TECHNISCHE UNIVERSITÄT MÜNCHEN

Lehrstuhl für Ernährungsmedizin

The effect of reducing the n-6/n-3 fatty acid ratio during pregnancy and lactation on maternal and fetal adipokines

Daniela Schmid

Vollständiger Abdruck der von der Fakultät Wissenschaftszentrum Weihenstephan für Ernährung, Landnutzung und Umwelt der Technischen Universität München zur Erlangung des akademischen Grades eines

Doktors der Naturwissenschaften

genehmigten Dissertation.

Vorsitzender: Univ.-Prof. Dr. M. Klingenspor

Prüfer der Dissertation:

1. Univ.-Prof. Dr. J. J. Hauner
2. apl. Prof. Dr. J. Kratzsch, Universität Leipzig
   (nur schriftliche Beurteilung)
3. Priv.-Doz. Dr. Th. Skurk (mündliche Prüfung)

Die Dissertation wurde am 06.04.2011 bei der Technischen Universität München eingereicht und durch die Fakultät Wissenschaftszentrum Weihenstephan für Ernährung, Landnutzung und Umwelt am 23.08.2011 angenommen.

Summary

During recent years nutritional programming of fetal development and its long-term health consequences has become an important issue. Animal data suggest that the dietary ratio of n-6/n-3 polyunsaturated fatty acids (PUFAs) during pregnancy is critical for early adipose tissue growth. Moreover, it was shown in animals that the impact of changes in the n-6/n-3 PUFA ratio in the maternal diet not only affects body weight and adipose tissue growth of their offspring but also modifies their adipokine profile and has a long-lasting effect on metabolic parameters in later life. To prove these observations in humans randomized controlled intervention studies are required.

The INFAT- ("The ***I***mpact of ***N***utritional ***F***atty acids during pregnancy and lactation for early human ***A***dipose ***T***issue development") study conducted at the Else Kröner-Fresenius centre of the Technische Universität München is the first study to investigate in a randomized controlled study design the impact of a reduced ratio in n-6/n-3 long-chain polyunsaturated fatty acids (LC-PUFAs) in the maternal diet on adipose tissue development of the infant. The intervention group received a supplementation with docosahexaenoic acid (DHA)/eicosapentaenoic acid (EPA) and reduced the dietary arachidonic acid (AA) intake. The women of the control group kept a diet according to the guidelines of the Deutsche Gesellschaft für Ernährung (DGE 2004) for a healthy diet during pregnancy and lactation.

The aim of this thesis was a) to investigate in a subgroup of the INFAT-study population the effect of a reduction in the ratio of n-6/n-3 LC-PUFAs in the maternal diet during pregnancy and lactation on adipokines and insulin in maternal plasma (n = 41/41 Intervention/Control) and breast milk (n = 40/38 Intervention/Control) as well as in cord blood (n = 55/53 Intervention/Control) b) to find in an explorative approach new biomarkers in cord blood using the Multi-Analyte profiling (MAP) technology by Rules based Medicine Inc. (RBM, Texas, USA) and performing a validation analysis of two interesting candidates using the Enzyme Linked Immunosorbent Assay (ELISA) method.

In a longitudinal analysis maternal leptin, soluble leptin receptor (sOB-R), free leptin index (FLI) and high molecular weight (HMW) adiponectin concentrations were

measured in plasma samples at the time points 15th and 32nd week of gestation, 6th week and 16th week post partum (PP) and in breast milk samples at the time points 6th week PP and 16th week PP. Furthermore, data of maternal plasma insulin concentrations, homeostasis model assessment for insulin resistance (HOMA-IR) and triacylglycerols (TAGs), total cholesterol, high density lipoprotein (HDL)-cholesterol and low density lipoprotein (LDL)-cholesterol levels were analyzed at the same time points. Correlations between the adipokines analyzed and blood lipids during pregnancy were calculated. In cord blood, leptin, sOB-R, FLI, HMW adiponectin and insulin concentrations were determined. The clinical parameters were measured in plasma samples under standardized conditions using ELISAs and radioimmunoassays (RIAs). The statistical analysis including defined inclusion criteria is based on tests of distribution and correlation models.

To discover novel biomarkers in fetal plasma which might be affected by the maternal dietary intervention, 86 metabolic and inflammatory biomarkers were determined in an explorative analysis by RBM Inc. using a Multi-Analyte Profiling (MAP) approach. As interesting candidates, agouti-related protein (AgRP) and brain-derived neurotrophic factor (BDNF) -both involved in hypothalamic energy regulation- were then validated in cord blood plasma by ELISA.

The measurement data evaluation and the statistical analysis showed no difference in the concentrations of maternal leptin, sOB-R, FLI, HMW adiponectin, insulin and HOMA-IR between the intervention and the control group at each time point during pregnancy and lactation. Furthermore, there was no group effect in leptin and total adiponectin concentrations in breast milk. Significant correlations between maternal adipokines and blood lipids at the time point 32th week of gestation were observed. Cord blood leptin, sOB-R, FLI and HMW adiponectin concentrations did not differ significantly between the groups ($P > 0.05$), whereas fetal insulin concentrations tended to be lower in the intervention group compared with the control group ($P = 0.06$). This difference was significant after exclusion of gestational mellitus diabetes (GDM) pregnancies and extreme outliers ($P = 0.013$).

Plasma BDNF concentrations appear to be lower in the girls of the intervention group than in the girls of the control group, whereas in boys the opposite result was observed. However, the group effect was statistically not significant, neither in girls nor in boys. Furthermore, AgRP levels did not significantly differ between the groups.

In conclusion, this study could not demonstrate a significant effect of a reduction in dietary n-6/n-3 LC-PUFA intake during pregnancy and lactation on maternal and cord blood adipokines and insulin. Further studies on dietary n-6 and n-3 LC-PUFAs during pregnancy and lactation are of great interest to explore their roles on infant adipokines and metabolic parameters in the perinatal period, but also to extend their investigations in the long-term follow-up of the infants.

Zusammenfassung

In den letzten Jahren konnte zunehmend gezeigt werden, dass die Ernährung einer schwangeren Frau die Entwicklung des Föten und die Gesundheit des Kindes, sogar bis in das Erwachsenenalter beeinflussen kann. An Tiermodellen wurde gezeigt, dass der n-6/n-3 Fettsäure-Quotient in der mütterlichen Ernährung während der Schwangerschaft und Laktationsperiode einen Einfluss auf die Fettgewebsentwicklung der Nachkommen hat. Ferner wurde in tierexperimentellen Studien gezeigt, dass eine Veränderung des n-6/n-3 Fettsäure-Quotienten in der mütterlichen Ernährung das Adipokinprofil bei den Nachkommen beeinflusst und Veränderungen im Metabolismus im Erwachsenenalter zur Folge haben kann. In diesem Kontext sind Humanstudien wünschenswert, um die Beobachtungen am Tiermodell auf den Menschen übertragen zu können.

Die INFAT- („The ***I***mpact of ***N***utritional ***F***atty acids during pregnancy and lactation for early human ***A***dipose ***T***issue development“) Studie ist die erste Humanstudie, die in einem randomisierten kontrollierten Ansatz den Einfluss einer Senkung des Verhältnisses der langkettigen mehrfach ungesättigten n-6/n-3 Fettsäuren (long-chain polyunsaturated fatty acids (LC-PUFAs)) in der mütterlichen Ernährung während der Schwangerschaft und Stillzeit auf die Fettgewebsentwicklung bei den Neugeborenen untersucht. Die Interventionsgruppe wurde mit Docosahexaensäure (DHA) und Eicosapentaensäure (EPA) supplementiert bei gleichzeitiger Einschränkung der Aufnahme der Arachidonsäure (AA) über die Ernährung. In der Kontrollgruppe ernährten sich die Frauen nach den Richtlinien der Deutschen Gesellschaft für Ernährung für eine gesunde Ernährung (DGE 2004) in der Schwangerschaft und Stillzeit.

Das Ziel dieser Arbeit war es in einer Subgruppe der INFAT- Studie a) den Einfluss der mütterlichen n-6/n-3 LC-PUFA Intervention auf den Verlauf der Konzentrationen von verschiedenen Adipokinen im mütterlichen Blut (n = 41/41 Intervention/Kontrolle) und Muttermilch (n = 40/38 Intervention/Kontrolle) während der Schwangerschaft und Stillperiode sowie im Nabelschnurblut zu untersuchen (n = 55/53 Intervention/Kontrolle) b) die Entdeckung neuer Biomarker in einem explorativen Ansatz. Dazu wurden Daten des Multi-Analyte Profilings (MAP) der Firma Rules

based Medicine Inc. (RDM) ausgewertet und zwei Kandidaten mittels Enzyme Linked Immunosorbent Assay (ELISA) validiert.

In mütterlichen Plasmaproben wurden Leptin/löslicher Leptinrezeptor (sOB-R), Free Leptin Index (FLI) und high molecular weight (HMW) Adiponektin in einer longitudinalen Analyse in der 15. und 32. Schwangerschaftswoche sowie in der 6. und 16. Woche post partum (PP) bestimmt. In der Muttermilch wurden Leptin und Adiponektin zu den Zeitpunkten 6. und 16. Woche PP bestimmt. Daten zu Insulin, homeostasis model assessment for insulin resistance (HOMA-IR) und Triacylglycerine (TAGs), Gesamtcholesterin, high density lipoprotein (HDL)-Cholesterin, low density lipoprotein (LDL)-Cholesterin wurden zu den gleichen Zeitpunkten in den Plasmaproben analysiert. Korrelationen zwischen den mütterlichen Adipokinen und Blutlipiden wurden berechnet. In den Nabelschnurblutproben wurden Konzentrationen von Leptin, sOB-R, FLI und HMW Adiponektin gemessen. Die Messungen wurden mittels ELISA oder Radioimmunoassay (RIA) unter standardisierten Bedingungen durchgeführt. Für die statistische Auswertung wurden unter definierten Einschlusskriterien Vergleichs- und Korrelationsanalysen durchgeführt.

Zur Identifizierung von neuen fötalen Biomarkern, die möglicherweise durch die n-6/n-3 LC-PUFA Intervention reguliert werden, wurden in einem explorativen Ansatz 86 metabolische und inflammatorische Marker im Nabelschnurblut gemessen. Anschließend wurden die Agouti-related protein (AgRP)- und brain-derived neurotrophic factor (BDNF)- Messungen mittels ELISAs validiert.

Bei Leptin, sOB-R, FLI, HMW Adiponektin, Insulin und HOMA-IR gab es keine signifikanten Unterschiede zwischen der Interventions- und Kontrollgruppe während der Schwangerschaft und Stillzeit. Ferner wurde kein Unterschied zwischen den Gruppen bezüglich Leptin und Adiponektin in der Muttermilch während der Laktationsperiode festgestellt. Signifikante Korrelationen zwischen den mütterlichen Adipokinen und Blutlipiden in der 32. Schwangerschaftswoche wurden beobachtet.

Im Nabelschnurblut wurde kein Unterschied in den Konzentrationen von Leptin, sOB-R, FLI, HMW Adiponektin zwischen den beiden Gruppen beobachtet ($P > 0.05$). Die Interventionsgruppe zeigte einen Trend zu niedrigeren Insulinwerten verglichen mit der Kontrollgruppe ($P = 0.06$). Nach Ausschluss von Schwangerschaften mit Gestationsdiabetes mellitus (GDM) und extremen Ausreißern war dieser Effekt auf

Insulin statistisch signifikant ($P = 0.013$). Bei den Mädchen wurden niedrigere Plasma BDNF- Konzentrationen in der Interventionsgruppe im Vergleich zur Kontrollgruppe nachgewiesen, während bei den Jungen die Plasma BDNF-Konzentrationen in der Interventionsgruppe höher waren als in der Kontrollgruppe. Allerdings war der Unterschied zwischen den Gruppen bei beiden Geschlechtern nicht signifikant. Bei den Plasma AgRP-Konzentrationen war kein signifikanter Unterschied zwischen den beiden Gruppen zu erkennen.

Zusammenfassend konnte gezeigt werden, dass eine Reduktion des n-6/n-3 Fettsäure-Quotienten in der mütterlichen Ernährung in der Schwangerschaft und Stillzeit keinen wesentlichen Einfluss auf Leptin, sOBR und FLI, HMW Adiponektin- und Insulin im mütterlichen und fötalen Plasma hat. Weitere Studien sind erforderlich, um neue Erkenntnisse über die Bedeutung einer Modifikation des n-6/n-3 Fettsäuren-Quotienten in der mütterlichen Ernährung in der Schwangerschaft und Stillperiode für die kindliche Adipokinsekretion zu gewinnen.

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Abbreviations

AA	Arachidonic acid (C20:4n-6)
ADIPOQ	Adiponectin gene
AgRP	Agouti-related protein
ALA	α-linolenic acid (C18:3n-3)
AMP	adenosine monophosphate-activated protein
ARA	Arachidonic acid
AT	Adipose tissue
BAT	Brown adipose tissue
BF	Body fat
BMI	Body mass index
BDNF	Brain derived neurotrophic factor
BSA	bovine serum albumin
BW	Birth weight
cAMP	Cyclic adenosine monophosphate
CART	cocaine and amphetamine regulated transcript
C/EBP	CCAAT/enhancer-binding protein
CG	Control group
CNS	Central nervous system
COX	cyclooxygenase
DGE	Deutsche Gesellschaft für Ernährung
DHA	Docosahexaenoic acid (C22:6n-3)
DHyLA	dihomo gamma linolenic acid (C20:3n-6)
EDTA	Ethylenediamine tetraacetic acid
ELISA	enzyme-linked immunosorbent assay

EPA	Eicosapentaenoic acid (C20:5n-3)
EFA	essential fatty acids
FA	Fatty acid
FAME	fatty acid methyl ester
fAd	full-length adiponectin
FFA	Free fatty acids
FLI	free leptin index
fAd	full- length adiponectin
FFM	Fat free mass
FM	Fat mass
GDM	Gestational diabetes mellitus
gest	gestation
GLC	Gas liquid chromatography
gAd	gobular adiponectin
GWG	Gestational weight gain
HCS	human chorionic somatomammotropin
HDL	High density lipoprotein
HMW	high molecular weight
HMW-Ad	high molecular weight adiponectin
5-HPETE	(arachidonic acid 5-hydroperoxide)
HPLC	high pressure liquidchromatography
5- HT_{2C}	Subtype 2C serotonin receptor
IG	Intervention group
IGF-1	Insulin-like growth factor 1
IRS-1	insulin receptor substrat
IOM	Institute of Medicine (USA)
JAK-STAT	Janus kinase-signal transducers and activators of transcription

LA	Linoleic acid (C18:2n-6)
LBM	Lean body mass
LIFA	ligand- immunofunctional assay
LC-PUFA	Long-chain polyunsaturated fatty acid
LDL	Low density lipoprotein
LGA	Large for gestational age
LMW	Low molecular weight
LOX	lipoxygenase
LPL	lipoprotein lipase
MAP	Multi-Analyte Profiling
MAPK	mitogen-activated protein kinase
MCH	Melanin-concentrating Hormone
MC4R	Melanoncortin 4 receptor
MMW	Medium molecular weight
MRI	Magnetic resonance imaging
mRNA	messenger RNA
α- MSH	melanocyte-stimulating hormones
NEFA	non-esterified fatty acids
NGT	normal glucose tolerance
NPY	Neuropeptid Y
PGI2	Prostacyclin
PGE_2	Prostaglandin E_2
$PGF_{2\alpha}$	Prostaglandin $F_{2\alpha}$
PI3’K	phosphatidylinositol 3’-kinase
PI	Ponderal Index
PLA2	phospholipase A2
PL	Phospholipid

POMC	Proopiomelanocortin
PP	Post partum
PPAR	Peroxisome proliferator-activated receptor
PTP	protein tyrosinephosphatase
PUFA	Polyunsaturated fatty acid
RBC	Red blood cells
RIA	radioimmunoassay
SD	Standard deviation
SFA	Saturated fatty acid
SFT	Skinfold thickness
SGA	Small for gestational age
SREBP-1c	sterol regulatory element-binding protein 1c
SNP	Single nucleotide polymorphism
sOB-R	soluble leptin receptor
SOCS-3	suppressor of cytokine signalling
T-Ad	total adiponectin
TAG	Triacylglycerid
TFA	Trans fatty acid
TNFα	Tumour necrosis factor α
TLR	Toll like receptors
UCP-1	uncoupling protein-1
VLDL	Very low densitiy lipoprotein
VMN	ventromedial nucleus
WAT	White adipose tissue
WHO	World Health Organization
wk	Week

1 Introduction

The prevalence of overweight and obesity among children and adolescence has increased continuously during the last two decades (Lobstein and Frelut 2003). In Germany 15% of children between the ages of 3 and 17 years are overweight and 6.3 % are obese (Kurth and Schaffrath Rosario 2007). Recently, it has been shown that perinatal period is a vulnerable period for programming of disease in later life by external factors (Nijland, Ford and Nathanielsz 2008). Influences from maternal diet during this early development time window are suggested to be critical for adipose tissue development (Ailhaud et al. 2006). In animal studies, it was observed that a high ratio of dietary n-6/n3- fatty acids (FAs) (n-6/n-3 ratio 59:1) in mice during pregnancy and lactation increased body weight and fat mass in their offspring compared with the offspring of mice receiving a diet with a low ratio of n-6/n-3 FAs (n-6/n-3 ratio 2:1) (Massiera et al. 2003). Furthermore, bioactive proteins, known as adipokines, secreted from adipose tissue which have a major role in coordinating a variety of biological processes (Kershaw and Flier 2004) were shown to be influenced by the maternal n-6/n-3 FA ratio in early life (Korotkova et al. 2002, Korotkova et al. 2005). Human intervention studies are required to clarify the role of maternal n-6/n-3 FA ratio during pregnancy and lactation on adipose tissue development, adipokine secretion and other metabolic parameters in the infants.

1.1 Physiological adaptations and metabolism during pregnancy

Maternal physiological changes can be divided into two parts: those occurring in the first half of pregnancy and those of the second half of pregnancy. Physiological changes in the first half of pregnancy are considered as maternal anabolic changes, because they build the capacity of the maternal body to deliver an adequate amount of nutrients to the fetus in the second half of pregnancy (Lain and Catalano 2007). The second half is a time of maternal catabolic changes in which the increased capacity to deliver stored energy and nutrients to the fetus, predominate (King 2000, Lain and Catalano 2007).

1.1.1 Energy requirement and gestational weight gain

Pregnant women require additional energy and nutrients to cover the demands for maternal and fetal tissue growth, and the increase in energy expenditure attributable to basal metabolism and physical activity (Butte et al. 2004). Gestational weight gain (GWG) is an important determinant of the increasing energy needs and comprises the product of conception (amniotic fluid, the placenta and the fetus) and maternal growth of tissues (expansion of blood and extracellular fluid, enlargement of uterus and maternal fat stores) (Butte et al. 2004). Low weight gain is related to higher risk of preterm delivery (Abrams, Altman and Pickett 2000). High weight gain is associated with increased risk of complications during pregnancy and at delivery and with high birth weight (Cedergren 2006, Kiel et al. 2007, DeVader et al. 2007, Cnattingius et al. 1998). Recommendations for weight gain during pregnancy are based on an optimal outcome for the mother and adequate development and growth of the fetus, and on the prevention of gestational and perinatal morbidity and mortality. The re-examined IOM (1990) guidelines for weight gain during pregnancy are shown as a range for each category of pre-pregnancy body mass index (BMI) based on the World Health Organization (WHO) cut-off points for BMI categories. It recommends different ranges of GWG for women with low BMI (< 18.5 kg/m²: 12.5 - 18 kg), normal BMI (18.5 - 24.9 kg/m²: 11.5 - 16 kg) and high BMI (overweight: BMI 24.9 – 29.9 kg/m²: 7 - 11.5 kg or obese: BMI > 29.9 kg/m²: 5 - 9 kg) (Rasmussen and Yaktine 2009).

1.1.2 Metabolic changes during pregnancy

During pregnancy physiologic changes affect the metabolism of all nutrients (King 2000). Changes in carbohydrate and lipid metabolism occur to maintain an adequate supply of energy to the fetus. In early pregnancy, maternal fat stores are increased, and basal endogenous hepatic glucose production rises during pregnancy (Catalano et al. 1999, Lain and Catalano 2007). Late gestation is characterized by insulin resistance (Zavalza-Gomez et al. 2008). Maternal adipose tissue depots decrease, whereas free fatty acid (FFA) levels increase and insulin-mediated glucose disposal is decreased by 40-60% (Catalano et al. 1999). Placental-derived hormones are considered as important factors in alteration of maternal physiology to maintain an insulin-resistant state (Barbour et al. 2007). However, synergy effects with other obesity or pregnancy-related factors such as cytokines and adipokines should also

be considered during the development of gestational insulin resistance (Zavalza-Gomez et al. 2008). Impaired glucose tolerance (IGT) and gestational diabetes mellitus (GDM) are common diseases occurring in pregnancy (Berger et al. 2002, Landon 2010).

Alterations in hepatic and adipose tissue metabolism during pregnancy lead to an increase in concentrations of triacylglycerols (TAGs), total cholesterol, FFA, cholesterolester and phospholipids (PLs) in pregnancy in the first two months of gestation (Darmady and Postle 1982, Butte 2000). The changes in total cholesterol concentrations reflect changes in lipoprotein fractions. In early pregnancy, there is a decrease in total and low density lipoprotein (LDL)-cholesterol but with advancing gestation the levels increase. Elevated high density lipoprotein (HDL)-cholesterol levels were reported in the second trimester in pregnancy (Mankuta et al. 2010). After an initial decrease very low density lipoprotein (VLDL) levels increase continuously until term. In the second half of pregnancy, VLDL clearance is altered by reduced lipoprotein lipase (LPL) activity in the adipose tissue and liver and, in the placenta, its activity is increased (Butte 2000). Additionally, the fatty acid pattern of the blood lipids alters during pregnancy. The proportion of total saturated fatty acids in maternal plasma increases during pregnancy, linoleic acid (LA; C18:2n-6), eicosapentaenoic acid (EPA, C:20:5n-3) and oleic acid (C18:1n-9) remain unchanged, and the proportion of arachidonic acid (AA; C20:4n-6) and docosahexaenoic acid (DHA, C22:6n-3) decreases throughout pregnancy (Herrera et al. 2004).

Alterations in lipid metabolism promote increase of maternal fat stores in early and mid pregnancy and enhance fat mobilization in late pregnancy (Butte 2000). During the first and second trimester, increased estrogen, progesterone, and insulin concentrations are considered to favor lipid deposition and inhibit lipolysis. In late gestation, human chorionic somatotropin (HCS) promotes lipolysis and fat mobilization. This metabolic shift promotes the use of lipids as a maternal energy source while maintaining glucose and amino acids for the fetus (Butte 2000).

Shifts in protein metabolism occur throughout gestation to provide nitrogen for fetal growth during the last quarter of pregnancy (King 2000). Denne et al. (1991) showed the complexity of metabolic adjustments in nitrogenous compounds with the use of

nitrogen and carbon stable isotopes, and Calloway (1974) summarized the results of 17 nitrogen balance studies (Calloway 1974, Denne, Rossi and Kalhan 1991).

1.2 Long-chain polyunsaturated fatty acids and formation of their metabolites

Long-chain polyunsaturated fatty acids (LC-PUFAs) are essential FAs and have to be provided by diet. LA is a precursor for the synthesis of the n-6 LC-PUFA AA and α-linolenic acid (ALA; 18:3 n-3) for synthesis of the n-3 LC-PUFAs DHA and EPA (Herrera 2002). Through alternate elongation and desaturation steps in the endoplasmatic reticulum they are converted into their long-chain, unsaturated metabolites. LA and ALA compete for the same enzyme Δ6 desaturase and the enzymatic affinity is higher for n-3 fatty acids (FAs) than for n-6 FAs (Flachs et al. 2009). An excessive amount of LA slows down the formation of EPA and DHA, but even without this inhibitory effect the synthesis of EPA and DHA from ALA is low (Gerster 1998, Emken, Adlof and Gulley 1994). EPA, DHA, ALA and AA are incorporated into cellular membranes and bind to the sn-2 position in the phospholipid molecule (Flachs et al. 2009). Many effects of LC-PUFAs depend on the formation of their active metabolites, eicosanoids and other lipid mediators. These molecules are produced after the release of LC-PUFAs from phospholipids by phospholipase A2 (PLA2) (Figure 1) and exert different tissue-dependent biological effects (Flachs et al. 2009). The activity of cyclooxygenases (COXs) and peroxidases on AA leads to the synthesis of the 2-series prostanoids (prostaglandins, prostacyclins and thromboxane), and the activity of 5-lipoxygenase (5-LOX) leads to the formation of arachidonic acid 5-hydroperoxide (5-HPETE), which produces the 4-series leukotrienes (Figure 1) (Russo 2009). EPA and DHA are converted by the same enzymes COX and LOX to the 3-series prostanoids (prostaglandins E3, prostacyclin I3 and thromboxane A3), and 5-series leukotrienes (Jump 2002, Russo 2009). In general, eicosanoids derived from n−3 PUFAs exert mainly anti-inflammatory effects, whereas eicosanoids derived from n−6 PUFAs promote inflammation (James, Gibson and Cleland 2000). Moreover, novel families of lipid mediators derived from EPA and DHA, have anti-inflammatory pro-resolving effects, and protect against tissue damage (Schwab et al. 2007).

In vitro, AA is a precursor of prostacyclin (PGI_2), a prostaglandin which promotes adipogenesis (Massiera et al. 2003). DHA and AA which are important structural components in the membrane lipids of the human central nervous system (CNS) and retinal cell membrane structure are important for visual function and cognitive development (Brenna and Lapillonne 2009, Fernstrom 2000).

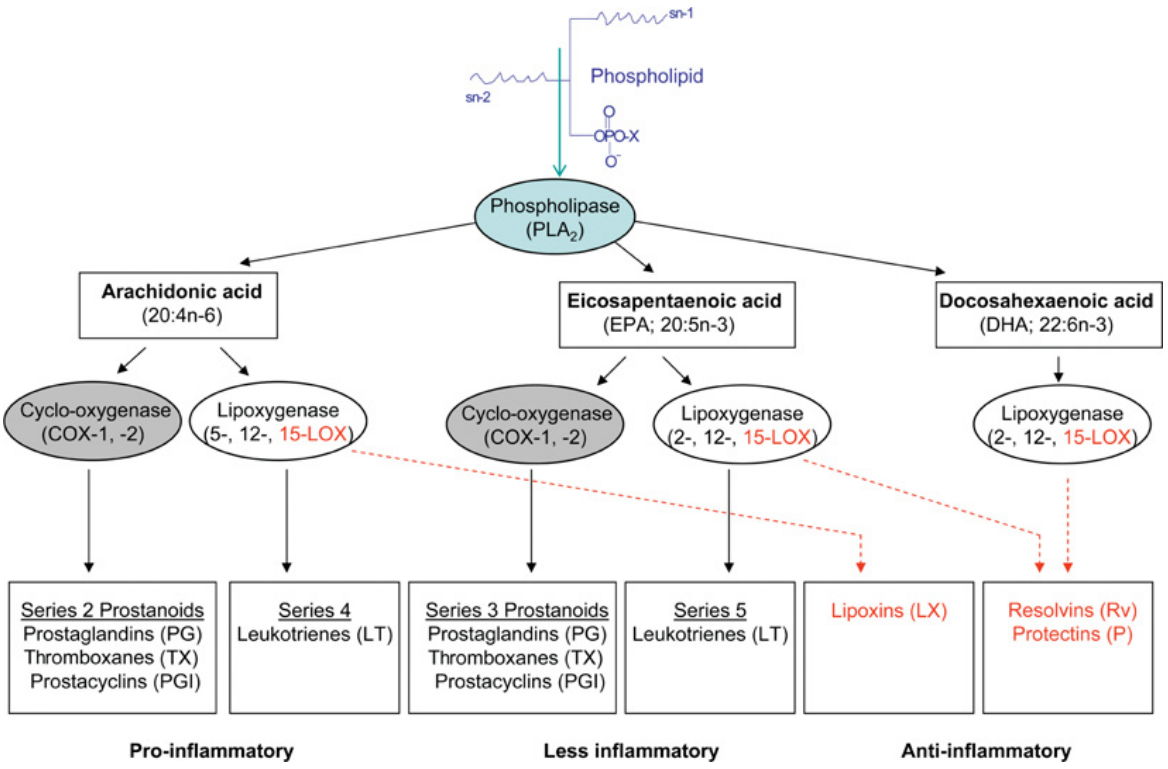


Figure 1 Metabolites derived from PUFAs AA, EPA and DHA (Flachs et al. 2009)

1.3 Human adipose tissue

1.3.1 PUFAs and adipocyte differentiation

First adipocytes in the human fetus have been demonstrated between the 14th and 16th week of gestation (Poissonnet, Burdi and Bookstein 1983, Burdi et al. 1985).

The percentage of total body fat in full-term newborns has been reported to be about 15-16% (Picaud et al. 1996, Harrington et al. 2002). In full-term newborns nearly 90% of adipose tissue was observed in the subcutaneous region and 4% in the visceral region using Magnetic Resonance Imaging (MRI) method (Harrington et al. 2002).

Poissonet et al. (1983) studied early prenatal fat development in human embryos and fetuses and suggested that the timeframe between the 14th and 23rd week constitutes a sensitive period in fat lobule development, and that disturbances in normal adipogenesis during this period may play a role in the etiology of obesity in later life (Poissonnet et al. 1983). The contribution of hyperplasia and hypertrophy to adipose tissue development was explored in several studies (Hauner 1989, Knittle, Ginsberg-Fellner and Brown 1977, Baum et al. 1986).

Recently, it was shown that the composition of food intake in early life may affect early adipose tissue development. Animal data have suggested a role of the n-6/n-3 FA ratio for early adipose tissue growth (Azain 2004). Wild-type mice were fed with either a high-fat diet rich in LA or a diet enriched in LA and ALA during gestation and the suckling period leading to a n-6/n-3 FA ratio of 59/1 and 2/1, respectively. From weaning onwards body weight and fat mass at 8 weeks of age were higher in pups with the LA diet compared to the LA/ALA diet (Massiera et al. 2003).

Data from *in vitro* studies confirmed the adipogenic effect of AA (Massiera et al. 2003). Several transcription factors from the Peroxisome proliferator-activated receptor (PPAR) and CCAAT/enhancer-binding protein (C/EBP) families are involved in the adipocyte differentiation process (Morrison and Farmer 2000, Rosen et al. 2000). AA up-regulates the expression of CCAAT/enhancer binding protein β (C/EBPβ) and C/EBPδ via the prostacyclin/IP receptor system and the protein kinase A (PKA) pathway (Belmonte et al. 2001). C/EBPβ and C/EBPδ up-regulate the expression of PPARγ known to be crucial for adipogenesis (Wu et al. 1995, Rosen et al. 1999). AA may also act through its COXs -synthesized metabolites, namely prostacyclin as activator/ligand of PPAR β/δ which up-regulates the expression of PPARγ (Figure 2) (Matsusue, Peters and Gonzalez 2004). Thus, AA via prostacyclin activates the PKA pathway through stimulation of cyclic adenosine monophosphate (cAMP) production, and enhances the differentiation process (Massiera et al. 2003). In addition, AA metabolites synthesized through LOXs as ligands of PPARγ are also implicated in adipogenesis at later steps. EPA and DHA, which are inactive as cAMP-elevating mediators, inhibit the stimulatory effect of AA on cAMP production (Massiera et al. 2003).

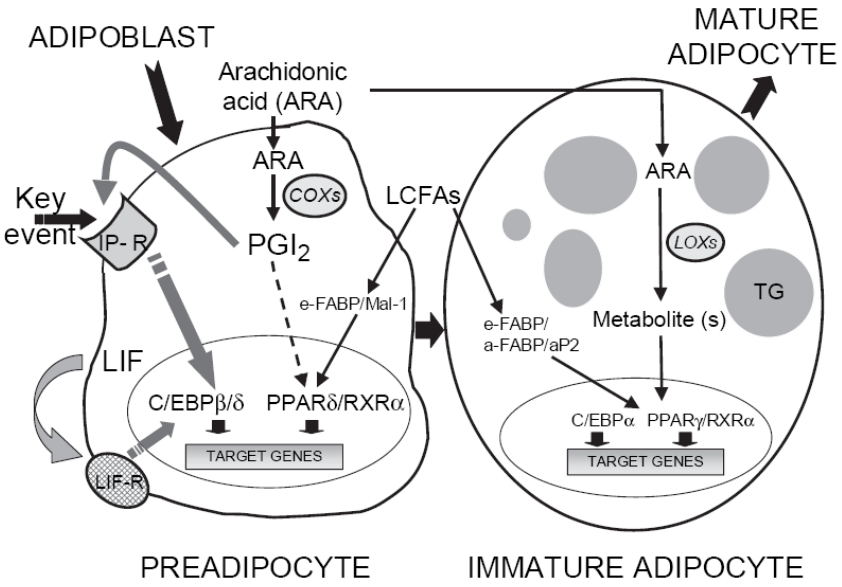


Figure 2 Pathways and PUFAs promoting adipogenesis (Ailhaud et al. 2006). Prostacyclin receptor (IP)/prostacyclin and leukaemia inhibitory factor (LIF) receptor/LIF, concur to up-regulate the expression of CCAAT/enhancer binding protein β/δ (C/EBPβ/δ) and to promote adipogenesis by dietary linoleic acid (LA) and/or arachidonic acid (ARA = AA). Furthermore, prostacyclin is assumed to bind to PPAR β/δ. In addition to AA metabolites synthesized through cyclooxygenases (COXs) at early step(s), involvement of AA metabolites synthesized through lipoxygenases (LOXs) as ligands of PPARγ is also implicated at later step(s). Epidermal (keratinocyte) fatty acid binding protein (e-FABP/Mal1) in preadipocytes and also adipocyte fatty acid binding protein (a-FABP/aP2) in adipocytes are assumed to bind and transport long-chain fatty acids (LCFAs); TG = triglycerides

1.3.2 Human adipose tissue as an endocrine organ

Adipogenesis is termed as the differentiation of precursor cells into mature, terminally-differentiated adipocytes (Smas and Sul 1995). Adipocytes derive from multipotent mesenchymal stem cells. In the first phase, known as determination, stem cells are converted to preadipocytes. In the second phase, which is known as terminal differentiation, the preadipocyte are differentiated into mature adipocytes. Several transciption factors are involved in the process and PPARγ and C/EBPs are

crucial transcription factors that promote adipogenesis (Rosen and MacDougald 2006).

There are two types of AT: brown AT (BAT) and white AT (WAT) which vary in their morphology and physiological functions. BAT regulates non-shivering thermogenesis, mediated by uncoupling protein-1 (UCP-1) in mitochondria, which are enriched in multilocular brown adipocytes (Cannon and Nedergaard 2004). In contrast, large unilocular adipocytes of WAT are filled with TAGs. The remaining stromal-vascular fraction of WAT comprises blood and endothelial cells, macrophages, fibroblasts, pericytes, fibroblasts, and AT precursor cells (Ailhaud et al. 2006).

The AT is not only a fat depot but also reflects a dynamic tissue which communicates with other tissues. WAT functions as an endocrine organ, integrating hormonal signals from different parts of the body in response to changes in energy balance and secreting a large number of various adipokines, bioactive proteins acting in an autocrine/paracrine and endocrine manner (Wang et al. 2008). Adipokines which have beneficial or deleterious effects on the homeostasis of the whole body, have an essential role in coordinating a variety of biological processes. Through the secretion of adipokines, WAT is involved in several functions such as the control of body temperature, immune response, bone mass, reproductive and thyroid functions (Flachs et al. 2009). The adipokines leptin (Chapter 1.4) and adiponectin (Chapter 1.5) as the most abundant adipocyte-derived proteins are capable to act via the blood circulation on the regulation of several metabolic processes and are linked with the development of obesity-associated co-morbidities (Tilg and Moschen 2006, Anghel and Wahli 2007, Fasshauer and Paschke 2003). Moreover, hypertrophic AT secretes various proinflammatory cytokines, chemokines and certain complement factors (Trayhurn and Wood 2004).

1.4 Leptin and leptin receptor

1.4.1 Biology and function of leptin

Leptin, encoded by the *LEP* gene, circulates in the blood as a protein of 146 amino acid residues (Zhang et al. 1994). It is mainly produced in WAT and circulating leptin levels in humans are strongly associated with fat mass (Frederich et al. 1995, Considine et al. 1996b, Banks 2004). There is some evidence that leptin is also produced in BAT (Klingenspor et al. 1996, Dessolin et al. 1997, Siegrist-Kaiser et al. 1997) and other tissues (Harris 2000, Challier et al. 2003, Ahima and Osei 2004). It is well known that leptin interacts with pathways in the CNS and through peripheral mechanisms (Margetic et al. 2002).

The leptin receptor OB-R belongs to the gp 130 family of cytokine class I receptors (Tartaglia et al. 1995). There are spliced isoforms (OB-Ra,-Rb,-Rc,-Rd,-Re and -Rf) which can be divided into long, short, and soluble isoforms (Gorska et al. 2010, Lee et al. 1996). The long isoform (OB-Rb) is the signalling isoform and transmits the leptin signal via the complete Janus kinase-signal transducers and activators of transcription (JAK-STAT) pathway (JAK 2 and STAT 3, 5 and 6) (Bjorbaek and Kahn 2004) (Figure 3). Upon leptin binding to OB-Rb and activation of Janus tyrosine kinases, the receptor provides a docking site for STAT (Baumann et al. 1996). STATs translocate to the nucleus and induce expression of other genes, including the suppressor of cytokine signalling 3 (SOCS)-3 (Bjorbaek et al. 1999) and the protein tyrosinephosphatase (PTP) 1B (Cheng et al. 2002). In addition to the JAK-2-STAT-3 pathway, the mitogen-activated protein kinase (MAPK), the insulin receptor substrat (IRS)-1, and the phosphatidylinositol 3'-kinase (PI3'K) pathways are also involved in leptin mediation (Bjorbaek and Kahn 2004). OB-R is not only sited in the hypothalamus, but is expressed ubiquitously (Frühbeck 2006). The function of the short isoform (OB-Ra) of the leptin receptor is less clear (Bjorbaek et al. 1997). It is ubiquitously present in human tissues and is proposed to transfer leptin across the blood-brain barrier (Bjorbaek and Kahn 2004). The OB-Re circulates as soluble receptor (sOB-R) which is thought to be the main leptin-binding activity in human blood (Lammert et al. 2001). Its physiological function in human circulation has not yet been fully elucidated, but the soluble form might act as a modulating factor of leptin action through different mechanisms (Sinha et al. 1996).

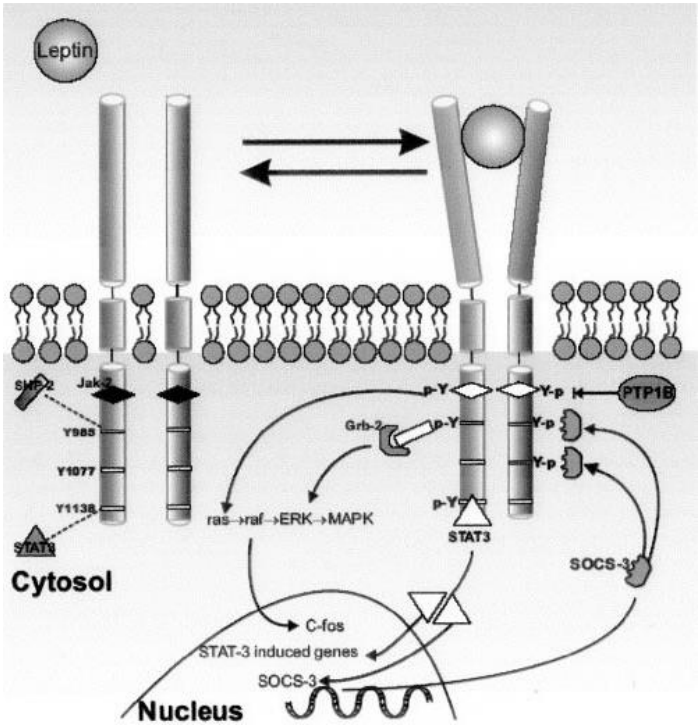


Figure 3 Neuronal signalling by the leptin receptor (Bjorbaek and Kahn 2004)

Well known is the neuroendocrine effect of leptin to regulate energy balance by its binding to leptin receptors in the hypothalamus (Ahima et al. 2000). Studies showed that administration of low doses of leptin into the brain ventricles of rodents reduced food intake and body weight (Campfield et al. 1995, Stephens et al. 1995). However, in overweight and obese subjects, leptin concentrations are higher than in normal weight subjects assuming leptin resistance under these conditions (Considine et al. 1996b, Hansen et al. 2010). The molecular mechanisms for leptin resistance in obesity remain to be determined.

DNA single nucleotide polymorphisms (SNPs) in the *LEP* gene may be associated with obesity. Sequence variants found in the 5' region of *LEP* gene were found to be related to obesity or to a reduction in BMI following a low calorie diet (Clement et al. 1998, Li et al. 1999, Mammes et al. 2000). Furthermore, several polymorphisms in the leptin receptor (*LEPR*) gene have been identified. Most studies on these *LEPR* polymorphisms were not able to show a major effect on body weight or fat mass

(Considine et al. 1996a, Rolland et al. 1998, Chung et al. 1997, Echwald et al. 1997, Heo et al. 2001).

In addition to the neuroendocrine effects of leptin in the control of food intake and energy expenditure, binding of this hormone in other tissues suggests additional pleiotropic functions of leptin. There is a line of evidence that supports a role for leptin in immune cells (Martin-Romero et al. 2000, Fernandez-Riejos et al. 2010, Matarese, Moschos and Mantzoros 2005). Furthermore, a number of studies of isolated pancreatic islets from rodents has shown that leptin can inhibit insulin secretion (Zhao, Feng and Chen 2006, Kieffer et al. 1997) although the opposite effect has been reported (Shimizu et al. 1997, Havel 2000). A plenty of studies have reported effects on intracellular signalling and metabolism in pancreatic cells, pituitary cells, kidney and insulin-sensitive cells like hepatocytes and muscle cells (Tuduri et al. 2009, Bjorbaek and Kahn 2004). There is a known link between leptin and reproductive function (Ahima et al. 1997, Donato et al. 2011). Furthermore, leptin stimulates angiogenesis and hematopoiesis and regulates the extent of bone formation (Harris 2000). An important aspect of leptin biology has been discovered with the finding that leptin is expressed in the placenta and ovary. This has been demonstrated in animals and humans (Ashworth et al. 2000, Spicer and Francisco 1997, Maymo et al. 2011).

1.4.2 Leptin during pregnancy

In pregnancy plasma leptin concentrations are increased in comparison to maternal pre-gravid levels and after delivery maternal leptin level declines rapidly to pregravid level (Helland et al. 1998, Schubring et al. 1998). The origin of the pregnancy induced rise in leptin is not known. The placenta is considered to be a main source for leptin synthesis and expresses high amounts of leptin messenger RNA (mRNA) and protein during pregnancy (Masuzaki et al. 1997, Henson, Swan and O'Neil 1998) which is identical to leptin expressed in adipose tissue (Senaris et al. 1997).

Linnemann et al. (2000) reported that only 1-2% of placental leptin enters the fetal circulation, while approximately 98% enters the maternal circulation (Linnemann et al. 2000). In addition, changes occur in the levels of other hormones that may influence leptin (Mantzoros 2000). It is suggested that the increase of leptin over pre-gravid plasma values may has a function different from the hypothalamic regulation of appetite suppression (Lage et al. 1999). Although it is obvious that pregnancy is

associated with specific adaptations of leptin homeostasis, the role of leptin during pregnancy is not fully understood yet.

Cord serum leptin concentrations are two to three times higher compared to those of adults (Petridou et al. 2005) and no relationship between plasma leptin concentration in maternal blood and umbilical blood at delivery exists (Schubring et al. 1998). This may lead to the assumption that cord blood leptin arises from placental and fetal tissues. An association between fetal leptin and birth weight and adiposity was shown suggesting leptin as a good marker of perinatal obesity (Schubring et al. 1999, Tsai et al. 2004). Furthermore, leptin seems to be involved in establishing the neural networks that regulate feeding behaviour in postnatal life and the establishment of neural pathways important for energy balance in later life (McMillen, Adam and Muhlhausler 2005, Bouret and Simerly 2006).

In animal studies, it was shown that leptin is transferred from the maternal blood to breast milk and then passes to neonatal blood, suggesting that maternal breast milk leptin may have biological effects on the infant (Casabiell et al. 1997). Although this was not proved in humans so far leptin was shown to be produced in human mammary epithelial cells (Smith-Kirwin et al. 1998) and it was suggested that milk leptin might exert a function on infant growth (Savino and Liguori 2008).

1.5 Adiponectin

1.5.1 Biology and function of adiponectin

Adiponectin is exclusively expressed by mature adipocytes (Hu, Liang and Spiegelman 1996, Scherer et al. 1995). The 3-kDa monomer protein of 247 amino acids consists of a collagen-like domain and a C-terminal globular domain (Scherer et al. 1995) that is responsible for exerting adiponectin effects. It forms trimeric low molecular weight (LMW) or higher order complexes including hexamers with medium molecular weight (MMW) and oligomers with high molecular weight (HMW), which each have different affinity for tissues and end-point responses in different tissues (Tsao et al. 2002, Tsao et al. 2003). In addition, full-length adiponectin (fAd) multimers are cleaved to release a fragment containing the C-terminal globular domain (gAd) (Scherer et al. 1995). Circulating adiponectin has been found primarily

in the HMW and MMW forms and to a lesser amount in the LMW form, whereas the presence of circulating gAd is controversial (Ceddia et al. 2005, Fang et al. 2005).

The adiponectin receptors were identified predominantly on muscle cells (AdipoR1) and liver cells (AdipoR2), although in humans they seem to be expressed ubiquitously throughout the body (Buechler, Wanninger and Neumeier 2010). Adiponectin exerts insulin-sensitizing properties. An inverse correlation between insulin resistance indices and plasma adiponectin concentrations in humans has been found (Hotta et al. 2000, Weyer et al. 2001), and SNPs in the *ADIPOQ* gene have been associated with risk of type 2 diabetes mellitus (Zacharova, Chiasson and Laakso 2005, Hara et al. 2002). Administration of adiponectin to mice improves glucose tolerance and insulin sensitivity (Yamauchi et al. 2001, Berg et al. 2001). Low adiponectin concentrations have been found in obesity, type 2 diabetes mellitus, and insulin resistance states (Fruebis et al. 2001, Mantzoros et al. 2005, Steffes et al. 2004). In addition to its role in glucose metabolism, adiponectin exerts anti-atherogenic (Okamoto et al. 2002, Ouchi et al. 2000), anti-inflammatory (Wulster-Radcliffe et al. 2004, Ouchi et al. 2000) and angiogenic (Shibata et al. 2004, Ouchi et al. 2004) properties. Most of the metabolic actions of adiponectin are mediated via activation of adenosine monophosphate-activated protein kinase (AMP) kinase leading to expression of PPARα and increased gene expression of enzymes of FA oxidation and glucose uptake, which increases insulin sensitivity (Gil-Campos, Canete and Gil 2004).

Adiponectin itself is controlled by a number of hormones (e.g., insulin) and factors involved in regulation of metabolic and/or immune function and in conditions of metabolic stress (Maahs et al. 2005). Thiazolidinediones, as known PPARγ agonists, have been reported to increase plasma adiponectin levels by transcriptional induction in adipose tissue (Iwaki et al. 2003, Lihn, Pedersen and Richelsen 2005).

Synthesis and secretion of total adiponectin and HMW adiponectin by n-3 FAs as agonists of PPARγ are reported (Banga et al. 2009). Other factors including catecholamines, glucocorticoids, cytokines (Il-6 and TNFα), prolactin, growth hormone, and androgens exert inhibitory effects on adiponectin regulation (Degawa-Yamauchi et al. 2005, Nilsson et al. 2005, Xu et al. 2005, Bottner et al. 2004).

The isoforms differ in their biological function, depending on tissue and receptor isotype. It was suggested that HMW adiponectin may be the most biologically active

form; low HMW adiponectin concentrations are closely associated with obesity-related complications (Pajvani et al. 2004) and better correlate with metabolic indices (e.g., HDL cholesterol and total cholesterol concentrations) and endothelial dysfunction than total adiponectin (Fisher et al. 2005, Bobbert et al. 2005, Lara-Castro et al. 2006, Retnakaran et al. 2007). Weight reduction and treatment with insulin sensitizing drugs (e.g., thiazolidinediones) preferentially enhance HMW adiponectin in comparison to the other MMW or LMW isoforms (Pajvani et al. 2004, Tonelli et al. 2004) or to total adiponectin concentration (Swarbrick et al. 2006, Salani et al. 2006). In line with data from epidemiological studies, evidence from genetic analysis is supporting the role of HMW adiponectin as a major insulin-sensitizing isoform in humans (Waki et al. 2003).

1.5.2 Adiponectin during pregnancy

Pregnancy is characterized by hypoadiponectinemia (Catalano et al. 2006, Cseh et al. 2004, Fuglsang et al. 2006). However, some authors have reported no significant difference in total adiponectin concentrations between non-pregnant and pregnant women (Naruse et al. 2005, Suwaki et al. 2006).

It has been reported that adiponectin plays a regulatory role in metabolic adaptation during pregnancy (Catalano et al. 2006, Nien et al. 2007, Mazaki-Tovi et al. 2007). During recent years adiponectin has been implicated in the physiology of insulin resistance during pregnancy (Kirwan et al. 2002, McLachlan et al. 2006, Retnakaran et al. 2004).

Only a few studies have addressed the changes in adiponectin multimer concentrations during pregnancy. The principal finding of a study by Mazaki-Tovi et al. (2008) was that HMW constitutes the most prevalent adiponectin isoform, regardless of gestational age or BMI status (Mazaki-Tovi et al. 2008). Furthermore, data from Retanakaran et al. (2007) suggested that the absolute level of HMW adiponectin is the most prevalent adiponectin form during pregnancy and may be the more relevant mediator of glucose tolerance in pregnancy. While both HMW adiponectin and the adiponectin sensitivity index (SA = HMW-to-total adiponectin ratio) were decreased in women with GDM, only the absolute HMW form was independently correlated with glycemia, insulin sensitivity and B-cell function and SA was not significantly associated with any of these measures (Retnakaran et al. 2007).

It was demonstrated that *ADIPOQ* gene expression and adiponectin protein are present in a number of tissues of the human fetus, whereas the expression of adiponectin in human placenta is still controversial (Chen et al. 2006, Caminos et al. 2005, Corbetta et al. 2005, Pinar et al. 2008). Pinar et al. (2008) have reported that *ADIPOQ* gene expression and adiponectin protein are not only present in adipose tissue but also in other tissues at mid- and late gestation including vascular endothelial cells of fetal organs, skeletal muscle, kidney, and brain (Pinar et al. 2008). Serum total adiponectin levels in umbilical cord are significantly higher compared to those of the infants' mothers (Vega-Sanchez et al. 2010, Kotani et al. 2004, Chan et al. 2004) and do not seem to be related to maternal concentrations (Chan et al. 2004, Weyermann et al. 2006). Several studies show that the HMW adiponectin is the main adiponectin form circulating in fetal plasma (Ibanez et al. 2008, Odden and Morkrid 2007, Pinar et al. 2008, Araki et al. 2006). Pinar et al. (2008) suggested that the high adiponectin levels with increased HMW form found in the fetus may have different metabolic effects *in utero* than in adult and may play a multiple role in tissue differentiation and growth during fetal development (Pinar et al. 2008). However, the origins of the high adiponectin concentrations in the perinatal period remain elusive.

Recently, adiponectin was detected in human milk in amounts that may have potential physiologic effects (Martin et al. 2006, Bronsky et al. 2006, Weyermann et al. 2006). Considering the biological properties of adiponectin and the fact that AdipoR1 is expressed in the small intestine of neonatal mice (Zhou et al. 2005), adiponectin in the milk is supposed to play a role in neonate development (Savino et al. 2009). However, no data exists so far showing that adiponectin from breast milk enters the circulation of the infant.

1.6 Biological molecules involved in the appetite-regulatory network system

It is postulated that maternal nutrition during pregnancy can profoundly alter the expression of major hypothalamic neuropeptides of energy balance in the infant (Wang, Storlien and Huang 2002, Muhlhausler et al. 2006).

The hypothalamus is the main site for integration of signals that influence energy balance (Walley, Asher and Froguel 2009) (Figure 4). The melanocortin pathway plays a major role in energy homeostasis. This pathway includes neurons within the arcuate nucleus that express proopiomelanocortin (POMC), from which the peptide α-melanocyte-stimulating hormone (α-MSH) is cleaved (Fan et al. 1997). Leptin increases POMC expression in these neurons (Cowley et al. 2001). α-MSH is an agonist for the melanoncortin 4 receptor (MC4R), which is a key receptor expressed within the CNS, and administration of α-MSH centrally suppresses feeding (Mountjoy 2010). MC4R receives not only the agonist ligand α-MSH, whose expression is induced by leptin, but also the antagonistic ligand Agouti-related Protein (AgRP) that promotes feeding (Ollmann et al. 1997) and is expressed by arcuate neurons. Moreover, the MC4R pathway seems to be involved in the regulation of appetite and body weight mediated through Subtype 2C serotonin receptor (5- HT_{2C}) (Heisler et al. 2002).

Neuropetide Y (NPY) is another neuropeptide whose expression within the arcuate nucleus (coexpressed with AgRP) is linked to energy balance (Hillebrand, de Wied and Adan 2002). Its expression is negatively regulated by leptin and insulin, and central NPY administration induces feeding and suppresses energy expenditure (Billington and Levine 1992).

Brain derived neurotrophic factor (BDNF) and its receptor, the tropomyosin-related kinase receptor B (TrkB), have been identified in hypothalamic neurons associated with satiety (Kernie, Liebl and Parada 2000). Mice heterozygous for BDNF deficiency are obese and hyperphagic and this could be reverted by central BDNF injection (Kernie et al. 2000). Conditional deletion of BDNF in the brain also results in obesity, suggesting that this neurotrophin may have a role in the central energy balance regulation (Rios et al. 2001). BDNF is highly expressed in the ventromedial nucleus (VMN) and its expression is regulated by nutrition and MC4R signalling (Xu et al. 2003). Recently, it was shown that BDNF exerts an important role during placental

development and fetal growth (Mayeur et al. 2010, Kawamura et al. 2009). From their animal experiment, Tozuka et al. (2010) suggested that the maternal energetic and nutritional status affects the BDNF/TrkB system in the offspring which may have long-lasting consequences (Tozuka et al. 2010).

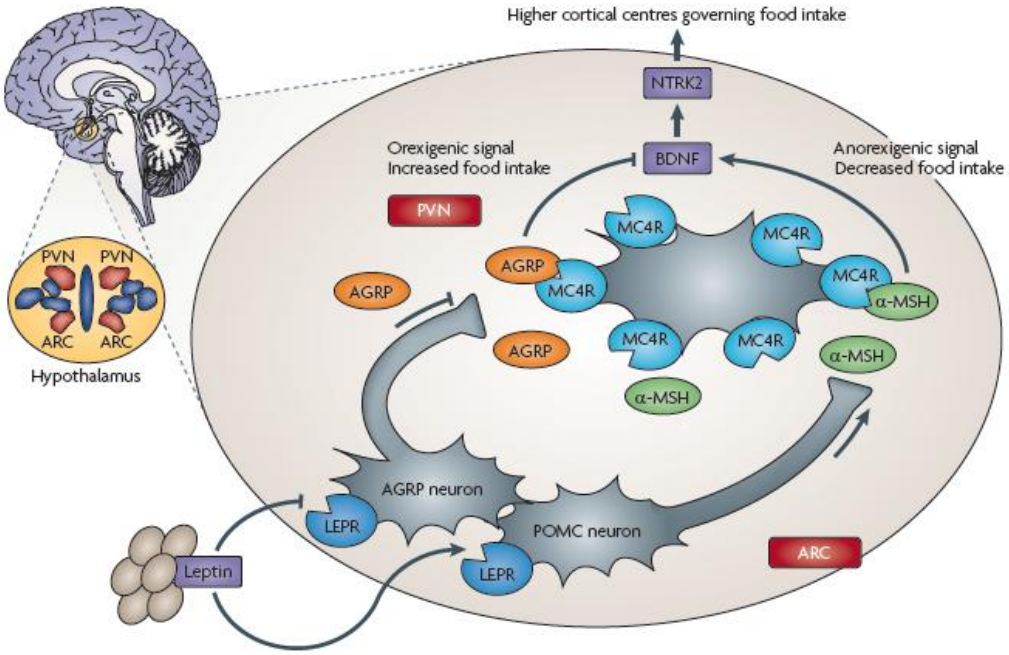


Figure 4 Leptin-regulated hypothalamic circuits (Walley et al. 2009).
MCH = Melanin-concentrating Hormone, POMC = Proopiomelanocortin, AgRP = Agouti-related protein, BDNF = Brain-derived neurotrophic factor, MC4R = Melanoncortin 4 receptor, α-MSH = melanocyte-stimulating hormones, NTRK2 = Neurotrophic tyrosine receptor Type 2

1.7 Nutrition and ‘perinatal programming’

Alterations of the intrauterine and early postnatal nutritional, metabolic, and hormonal environment to predispositions for developing diseases in adult life are defined as ‘perinatal programming’. Dörner (1975) has introduced the ‘perinatal programming’ of neuroendocrine networks (Dörner 1975) and during recent years this field was extended by the ‘Barker hypothesis’ (Barker 1998, Hales and Barker 1992). According to the ‘Barker hypothesis’ adverse influences in early development and during intrauterine life can result in permanent changes in physiology and metabolism and increased disease risk in adulthood.

When hormones of neuroendocrine systems occur in non-physiological concentrations during critical periods of development, the system may become permanently changed and the susceptibility to develop diseases in later life is increased (Plagemann and Harder 2009).

Passos et al. (2009) have demonstrated that exogenous induction of hyperleptinemia during the first ten days of life in rats leads to leptin resistance in adulthood (Passos et al. 2009). In contrast to sex steroids or glucocorticoids the role of other peptides, proteohormones and cytokines in fetal programming has been rarely explored and first data came from experiments on perinatal hyperinsulinism (Plagemann and Harder 2009). Evidence supporting a long-term effect of nutrition during pregnancy arose from the findings of the Dutch Hunger Winter of 1944-1945, which showed that maternal energy restriction during different trimesters of gestation was associated with obesity, dyslipidaemia, insulin resistance and coronary heart disease in the adult offspring (Ravelli et al. 1998, Roseboom et al. 2000b, Roseboom et al. 2000a). Perinatal hyperinsulinism and hyperleptinism occur due to early postnatal overfeeding leading to a lasting malprogramming of neuroendocrine systems regulating body weight, food intake, and metabolism (Plagemann 2004).

In parallel with the increasing prevalence of overweight and obesity among children and adolescence over the last 20 years (Lobstein and Frelut 2003), the consumption of saturated fat from animal food and n-6 PUFAs has increased in the industrialized countries, whereas the dietary intake of n-3 PUFAs from plants and marine products has declined (Uauy, Mena and Valenzuela 1999, Sanders 2000), which is also reflected in the increasing ratio of n-6/n-3 PUFAs in breast milk (Spector 1999, Sanders 2000).

In this context, the role of LC-PUFAs on adipose tissue development in the newborn is of great interest. Massiera et al. (2003) have demonstrated a reduced body weight and fat mass in offspring by reduced maternal n-6/n-3 PUFA intake during the suckling period (Massiera et al. 2003). Furthermore, adipokines have been shown to be influenced by the maternal n-6/n-3 PUFA ratio in early life. Recently, Korotkova et al. (2002) have demonstrated that the ratio of n-6/n-3 PUFA in the maternal diet affects serum leptin levels, body weight and growth of the offspring (Korotkova et al. 2002) and they have found that alterations in metabolic parameters in adult male rats

were linked to a specific dietary n-6/n-3 PUFA ratio in the perinatal period and possibly to early leptin homeostasis (Korotkova et al. 2005).

This data suggest that a reduction in the maternal ratio of n-3/n-6 FAs during pregnancy and lactation may lead to less weight and fat mass of the infant and to an appropriate balance of adipokines and other metabolic parameters in early childhood and adulthood. Human intervention studies are needed to prove this hypothesis.

2 Aims of thesis

During recent years nutritional programming of fetal development and its long-term health consequences has become a major subject. Recent data generated the hypothesis that a reduction in the n-6/n-3 LC-PUFAs in the maternal diet during pregnancy and lactation may lead to a) a less expansive adipose tissue development of the infant and b) adequate maintenance of adipokines and other metabolic parameters in early childhood and adulthood and c) may provide a new insight into early programming and primary prevention of overweight. The aim of this thesis is to investigate the effect of a decrease in the ratio of n-3/n-6 LC-PUFA in maternal diet during pregnancy and lactation on maternal and fetal adipokines and metabolic parameters. Therefore, data and biological samples from the INFAT-study (The ***I***mpact of ***N***utritional ***F***atty acids during pregnancy and lactation for early human ***A***dipose ***T***issue development) were used. The INFAT-study examines in a randomized controlled intervention the impact of a reduction in the ratio of n-6/n-3 LC-PUFAs in the maternal diet during pregnancy and lactation on adipose tissue development of the infant. The study comprises two groups: an intervention group receiving n-3 LC-PUFAs and reducing dietary AA intake, and the control group in which women keep a normal healthy diet.

The major objectives of this thesis are:

1) To investigate whether a reduced n-6/n-3 LC-PUFA ratio in the maternal diet affects circulating leptin, sOB-R, FLI (free leptin index), HMW adiponectin, blood lipids, insulin and insulin resistance (homeostasis model assessment for insulin resistance (HOMA-IR)) in maternal plasma and breast milk during pregnancy and lactation, as well as in cord blood. Potential differences between the intervention group and control group should be explored.

2) To explore the course of these adipokines in a longitudinal analysis during pregnancy and lactation. Defined time points are the 15^{th} and 32^{nd} week of gestation, 6^{th} and 16^{th} week PP. Correlation analysis between adipokines and blood lipids during pregnancy was performed.

3) To search for novel plasma biomarkers in cord plasma that might be affected by the modification of n-3/n-6 LC-PUFA ratio in maternal diet. In an explorative analysis 86 inflammatory markers and hormones were determined. Differences between the intervention and control group were analyzed.

3 Materials and Methods

In this work analyses were performed in a subgroup of the INFAT-study population. Variables of fat mass and fatty acids will be further analyzed in the total amount of 208 participants of the INFAT-study in another work at a later time point.

The following analyses were performed in this thesis:

- Analysis of clinical parameters: As part of the INFAT-team screening, randomization of the women, sample collection and anthropometric measures including height, weight, skinfold thickness measurements were conducted at the study centre Else-Kröner Fresenius centre of nutritional medicine, Klinikum rechts der Isar, Technische Universität München (Prof. Dr. H. Hauner).
- Experimental analysis: Blood samples and breast milk samples were drawn, prepared and pre-treated at the study centre, and adipokines, insulin, AgRP and BDNF were determined by the group of Prof. Dr. J. Kratzsch at the Institute of Laboratory Medicine, Clinical Chemistry and Molecular Diagnostics, University of Leipzig.
- Statistical analysis: The statistical analysis included tests of distribution and correlation analysis and was conducted in collaboration with the Institute for Medical Statistics and Epidemiology, Technische Universität München (Prof. Dr. K. Ulm, Dr. T. Schuster).

3.1.1 Study design of the INFAT-study

The aim of the INFAT- study is to examine the hypothesis that a reduction in the ratio of the n-6/n-3 LC-PUFA in maternal diet during pregnancy and lactation by supplementing n-3 LC-PUFAs and reducing the AA intake is associated with a less expansive adipose tissue development in the newborns. The study is designed as a randomized, controlled intervention study in pregnant and lactating women and their newborns. The study design of the INFAT-study was recently published (Hauner et al. 2009).

Primary objective

The primary outcome parameter comprises the measurement of adipose tissue mass in the newborn by skinfold thickness (SFT) measurement at 3-5 days PP, 6 weeks PP, 16 weeks PP and 12 months PP. In addition, subcutaneous and visceral fat mass were measured by ultrasonography and in a subgroup by MRI at the time points 6 weeks PP and 16 weeks PP.

Secondary objectives

Secondary objectives include the development of body weight, body length, head and upper arm circumference of the infant 3-5 days, 6 weeks, and 4 and 12 months after birth. In the pregnant and lactating women blood lipid concentrations (TAGs, total cholesterol, HDL- and LDL-cholesterols) at 15th week of gestation, 32nd week of gestation and 6 weeks and 16 weeks PP were determined. In maternal blood samples as well as in cord blood adipokines and FA profile were determined.

3.1.2 Study criteria

Inclusion criteria women:

- Gestational age 15th weeks of gestation
- Age between 18 and 43 years at study entry
- BMI at conception between 18 and 30 kg/m²
- Sufficient German language skills and written informed consent

Exclusion criteria women:

- High-risk pregnancy
- Hypertension
- Chronic diseases (diabetes or gastrointestinal disorders); psychiatric diseases
- N-3 PUFA supplementation before randomization
- Alcohol abuse
- Hyperemesis gravidarum

Inclusion criteria for follow-up of the newborns:

- Gestational age at birth between 37th and 42nd weeks
- Appropriate size for gestational age
- APGAR score > 7 at 5 min PP

Exclusion criteria for follow-up of the newborns:

- Severe malformations or diseases
- Chromosomal anomaly
- Inborn metabolic diseases

3.1.3 Recruitment and Screening

The recruitment of the participants was conducted from July 2006 until June 2009. Pregnant women before the 14th week of gestation were referred by gynecologists in private practices and outpatient clinics to the study centre (Else Kröner-Fresenius centre, Klinikum rechts der Isar). In addition, the study was advertised in local newspapers and on internet pages as well as in the journal “Baby & Familie”. The first screening included a detailed history by telephone or personal interview. Provided that the woman meet all inclusion criteria, randomization was performed using a random envelope prepared by the Institute of Medical Statistics and Epidemiology at the Technische Universität München.

3.1.4 Intervention and control group

Intervention Group

The intervention protocol combines two components: (1) to increase n-3 PUFA intake the participants were supplemented three fishoil capsules containing 1.2 g of n-3 LC-PUFA [180 mg EPA, 1.020 g DHA] (Marinol D-40 TM, Lipid Nutrition, Wormerveer, The Netherlands), and (2) to reduce dietary AA intake to the recommended range of 50-90 mg per day the women in the intervention group were advised to keep a healthy balanced diet with a reduction in the consumption of AA-rich foods, particularly meat products and eggs.

To support the intake of fish oil capsules and to maintain the compliance for the low-AA diet, capsule intake was recorded and the women were called every 4-8 weeks by a member of the study team.

Control Group

Participants of the control group received a single counselling on a healthy diet according to the guidelines of the Deutsche Gesellschaft für Ernährung (DGE 2004) for a healthy balanced diet.

3.1.5 7-day dietary record

Participants of both groups obtained an individual dietary counselling based on the 7-day dietary record.

The women in both groups completed a 7-day dietary record at 15th week of gestation, 32nd week of gestation and 6 weeks PP.

The dietary record data were analyzed using Prodi® 5.3. Expert (Nutri Science GmbH, Wissenschaftliche Verlagsgesellschaft Stuttgart GmbH) and daily dietary intake of total energy (kcal/d), protein (%), carbohydrates (%), fat (%), as well as several micronutrients were assessed.

In addition information on all dietary supplements taken by the participants was recorded.

3.1.6 Visits

At Visit 1 the screening was performed. The study details were explained and the women received written information (Figure 5).

At *Visit 2* (14th-16th week of gestation) the women were randomly allocated to the control or the intervention group. A first 7-day dietary questionnaire was filled in and information on dietary supplements was obtained. SFT of the pregnant women was measured using a caliper. Fasting blood samples were collected.

At *Visit 3* (32nd week of gestation) fasting blood samples were collected and the women were asked to fill in the second 7-day dietary record.

At birth gestational age, birth weight and height, placental weight, mode of delivery, APGAR score and other data were recorded. Cord blood and maternal blood, a piece

of the umbilical cord and the placenta were collected and immediately frozen at -80 °C.

At Visit S1 (3-8 days after delivery) upper arm circumference and SFTs at four independent sites (triceps, biceps, suprailiac and subscapular) were measured in the newborns. In case of maternal inability to breastfeed the infant, infant formula was provided for both groups (Aptamil Pre/1 with LCP-Milupan® and Aptamil HA Pre/1 with LCP-Milupan® of Milupa, Friedrichsdorf, Germany).

Visit S2 (6 weeks after birth) includes blood and breast milk sample collection from the mothers. The third 7-day dietary record was requested. In the newborn, upper arm circumference of the newborn and SFT were measured at four defined sites (triceps, biceps, suprailiac and subscapular). In addition the subcutaneous and the visceral fat mass were assessed by ultrasonography. In a subsample of the newborns whole body composition was determined by MRI.

At *Visit S3* (4 months after birth) the same anthropometric measurements were performed as described for visit S2. Maternal fasting blood and breast milk samples were collected. In addition, in a subgroup of infants, 1- to 4-ml blood samples were collected depending on the consent of the mother.

At *Visit S4* (12 months after birth) the same anthropometric measurements were performed as described for visit S2. In follow-up examinations at 18, 24, 36, 48 and 60 months PP, anthropometric parameters are assessed.

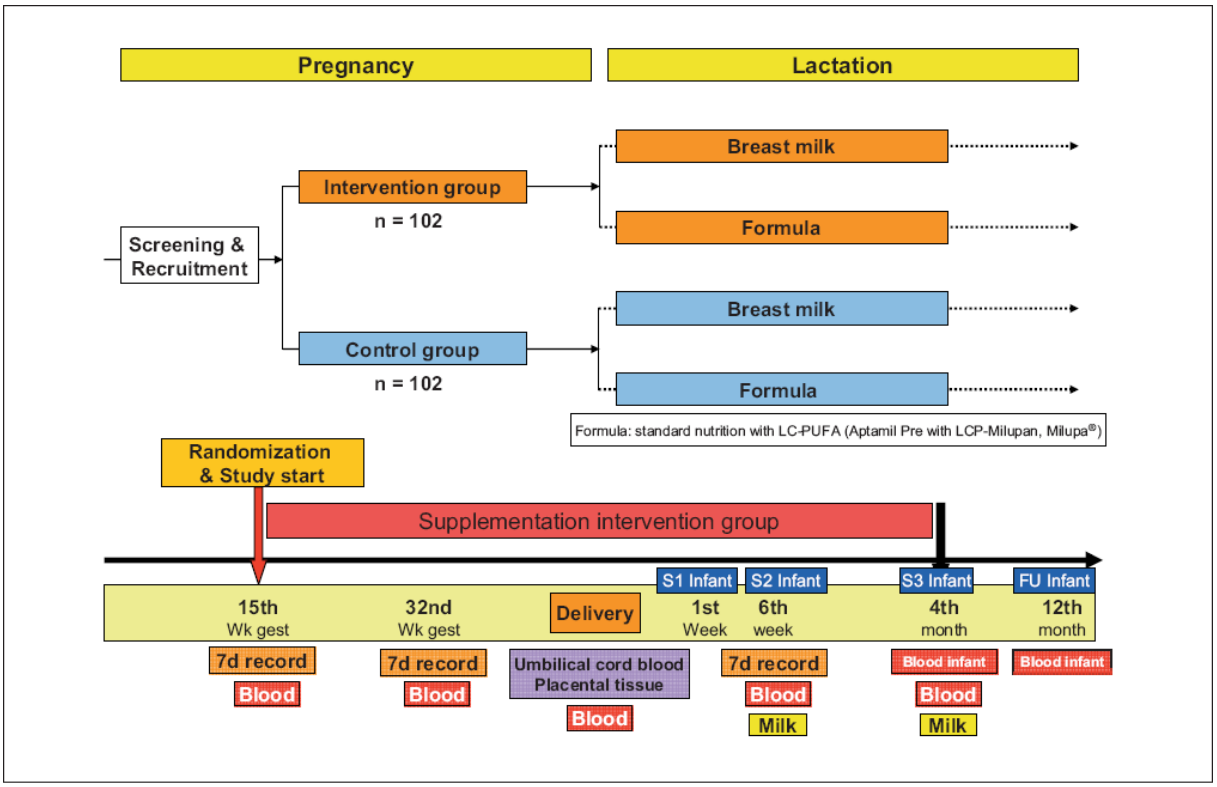


Figure 5 Study design of the INFAT-study (Hauner et al. 2009)
Wk gest = week of gestation

3.1.7 Sample size calculation

The calculation of sample size was based on SFT measurements. According to recent publications, a mean sum of the four skinfolds biceps, triceps, subscapular, suprailiacal of 30 ± 5 mm was expected (Schmelzle and Fusch 2002, Rodriguez et al. 2005). A difference of at least 5 mm in the sum of the four skin folds between the groups at 16 weeks PP was assumed. To be able to detect a clinically significant difference of difference of 5 mm between the groups at a two-sided α= 0.05 and a power of 80%, and assuming a dropout rate of 30%, a total of 102 women in each group had to be included into the study. The power calculation was performed using the nQuery (Version 5) software program.

3.1.8 Data management and legal requirements

The INFAT-study was approved by the Ethics Committee (Faculty of Medicine) of the Technische Universtität München (Nr.1479/06 / 2006/2/21).

Data from the participants were kept in coded form, randomly assigned to each participant. A list was prepared to link the data with the name of the participants.

Data management was performed according to the current data privacy laws. Data were collected using personal data questionnaires (Case Report Forms) and entered in a data base.

The trial was performed in accordance with the protocol, 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.

The study protocol was registered at ClinicalTrials.gov Protocol Registration System (NCT00362089).

3.2 Maternal measurements

3.2.1 Weight, height and skinfold thickness

Current body height, weight and para status were obtained from the maternity card and pre-pregnancy BMI [kg/m²] was calculated. SFT was measured at four independent sites (biceps, triceps, subscapular and suprailiacal) to the nearest 0.5 mm under standard conditions by using a skinfold caliper (Holtain Ltd. Croswell, Crymych, United Kingdom). The mean of three measurements per site was calculated.

3.2.2 Body fat mass

Maternal body fat (BF) in % of total body weight and maternal BF in kg were calculated according to Durnin & Womersley (1974) and Van Raaij et al (1988).

3.3 Measurements in infants

3.3.1 Weight and height

Birth weight, birth length and sex of the newborn were obtained from the maternity card and Ponderal Index (PI) in kg/m³ was calculated. Appropriate weight and length of the newborns for gestational age was defined using current age- and sex-related percentile charts (Voigt et al. 1996). At the defined visits weight was measured to the nearest 10 g by using a standard scale (Babywaage Ultra MBSC-55, myweight®).

Height was determined using a measuring stick (Säuglingsmessstab seca 207, seca, Pfaffenweiler) to the nearest 0.5 cm.

3.3.2 Skinfold thickness and body fat mass

Biceps, triceps, subscapular and suprailiacal SFT was measured to the nearest 0.5 mm in triplicate under standardized conditions by using a caliper (Holtain Ltd. Croswell, Crymych, United Kingdom). Infant BF in g and BF in % were estimated according to Deurenberg et al. (1990) using following equation (Deurenberg, Pieters and Hautvast 1990):

$$BF\ (\%) = \frac{\left(562 - 4 \cdot 2\,(age - 2)\right)}{body\ density} - \left(525 - 4 \cdot 7\,(age - 2)\right)$$

3.4 Measurement of biochemical parameters

3.4.1 Measured biomarkers in women and infants

A total of 208 women (n = 104 intervention group, n = 104 control group) were randomized in the INFAT-study.

In this thesis 82 women (n = 41 intervention group, n = 41 control group) were included for the adipokine analysis providing blood samples at each visit during pregnancy and lactation (15th week and 32nd week of gestation, 6 weeks and 16 weeks PP) and breast milk samples at each visit during lactation (6 weeks and 16 weeks PP). From 19 women (n = 9 intervention group, n = 10 control group) of them blood samples at delivery was obtained. 108 cord blood samples (n = 55 intervention group, n = 53 control group) were used for the determination of biomarkers in cord blood and 56 breast-fed infants (n = 29 intervention group, n = 27 control group) were included for adipokine analysis at time point 16th week PP.

Insulin, Leptin/sOB-R and adiponectin concentrations were measured in blood/breast milk of the mothers at different time points (15th week of gestation, 32nd week of gestation, 6th week PP and 16th week PP) as well as in cord blood samples and plasma samples of the infants at the 16th week PP. Furthermore, lipids (TAG, total cholesterol, HDL-cholesterol, LDL-cholesterol) and PUFAs were longitudinally analyzed in maternal blood and the latter was also determined in cord blood. For adipokine analysis the RIA and ELISA method was used.

As a result of the explorative analysis of biomarker analysis of AgRP and BDNF was performed using ELISAs.

3.4.2 Sample collection

Fresh blood samples were collected in Ethylenediamine tetraacetic acid (EDTA) containing tubes (Sarstedt, Germany) from each woman after an overnight fast and immediately centrifuged at 2000 x g for 10 minutes at 4°C to separate erythrocytes and plasma. Thereafter, the plasma was aliquotted and stored until analysis at -80°C.

At delivery, blood samples were collected from the umbilical cord. The samples were centrifuged at 2000 x g for 10 minutes at 4°C within 4 hours to separate erythrocytes and plasma, and after aliquotation stored at -80 °C until analysis.

Fresh breast milk samples were collected in a standardized manner after an overnight fast, aliquotted and immediately frozen until their use for analysis at -80°C.

3.4.3 Principles of ELISA and RIA

Principle of a RIA

Radioimmunoassay was first described by Yalow and Berson published in 1960 (Yalow and Berson 1960). A known quantity of an antigen is labeled with gamma-radioactive isotopes. This radiolabeled antigen is then mixed with a known amount of antibody for that antigen and the two chemically bind to one another. Then, an unknown quantity of the same antigen is added which competes with the radiolabeled antigen for the limited antibody binding sites. The amount of radiolabeled antigen bound to antibody decreases as the concentration of unlabeled antigen increases. This can be measured after separating antibody-bound from free labeled antigens and counting one or the other, or both fractions using a gamma counter. From a standard curve the amount of antigen in the unknown samples can be calculated.

Principle of an ELISA (Kramer 1997)

The principle of ELISA is the same as for RIA, whereas the antigen-antibody reaction is measured using colorimetric signals. This assay employs the quantitative sandwich enzyme immunoassay technique.

A monoclonal antibody specific for the antigen is pre-coated on the microplate. Standards and samples are pipetted into the wells and the antigen is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked monoclonal antibody specific for the antigen is added to the wells. Following a wash step to remove unbound antibody-enzyme reagents, a substrate solution is added to the wells and color develops in proportion to the amount of antigen bound in the initial step. The color development is stopped and the intensity of the color is measured.

3.4.4 Measurement of leptin in plasma and breast milk samples

Leptin concentrations were measured in plasma samples in a volume of 20 µl by an automated processing system (DYNEX Technologies, Chantilly, USA) using an ELISA (Mediagnost, Reutlingen, Germany). The concentrations were measured in ng/ml and the lower detection limit for leptin was 0.2 ng/ml. Intra- and interassay coefficients of variation were 4% and 2%, respectively.

For leptin measurement in breast milk three different milk samples in 1 ml tubes were thawed at room temperature and vortexed. The whole milk was sonicated (3 bursts, 5 sec duration/burst) by using a MS2-Stab UP50H/UP100H (Hielscher Ultrasonics GmbH, Teltow, Germany). Skim milk was prepared by centrifugation of whole milk at 14000 rpm for 30 min in an Eppendorf Centrifuge 5415C/D, to separate milk fat from the liquid phase. A 1 ml injection was used to extract the fat layer and the skim milk sample was used for further analysis.

For assay validation skim milk samples were spiked with human leptin standards to determine the recovery of the added mass.

Three breast milk samples with baseline leptin concentrations of 0.08, 0.27 and 0.29 ng/ml were enriched with various amounts of standards to increase the original leptin concentrations by +1.6, +3.2 and +4.8 ng/ml. The latter was 2-, 4-, and 8-fold diluted. Immunoreactive leptin was measured in milk samples using a human-leptin-sensitive RIA (Mediagnost, Reutlingen, Germany). A 125I-labeled of recombinant leptin was

used as tracer. The mean recovery was 70% with Standard deviation (SD) 3.4%. For the serial dilutions the average recovery was 82% with SD 2.3%. 100 µl of skim milk were used for measurement of leptin in breast milk samples. All samples were measured in duplicate using a human-leptin-sensitive RIA (Mediagnost, Reutlingen, Germany). Intra- and interassay coefficients of variation were 9.7% and 9.5%, respectively. The lower detection limit for leptin was 0.01 ng/ml.

3.4.5 Measurement of sOB-R in plasma samples

The concentration of sOB-R was determined by the Institute of Laboratory Medicine, Clinical Chemistry and Molecular Diagnostics, University Leipzig (Prof. Dr. Kratzsch) using an in-house ligand- immunofunctional assay (LIFA) (Kratzsch et al. 2005). The wells of the microtiter plate were coated with the anti sOB-R IgG in carbonate buffer. Blocking of potentially unspecific binding sites was performed by incubation with PBS, 1% bovine serum albumin (BSA) for 1 h at room temperature. 10 µl of rsOB-R standards or plasma samples were added to the wells and incubated with an excess of biotinylated leptin in assay buffer overnight at 4°C. The recombinant sOB-R preparation of Liu et al. (1997) was used for calibration (Liu et al. 1997).

The sOB-R and biotinylated leptin complex was detected by Europium-labelled streptavidin (Perkin-Elmer, Freiburg, Germany). Between coating, blocking, incubation and detection steps the plate was washed three times with PBS, 0.05% Tween 20. The solid phase linked fluorescence signal of Europium was detected by the Victor-system (Perkin-Elmer, Freiburg, Germany). The intra- and interassay coefficients of variation were 3.9%, respectively.

3.4.6 Measurement of adiponectin in plasma and breast milk samples

An adiponectin multimeric ELISA (ALPCO Diagnostics, Salem, USA) was used for the quantitative determination of HMW adiponectin in human plasma. The multimeric assay is proposed for the quantitative determination of HMW and total adiponectin in human serum or plasma and is based on the principle of an ELISA. To measure HMW adiponectin the samples were pre-treated with a protease which selectively digests LMW and MMW. The remaining HMW was measured according to the manufacturer’s instruction. HMW adiponectin concentrations were calculated in µg/ml. The intra-and interassay coefficients of variation were both 6%.

For determination of total adiponectin in breast milk, milk samples were pre-treated as described for leptin measurement (see 3.4.4) in breast milk and skim milk was used for further analysis. The recovery of total adiponectin was validated performing serial dilutions (1:2, 1:4, 1:8, 1:16). Additionally, skim milk samples were spiked with 50 ng/ml, 25 ng/ml and 10 ng/ml human adiponectin standard. Recovery of added mass averaged 118, 112 and 119%, respectively. Adiponectin concentrations in breast milk were determined by RIA (Millipor, St. Charles, USA). The adiponectin RIA assay utilizes 125I-labeled Murine Adiponectin as tracer.

Total adiponectin concentrations in skim breast milk samples were determined by RIA (Millipor, St. Charles, USA). A volume of 100 µl was used for the measurements (dilution 1:2). The results were calculated in ng/ml and the lower detection limit for total adiponectin was 2 ng/ml.

3.4.7 Measurement of AgRP in plasma samples

The plasma AgRP concentrations were measured by an ELISA for AgRP (R&D Systems, Inc. Minneapolis, USA) according to the manufacturer's instruction. For determination of AgRP 50 µl of maternal plasma at birth were used. Cord plasma samples were diluted (1:20) to receive concentrations lying within the standard curve. The intra- and interassay coefficients of variation were 4% and 8%, respectively.

3.4.8 Measurement of BDNF in plasma samples

BDNF concentrations were determined by a commercial ELISA for BDNF (R&D Systems, Inc. Minneapolis, USA) according to the manufacturer's instructions. The concentrations were measured in pg/ml. The intra-assay coefficient of variation was 5%.

3.4.9 Reagents ELISA/RIA

Leptin ELISA *(Cat. # E07)*

Antibody against leptin (coated plate)

Anti-human leptin antibody

Leptin RIA senstitiv *(Cat. # LEP-R40)*

Rabbit-anti-human leptin

Anti-rabbit IgG

BDNF *(Cat. # DBD00)*

Mouse monoclonal antibody against BDNF (coated plate)

Mouse monoclonal antibody against BDNF

AgRP *(Cat. # DAGR00)*

Mouse monoclonal antibody against AGRP (coated plate)

Polyclonal antibody against AgRP

Insulin *(LIAISON 310360)*

Suspension of magnetic particles coated with a mouse monoclonal antibody against insulin

Mouse monoclonal antibody against insulin

HMW adiponectin *(Cat. # 47-ADPHU-E01)*

Mouse monoclonal against Adiponectin (coated plate)

Biotin-conjugated anti-human adiponectin monoclonal antibody

Total adiponectin *(Cat. # HADP-61HK)*

Rabbit anti-Adiponectin Antibody

3.4.10 Explorative analysis of biomarkers in plasma samples

In an explorative manner 89 hormones and inflammation markers were measured in maternal plasma samples (10 intervention group; 10 control group) and in cord plasma samples (20 intervention group; 20 control group) using a Multi-Analyte Profiling (MAP) technology platform (Rules-Based Medicine, Inc. Austin, USA (appendices A.1 and A.2). 250 µl of each sample was used.

3.4.11 Measurement of insulin in plasma samples

Insulin was measured in 50 µl of each sample via an automated LIAISON® Analyzer (Diasorin GmbH, Dietzenbach, Germany) using a chemiluminescence immunoassay (LIAISON Insulin Diasurin, Saluggia, Italy). The results were calculated in pmol/l.

3.4.12 Maternal blood lipids and glucose

TAG, total cholesterol, HDL-cholesterol, LDL-cholesterol and glucose concentrations were measured in blood samples of pregnant and lactating women after fasting overnight. The analyses were carried out by an approved commercial laboratory (FutureLab, Munich, Germany).

3.4.13 Determination of FAs in plasma phospholipids and red blood cells

The FA pattern of maternal and fetal plasma phospholipids were performed by the laboratory of Milupa, (Friedrichsdorf, Germany) using gas chromatography (GC) according to published methods (Bligh and Dyer 1959, Lepage and Roy 1984). The method of fatty acid profile measurement in samples of the INFAT-study was described previously (Vollhardt 2010).

For determination of FAs in plasma phospholipids, frozen plasma was thawed at room temperature and total lipids were extracted with chloroform/methanol/water (10.10.9, by vol) (Bligh and Dyer 1959). The neutral and polar lipid classes were separated by high-pressure liquid chromatography (HPLC) using a HPLC Alliance 2695 Separation module from Waters (Waters GmbH, Eschborn, Germany) coupled with an PL-ELS 1000 evaporative light scattering detection system (Polymer Laboratories, Darmstadt, Germany) and fractionated via automatic fractionation sampling. The separated PL fraction of the HPLC fractionation was evaporated with nitrogen todryness. For derivatization, the samples were dissolved in 2 ml

methanol/hexane (4:1, vol/vol) plus 0.5% pyrogallol and were methylated according to Lepage & Roy (1984) with 200 µl acetylchloride at 100°C for 1 h. 5 ml K_2CO_3 (6%) were added and centrifuged for 10 min at 3200 x g. The upper hexane phase containing the fatty acid methyl ester (FAME) was separated and analyzed by capillary gas chromatography (CGC) performed on the 6890N gas chromatograph (Agilent Technologies, Waldbronn, Germany) fitted with a cold-on-column injector to prevent fatty acid discrimination (Beermann et al. 2005).

For determination of FAs in red blood cells frozen erythrocytes were thawed at room temperature. For derivatization, 200 µl of each sample was dissolved in 2 ml methanol/hexane (4:1, vol/vol) plus 0.5% pyrogallol and was methylated according to Lepage & Roy (1984) with 200 µl acetylchloride at 100°C for 1 h. 5 ml K_2CO_3 (6%) were added and centrifuged for 10 min at 3200 x g. The upper hexane phase containing the FAME was separated and analyzed by CGC.

3.4.14 Calculation of HOMA-IR and FLI

Insulin resistance was determined by HOMA-IR using the following equation:

HOMA-IR = fasting plasma insulin (mU/ml) x [fasting plasma glucose (mmol/l)/22.5]

Free leptin index **(FLI),** being presumed as an index representing the free leptin was calculated as the ratio of leptin to sOB-R.

3.5 Statistical Analysis

Due to the exploratory nature of the statistical analyses performed within this work non-parametric tests were used which were more conservative and more economic, since there was no need for particular proof of assumptions regarding the underlying data distribution (e.g., normality) (Altman 1991). Bonferroni correction of *P*-values was partially applied (variable wise for multiple group comparisons) to reduce the multiple testing issue. Subject characteristics, clinical and biochemical parameters between independent groups were compared using the Mann-Whitney-U-Test for quantitative data. Analysis of covariance models (ANCOVA) were employed to achieve confounder-adjusted estimates of group differences. The Chi-Square-Test, or if appropriate, the Fisher's exact test were used to compare categorical data between independent subgroups. Changes in parameters over time were statistically evaluated by using Wilcoxon-Test. Spearman's correlation coefficients were calculated to quantify the bivariate relationship between adipokine concentrations and clinical variables. Partial correlation coefficients adjusting for confounding variables were used to ascertain the association between plasma adipokines and other variables. All data were presented in mean±SD or median (interquartile range). A two-sided *P* value <0.05 was considered statistically significant.

Boxplots giving the median and quartiles are used to illustrate distribution of quantitative data. In the figures outliers and extreme values are presented as circles and asterisks.

Outliers were defined as follows:

Q1 (25^{th} percentile), Q3 (75^{th} percentile) and IQR (Interquartile range = 75^{th} -25^{th} percentiles) are used to define lower and upper limits of values in the data.

Upper limit = Q3 + 1.5 times the IQR

Lower limit = Q1 - 1.5 times the IQR

Values below the lower limit or above the higher limit are considered as outliers.

Extreme values are defined similarly to outliers, but using a higher upper limit and a smaller lower limit:

Extreme upper limit = Q3 + 3 times the IQR

Extreme lower limit = Q1 - 3 times the IQR

All statistical analysis analyses were performed with PASW Statistics software (Version, 17, SPSS Inc., Chicago, IL, USA).

4 Results

A total of 208 women were randomly assigned to the intervention group (n = 104) or the control group (n = 104). In this thesis, adipokine and insulin analysis were performed in a subgroup of the INFAT-study population.

Figure 6 presents the parameters analyzed in different biological samples of the women and infants. Insulin, leptin, sOB-R and HMW adiponectin concentrations were measured in maternal plasma at the 15th and 32nd week of gestation, 6 weeks PP and 16 weeks PP during pregnancy and lactation and in cord blood samples. Adiponectin and leptin concentrations were determined in maternal samples at birth and in breast milk samples at the 6th and 16th week PP.

Detailed information on parameters and sample size is given in table 1. The longitudinal analysis of maternal adipokines and insulin during pregnancy and lactation included 82 maternal blood samples. In 76 samples out of these 82 samples maternal adipokines at birth (n = 19) and in breast milk (n = 76) were determined. Leptin and insulin were measured in 107 and 108 cord blood samples, respectively. 57 cord blood samples were matched to the maternal samples. HMW adiponectin and sOB-R concentrations were determined in 50 and 57 cord plasma samples. Furthermore, HMW adiponectin was measured in 56 samples of the infant at the 16th week PP.

As a result of the MAP in cord plasma by RBM Inc. (20 intervention group, 20 control group) validation of AgRP and BDNF measurements was performed in 50 and 42 cord blood samples, respectively.

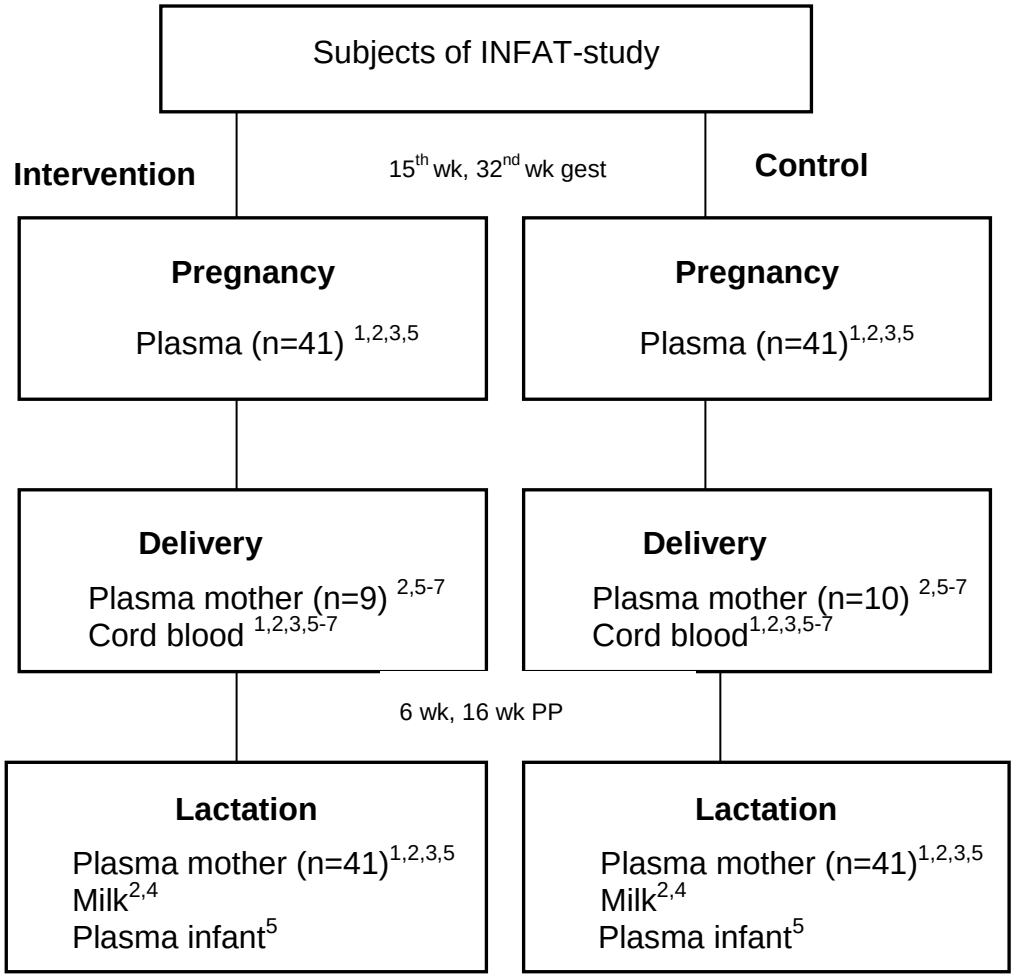


Figure 6 Overview of the analyzed parameters in maternal plasma, cord blood, infant blood and breast milk samples of the INFAT-study.
Insulin[1], leptin[2], sOB-R[3], T-Ad[4], HMW-Ad[5], AgRP[6] and BDNF[7]; HMW-Ad = high molecular weight adiponectin; T-Ad = total adiponectin; BDNF = brain-derived neurotrophic factor

Table 1 Sample size of adipokine and insulin measurements in different biological samples of the INFAT-study subjects

	Maternal samples							Infant samples	
	Plasma Pregnancy		Birth	Plasma Lactation		Milk Lactation		Cord	*Blood
	15th wk	32nd wk		6 wk PP	16 wk PP	6 wk PP	16 wk PP	blood	infant
Leptin (I/C)	41/41	41/41	9/10	41/41	41/41	37/39	38/38	55/52	
sOB-R (I/C)	41/41	41/41		41/41	41/41			31/26	
HMW-Ad (I/C)	41/41	41/41	9/9	41/41	41/41			24/26	29/27
T-Ad (I/C)						40/38	40/36		
Insulin (I/C)	41/41	41/41		41/41	41/41			55/53	
AgRP (I/C)			9/9					24/26	
BDNF (I/C)			8/8					22/20	

I = intervention, C = control, HMW-Ad = high molecular weight adiponectin, T-Ad = total adiponectin; * Blood infant = 4th month PP

4.1 Maternal characteristics and pregnancy outcome

The measurements of maternal SFT were performed at study entry (15th week of gestation) and 32nd week of gestation at the Else Kröner-Fresenius centre, Technische Universität München.

Baseline characteristics and pregnancy outcomes of the women included in the adipokine analysis during pregnancy and lactation (15th week of gestation, 32nd week of gestation, 6th and 16th week PP) are shown in tables 2 and 3. There was no significant difference in maternal age, parity, pre-pregnancy weight and pre-pregnancy BMI between the treatment groups. In the intervention group one woman developed GDM and one woman HELLP-syndrome during pregnancy. In the control group three women developed GDM.

Table 2 Maternal baseline characteristics and pregnancy outcome

	n	Intervention group	n	Control group	*P**
Maternal age	41	32.8 ± 4.8 (23-45)	41	31.4 ± 4.9 (20-40)	0.267
Para (0/1/2/3)	41	63.4%/34.1%/2.4%/0%	41	61%/24.4%/12.2%/2.4%	0.608
Pre-pregnancy weight [kg]	41	63.0 ± 8.7 (47-81)	41	62.7 ± 7.2 (50-77)	0.944
Height [m]	41	1.70 ± 5.9 (1.56-180)	41	1.67±6.1 (1.58-1.82)	0.063
Pre-pregnancy BMI [kg/m²]	41	21.9 ± 2.8 (17.6-29.7)	41	22.4 ± 2.6 (17.6-26.5)	0.214
Adverse events#	41	4.9%	41	7.3%	>0.99

Data are means ± SD (range), *$P < 0.05$ for difference between the groups; BMI = Body mass index, wk gest = week of gestation, # GDM or HELLP Syndrom

Mean BMI, BF [%] and BF [kg] are presented in table 3. No statistically significant difference in BMI, BF [%] and BF [kg] between the groups was found, neither at the 15th week gestation nor at 32nd week of gestation.

Table 3 Maternal BMI and BF at the 15th week (baseline visit) and 32nd week of gestation

		Wk gest 15			Wk gest 32			*P	#P	§P
		n	mean ±SD	(range)	n	mean± SD	(range)			
Body weight [kg]	IG	41	64.8 ± 9.0	(48-84)	40	74.2 ± 9.9	(58-96)	0.703	0.483	<0.001
	CG	41	64.9 ± 7.1	(51-81)	39	74.8 ± 8.5	(58-89)			<0.001
BMI [kg/m²]	IG	41	22.5 ± 3.0	(18.3-31.2)	40	25.7± 3.3	(20.8-35.7)	0.159	0.080	<0.001
	CG	41	23.2 ± 2.6	(18.0-27.3)	39	26.8 ± 3.2	(20.6-31.2)			<0.001
Body fat [kg]	IG	28	17.4 ± 4.2	(11.1-27.9)	27	19.6 ± 5.6	(11.2-34.4)	0.714	0.558	<0.001
	CG	29	17.3 ± 3.3	(12.3-27.0)	32	20.1 ± 5.0	(12.3-34.5)			<0.001
Body fat [%body weight]	IG	28	26.8 ± 3.5	(20.5-36.6)	27	25.8 ± 4.4	(17.7-36.4)	0.468	0.518	0.019
	CG	29	27.2 ± 2.8	(22.0-35.0)	32	26.4 ± 3.8	(19.2-36.4)			0.133

*P < 0.05 for difference between the groups at 15th wk gest
#P < 0.05 for difference between the groups at 32nd wk gest
§P < 0.05 for change between 15th and 32nd wk gest
IG = intervention group, CG = control group, wk gest = week of gestation, BMI = body mass index

4.2 Fetal outcome and anthropometry

The SFT measurements of the newborns were performed at an average of four days after birth at the maternity hospitals in the area of Munich.

Table 4 and 5 present the clinical and anthropometric data of the newborns. Gestational age was significantly higher in the intervention group in comparison to the control group (mean ± SD; 40.2 ± 1.1 weeks vs. 39.6 ± 1.2 weeks; P = 0.007). Placental weight, pH-values of cord blood, birth mode and sex did not differ significantly between the groups.

Birth weight was 3571 ± 414 g in the intervention group and 3444 ± 506 g in the control group, with no difference between the groups (P = 0.182). PI was slightly but not significantly higher in the intervention group compared to the control group (25.3 ± 2.5 kg/m^3 vs. 24.6 ± 2.5 kg/m^3; P = 0.096) (Table 4). No significant difference in BF [g], BF [%] and SFTs between the groups was found (Table 4).

Table 4 Clinical data and anthropometry of the newborns

	Intervention group			Control group			*P*
	n	mean ± SD (range)		n	mean ± SD (range)		
Gestational age [wk]	55	40.2 ± 1.1	(36.9-41.9)	53	39.6 ± 1.2*	(36.4-41.6)	0.007
pH-value cord blood	53	7.28 ± 0.09	(7.04-7.47)	49	7.30 ± 0.08	(7.06-7.49)	0.334
Placental weight [g]	51	537 ± 95	(300-772)	50	543 ± 105	(387-800)	0.965
Body weight [g]	55	3571 ± 414	(2450-4780)	53	3444 ± 506	(2200-4965)	0.182
Body length [cm]	55	52.0 ± 1.8	(49.0-57.0)	53	51.9 ± 2.6	(46.0-57.0)	0.830
PI [kg/m³]	55	25.3 ± 2.5	(19.6-30.4)	53	24.6 ± 2.5	(20.5-31.0)	0.096
Body fat [% BW]	53	11.7 ± 2.4	(7.1-17.7)	49	11.5 ± 2.4	(7.5-17.6)	0.592
Body fat [g]	53	421 ± 115	(206-757)	49	401 ± 124	(208-757)	0.291
Bizeps [mm]	53	3.5 ± 0.5	(2.5-4.6)	49	3.5 ± 0.8	(2.3-7.6)	0.332
Trizeps [mm]	53	4.7 ± 0.8	(2.5-6.9)	49	4.5 ± 0.9	(2.9-7.3)	0.249
Subscapular [mm]	53	4.5 ± 0.9	(2.8-7.3)	49	4.5 ± 0.9	(3.0-7.6)	0.989
Suprailiacal [mm]	53	3.3 ± 0.7	(2.4-5.3)	49	3.3 ± 0.9	(2.0-6.7)	0.394
Age at visit S1 [d]	53	4.3 ± 2.1	(1-11)	49	4.5 ± 2.1	(1-9)	0.716

*P < 0.05 for difference between the groups, PI = Ponderal Index

Table 5 Birth outcomes and weight percentiles of the newborns

		Intervention group (n=55)	Control group (n=53)	*P**
		%	%	
Birth mode	spontaneous	60.0	64.2	0.303
	CS	25.5	30.2	
	VE	14.5	5.7	
Infant sex	female	45.5	49.1	0.708
	male	54.5	50.9	
Birth weight <10th Percentile		1.8	9.4	
Birth weight >10th and <90th Percentile		89.1	86.8	0.161
Birth weight >90th Percentile		9.1	3.8	

*P < 0.05 for difference between the groups; CS = Caesarean Section; VE = Vacuum Extraction

4.3 Maternal and fetal LC-PUFA profile

4.3.1 Maternal blood

To investigate the compliance of the women the profile of LC-PUFAs in maternal and fetal plasma phospholipids (PLs) and red blood cells (RBCs) was determined by Milupa GmbH Friedrichsdorf, Germany.

Table 6 and figures Appendix C.2 present the profile of total n-3 LC-PUFAs, total n-6 LC-PUFAs, 20:4n-6 (AA), 20:5n-3 (EPA) and 22:6n-3 (DHA) in plasma PLs and RBCs of the women representing the subgroup analyzed during pregnancy and lactation. All values are recorded in % by weight.

At the 15th week of gestation the content of PL 20:5n-3, PL 20:4n-6, PL 22:6n-3, the sum of PL n-3 LC-PUFAs and the sum of PL n-6 LC-PUFAs were not significantly different between the groups. PL 20:5n-3, PL 22:6n-3, total PL n-3 LC-PUFAs contents increased in the women of the intervention group during pregnancy resulting into significantly higher levels at the 32nd week of gestation compared to those

women without treatment. These higher levels persisted throughout the lactation period. In contrast content of 20:4n-6 and total n-6 LC-PUFAs in plasma PLs were significant lower in the intervention group at the time points 32nd week of gestation, 6 weeks PP and 16 weeks PP in comparison to the control group. The ratio of PL 20:4n-6/22:6n-3 was similar in both groups at study entry, however during the time course of pregnancy and lactation the participants of the control group displayed a higher PL 20:4n-6/22:6n-3 ratio ($P < 0.05$) compared to the women exposed to the intervention. The LC-PUFAs in RBCs revealed a similar pattern to LC-PUFA profile in PLs (Table 6). There was no significant difference in LC-PUFA content in RBCs between the groups at study entry. However, RBC 20:5n-3, RBC 22:6n-3 and the sum of n-3 LC-PUFAs in RBCs were significantly higher in the intervention group than in the control group at the 32nd week of gestation and during the lactation period ($P < 0.05$). Furthermore, RBC 20:4n-6, the sum of n-6 LC-PUFAs in RBCs and the ratio of RBC 20:4n-6/22:6n-3 were significantly lower in the intervention group compared to the control group ($P < 0.05$).

Table 6 Maternal PUFA profile in PL and RBC during pregnancy and lactation

		Wk gest 15			Wk gest 32			6 wks PP			16 wks PP		
		n	mean±SD	(range)	n	mean±SD	(range)	n	mean±SD	(range)	n	mean±SD	(range)
PL n-3LCP	IG	41	6.8 ± 1.2	(4.5-10.5)	41	10.4 ± 1.6	(7.2-13.7)	40	9.7 ± 1.4	(6.6-13.4)	41	9.3 ± 1.6	(5.5-12.3)
	CG	41	6.9 ± 1.3	(4.6-10.9)	37	6.2 ± 1.1	(4.8-8.8)	40	5.1 ± 1.3	(2.9-9.1)	41	5.0 ± 1.5	(3.1-10.0)
PL n-6LCP	IG	41	15.1 ± 1.7	(11.5-18.7)	41	11.5 ± 1.6	(8.5-14.2)	40	11.5 ± 1.5	(7.9-13.6)	41	11.5 ± 1.5	(7.4-14.8)
	CG	41	15.5 ± 1.4	(12.3-18.2)	37	14.1 ± 1.5	(10.5-16.5)	40	14.5 ± 1.8	(9.3-17.3)	41	14.2 ± 2.0	(8.2-17.7)
PL 22:6n-3	IG	41	4.9 ± 0.8	(3.1-6.7)	41	8.2 ± 1.3	(5.6-11.0)	40	6.9 ± 1.2	(3.7-10.8)	41	6.6 ± 1.2	(3.4-9.0)
	CG	41	5.0 ± 1.1	(2.6-8.2)	37	4.7 ± 0.9	(3.0- 6.8)	40	3.1 ± 0.8	(1.6-5.1)	41	2.9 ± 1.1	(1.4-7.6)
PL 20:5n-3	IG	41	0.8 ± 0.6	(0.3-2.9)	41	1.3 ± 0.5	(0.5-3.0)	40	1.9 ± 0.9	(0.9-6.5)	41	1.7 ± 0.5	(0.7-3.5)
	CG	41	0.7 ± 0.3	(0.3-1.7)	37	0.6 ± 0.3	(0.1-1.3)	40	0.9 ± 0.4	(0.5-2.9)	41	1.0 ± 0.5	(0.5-2.8)
PL 20:4n-6	IG	41	10.2 ± 1.5	(7.8-13.7)	41	7.7 ± 1.1	(5.6-9.6)	40	8.4 ± 1.2	(4.9-10.1)	41	8.3 ± 1.2	(5.4-11.2)
	CG	41	10.5 ± 1.2	(7.8-13.3)	37	9.3 ± 2.0	(6.3-18.9)	40	10.3 ± 1.5	(7.0-13.3)	41	10.0 ± 1.7	(5.9-13.8)
PL 20:4n-6/22:6n-3	IG	41	2.1 ± 0.5	(1.4-3.3)	41	0.9 ± 0.2	(0.5-1.5)	40	1.3 ± 0.3	(0.8-2.3)	41	1.3 ± 0.4	(0.9-3.2)
	CG	41	2.2 ± 0.5	(1.1-3.9)	37	2.0 ± 0.5	(1.4-4.3)	40	3.5 ± 0.9	(1.3-5.3)	41	3.8 ± 1.2	(1.1-6.2)
RBC n-3LCP	IG	39	6.8 ± 2.3	(0.5- 8.9)	40	9.3 ± 3.7	(1.0-13.6)	40	9.1 ± 3.5	(1.2-12.3)	41	9.0 ± 3.4	(1.0-12.7)
	CG	40	6.9 ± 2.2	(1.4-10.1)	38	6.6 ± 2.9	(0.4-10.6)	35	6.3 ± 2.3	(0.4-9.8)	34	5.7 ± 2.6	(0.3-10.3)
RBC n-6LCP	IG	39	16.8 ± 4.7	(2.3-20.4)	40	12.3 ± 3.8	(2.9-17.1)	40	12.5 ± 3.9	(2.0-16.5)	41	12.7 ± 3.6	(2.8-16.9)
	CG	40	17.6 ± 3.8	(6.5-21.3)	38	15.5 ± 5.4	2.6-20.1	35	16.3 ± 4.5	(2.6-20.1)	34	16.1 ± 5.5	(2.0-21.5)
RBC 22:6n-3	IG	39	4.5 ± 1.5	(0.3-6.0)	40	7.2 ± 2.9	(0.8-10.6)	40	6.8 ± 2.7	(0.9-9.6)	41	6.5 ± 2.5	(0.8-9.3)
	CG	40	4.4 ± 1.5	(0.9-6.9)	38	4.4 ± 2.0	(0.3-6.9)	35	4.0 ± 1.5	(0.3-6.3)	34	3.2 ± 1.5	(0.1-6.21)
RBC 20:5n-3	IG	38	0.4 ± 0.2	(0.03-0.9)	40	0.7 ± 0.3	(0.1-1.5)	40	0.9 ± 0.6	(0.1-3.7)	41	1.0 ± 0.4	(0.1-1.7)
	CG	40	0.4 ± 0.2	(0.1-0.7)	36	0.4 ± 0.2	(0.04-0.7)	34	0.5 ± 0.2	(0.1-1.3)	34	0.5 ± 0.3	(0.01-1.3)
RBC 20:4n-6	IG	39	11.6 ± 3.3	(1.6-14.9)	40	8.7 ± 2.7	(2.0-12.0)	40	9.1 ± 2.8	(1.4-12.0)	41	9.5 ± 2.8	(1.9-20.2)
	CG	40	12.1 ± 2.8	(4.3-15.2)	38	10.4 ± 3.6	(1.7-13.7)	35	11.4 ± 3.3	(1.8-14.1)	34	11.5 ± 4.0	(1.2-15.4)
RBC 20:4n-6/22:6n-3	IG	39	2.8 ± 0.9	(1.9-7.4)	40	1.4 ± 0.4	(0.9-2.8)	40	1.5 ± 0.4	(1.0-2.5)	41	1.6 ± 0.4	(1.2-2.9)
	CG	40	3.0 ± 0.8	(1.7-4.9)	38	2.8 ± 1.1	(1.5-6.1)	35	3.2 ± 0.9	(1.8-6.4)	34	4.2 ± 1.5	(1.9-9.7)

Data are presented as mean±SD; Values for FAs are expressed as % of total fatty acids [wt %]; IG = intervention group; CG = control group; wk gest = week of gestation,
n-6LCP: C20:2n6; DHyLA; 20:4n-6; C22:2n-6; C22:4n-6; C22:5n-6;
n-3LCP: C20:3n3; C20:4n-3; 20:5n-3; C21:5n-3; C22:3n-3; n-3-DPA; 20:4n-6;
*****P < 0.05 significantly different distribution between the groups after Bonferroni correction

4.3.2 Cord blood

The LC-PUFA content in cord plasma phospholipids is shown in table 7 and appendix C.3. The maternal intervention resulted in significantly lower PL 20:4n-6 and total PL n-6 LC-PUFA in cord blood ($P < 0.05$) in comparison to the control group. Moreover, significantly higher levels of PL 20:5n-3, PL 22:6n-3 and total n-3 LC-PUFA was observed in cord plasma of the intervention group compared to the control group ($P < 0.05$). In RBCs 20:5n-3, 22:6n-3 and total n-3 LC-PUFA contents were also higher in the intervention group compared to the control group ($P < 0.05$) (Table 7). However, there was no significant difference in RBC 20:4n-6 and total RBC n-6 LC-PUFA content between the groups.

Table 7 PUFA profile in cord plasma PLs and RBCs

	group	n	mean ± SD	(range)
PL 20:4n-6	Intervention	23	14.9 ± 1.6*	12.4-17.7
	Control	24	17.9 ± 2.0	(12.9-21.9)
PL 22:6n-3	Intervention	23	8.3 ± 1.3*	(5.9-11.4)
	Control	24	6.2 ± 1.5	(3.8-9.6)
PL 20:5n-3	Intervention	23	0.9 ± 0.4*	(0.2-1.5)
	Control	24	0.3 ± 0.1	(0.1-0.5)
PL n-3 LCP	Intervention	23	9.9 ± 1.5*	(7.4-13.0)
	Control	24	6.8 ± 1.6	(4.3-10.5)
PL n-6 LCP	Intervention	23	21.3 ± 1.5*	(18.9-24.4)
	Control	24	25.0 ± 1.4	(22.3-27.8)
RBC 20:4n-6	Intervention	50	8.0 ± 4.3	(1.7-14.7)
	Control	49	8.4 ± 4.7	(1.8-16.7)
RBC 22:6n-3	Intervention	50	4.0 ± 2.7*	(0.4-9.2)
	Control	49	2.8 ± 2.1	(0.3-7.1)
RBC 20:5n-3	Intervention	50	0.21 ± 0.17*	(0.01-0.79)
	Control	44	0.08 ± 0.06	(0.02-0.22)
RBC n-3LCP	Intervention	50	4.7 ± 3.2*	(0.42-10.47)
	Control	49	3.2 ± 2.3	(0.36-8.03)
RBC n-6LCP	Intervention	50	12.8 ± 6.7	(2.4-24.2)
	Control	49	13.2 ± 7.1	(3.3-25.4)

*$P < 0.05$ for difference between the groups

4.4 Maternal insulin, glucose, HOMA-IR and lipids during pregnancy and lactation

4.4.1 Maternal insulin concentrations

In a subgroup of the INFAT-study maternal plasma insulin concentrations were measured during pregnancy and lactation to explore in a longitudinal analysis the effect of the n-6/n-3 LC-PUFA intervention. The insulin measurements were performed at the Institute of Laboratory Medicine, Clinical Chemistry and Molecular Diagnostics, University of Leipzig (Prof. Dr. Kratzsch) using an automatic analyzer.

Table 8 and figure 7 present the insulin concentrations in maternal plasma at the time points 15th week of gestation, 32nd week of gestation, 6th week PP and 16th week PP. Insulin concentrations increased significantly between 15th week of gestation and 32nd week of gestation from 37.7 ± 18.4 pmol/l to 61.0 ± 28.4 pmol/l ($P < 0.001$) in the intervention group and from 46.6 ± 24.9 pmol/l to 77.7 ± 50.0 ($P < 0.001$) in the control group (Table 8, Appendix B.1). Six weeks after birth lower plasma insulin levels compared with levels during pregnancy were observed in both, the intervention group (31.9 ± 24.7 pmol/l) and the control group (36.3 ± 18.8 pmol/l). Ten weeks later insulin concentrations were similar to the initial values at study entry.

To evaluate the group effect extreme outliers were excluded from the statistical analysis. As the initial values at study entry were higher in the control group further group effect adjusting for the initial values was computed. The insulin levels were not significantly different between the groups at all sampling points during pregnancy and lactation (Table 8). After exclusion of women who developed GDM during pregnancy, no significant effect of intervention at each time point during pregnancy and lactation was found either (Appendix B.5).

Table 8 Insulin concentrations [pmol/l] during pregnancy and lactation

Time		n	mean ± SD	(range)
15 wk gest	Intervention	41	37.7 ± 18.4	(11.8-78.3)
	Control	41	46.6 ± 24.9	(12.8-111.8)
32 wk gest#	Intervention	41	61.0 ± 28.4	(16.5-133.5)
	Control	41	77.7 ± 50.0	(21.5-335.4)
6 wk PP#	Intervention	41	31.9 ± 24.7	(8.3-153.9)
	Control	41	36.3 ± 18.8	(8.5-89.9)
16 wk PP#	Intervention	41	39.7 ± 24.1	(11.7-109.6)
	Control	41	45.9 ± 25.5	(10.4-125.8)

* P < 0.05 significantly different distribution between the groups after Bonferroni correction; # adjusted for baseline value (15th week of gestation); wk gest = week of gestation

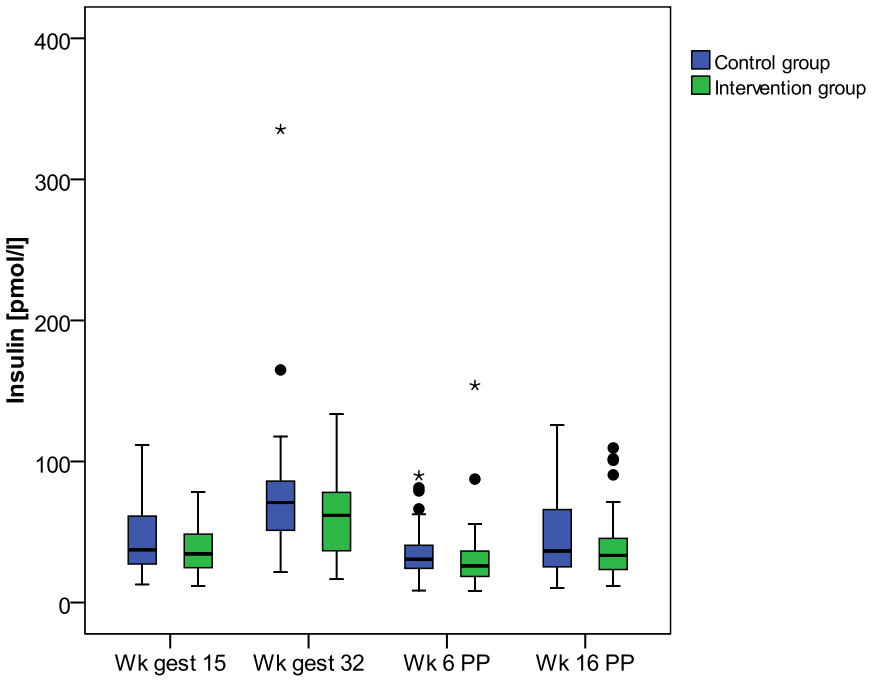


Figure 7 Longitudinal analysis of maternal insulin concentrations [pmol/l] during pregnancy and lactation; median, 25th and 75th centiles, ranges, outliers (sphericals), extreme outliers (asterisks) are shown

4.4.2 Maternal fasting glucose concentrations and HOMA-IR

Fasting glucose concentrations were measured at 15th week and 32nd week of gestation, 6th week and 16th week PP by a commercial laboratory (FutureLab, Munich, Germany) and HOMA-IR was calculated.

In table 9 maternal fasting glucose concentrations and insulin resistance expressed as HOMA-IR are shown. In the pregnant women glucose concentrations were 80 ± 10 mg/dl (Intervention) and 82 ± 12 mg/dl (Control) at 15th week of gestation and 82 ±

11 mg/dl (Intervention) and 80 ± 12 mg/dl (Control) at the 32nd week of gestation, respectively. Fasting glucose concentrations during lactation period were similar to baseline values at study entry in both groups. No difference in glucose levels between the groups was observed (Table 9).

HOMA-IR increased significantly during pregnancy from the 15th week of gestation to the 32nd week of gestation from 1.12 ± 0.49 to 1.81 ± 0.88 ($P < 0.001$) in the intervention group and from 1.39 ± 0.79 to 2.07 ± 0.99 ($P < 0.001$) in the control group, and declined after parturition to 0.91 ± 0.77 in the intervention group and to 1.06 ± 0.59 in the control group measured at 6 weeks PP (Table 9, Appendix B.2 and C.1). At 16 weeks PP HOMA-IR increased significantly to 1.13 ± 0.67 in the intervention group and to 1.39 ± 0.93 in the control group and reached values close to those measured at study entry (Table 9). For investigation of the group effect, two outliers from the intervention group were excluded. HOMA-IR levels were marginally but non-significant lower in the intervention group than in the control group at the 6th week PP. After adjustment for baseline values and exclusion of those women with GDM and HELLP syndrome the group effect was more attenuated (Appendix B.5).

Table 9 Maternal metabolic variables during pregnancy and lactation

		Wk gest 15			Wk gest 32			6 wks PP			16 wks PP		
		n	mean ±SD	(range)	n	mean ±SD	(range)	n	mean ±SD	(range)	n	mean ±SD	(range)
Glucose [mg/dl]	IG	31	80 ± 10	(64-106)	39	82 ± 11	(65-115)	41	80 ± 8	(64-101)	41	80 ± 8	(66-98)
	CG	40	82 ± 12	(65-107)	39	80 ± 12	(63-115)	41	82 ± 11	(63-112)	39	81 ± 11	(64-117)
HOMA-IR	IG	31	1.12 ± 0.49	(0.41-2.66)	39	1.81 ± 0.88	(0.59-3.94)	41	0.91 ± 0.77	(0.22-4.76)	41	1.13 ± 0.67	(0.37-3.11)
	CG	40	1.39 ± 0.79	(0.45-3.48)	39	2.07 ± 0.99	(0.66-4.98)	41	1.06 ± 0.59	(0.25-2.68)	39	1.39 ± 0.93	(0.24-5.23)
Total cholesterol [mg/dl]	IG	39	192 ± 31	(130-286)	41	268 ± 47	(194-378)	41	217 ± 39	(143-358)	40	197 ± 33	(123-309)
	CG	41	198 ± 37	(102-282)	40	269 ± 52	(150-364)	38	224 ± 42	(110-324)	41	202 ± 46	(99-315)
LDL-cholesterol [mg/dl]	IG	39	103 ± 24	(66-183)	41	165 ± 41	(98-272)	41	127 ± 36	(82-227)	39	113 ± 25	(63-185)
	CG	41	109 ± 33	(29-181)	40	166 ± 47	(45-261)	38	137 ± 42	34-228)	41	120 ± 39	(26-220)
HDL-cholesterol [mg/dl]	IG	39	79 ± 14	(45-107)	41	83 ± 17	(52-127)	41	79 ± 19	(34-127)	39	77 ± 18	(43-123)
	CG	41	76 ± 14	(40-105)	40	82 ± 17	(54-123)	38	71 ± 13	(53-115)	41	72 ± 15	(47-115)
TAG [mg/dl]	IG	39	102 ± 38	(43-232)	41	180 ± 66	(96-395)	40	66 ± 36*	(35-211)	40	55 ± 21*	(35-140)
	CG	41	117 ± 54	(42-335)	40	207 ± 64	(90-354)	38	81 ± 35	(35-205)	41	73 ± 39	(35-222)

adjusted for baseline values (= 15 wk gest); IG = intervention group, CG = control group; wk gest = week of gestation
*$P < 0.05$ significantly different distribution between the groups after Bonferroni correction

4.4.3 Maternal total cholesterol, HDL-cholesterol, LDL-cholesterol and TAGs

Total cholesterol, LDL-cholesterol, HDL-cholesterol and TAG concentrations were measured at the time points 15th week of gestation, 32nd week of gestation, 6 weeks PP and 16 weeks PP by a commercial laboratory (FutureLab, Munich, Germany). Differences between the groups and changes over time were explored (Table 9, Appendices B.2 and C.1)

In the intervention group total cholesterol levels increased from 192 ± 31 mg/dl to 268 ± 47 mg/dl during pregnancy (Table 9). In the control group total cholesterol levels changed from 198 ± 37 mg/dl to 269 ± 52 mg/dl during pregnancy. Sixteen weeks after birth total cholesterol levels of both groups were close to baseline values measured at the 15th week of gestation.

Pregnancy LDL-cholesterol levels increased from 103 ± 24 mg/dl to 165 ± 41 mg/dl in the intervention group and from 109 ± 33 mg/dl to 166 ± 47 mg/dl in the control group (Table 9). During the PP period LDL-cholesterol concentrations declined constantly and reached concentrations of 113 ± 25 mg/dl in the intervention group and 120 ± 39 mg/dl in the control group measured at 16 weeks PP. The LDL-cholesterol concentrations did not differ significantly between the groups at the 15th week of gestation, 32nd week of gestation, 6 weeks PP and 16 weeks PP.

At the 15th week of gestation mean HDL-cholesterol concentration was 79 ± 14 mg/dl in the intervention group and 76 ± 14 mg/dl in the control group. At the 32th week of gestation HDL-cholesterol concentrations reached 83 ± 17 mg/dl in the intervention group and 82 ± 17 mg/dl in the control group. There was no significant difference between the groups at any time point during pregnancy and lactation, although HDL-cholesterol levels were slightly higher in the intervention group in comparison to the control group at the 6th week PP (Table 9).

In both groups TAG concentrations increased considerably during the course of pregnancy and declined markedly PP (Table 9, Appendix B.2 and C.1). In the intervention group, TAG concentrations increased from 102 ± 38 mg/dl to 180 ± 66 mg/l during pregnancy and decreased to 66 ± 36 mg/dl at 6 weeks PP and to 55 ± 21 mg/dl at 16 weeks PP. In the control group, TAG concentrations increased from 117 ± 54 mg/dl to 207 ± 64 mg/dl during pregnancy and decreased to 73 ± 39 mg/dl measured at 16th week PP, respectively. TAG levels were lower in the intervention

group than in the control group throughout pregnancy and lactation, but only significant at 6 weeks and 16 weeks PP (Table 9). Without Bonferroni correction the TAG levels were significantly lower in the intervention group compared with the control group at the 32nd week of gestation and during the lactation period.

4.5 Maternal plasma leptin, FLI, sOB-R and HMW adiponectin concentrations

The course of plasma leptin, sOB-R, FLI and HMW adiponectin concentrations during pregnancy and lactation (15th week of gestation, 32nd week of gestation, 6 weeks PP and 16 weeks PP) was investigated and the group effect was explored after exclusion of extreme outliers. The measurements were performed at the Institute of Laboratory Medicine, Clinical Chemistry and Molecular Diagnostics, University of Leipzig (Prof. Dr. Kratzsch).

Maternal leptin concentrations increased continuously throughout pregnancy (Table 10 and Figure 8). In the intervention group leptin concentrations increased from 13.3 ± 7.7 ng/ml to 18.5 ± 9.5 ng/ml between the 15th week of gestation and the 32nd week of gestation and from 17.8 ± 10.0 ng/ml to 26.6 ± 17.2 ng/ml in the control group. The baseline leptin concentrations at the 15th week of gestation were higher in the control group compared to the intervention group. After adjustment for baseline values no significant difference between the groups at the time point 32nd week of gestation was observed (Table 10). In both groups leptin concentrations were significantly lower during lactation compared to pregnancy (Table 10, Appendix B.3). At the 6th week PP and 16th week PP leptin concentrations were 8.9 ± 6.3 ng/ml and 10.6 ± 9.00 ng/ml in the intervention group and 12.0 ± 7.1 ng/ml and 12.6 ± 9.9 ng/ml in the control group, respectively (Table 10). After adjustment for baseline values no significant difference between the groups at 6 weeks PP and 16 weeks PP was observed. Exclusion of women with GDM did not change the results (Appendix B.5).

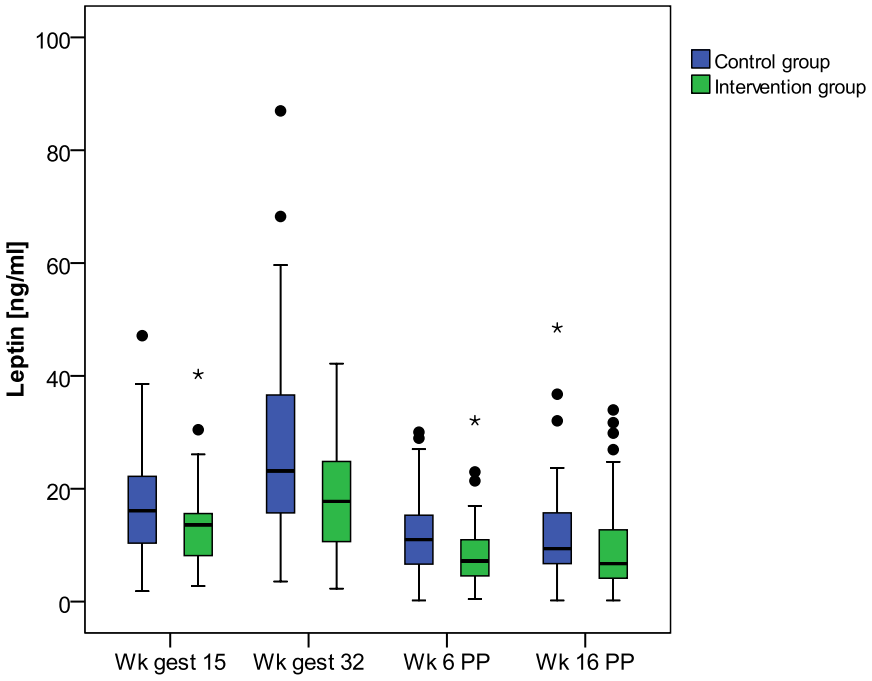


Figure 8 Longitudinal analysis of maternal leptin concentrations at 15th and 32nd week of gestation, 6 weeks PP and 16 weeks PP in the intervention and control group; median, 25th and 75th centiles, ranges, outliers (sphericals), extreme outliers (asterisks) are shown

Table 10 Leptin concentrations, sOB-R concentrations and FLI during pregnancy and lactation

			leptin#			sOB-R			FLI#	
15 wk gest	IG	41	13.3 ± 7.7	(2.8-40.3)	41	33.1 ± 8.3	(16.5-53.3)	41	0.44 ± 0.32	(0.06-1.48)
	CG	41	17.8 ± 10.0	(1.8-47.1)	41	32.5 ± 10.9	(18.2-88.2)	41	0.59 ± 0.38	(0.07-1.59)
32 wk gest	IG	41	18.5 ± 9.5	(2.3-42.2)	41	36.3 ± 10.0	(20.0-67.3)	41	0.58 ± 0.40	(0.03-1.74)
	CG	41	26.6 ± 17.2	(3.6-87.0)	41	33.8 ± 13.4	(17.7-99.6)	41	0.92 ± 0.77	(0.08-3.47)
6 wk PP	IG	41	8.9 ± 6.3	(0.4-32.1)	41	27.3 ± 5.6	(17.4-43.7)	41	0.35 ± 0.28	(0.01-1.32)
	CG	41	12.0 ± 7.1	(0.2-30.0)	41	27.6 ± 5.6	(17.9-46.8)	41	0.46 ± 0.32	(0.01-1.36)
16 wk PP	IG	41	10.6 ± 9.0	(0.2-34.0)	41	25.9 ± 5.7	(14.3-40.1)	41	0.47 ± 0.47	(0.01-1.88)
	CG	41	12.6 ± 9.9	(0.2-48.5)	41	26.2 ± 5.2	(13.8-40.1)	41	0.51 ± 0.43	(0.01-1.91)

*$P < 0.05$ significantly different distribution between the groups after Bonferroni correction

adjusted for baseline value (= 15 wk gest)

IG = intervention group, CG = control group, wk gest = week of gestation

As shown in figure 9 an increase similar and almost parallel to leptin was observed for the FLI during pregnancy and lactation. No significant difference in FLI was found between both groups at any time point during pregnancy and lactation (Table 10). Moreover, the group effect over time was not significant after adjustment for baseline levels.

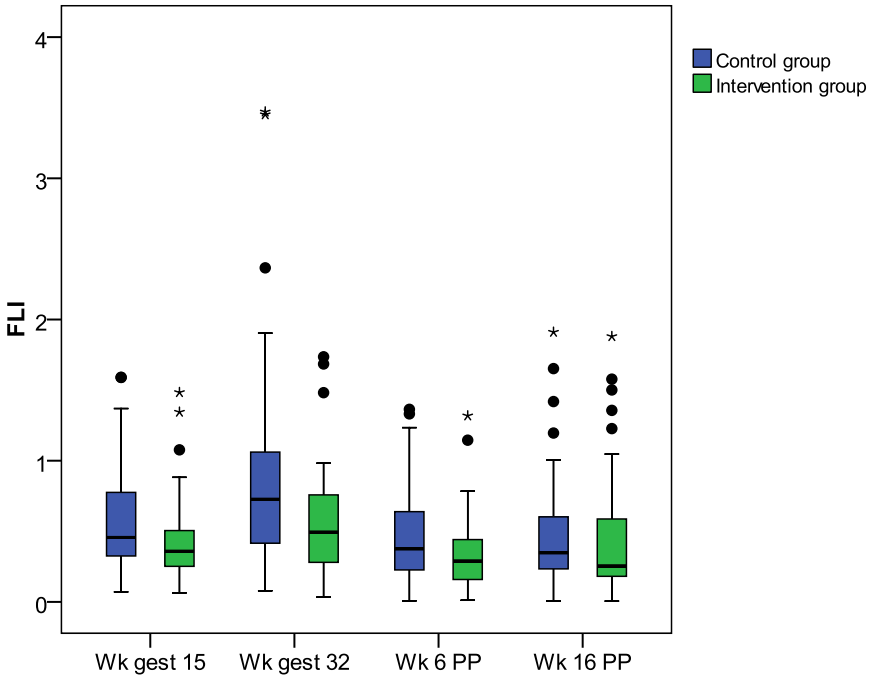


Figure 9 Longitudinal analysis of maternal FLI at 15th and 32nd week of gestation, 6 weeks PP and 16 weeks PP in the intervention and control group; median, 25th and 75th centiles, ranges, outliers (sphericals), extreme outliers (asterisks) are shown

In the intervention group, sOB-R concentrations increased significantly from 33.1 ± 8.3 ng/ml at the 15th week of gestation to 36.3 ± 10.0 ng/ml at the 32th week of gestation (Figure 10). In the control group there was no significant increase in mean sOB-R concentration from the 15th week of gestation (32.5 ±10.9 ng/ml) to the 32th week of gestation (33.8 ± 13.4 ng/ml) (Table 10). The sOB-R concentrations were lower during the PP period in both groups (Table 10, Appendix B.3). Until 6th week after birth mean sOB-R concentration dropped to 27.3 ± 5.6 ng/ml in the intervention group and reached a mean of 25.9 ± 5.7 ng/ml at 16th week after birth. In the control

group a mean sOB-R concentration of 27.6 ± 5.6 ng/ml at the 6th week PP and 26.2 ± 5.2 ng/ml at the 16th week PP was detected. No significant difference between both groups at each time point during pregnancy and lactation was observed (Table 10) which remained insignificant after exclusion of women with GDM (Appendix B.5).

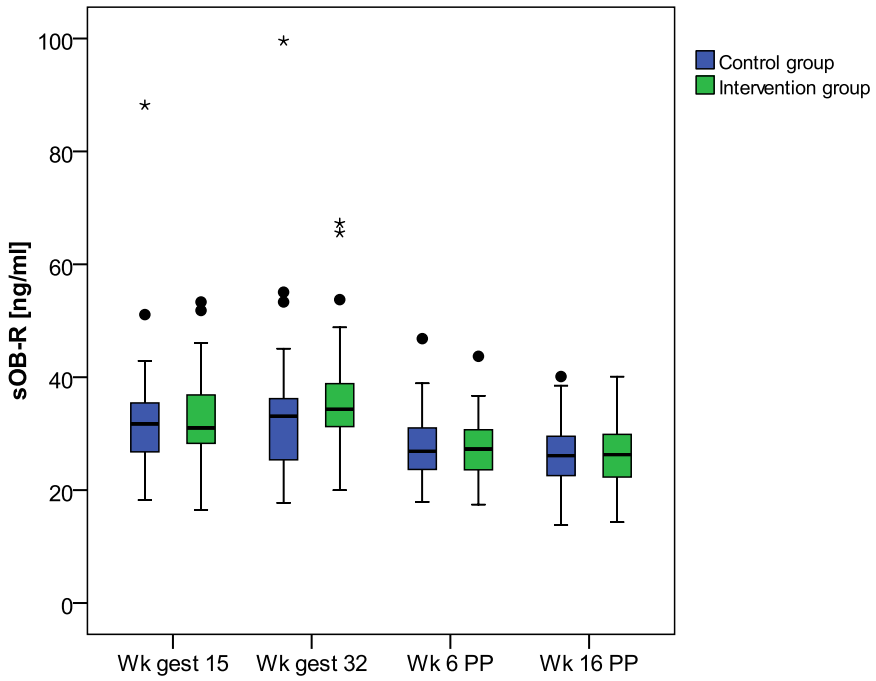


Figure 10 Longitudinal analysis of maternal sOB-R concentrations [ng/ml] at 15th and 32nd week of gestation, 6 weeks PP and 16 weeks PP in the intervention and control group; median, 25th and 75th centiles, ranges, outliers (sphericals), extreme outliers (asterisks) are shown

Figure 11 illustrates the course of HMW adiponectin levels during pregnancy and lactation. In both groups maternal HMW adiponectin concentrations decreased significantly during pregnancy (Table 11, Appendix B.4). In the intervention group mean HMW adiponectin concentration decreased significantly from 2.95 ± 1.19 µg/ml at the time point 15th week of gestation to 2.18 ± 1.04 µg/ml at the time point 32nd week of gestation. In the control group mean HMW adiponectin concentration was also significantly higher at the 15th week of gestation in comparison to the 32nd week of gestation (2.90 ± 1.61 µg/ml vs. 2.22 ± 1.27 µg/ml, $P < 0.001$). HMW adiponectin levels remained low at the 6th week PP in the intervention group (mean±SD 2.08 ±

0.77 µg/ml) as well as in the control group (mean±SD 2.11 ± 1.28 µg/ml) and increased until the 16th week PP in both groups, with a significant change only observed in the intervention group. There was no significant difference between both groups, neither during gestation nor during the lactation period, even after exclusion of women with GDM (Appendix B.5).

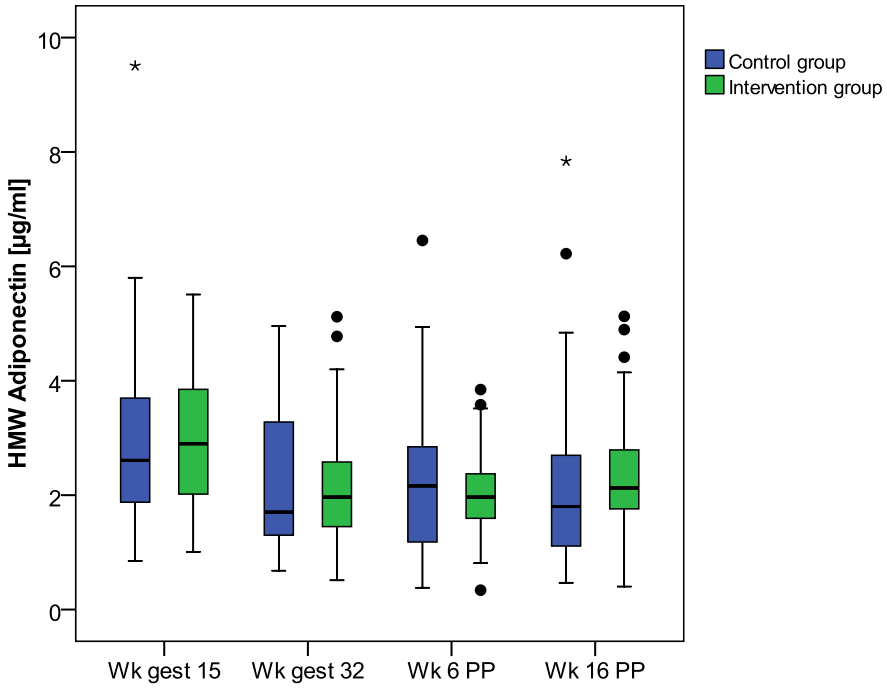


Figure 11 Longitudinal analysis of maternal HMW adiponectin concentrations at 15th week of gestation, 32 nd week of gestation, 6 weeks PP and 16 weeks PP in intervention and control group; median, 25th and 75th centiles, ranges, outliers (sphericals), extreme outliers (asterisks) are shown

Table 11 Maternal plasma HMW adiponectin concentrations [µg/ml] during pregnancy and lactation

Time		n	mean ± SD	(range)
15 wk gest	Intervention	41	2.95 ± 1.19	(1.01-5.51)
	Control	41	2.90 ± 1.61	(0.85-9.51)
32 wk gest	Intervention	41	2.18 ± 1.04	(0.51-5.12)
	Control	41	2.22 ± 1.27	(0.68-4.96)
6 wk PP	Intervention	41	2.08 ± 0.77	(0.34-3.85)
	Control	41	2.11 ± 1.28	(0.38-6.45)
16 wk PP	Intervention	41	2.34 ± 1.03	(0.40-5.13)
	Control	41	2.23 ± 1.58	(0.47-7.84)

*$P < 0.05$ significantly different distribution between the groups after Bonferroni correction; wk gest= week of gestation

4.6 Relation between maternal plasma concentrations of adipokines and BF measures and metabolic parameters during pregnancy

Extreme outliers and women with GDM/HELLP syndrome were excluded from the correlation analysis and the data of both groups were analyzed together. Partial correlation coefficients were calculated to elucidate the relation between maternal leptin system (leptin, sOB-R and FLI), HMW adiponectin concentrations, insulin concentrations, HOMA-IR, body fat and blood lipids (TAG, total cholesterol, HDL-cholesterol, LDL-cholesterol) during pregnancy. Age, pre-pregnancy BMI and group were considered as covariates (Tables 12 and 13).

4.6.1 Relation between maternal plasma leptin, sOB-R, BF measures, insulin, HOMA-IR and blood lipids during pregnancy

Pre-pregnancy BMI, pregnancy BMI and BF [%] were significantly positively associated with leptin levels and FLI during pregnancy ($P < 0.001$) (Table 12). sOB-R levels were inversely and significantly associated with BMI and BF [%] during pregnancy (Table 12).

Leptin levels were not significantly correlated with insulin, HOMA-IR and TAGs levels at the 15th week of gestation but showed a significant positive correlation at the 32nd

of gestation. No correlation between leptin levels and total cholesterol, LDL-cholesterol, HDL-cholesterol levels was observed (Table 12).

FLI was positively correlated with insulin and HOMA-IR levels during pregnancy. No correlations between FLI and TAGs, total cholesterol and HDL-cholesterol was found, except a weak correlation between FLI and TAG levels at the 32nd week of gestation (Table 12).

There was no correlation between sOB-R levels and HOMA-IR, total cholesterol, HDL-cholesterol, LDL-cholesterol levels during pregnancy (Table 12). sOB-R levels were weakly inversely correlated with insulin and TAG levels at time point 32nd week of gestation.

No statistically significant relation was found between plasma leptin levels and maternal HMW adiponectin concentrations at all sampling points during gestation. FLI was borderline inversely correlated with HMW adiponectin levels at the time point 32nd week of gestation ($r = -0.235$, $P = 0.046$). Interestingly, sOB-R levels showed a significant positive correlation with HMW adiponectin concentrations at the 15th week of gestation ($r = 0.273$, $P = 0.020$) and at the 32nd week of gestation ($r = 0.290$, $P = 0.013$).

Table 12 Correlations between maternal leptin levels, sOB-R levels, FLI, maternal BF and metabolic parameters

		leptin						sOB-R						FLI					
		15 wk gest			32 wk gest			15 wk gest			32 wk gest			15 wk gest			32 wk gest		
		n	r	*P*	n	r	*P*	n	r	*P*	n	r	*P*	n	r	*P*	n	r	*P*
Pre-pregnancy BMI[a]	all	76	0.559	**<0.001**	77	0.514	**<0.001**	76	-0.294	**0.010**	75	-0.260	**0.025**	75	0.562	**<0.001**	76	0.475	**<0.001**
Pregnancy BMI[a]	all	76	0.595	**<0.001**	74	0.669	**<0.001**	76	-0.310	**0.007**	72	-0.350	**0.003**	75	0.603	**<0.001**	73	0.649	**<0.001**
Pregnancy BF in %[a]	all	53	0.568	**<0.001**	56	0.613	**<0.001**	53	-0.359	**0.009**	54	-0.343	**0.012**	52	0.581	**<0.001**	55	0.628	**<0.001**
Insulin[b]	all	76	0.119	0.316	77	0.420	**<0.001**	76	-0.225	0.055	75	-0.233	**0.049**	75	0.237	**0.045**	76	0.417	**<0.001**
HOMA-IR[b]	all	66	0.179	0.161	75	0.469	**<0.001**	66	-0.217	0.088	73	-0.113	0.351	65	0.303	**0.017**	74	0.488	**<0.001**
HMW -Ad[b]	all	75	0.072	0.547	77	-0.172	0.143	75	0.273	**0.020**	75	0.290	**0.013**	74	-0.023	0.849	76	-0.235	**0.046**
TAG[b]	all	74	0.191	0.110	76	0.300	**0.010**	74	-0.063	0.604	74	-0.254	**0.032**	73	0.191	0.113	75	0.237	**0.045**
Total cholesterol[b]	all	74	0.140	0.244	76	0.173	0.143	74	0.027	0.825	74	0.129	0.285	73	0.112	0.355	75	-0.011	0.929
HDL-cholesterol[b]	all	74	0.077	0.524	76	0.117	0.326	74	0.160	0.183	74	0.038	0.750	73	0.001	>0.99	75	-0.018	0.881
LDL-cholesterol[b]	all	74	0.084	0.484	76	0.076	0.525	74	0.057	0.636	74	0.231	0.053	73	0.061	0.753	75	-0.080	0.502

Values at $P < 0.05$ are in boldface; [a)] adjusted for group [b)] adjusted for age, pre-pregnancy BMI and group; wk gest = week of gestation

4.6.2 Relation between maternal plasma HMW adiponectin, BF measures, insulin, HOMA-IR and blood lipids during pregnancy

Plasma HMW adiponectin concentrations were significantly inversely associated with BMI and BF [%] during pregnancy, albeit the correlation with BF was weak and not significant at time point 15th week of gestation (Table 13).

HMW adiponectin concentrations were inversely related to insulin levels, HOMA-IR, and TAG levels at the timepoint 32nd week of gestation. Total cholesterol was weakly positively associated with HMW adiponectin concentrations. HDL-cholesterol levels were significantly positively correlated with HMW adiponectin levels at the 15th week of gestation as well as at the 32nd week of gestation. LDL-cholesterol levels were not significantly associated with HMW adiponectin levels during pregnancy (Table 13).

Table 13 Correlations between maternal plasma HMW adiponectin levels and maternal BF and metabolic parameters

		15 wk gest			32 wk gest		
		n	r	*P*	n	r	*P*
Pre-pregnancy BMI[a]	all	76	-0.245	**0.034**	77	-0.165	0.154
Body fat in %[a]	all	53	-0.198	0.160	56	-0.385	**0.004**
BMI[a]	all	76	-0.265	**0.022**	74	-0.281	**0.016**
TAG[b]	all	74	-0.157	0.190	76	-0.292	**0.012**
Total cholesterol[b]	all	74	0.207	0.083	76	0.243	**0.038**
HDL-cholesterol[b]	all	74	0.267	**0.025**	76	0.283	**0.015**
LDL-cholesterol[b]	all	74	0.200	0.094	76	0.216	0.066
Insulin[b]	all	76	-0.042	0.721	77	-0.368	**0.001**
HOMA-IR[b]	all	66	0.028	0.828	75	-0.322	**0.006**

Values at $P < 0.05$ are in boldface; [a)] adjusted for group, [b)] adjusted for age, pre-pregnancy BMI and group; wk gest = week of gestation

4.7 Relation between maternal plasma insulin, adipokines and LC-PUFAs in plasma PLs during pregnancy

Potential correlations between maternal insulin levels, HOMA-IR, leptin, sOB-R, HMW adiponectin levels and LC-PUFAs in plasma PLs during pregnancy were explored with adjustment for age, pre-pregnancy BMI and group (pooled data) (Tables 14-17). Extreme outliers and women with GDM and HELLP syndrome were excluded from the correlation analysis and groups were analyzed together as well as separately.

4.7.1 Relation between maternal insulin, HOMA-IR, AA, EPA, DHA, n-3 and n-6 LC-PUFAs during pregnancy

A weak positive correlation between total PL n-6 LC-PUFA and maternal plasma insulin levels at time point 15th week of gestation in combined groups (r = 0.208, *P* = 0.075) and in the intervention group (r = 0.332, *P* = 0.044) was present (Table 14). No significant correlations between PL 20:4n-6, PL 20:5n-3, PL 22:6n-3, PL total n-3 LC-PUFA and insulin levels during pregnancy were observed (Table 14).

Table 14 Correlations between maternal plasma insulin and maternal PL LC-PUFAs in all subjects[a], intervention group[b] and control group[b] during pregnancy

		15 wk gest			32 wk gest		
		n	r	*P*	n	r	*P*
PL 20:4n-6	all	77	0.085	0.473	75	-0.074	0.537
	intervention	39	0.277	0.096	39	0.005	0.977
	control	38	-0.110	0.524	36	-0.097	0.586
PL 20:5n-3	all	77	0.006	0.957	75	-0.033	0.784
	intervention	39	-0.153	0.367	39	-0.116	0.494
	control	38	0.173	0.313	36	0.198	0.263
PL 22:6n-3	all	77	-0.028	0.811	75	-0.048	0.687
	intervention	39	-0.143	0.399	39	-0.062	0.716
	control	38	-0.009	0.956	36	0.001	>0.99
PL n-3 LC-PUFA	all	77	0.004	0.972	75	-0.055	0.645
	intervention	39	-0.175	0.300	39	-0.109	0.522
	control	38	0.077	0.657	36	0.067	0.706
PL n-6 LC-PUFA	all	77	0.208	0.075	75	0.049	0.683
	intervention	39	0.332	**0.044**	39	0.046	0.786
	control	38	0.114	0.508	36	0.042	0.813

Values at $P < 0.05$ are in boldface and denote significant correlation with insulin levels [a)] adjusted for age, pre-pregnancy BMI and group [b)] adjusted for age and pre-pregnancy BMI; wk gest = week of gestation

Potential associations between HOMA-IR and PUFAs during pregnancy were evaluated using the pooled data (Table 15). As seen for insulin levels there was a positive trend between the sum of PL n-6 LC-PUFA content and HOMA-IR (0.215, *P* = 0.087) at the 15th week of gestation. No relation between HOMA-IR and PL 20:4n-6, PL 20:5n-3, PL 22:6n-3 or PL n-3 LC-PUFA during pregnancy was found.

Table 15 Correlations between maternal HOMA-IR and maternal PL LC-PUFAs during pregnancy

		15 wk gest			32 wk gest		
		n	r	*P*	n	r	*P*
PL 20:4n-6	all	67	0.079	0.536	73	-0.106	0.382
PL 20:5n-3	all	67	0.068	0.592	73	0.058	0.634
PL 22:6n-3	all	67	-0.049	0.700	73	-0.013	0.914
PL n-3 LC-PUFA	all	67	0.005	0.970	73	0.005	0.966
PL n-6 LC-PUFA	all	67	0.215	0.087	73	-0.014	0.907

Values at *P* < 0.05 are in boldface and denote significant correlation with HOMA-IR adjusted for age, pre-pregnancy BMI and group; wk gest = week of gestation

4.7.2 Relation between maternal leptin, sOB-R, AA, EPA, DHA, n-3 and n-6 LC-PUFAs during pregnancy

PL 20:4n-6 FAs of all women and those of the control group (but not of the intervention group) were inversely associated with plasma leptin levels at study entry (Table 16). In both groups combined and the control group a weak inverse correlation between leptin concentrations and PL 22:6n-3 at the time point 32nd week of gestation was seen. There was no significant correlation between leptin concentrations and PL 20:5n-3, PL n-3 LC-PUFA and PL n-6 LC-PUFA content during pregnancy in any of the groups studied (Table 16).

sOB-R levels showed a significantly negative correlation with PL 20:4n-6 FAs in the intervention group at study entry (r = -0.360, *P* = 0.029). PL 20:5n-3 FAs were significantly positively correlated with sOB-R levels at the time point 15th week of gestation in all subjects (r = 0.326, *P* = 0.005) and in the intervention group (r = 0.505, *P* = 0.001). At study entry sOB-R levels were weakly positively associated with PL n-3-LC-PUFAs in both groups analyzed together and significantly associated with PL n-3-LC-PUFAs in the intervention group (Table 16).

Table 16 Correlations between maternal leptin, sOB-R and maternal PL LC-PUFAs in all subjects[a], intervention group[b] and control group[b]

		leptin						sOB-R					
		15th wk gest			32nd wk gest			15th wk gest			32nd wk gest		
		n	r	*P*	n	r	*P*	n	r	*P*	n	r	*P*
PL 20:4n-6	all	76	-0.289	**0.013**	75	-0.204	0.085	76	-0.104	0.381	73	-0.023	0.848
	IG	38	-0.133	0.439	39	-0.241	0.151	39	-0.360	**0.029**	38	-0.286	0.114
	CG	38	-0.413	**0.012**	36	-0.206	0.243	37	0.240	0.165	35	0.042	0.817
PL 20:5n-3	all	76	-0.226	0.054	75	-0.041	0.734	76	0.326	**0.005**	73	0.176	0.146
	IG	38	-0.253	0.137	39	-0.202	0.231	39	0.505	**0.001**	38	0.228	0.181
	CG	38	-0.253	0.136	36	0.050	0.780	37	-0.077	0.659	35	-0.052	0.774
PL 22:6n-3	all	76	-0.211	0.072	75	-0.203	0.087	76	0.085	0.475	73	0.132	0.276
	IG	38	-0.111	0.518	36	-0.187	0.269	39	0.156	0.355	38	0.185	0.280
	CG	38	0.206	0.227	39	-0.354	**0.040**	37	0.020	0.908	35	0.073	0.688
PL n-3LC-PUFA	all	76	-0.190	0.108	75	-0.154	0.196	76	0.214	0.069	73	0.148	0.222
	IG	38	-0.145	0.398	39	-0.200	0.235	39	0.404	**0.013**	38	0.238	0.161
	CG	38	-0.170	0.322	36	-0.240	0.172	36	-0.006	0.971	35	0.001	0.996
PL n-6LC-PUFA	all	76	-0.137	0.247	75	-0.032	0.790	75	-0.050	0.676	73	-0.197	0.103
	IG	38	0.064	0.711	39	-0.038	0.839	39	-0.238	0.157	39	-0.280	0.099
	CG	38	-0.306	0.070	36	-0.070	0.695	36	0.171	0.327	35	-0.112	0.535

Values at $P < 0.05$ are in boldface and denote significant correlations [a)] adjusted for age, pre-pregnancy BMI and group [b)] adjusted for age and pre-pregnancy BMI; IG = intervention group, CG = control group, wk gest = week of gestation

4.7.3 Relation between maternal HMW adiponectin, AA, EPA, DHA, n-3 and n-6 LC-PUFAs during pregnancy

PL 22:6n-3 was negatively and significantly correlated with HMW adiponectin concentrations at the 15th week of gestation in the combined groups (Table 17).

A negative trend between PL 20:4n-6 content and HMW adiponectin concentrations in the intervention group at 32nd week of gestation was observed (r = -0.307, P = 0.065) (Table 17).

PL n-6 LC-PUFAs showed a significantly negative association with HMW adiponectin concentrations in all subjects (r = -0.308, P = 0.009) and in the intervention group (r = -0.348, P = 0.035) at the time point 32nd week of gestation. The association between HMW adiponectin levels and PL n-6 LC-PUFA was less pronounced and not significant in the control group (P = 0.095).

Table 17 Correlations between maternal plasma HMW adiponectin concentrations and maternal PL LC-PUFAs in all subjects[a], intervention group[b] and control group[b]

		15 wk gest			32 wk gest		
		n	r	*P*	n	r	*P*
PL 20:4n-6	all	76	0.007	0.954	75	-0.014	0.905
	intervention	39	-0.052	0.760	39	-0.307	0.065
	control	37	0.075	0.671	36	0.098	0.581
PL 20:5n-3	all	76	0.025	0.835	75	-0.031	0.796
	intervention	39	0.099	0.560	39	-0.021	0.902
	control	37	-0.115	0.509	36	-0.175	0.323
PL 22:6n-3	all	76	-0.292	**0.012**	75	0.128	0.282
	intervention	39	-0.285	0.087	39	0.225	0.180
	control	37	-0.301	0.079	36	-0.022	0.904
PL n-3 LC-PUFA	all	76	-0.207	0.218	75	0.092	0.444
	intervention	39	-0.121	0.475	39	0.185	0.272
	control	37	-0.289	0.092	36	-0.081	0.649
PL n-6 LC-PUFA	all	76	0.036	0.761	75	-0.308	**0.009**
	intervention	39	-0.014	0.935	39	-0.348	**0.035**
	control	37	0.079	0.650	36	-0.291	0.095

Values at $P < 0.05$ are in boldface and denote significant correlations with HMW adiponectin concentrations [a)] adjusted for age, pre-pregnancy BMI and group [b)] adjusted for age and pre-pregnancy BMI; wk gest = week of gestation

4.8 Maternal plasma leptin and HMW adiponectin concentrations at delivery

In a subgroup, maternal plasma leptin and HMW adiponectin concentrations at birth were measured at the Institute of Laboratory Medicine, Clinical Chemistry and Molecular Diagnostics, University of Leipzig (Prof. Dr. Kratzsch).

Mean leptin concentration of the mothers at birth was 30.8 ± 17.1 ng/ml in the intervention group (n = 9) and 24.9 ± 15.7 ng/ml in the control group (n = 10), respectively. There was no significant difference between both groups ($P = 0.462$). Mean maternal HMW adiponectin concentration at birth was 0.62 ± 0.32 µg/ml in the intervention group (n = 9) and 0.82 ± 0.43 µg/ml in the control group (n = 9), but no significant group effect was observed ($P = 0.414$).

4.9 Insulin concentrations in cord blood

Analysis of insulin concentrations in cord plasma was performed at the Institute of Laboratory Medicine, Clinical Chemistry and Molecular Diagnostics, University of Leipzig (Prof. Dr. Kratzsch).

Cord plasma insulin median (25^{th}; 75^{th} centiles) was 24.9 (16.1; 43.3) pmol/l in the intervention group and 29.0 (22.5; 57.3) pmol/l in the control group, respectively. Cord blood insulin concentrations in the intervention group tended to be lower in the

intervention group compared to the control group (*P* = 0.060). However, after adjustment for gestational age and PI this effect was attenuated and not significant anymore (*P* = 0.868). The difference was significant after exclusion of extreme outliers and infants whose mothers had GDM pregnancies (*P* = 0.013) and remained significant after adjustment for gestational age and PI (Table 18, Appendix B.6).

Analyzing the effect in girls and boys separately, the values tended to be lower in the boys of the intervention group than in the boys of the control group (Figure 12), but failed to be statistically significant even after exclusion of extreme outliers and/or infants of mothers with GDM (Table 18, Appendix B.6).

Table 18 Insulin concentrations in cord plasma [pmol/l]

		Intervention group		Control group		*P*	*#P*
		n	median (25^{th}; 75^{th})	n	median (25^{th}; 75^{th})		
Insulin [pmol/l]	all	55	24.9 (16.1; 43.3)	53	29.0 (22.9; 57.4)	0.060	0.868
	girls	25	28.1 (17.5; 70.3)	26	31.2 (24.8; 63.8)	0.510	0.414
	boys	30	19.3 (14.1; 31.3)	27	28.1 (20.0; 53.8)	0.064	0.300
exclusion of GDM & extreme outliers		50	21.0 (15.1; 34.8)*	48	28.6 (22.2; 56.6)	0.013	0.042
	girls	21	27.8 (16.9; 44.2)	24	31.2 (24.5; 57.5)	0.101	0.182
	boys	29	18.4 (13.9; 30.2)	24	27.9 (19.6; 52.5)	0.080	0.159

**P* < 0.05 for difference between groups; # adjusted for gestational age and PI

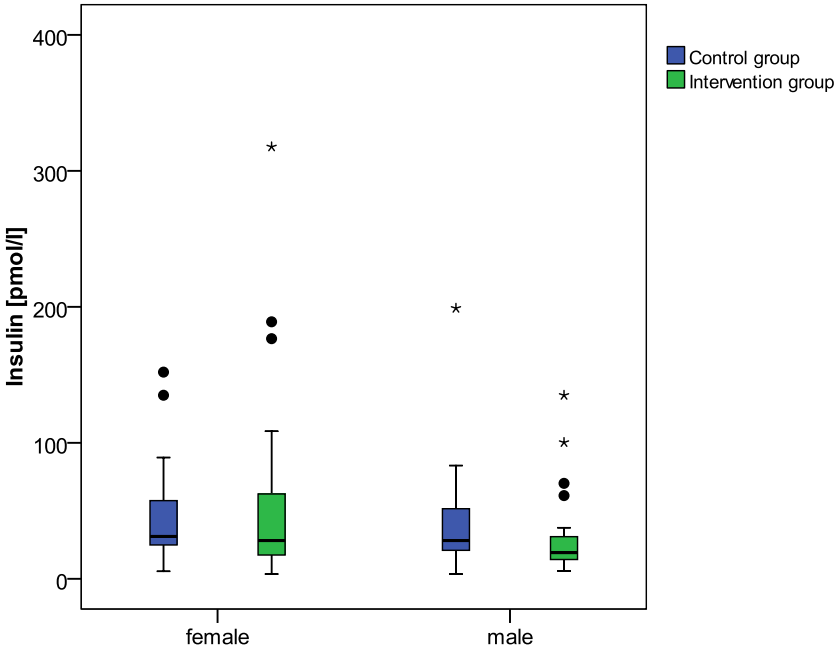


Figure 12 Cord plasma insulin concentrations [pmol/l] in girls vs. boys in the intervention and control group

4.10 Leptin, sOB-R, FLI and HMW adiponectin concentrations in cord blood

In a subgroup of the INFAT-study leptin, sOB-R, and HMW adiponectin concentrations were measured in cord plasma samples at the Institute of Laboratory Medicine, Clinical Chemistry and Molecular Diagnostics, University of Leipzig (Prof. Dr. Kratzsch).

Table 19 presents the mean leptin, sOB-R and HMW adiponectin concentration in cord blood. Mean leptin concentrations in cord blood were 12.6 ± 11.4 ng/ml in the intervention group and 10.0 ± 6.9 ng/ml in the control group, respectively (Table 19). For evaluation of the group effect one extreme outlier was excluded from the intervention group (Figure 13). No significant difference in leptin concentrations between both groups was found (P = 0.348). After further adjustment for gestational age and PI this difference remained insignificant (P = 0.744). Further exclusion of infants of mothers with GDM showed no significant group effect (Appendix B.6).

In consideration of potential effects between the sexes leptin concentrations in girls and boys were investigated separately. Cord plasma leptin concentrations were significantly higher in girls (n = 50) than in boys (n = 57) (mean±SD 14.5 ± 11.8 ng/ml vs. 8.6 ± 5.7 ng/ml, $P < 0.05$) (not shown in table 19). In the intervention group mean leptin concentration in girls was 17.4 ± 14.1 ng/ml and in boys 8.7 ± 6.3 ng/ml, respectively. In the control group mean leptin concentration of 11.6 ± 8.1 ng/ml was measured in girls and 8.6 ± 5.1 ng/ml in boys. No significant group effect was observed neither in females nor in males (one extreme outlier was excluded from the intervention group). After further exclusion of infants whose mothers had GDM, cord plasma leptin concentrations were significantly higher in girls of the intervention group compared to the control group (P = 0.029). However, this effect was not significant anymore after adjustment for gestational age and PI (Appendix B.6).

Mean sOB-R concentration, mean FLI and mean HMW adiponectin concentration did not differ between the groups even after adjustment for gestational age and PI (Table 19). Further exclusion of infants from GDM pregnancies revealed no significant difference between the groups (Appendix B.6).

Table 19 Leptin system and HMW adiponectin concentrations in cord plasma

		Intervention		Control		*P*	*#P*
		n	mean± SD (range)	n	mean± SD (range)		
Leptin [ng/ml]	*all*	55	12.6 ± 11.4 (0.9-72.2)	52	10.0 ± 6.9 (1.3-37.2)	0.384	0.744
	girls	25	17.4 ± 14.1 (5.4-72.2)	25	11.6 ± 8.1 (2.1-37.2)	0.101	0.300
	boys	30	8.7 ± 6.3 (0.9-28.0)	27	8.6 ± 5.1 (1.3-19.1)	0.860	0.498
sOBR [ng/ml]		31	14.8 ± 3.8 (8.8-22.7)	26	14.3 ± 4.3 (8.4-23.5)	0.781	0.654
FLI		31	0.9 ± 0.8 (0.1-3.4)	26	0.7 ± 0.6 (0.1-2.1)	0.204	0.347
HMW-Ad [µg/ml]		24	15.1 ± 5.9 (7.7-27.1)	26	13.5 ± 5.3 (6.7-28.6)	0.356	0.586

P < 0.05 for difference between the groups, # adjusted for gestational age and PI

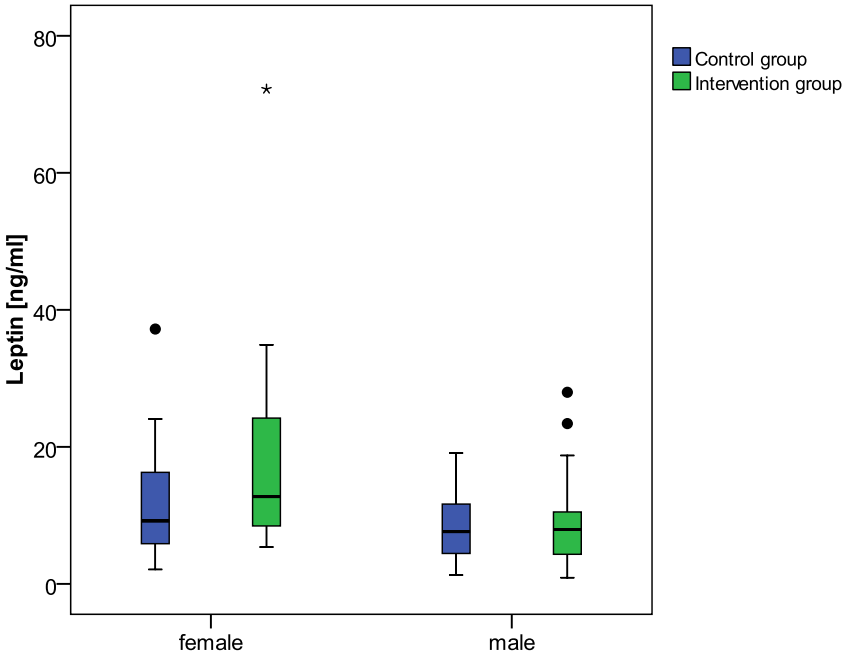


Figure 13 Cord plasma leptin concentrations [ng/ml] in girls vs. boys in the intervention and control group

4.11 Relation between cord blood insulin, adipokines, BF measures and anthropometric data of the newborns

Extreme outliers and values from infants of GDM pregnancies were excluded from the statistical analysis. Data of both groups were studied combined or separately. Infant sex, gestational age and group (pooled data) were considered as confounding variables.

4.11.1 Relation between cord blood insulin and BF measures and anthropometric data of the newborns

Cord plasma insulin concentrations were significantly inversely correlated with gestational age (r = -0.340, *P* = 0.001) (Table 20).

Birth weight was significantly positively correlated with cord plasma insulin concentrations in all subjects (r = 0.319, *P* = 0.002) and in the control group (r = 0.361, *P* = 0.014), and a weak association was seen in the intervention group (r = 0.268, *P* = 0.066). No significant correlation between PI and cord blood insulin concentrations was found, although there was a positive trend in both groups analyzed together and in the intervention group (Table 20).

Cord blood insulin was positively correlated with BF [%] in all subjects (r = 0.349, *P* = 0.001), in the intervention group (r = 0.387, *P* = 0.008) and in the control group (r = 0.342, *P* = 0.026).

Biceps SFT was weakly correlated with cord blood insulin levels in all subjects (r = 0.184, *P* = 0.084) and significantly correlated with cord blood insulin levels in the intervention group (r = 0.343, *P* = 0.020).

Suprailiacal SFT was significantly related to cord blood insulin concentrations in the pooled samples (r = 0.240, *P* = 0.023), whereas a weak positive correlation was observed in the intervention group (r = 0.275, *P* = 0.064).

Triceps SFT was significantly correlated with cord plasma insulin levels in both groups analyzed together and in the intervention group.

In the control group there was no significant correlation with biceps SFT, suprailical SFT and triceps SFT observed.

Subscapular SFT showed a positive correlation with cord blood insulin levels in all groups (Table 20).

Table 20 Correlations between cord plasma insulin levels and anthropometric data of the newborns in all subjects[a], control group[b] and intervention group[b]

		n	r	*P*
Gestational age	all	98	-0.340	**0.001**
Placental weight	all	98	0.146	0.167
Infant sex	all	98		**0.018**
Body weight	all	98	0.319	**0.002**
	Intervention	50	0.268	0.066
	Control	48	0.361	**0.014**
Body length	all	98	0.175	0.090
	Intervention	50	0.025	0.867
	Control	48	0.261	0.080
PI	all	98	0.180	0.081
	Intervention	50	0.261	0.073
	Control	48	0.134	0.374
BF in g	all	92	0.364	**<0.001**
	Intervention	48	0.403	**0.005**
	Control	44	0.360	**0.019**
BF in % BW	all	92	0.349	**0.001**
	Intervention	48	0.387	**0.008**
	Control	44	0.342	**0.026**
Bizeps	all	92	0.184	0.084
	Intervention	48	0.343	**0.020**
	Control	44	0.127	0.423
Trizeps	all	92	0.265	**0.012**
	Intervention	48	0.327	**0.027**
	Control	44	0.235	0.134
Subscapular	all	92	0.338	**0.001**
	Intervention	48	0.347	**0.018**
	Control	44	0.347	**0.024**
Suprailiacal	all	92	0.240	**0.023**
	Intervention	48	0.275	0.064
	Control	44	0.230	0.143

Values at $P < 0.05$ are in boldface and denote significant correlation with insulin levels [a)] adjusted for gestational age, sex and group [b)] adjusted for gestational age and sex

4.11.2 Relation between cord blood leptin, sOB-R, BF measures and anthropometric data of the newborns

Cord blood leptin levels were significantly associated with sex ($P = 0.002$) and gestational age (r = 0.299, $P = 0.003$) (Table 21). Cord blood leptin concentrations were significantly positively related to birth weight (r = 0.456, $P < 0.001$), birth length (r = 0.302, $P = 0.003$), BF [%] (r = 0.512, $P < 0.001$) and BF [g] (r = 0.560, P <0.001) of the newborn after adjustment for sex and gestational age (Table 21). No significant correlation between PI and cord blood leptin concentrations was found, although there was a positive trend (r = 0.171, $P = 0.095$). Biceps SFT, triceps SFT, subscapular SFT and suprailiacal SFT showed a significant positive correlation with cord blood leptin levels (Table 21).

FLI was significantly and positively associated with birth weight, birth length, BF [%], BF [g] and SFTs (except with SFT biceps) (Table 21).

A slight, but significant correlation between cord plasma sOB-R concentrations and gestational age (r = 0.281, *P* = 0.039) was found. Spearman's correlation analysis revealed no significant correlation between cord plasma sOB-R levels and birth weight (not shown in table 21). When adjusted for infant sex and gestational age, a borderline significant association between cord blood sOB-R levels and birth weight was found (r = -0.293, *P* = 0.035) (Table 21). Cord blood sOB-R concentrations were marginally inversely correlated with birth length (r = -0.264, *P* = 0.059) and BF [g] (r = -0.272, *P* = 0.056). PI was not related to sOB-R levels in cord blood. Furthermore, no significant association between SFTs and cord blood sOB-R levels was found (Table 21).

4.11.3 Relation between cord blood HMW adiponectin and BF measures and anthropometric data of the newborns

Cord blood HMW adiponectin levels were not correlated with birth weight (r = 0.187, *P* = 0.198), however a significant correlation was found between cord plasma HMW adiponectin levels and PI at birth (r = 0.324, *P* = 0.023) (Table 21). No statistically significant relation was found between cord blood HMW adiponectin concentrations and birth length, BF [%] and BF [g]. Furthermore, no association between fetal HMW adiponectin concentrations and SFT biceps, SFT triceps, SFT subscapular and SFT suprailiacal was observed (Table 21).

Table 21 Correlations between cord plasma leptin[a], sOB-R[b], FLI[b], HMW adiponectin[c] and clinical and anthropometric data of the newborns

	leptin			sOB-R			FLI			HMW adiponectin		
	n	r	*P*	n	r	*P*	n	r	*P*	n	r	*P*
GA	99	0.299	**0.003**	54	0.281	0.039	54	0.293	**0.032**	50	0.134	0.357
PW	99	0.417	**< 0.001**	53	0.003	0.980	53	0.415	**0.002**	50	-0.068	0.666
Sex	99		**0.002**	54		0.707	54		**0.033**	50		0.520
BW	99	0.456	**< 0.001**	54	-0.293	**0.035**	54	0.451	**0.001**	50	0.187	0.198
BL	99	0.302	**0.003**	54	-0.264	0.059	54	0.329	**0.017**	50	-0.057	0.696
PI	99	0.171	0.095	54	-0.023	0.872	54	0.120	0.396	50	0.324	**0.023**
BF [g]	93	0.560	**< 0.001**	52	-0.272	0.056	52	0.631	**<0.001**	46	0.192	0.206
BF [%]	93	0.512	**< 0.001**	52	-0.225	0.116	52	0.628	**<0.001**	46	0.112	0.462
Bizeps	93	0.223	**0.035**	52	-0.226	0.115	52	0.231	0.106	46	0.141	0.355
Trizeps	93	0.378	**< 0.001**	52	-0.204	0.155	52	0.322	**0.023**	46	0.191	0.209
Subscapular	93	0.514	**< 0.001**	52	-0.139	0.337	52	0.418	**0.003**	46	0.156	0.305
Suprailiacal	93	0.393	**< 0.001**	52	-0.248	0.083	52	0.578	**<0.001**	46	0.090	0.557

Values at *P* < 0.05 are in boldface; [a)] adjusted for gestational age, sex and group [b)] adjusted for gestational age and group [c)] adjusted for group; GA = gestational age, PW = placental weight, BW = birth weight, BL = birth length, PI = Ponderal Index, BF = Body fat

4.12 Relation between cord blood insulin, adipokines and LC-PUFAs in cord plasma PLs

Infants of GDM pregnancies and extreme outliers were excluded from the statistical analysis. Potential associations between cord plasma insulin levels, leptin system, HMW adiponectin levels and PUFAs (20:4n-6, 20:5n-3, 22:6n-3 FAs, the sum of n-3 LC-PUFAs and the sum of n-6 LC-PUFAs) in cord plasma PLs were investigated.

4.12.1 Relation between cord blood insulin, AA, DHA, EPA, n-3 and n-6 LC-PUFAs

Potential associations between cord plasma insulin levels and LC-PUFAs in cord plasma PLs were explored with adjustment for infant sex, gestational age and group (pooled data).

Combining both groups, cord blood insulin levels were not significantly associated with PL 20:4n-6, PL 22:6n-3, PL 20:5n-3, the sum of PL n-3 LC-PUFA and the sum of PL n-6 LC-PUFA (Table 22). Studying the groups separately, there was no indication for significant associations between these variables (Table 22), except in case of PL 20:5n-3 FAs. There was a negative trend between PL 20:5n-3 FAs and insulin concentrations in pooled samples (r = -0.304, *P* = 0.064) and in the intervention group the association was significant (r = -0.476, *P* = 0.039).

Table 22 Correlations between cord blood insulin levels and cord plasma PL LC-PUFAs in all subjects[a], intervention group[b] or control group[b]

		n	r	*P*
PL 20:4n-6	all	41	-0.105	0.530
	Intervention	21	-0.219	0.369
	Control	20	0.070	0.783
PL 20:5n-3	all	41	-0.304	0.064
	Intervention	21	-0.476	**0.039**
	Control	20	-0.060	0.813
PL 22:6n-3	all	41	-0.107	0.523
	Intervention	21	-0.043	0.861
	Control	20	-0.080	0.752
PL n3- LCP	all	41	-0.153	0.361
	Intervention	21	-0.175	0.473
	Control	20	-0.061	0.811
PL n6- LCP	all	41	-0.128	0.443
	Intervention	21	-0.193	0.429
	Control	20	0.001	0.999

Values at $P < 0.05$ are in boldface and denote significant correlation with insulin [a)] adjusted for gestational age, sex and group [b)] adjusted for gestational age and sex

4.12.2 Relation between cord blood leptin, sOB-R, AA, DHA, EPA, n-3 and n-6 LC-PUFAs

Partial correlation was performed between LC-PUFAs (20:4n-6, 20:5n-3, 22:6n-3 FAs, the sum of n-3 LC-PUFAs and the sum of n-6 LC-PUFAs) in cord plasma PLs and cord plasma leptin levels, FLI and sOB-R levels (Table 23).

There was a negative trend between cord plasma leptin levels and PL n-3 LC-PUFA (r = -0.299, *P* = 0.064) after adjustment for infant sex, gestational age and group. Cord blood leptin was not significantly associated with PL 20:4n-6, PL 20:5n-3, PL 22:6n-3, PL n-3 LC-PUFA and PL n-6 LC-PUFA (Table 23).

A negative trend between PL 22:6n-3 FA and FLI was observed after adjustment for gestational age and group. The other PUFAs were not related to FLI.

sOB-R levels were positively associated with PL 22:6n-3 (r = 0.463, *P* = 0.026) and PL n-3 LC-PUFA (r = 0.461, *P* = 0.027) after adjustment for gestational age and group (Table 23).

Table 23 Correlations between cord plasma leptin[a], sOB-R[b], FLI[b] and HMW adiponectin[c] and LC-PUFAs in cord plasma PLs

	leptin			sOB-R			FLI			HMW adiponectin		
	n	r	*P*	n	r	*P*	n	r	*P*	n	r	*P*
PL 20:4n-6	42	-0.117	0.479	25	0.257	0.237	25	-0.206	0.346	20	0.144	0.570
PL 20:5 n-3	42	-0.218	0.183	25	0.021	0.923	25	-0.049	0.825	20	0.114	0.652
PL 22:6n-3	42	-0.268	0.099	25	0.463	**0.026**	25	-0.397	0.060	20	0.479	**0.044**
PL n-3 LC-PUFA	42	-0.299	0.064	25	0.461	**0.027**	25	-0.371	0.081	20	0.453	0.059
PL n- 6 LC-PUFA	42	-0.083	0.617	25	0.085	0.700	25	-0.092	0.677	20	-0.025	0.922

Values at *P* < 0.05 are in boldface; [a)] adjusted for gestational age, sex and group, [b)] adjusted for gestational age and group
[c)] adjusted for Ponderal index and group

4.12.3 Relation between cord blood HMW adiponectin, AA, DHA, EPA, n-3 and n-6 LC-PUFAs

There was a weak positive relation between HMW adiponectin levels and PL 22:6n-3 content after adjustment for PI and group (r = 0.479, *P* = 0.044) (Table 23). Furthermore, cord blood HMW adiponectin levels showed a borderline correlation with PL n-3-LC-PUFA (r = 0.453, P = 0.059). PL 20:4n-6, PL 20:5n-3 and PL n-6 LC-PUFA were not significantly correlated with HMW adiponectin levels.

4.13 Maternal leptin and total adiponectin concentrations in breastmilk

Leptin and total adiponectin concentrations were measured in breast milk samples (6^{th} week PP and 16^{th} week PP) at the Institute of Laboratory Medicine, Clinical Chemistry and Molecular Diagnostics, University of Leipzig (Prof. Dr. Kratzsch). Due to methodological difficulties in measurement of the HMW adiponectin form total adiponectin was determined in breast milk samples.

The median (25^{th}, 75^{th} centiles) leptin concentration in breast milk at the time point 6 weeks PP was 0.10 (0.04, 0.16) ng/ml in the intervention group (n = 37) and 0.10 (0.05, 0.24) ng/ml in the control group (n = 39) (Figure 14). At the 16^{th} week PP the median was 0.09 (0.02, 0.15) ng/ml in the intervention group (n = 38) and 0.11 (0.06, 0.23) ng/ml in the control group (n = 38). No significant difference between the groups was found neither 6 weeks PP (P = 0.444), nor 16 weeks PP (P = 0.191). There was no significant change in milk leptin concentrations over time within the groups.

At the 6^{th} week PP, mean total adiponectin concentrations in skim breast milk were 12.24 ± 7.21 ng/ml (n = 40) in the intervention group and 11.70 ± 5.52 ng/ml (n = 38) in the control group, respectively (Figure 15). At the 16^{th} week PP mean total adiponectin concentrations were 11.0 ± 6.62 ng/ml (n = 40) in the intervention group and 10.83 ± 5.95 ng/ml (n = 36) in the control group, respectively. There was no significant group effect, neither at the 6^{th} week PP (P = 0.853) nor at the 16^{th} week PP (P = 0.880). Furthermore, no significant change within the time course between the 6^{th} week PP and 16^{th} week PP in both groups was found.

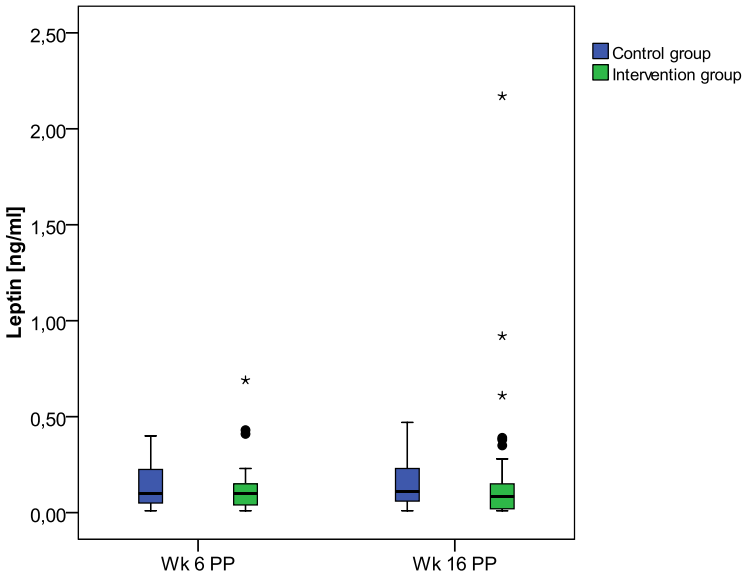


Figure 14 Leptin breast milk concentrations [ng/ml] at the 6th week PP and 16th week PP; median, 25th and 75th centiles, ranges, outliers (sphericals), extreme outliers (asterisks) are shown

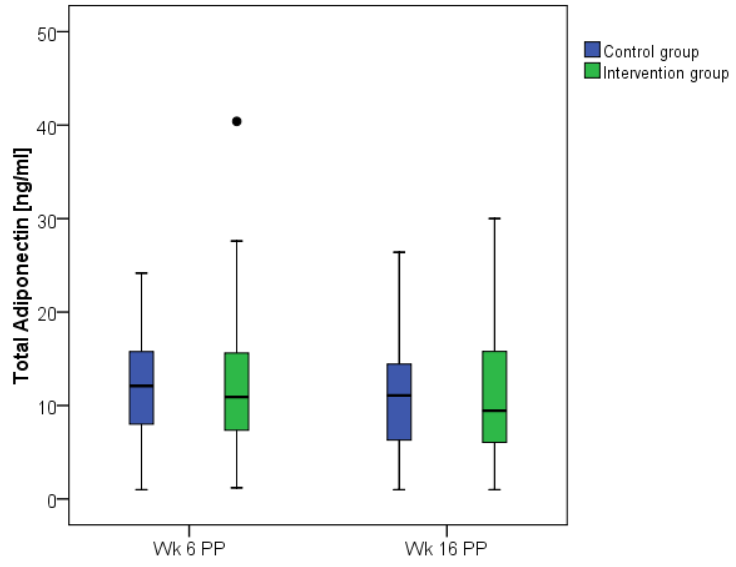


Figure 15 Total adiponectin breast milk concentrations [ng/ml] at the 6th week PP and 16th week PP; median, 25th and 75th centiles, ranges, outliers (sphericals), extreme outliers (asterisks) are shown

4.14 HMW adiponectin concentrations in infants 16 weeks PP

HMW adiponectin concentrations in plasma of breast-fed infants at the time point 16th week PP were measured at the Institute of Laboratory Medicine, Clinical Chemistry and Molecular Diagnostics, University of Leipzig (Prof. Dr. Kratzsch).

HMW adiponectin concentrations were 16.2 ± 5.9 µg/ml in the intervention group and 15.3 ± 5.1 µg/ml in the control group, respectively (Table 24). No difference between the groups was found (*P* = 0.380). Other variables such as infant sex, age, body weight, body length and PI did not differ between the groups in this subsample (Table 24).

Table 24 Anthropometric data and HMW adiponectin concentrations [µg/ml] of the infants 16 weeks PP

		n	mean±SD (range)	*P*
Sex	Intervention girls/boys	29	44.8%/55.2%	0.757
	Control girls/boys	27	40.7% /59.3%	
Age [weeks]	Intervention	29	15.8 ±1.1 (14-19)	0.566
	Control	27	15.7 ±1.1 (14-19)	
Body weight [g]	Intervention	29	6336 ± 553 (5416-7390)	0.774
	Control	27	6374 ± 806 (4660-7968)	
Body length [cm]	Intervention	29	62.0 ± 2.0 (58.0-65.5)	0.274
	Control	27	62.6 ± 2.4 (57.2-67.0)	
PI [kg/m³]	Intervention	29	26.7 ± 2.6 (21.7-31.8)	0.238
	Control	27	26.0 ± 2.3 (22.3-31.6)	
HMW-Adiponectin [µg/ml]	Intervention	29	16.2 ± 5.9 (0.73-26.6)	0.380
	Control	27	15.3 ± 5.1 (8.7-25.7)	

**P* < 0.05 for difference between the groups

4.15 Explorative analysis of biomarkers in cord blood

In an explorative analysis 86 metabolic, hormonal and inflammatory markers in cord plasma and in maternal plasma at the 32nd week of gestation were determined in the laboratory of RBM Inc. using an inflammation MAP and a metabolic/hormonal MAP. The aim was to identify biomarkers which might be influenced by the lower n-6/n-3 LC-PUFA ratio in maternal diet during pregnancy. Table 25 shows the variables which were significantly or marginally different in cord plasma between both groups. Group effects of all variables are shown in appendices B.7-8 and C.4-5.

As shown in table 25 Interleukin (IL)-1α, IL-17, Matrix metalloproteinase (MMP)-3, haptoglobin, VDBP (Vitamin D binding protein) derived from the inflammation MAP were significantly lower in the intervention group compared to the control group. In

addition, Glucagon like peptide (GLP)-1 and AgRP concentrations from the hormonal/metabolic MAP differed significantly between both groups. Higher concentrations of GLP-1 were found in the intervention group compared to the control group. AgRP concentrations were lower in the intervention group than in the control group. After adjustment for infant sex Apolipoprotein C-III (ApoC-III) levels were significantly higher in the control group than in the intervention group and BDNF concentrations tended to be higher in the control group. Analyzing girls and boys separately significantly lower BDNF concentrations were detected in females in the intervention group compared to the control group (P = 0.004). In males, BDNF levels were higher in the intervention group but the group effect was not significant.

In maternal plasma at the time point 32nd week of gestation none of the parameters from table 25 were different between both groups (data not shown), except in the case of fibrinogen lower maternal concentrations were observed in the intervention group than in the control group (mean±SD 3.1 ± 0.8 mg/ml vs. 4.6 ± 1.0 mg/ml, P = 0.007).

As interesting candidates AgRP and BDNF –both involved in hypothalamic energy regulation– were then validated using commercial ELISAs. The other variables could not be validated due to limited volume of samples and economic sources.

Table 25 Biomarkers of inflammation and hormonal/metabolic MAP with a significant or weak group effect in cord plasma

	Intervention group			Control group			*P**	*P#*
	n	mean ± SD	(range)	n	mean ± SD	(range)		
GLP-1[pg/ml]	20	18.0 ± 3.4	(11-25)	20	5.9 ± 3.9	(11-26)	0.033	0.061
AgRP [pg/ml]	20	880.5 ± 303.2	(518-1660)	20	1096.9 ± 357.0	(418-1820)	0.030	0.048
Calcitonin [pg/ml]	20	22.8 ± 6.9	(13-44)	20	26.2 ± 7.2	(14-40)	0.070	0.129
PYY [pg/ml]	20	338.7 ± 187.7	(66-765)	20	461.6 ± 221.5	(170-1000)	0.062	0.066
ApoCIII [µg/ml]	20	40.4 ± 14.1	(22-69)	20	50.0 ± 15.0	(21-84)	0.051	0.034
Cortisol [ng/ml]	20	207.6 ± 101.7	(51-389)	20	153.9 ± 83.4	(53-356)	0.051	0.075
Glucagon [pg/ml]	20	252.9 ± 113.9	(92-454)	20	319.8 ± 125.7	(134-636)	0.058	0.087
IGF-1 [ng/ml]	20	12.5 ± 3.8	(8-21)	20	15.2 ± 5.0	(4-24)	0.060	0.060
MDA-LDL [ng/ml]	20	170 ± 41	(92-270)	20	201 ± 64	(97-332)	0.078	0.069
IL-1α [ng/ml]	20	0.012 ± 0.006	(0.004-0.024)	18	0.016 ± 0.005	(0.01-0.023)	0.023	0.022
Fibrinogen [mg/ml]	20	2.2 ± 0.8	(0.6-3.6)	20	1.7 ± 0.8	(0.2-2.8)	0.070	0.055
IL-17 [pg/ml]	20	7.4 ± 2.7	(5.1-17.8)	20	9.1 ± 2.1	(5.5-16.1)	<0.001	0.035
BDNF [pg/ml]	20	4404 ± 2454	(817-9930)	20	6990 ± 5849	(264-19500)	0.317	0.080
MMP-3 [ng/ml]	20	0.09 ± 0.01	(0.06-0.1)	20	0.11 ± 0.02	(0.06-0.15)	0.001	<0.001
Haptoglobin [µg/ml]	18	5.49 ± 2.09	(4.03-10.9)	18	9.8 ± 3.5	(4.0-17.3)	<0.001	<0.001
VDBP [µg/ml]	20	159.0 ± 76	(67-325)	20	288.9 ± 79.3	(94-346)	0.008	0.044

*P < 0.05 for difference between the groups (without adjustment)
#P < 0.05 for difference between the groups adjusted for sex

4.16 AgRP and BDNF concentrations at birth

Cord blood AgRP and BDNF measurements were validated at the Institute of Laboratory Medicine, Clinical Chemistry and Molecular Diagnostics, University of Leipzig (Prof. Dr. Kratzsch) using commercial ELISAs. Furthermore, in a subsample maternal AgRP and BDNF concentrations at delivery were determined. The mean maternal plasma AgRP concentration at delivery was 59.4 ± 20.0 pg/ml in the intervention group and 64.9 ± 34.3 pg/ml in the control group (Table 26). The maternal AgRP concentrations did not differ significantly between both groups (*P* = 0.965).

AgRP concentrations in cord blood in the intervention group were 1990.3 ± 386.8 pg/ml in the intervention group and 1788.4 ± 627.8 pg/ml in the control group (Table 26). No significant difference between the groups was observed (*P* = 0.156). When AgRP concentrations were examined separately in girls and boys there was also no significant difference between both groups (Figure 16).

Mean maternal BDNF concentrations at delivery were 5026.8 ± 2264.7 pg/ml in the intervention group and 5439.1 ± 1687.8 pg/ml in the control group (Table 26). No significant difference between both groups was found (*P* = 0.958).

Mean cord plasma BDNF concentrations were 3857.5 ± 2190.5 pg/ml in the intervention group and 3965.8 ± 2352.4 pg/ml in the control group. No group effect was observed (*P* = 0.880). Analyzing girls and boys separately no significant difference between both groups was found (Figure 17).

Table 26 AgRP and BDNF concentrations in maternal and cord plasma

		Intervention group		Control group		*P*
		n	mean ± SD (range)	n	mean ± SD (range)	
Maternal						
AgRP [pg/ml]		9	59.4 ± 20.0 (32.8-100.3)	9	64.9 ± 34.3 (30.4-123.5)	0.965
BDNF [pg/ml]		8	5026.8 ± 2264.7 (1108-8000)	8	5439.1 ± 1687.8 (3500-8000)	0.958
Cord blood						
AgRP [pg/ml]		24	1990.3 ± 386.8 (1200-2700)	26	1788.4 ± 627.8 (564-3000)	0.156
	girls	10	2086.8 ± 430.2 (1200-2700)	10	1983.8 ± 649.3 (724-2880)	0.762
	boys	14	1921.4 ± 352.6 (1420-2500)	16	1666.3 ± 602.1 (564-3000)	0.129
BDNF [pg/ml]		22	3857.5 ± 2190.5 (907-8000)	20	3965.8 ± 2352.4 (1214-8000)	0.880
	girls	8	4346.5 ± 3013.7 (907-8000)	10	4916.9 ± 2208.3 (1778-8000)	0.593
	boys	14	3578.1 ± 1621.5 (1159-6540)	10	3014.6 ± 2189. 8 (1214-8000)	0.266

*$P < 0.05$ significantly different distribution between the groups

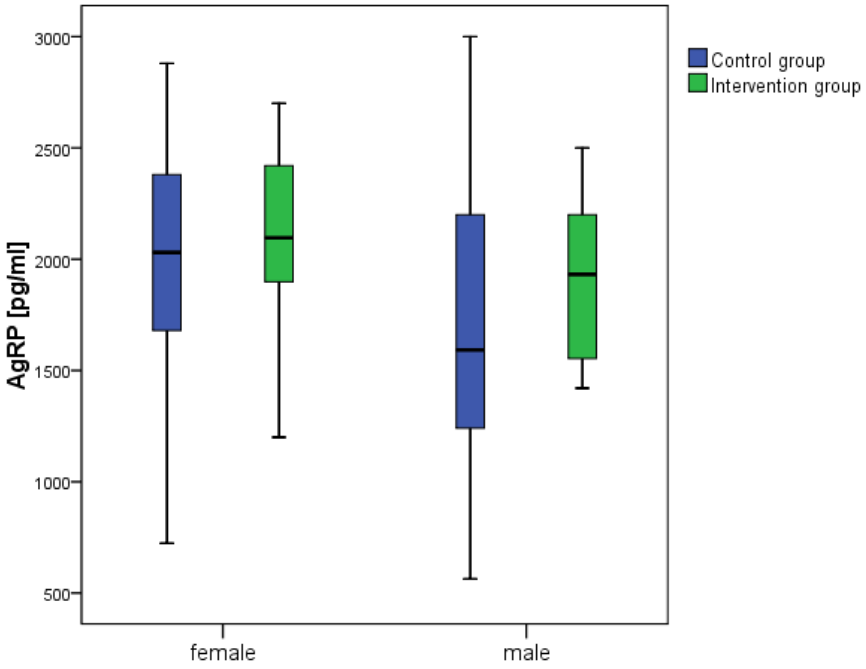


Figure 16 Cord blood AgRP concentrations [pg/ml] of the intervention and control group

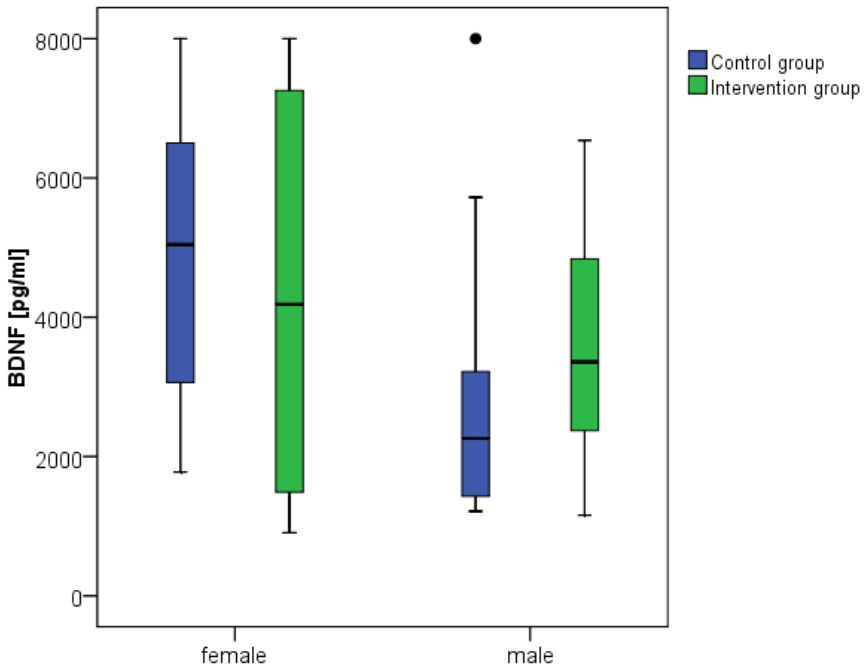


Figure 17 Cord blood BDNF concentrations [pg/ml] of the intervention and control group

5 Discussion

5.1 Pregnancy outcome, measures of fat mass and anthropometry

In the present study, no difference in maternal BMI, BF and SFTs between the groups during pregnancy was found. Furthermore, pregnancy outcome and clinical data of the infants were similar in both groups, except gestational age which was significantly higher in the intervention group compared to the control group. This result is consistent with other studies, showing that a higher n-3 LC-PUFA intake during pregnancy may result in an increase in pregnancy duration (Brenna and Lapillonne 2009, Judge, Harel and Lammi-Keefe 2007, Cetin and Koletzko 2008). It is suggested that n-3 LC-PUFAs may inhibit the production of the AA-derived prostaglandins PGE_2 and $PGF_{2\alpha}$ which are involved in the initiation of delivery and thereby leading to a prolongation of gestation (Olsen et al. 1986). In the subgroup analyzed a slightly increased PI was observed in the intervention group ($P = 0.096$) compared to the control group. This finding might be ascribed to the prolonged pregnancy duration. BF and SFTs of the newborns are in accordance with previous published data (Schmelzle and Fusch 2002, Catalano et al. 2003, Koo, Walters and Hockman 2004, Sewell et al. 2006).

The results of the present thesis are based on a subsample of the INFAT-study and do not represent the final results of the primary outcome. The higher sample size included in the final analysis may change the results of the primary outcome.

5.2 Maternal blood lipids, insulin and HOMA-IR during pregnancy and lactation

The blood LC-PUFA profile in plasma PLs and RBCs of this subgroup reflected the higher dietary intake of total n-3- LC-PUFAs and lower intake of AA in the intervention group during pregnancy and lactation. As expected decreased contents of AA and total n-6 LC-PUFA and a lower ratio of AA/DHA were achieved in the intervention group compared with the control group. Furthermore, cord blood PL and RBC DHA, EPA and total n-3 LC-PUFA content was significantly higher in the intervention group compared with the control group. These results confirm the

compliance of the participating women. In a previous work, maternal and fetal FA profile in plasma PLs and RBCs was analyzed in a subgroup of the INFAT- study and the results are consistent with the findings from the present study (Vollhardt 2010).

Maternal TAGs during pregnancy and lactation

In a subgroup of the INFAT-study changes in parameters of the carbohydrate and lipid metabolism during pregnancy and lactation was investigated. Without Bonferroni correction TAG levels were significantly lower in the intervention group than in the control group at the 32nd week of gestation and during lactation. Applying Bonferroni correction this group effect was attenuated and only significant at 6 weeks and 16 weeks PP. In a previous work, TAG levels were analyzed at the 32nd week of gestation in a higher sample size of the INFAT-study and significant lower TAG levels at this time point were reported in the intervention group compared with the control group (Vollhardt 2010). In non-pregnant healthy subjects, a TAG reduction was seen after DHA supplementation in the range of 0.9 g/d-1.7 g/d and 2.3 g/d DHA plus EPA supplementation, respectively (Agren et al. 1996, Geppert et al. 2006). Presumably the underlying mechanism for this TAG-lowering effect is by inhibition of hepatic TAG synthesis mediated via activation of PPARα by n-3 LC-PUFAs, increased FA oxidation and altered metabolism of enzymes (e. g. lipoprotein lipase) which are involved in the uptake and esterification of FAs (Nestel 2000).

Maternal total cholesterol, HDL-cholesterol and LDL-cholesterol during pregnancy and lactation

Total cholesterol increased significantly in both groups during pregnancy and declined thereafter reaching concentrations at 16 weeks PP close to baseline levels. HDL-cholesterol and LDL-cholesterol increased slightly over the course of pregnancy. There was no difference between the groups at any time point, although HDL-levels were slightly higher in the intervention group compared with the control group at 6 weeks PP. These findings are in accordance with those from other studies showing that n-3 LC-PUFA supplementation has no effect on serum cholesterol concentrations, and that HDL-cholesterol concentrations slightly increase after DHA supplementation in the non-pregnant status (Agren et al. 1996, Mori et al. 2000, Nestel 2000). It is suggested that fish oil reduces the activity of cholesterol ester

transfer protein which transfers cholesterol esters from HDL to VLDL and LDL, largely in exchange for VLDL triacylglycerols (Abbey et al. 1990).

Maternal glucose, insulin and HOMA-IR during pregnancy and lactation

The reduction in the ratio of n-6/n-3 LC-PUFA in maternal diet during pregnancy and lactation did not affect blood glucose concentrations. In both groups insulin increased significantly during pregnancy and declined thereafter. Insulin resistance calculated as HOMA-IR increased during pregnancy. Due to the increasing demand of glucose by the fetus maternal insulin sensitivity in late pregnancy is decreased. Declined maternal insulin sensitivity is compensated by an increased insulin production and release (Butte 2000, Catalano et al. 1999). In addition, from the third trimenon hepatic glucose production of the mother is increased to cover the demand of the fetus and the placenta (Catalano et al. 1992).

In this study, no significant intervention effect on maternal plasma insulin levels during pregnancy was found. HOMA-IR was marginally lower in the intervention group compared to the control group at the 6th week PP. This might be ascribed to the higher initial insulin values in the control group. Adjustment for the initial values attenuated the effect. Exclusion of GDM pregnancies had no impact on this result.

In animal studies, fish oil feeding was associated with lower insulin levels (Cha and Jones 1998, Luo et al. 1996). However, in a study of Kratz et al. (2008) in overweight/obese female and male adults a diet enriched with n-3 PUFA (1.4% of total daily energy intake) had no effect on insulin levels and HOMA-IR (Kratz et al. 2008). In the reviews of Galgani et al. (2008) and Fedor & Kelley (2009) inconclusive results about the impact of PUFAs on insulin resistance were reported. Galgani et al. (2008) summarized that human studies have failed to demonstrate a consistent effect of dietary fat quality on insulin sensitivity, which contrasts observations from animal studies, where n-6 PUFA in comparison with n-3 PUFA decrease insulin sensitivity (Galgani et al. 2008). Fedor & Kelley (2009) reported that a number of epidemiological studies proposed improved insulin sensitivity by increased consumption of n-3 PUFAs. However, results of intervention studies are inconsistent (Fedor and Kelley 2009). Two meta-analyses did not show any effects of fish oil supplementation on insulin resistance, and a few studies reported improved insulin sensitivity (Montori et al. 2000, Friedberg et al. 1998). In the present study, age- and pre-pregnancy- adjusted partial correlation showed no relationship between maternal

insulin/HOMA-IR levels and plasma PL AA, EPA, DHA, total n-3 LC-PUFA and total n-6 LC-PUFA during pregnancy. To the best of my knowledge, there is no human study available so far, investigating correlations between insulin/HOMA-IR and PUFAs in pregnant subjects receiving a reduced n-6/n-3 LC-PUFA diet. In Chinese diabetic adults, sex, age and BMI controlled partial correlation revealed a significantly negative correlation between HOMA-IR/insulin and plasma PL n-3 PUFA, PL 20:5n-3 and PL 22:6n-3 and a positive correlation between HOMA-IR and n-6 PUFAs. In the healthy control group only a negative correlation between HOMA-IR and total n-3 PUFA and 22:6n-3 was observed (Huang et al. 2010).

Fedor & Kelly (2008) discussed several mechanisms by which n-3 PUFA might reduce insulin resistance. Increased inflammation is supposed to be one of the major factors leading to the development of insulin resistance. DHA may counteract this process by reducing circulating TAGs and FFAs and by inhibiting the production of inflammatory cytokines (Fedor and Kelley 2009). N-3 PUFAs inhibit Toll like receptors (TLR) -2 and TLR-4 known to activate the NF-kB pathway for the production of inflammatory cytokines (Lee et al. 2004). In addition, n-3 PUFAs reduce the production of inflammatory eicosanoids from AA and serve as agents for the production of anti-inflammatory products (Calder 2006). N-3 PUFA regulate the expression of a number of genes involved in carbohydrate and lipid metabolism by modulating the activity or expression of a number of transcription factors (Jump et al. 2005). Sterol regulatory element-binding protein (SREBP)-1c stimulates fatty acid synthesis and inhibits insulin signaling (Shimano et al. 2007). It has been reported that EPA activates PPAR-α dependent genes, and both EPA and DHA suppress expression of SREBP-1c (Deckelbaum, Worgall and Seo 2006, Botolin et al. 2006). Activation of PPARγ by DHA metabolites accompanied by the increased clearance of glucose in db/db mice was reported earlier (Yamamoto et al. 2005). Thus, n-3 PUFAs increase glucose clearance, and fatty acid oxidation, and inhibit fatty acid synthesis. In addition, n-3 PUFAs may decrease insulin resistance through other effects including decrease in triglycerides, membrane fluidity and signal transduction (Fedor and Kelley 2009). The discrepancies in the results about the effect of n-3 FA on insulin sensitivity may be due to differences in health status of participants, amount, duration, and fatty acid composition of n-3 PUFA supplementation, and methods used to assess insulin resistance. There might be also different effects of EPA and

DHA on insulin sensitivity (Fedor and Kelley 2009). Moreover, the response to n-3 FA supplementation might be different between pregnant and non-pregnant subjects.

5.3 Maternal leptin, sOB-R and FLI during pregnancy and lactation

Longitudinal analysis of leptin, sOB-R and FLI during pregnancy and lactation

In this study, maternal leptin concentrations increased during pregnancy and were highest at term and declined thereafter during the lactation period in both groups. These findings are in accordance with data from other studies (Misra and Trudeau 2011, Schubring et al. 1998, Helland et al. 1998). The same pattern in pregnancy and lactation was observed for the FLI. Although the exact origin and role of the pregnancy-induced rise in leptin is not known, it has been proposed that the increase in leptin concentration is important for modulating maternal and fetal substrate availability and supply (Mounzih et al. 1998, Schubring et al. 1998). At present there are a number of possible explanations for the hyperleptinemia during pregnancy. There is strong evidence suggesting that the placenta makes a substantial contribution to the rise in maternal leptin concentrations. The human placenta expresses high amounts of leptin mRNA during gestation (Masuzaki et al. 1997, Henson, Swan and O'Neil 1998) which is identical to leptin of adipose tissue origin (Senaris et al. 1997). Circulating leptin rapidly declines after delivery in both the mother and the neonate (Hytinantti et al. 2001) supporting the role of the placenta as an important source for leptin production during pregnancy. Linnemann et al. (2000) reported that only 1-2% of placental leptin enters the fetal circulation, whereas approximately 98% is released into the maternal circulation (Linnemann et al. 2000). In the present study, a positive correlation between maternal leptin, BMI and BF in both groups at both time points during pregnancy was observed. This is in accordance to the findings of others (Fattah et al. 2010, Schubring et al. 1998) suggesting that leptin is produced in maternal white adipose tissue during pregnancy and that the increasing fat mass during pregnancy contributes to the elevated leptin levels. Considering the rapid increase in leptin concentration during the first weeks of pregnancy, other sources of leptin might be also involved and the release of leptin might be stimulated by other factors during pregnancy. As hyperleptinemia during pregnancy is not associated with decreased food intake Margetic et al. (2002) suggest that there might be a pregnancy-induced leptin resistance, or a change in

bioavailability of leptin (Margetic et al. 2002). It has been hypothesized that the sOB-R mediates leptin actions during pregnancy as the circulating concentrations of this protein are increased, particularly in animals (Gavrilova et al. 1997, Lewandowski et al. 1999, Barb 1999). However in humans, the course of sOB-R throughout pregnancy is controversially discussed. Nuamah et al. (2003) found an increase in sOB-R levels from the first to the second trimester (Nuamah et al. 2003), whereas Krizova et al. (2004) did not observe any change in sOB-R during pregnancy (Krizova et al. 2004). In the present study, sOB-R levels increase significantly during pregnancy from 15th week to 32nd week of gestation in the intervention group, but not in the control group. In both groups sOB-R levels were lower during lactation compared to pregnancy. In contrast to leptin levels it has been reported that serum sOB-R levels are low in obese individuals (Ogier et al. 2002, Chan et al. 2002). In the present study, a negative correlation between sOB-R and BMI during pregnancy was observed. Nuamah et al. (2003) also found a negative correlation between sOBR-levels and BMI in pregnant women, but not in the non-pregnant state (Nuamah et al. 2003). To date the physiological function of sOB-R in the human circulation has not yet been fully elucidated. As the binding affinity of the sOB-R for leptin is in the same range as the binding affinity of leptin for its membrane receptor, it is suggested that the soluble form might act as a modulating factor of leptin action through different mechanisms (Sinha et al. 1996).

Effect of intervention on maternal leptin, sOB-R and FLI

Differences in leptin, sOB-R and FLI levels between the groups were explored. As in this study baseline leptin concentrations were higher in the control group compared with the intervention group we adjusted for baseline levels. We did not observe a difference in leptin or FLI between the intervention and the control group during pregnancy and lactation. Moreover, sOB-R levels did not differ significantly between the groups.

Helland et al. (1998) studied the effect of n-3 FA supplementation in 180 pregnant women, providing either 10 ml of cod liver oil daily or the same amount of corn oil starting from 18th week of gestation. They did not observe a significant difference between the groups at the 35th week of gestation which is in agreement with our findings (Helland et al. 1998). There are some studies available investigating the

effect of PUFAs on leptin levels in non-pregnant subjects. Reseland et al. (2001) examined the effect of dietary supplementation with n-3 FA and/or antioxidants on plasma leptin concentrations in male smokers. They found no significant effects of the dietary intervention on plasma leptin concentrations (Reseland et al. 2001). Ramel et al. (2009) reported that seafood consumption (~2.1 g EPA and DHA) in combination with weight loss reduced circulating leptin only in males but not in females (Ramel et al. 2009). Increased leptin levels or lower plasma leptin concentrations and leptin mRNA expression have been reported in n-3 PUFA feeding studies with mice and rats (Ukropec et al. 2003, Hun et al. 1999, Cha and Jones 1998). Furthermore, *in vitro* experiments show inconsistent results whether n-3 PUFAs reduce or increase the expression of leptin (Hun et al. 1999, Perez-Matute et al. 2005).

The effect of PUFAs on leptin might be dependent on the health status and the dosis/duration of n-3 FA supplementation in humans. It was also suggested that n-3 FA supplementation might affect leptin levels via weight loss (Ramel et al. 2009). Pregnancy is characterized by weight gain and hyperleptinemia which represents a different physiological state than in non-pregnant subjects. Thus, the association between PUFAs and leptin levels during pregnancy might be different from non-pregnant women.

5.4 Maternal HMW adiponectin during pregnancy and lactation

Longitudinal analysis of HMW adiponectin during pregnancy and lactation

Maternal HMW adiponectin levels decreased during pregnancy and were lowest at delivery. Hypoadiponectinemia has already been demonstrated in animals and humans by others (Kondo et al. 2004, Combs et al. 2003, Catalano et al. 2006) although contradictory results have also been reported (Mazaki-Tovi et al. 2008). Catalano et al. reported (2006) that the hypoadiponectinemia during human pregnancy was reflected in a 2.5-fold decrease in adiponectin mRNA in WAT. Furthermore, they have shown that hypoadiponectinemia is reflected in a lower amount of the HMW adiponectin (Catalano et al. 2006). Indeed, HMW adiponectin has been reported to account for the most relevant adiponectin form in pregnancy (Retnakaran et al. 2007, Mazaki-Tovi et al. 2008).

In the present study, a significant negative correlation between HMW adiponectin levels and BMI at both time points during pregnancy was observed. Maternal BF [%] was borderline correlated with maternal HMW adiponectin at the 15th week of gestation but significantly at the 32nd week of gestation. This observation is in agreement with findings from Catalano et al. (2006) in pregnant women. Catalano et al. (2006) suggest that adipose tissue accretion is associated with signals for lowering adiponectin production or secretion even in the absence of obesity. Furthermore, parameters of lipid and carbohydrate metabolism are considered to interact with adiponectin regulation during pregnancy which will be discussed in Chapter 5.5.

Effect of intervention on maternal HMW adiponectin

In this study, there was no difference in maternal HMW adiponectin concentrations between the groups at any time point during pregnancy and lactation. Interestingly, we found a negative correlation between total n-6 LC-PUFAs and HMW adiponectin levels at the 32nd week of gestation and this association was more pronounced in the intervention group compared to the control group. Furthermore, PL 20:5 n-3 was positively correlated with HMW adiponectin levels in the intervention group. To the best of my knowledge, there is no human study available investigating the impact of a reduction in the ratio of n-6/n-3 LC-PUFA in maternal diet during pregnancy and lactation on maternal HMW adiponectin concentations. There are a few animal and human studies available with non-pregnant subjects which have explored the effect of n-3 PUFAs on total adiponectin levels and inconsistent results have been reported. Flachs et al. (2006) studied the effect of n-3 PUFAs on total adiponectin levels in mice and found a significant increase in total adiponectin levels (Flachs et al. 2006). The major finding of the study by Kratz et al. (2008) including overweight and obese humans was that neither total nor HMW adiponectin concentrations differed significantly between subjects consuming a n-3 PUFA-enriched diet and control subjects consuming a diet with low n-3 PUFA content (Kratz et al. 2008). Lara et al. (2007) observed a trend towards moderately higher plasma total adiponectin concentrations when subjects consumed a daily portion of salmon providing 2.4 g n-3 PUFAs – equivalent to ~1.1% of total energy intake – for four weeks compared to no fish consumption (Lara et al. 2007). The mechanism by which EPA and DHA might

influence adiponectin is thought to be the same as for thiazolidinediones. EPA and DHA may upregulate *ADIPOQ* by acting as ligands of the transcriptional regulator PPARγ (Iwaki et al. 2003). Flachs et al. (2005) proposed that the stimulation of *ADIPOQ* may also depend on the activation of AMPK, which stimulates *ADIPOQ* (Lihn et al. 2004) and is activated by EPA and DHA (Suchankova et al. 2005). Banga et al. (2008) have demonstrated an increase in HMW adiponectin secretion in rat adipocytes with no increase in adiponectin mRNA by DHA/EPA (Banga et al. 2009). To understand the mechanism underlying the regulation of adiponectin by PUFAs it is desirable to explore the effect of n-3 and n-6 PUFAs on different adiponectin isoforms in further functional and mechanistic studies.

Laumen et al. (2009) demonstrated that promoter variants associated with hypoadiponectinemia in humans substantially affect adiponectin promoter activity in adipocytes (Laumen et al. 2009). It was reported that variation in the *ADIPOQ* gene is associated with obesity, insulin resistance, and adiponectin levels. The SNPs G276T (intron 2) and T45G (exon 2) have been associated with varying levels of adiponectin, risk of type 2 diabetes mellitus as well as BMI, fasting insulin, and glucose (Menzaghi et al. 2002, Woo et al. 2006). A recent study found a decrease in adiponectin levels in overweight adults supplemented with n-3 PUFAs compared with unsupplemented subjects, with the greatest decrease among those carrying the rare T allele of SNP 276 (Nelson, Stevens and Hickey 2007). Verduci et al. (2009) disclosed that an interaction of SNP 276G> T and plasma FAs may exist in obese children (Verduci et al. 2009).

Thus, genetic polymorphisms might be involved in the relevant pathways with specific responses to PUFAs and should also be taken into account.

5.5 Correlation between maternal leptin, sOB-R, HMW adiponectin and metabolic parameters

It was suggested that leptin and adiponectin are involved in hormonal and metabolic alterations during pregnancy (Ritterath et al. 2009, Catalano et al. 2006, Retnakaran et al. 2004). In the present study, correlation analysis between the leptin, sOB-R, HMW adiponectin, insulin, HOMA-IR and blood lipids was performed. HOMA-IR levels did not significantly correlate with HMW adiponectin levels in early pregnancy but showed a significant correlation at the 32nd week of gestation adjusted for pre-

pregnancy BMI and age. These findings are in agreement with previous reports showing an association between total adiponectin and insulin resistance in the third trimenon (McLachlan et al. 2006, Retnakaran et al. 2004). Catalano et al. (2006) proposed that the changes in adiponectin during pregnancy relate to decreased insulin sensitivity of glucose disposal rather than alterations of lipid metabolism. They observed a strong negative correlation between fasting insulin and adiponectin concentrations throughout pregnancy. Furthermore, they found a significant relationship between HMW adiponectin and insulin sensitivity (Catalano et al. 2006). It has been extensively debated whether impaired insulin action is the cause or the consequence of hypoadiponectinemia (Catalano et al. 2006). Studies have suggested interactions between circulating non-esterified fatty acids (NEFAs) and adiponectin (Bernstein et al. 2004). In the present study, NEFAs were not measured. Furthermore, TNF-α was regarded as an important functional link between hypoadiponectinemia and insulin resistance (Kirwan et al. 2002).

Ritterath et al. (2009) found no correlation between total adiponectin and carbohydrate metabolism during pregnancy (Ritterath et al. 2009). In contrast to Catalano et al. (2009) they postulated that the decrease in adiponectin concentrations in pregnancy might be more associated with changes in fat metabolism than with an increase in insulin resistance or body weight. In the present study, TAGs were significantly and positively correlated with HMW adiponectin at the 32nd week of gestation, but not in early pregnancy after adjustment for pre-pregnancy BMI and age. Furthermore, HMW adiponectin levels were significantly positively correlated with HDL-cholesterol throughout pregnancy when adjusting for the same covariates. No correlation between LDL-cholesterol levels and HMW adiponectin levels during pregnancy was found. These findings are in agreement with those reported by Ritterrath et al. (2009) on total adiponectin. Furthermore, in non-pregnant subjects associations between HDL-cholesterol, TAGs and total adiponectin concentrations independent of BMI and fat mass were reported (Schulze et al. 2004, Matsubara, Maruoka and Katayose 2002, Baratta et al. 2004). The mechanisms by which adiponectin may affect blood lipids are largely unknown. Effects of adiponectin on hepatic lipase activity, which is increased in central obesity and insulin resistance, are discussed (Cnop et al. 2003). In this study, maternal leptin and FLI levels were positively correlated with insulin levels, HOMA-IR and TAGs at the time point 32nd of gestation after adjustment for age and pre-pregnancy BMI. In the literature, the

reported data on the associations between insulin and leptin during pregnancy are consistent with the findings from this study (Laivuori et al. 2000, Kautzky-Willer et al. 2001, McLachlan et al. 2006).

sOB-R levels were not related to insulin levels but showed a negative correlation with TAGs levels at the 32nd week of gestation. In a study by Ogawa et al. (2004) in healthy Japanese subjects serum sOB-R levels were negatively correlated with fasting insulin, HOMA-IR and TAGs and positively with HDL-cholesterol after adjustment for age, sex and BMI (Ogawa et al. 2004).

In summary, the results from the correlation analysis suggest that HMW adiponectin and leptin might be involved in carbohydrate and lipid metabolism during late pregnancy independent of age and pre-pregnancy BMI. However, no causation can be derived from this correlation analysis and the mechanisms remain to be elucidated in further studies.

5.6 Insulin in cord blood

In this study, insulin concentrations were lower in cord plasma compared to maternal plasma during pregnancy and lactation. There was a trend towards lower cord plasma insulin in the intervention group compared with the control group (P = 0.060). As gestational age was significantly higher in the intervention group compared to the control group and PI tended to higher levels in the intervention group we adjusted for these variables resulting in an attenuated group effect (P = 0.868). After exclusion of infants whose mothers suffered from GDM and extreme outliers the insulin levels were significantly lower in the intervention group compared to the control group (P = 0.013) even after adjustment for gestational age and PI (P = 0.042). A weak inverse correlation between cord blood insulin and EPA in the pooled data and in the intervention group was observed after adjustment for gestational age and sex. To my knowledge, no study in animals or humans is available investigating the effect of a reduced n-6/n-3 FA ratio in maternal diet on fetal insulin levels. Ibrahim et al. (2009) investigated in rats the effects of maternal diet high in LA, ALA, n-3 PUFA or TFA on glucose metabolism, insulin sensitivity and FA profile in male offspring. At the age of 105 d, in all groups the offspring displayed similar plasma insulin although maternal intake of n-3 PUFA resulted in higher n-3 PUFA in the offspring (Ibrahim, Basak and Ehtesham 2009). Korotkova et al. (2005) fed rats a n-3 FA diet, n-6 FA or n-3 plus n-

6 FA diet during late gestation and suckling period. They found higher insulin levels in the male offspring at adult age of n-6 plus n-3 FA group compared to the n-3 or n-6 FA groups, which were accompanied by higher serum TAGs and body weight (Korotkova et al. 2005). We did not explore lipid and glucose concentrations in cord blood and consequently could not determine HOMA-IR. Interestingly, according to Korotkova et al. (2005) we found that the intervention effect was more pronounced in males than in females assuming that there might be differences between the sexes (Korotkova et al. 2005).

Gestational age might have an impact on insulin levels as the group effect in the present study was attenuated after adjustment for gestational age. In the present study, no significant difference in body weight and fat mass between the groups was observed. Birth mode is also considered to have an impact on cord blood insulin levels albeit this is controversially discussed in the literature (Godfrey et al. 1996, Chiesa et al. 2008). As there were no differences neither in infant weight and anthropometric data nor in birth mode between the groups the observed lower insulin levels in the intervention group can probably not be ascribed to those factors. However, other factors that may affect fetal insulin levels and which were not considered in this study cannot be excluded. Follow-up observations are desirable to explore potential effects at a later age.

5.7 Leptin, sOB-R and FLI in cord blood

In the present study, cord plasma leptin concentrations were lower compared to maternal plasma leptin levels during pregnancy and at delivery, and there was no association between fetal and maternal leptin concentrations (data not shown). This was also reported by others (Butte, Hopkinson and Nicolson 1997, Schubring et al. 1998, Hassink et al. 1997) and may lead to the assumption that cord plasma leptin arises from placental and fetal tissues. The recognition of the placenta as a source of leptin production may suggest an active role for leptin in fetal growth. In this study, cord blood leptin was significantly correlated with birth weight, BF and SFTs at birth which was also demonstrated by others (Geary et al. 1999, Schubring et al. 1998). This association may reflect a relationship with AT mass or an active role for leptin in fetal growth, but the mechanisms underlying this association are still largely unknown.

In this study, the relation of FLI with parameters reflecting BF was similar to the relation of leptin with these parameters. This observation was also reported by others (Kratzsch et al. 2002, Misra et al. 2004). Cord plasma leptin concentrations and FLI were significantly higher in females compared to males which is in line with the findings of other studies (Helland et al. 1998, Schubring et al. 1998). However, the origin of the higher leptin levels in female neonates is not clear.

Lower sOB-R concentrations were found in cord blood compared to maternal blood during pregnancy and lactation. The sOB-R concentrations in cord blood were not associated with maternal sOB-R levels during gestation and lactation (data not shown) which is in agreement with the report of Kratzsch et al. (2005). Thus, similar to leptin sOB-R might be originated from fetal tissues. We found no sex differences in cord blood sOB-R concentrations. This finding supports the data from Kratzsch et al. (2005) suggesting that levels of sOB-R at birth are independent of sex (Kratzsch et al. 2005). A borderline significant inverse correlation between cord blood sOB-R levels and birth weight was found. In contrast to leptin which is significantly related to birth weight and skinfold thickness at birth, sOB-R seems to be weakly inversely correlated with these parameters, suggesting a regulation different from leptin. It was reported that there might be an inverse regulation of leptin and sOB-R and suppressive effects of sOB-R on leptin may be important for energy intake in conditions with a high energy requirement, such as during the first days of life (Kratzsch et al. 2005). Kratzsch et al. (2002) found high sOB-R concentrations in the first years of life, with a peak approximately two years after birth in both girls and boys compared to late childhood and pre-puberty. Thus, the sOB-R may behave differentially in children compared to adults (Kratzsch et al. 2002). To date, the molecular mechanism involved in the function of sOB-R is not clear. It would be of great interest to investigate the change of sOB-R during early childhood in the follow-up of the INFAT-study.

No difference in cord blood leptin levels and sOB-R levels between the intervention group and control group was observed. After exclusion of GDM pregnancies and extreme outliers leptin levels were significantly higher in females of the intervention group compared to the control group. This effect might be due to the prolonged pregnancy duration and consequently higher PI of the newborns in the intervention group. After adjustment for gestational age and PI this effect was not significant anymore. There was no correlation between cord blood leptin and LC-PUFAs. For

sOB-R a weak correlation with PL DHA was observed. Helland et al. (1998) found no difference in cord blood leptin concentrations between the groups with maternal cod liver oil or corn oil supplementation during pregnancy and the lactation period. Furthermore, they found no difference in leptin concentrations between the groups at 4 weeks PP and 16 weeks PP (Helland et al. 1998). In rats, Korotkova et al. (2002) found lower body weight, length, adipocyte size and serum leptin levels in suckling pups of mothers receiving a n-3 FA diet during gestation and lactation compared to n-3 plus n-6 FA group (Korotkova et al. 2002).

5.8 HMW adiponectin in cord plasma and plasma of the infant 16 weeks PP

In the present study, cord blood HMW adiponectin concentrations were higher compared to maternal concentrations during pregnancy. In the literature, high fetal adiponectin concentrations and no correlation between cord blood adiponectin and maternal adiponectin levels was reported suggesting that adiponectin may arise from other sources than maternal circulation (Sivan et al. 2003, Tsai et al. 2004, Mantzoros et al. 2004). Several studies have shown that the HMW oligomers represent the main adiponectin form circulating in fetal plasma (Ibanez et al. 2008, Odden and Morkrid 2007, Pinar et al. 2008, Araki et al. 2006). Data on adiponectin production by the placenta are controversial (Chen et al. 2006, Mazaki-Tovi et al. 2005, Caminos et al. 2005, Corbetta et al. 2005, Pinar et al. 2008). Recently, Pinar et al. (2008) have demonstrated that in addition to adipose tissue, *ADIPOQ* is highly expressed in vascular endothelial cells of fetal capillaries and in the media of larger fetal blood vessels of many tissues, including muscle, WAT, brain, and kidney assuming that the high plasma adiponectin concentrations found in the fetus could potentially be caused by pleiotropic adipokine tissue production (Pinar et al. 2008).

It is suggested that adiponectin may have different metabolic effects *in utero* than in adults. It is known that neonates are more sensitive to insulin than adults (Farrag et al. 1997), which might be consistent with a high adiponectin concentration at birth (Farrag et al. 1997). Pinar et al. (2008) have shown that higher proportions of the HMW multimers were associated with lower insulin concentrations and HOMA-IR in cord plasma compared to adult plasma, reflecting a high insulin sensitivity of the fetus (Pinar et al. 2008). In the present study, a weak inverse correlation between

insulin and HMW adiponectin was observed, which was not significant after controlling for gestational age and sex. No correlation between cord blood HMW adiponectin, birth weight, birth length and BF was found when adjusted for gestational age, sex and group. However, a positive correlation between cord blood HMW adiponectin levels and PI was found controlling for the same variables. In the literature, the association between cord blood adiponectin, birth weight and fat mass-related anthropometry is controversially discussed. Several studies reported a positive association between cord blood total adiponectin and birth weight (Chan et al. 2004, Tsai et al. 2004, Weyermann et al. 2006, Lindsay et al. 2003). However, other studies could not confirm these findings (Cortelazzi et al. 2007, Kotani et al. 2004, Odden and Morkrid 2007). Martos-Moreno et al. (2009) found no significant correlation between total adiponectin and birth weight when controlling for the effect of gestational age (Martos-Moreno et al. 2009). Pinar et al. (2008) measured the HMW form and observed no association with birth weight. The mechanisms of regulating plasma adiponectin levels and its isoforms in the fetus are still largely unknown and remain to be defined. There might be differences between the different adiponectin forms. In contrast to what is reported in adult studies (Arita et al. 1999, Yannakoulia et al. 2003) no sex differences in HMW adiponectin concentrations were observed in the present study which is in accordance with previous reports (Odden and Morkrid 2007, Inami et al. 2007, Sivan et al. 2003, Inoue et al. 2008). The difference between the sexes in adults might reflect an effect of the sex hormones or other mediators that are absent in newborns (Nishizawa et al. 2002).

Mean HMW adiponectin concentration in infants measured at the 16th week PP was similar to the mean concentration observed in cord blood. We found no difference in cord blood HMW adiponectin concentrations between the groups. Furthermore, no difference in HMW adiponectin concentrations of the infant measured at the 16th week of gestation was found. As seen for HMW adiponectin concentrations in mothers, the reduced maternal n-6/n-3 FA ratio may not affect adiponectin.

5.9 AgRP concentrations at birth

In this study, cord blood AgRP concentrations assessed by RBM Inc. using MAP technology were significantly lower in the intervention group compared to the control group (880.5 ± 303.2 pg/ml vs. 1096.9 ± 357.0 pg/ml). The AgRP measurements were further validated using a commercially available ELISA. Measurement by ELISA revealed higher mean concentrations in both, the intervention group (1990.3 ± 386.8 pg/ml) and the control group (1788.4 ± 627.8 pg/ml) compared to RBM MAP, with no significant difference between the groups. These discrepancies in the results may be due to methodological differences.

In the present study, mean maternal AgRP concentration was ~ 30-fold lower compared to cord blood. Bienertova-Vasku et al. (2010) measured maternal plasma AgRP concentrations during gestation in a preeclamptic cohort and in a non-preeclamptic cohort and found 11- and 19-fold lower concentrations in the maternal samples compared to cord blood AgRP concentrations (Bienertova-Vasku et al. 2010). The high fetal AgRP concentrations over maternal AgRP concentrations at birth may stimulate food intake in the infant and have a function in fetal growth.

Recently, it was reported that AgRP is expressed in the placenta in rats and that AgRP levels rise during pregnancy (Szczepankiewicz et al. 2009) with highest levels at the end of the gestation period in rats (Caminos et al. 2008). Rocha et al. (2003) observed an increased expression in AgRP in parallel with an increase in weight gain and leptin during pregnancy and they suggest that AgRP could be responsible for the increased food intake during pregnancy (Rocha et al. 2003). To my knowledge, there are no studies available investigating the effect of maternal n-3 PUFA supplementation on fetal AgRP in humans. Wang et al. (2002) found a significant reduction of AgRP mRNA in the hypothalamic arcuate nucleus in mice fed a diet rich in saturated fat compared to mice fed a n-6 PUFA, n-3 PUFA, or low-fat diet (Wang et al. 2002). Thus, changing the fatty acid profile of the diet alone can profoundly alter the expression of major hypothalamic neuropeptides involved in energy balance.

AgRP might play a considerable role during pregnancy and in fetal programming of central appetite regulatory network. There are only a few published data and more studies are required to explore the effect of maternal fatty acid profile on fetal neuropeptides of the appetite regulatory network.

5.10 BDNF concentrations at birth

BDNF is an important member of the neurotrophic family of growth factors, abundant in the brain and periphery (Xu et al. 2003). It is found in both, human serum and plasma (Fujimura et al. 2002, Radka et al. 1996, Rosenfeld et al. 1995) and serum levels have been found to be 200-fold higher than plasma levels (Rosenfeld et al. 1995). As human platelets contain a large amount of BDNF present in blood (Fujimura et al. 2002, Pliego-Rivero et al. 1997) the difference between serum and plasma levels of BDNF could reflect the amount of BDNF stored in circulating platelets. Studies provided considerable evidence supporting a role for BDNF in energy homeostasis and obesity (Rios et al. 2001, Lyons et al. 1999). Recently, in animal studies it was shown that maternal nutrition during pregnancy has an impact on the hippocampal development and BDNF production of the offspring (Tozuka et al. 2010). In the present study, BDNF concentrations were measured in cord plasma and maternal plasma at birth. The BDNF concentrations were first determined using a MAP by RBM Inc. and these measurements were then validated using a commercial ELISA. In females, cord blood BDNF concentrations measured by RBM Inc. were significantly lower in the intervention group compared to the control group. In males, non-significant higher BDNF levels were observed in the intervention group compared to the control group. BDNF levels measured by a commercially available ELISA were in general lower compared to the levels measured by the MAP technology. However, the same pattern was observed for the values measured by RBM Inc. with lower BDNF concentrations in females of the intervention vs. control group and higher levels in the intervention group compared to the control group in males. However, the group effect was not significant, neither in boys nor in girls. Indeed, higher plasma and whole-blood BDNF levels were found in women compared to men, which may be affected by body weight (Trajkovska et al. 2007). In the present study, higher plasma BDNF levels were observed in females than in males, although this difference was not significant.

In our study, BDNF concentrations in cord blood were ~ 1.3-fold lower in comparison to maternal BDNF levels at birth. BDNF has been shown to exert an important role in placental development and fetal growth (Mayeur et al. 2010). However, during development, little is known about the regulation of the BDNF/TrkB signaling system by nutritional status in the nervous system and also in the other non-neuronal tissues that express this neurotrophin. Recently, it has been reported that maternal

undernutrition modifies BDNF levels in the CNS of growth-restricted rat fetuses indicating that BDNF is sensitive to a reduction of the availability of nutrients and restriction of fetal growth (Coupe et al. 2009). Tozuka et al. (2010) examined the effect of diet-induced maternal obesity on hippocampal development and function in the mouse offspring and found that HFD offspring had less BDNF in the hippocampus compared to normal diet (ND) offspring (Tozuka et al. 2010). There is no study available investigating the effect of a modification of PUFA composition in the maternal diet on offspring BDNF.

The discrepancies in BDNF measurements (MAP by RBM Inc. vs. R&D ELISA) might be explained by the different methods used. Previous studies measuring plasma BDNF have reported different ranges of plasma BDNF levels in healthy subjects which were likely due to different assay methods. Recently, Karege et al. (2005) showed that the stability of BDNF in whole blood, serum, and plasma varied among different laboratories (Karege et al. 2005). Thus, different methods might lead to differences in measured BDNF level.

5.11 Leptin in breast milk

Despite higher n-3 LC-PUFA levels in the breast milk of fish oil supplemented women compared to the control group which was previously reported (Vollhardt 2010), there was no difference in leptin in breast milk between the study groups. To the best of my knowledge, no study is available investigating the impact of a reduced n-6/n-3 LC-PUFA ratio in maternal diet during pregnancy and lactation on leptin levels in breast milk. In an animal study, Korotkova et al. (2002) found no effect of various ratios of the dietary n-6 and n-3 PUFA during the perinatal period on breast milk leptin. It was postulated that breast milk leptin might have an impact on the development and adiposity of the infant (Miralles et al. 2006). In the present study, breast milk leptin levels were ~15-fold lower compared to maternal plasma leptin levels. A study by Smith-Kirwin et al. (1998) showed that human mammary epithelial cells express *LEP* (Smith-Kirwin et al. 1998). It was postulated that during neonatal development, leptin levels might be attributable to endogenous leptin production and/or to leptin derived from maternal milk (Casabiell et al. 1997, Devaskar et al. 1997). In an animal study, it was shown that leptin is transferred from the maternal blood to breast milk and then passes to neonatal blood, suggesting that maternal leptin may exert biological effects

on the infant (Casabiell et al. 1997). In human beings, this was not explored so far. Furthermore, there are controversially discussions about higher or lower concentrations of leptin in formula-fed vs. breast-fed infants (Lönnerdal and Havel 2000, Savino et al. 2004, Ucar et al. 2000).

It has been shown that leptin concentrations of whole milk were much higher than of skim milk and fat in human milk interfered with the leptin assay which might explain reported results of high leptin concentrations in whole milk (Lönnerdal and Havel 2000). We measured leptin in skim milk and our values are consistent with those from Lönnerdal et al. (2000). Lönnerdal et al. (2000) demonstrated ~10-fold lower breast milk leptin values compared to infant leptin serum concentrations and suggest that it is unlikely that breast milk leptin would significantly contribute to infant circulating serum leptin (Lönnerdal and Havel 2000).

5.12 Total adiponectin in breast milk

In breast milk samples, total adiponectin was measured as the HMW form could not be determined due to methodological difficulties. There was no significant difference in total adiponectin concentrations in skim milk between the groups neither at time point 6 week PP nor at time point 16th week PP. To the best of my knowledge, there are no studies available investigating the effect of LC-PUFAs on adiponectin in breast milk. Martin et al. (2006) reported that adiponectin levels in human breast milk decrease with the duration of lactation (Martin et al. 2006). Adiponectin concentrations were detected in a considerably high amount in breast milk when compared to leptin showing much lower concentrations in breast milk than in maternal plasma. It is suggested that milk adiponectin might have physiological relevance for the infant (Newburg, Woo and Morrow 2010). Considering the biological properties of adiponectin and the fact that AdipoR1 is expressed in the small intestine of neonatal mice (Zhou et al. 2005), it is speculated that adiponectin in breast milk may play a role in neonate development, even though it is still not well defined whether it is resistant to proteolysis and whether the receptors can mediate its absorption and action (Savino et al. 2009). No data exist so far showing that adiponectin from breast milk enters the circulation of the infant. Gillmann et al. (2007) postulated that adipokine exposure in infancy might determine later weight status (Gillman and Mantzoros 2007). Weyermann et al. (2007) reported that higher levels

of milk total adiponectin measured at 6 weeks PP were associated with increased risk for overweight at two years of age, particularly in those breastfed for at least 6 months (Weyermann et al. 2006). In contrast to these findings, Woo et al. (2009) found that higher total adiponectin concentrations in human milk were associated with significantly lower weight over the first six months of life in breast-fed infants (Woo et al. 2009). The authors hypothesized that milk adiponectin may serve as a biomarker for other factors in human milk with physiological impact on infant growth, such as IGF-1. Further analyses of milk adiponectin are required to clarify its respective physiological effects and possible impact on infant growth.

6 Conclusion and perspectives

In the present study, longitudinal analyses of plasma insulin levels and HOMA-IR as well as leptin, sOB-R, FLI, HMW adiponectin concentrations during pregnancy and lactation showed no significant difference between the intervention and control group. Furthermore, no significant difference in leptin and total adiponectin concentrations in breast milk between the groups was found. These results suggest that a reduced n-6/n-3 LC-PUFA ratio in maternal diet during pregnancy and lactation might not affect maternal adipokines. To the best of my knowledge, there is no human study available investigating a reduction in n-6/n-3 FA ratio during pregnancy and lactation on leptin and HMW adiponectin concentrations. Helland et al. (1998) supplemented healthy women with n-3 FA during pregnancy and lactation and found no intervention effect (Helland et al. 1998). In non-pregnant humans, no effect of n-3 FA supplementation on leptin levels or a decrease in leptin levels was found suggesting that this effect might be associated with weight loss (Ramel et al. 2009, Reseland et al. 2001). It was reported that n-3 FA supplementation had no effect on adiponectin levels or increased adiponectin secretion and the latter might be induced via PPARy (Kratz et al. 2008, Lara et al. 2007). The apparent discrepancies between different studies on the effect of n-3 PUFA supplementation on adipokines and insulin resistance in non-pregnant subjects may be partly a result of differences in study design and dietary exposure. There might be also different responses to n-3 PUFA supplementation due to different subjects and health status. Pregnancy describes a physiologic state different from non-pregnant women which is characterized by insulin resistance in late pregnancy. Furthermore, pregnancy is a state of hyperleptinemia and hypoadiponectinemia (Schubring et al. 1998, Catalano et al. 2006) which could be confirmed in this study. Thus, these adipokines might be insensitive to the dietary intervention during pregnancy. Leptin and adiponectin were suggested to be involved in carbohydrate and lipid metabolism during pregnancy (Catalano et al. 2006, Ritterath et al. 2009), albeit the mechanism is still unclear. Although no causality can be derived from this analysis, it is noteworthy that in the present study correlations between leptin, HMW adiponectin and metabolic parameters in late pregnancy were found independent of age and pre-pregnancy BMI. This suggests that adipokines are involved when lipolysis takes place and might play an important role in adaptation of

the metabolic state. Our study population consists of healthy pregnant women and it would be of major interest to investigate the effect of the reduced n-6/n-3 FA ratio in pathological states during pregnancy, e.g. GDM or in overweight and obese women as there might be an improvement in pathological state by reduced n-6/n-3 FA composition.

Variation in the *ADIPOQ* gene has been associated with obesity, insulin resistance, and adiponectin levels (Menzaghi et al. 2002, Woo et al. 2006). Thus, genetic polymorphisms might be involved in the relevant pathways with specific responses to PUFAs and should be taken into account. Furthermore, studies investigating the effect of PUFAs on the different adiponectin forms are desirable.

From this analysis it can be concluded that n-3 PUFA supplementation in combination with a reduction in AA intake during pregnancy and lactation does not considerably affect the leptin system and HMW adiponectin in the newborns. Korotkova et al. (2002) have demonstrated that the ratio of n-6/n-3 PUFA in the maternal diet affects serum leptin levels, body weight and growth of the offspring and they have found that alterations in metabolic parameters in adult male rats were linked to a specific dietary n-6/n-3 PUFA ratio in the perinatal period and possibly to early leptin homeostasis (Korotkova et al. 2005). Cord blood insulin showed a trend towards lower concentrations in the intervention group compared to the control group, which became significant after exclusion of GDM pregnancies. No causality can be derived from this observation so far and further studies are needed to confirm this finding. In the present study, glucose in cord blood was not measured and therefore fetal HOMA-IR could not be calculated. It would be desirable to measure insulin sensitivity and lipids, especially TAGs in cord blood as well. Furthermore, follow up investigations should be taken into account. The INFAT-study provides a follow up of the infants and has the potential to investigate these parameters at a later time point.

In the present study, the effect of a reduction in the n-6/n-3 LC-PUFA ratio in maternal diet on AgRP and BDNF levels was explored. Analysis using MAP technology showed higher AgRP levels in the intervention group compared to the control group and lower BDNF levels in females of the intervention group than in the control group. However, these results could not be confirmed using ELISAs. This suggests potential problems in stability and methodology and requires further studies

on this issue. There are only a few animals studies available exploring the impact of perinatal nutrition on these factors. It was postulated that AgRP might play an important role in the adaptation of energy regulation in perinatal period (Muhlhausler et al. 2006) and BDNF was found to be associated with childhood obesity (Rios et al. 2001). This highlights the need for more studies to understand the mechanisms and their role in fetal programming.

In the last years, supplementation with n-3 LC-PUFAs during pregnancy has been focused on cognitive function of the infant and was shown to have beneficial effects (Helland et al. 2003). As far as we know this is the first study to investigate longitudinally the effect a reduced n-6/n-3 LC-PUFA ratio during pregnancy and lactation on the maternal and fetal leptin and HMW adiponectin. A particular strength of the study was to be able to analyze these adipokines in various biological samples including maternal blood, cord blood, breast milk as well as infant blood. There was the opportunity to conduct this longitudinal analysis within a well designed, randomized study including a clearly defined dietary intervention and a well-characterized study population. The lack of an apparent impact on the adipokines of the newborns and other biomarkers at the present point of time should not be regarded as an absence so far. The impact of altered ratio of n-6/n-3 LC-PUFAs in maternal nutrition during pregnancy and lactation may not appear until childhood or adolescence. We are only just beginning to unravel how nutritional factors can influence epigenetic programming during pregnancy, altering genetic expression and potentially modifying disease risk. More studies on this topic are desirable to get additional insight into the impact of maternal nutritional factors, especially FAs on different adipokines in the context of fetal programming.

Appendix

Appendix A: Analytics

A.1 Metabolic/hormonal MAP (Rules based medicines, Texas, USA)

1. Adiponectin
2. Adrenocorticoptropic Hormone (ACTH)
3. Agouti-Related Protein (AgRP)
4. Angiopoietin 2 (ANG-2)
5. Angiotensin Converting Enzyme (ACE)
6. Angiotensinogen
7. Apolipoprotein A1
8. Apolipoprotein B
9. Apolipoprotein CIII
10. Apolipoprotein D
11. Apolipoprotein E
12. Apolipoprotein H
13. Apolipoprotein J
14. Calcitonin
15. Cortisol
16. Follicle Stimulating Hormone (FSH)
17. Galanin
18. Glucagon
19. Glucagon-like Peptide 1, total (GLP-1)
20. Insulin
21. Insulin-like Growth Factor-1 (IGF-1)
22. Insulin-like Growth Factor-Binding Protein 2 (IGFBP-2)
23. Insulin-like Growth Factor-Binding Protein 3 (IGFBP-3)
24. Lectin-like Oxidized low density lipoprotein receptor (LOX-1)
25. Leptin
26. Lipoprotein (a)
27. Luteinizing Hormone (LH)
28. Modified Low Density Lipoprotein (m-LDL)
29. Panceatic Polypeptide
30. Peptide YY
31. Pepsinogen
32. Progesterone
33. Prolactin
34. Resistin
35. S100 Calcium-binding protein, beta (S100B)
36. Secretin
37. Sex Hormone Binding Globulin
38. Testosterone
39. Thyroid Stimulating Hormone (TSH)
40. Thyroxine Binding Globulin (TBG)

A. 2 Inflammtion MAP (Rules based medicines, Texas, USA)

	UNITS	LDD	RBM RANGE		SERUM	SPIKE RECOVERY	PLASMA / SERUM RATIO	LINEARITY	PRECISION					
								% of Expected	LEVEL 1	%CV	LEVEL 2	%CV	LEVEL 3	%CV
AAT (Alpha-1 Antitrypsin)	mg/mL	0,011	1,1	-	3,1	99%	0,9	90%	0,80	5%	1,2	7%	1,4	9%
Adiponectin	ug/mL	0,20	1,6	-	14	96%	1,00	144%	0,26	22%	0,91	14%	3,2	3%
Alpha-2 Macroglobulin	mg/mL	0,061	0,35	-	7,3	131%	0,79	137%	0,49	11%	1,8	7%	7,8	11%
APO A1 (Apolipoprotein A1)	mg/mL	0,0066	0,29	-	1,6	95%	0,94	105%	0,11	12%	0,22	10%	0,24	7%
APO CIII (Apolipoprotein CIII)	ug/mL	2,7	31	-	298	101%	0,97	92%	68	17%	142	9%	174	13%
APO H (Apolipoprotein H)	ug/mL	8,8	81	-	579	104%	0,99	98%	105	9%	187	9%	214	8%
BDNF (Brain-Derived Neurotrophic Factor)	ng/mL	0,029	0,17	-	49	95%	0,76	123%	0,70	5%	2,7	7%	28	6%
Beta-2 Microglobulin	ug/mL	0,013	1,2	-	5,3	93%	1,0	121%	0,100	9%	0,36	4%	1,3	5%
C3 (Complement 3)	mg/mL	0,0053	0,82	-	3,0	99%	0,94	88%	0,88	5%	1,7	4%	2,0	8%
Calcitonin	pg/mL	6,0		<	23	65%	3,5	114%	4,8	30%	117	8%	235	11%
CRP (C Reactive Protein)	ug/mL	0,0440	0,08	-	3	134%	1,2	100%	22	13%	44	8%	53	10%
Eotaxin	pg/mL	41		<	305	75%	Indeterminant	109%	513	7%	1.187	4%	2.850	8%
Factor VII	ng/mL	1,0	129	-	831	91%	0,60	101%	89	8%	193	6%	215	11%
Ferritin	ng/mL	1,4	8,8	-	674	96%	1,1	119%	34	12%	124	10%	472	10%
Fibrinogen	mg/mL	0,0098		<	###	104%	209	81%	0,11	8%	0,33	8%	1,1	6%
GM-CSF (Granulocyte Macrophage Colony Stimulating Factor)	pg/mL	57		<	63	85%	0,65	110%	57	19%	2.969	5%	21.567	8%
Haptoglobin	mg/mL	0,0250	0,034	-	50	102%		87%	0,80	7%	1,80	7%	2,1	6%
ICAM-1 (Intercellular Adhesion Molecule 1)	ng/mL	3,2	51	-	325	70%	1,1	118%	141	10%	148	6%	133	8%

IL-10 (Interleukin 10)	pg/mL	15		<	24	94%	1,4	115%	10	17%	43	13%	202	9%
IL-12p40 (Interleukin 12p40)	ng/mL	1,2		<	2,2	52%	1,3	121%	8,2	11%	43	6%	376	7%
IL-12p70 (Interleukin 12p70)	pg/mL	94		<	94	91%	1,3	114%	563	8%	4.775	6%	31.780	7%
IL-13 (Interleukin 13)	pg/mL	57		<	126	89%	1,1	117%	194	4%	466	6%	2.057	5%
IL-15 (Interleukin 15)	ng/mL	1,3		<	5,0	70%	1,2	109%	5,1	12%	59	3%	138	6%
IL-17 (Interleukin 17)	pg/mL	66	195	-	###	91%	2,1	108%	1.318	7%	6.607	6%	26.600	4%
IL-18 (Interleukin 18)	pg/mL	54		<	800	72%	1,1	124%	1.322	7%	6.096	9%	23.200	7%
IL-1alpha (Interleukin 1alpha)	ng/mL	0,16		<	0,44	71%	1,4	108%	0,90	10%	1,7	8%	17	9%
IL-1beta (Interleukin 1beta)	pg/mL	1,5		<	9,9	77%	1,3	127%	44	10%	615	6%	1.231	8%
IL-2 (Interleukin 2)	pg/mL	60		<	60	70%	1,0	111%	214	9%	3.054	6%	6.683	5%
IL-3 (Interleukin 3)	ng/mL	0,17		<	2,50	81%	0,68	114%	1,6	8%	4,8	6%	24	6%
IL-4 (Interleukin 4)	pg/mL	104		<	104	91%	0,92	110%	699	8%	6.762	7%	14.360	7%
IL-5 (Interleukin 5)	pg/mL	33		<	48	69%	0,75	115%	211	9%	1.472	7%	5.174	6%
IL-6 (Interleukin 6)	pg/mL	12		<	78	78%	0,92	120%	361	10%	1.539	8%	3.570	8%
IL-7 (Interleukin 7)	pg/mL	53		<	82	92%	2,0	112%	117	22%	259	9%	539	8%
IL-8 (Interleukin 8)	pg/mL	3,5		<	244	101%	0,50	119%	103	10%	818	5%	5.016	3%
Insulin	uIU/mL	0,86		<	45	78%	1,8	108%	3,9	10%	35	8%	100	6%
Leptin	ng/mL	0,10	0,47	-	43	89%	1,1	117%	0,10	23%	7,3	7%	4,9	8%
Lipoprotein (a)	ug/mL	3,7		<	537	95%	0,87	131%	48	11%	157	7%	525	10%
MCP-1 (Monocyte Chemotactic Protein 1)	pg/mL	52		<	###	101%	1,0	118%	620	4%	4.152	9%	13.960	8%
MIP-1alpha (Macrophage Inflammatory Protein 1 alpha)	pg/mL	13		<	91	73%	0,20	79%	89	11%	395	5%	1.504	6%

MIP-1beta (Macrophage Inflammatory Protein 1 beta)	pg/mL	38		<	891	96%	0,30	108%	279	7%	999	7%	3.608	9%
MMP-2 (Matrix Metalloproteinase 2)	ng/mL	150		<	337	108%	Indeterminant	116%	300	5%	825	4%	2.213	3%
MMP-3 (Matrix Metalloproteinase 3)	ng/mL	0,20	1,6	-	55	93%	0,40	105%	2,5	11%	24	6%	62	11%
MMP-9 (Matrix Metalloproteinase 9)	ng/mL	37		<	436	91%	5,8	126%	168	17%	399	5%	921	10%
RANTES (Regulated Upon Activation, Normal T-cell Expressed and Secreted)	ng/mL	0,048	0,76	-	91	108%	1,0	133%	1,8	5%	6,2	6%	21	7%
SCF (Stem Cell Factor)	pg/mL	56		<	356	68%	3,0	117%	317	8%	1.051	6%	3.823	7%
SGOT (Serum Glutamic Oxaloacetic Transaminase, Total)	ug/mL	3,7		<	16	115%	5,0	84%	11,0	13%	34	9%	150	10%
TBG (Thyroxine Binding Globulin)	ug/mL	0,34	42	-	133	120%	1,0	116%	28	3%	47	6%	50	5%
TF (Tissue Factor)	ng/mL	0,84		<	5,3	68%	6,9	121%	14	10%	308	8%	570	8%
TIMP-1 (Tissue Inhibitor of Metalloproteinase 1)	ng/mL	8,4	63	-	420	97%	1,6	100%	8	23%	33	6%	124	6%
TNF-alpha (Tumor Necrosis Factor alpha)	pg/mL	4,0		<	116	79%	2,4	115%	70	12%	473	8%	1.259	9%
TNF-beta (Tumor Necrosis Factor beta)	pg/mL	46		<	61	80%	Not Detected	121%	642	8%	2.718	6%	6.519	9%
TNF-RII (Tumor Necrosis Factor Receptor, Type II)	ng/mL	0,1	3	-	10	99%	0,88	114%	7,9	10%	27	6%	99	7%
TSH (Thyroid Stimulating Hormone)	uIU/mL	0,028	0,44	-	5,3	85%	1,0	118%	0,30	15%	3,5	8%	18	9%
VCAM-1 (Vascular Cell Adhesion Molecule 1)	ng/mL	2,6	354	-	###	104%	1,0	129%	89	10%	346	5%	1.241	7%
VEGF (Vascular Endothelial Growth Factor)	pg/mL	7,5	97	-	###	84%	0,45	104%	382	7%	1.170	6%	2.916	8%
vWF (von Willebrand Factor)	ug/mL	3,80	1,8	-	54	97%	2,6	147%	19,00	17%	61,0	10%	175	4%

Appendix B: Tables

B.1 Change over time in maternal plasma insulin concentrations within each group

Source		*P* (intervention group)	*P* (control group)
time	level 2 vs. level 1	<0.001*	<0.001*
	level 3 vs. level 1	0.008	0.003*
	level 4 vs. level 1	ns	ns
time	level 1 vs. level 2	<0.001*	<0.001*
	level 2 vs. level 3	<0.001*	<0.001*
	level 3 vs. level 4	0.004	0.030*

**P* < 0.05 for change over defined time frame; level 1 = 15th wk gest, level 2 = 32nd wk gest; level 3 = 6th wk PP; level 4 = 16th wk PP; ns = not significant; wk gest = week of gestation

B.2 Change over time in metabolic parameters during pregnancy and lactation

	Group	*P*§	*P*#	*P*†	*P*‡
Glucose [mg/dl]	Intervention	ns	ns	ns	ns
	Control	ns	ns	ns	ns
HOMA	Intervention	< 0.001*	< 0.001*	0.004*	ns
	Control	< 0.001*	< 0.001*	ns	ns
Total cholesterol [mg/dl]	Intervention	< 0.001*	< 0.001**	< 0.001*	ns
	Control	< 0.001*	< 0.001*	< 0.001*	ns
HDL Cholesterol [mg/dl]	Intervention	ns	ns	ns	ns
	Control	0.007*	< 0.001*	ns	ns
LDL Cholesterol [mg/dl]	Intervention	< 0.001*	< 0.001*	0.002*	< 0.001*
	Control	< 0.001*	< 0.001*	< 0.001*	0.030
TAG [mg/dl]	Intervention	< 0.001*	< 0.001*	0.038	< 0.001*
	Control	< 0.001*	< 0.001*	0.029	< 0.001*

* *P* < 0.05 significant change over time
§ 15th wk gest vs.32 nd wk gest
# 32 nd wk gest vs. 6 th wk PP
† 6th wk PP vs.16 th wk PP,
‡ 16th wk PP vs. 15th wk gest ; wk = week

B.3 Change over time in leptin concentrations, sOB-R concentrations and FLI within each group

time	leptin		sOB-R		FLI	
	P		*P*		*P*	
	Intervention	Control	Intervention	Control	Intervention	Control
level 2 vs. level 1	<0.001*	<0.001*	0.001*	ns	<0.001*	<0.001*
level 3 vs. level 1	<0.001*	<0.001*	<0.001*	<0.001*	0.005*	<0.001*
level 4 vs. level 1	0.010	<0.001*	<0.001*	<0.001*	ns	0.013
level 1 vs. level 2	<0.001*	<0.001*	0.001*	ns	<0.001*	<0.001*
level 2 vs. level 3	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	<0.001*
level 3 vs. level 4	ns	ns	0.003*	0.013*	0.023	<0.001

**P* < 0.05 for change over time; level 1 = 15th wk gest, level 2 = 32th wk gest; level 3 = 6th wk PP; level 4 = 16th wk PP; wk gest = week of gestation

B.4 Change over time in maternal plasma HMW adiponectin concentrations within each group

Source		*P* (intervention)	*P* (control)
time	level 2 vs. level 1	<0.001*	<0.001*
	level 3 vs. level 1	<0.001*	<0.001*
	level 4 vs. level 1	<0.001*	<0.001*
time	level 1 vs. level 2	<0.001*	<0.001*
	level 2 vs. level 3	ns	ns
	level 3 vs. level 4	0.002*	ns

*$P < 0.05$ for change over time; level 1 = 15th wk gest, level 2 = 32th wk gest, level 3 = 6th wk PP, level 4 = 16th wk PP; wk gest = week of gestation

B.5 Maternal group effect after exclusion of outliers and GDM pregnancies

	15th wk gest		32nd wk gest		6th wk PP		16th wk PP	
	n (I/C)	*P*	n (I/C)	*P*	n (I/C)	*P*	n (I/C)	*P*
Insulin [pmol/l]	39/38	ns	39/38	ns	38/37	ns	39/38	ns
adjusted for baseline			39/38	ns	38/37	ns	39/38	ns
HOMA-IR	30/37	ns	37/38	ns	37/38	ns	39/36	ns
adjusted for baseline			30/37	ns	29/37	ns	30/35	ns
Leptin [ng/ml]	38/38	ns	39/38	ns	38/38	0.044*	39/37	ns
adjusted for baseline			38/38	ns	38/38	ns	38/37	ns
sOB- R [ng/ml]	39/37	ns	38/37	ns	39/38	ns	39/38	ns
FLI	37/38	ns	39/37	ns	38/38	ns	38/37	ns
adjusted for baseline			37/37	ns	36/38	ns	36/37	ns
HMW -Ad [µg/ml]	39/37	ns	39/38	ns	39/38	ns	39/37	ns

*$P < 0.05$ between the groups after Bonferroni correction; # adjusted for baseline values (15th wk gest)

B.6 Fetal group effect after exclusion of outliers and/or GDM pregnancies

	all			girls			boys		
	N (I/C)	P	#P	N (I/C)	*P*	#*P*	N (I/C)	*P*	#*P*
Insulin	55/53	ns	ns	25/26	ns	ns	30/27	ns	ns
-without outliers	51/52	0.012*	ns	22/26	ns	ns	29/26	ns	ns
-without GDM preg.	53/49	0.004*	ns	24/24	ns	ns	29/25	ns	ns
-without outliers&GDM	50/48	0.013*	0.042*	21/24	ns	ns	29/24	ns	ns
leptin	55/52	ns	ns	25/25	ns	ns	30/27	ns	ns
-without outliers	54/52	ns	ns	24/25	ns	ns	30/27	ns	ns
-without GDM preg.	53/48	ns	ns	24/24	0.041*	ns	29/25	ns	ns
-without outliers&GDM	52/47	ns	ns	23/22	0.029*	ns	29/25	ns	ns
sOB-R	31/26	ns	ns	15/14	ns	ns	16/12	ns	ns
-wihout GDM preg.	30/24	ns	ns	14/12	ns	ns	16/12	ns	ns
FLI	31/26	ns	ns	15/14	ns	ns	16/12	ns	ns
-wihout GDM preg.	30/24	ns	ns	14/12	ns	ns	16/12	ns	ns
HMW-Ad	24/26	ns	ns	10/11	ns	ns	14/15	ns	ns
-wihout GDM preg.	23/24	ns	ns	10/11	ns	ns	13/13	ns	ns

*$P < 0.05$ considered significant; # adjusted for gestational age and Ponderal index

B.7 Measures of location and group effect of biomarkers from inflammation MAP

	Control group			Intervention group			
	n	Mean ± SD	range	n	Mean ± SD	range	*P*
α2M [mg/ml]	20	0.7 ± 0.1	0.6-0.8	20	0.7 ± 0.1	0.6-0.8	0.208
α1 Antitrypsin [mg/ml]	20	1.6 ± 0.3	1.1-2.3	20	1.8 ± 0.5	1.0-2.8	0.228
B2M [µg/ml]	20	2.5 ± 0.4	1.8-3.2	20	2.7 ± 0.6	1.7-4.1	0.685
BDNF [pg/ml]	20	6990 ± 5849	264-19500	20	4404 ± 2454	817-9930	0.317
C3 [mg/ml]	20	0.6 ± 0.1	0.4-0.8	20	0.6 ± 0.1	0.4-0.9	0.168
CRP [µg/ml]	20	0.1 ± 0.1	0.04-0.2	20	0.61± 0.03	0.04-0.2	0.168
Eotaxin [pg/ml]	20	162 ± 74	60-324	20	142 ± 89	43-420	0.234
Factor VII [ng/ml]	20	425 ± 102	248-604	20	489 ± 167	249-920	0.204
Ferritin [ng/ml]	20	222 ± 162	20-592	20	214 ± 124	54-472	0.725
Fibrinogen [mg/ml]	20	1.7 ± 0.8	0.2-2.8	20	2.2 ± 0.8	0.6-3.6	0.070
GM-CSF [mg/ml]	20	n.d.		20	n.d.		
Haptoglobin [µg/ml]	18	9.8 ± 3.5	4.0-17.3	18	5.5 ± 2.1	4.0-10.9	<0.001
ICAM-1 [ng/ml]	20	84.4 ± 14.9	65.0-135.0	20	87.0 ± 16.5	63.6-116.0	0.626
IFN-gamma [pg/ml]	20	177 ± 56	91-290	20	184 ± 74	77-354	0.903
IL-10 [pg/ml]	20	9.0 ± 7.0	3.8-33.2	20	11.6 ± 19.4	3.8-92.5	0.806
IL-12p40 [ng/ml]	20	n.d		20	n.d		
IL-12p70 [pg/ml]	20	30.6 ± 8.5	16.0-52.1	20	25.2 ± 6.1	16.0-37.2	0.032
IL-15 [ng/ml]	20	0.3 ± 0.1	0.2-0.6	15	0.3 ± 0.2	0.1-0.7	0.787
IL-17 [pg/ml]	20	9.1 ± 2.1	5.5-16.1	20	7.4 ± 2.7	5.1-17.8	<0.001
IL-18 [pg/ml]	20	73.5 ± 27.8	22.5-139.0	20	78.1 ± 37.0	42-209	0.978
IL-1alpha [ng/ml]	18	0.02 ± 0.005	0.01-0.02	20	0.01 ± 0.006	0.004-0.02	0.023
IL-1beta [pg/ml]	17	1.4 ± 0.3	1.2-2.0	16	1.6 ± 0.9	0.9-4.7	0.703
IL-1ra [pg/ml]	20	258 (177-1225)#		20	215 (170,481)#		0.372
IL-2 [pg/ml]	18	14.7 ± 4.7	9-24	12	10.8 ± 3.2	7-16	0.018
IL-23 [ng/ml]	20	2.4 ± 0.6	1.6-4.2	20	2.3 ± 0.9	1.4-4.9	0.096
IL-3 [ng/ml]	13	0.02 ± 0.02	0.01-0.07	9	0.02 ± 0.01	0.01-0.03	0.813
IL-4 [pg/ml]	20	173 ± 63	54-290	20	187 ± 85	55-378	0.607
IL-5 [pg/ml]	20	n.d		20	n.d.		
IL-6 [pg/ml]	14	9.0 ± 10.9	2.7-45.0	10	5.7 ± 7.2	1.9-45.0	0.083
IL-7 [pg/ml]	20	n.d.		20	n.d.		
IL-8 [pg/ml]	20	17 (12, 29)#		20	19 (11, 25)#		0.570
MCP-1 [pg/ml]	20	152 (105, 237)#		20	120 (90,188)#		0.298
MIP-1alpha [pg/ml]	20	33.4 ± 7.4	23-50	20	31.1± 5.4	21-45	0.455
MIP-1beta [pg/ml]	20	481 ± 168	249-802	20	464 ± 210	181-890	0.776
MMP-2 [ng/ml]	20	2197 ± 422	1420-2960	20	2293 ± 518	1450-3506	0.570
MMP-3 [ng/ml]	20	0.1 ± 0.02	0.1-0.2	20	0.1 ± 0.01	0.06-0.1	0.001
MMP-9 [ng/ml]	20	629 ± 380	247-1600	20	715 ± 499	121-1810	0.839
RANTES [ng/ml]	20	22.2 ± 16.7	1.1-68.8	20	25.7 ± 15.7	1.4-54.6	0.365
SCF [pg/ml]	20	432 ± 120	170-633	20	459 ± 80	302-620	0.393
TIMP-1 [pg/ml]	20	134 ± 63	15-280	20	139 ± 48	66-259	0.745
TNF-alpha [pg/ml]	20	8.3 ± 2.2	5-12	20	7.5 ± 2.6	4.3-14.0	0.201
TNF-beta [pg/ml]	20	n.d.		20	n.d.		
TNF RII [ng/ml]	20	6.1 ± 1.0	4.3-7.7	20	6.7 ± 1.9	4.7-12.3	0.797
VCAM-1 [ng/ml]	20	1482 ± 294	1080-2370	20	1555 ± 367	1130-2360	0.655
VDBP [µg/ml]	20	229 ± 79	94-346	20	159 ± 76	67-325	0.008
VEGF [pg/ml]	20	862 ± 225	609-1470	20	922 ± 235	668-1680	0.267
vWF [µg/ml]	20	32.9 ± 13.3	5-53	20	34.6 ± 14.8	6-53	0.698

*$P < 0.05$ between the groups; # Data are median (25^{th}, 75^{th} centiles); n.d = not detectable

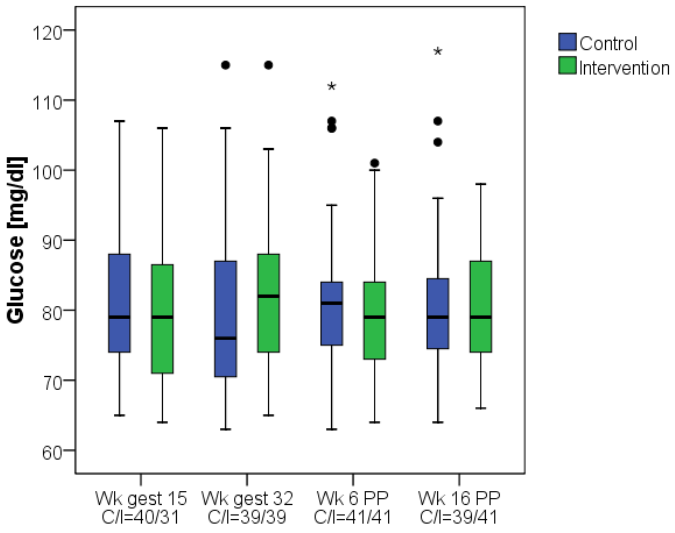
B.8 Measures of location and group effect of biomarkers from metabolic/hormonal MAP

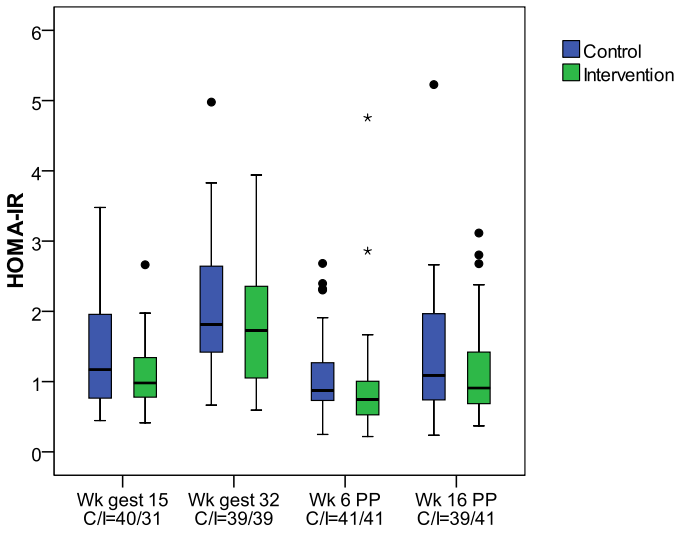
	Control group			Intervention group			
	n	mean±SD	range	n	mean±SD	range	*P*
ACE [ng/ml]	20	95.8 ± 25.5	59.7-143.0	20	103.1 ± 34.1	55.8-166.0	0.655
ACTH [ng/ml]	20	0.6 ± 0.3	0.2-1.3	17	0.5 ± 0.3	0.1-1.3	0.143
Adiponectin [µg/ml]	20	8.5 ± 2.4	5.0-13.9	20	10.5 ± 2.6*	8.0-17.2	0.009
AgRP [pg/ml]	20	1097 ± 357	418-1820	20	881 ± 303*	518-1660	0.030
ANG-2 [ng/ml]	20	14.6 ± 2.9	9.8-22.5	20	13.8 ± 4.1	5.1-23.1	0.570
Angiotensinogen [ng/ml]	20	5.54 (3.9,54.9)#		20	11.1 (2.2,57.4)#		0.766
Apo A1 [mg/ml]	20	0.4 ± 0.1	0.3-0.5	20	0.3 ± 0.1	0.3-0.5	0.105
Apo CIII [µg/ml]	20	50 ± 15	21.4-84.2	20	40 ± 14	22-67	0.051
Apo H [µg/ml]	20	132 ± 29	56-186	19	121 ± 29	49-171	0206
Apo B [µg/ml]	20	n.d		20	n.d.		.
Apo D [µg/ml]	20	24.6 ± 9.2	8.5-45.5	20	28.6 ± 7.7	11.8-46.7	0.133
Apo E [µg/ml]	20	71 ± 30	30-131	20	77 ± 45	13-200	0.914
Clusterin (Apo J) [µg/ml]	20	84.8 ± 14.3	54.7-117	20	89.0 ± 22.0	53.9-139.0	0.507
Calcitonin [pg/ml]	20	26.2 ± 7.2	14.0-40.0	20	22.7 ± 6.9	13.4-43.5	0.070
Cortisol [ng/ml]	20	154 ± 83.	53-354	20	208 ± 108	50.6-389.0	0.051
FSH [ng/ml]	20	9.8 ± 18.1	3.7- 85.2	20	4.8 ± 1.7*	3.4-10.4	0.045
Galanin [ng/ml]	20	n.d.		20	n.d.		
GLP-1 total [pg/ml]	20	17.9 ± 3.4	10.8-25.8	20	15.8 ± 3.9	10.6-24.5	0.033
Glucagon [pg/ml]	20	319 ± 126	134-636	20	253 ± 114	92-636	0.058
IGF-1 [ng/ml]	20	15.2 ± 5.0	4.4-23.7	20	12.5 ± 3.8	8.3-20.6	0.060
IGF BP-2 [ng/ml]	20	716 ± 250	301-1270	20	782 ± 301	245-1351	0.516
IGFBP-3 [ng/ml]	20	967 ± 251	543-1470	20	909 ± 237	533-1570	0.394
Insulin [uIU/ml]	20	2.0 ± 1.0	1.0-4.7	20	2.1 ± 2.9	0.7-14.0	0.076
Leptin [ng/ml]	20	10.1 ± 7.8	1.4-28.4	20	7.9 ± 5.7	2.2-24.8	0.745
LH [ng/ml]	20	0.5 ± 0.2	0.3-0.9	20	0.5 ± 0.1	0.3-0.8	0.262
LOX-1 [ng/ml]	20	1.9 ± 1.3	0.7-7.0	20	1.7 ± 0.7	0.7-3.2	0.755
Lp (a) [µg/ml]	17	12.3 ± 14.2	0.6-46.1	19	7.8 ± 7.8	0.5-32.8	0.516
MDA LDL [ng/ml]	20	201 ± 65	97-332	20	170 ± 41	92-270	0.078
PP [pg/ml]	20	31.1 ± 14.1	13.8-67.0	20	33.7 ± 19.6	13.1-84.3	0.957
Progesterone [ng/ml]	20	1424 ± 706	464-2244	20	1572 ± 602	687-2244	0.420
Prolactin [ng/ml]	20	141 ± 57.4	66.2-253.0	20	118.5 ± 45.8	58.3-219.0	0.213
PYY [pg/ml]	20	461 ± 221	170-1000	20	339 ± 188	65.8-765.0	0.062
Resistin [ng/ml]	20	7.8 ± 2.5	4.1-13.6	20	8.7 ± 3.8	3.2-17.6	0.685
S100b [ng/ml]	20	n.d		20	n.d		
Secretin [ng/ml]	20	n.d		20	n.d		
SHBG [nmol/l]	20	25.3 ± 7.2	18.9-47.2	20	25.8 ± 10.5	12.1-60.8	0.850
TBG [µg/ml]	20	64.7 ± 9.1	41.1-82.6	20	62.0 ± 6.6	51.5-76.1	0.176
Testosterone [ng/ml]	20	1.3 ± 0.3	0.8-1.8	20	1.5 ± 0.4	1.0-2.4	0.310
TSH [uIU/ml]	20	10.6 ± 10.3	2.5-47.5	20	7.1 ± 3.9	2.8-18.8	0.351

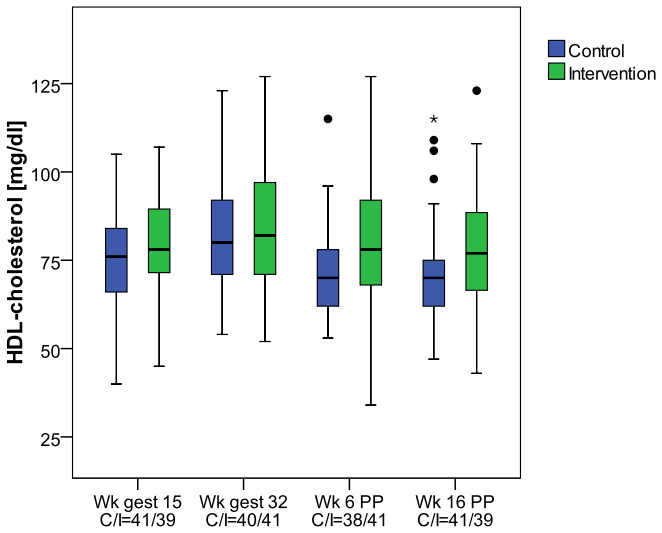
*$P < 0.05$ between the groups; # Data are median (25^{th}, 75^{th} centile); n.d = not detectable

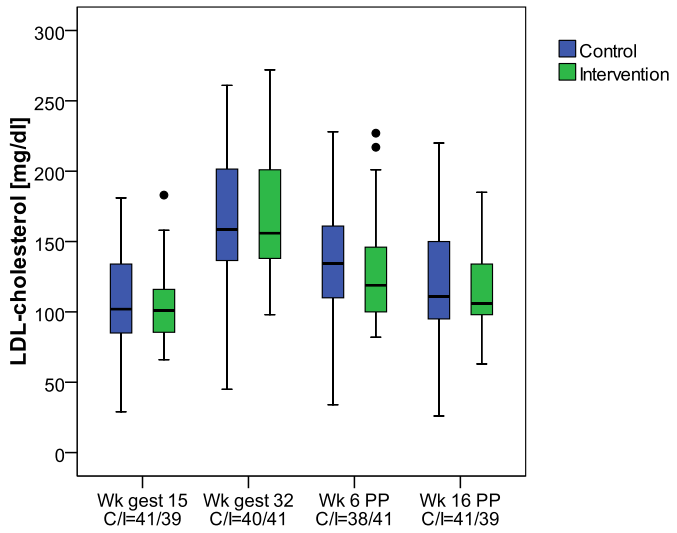
Appendix C: Figures

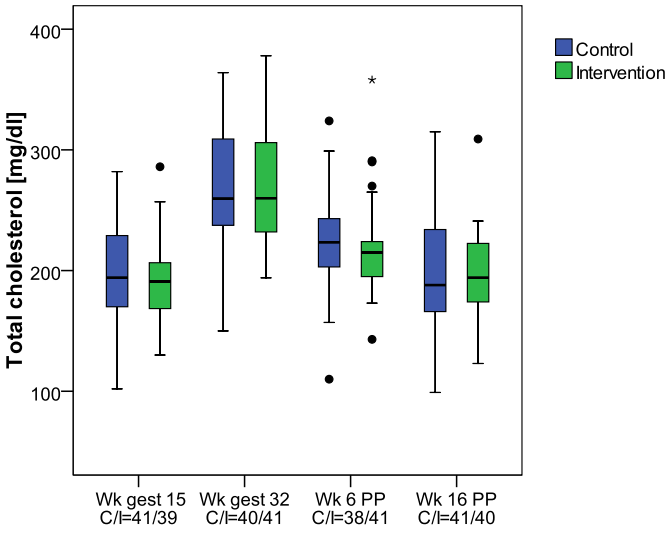
C.1 Comparison of metabolic parameters and indices between the control and intervention group (C/I) during pregnancy and lactation

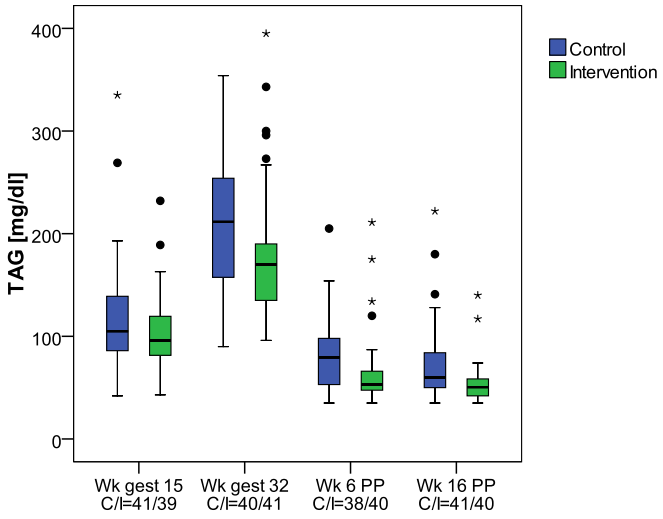




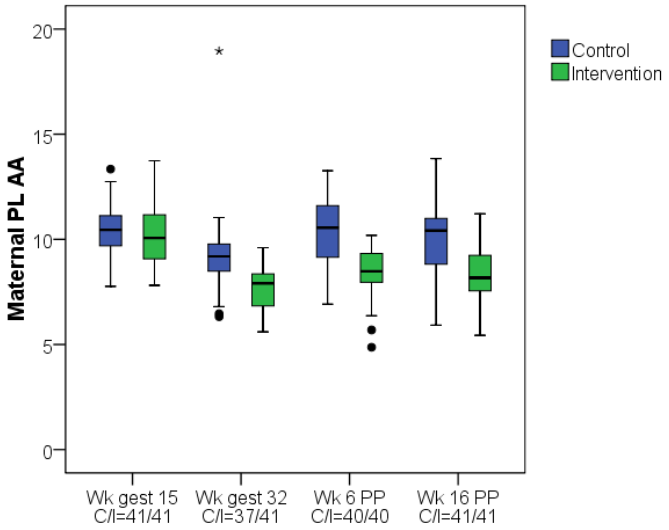


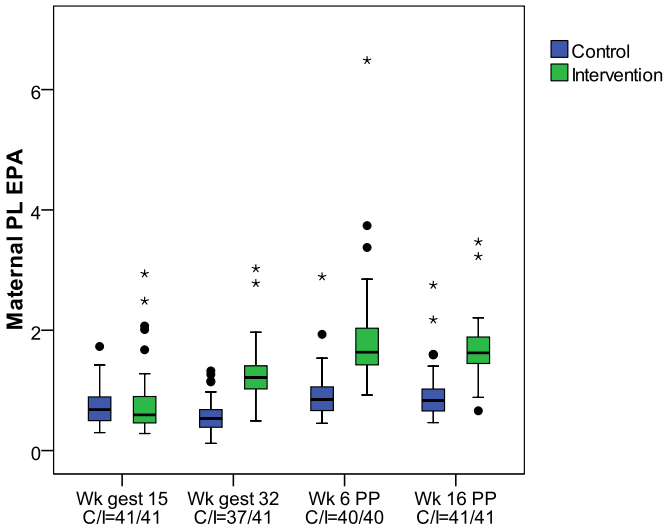


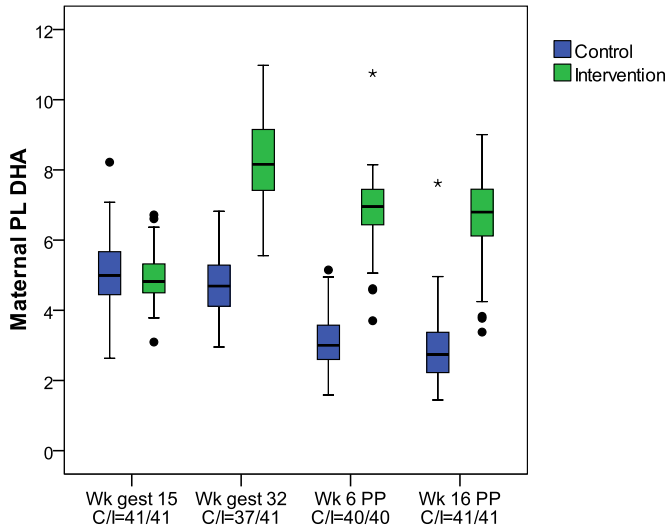

Total cholesterol [mg/dl]
400
300
200
100
Wk gest 15 C/I=41/39
Wk gest 32 C/I=40/41
Wk 6 PP C/I=38/41
Wk 16 PP C/I=41/40
Control
Intervention

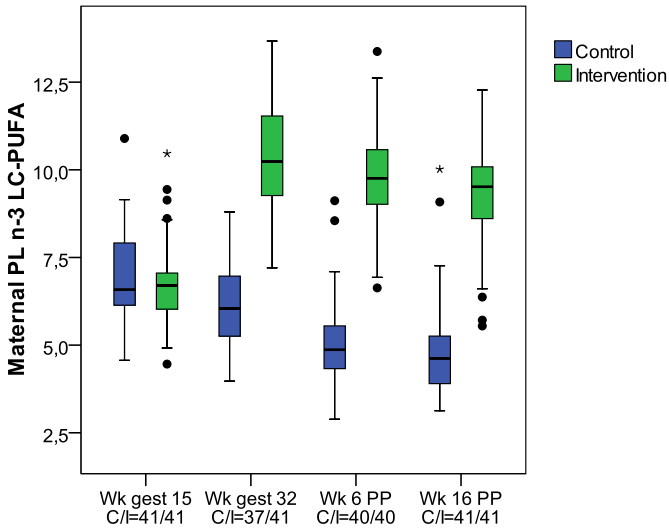

TAG [mg/dl]
400
300
200
100
0
Wk gest 15 C/I=41/39
Wk gest 32 C/I=40/41
Wk 6 PP C/I=38/40
Wk 16 PP C/I=41/40
Control
Intervention

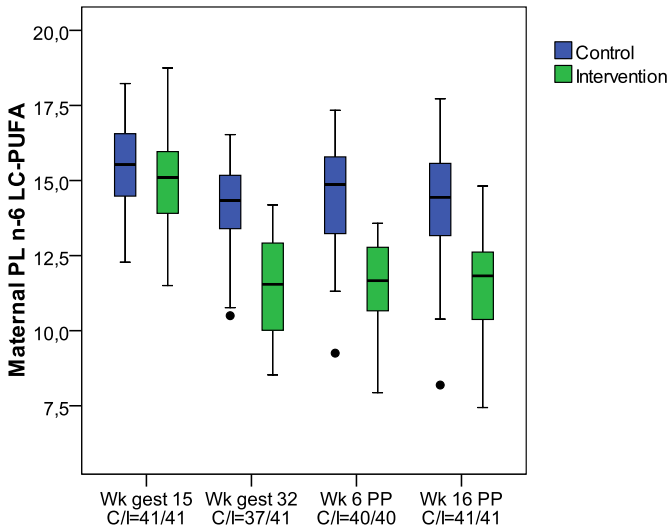
C.2 Maternal PUFA profile in plasma PLs and RBCs

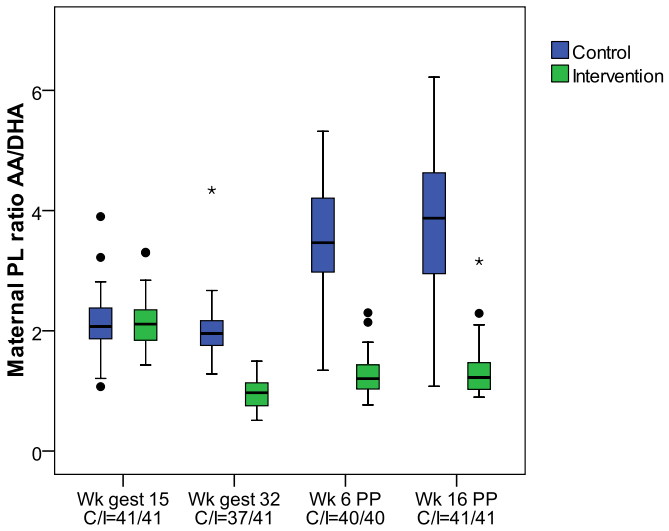


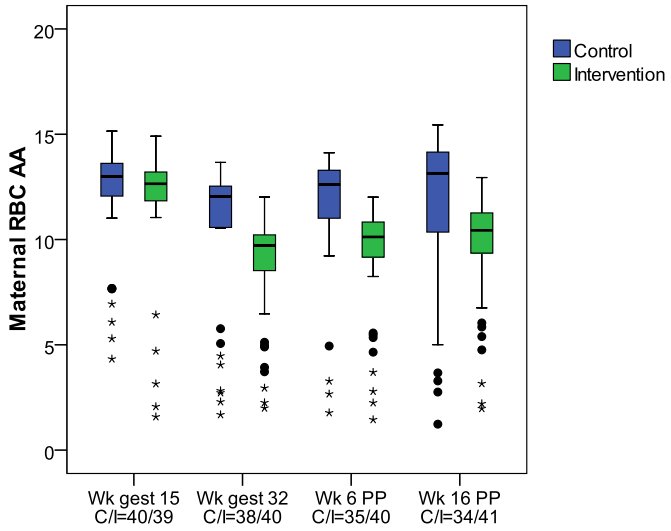


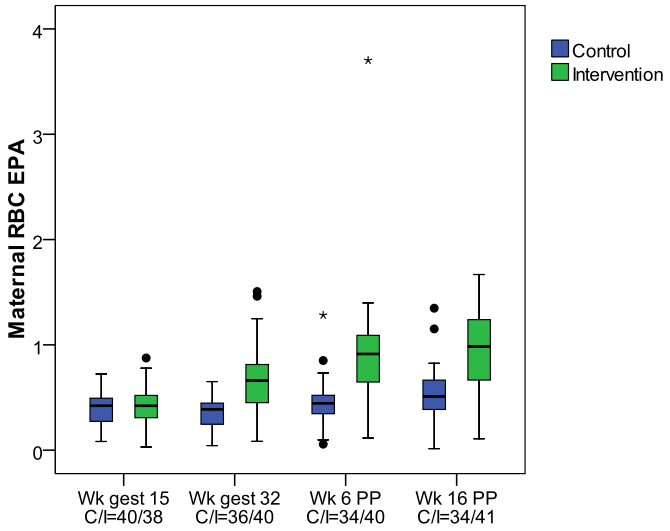


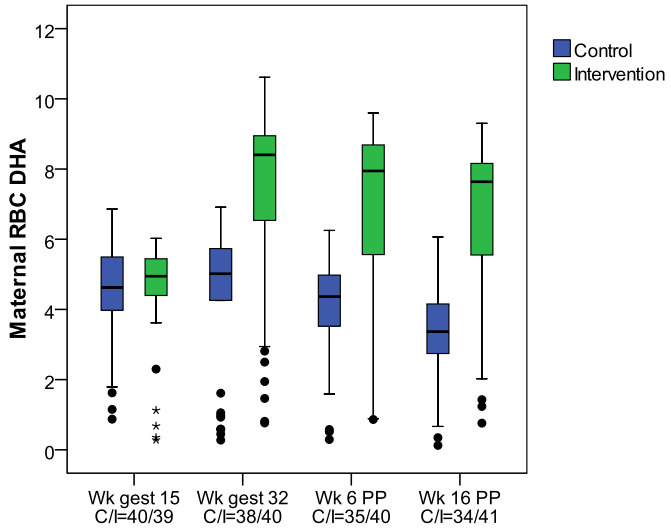


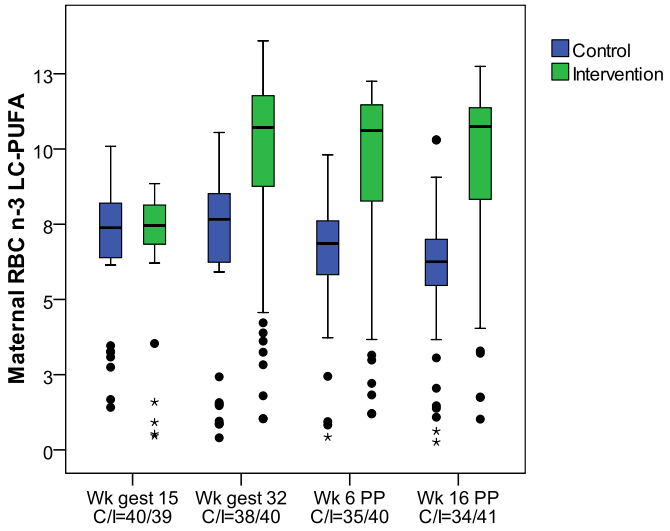


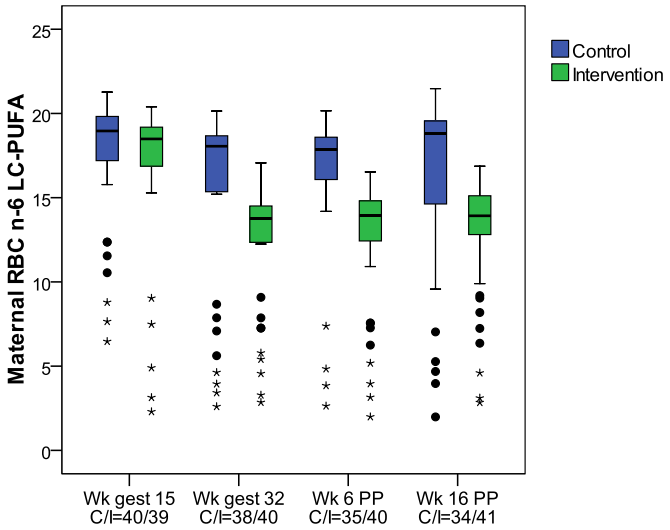


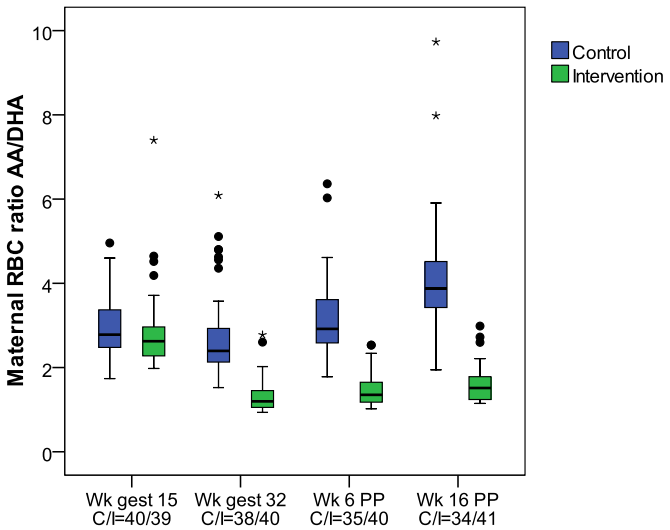

Maternal RBC AA
Control
Intervention
Wk gest 15 C/I=40/39
Wk gest 32 C/I=38/40
Wk 6 PP C/I=35/40
Wk 16 PP C/I=34/41


Maternal RBC EPA
Control
Intervention
Wk gest 15 C/I=40/38
Wk gest 32 C/I=36/40
Wk 6 PP C/I=34/40
Wk 16 PP C/I=34/41

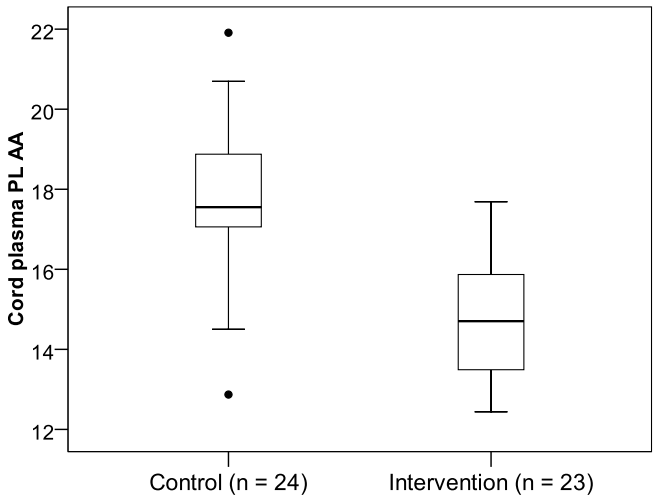

Maternal RBC DHA
Control
Intervention
Wk gest 15 C/I=40/39
Wk gest 32 C/I=38/40
Wk 6 PP C/I=35/40
Wk 16 PP C/I=34/41

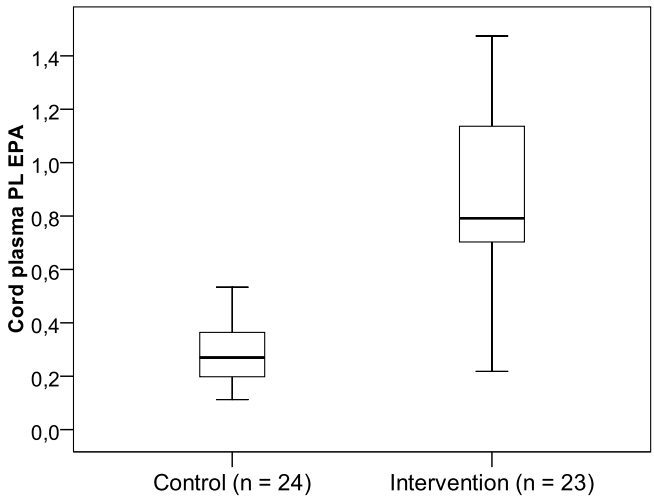

Maternal RBC n-3 LC-PUFA
Control
Intervention
Wk gest 15 C/I=40/39
Wk gest 32 C/I=38/40
Wk 6 PP C/I=35/40
Wk 16 PP C/I=34/41

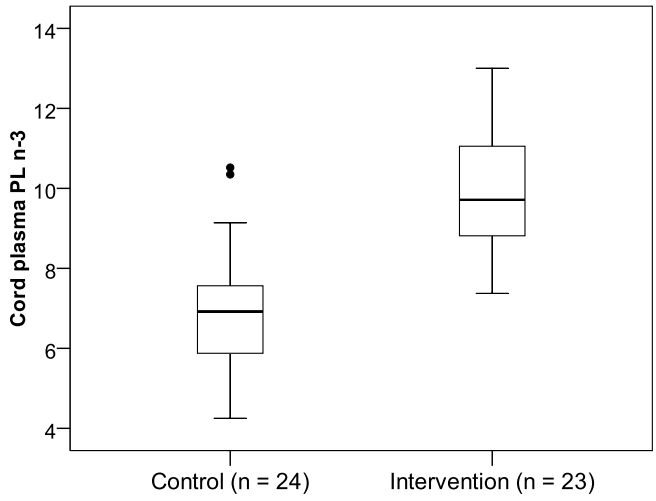

Maternal RBC n-6 LC-PUFA
Control
Intervention
Wk gest 15 C/I=40/39
Wk gest 32 C/I=38/40
Wk 6 PP C/I=35/40
Wk 16 PP C/I=34/41

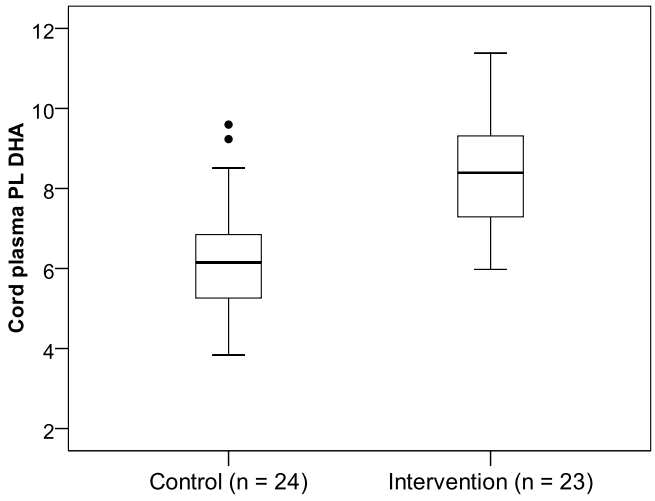

Maternal RBC ratio AA/DHA
Control
Intervention
Wk gest 15 C/I=40/39
Wk gest 32 C/I=38/40
Wk 6 PP C/I=35/40
Wk 16 PP C/I=34/41

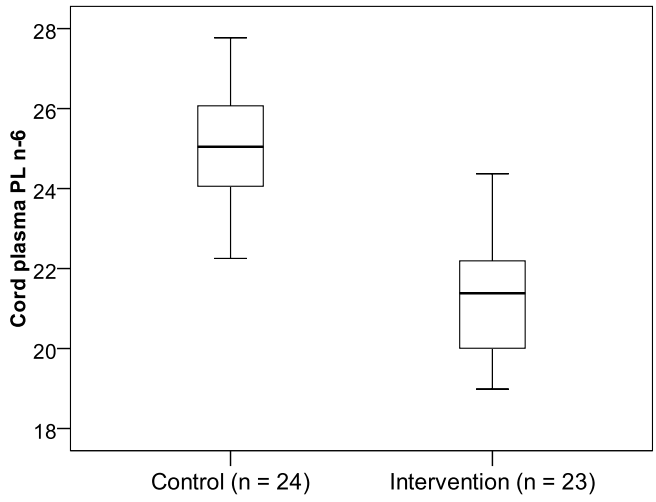
C.3 Fetal PUFA profile in plasma PLs and RBCs

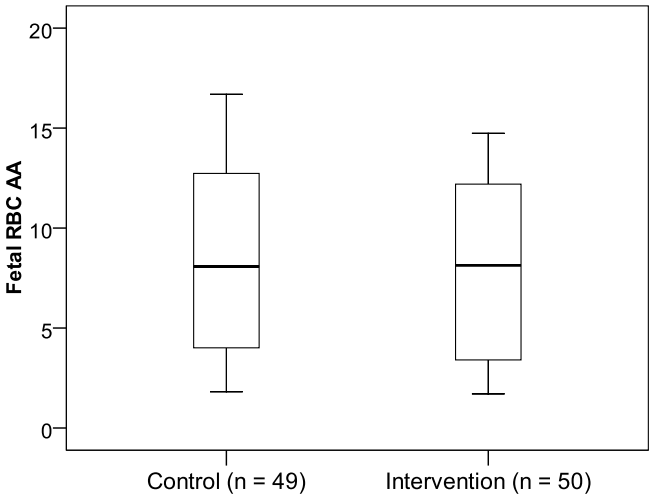


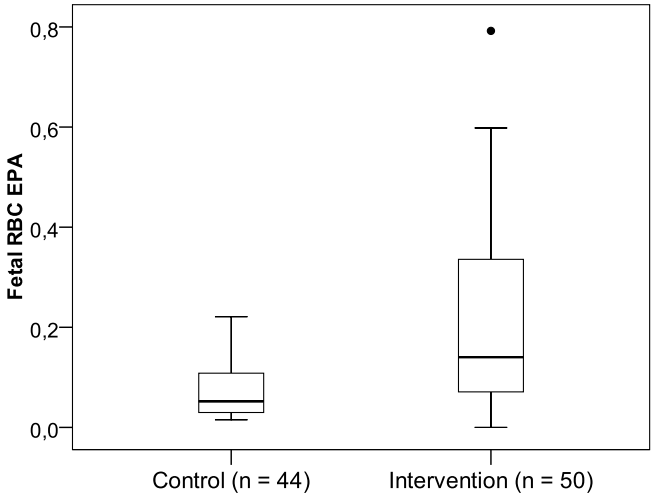


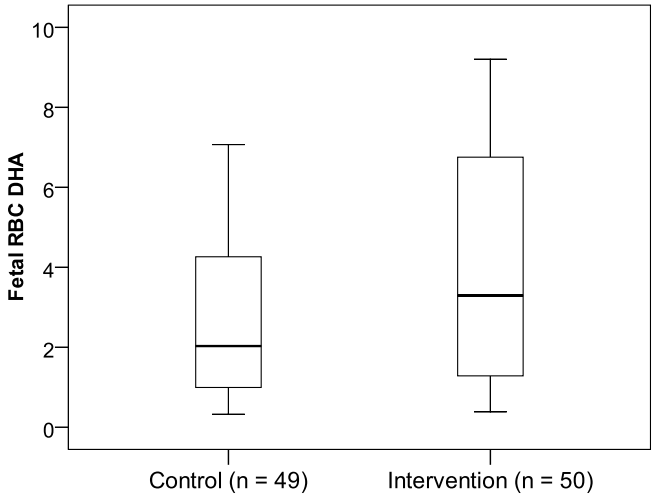


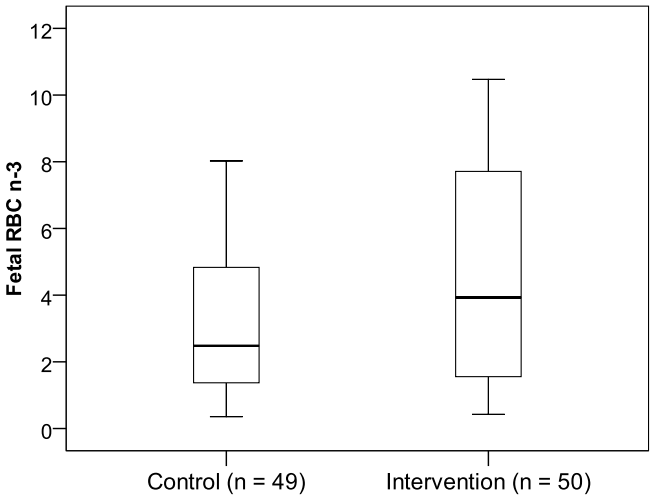


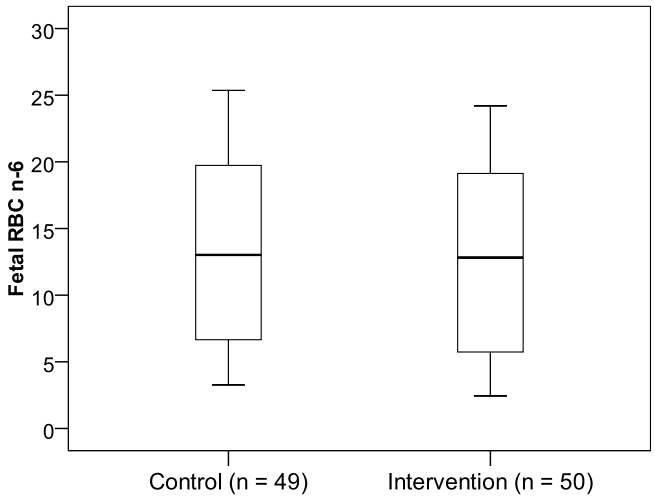



Fetal RBC AA
20
15
10
5
0
Control (n = 49)
Intervention (n = 50)

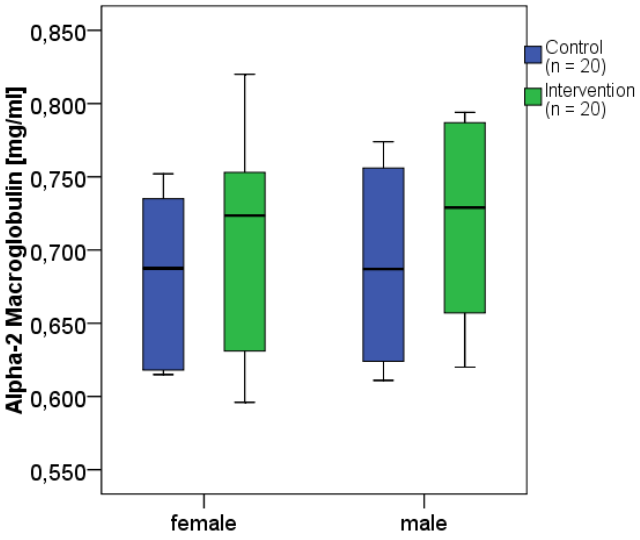

Fetal RBC EPA
0,8
0,6
0,4
0,2
0,0
Control (n = 44)
Intervention (n = 50)

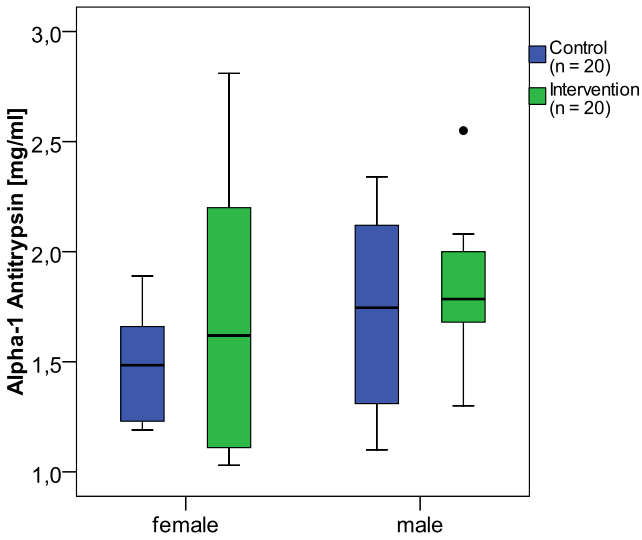

Fetal RBC DHA
10
8
6
4
2
0
Control (n = 49)
Intervention (n = 50)

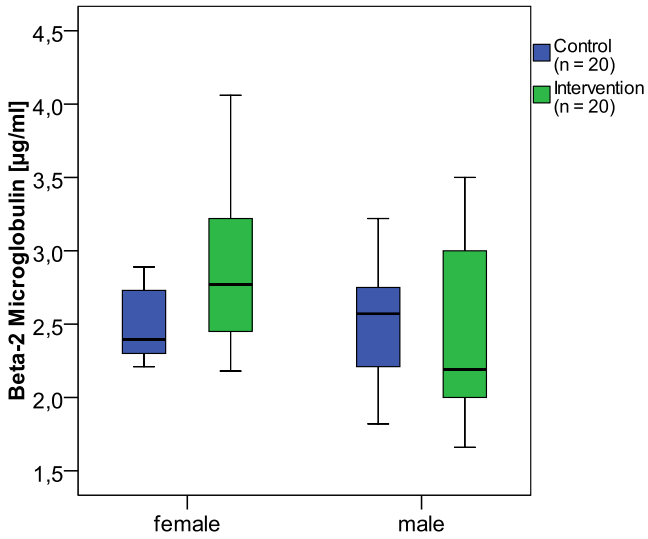

Fetal RBC n-3
12
10
8
6
4
2
0
Control (n = 49)
Intervention (n = 50)

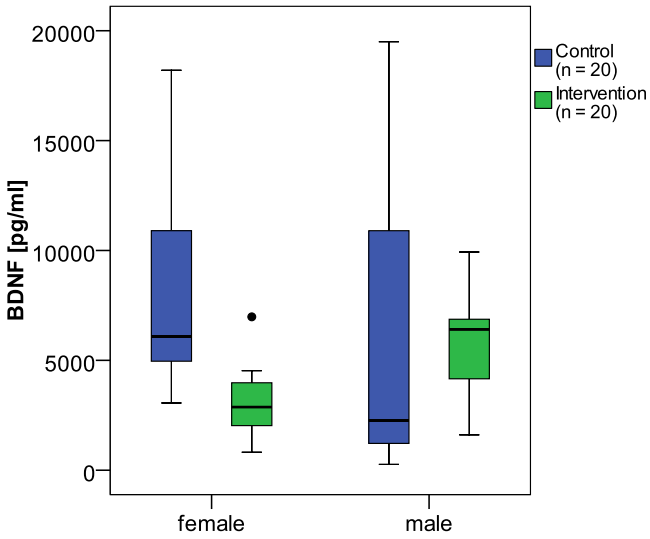

Fetal RBC n-6
30
25
20
15
10
5
0
Control (n = 49)
Intervention (n = 50)

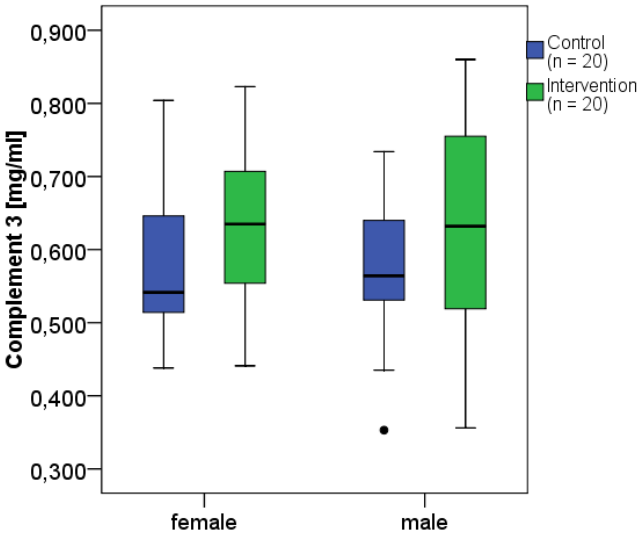
C. 4 Biomarkers from inflammation MAP

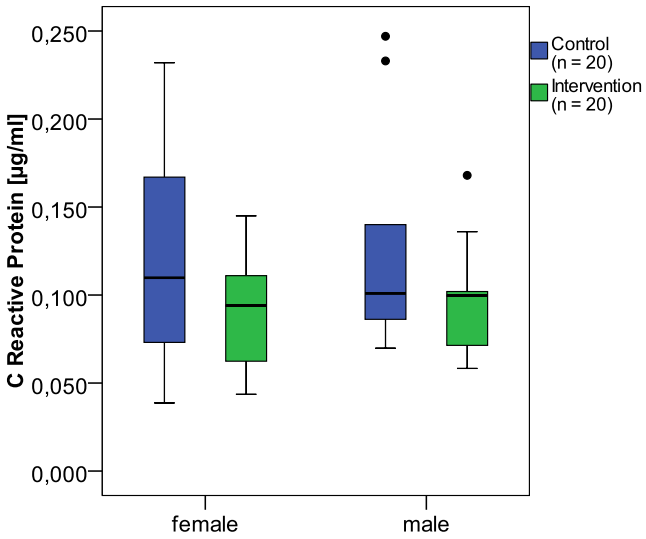


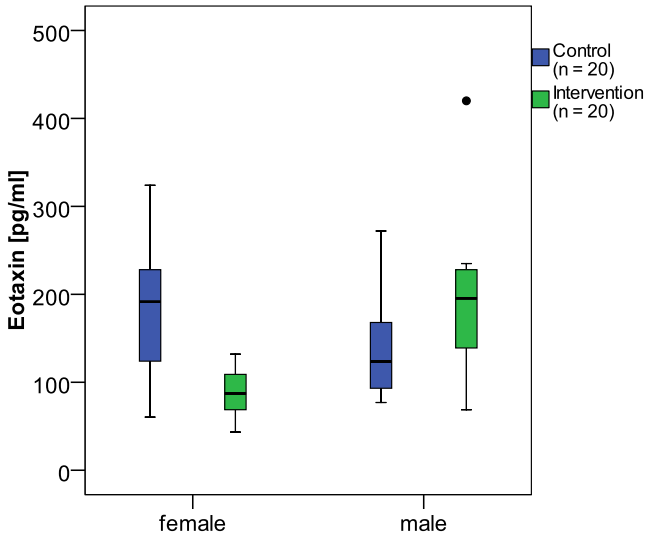


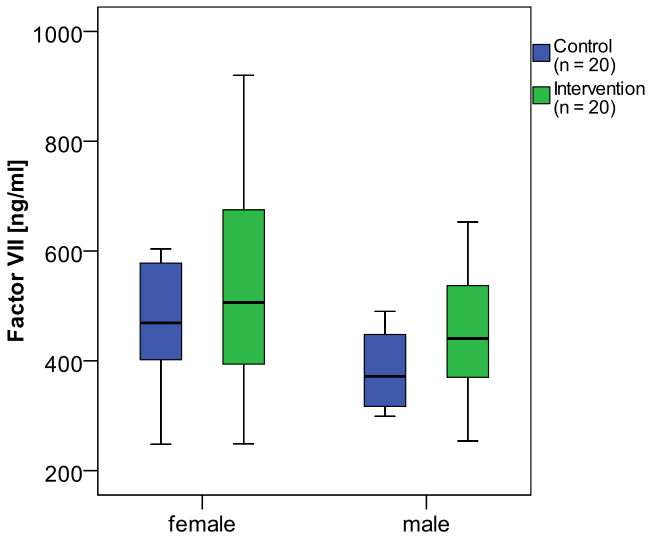


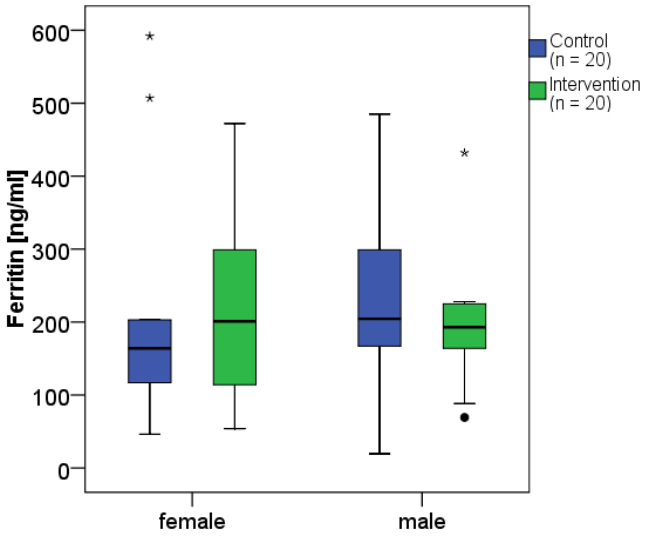


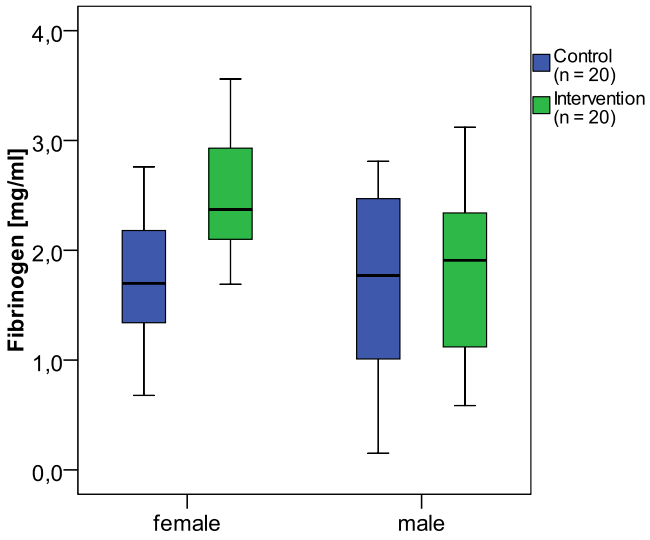


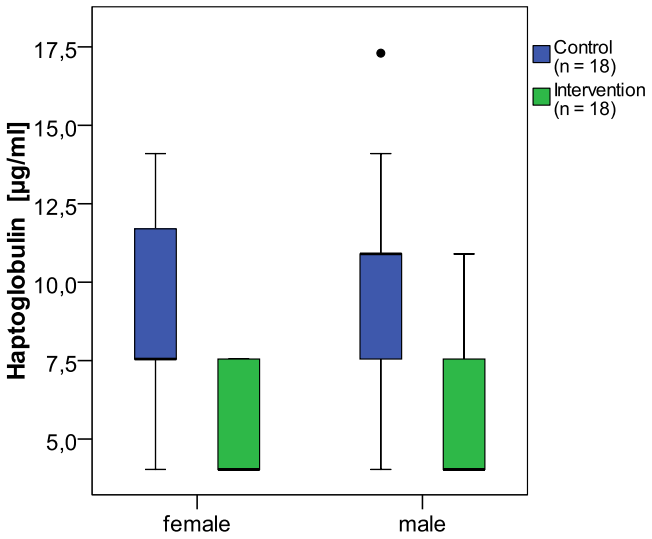


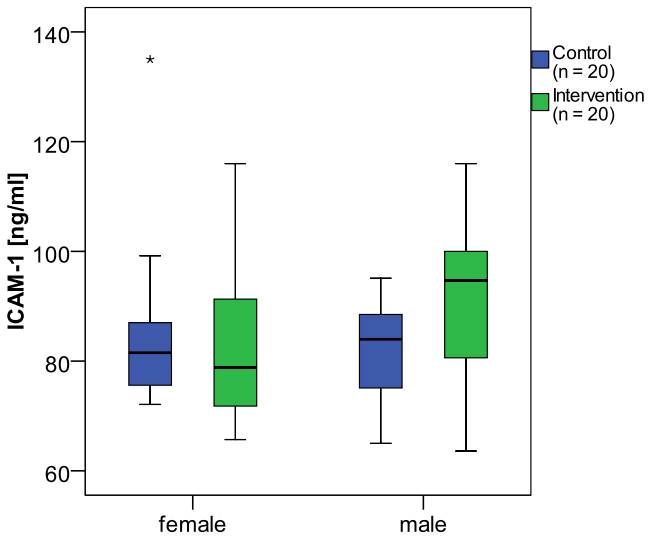

Eotaxin [pg/ml]
500
400
300
200
100
0
female
male
Control (n = 20)
Intervention (n = 20)

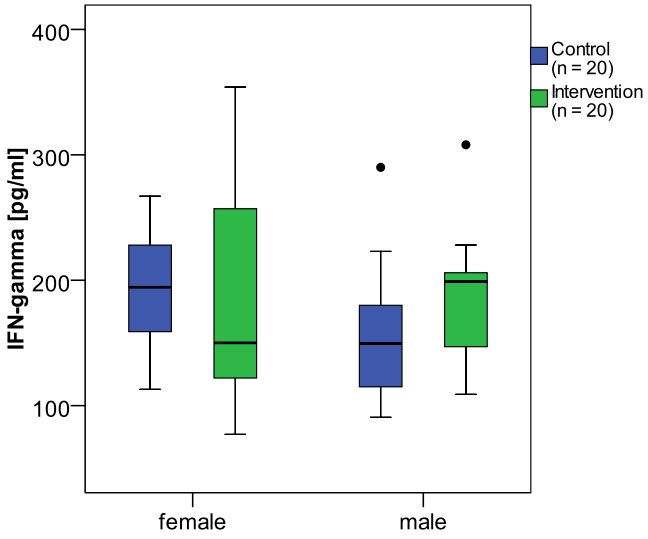

Factor VII [ng/ml]
1000
800
600
400
200
female
male
Control (n = 20)
Intervention (n = 20)

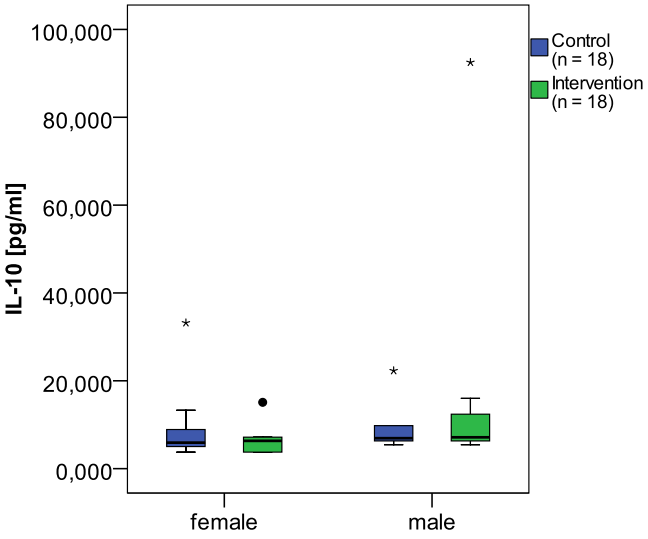

Ferritin [ng/ml]
600
500
400
300
200
100
0
female
male
Control (n = 20)
Intervention (n = 20)

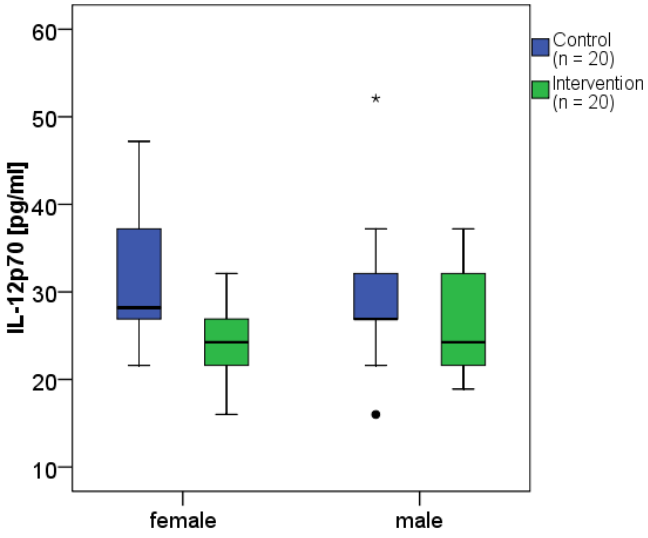

Fibrinogen [mg/ml]
4,0
3,0
2,0
1,0
0,0
female
male
Control (n = 20)
Intervention (n = 20)

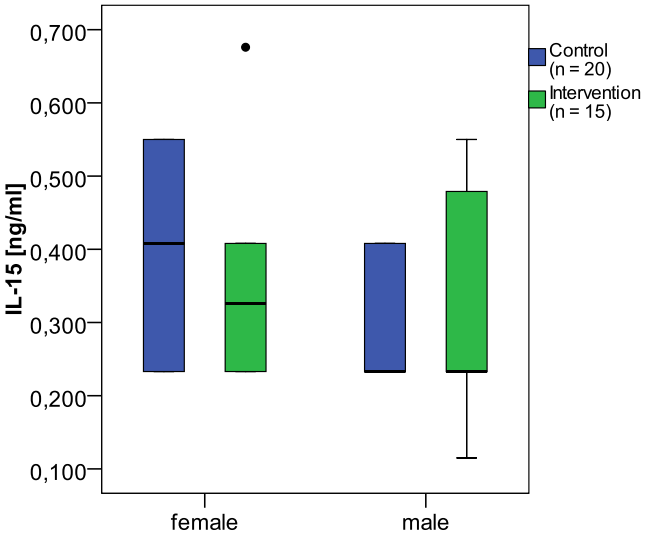

Haptoglobulin [µg/ml]
17,5
15,0
12,5
10,0
7,5
5,0
female
male
Control (n = 18)
Intervention (n = 18)

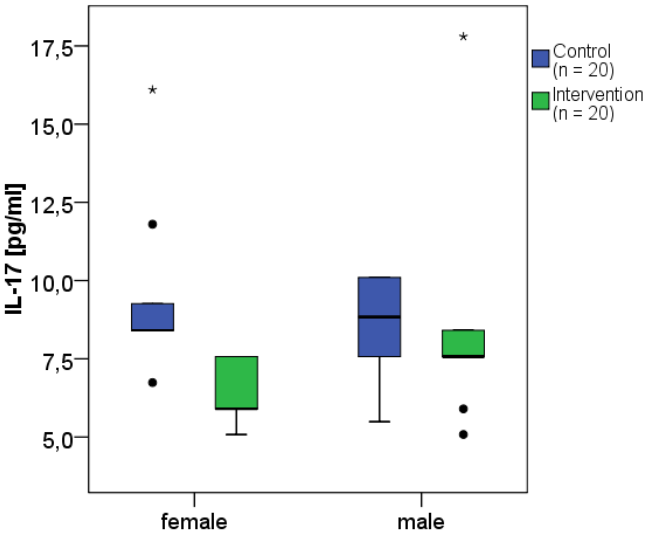

ICAM-1 [ng/ml]
140
120
100
80
60
female
male
Control (n = 20)
Intervention (n = 20)

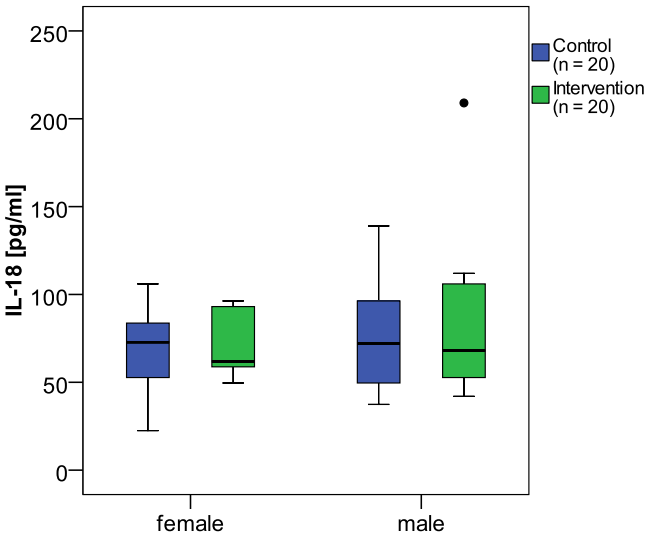

IFN-gamma [pg/ml]
400
300
200
100
female
male
Control (n = 20)
Intervention (n = 20)

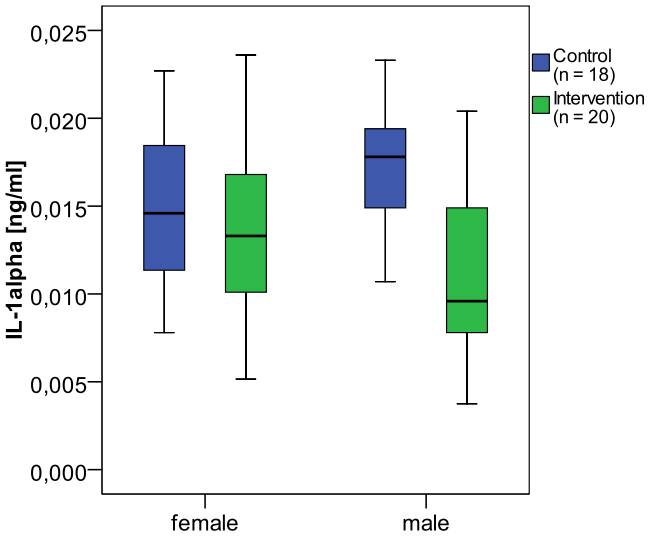

IL-10 [pg/ml]
100,000
80,000
60,000
40,000
20,000
0,000
female
male
Control (n = 18)
Intervention (n = 18)

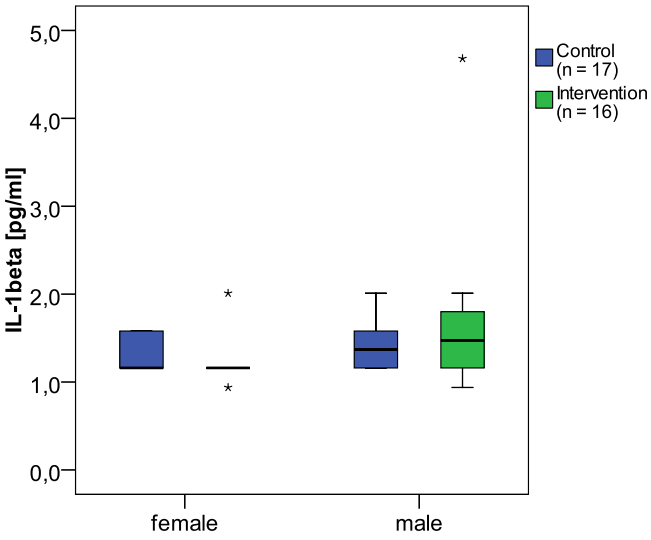

IL-12p70 [pg/ml]
60
50
40
30
20
10
female
male
Control (n = 20)
Intervention (n = 20)

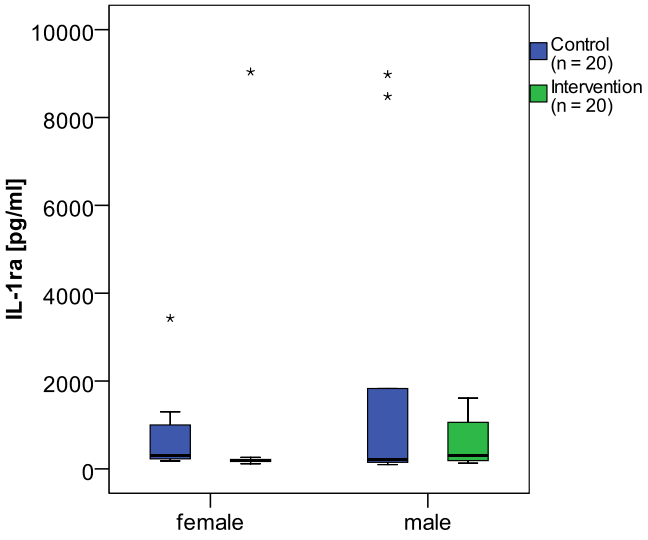

IL-15 [ng/ml]
0,700
0,600
0,500
0,400
0,300
0,200
0,100
female
male
Control (n = 20)
Intervention (n = 15)

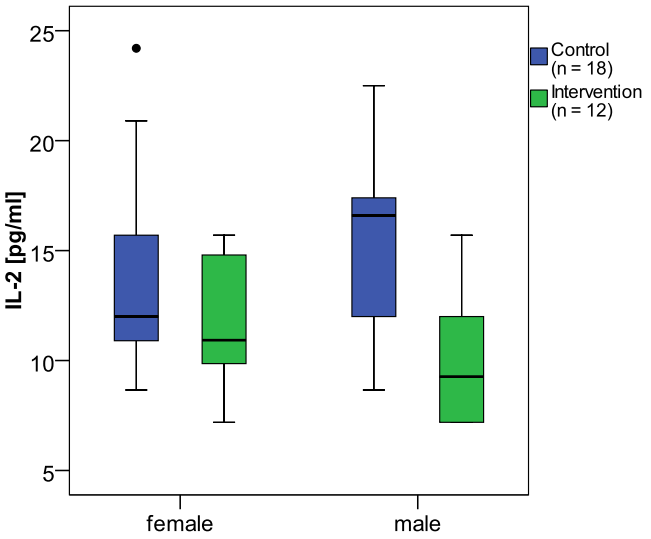

IL-17 [pg/ml]
17,5
15,0
12,5
10,0
7,5
5,0
female
male
Control (n = 20)
Intervention (n = 20)

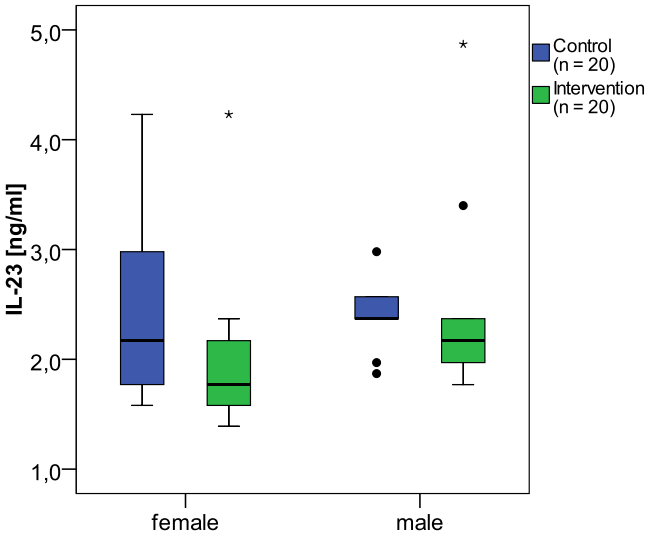

IL-18 [pg/ml]
250
200
150
100
50
0
female
male
Control (n = 20)
Intervention (n = 20)

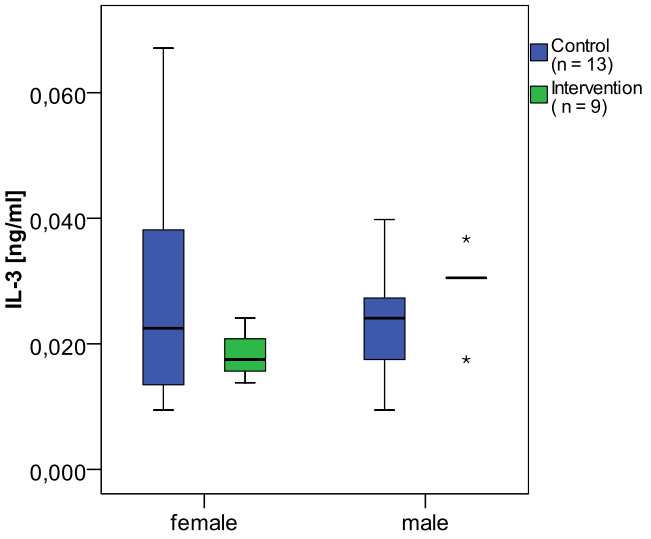

IL-1alpha [ng/ml]
0,025
0,020
0,015
0,010
0,005
0,000
female
male
Control (n = 18)
Intervention (n = 20)

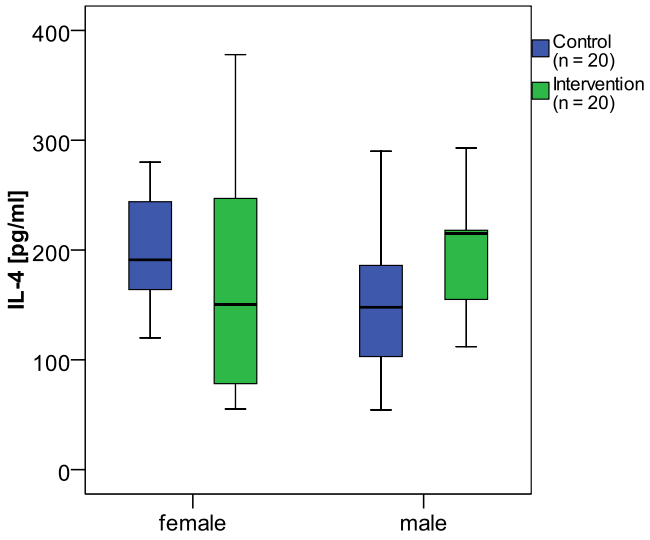

IL-1beta [pg/ml]
5,0
4,0
3,0
2,0
1,0
0,0
female
male
Control (n = 17)
Intervention (n = 16)

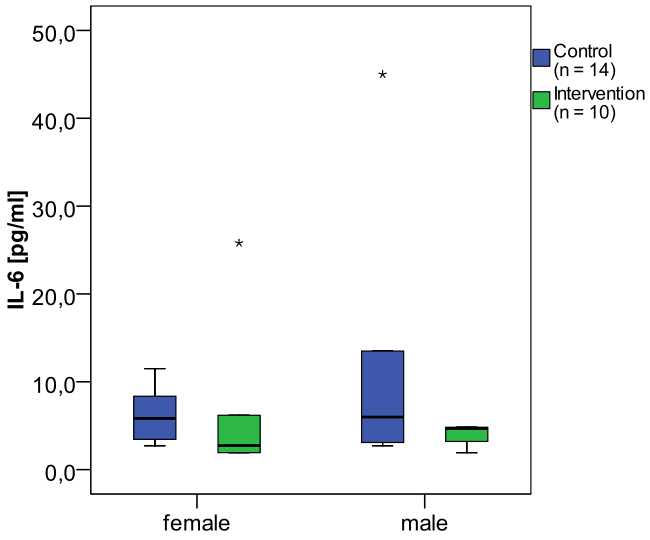

IL-1ra [pg/ml]
10000
8000
6000
4000
2000
0
female
male
Control (n = 20)
Intervention (n = 20)

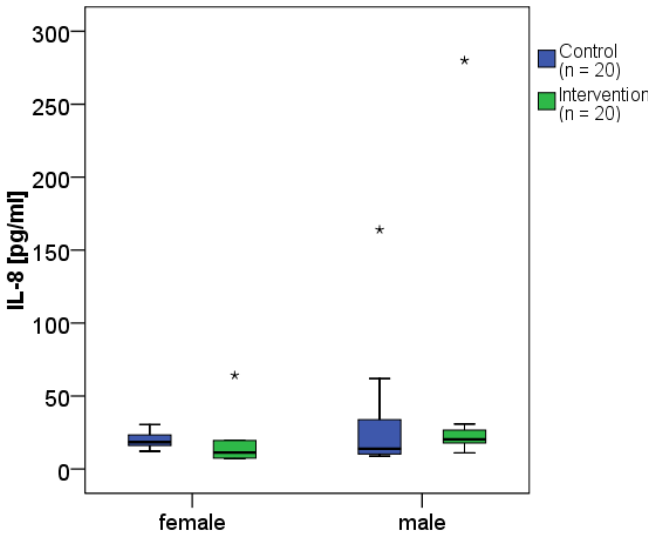

IL-2 [pg/ml]
25
20
15
10
5
female
male
Control (n = 18)
Intervention (n = 12)

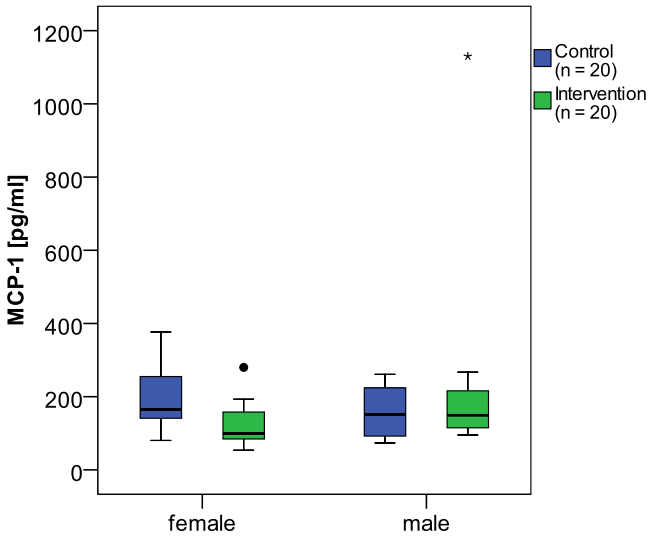

IL-23 [ng/ml]
5,0
4,0
3,0
2,0
1,0
female
male
Control (n = 20)
Intervention (n = 20)

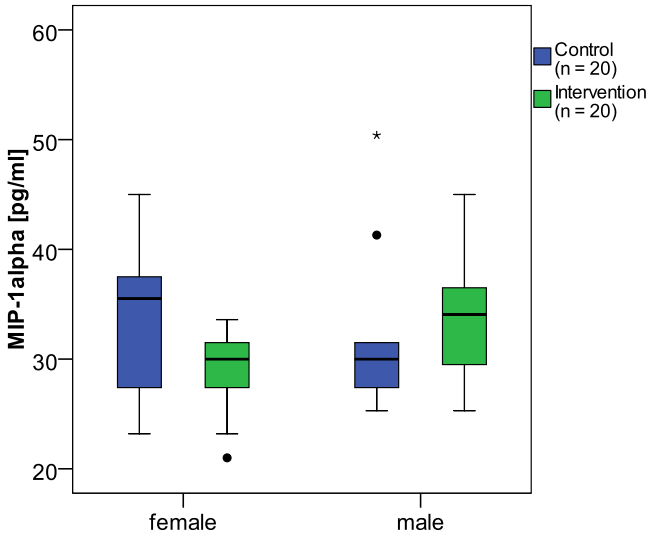

IL-3 [ng/ml]
0,060
0,040
0,020
0,000
female
male
Control (n = 13)
Intervention (n = 9)

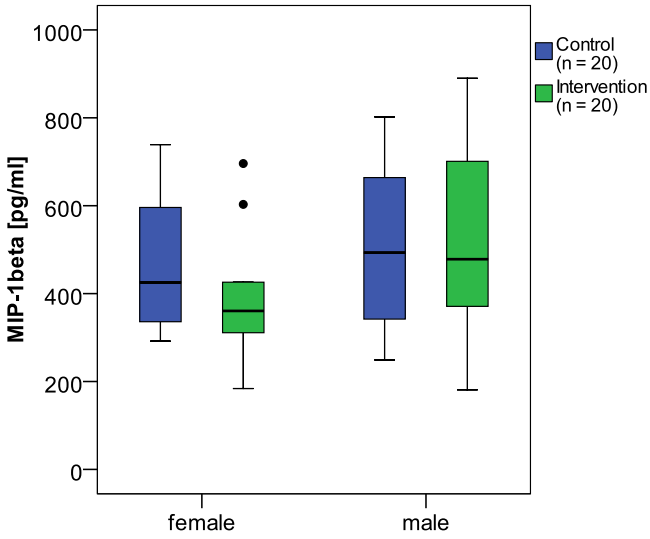

IL-4 [pg/ml]
400
300
200
100
0
female
male
Control (n = 20)
Intervention (n = 20)

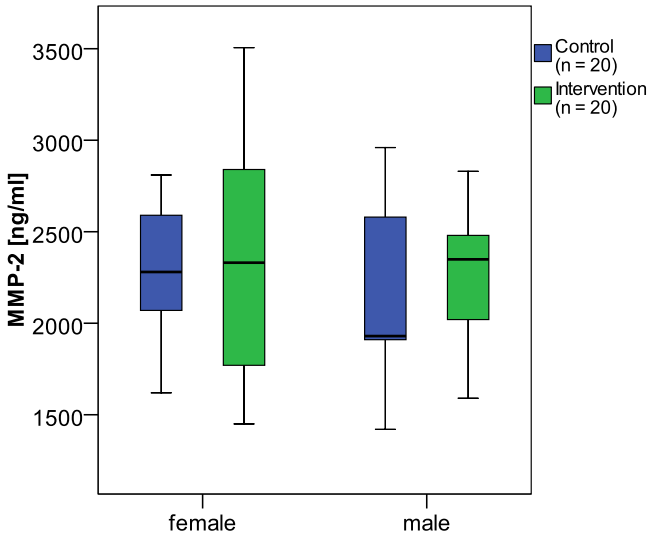

IL-6 [pg/ml]
50,0
40,0
30,0
20,0
10,0
0,0
female
male
Control (n = 14)
Intervention (n = 10)

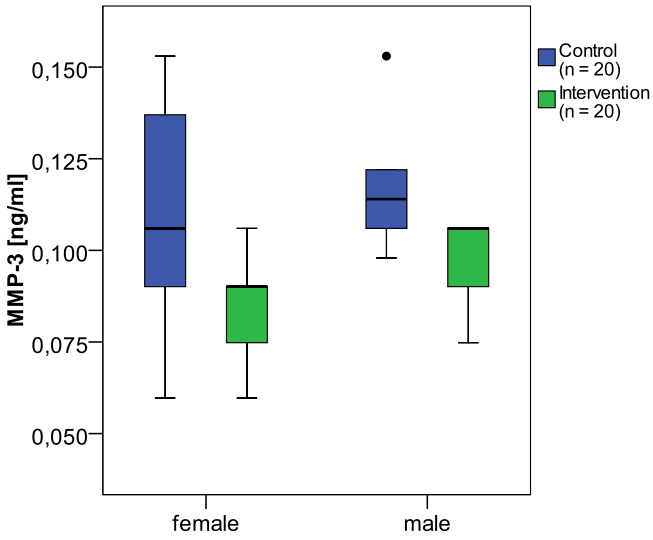

IL-8 [pg/ml]
300
250
200
150
100
50
0
female
male
Control (n = 20)
Intervention (n = 20)

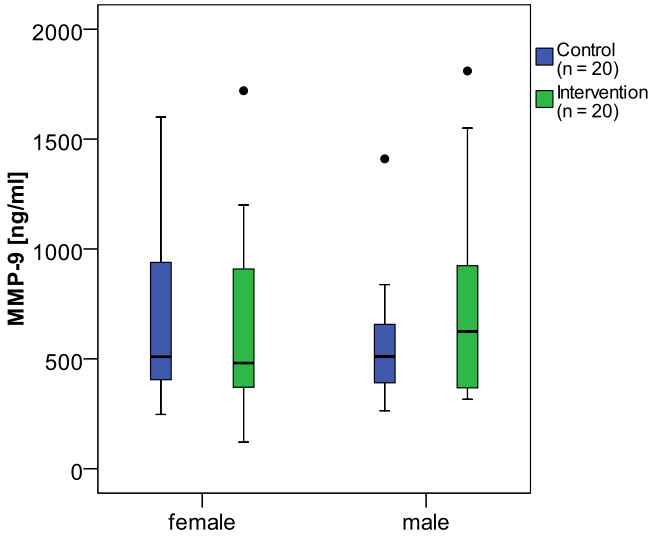

MCP-1 [pg/ml]
1200
1000
800
600
400
200
0
female
male
Control (n = 20)
Intervention (n = 20)

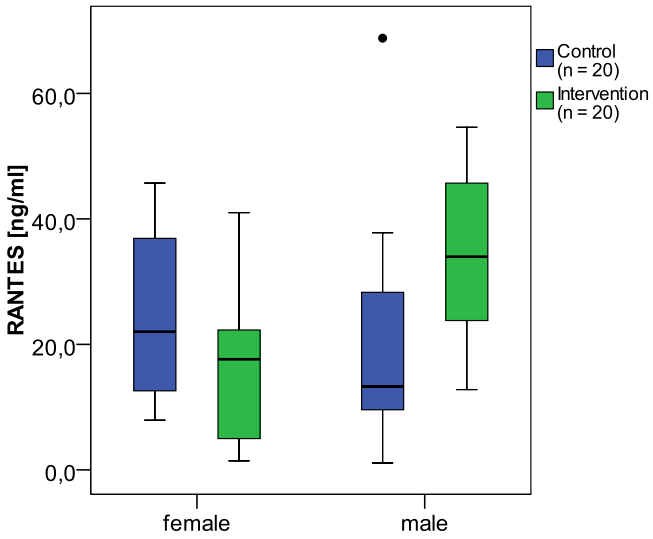

MIP-1alpha [pg/ml]
60
50
40
30
20
female
male
Control (n = 20)
Intervention (n = 20)

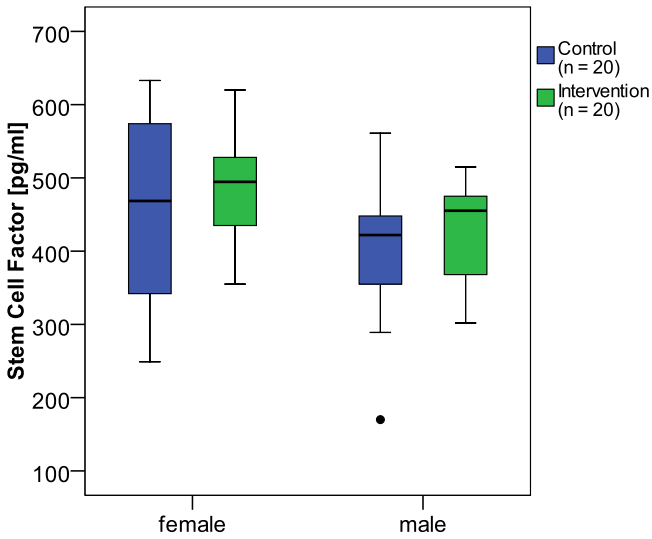

MIP-1beta [pg/ml]
1000
800
600
400
200
0
female
male
Control (n = 20)
Intervention (n = 20)

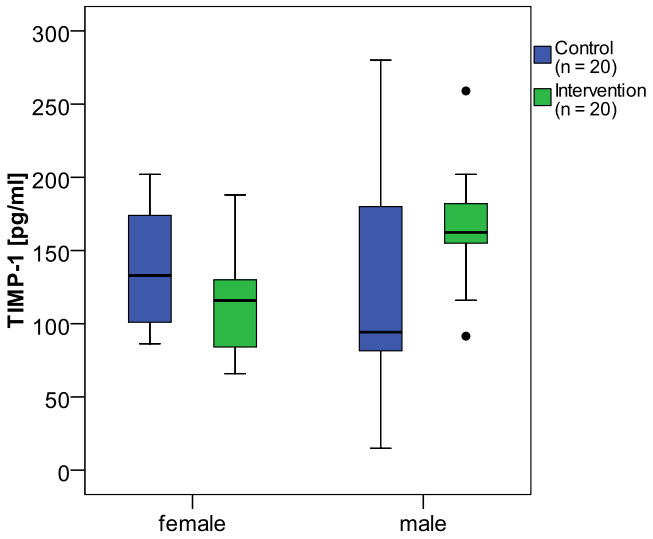

MMP-2 [ng/ml]
3500
3000
2500
2000
1500
female
male
Control (n = 20)
Intervention (n = 20)

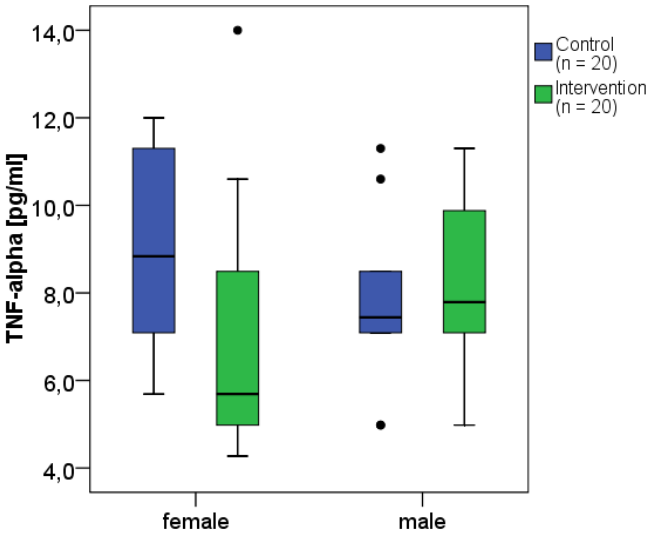

MMP-3 [ng/ml]
0,150
0,125
0,100
0,075
0,050
female
male
Control (n = 20)
Intervention (n = 20)

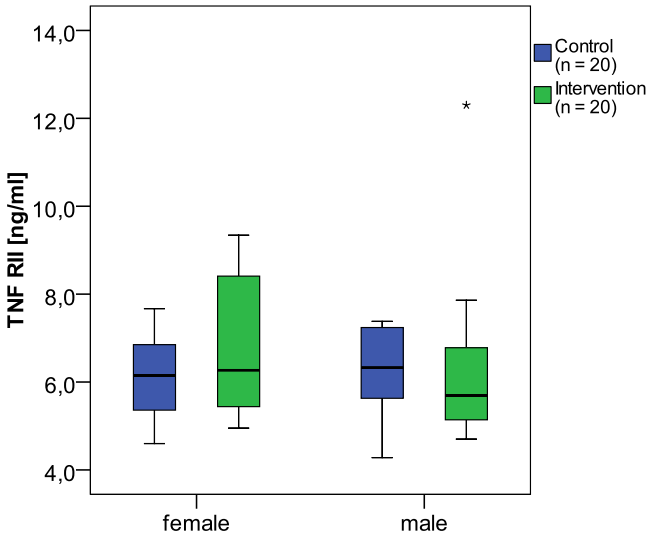

MMP-9 [ng/ml]
2000
1500
1000
500
0
female
male
Control (n = 20)
Intervention (n = 20)

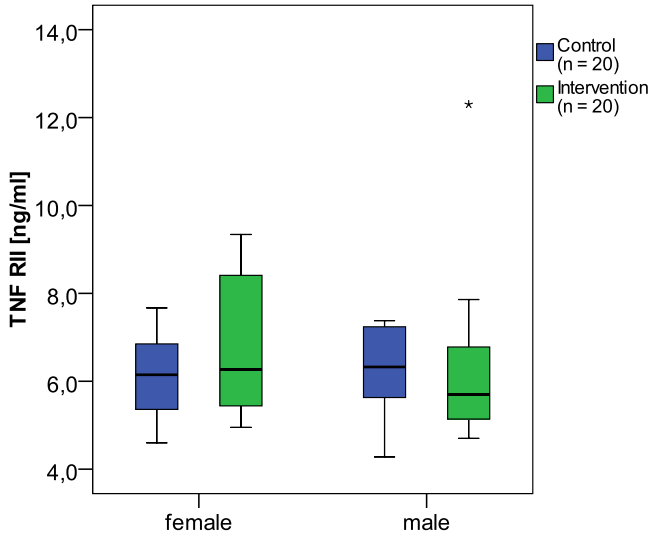

RANTES [ng/ml]
60,0
40,0
20,0
0,0
female
male
Control (n = 20)
Intervention (n = 20)

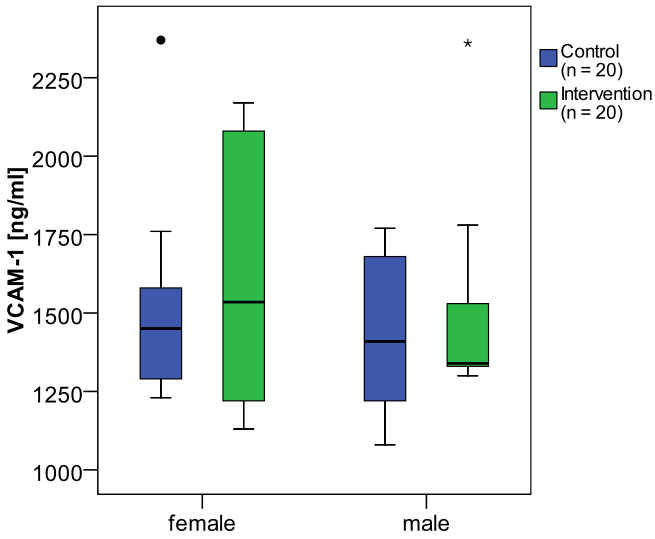

Stem Cell Factor [pg/ml]
700
600
500
400
300
200
100
female
male
Control (n = 20)
Intervention (n = 20)

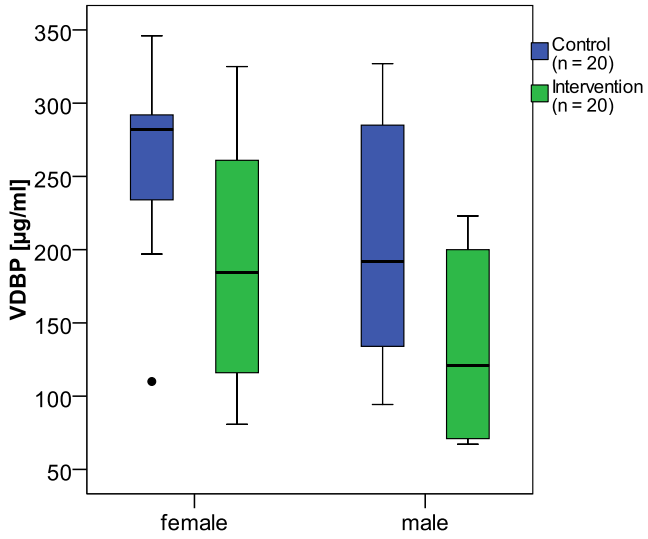

TIMP-1 [pg/ml]
300
250
200
150
100
50
0
female
male
Control (n = 20)
Intervention (n = 20)

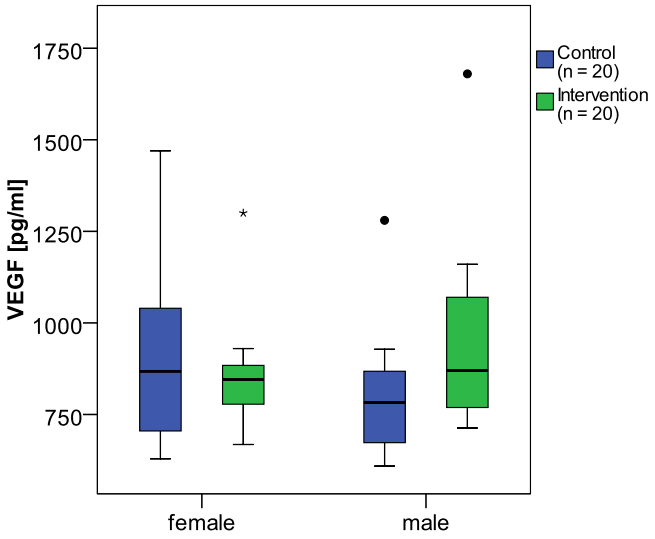

TNF-alpha [pg/ml]
14,0
12,0
10,0
8,0
6,0
4,0
female
male
Control (n = 20)
Intervention (n = 20)

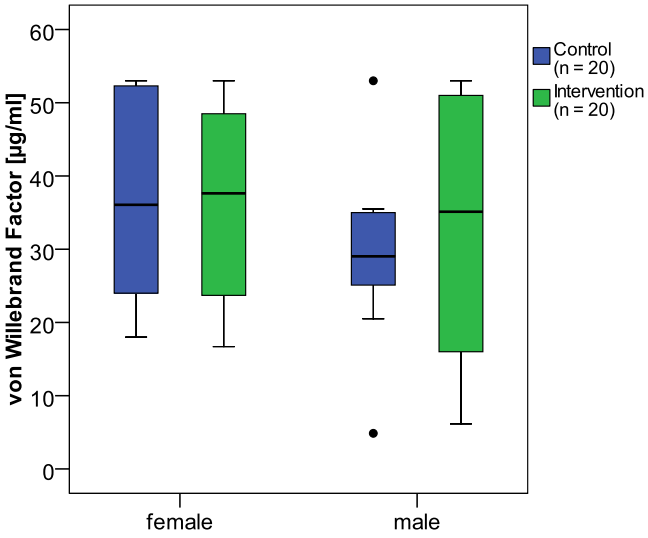

TNF RII [ng/ml]
14,0
12,0
10,0
8,0
6,0
4,0
female
male
Control (n = 20)
Intervention (n = 20)


TNF RII [ng/ml]
14,0
12,0
10,0
8,0
6,0
4,0
female
male
Control (n = 20)
Intervention (n = 20)

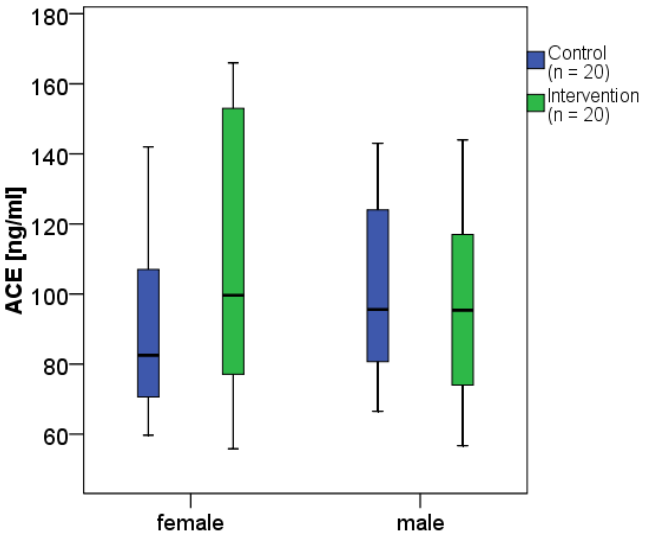

VCAM-1 [ng/ml]
2250
2000
1750
1500
1250
1000
female
male
Control (n = 20)
Intervention (n = 20)

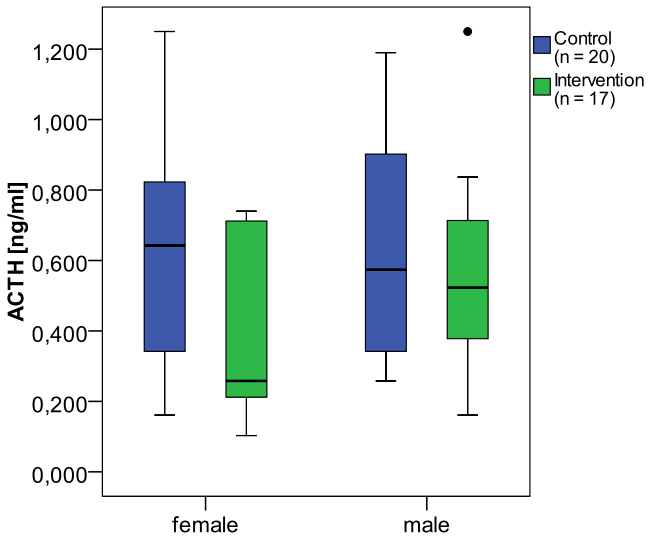

VDBP [µg/ml]
350
300
250
200
150
100
50
female
male
Control (n = 20)
Intervention (n = 20)

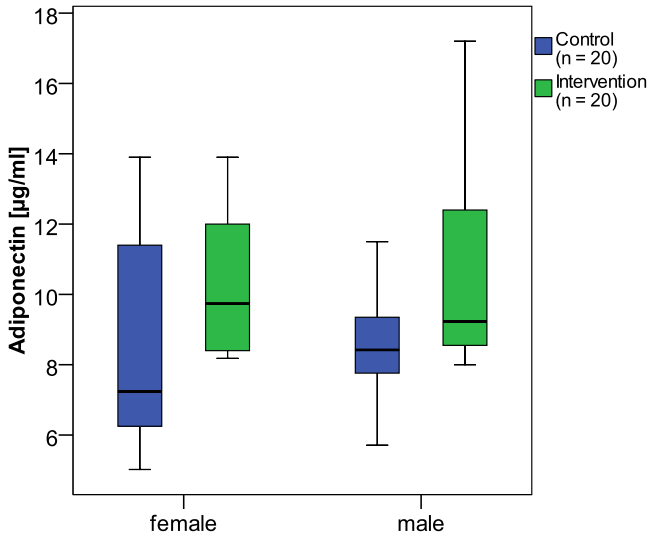

VEGF [pg/ml]
1750
1500
1250
1000
750
female
male
Control (n = 20)
Intervention (n = 20)

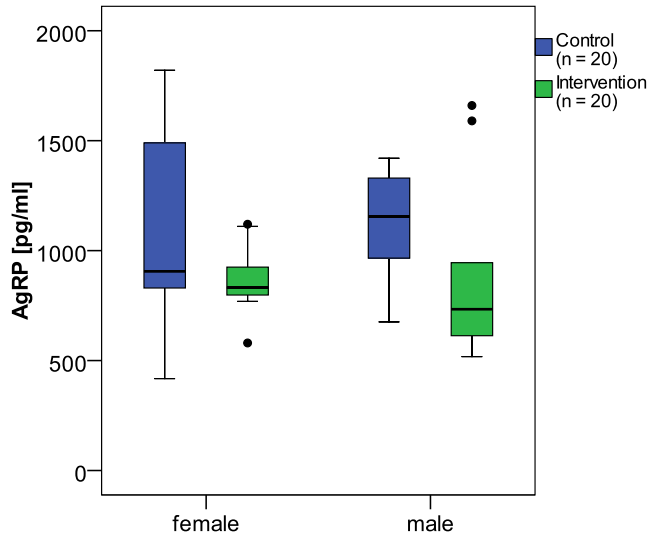

von Willebrand Factor [µg/ml]
60
50
40
30
20
10
0
female
male
Control
(n = 20)
Intervention
(n = 20)

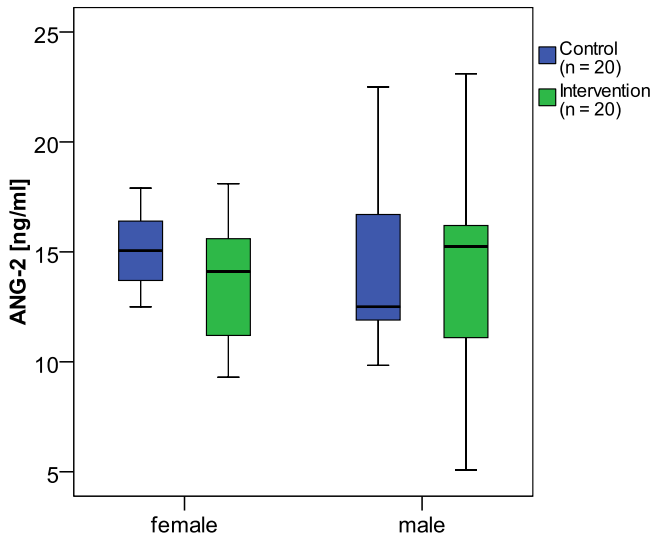
C. 5 Biomarkers from metabolic/hormonal MAP

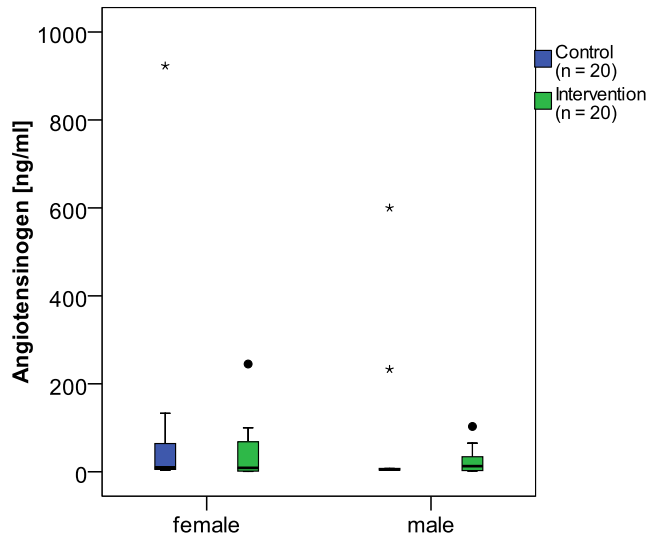


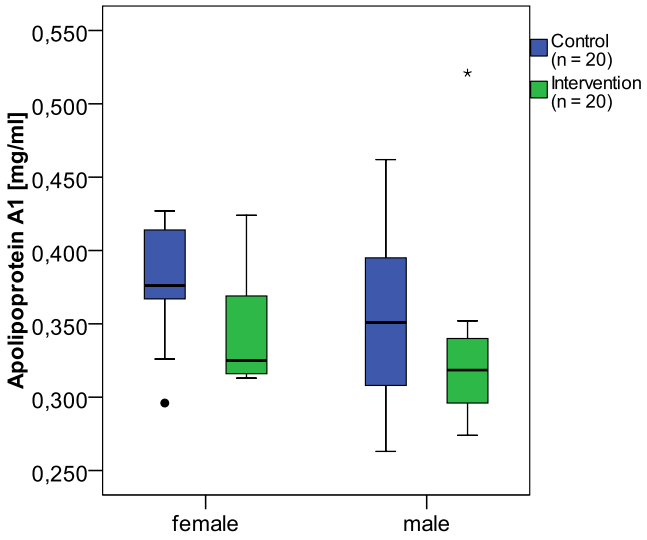


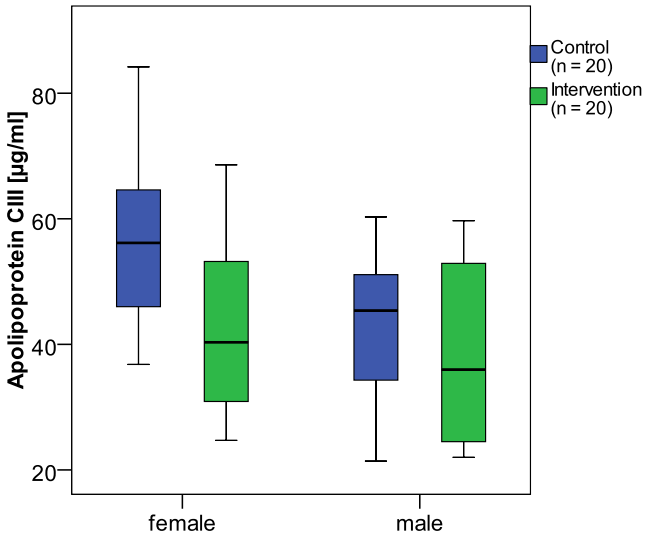


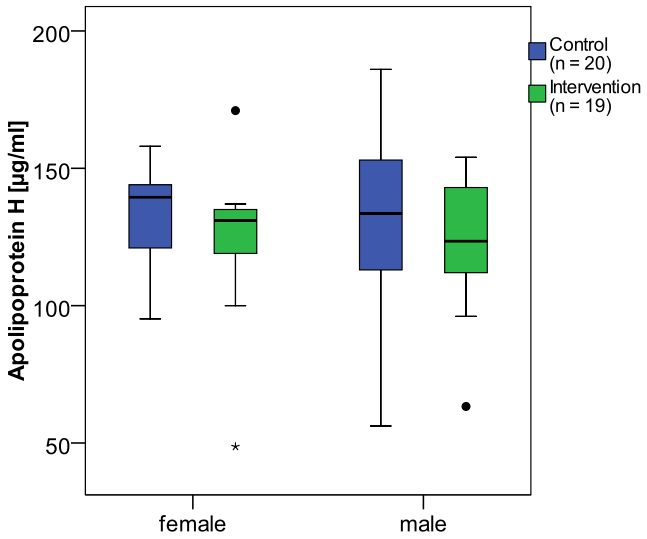


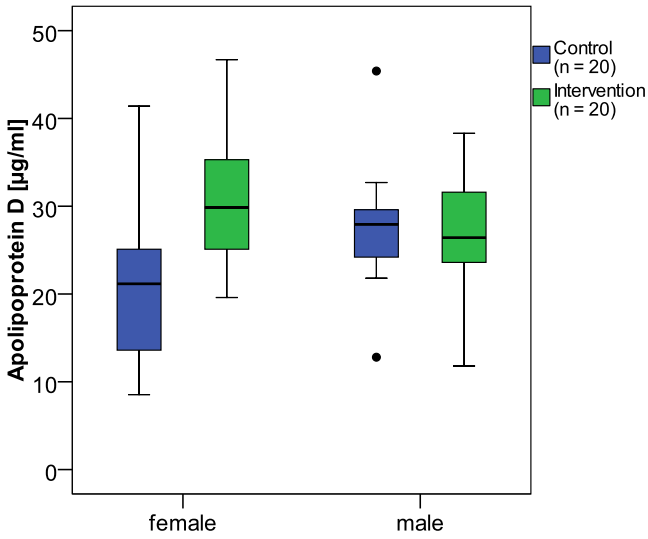


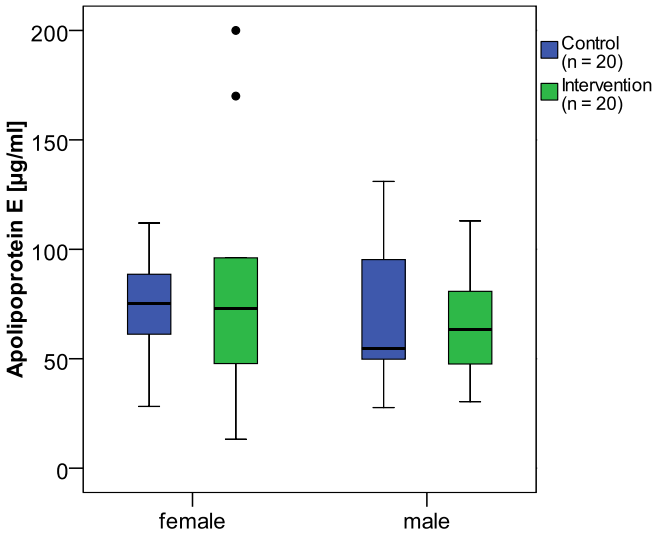


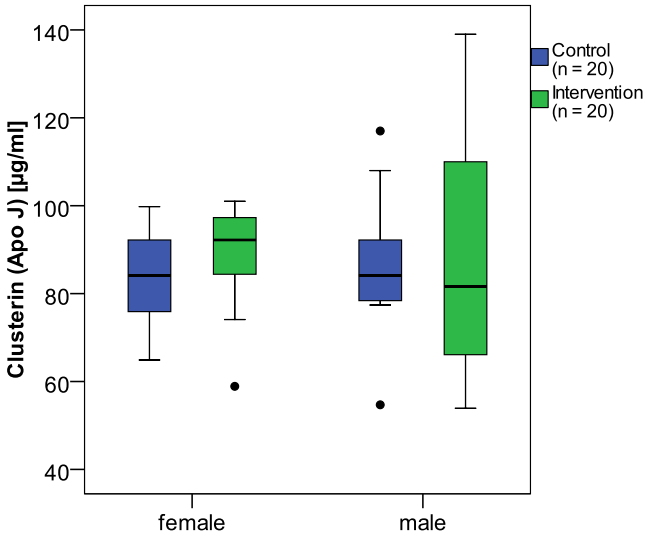

Apolipoprotein A1 [mg/ml]
0,550
0,500
0,450
0,400
0,350
0,300
0,250
female
male
Control (n = 20)
Intervention (n = 20)

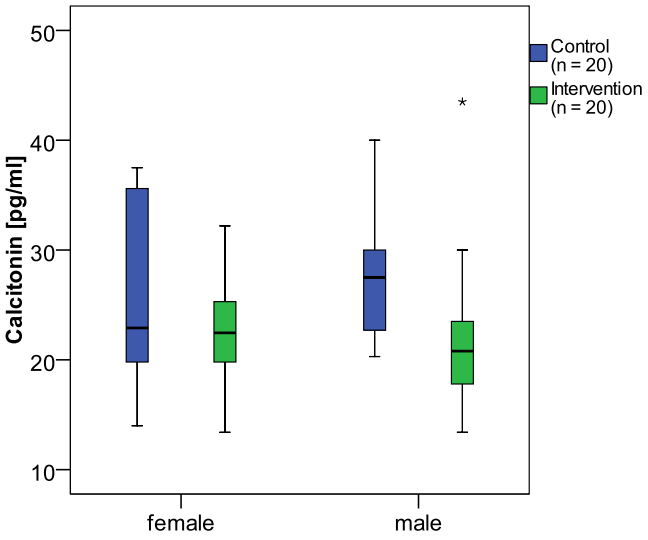

Apolipoprotein CIII [µg/ml]
80
60
40
20
female
male
Control (n = 20)
Intervention (n = 20)

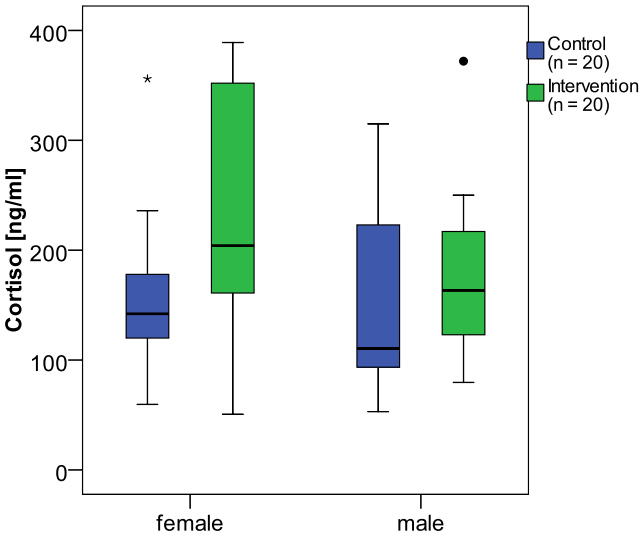

Apolipoprotein H [µg/ml]
200
150
100
50
female
male
Control (n = 20)
Intervention (n = 19)

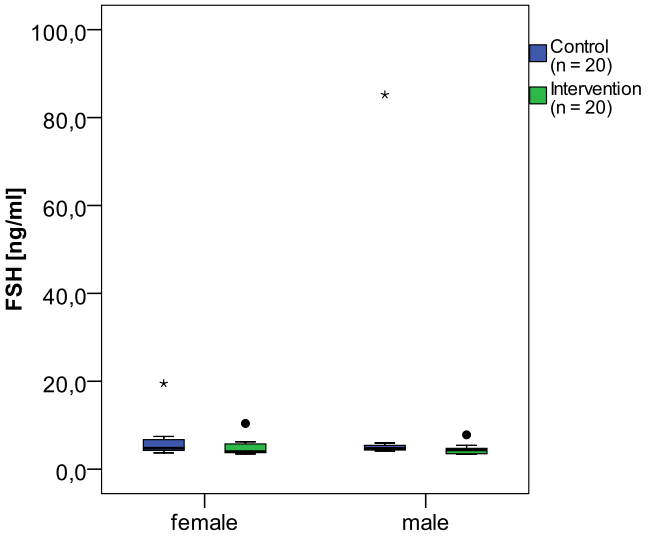

Apolipoprotein D [µg/ml]
50
40
30
20
10
0
female
male
Control (n = 20)
Intervention (n = 20)

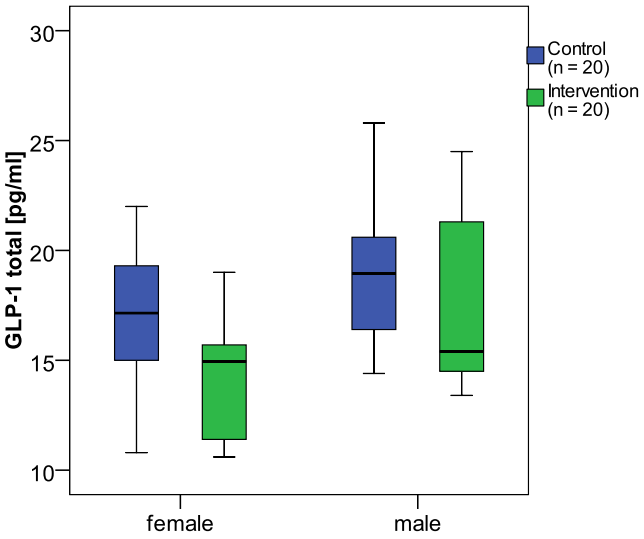

Apolipoprotein E [µg/ml]
200
150
100
50
0
female
male
Control (n = 20)
Intervention (n = 20)

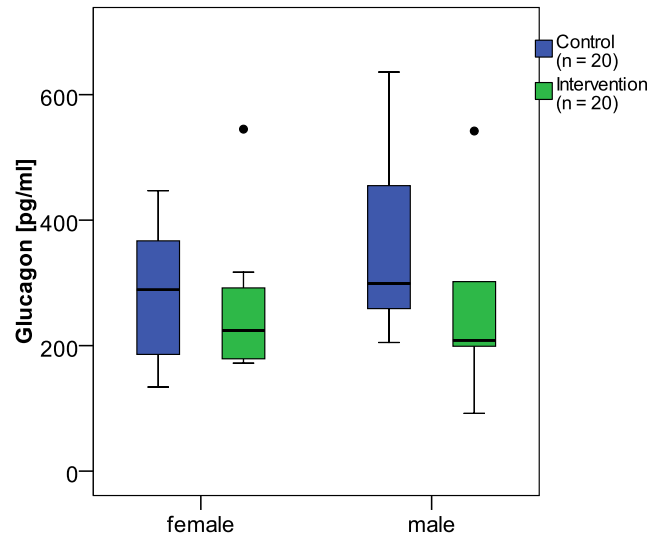

Clusterin (Apo J) [µg/ml]
140
120
100
80
60
40
female
male
Control (n = 20)
Intervention (n = 20)

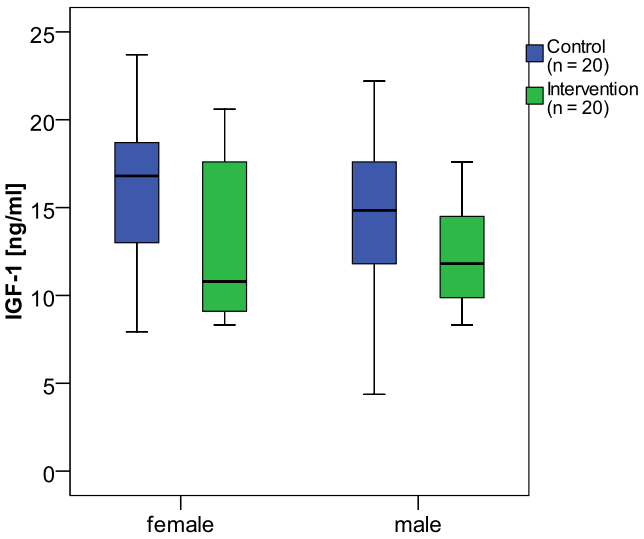

Calcitonin [pg/ml]
50
40
30
20
10
female
male
Control (n = 20)
Intervention (n = 20)

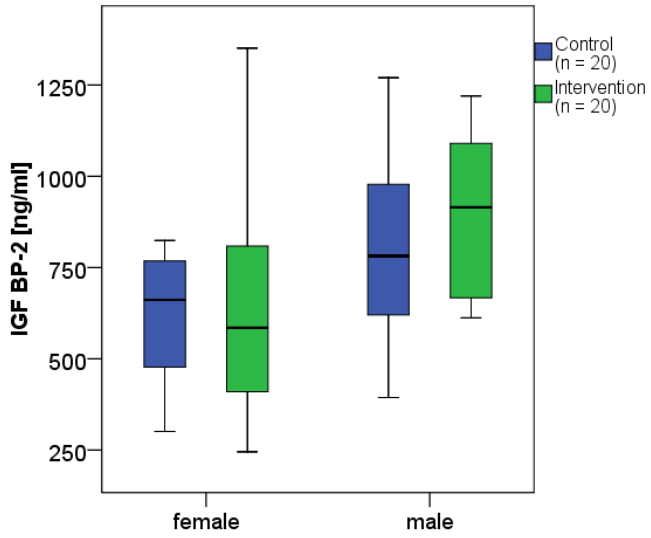

Cortisol [ng/ml]
400
300
200
100
0
female
male
Control (n = 20)
Intervention (n = 20)

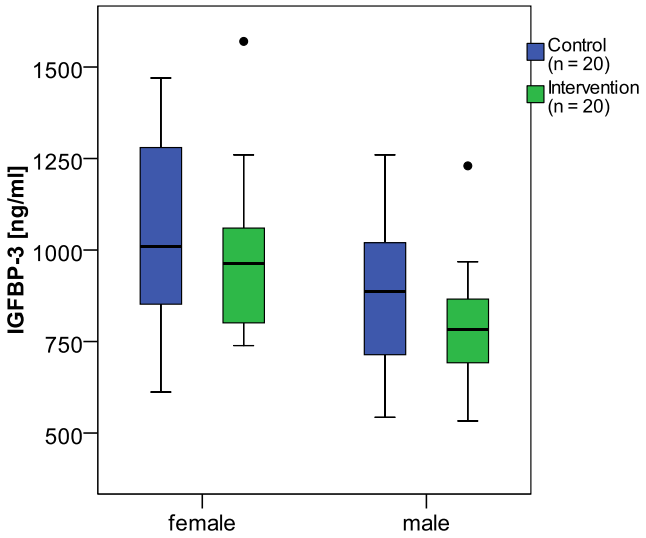

FSH [ng/ml]
100,0
80,0
60,0
40,0
20,0
0,0
female
male
Control (n = 20)
Intervention (n = 20)

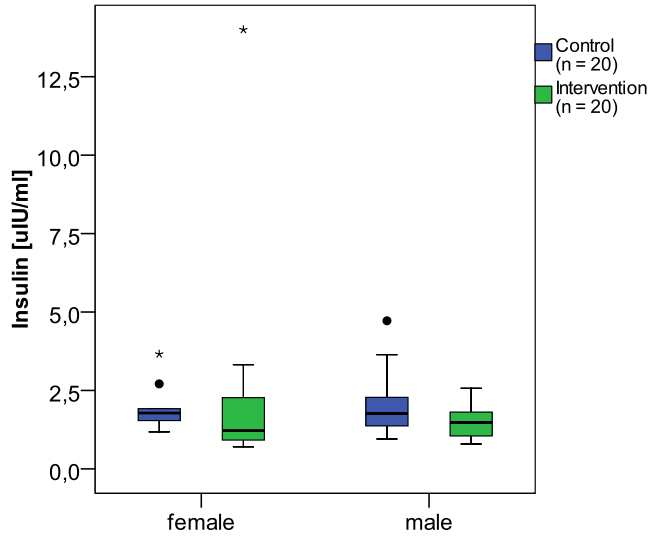

GLP-1 total [pg/ml]
30
25
20
15
10
female
male
Control (n = 20)
Intervention (n = 20)

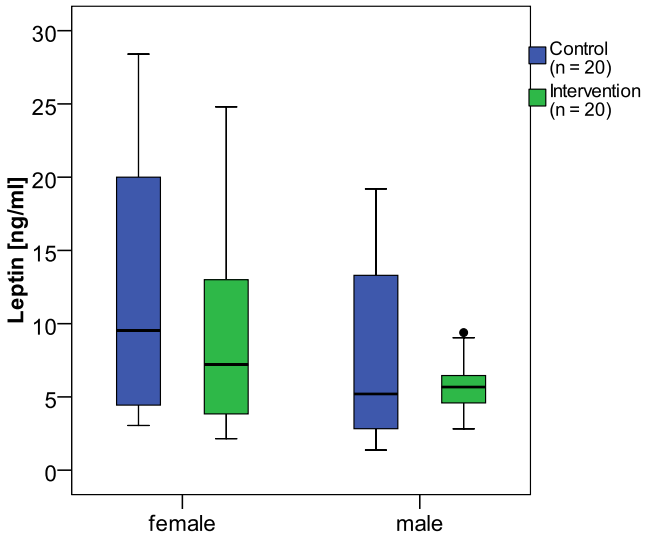

Glucagon [pg/ml]
600
400
200
0
female
male
Control (n = 20)
Intervention (n = 20)

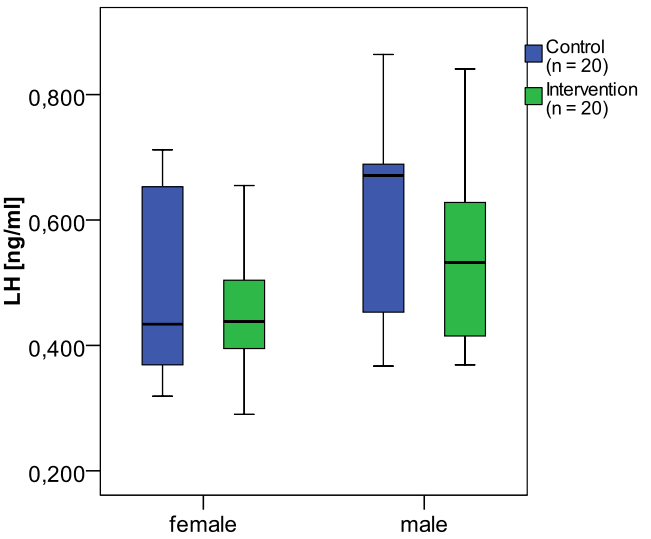

IGF-1 [ng/ml]
25
20
15
10
5
0
female
male
Control (n = 20)
Intervention (n = 20)

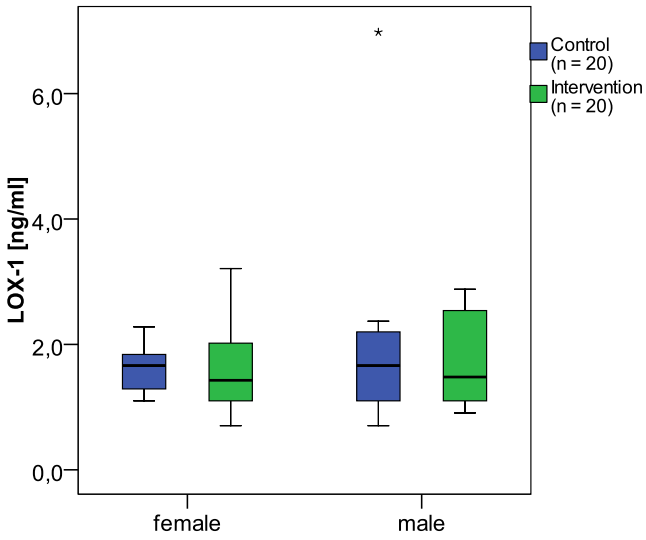

IGF BP-2 [ng/ml]
1250
1000
750
500
250
female
male
Control (n = 20)
Intervention (n = 20)

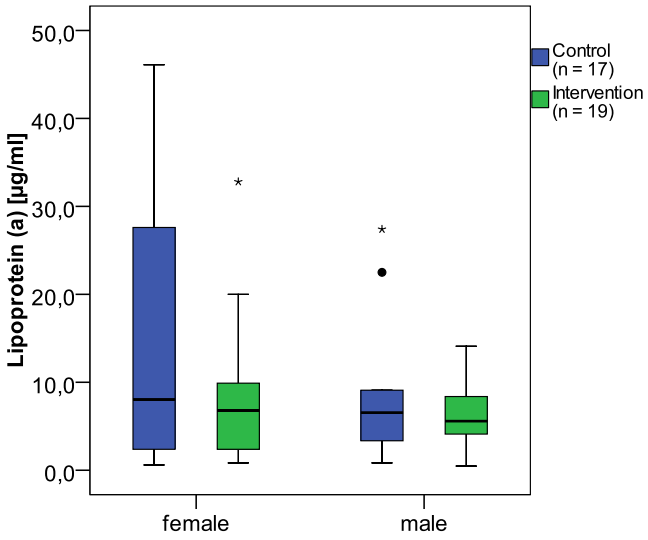

IGFBP-3 [ng/ml]
1500
1250
1000
750
500
female
male
Control (n = 20)
Intervention (n = 20)

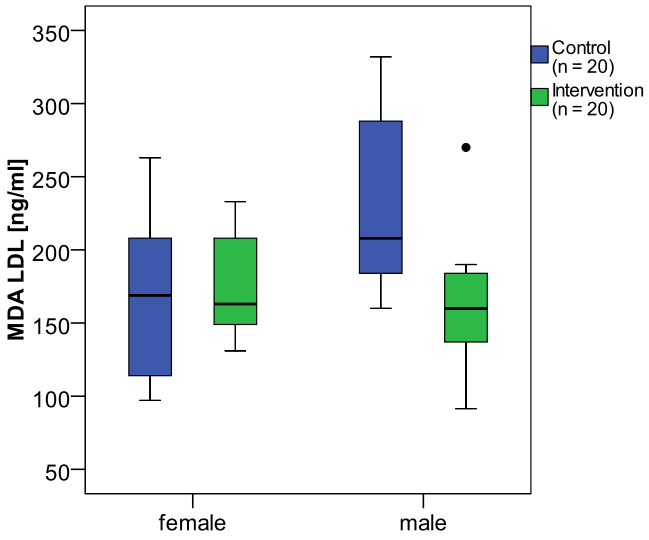

Insulin [uIU/ml]
12,5
10,0
7,5
5,0
2,5
0,0
female
male
Control (n = 20)
Intervention (n = 20)

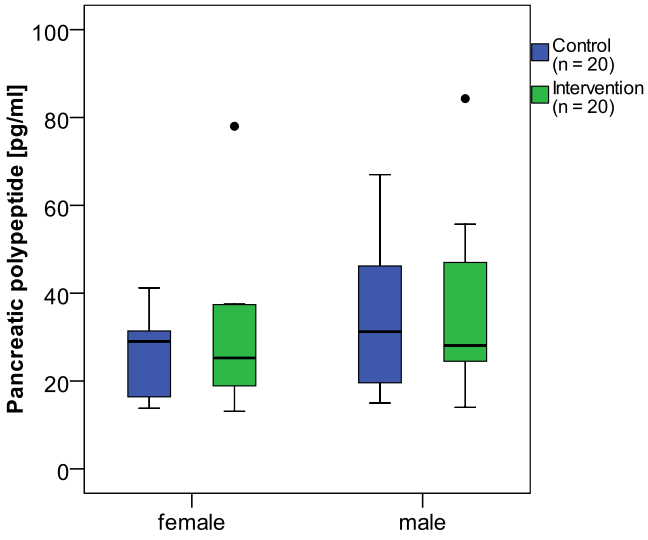

Leptin [ng/ml]
30
25
20
15
10
5
0
female
male
Control (n = 20)
Intervention (n = 20)

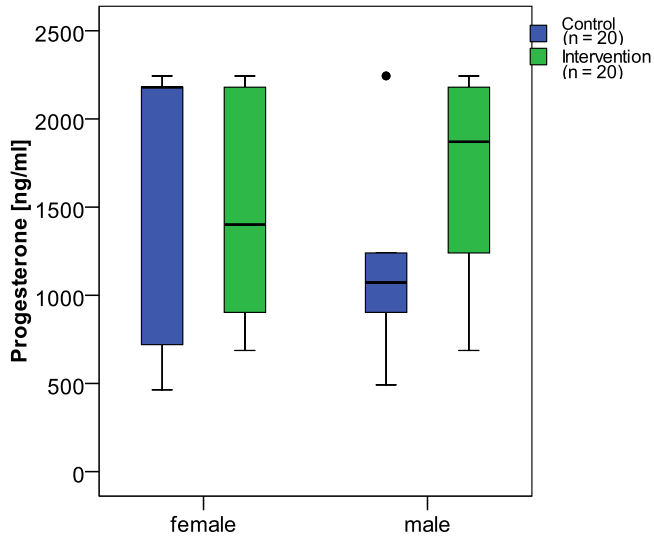

LH [ng/ml]
0,800
0,600
0,400
0,200
female
male
Control (n = 20)
Intervention (n = 20)

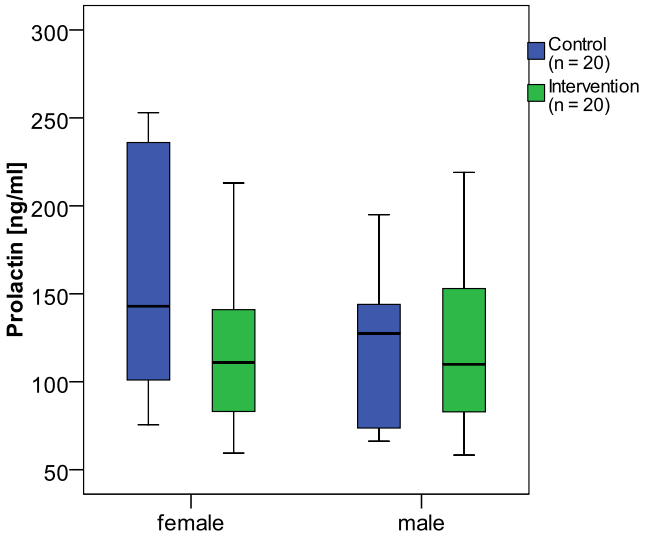

LOX-1 [ng/ml]
6,0
4,0
2,0
0,0
female
male
Control (n = 20)
Intervention (n = 20)

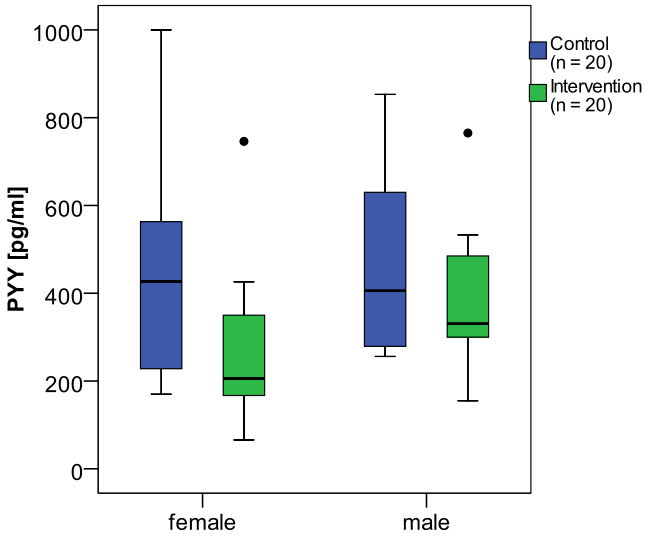

Lipoprotein (a) [µg/ml]
50,0
40,0
30,0
20,0
10,0
0,0
female
male
Control (n = 17)
Intervention (n = 19)

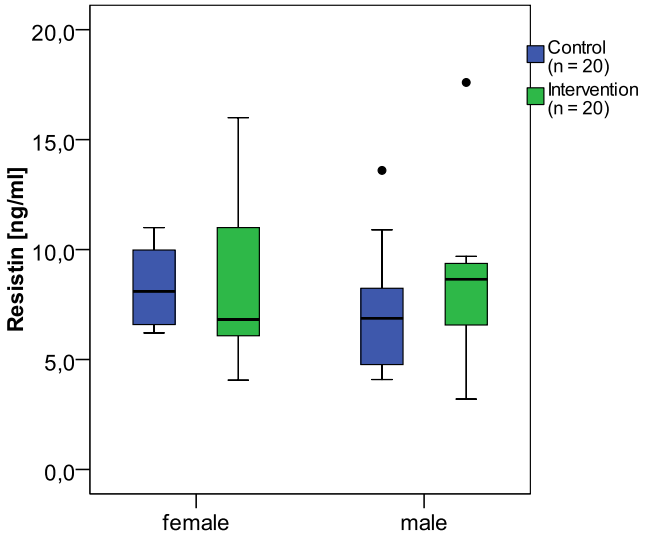

MDA LDL [ng/ml]
350
300
250
200
150
100
50
female
male
Control (n = 20)
Intervention (n = 20)

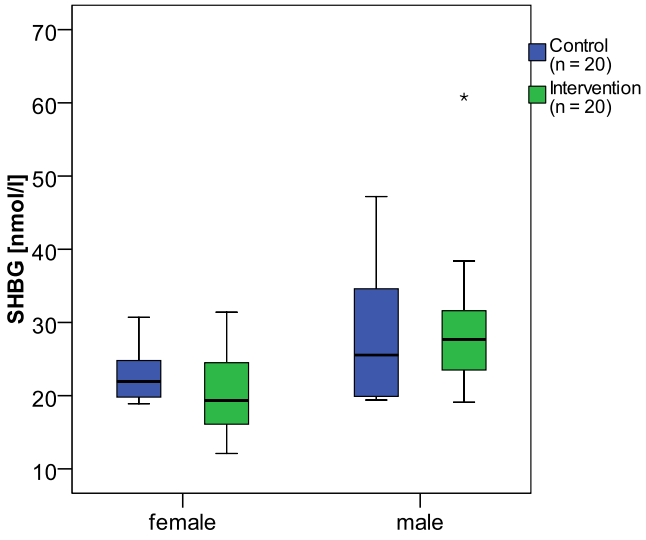

Pancreatic polypeptide [pg/ml]
100
80
60
40
20
0
female
male
Control (n = 20)
Intervention (n = 20)

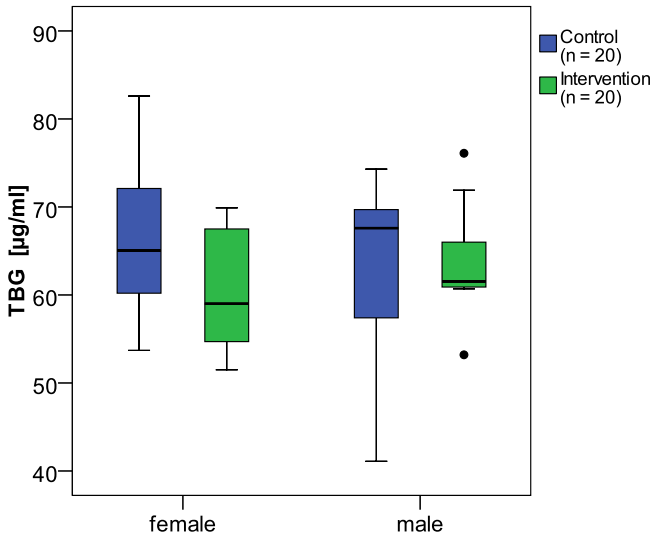

Progesterone [ng/ml]
2500
2000
1500
1000
500
0
female
male
Control (n = 20)
Intervention (n = 20)

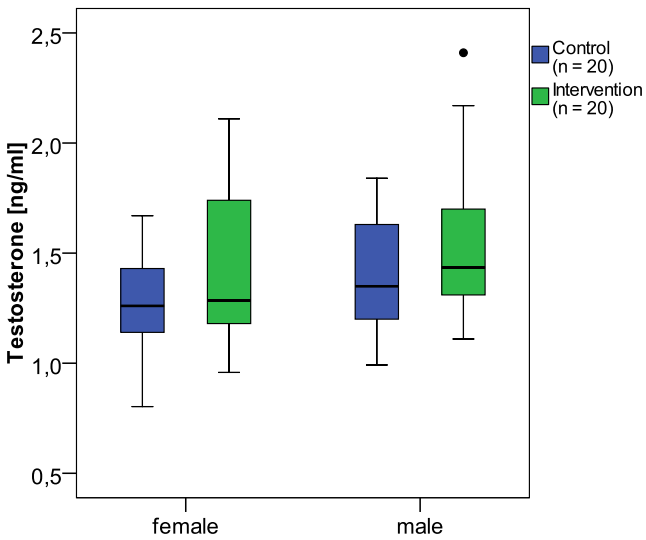

Prolactin [ng/ml]
300
250
200
150
100
50
female
male
Control (n = 20)
Intervention (n = 20)

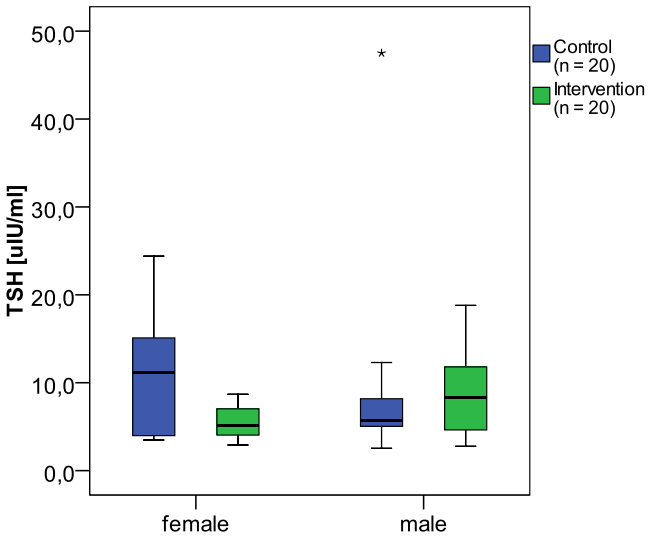

PYY [pg/ml]
1000
800
600
400
200
0
female
male
Control (n = 20)
Intervention (n = 20)


Resistin [ng/ml]
20,0
15,0
10,0
5,0
0,0
female
male
Control (n = 20)
Intervention (n = 20)


SHBG [nmol/l]
70
60
50
40
30
20
10
female
male
Control (n = 20)
Intervention (n = 20)


TBG [µg/ml]
90
80
70
60
50
40
female
male
Control (n = 20)
Intervention (n = 20)


Testosterone [ng/ml]
2,5
2,0
1,5
1,0
0,5
female
male
Control (n = 20)
Intervention (n = 20)


TSH [uIU/ml]
50,0
40,0
30,0
20,0
10,0
0,0
female
male
Control (n = 20)
Intervention (n = 20)

References

Abbey, M., P. Clifton, M. Kestin, B. Belling & P. Nestel (1990) Effect of fish oil on lipoproteins, lecithin:cholesterol acyltransferase, and lipid transfer protein activity in humans. *Arteriosclerosis,* 10**,** 85-94.

Abrams, B., S. L. Altman & K. E. Pickett (2000) Pregnancy weight gain: still controversial. *Am J Clin Nutr,* 71**,** 1233S-41S.

Agren, J. J., O. Hanninen, A. Julkunen, L. Fogelholm, H. Vidgren, U. Schwab, O. Pynnonen & M. Uusitupa (1996) Fish diet, fish oil and docosahexaenoic acid rich oil lower fasting and postprandial plasma lipid levels. *Eur J Clin Nutr,* 50**,** 765-71.

Ahima, R. S., J. Dushay, S. N. Flier, D. Prabakaran & J. S. Flier (1997) Leptin accelerates the onset of puberty in normal female mice. *J Clin Invest,* 99**,** 391-5.

Ahima, R. S. & S. Y. Osei (2004) Leptin signaling. *Physiol Behav,* 81**,** 223-41.

Ahima, R. S., C. B. Saper, J. S. Flier & J. K. Elmquist (2000) Leptin regulation of neuroendocrine systems. *Front Neuroendocrinol,* 21**,** 263-307.

Ailhaud, G., F. Massiera, P. Weill, P. Legrand, J. M. Alessandri & P. Guesnet (2006) Temporal changes in dietary fats: role of n-6 polyunsaturated fatty acids in excessive adipose tissue development and relationship to obesity. *Prog Lipid Res,* 45**,** 203-36.

Altman (1991) Practical Statistics for Medical Research. *Chapman & Hall, London.*

Anghel, S. I. & W. Wahli (2007) Fat poetry: a kingdom for PPAR gamma. *Cell Res,* 17**,** 486-511.

Araki, S., K. Dobashi, K. Kubo, K. Asayama & A. Shirahata (2006) High molecular weight, rather than total, adiponectin levels better reflect metabolic abnormalities associated with childhood obesity. *J Clin Endocrinol Metab,* 91**,** 5113-6.

Arita, Y., S. Kihara, N. Ouchi, M. Takahashi, K. Maeda, J. Miyagawa, K. Hotta, I. Shimomura, T. Nakamura, K. Miyaoka, H. Kuriyama, M. Nishida, S. Yamashita, K. Okubo, K. Matsubara, M. Muraguchi, Y. Ohmoto, T. Funahashi & Y. Matsuzawa (1999) Paradoxical decrease of an adipose-specific protein, adiponectin, in obesity. *Biochem Biophys Res Commun,* 257**,** 79-83.

Ashworth, C. J., N. Hoggard, L. Thomas, J. G. Mercer, J. M. Wallace & R. G. Lea (2000) Placental leptin. *Rev Reprod,* 5**,** 18-24.

Azain, M. J. (2004) Role of fatty acids in adipocyte growth and development. *J Anim Sci,* 82**,** 916-24.

Banga, A., R. Unal, P. Tripathi, I. Pokrovskaya, R. J. Owens, P. A. Kern & G. Ranganathan (2009) Adiponectin translation is increased by the PPARgamma agonists pioglitazone and omega-3 fatty acids. *Am J Physiol Endocrinol Metab,* 296**,** E480-9.

Banks, W. A. (2004) The many lives of leptin. *Peptides,* 25**,** 331-8.

Baratta, R., S. Amato, C. Degano, M. G. Farina, G. Patane, R. Vigneri & L. Frittitta (2004) Adiponectin relationship with lipid metabolism is independent of body fat mass: evidence from both cross-sectional and intervention studies. *J Clin Endocrinol Metab,* 89**,** 2665-71.

Barb, C. R. (1999) The brain-pituitary-adipocyte axis: role of leptin in modulating neuroendocrine function. *J Anim Sci,* 77**,** 1249-57.

Barbour, L. A., C. E. McCurdy, T. L. Hernandez, J. P. Kirwan, P. M. Catalano & J. E. Friedman (2007) Cellular mechanisms for insulin resistance in normal pregnancy and gestational diabetes. *Diabetes Care,* 30 Suppl 2**,** S112-9.

Barker, D. J. (1998) In utero programming of chronic disease. *Clin Sci (Lond),* 95**,** 115-28.

Baum, D., R. Q. Beck, L. D. Hammer, J. A. Brasel & M. R. Greenwood (1986) Adipose tissue thymidine kinase activity in man. *Pediatr Res,* 20**,** 118-21.

Baumann, H., K. K. Morella, D. W. White, M. Dembski, P. S. Bailon, H. Kim, C. F. Lai & L. A. Tartaglia (1996) The full-length leptin receptor has signaling capabilities of interleukin 6-type cytokine receptors. *Proc Natl Acad Sci U S A,* 93**,** 8374-8.

Beermann, C., M. Mobius, N. Winterling, J. J. Schmitt & G. Boehm (2005) sn-position determination of phospholipid-linked fatty acids derived from erythrocytes by liquid chromatography electrospray ionization ion-trap mass spectrometry. *Lipids,* 40**,** 211-8.

Belmonte, N., B. W. Phillips, F. Massiera, P. Villageois, B. Wdziekonski, P. Saint-Marc, J. Nichols, J. Aubert, K. Saeki, A. Yuo, S. Narumiya, G. Ailhaud & C. Dani (2001) Activation of extracellular signal-regulated kinases and CREB/ATF-1 mediate the expression of CCAAT/enhancer binding proteins beta and -delta in preadipocytes. *Mol Endocrinol,* 15**,** 2037-49.

Berg, A. H., T. P. Combs, X. Du, M. Brownlee & P. E. Scherer (2001) The adipocyte-secreted protein Acrp30 enhances hepatic insulin action. *Nat Med,* 7**,** 947-53.

Berger, H., J. Crane, D. Farine, A. Armson, S. De La Ronde, L. Keenan-Lindsay, L. Leduc, G. Reid & J. Van Aerde (2002) Screening for gestational diabetes mellitus. *J Obstet Gynaecol Can,* 24**,** 894-912.

Bernstein, E. L., P. Koutkia, K. Ljungquist, J. Breu, B. Canavan & S. Grinspoon (2004) Acute regulation of adiponectin by free fatty acids. *Metabolism,* 53**,** 790-3.

Bienertova-Vasku, J., P. Bienert, J. Tomandl, J. Lipkova, F. Zlamal, Z. Dostalova & A. Vasku (2010) Comparison of agouti-related peptide levels in peripheral blood of postpartum pre-eclamptic and non pre-eclamptic women and in umbilical cord blood from their pregnancies. *Diabetes Res Clin Pract,* 89**,** e53-5.

Billington, C. J. & A. S. Levine (1992) Hypothalamic neuropeptide Y regulation of feeding and energy metabolism. *Curr Opin Neurobiol,* 2**,** 847-51.

Bjorbaek, C., K. El-Haschimi, J. D. Frantz & J. S. Flier (1999) The role of SOCS-3 in leptin signaling and leptin resistance. *J Biol Chem,* 274**,** 30059-65.

Bjorbaek, C. & B. B. Kahn (2004) Leptin signaling in the central nervous system and the periphery. *Recent Prog Horm Res,* 59**,** 305-31.

Bjorbaek, C., S. Uotani, B. da Silva & J. S. Flier (1997) Divergent signaling capacities of the long and short isoforms of the leptin receptor. *J Biol Chem,* 272**,** 32686-95.

Bligh, E. G. & W. J. Dyer (1959) A rapid method of total lipid extraction and purification. *Can J Biochem Physiol,* 37**,** 911-7.

Bobbert, T., H. Rochlitz, U. Wegewitz, S. Akpulat, K. Mai, M. O. Weickert, M. Mohlig, A. F. Pfeiffer & J. Spranger (2005) Changes of adiponectin oligomer composition by moderate weight reduction. *Diabetes,* 54**,** 2712-9.

Botolin, D., Y. Wang, B. Christian & D. B. Jump (2006) Docosahexaneoic acid (22:6,n-3) regulates rat hepatocyte SREBP-1 nuclear abundance by Erk- and 26S proteasome-dependent pathways. *J Lipid Res,* 47**,** 181-92.

Bottner, A., J. Kratzsch, G. Muller, T. M. Kapellen, S. Bluher, E. Keller, M. Bluher & W. Kiess (2004) Gender differences of adiponectin levels develop during the progression of puberty and are related to serum androgen levels. *J Clin Endocrinol Metab,* 89**,** 4053-61.

Bouret, S. G. & R. B. Simerly (2006) Developmental programming of hypothalamic feeding circuits. *Clin Genet,* 70**,** 295-301.

Brenna, J. T. & A. Lapillonne (2009) Background paper on fat and fatty acid requirements during pregnancy and lactation. *Ann Nutr Metab,* 55**,** 97-122.

Bronsky, J., M. Karpisek, E. Bronska, M. Pechova, B. Jancikova, H. Kotolova, D. Stejskal, R. Prusa & J. Nevoral (2006) Adiponectin, adipocyte fatty acid binding protein, and epidermal fatty acid binding protein: proteins newly identified in human breast milk. *Clin Chem,* 52**,** 1763-70.

Buechler, C., J. Wanninger & M. Neumeier (2010) Adiponectin receptor binding proteins--recent advances in elucidating adiponectin signalling pathways. *FEBS Lett,* 584**,** 4280-6.

Burdi, A. R., C. M. Poissonnet, S. M. Garn, M. Lavelle, M. D. Sabet & P. Bridges (1985) Adipose tissue growth patterns during human gestation: a histometric comparison of buccal and gluteal fat depots. *Int J Obes,* 9**,** 247-56.

Butte, N. F. (2000) Carbohydrate and lipid metabolism in pregnancy: normal compared with gestational diabetes mellitus. *Am J Clin Nutr,* 71**,** 1256S-61S.

Butte, N. F., J. M. Hopkinson & M. A. Nicolson (1997) Leptin in human reproduction: serum leptin levels in pregnant and lactating women. *J Clin Endocrinol Metab,* 82**,** 585-9.

Butte, N. F., W. W. Wong, M. S. Treuth, K. J. Ellis & E. O'Brian Smith (2004) Energy requirements during pregnancy based on total energy expenditure and energy deposition. *Am J Clin Nutr,* 79**,** 1078-87.

Calder, P. C. (2006) n-3 polyunsaturated fatty acids, inflammation, and inflammatory diseases. *Am J Clin Nutr,* 83**,** 1505S-1519S.

Calloway, D. H. (1974) Nitrogen balance during pregnancy. *Curr Concepts Nutr,* 2**,** 79-94.

Caminos, J. E., R. Nogueiras, R. Gallego, S. Bravo, S. Tovar, T. Garcia-Caballero, F. F. Casanueva & C. Dieguez (2005) Expression and regulation of adiponectin and receptor in human and rat placenta. *J Clin Endocrinol Metab,* 90**,** 4276-86.

Campfield, L. A., F. J. Smith, Y. Guisez, R. Devos & P. Burn (1995) Recombinant mouse OB protein: evidence for a peripheral signal linking adiposity and central neural networks. *Science,* 269**,** 546-9.

Cannon, B. & J. Nedergaard (2004) Brown adipose tissue: function and physiological significance. *Physiol Rev,* 84**,** 277-359.

Casabiell, X., V. Pineiro, M. A. Tome, R. Peino, C. Dieguez & F. F. Casanueva (1997) Presence of leptin in colostrum and/or breast milk from lactating mothers: a potential role in the regulation of neonatal food intake. *J Clin Endocrinol Metab,* 82**,** 4270-3.

Catalano, P. M., M. Hoegh, J. Minium, L. Huston-Presley, S. Bernard, S. Kalhan & S. Hauguel-De Mouzon (2006) Adiponectin in human pregnancy: implications for regulation of glucose and lipid metabolism. *Diabetologia,* 49**,** 1677-85.

Catalano, P. M., L. Huston, S. B. Amini & S. C. Kalhan (1999) Longitudinal changes in glucose metabolism during pregnancy in obese women with normal glucose tolerance and gestational diabetes mellitus. *Am J Obstet Gynecol,* 180**,** 903-16.

Catalano, P. M., A. Thomas, L. Huston-Presley & S. B. Amini (2003) Increased fetal adiposity: a very sensitive marker of abnormal in utero development. *Am J Obstet Gynecol,* 189**,** 1698-704.

Catalano, P. M., E. D. Tyzbir, R. R. Wolfe, N. M. Roman, S. B. Amini & E. A. Sims (1992) Longitudinal changes in basal hepatic glucose production and suppression during insulin infusion in normal pregnant women. *Am J Obstet Gynecol,* 167**,** 913-9.

Ceddia, R. B., R. Somwar, A. Maida, X. Fang, G. Bikopoulos & G. Sweeney (2005) Globular adiponectin increases GLUT4 translocation and glucose uptake but reduces glycogen synthesis in rat skeletal muscle cells. *Diabetologia,* 48**,** 132-9.

Cedergren, M. (2006) Effects of gestational weight gain and body mass index on obstetric outcome in Sweden. *Int J Gynaecol Obstet,* 93**,** 269-74.

Cetin, I. & B. Koletzko (2008) Long-chain omega-3 fatty acid supply in pregnancy and lactation. *Curr Opin Clin Nutr Metab Care,* 11**,** 297-302.

Cha, M. C. & P. J. Jones (1998) Dietary fat type and energy restriction interactively influence plasma leptin concentration in rats. *J Lipid Res,* 39**,** 1655-60.

Challier, J., M. Galtier, T. Bintein, A. Cortez, J. Lepercq & S. Hauguel-de Mouzon (2003) Placental leptin receptor isoforms in normal and pathological pregnancies. *Placenta,* 24**,** 92-9.

Chan, J. L., S. Bluher, N. Yiannakouris, M. A. Suchard, J. Kratzsch & C. S. Mantzoros (2002) Regulation of circulating soluble leptin receptor levels by gender, adiposity, sex steroids, and leptin: observational and interventional studies in humans. *Diabetes,* 51**,** 2105-12.

Chan, T. F., S. S. Yuan, H. S. Chen, C. F. Guu, L. C. Wu, Y. T. Yeh, Y. F. Chung, S. B. Jong & J. H. Su (2004) Correlations between umbilical and maternal serum adiponectin levels and neonatal birthweights. *Acta Obstet Gynecol Scand,* 83**,** 165-9.

Chen, J., B. Tan, E. Karteris, S. Zervou, J. Digby, E. W. Hillhouse, M. Vatish & H. S. Randeva (2006) Secretion of adiponectin by human placenta: differential modulation of adiponectin and its receptors by cytokines. *Diabetologia,* 49**,** 1292-302.

Cheng, A., N. Uetani, P. D. Simoncic, V. P. Chaubey, A. Lee-Loy, C. J. McGlade, B. P. Kennedy & M. L. Tremblay (2002) Attenuation of leptin action and regulation of obesity by protein tyrosine phosphatase 1B. *Dev Cell,* 2**,** 497-503.

Chiesa, C., J. F. Osborn, C. Haass, F. Natale, M. Spinelli, E. Scapillati, A. Spinelli & L. Pacifico (2008) Ghrelin, leptin, IGF-1, IGFBP-3, and insulin concentrations at birth: is there a relationship with fetal growth and neonatal anthropometry? *Clin Chem,* 54**,** 550-8.

Chung, W. K., L. Power-Kehoe, M. Chua, F. Chu, L. Aronne, Z. Huma, M. Sothern, J. N. Udall, B. Kahle & R. L. Leibel (1997) Exonic and intronic sequence variation in the human leptin receptor gene (LEPR). *Diabetes,* 46**,** 1509-11.

Clement, K., C. Vaisse, N. Lahlou, S. Cabrol, V. Pelloux, D. Cassuto, M. Gourmelen, C. Dina, J. Chambaz, J. M. Lacorte, A. Basdevant, P. Bougneres, Y. Lebouc, P. Froguel & B. Guy-Grand (1998) A mutation in the human leptin receptor gene causes obesity and pituitary dysfunction. *Nature,* 392**,** 398-401.

Cnattingius, S., R. Bergstrom, L. Lipworth & M. S. Kramer (1998) Prepregnancy weight and the risk of adverse pregnancy outcomes. *N Engl J Med,* 338**,** 147-52.

Cnop, M., P. J. Havel, K. M. Utzschneider, D. B. Carr, M. K. Sinha, E. J. Boyko, B. M. Retzlaff, R. H. Knopp, J. D. Brunzell & S. E. Kahn (2003) Relationship of adiponectin to body fat distribution, insulin sensitivity and plasma lipoproteins: evidence for independent roles of age and sex. *Diabetologia,* 46**,** 459-69.

Combs, T. P., A. H. Berg, M. W. Rajala, S. Klebanov, P. Iyengar, J. C. Jimenez-Chillaron, M. E. Patti, S. L. Klein, R. S. Weinstein & P. E. Scherer (2003) Sexual differentiation, pregnancy, calorie restriction, and aging affect the adipocyte-specific secretory protein adiponectin. *Diabetes,* 52**,** 268-76.

Considine, R. V., E. L. Considine, C. J. Williams, T. M. Hyde & J. F. Caro (1996a) The hypothalamic leptin receptor in humans: identification of incidental sequence polymorphisms and absence of the db/db mouse and fa/fa rat mutations. *Diabetes,* 45**,** 992-4.

Considine, R. V., M. K. Sinha, M. L. Heiman, A. Kriauciunas, T. W. Stephens, M. R. Nyce, J. P. Ohannesian, C. C. Marco, L. J. McKee, T. L. Bauer & et al. (1996b) Serum immunoreactive-leptin concentrations in normal-weight and obese humans. *N Engl J Med,* 334**,** 292-5.

Corbetta, S., G. Bulfamante, D. Cortelazzi, V. Barresi, I. Cetin, G. Mantovani, S. Bondioni, P. Beck-Peccoz & A. Spada (2005) Adiponectin expression in human fetal tissues during mid- and late gestation. *J Clin Endocrinol Metab,* 90**,** 2397-402.

Cortelazzi, D., S. Corbetta, S. Ronzoni, F. Pelle, A. Marconi, V. Cozzi, I. Cetin, R. Cortelazzi, P. Beck-Peccoz & A. Spada (2007) Maternal and foetal resistin and adiponectin concentrations in normal and complicated pregnancies. *Clin Endocrinol (Oxf),* 66**,** 447-53.

Coupe, B., I. Dutriez-Casteloot, C. Breton, F. Lefevre, J. Mairesse, A. Dickes-Coopman, M. Silhol, L. Tapia-Arancibia, J. Lesage & D. Vieau (2009) Perinatal undernutrition modifies cell proliferation and brain-derived neurotrophic factor levels during critical time-windows for hypothalamic and hippocampal development in the male rat. *J Neuroendocrinol,* 21**,** 40-8.

Cowley, M. A., J. L. Smart, M. Rubinstein, M. G. Cerdan, S. Diano, T. L. Horvath, R. D. Cone & M. J. Low (2001) Leptin activates anorexigenic POMC neurons through a neural network in the arcuate nucleus. *Nature,* 411**,** 480-4.

Cseh, K., E. Baranyi, Z. Melczer, E. Kaszas, E. Palik & G. Winkler (2004) Plasma adiponectin and pregnancy-induced insulin resistance. *Diabetes Care,* 27**,** 274-5.

Darmady, J. M. & A. D. Postle (1982) Lipid metabolism in pregnancy. *Br J Obstet Gynaecol,* 89**,** 211-5.

Deckelbaum, R. J., T. S. Worgall & T. Seo (2006) n-3 fatty acids and gene expression. *Am J Clin Nutr,* 83**,** 1520S-1525S.

Degawa-Yamauchi, M., K. A. Moss, J. E. Bovenkerk, S. S. Shankar, C. L. Morrison, C. J. Lelliott, A. Vidal-Puig, R. Jones & R. V. Considine (2005) Regulation of adiponectin expression in human adipocytes: effects of adiposity, glucocorticoids, and tumor necrosis factor alpha. *Obes Res,* 13**,** 662-9.

Denne, S. C., E. M. Rossi & S. C. Kalhan (1991) Leucine kinetics during feeding in normal newborns. *Pediatr Res,* 30**,** 23-7.

Dessolin, S., M. Schalling, O. Champigny, F. Lonnqvist, G. Ailhaud, C. Dani & D. Ricquier (1997) Leptin gene is expressed in rat brown adipose tissue at birth. *FASEB J,* 11**,** 382-7.

Deurenberg, P., J. J. Pieters & J. G. Hautvast (1990) The assessment of the body fat percentage by skinfold thickness measurements in childhood and young adolescence. *Br J Nutr,* 63**,** 293-303.

DeVader, S. R., H. L. Neeley, T. D. Myles & T. L. Leet (2007) Evaluation of gestational weight gain guidelines for women with normal prepregnancy body mass index. *Obstet Gynecol,* 110**,** 745-51.

Devaskar, S. U., C. Ollesch, R. A. Rajakumar & P. A. Rajakumar (1997) Developmental changes in ob gene expression and circulating leptin peptide concentrations. *Biochem Biophys Res Commun,* 238**,** 44-7.

DGE, D. G. f. E. (2004) Ernährungsbericht.

Donato, J., Jr., R. M. Cravo, R. Frazao & C. F. Elias (2011) Hypothalamic sites of leptin action linking metabolism and reproduction. *Neuroendocrinology,* 93**,** 9-18.

Dörner (1975) Perinatal hormone levels and brain organization. In. *Anatomical Neuroendocrinology***,** 245–252 [WE Stumpf and LD Grant, editors]. Basel: Karger.

Echwald, S. M., T. D. Sorensen, T. I. Sorensen, A. Tybjaerg-Hansen, T. Andersen, W. K. Chung, R. L. Leibel & O. Pedersen (1997) Amino acid variants in the human leptin receptor: lack of association to juvenile onset obesity. *Biochem Biophys Res Commun,* 233**,** 248-52.

Emken, E. A., R. O. Adlof & R. M. Gulley (1994) Dietary linoleic acid influences desaturation and acylation of deuterium-labeled linoleic and linolenic acids in young adult males. *Biochim Biophys Acta,* 1213**,** 277-88.

Fan, W., B. A. Boston, R. A. Kesterson, V. J. Hruby & R. D. Cone (1997) Role of melanocortinergic neurons in feeding and the agouti obesity syndrome. *Nature,* 385**,** 165-8.

Fang, X., R. Palanivel, X. Zhou, Y. Liu, A. Xu, Y. Wang & G. Sweeney (2005) Hyperglycemia- and hyperinsulinemia-induced alteration of adiponectin receptor expression and adiponectin effects in L6 myoblasts. *J Mol Endocrinol,* 35**,** 465-76.

Farrag, H. M., L. M. Nawrath, J. E. Healey, E. J. Dorcus, R. E. Rapoza, W. Oh & R. M. Cowett (1997) Persistent glucose production and greater peripheral sensitivity to insulin in the neonate vs. the adult. *Am J Physiol,* 272**,** E86-93.

Fasshauer, M. & R. Paschke (2003) Regulation of adipocytokines and insulin resistance. *Diabetologia,* 46**,** 1594-603.

Fattah, C., S. Barry, N. O'Connor, N. Farah, B. Stuart & M. J. Turner (2010) Maternal leptin and body composition in the first trimester of pregnancy. *Gynecol Endocrinol*.

Fedor, D. & D. S. Kelley (2009) Prevention of insulin resistance by n-3 polyunsaturated fatty acids. *Curr Opin Clin Nutr Metab Care,* 12**,** 138-46.

Fernandez-Riejos, P., S. Najib, J. Santos-Alvarez, C. Martin-Romero, A. Perez-Perez, C. Gonzalez-Yanes & V. Sanchez-Margalet (2010) Role of leptin in the activation of immune cells. *Mediators Inflamm,* 2010**,** 568343.

Fernstrom, J. D. (2000) Can nutrient supplements modify brain function? *Am J Clin Nutr,* 71**,** 1669S-75S.

Fisher, F. F., M. E. Trujillo, W. Hanif, A. H. Barnett, P. G. McTernan, P. E. Scherer & S. Kumar (2005) Serum high molecular weight complex of adiponectin correlates better with glucose tolerance than total serum adiponectin in Indo-Asian males. *Diabetologia,* 48**,** 1084-7.

Flachs, P., V. Mohamed-Ali, O. Horakova, M. Rossmeisl, M. J. Hosseinzadeh-Attar, M. Hensler, J. Ruzickova & J. Kopecky (2006) Polyunsaturated fatty acids of marine origin induce adiponectin in mice fed a high-fat diet. *Diabetologia,* 49**,** 394-7.

Flachs, P., M. Rossmeisl, M. Bryhn & J. Kopecky (2009) Cellular and molecular effects of n-3 polyunsaturated fatty acids on adipose tissue biology and metabolism. *Clin Sci (Lond),* 116**,** 1-16.

Frederich, R. C., B. Lollmann, A. Hamann, A. Napolitano-Rosen, B. B. Kahn, B. B. Lowell & J. S. Flier (1995) Expression of ob mRNA and its encoded protein in rodents. Impact of nutrition and obesity. *J Clin Invest,* 96**,** 1658-63.

Friedberg, C. E., M. J. Janssen, R. J. Heine & D. E. Grobbee (1998) Fish oil and glycemic control in diabetes. A meta-analysis. *Diabetes Care,* 21**,** 494-500.

Fruebis, J., T. S. Tsao, S. Javorschi, D. Ebbets-Reed, M. R. Erickson, F. T. Yen, B. E. Bihain & H. F. Lodish (2001) Proteolytic cleavage product of 30-kDa adipocyte complement-related protein increases fatty acid oxidation in muscle and causes weight loss in mice. *Proc Natl Acad Sci U S A,* 98**,** 2005-10.

Frühbeck, G. (2006) Intracellular signalling pathways activated by leptin. *Biochem J,* 393**,** 7-20.

Fuglsang, J., C. Skjaerbaek, J. Frystyk, A. Flyvbjerg & P. Ovesen (2006) A longitudinal study of serum adiponectin during normal pregnancy. *BJOG,* 113**,** 110-3.

Fujimura, H., C. A. Altar, R. Chen, T. Nakamura, T. Nakahashi, J. Kambayashi, B. Sun & N. N. Tandon (2002) Brain-derived neurotrophic factor is stored in human platelets and released by agonist stimulation. *Thromb Haemost,* 87**,** 728-34.

Galgani, J. E., R. D. Uauy, C. A. Aguirre & E. O. Diaz (2008) Effect of the dietary fat quality on insulin sensitivity. *Br J Nutr,* 100**,** 471-9.

Gavrilova, O., V. Barr, B. Marcus-Samuels & M. Reitman (1997) Hyperleptinemia of pregnancy associated with the appearance of a circulating form of the leptin receptor. *J Biol Chem,* 272**,** 30546-51.

Geary, M., R. Herschkovitz, P. J. Pringle, C. H. Rodeck & P. C. Hindmarsh (1999) Ontogeny of serum leptin concentrations in the human. *Clin Endocrinol (Oxf),* 51**,** 189-92.

Geppert, J., V. Kraft, H. Demmelmair & B. Koletzko (2006) Microalgal docosahexaenoic acid decreases plasma triacylglycerol in normolipidaemic vegetarians: a randomised trial. *Br J Nutr,* 95**,** 779-86.

Gerster, H. (1998) Can adults adequately convert alpha-linolenic acid (18:3n-3) to eicosapentaenoic acid (20:5n-3) and docosahexaenoic acid (22:6n-3)? *Int J Vitam Nutr Res,* 68**,** 159-73.

Gil-Campos, M., R. R. Canete & A. Gil (2004) Adiponectin, the missing link in insulin resistance and obesity. *Clin Nutr,* 23**,** 963-74.

Gillman, M. W. & C. S. Mantzoros (2007) Breast-feeding, adipokines, and childhood obesity. *Epidemiology,* 18**,** 730-2.

Godfrey, K. M., C. N. Hales, C. Osmond, D. J. Barker & K. P. Taylor (1996) Relation of cord plasma concentrations of proinsulin, 32-33 split proinsulin, insulin and C-peptide to placental weight and the baby's size and proportions at birth. *Early Hum Dev,* 46**,** 129-40.

Gorska, E., K. Popko, A. Stelmaszczyk-Emmel, O. Ciepiela, A. Kucharska & M. Wasik (2010) Leptin receptors. *Eur J Med Res,* 15 Suppl 2**,** 50-4.

Hales, C. N. & D. J. Barker (1992) Type 2 (non-insulin-dependent) diabetes mellitus: the thrifty phenotype hypothesis. *Diabetologia,* 35**,** 595-601.

Hansen, D., P. Dendale, M. Beelen, R. A. Jonkers, A. Mullens, L. Corluy, R. Meeusen & L. J. van Loon (2010) Plasma adipokine and inflammatory marker concentrations are altered in obese, as opposed to non-obese, type 2 diabetes patients. *Eur J Appl Physiol,* 109**,** 397-404.

Hara, K., P. Boutin, Y. Mori, K. Tobe, C. Dina, K. Yasuda, T. Yamauchi, S. Otabe, T. Okada, K. Eto, H. Kadowaki, R. Hagura, Y. Akanuma, Y. Yazaki, R. Nagai, M. Taniyama, K. Matsubara, M. Yoda, Y. Nakano, M. Tomita, S. Kimura, C. Ito, P. Froguel & T. Kadowaki (2002) Genetic variation in the gene encoding adiponectin is associated with an increased risk of type 2 diabetes in the Japanese population. *Diabetes,* 51**,** 536-40.

Harrington, T. A., E. L. Thomas, N. Modi, G. Frost, G. A. Coutts & J. D. Bell (2002) Fast and reproducible method for the direct quantitation of adipose tissue in newborn infants. *Lipids,* 37**,** 95-100.

Harris, R. B. (2000) Leptin--much more than a satiety signal. *Annu Rev Nutr,* 20**,** 45-75.

Hassink, S. G., E. de Lancey, D. V. Sheslow, S. M. Smith-Kirwin, D. M. O'Connor, R. V. Considine, I. Opentanova, K. Dostal, M. L. Spear, K. Leef, M. Ash, A. R. Spitzer & V. L. Funanage (1997) Placental leptin: an important new growth factor in intrauterine and neonatal development? *Pediatrics,* 100**,** E1.

Hauner, H., M. Wabitsch, E. Pfeiffer (1989) Proliferation and differentiation of adipose tissue derived stromal-vascular cells from children at different ages. First European Congress on Obesity, Libbey, London.

Hauner, H., C. Vollhardt, K. T. Schneider, A. Zimmermann, T. Schuster & U. Amann-Gassner (2009) The impact of nutritional fatty acids during pregnancy and lactation on early human adipose tissue development. Rationale and design of the INFAT study. *Ann Nutr Metab,* 54**,** 97-103.

Havel, P. J. (2000) Role of adipose tissue in body-weight regulation: mechanisms regulating leptin production and energy balance. *Proc Nutr Soc,* 59**,** 359-71.

Heisler, L. K., M. A. Cowley, L. H. Tecott, W. Fan, M. J. Low, J. L. Smart, M. Rubinstein, J. B. Tatro, J. N. Marcus, H. Holstege, C. E. Lee, R. D. Cone & J. K. Elmquist (2002) Activation of central melanocortin pathways by fenfluramine. *Science,* 297**,** 609-11.

Helland, I. B., J. E. Reseland, O. D. Saugstad & C. A. Drevon (1998) Leptin levels in pregnant women and newborn infants: gender differences and reduction during the neonatal period. *Pediatrics,* 101**,** E12.

Helland, I. B., L. Smith, K. Saarem, O. D. Saugstad & C. A. Drevon (2003) Maternal supplementation with very-long-chain n-3 fatty acids during pregnancy and lactation augments children's IQ at 4 years of age. *Pediatrics,* 111**,** e39-44.

Henson, M. C., K. F. Swan & J. S. O'Neil (1998) Expression of placental leptin and leptin receptor transcripts in early pregnancy and at term. *Obstet Gynecol,* 92**,** 1020-8.

Heo, M., R. L. Leibel, B. B. Boyer, W. K. Chung, M. Koulu, M. K. Karvonen, U. Pesonen, A. Rissanen, M. Laakso, M. I. Uusitupa, Y. Chagnon, C. Bouchard, P. A. Donohoue, T. L. Burns, A. R. Shuldiner, K. Silver, R. E. Andersen, O. Pedersen, S. Echwald, T. I. Sorensen, P. Behn, M. A. Permutt, K. B. Jacobs, R. C. Elston, D. J. Hoffman & D. B. Allison (2001) Pooling analysis of genetic data: the association of leptin receptor (LEPR) polymorphisms with variables related to human adiposity. *Genetics,* 159**,** 1163-78.

Herrera, E. (2002) Implications of dietary fatty acids during pregnancy on placental, fetal and postnatal development--a review. *Placenta,* 23 Suppl A**,** S9-19.

Herrera, E., H. Ortega, G. Alvino, N. Giovannini, E. Amusquivar & I. Cetin (2004) Relationship between plasma fatty acid profile and antioxidant vitamins during normal pregnancy. *Eur J Clin Nutr,* 58**,** 1231-8.

Hillebrand, J. J., D. de Wied & R. A. Adan (2002) Neuropeptides, food intake and body weight regulation: a hypothalamic focus. *Peptides,* 23**,** 2283-306.

Hotta, K., T. Funahashi, Y. Arita, M. Takahashi, M. Matsuda, Y. Okamoto, H. Iwahashi, H. Kuriyama, N. Ouchi, K. Maeda, M. Nishida, S. Kihara, N. Sakai, T. Nakajima, K. Hasegawa, M. Muraguchi, Y. Ohmoto, T. Nakamura, S. Yamashita, T. Hanafusa & Y. Matsuzawa (2000) Plasma concentrations of a novel, adipose-specific protein, adiponectin, in type 2 diabetic patients. *Arterioscler Thromb Vasc Biol,* 20**,** 1595-9.

Hu, E., P. Liang & B. M. Spiegelman (1996) AdipoQ is a novel adipose-specific gene dysregulated in obesity. *J Biol Chem,* 271**,** 10697-703.

Huang, T., M. L. Wahlqvist, T. Xu, A. Xu, A. Zhang & D. Li (2010) Increased plasma n-3 polyunsaturated fatty acid is associated with improved insulin sensitivity in type 2 diabetes in China. *Mol Nutr Food Res,* 54 Suppl 1**,** S112-9.

Hun, C. S., K. Hasegawa, T. Kawabata, M. Kato, T. Shimokawa & Y. Kagawa (1999) Increased uncoupling protein2 mRNA in white adipose tissue, and decrease in leptin, visceral fat, blood glucose, and cholesterol in KK-Ay mice fed with eicosapentaenoic and docosahexaenoic acids in addition to linolenic acid. *Biochem Biophys Res Commun,* 259**,** 85-90.

Hytinantti, T. K., M. Juntunen, H. A. Koistinen, V. A. Koivisto, S. L. Karonen & S. Andersson (2001) Postnatal changes in concentrations of free and bound leptin. *Arch Dis Child Fetal Neonatal Ed,* 85**,** F123-6.

Ibanez, L., G. Sebastiani, A. Lopez-Bermejo, M. Diaz, M. D. Gomez-Roig & F. de Zegher (2008) Gender specificity of body adiposity and circulating adiponectin, visfatin, insulin, and insulin growth factor-I at term birth: relation to prenatal growth. *J Clin Endocrinol Metab,* 93**,** 2774-8.

Ibrahim, A., S. Basak & N. Z. Ehtesham (2009) Impact of maternal dietary fatty acid composition on glucose and lipid metabolism in male rat offspring aged 105 d. *Br J Nutr,* 102**,** 233-41.

Inami, I., T. Okada, H. Fujita, M. Makimoto, S. Hosono, M. Minato, S. Takahashi, K. Harada & T. Yamamoto (2007) Impact of serum adiponectin concentration on birth size and early postnatal growth. *Pediatr Res,* 61**,** 604-6.

Inoue, M., K. Itabashi, Y. Nakano & T. Tobe (2008) High-molecular-weight adiponectin and leptin levels in cord blood are associated with anthropometric measurements at birth. *Horm Res,* 70**,** 268-72.

Iwaki, M., M. Matsuda, N. Maeda, T. Funahashi, Y. Matsuzawa, M. Makishima & I. Shimomura (2003) Induction of adiponectin, a fat-derived antidiabetic and antiatherogenic factor, by nuclear receptors. *Diabetes,* 52**,** 1655-63.

James, M. J., R. A. Gibson & L. G. Cleland (2000) Dietary polyunsaturated fatty acids and inflammatory mediator production. *Am J Clin Nutr,* 71**,** 343S-8S.

Judge, M. P., O. Harel & C. J. Lammi-Keefe (2007) A docosahexaenoic acid-functional food during pregnancy benefits infant visual acuity at four but not six months of age. *Lipids,* 42**,** 117-22.

Jump, D. B. (2002) The biochemistry of n-3 polyunsaturated fatty acids. *J Biol Chem,* 277**,** 8755-8.

Jump, D. B., D. Botolin, Y. Wang, J. Xu, B. Christian & O. Demeure (2005) Fatty acid regulation of hepatic gene transcription. *J Nutr,* 135**,** 2503-6.

Karege, F., G. Bondolfi, N. Gervasoni, M. Schwald, J. M. Aubry & G. Bertschy (2005) Low brain-derived neurotrophic factor (BDNF) levels in serum of depressed patients probably results from lowered platelet BDNF release unrelated to platelet reactivity. *Biol Psychiatry,* 57**,** 1068-72.

Kautzky-Willer, A., G. Pacini, A. Tura, C. Bieglmayer, B. Schneider, B. Ludvik, R. Prager & W. Waldhausl (2001) Increased plasma leptin in gestational diabetes. *Diabetologia,* 44**,** 164-72.

Kawamura, K., N. Kawamura, W. Sato, J. Fukuda, J. Kumagai & T. Tanaka (2009) Brain-derived neurotrophic factor promotes implantation and subsequent placental development by stimulating trophoblast cell growth and survival. *Endocrinology,* 150**,** 3774-82.

Kernie, S. G., D. J. Liebl & L. F. Parada (2000) BDNF regulates eating behavior and locomotor activity in mice. *EMBO J,* 19**,** 1290-300.

Kershaw, E. E. & J. S. Flier (2004) Adipose tissue as an endocrine organ. *J Clin Endocrinol Metab,* 89**,** 2548-56.

Kieffer, T. J., R. S. Heller, C. A. Leech, G. G. Holz & J. F. Habener (1997) Leptin suppression of insulin secretion by the activation of ATP-sensitive K+ channels in pancreatic beta-cells. *Diabetes,* 46**,** 1087-93.

Kiel, D. W., E. A. Dodson, R. Artal, T. K. Boehmer & T. L. Leet (2007) Gestational weight gain and pregnancy outcomes in obese women: how much is enough? *Obstet Gynecol,* 110**,** 752-8.

King, J. C. (2000) Physiology of pregnancy and nutrient metabolism. *Am J Clin Nutr,* 71**,** 1218S-25S.

Kirwan, J. P., S. Hauguel-De Mouzon, J. Lepercq, J. C. Challier, L. Huston-Presley, J. E. Friedman, S. C. Kalhan & P. M. Catalano (2002) TNF-alpha is a predictor of insulin resistance in human pregnancy. *Diabetes,* 51**,** 2207-13.

Klingenspor, M., A. Dickopp, G. Heldmaier & S. Klaus (1996) Short photoperiod reduces leptin gene expression in white and brown adipose tissue of Djungarian hamsters. *FEBS Lett,* 399**,** 290-4.

Knittle, J. L., F. Ginsberg-Fellner & R. E. Brown (1977) Adipose tissue development in man. *Am J Clin Nutr,* 30**,** 762-6.

Kondo, E., T. Sugiyama, H. Kusaka & N. Toyoda (2004) Adiponectin mRNA levels in parametrial adipose tissue and serum adiponectin levels are reduced in mice during late pregnancy. *Horm Metab Res,* 36**,** 465-9.

Koo, W. W., J. C. Walters & E. M. Hockman (2004) Body composition in neonates: relationship between measured and derived anthropometry with dual-energy X-ray absorptiometry measurements. *Pediatr Res,* 56**,** 694-700.

Korotkova, M., B. Gabrielsson, M. Lonn, L. A. Hanson & B. Strandvik (2002) Leptin levels in rat offspring are modified by the ratio of linoleic to alpha-linolenic acid in the maternal diet. *J Lipid Res,* 43**,** 1743-9.

Korotkova, M., B. G. Gabrielsson, A. Holmang, B. M. Larsson, L. A. Hanson & B. Strandvik (2005) Gender-related long-term effects in adult rats by perinatal dietary ratio of n-6/n-3 fatty acids. *Am J Physiol Regul Integr Comp Physiol,* 288**,** R575-9.

Kotani, Y., I. Yokota, S. Kitamura, J. Matsuda, E. Naito & Y. Kuroda (2004) Plasma adiponectin levels in newborns are higher than those in adults and positively correlated with birth weight. *Clin Endocrinol (Oxf),* 61**,** 418-23.

Kramer (1997) Immunologie und Immunpathologie. Stuttgart:Ferdinand Enke Verlag.

Kratz, M., M. M. Swarbrick, H. S. Callahan, C. C. Matthys, P. J. Havel & D. S. Weigle (2008) Effect of dietary n-3 polyunsaturated fatty acids on plasma total and high-molecular-weight adiponectin concentrations in overweight to moderately obese men and women. *Am J Clin Nutr,* 87**,** 347-53.

Kratzsch, J., A. Lammert, A. Bottner, B. Seidel, G. Mueller, J. Thiery, J. Hebebrand & W. Kiess (2002) Circulating soluble leptin receptor and free leptin index during childhood, puberty, and adolescence. *J Clin Endocrinol Metab,* 87**,** 4587-94.

Kratzsch, J., C. Schubring, B. Stitzel, A. Bottner, A. Berthold, J. Thiery & W. Kiess (2005) Inverse changes in the serum levels of the soluble leptin receptor and leptin in neonates: relations to anthropometric data. *J Clin Endocrinol Metab,* 90**,** 2212-7.

Krizova, J., V. Eretova, D. Haluzikova, K. Anderlova, J. Housova, E. Kotrlikova & M. Haluzik (2004) Soluble leptin receptor and leptin levels in pregnant women before and after delivery. *Endocr Res,* 30**,** 379-85.

Kurth, B. M. & A. Schaffrath Rosario (2007) [The prevalence of overweight and obese children and adolescents living in Germany. Results of the German Health Interview and Examination Survey for Children and Adolescents (KiGGS)]. *Bundesgesundheitsblatt Gesundheitsforschung Gesundheitsschutz,* 50**,** 736-43.

Lage, M., R. V. Garcia-Mayor, M. A. Tome, F. Cordido, F. Valle-Inclan, R. V. Considine, J. F. Caro, C. Dieguez & F. F. Casanueva (1999) Serum leptin levels in women throughout pregnancy and the postpartum period and in women suffering spontaneous abortion. *Clin Endocrinol (Oxf),* 50**,** 211-6.

Lain, K. Y. & P. M. Catalano (2007) Metabolic changes in pregnancy. *Clin Obstet Gynecol,* 50**,** 938-48.

Laivuori, H., R. Kaaja, H. Koistinen, S. L. Karonen, S. Andersson, V. Koivisto & O. Ylikorkala (2000) Leptin during and after preeclamptic or normal pregnancy: its relation to serum insulin and insulin sensitivity. *Metabolism,* 49**,** 259-63.

Lammert, A., W. Kiess, A. Bottner, A. Glasow & J. Kratzsch (2001) Soluble leptin receptor represents the main leptin binding activity in human blood. *Biochem Biophys Res Commun,* 283**,** 982-8.

Landon, M. B. (2010) Is there a benefit to the treatment of mild gestational diabetes mellitus? *Am J Obstet Gynecol,* 202**,** 649-53.

Lara-Castro, C., N. Luo, P. Wallace, R. L. Klein & W. T. Garvey (2006) Adiponectin multimeric complexes and the metabolic syndrome trait cluster. *Diabetes,* 55**,** 249-59.

Lara, J. J., M. Economou, A. M. Wallace, A. Rumley, G. Lowe, C. Slater, M. Caslake, N. Sattar & M. E. Lean (2007) Benefits of salmon eating on traditional and novel vascular risk factors in young, non-obese healthy subjects. *Atherosclerosis,* 193**,** 213-21.

Laumen, H., A. D. Saningong, I. M. Heid, J. Hess, C. Herder, M. Claussnitzer, J. Baumert, C. Lamina, W. Rathmann, E. M. Sedlmeier, N. Klopp, B. Thorand, H. E. Wichmann, T. Illig & H. Hauner (2009) Functional characterization of promoter variants of the adiponectin gene complemented by epidemiological data. *Diabetes,* 58**,** 984-91.

Lee, G. H., R. Proenca, J. M. Montez, K. M. Carroll, J. G. Darvishzadeh, J. I. Lee & J. M. Friedman (1996) Abnormal splicing of the leptin receptor in diabetic mice. *Nature,* 379**,** 632-5.

Lee, J. Y., L. Zhao, H. S. Youn, A. R. Weatherill, R. Tapping, L. Feng, W. H. Lee, K. A. Fitzgerald & D. H. Hwang (2004) Saturated fatty acid activates but polyunsaturated fatty acid inhibits Toll-like receptor 2 dimerized with Toll-like receptor 6 or 1. *J Biol Chem,* 279**,** 16971-9.

Lepage, G. & C. C. Roy (1984) Improved recovery of fatty acid through direct transesterification without prior extraction or purification. *J Lipid Res,* 25**,** 1391-6.

Lewandowski, K., R. Horn, C. J. O'Callaghan, D. Dunlop, G. F. Medley, P. O'Hare & G. Brabant (1999) Free leptin, bound leptin, and soluble leptin receptor in normal and diabetic pregnancies. *J Clin Endocrinol Metab,* 84**,** 300-6.

Li, W. D., D. R. Reed, J. H. Lee, W. Xu, R. L. Kilker, B. R. Sodam & R. A. Price (1999) Sequence variants in the 5' flanking region of the leptin gene are associated with obesity in women. *Ann Hum Genet,* 63**,** 227-34.

Lihn, A. S., N. Jessen, S. B. Pedersen, S. Lund & B. Richelsen (2004) AICAR stimulates adiponectin and inhibits cytokines in adipose tissue. *Biochem Biophys Res Commun,* 316**,** 853-8.

Lihn, A. S., S. B. Pedersen & B. Richelsen (2005) Adiponectin: action, regulation and association to insulin sensitivity. *Obes Rev,* 6**,** 13-21.

Lindsay, R. S., J. D. Walker, P. J. Havel, B. A. Hamilton, A. A. Calder & F. D. Johnstone (2003) Adiponectin is present in cord blood but is unrelated to birth weight. *Diabetes Care,* 26**,** 2244-9.

Linnemann, K., A. Malek, R. Sager, W. F. Blum, H. Schneider & C. Fusch (2000) Leptin production and release in the dually in vitro perfused human placenta. *J Clin Endocrinol Metab,* 85**,** 4298-301.

Liu, C., X. J. Liu, G. Barry, N. Ling, R. A. Maki & E. B. De Souza (1997) Expression and characterization of a putative high affinity human soluble leptin receptor. *Endocrinology,* 138**,** 3548-54.

Lobstein, T. & M. L. Frelut (2003) Prevalence of overweight among children in Europe. *Obes Rev,* 4**,** 195-200.

Lönnerdal, B. & P. J. Havel (2000) Serum leptin concentrations in infants: effects of diet, sex, and adiposity. *Am J Clin Nutr,* 72**,** 484-9.

Luo, J., S. W. Rizkalla, J. Boillot, C. Alamowitch, H. Chaib, F. Bruzzo, N. Desplanque, A. M. Dalix, G. Durand & G. Slama (1996) Dietary (n-3) polyunsaturated fatty acids improve adipocyte insulin action and glucose metabolism in insulin-resistant rats: relation to membrane fatty acids. *J Nutr,* 126**,** 1951-8.

Lyons, W. E., L. A. Mamounas, G. A. Ricaurte, V. Coppola, S. W. Reid, S. H. Bora, C. Wihler, V. E. Koliatsos & L. Tessarollo (1999) Brain-derived neurotrophic

factor-deficient mice develop aggressiveness and hyperphagia in conjunction with brain serotonergic abnormalities. *Proc Natl Acad Sci U S A,* 96**,** 15239-44.

Maahs, D. M., L. G. Ogden, G. L. Kinney, P. Wadwa, J. K. Snell-Bergeon, D. Dabelea, J. E. Hokanson, J. Ehrlich, R. H. Eckel & M. Rewers (2005) Low plasma adiponectin levels predict progression of coronary artery calcification. *Circulation,* 111**,** 747-53.

Mammes, O., D. Betoulle, R. Aubert, B. Herbeth, G. Siest & F. Fumeron (2000) Association of the G-2548A polymorphism in the 5' region of the LEP gene with overweight. *Ann Hum Genet,* 64**,** 391-4.

Mankuta, D., M. Elami-Suzin, A. Elhayani & S. Vinker (2010) Lipid profile in consecutive pregnancies. *Lipids Health Dis,* 9**,** 58.

Mantzoros, C., E. Petridou, D. M. Alexe, A. Skalkidou, N. Dessypris, E. Papathoma, H. Salvanos, G. Shetty, A. Gavrila, S. Kedikoglou, G. Chrousos & D. Trichopoulos (2004) Serum adiponectin concentrations in relation to maternal and perinatal characteristics in newborns. *Eur J Endocrinol,* 151**,** 741-6.

Mantzoros, C. S. (2000) Role of leptin in reproduction. *Ann N Y Acad Sci,* 900**,** 174-83.

Mantzoros, C. S., T. Li, J. E. Manson, J. B. Meigs & F. B. Hu (2005) Circulating adiponectin levels are associated with better glycemic control, more favorable lipid profile, and reduced inflammation in women with type 2 diabetes. *J Clin Endocrinol Metab,* 90**,** 4542-8.

Margetic, S., C. Gazzola, G. G. Pegg & R. A. Hill (2002) Leptin: a review of its peripheral actions and interactions. *Int J Obes Relat Metab Disord,* 26**,** 1407-33.

Martin-Romero, C., J. Santos-Alvarez, R. Goberna & V. Sanchez-Margalet (2000) Human leptin enhances activation and proliferation of human circulating T lymphocytes. *Cell Immunol,* 199**,** 15-24.

Martin, L. J., J. G. Woo, S. R. Geraghty, M. Altaye, B. S. Davidson, W. Banach, L. M. Dolan, G. M. Ruiz-Palacios & A. L. Morrow (2006) Adiponectin is present in human milk and is associated with maternal factors. *Am J Clin Nutr,* 83**,** 1106-11.

Martos-Moreno, G. A., V. Barrios, M. Saenz de Pipaon, J. Pozo, I. Dorronsoro, M. Martinez-Biarge, J. Quero & J. Argente (2009) Influence of prematurity and growth restriction on the adipokine profile, IGF1, and ghrelin levels in cord blood: relationship with glucose metabolism. *Eur J Endocrinol,* 161**,** 381-9.

Massiera, F., P. Saint-Marc, J. Seydoux, T. Murata, T. Kobayashi, S. Narumiya, P. Guesnet, E. Z. Amri, R. Negrel & G. Ailhaud (2003) Arachidonic acid and prostacyclin signaling promote adipose tissue development: a human health concern? *J Lipid Res,* 44**,** 271-9.

Masuzaki, H., Y. Ogawa, N. Sagawa, K. Hosoda, T. Matsumoto, H. Mise, H. Nishimura, Y. Yoshimasa, I. Tanaka, T. Mori & K. Nakao (1997) Nonadipose tissue production of leptin: leptin as a novel placenta-derived hormone in humans. *Nat Med,* 3**,** 1029-33.

Matarese, G., S. Moschos & C. S. Mantzoros (2005) Leptin in immunology. *J Immunol,* 174**,** 3137-42.

Matsubara, M., S. Maruoka & S. Katayose (2002) Decreased plasma adiponectin concentrations in women with dyslipidemia. *J Clin Endocrinol Metab,* 87**,** 2764-9.

Matsusue, K., J. M. Peters & F. J. Gonzalez (2004) PPARbeta/delta potentiates PPARgamma-stimulated adipocyte differentiation. *FASEB J,* 18**,** 1477-9.

Mayeur, S., M. Silhol, E. Moitrot, S. Barbaux, C. Breton, A. Gabory, D. Vaiman, I. Dutriez-Casteloot, I. Fajardy, A. Vambergue, L. Tapia-Arancibia, B. Bastide, L. Storme, C. Junien, D. Vieau & J. Lesage (2010) Placental BDNF/TrkB signaling system is modulated by fetal growth disturbances in rat and human. *Placenta,* 31**,** 785-91.

Maymo, J. L., A. P. Perez, Y. Gambino, J. C. Calvo, V. Sanchez-Margalet & C. L. Varone (2011) Review: Leptin gene expression in the placenta--regulation of a key hormone in trophoblast proliferation and survival. *Placenta,* 32 Suppl 2**,** S146-53.

Mazaki-Tovi, S., H. Kanety, C. Pariente, R. Hemi, A. Wiser, E. Schiff & E. Sivan (2007) Maternal serum adiponectin levels during human pregnancy. *J Perinatol,* 27**,** 77-81.

Mazaki-Tovi, S., R. Romero, J. P. Kusanovic, O. Erez, E. Vaisbuch, F. Gotsch, P. Mittal, G. N. Than, C. Nhan-Chang, T. Chaiworapongsa, S. Edwin, N. Camacho, J. K. Nien & S. S. Hassan (2008) Adiponectin multimers in maternal plasma. *J Matern Fetal Neonatal Med,* 21**,** 796-815.

McLachlan, K. A., D. O'Neal, A. Jenkins & F. P. Alford (2006) Do adiponectin, TNFalpha, leptin and CRP relate to insulin resistance in pregnancy? Studies in women with and without gestational diabetes, during and after pregnancy. *Diabetes Metab Res Rev,* 22**,** 131-8.

McMillen, I. C., C. L. Adam & B. S. Muhlhausler (2005) Early origins of obesity: programming the appetite regulatory system. *J Physiol,* 565**,** 9-17.

Menzaghi, C., T. Ercolino, R. Di Paola, A. H. Berg, J. H. Warram, P. E. Scherer, V. Trischitta & A. Doria (2002) A haplotype at the adiponectin locus is associated with obesity and other features of the insulin resistance syndrome. *Diabetes,* 51**,** 2306-12.

Miralles, O., J. Sanchez, A. Palou & C. Pico (2006) A physiological role of breast milk leptin in body weight control in developing infants. *Obesity (Silver Spring),* 14**,** 1371-7.

Misra, M., K. K. Miller, C. Almazan, K. Ramaswamy, A. Aggarwal, D. B. Herzog, G. Neubauer, J. Breu & A. Klibanski (2004) Hormonal and body composition predictors of soluble leptin receptor, leptin, and free leptin index in adolescent girls with anorexia nervosa and controls and relation to insulin sensitivity. *J Clin Endocrinol Metab,* 89**,** 3486-95.

Misra, V. K. & S. Trudeau (2011) The influence of overweight and obesity on longitudinal trends in maternal serum leptin levels during pregnancy. *Obesity (Silver Spring),* 19**,** 416-21.

Montori, V. M., A. Farmer, P. C. Wollan & S. F. Dinneen (2000) Fish oil supplementation in type 2 diabetes: a quantitative systematic review. *Diabetes Care,* 23**,** 1407-15.

Mori, T. A., V. Burke, I. B. Puddey, G. F. Watts, D. N. O'Neal, J. D. Best & L. J. Beilin (2000) Purified eicosapentaenoic and docosahexaenoic acids have differential effects on serum lipids and lipoproteins, LDL particle size, glucose, and insulin in mildly hyperlipidemic men. *Am J Clin Nutr,* 71**,** 1085-94.

Morrison, R. F. & S. R. Farmer (2000) Hormonal signaling and transcriptional control of adipocyte differentiation. *J Nutr,* 130**,** 3116S-3121S.

Mountjoy, K. G. (2010) Functions for pro-opiomelanocortin-derived peptides in obesity and diabetes. *Biochem J,* 428**,** 305-24.

Mounzih, K., J. Qiu, A. Ewart-Toland & F. F. Chehab (1998) Leptin is not necessary for gestation and parturition but regulates maternal nutrition via a leptin resistance state. *Endocrinology,* 139**,** 5259-62.

Muhlhausler, B. S., C. L. Adam, P. A. Findlay, J. A. Duffield & I. C. McMillen (2006) Increased maternal nutrition alters development of the appetite-regulating network in the brain. *FASEB J,* 20**,** 1257-9.

Naruse, K., M. Yamasaki, H. Umekage, T. Sado, Y. Sakamoto & H. Morikawa (2005) Peripheral blood concentrations of adiponectin, an adipocyte-specific plasma protein, in normal pregnancy and preeclampsia. *J Reprod Immunol,* 65**,** 65-75.

Nelson, T. L., J. R. Stevens & M. S. Hickey (2007) Adiponectin levels are reduced, independent of polymorphisms in the adiponectin gene, after supplementation with alpha-linolenic acid among healthy adults. *Metabolism,* 56**,** 1209-15.

Nestel, P. J. (2000) Fish oil and cardiovascular disease: lipids and arterial function. *Am J Clin Nutr,* 71**,** 228S-31S.

Newburg, D. S., J. G. Woo & A. L. Morrow (2010) Characteristics and potential functions of human milk adiponectin. *J Pediatr,* 156**,** S41-6.

Nien, J. K., S. Mazaki-Tovi, R. Romero, O. Erez, J. P. Kusanovic, F. Gotsch, B. L. Pineles, R. Gomez, S. Edwin, M. Mazor, J. Espinoza, B. H. Yoon & S. S. Hassan (2007) Plasma adiponectin concentrations in non-pregnant, normal and overweight pregnant women. *J Perinat Med,* 35**,** 522-31.

Nijland, M. J., S. P. Ford & P. W. Nathanielsz (2008) Prenatal origins of adult disease. *Curr Opin Obstet Gynecol,* 20**,** 132-8.

Nilsson, L., N. Binart, Y. M. Bohlooly, M. Bramnert, E. Egecioglu, J. Kindblom, P. A. Kelly, J. J. Kopchick, C. J. Ormandy, C. Ling & H. Billig (2005) Prolactin and growth hormone regulate adiponectin secretion and receptor expression in adipose tissue. *Biochem Biophys Res Commun,* 331**,** 1120-6.

Nishizawa, H., I. Shimomura, K. Kishida, N. Maeda, H. Kuriyama, H. Nagaretani, M. Matsuda, H. Kondo, N. Furuyama, S. Kihara, T. Nakamura, Y. Tochino, T. Funahashi & Y. Matsuzawa (2002) Androgens decrease plasma adiponectin, an insulin-sensitizing adipocyte-derived protein. *Diabetes,* 51**,** 2734-41.

Nuamah, M. A., N. Sagawa, S. Yura, H. Mise, H. Itoh, Y. Ogawa, K. Nakao & S. Fujii (2003) Free-to-total leptin ratio in maternal plasma is constant throughout human pregnancy. *Endocr J,* 50**,** 421-8.

Odden, N. & L. Morkrid (2007) High molecular weight adiponectin dominates in cord blood of newborns but is unaffected by pre-eclamptic pregnancies. *Clin Endocrinol (Oxf),* 67**,** 891-6.

Ogawa, T., H. Hirose, Y. Yamamoto, K. Nishikai, K. Miyashita, H. Nakamura, I. Saito & T. Saruta (2004) Relationships between serum soluble leptin receptor level and serum leptin and adiponectin levels, insulin resistance index, lipid profile, and leptin receptor gene polymorphisms in the Japanese population. *Metabolism,* 53**,** 879-85.

Ogier, V., O. Ziegler, L. Mejean, J. P. Nicolas & A. Stricker-Krongrad (2002) Obesity is associated with decreasing levels of the circulating soluble leptin receptor in humans. *Int J Obes Relat Metab Disord,* 26**,** 496-503.

Okamoto, Y., S. Kihara, N. Ouchi, M. Nishida, Y. Arita, M. Kumada, K. Ohashi, N. Sakai, I. Shimomura, H. Kobayashi, N. Terasaka, T. Inaba, T. Funahashi & Y. Matsuzawa (2002) Adiponectin reduces atherosclerosis in apolipoprotein E-deficient mice. *Circulation,* 106**,** 2767-70.

Ollmann, M. M., B. D. Wilson, Y. K. Yang, J. A. Kerns, Y. Chen, I. Gantz & G. S. Barsh (1997) Antagonism of central melanocortin receptors in vitro and in vivo by agouti-related protein. *Science,* 278**,** 135-8.

Olsen, S. F., H. S. Hansen, T. I. Sorensen, B. Jensen, N. J. Secher, S. Sommer & L. B. Knudsen (1986) Intake of marine fat, rich in (n-3)-polyunsaturated fatty acids, may increase birthweight by prolonging gestation. *Lancet,* 2**,** 367-9.

Ouchi, N., S. Kihara, Y. Arita, Y. Okamoto, K. Maeda, H. Kuriyama, K. Hotta, M. Nishida, M. Takahashi, M. Muraguchi, Y. Ohmoto, T. Nakamura, S. Yamashita, T. Funahashi & Y. Matsuzawa (2000) Adiponectin, an adipocyte-derived plasma protein, inhibits endothelial NF-kappaB signaling through a cAMP-dependent pathway. *Circulation,* 102**,** 1296-301.

Ouchi, N., H. Kobayashi, S. Kihara, M. Kumada, K. Sato, T. Inoue, T. Funahashi & K. Walsh (2004) Adiponectin stimulates angiogenesis by promoting cross-talk between AMP-activated protein kinase and Akt signaling in endothelial cells. *J Biol Chem,* 279**,** 1304-9.

Pajvani, U. B., M. Hawkins, T. P. Combs, M. W. Rajala, T. Doebber, J. P. Berger, J. A. Wagner, M. Wu, A. Knopps, A. H. Xiang, K. M. Utzschneider, S. E. Kahn, J. M. Olefsky, T. A. Buchanan & P. E. Scherer (2004) Complex distribution, not absolute amount of adiponectin, correlates with thiazolidinedione-mediated improvement in insulin sensitivity. *J Biol Chem,* 279**,** 12152-62.

Passos, M. C., F. P. Toste, S. C. Dutra, P. A. Trotta, P. C. Lisboa & E. G. de Moura (2009) Role of neonatal hyperleptinaemia on serum adiponectin and suppressor of cytokine signalling-3 expression in young rats. *Br J Nutr,* 101**,** 250-6.

Perez-Matute, P., A. Marti, J. A. Martinez, M. P. Fernandez-Otero, K. L. Stanhope, P. J. Havel & M. J. Moreno-Aliaga (2005) Eicosapentaenoic fatty acid increases leptin secretion from primary cultured rat adipocytes: role of glucose metabolism. *Am J Physiol Regul Integr Comp Physiol,* 288**,** R1682-8.

Petridou, E., C. S. Mantzoros, M. Belechri, A. Skalkidou, N. Dessypris, E. Papathoma, H. Salvanos, J. H. Lee, S. Kedikoglou, G. Chrousos & D. Trichopoulos (2005) Neonatal leptin levels are strongly associated with female gender, birth length, IGF-I levels and formula feeding. *Clin Endocrinol (Oxf),* 62**,** 366-71.

Picaud, J. C., J. Rigo, K. Nyamugabo, J. Milet & J. Senterre (1996) Evaluation of dual-energy X-ray absorptiometry for body-composition assessment in piglets and term human neonates. *Am J Clin Nutr,* 63**,** 157-63.

Pinar, H., S. Basu, K. Hotmire, L. Laffineuse, L. Presley, M. Carpenter, P. M. Catalano & S. Hauguel-de Mouzon (2008) High molecular mass multimer complexes and vascular expression contribute to high adiponectin in the fetus. *J Clin Endocrinol Metab,* 93**,** 2885-90.

Plagemann, A. (2004) 'Fetal programming' and 'functional teratogenesis': on epigenetic mechanisms and prevention of perinatally acquired lasting health risks. *J Perinat Med,* 32**,** 297-305.

Plagemann, A. & T. Harder (2009) Hormonal programming in perinatal life: leptin and beyond. *Br J Nutr,* 101**,** 151-2.

Pliego-Rivero, F. B., N. Bayatti, X. Giannakoulopoulos, V. Glover, H. F. Bradford, G. Stern & M. Sandler (1997) Brain-derived neurotrophic factor in human platelets. *Biochem Pharmacol,* 54**,** 207-9.

Poissonnet, C. M., A. R. Burdi & F. L. Bookstein (1983) Growth and development of human adipose tissue during early gestation. *Early Hum Dev,* 8**,** 1-11.

Radka, S. F., P. A. Holst, M. Fritsche & C. A. Altar (1996) Presence of brain-derived neurotrophic factor in brain and human and rat but not mouse serum detected by a sensitive and specific immunoassay. *Brain Res,* 709**,** 122-301.

Ramel, A., D. Parra, J. A. Martinez, M. Kiely & I. Thorsdottir (2009) Effects of seafood consumption and weight loss on fasting leptin and ghrelin concentrations in overweight and obese European young adults. *Eur J Nutr,* 48**,** 107-14.

Rasmussen, K. M. & A. L. Yaktine (2009) Weight Gain During Pregnancy: Reexamining the Guidelines. *National Academy Press, Washington, DC*.

Ravelli, A. C., J. H. van der Meulen, R. P. Michels, C. Osmond, D. J. Barker, C. N. Hales & O. P. Bleker (1998) Glucose tolerance in adults after prenatal exposure to famine. *Lancet,* 351**,** 173-7.

Reseland, J. E., F. Haugen, K. Hollung, K. Solvoll, B. Halvorsen, I. R. Brude, M. S. Nenseter, E. N. Christiansen & C. A. Drevon (2001) Reduction of leptin gene expression by dietary polyunsaturated fatty acids. *J Lipid Res,* 42**,** 743-50.

Retnakaran, R., P. W. Connelly, G. Maguire, M. Sermer, B. Zinman & A. J. Hanley (2007) Decreased high-molecular-weight adiponectin in gestational diabetes: implications for the pathophysiology of Type 2 diabetes. *Diabet Med,* 24**,** 245-52.

Retnakaran, R., A. J. Hanley, N. Raif, P. W. Connelly, M. Sermer & B. Zinman (2004) Reduced adiponectin concentration in women with gestational diabetes: a potential factor in progression to type 2 diabetes. *Diabetes Care,* 27**,** 799-800.

Rios, M., G. Fan, C. Fekete, J. Kelly, B. Bates, R. Kuehn, R. M. Lechan & R. Jaenisch (2001) Conditional deletion of brain-derived neurotrophic factor in the postnatal brain leads to obesity and hyperactivity. *Mol Endocrinol,* 15**,** 1748-57.

Ritterath, C., N. T. Rad, T. Siegmund, T. Heinze, G. Siebert & K. J. Buhling (2009) Adiponectin during pregnancy: correlation with fat metabolism, but not with carbohydrate metabolism. *Arch Gynecol Obstet*.

Rocha, M., C. Bing, G. Williams & M. Puerta (2003) Pregnancy-induced hyperphagia is associated with increased gene expression of hypothalamic agouti-related peptide in rats. *Regul Pept,* 114**,** 159-65.

Rodriguez, G., M. P. Samper, J. L. Olivares, P. Ventura, L. A. Moreno & J. M. Perez-Gonzalez (2005) Skinfold measurements at birth: sex and anthropometric influence. *Arch Dis Child Fetal Neonatal Ed,* 90**,** F273-5.

Rolland, V., K. Clement, I. Dugail, B. Guy-Grand, A. Basdevant, P. Froguel & M. Lavau (1998) Leptin receptor gene in a large cohort of massively obese subjects: no indication of the fa/fa rat mutation. Detection of an intronic variant with no association with obesity. *Obes Res,* 6**,** 122-7.

Roseboom, T. J., J. H. van der Meulen, C. Osmond, D. J. Barker, A. C. Ravelli & O. P. Bleker (2000a) Plasma lipid profiles in adults after prenatal exposure to the Dutch famine. *Am J Clin Nutr,* 72**,** 1101-6.

Roseboom, T. J., J. H. van der Meulen, C. Osmond, D. J. Barker, A. C. Ravelli, J. M. Schroeder-Tanka, G. A. van Montfrans, R. P. Michels & O. P. Bleker (2000b) Coronary heart disease after prenatal exposure to the Dutch famine, 1944-45. *Heart,* 84**,** 595-8.

Rosen, E. D. & O. A. MacDougald (2006) Adipocyte differentiation from the inside out. *Nat Rev Mol Cell Biol,* 7**,** 885-96.

Rosen, E. D., P. Sarraf, A. E. Troy, G. Bradwin, K. Moore, D. S. Milstone, B. M. Spiegelman & R. M. Mortensen (1999) PPAR gamma is required for the differentiation of adipose tissue in vivo and in vitro. *Mol Cell,* 4**,** 611-7.

Rosen, E. D., C. J. Walkey, P. Puigserver & B. M. Spiegelman (2000) Transcriptional regulation of adipogenesis. *Genes Dev,* 14**,** 1293-307.

Rosenfeld, R. D., L. Zeni, M. Haniu, J. Talvenheimo, S. F. Radka, L. Bennett, J. A. Miller & A. A. Welcher (1995) Purification and identification of brain-derived neurotrophic factor from human serum. *Protein Expr Purif,* 6**,** 465-71.

Russo, G. L. (2009) Dietary n-6 and n-3 polyunsaturated fatty acids: from biochemistry to clinical implications in cardiovascular prevention. *Biochem Pharmacol,* 77**,** 937-46.

Salani, B., L. Briatore, G. Andraghetti, G. F. Adami, D. Maggi & R. Cordera (2006) High-molecular weight adiponectin isoforms increase after biliopancreatic diversion in obese subjects. *Obesity (Silver Spring),* 14**,** 1511-4.

Sanders, T. A. (2000) Polyunsaturated fatty acids in the food chain in Europe. *Am J Clin Nutr,* 71**,** 176S-8S.

Savino, F. & S. A. Liguori (2008) Update on breast milk hormones: leptin, ghrelin and adiponectin. *Clin Nutr,* 27**,** 42-7.

Savino, F., S. A. Liguori, M. F. Fissore & R. Oggero (2009) Breast milk hormones and their protective effect on obesity. *Int J Pediatr Endocrinol,* 2009**,** 327505.

Savino, F., G. E. Nanni, S. Maccario, M. Costamagna, R. Oggero & L. Silvestro (2004) Breast-fed infants have higher leptin values than formula-fed infants in the first four months of life. *J Pediatr Endocrinol Metab,* 17**,** 1527-32.

Scherer, P. E., S. Williams, M. Fogliano, G. Baldini & H. F. Lodish (1995) A novel serum protein similar to C1q, produced exclusively in adipocytes. *J Biol Chem,* 270**,** 26746-9.

Schmelzle, H. R. & C. Fusch (2002) Body fat in neonates and young infants: validation of skinfold thickness versus dual-energy X-ray absorptiometry. *Am J Clin Nutr,* 76**,** 1096-100.

Schubring, C., P. Englaro, T. Siebler, W. F. Blum, T. Demirakca, J. Kratzsch & W. Kiess (1998) Longitudinal analysis of maternal serum leptin levels during pregnancy, at birth and up to six weeks after birth: relation to body mass index, skinfolds, sex steroids and umbilical cord blood leptin levels. *Horm Res,* 50**,** 276-83.

Schubring, C., T. Siebler, J. Kratzsch, P. Englaro, W. F. Blum, K. Triep & W. Kiess (1999) Leptin serum concentrations in healthy neonates within the first week of life: relation to insulin and growth hormone levels, skinfold thickness, body mass index and weight. *Clin Endocrinol (Oxf),* 51**,** 199-204.

Schulze, M. B., E. B. Rimm, I. Shai, N. Rifai & F. B. Hu (2004) Relationship between adiponectin and glycemic control, blood lipids, and inflammatory markers in men with type 2 diabetes. *Diabetes Care,* 27**,** 1680-7.

Schwab, J. M., N. Chiang, M. Arita & C. N. Serhan (2007) Resolvin E1 and protectin D1 activate inflammation-resolution programmes. *Nature,* 447**,** 869-74.

Senaris, R., T. Garcia-Caballero, X. Casabiell, R. Gallego, R. Castro, R. V. Considine, C. Dieguez & F. F. Casanueva (1997) Synthesis of leptin in human placenta. *Endocrinology,* 138**,** 4501-4.

Sewell, M. F., L. Huston-Presley, D. M. Super & P. Catalano (2006) Increased neonatal fat mass, not lean body mass, is associated with maternal obesity. *Am J Obstet Gynecol,* 195**,** 1100-3.

Shibata, R., N. Ouchi, S. Kihara, K. Sato, T. Funahashi & K. Walsh (2004) Adiponectin stimulates angiogenesis in response to tissue ischemia through stimulation of amp-activated protein kinase signaling. *J Biol Chem,* 279**,** 28670-4.

Shimano, H., M. Amemiya-Kudo, A. Takahashi, T. Kato, M. Ishikawa & N. Yamada (2007) Sterol regulatory element-binding protein-1c and pancreatic beta-cell dysfunction. *Diabetes Obes Metab,* 9 Suppl 2**,** 133-9.

Shimizu, H., K. Ohtani, T. Tsuchiya, H. Takahashi, Y. Uehara, N. Sato & M. Mori (1997) Leptin stimulates insulin secretion and synthesis in HIT-T 15 cells. *Peptides,* 18**,** 1263-6.

Siegrist-Kaiser, C. A., V. Pauli, C. E. Juge-Aubry, O. Boss, A. Pernin, W. W. Chin, I. Cusin, F. Rohner-Jeanrenaud, A. G. Burger, J. Zapf & C. A. Meier (1997) Direct effects of leptin on brown and white adipose tissue. *J Clin Invest,* 100**,** 2858-64.

Sinha, M. K., I. Opentanova, J. P. Ohannesian, J. W. Kolaczynski, M. L. Heiman, J. Hale, G. W. Becker, R. R. Bowsher, T. W. Stephens & J. F. Caro (1996) Evidence of free and bound leptin in human circulation. Studies in lean and obese subjects and during short-term fasting. *J Clin Invest,* 98**,** 1277-82.

Sivan, E., S. Mazaki-Tovi, C. Pariente, Y. Efraty, E. Schiff, R. Hemi & H. Kanety (2003) Adiponectin in human cord blood: relation to fetal birth weight and gender. *J Clin Endocrinol Metab,* 88**,** 5656-60.

Smas, C. M. & H. S. Sul (1995) Control of adipocyte differentiation. *Biochem J,* 309 (Pt 3)**,** 697-710.

Smith-Kirwin, S. M., D. M. O'Connor, J. De Johnston, E. D. Lancey, S. G. Hassink & V. L. Funanage (1998) Leptin expression in human mammary epithelial cells and breast milk. *J Clin Endocrinol Metab,* 83**,** 1810-3.

Spector, A. A. (1999) Essentiality of fatty acids. *Lipids,* 34 Suppl**,** S1-3.

Spicer, L. J. & C. C. Francisco (1997) The adipose obese gene product, leptin: evidence of a direct inhibitory role in ovarian function. *Endocrinology,* 138**,** 3374-9.

Steffes, M. W., M. D. Gross, P. J. Schreiner, X. Yu, J. E. Hilner, R. Gingerich & D. R. Jacobs, Jr. (2004) Serum adiponectin in young adults--interactions with central adiposity, circulating levels of glucose, and insulin resistance: the CARDIA study. *Ann Epidemiol,* 14**,** 492-8.

Stephens, T. W., M. Basinski, P. K. Bristow, J. M. Bue-Valleskey, S. G. Burgett, L. Craft, J. Hale, J. Hoffmann, H. M. Hsiung, A. Kriauciunas & et al. (1995) The role of neuropeptide Y in the antiobesity action of the obese gene product. *Nature,* 377**,** 530-2.

Suchankova, G., M. Tekle, A. K. Saha, N. B. Ruderman, S. D. Clarke & T. W. Gettys (2005) Dietary polyunsaturated fatty acids enhance hepatic AMP-activated protein kinase activity in rats. *Biochem Biophys Res Commun,* 326**,** 851-8.

Suwaki, N., H. Masuyama, H. Nakatsukasa, A. Masumoto, Y. Sumida, N. Takamoto & Y. Hiramatrsu (2006) Hypoadiponectinemia and circulating angiogenic factors in overweight patients complicated with pre-eclampsia. *Am J Obstet Gynecol,* 195**,** 1687-92.

Swarbrick, M. M., I. T. Austrheim-Smith, K. L. Stanhope, M. D. Van Loan, M. R. Ali, B. M. Wolfe & P. J. Havel (2006) Circulating concentrations of high-molecular-weight adiponectin are increased following Roux-en-Y gastric bypass surgery. *Diabetologia,* 49**,** 2552-8.

Szczepankiewicz, D., E. Pruszynska-Oszmalek, P. Kaczmarek, M. Skrzypski, K. Andralojc, T. Wojciechowicz, M. Sassek & K. W. Nowak (2009) Changes of agouti-related protein in hypothalamus, placenta, and serum during pregnancy in the rat. *J Endocrinol,* 202**,** 35-41.

Tartaglia, L. A., M. Dembski, X. Weng, N. Deng, J. Culpepper, R. Devos, G. J. Richards, L. A. Campfield, F. T. Clark, J. Deeds, C. Muir, S. Sanker, A. Moriarty, K. J. Moore, J. S. Smutko, G. G. Mays, E. A. Wool, C. A. Monroe & R. I. Tepper (1995) Identification and expression cloning of a leptin receptor, OB-R. *Cell,* 83**,** 1263-71.

Tilg, H. & A. R. Moschen (2006) Adipocytokines: mediators linking adipose tissue, inflammation and immunity. *Nat Rev Immunol,* 6**,** 772-83.

Tonelli, J., W. Li, P. Kishore, U. B. Pajvani, E. Kwon, C. Weaver, P. E. Scherer & M. Hawkins (2004) Mechanisms of early insulin-sensitizing effects of thiazolidinediones in type 2 diabetes. *Diabetes,* 53**,** 1621-9.

Tozuka, Y., M. Kumon, E. Wada, M. Onodera, H. Mochizuki & K. Wada (2010) Maternal obesity impairs hippocampal BDNF production and spatial learning performance in young mouse offspring. *Neurochem Int,* 57**,** 235-47.

Trajkovska, V., A. B. Marcussen, M. Vinberg, P. Hartvig, S. Aznar & G. M. Knudsen (2007) Measurements of brain-derived neurotrophic factor: methodological aspects and demographical data. *Brain Res Bull,* 73**,** 143-9.

Trayhurn, P. & I. S. Wood (2004) Adipokines: inflammation and the pleiotropic role of white adipose tissue. *Br J Nutr,* 92**,** 347-55.

Tsai, P. J., C. H. Yu, S. P. Hsu, Y. H. Lee, C. H. Chiou, Y. W. Hsu, S. C. Ho & C. H. Chu (2004) Cord plasma concentrations of adiponectin and leptin in healthy term neonates: positive correlation with birthweight and neonatal adiposity. *Clin Endocrinol (Oxf),* 61**,** 88-93.

Tsao, T. S., H. E. Murrey, C. Hug, D. H. Lee & H. F. Lodish (2002) Oligomerization state-dependent activation of NF-kappa B signaling pathway by adipocyte complement-related protein of 30 kDa (Acrp30). *J Biol Chem,* 277**,** 29359-62.

Tsao, T. S., E. Tomas, H. E. Murrey, C. Hug, D. H. Lee, N. B. Ruderman, J. E. Heuser & H. F. Lodish (2003) Role of disulfide bonds in Acrp30/adiponectin structure and signaling specificity. Different oligomers activate different signal transduction pathways. *J Biol Chem,* 278**,** 50810-7.

Tuduri, E., L. Marroqui, S. Soriano, A. B. Ropero, T. M. Batista, S. Piquer, M. A. Lopez-Boado, E. M. Carneiro, R. Gomis, A. Nadal & I. Quesada (2009) Inhibitory effects of leptin on pancreatic alpha-cell function. *Diabetes,* 58**,** 1616-24.

Uauy, R., P. Mena & A. Valenzuela (1999) Essential fatty acids as determinants of lipid requirements in infants, children and adults. *Eur J Clin Nutr,* 53 Suppl 1**,** S66-77.

Ucar, B., B. Kirel, O. Bor, F. S. Kilic, N. Dogruel, S. D. Aydogdu & N. Tekin (2000) Breast milk leptin concentrations in initial and terminal milk samples: relationships to maternal and infant plasma leptin concentrations, adiposity, serum glucose, insulin, lipid and lipoprotein levels. *J Pediatr Endocrinol Metab,* 13**,** 149-56.

Ukropec, J., J. E. Reseland, D. Gasperikova, E. Demcakova, L. Madsen, R. K. Berge, A. C. Rustan, I. Klimes, C. A. Drevon & E. Sebokova (2003) The hypotriglyceridemic effect of dietary n-3 FA is associated with increased beta-oxidation and reduced leptin expression. *Lipids,* 38**,** 1023-9.

Vega-Sanchez, R., H. A. Barajas-Vega, G. Rozada, A. Espejel-Nunez, J. Beltran-Montoya & F. Vadillo-Ortega (2010) Association between adiposity and inflammatory markers in maternal and fetal blood in a group of Mexican pregnant women. *Br J Nutr***,** 1-5.

Verduci, E., S. Scaglioni, C. Agostoni, G. Radaelli, M. Biondi, A. S. Manso, E. Riva & M. Giovannini (2009) The relationship of insulin resistance with SNP 276G>T at adiponectin gene and plasma long-chain polyunsaturated fatty acids in obese children. *Pediatr Res,* 66**,** 346-9.

Vollhardt, C. (2010) The effect of lowering the ω-6/ω-3 long-chain polyunsaturated fatty acid ratio in the diet of pregnant and lactating women on fatty acid levels and body composition of the women and their newborns. Dissertation. Fakultät Wissenschaftszentrum Weihenstephan. Technische Universität München.

Waki, H., T. Yamauchi, J. Kamon, Y. Ito, S. Uchida, S. Kita, K. Hara, Y. Hada, F. Vasseur, P. Froguel, S. Kimura, R. Nagai & T. Kadowaki (2003) Impaired multimerization of human adiponectin mutants associated with diabetes. Molecular structure and multimer formation of adiponectin. *J Biol Chem,* 278**,** 40352-63.

Walley, A. J., J. E. Asher & P. Froguel (2009) The genetic contribution to non-syndromic human obesity. *Nat Rev Genet,* 10**,** 431-42.

Wang, H., L. H. Storlien & X. F. Huang (2002) Effects of dietary fat types on body fatness, leptin, and ARC leptin receptor, NPY, and AgRP mRNA expression. *Am J Physiol Endocrinol Metab,* 282**,** E1352-9.

Wang, P., E. Mariman, J. Renes & J. Keijer (2008) The secretory function of adipocytes in the physiology of white adipose tissue. *J Cell Physiol,* 216**,** 3-13.

Weyer, C., T. Funahashi, S. Tanaka, K. Hotta, Y. Matsuzawa, R. E. Pratley & P. A. Tataranni (2001) Hypoadiponectinemia in obesity and type 2 diabetes: close association with insulin resistance and hyperinsulinemia. *J Clin Endocrinol Metab,* 86**,** 1930-5.

Weyermann, M., C. Beermann, H. Brenner & D. Rothenbacher (2006) Adiponectin and leptin in maternal serum, cord blood, and breast milk. *Clin Chem,* 52**,** 2095-102.

Woo, J. G., L. M. Dolan, R. Deka, R. D. Kaushal, Y. Shen, P. Pal, S. R. Daniels & L. J. Martin (2006) Interactions between noncontiguous haplotypes in the adiponectin gene ACDC are associated with plasma adiponectin. *Diabetes,* 55**,** 523-9.

Woo, J. G., M. L. Guerrero, M. Altaye, G. M. Ruiz-Palacios, L. J. Martin, A. Dubert-Ferrandon, D. S. Newburg & A. L. Morrow (2009) Human milk adiponectin is associated with infant growth in two independent cohorts. *Breastfeed Med,* 4**,** 101-9.

Wu, Z., Y. Xie, N. L. Bucher & S. R. Farmer (1995) Conditional ectopic expression of C/EBP beta in NIH-3T3 cells induces PPAR gamma and stimulates adipogenesis. *Genes Dev,* 9**,** 2350-63.

Wulster-Radcliffe, M. C., K. M. Ajuwon, J. Wang, J. A. Christian & M. E. Spurlock (2004) Adiponectin differentially regulates cytokines in porcine macrophages. *Biochem Biophys Res Commun,* 316**,** 924-9.

Xu, A., K. W. Chan, R. L. Hoo, Y. Wang, K. C. Tan, J. Zhang, B. Chen, M. C. Lam, C. Tse, G. J. Cooper & K. S. Lam (2005) Testosterone selectively reduces the high molecular weight form of adiponectin by inhibiting its secretion from adipocytes. *J Biol Chem,* 280**,** 18073-80.

Xu, B., E. H. Goulding, K. Zang, D. Cepoi, R. D. Cone, K. R. Jones, L. H. Tecott & L. F. Reichardt (2003) Brain-derived neurotrophic factor regulates energy balance downstream of melanocortin-4 receptor. *Nat Neurosci,* 6**,** 736-42.

Yalow, R. S. & S. A. Berson (1960) Immunoassay of endogenous plasma insulin in man. *J Clin Invest,* 39**,** 1157-75.

Yamamoto, K., T. Itoh, D. Abe, M. Shimizu, T. Kanda, T. Koyama, M. Nishikawa, T. Tamai, H. Ooizumi & S. Yamada (2005) Identification of putative metabolites of docosahexaenoic acid as potent PPARgamma agonists and antidiabetic agents. *Bioorg Med Chem Lett,* 15**,** 517-22.

Yamauchi, T., J. Kamon, H. Waki, Y. Terauchi, N. Kubota, K. Hara, Y. Mori, T. Ide, K. Murakami, N. Tsuboyama-Kasaoka, O. Ezaki, Y. Akanuma, O. Gavrilova, C. Vinson, M. L. Reitman, H. Kagechika, K. Shudo, M. Yoda, Y. Nakano, K. Tobe, R. Nagai, S. Kimura, M. Tomita, P. Froguel & T. Kadowaki (2001) The

fat-derived hormone adiponectin reverses insulin resistance associated with both lipoatrophy and obesity. *Nat Med,* 7**,** 941-6.

Yannakoulia, M., N. Yiannakouris, S. Bluher, A. L. Matalas, D. Klimis-Zacas & C. S. Mantzoros (2003) Body fat mass and macronutrient intake in relation to circulating soluble leptin receptor, free leptin index, adiponectin, and resistin concentrations in healthy humans. *J Clin Endocrinol Metab,* 88**,** 1730-6.

Zacharova, J., J. L. Chiasson & M. Laakso (2005) The common polymorphisms (single nucleotide polymorphism [SNP] +45 and SNP +276) of the adiponectin gene predict the conversion from impaired glucose tolerance to type 2 diabetes: the STOP-NIDDM trial. *Diabetes,* 54**,** 893-9.

Zavalza-Gomez, A. B., R. Anaya-Prado, A. R. Rincon-Sanchez & J. M. Mora-Martinez (2008) Adipokines and insulin resistance during pregnancy. *Diabetes Res Clin Pract,* 80**,** 8-15.

Zhang, Y., R. Proenca, M. Maffei, M. Barone, L. Leopold & J. M. Friedman (1994) Positional cloning of the mouse obese gene and its human homologue. *Nature,* 372**,** 425-32.

Zhao, Y. F., D. D. Feng & C. Chen (2006) Contribution of adipocyte-derived factors to beta-cell dysfunction in diabetes. *Int J Biochem Cell Biol,* 38**,** 804-19.

Zhou, Y., X. Sun, L. Jin, T. Stringfield, L. Lin & Y. Chen (2005) Expression profiles of adiponectin receptors in mouse embryos. *Gene Expr Patterns,* 5**,** 711-5.

Danksagung

Mein herzlicher Dank gilt allen, die zum Entstehen dieser Arbeit beigetragen haben.

Mein besonderer Dank gilt

Herrn Prof. Dr. H. Hauner für die Überlassung dieses interessanten Themas, für seine uneingeschränkte Unterstützung und die wertvollen Ratschläge sowie für die gute Betreuung der Arbeit.

Frau Dr. U. Amann-Gassner und Herrn Dr. B. Bader für die wertvolle Unterstützung bei dieser Arbeit und die hilfreichen wissenschaftlichen Diskussionen.

Herrn Prof. Dr. Kratzsch von der Universität Leipzig und seinem Team für die gute Zusammenarbeit bei der Adipokinanalytik und für die wertvollen Ideen.

Dem INFAT-Team für das freundliche Arbeitsklima, die stetige Diskussions- und Hilfsbereitschaft sowie Frau Tholl für die Unterstützung bei den Blutabnahmen.

Herrn Dr. T. Schuster vom Institut für medizinische Statistik und Epidemiologie der Technischen Universität München für die kompetente Beratung in statistischen Fragen.

Den Kooperationspartnern der INFAT-Studie, Herrn Prof. Dr. KTM Schneider und Frau Dr. A. Zimmermann vom Klinikum rechts der Isar der Technischen Universität München, der Firma Milupa GmbH, Friedrichsdorf und dem EARNEST („The Early Nutritional Programming Project“) Konsortium.

Dem Cusanuswerk, das mich mit einem Stipendium gefördert hat.

Den Geburtskliniken, Hebammen und Gynäkologen für die produktive und freundliche Kooperation, ohne deren Hilfe die erfolgreiche Durchführung der Studie nicht möglich gewesen wäre.

Den Korrekturlesern dieser Arbeit sowie meiner Familie und Freunden für die Unterstützung und die aufmunternden Worte.

Zu guter Letzt allen Müttern mit ihren Kindern für die Teilnahme an der Studie und für das entgegengebrachte Vertrauen.

Curriculum Vitae

Aus datenschutzrechtlichen Gründen wir mein Lebenslauf in der elektronischen Version der Dissertation nicht veröffentlicht.