 3	50.5%
C14:0	2.0%
C16:0	10.4%
C16:1	2.8%
C18:0	3.09%
C18:1	9.99%
C18:2	1.0%
C20:0	0.4%
C20:3	0.2%
C20:4	2.2%
C20:1	1.8%
C20:2	0.2%
C22:1	0.4%
C24:1	1.0%
Fatty acid composition EPA (mg/g)	54.2
Fatty acid composition DHA (mg/g)	386.6

11.2. Checklist for MIQE guidelines according to Bustin *et al.* (2009. Clin. Chem 55 (4):611-22) for gene expression analysis

ITEM TO CHECK	IMPOR-TANCE	CHECKLIST
EXPERIMENTAL DESIGN		
Definition of experimental and control groups	**E**	**Experimental groups:** male (CM) and female (CF) placentas from control group (counseling for healthy nutrition during pregnancy) as well as male (IM) and female (IF) placentas from n-6/n-3 LCPUFA intervention group
Number within each group	**E**	CM: n = 9, CF: n = 11, IM: n = 11 and IF: n = 10
Assay carried out by core lab or investigator's lab?	D	Assay carried out by investigator's lab
Acknowledgement of authors' contributions	D	Assays carried out by ES
SAMPLE		
Description	**E**	Human placenta villous fraction. 1 cm^3 was sampled from each of the four quadrants, with the same distance from placental middle. Maternal basal plate and fetal chorionic plate were removed and villous fraction was stored at -80℃. For nucleic acid extraction the four quadrant s per placenta were pooled.
Volume/mass of sample processed	D	Per placenta 1 cm^3 per quadrant
Microdissection or macrodissection	**E**	Macrodissection
Processing procedure	**E**	Placentas were stored at 4℃ until processed as soo n as possible, Samples were frozen in liquid nitrogen and stored at -80℃
If frozen - how and how quickly?	**E**	Frozen as soon as possible
If fixed - with what, how quickly?	**E**	no fixation was done
Sample storage conditions and duration (especially for FFPE samples)	**E**	Samples were stored at -80℃ from 02.2007 until 11. 2009 (first to last birth of the INFAT study)
NUCLEIC ACID EXTRACTION		
Procedure and/or instrumentation	**E**	Total RNA was extracted with a combined approach of TRIzol Reagent and midiRNeasy Kit. The placental pieces from the four quadrants were each homogenized in 1 ml TRIzol per 10 mg tissue by a rotor-stator homogenizer. Purified total RNA was eluted in RNAse-free H2O supplied by the midi RNeasy kit.
Name of kit and details of any modifications	**E**	TRIzol Reagent (Invitrogen, Karlsruhe, Germany) and midiRNeasy Kit (Qiagen, Hilden, Germany). We exactly followed the instructions of the TRIzol manual until separation of watery and organic phases. Afterwards we exactly followed the manual of the midiRNeasy kit.
Source of additional reagents used	D	Chloroform (Carl Roth GmbH & Co. KG, Karlsruhe, Germany), 96% Ethanol (J.T. Baker, Mallinckrodt , Deventer, Netherlands), RNAse ZAP (Sigma-Aldrich Chemie GmbH, Taufkirchen, Germany)
Details of DNase or RNAse treatment	**E**	no Dnase treatment was used
Contamination assessment (DNA or RNA)	**E**	Reverse transcription controls with no RT enzyme (-RTs) were used to assess DNA contamination in four pool á 10 total RNA samples for each primer.
Nucleic acid quantification	**E**	Total RNA concentration was determined by measuring absorbance at 260 nm UV-light
Instrument and method	**E**	Total RNA concentration was measured by NanodropTM 1000 Spectrophotomerter (Peqlab biotechnology GmbH, Erlangen, Germany)
Purity (A260/A280)	D	Almost all total RNA samples showed a 260/280 ratio of 2, but three tRNA samples (1017, 1087, 0082) showed a 260/280 ratio of 1.4, but were included.
Yield	D	Yield of total RNA samples were in the range of 306 - 1425 ng/µl
RNA integrity method/instrument	**E**	Agilent 2100 Bioanalyzer (Agilent Technologies GmbH, Böblingen, Germany)
RIN/RQI or Cq of 3' and 5' transcripts	**E**	RIN of all samples was above 5.
Electrophoresis traces	D	Electropherograms of the Agilent 2100 Bioanalyzer were checked
Inhibition testing (Cq dilutions, spike or other)	**E**	no inhibition testing was done
REVERSE TRANSCRIPTION		
Complete reaction conditions	**E**	One-step RT-qPCR was used.
Amount of RNA and reaction volume	**E**	10ng in 20µl reaction volume
Priming oligonucleotide (if using GSP) and concentration	**E**	Specific primers were used in one-step RT-qPCR. Commercial Qiagen primer (10x) were used with 2µl in 20µl reaction volume and self-designed primer pairs (10 pmol/µl) were used with 1µl in 20µl reaction volume.
Reverse transcriptase and concentration	**E**	Omniscript and Sensiscript Reverse Transcriptases from QuantiTect SYBR Green RT-PCR Kit; 0,2 µl in 20µl reaction volume.

Temperature and time	**E**	50.0℃ for 30 min, 95℃ for 15 min
Manufacturer of reagents and catalogue numbers	D	QuantiTect SYBR Green RT-PCR Kit (Qiagen, Hilden, Germany), cat. no: 204245
Cqs with and without RT	D*	All used primer pairs showed no amplification or differences between total RNA pool and the 4 -RT pools above 6.5 ΔCq
Storage conditions of cDNA	D	There was no storage of cDNA because of the one-step RT-qPCR.
qPCR TARGET INFORMATION		
If multiplex, efficiency and LOD of each assay.	**E**	We did not use a multiplex approach.
Sequence accession number	**E**	provided in supplementary table 1
Location of amplicon	D	provided in supplementary table 1
Amplicon length	**E**	provided in supplementary table 1
In silico specificity screen (BLAST, etc.)	**E**	All primers were blasted with Oligo Calc: Oligonucleotide Properties Calculator on http://www.basic.northwestern. edu/biotools/oligocalc.html and in silico-PCR was performed with http://genome.ucsc.edu/cgi-bin/hgPcr
Pseudogenes, retropseudogenes or other homologs?	D	
Sequence alignment	D	
Secondary structure analysis of amplicon	D	Secondary structure of self-designed primer-pairs was done with Oligo Calc: Oligonucleotide Properties Calculator on http://www.basic.northwestern.edu/biotools/oligocalc.html
Location of each primer by exon or intron (if applicable)	**E**	provided in supplementary table 1
What splice variants are targeted?	**E**	As many splice variants as possible by one primer-pair were targeted
qPCR OLIGONUCLEOTIDES		
Primer sequences	**E**	provided in supplementary table 1
RTPrimerDB Identification Number	D	was not used
Probe sequences	D**	No probes were used
Location and identity of any modifications	**E**	No modifications were used
Manufacturer of oligonucleotides	D	Self-designed primer-pairs were purchased from metabion GmbH (Martinsried, Germany) and commercial primer-pairs were purchased from Qiagen (Hilden, Germany)
Purification method	D	Self-designed primer-pairs: standard grade, deprotected and desalted (0.02); commercial primer-pairs: unknown
qPCR PROTOCOL		
Complete reaction conditions	**E**	One-step RT-qPCR was carried out on a "Mastercycler ep Realplex" using QuantiTect SYBR Green RT-PCR Kit in a final volume of 20µl. Reaction mix consisted of 10µl 2x Quantitect SYBR Green RT-PCR Master Mix, 6,8µl RNase-free H_2O, 1µl Primer-mix 10pmol/µl per primer, 0,2µl QuantiTect RT Mix and 2µl of 5ng/µl tRNA. The RT-qPCR was initiated with 50℃ for 30 min and 95℃ for 15 min. 40 Cycles with 95℃ for 15 sec , 60℃ (or as stated in the supplementary table 1) for 30 sec. and 72℃ for 30 sec. were followed by 95℃ for 15 sec as well as a melting curve from 60 to 95℃ with ramp duration of 20 min. The program ended with 95℃ for 15 sec. All re actions were done in duplicate.
Reaction volume and amount of cDNA/DNA	**E**	Reaction volume: 20µl; Amount of total RNA: 10ng/µl because of one-step RT-qPCR
Primer, (probe), Mg++ and dNTP concentrations	**E**	Concentration of each primer: 200pmol; Mg++: 2.5 mM as provided in 2x QuantiTect SYBR Green RT-PCR Master Mix; dNTP: unknown, provided in 2x QuantiTect SYBR Green RT-PCR Master Mix.
Polymerase identity and concentration	**E**	HotStarTaq DNA Polymerase included in 2x QuantiTect SYBR Green RT-PCR Master Mix, concentration unknown
Buffer/kit identity and manufacturer	**E**	QuantiTect SYBR Green RT-PCR Kit (Qiagen, Hilden, Germany), cat. no: 204245
Exact chemical constitution of the buffer	D	QuantiTect SYBR Green RT-PCR Buffer contains Tris, KCl, (NH4)2SO4, 5 mM MgCl2, pH 8.7 (20℃) included in 2x QuantiTect SYB R Green RT-PCR Master Mix
Additives (SYBR Green I, DMSO, etc.)	**E**	SYBR Green I and ROX as included in 2x QuantiTect SYBR Green RT-PCR Master Mix; no other additives
Manufacturer of plates/tubes and catalog number	D	Eppendorf twin.tec real-time PCR plate 96well, with white wells (cat. no. 0030 132.530, Eppendorf, Hamburg, Germany), Eppendorf Heat Sealing Film (cat. no. 0030 127.838, Eppendorf, Hamburg, Germany)
Complete thermocycling parameters	**E**	1x 50℃ for 30 min, 1x 95℃ for 15 min, 40x : 95℃ 15sec. / 60℃ (or as stated in supplementary table 1) for 30 sec. / 72℃ for 30 sec, 95℃ 15 sec., Melting curve: 60 -95℃ in 20 min, 95℃ 15 min.
Reaction setup (manual/robotic)	D	manual
Manufacturer of qPCR instrument	**E**	Mastercycler ep Realplex (Eppendorf, Hamburg, Germany)

qPCR VALIDATION		
Evidence of optimization (from gradients)	D	no
Specificity (gel, sequence, melt, or digest)	**E**	Melting curve analysis for one single peak and agarose gel electrophoresis (2%) with ethidiumbromide for one single band at the marker height of the calculated amplicon length. No template controls (no tRNA in RT-qPCR reaction) in each run were used to detect primer dimerization and unspecific amplification.
For SYBR Green I, Cq of the NTC	**E**	In general there were no Cqs in the NTCs or the Cq of the NTC was more than 5 Cqs higher than Cqs of the pooled tRNAs. In seldom cases, when the difference between NTCS and pooled tRNAs was lower this could be attributed to artifacts (Cq value although no amplification curve was observed; B2M, ANAPC4, GAS1, SFRP1) or primer dimerization (ANAPC4). Cq values of NTCs are available upon request
Standard curves with slope and y-intercept	**E**	We did not use standard curves
PCR efficiency calculated from slope	**E**	PCR efficiencies were calculated with LinReg PCR (J. M. Ruijter, C. Ramakers, W. M. H. Hoogaars *et al*. 2009 Nucleic acid research)
Confidence interval for PCR efficiency or standard error	D	PCR efficiency 87.0 ± 3.8%
r2 of standard curve	**E**	We did not use standard curves
Linear dynamic range	**E**	We did not use standard curves
Cq variation at lower limit	**E**	-
Confidence intervals throughout range	D	-
Evidence for limit of detection	**E**	-
If multiplex, efficiency and LOD of each assay.	**E**	-
DATA ANALYSIS		
qPCR analysis program (source, version)	**E**	We did not use a RT-qPCR analysis program
Cq method determination	**E**	Threshold was determined by CalqPlex in the realplex 2.0 software used by "Mastercycler ep Realplex"
Outlier identification and disposition	**E**	Cq values were duplicated differed by more than 0.5 Cq were discarded, biological outliers were included in the analysis
Results of NTCs	**E**	In general there were no Cqs in the NTCs or the Cq of the NTC was more than 5 Cqs higher than Cqs of the pooled tRNAs. In seldom cases, when the difference between NTCS and pooled tRNAs was lower this could be attributed to artifacts (Cq value although no amplification curve was observed; B2M, ANAPC4, GAS1, SFRP1) or primer dimerization (ANAPC4). Cq values of NTCs are available upon request
Justification of number and choice of reference genes	**E**	ACTB, POLR2A, B2M, and TOP1 were tested with BestKeeper (M.W. Pfaffl, A. Tichopád, C. Prgomet *et al*. 2004 Biotechnology letters) and for differences in mean Cq value between the analysis groups. All four genes were suitable as reference genes
Description of normalization method	**E**	The geometric mean of ACTB, POLR2A, B2M, and TOP1 was used for normalization.
Number and concordance of biological replicates	D	Ten to eleven biological replicates were analyzed.
Number and stage (RT or qPCR) of technical replicates	**E**	Technical replicates: RT and qPCR duplicates
Repeatability (intra-assay variation)	E	not determined
Reproducibility (inter-assay variation, %CV)	D	not determined
Power analysis	D	not determined
Statistical methods for result significance	**E**	Normal distribution of ΔCq values: Two-way ANOVA, not normal distribution or heteroscedascity: Two-way ANOVA on ranks (described in subjects and methods)
Software (source, version)	E	Sigma Plot 11.0 (Systat Software GmbH, Ekrath, Germany)
Cq or raw data submission using RDML	**D**	Cq values are available upon request

Table 1. MIQE checklist for authors, reviewers and editors. All essential information (E) must be submitted with the manuscript. Desirable information (D) should be submitted if available. If using primers obtained from RTPrimerDB, information on qPCR target, oligonucleotides, protocols and validation is available from that source.*: Assessing the absence of DNA using a no RT assay is essential when first extracting RNA. Once the sample has been validated as RDNA-free, inclusion of a no-RT control is desirable, but no longer essential. **: Disclosure of the probe sequence is highly desirable and strongly encouraged. However, since not all commercial pre-designed assay vendors provide this information, it cannot be an essential requirement. Use of such assays is advised against.

11.3. Checklist for MIQE guidelines according to Bustin *et al*. (2009. Clin. Chem 55 (4):611-22) for microRNA expression analysis

ITEM TO CHECK	IMPOR-TANCE	CHECKLIST
EXPERIMENTAL DESIGN		
Definition of experimental and control groups	**E**	**Experimental groups:** male (CM) and female (CF) placentas from control group (counseling for healthy nutrition during pregnancy) as well as male (IM) and female (IF) placentas from n-6/n-3 LCPUFA intervention group
Number within each group	**E**	CM: n = 9, CF: n = 11, IM: n = 11 and IF: n = 10
Assay carried out by core lab or investigator's lab?	D	Assay carried out by investigator's lab
Acknowledgement of authors' contributions	D	Assays carried out by ES
SAMPLE		
Description	**E**	Human placenta villous fraction. 1 cm^3 was sampled from each of the four quadrants, with the same distance from placental middle. Maternal basal plate and fetal chorionic plate were removed and villous fraction was stored at -80℃. For nucleic acid extraction the four quadrant s per placenta were pooled.
Volume/mass of sample processed	D	Per placenta 1 cm^3 per quadrant
Microdissection or macrodissection	**E**	Macrodissection
Processing procedure	**E**	Placentas were stored at 4℃ until processed as soo n as possible. Samples were frozen in liquid nitrogen and stored at -80℃
If frozen - how and how quickly?	**E**	As soon as possible
If fixed - with what, how quickly?	**E**	no fixation was done
Sample storage conditions and duration (especially for FFPE samples)	**E**	Samples were stored at -80℃ from 02.2007 until 11. 2009 (first to last birth of the INFAT study)
NUCLEIC ACID EXTRACTION		
Procedure and/or instrumentation	**E**	Total RNA including smallRNA (microRNA) was extracted with a combined approach of TRIzol Reagent and mirPremier® microRNA isolation Kit. The placental pieces from the four quadrants were each homogenized in 1 ml TRIzol per 10 mg tissue by a rotor-stator homogenizer. Purified total RNA was eluted in RNAse-free H2O supplied by the mirPremier® microRNA isolation Kit.
Name of kit and details of any modifications	**E**	TRIzol Reagent (Invitrogen, Karlsruhe, Germany) and mirPremier® microRNA isolation Kit (Sigma-Aldrich, Taufkirchen, Germany). We exactly followed the instructions of the TRIzol manual until separation of watery and organic phases. Afterwards we exactly followed the manual of the mirPremier® microRNA isolation Kit.
Source of additional reagents used	D	Chloroform (Carl Roth GmbH & Co. KG, Karlsruhe, Germany), 96% Ethanol (J.T. Baker, Mallinckrodt , Deventer, Netherlands), RNAse ZAP (Sigma-Aldrich Chemie GmbH, Taufkirchen, Germany)
Details of DNase or RNAse treatment	**E**	no Dnase treatment was used
Contamination assessment (DNA or RNA)	**E**	Reverse transcription controls with no RT enzyme (-RTs) were used to assess DNA contamination in four pool á 10 total RNA samples for each primer.
Nucleic acid quantification	**E**	Total RNA concentration was determined by measuring absorbance at 260 nm UV-light
Instrument and method	**E**	Total RNA concentration was measured by NanodropTM 1000 Spectrophotomerter (Peqlab biotechnology GmbH, Erlangen, Germany)
Purity (A260/A280)	D	Almost all RNA samples showed a 260/280 ratio of about 2. Only one sample (1023) showed a 260/280 ratio of 1.8, but was included.
Yield	D	Yield of tRNA samples were in the range of 290- 1732 ng/µl
RNA integrity method/instrument	**E**	Agilent 2100 Bioanalyzer (Agilent Technologies GmbH, Böblingen, Germany)
RIN/RQI or Cq of 3' and 5' transcripts	**E**	RIN of all samples was above 5.
Electrophoresis traces	D	Electropherograms of the Agilent 2100 Bioanalyzer were checked
Inhibition testing (Cq dilutions, spike or other)	**E**	no inhibition testing was done
REVERSE TRANSCRIPTION		
Complete reaction conditions	**E**	Two-step RT-qPCR was used.
Amount of RNA and reaction volume	**E**	10ng in 15µl reaction volume
Priming oligonucleotide (if using GSP) and concentration	**E**	Specific primers were used in two-step RT-qPCR. Commercial TaqMan® MicroRNA Assays (5x) were used with 3µl in 15µl reaction volume

Reverse transcriptase and concentration	**E**	MultiScribe TM Reverse Transcriptase 50U/µl from TaqMan® MicroRNA Reverse Transcription Kit; 1.0 µl in 15µl reaction volume.
Temperature and time	**E**	1 cycle at 16℃ for 30 min, 1 cycle at 42℃ for 30 min, and 1 cycle at 85℃ for 5 min
Manufacturer of reagents and catalogue numbers	D	TaqMan® MicroRNA Reverse Transcription Kit (Applied Biosystems / Life technologies, Darmstadt, Germany), cat. no: 4366596
Cqs with and without RT	D*	All used primer assays showed no amplification
Storage conditions of cDNA	D	There was no storage of cDNA.
qPCR TARGET INFORMATION		
If multiplex, efficiency and LOD of each assay.	**E**	We did not use a multiplex approach.
Sequence accession number	**E**	provided in supplementary table 1
Location of amplicon	D	Complete microRNA sequence
Amplicon length	**E**	Complete microRNA sequence
In silico specificity screen (BLAST, etc.)	**E**	Commercially available microRNA assays
Pseudogenes, retropseudogenes or other homologs?	D	Commercially available microRNA assays
Sequence alignment	D	Commercially available microRNA assays
Secondary structure analysis of amplicon	D	Commercially available microRNA assays
Location of each primer by exon or intron (if applicable)	**E**	Commercially available microRNA assays
What splice variants are targeted?	**E**	microRNA sequence
qPCR OLIGONUCLEOTIDES		
Primer sequences	**E**	provided in supplementary table 1
RTPrimerDB Identification Number	D	was not used
Probe sequences	D**	Commercially available microRNA assays
Location and identity of any modifications	**E**	No known modifications
Manufacturer of oligonucleotides	D	Applied Biosystems / Life technologies, Darmstadt, Germany
Purification method	D	Commercial primer-pairs: unknown
qPCR PROTOCOL		
Complete reaction conditions	**E**	Two-step RT-qPCR was carried out on a "Mastercycler ep Realplex" using TaqMan 2x Universal PCR Mastermix (no AmpErase UNG) in a final volume of 20µl. Reaction mix consisted of 10µl TaqMan 2x Universal PCR Mastermix (no AmpErase UNG), 6,0µl RNase-free H_2O, 1µl TaqMan Assay (20x), and 3,0 l of RT product. The qPCR was initiated with 1 cycle at 95℃ for 10 min and 40 cycles with 95℃ for 15 sec and 60℃ for 30 sec. Th e program ended with 95℃ for 15 sec. All reactions were done in duplica te.
Reaction volume and amount of cDNA/DNA	**E**	Reaction volume: 20µl; Amount of cDNA: 3µl
Primer, (probe), Mg++ and dNTP concentrations	**E**	Concentration of each primer assay: 20x; Mg++: unknown in TaqMan 2x Universal PCR Mastermix (no AmpErase UNG); dNTP: unknown, provided in TaqMan 2x Universal PCR Mastermix (no AmpErase UNG)
Polymerase identity and concentration	**E**	AmpliTaq Gold enzyme included in TaqMan 2x Universal PCR Mastermix (no AmpErase UNG), concentration unknown
Buffer/kit identity and manufacturer	**E**	TaqMan 2x Universal PCR Mastermix (no AmpErase UNG; Applied Biosystems / Life technologies, Darmstadt, Germany), cat. no: 4324018
Exact chemical constitution of the buffer	D	Included in TaqMan 2x Universal PCR Mastermix, exact concentration not stated
Additives (SYBR Green I, DMSO, etc.)	**E**	Passive reference dye (ROX) as included in TaqMan 2x Universal PCR Mastermix; no other additives
Manufacturer of plates/tubes and catalog number	D	Eppendorf twin.tec real-time PCR plate 96well, with white wells (cat. no. 0030 132.530, Eppendorf, Hamburg, Germany), Eppendorf Heat Sealing Film (cat. no. 0030 127.838, Eppendorf, Hamburg, Germany)
Complete thermocycling parameters	**E**	1 cycle at 95℃ for 10 min and 40 cycles with 95℃ for 15 sec and 60℃ for 30 sec.
Reaction setup (manual/robotic)	D	manual
Manufacturer of qPCR instrument	**E**	Mastercycler ep Realplex (Eppendorf, Hamburg, Germany)
qPCR VALIDATION		
Evidence of optimization (from gradients)	D	no
Specificity (gel, sequence, melt, or digest)	**E**	Specific amplicon identification by TaqMan probes, no template controls (no RNA in RT-qPCR reaction) in each run were used to detect primer dimerization and unspecific amplification.
For SYBR Green I, Cq of the NTC	**E**	No SYBR green was used

Standard curves with slope and y-intercept	**E**	We did not use standard curves
PCR efficiency calculated from slope	**E**	PCR efficiencies were calculated with LinReg PCR (J. M. Ruijter, C. Ramakers, W. M. H. Hoogaars *et al*. 2009 Nucleic acid research)
Confidence interval for PCR efficiency or standard error	D	PCR efficiency 88.2 ± 4.1%
r2 of standard curve	**E**	We did not use standard curves
Linear dynamic range	**E**	We did not use standard curves
Cq variation at lower limit	**E**	-
Confidence intervals throughout range	D	-
Evidence for limit of detection	**E**	-
If multiplex, efficiency and LOD of each assay.	**E**	-
DATA ANALYSIS		
qPCR analysis program (source, version)	**E**	We did not use a RT-qPCR analysis program
Cq method determination	**E**	Threshold was determined by CalqPlex in the realplex 2.0 software used by "Mastercycler ep Realplex"
Outlier identification and disposition	**E**	Cq values were duplicated differed by more than 0.5 Cq were discarded, biological outliers were included in the analysis
Results of NTCs	**E**	In general there were no Cqs in the NTCs
Justification of number and choice of reference genes	**E**	RNU24, RNU6b, and miR-26 were tested with BestKeeper (M.W. Pfaffl, A. Tichopád, C. Prgomet *et al*. 2004 Biotechnology letters) and for differences in mean Cq value between the analysis groups. All four genes were suitable as reference genes
Description of normalization method	**E**	The geometric mean of RNU24, RNU6b, and miR-26 was used for normalization.
Number and concordance of biological replicates	D	Ten to eleven biological replicates were analyzed.
Number and stage (RT or qPCR) of technical replicates	**E**	Technical replicates: RT duplicates
Repeatability (intra-assay variation)	E	not determined
Reproducibility (inter-assay variation, %CV)	D	not determined
Power analysis	D	not determined
Statistical methods for result significance	**E**	Normal distribution of ΔCq values: Two-way ANOVA, not normal distribution or heteroscedascity: Two-way ANOVA on ranks (described in subjects and methods)
Software (source, version)	E	Sigma Plot 11.0 (Systat Software GmbH, Ekrath, Germany)
Cq or raw data submission using RDML	**D**	Cq values are available upon request

Table 1. MIQE checklist for authors, reviewers and editors. All essential information (E) must be submitted with the manuscript. Desirable information (D) should be submitted if available. If using primers obtained from RTPrimerDB, information on qPCR target, oligonucleotides, protocols and validation is available from that source.*: Assessing the absence of DNA using a no RT assay is essential when first extracting RNA. Once the sample has been validated as DNA-free, inclusion of a no-RT control is desirable, but no longer essential. **: Disclosure of the probe sequence is highly desirable and strongly encouraged. However, since not all commercial pre-designed assay vendors provide this information, it cannot be an essential requirement. Use of such assays is advised against.

11.4. Additional offspring growth and fat mass parameters

Additional offspring growth and fat mass parameters		male		female			p*	p#	p*#
		n	mean ± SD	n	mean ± SD	CM vs CF	IM vs IF	IF vs CF	IM vs CM
weight 6 weeks (g)	CG	9	5041.1 ± 351.4	11	4858.2 ± 547.1		0.183	0.281	0.973
	IG	11	4812.7 ± 716.4	10	4618.0 ± 482.7				
weight 4 month (g)	CG	9	6635.6 ± 577.9	11	6110.9 ± 711.5		0.638	0.121	0.409
	IG	11	6557.3 ± 876.9	9	6394.4 ± 398.9				
BMI 6 weeks (kg/m^2)†	CG	9	15.6 (15.0-16.4)	11	15.5 (14.6-16.5)		0.131	0.535	0.840
	IG	11	15.4 (13.7-16.5)	10	14.9 (13.9-15.8)				
BMI 4 month (kg/m^2)†	CG	9	16.5 (15.8-17.3)	11	15.9 (14.4-16.8)		0.893	0.113	0.907
	IG	11	16.5 (15.0-18.0)	9	16.0 (15.6-17.1)				
BMI 1 year (kg/m^2)	CG	8	16.5 ± 1.0	11	16.8 ± 1.8		0.640	0.899	0.494
	IG	11	17.0 ± 0.9	10	16.7 ± 1.6				
head circumference 6 weeks (cm)†	CG	9	39.0 (39.0-39.5)	11	39.0 (38.0-40.0)		0.088	**0.023**	0.544
	IG	11	39.0 (38.0-39.0)	10	38.0 (37.0-39.0)	0.229	**0.039**	0.098	0.431
head circumference 4 month (cm)	CG	9	42.1 ± 0.9	11	40.6 ± 1.2		0.832	**<0.001**	0.493
	IG	11	41.8 ± 0.9	10	40.7 ± 1.1	**0.002**	**0.018**	0.734	0.531
head circumference 1 year (cm)	CG	8	47.3 ± 0.8	11	45.3 ± 1.0		0.681	**<0.001**	0.339
	IG	11	47.1 ± 1.1	10	45.8 ± 1.1	**<0.001**	**0.005**	0.319	0.705
Sum of 4 SFT[1] 6 weeks (mm^2)	CG	9	22.7 ± 4.4	11	22.3 ± 3.0		0.237	0.447	0.623
	IG	11	21.8 ± 5.2	10	20.2 ± 3.2				
Sum of 4 SFT[1] 4 months (mm^2)	CG	9	23.3 ± 3.7	11	25.4 ± 3.6		0.859	0.790	0.112
	IG	11	25.3 ± 3.7	10	23.8 ± 3.3				
Age at S1 (d)†	CG	8	3.0 (2.3-4.8)	11	3.0 (3.0-5.0)		0.694	0.052	0.409
	IG	11	3.0 (2.0-5.0)	9	6.0 (3.0-6.0)				
Age at S2 (d)†	CG	9	41.0 (39.0-49.5)	11	47.0 (44.0-50.0)		0.572	0.080	0.718
	IG	11	43.0 (40.0-45.0	10	46.0 (42.5-47.5)				
Age at S3 (d)†	CG	9	111.0 (105.0-118.0)	11	107.0 (102.0-115.0)		0.673	0.768	0.372
	IG	11	111.0 (104.0-112.0)	10	112.5 (104.0-114.0)				
Age at S4 (d)†	CG	8	372.0 (370.3-384.8)	11	381.0 (377.0-385.0)		0.919	0.433	0.184
	IG	11	376.0 (373.0-386.0)	10	376.5 (371.8-383.5)				
Weight / length 6 weeks (g/cm)	CG	9	89.1 ± 5.0	11	87.3 ± 7.2		0.115	0.403	0.915
	IG	11	85.3 ± 10.8	10	82.9 ± 7.3				
Weight / length 4 months (g/cm)	CG	9	104.7 ± 7.9	11	98.4 ± 9.6		0.577	0.141	0.499
	IG	11	104.4 ± 11.6	9	102.0 ± 5.0				
Weight / length 12 months (g/cm)	CG	8	126.3 ± 10.6	11	123.4 ± 11.6		0.417	0.374	0.893
	IG	11	129.8 ± 11.1	10	126.0 ± 12.6				
weight gain birth - 6 weeks (g)	CG	9	1553.9 ± 501.5	11	1313.6 ± 468.8		0.171	0.249	0.800
	IG	11	1275.9 ± 711.2	10	1121.5 ± 397.6				
weight gain 6 weeks - 4 month (g)	CG	9	1594.4 ± 352.1	11	1252.7 ± 259.4		**0.006**	0.269	0.104
	IG	11	1744.6 ± 509.3	9	1811.1 ± 368.3	0.056	0.703	**0.003**	0.392
weight gain 4 month - 1 year (g)	CG	8	3058.8 ± 523.6	11	2947.3 ± 631.4		0.228	0.813	0.804
	IG	11	3281.8 ± 782.9	9	3284.4 ± 821.8				
weight gain birth - 1 year (g)	CG	8	6189.4 ± 1221.6	11	5513.6 ± 825.3		0.387	0.150	0.593
	IG	11	6302.3 ± 1198.2	10	5989.5 ± 965.4				
Fat mass[2] 3-5 d pp. (g)	CG	8	760.2 ± 62.2	11	779.8 ± 65.6		0.800	0.736	0.525
	IG	11	778.1 ± 36.9	9	772.1 ± 78.3				
Fat mass[2] 6 weeks (g)	CG	9	1109.0 ± 77.3	11	1068.8 ± 120.4		0.183	0.281	0.973
	IG	11	1058.8 ± 157.6	10	1016.0 ± 106.2				
Fat mass[2] 4 months (g)	CG	9	1459.8 ± 127.1	11	1344.4 ± 156.5		0.638	0.121	0.409
	IG	11	1442.6 ± 192.9	9	1406.8 ± 87.8				
Fat mass[2] 12 months (g)	CG	8	2120.1 ± 236.2	11	1992.7 ± 203.0		0.366	0.184	0.744
	IG	11	2164.5 ± 277.1	10	2086.9 ± 224.6				
Fat mass[2] 3-5 d pp. (% body weight)†	CG	8	12.6 (10.6-14.2)	11	13.8 (9.8-14.4)		0.092	0.115	0.705
	IG	11	13.8 (11.6-14.7)	9	15.1 (12.8-16.1)				
Fat mass[2] 6 weeks (% body weight)	CG	9	19.4 ± 3.3	11	19.3 ± 2.2		0.202	0.591	0,669
	IG	11	18.5 ± 4.6	10	17.5 ± 2.8				
Fat mass[2] 4 months (% body weight)	CG	9	19.8 ± 2.6	11	21.3 ± 2.3		0.848	0.767	0,106
	IG	11	21.2 ± 2.6	10	20.2 ± 2.5				

Additional offspring growth and fat mass parameters		male		female			p*	p#	p*#
		n	mean ± SD	n	mean ± SD	CM vs CF	IM vs IF	IF vs CF	IM vs CM
Fat mass[2] 12 months (% body weight)[†]	CG	8	19.2 (16.3-21.0)	11	20.9 (17.7-21.8)		0.986	0.325	0.502
	IG	11	19.8 (18.3-20.6)	10	20.4 (16.6-22.3)				
Lean body mass 3-5 d pp. (g)	CG	8	2695.4 ± 220.4	11	2764.7 ± 232.4		0.800	0.736	0.525
	IG	11	2758.7 ± 130.9	9	2737.4 ± 277.6				
Lean body mass 6 weeks (g)	CG	9	3932.1 ± 274.1	11	3789.4 ± 426.8		0.183	0.281	0.973
	IG	11	3753.9 ± 558.8	10	3602.0 ± 376.5				
Lean body mass 4 months (g)	CG	9	5175.7 ± 450.7	11	4766.5 ± 554.9		0.638	0.121	0.409
	IG	11	5114.7 ± 684.0	9	4987.7 ± 311.1				
Lean body mass 12 months (g)	CG	8	7516.1 ± 837.5	11	7065.5 ± 719.4		0.365	0.184	0.746
	IG	11	7674.6 ± 982.4	10	7399.1 ± 796.1				
Lean body mass 3-5 d pp. (% body weight)[†]	CG	8	87.5 (85.8-89.4)	11	86.2 (85.6-90.2)		0.092	0.115	0.705
	IG	11	86.2 (85.3-88.4)	9	84.9 (84.0-87.3)				
Lean body mass 6 weeks (% body weight)	CG	9	80.6 ± 3.3	11	80.7 ± 2.2		0.202	0.591	0.669
	IG	11	81.5 ± 4.6	10	82.5 ± 2.8				
Lean body mass 4 months (% body weight)	CG	9	80.2 ± 2.6	11	78.7 ± 2.3		0.848	0.767	0.106
	IG	11	78.8 ± 2.6	10	79.8 ± 2.5				
Lean body mass 12 months (% body weight)	CG	8	81.2 ± 2.5	11	80.2 ± 2.9		0.986	0.325	0.502
	IG	11	80.5 ± 1.9	10	80.6 ± 2.9				
Ratio Subscapular/Triceps SF 3-5 d pp.	CG	8	1.0 ± 0.2	11	1.0 ± 0.2		0.060	0.335	0.859
	IG	11	0.9 ± 0.1	9	0.9 ± 0.1				
Ratio Subscapular/Triceps SF 6 weeks	CG	9	1.0 ± 0.3	11	1.0 ± 0.2		0.352	0.781	0.727
	IG	11	0.9 ± 0.2	10	0.9 ± 0.2				
Ratio Subscapular/Triceps SF 4 months	CG	9	0.8 ± 0.2	11	0.9 ± 0.2		0.479	0.586	0.604
	IG	11	0.8 ± 0.1	10	0.8 ± 0.2				
Ratio Subscapular/Triceps SF 12 months	CG	8	0.8 ± 0.2	11	0.8 ± 0.1		0.569	0.434	0.470
	IG	11	0.8 ± 0.2	10	0.8 ± 0.1				
Trunk-to-total skinfolds[3] 3-5 d pp. (%)	CG	8	48.9 ± 4.1	11	50.2 ± 3.6		0.216	0.329	0.904
	IG	11	47.7 ± 2.3	9	48.6 ± 3.7				
Trunk-to-total skinfolds[3] 6 weeks (%)	CG	9	49.2 ± 4.6	11	52.1 ± 2.0		0.092	**0.042**	0.619
	IG	11	47.8 ± 4.1	10	49.6 ± 3.3	0.077	0.257	0.118	0.397
Trunk-to-total skinfolds[3] 4 months (%)	CG	9	47.3 ± 4.4	11	50.9 ± 3.4		0.430	0.147	0.217
	IG	11	47.9 ± 3.4	10	48.2 ± 5.4				
Trunk-to-total skinfolds[3] 12 months (%)	CG	8	44.3 ± 4.6	11	45.1 ± 3.4		0.455	0.607	0.890
	IG	10	45.8 ± 4.0	10	45.8 ± 4.0				
Subcutaneous-area sagittal[4] 6 weeks (mm^2)	CG	8	0.3 ± 0.1	9	0.3 ± 0.1		0.989	0.821	0.439
	IG	9	0.3 ± 0.2	8	0.3 ± 0.1				
Subcutaneous-area sagittal[4] 4 months (mm^2)[†]	CG	9	0.3 (0.3-0.5)	11	0.5 (0.3-0.6)		0.397	0.415	0.311
	IG	10	0.5 (0.2-0.6)	8	0.5 (0.3-0.6)				
Subcutaneous-area sagittal[4] 12 months (mm^2)	CG	8	0.2 ± 0.1	10	0.3 ± 0.1		0.274	0.197	0.358
	IG	9	0.3 ± 0.1	10	0.3 ± 0.1				
Subcutaneous-area axial[5] 6 weeks (mm^2)	CG	8	0.3 ± 0.1	9	0.3 ± 0.1		0.534	0.898	0.255
	IG	9	0.3 ± 0.2	8	0.3 ± 0.1				
Subcutaneous-area axial[5] 4 months (mm^2)	CG	9	0.4 ± 0.1	11	0.5 ± 0.2		0.588	0.459	0.176
	IG	10	0.5 ± 0.2	8	0.5 ± 0.2				
Subcutaneous-area axial[5] 12 months (mm^2)[†]	CG	8	0.2 (0.1-0.3)	11	0.3 (0.3-0.4)		0.416	0.230	0.179
	IG	9	0.3 (0.2-0.4)	10	0.3 (0.2-0.4)				

Additional offspring growth and fat mass parameters		male		female			p*	p#	p*#
		n	mean ± SD	n	mean ± SD	CM vs CF	IM vs IF	IF vs CF	IM vs CM
Preperitoneal-area sagittal[4] 6 weeks (mm^2)	CG	8	0.1 ± 0.0	7	0.1 ± 0.0		0.185	0.762	0.151
	IG	9	0.1 ± 0.0	8	0.1 ± 0.0				
Preperitoneal-area sagittal[4] 4 months (mm^2)	CG	9	0.1 ± 0.0	10	0.1 ± 0.0		0.955	0.789	0.696
	IG	10	0.1 ± 0.1	8	0.1 ± 0.0				
Preperitoneal-area sagittal[4] 12 months (mm^2)	CG	8	0.1 ± 0.1	10	0.2 ± 0.0		0.102	0.445	0.112
	IG	9	0.2 ± 0.0	10	0.2 ± 0.1				
Ratio [SC/PP][6] 4 months†	CG	9	2.9 (2.1-3.6)	10	3.5 (2.6-4.8)		0.596	0.684	0.220
	IG	10	3.7 (2.7-4.5)	8	3.2 (2.3-3.9)				
Age at US1 (d)†	CG	8	41.5 (39.0-50.2)	9	48.0 (45.0-50.0)		0.579	0.272	0.579
	IG	9	43.0 (38.5-52.5)	8	45.0 (41.5-48.5)				
Age at US21 (d)†	CG	9	111.0 (103.0-118.0)	11	111.0 (103.0-115.0)		0.787	0.670	0.666
	IG	10	110.5 (105.3-113.5)	8	113.0 (105.0-116.5)				
Age at US3 (d)†	CG	8	372.0 (370.3-384.8)	11	382.0 (377.0-386.0)		0.823	0.615	0.089
	IG	9	377.0 (373.5-389.0)	10	376.5 (371.8-383.5)				

*Data are presented as n, mean ± SD or percentage of the group. For quantitative variables p values of two-way ANOVA are presented. † Not normal distributed variables or variables that violated homoscedascity were analyzed with ANOVA on ranks and presented as median (25th-75th percentile). Holm-Sidak post hoc test were carried out in case of significance in the two-way ANONA and the results are grey shaded. P values < 0.05 are marked bold. [1] Sum of the four skinfold thickness (SFT) was calculated as: biceps + triceps + subscapular + suprailiac SFT, [2] Fat mass and percent body fat were estimated from the sum of 4 SFT using the equations of Weststrate et al. (reported in [124]), [3] Trunk-to-total skinfolds was calculated as: ((subscapular + suprailiac) / sum of 4SFT)*100 using the equations of Weststrate et al. (reported in [124]), [4] Sagittal subcutaneous and preperitoneal fat were measured as areas of 1 cm length according to the method of Holzhauer et al. as described in [124]), [5] Axial subcutaneous fat was measured between the middle of the xiphoid process and the navel, directly above the linea alba, [6] The ratio of subcutaneous to preperitoneal (SC/PP) fat was calculated as [(subcutaneous-area sagittal + axial)/2]/preperitoneal-area sagittal*

11.5. Additional offspring biomarkers

		male (M)		female (F)		p values	p*	p#	p*#
		n	mean ± SD	n	mean ± SD	CM vs CF	IM vs IF	IF vs CF	IM vs CM
EPA placenta tissue total (20:5n3)†	C	9	0.09 (0.07-0.13)	11	0.12 (0.07-0.16)		**< 0.001**	0.692	0.391
	I	11	0.28 (0.18-0.38)	10	0.27 (0.20-0.38)	0.382	0.738	**0.004**	**< 0.001**
DHA placenta tissue total (22:6n3)†	C	9	3.07 (2.22-3.73)	11	3.03 (2.25-4.40)		**0.009**	0.926	0.857
	I	11	4.65 (3.24-5.76)	10	4.44 (2.42-6.80)	0.951	0.845	0.071	**0.046**
AA placenta tissue total (20:4n6)	C	9	15.67 ± 4.53	11	16.09 ± 3.13		0.113	0.739	0.988
	I	11	13.73 ± 3.39	10	14.12 ± 4.35				
n-6 /n-3 LCPUFA ratio placenta tissue total†,1	C	9	5.79 (5.43-7.24)	11	5.59 (4.58-6.88)		**< 0.001**	0.903	0.377
	I	11	3.67 (3.06-4.01)	10	3.57 (3.09-4.95)	0.482	0.583	**0.002**	**< 0.001**
EPA placenta tissue TG (20:5n3)†	C	9	0.23 (0.19-0.25)	11	0.19 (0.16-0.31)		**0.005**	0.566	0.550
	I	11	0.60 (0.37-0.71)	10	0.48 (0.35-0.58)	0.987	0.402	0.091	**0.016**
DHA placenta tissue TG (22:6n3)†	C	8	1.70 (1.00-2.25)	11	1.67 (1.34-2.14)		**< 0.001**	0.517	0.815
	I	11	3.68 (1.63-4.78)	10	2.69 (1.34-3.92)	0.775	0.520	**0.005**	**0.004**
AA placenta tissue TG (20:4n6)	C	9	7.36 ± 2.79	11	7.15 ± 1.27		0.078	0.682	0.927
	I	11	6.22 ± 1.85	10	5.89 ± 2.39				
n-6 /n-3 LCPUFA ratio placenta tissue TG[1]	C	9	5.18 ± 0.84	11	4.86 ± 1.07		**< 0.001**	0.903	0.234
	I	11	2.47 ± 0.80	10	2.86 ± 0.95	0.453	0.345	**< 0.001**	**< 0.001**

LCPUFA fatty acid percentages of placental tissue in total (PL and TG fraction) and from TG fraction. Values for fatty acids are expressed as % of total fatty acids (wt%). Data are presented as n, mean ± SD for each of the four analysis groups (CM, CF, IM, IF). For quantitative variables p-values of two-way ANOVA are presented. † Not normal distributed variables or variables that violated homoscedascity were presented as median (25^{th}-75^{th} percentile) and p-values from two-way ANOVA on ranks are reported. Significance level for post-hoc tests were adjusted with Holm-Sidak test. P values < 0.05 are considered as significant and marked bold; p < 0.05 means significant difference between the IG and the CG, p# < 0.05 means significant difference between male and female placenta; p*# < 0.05 means significant interaction between sex and n-3/n-6 LCPUFA intervention; [1]n-6/n-3 LCPUFA ratio: (C20:2n-6 + C20:3n-6 + C20:4n-6 +C22:2n-6 + C22:4n-6 +C22:5n-6) / (C20:3n-3 + C20:4n-3 + C20:5n-3 + C22:3n-3 + C22:5n-3 + C22:6n-3).*

11.6. Supplementary data of DNA microarray analysis

The DNA microarray data are confidential for publication purposes. Access to these data will be provided for the PhD thesis committee by a compact disc attached to the thesis. In the context of publication these data will be available in the publicly available database gene expression omnibus.

11.6.1. List of significantly regulated genes between n-6/n-3 LCPUFA intervention group and control group in female and male placentas independent of sex from the DNA microarray analysis (IM+IF vs. CM+CF)

11.6.2. List of significantly regulated genes between n-6/n-3 LCPUFA intervention group and control group in female and male placentas under consideration of sex from the DNA microarray analysis (IM+IF vs. CM+CF)

11.6.3. List of significantly regulated genes between male and female placentas in the control group from the DNA microarray analysis (CM vs. CF)

11.6.4. List of significantly regulated genes between male and female placentas in the n-6/n-3 LCPUFA intervention group from the DNA microarray analysis (IM vs. IF)

11.6.5. List of significantly regulated genes between n-6/n-3 LCPUFA intervention group and control group in male placentas from the DNA microarray analysis (IM vs. CM)

11.6.6. List of significantly regulated genes between n-6/n-3 LCPUFA intervention group and control group in female placentas from the DNA microarray analysis (IF vs. CF)

11.7. Genes involved in lipid metabolism compared to the transcriptome data of the n-6/n-3 LCPUFA intervention

Gene symbol	Gene description	FC IM+IF vs CM+CF	p value IM+IF vs CM+CF	FC IM vs CM	p value IM vs CM	FC IF vs CF	p value IF vs CF
	Fatty acid lipases						
LPL	lipoprotein lipase	-1.61	>0.05	-3.62	>0.05	2.27	0.0381
LIPE§	lipase, hormone-sensitive	1.06	>0.05	-1.02	>0.05	1.11	>0.05
LIPC§	lipase, hepatic	-1.03	>0.05	-1.03	>0.05	1.00	>0.05
PNLIP§	pancreatic lipase	1.04	>0.05	-1.10	>0.05	1.09	>0.05
	Long-chain fatty acid receptors						
GPR120§	omega-3 fatty acid receptor 1	-1.10	>0.05	-1.02	>0.05	-1.04	>0.05
FFAR1§	free fatty acid receptor 1	1.23	0.0304	1.16	0.0452	1.07	>0.05
	Fatty acid transport						
CD36	**CD36** molecule (thrombospondin receptor)	1.26	>0.05	1.55	>0.05	-1.21	>0.05
FABP1§	fatty acid binding protein 1, liver	1.03	>0.05	1.05	>0.05	-1.04	>0.05
FABP2§	fatty acid binding protein 2, intestinal	1.09	>0.05	1.02	>0.05	1.00	>0.05
FABP3§	fatty acid binding protein 3, muscle and heart (mammary-derived growth inhibitor)	-1.07	>0.05	1.05	>0.05	-1.09	>0.05
FABP4	fatty acid binding protein 4, adipocyte	1.42	>0.05	1.20	>0.05	1.20	>0.05
FABP5	fatty acid binding protein 5 (psoriasis-associated)	not annotated					
FABP6§	fatty acid binding protein 6, ileal	1.07	>0.05	-1.02	>0.05	-1.01	>0.05
FABP7§	fatty acid binding protein 7, brain	1.04	>0.05	1.00	>0.05	1.03	>0.05
FABP9	fatty acid binding protein 9, testis	no probe sets on DNA microarray					
GOT2§	glutamic-oxaloacetic transaminase 2, mitochondrial (aspartate aminotransferase 2)	-1.18	>0.05	1.15	>0.05	-1.11	>0.05
SLC27A1§	solute carrier family 27 (fatty acid transporter), member 1	-1.03	>0.05	-1.02	>0.05	1.01	>0.05
SLC27A2	solute carrier family 27 (fatty acid transporter), member 2	-1.58	>0.05	1.05	>0.05	-1.62	>0.05
SLC27A3	solute carrier family 27 (fatty acid transporter), member 3	1.06	>0.05	1.15	>0.05	-1.04	>0.05
SLC27A4§	solute carrier family 27 (fatty acid transporter), member 4	1.20	0.0474	1.12	>0.05	1.09	>0.05
SLC27A5§	solute carrier family 27 (fatty acid transporter), member 5	-1.16	>0.05	-1.04	>0.05	-1.10	>0.05
SLC27A6	solute carrier family 27 (fatty acid transporter), member 6	-1.55	>0.05	-1.24	>0.05	-1.25	>0.05
	Acyl CoA Synthases						
ACSL1	acyl-CoA synthetase long-chain family member 1	-1.19	>0.05	-1.08	>0.05	-1.18	>0.05
ACSL3	acyl-CoA synthetase long-chain family member 3	no probe sets on DNA microarray					
ACSL4	acyl-CoA synthetase long-chain family member 4	-1.48	>0.05	-1.66	>0.05	1.22	>0.05
ACSL5§	acyl-CoA synthetase long-chain family member 5	1.03	>0.05	-1.01	>0.05	1.02	>0.05
ACSL6	acyl-CoA synthetase long-chain family member 6	no probe sets on DNA microarray					
	Acyl-CoA binding protein (DBI)						
DBI	diazepam binding inhibitor (GABA receptor modulator, acyl-CoA binding protein)	1.04	>0.05	1.22	>0.05	-1.13	>0.05
PECI	enoyl-CoA delta isomerase 2	-1.57	>0.05	-1.09	>0.05	-1.50	>0.05
ACBP3-7	acyl-CoA-binding protein 3-7	no probe sets on DNA microarray					
	TG Biosynthesis						
DGAT1§	diacylglycerol O-acyltransferase 1	1.15	>0.05	1.07	>0.05	1.10	>0.05
DGAT2§	diacylglycerol O-acyltransferase 2	1.28	>0.05	1.11	>0.05	1.15	>0.05
GPAM§	glycerol-3-phosphate acyltransferase, mitochondrial	-1.15	>0.05	1.10	>0.05	-1.19	>0.05
LPIN1	lipin 1	-1.38	>0.05	-1.64	>0.05	1.24	>0.05
LPIN2	lipin 2	1.10	>0.05	1.09	>0.05	1.06	>0.05
MOGAT1§	monoacylglycerol O-acyltransferase 1	1.07	>0.05	1.18	0.0241	-1.06	>0.05
MOGAT2§	monoacylglycerol O-acyltransferase 2	1.48	>0.05	-1.03	>0.05	1.45	0.0283
	ß-oxidation						
ACAA2§	acetyl-CoA acyltransferase 2	1.19	>0.05	1.15	0.0343	1.10	>0.05
ACAA1§	acetyl-CoA acyltransferase 1	-1.08	>0.05	-1.10	>0.05	-1.03	>0.05
ACADM	acyl-CoA dehydrogenase, C-4 to C-12 straight chain	-1.67	0.0244	-1.22	>0.05	-1.39	>0.05
ACADS§	acyl-CoA dehydrogenase, C-2 to C-3 short chain	1.03	>0.05	1.01	>0.05	1.12	>0.05
ACAD9	acyl-CoA dehydrogenase family, member 9	1.01	>0.05	-1.22	>0.05	1.30	>0.05
ACAD11	acyl-CoA dehydrogenase family, member 11	-1.16	>0.05	-1.26	>0.05	1.05	>0.05

Gene symbol	Gene description	FC IM+IF vs CM+CF	p value IM+IF vs CM+CF	FC IM vs CM	p value IM vs CM	FC IF vs CF	p value IF vs CF
ACADVL	acyl-CoA dehydrogenase, very long chain	1.30	>0.05	-1.08	>0.05	1.35	>0.05
ACOX1	acyl-CoA oxidase 1, palmitoyl	-1.11	>0.05	-1.00	>0.05	-1.10	>0.05
CPT1A	carnitine palmitoyltransferase 1A (liver)	-1.36	>0.05	-1.05	>0.05	-1.29	>0.05
CPT1B	carnitine palmitoyltransferase 1B (muscle)	no probe sets on DNA microarray					
CPT1C§	carnitine palmitoyltransferase 1C	1.00	>0.05	1.00	>0.05	-1.01	>0.05
CPT2	carnitine palmitoyltransferase 2	-1.10	>0.05	1.01	>0.05	-1.13	>0.05
ECH1	enoyl CoA hydratase 1, peroxisomal	-1.12	>0.05	-1.05	>0.05	-1.07	>0.05
ECHS1	enoyl CoA hydratase, short chain, 1, mitochondrial	-1.16	>0.05	1.16	>0.05	-1.28	0.0223
HADHA	hydroxyacyl-CoA dehydrogenase/3-ketoacyl-CoA thiolase/enoyl-CoA hydratase (trifunctional protein), alpha subunit	-1.01	>0.05	-1.08	>0.05	1.10	>0.05
HADHB	hydroxyacyl-CoA dehydrogenase/3-ketoacyl-CoA thiolase/enoyl-CoA hydratase (trifunctional protein), beta subunit	1.01	>0.05	1.06	>0.05	-1.05	>0.05
NDUFAB1	NADH dehydrogenase (ubiquinone) 1, alpha/beta subcomplex, 1, 8kDa	-1.05	>0.05	1.03	>0.05	1.00	>0.05
SLC25A20§	solute carrier family 25 (carnitine/acylcarnitine translocase), member 20	1.14	>0.05	1.05	>0.05	-1.07	>0.05
	ß oxidation uneven fatty acids						
PCCA	propionyl CoA carboxylase, alpha polypeptide	-1.02	>0.05	-1.08	>0.05	1.04	>0.05
PCCB	propionyl CoA carboxylase, beta polypeptide	1.04	>0.05	-1.02	>0.05	1.00	>0.05
MCEE	methylmalonyl CoA epimerase	1.05	>0.05	1.11	>0.05	-1.14	>0.05
	ß oxidation unsaturated fatty acids						
EHHADH§	enoyl-CoA, hydratase/3-hydroxyacyl CoA	1.10	>0.05	1.12	>0.05	1.06	>0.05
ACADL§	acyl-CoA dehydrogenase, long chain	1.00	>0.05	-1.15	>0.05	1.04	>0.05
	Biosynthesis of saturated fatty acids						
ACACA	acetyl-CoA carboxylase alpha	1.20	>0.05	1.07	>0.05	1.18	>0.05
ACACB§	acetyl-CoA carboxylase beta	1.12	>0.05	1.04	>0.05	1.07	>0.05
FASN§	fatty acid synthase	1.29	>0.05	-1.18	>0.05	1.49	0.0003
	Biosynthesis of unsaturated fatty acids						
FADS6§	fatty acid desaturase domain family, member 6	-1.05	>0.05	-1.03	>0.05	-1.01	>0.05
FADS2§	fatty acid desaturase 2	-1.06	>0.05	1.12	>0.05	-1.12	>0.05
ELOVL1	ELOVL fatty acid elongase 1	1.00	>0.05	1.04	>0.05	-1.03	>0.05
ELOVL2§	ELOVL fatty acid elongase 2	-1.14	>0.05	1.03	>0.05	-1.22	>0.05
ELOVL3§	ELOVL fatty acid elongase 3	1.03	>0.05	-1.05	>0.05	1.06	>0.05
ELOVL4§	ELOVL fatty acid elongase 4	-1.04	>0.05	-1.03	>0.05	-1.17	>0.05
ELOVL5	ELOVL fatty acid elongase 5	-1.23	>0.05	-1.11	>0.05	1.01	>0.05
ELOVL6§	ELOVL fatty acid elongase 6	1.01	>0.05	-1.01	>0.05	-1.03	>0.05
ELOVL7§	ELOVL fatty acid elongase 7	-1.30	>0.05	-1.42	>0.05	1.04	>0.05
SCD1	stearoyl-CoA desaturase (delta-9-desaturase)	1.02	>0.05	-1.21	>0.05	1.14	>0.05
	Biosynthesis of Eicosanoides						
PLA2G4A§	phospholipase A2, group IVA (cytosolic, calcium-dependent)	-1.35	>0.05	-1.24	>0.05	-1.03	>0.05
PLA2G4D§	phospholipase A2, group IVD (cytosolic)	-1.18	>0.05	-1.14	>0.05	1.00	>0.05
PLA2G4E	phospholipase A2, group IVE	not annotated					
PLA2G4F§	phospholipase A2, group IVF	1.04	>0.05	1.10	>0.05	1.02	>0.05
PTGS1§	prostaglandin-endoperoxide synthase 1 (prostaglandin G/H synthase and cyclooxygenase)	1.03	>0.05	1.16	>0.05	-1.01	>0.05
PTGS2§	prostaglandin-endoperoxide synthase 2 (prostaglandin G/H synthase and cyclooxygenase)	1.37	>0.05	-1.16	>0.05	1.47	>0.05
ALOX12§	arachidonate 12-lipoxygenase	1.01	>0.05	1.01	>0.05	1.03	>0.05
ALOX12B§	arachidonate 12-lipoxygenase, 12R type	-1.05	>0.05	1.05	>0.05	-1.06	>0.05
ALOX15§	arachidonate 15-lipoxygenase	-1.03	>0.05	-1.02	>0.05	-1.03	>0.05
ALOX15B§	arachidonate 15-lipoxygenase, type B	1.07	>0.05	1.05	>0.05	1.02	>0.05
ALOX5	arachidonate 5-lipoxygenase	-1.00	>0.05	1.02	>0.05	-1.03	>0.05
ALOX5AP	arachidonate 5-lipoxygenase-activating protein	-1.31	>0.05	-1.24	>0.05	-1.02	>0.05
ALOXE3§	arachidonate lipoxygenase 3	1.16	>0.05	-1.08	>0.05	1.12	>0.05
	Storage and release of PUFA in TG and PL						
AGPAT1	1-acylglycerol-3-phosphate O-acyltransferase 1 (lysophosphatidic acid acyltransferase, alpha)	1.03	>0.05	1.05	>0.05	-1.08	>0.05

Gene symbol	Gene description	FC IM+IF vs CM+CF	p value IM+IF vs CM+CF	FC IM vs CM	p value IM vs CM	FC IF vs CF	p value IF vs CF
PPAP2A	phosphatidic acid phosphatase type 2A	-1.39	>0.05	-1.03	>0.05	-1.39	>0.05
PPAP2B	phosphatidic acid phosphatase type 2B	1.13	>0.05	1.09	>0.05	1.04	>0.05
PPAP2C§	phosphatidic acid phosphatase type 2C	1.06	>0.05	1.03	>0.05	1.08	>0.05
ACAT1	acetyl-CoA acetyltransferase 1	-1.10	>0.05	-1.10	>0.05	-1.04	>0.05
CAV1	caveolin 1, caveolae protein, 22kDa	-1.08	>0.05	1.05	>0.05	-1.17	>0.05
PLIN1§	perilipin 1	1.09	>0.05	-1.01	>0.05	1.03	>0.05
PLIN2	perilipin 2	1.76	>0.05	-1.08	>0.05	1.97	>0.05
PPARA§	peroxisome proliferator-activated receptor alpha	-1.00	>0.05	1.04	>0.05	-1.02	>0.05
PPARG	peroxisome proliferator-activated receptor gamma	-1.27	>0.05	1.03	>0.05	-1.21	>0.05
PPARGC1§A	peroxisome proliferator-activated receptor gamma, coactivator 1 alpha	1.25	0.0459	1.06	>0.05	1.17	>0.05
RXRA	retinoid X receptor, alpha	-1.02	>0.05	-1.20	0.0148	1.21	0.0075
RXRB	retinoid X receptor, beta	1.11	>0.05	-1.08	>0.05	1.12	>0.05
RXRG§	retinoid X receptor, gamma	1.04	>0.05	-1.02	>0.05	1.04	>0.05
HNF4A	hepatocyte nuclear factor 4, alpha	not annotated					
HNF4B	hepatocyte nuclear factor 4, beta	no probe sets on DNA microarray					
HNF4G§	hepatocyte nuclear factor 4, gamma	1.18	>0.05	1.01	>0.05	1.13	>0.05
NR1H3	nuclear receptor subfamily 1, group H, member 3	1.04	>0.05	1.04	>0.05	1.04	>0.05
SREBF1§	sterol regulatory element binding transcription factor 1	1.32	>0.05	-1.23	>0.05	1.57	>0.05
SREBF2	sterol regulatory element binding transcription factor 2	no probe sets on DNA microarray					
NFKB1	nuclear factor of kappa light polypeptide gene enhancer in Bells 1	1.03	>0.05	1.02	>0.05	-1.03	>0.05

Data are presented as median (interquartile range IQR = 25th-75th percentile). CM: Control male = placentas of male offspring in the control group (n_{CM} = 3), CF: Control female = placentas of female offspring in the control group (n_{CF} = 4), IM: intervention male = placentas of male offspring in the n-3 LCPUFA intervention group (n_{IM} = 5), IF: intervention female = placentas of female offspring in the n-3 LCPUFA intervention group (n_{IF} = 4). The applied significance criteria were FC ≥ +1.5 and p < 0.05 and FC ≤ -1.5 and p < 0.05. §, average intensity below 20; FC, fold change.

11.8. PPARγ target genes selected from literature compared to the transcriptome data of the n-6/n-3 LCPUFA intervention

gene symbol	Gene description	FC IM+IF vs CM+CF	p value IM+IF vs CM+CF	FC IM vs CM	p value IM vs CM	FC IF vs CF	p value IF vs CF
ADIPOQ§	adiponectin, C1Q and collagen domain containing	-1.07	>0.05	-1.09	>0.05	1.04	>0.05
ANGPTL4	angiopoietin-like 4	1.15	>0.05	-1.13	>0.05	1.37	>0.05
APOE	apolipoprotein E	1.45	>0.05	-1.02	>0.05	1.30	>0.05
AQP7§	aquaporin 7	1.03	>0.05	1.08	>0.05	-1.02	>0.05
BCMO1§	beta-carotene 15,15'-monooxygenase 1	1.09	>0.05	1.11	>0.05	-1.01	>0.05
CAT	catalase	-1.39	>0.05	1.02	>0.05	-1.34	>0.05
CD36	**CD36** molecule (thrombospondin receptor)	1.26	>0.05	1.55	>0.05	-1.21	>0.05
CIDEA§	cell death-inducing DFFA-like effector a	-1.07	>0.05	-1.01	>0.05	-1.06	>0.05
CPT1A	carnitine palmitoyltransferase 1A (liver)	-1.36	>0.05	-1.05	>0.05	-1.29	>0.05
DBI	diazepam binding inhibitor (GABA receptor modulator, acyl-CoA binding protein)	1.04	>0.05	1.22	>0.05	-1.13	>0.05
FABP4	fatty acid binding protein 4, adipocyte	1.42	>0.05	1.20	>0.05	1.20	>0.05
G0S2	G0/G1switch 2	1.18	>0.05	1.28	>0.05	-1.05	>0.05
GCK§	glucokinase (hexokinase 4)	1.06	>0.05	-1.01	>0.05	1.09	>0.05
IGFBP1*	insulin-like growth factor binding protein 1	1.60	>0.05	10.87	0.04334	-7.30	>0.05
IGFBP2	insulin-like growth factor binding protein 2, 36kDa	-1.38	>0.05	1.33	>0.05	-1.69	>0.05
IGFBP5	insulin-like growth factor binding protein 5	-1.18	>0.05	-1.25	>0.05	-1.01	>0.05
INSIG1	insulin induced gene 1	1.18	>0.05	-1.65	>0.05	2.00	>0.05
LPL*	lipoprotein lipase	-1.61	>0.05	-3.62	>0.05	2.27	0.03809
LRP1	low density lipoprotein receptor-related protein 1	-1.18	>0.05	-1.03	>0.05	-1.09	>0.05
MC2R§	melanocortin 2 receptor (adrenocorticotropic hormone)	1.02	>0.05	1.03	>0.05	1.02	>0.05
MUC1	mucin 1, cell surface associated	1.20	>0.05	-1.34	>0.05	1.47	0.0460
NPHS1	nephrosis 1, congenital, Finnish type (nephrin)	no probe sets on DNA microarray					
NR1D1§	nuclear receptor subfamily 1, group D, member 1	1.19	>0.05	1.13	>0.05	1.09	>0.05
NR1H3	nuclear receptor subfamily 1, group H, member 3	1.04	>0.05	1.04	>0.05	1.04	>0.05
PCK1§	phosphoenolpyruvate carboxykinase 1 (soluble)	-1.06	>0.05	-1.18	>0.05	1.22	0.0293
PLIN1§	perilipin 1	1.09	>0.05	-1.01	>0.05	1.03	>0.05
PLIN2	perilipin 2	1.76	>0.05	-1.08	>0.05	1.97	>0.05
PTGS2	prostaglandin-endoperoxide synthase 2 (prostaglandin G/H synthase and cyclooxygenase)	1.37	>0.05	-1.16	>0.05	1.47	>0.05
SCARB1	scavenger receptor class B, member 1	1.01	>0.05	-1.29	0.0426	1.36	>0.05
SCNN1G§	sodium channel, non-voltage-gated 1, gamma subunit	1.10	>0.05	-1.01	>0.05	1.11	>0.05
SERPINE1	serpin peptidase inhibitor, clade E (nexin, plasminogen	-1.02	>0.05	1.14	>0.05	-1.17	>0.05
SLC1A2§	solute carrier family 1 (glial high affinity glutamate	-1.09	>0.05	1.05	>0.05	-1.04	>0.05
SLC22A1§	solute carrier family 22 (organic cation transporter),	-1.02	>0.05	1.01	>0.05	-1.06	>0.05
SLC27A1§	solute carrier family 27 (fatty acid transporter), member	-1.03	>0.05	-1.02	>0.05	1.01	>0.05
SLC27A4§	solute carrier family 27 (fatty acid transporter), member	1.20	0.0474	1.12	>0.05	1.09	>0.05
SLC2A2	solute carrier family 2 (facilitated glucose transporter),	no probe sets on DNA microarray					
SORBS1§	sorbin and SH3 domain containing 1	1.08	>0.05	1.02	>0.05	1.15	>0.05
TFF2§	trefoil factor 2	1.03	>0.05	-1.05	>0.05	1.16	0.0193
UCP1§	uncoupling protein 1 (mitochondrial, proton carrier)	1.11	>0.05	1.14	>0.05	-1.01	>0.05

*Data are presented as median (interquartile range IQR = 25th-75th percentile). CM: Control male = placentas of male offspring in the control group (n_{CM} = 3), CF: Control female = placentas of female offspring in the control group (n_{CF} = 4), IM: intervention male = placentas of male offspring in the n-3 LCPUFA intervention group (n_{IM} = 5), IF: intervention female = placentas of female offspring in the n-3 LCPUFA intervention group (n_{IF} = 4). The applied significance criteria were FC ≥ +1.5 and p < 0.05 and FC ≤ -1.5 and p < 0.05. Genes marked in bold are experimentally validated PPARγ target genes in the placenta, *, significantly differential expressed gene; §, average intensity below 20; FC, fold change.*

11.9. PPAR ß/d target from literature compared to the transcriptome data of the n-6/n-3 LCPUFA intervention

gene symbol	gene description	FC IM+IF vs CM+CF	p value IM+IF vs CM+CF	FC IM vs CM	p value IM vs CM	FC IF vs CF	p value IF vs CF
ABCA1	ATP-binding cassette, sub-family A (ABC1), member 1	-1.02	>0.05	1.02	>0.05	-1.09	>0.05
ACAA2§	acetyl-CoA acyltransferase 2	1.19	>0.05	1.15	0.0343	1.10	>0.05
ACSL5§	acyl-CoA synthetase long-chain family member 5	1.03	>0.05	-1.01	>0.05	1.02	>0.05
PLIN2	perilipin 2	1.76	>0.05	-1.08	>0.05	1.97	>0.05
AHNAK	AHNAK nucleoprotein	-1.06	>0.05	-1.10	>0.05	-1.00	>0.05
AK3	adenylate kinase 3	-1.05	>0.05	-1.00	>0.05	-1.08	>0.05
ANGPTL4	angiopoietin-like 4	1.15	>0.05	-1.13	>0.05	1.37	>0.05
AP2A1§	adaptor-related protein complex 2, alpha 1 subunit	1.08	>0.05	-1.07	>0.05	1.15	>0.05
APH1B§	anterior pharynx defective 1 homolog B (C. elegans)	1.01	>0.05	-1.02	>0.05	-1.04	>0.05
BIRC3	baculoviral IAP repeat containing 3	no probe sets on DNA microarray					
CDKN2C§	cyclin-dependent kinase inhibitor 2C (p18, inhibits CDK4)	-1.01	>0.05	1.09	>0.05	-1.06	>0.05
CNO	biogenesis of lysosomal organelles complex-1, subunit 4, cappuccino	1.30	>0.05	-1.07	>0.05	1.40	0.0068
CPT1a	carnitine palmitoyltransferase 1A (liver)	-1.36	>0.05	-1.05	>0.05	-1.29	>0.05
CPT2	carnitine palmitoyltransferase 2	-1.10	>0.05	1.01	>0.05	-1.13	>0.05
CSNK1G2§	casein kinase 1, gamma 2	-1.07	>0.05	-1.06	>0.05	1.00	>0.05
CTBS§	chitobiase, di-N-acetyl-	-1.13	>0.05	1.09	>0.05	-1.25	>0.05
CYB5D2§	cytochrome b5 domain containing 2	1.08	>0.05	1.07	>0.05	-1.03	>0.05
DCP1A	DCP1 decapping enzyme homolog A (S. cerevisiae)	-1.15	>0.05	-1.35	>0.05	1.20	0.0120
DIAPH1	diaphanous homolog 1 (Drosophila)	1.41	>0.05	-1.15	>0.05	1.48	0.0135
DOCK4	dedicator of cytokinesis 4	-1.65	>0.05	1.02	>0.05	-1.68	>0.05
ECH1	enoyl CoA hydratase 1, peroxisomal	-1.12	>0.05	-1.05	>0.05	-1.07	>0.05
ETFB	electron-transfer-flavoprotein, beta polypeptide	-1.10	>0.05	1.15	>0.05	-1.23	>0.05
ETFDH§	electron-transferring-flavoprotein dehydrogenase	1.07	>0.05	-1.02	>0.05	1.16	>0.05
EXOC6B§	exocyst complex component 6B	1.06	>0.05	-1.04	>0.05	1.08	>0.05
GPR180§	G protein-coupled receptor 180	-1.27	>0.05	-1.06	>0.05	-1.27	>0.05
GRAMD3	GRAM domain containing 3	1.30	>0.05	1.05	>0.05	1.26	>0.05
HMOX1	heme oxygenase (decycling) 1	-1.44	>0.05	-1.30	>0.05	-1.15	>0.05
HSD11B2	hydroxysteroid (11-beta) dehydrogenase 2	1.66	>0.05	1.24	>0.05	1.35	>0.05
HSDL2	hydroxysteroid dehydrogenase like 2	-1.77	>0.05	-1.25	>0.05	-1.54	>0.05
IFIT2	interferon-induced protein with tetratricopeptide repeats 2	-1.16	>0.05	-1.05	>0.05	-1.06	>0.05
IMPA2	inositol(myo)-1(or 4)-monophosphatase 2	1.49	>0.05	1.11	>0.05	1.32	>0.05
ISCA1	iron-sulfur cluster assembly 1 homolog (S. cerevisiae)	-1.55	>0.05	-1.10	>0.05	-1.41	0.0356
KLF10	Kruppel-like factor 10	-1.07	>0.05	1.17	>0.05	-1.22	0.0045
KLF11	Kruppel-like factor 11	-1.69	0.0398	-1.15	>0.05	-1.54	0.0144
MLYCD§	malonyl-CoA decarboxylase	1.11	>0.05	1.17	>0.05	-1.05	>0.05
MYO18A	myosin XVIIIA	not annotated					
NUDT13§	nudix (nucleoside diphosphate linked moiety X)-type motif 13	-1.01	>0.05	-1.02	>0.05	1.04	>0.05
NUDT9	nudix (nucleoside diphosphate linked moiety X)-type motif 9	-1.04	>0.05	-1.11	>0.05	1.07	>0.05
PDK4	pyruvate dehydrogenase kinase, isozyme 4	-1.25	>0.05	1.02	>0.05	-1.25	>0.05
PLIN1§	perilipin 1	1.09	>0.05	-1.01	>0.05	1.03	>0.05
PPARG	peroxisome proliferator-activated receptor gamma	-1.27	>0.05	1.03	>0.05	-1.21	>0.05
RUNX1§	runt-related transcription factor 1	1.01	>0.05	-1.07	>0.05	1.11	>0.05
SLC25A20§	solute carrier family 25 (carnitine/acylcarnitine translocase), member 20	1.14	>0.05	1.05	>0.05	-1.07	>0.05
SYTL3§	synaptotagmin-like 3	1.02	>0.05	1.06	>0.05	1.04	>0.05
TALDO1	transaldolase 1	1.05	>0.05	1.03	>0.05	-1.04	>0.05
TIMP4	TIMP metallopeptidase inhibitor 4	no probe sets on DNA microarray					
TMEM135	transmembrane protein 135	no probe sets on DNA microarray					
TNFRSF1A	tumor necrosis factor receptor superfamily, member 1A	-1.09	>0.05	-1.03	>0.05	-1.05	>0.05

gene symbol	**gene description**	**FC** IM+IF vs CM+CF	**p value** IM+IF vs CM+CF	**FC** IM vs CM	**p value** IM vs CM	**FC** IF vs CF	**p value** IF vs CF
TTC33	tetratricopeptide repeat domain 33	1.01	>0.05	1.02	>0.05	1.01	>0.05
UROS§	uroporphyrinogen III synthase	1.06	>0.05	1.01	>0.05	-1.05	>0.05
VAMP8	vesicle-associated membrane protein 8	1.13	>0.05	1.08	>0.05	1.02	>0.05
ZNF354A	zinc finger protein 354A	1.01	>0.05	-1.09	>0.05	1.04	>0.05
ZNF367§	zinc finger protein 367	-1.02	>0.05	1.24	0.0107	-1.26	0.0262

Data are presented as median (interquartile range IQR = 25th-75th percentile). CM: Control male = placentas of male offspring in the control group (n_{CM} = 3), CF: Control female = placentas of female offspring in the control group (n_{CF} = 4), IM: intervention male = placentas of male offspring in the n-3 LCPUFA intervention group (n_{IM} = 5), IF: intervention female = placentas of female offspring in the n-3 LCPUFA intervention group (n_{IF} = 4). The applied significance criteria were FC ≥ +1.5 and p < 0.05 and FC ≤ -1.5 and p < 0.05. Genes marked in bold are experimentally validated PPARβ/δ target genes in the placenta, *, significantly differential expressed gene; *§, average intensity below 20; FC, fold change.*

11.10. Other target genes of n-3 LCPUFAs compared to the transcriptome datasets for the n-6/n-3 LCPUFA intervention

Gene name	Gene description	FC IM+IF vs CM+CF	p value IM+IF vs CM+CF	FC IM vs CM	p value IM vs CM	FC IF vs CF	p value IF vs CF
ABCA1	ATP-binding cassette, sub-family A (ABC1), member 1	-1.02	>0.05	1.02	>0.05	-1.09	>0.05
ACACA	acetyl-Coenzyme A carboxylase alpha	1.20	>0.05	1.07	>0.05	1.18	>0.05
ACLY	ATP citrate lyase	1.16	>0.05	1.02	>0.05	1.14	>0.05
ACOT1	acyl-CoA thioesterase 1	no probe sets on DNA microarray					
ACOT2	acyl-CoA thioesterase 2	no probe sets on DNA microarray					
ACOT8§	acyl-CoA thioesterase 8	1.22	>0.05	1.11	>0.05	1.08	>0.05
ACOX1	acyl-Coenzyme A oxidase 1, palmitoyl	-1.11	>0.05	-1.00	>0.05	-1.10	>0.05
ACSL1	acyl-CoA synthetase long-chain family member 1	-1.19	>0.05	-1.08	>0.05	-1.18	>0.05
ADIPOQ§	adiponectin, C1Q and collagen domain containing	-1.07	>0.05	-1.09	>0.05	1.04	>0.05
APOC3§	apolipoprotein C-III	-1.13	>0.05	-1.18	>0.05	1.20	0.0334
APOE	apolipoprotein E	1.45	>0.05	-1.02	>0.05	1.30	>0.05
CAV1	caveolin 1, caveolae protein, 22kDa	-1.08	>0.05	1.05	>0.05	-1.17	>0.05
CAV2	caveolin 2	-1.20	>0.05	-1.04	>0.05	-1.17	>0.05
CCL2	chemokine (C-C motif) ligand 2	1.34	>0.05	1.49	>0.05	-1.01	>0.05
CD36	CD36 molecule (thrombospondin receptor)	1.26	>0.05	1.55	>0.05	-1.21	>0.05
CEBPA	CCAAT/enhancer binding protein (C/EBP), alpha	no probe sets on DNA microarray					
CFD	complement factor D (adipsin)	-1.12	>0.05	-1.06	>0.05	-1.01	>0.05
CHUK	conserved helix-loop-helix ubiquitous kinase	-1.18	>0.05	-1.17	>0.05	-1.03	>0.05
CPT1A	carnitine palmitoyltransferase 1A (liver)	-1.36	>0.05	-1.05	>0.05	-1.29	>0.05
CRP§	C-reactive protein, pentraxin-related	1.06	>0.05	1.02	>0.05	1.06	>0.05
CYP4A11	cytochrome P450, family 4, subfamily A, polypeptide 11	not annotated					
CYP4A22	cytochrome P450, family 4, subfamily A, polypeptide 22	no probe sets on DNA microarray					
CYP7A1§	cytochrome P450, family 7, subfamily A, polypeptide 1	1.11	>0.05	1.10	>0.05	-1.02	>0.05
EGR1	early growth response 1	-1.20	>0.05	1.07	>0.05	-1.25	>0.05
ELOVL5	ELOVL family member 5, elongation of long chain fatty acids (FEN1/Elo2, SUR4/Elo3-like, yeast)	-1.23	>0.05	-1.11	>0.05	1.01	>0.05
ELOVL6§	ELOVL family member 6, elongation of long chain fatty acids (FEN1/Elo2, SUR4/Elo3-like, yeast)	1.01	>0.05	-1.01	>0.05	-1.03	>0.05
FADS2§	fatty acid desaturase 2	-1.06	>0.05	1.12	>0.05	-1.12	>0.05
FADS6§	fatty acid desaturase domain family, member 6	-1.05	>0.05	-1.03	>0.05	-1.01	>0.05
FASN§	fatty acid synthase	1.29	>0.05	-1.18	>0.05	1.49	0.0003
FOS	FBJ murine osteosarcoma viral oncogene homolog	-1.24	>0.05	1.16	>0.05	-1.41	>0.05
G6PC§	glucose-6-phosphatase, catalytic subunit	1.09	>0.05	1.07	>0.05	1.05	>0.05
G6PC2§	glucose-6-phosphatase, catalytic, 2	1.11	>0.05	1.03	>0.05	1.03	>0.05
G6PC3	glucose 6 phosphatase, catalytic, 3	-1.13	>0.05	-1.08	>0.05	-1.09	>0.05
G6PD§	glucose-6-phosphate dehydrogenase	-1.06	>0.05	1.03	>0.05	1.00	>0.05
GCK§	glucokinase (hexokinase 4)	1.06	>0.05	-1.01	>0.05	1.09	>0.05
HMGCS1	3-hydroxy-3-methylglutaryl-Coenzyme A synthase 1 (soluble)	-1.14	>0.05	-1.18	>0.05	1.05	>0.05
HMGCS2	3-hydroxy-3-methylglutaryl-CoA synthase 2 (mitochondrial)	no probe sets on DNA microarray					
ICAM1§	intercellular adhesion molecule 1	1.16	>0.05	1.11	>0.05	-1.03	>0.05
IFNG§	interferon, gamma	1.29	>0.05	1.07	>0.05	1.09	>0.05
IKBKB	inhibitor of kappa light polypeptide gene enhancer in B-cells, kinase beta	1.12	>0.05	-1.03	>0.05	1.06	>0.05
IKBKE§	inhibitor of kappa light polypeptide gene enhancer in B-cells, kinase epsilon	1.27	0.0480	-1.01	>0.05	1.18	0.0078
IL12A§	interleukin 12A (natural killer cell stimulatory factor 1, cytotoxic lymphocyte maturation factor 1, p35)	-1.08	>0.05	-1.05	>0.05	1.03	>0.05
IL12B§	interleukin 12B (natural killer cell stimulatory factor 2, cytotoxic lymphocyte maturation factor 2, p40)	-1.02	>0.05	-1.05	>0.05	-1.01	>0.05
IL1B§	interleukin 1, beta	1.16	>0.05	1.19	>0.05	1.05	>0.05
IL2§	interleukin 2	-1.01	>0.05	-1.22	0.0359	1.15	>0.05
IL6§	interleukin 6 (interferon, beta 2)	-1.27	>0.05	-1.06	>0.05	-1.13	>0.05
IL8§	interleukin 8	-1.19	>0.05	1.65	>0.05	-2.16	0.0212

LEP	leptin	1.07	>0.05	-5.66	>0.05	6.28	>0.05
LIPE§	lipase, hormone-sensitive	1.06	>0.05	-1.02	>0.05	1.11	>0.05
LPL	lipoprotein lipase	-1.61	>0.05	-3.62	>0.05	2.27	0.0381
ME1§	malic enzyme 1, NADP(+)-dependent, cytosolic	-1.73	>0.05	-1.19	>0.05	-1.47	>0.05
MMP9§	matrix metallopeptidase 9 (gelatinase B, 92kDa gelatinase, 92kDa type IV collagenase)	1.13	>0.05	-1.01	>0.05	1.03	>0.05
NFKB1	nuclear factor of kappa light polypeptide gene enhancer in B-cells 1	1.03	>0.05	1.02	>0.05	-1.03	>0.05
NOS2§	nitric oxide synthase 2, inducible	-1.11	>0.05	1.03	>0.05	-1.18	>0.05
NRF1	nuclear respiratory factor 1	1.02	>0.05	-1.21	>0.05	1.15	>0.05
PCK1§	phosphoenolpyruvate carboxykinase 1 (soluble)	-1.06	>0.05	-1.18	>0.05	1.22	0.0293
PCK2§	phosphoenolpyruvate carboxykinase 2 (mitochondrial)	-1.02	>0.05	-1.08	>0.05	1.09	>0.05
PDGFA§	platelet-derived growth factor alpha polypeptide	-1.02	>0.05	-1.01	>0.05	1.05	>0.05
PDK4	pyruvate dehydrogenase kinase, isozyme 4	-1.25	>0.05	1.02	>0.05	-1.25	>0.05
PKLR§	pyruvate kinase, liver and RBC	1.02	>0.05	1.10	>0.05	-1.04	>0.05
PPARA§	peroxisome proliferator-activated receptor alpha	-1.00	>0.05	1.04	>0.05	-1.02	>0.05
PPARD	peroxisome proliferator-activated receptor delta	1.04	>0.05	-1.09	>0.05	1.13	>0.05
PPARG	peroxisome proliferator-activated receptor gamma	-1.27	>0.05	1.03	>0.05	-1.21	>0.05
PTGS2§	prostaglandin-endoperoxide synthase 2 (prostaglandin G/H synthase and cyclooxygenase)	1.37	>0.05	-1.16	>0.05	1.47	>0.05
RETN§	resistin	1.03	>0.05	1.01	>0.05	1.08	>0.05
SCD1	stearoyl-CoA desaturase (delta-9-desaturase)	1.02	>0.05	-1.21	>0.05	1.14	>0.05
SCD5§	stearoyl-CoA desaturase 5	1.31	>0.05	1.15	>0.05	1.13	>0.05
SELE§	selectin E	-1.04	>0.05	1.15	>0.05	-1.23	>0.05
SLC27A1§	solute carrier family 27 (fatty acid transporter), member 1	-1.03	>0.05	-1.02	>0.05	1.01	>0.05
SLC27A2	solute carrier family 27 (fatty acid transporter), member 2	-1.58	>0.05	1.05	>0.05	-1.62	>0.05
SLC27A3	solute carrier family 27 (fatty acid transporter), member 3	1.06	>0.05	1.15	>0.05	-1.04	>0.05
SLC27A4§	solute carrier family 27 (fatty acid transporter), member 4	1.20	0.0474	1.12	>0.05	1.09	>0.05
SLC27A5§	solute carrier family 27 (fatty acid transporter), member 5	-1.16	>0.05	-1.04	>0.05	-1.10	>0.05
SLC27A6	solute carrier family 27 (fatty acid transporter), member 6	-1.55	>0.05	-1.24	>0.05	-1.25	>0.05
SLC2A1	solute carrier family 2 (facilitated glucose transporter), member 1	-1.20	>0.05	-1.67	>0.05	1.37	0.0188
SLC2A2	solute carrier family 2 (facilitated glucose transporter), member 2	no probe sets on DNA microarray					
SLC2A4§	solute carrier family 2 (facilitated glucose transporter), member 4	1.14	>0.05	1.04	>0.05	1.03	>0.05
SREBF1	sterol regulatory element binding transcription factor 1	1.32	>0.05	-1.23	>0.05	1.57	>0.05
THRSP§	thyroid hormone responsive (SPOT14 homolog, rat)	1.10	>0.05	1.11	>0.05	1.04	>0.05
TNF§	tumor necrosis factor (TNF superfamily, member 2)	-1.05	>0.05	-1.10	>0.05	1.02	>0.05
UCP1§	uncoupling protein 1 (mitochondrial, proton carrier)	1.11	>0.05	1.14	>0.05	-1.01	>0.05
UCP2	uncoupling protein 2 (mitochondrial, proton carrier)	1.46	>0.05	1.22	>0.05	1.18	>0.05
VCAM1	vascular cell adhesion molecule 1	1.17	>0.05	1.48	>0.05	-1.26	>0.05
VWF	von Willebrand factor	-1.44	>0.05	-1.23	>0.05	-1.21	>0.05

Data are presented as median (interquartile range IQR = 25th-75th percentile). CM: Control male = placentas of male offspring in the control group (n_{CM} = 3), CF: Control female = placentas of female offspring in the control group (n_{CF} = 4), IM: intervention male = placentas of male offspring in the n-3 LCPUFA intervention group (n_{IM} = 5), IF: intervention female = placentas of female offspring in the n-3 LCPUFA intervention group (n_{IF} = 4). The applied significance criteria were FC ≥ +1.5 and p < 0.05 and FC ≤ -1.5 and p < 0.05. *, significantly differential expressed gene; *§, average intensity below 20; FC, fold change.* §

11.11. Complete lists of significantly overrepresented pathways in the gene lists of placentas of male children compared to placentas of female children

Pathways containing significantly overrepresented genes from the gene dataset of male compared to female placentas within the control group

regulated Pathways	pos.	meas.	total	%	Z Score	regulated genes in the pathway
Fatty Acid Beta Oxidation	5	30	43	16.67	4.26	**PNPLA2** 1.56 p:0.02, **LPL** 5.60 p.0.00002, **GK** -1.69 p:0.03, **ACADVL** 1.57 p:0.03, **DECR1** -1.51 p:0.04
Sulfation	3	14	29	21.43	3.93	**SULT2B1** 1.75 p:0.0007, **PAPSS1** -1.80 p:0.02, **PAPSS2** -1.77 p:0.02
Adipogenesis	11	118	131	9.32	3.91	**SREBF1** 1.63 p:0.04, **MBNL1** -4.12 p:0.03, **NRIP1**-2.91 p:0.006, **LPL** 5.60 p.0.00002, **LPIN1** 2.25 p:0.04, **CDKN1A** 2.59 p.0.03, **ID3** -1.57 p:0.04, **LIFR** -1.70 p:0.01, **IL6ST** 3.36 p.0.02, **DVL1** 1.51 p:0.004, **FRZB** 3.02 p:0.01
Triacylglyceride Synthesis	4	24	37	16.67	3.81	**PNPLA2** 1.56 p:0.02, **LPL** 5.60 p.0.00002, **GK** -1.69 p:0.03, **GPAM** -1.52 p.0.009
Glycogen Metabolism	4	32	43	12.50	3.04	**CALM1** (805) -1.50 p.0.005 (3x), **GSK3B** -1.77 p:0.005
MicroRNAs in cardiomyocyte hypertrophy	6	77	109	7.79	2.36	**CALM1** (805) -1.50 p.0.005, **CALM1** (801) 1.52 p.0.01, **PPP3CA** -1.97 p:0.005, **GSK3B** -1.77 p:0.005, **ROCK1** -2.23 p:0.03, **IL6ST** 3.36 p.0.02,
Wnt Signaling Pathway NetPath	7	99	110	7.07	2.27	**SFRP1** -1.94 p:0.03, **FZD6** -1.73 p:0.03, **DVL1** 1.51 p:0.004, **GSK3B** -1.77 p:0.005, **DLG1** -1.58 p.0.03, **CAMK2G** 1.74 p.0.03, **SALL1** -2.01 p.0.01
B Cell Receptor Signaling Pathway	9	144	159	6.25	2.18	**RAP2A** -5.63 p.0.04, **PIK3AP1** 1.74 p:0.05, **SH2B2** 2.02 p.0.02, **ATP2B4** -1.55 p.0.03, **GSK3B** -1.77 p:0.005, **ACTR2** -1.71 p:0.02, **RASGRP3** -1.50 p:0.007, **CDK6** -4.43 p:0.03, **PPP3CA** -1.97 p:0.005
Myometrial Relaxation and Contraction Pathways	9	146	161	6.16	2.13	**GUCY1A3** -1.64 p:0.04, **GNB1** -2.71 p:0.02, **ACTB** -1.95 p:0.03, **CALM1** (801) 1.52 p:0.01, **CALM1** (805) -1.50 p:0.005, **RGS5** -2.10 p:0.02, **YWHAB** -1.96 p:0.03, **CAMK2G** 1.74 p.0.03, **GNB1** -2.71 p:0.02
Senescence and Autophagy	6	92	102	6.52	1.88	**CDKN1A** 2.59 p:0.03, **IGFBP7** -2.30 p:0.05, **IFI16** -1.55 p:0.004, **FN1** -2.88 p.0.03, **IL6ST** -3.36 p:0.02, **GSK3B** -1.77 p:0.005,
Type II interferon signaling (IFNG)	3	37	37	8.11	1.74	**IFI6** -2.29 p:0.02, **HIST2H4A** (8364) -7.54 p:0.02, **CYBB** -1.98 p:0.04
Translation Factors	3	38	50	7.89	1.69	**EIF1AX** -1.67 p:0.04, **EIF1AY** 4.02 p:0.000005, **EEF1A1** -1.87 p:0.01
Alpha6-Beta4 Integrin Signaling Pathway	4	61	67	6.56	1.54	**PLEC** 1.65 p:0.02, **YWHAB** -1.96 p:0.03, **PAK1** 1.59 p:0.01, **CDKN1A** 2.59 p:0.03
Endochondral Ossification	4	61	68	6.56	1.54	**FRZB** 3.01 p:0.01, **CALM1** (801) 1.52 p:0.01, **CALM1** (805) -1.50 p:0.005, **TIMP3** -3.45 p:0.02, **SERPINH1** -1.77 p:0.01
Calcium Regulation in the Cardiac Cell	7	136	153	5.15	1.36	**RGS5** -2.10 p:0.02, **YWHAB** -1.96 p:0.03, **CAMK2G** 1.74 p.0.03, **CALM1** (801) 1.52 p:0.01, **CALM1** (805) -1.50 p:0.005, **GNB1** -2.71 p:0.02
Diurnally regulated genes with circadian orthologs	3	45	48	6.67	1.36	**PER2** 1.64 p:0.04, **CRY1** 1.66 p:0.03, **CRY2** 1.64 p:0.007
Id Signaling Pathway	3	47	53	6.38	1.28	**IFI16** -1.55 p:0.004, **ID3** -1.57 p:0.04, **SREBF1** 1.63 p:0.04
Cell cycle	4	81	94	4.94	0.93	**GSK3B** -1.77 p:0.005, **CDK6** -4.43 p:0.03, **CDKN1A** 2.59 p:0.03, **CCNH** -1.55 p:0.005
TGF-beta Receptor Signaling Pathway	6	134	152	4.48	0.9	**ROCK1** -2.23 p:0.03, **CAMK2G** 1.74 p.0.03, **DVL1** 1.51 p:0.004, **YAP1**-1.69 p:0.03, **CDK6** -4.43 p:0.03, **CDKN1A** 2.59 p:0.03
G1 to S cell cycle control	3	59	69	5.08	0.86	**CDK6** -4.43 p:0.03, **CCNH** -1.55 p:0.005, **CDKN1A** 2.59 p:0.03
Wnt Signaling Pathway	3	59	61	5.08	0.86	**FZD6** -1.73 p:0.03, **DVL1** 1.51 p:0.004, **GSK3B** -1.77 p:0.005,

regulated Pathways	pos.	meas.	total	%	Z Score	regulated genes in the pathway
mRNA processing	5	110	131	4.55	0.85	**HNRNPR** -1.85 p:0.02, **SNRNP70** 1.68 p:0.009, **SNRPF** -1.56 p:0.02, **SF3A2** 1.63 p:0.04, **SFRS16** 1.53 p:0.02
AMPK signaling	3	63	77	4.76	0.74	**SREBF1** 1.63 p:0.04, **FASN** 1.66 p:0.03, **CDKN1A** 2.59 p:0.03
IL-5 Signaling Pathway	3	66	69	4.55	0.66	**GSK3B** -1.77 p:0.005, **SOX4** -1.81 p:0.005, **SH2B2** 2.02 p:0.02
Regulation of Actin Cytoskeleton	5	130	157	3.85	0.47	**FN1** -2.88 p:0.03, **ACTB** -1.95 p:0.03, **PAK1** 1.59 p:0.01, **ROCK1** -2.23 p:0.03, **MSN** -1.64 p:0.05
Androgen Receptor Signaling Pathway	4	105	114	3.81	0.4	**IL6ST** -3.36 p:0.02, **NRIP1** -2.91 p:0.006, **POU2F1** 1.57 p:0.002, **CCNH** 1.55 p:0.005,
Focal Adhesion	6	168	190	3.57	0.32	**COL5A1** -1.88 p:0.01, **FN1** -2.88 p:0.03, **ROCK1** -2.23 p.0.03, **ACTB** -1.95 p:0.03, **PAK1** 1.59 p:0.01, **GSK3B** -1.77 p:0.005,
G Protein Signaling Pathways	3	82	96	5.15	5.15	**GNB1** -2.71 p:0.02, **PPP3CA** -1.97 p:0.005, **CALM1** (801) 1.52 p:0.01, **CALM1** (805) -1.50 p:0.005
DNA damage response (only ATM dependent)	3	84	97	3.57	0.23	**CDKN1A** 2.59 p:0.03, **GSK3B** -1.77 p:0.005, **DVL1** 1.51 p:0.004,
Wnt Signaling Pathway and Pluripotency	3	89	101	3.37	0.12	**DVL1** 1.51 p:0.004, **FZD6** -1.73 p:0.03, **GSK3B** -1.77 p:0.005
IL-3 Signaling Pathway	3	95	102	3.16	0.01	**GSK3B** -1.77 p:0.005, **YWHAB** -1.96 p:0.03, **PAK1** 1.59 p:0.01

Pathways containing significantly overrepresented genes from the gene list of male compared to female placentas within the n-3 LCPUFA intervention group

regulated Pathways	pos.	meas.	total	%	Z Score	regulated genes in the pathway
Adipogenesis	3	118	131	2.54	2.63	**BMP2** 1.72 p:0.02, **SOCS1** -1.56 p:0.03, **LEP** -9.85 p:0.03
Myometrial Relaxation and Contraction Pathways	3	146	161	2.05	2.19	**CXCR7** -1.74 p:0.03, **IGFBP1** 16.07 p:0.03, **IGFBP2** 1.72 p:0.03

Pathways containing significantly overrepresented genes from the gene list of male compared to female placentas within the control and the n-3 LCPUFA intervention group

regulated Pathways	pos.	meas.	total	%	Z Score	regulated genes in the pathway
Sulfation	4	14	29	28.57	4,21	**SULT1A1 -**2.08 p:0.03, **PAPSS1** -2.35 p:0.005, **PAPSS2** -2.30 p:0.008, **SULT2B1** 1.74 p:0.0004
mRNA processing	12	110	131	10.91	3,10	**RNMT** 1.54 p:0.006, **HNRNPC** 1.63 p:0.03, **HNRNPR** -2.05 p:0.02, **SNRNP70** 1.70 p:0.008, **SNRPD1** -1.73 p:0.001, **SMC1A** -2.22 p:0.000005, **SF3A1** -1.55 p:0.001, **SF3A2** 2.18 p:0.006, **CLK3** 1.61 p:0.004, **CELF2** -1.69 p:0.03, **RBM39** 1.60 p:0.007, **NONO** -1.50 p:0.02
miRNAs involved in DDR	3	15	70	20.00	2,79	**TP53** 1.53 p:0.002, **CDK6** -4.19 p:0.04, **CDKN1A** 2.36 p:0.03
Nucleotide Metabolism	3	17	36	17.65	2,51	**MTHFD2** -2.28 p:0.008, **HPRT1** -1.65 p:0.03, **RRM2B** 1.51 p:0.04
Translation Factors	5	38	50	13.16	2,46	**EIF1AX** -2.43 p:0.0007, **EIF1AY** 12.4 p:0.00000003, **EIF2S3** -1.83 p:0.003, **EIF2B5** 1.54 p:0.02, **EEF1A1** -1.89 p:0.01
Vitamin A and carotenoid metabolism	5	39	66	12.82	2,39	**ADH4** -1.59 p:0.03, **RDH10** -1.84 p.0.01, **RXRB** 1.65 p:0.0003, **SULT1A1** -2.08 p:0.03, **SULT2B1** 1.74 p:0.0004
MicroRNAs in cardiomyocyte hypertrophy	8	77	109	10.39	2,36	**CALM2** (805) -1.69 p:0.0004, **PPP3CA** -2.20 p:0.0002, **MAP2K1** -1.70 p:0.02, **IKBKB** 1.51 p:0.007, **RHOA** -1.70 p:0.04, **IL6ST** -3.19 p:0.02, **FZD1** -1.65 p:0.02, **EIF2B5** 1.54 p:0.02
Androgen Receptor Signaling Pathway	10	105	114	9.52	2,35	**IL6ST** -3.19 p:0.02, **MAP2K1** -1.70 p:0.02, **RCHY1** -1.61 p:0.04, **YWHAH** -1.68 p:0.03, **NRIP1** -3.24 p:0.04, **POU2F1** 1.53 p:0.05, **CCNH** -1.67 p:0.01, **NCOA3** -1.68 p:0.05, **TP53** 1.53 p:0.002, **HMGB1** -1.52 p:0.03
estrogen signalling	3	19	23	15.79	2,28	**GNB1** -3.31 p:0.008, **BRAF** -1.60 p:0.01, **MAP2K1** -1.70 p:0.02
Glycogen Metabolism	4	32	43	12.50	2,08	**PYGL** -1.80 p:0.04, **CALM2** (805) -1.69 p:0.0004 (3x)
Alpha6-Beta4 Integrin Signaling Pathway	6	61	67	9.84	1,89	**RHOA** -1.70 p:0.04, **PLEC** 1.51 p:0.03, **YWHAB** -2.18 p:0.01, **YWHAH** -1.68 p:0.03, **PAK1** 1.87 p:0.01, **CDKN1A** 2.36 p:0.03

regulated Pathways	pos.	meas.	total	%	Z Score	regulated genes in the pathway
Type II interferon signaling (IFNG)	4	37	37	10.81	1,75	**SOCS1** -2.06 p:0.02, **IFI6** -2.10 p:0.04, **CYBB** -2.56 p:0.006
DNA damage response	6	66	71	9.09	1,68	**LRDD** 1.54 p:0.0005, **RRM2B** 1.51 p:0.04, **CDKN1A** 2.36 p:0.03, **TP53** 1.53 p:0.002, **SMC1A** -2.22 p:0.000005, **CDK6** -4.19 p:0.04
Cell cycle	7	81	94	8.64	1,68	**SMC1A** -2.22 p:0.000005, **TP53** 1.53 p:0.002, **CDKN1A** 2.36 p:0.03, **CCNH** -1.67 p:0.01, **CDK6** -4.19 p:0.04, **DBF4** -1.85 p:0.0001, **E2F3** -1.59 p:0.002
DNA damage response (only ATM dependent)	7	84	97	8.33	1,57	**SCP2** -1.60 p:0.01, **RAC2** -1.58 p:0.04, **HMGB1** -1.52 p:0.03, **TP53** 1.53 p:0.002, **CDKN1A** 2.36 p:0.03, **DVL2** 1.52 p:0.005, **RHOA** -1.70 p:0.04
Ovarian Infertility Genes	3	27	32	11.11	1,57	**SMPD1** 1.53 p:0.04, **INHA** 4.65 p:0.008, **NRIP1** -3.24 p:0.04
G1 to S cell cycle control	5	59	69	8.47	1,36	**CDK6** -4.19 p:0.04, **CCNH** -1.67 p:0.01, **TP53** 1.53 p:0.002, **CDKN1A** 2.36 p:0.03, **E2F3** -1.59 p:0.002
Fatty Acid Beta Oxidation	3	30	43	10.00	1,36	**PNPLA2** 1.73 p:0.003, **ACADVL** 1.68 p:0.008, **GK** -1,88 p:0.01
Endochondral Ossification	5	61	68	8.20	1,28	**FRZB** 2.25 p:0.04, **KIF3A** -2.54 p:0.04, **SCIN** 1.71 p:0.02, **CALM2** (805) -1.69 p:0.0004, **TIMP3** -3.16 p:0.04
EGFR1 Signaling Pathway	11	164	177	6.71	1,22	**PAK1** 1.87 p:0.01, **PLSCR1** -1.55 p:0.04, **WNK1** -2.83 p:0.05, **TNK2** 1.52 p:0.008, **SOCS1** -2.06 p:0.02, **RGS16** 1.81 p:0.03, **KRT7** 1.64 p:0.03, **EEF1A1** -1.89 p:0.01, **MAP2K1** -1.70 p:0.02, **YWHAB** -2.18 p:0.01, **PLEC** 1.51 p:0.03
Regulation of Actin Cytoskeleton	9	130	157	6.92	1,20	**RRAS2** 1.66 p:0.04, **BRAF** -1.59 p:0.01, **MAP2K1** -1.70 p:0.02, **ACTB** -2.65 p:0.05, **RHOA** -1.70 p:0.04, **RAC2** -1.58 p:0.04, **MSN** -1.98 p:0.01, **PAK1** 1.87 p:0.01, **C3orf10** -1.53 p:0.03
Type II diabetes mellitus	2	19	26	10.53	1,19	**IKBKB** 1.51 p:0.007, **GK** -1,88 p:0.01
Wnt Signaling Pathway NetPath	7	99	110	7.07	1,11	**FZD1** -1.65 p:0.02, **DVL2** 1.52 p:0.005, **DLG1** -2.03 p:0.03, **CAMK2G** 1.92 p:0.03, **RHOA** -1.70 p:0.04, **TCF4** -2.13 p:0.02, **SALL1** -2.05 p:0.02
G13 Signaling Pathway	3	35	38	8.57	1,07	**RHOA** -1.70 p:0.04, **CALM2** (805) -1.69 p:0.0004, **TNK2** 1.52 p:0.008
Adipogenesis	8	118	131	6.78	1,06	**MBNL1** -5.16 p:0.05, **NRIP1** -3.24 p:0.04, **SOCS1** -2.06 p:0.02, **FZD1** -1.65 p:0.02, **IL6ST** -3.19 p:0.02, **FRZB** 2.25 p:0.04, **CDKN1A** 2.36 p:0.03, **LPIN1** 2.59 p:0.02,
Calcium Regulation in the Cardiac Cell	9	136	153	6.62	1,06	**RGS16** 1.81 p:0.03, **CAMK2G** 1.92 p:0.03, **YWHAB** -2.18 p:0.01, **YWHAH** -1.68 p:0.03, **CALM2** (805) -1.69 p:0.0004 (3x), **GNB1** -3.31 p:0.008, **GNAI3** -1.63 p:0.01
B Cell Receptor Signaling Pathway	9	144	159	6.25	0,88	**RAP2A** -5.59 p:0.01, **SH2B2** 2.00 p:0.02, **BRAF** -1.60 p:0.01, **PPP3CA** -2.20 p:0.0002, **IKBKB** 1.51 p:0.007, **MAP2K1** -1.70 p:0.02, **CDK6** -4.19 p:0.04, **ACTR2** -2.11 p:0.004, **RHOA** -1.70 p:0.04,
T Cell Receptor Signaling Pathway	8	126	135	6.35	0,87	**DLG1** -2.03 p:0.03, **DNM2** 1.58 p:0.0005, **PAK1** 1.87 p:0.01, **RAC2** -1.58 p:0.04, **MAP2K1** -1.70 p:0.02, **PRKD2** 1.71 p:0.0002, **BRAF** -1.60 p:0.01, **CABIN1** 1.59 p:0.02,
MAPK signaling pathway	9	146	167	6.16	0,83	**BRAF** -1.60 p:0.01, **MAP2K1** -1.70 p:0.02, **IKBKB** 1.51 p:0.007, **PPP3CA** -2.20 p:0.0002, **PAK1** 1.87 p:0.01, **RAC2** -1.58 p:0.04, **TP53** 1.53 p:0.002, **MAPKAPK5** 1.52 p:0.009, **TAOK1** -3.25 p:0.01
Myometrial Relaxation and Contraction Pathways	9	146	161	6.16	0,83	**GNB1** -3.31 p:0.008, **ACTB** -2.66 p:0.05, **CALM2** (805) -1.69 p:0.0004 (3x), **CAMK2G** 1.92 p:0.03, **YWHAB** -2.18 p:0.01, **YWHAH** -1.68 p:0.03, **RGS16** 1.81 p:0.03,
Diurnally regulated genes with circadian orthologs	3	45	48	6.67	0,61	**ARNTL** -1.74 p:0.006, **CRY2** 1.64 p:0.002, **DAZAP2** -1.58 p:0.03
G Protein Signaling Pathways	5	82	96	6.10	0,59	**GNB1** -3.31 p:0.008, **GNAI3** -1.63 p:0.01, **PPP3CA** -2.20 p:0.0002, **CALM2** (805) -1.69 p:0.0004, **RHOA** -1.70 p:0.04
Cytoplasmic Ribosomal Proteins	3	46	88	6.52	0,58	**RPL23** -1.65 p:0.003, **RPL31** -2.64 p:0.008, **RPS4Y1** 1957.69 p:0.000000000000004

regulated Pathways	pos.	meas.	total	%	Z Score	regulated genes in the pathway
TNF-alpha/NF-kB Signaling Pathway	9	170	188	5.29	0,35	**PEG3** -3.34 p:0.04, **YWHAB** -2.18 p:0.01, **YWHAH** -1.68 p:0.03, **IKBKB** 1.51 p:0.007, **GLG1** 1.73 p:0.03, **DDX3X** -1.69 p:0.0001, **POLR1D** 1.76 p:0.003, **NFKBIZ** -4.52 p.0.03, **KPNA6** 1.51 p:0.007
cytochrome P450	3	54	72	5.56	0,29	**CYB5A** -1.59 p:0.007, **CYB5R2** 1.67 p.0.02, **CYP4F12** -1.52 p.0.002
TGF-beta Receptor Signaling Pathway	7	134	152	5.22	0,27	**HGS** 1.72 p:0.004, **CAMK2G** 1.92 p.0.03, **CDK6** -4.19 p:0.04, **CDKN1A** 2.36 p.0.03, **JUNB** 1.66 p:0.04, **EID2** 1.83 p:0.01, **TP53** 1.53 p:0.002
Wnt Signaling Pathway	3	59	61	5.08	0,13	**FZD1** -1.65 p:0.02, **DVL2** 1.52 p:0.005, **RHOA** -1.70 p:0.04
Toll-like receptor signaling pathway	4	84	108	4.76	0,01	**LY96** -1.73 p:0.01, **IKBKB** 1.51 p:0.007, **MAP2K1** -1.70 p:0.02, **IFNA7** 1.62 p:0.0003
AMPK signaling	3	63	77	4.76	0,01	**CDKN1A** 2.36 p.0.03, **TP53** 1.53 p:0.002, **FASN** 1.99 p.0.002
Kit Receptor Signaling Pathway	3	63	67	4.76	0,01	**MAP2K1** -1.70 p:0.02, **SOCS1** -2.06 p.0.02, **SH2B2** 2.00 p:0.02

Pos: positive, number of genes in the pathway fulfilling the significance criteria FC ≥ +1.5 and p < 0.05 and FC ≤ -1.5 and p < 0.05 in the transcriptome analysis; meas: measured, number of genes within the pathway measured by the transcriptome analysis; total: number of genes belonging to the pathway; %,number of genes measured d divided by number of positive genes. The Z-scores were calculated by subtracting the number of expected genes to be regulated within the pathway from the observed number of significantly regulated genes in the pathway (pos) and dividing this difference by the SD of the significantly regulated genes in the transcriptome analysis (pos) [135]. Z-score >0.0 means a significant overrepresentation of regulated genes in the transcriptome analysis within the respective pathway. The genes with significant regulations in the transcriptome analysis shown along with their fold changes and p values.

11.12. Complete lists of significantly overrepresented pathways in the datasets of the n-6/n-3 LCPUFA intervention compared to the control group

Pathways containing significantly overrepresented genes from the gene list of placentas of male and female children

regulated Pathways	pos.	meas.	total	%	Z Score	regulated genes in the pathway
Hedgehog Signaling Pathway	3	21	22	14.29	5.25	**GAS1** -2.2 p:0.04, **CCNB1** -1.6 p:0.02, **HHIP** 1.5 p: 0.005
DNA Replication	4	38	49	10.53	5.03	**ASK** -1.7 p:0.0005, **RFC4** -1.6 p:0.045, **RFC3** -1,7 p:0.006, **MCM3** -1.5 p:0.02
DNA damage response	4	66	71	6.06	3.43	**APAF1** -1.7 p: 0.005, **CDK4** -1.6 p:0.01, **CCNB1** -1.6 p:0.02, **RAD52** -1.5 p:0.03
Cell cycle	4	81	94	4.94	2.91	**CDK4** -1.6 p:0.01, **CCNB1** -1.6 p:0.02, **MCM3** -1.5 p:0.02, **ASK** -1.7 p:0.0005
G1 to S cell cycle control	3	59	69	5.08	2.57	**CDK4** -1.6 p:0.01, **CCNB1** -1.6 p:0.02, **MCM3** -1.5 p:0.02
Apoptosis	3	77	84	3.90	2.02	**APAF1** -1.7 p: 0.005, **BIRCA** -1.6 p:0.047, **BOK** -1.7 p:0.009
G Protein Signaling Pathways	3	82	96	3.66	1.89	**AKAP1** -1.5 p:0.004, **PRKD3** -2.1 p:0.04, **GNAL** 1.5 p:0.02
GPCRs, Class A Rhodopsin-like	3	225	269	1.33	0.03	**CX3CR1** 3.8 p:0.02, **F2RL1**-1.8 p:0.01, **GPR1** 1.5 p:0.02

Pathways containing significantly overrepresented genes from the gene list of placentas of male children

regulated Pathways	pos.	meas.	total	%	Z Score	regulated genes in the pathway
Myometrial Relaxation and Contraction Pathways	5	146	161	3.42	3.82	**IGFBP1** 10.9 p:0.04, **CMKOR1** -1.6 p:0.05, **CALM1** -1.5 p:0.01(3x)

Pathways containing significantly overrepresented genes from the gene list of placentas of female children

regulated Pathways	pos.	meas.	total	%	Z Score	regulated genes in the pathway
Cell cycle	5	81	94	6.17	3.02	**TGFß** 2.0 p:0.03, **HDAC5** 1.6 p:0.02, **CDK6** -2.0 p:0.02, **CDC2** -1.7 p:0.02, **MAD2L1** -1.55 p:0.02
Endochondral Ossification	4	61	68	6.56	2.84	**FRZB1** 2.6 p:0.005, **TGFß1** 2.0 p:0.03, **MEF2c** -1.7 p: 0.025, **TIMP3** -1.9 p:0.04
TGF-beta Receptor Signaling Pathway	6	134	152	4.48	2.40	**TGFß1** 2.0 p:0.03, **ANAPC4** -1.7 p:0.009, **MEF2c** -1.7 p: 0.025, **CDK6** -2.0 p:0.02, **CDC2** -1.7 p:0.02, **TCF8** -1.8 p:0.045
MicroRNAs in cardiomyocyte hypertrophy	4	77	109	5.19	2.29	**HDAC5** 1.6 p:0.02 (--> miR 30e),**TGFß** 2.0 p:0.03, **PPP3CA** -1.5 p:0.003, **CaINA** -1.7 p:0.003
B Cell Receptor Signaling Pathway	6	144	159	4.17	2.21	**PIK3AP1** 1.6 p:0.01, **HDAC5** 1.6 p:0.02, **BCL6** 1.6 p:0.04, **CDK6** -2.0 p:0.02, **PPP3CA** -1.5 p:0.003
DNA damage response (only ATM dependent)	4	84	97	4.76	2.09	**BCL6** 1.6 p:0.04, **TGFß1** 2.0 p:0.03, **TGF1** 2.0 p:0.03, **PDK1** -1.5 p:0.006,
Adipogenesis	5	118	131	4.24	2.05	**TGFß1** 2.0 p:0.03, **LPL** 2.3 p:0,04 , **FRZB** 2.6 p:0.005 , **BMP1** 2.5 p:0.047, **MEF2c** -1.7 p: 0.025,
IL-5 Signaling Pathway	3	66	69	4.55	1.71	**RAP1GAP** 1.55 p:0.03, **APS**: 1.6 p:0.04, **SOX4** -1.7 p: 0.0003
Wnt Signaling Pathway NetPath	3	99	110	3.03	0.95	**LRP6** -1.5 p:0.04, **SFRB1** -1.9 p:0.01 , **CDC2 (CDK1)** -1.7 p:0.02
Calcium Regulation in the Cardiac Cell	3	136	153	2.21	0.38	**RGS5** -2.0 p:0.03, **PRKACB** -1.5 p:0.04, **ATP2B1** -1.7 p:0.002
MAPK signaling pathway	3	146	167	2.05	0.25	**TGFß1** 2.0 p:0.03, **MEF2c** -1.7 p: 0.025 , **PPP3CA** -1.5 p:0.003
Myometrial Relaxation and Contraction Pathways	3	146	161	2.05	0.25	**PRKACB** -1.5 p:0.04, **EDG2** -1.9 p:0.008, **RGS5** -2.0 p:0.03,
Insulin Signaling	3	152	161	1.97	0.18	**APS**: 1.6 p:0.04, **MAP4K5** -1.7 p:0.006, **RHOQ** -1.6 p:0.04

Pos: positive, number of genes in the pathway fulfilling the significance criteria FC ≥ +1.5 and p < 0.05 and FC ≤ -1.5 and p < 0.05 in the transcriptome analysis; meas: measured, number of genes within the pathway measured by the transcriptome analysis; total: number of genes belonging to the pathway; %,number of genes measured d divided by number of positive genes. The Z-scores were calculated by subtracting the number of expected genes to be regulated within the pathway from the observed number of significantly regulated genes in the pathway (pos) and dividing this difference by the SD of the significantly regulated genes in the transcriptome analysis (pos) [135]. Z-score >0.0 means a significant overrepresentation of regulated genes in the transcriptome analysis within the respective pathway. The genes with significant regulations in the transcriptome analysis shown along with their fold changes and p values.

11.13. Raw data and statistical analysis of miRNome profiling

The DNA microarray data are confidential for publication purposes. Access to these data will be provided for the PhD thesis committee by a compact disc attached to the thesis.

11.14. Explorative microRNA profiling data: Expression of microRNAs in the placenta-specific C19MC microRNA cluster

Plate	Detector	Flag CF	Flag IF	Raw Cq		Norm. Cq		median Cq	log RQ	threshold		Ex-treme	FC
				CF	IF	CF	IF			high	low		
B	hsa-miR-498-4373223	Yes	Yes	Inf	Inf	Inf	Inf	NA	NA	NA	-NA	NA	NA
A	hsa-miR-512-3p-4381034	No	No	14.9	12.9	13.1	13.1	13.1	0.01	0.22	-0.30	no	1.01
A	hsa-miR-512-5p-4373238	No	No	18.8	18.2	18.5	18.5	18.5	-0.04	0.42	-0.54	no	-1.03
A	hsa-miR-515-3p-4395480	No	No	17.0	16.5	16.7	16.7	16.7	-0.09	0.34	-0.45	no	-1.07
A	hsa-miR-515-5p-4373242	No	No	16.6	15.6	15.6	15.9	15.8	-0.26	0.30	-0.40	no	-1.20
B	hsa-miR-516a-3p-4373183	No	No	23.9	23.5	23.7	23.7	23.7	-0.04	0.77	-0.95	no	-1.03
A	hsa-miR-516a-5p-4395527	No	No	22.4	22.2	22.6	22.4	22.5	0.21	0.67	-0.84	no	1.15
A	hsa-miR-516b-4395172	No	No	16.0	15.3	15.4	15.5	15.5	-0.14	0.29	-0.39	no	-1.10
B	hsa-miR-517*-4378078	No	No	24.5	23.7	24.2	23.9	24.1	0.31	0.80	-0.99	no	1.24
A	hsa-miR-517a-4395513	No	No	14.5	11.6	11.8	11.7	11.8	0.08	0.19	-0.27	no	1.06
A	**hsa-miR-517b-4373244**	**No**	**No**	**18.6**	**16.5**	**17.3**	**16.8**	**17.1**	**0.50**	**0.36**	**-0.46**	**yes**	**1.41**
A	hsa-miR-517c-4373264	No	No	14.7	12.0	12.1	12.2	12.1	-0.07	0.20	-0.28	no	-1.05
A	hsa-miR-518a-3p-4395508	No	No	17.9	17.1	17.7	17.4	17.6	0.30	0.38	-0.49	no	1.23
A	hsa-miR-518a-5p-4395507	No	No	25.2	25.5	25.3	25.8	25.6	-0.50	0.94	-1.15	no	-1.41
A	hsa-miR-518b-4373246	No	No	16.4	15.5	15.9	15.8	15.8	0.07	0.30	-0.41	no	1.05
B	hsa-miR-518c*-4378082	No	No	21.3	21.1	21.0	21.3	21.2	-0.32	0.58	-0.73	no	-1.25
A	hsa-miR-518c-4395512	No	No	19.3	19.0	19.1	19.2	19.2	-0.19	0.46	-0.58	no	-1.14
A	hsa-miR-518d-3p-4373248	No	No	24.3	24.5	24.5	24.8	24.6	-0.27	0.85	-1.05	no	-1.21
B	hsa-miR-518e*-4395482	No	No	22.7	22.3	22.5	22.5	22.5	-0.06	0.67	-0.84	no	-1.04
A	hsa-miR-518e-4395506	No	No	15.4	14.5	14.8	14.7	14.8	0.07	0.27	-0.36	no	1.05
B	hsa-miR-518f*-4395498	No	No	26.4	26.3	26.3	26.5	26.4	-0.20	1.02	-1.25	no	-1.15
A	hsa-miR-518f-4395499	No	No	15.7	14.8	15.0	15.0	15.0	-0.01	0.28	-0.37	no	-1.01
B	hsa-miR-519b-3p-4395495	No	No	17.6	17.1	17.1	17.4	17.3	-0.33	0.36	-0.47	no	-1.25
A	hsa-miR-519c-3p-4373251	No	No	20.8	20.9	20.6	21.2	20.9	-0.56	0.56	-0.71	no	-1.48
A	hsa-miR-519d-4395514	No	No	15.0	13.5	13.9	13.7	13.8	0.23	0.24	-0.33	no	1.18
B	hsa-miR-519e*-4378084	No	No	20.6	20.2	20.4	20.4	20.4	-0.06	0.53	-0.67	no	-1.04
A	hsa-miR-519e-4395481	No	No	20.7	20.2	20.7	20.5	20.6	0.25	0.54	-0.68	no	1.19
A	hsa-miR-520a-3p-4373268	No	No	19.2	18.7	19.1	19.0	19.1	0.09	0.45	-0.58	no	1.06
A	hsa-miR-520a-5p-4378085	No	No	20.1	19.9	20.1	20.1	20.1	0.00	0.51	-0.65	no	1.00
A	hsa-miR-520b-4373252	No	No	24.1	24.7	24.8	25.0	24.9	-0.15	0.87	-1.08	no	-1.11
B	hsa-miR-520c-3p-4395511	No	No	17.3	16.9	16.7	17.1	16.9	-0.38	0.35	-0.46	no	-1.30
	hsa-miR-520d-3p	No primer present on the low-density array											
A	hsa-miR-520e-4373255	No	No	29.6	29.8	30.6	30.0	30.3	0.58	1.49	-1.80	no	1.50
A	hsa-miR-520f-4373256	No	No	23.1	22.9	23.2	23.1	23.2	0.12	0.73	-0.90	no	1.08
A	hsa-miR-520g-4373257	No	No	18.1	17.5	17.9	17.8	17.8	0.05	0.39	-0.51	no	1.03
B	hsa-miR-520h-4373258	No	No	18.9	18.3	18.5	18.5	18.5	0.03	0.42	-0.54	no	1.02
A	hsa-miR-521-4373259	No	No	19.1	18.9	19.0	19.2	19.1	-0.17	0.45	-0.58	no	-1.12
A	**hsa-miR-522-4395524**	**No**	**No**	**16.2**	**15.5**	**15.4**	**15.8**	**15.6**	**-0.40**	**0.29**	**-0.39**	**yes**	**-1.32**
A	hsa-miR-523-4395497	No	No	18.4	17.9	18.3	18.2	18.2	0.07	0.41	-0.53	no	1.05
B	hsa-miR-524-3p-4378087	No	No	20.0	19.4	19.7	19.7	19.7	0.06	0.49	-0.62	no	1.04
B	hsa-miR-524-3p-4378087§	No	No	20.0	19.5	19.7	19.6	19.7	0.12	0.49	-0.62	no	1.09
A	hsa-miR-525-3p-4395496	No	No	16.4	15.4	15.7	15.7	15.7	0.05	0.30	-0.40	no	1.03
A	hsa-miR-525-5p-4378088	No	No	19.2	18.9	19.1	19.2	19.2	-0.13	0.46	-0.58	no	-1.10
B	hsa-miR-526b*-4395494	No	No	19.8	19.1	19.5	19.3	19.4	0.25	0.47	-0.60	no	1.19
A	hsa-miR-526b-4395493	No	No	18.5	18.0	18.4	18.3	18.4	0.03	0.41	-0.53	no	1.02
	Hsa-miR-1283	No primer present on the low-density array											
	Hsa-miR-1323	No primer present on the low-density array											

MicroRNA profiling of n-6/n-3 LCPUFA intervention group compared to the control group in female placentas (IF vs. CF, pool of n=3 in each analysis group). The microRNA profiling was conducted on two plates, depicted as plate A or B. The columns Flag CF and Flag IF indicate whether there was a problem in the amplification of the RT-qPCR. No = no problem, yes = flagged, problem in the amplification (often flagged when there is no amplification). The raw Cq values and the normalized Cq (norm. Cq) after loess normalization are shown. Median Cq was calculated from normalized Cq values. Log RQ was calculated by (norm. Cq IF – norm. Cq CF). The high and low thresholds were calculated with quantile regression with a quadratic model. LogRQ values below the 5^{th} and above the 95^{th} percentile were marked with yes in the column extreme. LogRQ values within the 5^{th}-95^{th} percentile were marked with no in the column extreme. LogRQ values below the 5^{th} and 95^{th} were considered to be regulated. Fold changes (FC) were calculated by 2^{logRQ} or -2^{logRQ} (in case of negative logRQ). Inf = infinite, NA = not applicable, §, duplicate

11.15. MicroRNAs and their significantly regulated genes with microRNA binding sites from transcriptome datasets analyzed by DIANA miR-ExTra

microRNA ID	Gene names
hsa-miR-888	MAP4K5, MAPK6, ATP8B1, ESF1, MKNK2, SLCO4A1, CEP192, SETD6, **CDK6, HDAC5**, COL12A1, MCM3, LMNB1, BCL6, CSF3R, SSB, IGFBP1, ZEB1, SERPINH1, DNAJB4, GNL3, **MAD2L1**, HHIP, CX3CR1, IL8, PTGER4, BOK, SEPW1, SERTAD2, GAS1, FAM46C, BRWD1, ZNF682
hsa-miR-375	CRISPLD1, VPS13B, ID4, PRR16
hsa-miR-586	LPGAT1
hsa-miR-130b	PDIA5, ERC1, SMOC2, ENPP5, PRKD3, RBM25, HOXB3, AKAP1, SOX4, **SLC6A6**, CNTN4, PDK1, FRZB, FBN1, BMP1, NPNT, PRNP, GAP43, MOB1B, SERTAD2, BRWD1, SESTD1
hsa-miR-320	BCAT1, **CDK6,** SC5DL, WLS, SOX4, VPS13B, WWC2, PDK1, RACGAP1, PROK2, HECTD2, CD47, ZNF138
hsa-miR-21	MSH2, TIMP3, NAGK, TRIP10, GLIS2, MYCN, **FZD7**, NUP35, STOX2, PRR16, SH2D5
hsa-miR-522	TIMP3, SC5DL, AKAP1, TULP4, RFC3, FGF2, OSBPL10, DACH1, MOB1B, BRWD1
hsa-miR-30d	DBF4, ARID4A, PI4K2B, LIMCH1, EML1, ATP2B1, PGM1, ATP8B1, SNX10, SEMA6A, TIMP3, ZMYND8, TBL1X, DNMBP, **HDAC5**, COL12A1, BCL6, RND3, S100PBP, B4GALT6, RBM25, LPGAT1, SOX4, TUBGCP3, TULP4, **SLC6A6**, WDR44, MEIS2, AGTPBP1, RCBTB1, PDCL, PP3CA,CNTN4, ABI3BP, CA10, EPB41, FRZB, WDR43, HECTD2, ING2, ZNF318, STOX2, CHST2, BCOR, FAM46C, BRWD1, DMD
hsa-miR-451	-
hsa-miR-495	SKAP2, CD9, MAP4K5, XK, BCAT1, MAPK6, ATP2B1, GPC4, MEF2C, ERC1, RAB10, ARHGAP28, MKNK2, PAPLN, TBL1X, ZDHHC2, **CDK6, HDAC5**, SLC35F2, KCTD10, KIAA0240, PRKD3, SMG7, B4GALT6, ANKRD13C, APAF1, SOX4, METTL16, POT1, TULP4, VPS13B, AGTPBP1, PDCL, MDC1, FGF2, PPP3CA, PRKACB, PRSS23, PIK3AP1**, FZD7**, SLC26A2, FAM122B, CUL4B, RAPGEF6, ATXN7L2, CITED2, HECTD2, DACH1, MLLT3, RAB33B, GAP43, ID4, SLC2A14, MOB1B, GAS1, TMEM45A, MXRA7, BCOR, DAPK1, CD47, SMC5, DMD
hsa-miR-517b	SPRTN, ARID4A, ATP2B1, MEF2C, RAB10, MKNK2, TIMP3, RASSF2, TBL1X, **CDK6**, MAP3K8, SMC3, KIAA0240, ENPP5, BCL6, ECT2, RND3, SMG7, NAGK, TRIP10, MBD2, MYCN, PRRG4, RAB11FIP5, SERPINE2, PPP3CA, PRKACB, TNFRSF21, AP1S3, CA10, ATAD2, EPB41, RACGAP1, OLFML2B, NUP35, WDR43, IL8, KLF11, SERTAD2, NPM1, FAM46C, PRR16, WDR45
hsa-miR-139-5p	PGM1, ATP8B1, SNX10, **CDK6**, TUBGCP3, MEIS2, PPP3CA, FRZB, WDR43, HECTD2, ING2, ZNF318, CHST2
hsa-miR-99a	PLEKHG6, ARID4A, PDIA5, MAPK6, ARHGAP28, RBFOX2, PPP3CA, LRIG3, ATXN7L2, HECTD2, ST5, ING2, ZNF318, GAP43, SULF2, LRIG2
hsa-miR-100	PLEKHG6, ARID4A, PDIA5, MAPK6, ARHGAP28, RBFOX2, PPP3CA, LRIG3, ATXN7L2, HECTD2, ST5, ING2, ZNF318, GAP43, SULF2, LRIG2
hsa-miR-668	SKAP2, CYBA, ESF1, INHA, PEPD, VPS13B
hsa-miR-641	ATP2B1, **CDK6**, SC5DL, LMNB1, B4GALT6, RHOQ, PRKACB, RAPGEF6
hsa-miR-302b	VEZT, ARID4A, PDIA5, MKNK2, RASSF2, SC5DL, KIAA0240, BCL6, PLCL1, RND3, VPS13B, MBD2, PRRG4, RAPGEF5, ATAD2, IL8, FAM46C, SH2D5
hsa-miR-367	SNAPC1, VEZT, ARID4A, TMCC3, BCAT1, PDIA5, ASPN, COL12A1, BCL6, ANKRD13C, SOX4, TULP4, VPS13B, PPP1R3D, PRRG4, AP1S3, RAPGEF6, MORC3, FBN1, GAP43, SERTAD2, SH2D5, DMD
hsa-miR-649	VPS13B, DMD
hsa-miR-302a	VEZT, ARID4A, SCML1, LIMCH1, PDIA5, MKNK2, RASSF2, SC5DL, KIAA0240, BCL6, PLCL1, RND3, VPS13B, MBD2, PRRG4, RAPGEF5, SERPINH1, ATAD2, IL8, FAM46C, SH2D5
hsa-miR-569	RGS5, SPRED2
hsa-miR-630	SKAP2, PLEKHG6, HSD17B6, ARID4A, MRPS35, LIMCH1, ATP8B1, TBL1X, ABHD11, TXNDC15, NDUFS7, TRIP10, METTL16, WDR44, TNS4, PRRG4, FLNB, ATAD2, GDPD5, DAGLB, RAB43, OVOL1, ZNF816, H1F0, SH2D5, SULF2, AP1G2
hsa-miR-155	MAP4K5, SNAPC1, BCAT1, S100PBP, ZEB1, ING2, MOB1B, BASP1, SERTAD2, ZNF138

11.16. Summary of data for biological validation of selected mRNAs by RT-qPCR

mRNA		n	FC ± SD (n)	Cq_{min} - Cq_{max}	no template controls		Statistical analysis		
CDK6	CF	10	100 ± 37	24.68 - 27.05	x	x	Two-way ANOVA on ranks	p*	**0.027**
	CM	8	106 ± 39	25.57 - 26.86	x	x		p#	0.757
	IF	10	132 ± 49	25.29 - 26.77	**no RTs**			p*#	0.607
	IM	10	120 ± 43	25.20 - 26.57	x	x	Post-hoc tests Holm-Sidak	p# CM vs CF	0.887
	CG	18	100 ± 36	24.68 - 27.05	x	x		p# IM vs IF	0.549
	IG	20	123 ± 44	25.20 - 26.77	Δno RT- mean Cq			p* IF vs CF	**0.046**
	Primer efficiency			85.9%	nd			p* IM vs CM	0.225
CDK1	CF	11	100 ± 23	26.33 - 27.59	38.71§	36.87§	Two-way ANOVA	p*	0.304
	CM	9	91 ± 28	26.69 - 28.31	37.65§	40.24§		p#	0.230
	IF	10	101 ± 58	26.45 - 28.21	**no RTs**			p*#	0.830
	IM	10	99 ± 23	26.07 - 28.21	x	x	Post-hoc tests Holm-Sidak	p# CM vs CF	nd
	CG	20	100 ± 30	26.33 - 28.31	x	x		p# IM vs IF	nd
	IG	20	104 ± 47	26.07 - 28.21	Δno RT- mean Cq			p* IF vs CF	nd
	Primer efficiency			83.5%	nd			p* IM vs CM	nd
TGFB1	CF	11	100 ± 45	22.31 - 24.29	34.85§	35.15§	Two-way ANOVA	p*	0.925
	CM	9	160 ± 71	21.73 - 23.78	34.55§	34.84§		p#	0.086
	IF	10	132 ± 48	22.44 - 23.88	**no RTs**			p*#	**0.001**
	IM	11	116 ± 50	22.21 - 24.29	nd	nd	Post-hoc tests Holm-Sidak	p# CM vs CF	**<0.001**
	CG	20	100 ± 56	21.73 - 24.29	nd	nd		p# IM vs IF	0.211
	IG	21	97 ± 45	22.21 - 24.29	Δno RT- mean Cq			p* IF vs CF	**0.018**
	Primer efficiency			89.3%	nd			p* IM vs CM	**0.016**
MAD2L1	CF	11	100 ± 16	27.95 - 29.11	x	nd	Two-way ANOVA on ranks	p*	0.746
	CM	9	98 ± 24	28.15 - 29.39	x	nd		p#	0.146
	IF	10	114 ± 41	28.13 - 29.04	**no RTs**			p*#	0.533
	IM	11	95 ± 16	27.64 - 29.44	35.69	36.44	Post-hoc tests Holm-Sidak	p# CM vs CF	nd
	CG	20	100 ± 23	27.95 - 29.39	37.30	36.35		p# IM vs IF	nd
	IG	21	105 ± 34	27.64 - 29.44	Δno RT- mean Cq			p* IF vs CF	nd
	Primer efficiency			90.8%	36.45-28.52=7.93			p* IM vs CM	nd
ANAPC4	CF	11	100 ± 25	25.91 - 27.38	37.72§	x	Two-way ANOVA	p*	0.416
	CM	9	99 ± 21	26.55 - 32.14	x	x		p#	0.885
	IF	10	94 ± 27	26.63 - 28.02	**no RTs**			p*#	0.724
	IM	11	97 ± 32	26.30 - 27.76	x	x	Post-hoc tests Holm-Sidak	p# CM vs CF	nd
	CG	20	100 ± 21	25.91 - 32.14	x	x		p# IM vs IF	nd
	IG	21	96 ± 28	26.30 - 28.02	Δno RT- mean Cq			p* IF vs CF	nd
	Primer efficiency			87.6%	nd			p* IM vs CM	nd
HDAC5	CF	11	100 ± 40	24.14 - 25.96	37.06§	35.49§	Two-way ANOVA	p*	0.850
	CM	9	135 ± 52	24.04 - 25.71	35.47§	nd		p#	0.148
	IF	10	122 ± 50	24.45 - 25.44	**no RTs**			p*#	**0.040**
	IM	11	114 ± 41	23.74 - 25.61	nd	nd	Post-hoc tests Holm-Sidak	p# CM vs CF	**0.016**
	CG	20	100 ± 43	24.04 - 25.96	nd	nd		p# IM vs IF	0.644
	IG	21	102 ± 41	23.74 - 25.61	Δno RT- mean Cq			p* IF vs CF	0.105
	Primer efficiency			88.7%	nd			p* IM vs CM	0.185
PCNA	CF	11	100 ± 17	24.49 - 25.96	35.14§	35.13§	Two-way ANOVA on ranks	p*	**0.002**
	CM	9	102 ± 23	25.06 - 26.36	34.86§	nd		p#	0.873
	IF	10	134 ± 55	24.61 - 25.63	**no RTs**			p*#	0.364
	IM	11	114 ± 18	24.35 - 26.03	32.87	34.26	Post-hoc tests Holm-Sidak	p# CM vs CF	0.600
	CG	20	100 ± 21	24.49 - 26.36	35.87	33.81		p# IM vs IF	0.443
	IG	21	122 ± 42	24.35 - 26.03	Δno RT- mean Cq			p* IF vs CF	**0.005**
	Primer efficiency			94.1%	34.20-25.28=8.92			p* IM vs CM	0.106
DKK1	CF	11	100 ± 133	28.06 - 32.83	36.86§	36.71§	Two-way ANOVA	p*	0.666
	CM	9	111 ± 122	28.99 - 31.61	37.44§	37.76§		p#	0.347
	IF	10	81 ± 85	29.63 - 32.29	**no RTs**			p*#	0.986
	IM	11	111 ± 126	28.22 - 32.76	37.40	37.80	Post-hoc tests Holm-Sidak	p# CM vs CF	nd
	CG	20	100 ± 107	28.06 - 32.83	x	x		p# IM vs IF	nd
	IG	21	92 ± 89	28.22 - 32.76	Δno RT- mean Cq			p* IF vs CF	nd
	Primer efficiency			82.4%	37.60-30.48=7.12			p* IM vs CM	nd

mRNA		n	FC ± SD (n)	Cq_{min} - Cq_{max}	no template controls		Statistical analysis		
SFRP1	CF	11	100 ± 50	27.23 - 28.90	x	x	Two-way ANOVA	p*	0.065
	CM	9	99 ± 53	27.02 - 28.70	x	x		p#	0.386
	IF	10	155 ± 121	25.73 - 28.75	**no RTs**			p*#	0.446
	IM	11	115 ± 66	26.68 - 29.22	x	x	Post-hoc tests Holm-Sidak	p# CM vs CF	nd
	CG	20	100 ± 52	27.02 - 28.90	x	x		p# IM vs IF	nd
	IG	21	135 ± 98	25.73 - 29.22	Δno RT- mean Cq			p* IF vs CF	nd
	Primer efficiency			90.4%	nd			p* IM vs CM	nd
FZD7	CF	11	100 ± 45	26.26 - 28.35	39.45§	37.43	Two-way ANOVA	p*	0.069
	CM	9	120 ± 44	26.78 - 28.13	36.64	35.88§		p#	0.702
	IF	10	141 ± 60	26.73 - 28.09	**no RTs**			p*#	0.065
	IM	11	124 ± 55	26.30 - 28.20	33.60	34.34	Post-hoc tests Holm-Sidak	p# CM vs CF	nd
	CG	20	100 ± 38	26.26 - 28.35	35.70	34.09		p# IM vs IF	nd
	IG	21	121 ± 48	26.30 - 28.20	Δno RT- mean Cq			p* IF vs CF	nd
	Primer efficiency			85.7%	34.34-27.40=6.94			p* IM vs CM	nd
LRP6	CF	11	100 ± 33	25.07 - 26.69	34.99§	34.53§	Two-way ANOVA	p*	**0.024**
	CM	9	94 ± 26	25.64 - 26.94	34.79§	34.21§		p#	0.861
	IF	10	84 ± 30	25.71 - 28.37	**no RTs**			p*#	0.633
	IM	11	84 ± 25	25.52 - 27.25	x	x	Post-hoc tests Holm-Sidak	p# CM vs CF	0.649
	CG	20	100 ± 28	25.07 - 26.94	x	x		p# IM vs IF	0.828
	IG	21	86 ± 26	25.52 - 28.37	Δno RT- mean Cq			p* IF vs CF	**0.049**
	Primer efficiency			87.8%	nd			p* IM vs CM	0.201
DVL1	CF	11	100 ± 41	25.88 - 27.53	36.54§	37.52§	Two-way ANOVA	p*	0.298
	CM	8	122 ± 41	25.60 - 27.28	36.95§	35.65§		p#	**0.025**
	IF	10	103 ± 36	26.21 - 28.03	**no RTs**			p*#	0.113
	IM	11	107 ± 34	25.51 - 27.14	x	x	Post-hoc tests Holm-Sidak	p# CM vs CF	**0.009**
	CG	19	100 ± 36	25.60 - 27.53	x	x		p# IM vs IF	0.608
	IG	21	96 ± 29	25.51 - 28.03	Δno RT- mean Cq			p* IF vs CF	0.687
	Primer efficiency			86.7%	nd			p* IM vs CM	0.070
LPL	CF	10	100 ± 141	23.54 - 27.55	36.30	36.73	Two-way ANOVA	p*	0.608
	CM	9	120 ± 140	24.04 - 26.86	x	37.46§		p#	0.243
	IF	10	84 ± 106	23.82 - 29.00	**no RTs**			p*#	0.909
	IM	10	110 ± 138	23.39 - 27.56	x	x	Post-hoc tests Holm-Sidak	p# CM vs CF	nd
	CG	19	100 ± 111	23.54 - 27.55	x	x		p# IM vs IF	nd
	IG	20	89 ± 97	23.39 - 29.00	Δno RT- mean Cq			p* IF vs CF	nd
	Primer efficiency			80.4%	nd			p* IM vs CM	nd
MTOR	CF	11	100 ± 13	26.33 - 27.81	37.40§	nd	Two-way ANOVA	p*	**0.008**
	CM	9	119 ± 21	26.32 - 27.64	37.50§	nd		p#	0.297
	IF	10	136 ± 38	26.46 - 27.74	**no RTs**			p*#	0.098
	IM	11	129 ± 28	25.99 - 28.09	x	x	Post-hoc tests Holm-Sidak	p# CM vs CF	0.062
	CG	20	100 ± 22	26.32 - 27.81	x	x		p# IM vs IF	0.649
	IG	21	122 ± 34	25.99 - 28.09	Δno RT- mean Cq			p* IF vs CF	**0.003**
	Primer efficiency			86.0%	nd			p* IM vs CM	0.441
RPTOR	CF	11	100 ± 24	27.05 - 28.44	-	-	Two-way ANOVA	p*	0.950
	CM	9	135 ± 37	27.06 - 27.97	-	nd		p#	**0.049**
	IF	10	117 ± 34	27.06 - 38.23	**no RTs**			p*#	**0.050**
	IM	11	117 ± 35	26.89 - 28.52	x	x	Post-hoc tests Holm-Sidak	p# CM vs CF	**0.007**
	CG	20	100 ± 35	27.05 - 28.44	x	x		p# IM vs IF	0.991
	IG	21	101 ± 35	26.89 - 38.23	Δno RT- mean Cq			p* IF vs CF	0.168
	Primer efficiency			87.9%	nd			p* IM vs CM	0.154
LAT1	CF	11	100 ± 60	24.12 - 27.05	37.29§	36.57§	Two-way ANOVA	p*	**0.044**
	CM	9	183 ± 104	23.52 - 25.42	37.44§	36.75§		p#	**0.010**
	IF	10	165 ± 80	23.74 - 25.57	**no RTs**			p*#	**0.012**
	IM	11	170 ± 90	23.07 - 25.76	34.17	34.06	Post-hoc tests Holm-Sidak	p# CM vs CF	**<0.001**
	CG	20	100 ± 71	23.52 - 27.05	36.38	33.38		p# IM vs IF	0.955
	IG	21	122 ± 70	23.07 - 25.76	Δno RT- mean Cq			p* IF vs CF	**0.002**
	Primer efficiency			87.2%	9.85			p* IM vs CM	0.704
SLC3A2	CF	11	100 ± 39	23.19 - 25.75	37.21§	36.51§	Two-way ANOVA on ranks	p*	0.639
	CM	9	119 ± 39	23.27 - 24.75	37.17§	36.67§		p#	0.166
	IF	10	115 ± 47	23.49 - 24.75	**no RTs**			p*#	0.455
	IM	11	119 ± 41	22.68 - 24.56	x	x	Post-hoc	p# CM vs CF	nd
	CG	20	100 ± 34	23.19 - 25.75	x	x		p# IM vs IF	nd

mRNA		n	FC ± SD (n)	Cq_{min} - Cq_{max}	no template controls		Statistical analysis		
	IG	21	108 ± 38	22.68 - 24.75	Δno RT- mean Cq		**tests Holm-Sidak**	p* IF vs CF	nd
	Primer efficiency			86.8%	nd			p* IM vs CM	nd
TAUT	**CF**	11	100 ± 40	27.19 - 29.08	x	x	**Two-way ANOVA on ranks**	p*	**<0.001**
	CM	9	109 ± 67	27.19 - 28.93	x	x		p#	0.587
	IF	9	57 ± 22	28.13 - 30.36	**no RTs**			p*#	0.760
	IM	11	54 ± 18	28.24 - 29.59	x	x	**Post-hoc tests Holm-Sidak**	p# CM vs CF	0.865
	CG	20	100 ± 60	27.19 - 29.08	x	x		p# IM vs IF	0.549
	IG	20	53 ± 25	28.13 - 30.36	Δno RT- mean Cq			p* IF vs CF	**<0.001**
	Primer efficiency			82.5%	nd			p* IM vs CM	**<0.001**
FATP4	**CF**	11	100 ± 19	28.31 - 29.59	32.69§	33.03§	**Two-way ANOVA**	p*	0.415
	CM	9	116 ± 25	28.17 - 29.43	32.93§	33.23§		p#	0.105
	IF	10	113 ± 33	28.23 - 29.58	**no RTs**			p*#	0.472
	IM	11	117 ± 25	27.68 - 29.28	38.23	38.65	**Post-hoc tests Holm-Sidak**	p# CM vs CF	nd
	CG	20	100 ± 24	28.17 - 29.59	40.51	38.05		p# IM vs IF	nd
	IG	21	107 ± 29	27.68 - 29.58	Δ no RT- mean Cq			p* IF vs CF	nd
	Primer efficiency			84.2%	38.87-28.78=10.09			p* IM vs CM	nd
ACTB	**CF**	11	Reference gene. r=0.858 / p=0.001 with bestkeeper of ACTB. POLR2a. TOP1 .B2M	19.26 - 20.36	36.50§	37.27§	**Two-way ANOVA on ranks**	p*	0.420
	CM	9		19.32 - 20.55	36.86§	37.22§		p#	0.519
	IF	10		19.51 - 21.42	**no RTs**			p*#	0.455
	IM	11		18.58 - 20.52	31.63	32.24	**Post-hoc tests Holm-Sidak**	p# CM vs CF	nd
	CG	20		19.26 - 20.55	33.73	32.11		p# IM vs IF	nd
	IG	21		18.58 - 21.42	Δno RT- mean Cq			p* IF vs CF	nd
	Primer efficiency			81.6%	32.43-19.85=12.58			p* IM vs CM	nd
POLR2a	**CF**	11	Reference gene. r=0.892 / p=0.001 with bestkeeper of ACTB. POLR2a. TOP1 .B2M	25.92 - 27.24	37.25§	37.15§	**Two-way ANOVA**	p*	0.073
	CM	9		26.06 - 27.10	36.75§	37.69§		p#	0.193
	IF	10		25.72 - 27.81	**no RTs**			p*#	0.364
	IM	11		25.18 - 27.25	x	x	**Post-hoc tests Holm-Sidak**	p# CM vs CF	nd
	CG	20		25.92 - 27.24	x	x		p# IM vs IF	nd
	IG	21		25.18 - 27.81	Δno RT- mean Cq			p* IF vs CF	nd
	Primer efficiency			82.5%	nd			p* IM vs CM	nd
TOP1	**CF**	11	Reference gene. r=0.783 / p=0.001 with bestkeeper of ACTB. POLR2a. TOP1 .B2M	25.04 - 26.77	37.86§	37.98§	**Two-way ANOVA**	p*	0.702
	CM	9		25.06 - 26.23	x	nd		p#	**0.027**
	IF	10		25.28 - 26.98	**no RTs**			p*#	0.752
	IM	11		24.57 - 26.30	x	x	**Post-hoc tests Holm-Sidak**	p# CM vs CF	0.075
	CG	20		25.04 - 38.79	x	x		p# IM vs IF	0.162
	IG	21		24.57 - 26.98	Δno RT- mean Cq			p* IF vs CF	0.962
	Primer efficiency			87.3%	nd			p* IM vs CM	0.626
B2M	**CF**	11	Reference gene. r=0.783 / p=0.001 with bestkeeper of ACTB. POLR2a. TOP1 .B2M	20.16 - 21.20	x	x	**Two-way ANOVA**	p*	0.420
	CM	9		20.57 - 21.77	38.98§	x		p#	**0.048**
	IF	10		19.86 - 21.83	**no RTs**			p*#	0.338
	IM	11		20.37 - 21.80	x	x	**Post-hoc tests Holm-Sidak**	p# CM vs CF	**0.042**
	CG	20		20.16 - 21.77	x	x		p# IM vs IF	0.445
	IG	21		19.86 - 21.83	Δno RT- mean Cq			p* IF vs CF	0.912
	Primer efficiency			88.1%	nd			p* IM vs CM	0.221
Bestkeeper of ACTB. POLR2a. TOP1 and B2M	**CF**	11	Used for normalization	20.16 - 21.20			**Two-way ANOVA**	p*	0.707
	CM	9		20.57 - 21.77				p#	0.708
	IF	10		19.86 - 21.83				p*#	0.417
	IM	11		20.37 - 21.80			**Post-hoc tests Holm-Sidak**	p# CM vs CF	nd
	CG	20		20.16 - 21.77				p# IM vs IF	nd
	IG	21		19.86 - 21.83				p* IF vs CF	nd
								p* IM vs CM	nd

FC =FC in %. SD = SD with error propagation; 'nd = not determined (in two-way ANOVA: nd because it is not allowed to conduct post-hoc test when there is no significance in the two-way ANOVA); § amplification is due to primer dimers which are only apparent in the H2O control (melting curve analysis); x = no amplification; H_2O controls that are not primer dimers should differ from the templates by more than five Cq values; no RT were analyzed in four pools à 10-11 samples; if amplified. Mean Cq value of duplicates are shown per pool and difference between mean template Cq and mean no-RT Cq is calculated. This difference should be more than five to exclude genomic contribution to the template Cq values.

11.17. Summary of data for biological validation of regulated microRNAs of the explorative profiling by RT-qPCR

microRNA		n	FC ± SD	Cq_{min} - Cq_{max}	No template controls		Statistical analysis		
miR-30d	**CF**	9	100 ± 35	23.73 - 25.24	x	x	**Two-way ANOVA**	p*	0.656
	CM	7	97 ± 29	23.94 - 25.28	x	x		p#	0.792
	IF	10	94 ± 38	24.08 - 25.48	**no RT controls**			p*#	0.652
	IM	9	98 ± 31	24.11 - 25.29	x	x	**Post-hoc tests Holm-Sidak**	p# CM vs CF	nd
	CG	16	100 ± 30	23.73 - 25.28	x	x		p# IM vs IF	nd
	IG	19	97 ± 33	24.08 - 25.48	Δno RT- mean Cq			p* IF vs CF	nd
	Primer efficiency			98.6%	nd			p* IM vs CM	nd
miR-99a	**CF**	11	100 ± 41	26.77 - 28.02	x	nd	**Two-way ANOVA**	p*	**0.001**
	CM	8	142 ± 63	26.21 - 27.91	x	nd		p#	0.47
	IF	10	186 ± 70	25.82 - 27.05	**no RT controls**			p*#	**0.026**
	IM	9	164 ± 79	25.55 - 27.88	x	x	**Post-hoc tests Holm-Sidak**	p# CM vs CF	**0.039**
	CG	19	100 ± 51	26.21 - 28.02	x	x		p# IM vs IF	0.266
	IG	19	149 ± 70	25.55 - 28.02	Δno RT- mean Cq			p* IF vs CF	**<0.001**
	Primer efficiency			83.0%	nd			p* IM vs CM	0.452
miR-100	**CF**	11	100 ± 33	24.58 - 25.61	x	x	**Two-way ANOVA on ranks**	p*	0.441
	CM	8	108 ± 37	24.55 - 26.00	x	x		p#	0.851
	IF	9	115 ± 41	24.08 - 26.17	**no RT controls**			p*#	0.45
	IM	9	107 ± 42	24.03 - 25.96	x	x	**Post-hoc tests Holm-Sidak**	p# CM vs CF	nd
	CG	19	100 ± 34	24.55 - 26.00	x	x		p# IM vs IF	nd
	IG	18	107 ± 40	24.03 - 26.17	Δno RT- mean Cq			p* IF vs CF	nd
	Primer efficiency			87.6%	nd			p* IM vs CM	nd
miR-320	**CF**	10	100 ± 70	26.85 - 29.89	x	x	**Two-way ANOVA**	p*	0.447
	CM	8	92 ± 59	27.10 - 29.85	x	x		p#	0.53
	IF	10	111 ± 68	27.07 - 28.57	**no RT controls**			p*#	0.65
	IM	9	95 ± 57	26.98 - 29.36	x	x	**Post-hoc tests Holm-Sidak**	p# CM vs CF	nd
	CG	18	100 ± 64	26.85 - 29.89	x	x		p# IM vs IF	nd
	IG	19	108 ± 61	26.98 - 29.89	Δno RT- mean Cq			p* IF vs CF	nd
	Primer efficiency			87.2%	nd			p* IM vs CM	nd
miR-375	**CF**	9	100 ± 102	33.48 - 36.24	x	x	**Two-way ANOVA on ranks**	p*	0.808
	CM	8	94 ± 106	33.39 - 36.72	x	x		p#	0.860
	IF	10	98 ± 123	33.12 - 36.47	**no RT controls**			p*#	0.639
	IM	10	113 ± 127	33.02 - 36.50	x	x	**Post-hoc tests Holm-Sidak**	p# CM vs CF	nd
	CG	17	100 ± 108	33.39 - 36.72	x	x		p# IM vs IF	nd
	IG	20	109 ± 129	33.02 - 36.72	Δno RT- mean Cq			p* IF vs CF	nd
	Primer efficiency			105.9%	nd			p* IM vs CM	nd
miR-26b	**CF**	11	Reference gene. r=0.603 / p=0.001 with bestkeeper of miR-26b. RNU6b. RNU24	25.14 - 26.20	x	x	**Two-way ANOVA**	p*	0.132
	CM	8		25.24 - 27.00	x	x		p#	**0.012**
	IF	10		25.44 - 26.42	**no RT controls**			p*#	0.133
	IM	10		25.51 - 26.30	x	x	**Post-hoc tests Holm-Sidak**	p# CM vs CF	**0.006**
	CG	19		25.14 - 27.00	x	x		p# IM vs IF	0.434
	IG	20		25.44 - 27.00	Δno RT- mean Cq			p* IF vs CF	0.997
	Primer efficiency			85.3%	nd			p* IM vs CM	**0.043**
RNU6b	**CF**	11	Reference gene. r=0.576 / p=0.001 with bestkeeper of miR-26b. RNU6b. RNU24	27.49 - 29.44	x	x	**Two-way ANOVA**	p*	0.286
	CM	8		27.73 - 29.86	x	x		p#	0.481
	IF	10		28.33 - 29.84	**no RT controls**			p*#	0.225
	IM	10		27.51 - 29.58	x	x	**Post-hoc tests Holm-Sidak**	p# CM vs CF	nd
	CG	19		27.49 - 29.86	x	x		p# IM vs IF	nd
	IG	20		27.51 - 29.86	Δno RT- mean Cq			p* IF vs CF	nd
	Primer efficiency			88.8%	nd			p* IM vs CM	nd

microRNA		n	FC ± SD	Cq_{min} - Cq_{max}	No template controls		Statistical analysis		
RNU24	**CF**	11	Reference gene. r=0.558 / p=0.001 with bestkeeper of miR-26b. RNU6b. RNU24	27.42 - 28.36	x	x	**Two-way ANOVA on ranks**	p*	0.236
	CM	8		27.20 - 28.15	x	x		p#	0.122
	IF	10		27.25 - 29.05	**no RT controls**			p*#	0.811
	IM	10		27.28 - 27.80	x	x	**Post-hoc tests Holm-Sidak**	p# CM vs CF	nd
	CG	19		27.20 - 28.36	x	x		p# IM vs IF	nd
	IG	20		27.25 - 29.05	Δno RT- mean Cq			p* IF vs CF	nd
	Primer efficiency			86.8%	nd			p* IM vs CM	nd
Bestkeeper of miR-26b. RNU6b and RNU24	**CF**	11	Used for normalization	26.81 - 27.81			**Two-way ANOVA**	p*	0.895
	CM	8		26.84 - 28.14				p#	0.485
	IF	10		27.07 - 28.40				p*#	0.184
	IM	10		26.97 - 27.69			**Post-hoc tests Holm-Sidak**	p# CM vs CF	nd
	CG	19		26.81 - 28.14				p# IM vs IF	nd
	IG	20		26.97 - 28.40				p* IF vs CF	nd
								p* IM vs CM	nd

FC =FC in %, SD = SD with error propagation; 'nd = not determined (in two-way ANOVA: nd because it is not allowed to conduct post-hoc test when there is no significance in the two-way ANOVA); x = no amplification; no RT controls were analyzed in four pools à 10-11 samples.

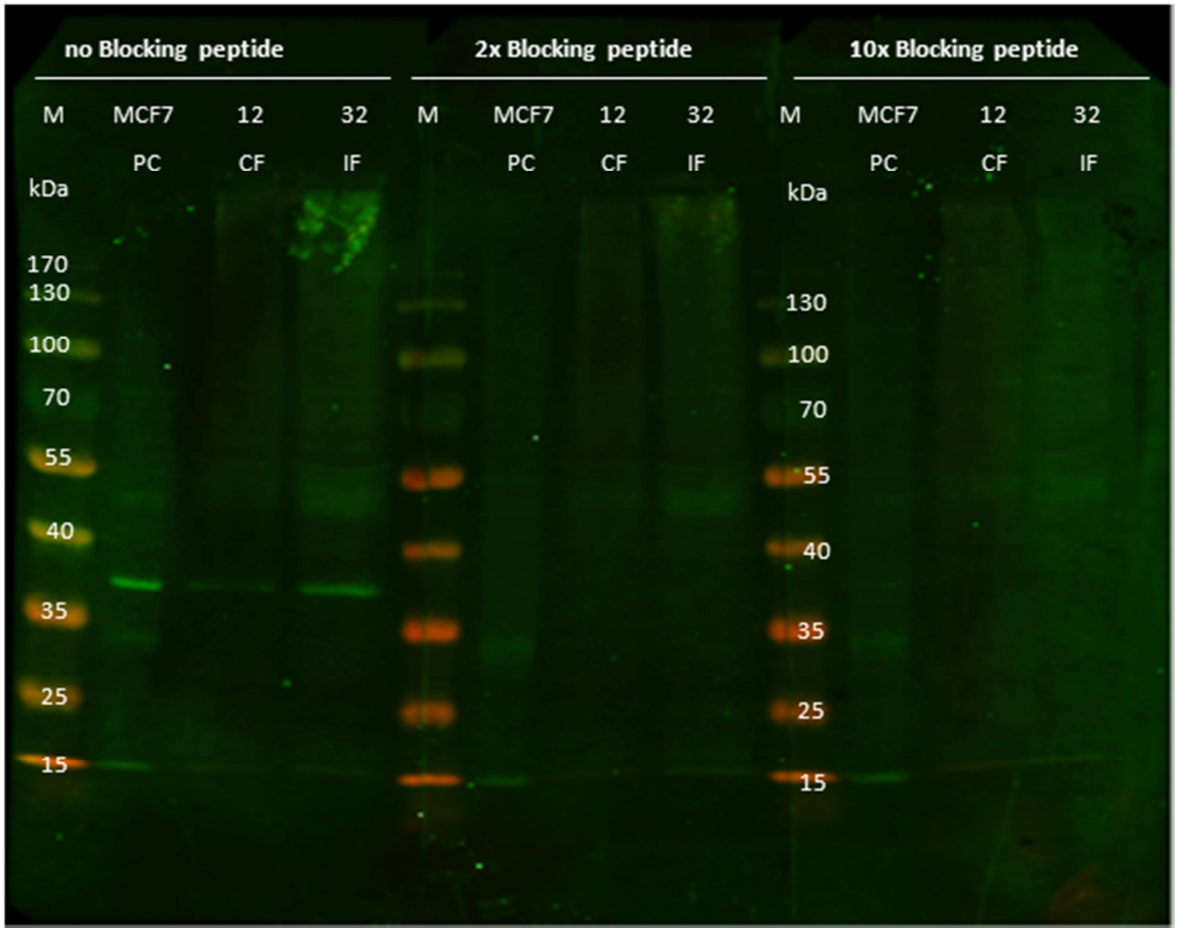
11.18. Pictures of scanned fluorescent western blot membranes.

11.18.1. Blocking peptide for *LAT1* to test *LAT1* specificity (12.07.2011)

10 % SDS-Gel, blotted onto nitrocellulose membrane and blocked in 2% ECL-TBS.

1. Antibody (AB): *LAT1* (Sigma SAB2501232, 35 kDa) in 2% ECL-TBS-T diluted 1:1000
2. AB: *Donkey anti-goat 304* (*Odyssey*, channel 800) in TBS-T diluted 1:10000

Scan: Channel 700: 2.0 / Channel 800: 5.0, both channels contrast (CON): 50, brightness (BRI): 50, linear manual (LM): 5

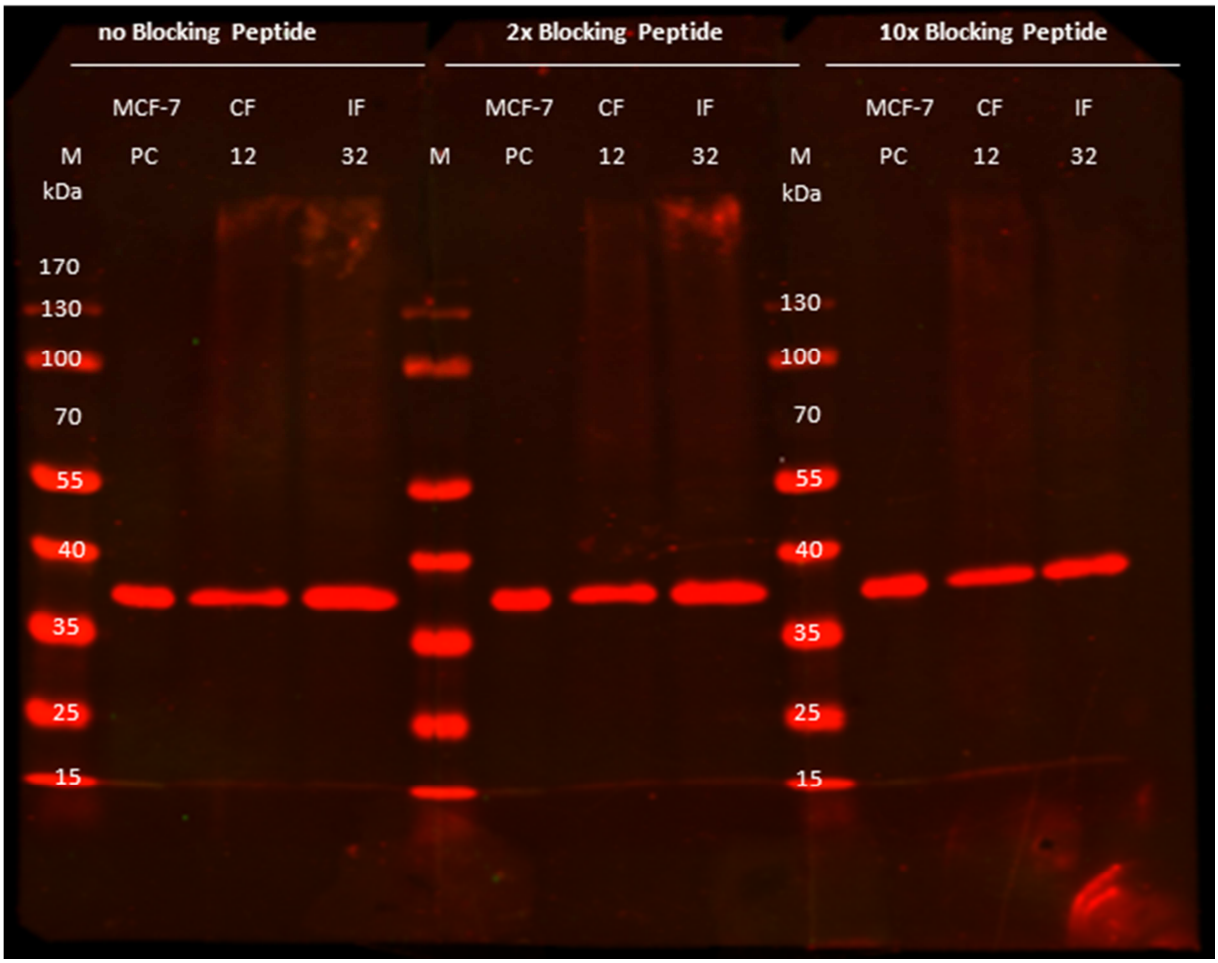


M = marker (3 µl loaded), PC = positive control MCF7 untreated (2µl loaded), 12 and 32 = placental villous fraction from the INFAT study (50 µg protein extract loaded). Blocking peptide was added to the LAT1 antibody 2 times and 10 times the concentration of the antibody. Blocking peptide and antibody were incubated together at room temerature for 30 min. before incubating the membrane.

Comment: The bands at approx. 37 kDa, detected with no blocking peptide, disappear upon incubation of the *LAT1* antibody with blocking peptide at 2x and 10x concentration. Therefore the bands at approx. 37 kDA are specific for *LAT1* (Ritchie JWA and Taylor PM. Role of the System L permease *LAT1* in amino acid and iodothyronine transport in placenta. 2001. Biochem. J. 356:719-725)

11.18.2. Re-incubation of membrane probed for *LAT1* with *GAPDH* as loading control

1. AB: *GAPDH* (Ambion AM3400, 36 kDa) 1:4000 in 2% ECL-TBS-T
2. AB: *Goat anti-mouse 303* (*Odyssey*, channel 700) 1:10.000 in TBS-T
Scan: Channel 700: 5.0 / Channel 800: 5.0, both channels CON: 50, BRI: 50, LM:5



M = marker (3 µl loaded), PC = positive control MCF7 untreated (2µl loaded), 12 and 32 = placental villous fraction from the INFAT study (50 µg protein extract loaded).

Comment: *GAPDH* intensity was equal in every lane, indicating equal loading in every lane.

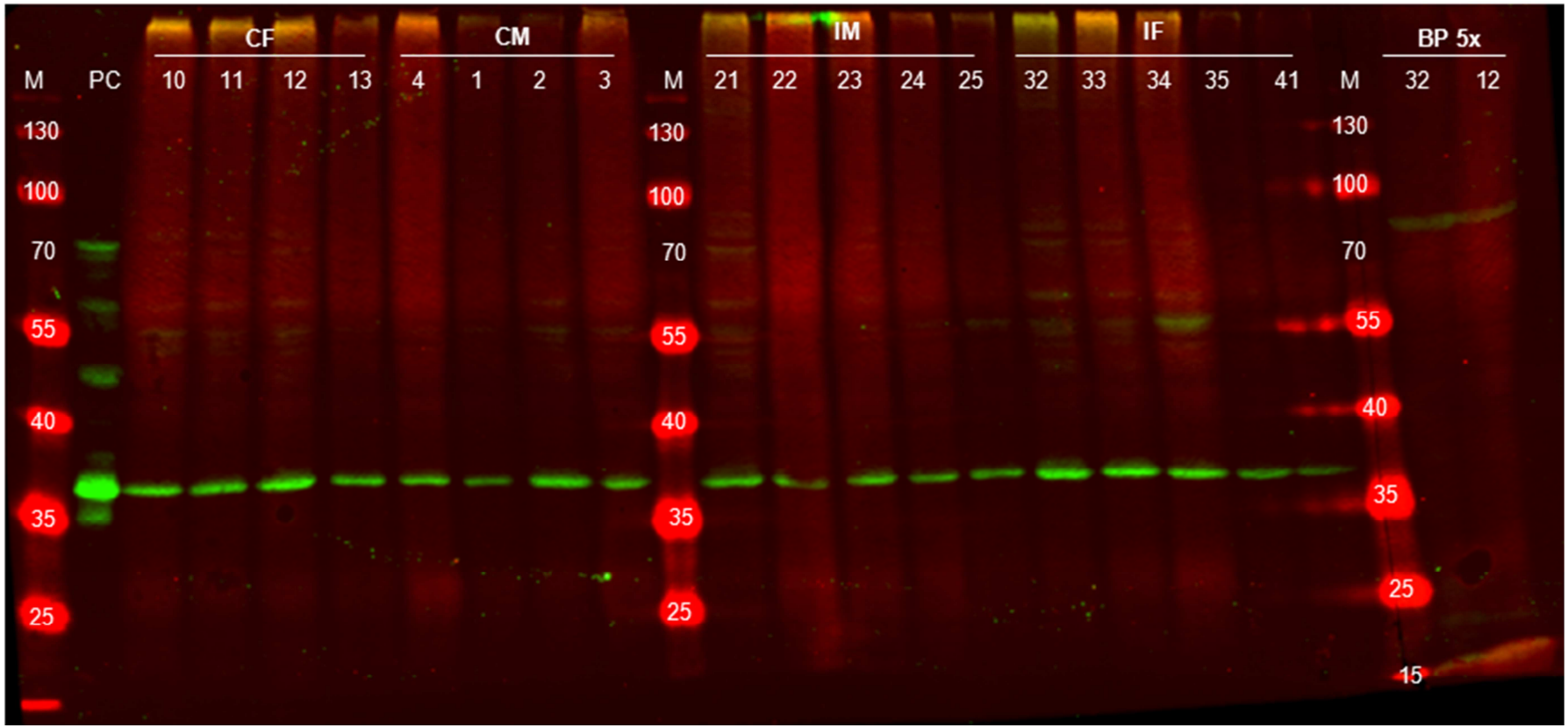
In summary the experiment with the blocking peptide demonstrated the specificity of the *LAT1* antibody detecting a specific band which can be blocked by blocking peptide at approx. 37 kDA.

11.18.3. *LAT1* quantification in placentas of spontaneous birth without anesthetics or analgesics (16.05.2011)

10 % SDS-Gel, blotted onto nitrocellulose membrane and blocked in 2% ECL-TBS.

1. AB: *LAT1* (*Sigma* SAB2501232, 35 kDa) in 2% ECL-TBS-T diluted 1:1000
2. AB: *Donkey anti-goat 304* (*Odyssey*, channel 800) in TBS-T diluted 1:10000

Scan: Channel 700: 2.0 / Channel 800: 5.0, both channels contrast (CON): 50, brightness (BRI): 50, linear manual (LM): 5



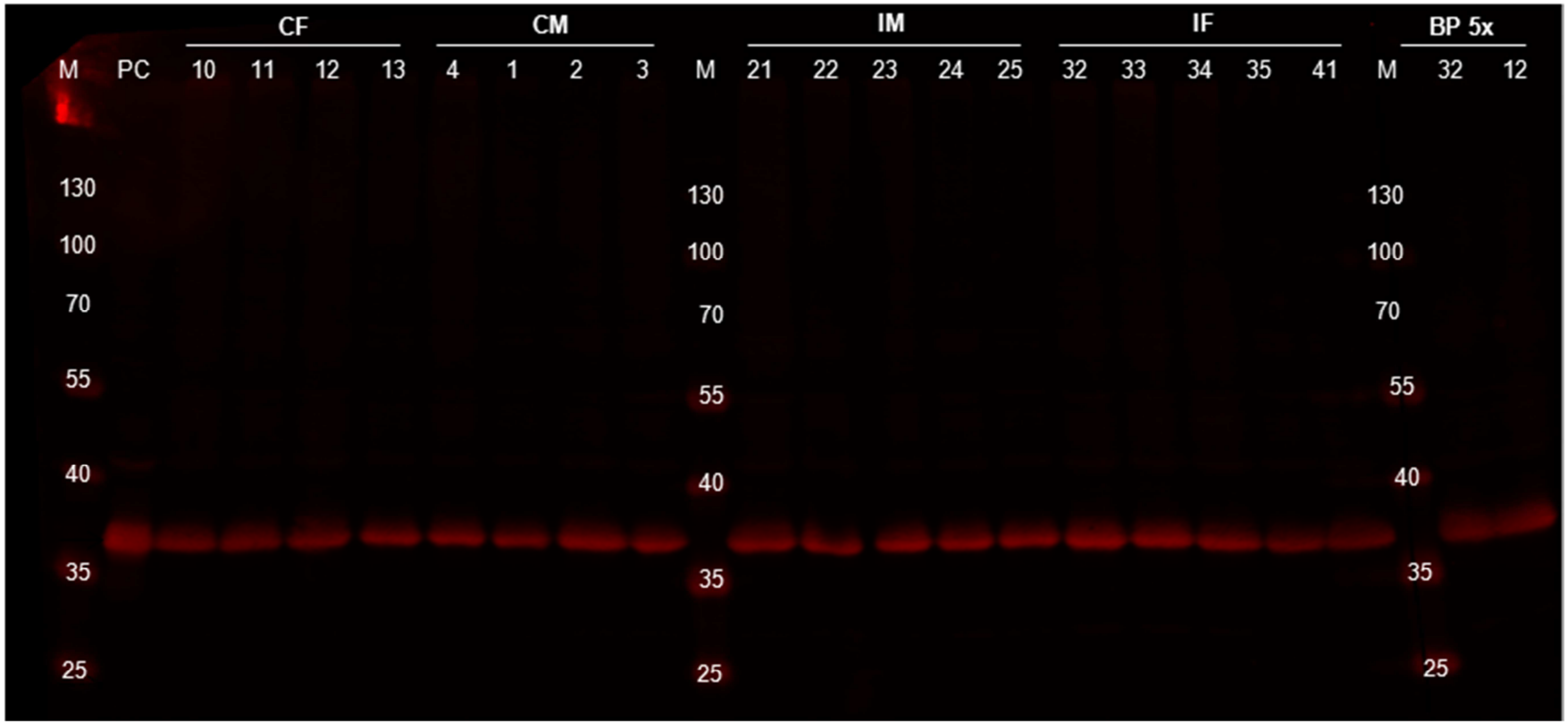
1-41 = placental villous fraction from the INFAT study (50 µg loaded); CF = villous fraction of female placentas from the control group (CG); CM = villous fraction of male placentas from the CG; IM = villous fraction of male placentas from the n-6/n-3 LCPUFA intervention group (IG); IF = villous fraction from female placentas from the IG; M = marker (3 µl loaded), PC = positive control MCF7 untreated (4µl loaded).

Comment: The PC shows a strong band at approx. 37 kDA. This band was also observed in the placental villous fractions of the INFAT study. These bands at approx. 37 kDa disappear upon treatment with blocking peptide 5 times the antibody concentration. Therefore the bands at approx. 37 kDA are specific for *LAT1* and were quantified.

11.18.4. Re-incubation of membrane probed for *LAT1* with *GAPDH* as loading control:

1. AB: *GAPDH* (*Ambion* AM4300, 36 kDa) 1:4000 in 2% ECL-TBS-T
2. AB*: Goat anti-mouse 303* (*Odyssey*, channel 700) 1:10.000 in TBS-T

Scan: Channel 700: 1.0 / Channel 800: 0.0, both channels CON: 50, BRI: 50, LM:5



M = marker (3 µl loaded) , PC = positive control MCF7 untreated (4µl loaded). 1-41 = placental villous fraction from the INFAT study (50 µg loaded); CF = villous fraction of female placentas from the control group (CG); CM = villous fraction of male placentas from the CG; IM = villous fraction of male placentas from the n-6/n-3 LCPUFA intervention group (IG); IF = villous fraction from female placentas from the IG;

Comment: *GADPH* at 36 kDA was measured in the second channel and detected in every lane. In the INFAT placental samples the loading is equal. Also in the lanes previously incubated with the blocking peptide *GAPDH* was detected, therefore the loss of *LAT1* signal in the first blot is due to the blocking peptide and not to unequal protein load.

11.18.5. Raw integrated intensity data for *LAT1* quantification, calculation of relative density and statistics

ID	Analysis group	Integrated intensity		relative density
		LAT1	GAPDH	LAT1 / GAPDH
10	CF	2.11	7.43	0.28
11	CF	2.02	7.30	0.28
12	CF	2.45	7.24	0.34
4	CM	1.50	7.38	0.20
1	CM	1.00	6.50	0.15
2	CM	2.22	9.36	0.24
3	CM	1.38	7.00	0.20
13	CF	1.66	7.64	0.22
21	IM	1.72	9.12	0.19
22	IM	1.05	7.41	0.14
23	IM	1.43	8.21	0.17
32	IF	2.20	10.20	0.22
33	IF	2.33	9.64	0.24
34	IF	2.07	8.41	0.25
24	IM	1.19	8.13	0.15
25	IM	1.35	8.58	0.16
35	IF	1.27	6.43	0.20
41	IF	0.87	6.53	0.13

Results for the statistical analysis of LAT1 protein expression in Western blot		
Two-way ANOVA on ranks	p*	**0.010**
	p#	0.004
	p*#	0.341
Post-hoc tests Holm-Sidak	p# CM vs CF	0.010
	p# IM vs IF	0.085
	p* IF vs CF	**0.014**
	p* IM vs CM	0.183

*Integrated intensity = raw data read out from the Odyssey Infrared Imaging System (Li-cor) software, relative density: target protein normalized to GAPDH. Significant effects for the factor offspring sex were marked with #, significant effects for the n-3 LCPUFA treatment with * and significant interactions with * #. CM: Control male = placentas of male offspring in the control group (n_{CM} = 4), CF: Control female = placentas of female offspring in the control group (n_{CF} = 4), IM: intervention male = placentas of male offspring in the n-3 LCPUFA intervention group (n_{IM} = 5), IF: intervention female = placentas of female offspring in the n-3 LCPUFA intervention group (n_{IF} = 5)*

11.19. Sex steroid analysis in placental tissue and umbilical cord plasma

Sex hormones		male			female				P*	P#	P*#
		n	median	(25^{th}-75^{th} percentile)	n	median	(25^{th}-75^{th} percentile)	CM vs CF	IM vs IF	IF vs CF	IM vs CM
Plasma UC free estradiol-17ß ng/ml†	C	7	13.4	(11.4-38.0)	8	17.2	(13.1-32.0)		0.319	0.709	0.367
	I	9	20.9	(18.7-24.3)	9	18.5	(11.7-26.0)				
Plasma UC conjugated estradiol-17ß ng/ml†	C	7	272.5	(219.0-318.0)	8	247.8	(174.6-380.0)		0.188	0.778	0.190
	I	9	158.0	(141.0-270.8)	9	281.5	(170.0-324.3)				
Plasma UC total free estrogen ng/ml	C	7	26.9	(24.1-57.0)	8	42.6	(31.7-69.7)		0.290	0.495	0.210
	I	9	43.1	(36.7-65.4)	9	49.1	(26.1-63.9)				
Plasma UC conjugated total estrogen ng/ml†	C	7	132.8	(110.4-298.9)	8	111.0	(74.2-192.6)		0.083	0.847	0.094
	I	9	79.3	(63.4-106.6)	9	120.0	(76.2-148.5)				
Plasma UC testosterone ng/ml†	C	7	1.3	(1.0-1.3)	8	1.1	(0.8-1.3)		0.519	**0.049**	0.762
	I	9	1.2	(1.0-1.4)	9	0.9	(0.8-1.1)	0.246	0.090	0.496	0.811
Plasma UC progesterone ng/ml†	C	7	435.0	(358.0-803.0)	8	713.5	(367.0-857.5)		0.222	0.965	0.232
	I	9	717.0	(481.0-1154.5)	9	629.0	(439.0-799.0)				
Plasma UC free estradiol-17ß / testosterone ratio†	C	7	12.9	(9.5-28.5)	8	17.0	(13.9-27.0)		0.619	0.563	0.428
	I	9	18.4	(16.3-21.9)	9	19.6	(13.7-25.2)				
Placental free estradiol-17ß ng/g†	C	9	53.2	(40.1-86.1)	11	50.9	(42.7-70.3)		0.830	0.396	0.616
	I	11	65.4	(35.4-82.4)	10	50.6	(25.5-74.8)				
Placental conjugated estradiol-17ß ng/g†	C	9	23.3	(16.0-26.4)	11	19.4	(17.5-31.7)		0.192	0.609	0.800
	I	11	25.0	(19.8-34.8)	10	24.6	(19.9-40.6)				
Placental free total estrogen ng/g†	C	9	190.4	(128.6-330.8)	11	218.8	(167.9-278.7)		0.633	0.953	0.491
	I	11	278.3	(126.3-389.8)	10	234.0	(111.5-324.0)				
Placental conjugated total estrogen ng/g†	C	9	11.8	(8.7-15.2)	11	11.2	(10.0-15.0)		0.979	0.984	0.920
	I	11	13.2	(7.9-14.4)	10	12.4	(8.0-15.2)				
Placental testosterone ng/g	C	9	10.6	(7.9-16.6)	11	14.8	(10.2-20.1)		0.252	**0.008**	0.278
	I	11	10.7	(9.7-14.4)	10	19.7	(16.6-22.6)	0.249	**0.008**	0.113	0.965
Placental progesterone ng/g†	C	9	2837.0	(1398.5-1398.5)	11	3074.0	(2668.0-3312.0)		0.926	0.452	0.400
	I	11	3432.0	(2326.0-3980.0)	10	2239.5	(1413.3-3600.8)				
Placental free estradiol-17ß / testosterone ratio†	C	9	5.0	(3.0-6.6)	11	4.2	(2.5-6.1)		0.398	**0.013**	**0.045**
	I	11	5.7	(3.6-6.3)	10	2.1	(1.7-3.7)	0.719	**0.002**	**0.042**	0.401

*Sex steroid data are presented as median with interquartile range (IQR: 25th - 75th percentiles). Statistical significance was calculated by two-way ANOVA with Holm-Sidak post-hoc test. Not normal distributed sex steroid parameters were tested for statistical significance by two-way ANOVA on ranks with Holm-Sidak post-hoc test. Significant effects for sex differences are marked with #, significant effects for the n-3 LCPUFA treatment with * and significant interactions with * #. P values < 0.05 are marked in bold. C, control group; I, intervention group; UC, umbilical cord blood; CM: Control male = placentas of male offspring in the control group, CF: Control female = placentas of female offspring in the control group, IM: intervention male = placentas of male offspring in the n-3 LCPUFA intervention group, IF: intervention female = placentas of female offspring in the n-3 LCPUFA intervention group*

11.20. Correlation of significantly regulated genes (ΔCg) with selected weight and fat distribution measurements up to one year

	LAT1	TAUT	LRP6	DVL1	PCNA	CDK6	HDAC5	TGFB1	MTOR	RPTOR	miR-99a	LAT1 protein
with birth weight												
R	-0.04	-0.01	0.06	-0.02	**0.31**	**0.33**	0.06	-0.15	0.10	-0.03	**-0.33**	0.25
p	0.805	0.957	0.719	0.918	**0.049**	**0.042**	0.717	0.339	0.523	0.834	**0.041**	0.309
n	41	40	41	41	**41**	**38**	41	41	41	41	**38**	18
with ponderal index												
R	-0.18	0.00	-0.06	0.03	0.16	-0.02	-0.05	-0.23	-0.13	-0.20	-0.31	0.18
p	0.261	0.988	0.730	0.831	0.328	0.915	0.769	0.151	0.428	0.209	0.062	0.484
n	41	40	41	41	41	38	41	41	41	41	38	18
with weight / length ratio												
R	-0.11	0.01	0.06	0.01	**0.32**	0.26	0.04	-0.19	0.03	-0.12	**-0.37**	0.24
p	0.498	0.947	0.721	0.931	**0.039**	0.111	0.825	0.232	0.876	0.443	**0.023**	0.341
n	41	40	41	41	**41**	38	41	41	41	41	**38**	18
with birth weight / placental weight ratio												
R	0.23	0.06	-0.12	0.16	0.08	0.26	0.28	0.18	**0.37**	0.25	-0.10	-0.29
p	0.149	0.705	0.471	0.325	0.626	0.111	0.079	0.250	**0.017**	0.117	0.565	0.250
n	41	40	41	41	41	38	41	41	**41**	41	38	18
with placental weight												
R	-0.28	-0.03	0.13	-0.15	0.12	-0.09	-0.23	-0.30	-0.30	**-0.31**	-0.05	0.28
p	0.074	0.860	0.402	0.366	0.462	0.595	0.151	0.055	0.056	**0.045**	0.755	0.254
n	41	40	41	41	41	38	41	41	41	**41**	38	18
with weight at 1 year												
R	**0.38**	-0.08	0.03	**0.49**	0.15	0.22	**0.48**	0.20	0.28	0.21	0.15	-0.19
p	**0.014**	0.651	0.834	**0.001**	0.350	0.193	**0.002**	0.210	0.085	0.205	0.383	0.468
n	**40**	39	40	**40**	40	37	**40**	40	40	40	37	17
with sum of four skin fold thicknesses (SFT) 3-5 days												
R	0.02	-0.09	-0.02	-0.31	0.29	0.31	-0.14	-0.07	0.20	-0.08	-0.09	0.32
p	0.921	0.589	0.924	0.056	0.074	0.067	0.387	0.661	0.223	0.611	0.592	0.200
n	39	38	39	39	39	36	39	39	39	39	36	18
with sum of four skin fold thicknesses (SFT) 1 year												
R	0.03	0.02	0.13	0.07	0.04	0.04	-0.01	-0.16	0.04	-0.13	0.06	-0.11
p	0.860	0.905	0.422	0.665	0.832	0.802	0.956	0.336	0.791	0.429	0.711	0.687
n	40	39	40	40	40	37	40	40	40	40	37	17
with Subcutaneous-to-preperitoneal fat mass ratio (SC/PP) 6 weeks												
R	-0.15	0.21	0.20	0.01	-0.08	0.06	0.01	-0.09	-0.07	-0.04	-0.05	**0.49**
p	0.428	0.253	0.273	0.961	0.669	0.758	0.971	0.619	0.717	0.820	0.802	**0.045**
n	32	31	32	32	32	29	32	32	32	32	30	**17**
with Subcutaneous-to-preperitoneal fat mass ratio (SC/PP) 1 year												
R	-0.11	-0.08	0.32	-0.04	-0.14	0.25	-0.08	-0.07	-0.01	-0.04	0.32	-0.11
p	0.505	0.665	0.057	0.800	0.416	0.149	0.643	0.673	0.973	0.796	0.067	0.663
n	37	36	37	37	37	34	37	37	37	37	34	17

Correlation coefficients between 0.0 - 0.4, 0.4 – 0.7, and 0.7 – 1.0 were considered as weak, moderate and strong correlations respectively. A negative value indicates an inverse correlation, whereas a positive value depicts a positive correlation. The correlation analysis was conducted independent of n-3 LCPUFA intervention status. P values < 0.05 were considered as significant correlations and are marked in bold. Rs, spearman-rho correlation coefficient; p, p-value for the respective correlation;

11.21. Summary of transcriptome data, analyzed for the effect of n-6/n-3 LCPUFA intervention, for amino acid transporters reported to be expressed in placenta

gene name	Gene description	Protein name	System	FC IM+IF vs. CM+CF	p-value IM+IF vs. CM+CF	FC IM vs. CM	p-value IM vs. CM	FC IF vs. CF	p-value IF vs. CF
SLC1A1	solute carrier family 1 (neuronal/epithelial high affinity glutamate transporter, system Xag), memb 1	EAAT3	X^-_{AG}	1.36	>0.05	1.10	>0.05	1.25	>0.05
SLC1A2§	solute carrier family 1 (glial high affinity glutamate transporter), member 2	EAAT2	X^-_{AG}	-1.09	>0.05	1.05	>0.05	-1.04	>0.05
SLC1A3	solute carrier family 1 (glial high affinity glutamate transporter), member 3	EAAT1	X^-_{AG}	-1.03	>0.05	-1.10	>0.05	1.14	>0.05
SLC1A4§	solute carrier family 1 (glutamate/neutral amino acid transporter), member 4	ASCT1	ASC	-1.45	>0.05	-1.10	>0.05	-1.25	>0.05
SLC1A5	solute carrier family 1 (neutral amino acid transporter), member 5	ASCT2	B^0	-1.03	>0.05	1.16	>0.05	-1.14	>0.05
SLC3A2	solute carrier family 3 (activators of dibasic and neutral amino acid transport), member 2	**4F2hc**		1.31	>0.05	-1.13	>0.05	1.49	>0.05
SLC6A6	solute carrier family 6 (neurotransmitter transporter, taurine), member 6	**TAUT**	**ß**	**-1.72**	**0.0311**	**-1.84**	**0.0027**	1.09	>0.05
SLC7A1§	solute carrier family 7 (cationic amino acid transporter, y+ system), member 1	CAT1	y^+	1.23	>0.05	1.16	>0.05	1.05	>0.05
SLC7A2§	solute carrier family 7 (cationic amino acid transporter, y+ system), member 2	CAT2B	y^+	-1.06	>0.05	-1.10	>0.05	1.01	>0.05
SLC7A3	solute carrier family 7 (cationic amino acid transporter, y+ system), member 3	CAT3							
SLC7A4§	solute carrier family 7 (cationic amino acid transporter, y+ system), member 4	CAT4	y^+	1.32	>0.05	1.22	>0.05	1.06	>0.05
SLC7A5	solute carrier family 7 (cationic amino acid transporter, y+ system), member 5	**LAT1**	**L**	1.71	>0.05	-1.52	>0.05	**2.45**	**0.0206**
SLC7A6	solute carrier family 7 (cationic amino acid transporter, y+ system), member 6	y^+ LAT2	y^+ L	1.18	>0.05	1.16	>0.05	-1.01	>0.05
SLC7A7§	solute carrier family 7 (cationic amino acid transporter, y+ system), member 7	y^+ LAT1	y^+ L	-1.44	>0.05	-1.22	>0.05	-1.20	>0.05
SLC7A8	solute carrier family 7 (cationic amino acid transporter, y+ system), member 8	LAT2	L	1.94	>0.05	1.16	>0.05	1.69	>0.05
SLC7A9§	solute carrier family 7 (cationic amino acid transporter, y+ system), member 9	$b^{0,+}$	$b^{0,+}$	1.15	>0.05	-1.03	>0.05	1.10	>0.05
SLC7A10§	solute carrier family 7, (neutral amino acid transporter, y+ system) member 10	ASCT1	asc	1.19	>0.05	-1.04	>0.05	1.25	>0.05
SLC7A12	solute carrier family 7 (cationic amino acid transporter, y+ system), member 12	ASCT2	asc						
SLC16A10	solute carrier family 16, member 10 (aromatic amino acid transporter)	TAT1	T						
SLC38A1	solute carrier family 38, member 1	SNAT1	A	1.70	>0.05	1.10	>0.05	1.48	>0.05
SLC38A2	solute carrier family 38, member 2	SNAT2	A	1.09	>0.05	1.07	>0.05	1.03	>0.05
SLC38A4§	solute carrier family 38, member 4	SNAT4	A	1.06	>0.05	1.09	>0.05	-1.04	>0.05
SLC43A2	solute carrier family 43, member 2	LAT4		1.55	>0.05	-1.08	>0.05	1.68	>0.05

CM: Control male = placentas of male offspring in the control group (n_{CM} = 3), CF: Control female = placentas of female offspring in the control group (n_{CF} = 4), IM: intervention male = placentas of male offspring in the n-3 LCPUFA intervention group (n_{IM} = 5), IF: intervention female = placentas of female offspring in the n-3 LCPUFA intervention group (n_{IF} = 4). The applied significance criteria were FC ≥ +1.5 and p < 0.05 and FC ≤ -1.5 and p < 0.05. Significantly differential expressed genes are marked in bold. *§, average intensity below 20; FC, fold change.*

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Benirschke, K., Burton, G.J., Baergen, R. N.
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Scientific communications

Original peer reviewed scientific publications related to the topic of this thesis

Brunner S, Schmid D, Hüttinger K, Much D, Heimberg E, **Sedlmeier EM**, Brüderl M, Kratzsch J, Bader BL, Amann-Gassner U, Hauner H. Maternal insulin resistance, triglycerides and cord blood insulin in relation to post-natal weight trajectories and body composition in the offspring up to 2 years. Diabet Med. 2013 Aug 3. doi: 10.1111/dme.12298. [Epub ahead of print]

Much D, Brunner S, Vollhardt C, Schmid D, **Sedlmeier EM**, Brüderl M, Heimberg E, Bartke N, Boehm G, Bader BL, Amann-Gassner U, Hauner H. Breast milk fatty acid profile in relation to infant growth and body composition: results from the INFAT study. Pediatr Res. 2013 May 28. doi: 10.1038/pr.2013.82. [Epub ahead of print]

Brunner S, Schmid D, Hüttinger K, Much D, Brüderl M, **Sedlmeier EM**, Kratzsch J, Amann-Gassner U, Bader BL, Hauner H. Effect of reducing the n-6/n-3 fatty acid ratio on the maternal and fetal leptin axis in relation to infant body composition. Obesity (Silver Spring). 2013 Apr 17. doi: 10.1002/oby.20481. [Epub ahead of print]

Much D, Brunner S, Vollhardt C, Schmid D, **Sedlmeier EM**, Brüderl M, Heimberg E, Bartke N, Boehm G, Bader BL, Amann-Gassner U, Hauner H. Effect of dietary intervention to reduce the n-6/n-3 fatty acid ratio on maternal and fetal fatty acid profile and its relation to offspring growth and body composition at 1 year of age. Eur J Clin Nutr. 2013 Mar; 67(3):282-8.

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Laumen H, Saningong AD, Heid IM, Hess J, Herder C, Claussnitzer M, Baumert J, Lamina C, Rathmann W, **Sedlmeier EM**, Klopp N, Thorand B, Wichmann HE, Illig T, Hauner H. Functional characterization of promoter variants of the adiponectin gene complemented by epidemiological data. Diabetes. 2009 Apr;58(4):984-91.

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Non peer-reviewed publications

Sedlmeier EM, Dahlhoff C, Fürst RW, Ruhlig K, Bader BL. Epigenetik und Ernährung. Ernährung. 2008; 2:116-124. * all authors contributed equally

Oral scientific presentations

Sedlmeier EM: Nutrigenomics applications to omega-3-PUFA in pregnancy and obesity. At the 10th NuGOweek 2013 Nutrigenomics & more, Freising-Weihenstephan, Germany, 11.09.2013

Poster presentation

Sedlmeier EM, Much D, Brunner S, Schmid D, Vollhardt C, Fleischmann K, Pagel P, Roscher A, Amann-Gassner U, Hauner H and Bader BL. Impact of a reduced n6/n3-LCPUFA ratio in the human maternal diet during pregnancy on placental gene expression and epigenetic mechanisms. At the 49. Wissenschaftlichen Kongress der Deutschen Gesellschaft für Ernährung e.V. (Poster prize 2012)

Data on compact disc

11.6 Supplementary data of DNA microarray analysis

11.6.1 List of significantly regulated genes between n-6/n-3 LCPUFA intervention group and control group in female and male placentas independent of sex from the microarray analysis (IM+IF vs. CM+CF)

Data are presented as median (interquartile range IQR = 25th-75th percentile). Control = placentas of female and male offspring

Table 19: 22 significantly regulated genes upon the n-3 LCPUFA intervention independent of offspring sex.

gene name	FC	raw p-value	Intensity normalized by gcRMA(slow) in unlogged scale		description
			control	n-3 LCPUFA intervention	
CBR1	-1.74	0.04048	22 (16-39)	17 (14-19)	carbonyl reductase 1
LPGAT1	-1.65	0.00120	92 (85-101)	51 (47-75)	lysophosphatidylglycerol acyltransferase 1
PRR16	-1.62	0.00682	40 (33-43)	23 (17-26)	proline rich 16
LACTB2	-1.61	0.00172	26 (19-34)	17 (13-18)	lactamase, beta 2
CSTA	-1.61	0.02346	183 (123-226)	110 (90-121)	cystatin A (stefin A)
CENPK	-1.59	0.01885	37 (36-43)	24 (20-37)	centromere protein K
ANXA3	-1.56	0.00111	493 (438-629)	338 (304-374)	annexin A3
SFRP1	-1.56	0.01890	30 (24-39)	19 (18-27)	secreted frizzled-related protein 1
PPAT	-1.51	0.03710	18 (17-20)	11 (8-19)	phosphoribosyl pyrophosphate amidotransferase
PRSS23	-1.50	0.03012	177 (138-246)	125 (109-149)	protease, serine, 23
THOP1	1.58	0.00374	16 (12-19)	22 (21-25)	thimet oligopeptidase 1
CCDC69	1.62	0.04745	82 (46-108)	109 (102-119)	coiled-coil domain containing 69
TPPP3	1.66	0.04968	147 (130-189)	230 (177-354)	tubulin polymerization-promoting protein family member 3
FURIN	1.66	0.03882	213 (191-245)	290 (287-454)	furin (paired basic amino acid cleaving enzyme)
LOC100288618	1.67	0.00001	8 (7-8)	13 (12-15)	hypothetical protein LOC100288618
PAFAH2	1.70	0.00177	20 (20-25)	42 (29-42)	platelet-activating factor acetylhydrolase 2, 40kDa
PRRG4	1.80	0.02551	51 (39-61)	87 (63-105)	proline rich Gla (G-carboxyglutamic acid) 4 (transmembrane)
SH3GLB2	1.85	0.04398	110 (87-209)	224 (173-256)	SH3-domain GRB2-like endophilin B2
CX3CR1	1.87	0.01984	17 (15-37)	40 (32-51)	chemokine (C-X3-C motif) receptor 1
TC2N	2.00	0.04455	77 (64-126)	124 (101-192)	tandem C2 domains, nuclear
CORO6	2.10	0.01762	210 (139-279)	458 (283-667)	coronin 6
HINT3	3.03	0.01622	13 (10-15)	27 (16-97)	histidine triad nucleotide binding protein 3

in the control group, intervention = placentas of female and male offspring in the n-3 LCPUFA intervention group. The genes are sorted according to their fold change. FC, fold change;

11.6.2 List of significantly regulated genes between n-6/n-3 LCPUFA intervention group and control group in female and male placentas under consideration of sex from the microarray analysis (IM+IF vs. CM+CF)

gene name	FC	raw p value	Intensity normalized by gcRMA(slow) in unlogged scale				description
			control female	control male	intervention female	Intervention male	
CBR1	-3.30	0.03148	24 (15-36)	22 (20-70)	14 (12-16)	17 (17-21)	carbonyl reductase 1
LPGAT1	-2.56	0.00054	94 (90-99)	84 (76-98)	76 (68-81)	48 (47-51)	lysophosphatidylglycerol acyltransferase 1
LACTB2	-2.55	0.00367	28 (24-32)	19 (19-29)	18 (16-18)	16 (13-17)	lactamase, beta 2
CENPK	-2.53	0.02357	43 (34-55)	36 (36-36)	22 (18-27)	32 (23-37)	centromere protein K
PRR16	-2.53	0.01080	40 (39-43)	28 (25-38)	20 (16-28)	23 (21-26)	proline rich 16
CSTA	-2.46	0.03406	226 (184-250)	135 (113-159)	100 (81-131)	112 (104-121)	cystatin A (stefin A)
ANXA3	-2.40	0.00057	607 (484-729)	422 (394-480)	292 (244-347)	374 (333-394)	annexin A3
SFRP1	-2.24	0.01855	39 (34-51)	21 (21-25)	19 (18-23)	19 (18-27)	secreted frizzled-related protein 1
GAS1	-2.22	0.04398	379 (330-561)	305 (297-327)	195 (169-236)	276 (270-349)	growth arrest-specific 1
PRSS23	-2.20	0.04579	207 (150-304)	177 (144-209)	124 (120-131)	147 (107-155)	protease, serine, 23
ADARB1	-2.19	0.02208	20 (19-28)	32 (25-36)	16 (15-19)	17 (15-21)	adenosine deaminase, RNA-specific, B1 (RED1 homolog rat)
ANPEP	-2.18	0.03950	36 (33-38)	48 (41-84)	32 (28-34)	34 (28-41)	alanyl (membrane) aminopeptidase
ANAPC4	-2.17	0.00335	133 (111-153)	129 (117-132)	76 (64-89)	99 (86-106)	anaphase promoting complex subunit 4
SMOC2	-2.15	0.02250	15 (13-19)	18 (16-26)	13 (11-13)	13 (11-13)	SPARC related modular calcium binding 2
ATP8B1	-2.13	0.02562	112 (81-146)	90 (85-132)	77 (72-87)	65 (61-92)	ATPase, class I, type 8B, member 1
CTSC	-2.13	0.03420	171 (131-216)	97 (96-123)	81 (78-90)	114 (73-123)	cathepsin C
RNFT1	-2.13	0.00130	28 (24-31)	17 (17-19)	14 (13-15)	15 (14-18)	ring finger protein, transmembrane 1
PRKD3	-2.12	0.04280	154 (128-178)	104 (85-179)	99 (85-111)	89 (88-101)	protein kinase D3
VEZT	-2.08	0.00209	40 (35-44)	53 (51-57)	34 (30-37)	26 (25-32)	vezatin, adherens junctions transmembrane protein
LPAR1	-2.07	0.04996	84 (75-91)	92 (64-92)	46 (35-57)	53 (51-83)	lysophosphatidic acid receptor 1
HAUS1	-2.04	0.00621	111 (91-124)	80 (79-85)	61 (55-68)	74 (61-74)	HAUS augmin-like complex, subunit 1
C1orf124	-2.02	0.00629	26 (26-27)	22 (20-23)	17 (13-20)	17 (13-21)	chromosome 1 open reading frame 124
FLJ32065	-2.02	0.02032	18 (16-20)	21 (18-24)	12 (10-14)	15 (13-20)	hypothetical protein FLJ32065
RAB33B	-2.02	0.04184	91 (82-103)	102 (83-153)	61 (58-67)	80 (62-106)	RAB33B, member RAS oncogene family
C4orf43	-2.01	0.01054	30 (29-37)	31 (27-37)	24 (22-27)	19 (19-23)	chromosome 4 open reading frame 43
MEIS2	-2.01	0.04333	52 (43-62)	34 (27-48)	29 (23-35)	31 (30-41)	Meis homeobox 2
NPNT	-2.00	0.03394	21 (17-24)	20 (19-22)	14 (12-16)	14 (10-15)	nephronectin
CEP192	-2.00	0.00379	21 (17-24)	19 (17-19)	12 (12-13)	14 (13-14)	centrosomal protein 192kDa
SYCP2	-1.99	0.00077	12 (10-13)	11 (11-11)	7 (6-9)	8 (7-9)	synaptonemal complex protein 2
BCAT1	-1.98	0.01344	373 (352-399)	279 (269-304)	232 (211-243)	217 (197-352)	branched chain aminotransferase 1, cytosolic
C3orf64	-1.98	0.01666	170 (158-209)	208 (183-227)	132 (123-159)	126 (111-147)	chromosome 3 open reading frame 64
CNTN4	-1.97	0.00896	14 (12-15)	10 (10-15)	10 (9-10)	10 (8-10)	contactin 4
ECT2	-1.96	0.03942	93 (68-119)	85 (73-86)	59 (50-68)	66 (62-74)	epithelial cell transforming sequence 2 oncogene
C1QTNF7	-1.93	0.02806	22 (21-26)	23 (20-24)	17 (12-21)	14 (14-18)	C1q and tumor necrosis factor related protein 7
TUBGCP3	-1.93	0.02289	51 (42-65)	42 (40-45)	36 (33-39)	33 (28-39)	tubulin, gamma complex associated protein 3
C12orf29	-1.93	0.00340	60 (57-64)	42 (40-49)	33 (31-37)	40 (38-43)	chromosome 12 open reading frame 29
ZNF823	-1.92	0.00036	58 (55-59)	83 (80-92)	43 (42-49)	51 (50-55)	zinc finger protein 823

gene name	FC	raw p value	Intensity normalized by gcRMA(slow) in unlogged scale				description
			control female	control male	intervention female	Intervention male	
SLC25A13	-1.92	0.00913	147 (115-166)	127 (103-147)	106 (95-115)	127 (78-127)	solute carrier family 25, member 13 (citrin)
COL12A1	-1.91	0.02632	11 (9-15)	8 (7-11)	7 (7-8)	8 (7-8)	collagen, type XII, alpha 1
TCEAL1	-1.91	0.04786	168 (147-179)	162 (131-187)	103 (93-123)	129 (81-141)	transcription elongation factor A (SII)-like 1
MYO5C	-1.90	0.00439	13 (13-15)	11 (10-13)	9 (8-10)	9 (9-10)	myosin VC
ITGB3BP	-1.90	0.02702	25 (23-25)	21 (17-22)	13 (12-15)	15 (14-16)	integrin beta 3 binding protein (beta3-endonexin)
CCDC14	-1.89	0.00651	37 (34-40)	41 (37-47)	22 (21-25)	31 (30-39)	coiled-coil domain containing 14
ATAD2	-1.88	0.00568	24 (22-27)	17 (16-20)	14 (12-16)	16 (15-19)	ATPase family, AAA domain containing 2
AGTPBP1	-1.88	0.00518	32 (31-33)	27 (24-30)	20 (18-21)	21 (19-23)	ATP/GTP binding protein 1
C6orf170	-1.87	0.02057	33 (29-36)	31 (24-32)	20 (19-22)	20 (19-20)	chromosome 6 open reading frame 170
PEPD	-1.87	0.02894	52 (48-56)	74 (70-82)	42 (38-51)	43 (43-55)	peptidase D
CD9	-1.86	0.02752	124 (94-159)	70 (63-80)	62 (55-67)	87 (64-88)	CD9 molecule
STIL	-1.86	0.01735	26 (23-29)	28 (21-30)	19 (17-20)	17 (16-18)	SCL/TAL1 interrupting locus
DMD	-1.85	0.04423	361 (327-391)	336 (266-338)	248 (213-258)	255 (199-320)	dystrophin
PYGL	-1.82	0.03442	83 (77-99)	58 (56-62)	56 (54-62)	62 (39-68)	phosphorylase, glycogen, liver
F2RL1	-1.81	0.01258	13 (12-14)	11 (10-11)	8 (7-9)	9 (8-11)	coagulation factor II (thrombin) receptor-like 1
CYP51A1	-1.80	0.00736	175 (151-200)	205 (182-207)	148 (134-161)	118 (108-124)	cytochrome P450, family 51, subfamily A, polypeptide 1
RAB10	-1.80	0.01464	332 (294-373)	243 (204-271)	223 (214-226)	235 (163-239)	RAB10, member RAS oncogene family
TUBB2B	-1.79	0.03649	19 (13-24)	15 (14-16)	13 (10-15)	11 (11-12)	tubulin, beta 2B
DNAJB4	-1.79	0.02925	386 (354-415)	309 (307-344)	256 (239-265)	297 (291-311)	DnaJ (Hsp40) homolog, subfamily B, member 4
KDELR3	-1.79	0.00462	33 (28-38)	15 (14-16)	16 (16-18)	15 (14-17)	KDEL (Lys-Asp-Glu-Leu) endoplasmic reticulum protein retention receptor 3
CUL4B	-1.78	0.01841	24 (22-27)	17 (16-22)	18 (17-19)	15 (14-18)	cullin 4B
POT1	-1.78	0.00503	73 (64-83)	55 (49-63)	46 (44-48)	50 (44-56)	POT1 protection of telomeres 1 homolog (S. pombe)
SETMAR	-1.77	0.01624	15 (15-16)	17 (16-24)	11 (10-11)	16 (15-18)	SET domain and mariner transposase fusion gene
KCNK6	-1.77	0.03203	13 (12-16)	16 (13-18)	11 (11-12)	9 (9-12)	potassium channel, subfamily K, member 6
ZDHHC2	-1.77	0.03199	57 (55-64)	84 (65-90)	47 (43-51)	50 (46-70)	zinc finger, DHHC-type containing 2
OMA1	-1.77	0.00810	68 (58-79)	63 (59-66)	49 (40-56)	55 (49-55)	OMA1 homolog, zinc metallopeptidase (S. cerevisiae)
PGCP	-1.76	0.02415	89 (75-98)	85 (77-85)	62 (53-69)	65 (54-73)	plasma glutamate carboxypeptidase
ZNF318	-1.75	0.00252	12 (11-14)	14 (12-16)	9 (9-10)	11 (10-11)	zinc finger protein 318
FGF2	-1.75	0.00247	11 (10-12)	9 (8-10)	7 (7-8)	7 (7-7)	fibroblast growth factor 2 (basic)
APAF1	-1.75	0.00454	42 (34-51)	36 (33-37)	27 (26-29)	30 (26-31)	apoptotic peptidase activating factor 1
BOK	-1.72	0.00917	15 (14-17)	18 (17-25)	14 (13-14)	13 (13-13)	BCL2-related ovarian killer
SCML1	-1.72	0.00858	41 (38-47)	71 (63-83)	45 (40-48)	40 (40-43)	sex comb on midleg-like 1 (Drosophila)
TOP2A	-1.72	0.04523	145 (110-173)	84 (80-108)	88 (78-93)	84 (82-85)	topoisomerase (DNA) II alpha 170kDa
SLC6A6	-1.72	0.03112	294 (251-339)	394 (334-494)	330 (276-378)	221 (192-226)	solute carrier family 6 (neurotransmitter transporter, taurine), member 6
WDR43	-1.71	0.03220	37 (35-42)	46 (45-50)	31 (29-33)	33 (30-37)	WD repeat domain 43
ZNF443	-1.71	0.01044	48 (43-54)	41 (40-43)	29 (26-32)	39 (35-54)	zinc finger protein 443
NFXL1	-1.71	0.01635	28 (24-30)	35 (33-44)	22 (20-24)	25 (24-26)	nuclear transcription factor, X-box binding-like 1
HOXB3	-1.71	0.01421	19 (16-24)	21 (21-22)	15 (14-17)	16 (15-17)	homeobox B3

gene name	FC	raw p value	Intensity normalized by gcRMA(slow) in unlogged scale				description
			control female	control male	intervention female	Intervention male	
RFC3	-1.70	0.00595	56 (53-63)	44 (40-49)	39 (36-40)	43 (43-46)	replication factor C (activator 1) 3, 38kDa
MTRR	-1.70	0.00189	41 (39-43)	46 (42-54)	32 (29-35)	37 (36-38)	5-methyltetrahydrofolate-homocysteine methyltransferase reductase
DBF4	-1.69	0.00050	27 (26-30)	21 (18-21)	20 (19-21)	16 (15-17)	DBF4 homolog (S. cerevisiae)
SPRED2	-1.69	0.02887	49 (48-52)	33 (30-36)	26 (24-30)	36 (32-41)	sprouty-related, EVH1 domain containing 2
MRPL23	-1.69	0.04378	45 (44-46)	37 (37-62)	31 (25-36)	44 (44-46)	mitochondrial ribosomal protein L23
GNL3	-1.69	0.04096	72 (66-75)	56 (56-56)	45 (40-52)	55 (50-61)	guanine nucleotide binding protein-like 3 (nucleolar)
KLF11	-1.69	0.03982	41 (37-50)	46 (40-48)	32 (30-33)	38 (33-44)	Kruppel-like factor 11
EML1	-1.68	0.03393	18 (17-24)	23 (23-24)	18 (17-21)	15 (15-16)	echinoderm microtubule associated protein like 1
ZCCHC7	-1.68	0.02434	37 (28-45)	36 (31-41)	26 (25-27)	26 (26-30)	zinc finger, CCHC domain containing 7
DLGAP5	-1.68	0.01348	9 (8-9)	7 (6-9)	6 (6-6)	6 (6-7)	discs, large (Drosophila) homolog-associated protein 5
BCOR	-1.68	0.01569	32 (31-36)	27 (23-31)	22 (20-24)	24 (22-27)	BCL6 co-repressor
RAD51AP1	-1.68	0.02292	23 (23-25)	15 (15-16)	15 (12-17)	15 (15-18)	RAD51 associated protein 1
NUDT12	-1.68	0.00746	22 (22-23)	23 (22-23)	19 (16-21)	16 (16-19)	nudix (nucleoside diphosphate linked moiety X)-type motif 12
ZNF506	-1.67	0.01647	41 (40-43)	52 (51-52)	37 (35-40)	36 (25-38)	zinc finger protein 506
C17orf58	-1.67	0.04807	353 (335-415)	321 (295-437)	267 (222-314)	317 (296-319)	chromosome 17 open reading frame 58
ASAP2	-1.67	0.03378	81 (70-96)	65 (52-80)	50 (46-54)	64 (56-71)	ArfGAP with SH3 domain, ankyrin repeat and PH domain 2
ACADM	-1.67	0.02443	212 (201-217)	196 (184-219)	161 (127-189)	162 (156-178)	acyl-Coenzyme A dehydrogenase, C-4 to C-12 straight chain
DZIP3	-1.66	0.01943	22 (20-25)	22 (20-26)	17 (16-19)	20 (18-20)	DAZ interacting protein 3, zinc finger
TMEM192	-1.66	0.03763	35 (34-39)	24 (22-30)	24 (23-26)	25 (21-29)	transmembrane protein 192
ANKRD13C	-1.66	0.00661	31 (29-32)	28 (28-31)	24 (21-26)	26 (20-27)	ankyrin repeat domain 13C
STOX2	-1.65	0.04254	19 (17-23)	14 (14-16)	13 (12-13)	15 (12-15)	storkhead box 2
RASSF2	-1.65	0.01262	15 (13-17)	15 (14-18)	13 (12-13)	11 (10-12)	Ras association (RalGDS/AF-6) domain family member 2
AGAP1	-1.65	0.02753	14 (11-17)	18 (16-19)	12 (11-12)	11 (10-15)	ArfGAP with GTPase domain, ankyrin repeat and PH domain 1
SAMD9	-1.65	0.02733	45 (42-48)	30 (24-32)	24 (23-26)	30 (26-33)	sterile alpha motif domain containing 9
MORC3	-1.65	0.00734	360 (340-390)	458 (391-546)	324 (312-343)	312 (290-329)	MORC family CW-type zinc finger 3
PLD1	-1.64	0.01325	12 (11-15)	11 (10-12)	9 (9-10)	10 (9-10)	phospholipase D1, phosphatidylcholine-specific
TUBB2A	-1.64	0.03903	657 (584-771)	504 (483-569)	434 (386-484)	531 (410-597)	tubulin, beta 2A
ENPP5	-1.64	0.00486	10 (10-10)	12 (11-12)	8 (7-9)	9 (8-9)	ectonucleotide pyrophosphatase/phosphodiesterase 5 (putative function)
KCTD10	-1.64	0.03571	56 (51-61)	66 (55-71)	39 (36-47)	44 (43-53)	potassium channel tetramerisation domain containing 10
VPS13B	-1.64	0.00651	81 (74-85)	69 (66-81)	51 (49-56)	65 (64-68)	vacuolar protein sorting 13 homolog B (yeast)
PYROXD2	-1.63	0.01934	13 (13-15)	15 (12-16)	10 (9-12)	10 (10-12)	pyridine nucleotide-disulphide oxidoreductase domain 2
GRAMD4	-1.62	0.00321	18 (17-18)	33 (28-35)	18 (17-18)	19 (19-20)	GRAM domain containing 4

gene name	FC	raw p value	Intensity normalized by gcRMA(slow) in unlogged scale				description
			control female	control male	intervention female	intervention male	
ASPN	-1.62	0.02681	14 (13-16)	12 (11-13)	9 (7-11)	11 (11-11)	asporin
SLC15A4	-1.62	0.02276	63 (60-65)	61 (54-63)	48 (44-52)	43 (39-45)	solute carrier family 15, member 4
RFC4	-1.62	0.04586	45 (43-50)	52 (45-61)	37 (35-40)	41 (36-50)	replication factor C (activator 1) 4, 37kDa
MOBKL1A	-1.62	0.03180	62 (58-75)	46 (45-55)	41 (39-45)	48 (47-61)	MOB1, Mps One Binder kinase activator-like 1A (yeast)
TMEM14A	-1.62	0.02808	41 (35-49)	28 (27-35)	26 (25-27)	35 (34-35)	transmembrane protein 14A
KIAA0406	-1.62	0.02150	126 (115-138)	154 (151-197)	117 (113-122)	125 (98-133)	KIAA0406
FBXO5	-1.62	0.00179	25 (22-28)	22 (22-23)	17 (16-20)	19 (19-20)	F-box protein 5
CCNB1	-1.62	0.02195	17 (14-21)	17 (16-20)	13 (13-14)	14 (13-17)	cyclin B1
ATMIN	-1.61	0.03771	343 (308-355)	302 (246-348)	251 (228-265)	240 (214-245)	ATM interactor
ZMYM1	-1.60	0.00487	12 (12-12)	14 (13-15)	11 (10-12)	10 (9-12)	zinc finger, MYM-type 1
RICH2	-1.60	0.04688	22 (19-24)	19 (16-20)	13 (12-15)	17 (17-18)	Rho-type GTPase-activating protein RICH2
MSH2	-1.60	0.04151	36 (31-39)	19 (19-23)	20 (18-22)	24 (21-26)	mutS homolog 2, colon cancer, nonpolyposis type 1 (E. coli)
MRPS30	-1.60	0.00090	16 (16-17)	19 (18-22)	14 (13-15)	14 (13-15)	mitochondrial ribosomal protein S30
TULP4	-1.60	0.01876	34 (33-41)	38 (36-43)	28 (27-31)	31 (31-33)	tubby like protein 4
NR2C1	-1.59	0.04400	90 (83-108)	111 (104-116)	98 (89-104)	71 (60-87)	nuclear receptor subfamily 2, group C, member 1
PTER	-1.59	0.04879	26 (24-32)	23 (22-27)	20 (18-22)	24 (21-25)	phosphotriesterase related
PDCL	-1.59	0.04732	105 (88-120)	106 (102-144)	87 (81-101)	81 (80-89)	phosducin-like
S100PBP	-1.59	0.04360	33 (31-38)	35 (35-47)	27 (26-31)	32 (29-33)	S100P binding protein
PRNP	-1.59	0.00189	1287 (1243-1321)	1203 (1124-1312)	857 (770-988)	1107 (1051-1142)	prion protein
MXRA7	-1.58	0.03233	16 (16-19)	15 (15-17)	15 (14-16)	12 (11-13)	matrix-remodelling associated 7
OSBPL10	-1.58	0.02152	356 (328-367)	334 (312-411)	262 (257-283)	265 (250-270)	oxysterol binding protein-like 10
XIAP	-1.58	0.04744	1015 (990-1060)	1056 (1025-1126)	944 (920-978)	796 (710-958)	X-linked inhibitor of apoptosis
ARID4A	-1.57	0.02650	29 (25-32)	37 (36-41)	25 (23-28)	26 (25-30)	AT rich interactive domain 4A (RBP1-like)
WDR44	-1.57	0.00242	21 (21-23)	22 (21-23)	19 (17-20)	19 (17-19)	WD repeat domain 44
CDK17	-1.57	0.04486	575 (533-634)	436 (397-441)	357 (352-392)	414 (393-426)	cyclin-dependent kinase 17
ST6GALNAC1	-1.56	0.02453	11 (9-13)	10 (9-11)	9 (8-10)	8 (7-8)	ST6 (alpha-N-acetyl-neuraminyl-2,3-beta-galactosyl-1,3)-N-acetylgalactosaminide alpha-2,6-sialyltransferase 1
HIBCH	-1.56	0.04667	81 (69-96)	78 (74-83)	59 (52-67)	74 (61-78)	3-hydroxyisobutyryl-Coenzyme A hydrolase
PI4K2B	-1.56	0.01732	22 (19-26)	19 (18-21)	17 (14-20)	17 (17-17)	phosphatidylinositol 4-kinase type 2 beta
MRPS35	-1.56	0.01933	182 (173-205)	207 (201-214)	150 (143-162)	172 (134-189)	mitochondrial ribosomal protein S35
MMAA	-1.56	0.00544	16 (15-17)	14 (13-17)	12 (11-13)	13 (13-15)	methylmalonic aciduria (cobalamin deficiency) cblA type
ZNF721	-1.55	0.04260	60 (57-70)	85 (83-92)	57 (54-63)	65 (52-74)	zinc finger protein 721
CDK4	-1.55	0.00705	33 (33-34)	30 (28-34)	25 (22-27)	28 (27-32)	cyclin-dependent kinase 4
FAM76A	-1.55	0.04328	12 (10-15)	11 (10-12)	9 (9-10)	10 (10-11)	family with sequence similarity 76, member A
PAPLN	-1.55	0.04204	10 (9-11)	14 (11-14)	7 (7-8)	9 (8-10)	papilin, proteoglycan-like sulfated glycoprotein
BASP1	-1.55	0.01825	1336 (1285-1414)	1795 (1707-2171)	1257 (1190-1326)	1332 (1185-1524)	brain abundant, membrane attached signal protein 1
MCM3	-1.54	0.01528	57 (56-58)	51 (49-63)	44 (42-47)	48 (44-52)	minichromosome maintenance complex component 3
PDIA5	-1.54	0.03513	397 (382-405)	226 (216-237)	220 (191-237)	319 (221-339)	protein disulfide isomerase family A, member 5

gene name	FC	raw p value	Intensity normalized by gcRMA(slow) in unlogged scale				description
			control female	control male	intervention female	Intervention male	
CCDC99	-1.54	0.00264	18 (16-20)	15 (15-18)	15 (14-15)	14 (13-14)	coiled-coil domain containing 99
CYTH1	-1.54	0.00912	24 (23-26)	26 (22-28)	19 (18-20)	20 (19-23)	cytohesin 1
SETD6	-1.54	0.03173	16 (16-18)	13 (11-15)	14 (13-15)	11 (10-11)	SET domain containing 6
TSEN15	-1.53	0.01617	102 (98-103)	62 (62-74)	72 (64-77)	64 (60-78)	tRNA splicing endonuclease 15 homolog (S. cerevisiae)
RIOK1	-1.53	0.04560	33 (32-35)	35 (32-50)	31 (29-34)	27 (26-32)	RIO kinase 1 (yeast)
RAD52	-1.53	0.03455	8 (7-8)	9 (8-9)	6 (5-7)	8 (6-8)	RAD52 homolog (S. cerevisiae)
RCBTB1	-1.53	0.04032	141 (139-150)	116 (111-121)	104 (94-114)	103 (90-137)	regulator of chromosome condensation (RCC1) and BTB (POZ) domain containing protein 1
MDC1	-1.52	0.00947	25 (23-27)	32 (29-33)	21 (19-23)	22 (22-26)	mediator of DNA-damage checkpoint 1
CPOX	-1.52	0.01399	43 (41-44)	39 (38-43)	32 (31-35)	35 (28-36)	coproporphyrinogen oxidase
DNMBP	-1.52	0.02562	44 (42-47)	58 (47-64)	41 (40-41)	39 (37-41)	dynamin binding protein
KIAA1671	-1.52	0.03608	21 (19-24)	20 (17-24)	17 (16-18)	18 (17-18)	KIAA1671
HACL1	-1.51	0.01800	15 (14-16)	17 (17-18)	12 (11-13)	14 (12-15)	2-hydroxyacyl-CoA lyase 1
TRIM44	-1.51	0.02382	194 (178-211)	218 (211-250)	152 (147-174)	176 (172-185)	tripartite motif-containing 44
CLK4	-1.51	0.04853	77 (68-88)	64 (63-89)	61 (56-65)	63 (59-65)	CDC-like kinase 4
KIAA0240	-1.51	0.04673	30 (27-37)	33 (26-33)	21 (20-24)	27 (26-28)	KIAA0240
AKAP1	-1.50	0.00432	39 (37-41)	38 (38-38)	30 (27-36)	32 (30-32)	A kinase (PRKA) anchor protein 1
HHIP	1.50	0.00497	5 (5-5)	5 (5-5)	6 (6-6)	5 (5-6)	hedgehog interacting protein
FAM167A	1.50	0.00868	13 (13-14)	13 (12-13)	17 (15-19)	15 (13-16)	family with sequence similarity 167, member A
GNAL	1.50	0.01877	4 (4-5)	4 (4-4)	5 (5-5)	6 (5-6)	guanine nucleotide binding protein (G protein), alpha activating activity polypeptide, olfactory type
GPR1	1.51	0.01560	275 (269-292)	252 (250-267)	358 (315-404)	315 (298-329)	G protein-coupled receptor 1
TBC1D17	1.52	0.04318	15 (14-16)	19 (17-19)	17 (16-19)	22 (20-24)	TBC1 domain family, member 17
LRIG2	1.52	0.00911	15 (15-16)	17 (15-17)	22 (19-26)	18 (17-18)	leucine-rich repeats and immunoglobulin-like domains 2
PPP2R1A	1.52	0.00315	36 (35-37)	32 (30-34)	38 (36-38)	47 (43-50)	protein phosphatase 2 (formerly 2A), regulatory subunit A, alpha isoform
PCGF1	1.53	0.04269	67 (56-79)	63 (57-68)	81 (73-90)	84 (83-85)	polycomb group ring finger 1
NDUFS7	1.53	0.01983	7 (6-8)	8 (7-8)	8 (7-8)	10 (8-11)	NADH dehydrogenase (ubiquinone) Fe-S protein 7, 20kDa (NADH-coenzyme Q reductase)
PRR4	1.54	0.00087	13 (13-14)	11 (11-11)	15 (14-16)	15 (14-16)	proline rich 4 (lacrimal)
MKNK2	1.54	0.01743	36 (35-37)	52 (47-53)	52 (47-54)	54 (49-66)	MAP kinase interacting serine/threonine kinase 2
ATXN7L2	1.55	0.00660	10 (10-10)	10 (9-12)	12 (11-13)	13 (13-14)	ataxin 7-like 2
CD47	1.55	0.00452	389 (365-415)	292 (255-304)	388 (367-411)	403 (402-450)	CD47 molecule
C11orf17	1.56	0.01512	10 (9-11)	9 (9-11)	11 (11-12)	12 (12-15)	chromosome 11 open reading frame 17
WDR13	1.56	0.04035	68 (66-73)	113 (105-121)	105 (98-114)	120 (89-139)	WD repeat domain 13
STRA13	1.58	0.04589	87 (76-95)	61 (59-80)	96 (93-97)	92 (85-116)	stimulated by retinoic acid 13 homolog (mouse)
MS4A4A	1.58	0.04283	580 (488-645)	429 (381-430)	533 (511-588)	611 (561-652)	membrane-spanning 4-domains, subfamily A, member 4
C14orf19	1.59	0.04870	4 (4-4)	5 (5-5)	6 (5-6)	5 (5-6)	immunoglobulin (CD79A) binding protein 1 pseudogene
C20orf3	1.59	0.03202	523 (442-588)	533 (495-599)	603 (551-646)	776 (592-783)	chromosome 20 open reading frame 3
LOC441666	1.59	0.02138	5 (4-5)	6 (5-6)	7 (6-7)	6 (6-7)	zinc finger protein 91 pseudogene

gene name	FC	raw p value	Intensity normalized by gcRMA(slow) in unlogged scale				description
			Control female	Control male	intervention female	Intervention male	
CD79A	1.61	0.00110	13 (12-14)	14 (13-14)	18 (17-19)	16 (16-16)	CD79a molecule, immunoglobulin-associated alpha
ISY1	1.61	0.02085	96 (85-107)	85 (82-90)	108 (101-118)	122 (116-127)	ISY1 splicing factor homolog (S. cerevisiae)
MAFK	1.62	0.01361	10 (10-11)	13 (12-14)	14 (13-17)	14 (13-16)	v-maf musculoaponeurotic fibrosarcoma oncogene homolog K (avian)
METT10D	1.62	0.01328	6 (5-6)	5 (5-6)	7 (6-7)	7 (7-7)	methyltransferase 10 domain containing
CIRBP	1.62	0.02411	47 (46-53)	77 (74-77)	85 (78-91)	74 (61-77)	cold inducible RNA binding protein
ALDH16A1	1.62	0.04029	14 (13-14)	18 (17-20)	20 (17-22)	22 (17-25)	aldehyde dehydrogenase 16 family, member A1
HSD17B3	1.62	0.03523	6 (6-6)	7 (6-7)	9 (7-10)	8 (7-10)	hydroxysteroid (17-beta) dehydrogenase 3
APBA3	1.63	0.01531	27 (26-27)	23 (20-27)	34 (32-36)	30 (26-34)	amyloid beta (A4) precursor protein-binding, family A, member 3
C4orf29	1.63	0.03579	19 (17-21)	17 (15-17)	26 (23-28)	22 (18-23)	chromosome 4 open reading frame 29
LOC401859	1.64	0.01919	23 (21-24)	23 (21-23)	27 (24-31)	28 (24-31)	similar to TRIMCyp
SMG7	1.65	0.04122	61 (59-65)	92 (89-98)	93 (86-98)	102 (81-152)	Smg-7 homolog, nonsense mediated mRNA decay factor (C. elegans)
C1orf77	1.66	0.00094	26 (25-27)	25 (23-26)	30 (26-34)	36 (35-37)	chromosome 1 open reading frame 77
CERCAM	1.66	0.02585	43 (35-49)	39 (38-43)	45 (42-53)	54 (48-66)	cerebral endothelial cell adhesion molecule
hCG_17324	1.66	0.00163	5 (5-6)	4 (4-5)	6 (6-6)	6 (6-6)	primary ciliary dyskinesia protein 1
FAM47E	1.67	0.00428	7 (7-8)	8 (7-8)	11 (10-11)	8 (7-10)	family with sequence similarity 47, member E
SYMPK	1.68	0.00631	13 (13-15)	23 (22-24)	21 (19-24)	24 (21-26)	symplekin
RAB36	1.68	0.01146	10 (8-11)	13 (12-13)	15 (13-17)	13 (13-15)	RAB36, member RAS oncogene family
NAGK	1.68	0.04079	164 (141-176)	167 (162-226)	232 (214-254)	199 (190-247)	N-acetylglucosamine kinase
LYG2	1.69	0.00021	10 (10-10)	9 (8-9)	14 (13-14)	11 (10-11)	lysozyme G-like 2
ZSCAN18	1.72	0.01475	120 (111-130)	185 (170-221)	185 (180-208)	211 (174-236)	zinc finger and SCAN domain containing 18
PTGR1	1.75	0.01324	24 (20-30)	16 (14-16)	28 (26-28)	26 (23-29)	prostaglandin reductase 1
PROK2	1.82	0.04932	11 (8-13)	9 (9-12)	12 (11-13)	17 (17-22)	prokineticin 2
GAP43	1.82	0.00117	11 (11-11)	7 (7-7)	11 (10-11)	14 (12-16)	growth associated protein 43
GNL3L	1.84	0.04403	22 (16-27)	18 (15-21)	25 (23-27)	24 (22-26)	guanine nucleotide binding protein-like 3 (nucleolar)-like
ZNF321	1.89	0.02371	6 (4-7)	11 (9-11)	9 (9-9)	11 (10-13)	zinc finger protein 321
OVOL1	1.89	0.04886	39 (32-47)	48 (40-49)	60 (55-66)	48 (42-52)	ovo-like 1(Drosophila)
FLT4	2.04	0.02282	25 (22-28)	44 (39-50)	49 (43-57)	52 (37-58)	fms-related tyrosine kinase 4
TFRC	2.06	0.01020	6148 (5527-6241)	3532 (3117-5108)	6465 (5953-7235)	6891 (6666-7136)	transferrin receptor (p90, CD71)
CCDC69	2.31	0.03583	46 (35-63)	115 (99-133)	109 (107-111)	119 (91-122)	coiled-coil domain containing 69
THOP1	2.32	0.00329	12 (9-15)	20 (18-21)	22 (20-25)	22 (22-25)	thimet oligopeptidase 1
FURIN	2.51	0.04597	191 (147-209)	277 (245-360)	325 (275-399)	290 (287-454)	furin (paired basic amino acid cleaving enzyme)
LOC100288618	2.73	0.00003	8 (7-8)	9 (8-10)	13 (12-15)	14 (11-15)	hypothetical protein LOC100288618
PAFAH2	2.82	0.00069	20 (18-20)	29 (24-34)	42 (42-45)	29 (28-34)	platelet-activating factor acetylhydrolase 2, 40kDa
SH3GLB2	2.90	0.03864	87 (59-114)	282 (196-335)	230 (188-268)	224 (173-235)	SH3-domain GRB2-like endophilin B2
CPZ	2.98	0.04566	168 (111-256)	674 (498-680)	508 (462-611)	447 (326-530)	carboxypeptidase Z
FAM150B	3.18	0.04888	21 (16-30)	17 (14-18)	23 (20-30)	35 (29-81)	family with sequence similarity 150, member B

gene name	FC	raw p value	Intensity normalized by gcRMA(slow) in unlogged scale				description
			control female	control male	intervention female	Intervention male	
PRRG4	3.49	0.02213	59 (44-82)	51 (36-53)	87 (78-123)	86 (63-105)	proline rich Gla (G-carboxyglutamic acid) 4 (transmembrane)
CORO6	3.73	0.01491	139 (112-164)	340 (279-481)	408 (296-535)	458 (283-687)	coronin 6
CX3CR1	3.80	0.01587	27 (16-43)	16 (12-26)	45 (38-56)	36 (31-44)	chemokine (C-X3-C motif) receptor 1
LYZ	9.27	0.00368	12 (9-14)	7 (7-8)	69 (38-168)	9 (8-9)	lysozyme (renal amyloidosis)
HINT3	9.32	0.01581	11 (9-13)	16 (13-27)	88 (62-117)	27 (16-27)	histidine triad nucleotide binding protein 3

Data are presented as median (interquartile range IQR = 25th-75th percentile). Control female = placentas of female offspring in the control group, Control male = placentas of male offspring in the control group, Intervention female = placentas of female offspring in the n-3 LCPUFA intervention group, Intervention male = placentas of male offspring in the n-3 LCPUFA intervention group. The genes are sorted according to their fold change. FC, fold change;

11.6.3 List of significantly regulated genes between male and female placentas in the control group from the microarray analysis (CM vs. CF)

gene name	FC	raw p value	Intensity normalized by gcRMA(slow) in unlogged scale		description
			control female	control male	
PEG3	-8.23	0.03637	1155 (977-1215)	320 (167-348)	paternally expressed 3
HIST1H4C	-7.54	0.01985	616 (522-650)	82 (48-193)	histone cluster 1, H4c
NKTR	-6.75	0.02379	143 (131-211)	22 (14-71)	natural killer-tumor recognition sequence
PPIG	-6.44	0.01685	279 (195-374)	46 (28-101)	peptidylprolyl isomerase G (cyclophilin G)
RAP2A	-5.63	0.03508	299 (209-503)	35 (29-149)	RAP2A, member of RAS oncogene family
GALNT1	-4.76	0.04757	122 (107-208)	51 (29-63)	UDP-N-acetyl-alpha-D-galactosamine:polypeptide N-acetylgalactosaminyltransferase 1 (GalNAc-T1)
CDK6	-4.43	0.03083	338 (291-419)	106 (63-165)	cyclin-dependent kinase 6
MBNL1	-4.12	0.03084	113 (90-148)	39 (23-56)	muscleblind-like (Drosophila)
C8orf59	-3.79	0.00008	75 (71-80)	24 (19-24)	chromosome 8 open reading frame 59
NFKBIZ	-3.78	0.04497	115 (114-199)	48 (31-78)	nuclear factor of kappa light polypeptide gene enhancer in B-cells inhibitor, zeta
TRAK2	-3.72	0.00280	52 (44-58)	12 (10-16)	trafficking protein, kinesin binding 2
TIMP3	-3.45	0.01874	663 (605-1073)	243 (190-354)	TIMP metallopeptidase inhibitor 3
EPRS	-3.40	0.01805	66 (56-93)	22 (16-32)	glutamyl-prolyl-tRNA synthetase
IL6ST	-3.36	0.01833	44 (39-45)	9 (7-20)	interleukin 6 signal transducer (gp130, oncostatin M receptor)
LIN28B	-3.24	0.01990	70 (47-98)	18 (15-28)	lin-28 homolog B (C. elegans)
TAOK1	-3.10	0.01144	29 (24-32)	7 (6-12)	TAO kinase 1
NRIP1	-2.91	0.00636	644 (521-763)	282 (197-291)	nuclear receptor interacting protein 1
OXR1	-2.89	0.03187	85 (60-108)	35 (23-43)	oxidation resistance 1
FN1	-2.88	0.02625	293 (245-348)	102 (72-172)	fibronectin 1
SSB	-2.88	0.01718	485 (376-552)	182 (126-210)	Sjogren syndrome antigen B (autoantigen La)
ESF1	-2.84	0.00932	58 (48-65)	19 (15-25)	ESF1, nucleolar pre-rRNA processing protein, homolog (S. cerevisiae)
HSD11B1	-2.80	0.00727	135 (104-182)	46 (43-56)	hydroxysteroid (11-beta) dehydrogenase 1
ANGPT2	-2.76	0.03481	41 (29-71)	17 (16-18)	angiopoietin 2
MME	-2.76	0.04367	211 (143-284)	87 (59-107)	membrane metallo-endopeptidase
GNB1	-2.71	0.02182	643 (558-692)	258 (181-307)	guanine nucleotide binding protein (G protein), beta polypeptide 1
ARHGAP18	-2.71	0.03703	25 (21-42)	13 (10-13)	Rho GTPase activating protein 18
NT5C3	-2.63	0.00227	102 (91-119)	48 (37-50)	5'-nucleotidase, cytosolic III
SESTD1	-2.62	0.02488	91 (71-123)	32 (27-49)	SEC14 and spectrin domains 1
SEPW1	-2.46	0.00957	671 (490-880)	322 (248-332)	selenoprotein W, 1
TRIM13	-2.39	0.02689	43 (38-56)	16 (15-24)	tripartite motif-containing 13
MYO1B	-2.38	0.00314	76 (71-97)	36 (34-38)	myosin IB
BRCC3	-2.35	0.03018	21 (15-26)	7 (7-9)	BRCA1/BRCA2-containing complex, subunit 3
IGFBP7	-2.30	0.04598	714 (642-803)	273 (215-474)	insulin-like growth factor binding protein 7
BAT2L2	-2.29	0.03860	57 (48-68)	22 (19-29)	HLA-B associated transcript 2-like 2
IFI6	-2.29	0.01731	114 (102-135)	39 (38-68)	interferon, alpha-inducible protein 6
OLR1	-2.28	0.01462	269 (215-352)	118 (103-150)	oxidized low density lipoprotein (lectin-like) receptor 1
PRKX	-2.27	0.00764	75 (68-78)	30 (25-39)	protein kinase, X-linked
TAF9B	-2.27	0.01681	16 (13-20)	6 (6-8)	TAF9B RNA polymerase II, TATA box binding protein (TBP)-associated factor, 31kDa
SDCCAG1	-2.27	0.03694	30 (24-35)	18 (12-18)	serologically defined colon cancer antigen 1
ROCK1	-2.23	0.02695	45 (35-52)	22 (16-26)	Rho-associated, coiled-coil containing protein kinase 1
IFI27	-2.23	0.01599	826 (729-1001)	354 (309-487)	interferon, alpha-inducible protein 27
QSER1	-2.22	0.02385	16 (13-23)	8 (7-9)	glutamine and serine rich 1
ERC1	-2.21	0.03044	35 (28-43)	17 (13-21)	ELKS/RAB6-interacting/CAST family member 1
LAIR2	-2.19	0.03256	34 (30-48)	18 (17-18)	leukocyte-associated immunoglobulin-like receptor 2
CAB39L	-2.19	0.01649	15 (14-19)	7 (6-9)	calcium binding protein 39-like
IFIT1	-2.19	0.01002	70 (61-71)	24 (23-31)	interferon-induced protein with tetratricopeptide repeats 1
MOSPD1	-2.19	0.01693	29 (23-35)	15 (11-17)	motile sperm domain containing 1
CHN1	-2.17	0.04577	114 (99-180)	66 (62-68)	chimerin (chimaerin) 1
RNF115	-2.16	0.00447	36 (33-38)	19 (15-20)	ring finger protein 115

gene name	FC	raw p value	Intensity normalized by gcRMA(slow) in unlogged scale		description
			control female	control male	
EPB41	-2.14	0.02059	75 (64-93)	34 (30-46)	erythrocyte membrane protein band 4.1 (elliptocytosis 1, RH-linked)
OAS2	-2.13	0.02457	59 (35-81)	24 (23-26)	2'-5'-oligoadenylate synthetase 2, 69/71kDa
GALNT11	-2.12	0.00407	799 (720-862)	331 (322-384)	UDP-N-acetyl-alpha-D-galactosamine:polypeptide N-acetylgalactosaminyltransferase 11 (GalNAc-T11)
HOXA13	-2.10	0.04735	20 (13-27)	10 (8-12)	homeobox A13
RGS5	-2.10	0.02332	48 (38-58)	22 (18-30)	regulator of G-protein signaling 5
TBL1X	-2.10	0.01948	15 (14-18)	7 (6-9)	transducin (beta)-like 1X-linked
LMLN	-2.05	0.01242	22 (16-28)	10 (9-12)	leishmanolysin-like (metallopeptidase M8 family)
GPX8	-2.05	0.00066	421 (414-451)	198 (190-239)	glutathione peroxidase 8 (putative)
EPHA3	-2.04	0.01243	45 (43-57)	24 (22-29)	EPH receptor A3
BBX	-2.04	0.04432	235 (216-258)	146 (101-171)	bobby sox homolog (Drosophila)
RBM9	-2.02	0.02225	80 (66-95)	36 (31-52)	RNA binding motif protein 9
RAB8B	-2.01	0.02143	43 (37-55)	20 (19-28)	RAB8B, member RAS oncogene family
SALL1	-2.01	0.01355	11 (9-14)	6 (5-6)	sal-like 1 (Drosophila)
MTHFD2	-2.00	0.00338	163 (159-173)	90 (74-102)	methylenetetrahydrofolate dehydrogenase (NADP+ dependent) 2, methenyltetrahydrofolate cyclohydrolase
CYBB	-1.98	0.03653	52 (44-71)	31 (25-37)	cytochrome b-245, beta polypeptide
LGALS8	-1.98	0.01620	54 (42-67)	27 (25-27)	lectin, galactoside-binding, soluble, 8
PPP3CA	-1.97	0.00530	125 (101-149)	58 (54-71)	protein phosphatase 3 (formerly 2B), catalytic subunit, alpha isoform
KLF3	-1.97	0.00900	35 (31-41)	15 (15-22)	Kruppel-like factor 3 (basic)
PSMD10	-1.97	0.01269	69 (53-88)	33 (30-40)	proteasome (prosome, macropain) 26S subunit, non-ATPase, 10
GPR34	-1.97	0.04537	574 (466-699)	261 (232-336)	G protein-coupled receptor 34
YWHAB	-1.96	0.02816	576 (528-614)	323 (240-398)	tyrosine 3-monooxygenase/tryptophan 5-monooxygenase activation protein, beta polypeptide
ACTB	-1.95	0.03307	2071 (1716-2586)	1115 (910-1390)	actin, beta
SFRP1	-1.94	0.02787	44 (40-55)	21 (20-29)	secreted frizzled-related protein 1
ZNF138	-1.93	0.00772	16 (15-20)	9 (9-9)	zinc finger protein 138
KDELR3	-1.93	0.00234	33 (28-39)	18 (16-19)	KDEL (Lys-Asp-Glu-Leu) endoplasmic reticulum protein retention receptor 3
AFFX-HSAC07/X00351_M_at	-1.92	0.02433	3687 (3073-4394)	1819 (1594-2247)	NA
CCR1	-1.92	0.02497	84 (67-111)	39 (39-53)	chemokine (C-C motif) receptor 1
TMEM181	-1.91	0.02241	196 (181-209)	107 (83-133)	transmembrane protein 181
MAGOH	-1.91	0.01339	64 (55-73)	28 (26-40)	mago-nashi homolog, proliferation-associated (Drosophila)
SULF2	-1.90	0.02068	205 (189-247)	105 (104-121)	sulfatase 2
ITM2C	-1.90	0.03641	121 (105-140)	65 (56-69)	integral membrane protein 2C
PGD	-1.89	0.01719	89 (64-113)	47 (41-50)	phosphogluconate dehydrogenase
LGALS1	-1.89	0.01705	1658 (1436-2095)	980 (858-1080)	lectin, galactoside-binding, soluble, 1
COL5A1	-1.88	0.01005	154 (146-177)	84 (74-105)	collagen, type V, alpha 1
hCG_1990547	-1.88	0.04465	23 (22-33)	16 (14-17)	family with sequence similarity 86, member A pseudogene
EEF1A1	-1.87	0.01369	134 (102-175)	72 (71-74)	eukaryotic translation elongation factor 1 alpha 1
KDM6A	-1.87	0.00714	62 (56-68)	38 (30-39)	lysine (K)-specific demethylase 6A
ADH5	-1.87	0.00659	200 (167-242)	111 (105-112)	alcohol dehydrogenase 5 (class III), chi polypeptide
KIAA0802	-1.86	0.00893	31 (28-33)	15 (14-18)	KIAA0802
HNRNPR	-1.85	0.02234	42 (40-45)	29 (21-30)	heterogeneous nuclear ribonucleoprotein R
DNAJB14	-1.84	0.01004	678 (675-772)	383 (349-477)	DnaJ (Hsp40) homolog, subfamily B, member 14
CAPRIN2	-1.83	0.02986	45 (35-55)	22 (20-26)	caprin family member 2
P2RY1	-1.83	0.00542	15 (14-17)	7 (7-10)	purinergic receptor P2Y, G-protein coupled, 1
MOSPD2	-1.83	0.03551	97 (76-120)	47 (45-57)	motile sperm domain containing 2
LYVE1	-1.83	0.04365	856 (655-1018)	374 (359-473)	lymphatic vessel endothelial hyaluronan receptor 1
MYCN	-1.82	0.00065	224 (214-237)	113 (112-132)	v-myc myelocytomatosis viral related oncogene, neuroblastoma derived (avian)
SOX4	-1.81	0.00464	107 (97-115)	53 (51-62)	SRY (sex determining region Y)-box 4
CKS2	-1.81	0.00999	58 (53-65)	31 (28-36)	CDC28 protein kinase regulatory subunit 2
ZNF124	-1.81	0.00203	12 (11-13)	7 (6-7)	zinc finger protein 124
HDHD1A	-1.81	0.00133	221 (193-248)	134 (116-134)	haloacid dehalogenase-like hydrolase domain containing 1A

gene name	FC	raw p value	Intensity normalized by gcRMA(slow) in unlogged scale		description
			control female	control male	
VPS13A	-1.80	0.01195	35 (30-40)	19 (16-23)	vacuolar protein sorting 13 homolog A (S. cerevisiae)
PAPSS1	-1.80	0.01759	32 (24-40)	15 (15-18)	3'-phosphoadenosine 5'-phosphosulfate synthase 1
OAT	-1.79	0.00223	786 (679-887)	418 (392-470)	ornithine aminotransferase
TEAD1	-1.79	0.02513	801 (688-967)	379 (375-554)	TEA domain family member 1 (SV40 transcriptional enhancer factor)
ZNF573	-1.79	0.03956	46 (36-58)	25 (22-31)	zinc finger protein 573
CKAP2	-1.79	0.00825	78 (60-97)	40 (40-45)	cytoskeleton associated protein 2
MOCS2	-1.78	0.00264	60 (59-68)	36 (34-39)	molybdenum cofactor synthesis 2
ARRDC4	-1.77	0.02517	71 (70-90)	45 (44-51)	arrestin domain containing 4
WRB	-1.77	0.00935	231 (184-284)	127 (125-134)	tryptophan rich basic protein
PAPSS2	-1.77	0.01846	21 (16-26)	11 (10-13)	3'-phosphoadenosine 5'-phosphosulfate synthase 2
SERPINH1	-1.77	0.00990	529 (479-591)	304 (286-311)	serpin peptidase inhibitor, clade H (heat shock protein 47), member 1, (collagen binding protein 1)
GSK3B	-1.77	0.00471	103 (93-108)	51 (49-60)	glycogen synthase kinase 3 beta
CTSO	-1.76	0.00146	83 (75-86)	42 (41-47)	cathepsin O
CHD4	-1.75	0.03334	56 (39-74)	31 (29-34)	chromodomain helicase DNA binding protein 4
MAGED1	-1.75	0.03013	991 (930-1029)	683 (504-714)	melanoma antigen family D, 1
MED13L	-1.74	0.04602	43 (40-46)	30 (22-33)	mediator complex subunit 13-like
FZD6	-1.73	0.02742	350 (285-436)	223 (186-246)	frizzled homolog 6 (Drosophila)
FCER1G	-1.73	0.02299	338 (263-415)	201 (174-216)	Fc fragment of IgE, high affinity I, receptor for /// gamma polypeptide
STIM2	-1.72	0.00814	22 (20-25)	12 (12-14)	stromal interaction molecule 2
EDNRA	-1.72	0.03849	49 (46-62)	28 (28-37)	endothelin receptor type A
FYB	-1.72	0.02842	20 (17-22)	11 (9-12)	FYN binding protein (FYB-120/130)
ZNF440	-1.71	0.02395	17 (15-18)	10 (8-11)	zinc finger protein 440
C18orf10	-1.71	0.01658	39 (32-47)	25 (21-27)	chromosome 18 open reading frame 10
ACTR2	-1.71	0.02087	299 (278-315)	196 (154-211)	ARP2 actin-related protein 2 homolog (yeast)
PDIA5	-1.71	0.00024	439 (424-449)	260 (241-270)	protein disulfide isomerase family A, member 5
IKBIP	-1.70	0.01409	199 (184-225)	110 (105-134)	IKBKB interacting protein
TNFRSF21	-1.70	0.01872	82 (80-96)	57 (49-62)	tumor necrosis factor receptor superfamily, member 21
AFFX-HUMGAPDH/M33197_5_at	-1.70	0.00216	2764 (2456-3147)	1772 (1580-1802)	NA
ZBTB43	1.70	0.02742	17 (15-21)	11 (9-13)	zinc finger and BTB domain containing 43
LIFR	-1.70	0.01099	509 (461-612)	352 (306-362)	leukemia inhibitory factor receptor alpha
GIMAP7	-1.69	0.02337	349 (327-398)	209 (187-250)	GTPase, IMAP family member 7
GK	-1.69	0.02733	12 (10-15)	8 (7-8)	glycerol kinase
YAP1	-1.69	0.02994	413 (396-445)	282 (219-321)	Yes-associated protein 1, 65kDa
ZNF92	-1.69	0.00136	21 (20-24)	14 (12-14)	zinc finger protein 92
SAE1	-1.69	0.02487	53 (50-66)	38 (34-39)	SUMO1 activating enzyme subunit 1
CHST2	-1.69	0.00863	73 (70-87)	47 (46-50)	carbohydrate (N-acetylglucosamine-6-O) sulfotransferase 2
SC5DL	-1.69	0.01770	53 (45-58)	25 (25-32)	sterol-C5-desaturase (ERG3 delta-5-desaturase homolog, S. cerevisiae)-like
PTGR1	-1.67	0.02732	26 (22-33)	19 (16-19)	prostaglandin reductase 1
EIF1AX	-1.67	0.04470	209 (163-252)	108 (101-135)	eukaryotic translation initiation factor 1A, X-linked
CYSLTR1	-1.67	0.04550	10 (10-13)	7 (7-8)	cysteinyl leukotriene receptor 1
MAPRE1	-1.67	0.02602	79 (67-90)	42 (39-51)	microtubule-associated protein, RP/EB family, member 1
RUFY3	-1.67	0.01622	59 (51-71)	39 (34-40)	RUN and FYVE domain containing 3
C8orf84	-1.67	0.04384	17 (14-21)	11 (9-12)	chromosome 8 open reading frame 84
MAP7D2	-1.67	0.02243	125 (117-132)	83 (65-92)	MAP7 domain containing 2
GLIPR1	-1.67	0.04654	56 (53-71)	34 (33-44)	GLI pathogenesis-related 1
FMNL2	-1.67	0.03662	645 (579-722)	436 (340-498)	formin-like 2
MPP1	-1.67	0.02410	91 (85-101)	54 (47-69)	membrane protein, palmitoylated 1, 55kDa
SAMD9	-1.66	0.04137	44 (40-46)	31 (24-32)	sterile alpha motif domain containing 9
RNFT1	-1.66	0.00440	31 (29-33)	18 (17-20)	ring finger protein, transmembrane 1
TMEM126A	-1.65	0.01386	115 (96-133)	68 (60-78)	transmembrane protein 126A
IDO1	-1.65	0.04078	473 (381-621)	288 (281-321)	indoleamine 2,3-dioxygenase 1
TERF1	-1.65	0.04636	46 (37-51)	25 (23-28)	telomeric repeat binding factor (NIMA-interacting) 1

gene name	FC	raw p value	Intensity normalized by gcRMA(slow) in unlogged scale		description
			control female	control male	
LARP4	-1.65	0.00048	194 (186-198)	107 (107-119)	La ribonucleoprotein domain family, member 4
MSN	-1.64	0.04623	709 (692-921)	506 (492-535)	moesin
NOX4	-1.64	0.02462	10 (10-12)	6 (6-8)	NADPH oxidase 4
TMC5	-1.64	0.04007	37 (34-42)	26 (20-30)	transmembrane channel-like 5
GUCY1A3	-1.64	0.03707	99 (90-105)	53 (47-70)	guanylate cyclase 1, soluble, alpha 3
LGALS3	-1.64	0.01001	1426 (1199-1707)	891 (819-965)	lectin, galactoside-binding, soluble, 3
TMEM126B	-1.64	0.01989	121 (114-135)	67 (67-83)	transmembrane protein 126B
PPIL1	-1.63	0.04138	103 (99-109)	50 (49-79)	peptidylprolyl isomerase (cyclophilin)-like 1
CNOT8	-1.63	0.01665	43 (38-50)	29 (26-29)	CCR4-NOT transcription complex, subunit 8
HECTD1	-1.63	0.04820	17 (15-21)	13 (10-14)	HECT domain containing 1
APEX1	-1.63	0.00810	159 (154-175)	118 (98-119)	APEX nuclease (multifunctional DNA repair enzyme) 1
PRCP	-1.62	0.04115	896 (858-1148)	628 (612-674)	prolylcarboxypeptidase (angiotensinase C)
C4orf27	-1.62	0.02626	31 (29-33)	20 (17-24)	chromosome 4 open reading frame 27
SLFN5	-1.61	0.00746	10 (9-10)	6 (6-6)	schlafen family member 5
ARL2BP	-1.61	0.01274	35 (31-39)	22 (20-23)	ADP-ribosylation factor-like 2 binding protein
GIMAP2	-1.61	0.03052	130 (120-155)	79 (75-100)	GTPase, IMAP family member 2
TCEAL7	-1.61	0.01814	9 (8-10)	6 (5-6)	transcription elongation factor A (SII)-like 7
C1orf27	-1.61	0.01419	83 (72-95)	55 (49-55)	chromosome 1 open reading frame 27
TMEM192	-1.61	0.01317	45 (41-51)	27 (25-32)	transmembrane protein 192
MSH2	-1.61	0.01606	35 (31-39)	19 (18-24)	mutS homolog 2, colon cancer, nonpolyposis type 1 (E. coli)
EEF1E1	-1.60	0.04576	41 (41-51)	33 (28-35)	eukaryotic translation elongation factor 1 epsilon 1
IDH1	-1.60	0.02002	1199 (1112-1258)	650 (615-827)	isocitrate dehydrogenase 1 (NADP+), soluble
ZFP36L1	-1.60	0.02025	859 (800-940)	590 (514-597)	zinc finger protein 36, C3H type-like 1
IL33	-1.59	0.03369	754 (668-830)	545 (427-565)	interleukin 33
LYPLAL1	-1.59	0.01543	105 (86-122)	62 (62-64)	lysophospholipase-like 1
GPX7	-1.59	0.00346	20 (19-21)	11 (11-13)	glutathione peroxidase 7
CLEC1A	-1.59	0.00718	355 (344-405)	246 (235-254)	C-type lectin domain family 1, member A
ENO1	-1.59	0.00367	301 (269-346)	199 (187-207)	enolase 1, (alpha)
LY96	-1.59	0.00030	281 (268-291)	167 (167-179)	lymphocyte antigen 96
PCDHB16	-1.58	0.02614	8 (7-10)	5 (5-6)	protocadherin beta 16
MXD4	-1.58	0.01925	20 (17-23)	11 (11-14)	MAX dimerization protein 4
NUP37	-1.58	0.00058	81 (75-84)	50 (48-51)	nucleoporin 37kDa
DLG1	-1.58	0.02814	109 (103-113)	65 (57-81)	discs, large homolog 1 (Drosophila)
ID3	-1.57	0.03894	534 (480-640)	310 (309-406)	inhibitor of DNA binding 3, dominant negative helix-loop-helix protein
CTHRC1	-1.57	0.04162	1281 (1185-1459)	722 (722-941)	collagen triple helix repeat containing 1
SEL1L3	-1.57	0.04178	35 (32-39)	20 (20-25)	sel-1 suppressor of lin-12-like 3 (C. elegans)
NKRF	-1.56	0.00254	34 (34-37)	22 (21-24)	NFKB repressing factor
BAG2	-1.56	0.00304	96 (87-109)	61 (61-64)	BCL2-associated athanogene 2
PIPOX	-1.56	0.02702	19 (18-22)	12 (11-15)	pipecolic acid oxidase
SNRPF	-1.56	0.01840	53 (46-62)	34 (31-38)	small nuclear ribonucleoprotein polypeptide F
ATP2B4	-1.55	0.02806	639 (608-659)	369 (335-476)	ATPase, Ca++ transporting, plasma membrane 4
CCNH	-1.55	0.00498	39 (35-42)	25 (23-25)	cyclin H
TMEM165	-1.55	0.03852	386 (367-440)	284 (241-306)	transmembrane protein 165
BIN2	-1.55	0.00306	21 (20-22)	14 (12-14)	bridging integrator 2
IFI16	-1.55	0.00386	757 (730-830)	506 (499-523)	interferon, gamma-inducible protein 16
UBE2L6	-1.54	0.01634	102 (95-118)	72 (66-76)	ubiquitin-conjugating enzyme E2L 6
ANKH	-1.54	0.03905	74 (69-88)	54 (47-60)	ankylosis, progressive homolog (mouse)
EPSTI1	-1.53	0.03231	23 (21-26)	15 (14-16)	epithelial stromal interaction 1 (breast)
ADD3	-1.53	0.00663	794 (745-878)	576 (512-583)	adducin 3 (gamma)
ZNF69	-1.53	0.02796	15 (14-17)	10 (9-11)	zinc finger protein 69
MGAT2	-1.52	0.00113	33 (30-35)	21 (21-21)	mannosyl (alpha-1,6-)-glycoprotein beta-1,2-N-acetylglucosaminyltransferase
CASC4	-1.52	0.01971	637 (630-713)	478 (422-512)	cancer susceptibility candidate 4
RPL23	-1.52	0.00937	4619 (4285-5108)	3067 (2993-3179)	ribosomal protein L23
SEMA3A	-1.52	0.00763	13 (11-14)	8 (8-9)	sema domain, immunoglobulin domain (Ig), short basic domain, secreted, (semaphorin) 3A
GPAM	-1.52	0.00933	8 (8-9)	5 (5-6)	glycerol-3-phosphate acyltransferase, mitochondrial
OAS3	-1.51	0.03521	39 (34-41)	25 (22-26)	2'-5'-oligoadenylate synthetase 3, 100kDa

gene name	FC	raw p value	Intensity normalized by gcRMA(slow) in unlogged scale		description
			control female	control male	
USP10	-1.51	0.04343	178 (162-189)	117 (97-138)	ubiquitin specific peptidase 10
C1orf97	-1.51	0.00473	16 (15-17)	11 (10-12)	chromosome 1 open reading frame 97
DECR1	-1.51	0.03967	123 (104-144)	88 (74-97)	2,4-dienoyl CoA reductase 1, mitochondrial
PSMD14	-1.51	0.00577	306 (294-318)	208 (185-229)	proteasome (prosome, macropain) 26S subunit, non-ATPase, 14
NNT	1.51	0.02925	22 (20-24)	16 (14-17)	nicotinamide nucleotide transhydrogenase
FAR1	-1.51	0.03481	373 (367-425)	258 (239-307)	fatty acyl CoA reductase 1
MEST	-1.51	0.01280	5635 (5294-6256)	3595 (3467-4245)	mesoderm specific transcript homolog (mouse)
RASGRP3	-1.50	0.00722	20 (18-22)	13 (13-14)	RAS guanyl releasing protein 3 (calcium and DAG-regulated)
CALM2	-1.50	0.00469	7686 (7245-8230)	4902 (4809-5400)	calmodulin 2 (phosphorylase kinase, delta)
LRRC8E	1.50	0.04862	8 (7-8)	10 (9-13)	leucine rich repeat containing 8 family, member E
hCG_2008140	1.50	0.01675	17 (15-18)	25 (22-26)	hypothetical LOC729614
SIK3	1.50	0.00530	153 (140-157)	209 (204-225)	SIK family kinase 3
APOBEC3A	1.51	0.03831	13 (11-14)	19 (16-21)	apolipoprotein B mRNA editing enzyme, catalytic polypeptide-like 3A
RAB15	1.51	0.01109	30 (25-34)	43 (42-45)	RAB15, member RAS onocogene family
DVL1	1.51	0.00377	50 (46-54)	77 (73-79)	dishevelled, dsh homolog 1 (Drosophila)
AAK1	1.51	0.01942	638 (549-682)	869 (823-935)	AP2 associated kinase 1
TMUB1	1.51	0.01542	28 (24-31)	40 (37-44)	transmembrane and ubiquitin-like domain containing 1
PANK4	1.51	0.00382	34 (32-36)	51 (48-55)	pantothenate kinase 4
GRAMD4	1.51	0.00537	25 (24-27)	40 (36-44)	GRAM domain containing 4
ATP6V0B	1.51	0.02475	57 (47-68)	84 (81-89)	ATPase, H+ transporting, lysosomal 21kDa, V0 subunit b
FLJ41603	1.51	0.03520	39 (33-40)	51 (50-51)	FLJ41603 protein
MTA1	1.51	0.00440	87 (83-89)	132 (119-141)	metastasis associated 1
PRPF40B	1.52	0.00551	13 (12-14)	18 (18-21)	PRP40 pre-mRNA processing factor 40 homolog B (S. cerevisiae)
TRIM28	1.52	0.00767	165 (150-168)	224 (220-238)	tripartite motif-containing 28
CALM1	1.52	0.01321	243 (229-260)	383 (331-432)	calmodulin 1 (phosphorylase kinase, delta)
ACAD9	1.52	0.02523	30 (28-31)	40 (37-49)	acyl-Coenzyme A dehydrogenase family, member 9
AFG3L1	1.52	0.02330	128 (109-152)	200 (192-202)	AFG3 ATPase family gene 3-like 1 (S. cerevisiae)
RNF207	1.52	0.03890	18 (15-19)	24 (24-25)	ring finger protein 207
INO80E	1.52	0.00148	30 (29-32)	44 (43-50)	INO80 complex subunit E
CNO	1.53	0.00827	38 (35-42)	57 (54-64)	cappuccino homolog (mouse)
SFRS16	1.53	0.02136	66 (61-70)	108 (89-118)	splicing factor, arginine/serine-rich 16
MMP15	1.53	0.04785	26 (21-30)	38 (34-40)	matrix metallopeptidase 15 (membrane-inserted)
EDEM2	1.53	0.04586	39 (34-44)	59 (55-60)	ER degradation enhancer, mannosidase alpha-like 2
DHPS	1.53	0.00535	17 (16-18)	24 (24-28)	deoxyhypusine synthase
ZNF212	1.53	0.01658	55 (45-64)	85 (78-87)	zinc finger protein 212
C1QTNF1	1.53	0.01563	52 (44-56)	70 (69-76)	C1q and tumor necrosis factor related protein 1
GLIS2	1.53	0.00958	30 (28-33)	47 (42-53)	GLIS family zinc finger 2
HOOK2	1.54	0.00961	36 (33-40)	56 (55-58)	hook homolog 2 (Drosophila)
SMARCD2	1.54	0.04205	18 (16-20)	25 (24-27)	SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily d, member 2
ALAS1	1.55	0.00694	170 (160-181)	272 (245-288)	aminolevulinate, delta-, synthase 1
MCM3AP	1.55	0.00835	26 (25-27)	41 (36-45)	minichromosome maintenance complex component 3 associated protein
MAPRE3	1.55	0.02631	6 (6-6)	10 (8-11)	microtubule-associated protein, RP/EB family, member 3
LONP1	1.55	0.00559	55 (51-57)	80 (75-86)	lon peptidase 1, mitochondrial
CDC42SE1	1.55	0.02799	62 (53-68)	105 (85-108)	CDC42 small effector 1
ZNF879	1.55	0.01622	11 (9-13)	18 (16-19)	zinc finger protein 879
SPATA9	1.55	0.01278	6 (5-6)	8 (8-9)	spermatogenesis associated 9
LAD1	1.55	0.04651	16 (13-18)	22 (21-25)	ladinin 1
PNPLA2	1.56	0.02458	12 (11-12)	16 (15-20)	patatin-like phospholipase domain containing 2
PQLC2	1.56	0.00855	8 (7-9)	13 (12-14)	PQ loop repeat containing 2
IP6K3	1.56	0.03832	13 (12-14)	18 (17-25)	inositol hexakisphosphate kinase 3
MEPCE	1.57	0.00476	79 (70-85)	116 (115-120)	methylphosphate capping enzyme
TMEM115	1.57	0.01398	78 (69-83)	129 (108-130)	transmembrane protein 115
SLC26A11	1.57	0.01577	78 (67-89)	113 (112-124)	solute carrier family 26, member 11

gene name	FC	raw p value	Intensity normalized by gcRMA(slow) in unlogged scale		description
			control female	control male	
ACADVL	1.57	0.02647	737 (634-800)	1060 (1034-1089)	acyl-Coenzyme A dehydrogenase, very long chain
SRRM2	1.57	0.00062	372 (364-393)	592 (567-638)	serine/arginine repetitive matrix 2
POU2F1	1.57	0.00164	24 (23-26)	39 (39-40)	POU class 2 homeobox 1
AQP11	1.58	0.03869	6 (6-7)	12 (9-13)	aquaporin 11
TRIM56	1.58	0.00097	17 (16-18)	26 (26-27)	tripartite motif-containing 56
LOC113230	1.58	0.02446	19 (15-21)	28 (25-32)	hypothetical protein LOC113230
SHANK2	1.58	0.00323	9 (8-9)	15 (13-15)	SH3 and multiple ankyrin repeat domains 2
C1orf213	1.59	0.00230	14 (13-15)	23 (21-23)	chromosome 1 open reading frame 213
PACSIN3	1.59	0.01818	11 (9-12)	18 (16-18)	protein kinase C and casein kinase substrate in neurons 3
WDR45	1.59	0.02392	105 (90-117)	172 (152-174)	WD repeat domain 45
PAK1	1.59	0.01175	78 (68-86)	109 (108-123)	p21 protein (Cdc42/Rac)-activated kinase 1
ATG2A	1.59	0.01275	32 (29-34)	48 (44-54)	ATG2 autophagy related 2 homolog A (S. cerevisiae)
NT5DC3	1.60	0.04298	9 (8-9)	14 (12-15)	5'-nucleotidase domain containing 3
CDYL	1.60	0.03141	127 (108-136)	181 (175-188)	chromodomain protein, Y-like
TRIT1	1.62	0.04351	51 (40-64)	77 (76-88)	tRNA isopentenyltransferase 1
FER1L4	1.62	0.00291	18 (17-19)	29 (27-31)	fer-1-like 4 (C. elegans)
ZNF331	1.62	0.03608	214 (194-254)	349 (326-408)	zinc finger protein 331
UCKL1	1.62	0.01254	57 (51-65)	94 (90-94)	uridine-cytidine kinase 1-like 1
CD320	1.63	0.03571	12 (11-13)	20 (16-24)	CD320 molecule
SREBF1	1.63	0.04239	11 (9-12)	15 (14-18)	sterol regulatory element binding transcription factor 1
SF3A2	1.63	0.03548	11 (10-11)	14 (13-20)	splicing factor 3a, subunit 2, 66kDa
KCTD1	1.63	0.04494	24 (21-25)	34 (31-37)	potassium channel tetramerisation domain containing 1
RBM6	1.63	0.00851	39 (34-46)	64 (59-71)	RNA binding motif protein 6
ABHD11	1.64	0.01468	75 (70-86)	123 (111-149)	abhydrolase domain containing 11
PER2	1.64	0.03705	38 (33-40)	54 (50-64)	period homolog 2 (Drosophila)
CRY2	1.64	0.00701	14 (13-16)	23 (21-27)	cryptochrome 2 (photolyase-like)
RHBDF1	1.64	0.00583	132 (126-144)	208 (203-241)	rhomboid 5 homolog 1 (Drosophila)
SYMPK	1.64	0.00055	14 (13-15)	24 (24-24)	symplekin
PLEC	1.65	0.02006	31 (26-35)	55 (45-57)	plectin
LITAF	1.65	0.01881	419 (378-467)	665 (611-767)	lipopolysaccharide-induced TNF factor
ZNF321	1.65	0.04340	6 (5-8)	12 (10-13)	zinc finger protein 321
GSDMB	1.65	0.04583	11 (10-12)	16 (14-22)	gasdermin B
FASN	1.66	0.02889	12 (11-14)	20 (18-25)	fatty acid synthase
GKAP1	1.66	0.01119	21 (19-23)	42 (33-42)	G kinase anchoring protein 1
DAB2IP	1.66	0.02182	37 (32-40)	59 (50-68)	DAB2 interacting protein
CRY1	1.66	0.03248	440 (399-445)	747 (597-794)	cryptochrome 1 (photolyase-like)
KIFC2	1.66	0.00682	12 (10-14)	20 (18-21)	kinesin family member C2
L3MBTL	1.66	0.01962	16 (15-18)	31 (25-33)	l(3)mbt-like (Drosophila)
TOM1	1.66	0.00065	11 (11-12)	18 (18-19)	target of myb1 (chicken)
LSS	1.67	0.01080	15 (14-17)	27 (23-29)	lanosterol synthase (2,3-oxidosqualene-lanosterol cyclase)
RGL2	1.67	0.00753	284 (256-335)	500 (479-526)	ral guanine nucleotide dissociation stimulator-like 2
IGDCC4	1.67	0.03568	244 (212-269)	368 (356-395)	immunoglobulin superfamily, DCC subclass, member 4
SNRNP70	1.68	0.00946	113 (104-120)	199 (166-221)	small nuclear ribonucleoprotein 70kDa (U1)
SCML1	1.69	0.01724	51 (47-57)	86 (78-99)	sex comb on midleg-like 1 (Drosophila)
MLL4	1.69	0.00249	23 (22-24)	42 (36-44)	myeloid/lymphoid or mixed-lineage leukemia 4
POLRMT	1.69	0.01245	7 (7-7)	12 (10-14)	polymerase (RNA) mitochondrial (DNA directed)
PELI1	1.69	0.00211	294 (267-323)	508 (469-531)	pellino homolog 1 (Drosophila)
RAB40C	1.69	0.02294	54 (50-56)	71 (70-102)	RAB40C, member RAS oncogene family
BRD1	1.70	0.00234	67 (62-71)	111 (105-116)	bromodomain containing 1
CTSH	1.70	0.02448	49 (40-55)	81 (68-92)	cathepsin H
LDB1	1.70	0.02374	20 (18-23)	36 (32-37)	LIM domain binding 1
POU6F2	1.71	0.01273	47 (40-56)	86 (75-93)	POU class 6 homeobox 2
C21orf66	1.71	0.00262	51 (48-56)	93 (83-98)	chromosome 21 open reading frame 66
DGCR8	1.72	0.01051	38 (35-39)	53 (52-74)	DiGeorge syndrome critical region gene 8
FLCN	1.73	0.00833	23 (20-23)	35 (32-40)	folliculin
PKM2	1.74	0.01240	208 (191-216)	323 (289-405)	pyruvate kinase, muscle

gene name	FC	raw p value	Intensity normalized by gcRMA(slow) in unlogged scale		description
			control female	control male	
RAB24	1.74	0.01146	77 (64-85)	132 (115-135)	RAB24, member RAS oncogene family
FLT4	1.74	0.01618	21 (18-23)	37 (32-42)	fms-related tyrosine kinase 4
YIPF4	1.74	0.03936	212 (186-227)	364 (315-371)	Yip1 domain family, member 4
PIK3AP1	1.74	0.04861	98 (91-107)	145 (129-229)	phosphoinositide-3-kinase adaptor protein 1
CAMK2G	1.74	0.03189	34 (29-37)	55 (51-57)	calcium/calmodulin-dependent protein kinase II gamma
AP1G2	1.75	0.03540	63 (46-78)	94 (88-118)	adaptor-related protein complex 1, gamma 2 subunit
SULT2B1	1.75	0.00070	12 (11-12)	20 (19-21)	sulfotransferase family, cytosolic, 2B, member 1
SPAG4	1.75	0.00968	6 (6-6)	9 (8-12)	sperm associated antigen 4
SYT12	1.75	0.03046	19 (16-22)	25 (25-39)	synaptotagmin XII
FAM50A	1.76	0.00185	58 (55-64)	109 (100-116)	family with sequence similarity 50, member A
MICAL1	1.76	0.00488	14 (14-16)	26 (23-30)	microtubule associated monoxygenase, calponin and LIM domain containing 1
SERTAD2	1.76	0.02118	238 (232-243)	327 (322-509)	SERTA domain containing 2
FAM122B	1.77	0.02716	15 (14-16)	22 (21-30)	family with sequence similarity 122B
PLD3	1.77	0.04538	289 (251-302)	417 (382-511)	phospholipase D family, member 3
FAM100A	1.78	0.00706	31 (27-34)	51 (47-55)	family with sequence similarity 100, member A
H1F0	1.78	0.02473	285 (263-347)	499 (474-639)	H1 histone family, member 0
ANKHD1	1.79	0.04195	23 (17-27)	37 (33-38)	ankyrin repeat and KH domain containing 1
GLG1	1.79	0.00635	36 (30-43)	59 (58-70)	golgi glycoprotein 1
SIN3B	1.79	0.03073	66 (46-85)	109 (102-115)	SIN3 homolog B, transcription regulator (yeast)
PWWP2B	1.80	0.00411	18 (17-20)	30 (30-36)	PWWP domain containing 2B
DDT	1.80	0.02737	16 (13-19)	24 (23-34)	D-dopachrome tautomerase
PIGH	1.80	0.03125	48 (38-55)	77 (68-90)	phosphatidylinositol glycan anchor biosynthesis, class H
ARHGAP26	1.80	0.02176	54 (43-63)	81 (76-108)	Rho GTPase activating protein 26
ADSSL1	1.80	0.01506	26 (21-30)	42 (39-51)	adenylosuccinate synthase like 1
KIAA0195	1.81	0.03162	43 (34-49)	64 (60-76)	KIAA0195
CSF3R	1.81	0.03874	649 (532-697)	984 (864-1178)	colony stimulating factor 3 receptor (granulocyte)
SCAP	1.82	0.00088	63 (55-69)	112 (108-113)	SREBF chaperone
MED25	1.83	0.00103	55 (54-58)	106 (95-115)	mediator complex subunit 25
PI4KA	1.83	0.00076	47 (43-51)	83 (80-93)	phosphatidylinositol 4-kinase, catalytic, alpha
LENG8	1.84	0.00647	44 (39-51)	85 (75-96)	leukocyte receptor cluster (LRC) member 8
HINT3	1.90	0.04457	9 (8-10)	15 (12-24)	histidine triad nucleotide binding protein 3
ACOX3	1.91	0.03062	20 (14-27)	40 (35-44)	acyl-Coenzyme A oxidase 3, pristanoyl
SLC22A5	1.91	0.03106	41 (35-42)	67 (61-69)	solute carrier family 22 (organic cation/carnitine transporter), member 5
CD99L2	1.92	0.04609	39 (32-44)	62 (57-76)	CD99 molecule-like 2
PRR5	1.92	0.01094	24 (21-27)	38 (36-52)	proline rich 5 (renal)
CTDSP1	1.95	0.00052	346 (327-364)	627 (612-712)	CTD (carboxy-terminal domain, RNA polymerase II, polypeptide A) small phosphatase 1
TRIP10	1.98	0.03550	138 (103-160)	234 (201-275)	thyroid hormone receptor interactor 10
CARKD	2.00	0.00025	56 (52-59)	119 (104-123)	carbohydrate kinase domain containing
MBD2	2.00	0.02623	17 (15-20)	29 (26-40)	methyl-CpG binding domain protein 2
CCDC113	2.01	0.04181	20 (15-22)	29 (28-35)	coiled-coil domain containing 113
SH2B2	2.02	0.01663	18 (15-19)	33 (30-34)	SH2B adaptor protein 2
KCNN4	2.05	0.00805	89 (78-94)	156 (149-178)	potassium intermediate/small conductance calcium-activated channel, subfamily N, member 4
AKAP8L	2.07	0.02689	44 (33-54)	73 (66-106)	A kinase (PRKA) anchor protein 8-like
BRWD1	2.08	0.01645	45 (39-47)	94 (75-96)	bromodomain and WD repeat domain containing 1
METRNL	2.10	0.04715	133 (94-167)	265 (223-279)	meteorin, glial cell differentiation regulator-like
TLE2	2.11	0.02459	37 (32-45)	89 (75-90)	transducin-like enhancer of split 2 (E(sp1) homolog, Drosophila)
ANKRD9	2.18	0.00394	437 (331-530)	880 (861-906)	ankyrin repeat domain 9
CCDC120	2.18	0.02456	133 (110-139)	210 (209-253)	coiled-coil domain containing 120
ANO2	2.20	0.00839	16 (13-18)	35 (30-38)	anoctamin 2
ZMYND8	2.23	0.02908	37 (35-41)	68 (59-123)	zinc finger, MYND-type containing 8
GDPD5	2.23	0.00410	440 (344-519)	951 (833-1029)	glycerophosphodiester phosphodiesterase domain containing 5
KRTAP26-1	2.24	0.01174	8 (7-10)	21 (16-24)	keratin associated protein 26-1
ZNF711	2.25	0.04272	34 (33-42)	116 (78-130)	zinc finger protein 711

gene name	FC	raw p value	Intensity normalized by gcRMA(slow) in unlogged scale		description
			control female	control male	
CHCHD10	2.25	0.04005	140 (104-159)	209 (209-285)	coiled-coil-helix-coiled-coil-helix domain containing 10
LPIN1	2.25	0.04254	109 (82-132)	288 (206-289)	lipin 1
STK40	2.26	0.02917	65 (52-70)	113 (113-121)	serine/threonine kinase 40
TADA1	2.28	0.04137	94 (64-118)	159 (146-217)	transcriptional adaptor 1
HMBOX1	2.34	0.03954	57 (38-90)	139 (127-158)	homeobox containing 1
EMP2	2.35	0.04091	399 (322-436)	694 (670-806)	epithelial membrane protein 2
CCDC69	2.55	0.03576	45 (32-62)	109 (94-126)	coiled-coil domain containing 69
CDKN1A	2.59	0.02893	96 (57-136)	188 (173-288)	cyclin-dependent kinase inhibitor 1A (p21, Cip1)
SH3GLB2	2.66	0.04568	95 (64-119)	286 (191-317)	SH3-domain GRB2-like endophilin B2
SLC7A5	2.72	0.02956	199 (152-220)	474 (375-509)	solute carrier family 7 (cationic amino acid transporter, y+ system), member 5
CORO6	2.84	0.01816	150 (120-165)	314 (266-501)	coronin 6
FRZB	3.01	0.01123	279 (187-403)	814 (708-990)	frizzled-related protein
LTBP3	3.03	0.02316	62 (37-89)	147 (137-192)	latent transforming growth factor beta binding protein 3
HMG20B	3.15	0.02413	45 (33-51)	108 (87-143)	high-mobility group 20B
EPS8L2	3.23	0.00378	56 (50-61)	197 (149-220)	EPS8-like 2
CLMN	3.34	0.00341	12 (8-16)	34 (32-42)	calmin (calponin-like, transmembrane)
STRA6	3.38	0.00476	59 (44-74)	192 (169-196)	stimulated by retinoic acid gene 6 homolog (mouse)
CPZ	3.47	0.03035	185 (113-279)	743 (533-768)	carboxypeptidase Z
LIMCH1	3.48	0.04135	55 (43-62)	123 (104-200)	LIM and calponin homology domains 1
EIF1AY	4.02	4.6E-06	7 (7-7)	30 (27-34)	eukaryotic translation initiation factor 1A, Y-linked
HIST1H1C	4.16	0.03262	193 (149-218)	580 (488-655)	histone cluster 1, H1c
UTY	4.17	0.00006	8 (7-9)	30 (27-38)	ubiquitously transcribed tetratricopeptide repeat gene, Y-linked
LPL	5.60	0.00002	73 (60-84)	356 (335-445)	lipoprotein lipase
INHA	5.62	0.01936	24 (20-36)	150 (102-238)	inhibin, alpha
AQP3	7.19	0.00096	52 (28-78)	372 (307-390)	aquaporin 3 (Gill blood group)
KDM5D	12.34	0.00004	4 (4-4)	53 (40-76)	lysine (K)-specific demethylase 5D
ZFY	13.98	0.00038	7 (7-7)	84 (61-176)	zinc finger protein, Y-linked
USP9Y	18.56	5.5E-07	6 (5-6)	119 (93-122)	ubiquitin specific peptidase 9, Y-linked
RPS4Y1	44.69	3.6E-07	8 (8-8)	470 (342-487)	ribosomal protein S4, Y-linked 1
DDX3Y	265.45	2.6E-10	4 (4-5)	1143 (1120-1263)	DEAD (Asp-Glu-Ala-Asp) box polypeptide 3, Y-linked

Data are presented as median (interquartile range IQR = 25th-75th percentile). Control female = placentas of female offspring in the control group, Control male = placentas of male offspring in the control group. The genes are sorted according to their fold change. FC, fold change;

11.6.4 List of significantly regulated genes between male and female placentas in the n-6/n-3 LCPUFA intervention group from the microarray analysis (IM vs. IF)

gene name	FC	raw p value	Intensity normalized by gcRMA(slow) in unlogged scale		description
			intervention female	intervention male	
LEP	-9.85	0.03062	1889 (1366-2050)	148 (30-429)	leptin
LYZ	-7.37	0.00251	63 (33-141)	9 (7-9)	lysozyme (renal amyloidosis)
LYPD5	-2.35	0.00651	27 (23-34)	12 (8-14)	LY6/PLAUR domain containing 5
MFSD4	-1.90	0.00505	31 (25-39)	18 (14-18)	major facilitator superfamily domain containing 4
LIMCH1	-1.89	0.00574	120 (109-133)	50 (47-91)	LIM and calponin homology domains 1
KDM6A	-1.83	0.00132	69 (57-78)	39 (35-40)	lysine (K)-specific demethylase 6A
SSB	-1.80	0.02336	265 (258-273)	177 (172-189)	Sjogren syndrome antigen B (autoantigen La)
HDHD1A	-1.80	0.00191	148 (137-173)	97 (71-103)	haloacid dehalogenase-like hydrolase domain containing 1A
CXCR7	-1.74	0.02541	984 (742-1261)	603 (528-676)	chemokine (C-X-C motif) receptor 7
STS	-1.70	0.00555	3980 (3716-4273)	2540 (1807-2683)	steroid sulfatase (microsomal), isozyme S
KIF3A	-1.68	0.03908	29 (19-38)	16 (13-17)	kinesin family member 3A
CCR5	-1.67	0.00854	13 (11-15)	8 (8-9)	chemokine (C-C motif) receptor 5
SLC44A1	-1.65	0.02917	489 (379-601)	300 (211-401)	solute carrier family 44, member 1
LOC220115	-1.60	0.00102	9 (9-11)	6 (6-7)	TPTE and PTEN homologous inositol lipid phosphatase pseudogene
TCTE3	-1.57	0.00017	12 (12-13)	8 (7-8)	t-complex-associated-testis-expressed 3
LPGAT1	-1.56	0.01823	72 (55-91)	47 (44-51)	lysophosphatidylglycerol acyltransferase 1
SOCS1	-1.56	0.02528	39 (32-49)	26 (24-31)	suppressor of cytokine signaling 1
CRLF3	-1.55	0.03054	188 (183-196)	140 (103-141)	cytokine receptor-like factor 3
ZNF236	-1.55	0.00173	31 (28-35)	20 (19-22)	zinc finger protein 236
AP1S2	-1.54	0.04969	44 (42-55)	33 (27-42)	adaptor-related protein complex 1, sigma 2 subunit
LCE5A	-1.54	0.00771	23 (21-25)	17 (15-17)	late cornified envelope 5A
PVRL4	-1.53	0.03330	23 (20-28)	16 (13-18)	poliovirus receptor-related 4
IL13RA2	-1.53	0.03076	6 (6-7)	4 (4-4)	interleukin 13 receptor, alpha 2
NDUFB7	1.50	0.03792	102 (95-109)	169 (148-180)	NADH dehydrogenase (ubiquinone) 1 beta subcomplex, 7, 18kDa
KBTBD8	1.50	0.02229	20 (19-23)	30 (26-41)	kelch repeat and BTB (POZ) domain containing 8
TBPL1	1.51	0.03100	85 (61-104)	119 (101-122)	TBP-like 1
TXNDC15	1.52	0.00072	46 (41-51)	71 (64-75)	thioredoxin domain containing 15
ILF2	1.52	0.00048	152 (142-157)	235 (218-235)	interleukin enhancer binding factor 2, 45kDa
FKBP11	1.53	0.00464	13 (11-14)	20 (19-21)	FK506 binding protein 11, 19 kDa
RAB33B	1.53	0.03165	54 (49-57)	83 (60-98)	RAB33B, member RAS oncogene family
ZFP36	1.54	0.01821	81 (77-88)	150 (114-155)	zinc finger protein 36, C3H type, homolog (mouse)
CD36	1.55	0.03692	1521 (1318-1807)	2215 (1841-3115)	CD36 molecule (thrombospondin receptor)
ISL1	1.55	0.00705	73 (64-82)	117 (91-138)	ISL LIM homeobox 1
PROK2	1.55	0.03096	11 (10-12)	17 (15-18)	prokineticin 2
SELENBP1	1.56	0.00554	16 (15-18)	28 (21-30)	selenium binding protein 1
ZNF521	1.56	0.04585	13 (11-14)	17 (16-19)	zinc finger protein 521
MGST1	1.57	0.03596	8 (7-10)	10 (10-17)	microsomal glutathione S-transferase 1
LAMC3	1.60	0.04693	38 (34-50)	67 (61-72)	laminin, gamma 3
FKBP10	1.60	0.00054	16 (15-16)	26 (25-27)	FK506 binding protein 10, 65 kDa
SNAP29	1.62	0.01743	17 (14-20)	24 (22-32)	synaptosomal-associated protein, 29kDa
LOC729082	1.62	0.00348	21 (18-24)	34 (29-36)	hypothetical protein LOC729082
SPRY1	1.63	0.02262	110 (104-126)	188 (142-228)	sprouty homolog 1, antagonist of FGF signaling (Drosophila)
FBN1	1.64	0.00826	49 (46-53)	91 (79-94)	fibrillin 1
GAS1	1.65	0.03006	178 (150-216)	245 (244-325)	growth arrest-specific 1
CASQ1	1.68	0.04034	6 (5-6)	9 (7-13)	calsequestrin 1 (fast-twitch, skeletal muscle)
IGFBP2	1.72	0.02526	22 (20-24)	44 (37-47)	insulin-like growth factor binding protein 2, 36kDa
BMP2	1.72	0.01891	17 (15-19)	27 (25-35)	bone morphogenetic protein 2
CTGF	1.74	0.04748	884 (675-1059)	1807 (851-2107)	connective tissue growth factor
MASTL	1.75	0.00075	64 (54-74)	108 (103-109)	microtubule associated serine/threonine kinase-like
ID4	1.75	0.03759	166 (119-203)	281 (210-283)	inhibitor of DNA binding 4, dominant negative helix-loop-helix protein
RPS4Y2	1.79	0.00436	13 (11-14)	21 (20-24)	ribosomal protein S4, Y-linked 2

gene name	FC	raw p value	Intensity normalized by gcRMA(slow) in unlogged scale		description
			intervention female	intervention male	
HBEGF	1.94	0.01633	12 (9-14)	26 (19-30)	heparin-binding EGF-like growth factor
ABI3BP	1.96	0.00186	55 (50-70)	125 (98-143)	ABI family, member 3 (NESH) binding protein
HECW2	1.97	0.00852	70 (49-91)	128 (102-163)	HECT, C2 and WW domain containing E3 ubiquitin protein ligase 2
ZNF711	1.98	0.00373	29 (25-35)	52 (47-70)	zinc finger protein 711
LOC387763	2.01	0.01898	158 (123-205)	271 (231-496)	hypothetical protein LOC387763
HBM	2.12	0.02865	40 (38-59)	119 (106-144)	hemoglobin, mu
UTY	2.28	0.00000	7 (7-8)	17 (15-18)	ubiquitously transcribed tetratricopeptide repeat gene, Y-linked
EIF1AY	2.71	0.00025	9 (9-10)	25 (19-30)	eukaryotic translation initiation factor 1A, Y-linked
KDM5D	6.28	0.00000	4 (4-5)	25 (22-36)	lysine (K)-specific demethylase 5D
ZFY	9.44	0.00000	6 (5-6)	57 (43-82)	zinc finger protein, Y-linked
USP9Y	11.83	0.00000	4 (4-4)	50 (42-61)	ubiquitin specific peptidase 9, Y-linked
IGFBP1	16.07	0.03443	485 (14-1774)	2520 (2176-4340)	insulin-like growth factor binding protein 1
RPS4Y1	44.40	0.00000	35 (32-37)	1520 (1485-1534)	ribosomal protein S4, Y-linked 1
DDX3Y	148.66	0.00000	5 (5-5)	768 (734-817)	DEAD (Asp-Glu-Ala-Asp) box polypeptide 3, Y-linked

Data are presented as median (interquartile range IQR = 25th-75th percentile). Intervention female = placentas of female offspring in the n-3 LCPUFA intervention group, Intervention male = placentas of male offspring in the n-3 LCPUFA intervention group. The genes are sorted according to their fold change. FC, fold change;

11.6.5 List of significantly regulated genes between n-6/n-3 LCPUFA intervention group and control group in male placentas from the microarray analysis (IM vs. CM)

gene name	FC	p value	Intensity normalized by gcRMA(slow) in unlogged scale		description
			control male	intervention male	
INHA	-4.29	0.00266	153 (102-201)	29 (21-43)	inhibin, alpha
HK2	-3.28	0.03960	13 (11-60)	7 (6-8)	hexokinase 2
LIMCH1	-2.64	0.00979	139 (116-223)	48 (44-85)	LIM and calponin homology domains 1
HIST1H1C	-2.55	0.03050	593 (515-708)	260 (160-401)	histone cluster 1, H1c
MAP3K8	-2.19	0.00469	470 (385-689)	221 (216-236)	mitogen-activated protein kinase kinase kinase 8
GPNMB	-2.15	0.02265	1787 (1640-1988)	682 (557-1324)	glycoprotein (transmembrane) nmb
MBD2	-2.13	0.00625	23 (21-35)	13 (10-16)	methyl-CpG binding domain protein 2
HMG20B	-2.01	0.02164	160 (125-213)	86 (78-91)	high-mobility group 20B
TRIP10	-1.98	0.01564	167 (161-198)	99 (60-115)	thyroid hormone receptor interactor 10
LPGAT1	-1.91	0.00380	90 (78-99)	50 (46-53)	lysophosphatidylglycerol acyltransferase 1
LYPD5	-1.85	0.00940	26 (21-29)	14 (10-14)	LY6/PLAUR domain containing 5
SLC6A6	-1.84	0.00274	364 (322-468)	222 (192-226)	solute carrier family 6 (neurotransmitter transporter, taurine), member 6
SH2D5	-1.84	0.02800	178 (176-182)	113 (87-131)	SH2 domain containing 5
PGM1	-1.80	0.03087	490 (471-540)	300 (252-373)	phosphoglucomutase 1
HTRA1	-1.79	0.01614	4379 (3155-4572)	1819 (1746-2240)	HtrA serine peptidase 1
MAGEA11	-1.78	0.04990	15 (12-21)	10 (8-11)	melanoma antigen family A, 11
FZD7	-1.77	0.03731	178 (177-266)	131 (89-166)	frizzled homolog 7 (Drosophila)
NGFR	-1.77	0.02874	23 (23-43)	19 (16-20)	nerve growth factor receptor (TNFR superfamily, member 16)
ST3GAL6	-1.73	0.02742	239 (221-337)	167 (152-195)	ST3 beta-galactoside alpha-2,3-sialyltransferase 6
IP6K3	-1.71	0.03845	22 (20-27)	12 (12-16)	inositol hexakisphosphate kinase 3
NDRG1	-1.70	0.03931	991 (812-1347)	661 (501-703)	N-myc downstream regulated 1
ADARB1	-1.69	0.02679	36 (28-39)	16 (16-22)	adenosine deaminase, RNA-specific, B1 (RED1 homolog rat)
HIST1H2BH	-1.69	0.03524	43 (34-48)	24 (17-28)	histone cluster 1, H2bh
LSS	-1.69	0.00599	27 (23-29)	15 (13-15)	lanosterol synthase (2,3-oxidosqualene-lanosterol cyclase)
ANO1	-1.68	0.01588	65 (55-67)	38 (30-42)	anoctamin 1, calcium activated chloride channel
PDLIM2	-1.66	0.01312	417 (359-495)	267 (209-289)	PDZ and LIM domain 2 (mystique)
PTGER4	-1.66	0.03956	40 (32-50)	23 (19-27)	prostaglandin E receptor 4 (subtype EP4)
EPS8L2	-1.65	0.01881	185 (147-201)	102 (83-114)	EPS8-like 2
PM20D2	-1.65	0.03445	357 (305-364)	211 (186-241)	peptidase M20 domain containing 2
SMOC2	-1.65	0.03879	28 (24-38)	16 (16-19)	SPARC related modular calcium binding 2
VEZT	-1.65	0.00553	50 (49-56)	32 (27-35)	vezatin, adherens junctions transmembrane protein
SCML1	-1.62	0.00316	67 (63-83)	44 (43-46)	sex comb on midleg-like 1 (Drosophila)
C3orf64	-1.61	0.01150	153 (133-155)	80 (79-95)	chromosome 3 open reading frame 64
STX3	-1.60	0.04942	281 (254-300)	198 (158-205)	syntaxin 3
LOC113230	-1.60	0.04498	26 (23-33)	19 (16-21)	hypothetical protein LOC113230
METTL7B	-1.60	0.04206	14 (13-16)	11 (7-11)	methyltransferase like 7B
MAPK6	-1.59	0.02102	74 (74-75)	49 (38-62)	mitogen-activated protein kinase 6
NFXL1	-1.58	0.00731	35 (35-48)	26 (26-29)	nuclear transcription factor, X-box binding-like 1
C14orf139	-1.58	0.04228	41 (31-41)	19 (18-23)	chromosome 14 open reading frame 139
SPAG4	-1.57	0.01773	10 (9-14)	7 (7-7)	sperm associated antigen 4
BOK	-1.57	0.01894	17 (16-24)	13 (12-13)	BCL2-related ovarian killer
TPBG	-1.57	0.01995	415 (326-432)	228 (200-265)	trophoblast glycoprotein
SEMA7A	-1.57	0.03326	126 (97-134)	70 (67-71)	semaphorin 7A, GPI membrane anchor (John Milton Hagen blood group)
CXCR7	-1.57	0.04850	979 (867-1011)	635 (570-694)	chemokine (C-X-C motif) receptor 7
CRTC3	-1.56	0.01481	317 (255-338)	175 (175-176)	CREB regulated transcription coactivator 3
ZNF721	-1.55	0.01447	184 (181-204)	116 (106-162)	zinc finger protein 721
GRAMD1C	-1.55	0.02522	78 (70-100)	54 (53-55)	GRAM domain containing 1C
SERTAD2	-1.55	0.04426	317 (305-502)	262 (250-289)	SERTA domain containing 2
NPNT	-1.54	0.03772	22 (21-24)	16 (11-16)	nephronectin

gene name	FC	p value	Intensity normalized by gcRMA(slow) in unlogged scale		description
			control male	intervention male	
CYP51A1	-1.53	0.01221	215 (185-219)	116 (114-127)	cytochrome P450, family 51, subfamily A, polypeptide 1
CALM1	-1.52	0.01204	390 (331-418)	228 (213-230)	calmodulin 1 (phosphorylase kinase, delta)
BRWD1	-1.52	0.03716	157 (134-174)	87 (86-125)	bromodomain and WD repeat domain containing 1
NEK11	-1.52	0.00567	13 (11-15)	8 (8-9)	NIMA (never in mitosis gene a)- related kinase 11
ING2	-1.52	0.00430	64 (61-75)	48 (39-48)	inhibitor of growth family, member 2
C4orf43	-1.51	0.00510	30 (28-38)	22 (21-23)	chromosome 4 open reading frame 43
EML1	-1.51	0.00067	27 (24-27)	16 (15-18)	echinoderm microtubule associated protein like 1
STRA6	-1.51	0.01588	166 (158-175)	120 (98-132)	stimulated by retinoic acid gene 6 homolog (mouse)
ALAS1	-1.51	0.01412	292 (268-298)	188 (157-227)	aminolevulinate, delta-, synthase 1
CCDC113	-1.51	0.04189	28 (26-35)	18 (17-22)	coiled-coil domain containing 113
CYBA	1.51	0.00452	76 (74-87)	124 (115-136)	cytochrome b-245, alpha polypeptide
NUBPL	1.53	0.00812	22 (21-24)	32 (31-34)	nucleotide binding protein-like
GALNT11	1.53	0.03764	396 (395-436)	671 (597-709)	UDP-N-acetyl-alpha-D-galactosamine:polypeptide N-acetylgalactosaminyltransferase 11 (GalNAc-T11)
TNFRSF21	1.54	0.01444	43 (39-47)	63 (61-77)	tumor necrosis factor receptor superfamily, member 21
AGA	1.54	0.01579	12 (9-12)	15 (13-18)	aspartylglucosaminidase
MS4A4A	1.55	0.01063	432 (381-454)	644 (585-672)	membrane-spanning 4-domains, subfamily A, member 4
PGD	1.55	0.03668	35 (34-37)	48 (46-68)	phosphogluconate dehydrogenase
CD47	1.56	0.00186	289 (246-300)	407 (385-436)	CD47 molecule
ZNF559	1.56	0.00908	25 (23-30)	44 (36-49)	zinc finger protein 559
SERPINH1	1.56	0.03217	306 (288-315)	403 (398-495)	serpin peptidase inhibitor, clade H (heat shock protein 47), member 1, (collagen binding protein 1)
CKS2	1.57	0.00440	21 (21-25)	36 (34-39)	CDC28 protein kinase regulatory subunit 2
IFI27	1.59	0.02994	327 (285-418)	521 (512-665)	interferon, alpha-inducible protein 27
SELL	1.60	0.04275	13 (11-13)	21 (16-23)	selectin L
AP1S3	1.62	0.04800	6 (6-7)	12 (10-14)	adaptor-related protein complex 1, sigma 3 subunit
TFRC	1.63	0.02425	3595 (3149-5084)	6707 (6230-6783)	transferrin receptor (p90, CD71)
SMAGP	1.63	0.00857	222 (202-268)	404 (367-409)	small cell adhesion glycoprotein
BHLHE41	1.64	0.03252	31 (25-32)	52 (34-55)	basic helix-loop-helix family, member e41
SEMA6A	1.65	0.03118	137 (112-152)	246 (184-252)	sema domain, transmembrane domain (TM), and cytoplasmic domain, (semaphorin) 6A
ZNF266	1.65	0.00303	46 (43-56)	80 (79-86)	zinc finger protein 266
PROK2	1.66	0.02158	10 (10-12)	19 (18-22)	prokineticin 2
SEPW1	1.66	0.02370	303 (233-332)	411 (368-488)	selenoprotein W, 1
C2orf74	1.68	0.02835	16 (15-16)	28 (22-28)	chromosome 2 open reading frame 74
PTGR1	1.73	0.00192	16 (14-17)	26 (24-30)	prostaglandin reductase 1
LGALS8	1.79	0.01914	28 (28-30)	59 (54-63)	lectin, galactoside-binding, soluble, 8
RBM9	1.80	0.02628	51 (39-61)	90 (79-96)	RNA binding motif protein 9
NUCKS1	1.81	0.03462	269 (247-494)	692 (568-697)	nuclear casein kinase and cyclin-dependent kinase substrate 1
GAP43	1.88	0.00054	7 (7-8)	15 (11-16)	growth associated protein 43
ISL1	2.03	0.00170	62 (57-77)	149 (113-153)	ISL LIM homeobox 1
PRRG4	2.11	0.02285	56 (38-57)	92 (63-105)	proline rich Gla (G-carboxyglutamic acid) 4 (transmembrane)
PRL	2.38	0.02759	8 (7-11)	20 (14-24)	prolactin
DKK1	2.53	0.01072	110 (100-143)	279 (223-401)	dickkopf homolog 1 (Xenopus laevis)
C8orf59	2.60	0.04579	30 (23-30)	57 (56-91)	chromosome 8 open reading frame 59
FAM150B	2.74	0.02613	18 (15-19)	34 (30-79)	family with sequence similarity 150, member B
IGFBP1	10.87	0.04334	390 (201-1584)	2510 (2083-3910)	insulin-like growth factor binding protein 1

Data are presented as median (interquartile range IQR = 25th-75th percentile. Control male = placentas of male offspring in the control group, Intervention male = placentas of male offspring in the n-3 LCPUFA intervention group. The genes are sorted according to their fold change. FC, fold change;

11.6.6 List of significantly regulated genes between n-6/n-3 LCPUFA intervention group and control group in female placentas from the microarray analysis (IF vs. CF)

gene name	FC	p value	Intensity normalized by gcRMA(slow) in unlogged scale		description
			control female	intervention female	
TAC3	-4.15	0.03614	741 (220-1295)	134 (70-204)	tachykinin 3
FAM46C	-3.13	0.02219	179 (154-292)	80 (45-123)	family with sequence similarity 46, member C
CRISPLD1	-2.65	0.02242	33 (25-42)	12 (8-16)	cysteine-rich secretory protein LCCL domain containing 1
DACH1	-2.43	0.04934	42 (31-65)	20 (16-25)	dachshund homolog 1 (Drosophila)
ABI3BP	-2.34	0.03725	118 (91-190)	51 (45-65)	ABI family, member 3 (NESH) binding protein
HEMGN	-2.31	0.04134	35 (21-56)	14 (12-18)	hemogen
C8orf59	-2.28	0.04554	70 (66-73)	22 (20-43)	chromosome 8 open reading frame 59
LOC387763	-2.21	0.01470	311 (268-421)	148 (119-191)	hypothetical protein LOC387763
GAS1	-2.21	0.02932	386 (316-588)	210 (172-251)	growth arrest-specific 1
ALAS2	-2.16	0.04652	44 (37-71)	27 (18-36)	aminolevulinate, delta-, synthase 2
RBM25	-2.16	0.03206	249 (192-322)	106 (86-132)	RNA binding motif protein 25
IL8	-2.16	0.02123	28 (19-38)	12 (10-14)	interleukin 8
FBN1	-2.14	0.00002	137 (129-138)	60 (58-64)	fibrillin 1
SESTD1	-2.14	0.01516	76 (58-101)	39 (28-49)	SEC14 and spectrin domains 1
GPR183	-2.12	0.03581	86 (68-95)	32 (27-41)	G protein-coupled receptor 183
CENPK	-2.11	0.02140	57 (39-73)	25 (19-32)	centromere protein K
PRR16	-2.09	0.00434	41 (38-45)	18 (15-24)	proline rich 16
EPB41	-2.08	0.03744	58 (50-80)	30 (22-43)	erythrocyte membrane protein band 4.1 (elliptocytosis 1, RH-linked)
ID4	-2.04	0.03645	327 (235-468)	183 (128-235)	inhibitor of DNA binding 4, dominant negative helix-loop-helix protein
RGS5	-2.04	0.02576	43 (32-58)	20 (18-25)	regulator of G-protein signaling 5
LAIR2	-2.04	0.03030	38 (34-53)	22 (19-25)	leukocyte-associated immunoglobulin-like receptor 2
CD9	-2.02	0.00885	140 (103-185)	70 (64-73)	CD9 molecule
CDK6	-2.02	0.02162	274 (229-368)	143 (128-163)	cyclin-dependent kinase 6
PLAC8	-2.01	0.00207	59 (52-65)	31 (24-37)	placenta-specific 8
PDIA5	-1.98	0.00055	379 (360-388)	195 (167-219)	protein disulfide isomerase family A, member 5
OLFML2B	-1.96	0.01892	86 (74-101)	47 (41-52)	olfactomedin-like 2B
ANXA3	-1.96	0.00240	545 (436-670)	286 (233-337)	annexin A3
KDELR3	-1.92	0.00051	33 (29-38)	16 (15-18)	KDEL (Lys-Asp-Glu-Leu) endoplasmic reticulum protein retention receptor 3
TIMP3	-1.91	0.03594	609 (559-945)	429 (386-453)	TIMP metallopeptidase inhibitor 3
CTSC	-1.90	0.00857	128 (94-165)	61 (58-70)	cathepsin C
SFRP1	-1.90	0.01164	40 (35-48)	21 (20-24)	secreted frizzled-related protein 1
HECTD2	-1.90	0.02871	19 (15-29)	12 (11-13)	HECT domain containing 2
HMMR	-1.89	0.01716	38 (31-45)	21 (17-24)	hyaluronan-mediated motility receptor (RHAMM)
F5	-1.88	0.01643	43 (37-52)	22 (18-30)	coagulation factor V (proaccelerin, labile factor)
XK	-1.87	0.00518	9 (8-11)	5 (5-6)	X-linked Kx blood group (McLeod syndrome)
TMEM45A	-1.87	0.03388	188 (139-275)	108 (105-109)	transmembrane protein 45A
FNIP1	-1.86	0.04668	27 (21-40)	17 (14-20)	folliculin interacting protein 1
NT5C3	-1.86	0.00195	70 (62-82)	40 (33-47)	5'-nucleotidase, cytosolic III
ESF1	-1.86	0.02628	57 (48-64)	27 (23-33)	ESF1, nucleolar pre-rRNA processing protein, homolog (S. cerevisiae)
TBL1X	-1.86	0.01687	12 (11-16)	7 (7-8)	transducin (beta)-like 1X-linked
LPAR1	-1.85	0.00771	106 (96-114)	59 (42-75)	lysophosphatidic acid receptor 1
IFI6	-1.85	0.01880	101 (87-121)	60 (46-73)	interferon, alpha-inducible protein 6
ARHGAP28	-1.83	0.04598	64 (38-91)	32 (26-40)	Rho GTPase activating protein 28
BCAT1	-1.82	0.00274	341 (332-365)	197 (170-228)	branched chain aminotransferase 1, cytosolic
RACGAP1	-1.82	0.02662	357 (322-376)	190 (126-261)	Rac GTPase activating protein 1
ZEB1	-1.82	0.04491	281 (245-365)	184 (136-234)	zinc finger E-box binding homeobox 1
SLC26A2	-1.81	0.02264	1011 (859-1250)	632 (489-761)	solute carrier family 26 (sulfate transporter), member 2
ZNF443	-1.80	0.00039	43 (40-47)	24 (22-26)	zinc finger protein 443
SAMD9	-1.80	0.00158	55 (51-58)	28 (27-31)	sterile alpha motif domain containing 9

gene name	FC	p value	Intensity normalized by gcRMA(slow) in unlogged scale		description
			control female	intervention female	
ERC1	-1.80	0.04191	30 (25-36)	18 (12-23)	ELKS/RAB6-interacting/CAST family member 1
RAD51AP1	-1.80	0.00236	25 (23-27)	14 (13-16)	RAD51 associated protein 1
FILIP1L	-1.78	0.01827	201 (178-240)	144 (121-148)	filamin A interacting protein 1-like
MEIS2	-1.77	0.00652	71 (61-83)	42 (32-52)	Meis homeobox 2
P2RY14	-1.77	0.01613	60 (59-66)	34 (27-45)	purinergic receptor P2Y, G-protein coupled, 14
MSH2	-1.77	0.00156	35 (33-35)	20 (18-21)	mutS homolog 2, colon cancer, nonpolyposis type 1 (E. coli)
PLCL1	-1.75	0.03662	27 (24-28)	17 (13-19)	phospholipase C-like 1
ANAPC4	-1.75	0.00942	122 (106-142)	70 (63-80)	anaphase promoting complex subunit 4
CTSO	-1.74	0.00283	69 (58-75)	35 (34-37)	cathepsin O
MYCN	-1.72	0.00904	139 (132-158)	89 (65-114)	v-myc myelocytomatosis viral related oncogene, neuroblastoma derived (avian)
TYMS	-1.72	0.00612	388 (351-430)	210 (194-242)	thymidylate synthetase
TXNDC15	-1.72	0.00014	98 (92-103)	55 (52-58)	thioredoxin domain containing 15
SOX4	-1.71	0.00038	92 (87-102)	54 (51-59)	SRY (sex determining region Y)-box 4
MEF2C	-1.71	0.02549	82 (78-108)	61 (50-69)	myocyte enhancer factor 2C
LMNB1	-1.71	0.00322	29 (25-30)	15 (14-16)	lamin B1
FAT1	-1.71	0.03693	168 (153-190)	111 (69-151)	FAT tumor suppressor homolog 1 (Drosophila)
MAP4K5	-1.71	0.00629	230 (186-291)	140 (125-156)	mitogen-activated protein kinase kinase kinase kinase 5
CCL13	-1.71	0.04810	14 (10-19)	8 (7-9)	chemokine (C-C motif) ligand 13
CDK1	-1.70	0.02121	84 (61-107)	49 (46-51)	cyclin-dependent kinase 1
DNAJB14	-1.69	0.02718	593 (587-668)	449 (388-468)	DnaJ (Hsp40) homolog, subfamily B, member 14
RNFT1	-1.69	0.00582	33 (28-37)	19 (17-20)	ring finger protein, transmembrane 1
ITGB3BP	-1.68	0.00455	31 (29-31)	17 (15-20)	integrin beta 3 binding protein (beta3-endonexin)
HAUS1	-1.68	0.01997	112 (89-128)	61 (56-67)	HAUS augmin-like complex, subunit 1
RFC3	-1.68	0.00629	50 (47-52)	31 (28-33)	replication factor C (activator 1) 3, 38kDa
SC5DL	-1.68	0.00564	34 (30-36)	20 (17-22)	sterol-C5-desaturase (ERG3 delta-5-desaturase homolog, S. cerevisiae)-like
DMD	-1.68	0.03208	367 (336-393)	256 (213-272)	dystrophin
NUSAP1	-1.67	0.03527	47 (40-49)	24 (19-31)	nucleolar and spindle associated protein 1
LACTB2	-1.67	0.01965	31 (26-37)	20 (18-20)	lactamase, beta 2
ATP2B1	-1.66	0.00198	47 (42-55)	30 (29-31)	ATPase, Ca++ transporting, plasma membrane 1
ATAD2	-1.66	0.00086	20 (18-23)	12 (11-13)	ATPase family, AAA domain containing 2
GPC4	-1.66	0.02022	34 (30-42)	21 (19-25)	glypican 4
SMC5	-1.64	0.02608	48 (42-54)	30 (22-39)	structural maintenance of chromosomes 5
ZNF138	-1.64	0.01777	14 (12-16)	9 (7-11)	zinc finger protein 138
DPH3	-1.63	0.00461	89 (73-104)	53 (47-59)	DPH3, KTI11 homolog (S. cerevisiae)
F2RL1	-1.63	0.00700	13 (12-14)	8 (7-9)	coagulation factor II (thrombin) receptor-like 1
FLJ32065	-1.63	0.01317	17 (15-19)	11 (9-12)	hypothetical protein FLJ32065
TMEM14A	-1.63	0.01343	41 (33-53)	26 (24-28)	transmembrane protein 14A
IMPA1	-1.63	0.00382	70 (65-78)	44 (38-51)	inositol(myo)-1(or 4)-monophosphatase 1
SPRED2	-1.63	0.00162	39 (39-41)	23 (22-26)	sprouty-related, EVH1 domain containing 2
TMCC3	-1.62	0.03575	242 (173-317)	152 (125-177)	transmembrane and coiled-coil domain family 3
MRPL23	-1.61	0.01607	44 (44-46)	31 (23-37)	mitochondrial ribosomal protein L23
SELENBP1	-1.61	0.00407	20 (20-22)	13 (12-15)	selenium binding protein 1
TOB1	-1.61	0.02197	106 (83-131)	66 (60-71)	transducer of ERBB2, 1
SLC2A3	-1.61	0.02180	281 (245-292)	158 (143-171)	solute carrier family 2 (facilitated glucose transporter), member 3
GPR177	-1.60	0.04016	143 (130-157)	95 (81-108)	G protein-coupled receptor 177
MOBKL1A	-1.60	0.01015	60 (56-72)	41 (38-44)	MOB1, Mps One Binder kinase activator-like 1A (yeast)
CKS2	-1.60	0.00984	55 (48-66)	36 (32-40)	CDC28 protein kinase regulatory subunit 2
PI4K2B	-1.60	0.00761	22 (19-26)	15 (13-16)	phosphatidylinositol 4-kinase type 2 beta
CKAP2	-1.59	0.01713	71 (56-86)	44 (41-47)	cytoskeleton associated protein 2
STOX2	-1.59	0.01718	16 (14-21)	11 (10-12)	storkhead box 2
C12orf29	-1.59	0.00285	53 (51-57)	32 (31-36)	chromosome 12 open reading frame 29
TUBB2A	-1.59	0.01328	619 (564-732)	410 (360-472)	tubulin, beta 2A
KIAA0240	-1.59	0.00459	30 (29-33)	19 (19-21)	KIAA0240
GALNT11	-1.59	0.01160	735 (665-787)	439 (380-512)	UDP-N-acetyl-alpha-D-galactosamine:polypeptide N-acetylgalactosaminyltransferase 11 (GalNAc-T11)
SSB	-1.59	0.03482	505 (390-575)	277 (265-287)	Sjogren syndrome antigen B (autoantigen La)

gene name	FC	raw p value	Intensity normalized by gcRMA(slow) in unlogged scale		description
			control female	intervention female	
ANTXR2	-1.58	0.03262	146 (114-162)	80 (77-82)	anthrax toxin receptor 2
RNF115	-1.58	0.03049	32 (30-34)	24 (19-26)	ring finger protein 115
PYGL	-1.58	0.01680	109 (93-132)	70 (65-78)	phosphorylase, glycogen, liver
SERPINE2	-1.58	0.02521	7195 (6291-7854)	4202 (3504-5069)	serpin peptidase inhibitor, clade E (nexin, plasminogen activator inhibitor type 1), member 2
RND3	-1.58	0.01161	661 (553-733)	382 (344-426)	Rho family GTPase 3
ECT2	-1.58	0.04695	79 (61-100)	52 (42-60)	epithelial cell transforming sequence 2 oncogene
ASAP2	-1.57	0.00214	75 (69-85)	49 (48-51)	ArfGAP with SH3 domain, ankyrin repeat and PH domain 2
ZNF573	-1.57	0.03650	39 (33-47)	23 (21-29)	zinc finger protein 573
VPS13A	-1.57	0.01511	39 (37-40)	26 (21-29)	vacuolar protein sorting 13 homolog A (S. cerevisiae)
DCN	-1.57	0.02694	868 (690-1109)	540 (520-597)	decorin
SULF2	-1.57	0.02091	198 (173-230)	118 (114-133)	sulfatase 2
ZNF682	-1.56	0.01744	26 (24-29)	17 (14-20)	zinc finger protein 682
DECR1	-1.56	0.02947	137 (120-161)	94 (80-107)	2,4-dienoyl CoA reductase 1, mitochondrial
MAD2L1	-1.56	0.02443	31 (26-36)	20 (18-21)	MAD2 mitotic arrest deficient-like 1 (yeast)
RHOQ	-1.56	0.03711	714 (536-882)	440 (392-481)	ras homolog gene family, member Q
RAB10	-1.56	0.00303	356 (304-415)	243 (228-246)	RAB10, member RAS oncogene family
ZFP36L1	-1.55	0.01836	1056 (889-1208)	646 (594-709)	zinc finger protein 36, C3H type-like 1
RAPGEF5	-1.55	0.02291	429 (381-474)	283 (240-316)	Rap guanine nucleotide exchange factor (GEF) 5
TOP2A	-1.55	0.03453	131 (97-160)	83 (74-88)	topoisomerase (DNA) II alpha 170kDa
TM7SF3	-1.55	0.00952	29 (27-30)	17 (16-19)	transmembrane 7 superfamily member 3
HSD17B6	-1.55	0.04089	19 (16-21)	11 (10-12)	hydroxysteroid (17-beta) dehydrogenase 6 homolog (mouse)
SKAP2	-1.55	0.02304	36 (29-41)	21 (19-25)	src kinase associated phosphoprotein 2
ARSJ	-1.55	0.03944	9 (8-12)	7 (6-7)	arylsulfatase family, member J
DNAJB4	-1.55	0.00019	403 (380-426)	262 (252-269)	DnaJ (Hsp40) homolog, subfamily B, member 4
CHML	-1.55	0.00417	638 (584-666)	393 (345-445)	choroideremia-like (Rab escort protein 2)
NUP35	-1.55	0.03475	79 (74-89)	62 (54-64)	nucleoporin 35kDa
CCDC14	-1.54	0.00432	32 (29-37)	22 (20-23)	coiled-coil domain containing 14
TMEM192	-1.54	0.00320	53 (50-60)	35 (34-37)	transmembrane protein 192
KLF11	-1.54	0.01436	48 (42-57)	33 (30-35)	Kruppel-like factor 11
FLNB	-1.54	0.02761	120 (106-138)	85 (63-105)	filamin B, beta
COL5A1	-1.54	0.01231	114 (99-134)	73 (69-80)	collagen, type V, alpha 1
CHST2	-1.54	0.02392	70 (65-83)	49 (45-56)	carbohydrate (N-acetylglucosamine-6-O) sulfotransferase 2
PRKACB	-1.54	0.03821	94 (84-104)	63 (50-73)	protein kinase, cAMP-dependent, catalytic, beta
LRP6	-1.53	0.04090	71 (64-94)	53 (47-60)	low density lipoprotein receptor-related protein 6
PPP1R3D	-1.53	0.02277	40 (36-44)	28 (25-30)	protein phosphatase 1, regulatory (inhibitor) subunit 3D
RICH2	-1.53	0.03194	20 (17-22)	12 (11-14)	Rho-type GTPase-activating protein RICH2
PPP3CA	-1.53	0.00346	75 (66-85)	49 (44-54)	protein phosphatase 3 (formerly 2B), catalytic subunit, alpha isoform
CITED2	-1.53	0.01298	584 (498-712)	400 (351-449)	Cbp/p300-interacting transactivator, with Glu/Asp-rich carboxy-terminal domain, 2
NPM1	-1.53	0.00462	128 (119-139)	87 (80-92)	nucleophosmin (nucleolar phosphoprotein B23, numatrin)
SH3D19	-1.53	0.01480	213 (181-232)	125 (119-134)	SH3 domain containing 19
SMC3	-1.53	0.03551	624 (554-761)	483 (424-511)	structural maintenance of chromosomes 3
ABHD10	-1.53	0.01135	19 (17-23)	14 (13-15)	abhydrolase domain containing 10
DNAJC24	-1.52	0.00763	38 (34-43)	25 (25-26)	DnaJ (Hsp40) homolog, subfamily C, member 24
SCUBE2	-1.52	0.03617	17 (16-20)	12 (11-14)	signal peptide, CUB domain, EGF-like 2
PDK1	-1.52	0.00645	43 (41-44)	28 (23-34)	pyruvate dehydrogenase kinase, isozyme 1
SETMAR	-1.52	0.00069	14 (13-15)	10 (9-10)	SET domain and mariner transposase fusion gene
CDK17	-1.51	0.01439	536 (494-608)	346 (335-380)	cyclin-dependent kinase 17
C8orf84	-1.51	0.04544	24 (22-25)	15 (11-19)	chromosome 8 open reading frame 84
TSEN15	-1.51	0.00162	106 (93-116)	68 (62-74)	tRNA splicing endonuclease 15 homolog (S. cerevisiae)
B4GALT6	-1.51	0.02037	21 (19-26)	15 (14-17)	UDP-Gal:betaGlcNAc beta 1,4- galactosyltransferase, polypeptide 6
LRIG3	-1.51	0.01184	29 (26-32)	19 (16-22)	leucine-rich repeats and immunoglobulin-like domains 3

gene name	FC	p value	Intensity normalized by gcRMA(slow) in unlogged scale		description
			control female	intervention female	
MLLT3	-1.51	0.00015	15 (15-16)	10 (10-10)	myeloid/lymphoid or mixed-lineage leukemia (trithorax homolog, Drosophila) /// translocated to, 3
POT1	-1.51	0.00209	66 (58-73)	43 (41-44)	POT1 protection of telomeres 1 homolog (S. pombe)
GNL3	-1.51	0.03875	72 (70-79)	57 (49-60)	guanine nucleotide binding protein-like 3 (nucleolar)
SNAPC1	-1.50	0.00845	38 (36-42)	27 (24-30)	small nuclear RNA activating complex, polypeptide 1, 43kDa
SYMPK	1.50	0.01088	13 (12-13)	19 (15-23)	symplekin
H1F0	1.50	0.04682	279 (259-338)	481 (428-525)	H1 histone family, member 0
ST5	1.51	0.01230	70 (57-80)	98 (90-106)	suppression of tumorigenicity 5
ARHGEF16	1.51	0.00530	17 (16-17)	24 (21-29)	Rho guanine exchange factor (GEF) 16
RAB24	1.51	0.01456	68 (57-74)	93 (85-102)	RAB24, member RAS oncogene family
HOOK2	1.53	0.00391	32 (31-35)	50 (47-53)	hook homolog 2 (Drosophila)
WDR45	1.53	0.03587	99 (79-120)	144 (133-157)	WD repeat domain 45
ABHD11	1.53	0.00663	71 (63-75)	103 (96-109)	abhydrolase domain containing 11
PLEKHG6	1.53	0.04299	22 (19-23)	30 (28-32)	pleckstrin homology domain containing, family G (with RhoGef domain) member 6
FAM122B	1.53	0.01822	15 (13-17)	21 (20-24)	family with sequence similarity 122B
RDH13	1.53	0.04366	44 (37-48)	57 (51-69)	retinol dehydrogenase 13 (all-trans/9-cis)
CDK16	1.53	0.00053	26 (26-28)	40 (39-42)	cyclin-dependent kinase 16
ZMYND8	1.54	0.01144	35 (34-37)	57 (52-62)	zinc finger, MYND-type containing 8
MEIG1	1.54	0.02594	8 (8-9)	12 (11-14)	meiosis expressed gene 1 homolog (mouse)
ZSCAN4	1.55	0.01185	12 (10-12)	16 (15-17)	zinc finger and SCAN domain containing 4
RAP1GAP	1.55	0.03275	40 (33-43)	56 (50-60)	RAP1 GTPase activating protein
WDR13	1.56	0.00265	72 (70-78)	115 (106-128)	WD repeat domain 13
SIN3B	1.57	0.04774	52 (38-65)	70 (65-83)	SIN3 homolog B, transcription regulator (yeast)
CIRBP	1.58	0.01066	46 (43-54)	81 (72-89)	cold inducible RNA binding protein
NAGK	1.58	0.00958	153 (137-161)	226 (204-248)	N-acetylglucosamine kinase
ZSCAN18	1.58	0.00708	95 (83-108)	139 (135-154)	zinc finger and SCAN domain containing 18
HDAC5	1.59	0.02017	21 (18-24)	31 (30-32)	histone deacetylase 5
CNKSR1	1.59	0.02484	47 (40-52)	69 (65-74)	connector enhancer of kinase suppressor of Ras 1
KIAA1549	1.60	0.04002	18 (13-21)	28 (24-30)	KIAA1549
ANKRD9	1.62	0.00761	488 (382-582)	775 (743-781)	ankyrin repeat domain 9
PIK3AP1	1.62	0.01267	136 (122-152)	247 (197-280)	phosphoinositide-3-kinase adaptor protein 1
ANKRD10	1.63	0.01992	325 (275-375)	486 (474-519)	ankyrin repeat domain 10
BCL6	1.64	0.03658	144 (121-170)	249 (198-293)	B-cell CLL/lymphoma 6
SH2B2	1.65	0.03995	16 (13-18)	23 (21-26)	SH2B adaptor protein 2
RGL2	1.65	0.00262	248 (231-294)	454 (421-480)	ral guanine nucleotide dissociation stimulator-like 2
SLC35F2	1.65	0.00868	16 (14-19)	25 (24-27)	solute carrier family 35, member F2
C10orf54	1.66	0.03204	662 (561-710)	1001 (886-1088)	chromosome 10 open reading frame 54
ZNF321	1.66	0.01788	6 (5-7)	10 (9-10)	zinc finger protein 321
CRIP2	1.67	0.04544	46 (39-48)	67 (57-78)	cysteine-rich protein 2
GLIS2	1.67	0.00125	33 (30-35)	50 (48-58)	GLIS family zinc finger 2
LOC100288618	1.67	0.00015	8 (8-8)	13 (12-14)	hypothetical protein LOC100288618
AP1G2	1.68	0.00378	56 (44-67)	92 (88-95)	adaptor-related protein complex 1, gamma 2 subunit
TADA1	1.68	0.04819	70 (52-87)	105 (92-122)	transcriptional adaptor 1
THOP1	1.69	0.02490	10 (8-13)	17 (15-18)	thimet oligopeptidase 1
LENG8	1.70	0.01357	37 (32-43)	66 (56-74)	leukocyte receptor cluster (LRC) member 8
KCNN4	1.71	0.01854	75 (61-83)	112 (105-121)	potassium intermediate/small conductance calcium-activated channel, subfamily N, member 4
CTDSP1	1.73	0.00101	252 (245-276)	467 (424-510)	CTD (carboxy-terminal domain, RNA polymerase II, polypeptide A) small phosphatase 1
DAGLB	1.73	0.02125	14 (11-16)	21 (18-25)	diacylglycerol lipase, beta
ISM1	1.73	0.04133	138 (104-169)	231 (180-283)	isthmin 1 homolog (zebrafish)
CD99L2	1.74	0.02130	27 (24-31)	45 (40-51)	CD99 molecule-like 2
CSF3R	1.75	0.00982	570 (465-618)	871 (784-950)	colony stimulating factor 3 receptor (granulocyte)
SNX10	1.77	0.03715	314 (267-318)	432 (395-498)	sorting nexin 10
AKAP8L	1.84	0.00587	41 (33-47)	69 (64-75)	A kinase (PRKA) anchor protein 8-like
CCDC120	1.85	0.03663	108 (88-117)	168 (158-174)	coiled-coil domain containing 120
TNS4	1.88	0.01912	85 (66-105)	175 (154-177)	tensin 4
RAB11FIP5	1.90	0.03584	48 (39-52)	74 (67-82)	RAB11 family interacting protein 5 (class I)

gene name	FC	p value	Intensity normalized by gcRMA(slow) in unlogged scale		description
			control female	intervention female	
GDPD5	1.96	0.00264	374 (301-433)	691 (590-793)	glycerophosphodiester phosphodiesterase domain containing 5
TGFB1	2.00	0.02987	59 (45-73)	103 (95-118)	transforming growth factor, beta 1
FLT4	2.03	0.00594	21 (19-24)	42 (34-52)	fms-related tyrosine kinase 4
IFI30	2.07	0.04729	317 (224-366)	525 (431-594)	interferon, gamma-inducible protein 30
LTBP3	2.08	0.03346	63 (40-87)	116 (105-127)	latent transforming growth factor beta binding protein 3
EPS8L2	2.12	0.00731	48 (43-54)	105 (88-118)	EPS8-like 2
DAPK1	2.16	0.01198	1893 (1432-2122)	3268 (2954-3586)	death-associated protein kinase 1
CFB	2.23	0.04035	78 (40-112)	131 (123-144)	complement factor B
LPL	2.27	0.03809	65 (56-75)	140 (90-234)	lipoprotein lipase
FURIN	2.28	0.02482	186 (147-195)	311 (253-384)	furin (paired basic amino acid cleaving enzyme)
ALDH3B2	2.29	0.02417	27 (23-30)	61 (37-89)	aldehyde dehydrogenase 3 family, member B2
CCDC69	2.30	0.02315	54 (44-69)	109 (107-117)	coiled-coil domain containing 69
STRA6	2.38	0.00642	48 (37-62)	111 (105-115)	stimulated by retinoic acid gene 6 homolog (mouse)
LIMCH1	2.44	0.04391	53 (40-61)	94 (85-106)	LIM and calponin homology domains 1
SLC7A5	2.45	0.02056	190 (147-206)	333 (288-398)	solute carrier family 7 (cationic amino acid transporter, y+ system), member 5
PAFAH2	2.48	0.00003	18 (16-20)	42 (41-45)	platelet-activating factor acetylhydrolase 2, 40kDa
BMP1	2.54	0.04683	135 (99-165)	239 (214-294)	bone morphogenetic protein 1
C20orf46	2.56	0.01841	22 (20-33)	68 (47-98)	chromosome 20 open reading frame 46
FRZB	2.60	0.00469	324 (250-434)	793 (746-924)	frizzled-related protein
TXK	2.66	0.02722	31 (25-36)	78 (44-114)	TXK tyrosine kinase
KRTAP26-1	2.84	0.00589	7 (6-8)	18 (16-25)	keratin associated protein 26-1
SH3GLB2	3.02	0.00342	96 (65-123)	263 (229-295)	SH3-domain GRB2-like endophilin B2
CORO6	3.07	0.00415	126 (99-148)	348 (259-462)	coronin 6
CPZ	3.27	0.01050	182 (109-277)	492 (440-601)	carboxypeptidase Z
SLCO4A1	3.31	0.00552	10 (7-14)	29 (25-43)	solute carrier organic anion transporter family, member 4A1
CA10	3.45	0.02131	20 (14-29)	77 (58-100)	carbonic anhydrase X
SLC2A11	3.73	0.04584	112 (55-174)	290 (219-378)	solute carrier family 2 (facilitated glucose transporter), member 11
LYZ	6.17	0.00858	12 (9-15)	56 (32-135)	lysozyme (renal amyloidosis)
HINT3	6.43	0.00646	10 (9-12)	93 (63-132)	histidine triad nucleotide binding protein 3

Data are presented as median (interquartile range IQR = 25th-75th percentile). Control female = placentas of female offspring in the control group, Intervention female = placentas of female offspring in the n-3 LCPUFA intervention group. The genes are sorted according to their fold change. FC, fold change;

11.13 Raw data and statistical analysis of miRNome profiling

Plate	Detector	Flag CF	Flag IF	Raw Cq		Norm. Cq		median Cq	log RQ	threshold		Ex-treme	FC
				CF	IF	CF	IF			high	low		
A	ath-miR159a-4373390	Yes	Yes	33.3	Inf	Inf	Inf	NA	NA	NA	-NA	NA	NA
B	ath-miR159a-4373390	Yes	Yes	Inf	Inf	Inf	Inf	NA	NA	NA	-NA	NA	NA
B	hsa-let-7a*-4395418	No	No	28.0	27.8	27.9	28.0	28.0	-0.12	1.19	-1.45	no	-1.09
A	hsa-let-7a-4373169	No	No	18.6	18.0	18.5	18.3	18.4	0.23	0.42	-0.54	no	1.18
B	hsa-let-7b*-4395515	Yes	Yes	34.9	Inf	Inf	Inf	NA	NA	NA	-NA	NA	NA
A	hsa-let-7b-4395446	No	No	19.1	18.4	19.2	18.7	18.9	0.43	0.45	-0.57	no	1.35
A	hsa-let-7c-4373167	No	No	22.6	22.0	22.7	22.2	22.5	0.53	0.67	-0.84	no	1.45
B	hsa-let-7d*-4378108	Yes	Yes	Inf	Inf	Inf	Inf	NA	NA	NA	-NA	NA	NA
A	hsa-let-7d-4395394	No	No	20.4	20.1	20.5	20.3	20.4	0.16	0.53	-0.67	no	1.12
B	hsa-let-7e*-4395518	No	No	26.3	26.5	26.1	26.7	26.4	-0.61	1.02	-1.25	no	-1.53
A	hsa-let-7e-4395517	No	No	16.5	15.7	15.7	16.0	15.9	-0.27	0.31	-0.41	no	-1.20
B	hsa-let-7f-1*-4395528	No	No	32.4	32.0	32.7	32.1	32.4	0.57	1.79	-2.16	no	1.48
B	hsa-let-7f-2*-4395529	No	No	27.4	26.8	27.2	27.0	27.1	0.24	1.10	-1.34	no	1.18
A	hsa-let-7f-4373164	No	No	22.4	22.3	22.7	22.5	22.6	0.18	0.68	-0.85	no	1.13
B	hsa-let-7g*-4395229	No	No	29.2	29.3	29.0	29.5	29.3	-0.43	1.35	-1.64	no	-1.34
A	hsa-let-7g-4395393	No	No	19.3	19.4	19.3	19.7	19.5	-0.42	0.48	-0.61	no	-1.34
B	hsa-let-7i*-4395283	No	No	30.0	30.2	29.9	30.3	30.1	-0.44	1.46	-1.77	no	-1.36
B	hsa-miR-100*-4395253	No	No	24.9	24.6	24.7	24.8	24.7	-0.06	0.86	-1.06	no	-1.04
A	**hsa-miR-100-4373160**	**No**	**No**	**16.8**	**14.9**	**15.8**	**15.2**	**15.5**	**0.63**	**0.29**	**-0.39**	**yes**	**1.55**
B	hsa-miR-101*-4395254	No	No	28.5	27.7	28.5	27.9	28.2	0.69	1.22	-1.49	no	1.61
A	hsa-miR-101-4395364	No	No	20.8	20.5	20.8	20.8	20.8	0.06	0.55	-0.70	no	1.05
A	hsa-miR-103-4373158	No	No	19.6	19.4	19.5	19.6	19.6	-0.17	0.48	-0.61	no	-1.12
B	hsa-miR-105*-4395279	Yes	Yes	Inf	Inf	Inf	Inf	NA	NA	NA	-NA	NA	NA
A	hsa-miR-105-4395278	No	No	28.2	28.1	28.7	28.3	28.5	0.43	1.26	-1.53	no	1.34
B	hsa-miR-106a*-4395281	Yes	Yes	Inf	Inf	Inf	Inf	NA	NA	NA	-NA	NA	NA
A	hsa-miR-106a-4395280	No	No	16.3	15.4	15.6	15.6	15.6	0.01	0.30	-0.40	no	1.01
B	hsa-miR-106b*-4395491	No	No	22.1	21.8	22.0	22.0	22.0	-0.03	0.63	-0.79	no	-1.02
A	hsa-miR-106b-4373155	No	No	18.1	17.7	17.9	18.0	18.0	-0.12	0.40	-0.51	no	-1.09
A	hsa-miR-107-4373154	No	No	26.2	26.1	27.2	26.4	26.8	0.83	1.06	-1.30	no	1.78
B	hsa-miR-10a*-4395399	No	No	30.2	30.6	30.3	30.7	30.5	-0.42	1.52	-1.83	no	-1.34
A	hsa-miR-10a-4373153	No	No	21.3	21.6	21.5	21.8	21.6	-0.39	0.61	-0.77	no	-1.31
B	hsa-miR-10b*-4395426	No	No	25.0	25.6	24.8	25.9	25.3	-1.03	0.92	-1.13	no	-2.05
A	**hsa-miR-10b-4395329**	**No**	**No**	**20.8**	**21.6**	**20.8**	**21.9**	**21.4**	**-1.04**	**0.59**	**-0.74**	**yes**	**-2.06**
B	hsa-miR-122*-4395241	Yes	Yes	Inf	Inf	Inf	Inf	NA	NA	NA	-NA	NA	NA
A	hsa-miR-122-4395356	No	No	28.4	31.1	29.4	31.1	30.3	-1.76	1.48	-1.80	no	-3.40
B	hsa-miR-124*-4395308	Yes	Yes	Inf	Inf	Inf	Inf	NA	NA	NA	-NA	NA	NA
A	hsa-miR-124-4373295	Yes	Yes	29.3	Inf	Inf	Inf	NA	NA	NA	-NA	NA	NA
A	hsa-miR-125a-3p-4395310	No	No	24.6	24.7	25.0	24.9	25.0	0.02	0.88	-1.08	no	1.02
A	hsa-miR-125a-5p-4395309	No	No	18.3	17.7	18.0	18.0	18.0	0.02	0.40	-0.51	no	1.01
B	hsa-miR-125b-1*-4395489	No	No	28.4	28.3	28.3	28.5	28.4	-0.18	1.24	-1.51	no	-1.13
B	hsa-miR-125b-2*-4395269	Yes	Yes	33.7	Inf	Inf	Inf	NA	NA	NA	-NA	NA	NA
A	hsa-miR-125b-4373148	No	No	16.2	15.2	15.3	15.4	15.4	-0.14	0.29	-0.39	no	-1.10
B	hsa-miR-126*-4373269	No	No	16.7	15.8	16.0	16.0	16.0	0.02	0.31	-0.41	no	1.01
A	hsa-miR-126-4395339	No	No	14.5	12.3	12.6	12.5	12.6	0.04	0.21	-0.29	no	1.03
A	hsa-miR-127-3p-4373147	No	No	17.5	17.0	17.3	17.3	17.3	-0.04	0.36	-0.47	no	-1.03
A	hsa-miR-127-5p-4395340	No	No	26.4	28.3	28.0	28.6	28.3	-0.56	1.23	-1.50	no	-1.47
A	hsa-miR-128-4395327	No	No	23.5	24.0	23.9	24.2	24.1	-0.36	0.80	-0.99	no	-1.28
A	hsa-miR-129-3p-4373297	Yes	Yes	30.0	Inf	Inf	Inf	NA	NA	NA	-NA	NA	NA
A	hsa-miR-129-5p-4373171	Yes	Yes	Inf	Inf	Inf	Inf	NA	NA	NA	-NA	NA	NA
B	**hsa-miR-130a*-4395242**	**Yes**	**No**	**Inf**	**33.2**	**Inf**	**33.2**	**NA**	**NA**	**NA**	**-NA**	**yes**	**NA**
A	hsa-miR-130a-4373145	No	No	18.0	17.4	17.9	17.7	17.8	0.15	0.39	-0.50	no	1.11
B	hsa-miR-130b*-4395225	No	No	26.3	27.0	26.1	27.2	26.6	-1.05	1.05	-1.28	no	-2.08
A	**hsa-miR-130b-4373144**	**No**	**No**	**20.9**	**21.8**	**21.0**	**22.0**	**21.5**	**-1.09**	**0.60**	**-0.75**	**yes**	**-2.12**
B	hsa-miR-132*-4395243	Yes	Yes	34.2	Inf	Inf	Inf	NA	NA	NA	-NA	NA	NA
A	hsa-miR-132-4373143	No	No	19.7	19.6	19.8	19.9	19.8	-0.11	0.49	-0.63	no	-1.08

Plate	Detector	Flag CF	Flag IF	Raw Cq		Norm. Cq		median Cq	log RQ	threshold		Ex-treme	FC
				CF	IF	CF	IF			high	low		
A	hsa-miR-133a-4395357	No	No	20.8	20.4	20.8	20.7	20.7	0.15	0.55	-0.69	no	1.11
A	hsa-miR-133b-4395358	No	No	23.8	23.8	24.1	24.1	24.1	0.03	0.80	-0.99	no	1.02
A	hsa-miR-134-4373299	No	No	24.3	24.3	24.4	24.6	24.5	-0.14	0.84	-1.03	no	-1.10
B	hsa-miR-135a*-4395343	No	No	21.0	20.7	20.8	20.9	20.8	-0.14	0.56	-0.70	no	-1.10
A	hsa-miR-135a-4373140	No	No	22.3	22.3	22.5	22.6	22.5	-0.08	0.67	-0.84	no	-1.06
B	hsa-miR-135b*-4395270	No	No	26.5	26.0	26.3	26.2	26.3	0.13	1.01	-1.23	no	1.09
A	hsa-miR-135b-4395372	No	No	16.9	16.3	16.6	16.6	16.6	-0.01	0.33	-0.44	no	-1.01
B	hsa-miR-136*-4395211	No	No	19.7	18.9	19.4	19.1	19.2	0.32	0.46	-0.59	no	1.25
A	hsa-miR-136-4373173	No	No	29.9	31.4	31.7	31.5	31.6	0.12	1.68	-2.02	no	1.09
A	hsa-miR-137-4373301	No	No	22.1	22.4	22.2	22.7	22.4	-0.49	0.67	-0.83	no	-1.40
B	hsa-miR-138-1*-4395273	No	No	26.1	25.3	25.9	25.5	25.7	0.37	0.95	-1.16	no	1.29
B	hsa-miR-138-2*-4395255	Yes	Yes	Inf	Inf	Inf	Inf	NA	NA	NA	-NA	NA	NA
A	hsa-miR-138-4395395	No	No	19.8	19.8	19.8	20.1	19.9	-0.33	0.50	-0.64	no	-1.26
A	hsa-miR-139-3p-4395424	No	No	25.4	25.1	25.7	25.4	25.6	0.36	0.94	-1.15	no	1.28
A	**hsa-miR-139-5p-4395400**	**No**	**No**	**19.1**	**18.2**	**19.0**	**18.5**	**18.8**	**0.59**	**0.44**	**-0.56**	**yes**	**1.51**
A	hsa-miR-140-3p-4395345	No	No	23.9	23.6	24.0	23.8	23.9	0.14	0.79	-0.97	no	1.10
A	hsa-miR-140-5p-4373374	No	No	17.6	17.0	17.4	17.3	17.3	0.10	0.37	-0.48	no	1.07
B	hsa-miR-141*-4395256	No	No	26.7	26.5	26.5	26.7	26.6	-0.22	1.04	-1.27	no	-1.17
A	hsa-miR-141-4373137	No	No	17.6	16.8	17.4	17.1	17.3	0.25	0.36	-0.47	no	1.19
A	hsa-miR-142-3p-4373136	No	No	16.8	16.1	16.4	16.4	16.4	0.04	0.33	-0.43	no	1.03
A	hsa-miR-142-5p-4395359	No	No	23.4	23.3	23.6	23.5	23.5	0.07	0.75	-0.94	no	1.05
B	hsa-miR-143*-4395257	Yes	Yes	26.6	Inf	Inf	Inf	NA	NA	NA	-NA	NA	NA
A	hsa-miR-143-4395360	No	No	16.1	15.4	15.6	15.6	15.6	-0.03	0.30	-0.40	no	-1.02
A	hsa-miR-1-4395333	No	No	22.8	23.3	23.3	23.6	23.4	-0.28	0.75	-0.92	no	-1.21
B	hsa-miR-144*-4395259	No	No	17.8	17.4	17.4	17.6	17.5	-0.21	0.37	-0.49	no	-1.16
B	hsa-miR-145*-4395260	No	No	21.6	21.4	21.4	21.6	21.5	-0.12	0.60	-0.75	no	-1.09
A	hsa-miR-145-4395389	No	No	15.3	13.9	14.1	14.1	14.1	-0.02	0.25	-0.34	no	-1.01
B	hsa-miR-146a*-4395274	Yes	Yes	Inf	Inf	Inf	Inf	NA	NA	NA	-NA	NA	NA
A	hsa-miR-146a-4373132	No	No	18.3	17.5	18.2	17.8	18.0	0.32	0.40	-0.51	no	1.25
A	hsa-miR-146b-3p-4395472	No	No	27.9	29.0	28.9	29.3	29.1	-0.35	1.33	-1.62	no	-1.28
A	hsa-miR-146b-5p-4373178	No	No	18.8	18.9	18.7	19.2	18.9	-0.46	0.44	-0.57	no	-1.37
A	hsa-miR-147-4373131	Yes	Yes	34.9	Inf	Inf	Inf	NA	NA	NA	-NA	NA	NA
A	hsa-miR-147b-4395373	No	No	30.0	30.9	31.9	31.1	31.5	0.86	1.66	-2.00	no	1.82
B	hsa-miR-148a*-4395245	Yes	Yes	Inf	Inf	Inf	Inf	NA	NA	NA	-NA	NA	NA
A	hsa-miR-148a-4373130	No	No	19.7	19.6	19.7	19.8	19.8	-0.09	0.49	-0.63	no	-1.07
B	hsa-miR-148b*-4395271	No	No	24.3	23.8	24.1	24.1	24.1	0.02	0.80	-0.99	no	1.02
A	hsa-miR-148b-4373129	No	No	22.4	22.4	22.6	22.7	22.6	-0.07	0.68	-0.85	no	-1.05
B	hsa-miR-149*-4395275	Yes	Yes	28.2	Inf	Inf	Inf	NA	NA	NA	-NA	NA	NA
A	hsa-miR-149-4395366	No	No	18.0	17.7	17.9	17.9	17.9	-0.01	0.39	-0.51	no	-1.01
A	hsa-miR-150-4373127	No	No	19.7	19.1	19.6	19.4	19.5	0.23	0.47	-0.61	no	1.17
B	hsa-miR-151-3p-4395365	No	No	20.2	19.9	19.9	20.1	20.0	-0.26	0.51	-0.64	no	-1.20
A	hsa-miR-152-4395170	No	No	19.2	19.1	19.1	19.4	19.3	-0.26	0.46	-0.59	no	-1.20
A	hsa-miR-153-4373305	No	Yes	31.8	36.5	33.8	36.3	35.1	-2.42	2.25	-2.70	no	-5.36
B	hsa-miR-154*-4378065	No	No	24.0	23.6	23.8	23.8	23.8	0.03	0.78	-0.96	no	1.02
A	hsa-miR-154-4373270	No	No	26.0	25.6	26.2	25.9	26.0	0.26	0.98	-1.21	no	1.19
A	**hsa-miR-155-4395459**	**Yes**	**No**	**36.5**	**32.1**	**Inf**	**32.1**	**NA**	**NA**	**NA**	**-NA**	**yes**	**NA**
B	hsa-miR-155*-4395398	Yes	Yes	Inf	Inf	Inf	Inf	NA	NA	NA	-NA	NA	NA
B	hsa-miR-15a*-4395530	No	No	24.9	24.5	24.7	24.7	24.7	-0.03	0.86	-1.05	no	-1.02
A	hsa-miR-15a-4373123	No	No	22.6	22.3	23.0	22.6	22.8	0.38	0.69	-0.86	no	1.30
B	hsa-miR-15b*-4395284	No	No	23.6	23.0	23.6	23.2	23.4	0.37	0.74	-0.92	no	1.29
A	hsa-miR-15b-4373122	No	Yes	18.6	18.1	18.3	18.4	18.4	-0.02	0.42	-0.54	no	-1.02
B	hsa-miR-16-1*-4395531	No	No	26.0	25.5	25.8	25.7	25.8	0.09	0.96	-1.18	no	1.06
B	hsa-miR-16-2*-4395282	Yes	Yes	Inf	Inf	Inf	Inf	NA	NA	NA	-NA	NA	NA
A	hsa-miR-16-4373121	No	No	16.0	14.9	15.3	15.2	15.3	0.14	0.28	-0.38	no	1.10
B	hsa-miR-17*-4395532	No	No	25.5	25.4	25.3	25.6	25.5	-0.31	0.93	-1.14	no	-1.24
A	hsa-miR-17-4395419	No	No	16.2	15.4	15.6	15.7	15.6	-0.06	0.30	-0.40	no	-1.04
B	hsa-miR-181a*-4373086	No	No	23.8	23.2	23.6	23.4	23.5	0.19	0.75	-0.94	no	1.14

Plate	Detector	Flag CF	Flag IF	Raw Cq		Norm. Cq		median Cq	log RQ	threshold		Ex-treme	FC
				CF	IF	CF	IF			high	low		
B	hsa-miR-181a-2*-4395428	No	No	22.4	22.1	22.1	22.3	22.2	-0.16	0.65	-0.82	no	-1.12
A	hsa-miR-181a-4373117	No	No	19.3	18.8	18.9	19.1	19.0	-0.16	0.45	-0.57	no	-1.12
B	hsa-miR-181c*-4395444	Yes	Yes	32.8	Inf	Inf	Inf	NA	NA	NA	-NA	NA	NA
A	hsa-miR-181c-4373115	No	No	24.1	23.9	24.4	24.2	24.3	0.27	0.82	-1.01	no	1.20
B	hsa-miR-182*-4378066	Yes	Yes	Inf	Inf	Inf	Inf	NA	NA	NA	-NA	NA	NA
A	hsa-miR-182-4395445	No	No	25.5	25.7	25.8	26.0	25.9	-0.23	0.97	-1.19	no	-1.17
B	hsa-miR-183*-4395381	No	No	26.8	26.0	26.7	26.2	26.4	0.54	1.02	-1.25	no	1.45
A	hsa-miR-183-4395380	No	No	26.7	26.8	27.3	27.1	27.2	0.14	1.11	-1.35	no	1.10
A	hsa-miR-184-4373113	No	No	21.6	20.9	21.7	21.1	21.4	0.59	0.60	-0.75	no	1.50
B	hsa-miR-185*-4395215	Yes	Yes	Inf	Inf	Inf	Inf	NA	NA	NA	-NA	NA	NA
A	hsa-miR-185-4395382	No	No	20.1	19.9	20.1	20.2	20.1	-0.07	0.51	-0.65	no	-1.05
B	hsa-miR-186*-4395216	Yes	Yes	Inf	Inf	Inf	Inf	NA	NA	NA	-NA	NA	NA
A	hsa-miR-186-4395396	No	No	18.3	17.6	18.2	17.9	18.0	0.34	0.40	-0.52	no	1.26
A	hsa-miR-187-4373307	Yes	Yes	33.5	Inf	Inf	Inf	NA	NA	NA	-NA	NA	NA
A	hsa-miR-188-3p-4395217	No	No	28.3	30.2	30.5	30.4	30.4	0.08	1.50	-1.82	no	1.05
B	hsa-miR-188-5p-4395431	No	No	25.1	24.3	25.0	24.5	24.8	0.54	0.86	-1.06	no	1.45
B	hsa-miR-18a*-4395534	No	No	29.1	28.4	29.1	28.6	28.8	0.48	1.30	-1.57	no	1.40
A	hsa-miR-18a-4395533	No	No	20.9	20.6	20.9	20.9	20.9	0.01	0.56	-0.71	no	1.00
B	hsa-miR-18b*-4395421	Yes	Yes	Inf	Inf	Inf	Inf	NA	NA	NA	-NA	NA	NA
A	hsa-miR-18b-4395328	No	No	25.0	25.3	25.3	25.5	25.4	-0.26	0.92	-1.13	no	-1.20
A	hsa-miR-190-4373110	No	No	25.7	26.1	25.9	26.4	26.1	-0.47	0.99	-1.22	no	-1.39
B	hsa-miR-190b-4395374	No	No	25.2	24.9	24.9	25.1	25.0	-0.13	0.88	-1.09	no	-1.10
A	hsa-miR-191-4395410	No	No	15.6	14.5	14.9	14.8	14.8	0.08	0.27	-0.36	no	1.05
B	hsa-miR-192*-4395383	No	No	27.1	26.5	27.0	26.7	26.8	0.24	1.07	-1.31	no	1.18
A	hsa-miR-192-4373108	No	No	21.4	21.3	21.5	21.5	21.5	-0.03	0.60	-0.76	no	-1.02
A	hsa-miR-193a-3p-4395361	No	No	26.4	26.6	26.9	26.9	26.9	0.02	1.07	-1.31	no	1.02
A	hsa-miR-193a-5p-4395392	No	No	21.8	22.0	21.8	22.2	22.0	-0.40	0.64	-0.80	no	-1.32
B	hsa-miR-193b*-4395477	No	No	24.2	23.5	24.0	23.7	23.9	0.25	0.78	-0.97	no	1.19
A	hsa-miR-193b-4395478	No	No	16.1	15.1	15.4	15.3	15.4	0.08	0.29	-0.39	no	1.05
B	hsa-miR-194*-4395490	Yes	Yes	Inf	Inf	Inf	Inf	NA	NA	NA	-NA	NA	NA
A	hsa-miR-194-4373106	No	No	20.3	20.4	20.4	20.7	20.5	-0.32	0.54	-0.68	no	-1.25
B	hsa-miR-195*-4395218	Yes	Yes	Inf	Inf	Inf	Inf	NA	NA	NA	-NA	NA	NA
A	hsa-miR-195-4373105	No	No	18.4	18.0	18.2	18.3	18.3	-0.10	0.41	-0.53	no	-1.07
A	hsa-miR-196b-4395326	No	No	21.5	22.0	21.6	22.2	21.9	-0.58	0.63	-0.79	no	-1.49
A	hsa-miR-197-4373102	No	No	21.4	21.2	21.6	21.5	21.5	0.16	0.60	-0.76	no	1.12
A	hsa-miR-198-4395384	No	No	26.8	27.0	27.3	27.3	27.3	-0.03	1.12	-1.36	no	-1.02
A	hsa-miR-199a-3p-4395415	No	No	16.3	15.5	15.8	15.8	15.8	0.04	0.30	-0.40	no	1.03
A	hsa-miR-199a-5p-4373272	No	No	22.9	22.7	23.0	22.9	23.0	0.11	0.71	-0.88	no	1.08
A	hsa-miR-199b-5p-4373100	No	No	23.8	23.8	24.1	24.0	24.0	0.08	0.80	-0.99	no	1.06
B	hsa-miR-19a*-4395535	No	No	33.8	32.5	33.6	32.5	33.0	1.02	1.90	-2.29	no	2.03
A	hsa-miR-19a-4373099	No	No	18.5	18.4	18.4	18.7	18.5	-0.28	0.42	-0.55	no	-1.21
B	hsa-miR-19b-1*-4395536	No	No	25.7	25.4	25.5	25.6	25.6	-0.07	0.94	-1.15	no	-1.05
B	hsa-miR-19b-2*-4395537	Yes	Yes	Inf	Inf	Inf	Inf	NA	NA	NA	-NA	NA	NA
A	hsa-miR-19b-4373098	No	No	15.8	14.5	14.5	14.7	14.6	-0.18	0.26	-0.36	no	-1.13
B	**hsa-miR-200a*-4373273**	**No**	**Yes**	**32.2**	**Inf**	**32.0**	**Inf**	**NA**	**-NA**	**NA**	**-NA**	**yes**	**NA**
A	hsa-miR-200a-4378069	No	No	26.2	27.6	26.6	27.9	27.3	-1.25	1.11	-1.36	no	-2.37
B	hsa-miR-200b*-4395385	Yes	Yes	Inf	Inf	Inf	Inf	NA	NA	NA	-NA	NA	NA
A	hsa-miR-200b-4395362	No	No	19.4	19.1	19.2	19.4	19.3	-0.11	0.46	-0.59	no	-1.08
B	hsa-miR-200c*-4395397	No	No	27.3	27.3	27.3	27.5	27.4	-0.17	1.13	-1.38	no	-1.12
A	hsa-miR-200c-4395411	No	No	15.8	14.2	14.6	14.5	14.5	0.11	0.26	-0.35	no	1.08
B	hsa-miR-202*-4395473	No	No	32.6	31.6	32.6	31.7	32.1	0.89	1.75	-2.12	no	1.85
A	hsa-miR-202-4395474	No	No	28.4	30.0	29.7	30.2	29.9	-0.52	1.44	-1.74	no	-1.44
A	hsa-miR-203-4373095	No	No	18.9	18.6	18.8	18.9	18.8	-0.11	0.44	-0.56	no	-1.08
A	hsa-miR-204-4373094	No	No	20.2	19.8	20.2	20.1	20.1	0.13	0.51	-0.65	no	1.09
A	hsa-miR-205-4373093	No	No	19.5	19.1	19.5	19.3	19.4	0.13	0.47	-0.60	no	1.09
B	hsa-miR-206-4373092	No	No	31.0	30.2	30.8	30.4	30.6	0.45	1.53	-1.85	no	1.36
A	**hsa-miR-208-4373091**	**Yes**	**No**	**37.2**	**32.3**	**Inf**	**32.3**	**NA**	**NA**	**NA**	**-NA**	**yes**	**NA**

Plate	Detector	Flag CF	Flag IF	Raw Cq		Norm. Cq		median Cq	log RQ	threshold		Ex-treme	FC
				CF	IF	CF	IF			high	low		
A	hsa-miR-208b-4395401	Yes	Yes	35.4	Inf	Inf	Inf	NA	NA	NA	-NA	NA	NA
B	hsa-miR-20a*-4395548	No	No	23.3	22.9	23.1	23.1	23.1	-0.04	0.72	-0.89	no	-1.03
A	hsa-miR-20a-4373286	No	No	15.8	15.1	15.1	15.4	15.3	-0.25	0.28	-0.38	no	-1.19
B	hsa-miR-20b*-4395422	No	No	31.2	32.7	31.1	32.7	31.9	-1.67	1.72	-2.07	no	-3.18
A	hsa-miR-20b-4373263	No	No	19.0	18.8	18.9	19.1	19.0	-0.17	0.45	-0.57	no	-1.13
B	hsa-miR-21*-4395549	No	No	26.9	27.3	26.7	27.4	27.1	-0.70	1.09	-1.34	no	-1.62
A	hsa-miR-210-4373089	No	No	21.7	21.7	21.7	22.0	21.9	-0.24	0.63	-0.78	no	-1.18
A	hsa-miR-211-4373088	Yes	Yes	28.6	Inf	Inf	Inf	NA	NA	NA	-NA	NA	NA
A	hsa-miR-212-4373087	No	No	25.7	25.9	25.9	26.1	26.0	-0.28	0.98	-1.20	no	-1.22
B	hsa-miR-214*-4395404	No	No	22.5	22.1	22.3	22.3	22.3	-0.02	0.66	-0.82	no	-1.01
A	**hsa-miR-21-4373090**	**No**	**No**	**15.9**	**15.1**	**14.9**	**15.4**	**15.2**	**-0.44**	**0.28**	**-0.38**	**yes**	**-1.36**
A	hsa-miR-214-4395417	No	No	18.9	18.5	18.8	18.8	18.8	-0.01	0.44	-0.56	no	-1.01
A	hsa-miR-215-4373084	No	Yes	25.9	Inf	Inf	Inf	NA	NA	NA	-NA	NA	NA
A	**hsa-miR-216a-4395331**	**No**	**No**	**28.5**	**32.5**	**29.8**	**32.7**	**31.2**	**-2.91**	**1.62**	**-1.96**	**yes**	**-7.52**
A	**hsa-miR-216b-4395437**	**Yes**	**No**	**35.0**	**34.1**	**Inf**	**34.2**	**NA**	**NA**	**NA**	**-NA**	**yes**	**NA**
A	hsa-miR-217-4395448	No	No	28.8	30.1	29.3	30.4	29.8	-1.10	1.42	-1.72	no	-2.15
B	hsa-miR-218-2*-4395405	No	No	31.7	31.1	31.5	31.2	31.4	0.33	1.64	-1.98	no	1.26
A	hsa-miR-218-4373081	No	No	18.0	17.6	17.9	17.9	17.9	-0.02	0.39	-0.51	no	-1.02
A	**hsa-miR-219-1-3p-4395206**	**Yes**	**No**	**33.6**	**32.9**	**Inf**	**32.9**	**NA**	**NA**	**NA**	**-NA**	**yes**	**NA**
A	hsa-miR-219-2-3p-4395501	Yes	Yes	Inf	Inf	Inf	Inf	NA	NA	NA	-NA	NA	NA
A	**hsa-miR-219-5p-4373080**	**Yes**	**Yes**	**30.5**	**32.0**	**Inf**	**32.0**	**NA**	**NA**	**NA**	**-NA**	**yes**	**NA**
B	hsa-miR-22*-4395412	No	No	22.1	21.8	21.9	22.0	21.9	-0.08	0.63	-0.79	no	-1.06
A	hsa-miR-220-4373078	Yes	Yes	Inf	Inf	Inf	Inf	NA	NA	NA	-NA	NA	NA
A	hsa-miR-220b-4395317	Yes	Yes	Inf	Inf	Inf	Inf	NA	NA	NA	-NA	NA	NA
A	hsa-miR-220c-4395322	Yes	Yes	Inf	Inf	Inf	Inf	NA	NA	NA	-NA	NA	NA
B	hsa-miR-221*-4395207	No	No	27.3	27.2	27.2	27.4	27.3	-0.20	1.11	-1.36	no	-1.15
A	hsa-miR-221-4373077	No	No	17.7	17.2	17.5	17.5	17.5	0.00	0.37	-0.49	no	1.00
B	hsa-miR-222*-4395208	No	No	23.3	22.7	23.2	22.9	23.0	0.24	0.71	-0.89	no	1.18
A	hsa-miR-222-4395387	No	No	15.1	13.8	14.3	14.1	14.2	0.24	0.25	-0.34	no	1.18
B	hsa-miR-223*-4395209	No	No	24.8	24.4	24.6	24.6	24.6	-0.02	0.85	-1.05	no	-1.01
A	**hsa-miR-223-4395406**	**No**	**No**	**15.5**	**14.8**	**14.5**	**15.1**	**14.8**	**-0.55**	**0.27**	**-0.36**	**yes**	**-1.46**
A	hsa-miR-22-4373079	No	No	18.4	18.4	18.3	18.7	18.5	-0.45	0.42	-0.54	no	-1.37
A	hsa-miR-224-4395210	No	No	16.9	16.1	16.6	16.4	16.5	0.14	0.33	-0.44	no	1.10
B	hsa-miR-23a*-4395550	No	No	30.0	30.6	30.0	30.7	30.3	-0.72	1.50	-1.81	no	-1.64
A	hsa-miR-23a-4373074	No	No	21.1	21.1	21.1	21.4	21.2	-0.27	0.58	-0.73	no	-1.20
B	hsa-miR-23b*-4395237	Yes	Yes	37.7	Inf	Inf	Inf	NA	NA	NA	-NA	NA	NA
A	hsa-miR-23b-4373073	No	No	21.1	20.7	20.9	21.0	20.9	0.00	0.56	-0.71	no	1.00
B	hsa-miR-24-1*-4395551	Yes	Yes	35.9	Inf	Inf	Inf	NA	NA	NA	-NA	NA	NA
A	hsa-miR-24-4373072	No	No	14.5	11.9	12.0	12.0	12.0	-0.07	0.19	-0.27	no	-1.05
B	hsa-miR-25*-4395553	Yes	Yes	Inf	Inf	Inf	Inf	NA	NA	NA	-NA	NA	NA
A	hsa-miR-25-4373071	No	No	19.9	19.4	19.9	19.7	19.8	0.16	0.49	-0.63	no	1.11
B	hsa-miR-26a-1*-4395554	No	No	25.8	25.5	25.6	25.7	25.7	-0.09	0.95	-1.17	no	-1.06
B	hsa-miR-26a-2*-4395226	No	No	26.5	26.2	26.4	26.4	26.4	-0.05	1.02	-1.25	no	-1.04
A	hsa-miR-26a-4395166	No	No	15.8	14.8	15.0	15.1	15.0	-0.06	0.28	-0.37	no	-1.04
B	hsa-miR-26b*-4395555	No	No	24.2	23.7	24.0	23.9	23.9	0.11	0.79	-0.98	no	1.08
A	hsa-miR-26b-4395167	No	No	16.4	15.7	16.0	16.0	16.0	-0.04	0.31	-0.41	no	-1.03
B	hsa-miR-27a*-4395556	No	No	22.1	21.3	21.9	21.5	21.7	0.46	0.61	-0.77	no	1.37
A	hsa-miR-27a-4373287	No	No	15.8	14.9	15.2	15.2	15.2	0.03	0.28	-0.38	no	1.02
B	hsa-miR-27b*-4395285	No	No	25.7	25.4	25.5	25.6	25.6	-0.09	0.94	-1.15	no	-1.06
A	hsa-miR-27b-4373068	No	No	17.8	17.4	17.6	17.7	17.7	-0.07	0.38	-0.50	no	-1.05
A	hsa-miR-28-3p-4395557	No	No	20.6	20.3	20.5	20.5	20.5	-0.01	0.54	-0.68	no	-1.01
A	hsa-miR-28-5p-4373067	No	No	19.9	19.9	19.9	20.2	20.0	-0.30	0.51	-0.64	no	-1.24
A	hsa-miR-296-3p-4395212	No	No	29.6	30.2	30.2	30.4	30.3	-0.23	1.49	-1.81	no	-1.17
A	hsa-miR-296-5p-4373066	No	No	24.3	23.9	24.5	24.1	24.3	0.36	0.82	-1.01	no	1.29
A	hsa-miR-298-4395301	Yes	Yes	Inf	Inf	Inf	Inf	NA	NA	NA	-NA	NA	NA
A	hsa-miR-299-3p-4373189	No	No	32.0	33.6	33.4	33.5	33.5	-0.10	1.97	-2.37	no	-1.07

Plate	Detector	Flag CF	Flag IF	Raw Cq		Norm. Cq		median Cq	log RQ	threshold		Ex-treme	FC
				CF	IF	CF	IF			high	low		
A	hsa-miR-299-5p-4373188	No	No	26.5	26.1	27.0	26.4	26.7	0.59	1.05	-1.28	no	1.50
B	hsa-miR-29a*-4395558	No	No	23.6	23.1	23.3	23.4	23.3	-0.02	0.74	-0.92	no	-1.01
A	hsa-miR-29a-4395223	No	No	15.4	14.6	14.6	14.9	14.8	-0.22	0.27	-0.36	no	-1.17
B	hsa-miR-29b-1*-4395276	No	No	29.9	29.7	29.9	29.9	29.9	0.07	1.43	-1.74	no	1.05
B	hsa-miR-29b-2*-4395277	No	No	27.1	26.7	26.9	26.8	26.9	0.06	1.07	-1.31	no	1.04
A	hsa-miR-29b-4373288	No	No	20.1	19.7	20.1	19.9	20.0	0.20	0.51	-0.64	no	1.15
B	hsa-miR-29c*-4381131	No	No	24.3	23.9	24.1	24.1	24.1	-0.01	0.80	-0.99	no	-1.01
A	hsa-miR-29c-4395171	No	No	18.6	18.3	18.3	18.6	18.5	-0.29	0.42	-0.54	no	-1.22
A	hsa-miR-301a-4373064	No	No	20.3	20.0	20.3	20.3	20.3	0.03	0.52	-0.66	no	1.02
A	hsa-miR-301b-4395503	No	No	24.0	24.8	24.1	25.0	24.5	-0.95	0.84	-1.04	no	-1.93
B	hsa-miR-302a*-4395492	Yes	Yes	Inf	Inf	Inf	Inf	NA	NA	NA	-NA	NA	NA
A	**hsa-miR-302a-4378070**	**No**	**Yes**	**33.5**	**Inf**	**36.4**	**Inf**	**NA**	**-NA**	**NA**	**-NA**	**yes**	**NA**
B	hsa-miR-302b*-4395230	Yes	Yes	Inf	Inf	Inf	Inf	NA	NA	NA	-NA	NA	NA
A	**hsa-miR-302b-4378071**	**No**	**No**	**31.5**	**35.7**	**39.6**	**35.2**	**37.4**	**4.41**	**2.71**	**-3.24**	**yes**	**21.22**
B	**hsa-miR-302c*-4373277**	**No**	**Yes**	**34.4**	**Inf**	**34.8**	**Inf**	**NA**	**-NA**	**NA**	**-NA**	**yes**	**NA**
A	hsa-miR-302c-4378072	Yes	Yes	37.8	Inf	Inf	Inf	NA	NA	NA	-NA	NA	NA
B	hsa-miR-302d*-4395231	Yes	Yes	Inf	Inf	Inf	Inf	NA	NA	NA	-NA	NA	NA
B	hsa-miR-302d-4373063	Yes	Yes	37.6	Inf	Inf	Inf	NA	NA	NA	-NA	NA	NA
B	hsa-miR-302d-4373063	Yes	Yes	Inf	Inf	Inf	Inf	NA	NA	NA	-NA	NA	NA
B	hsa-miR-30a*-4373062	No	No	16.2	15.2	15.6	15.4	15.5	0.20	0.29	-0.39	no	1.15
B	hsa-miR-30a-4373061	No	No	15.1	13.6	13.9	13.8	13.8	0.13	0.24	-0.33	no	1.10
B	hsa-miR-30b*-4395240	Yes	Yes	34.3	Inf	Inf	Inf	NA	NA	NA	-NA	NA	NA
A	hsa-miR-30b-4373290	No	No	14.8	12.9	13.3	13.1	13.2	0.14	0.22	-0.31	no	1.10
B	**hsa-miR-30c-1*-4395219**	**Yes**	**Yes**	**33.0**	**32.2**	**Inf**	**32.2**	**NA**	**NA**	**NA**	**-NA**	**yes**	**NA**
B	**hsa-miR-30c-2*-4395221**	**Yes**	**Yes**	**30.8**	**29.8**	**Inf**	**29.8**	**NA**	**NA**	**NA**	**-NA**	**yes**	**NA**
A	hsa-miR-30c-4373060	No	No	15.1	13.7	13.9	14.0	13.9	-0.07	0.24	-0.33	no	-1.05
B	hsa-miR-30d*-4395416	No	No	24.4	23.6	24.3	23.8	24.1	0.53	0.80	-0.99	no	1.45
B	**hsa-miR-30d-4373059**	**No**	**No**	**17.4**	**16.2**	**16.8**	**16.4**	**16.6**	**0.37**	**0.33**	**-0.44**	**yes**	**-1.29**
B	**hsa-miR-30d-4373059**	**No**	**No**	**17.5**	**16.3**	**16.9**	**16.5**	**16.7**	**0.34**	**0.34**	**-0.45**	**yes**	**-1.26**
B	**hsa-miR-30e*-4373057**	**No**	**No**	**17.0**	**15.7**	**16.5**	**16.0**	**16.2**	**0.54**	**0.32**	**-0.42**	**yes**	**-1.45**
B	hsa-miR-30e-4395334	No	No	17.0	16.3	16.5	16.5	16.5	-0.01	0.33	-0.44	no	-1.01
A	hsa-miR-31-4395390	No	No	19.0	18.9	19.0	19.1	19.1	-0.12	0.45	-0.58	no	-1.09
B	hsa-miR-32*-4395222	Yes	Yes	Inf	Inf	Inf	Inf	NA	NA	NA	-NA	NA	NA
A	**hsa-miR-320-4395388**	**No**	**No**	**17.0**	**16.9**	**16.6**	**17.1**	**16.9**	**-0.49**	**0.35**	**-0.46**	**yes**	**-1.40**
A	hsa-miR-323-3p-4395338	No	No	22.9	22.5	23.0	22.8	22.9	0.24	0.70	-0.88	no	1.18
A	hsa-miR-32-4395220	No	No	23.5	24.2	24.0	24.4	24.2	-0.42	0.81	-1.00	no	-1.34
A	hsa-miR-324-3p-4395272	No	No	22.0	21.8	22.1	22.1	22.1	0.00	0.64	-0.81	no	1.00
A	hsa-miR-324-5p-4373052	No	No	20.3	20.0	20.3	20.3	20.3	0.03	0.52	-0.66	no	1.02
A	hsa-miR-325-4373051	Yes	Yes	Inf	Inf	Inf	Inf	NA	NA	NA	-NA	NA	NA
A	hsa-miR-326-4373050	No	No	30.9	32.4	32.5	32.5	32.5	0.04	1.81	-2.19	no	1.03
A	hsa-miR-328-4373049	No	No	21.0	20.5	21.1	20.8	20.9	0.33	0.56	-0.71	no	1.26
A	hsa-miR-329-4373191	No	No	27.0	26.6	27.4	26.9	27.2	0.58	1.10	-1.35	no	1.49
A	hsa-miR-330-3p-4373047	No	No	24.9	25.7	25.7	26.0	25.8	-0.23	0.96	-1.18	no	-1.17
A	hsa-miR-330-5p-4395341	No	No	26.1	27.4	27.3	27.6	27.5	-0.30	1.14	-1.39	no	-1.23
A	hsa-miR-331-3p-4373046	No	No	17.4	17.0	17.0	17.3	17.1	-0.26	0.36	-0.47	no	-1.20
A	hsa-miR-331-5p-4395344	No	No	26.6	26.5	27.0	26.7	26.9	0.26	1.07	-1.31	no	1.20
B	hsa-miR-335*-4395296	No	No	18.8	18.2	18.4	18.4	18.4	-0.03	0.42	-0.54	no	-1.02
A	hsa-miR-335-4373045	No	No	17.7	17.0	17.5	17.3	17.4	0.27	0.37	-0.48	no	1.21
B	hsa-miR-337-3p-4395268	No	No	25.6	25.0	25.5	25.3	25.4	0.23	0.92	-1.13	no	1.17
A	hsa-miR-337-5p-4395267	No	No	21.8	21.7	21.9	21.9	21.9	-0.04	0.63	-0.79	no	-1.03
A	hsa-miR-338-3p-4395363	No	No	27.8	27.9	28.4	28.2	28.3	0.16	1.23	-1.50	no	1.11
A	hsa-miR-339-3p-4395295	No	No	23.1	23.1	23.2	23.4	23.3	-0.19	0.73	-0.91	no	-1.14
A	hsa-miR-339-5p-4395368	No	No	22.6	22.3	22.6	22.6	22.6	0.02	0.68	-0.85	no	1.01
B	hsa-miR-33a*-4395247	No	No	26.9	26.3	26.7	26.5	26.6	0.29	1.04	-1.28	no	1.22
A	hsa-miR-33b-4395196	No	Yes	30.0	34.0	33.5	33.8	33.6	-0.35	2.00	-2.40	no	-1.27
B	hsa-miR-340*-4395370	No	No	23.2	23.0	23.1	23.2	23.1	-0.10	0.72	-0.90	no	-1.07
A	hsa-miR-340-4395369	No	No	21.1	21.2	21.1	21.4	21.3	-0.28	0.59	-0.74	no	-1.22

Plate	Detector	Flag CF	Flag IF	Raw Cq		Norm. Cq		median Cq	log RQ	threshold		Ex-treme	FC
				CF	IF	CF	IF			high	low		
A	hsa-miR-342-3p-4395371	No	No	18.4	18.1	18.2	18.4	18.3	-0.16	0.41	-0.53	no	-1.12
A	hsa-miR-342-5p-4395258	No	No	27.2	27.1	27.9	27.4	27.7	0.52	1.16	-1.41	no	1.43
A	hsa-miR-345-4395297	No	No	22.3	22.6	22.4	22.8	22.6	-0.47	0.68	-0.85	no	-1.38
A	hsa-miR-346-4373038	Yes	Yes	33.4	Inf	Inf	Inf	NA	NA	NA	-NA	NA	NA
B	hsa-miR-34a*-4395427	No	No	22.6	21.8	22.4	22.0	22.2	0.36	0.65	-0.81	no	1.29
A	hsa-miR-34a-4395168	No	No	19.2	18.8	19.2	19.1	19.1	0.06	0.46	-0.58	no	1.04
B	hsa-miR-34b*-4373037	No	No	27.6	26.9	27.6	27.1	27.3	0.48	1.12	-1.37	no	1.39
A	hsa-miR-34c-5p-4373036	No	No	23.5	23.3	23.5	23.6	23.5	-0.03	0.76	-0.94	no	-1.02
B	**hsa-miR-361-3p-4395227**	**Yes**	**Yes**	**28.9**	**29.0**	**Inf**	**29.0**	**NA**	**NA**	**NA**	**-NA**	**yes**	**NA**
B	hsa-miR-361-3p-4395227	Yes	Yes	30.3	Inf	Inf	Inf	NA	NA	NA	-NA	NA	NA
A	hsa-miR-361-5p-4373035	No	No	21.2	21.1	21.3	21.4	21.3	-0.13	0.59	-0.74	no	-1.09
A	hsa-miR-362-3p-4395228	No	No	23.1	22.8	23.3	23.1	23.2	0.19	0.72	-0.90	no	1.14
A	hsa-miR-362-5p-4378092	No	No	22.0	21.8	22.1	22.1	22.1	-0.03	0.64	-0.80	no	-1.02
B	hsa-miR-363*-4380917	Yes	Yes	36.7	Inf	Inf	Inf	NA	NA	NA	-NA	NA	NA
A	hsa-miR-363-4378090	No	No	23.6	23.4	23.8	23.6	23.7	0.15	0.77	-0.96	no	1.11
A	hsa-miR-365-4373194	No	No	18.1	17.6	18.0	17.8	17.9	0.15	0.39	-0.51	no	1.11
B	hsa-miR-367*-4395232	Yes	Yes	Inf	Inf	Inf	Inf	NA	NA	NA	-NA	NA	NA
A	**hsa-miR-367-4373034**	**No**	**Yes**	**32.7**	**Inf**	**34.0**	**Inf**	**NA**	**-NA**	**NA**	**-NA**	**yes**	**NA**
A	hsa-miR-369-3p-4373032	No	No	23.8	23.4	23.9	23.7	23.8	0.23	0.78	-0.96	no	1.17
A	hsa-miR-369-5p-4373195	No	No	26.2	26.1	26.6	26.4	26.5	0.28	1.03	-1.26	no	1.21
A	hsa-miR-370-4395386	No	No	21.5	20.9	21.5	21.2	21.3	0.27	0.59	-0.74	no	1.20
A	hsa-miR-371-3p-4395235	No	No	22.3	22.3	22.3	22.6	22.5	-0.21	0.67	-0.84	no	-1.16
A	hsa-miR-372-4373029	No	No	19.1	18.9	19.0	19.2	19.1	-0.14	0.45	-0.58	no	-1.10
B	hsa-miR-373*-4373279	Yes	Yes	Inf	Inf	Inf	Inf	NA	NA	NA	-NA	NA	NA
A	hsa-miR-373-4378073	No	No	23.0	23.4	23.1	23.6	23.4	-0.49	0.74	-0.92	no	-1.40
B	hsa-miR-374a*-4395236	No	No	26.3	26.5	26.2	26.7	26.5	-0.51	1.03	-1.26	no	-1.43
A	hsa-miR-374a-4373028	No	No	17.3	16.8	17.0	17.1	17.0	-0.02	0.35	-0.46	no	-1.02
B	hsa-miR-374b*-4395502	No	No	28.5	28.7	28.5	28.9	28.7	-0.42	1.28	-1.56	no	-1.34
A	hsa-miR-374b-4381045	No	No	18.1	17.6	18.0	17.9	17.9	0.15	0.39	-0.51	no	1.11
A	**hsa-miR-375-4373027**	**No**	**No**	**23.4**	**26.2**	**23.7**	**26.5**	**25.1**	**-2.76**	**0.89**	**-1.10**	**yes**	**-6.75**
B	hsa-miR-376a*-4395238	No	No	24.6	24.4	24.5	24.6	24.5	-0.12	0.84	-1.04	no	-1.09
A	hsa-miR-376a-4373026	No	No	18.3	18.0	18.2	18.3	18.3	-0.08	0.41	-0.53	no	-1.06
A	hsa-miR-376b-4373196	Yes	Yes	29.7	Inf	Inf	Inf	NA	NA	NA	-NA	NA	NA
A	hsa-miR-376c-4395233	No	No	17.0	16.3	16.7	16.6	16.6	0.07	0.34	-0.44	no	1.05
B	hsa-miR-377*-4395239	No	No	27.0	26.0	26.8	26.2	26.5	0.66	1.03	-1.26	no	1.58
A	hsa-miR-377-4373025	No	No	27.4	27.6	28.0	27.8	27.9	0.15	1.19	-1.45	no	1.11
B	hsa-miR-378*-4373024	No	No	27.9	27.5	27.8	27.6	27.7	0.13	1.16	-1.42	no	1.09
B	hsa-miR-378-4395354	No	No	23.0	22.7	22.8	22.9	22.8	-0.12	0.70	-0.87	no	-1.08
B	hsa-miR-379*-4395244	No	No	27.1	26.7	26.9	26.9	26.9	0.05	1.08	-1.31	no	1.04
A	hsa-miR-379-4373349	No	No	21.7	21.5	21.8	21.7	21.8	0.10	0.62	-0.78	no	1.08
B	hsa-miR-380*-4373021	No	No	26.8	26.0	26.6	26.2	26.4	0.41	1.02	-1.25	no	1.32
A	hsa-miR-380-4373022	No	No	29.1	29.3	29.6	29.5	29.6	0.02	1.39	-1.69	no	1.02
A	hsa-miR-381-4373020	No	No	27.1	26.5	27.6	26.8	27.2	0.85	1.11	-1.35	no	1.80
A	hsa-miR-382-4373019	No	No	19.6	19.1	19.6	19.4	19.5	0.23	0.47	-0.61	no	1.17
A	hsa-miR-383-4373018	Yes	Yes	37.7	Inf	Inf	Inf	NA	NA	NA	-NA	NA	NA
A	hsa-miR-384-4373017	Yes	Yes	Inf	Inf	Inf	Inf	NA	NA	NA	-NA	NA	NA
B	hsa-miR-409-3p-4395443	No	No	19.6	18.8	19.2	19.0	19.1	0.23	0.45	-0.58	no	1.17
B	hsa-miR-409-3p-4395443	No	No	19.7	18.8	19.3	19.0	19.2	0.31	0.46	-0.59	no	1.24
A	hsa-miR-409-5p-4395442	No	No	25.1	24.8	25.2	25.1	25.2	0.16	0.90	-1.11	no	1.12
A	hsa-miR-410-4378093	No	No	21.2	20.9	21.3	21.1	21.2	0.17	0.58	-0.73	no	1.12
B	hsa-miR-411*-4395349	No	No	25.2	24.7	25.0	24.9	24.9	0.06	0.88	-1.08	no	1.04
A	hsa-miR-411-4381013	No	No	19.2	18.7	19.1	19.0	19.0	0.09	0.45	-0.58	no	1.06
A	hsa-miR-412-4373199	Yes	Yes	33.7	Inf	Inf	Inf	NA	NA	NA	-NA	NA	NA
A	hsa-miR-422a-4395408	No	No	25.1	25.5	25.4	25.7	25.5	-0.33	0.93	-1.15	no	-1.26
A	hsa-miR-423-5p-4395451	No	No	22.4	22.5	22.6	22.7	22.7	-0.17	0.68	-0.85	no	-1.13
B	hsa-miR-424*-4395420	No	No	20.2	19.7	19.9	19.9	19.9	0.00	0.50	-0.64	no	1.00
A	hsa-miR-424-4373201	No	No	18.8	18.1	18.6	18.4	18.5	0.28	0.42	-0.54	no	1.21

Plate	Detector	Flag CF	Flag IF	Raw Cq		Norm. Cq		median Cq	log RQ	threshold		Ex-treme	FC
				CF	IF	CF	IF			high	low		
B	hsa-miR-425*-4395413	No	No	25.6	25.3	25.4	25.5	25.4	-0.08	0.93	-1.14	no	-1.05
A	hsa-miR-425-4380926	No	No	20.4	20.0	20.4	20.3	20.4	0.14	0.53	-0.67	no	1.10
A	hsa-miR-429-4373203	No	No	28.9	31.7	29.8	31.7	30.8	-1.85	1.55	-1.88	no	-3.60
B	hsa-miR-431*-4395423	Yes	Yes	35.2	Inf	Inf	Inf	NA	NA	NA	-NA	NA	NA
A	hsa-miR-431-4395173	No	No	19.6	19.1	19.6	19.3	19.5	0.23	0.47	-0.60	no	1.18
B	hsa-miR-432*-4378076	Yes	Yes	33.8	Inf	Inf	Inf	NA	NA	NA	-NA	NA	NA
B	hsa-miR-432-4373280	No	No	21.6	21.0	21.3	21.3	21.3	0.05	0.59	-0.74	no	1.04
B	hsa-miR-432-4373280	No	No	21.6	21.1	21.4	21.2	21.3	0.19	0.58	-0.74	no	1.14
A	hsa-miR-433-4373205	No	No	21.3	20.9	21.4	21.2	21.3	0.27	0.59	-0.74	no	1.21
A	hsa-miR-448-4373206	Yes	Yes	Inf	Inf	Inf	Inf	NA	NA	NA	-NA	NA	NA
A	hsa-miR-449a-4373207	No	No	27.7	27.4	28.0	27.7	27.8	0.32	1.18	-1.43	no	1.25
A	hsa-miR-449b-4381011	No	No	27.5	28.2	28.3	28.4	28.3	-0.16	1.24	-1.51	no	-1.12
A	hsa-miR-450a-4395414	No	No	22.2	22.2	22.2	22.5	22.3	-0.25	0.66	-0.83	no	-1.19
A	hsa-miR-450b-3p-4395319	Yes	Yes	31.6	Inf	Inf	Inf	NA	NA	NA	-NA	NA	NA
A	hsa-miR-450b-5p-4395318	No	No	22.6	22.6	22.7	22.9	22.8	-0.21	0.69	-0.87	no	-1.15
A	**hsa-miR-451-4373360**	**No**	**No**	**15.6**	**15.1**	**15.0**	**15.3**	**15.2**	**-0.38**	**0.28**	**-0.38**	**yes**	**-1.30**
B	hsa-miR-452*-4395441	Yes	Yes	Inf	Inf	Inf	Inf	NA	NA	NA	-NA	NA	NA
A	hsa-miR-452-4395440	No	No	20.3	19.7	20.3	20.0	20.1	0.30	0.51	-0.65	no	1.23
A	hsa-miR-453-4395429	No	No	32.1	32.9	33.7	32.9	33.3	0.81	1.94	-2.34	no	1.75
B	hsa-miR-454*-4395185	No	No	28.5	27.9	28.3	28.1	28.2	0.16	1.22	-1.48	no	1.11
A	hsa-miR-454-4395434	No	No	18.1	18.0	18.0	18.3	18.1	-0.25	0.40	-0.52	no	-1.19
A	hsa-miR-455-3p-4395355	No	No	21.0	20.9	21.1	21.1	21.1	-0.06	0.57	-0.72	no	-1.04
A	hsa-miR-455-5p-4378098	No	No	20.1	19.7	20.1	19.9	20.0	0.13	0.50	-0.64	no	1.09
A	hsa-miR-483-5p-4395449	No	No	18.9	18.7	18.8	19.0	18.9	-0.21	0.44	-0.57	no	-1.16
A	hsa-miR-484-4381032	No	No	16.2	15.3	15.7	15.6	15.7	0.18	0.30	-0.40	no	1.13
A	hsa-miR-485-3p-4378095	No	No	22.6	22.2	22.7	22.5	22.6	0.20	0.68	-0.85	no	1.15
A	**hsa-miR-485-5p-4373212**	**Yes**	**Yes**	**28.0**	**27.6**	**Inf**	**27.6**	**NA**	**NA**	**NA**	**-NA**	**yes**	**NA**
A	hsa-miR-486-3p-4395204	No	No	26.9	26.7	27.3	27.0	27.1	0.31	1.10	-1.34	no	1.24
A	hsa-miR-486-5p-4378096	No	No	23.8	23.0	23.9	23.2	23.6	0.62	0.76	-0.94	no	1.54
A	hsa-miR-487a-4378097	No	No	25.1	24.7	25.2	24.9	25.1	0.31	0.89	-1.09	no	1.24
A	hsa-miR-487b-4378102	No	No	20.5	19.9	20.5	20.2	20.4	0.30	0.53	-0.67	no	1.23
B	hsa-miR-488*-4373213	Yes	Yes	36.8	38.8	36.9	38.1	37.5	-1.11	2.73	-3.26	no	-2.15
A	hsa-miR-488-4395468	No	No	26.6	28.4	27.5	28.6	28.1	-1.17	1.20	-1.47	no	-2.25
A	hsa-miR-489-4395469	No	No	21.2	21.0	21.2	21.3	21.2	-0.04	0.58	-0.73	no	-1.03
A	hsa-miR-490-3p-4373215	No	No	28.9	28.7	29.4	29.0	29.2	0.45	1.34	-1.63	no	1.37
A	hsa-miR-491-3p-4395471	Yes	Yes	32.5	Inf	Inf	Inf	NA	NA	NA	-NA	NA	NA
A	hsa-miR-491-5p-4381053	No	No	23.1	22.9	23.2	23.1	23.2	0.08	0.72	-0.90	no	1.06
A	hsa-miR-492-4373217	No	No	27.4	28.3	28.2	28.6	28.4	-0.38	1.24	-1.51	no	-1.30
B	hsa-miR-493*-4373218	No	No	24.1	23.7	23.9	23.9	23.9	0.00	0.78	-0.97	no	1.00
A	hsa-miR-493-4395475	No	No	23.4	23.0	23.5	23.2	23.4	0.29	0.74	-0.92	no	1.22
A	hsa-miR-494-4395476	No	No	21.2	21.0	21.2	21.3	21.3	-0.02	0.58	-0.74	no	-1.01
A	**hsa-miR-495-4381078**	**No**	**No**	**18.1**	**17.3**	**18.0**	**17.6**	**17.8**	**0.43**	**0.39**	**-0.50**	**yes**	**1.35**
A	hsa-miR-496-4386771	No	No	27.7	27.8	28.1	28.1	28.1	0.05	1.21	-1.47	no	1.04
B	**hsa-miR-497*-4395479**	**Yes**	**Yes**	**35.3**	**33.0**	**Inf**	**33.0**	**NA**	**NA**	**NA**	**-NA**	**yes**	**NA**
B	hsa-miR-497-4373222	No	No	24.4	24.3	24.2	24.5	24.4	-0.26	0.82	-1.02	no	-1.20
B	hsa-miR-497-4373222	No	No	24.5	24.4	24.3	24.6	24.5	-0.30	0.84	-1.03	no	-1.23
B	hsa-miR-498-4373223	Yes	Yes	Inf	Inf	Inf	Inf	NA	NA	NA	-NA	NA	NA
A	hsa-miR-499-3p-4395538	Yes	Yes	Inf	Inf	Inf	Inf	NA	NA	NA	-NA	NA	NA
A	hsa-miR-499-5p-4381047	No	No	29.3	29.9	30.4	30.1	30.3	0.29	1.49	-1.80	no	1.22
B	hsa-miR-500*-4373225	Yes	Yes	30.5	Inf	Inf	Inf	NA	NA	NA	-NA	NA	NA
A	hsa-miR-500-4395539	No	No	22.4	22.1	22.4	22.4	22.4	0.07	0.67	-0.83	no	1.05
A	hsa-miR-501-3p-4395546	Yes	Yes	30.9	Inf	Inf	Inf	NA	NA	NA	-NA	NA	NA
A	hsa-miR-501-5p-4373226	No	No	24.2	24.4	24.5	24.6	24.6	-0.11	0.84	-1.04	no	-1.08
A	hsa-miR-502-3p-4395194	No	No	23.8	23.6	24.0	23.8	23.9	0.10	0.79	-0.97	no	1.08
A	hsa-miR-502-5p-4373227	No	No	24.8	24.8	25.2	25.0	25.1	0.13	0.89	-1.10	no	1.10
A	hsa-miR-503-4373228	No	No	20.6	20.6	20.7	20.9	20.8	-0.16	0.55	-0.70	no	-1.12
A	hsa-miR-504-4395195	No	No	24.5	24.3	24.7	24.6	24.7	0.16	0.85	-1.05	no	1.12

Plate	Detector	Flag CF	Flag IF	Raw Cq		Norm. Cq		median Cq	log RQ	threshold		Ex-treme	FC
				CF	IF	CF	IF			high	low		
B	hsa-miR-505*-4395198	No	No	24.5	24.4	24.3	24.6	24.5	-0.27	0.83	-1.03	no	-1.20
A	hsa-miR-505-4395200	No	No	25.5	25.7	25.8	25.9	25.9	-0.06	0.97	-1.19	no	-1.04
A	hsa-miR-506-4373231	No	No	29.5	30.3	30.7	30.4	30.6	0.29	1.53	-1.85	no	1.22
A	hsa-miR-507-4373232	Yes	Yes	34.0	Inf	Inf	Inf	NA	NA	NA	-NA	NA	NA
A	hsa-miR-508-3p-4373233	No	No	25.2	25.4	25.5	25.7	25.6	-0.22	0.94	-1.15	no	-1.17
A	hsa-miR-508-5p-4395203	Yes	Yes	Inf	Inf	Inf	Inf	NA	NA	NA	-NA	NA	NA
A	hsa-miR-509-3-5p-4395266	Yes	Yes	33.5	Inf	Inf	Inf	NA	NA	NA	-NA	NA	NA
B	hsa-miR-509-3p-4395347	No	No	24.6	24.9	24.4	25.1	24.7	-0.73	0.86	-1.06	no	-1.66
A	hsa-miR-509-5p-4395346	No	No	27.3	26.4	27.6	26.6	27.1	1.01	1.10	-1.34	no	2.01
A	hsa-miR-510-4395352	Yes	Yes	38.3	Inf	Inf	Inf	NA	NA	NA	-NA	NA	NA
A	hsa-miR-511-4373236	No	No	23.8	23.7	23.9	24.0	23.9	-0.12	0.79	-0.97	no	-1.09
A	hsa-miR-512-3p-4381034	No	No	14.9	12.9	13.1	13.1	13.1	0.01	0.22	-0.30	no	1.01
A	hsa-miR-512-5p-4373238	No	No	18.8	18.2	18.5	18.5	18.5	-0.04	0.42	-0.54	no	-1.03
B	hsa-miR-513-3p-4395202	No	No	29.5	29.5	29.6	29.6	29.6	-0.02	1.40	-1.70	no	-1.01
A	hsa-miR-513-5p-4395201	Yes	Yes	32.3	Inf	Inf	Inf	NA	NA	NA	-NA	NA	NA
A	hsa-miR-515-3p-4395480	No	No	17.0	16.5	16.7	16.7	16.7	-0.09	0.34	-0.45	no	-1.07
A	hsa-miR-515-5p-4373242	No	No	16.6	15.6	15.6	15.9	15.8	-0.26	0.30	-0.40	no	-1.20
B	hsa-miR-516a-3p-4373183	No	No	23.9	23.5	23.7	23.7	23.7	-0.04	0.77	-0.95	no	-1.03
A	hsa-miR-516a-5p-4395527	No	No	22.4	22.2	22.6	22.4	22.5	0.21	0.67	-0.84	no	1.15
A	hsa-miR-516b-4395172	No	No	16.0	15.3	15.4	15.5	15.5	-0.14	0.29	-0.39	no	-1.10
B	hsa-miR-517*-4378078	No	No	24.5	23.7	24.2	23.9	24.1	0.31	0.80	-0.99	no	1.24
A	hsa-miR-517a-4395513	No	No	14.5	11.6	11.8	11.7	11.8	0.08	0.19	-0.27	no	1.06
A	**hsa-miR-517b-4373244**	**No**	**No**	**18.6**	**16.5**	**17.3**	**16.8**	**17.1**	**0.50**	**0.36**	**-0.46**	**yes**	**1.41**
A	hsa-miR-517c-4373264	No	No	14.7	12.0	12.1	12.2	12.1	-0.07	0.20	-0.28	no	-1.05
A	hsa-miR-518a-3p-4395508	No	No	17.9	17.1	17.7	17.4	17.6	0.30	0.38	-0.49	no	1.23
A	hsa-miR-518a-5p-4395507	No	No	25.2	25.5	25.3	25.8	25.6	-0.50	0.94	-1.15	no	-1.41
A	hsa-miR-518b-4373246	No	No	16.4	15.5	15.9	15.8	15.8	0.07	0.30	-0.41	no	1.05
B	hsa-miR-518c*-4378082	No	No	21.3	21.1	21.0	21.3	21.2	-0.32	0.58	-0.73	no	-1.25
A	hsa-miR-518c-4395512	No	No	19.3	19.0	19.1	19.2	19.2	-0.19	0.46	-0.58	no	-1.14
A	hsa-miR-518d-3p-4373248	No	No	24.3	24.5	24.5	24.8	24.6	-0.27	0.85	-1.05	no	-1.21
A	hsa-miR-518d-5p-4395500	No	No	17.9	17.6	17.8	17.9	17.8	-0.06	0.39	-0.50	no	-1.04
B	hsa-miR-518e*-4395482	No	No	22.7	22.3	22.5	22.5	22.5	-0.06	0.67	-0.84	no	-1.04
A	hsa-miR-518e-4395506	No	No	15.4	14.5	14.8	14.7	14.8	0.07	0.27	-0.36	no	1.05
B	hsa-miR-518f*-4395498	No	No	26.4	26.3	26.3	26.5	26.4	-0.20	1.02	-1.25	no	-1.15
A	hsa-miR-518f-4395499	No	No	15.7	14.8	15.0	15.0	15.0	-0.01	0.28	-0.37	no	-1.01
A	hsa-miR-519a-4395526	No	No	14.7	12.3	12.6	12.5	12.6	0.15	0.21	-0.29	no	1.11
B	hsa-miR-519b-3p-4395495	No	No	17.6	17.1	17.1	17.4	17.3	-0.33	0.36	-0.47	no	-1.25
B	hsa-miR-519b-3p-4395495	No	No	17.6	17.2	17.1	17.4	17.2	-0.23	0.36	-0.47	no	-1.17
A	hsa-miR-519c-3p-4373251	No	No	20.8	20.9	20.6	21.2	20.9	-0.56	0.56	-0.71	no	-1.48
A	hsa-miR-519d-4395514	No	No	15.0	13.5	13.9	13.7	13.8	0.23	0.24	-0.33	no	1.18
B	hsa-miR-519e*-4378084	No	No	20.6	20.2	20.4	20.4	20.4	-0.06	0.53	-0.67	no	-1.04
A	hsa-miR-519e-4395481	No	No	20.7	20.2	20.7	20.5	20.6	0.25	0.54	-0.68	no	1.19
A	hsa-miR-520a-3p-4373268	No	No	19.2	18.7	19.1	19.0	19.1	0.09	0.45	-0.58	no	1.06
A	hsa-miR-520a-5p-4378085	No	No	20.1	19.9	20.1	20.1	20.1	0.00	0.51	-0.65	no	1.00
A	hsa-miR-520b-4373252	No	No	24.1	24.7	24.8	25.0	24.9	-0.15	0.87	-1.08	no	-1.11
B	hsa-miR-520c-3p-4395511	No	No	17.3	16.9	16.7	17.1	16.9	-0.38	0.35	-0.46	no	-1.30
A	hsa-miR-520d-5p-4395504	No	No	23.1	22.5	23.1	22.8	23.0	0.35	0.71	-0.88	no	1.27
A	hsa-miR-520e-4373255	No	No	29.6	29.8	30.6	30.0	30.3	0.58	1.49	-1.80	no	1.50
A	hsa-miR-520f-4373256	No	No	23.1	22.9	23.2	23.1	23.2	0.12	0.73	-0.90	no	1.08
A	hsa-miR-520g-4373257	No	No	18.1	17.5	17.9	17.8	17.8	0.05	0.39	-0.51	no	1.03
B	hsa-miR-520h-4373258	No	No	18.9	18.3	18.5	18.5	18.5	0.03	0.42	-0.54	no	1.02
A	hsa-miR-521-4373259	No	No	19.1	18.9	19.0	19.2	19.1	-0.17	0.45	-0.58	no	-1.12
A	**hsa-miR-522-4395524**	**No**	**No**	**16.2**	**15.5**	**15.4**	**15.8**	**15.6**	**-0.40**	**0.29**	**-0.39**	**yes**	**-1.32**
A	hsa-miR-523-4395497	No	No	18.4	17.9	18.3	18.2	18.2	0.07	0.41	-0.53	no	1.05
B	hsa-miR-524-3p-4378087	No	No	20.0	19.4	19.7	19.7	19.7	0.06	0.49	-0.62	no	1.04
B	hsa-miR-524-3p-4378087	No	No	20.0	19.5	19.7	19.6	19.7	0.12	0.49	-0.62	no	1.09
A	hsa-miR-524-5p-4395174	No	No	28.4	28.2	29.2	28.4	28.8	0.78	1.30	-1.57	no	1.72

Plate	Detector	Flag CF	Flag IF	Raw Cq		Norm. Cq		median Cq	log RQ	threshold		Ex-treme	FC
				CF	IF	CF	IF			high	low		
A	hsa-miR-525-3p-4395496	No	No	16.4	15.4	15.7	15.7	15.7	0.05	0.30	-0.40	no	1.03
A	hsa-miR-525-5p-4378088	No	No	19.2	18.9	19.1	19.2	19.2	-0.13	0.46	-0.58	no	-1.10
B	hsa-miR-526b*-4395494	No	No	19.8	19.1	19.5	19.3	19.4	0.25	0.47	-0.60	no	1.19
A	hsa-miR-526b-4395493	No	No	18.5	18.0	18.4	18.3	18.4	0.03	0.41	-0.53	no	1.02
A	hsa-miR-532-3p-4395466	No	No	19.8	19.3	19.8	19.6	19.7	0.13	0.49	-0.62	no	1.09
A	hsa-miR-532-5p-4380928	No	No	18.2	17.8	18.0	18.0	18.0	0.00	0.40	-0.52	no	1.00
A	hsa-miR-539-4378103	No	No	19.5	19.0	19.5	19.3	19.4	0.20	0.47	-0.60	no	1.15
B	hsa-miR-541*-4395311	Yes	Yes	Inf	Inf	Inf	Inf	NA	NA	NA	-NA	NA	NA
A	hsa-miR-541-4395312	Yes	Yes	34.2	Inf	Inf	Inf	NA	NA	NA	-NA	NA	NA
A	hsa-miR-542-3p-4378101	No	No	22.1	22.1	22.2	22.3	22.3	-0.13	0.66	-0.82	no	-1.10
A	hsa-miR-542-5p-4395351	No	No	25.0	25.1	25.1	25.4	25.3	-0.24	0.91	-1.12	no	-1.18
B	hsa-miR-543-4395487	No	No	21.3	20.8	21.0	21.0	21.0	-0.06	0.57	-0.72	no	-1.04
A	hsa-miR-544-4395376	No	No	28.6	28.6	28.8	28.9	28.9	-0.09	1.30	-1.58	no	-1.06
B	hsa-miR-545*-4395377	No	No	26.5	26.8	26.4	27.0	26.7	-0.62	1.05	-1.29	no	-1.53
A	hsa-miR-545-4395378	No	No	23.9	23.9	24.0	24.1	24.1	-0.14	0.80	-0.99	no	-1.10
A	hsa-miR-548a-3p-4380948	Yes	Yes	Inf	Inf	Inf	Inf	NA	NA	NA	-NA	NA	NA
A	hsa-miR-548a-5p-4395523	Yes	Yes	Inf	Inf	Inf	Inf	NA	NA	NA	-NA	NA	NA
A	hsa-miR-548b-3p-4380951	Yes	Yes	38.8	Inf	Inf	Inf	NA	NA	NA	-NA	NA	NA
A	hsa-miR-548b-5p-4395519	No	No	28.4	28.7	29.1	28.9	29.0	0.13	1.32	-1.60	no	1.09
A	hsa-miR-548c-3p-4380993	No	No	33.8	34.8	34.5	34.5	34.5	-0.03	2.15	-2.58	no	-1.02
A	hsa-miR-548c-5p-4395540	No	No	29.6	30.1	30.1	30.3	30.2	-0.16	1.47	-1.79	no	-1.11
A	hsa-miR-548d-3p-4381008	No	No	30.4	30.0	31.3	30.2	30.8	1.11	1.55	-1.88	no	2.16
A	hsa-miR-548d-5p-4395348	No	No	28.9	29.0	29.4	29.3	29.3	0.17	1.36	-1.65	no	1.12
B	hsa-miR-549-4380921	Yes	Yes	34.2	34.1	34.2	34.0	34.1	0.26	2.08	-2.50	no	1.20
B	**hsa-miR-550*-4380954**	**No**	**No**	**32.2**	**30.6**	**32.4**	**30.7**	**31.5**	**1.66**	**1.66**	**-2.01**	**yes**	**3.17**
B	hsa-miR-550-4395521	No	No	30.8	30.4	30.6	30.6	30.6	0.02	1.53	-1.85	no	1.01
B	hsa-miR-551a-4380929	Yes	Yes	Inf	Inf	Inf	Inf	NA	NA	NA	-NA	NA	NA
B	hsa-miR-551b*-4395457	No	No	29.0	28.0	28.9	28.1	28.5	0.75	1.26	-1.53	no	1.68
A	hsa-miR-551b-4380945	No	No	22.9	22.6	23.1	22.9	23.0	0.20	0.71	-0.88	no	1.15
B	hsa-miR-552-4380930	Yes	Yes	Inf	Inf	Inf	Inf	NA	NA	NA	-NA	NA	NA
B	hsa-miR-553-4380931	Yes	Yes	Inf	Inf	Inf	Inf	NA	NA	NA	-NA	NA	NA
B	hsa-miR-554-4380932	No	No	29.2	29.2	29.1	29.4	29.2	-0.27	1.35	-1.64	no	-1.21
B	hsa-miR-555-4380933	Yes	Yes	Inf	Inf	Inf	Inf	NA	NA	NA	-NA	NA	NA
A	hsa-miR-556-3p-4395456	Yes	Yes	36.7	Inf	Inf	Inf	NA	NA	NA	-NA	NA	NA
A	hsa-miR-556-5p-4395455	Yes	Yes	37.5	Inf	Inf	Inf	NA	NA	NA	-NA	NA	NA
B	hsa-miR-557-4380935	Yes	Yes	Inf	Inf	Inf	Inf	NA	NA	NA	-NA	NA	NA
B	hsa-miR-558-4380936	Yes	Yes	Inf	Inf	Inf	Inf	NA	NA	NA	-NA	NA	NA
B	hsa-miR-559-4380937	Yes	Yes	Inf	Inf	Inf	Inf	NA	NA	NA	-NA	NA	NA
B	hsa-miR-559-4380937	Yes	Yes	Inf	Inf	Inf	Inf	NA	NA	NA	-NA	NA	NA
A	hsa-miR-561-4380938	No	No	29.2	30.3	30.5	30.4	30.5	0.02	1.51	-1.83	no	1.01
B	hsa-miR-562-4380939	Yes	Yes	Inf	Inf	Inf	Inf	NA	NA	NA	-NA	NA	NA
B	hsa-miR-562-4380939	Yes	Yes	Inf	Inf	Inf	Inf	NA	NA	NA	-NA	NA	NA
B	hsa-miR-563-4380940	Yes	Yes	Inf	Inf	Inf	Inf	NA	NA	NA	-NA	NA	NA
B	hsa-miR-564-4380941	No	No	29.1	28.3	28.9	28.5	28.7	0.42	1.28	-1.56	no	1.34
B	hsa-miR-565-4380942	No	No	23.1	23.6	23.0	23.8	23.4	-0.79	0.74	-0.92	no	-1.73
B	hsa-miR-566-4380943	Yes	Yes	32.0	Inf	Inf	Inf	NA	NA	NA	-NA	NA	NA
B	hsa-miR-566-4380943	Yes	Yes	32.4	Inf	Inf	Inf	NA	NA	NA	-NA	NA	NA
B	hsa-miR-567-4380944	Yes	Yes	38.4	Inf	Inf	Inf	NA	NA	NA	-NA	NA	NA
B	**hsa-miR-569-4380946**	**Yes**	**Yes**	**37.1**	**35.7**	**Inf**	**35.6**	**NA**	**NA**	**NA**	**-NA**	**yes**	**NA**
A	hsa-miR-570-4395458	No	No	27.1	27.4	27.5	27.7	27.6	-0.16	1.15	-1.41	no	-1.12
B	hsa-miR-571-4381016	Yes	Yes	32.9	Inf	Inf	Inf	NA	NA	NA	-NA	NA	NA
B	hsa-miR-571-4381016	Yes	Yes	33.8	Inf	Inf	Inf	NA	NA	NA	-NA	NA	NA
B	hsa-miR-572-4381017	No	No	28.7	29.0	28.5	29.2	28.9	-0.69	1.30	-1.58	no	-1.61
B	hsa-miR-572-4381017	No	No	29.2	29.1	29.0	29.1	29.1	-0.11	1.33	-1.61	no	-1.08
B	hsa-miR-573-4381018	Yes	Yes	35.8	Inf	Inf	Inf	NA	NA	NA	-NA	NA	NA
A	hsa-miR-574-3p-4395460	No	No	17.3	16.7	17.0	16.9	17.0	0.07	0.35	-0.46	no	1.05
B	hsa-miR-575-4381020	Yes	Yes	Inf	Inf	Inf	Inf	NA	NA	NA	-NA	NA	NA

Plate	Detector	Flag CF	Flag IF	Raw Cq		Norm. Cq		median Cq	log RQ	threshold		Ex-treme	FC
				CF	IF	CF	IF			high	low		
A	hsa-miR-576-3p-4395462	No	No	24.6	24.4	24.8	24.7	24.7	0.13	0.86	-1.06	no	1.09
A	hsa-miR-576-5p-4395461	No	Yes	28.5	Inf	Inf	Inf	NA	NA	NA	-NA	NA	NA
B	hsa-miR-578-4381022	Yes	Yes	Inf	Inf	Inf	Inf	NA	NA	NA	-NA	NA	NA
B	hsa-miR-578-4381022	Yes	Yes	Inf	Inf	Inf	Inf	NA	NA	NA	-NA	NA	NA
A	hsa-miR-579-4395509	No	No	24.4	24.1	24.6	24.3	24.4	0.25	0.83	-1.03	no	1.19
B	hsa-miR-580-4381024	No	No	30.3	30.1	30.2	31.0	30.6	-0.81	1.53	-1.85	no	-1.75
B	hsa-miR-580-4381024	No	No	31.2	30.8	31.0	30.3	30.6	0.69	1.54	-1.86	no	1.61
B	**hsa-miR-581-4386744**	**No**	**Yes**	**31.6**	**33.5**	**31.4**	**33.6**	**32.5**	**-2.18**	**1.81**	**-2.18**	**yes**	**-4.53**
B	hsa-miR-581-4386744	No	No	32.6	34.0	32.6	34.0	33.3	-1.41	1.94	-2.33	no	-2.65
A	hsa-miR-582-3p-4395510	No	No	29.7	29.5	30.6	29.7	30.1	0.84	1.47	-1.78	no	1.79
A	hsa-miR-582-5p-4395175	No	No	31.3	31.7	32.7	31.8	32.3	0.97	1.77	-2.14	no	1.96
B	hsa-miR-583-4381025	Yes	Yes	Inf	Inf	Inf	Inf	NA	NA	NA	-NA	NA	NA
B	hsa-miR-584-4381026	No	No	22.9	22.5	22.7	22.7	22.7	0.00	0.69	-0.86	no	1.00
B	hsa-miR-584-4381026	No	No	23.0	22.6	22.9	22.8	22.8	0.06	0.70	-0.87	no	1.04
B	hsa-miR-585-4381027	Yes	Yes	Inf	Inf	Inf	Inf	NA	NA	NA	-NA	NA	NA
B	**hsa-miR-586-4380949**	**No**	**No**	**31.0**	**33.0**	**30.9**	**33.1**	**32.0**	**-2.17**	**1.73**	**-2.09**	**yes**	**-4.50**
B	hsa-miR-587-4380950	Yes	Yes	Inf	Inf	Inf	Inf	NA	NA	NA	-NA	NA	NA
B	hsa-miR-588-4380952	Yes	Yes	Inf	Inf	Inf	Inf	NA	NA	NA	-NA	NA	NA
B	hsa-miR-588-4380952	Yes	Yes	Inf	Inf	Inf	Inf	NA	NA	NA	-NA	NA	NA
B	hsa-miR-589*-4380953	No	No	28.0	27.7	28.0	27.9	27.9	0.11	1.19	-1.45	no	1.08
A	hsa-miR-589-4395520	No	No	31.3	32.3	32.0	32.4	32.2	-0.38	1.77	-2.13	no	-1.30
A	hsa-miR-590-5p-4395176	No	No	19.0	18.8	19.0	19.1	19.1	-0.14	0.45	-0.58	no	-1.10
B	hsa-miR-591-4380955	Yes	Yes	34.3	32.9	34.9	32.8	33.8	2.01	2.03	-2.44	no	4.03
B	**hsa-miR-591-4380955**	**Yes**	**No**	**35.9**	**33.7**	**Inf**	**33.7**	**NA**	**NA**	**NA**	**-NA**	**yes**	**NA**
B	hsa-miR-592-4380956	No	No	27.7	28.1	27.5	28.3	27.9	-0.76	1.18	-1.44	no	-1.69
B	hsa-miR-593*-4380957	Yes	Yes	Inf	Inf	Inf	Inf	NA	NA	NA	-NA	NA	NA
B	hsa-miR-593-4395522	Yes	Yes	Inf	Inf	Inf	Inf	NA	NA	NA	-NA	NA	NA
B	hsa-miR-595-4395178	Yes	Yes	Inf	Inf	Inf	Inf	NA	NA	NA	-NA	NA	NA
B	hsa-miR-596-4380959	Yes	Yes	Inf	Inf	Inf	Inf	NA	NA	NA	-NA	NA	NA
B	hsa-miR-596-4380959	Yes	Yes	Inf	Inf	Inf	Inf	NA	NA	NA	-NA	NA	NA
A	hsa-miR-597-4380960	No	No	25.2	25.1	25.4	25.4	25.4	0.02	0.92	-1.13	no	1.02
A	hsa-miR-598-4395179	No	No	25.8	25.7	26.0	25.9	26.0	0.07	0.98	-1.20	no	1.05
B	hsa-miR-599-4380962	Yes	Yes	Inf	Inf	Inf	Inf	NA	NA	NA	-NA	NA	NA
B	hsa-miR-599-4380962	Yes	Yes	Inf	Inf	Inf	Inf	NA	NA	NA	-NA	NA	NA
B	hsa-miR-600-4380963	Yes	Yes	Inf	Inf	Inf	Inf	NA	NA	NA	-NA	NA	NA
B	hsa-miR-600-4380963	Yes	Yes	Inf	Inf	Inf	Inf	NA	NA	NA	-NA	NA	NA
B	hsa-miR-601-4380965	No	No	27.7	27.8	27.5	28.0	27.7	-0.44	1.17	-1.42	no	-1.36
B	hsa-miR-603-4380972	Yes	Yes	Inf	Inf	Inf	Inf	NA	NA	NA	-NA	NA	NA
B	hsa-miR-604-4380973	Yes	Yes	36.0	Inf	Inf	Inf	NA	NA	NA	-NA	NA	NA
B	hsa-miR-604-4380973	Yes	Yes	Inf	Inf	Inf	Inf	NA	NA	NA	-NA	NA	NA
B	hsa-miR-605-4386742	Yes	Yes	32.7	Inf	Inf	Inf	NA	NA	NA	-NA	NA	NA
B	hsa-miR-605-4386742	Yes	Yes	33.8	Inf	Inf	Inf	NA	NA	NA	-NA	NA	NA
B	hsa-miR-606-4380974	Yes	Yes	Inf	Inf	Inf	Inf	NA	NA	NA	-NA	NA	NA
B	hsa-miR-606-4380974	Yes	Yes	Inf	Inf	Inf	Inf	NA	NA	NA	-NA	NA	NA
B	hsa-miR-607-4380975	Yes	Yes	Inf	Inf	Inf	Inf	NA	NA	NA	-NA	NA	NA
B	hsa-miR-607-4380975	Yes	Yes	Inf	Inf	Inf	Inf	NA	NA	NA	-NA	NA	NA
B	hsa-miR-608-4380976	Yes	Yes	Inf	Inf	Inf	Inf	NA	NA	NA	-NA	NA	NA
B	hsa-miR-608-4380976	Yes	Yes	Inf	Inf	Inf	Inf	NA	NA	NA	-NA	NA	NA
B	hsa-miR-609-4380978	Yes	Yes	Inf	Inf	Inf	Inf	NA	NA	NA	-NA	NA	NA
B	hsa-miR-609-4380978	Yes	Yes	Inf	Inf	Inf	Inf	NA	NA	NA	-NA	NA	NA
B	hsa-miR-610-4380980	Yes	No	31.7	30.1	31.7	30.2	31.0	1.51	1.58	-1.91	no	2.84
B	hsa-miR-612-4380983	Yes	Yes	Inf	Inf	Inf	Inf	NA	NA	NA	-NA	NA	NA
B	hsa-miR-613-4380989	Yes	Yes	Inf	Inf	Inf	Inf	NA	NA	NA	-NA	NA	NA
B	hsa-miR-613-4380989	Yes	Yes	Inf	Inf	Inf	Inf	NA	NA	NA	-NA	NA	NA
B	hsa-miR-614-4380990	Yes	Yes	Inf	Inf	Inf	Inf	NA	NA	NA	-NA	NA	NA
B	hsa-miR-614-4380990	Yes	Yes	Inf	Inf	Inf	Inf	NA	NA	NA	-NA	NA	NA
A	hsa-miR-615-3p-4386777	Yes	Yes	33.6	Inf	Inf	Inf	NA	NA	NA	-NA	NA	NA

Plate	Detector	Flag CF	Flag IF	Raw Cq		Norm. Cq		median Cq	log RQ	threshold		Ex-treme	FC
				CF	IF	CF	IF			high	low		
A	hsa-miR-615-5p-4395464	Yes	Yes	37.8	Inf	Inf	Inf	NA	NA	NA	-NA	NA	NA
B	hsa-miR-616*-4380992	No	No	26.0	26.0	25.8	26.2	26.0	-0.41	0.98	-1.21	no	-1.33
A	hsa-miR-616-4395525	No	No	29.6	30.9	30.9	31.0	31.0	-0.11	1.58	-1.91	no	-1.08
B	hsa-miR-617-4380994	Yes	Yes	Inf	Inf	Inf	Inf	NA	NA	NA	-NA	NA	NA
A	hsa-miR-618-4380996	No	No	27.8	27.7	28.1	28.0	28.0	0.16	1.20	-1.46	no	1.11
B	hsa-miR-619-4380998	Yes	Yes	Inf	Inf	Inf	Inf	NA	NA	NA	-NA	NA	NA
B	hsa-miR-621-4381001	Yes	Yes	Inf	Inf	Inf	Inf	NA	NA	NA	-NA	NA	NA
B	hsa-miR-621-4381001	Yes	Yes	Inf	Inf	Inf	Inf	NA	NA	NA	-NA	NA	NA
B	hsa-miR-622-4380961	No	No	26.5	25.7	26.3	25.9	26.1	0.46	0.99	-1.22	no	1.37
B	hsa-miR-622-4380961	No	No	26.8	26.2	26.8	26.4	26.6	0.36	1.04	-1.27	no	1.28
B	hsa-miR-623-4386740	Yes	Yes	Inf	Inf	Inf	Inf	NA	NA	NA	-NA	NA	NA
B	hsa-miR-623-4386740	Yes	Yes	Inf	Inf	Inf	Inf	NA	NA	NA	-NA	NA	NA
B	hsa-miR-624*-4380964	No	No	28.2	27.5	28.1	27.6	27.9	0.50	1.19	-1.44	no	1.42
A	hsa-miR-624-4395541	Yes	Yes	Inf	Inf	Inf	Inf	NA	NA	NA	-NA	NA	NA
B	hsa-miR-625*-4395543	No	No	19.5	18.7	19.2	18.9	19.1	0.21	0.45	-0.58	no	1.16
A	hsa-miR-625-4395542	No	No	21.4	21.2	21.5	21.5	21.5	-0.03	0.60	-0.75	no	-1.02
B	hsa-miR-626-4380966	Yes	Yes	Inf	Inf	Inf	Inf	NA	NA	NA	-NA	NA	NA
B	hsa-miR-626-4380966	Yes	Yes	Inf	Inf	Inf	Inf	NA	NA	NA	-NA	NA	NA
A	hsa-miR-627-4380967	No	No	29.8	30.4	30.7	30.6	30.6	0.16	1.54	-1.86	no	1.12
B	hsa-miR-628-3p-4395545	No	No	27.7	27.4	27.6	27.6	27.6	-0.03	1.15	-1.40	no	-1.02
B	hsa-miR-628-3p-4395545	No	No	27.9	27.5	27.8	27.6	27.7	0.11	1.16	-1.42	no	1.08
A	hsa-miR-628-5p-4395544	No	No	25.1	25.0	25.3	25.3	25.3	-0.03	0.91	-1.12	no	-1.02
B	hsa-miR-629*-4380969	No	No	24.1	23.3	23.9	23.5	23.7	0.37	0.77	-0.95	no	1.29
A	hsa-miR-629-4395547	No	No	25.2	25.4	25.4	25.7	25.5	-0.30	0.93	-1.15	no	-1.23
B	**hsa-miR-630-4380970**	**Yes**	**No**	**33.2**	**32.5**	**Inf**	**32.5**	**NA**	**NA**	**NA**	**-NA**	**yes**	**NA**
B	hsa-miR-631-4380971	Yes	Yes	Inf	Inf	Inf	Inf	NA	NA	NA	-NA	NA	NA
B	hsa-miR-631-4380971	Yes	Yes	Inf	Inf	Inf	Inf	NA	NA	NA	-NA	NA	NA
B	hsa-miR-632-4380977	No	No	30.4	30.2	30.4	30.3	30.4	0.12	1.50	-1.82	no	1.08
B	hsa-miR-633-4380979	Yes	Yes	Inf	Inf	Inf	Inf	NA	NA	NA	-NA	NA	NA
B	hsa-miR-633-4380979	Yes	Yes	Inf	Inf	Inf	Inf	NA	NA	NA	-NA	NA	NA
B	hsa-miR-634-4380981	Yes	Yes	Inf	Inf	Inf	Inf	NA	NA	NA	-NA	NA	NA
B	hsa-miR-634-4380981	Yes	Yes	Inf	Inf	Inf	Inf	NA	NA	NA	-NA	NA	NA
B	hsa-miR-635-4380982	No	No	30.4	29.4	30.3	29.5	29.9	0.78	1.44	-1.74	no	1.72
B	hsa-miR-635-4380982	No	No	30.5	29.6	30.4	29.7	30.1	0.68	1.46	-1.76	no	1.60
A	hsa-miR-636-4395199	No	No	28.2	28.6	28.6	28.8	28.7	-0.17	1.29	-1.56	no	-1.13
B	hsa-miR-637-4380985	Yes	Yes	Inf	Inf	Inf	Inf	NA	NA	NA	-NA	NA	NA
B	hsa-miR-637-4380985	Yes	Yes	Inf	Inf	Inf	Inf	NA	NA	NA	-NA	NA	NA
B	hsa-miR-638-4380986	No	No	27.7	27.8	27.6	27.9	27.8	-0.32	1.17	-1.43	no	-1.25
B	hsa-miR-639-4380987	No	No	27.7	27.3	27.6	27.7	27.7	-0.13	1.16	-1.41	no	-1.09
B	hsa-miR-639-4380987	No	No	28.1	27.5	28.0	27.5	27.8	0.51	1.17	-1.43	no	1.42
B	hsa-miR-640-4386743	Yes	Yes	38.0	Inf	Inf	Inf	NA	NA	NA	-NA	NA	NA
B	hsa-miR-640-4386743	Yes	Yes	Inf	Inf	Inf	Inf	NA	NA	NA	-NA	NA	NA
B	**hsa-miR-641-4380988**	**No**	**No**	**31.9**	**29.9**	**31.8**	**30.0**	**30.9**	**1.76**	**1.58**	**-1.90**	**yes**	**3.39**
B	**hsa-miR-641-4380988**	**No**	**No**	**32.3**	**30.3**	**32.2**	**30.5**	**31.3**	**1.70**	**1.63**	**-1.97**	**yes**	**3.26**
A	hsa-miR-642-4380995	No	No	26.9	27.9	27.2	28.2	27.7	-0.98	1.16	-1.42	no	-1.98
B	hsa-miR-643-4380997	Yes	Yes	34.0	Inf	Inf	Inf	NA	NA	NA	-NA	NA	NA
B	hsa-miR-644-4380999	Yes	Yes	34.2	Inf	Inf	Inf	NA	NA	NA	-NA	NA	NA
B	hsa-miR-644-4380999	Yes	Yes	35.3	Inf	Inf	Inf	NA	NA	NA	-NA	NA	NA
B	hsa-miR-645-4381000	No	No	33.0	33.1	32.7	33.0	32.9	-0.34	1.87	-2.25	no	-1.27
B	hsa-miR-645-4381000	No	No	33.2	33.2	32.9	33.2	33.0	-0.34	1.90	-2.29	no	-1.26
B	hsa-miR-646-4381002	Yes	Yes	Inf	Inf	Inf	Inf	NA	NA	NA	-NA	NA	NA
B	hsa-miR-646-4381002	Yes	Yes	Inf	Inf	Inf	Inf	NA	NA	NA	-NA	NA	NA
B	hsa-miR-647-4381003	Yes	Yes	Inf	Inf	Inf	Inf	NA	NA	NA	-NA	NA	NA
B	hsa-miR-647-4381003	Yes	Yes	Inf	Inf	Inf	Inf	NA	NA	NA	-NA	NA	NA
B	hsa-miR-648-4381004	Yes	Yes	34.6	Inf	Inf	Inf	NA	NA	NA	-NA	NA	NA
B	hsa-miR-648-4381004	Yes	Yes	34.8	Inf	Inf	Inf	NA	NA	NA	-NA	NA	NA
B	**hsa-miR-649-4381005**	**Yes**	**Yes**	**33.0**	**Inf**	**33.6**	**Inf**	**NA**	**-NA**	**NA**	**-NA**	**yes**	**NA**

Plate	Detector	Flag CF	Flag IF	Raw Cq		Norm. Cq		median Cq	log RQ	threshold		Ex-treme	FC
				CF	IF	CF	IF			high	low		
B	hsa-miR-649-4381005	Yes	Yes	34.2	34.7	34.5	34.5	34.5	0.02	2.15	-2.58	no	1.01
B	hsa-miR-650-4381006	Yes	Yes	34.0	Inf	Inf	Inf	NA	NA	NA	-NA	NA	NA
B	hsa-miR-650-4381006	Yes	Yes	34.0	Inf	Inf	Inf	NA	NA	NA	-NA	NA	NA
A	hsa-miR-651-4381007	No	No	30.1	30.2	30.5	30.4	30.5	0.07	1.51	-1.83	no	1.05
A	hsa-miR-652-4395463	No	No	19.8	19.5	19.8	19.8	19.8	-0.04	0.49	-0.63	no	-1.02
A	hsa-miR-653-4395403	No	No	28.8	28.9	29.0	29.1	29.1	-0.08	1.33	-1.61	no	-1.06
A	hsa-miR-654-3p-4395350	No	No	24.2	24.2	24.5	24.4	24.4	0.10	0.83	-1.03	no	1.07
A	hsa-miR-654-5p-4381014	No	No	24.7	24.8	25.0	25.0	25.0	-0.04	0.88	-1.09	no	-1.03
A	hsa-miR-655-4381015	No	No	22.0	21.7	22.1	21.9	22.0	0.15	0.64	-0.80	no	1.11
B	hsa-miR-656-4380920	No	No	22.4	21.9	22.1	22.2	22.2	-0.02	0.65	-0.81	no	-1.02
B	hsa-miR-656-4380920	No	No	22.4	22.0	22.2	22.1	22.2	0.08	0.65	-0.81	no	1.06
B	hsa-miR-657-4380922	Yes	Yes	Inf	Inf	Inf	Inf	NA	NA	NA	-NA	NA	NA
B	hsa-miR-657-4380922	Yes	Yes	Inf	Inf	Inf	Inf	NA	NA	NA	-NA	NA	NA
B	hsa-miR-658-4380923	Yes	Yes	Inf	Inf	Inf	Inf	NA	NA	NA	-NA	NA	NA
B	hsa-miR-658-4380923	Yes	Yes	Inf	Inf	Inf	Inf	NA	NA	NA	-NA	NA	NA
B	hsa-miR-659-4380924	Yes	Yes	Inf	Inf	Inf	Inf	NA	NA	NA	-NA	NA	NA
A	hsa-miR-660-4380925	No	No	18.1	17.7	18.0	18.0	18.0	0.00	0.40	-0.51	no	1.00
B	hsa-miR-661-4381009	No	No	25.3	25.3	25.1	25.5	25.3	-0.41	0.91	-1.12	no	-1.33
B	hsa-miR-662-4381010	Yes	Yes	Inf	Inf	Inf	Inf	NA	NA	NA	-NA	NA	NA
B	hsa-miR-662-4381010	Yes	Yes	Inf	Inf	Inf	Inf	NA	NA	NA	-NA	NA	NA
B	**hsa-miR-668-4395181**	**No**	**No**	**27.0**	**25.0**	**26.8**	**25.1**	**26.0**	**1.65**	**0.98**	**-1.20**	**yes**	**3.14**
B	**hsa-miR-668-4395181**	**No**	**No**	**27.0**	**24.9**	**26.9**	**25.3**	**26.1**	**1.59**	**0.99**	**-1.21**	**yes**	**3.02**
A	hsa-miR-671-3p-4395433	No	No	24.5	24.5	24.6	24.7	24.6	-0.15	0.85	-1.05	no	-1.11
A	hsa-miR-672-4395438	Yes	Yes	37.6	Inf	Inf	Inf	NA	NA	NA	-NA	NA	NA
A	hsa-miR-674-4395193	Yes	Yes	Inf	Inf	Inf	Inf	NA	NA	NA	-NA	NA	NA
B	hsa-miR-675-4395192	No	No	21.9	21.5	21.7	21.7	21.7	-0.01	0.62	-0.77	no	-1.00
B	hsa-miR-675-4395192	No	No	22.0	21.6	21.7	21.7	21.7	-0.02	0.62	-0.77	no	-1.01
B	hsa-miR-708*-4395453	Yes	Yes	Inf	Inf	Inf	Inf	NA	NA	NA	-NA	NA	NA
A	hsa-miR-708-4395452	No	No	27.6	28.5	27.8	28.7	28.3	-0.92	1.23	-1.49	no	-1.89
B	hsa-miR-7-1*-4381118	No	No	22.6	21.8	22.3	22.0	22.2	0.39	0.65	-0.81	no	1.31
B	hsa-miR-7-2*-4395425	No	No	31.7	31.3	31.6	31.4	31.5	0.19	1.66	-2.00	no	1.14
B	hsa-miR-7-4378130	No	No	25.5	24.8	25.3	25.0	25.2	0.26	0.90	-1.11	no	1.20
B	hsa-miR-7-4378130	No	No	25.6	24.9	25.4	25.2	25.3	0.28	0.91	-1.12	no	1.21
B	hsa-miR-744*-4395436	No	No	26.3	26.0	26.1	26.2	26.2	-0.05	1.00	-1.22	no	-1.03
A	hsa-miR-744-4395435	No	No	20.0	19.9	20.0	20.1	20.1	-0.17	0.51	-0.65	no	-1.12
A	hsa-miR-758-4395180	No	No	23.9	23.7	23.9	24.0	24.0	-0.05	0.79	-0.98	no	-1.03
B	hsa-miR-760-4395439	No	No	25.8	25.8	25.7	26.0	25.8	-0.29	0.96	-1.18	no	-1.23
B	hsa-miR-766-4395177	No	No	21.9	21.5	21.7	21.7	21.7	-0.05	0.62	-0.77	no	-1.03
B	hsa-miR-766-4395177	No	No	22.2	21.6	22.0	21.8	21.9	0.16	0.63	-0.79	no	1.11
B	hsa-miR-767-3p-4395184	No	No	33.9	33.9	33.9	34.0	33.9	-0.14	2.05	-2.46	no	-1.10
B	**hsa-miR-767-3p-4395184**	**Yes**	**No**	**35.5**	**34.1**	**Inf**	**33.8**	**NA**	**NA**	**NA**	**-NA**	**yes**	**NA**
B	hsa-miR-767-5p-4395182	No	No	27.3	27.4	27.2	27.1	27.2	0.05	1.10	-1.35	no	1.04
B	hsa-miR-767-5p-4395182	No	No	27.7	26.9	27.6	27.6	27.6	0.03	1.15	-1.40	no	1.02
B	hsa-miR-768-3p-4395188	No	No	16.7	16.0	16.2	16.2	16.2	-0.06	0.32	-0.42	no	-1.05
B	hsa-miR-768-3p-4395188	No	No	16.8	16.1	16.3	16.3	16.3	-0.07	0.32	-0.43	no	-1.05
B	hsa-miR-769-3p-4395190	Yes	Yes	35.6	Inf	Inf	Inf	NA	NA	NA	-NA	NA	NA
B	hsa-miR-769-3p-4395190	Yes	Yes	39.0	Inf	Inf	Inf	NA	NA	NA	-NA	NA	NA
B	hsa-miR-769-5p-4395186	No	No	22.9	22.6	22.7	22.8	22.8	-0.12	0.69	-0.86	no	-1.08
B	hsa-miR-769-5p-4395186	No	No	23.0	22.7	22.8	22.9	22.8	-0.07	0.70	-0.87	no	-1.05
B	hsa-miR-770-5p-4395189	No	No	25.7	24.6	25.6	24.9	25.2	0.69	0.90	-1.11	no	1.61
B	hsa-miR-801-4395183	No	No	18.7	18.6	18.4	18.7	18.5	-0.33	0.42	-0.55	no	-1.26
B	hsa-miR-801-4395183	No	No	18.8	18.5	18.4	18.8	18.6	-0.42	0.43	-0.55	no	-1.34
A	hsa-miR-871-4395465	Yes	Yes	Inf	Inf	Inf	Inf	NA	NA	NA	-NA	NA	NA
A	hsa-miR-872-4395375	Yes	Yes	Inf	Inf	Inf	Inf	NA	NA	NA	-NA	NA	NA
A	hsa-miR-873-4395467	No	No	31.6	31.8	32.6	31.9	32.2	0.68	1.77	-2.13	no	1.60
A	hsa-miR-874-4395379	Yes	No	26.2	25.8	26.6	26.0	26.3	0.59	1.01	-1.24	no	1.50
A	hsa-miR-875-3p-4395315	Yes	Yes	Inf	Inf	Inf	Inf	NA	NA	NA	-NA	NA	NA

Plate	Detector	Flag CF	Flag IF	Raw Cq		Norm. Cq		median Cq	log RQ	threshold		Ex-treme	FC
				CF	IF	CF	IF			high	low		
B	hsa-miR-875-5p-4395314	No	No	28.9	29.2	28.8	29.4	29.1	-0.60	1.33	-1.61	no	-1.51
A	hsa-miR-876-3p-4395336	No	No	29.6	30.4	30.8	30.6	30.7	0.17	1.55	-1.87	no	1.12
A	hsa-miR-876-5p-4395316	No	No	31.6	31.2	32.3	31.2	31.7	1.09	1.69	-2.04	no	2.13
B	hsa-miR-877-4395402	No	No	25.4	25.1	25.2	25.3	25.3	-0.11	0.91	-1.12	no	-1.08
A	hsa-miR-885-3p-4395483	Yes	Yes	33.1	Inf	Inf	Inf	NA	NA	NA	-NA	NA	NA
A	hsa-miR-885-5p-4395407	No	No	28.8	29.8	29.1	30.1	29.6	-0.93	1.40	-1.69	no	-1.90
A	hsa-miR-886-3p-4395305	No	No	21.7	21.3	21.8	21.5	21.7	0.26	0.61	-0.77	no	1.20
A	hsa-miR-886-5p-4395304	No	No	22.8	22.6	23.0	22.9	22.9	0.10	0.70	-0.88	no	1.07
A	hsa-miR-887-4395485	Yes	Yes	Inf	Inf	Inf	Inf	NA	NA	NA	-NA	NA	NA
B	hsa-miR-888*-4395324	Yes	Yes	Inf	Inf	Inf	Inf	NA	NA	NA	-NA	NA	NA
A	**hsa-miR-888-4395323**	**No**	**No**	**30.7**	**35.8**	**32.2**	**35.7**	**34.0**	**-3.52**	**2.05**	**-2.47**	**yes**	**-11.45**
A	hsa-miR-889-4395313	No	No	22.6	22.2	22.7	22.5	22.6	0.22	0.68	-0.85	no	1.17
A	hsa-miR-890-4395320	Yes	Yes	Inf	Inf	Inf	Inf	NA	NA	NA	-NA	NA	NA
A	hsa-miR-891a-4395302	Yes	Yes	39.6	Inf	Inf	Inf	NA	NA	NA	-NA	NA	NA
A	hsa-miR-891b-4395321	Yes	Yes	Inf	Inf	Inf	Inf	NA	NA	NA	-NA	NA	NA
A	hsa-miR-892a-4395306	Yes	Yes	Inf	Inf	Inf	Inf	NA	NA	NA	-NA	NA	NA
B	hsa-miR-892b-4395325	Yes	Yes	Inf	Inf	Inf	Inf	NA	NA	NA	-NA	NA	NA
B	hsa-miR-9*-4395342	No	No	23.7	23.6	23.5	23.8	23.7	-0.24	0.77	-0.95	no	-1.18
B	hsa-miR-920-4395261	Yes	Yes	Inf	Inf	Inf	Inf	NA	NA	NA	-NA	NA	NA
B	hsa-miR-920-4395261	Yes	Yes	Inf	Inf	Inf	Inf	NA	NA	NA	-NA	NA	NA
B	hsa-miR-921-4395262	Yes	Yes	Inf	Inf	Inf	Inf	NA	NA	NA	-NA	NA	NA
B	hsa-miR-921-4395262	Yes	Yes	Inf	Inf	Inf	Inf	NA	NA	NA	-NA	NA	NA
B	hsa-miR-922-4395263	No	No	31.0	31.6	30.8	31.0	30.9	-0.17	1.58	-1.91	no	-1.13
B	hsa-miR-922-4395263	No	No	31.2	30.9	31.0	31.8	31.4	-0.77	1.64	-1.98	no	-1.71
B	**hsa-miR-923-4395264**	**No**	**No**	**20.1**	**21.1**	**19.8**	**20.9**	**20.4**	**-1.13**	**0.53**	**-0.67**	**yes**	**-2.18**
B	**hsa-miR-923-4395264**	**No**	**No**	**20.2**	**20.7**	**19.9**	**21.3**	**20.6**	**-1.48**	**0.54**	**-0.69**	**yes**	**-2.79**
B	hsa-miR-924-4395265	Yes	Yes	Inf	Inf	Inf	Inf	NA	NA	NA	-NA	NA	NA
B	hsa-miR-924-4395265	Yes	Yes	Inf	Inf	Inf	Inf	NA	NA	NA	-NA	NA	NA
B	hsa-miR-92a-1*-4395248	No	No	26.4	26.3	26.2	26.5	26.4	-0.30	1.02	-1.25	no	-1.23
B	hsa-miR-92a-2*-4395249	Yes	Yes	Inf	Inf	Inf	Inf	NA	NA	NA	-NA	NA	NA
A	hsa-miR-92a-4395169	No	No	18.3	17.8	18.2	18.1	18.1	0.16	0.40	-0.52	no	1.12
B	hsa-miR-92b*-4395454	Yes	Yes	39.8	Inf	Inf	Inf	NA	NA	NA	-NA	NA	NA
B	hsa-miR-93*-4395250	No	No	23.9	23.6	23.8	23.8	23.8	-0.06	0.78	-0.96	no	-1.04
B	hsa-miR-933-4395287	Yes	Yes	Inf	Inf	Inf	Inf	NA	NA	NA	-NA	NA	NA
B	hsa-miR-933-4395287	Yes	Yes	Inf	Inf	Inf	Inf	NA	NA	NA	-NA	NA	NA
A	hsa-miR-93-4373302	No	No	17.6	17.3	17.4	17.6	17.5	-0.20	0.37	-0.49	no	-1.15
B	hsa-miR-934-4395288	No	No	24.8	24.3	24.6	24.6	24.6	0.03	0.84	-1.04	no	1.02
B	hsa-miR-934-4395288	No	No	24.9	24.5	24.7	24.7	24.7	-0.01	0.86	-1.06	no	-1.01
B	hsa-miR-935-4395289	Yes	Yes	Inf	Inf	Inf	Inf	NA	NA	NA	-NA	NA	NA
B	hsa-miR-935-4395289	Yes	Yes	Inf	Inf	Inf	Inf	NA	NA	NA	-NA	NA	NA
B	**hsa-miR-936-4395290**	**Yes**	**No**	**Inf**	**33.5**	**Inf**	**33.5**	**NA**	**NA**	**NA**	**-NA**	**yes**	**NA**
B	hsa-miR-937-4395291	No	No	30.6	29.5	30.4	29.6	30.0	0.78	1.45	-1.75	no	1.72
B	hsa-miR-938-4395292	No	No	31.7	32.3	31.4	32.4	31.9	-1.03	1.72	-2.07	no	-2.05
B	hsa-miR-939-4395293	Yes	Yes	27.4	Inf	Inf	Inf	NA	NA	NA	-NA	NA	NA
B	hsa-miR-941-4395294	No	No	29.1	28.1	29.0	28.2	28.6	0.75	1.27	-1.55	no	1.68
B	hsa-miR-942-4395298	No	No	24.6	24.1	24.4	24.3	24.4	0.08	0.83	-1.02	no	1.06
B	hsa-miR-943-4395299	Yes	Yes	Inf	Inf	Inf	Inf	NA	NA	NA	-NA	NA	NA
A	hsa-miR-9-4373285	No	No	21.7	22.0	21.9	22.3	22.1	-0.36	0.64	-0.81	no	-1.28
B	hsa-miR-944-4395300	No	No	24.7	24.7	24.5	24.9	24.7	-0.43	0.86	-1.06	no	-1.35
A	hsa-miR-95-4373011	No	No	23.8	24.5	23.9	24.7	24.3	-0.79	0.82	-1.02	no	-1.73
B	hsa-miR-96*-4395251	Yes	Yes	Inf	Inf	Inf	Inf	NA	NA	NA	-NA	NA	NA
A	hsa-miR-96-4373372	No	No	28.1	28.5	28.7	28.7	28.7	-0.05	1.28	-1.56	no	-1.04
A	hsa-miR-98-4373009	Yes	Yes	26.0	Inf	Inf	Inf	NA	NA	NA	-NA	NA	NA
B	hsa-miR-99a*-4395252	No	No	27.1	26.1	27.2	26.3	26.7	0.82	1.06	-1.29	no	1.77
A	**hsa-miR-99a-4373008**	**No**	**No**	**18.8**	**16.9**	**17.8**	**17.2**	**17.5**	**0.61**	**0.37**	**-0.49**	**yes**	**1.53**
B	hsa-miR-99b*-4395307	No	No	24.7	24.3	24.5	24.5	24.5	0.03	0.84	-1.04	no	1.02
A	hsa-miR-99b-4373007	No	No	18.4	17.7	18.0	18.0	18.0	0.08	0.40	-0.51	no	1.06

Plate	Detector	Flag CF	Flag IF	Raw Cq		Norm. Cq		median Cq	log RQ	threshold		Ex-treme	FC
				CF	IF	CF	IF			high	low		
A	MammU6-4395470	No	No	15.2	12.4	12.4	12.6	12.5	-0.24	0.20	-0.29	no	-1.18
A	MammU6-4395470	No	No	15.3	13.2	12.5	12.4	12.5	0.09	0.20	-0.29	no	1.06
A	MammU6-4395470	No	No	15.3	12.2	12.5	12.6	12.5	-0.04	0.21	-0.29	no	-1.03
A	**MammU6-4395470**	**No**	**No**	**16.4**	**12.4**	**13.8**	**13.4**	**13.6**	**0.39**	**0.23**	**-0.32**	**yes**	**1.31**
B	MammU6-4395470	No	No	15.5	12.8	12.8	12.8	12.8	-0.05	0.21	-0.30	no	-1.04
B	MammU6-4395470	No	No	15.5	13.0	12.8	12.9	12.9	-0.05	0.21	-0.30	no	-1.03
B	MammU6-4395470	No	No	15.7	12.7	12.9	13.0	13.0	-0.07	0.22	-0.30	no	-1.05
B	MammU6-4395470	No	No	15.7	12.7	13.0	13.2	13.1	-0.21	0.22	-0.30	no	-1.16
B	RNU24-4373379	No	No	20.1	19.4	19.8	19.6	19.7	0.12	0.49	-0.62	no	1.09
B	RNU24-4373379	No	No	20.1	19.4	19.8	19.7	19.8	0.09	0.49	-0.63	no	1.07
B	RNU24-4373379	No	No	20.2	19.5	19.9	19.7	19.8	0.18	0.49	-0.63	no	1.13
B	RNU24-4373379	No	No	20.2	19.5	19.9	19.6	19.7	0.26	0.49	-0.62	no	1.20
B	RNU43-4373375	No	No	22.0	21.9	21.7	21.7	21.7	0.05	0.62	-0.77	no	1.04
B	RNU43-4373375	No	No	22.0	21.9	21.8	22.1	21.9	-0.33	0.63	-0.79	no	-1.26
B	RNU43-4373375	No	No	22.1	21.9	21.9	22.1	22.0	-0.19	0.63	-0.79	no	-1.14
B	RNU43-4373375	No	Yes	22.1	21.5	21.9	22.1	22.0	-0.16	0.64	-0.80	no	-1.12
A	RNU44-4373384	No	No	17.4	16.6	17.2	16.9	17.0	0.31	0.35	-0.46	no	1.24
B	RNU44-4373384	No	No	17.9	17.7	17.4	17.6	17.5	-0.20	0.37	-0.49	no	-1.15
B	RNU44-4373384	No	No	18.0	17.4	17.5	17.6	17.5	-0.06	0.38	-0.49	no	-1.04
B	RNU44-4373384	No	No	18.0	17.4	17.6	17.6	17.6	0.05	0.38	-0.49	no	1.03
B	RNU44-4373384	No	No	18.4	17.4	17.9	17.9	17.9	0.04	0.39	-0.51	no	1.03
A	RNU48-4373383	No	No	15.3	13.7	14.1	14.0	14.0	0.11	0.25	-0.33	no	1.08
B	RNU48-4373383	No	No	15.7	14.2	14.5	14.6	14.5	-0.09	0.26	-0.35	no	-1.07
B	RNU48-4373383	No	No	15.7	14.4	14.6	14.6	14.6	0.03	0.26	-0.35	no	1.02
B	RNU48-4373383	No	No	15.7	14.5	14.6	14.4	14.5	0.21	0.26	-0.35	no	1.15
B	RNU48-4373383	No	No	15.8	14.4	14.6	14.7	14.7	-0.01	0.26	-0.36	no	-1.01
B	RNU6B-4373381	No	No	22.1	22.0	21.9	22.2	22.0	-0.31	0.64	-0.80	no	-1.24
B	RNU6B-4373381	No	No	22.1	22.2	21.9	22.1	22.0	-0.15	0.64	-0.80	no	-1.11
B	RNU6B-4373381	No	No	22.2	22.1	22.0	22.4	22.2	-0.33	0.65	-0.82	no	-1.26
B	RNU6B-4373381	No	No	22.5	21.9	22.3	22.3	22.3	0.04	0.66	-0.82	no	1.02

MicroRNA profiling of n-6/n-3 LCPUFA intervention group compared to the control group in female placentas (IF vs CF, pool of n=3 in each analysis group). The microRNA profiling was conducted on two plates, depicted as plate A or B. The columns Flag CF and Flag IF indicate whether there was a problem in the amplification of the RT-qPCR. No = no problem, yes = flagged, problem in the amplification (often flagged when there is no amplification). The raw Cq values and the normalised Cq (norm. Cq) after loess normalisation are shown. Median Cq was calculated from normalised Cq values. Log RQ was calculated by (norm. Cq IF – norm. Cq CF). The high and low thresholds were calculated with quantile regression with a quadratic model. LogRQ values below the 5^{th} and above the 95^{th} percentile were were marked with yes in the column extreme. LogRQ values within the 5^{th}-95^{th} percentile were marked with no in the colum extreme. LogRQ values below th 5^{th} and 95^{th} were considered to be putatively regulated. Fold changes (FC) were calculated by 2^{logRQ} or -2^{logRQ} (in case of negative logRQ). Inf = infinite, NA = not applicable