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EFFECT OF WEIGHT LOSS ON ADIPOKINE AND CHEMOKINE SERUM CONCENTRATION IN OVERWEIGHT AND OBESE ADULTS

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to Klaus

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Summary

Background. Obesity is characterized by a chronic inflammatory status with increased blood levels of pro-inflammatory substances, *e.g.* chemokines. Chemokines are known to be secreted by adipose tissue and to act as chemoattractants for immune cells, *e.g.* macrophages and T-cells. The infiltration of immune cells into adipose tissue during obesity is proposed to contribute to a pro-inflammatory state favoring obesity-linked disorders like T2DM. Because of the increasing prevalence of obesity and associated metabolic consequences, there is a need to set up efficient weight loss programmes on a large scale and assess the impact on chronic inflammatory responses.

Objectives and methods. To study the efficacy of the commercial Weight Watchers programme compared to standard care provided by general practitioners, a multicenter, randomized controlled trial including 772 overweight and obese participants (mean BMI 31 kg/m^2) was conducted with clinic visits at baseline and after 2, 4, 6, 9 and 12 months in three countries (UK, Australia, Germany). Weight change was the primary outcome. The hypothesis that serum levels of the pro-inflammatory chemokines MCP-1, IP-10, RANTES and PGRN decrease during weight loss was tested. The involvement of the anti-inflammatory adipokine adiponectin and its HMW isoform in weight loss and inflammation was also investigated, since lower blood levels have been associated with obesity and T2DM. For these purposes, blood samples from 103 German adult subjects from the aforementioned study cohort were analysed using immunoassays in order to quantify circulating levels of MCP-1, IP-10, RANTES, PGRN, total and HMW adiponectin. In addition, changes in standard clinical and metabolic parameters were determined.

Results. After 1 year of intervention, subjects in the commercial group lost significantly more body weight compared to standard care (in Germany: -5.15 *vs.* 2.67 kg; $p = 0.001$; $n = 201$; all countries per protocol analysis: -6.65 *vs.* -3.26 kg; $p < 0.001$; $n = 444$). Because there was no difference between the two intervention groups regarding the measured adipokines/chemokines at baseline, data from both groups were combined for further analysis according to body weight loss. Among the four pro-inflammatory molecules measured, a body weight loss by -1 % was significantly associated with decreased MCP-1 levels (-0.7 %, $p = 0.045$). Body weight loss triggered also an increase in total and HMW adiponectin levels (+0.6 and 0.7 %, $p = 0.010$ and 0.027, respectively) as well as an improvement in glucose (-0.6 %, $p = 0.001$), insulin (-2.4 %, $p = 0.003$), HOMA-IR (-2.9 %,

p = 0.001), TG (-1.4 %, p = 0.002), total- and LDL-cholesterol (-0.5 and -0.8 %, p = 0.001 and < 0.001, respectively), blood pressure (-0.3 %, p = 0.038) and CRP (-4.5 %, p = 0.029). Grouping the subjects according to the degree of weight loss (< 5 %, 5-10 % and > 10 % of initial body weight) revealed that weight loss between 5 and 10 % already resulted in reduced MCP-1 and increased total and HMW adiponectin levels, while the other metabolic parameters (glucose, insulin, HOMA-IR, TG, total- and LDL-cholesterol) and blood pressure only improved in subjects losing > 10 % of initial body weight. Moreover, total and HMW adiponectin correlated negatively with HOMA-IR (r = -0.26 and -0.38, respectively) and TG (r = -0.28 and -0.25, respectively) and positively with HDL-cholesterol (r = 0.50 and 0.48, respectively) while MCP-1 levels correlated positively with IP-10 and RANTES concentrations (r = 0.25 and 0.33, respectively).

Conclusion. These findings suggest that at least a modest weight loss by 5-10 % of initial body weight is required to improve serum levels of adiponectin (ca. +0.9 µg/ml) and MCP-1 (ca. -10.8 pg/ml) whereas IP-10, RANTES and PGRN levels seem to be independent of weight loss. The observed improvement of metabolic risk factors associated with weight loss may favour a reduced risk of developing metabolic diseases in the long-term.

Zusammenfassung

Hintergrund: Adipositas wird als Zustand einer chronischen Entzündung angesehen, da inflammatorische Substanzen, wie z.B. Chemokine, im Blut in erhöhter Konzentration zirkulieren. Einige Chemokine werden von Fettzellen sezerniert und fungieren als chemische Botenstoffe für Immunzellen, wie Makrophagen und T-Zellen, die bei adipösen Menschen in erhöhtem Maße ins Fettgewebe infiltrieren. Dies führt zur Aufrechterhaltung einer entzündlichen Reaktion, die mit der Entstehung von Adipositas-assoziierten Erkrankungen, wie Typ 2 Diabetes in Verbindung gebracht wird. Da die Prävalenz von Adipositas mit ihren metabolischen Folgen ansteigt, besteht die Notwendigkeit effiziente Abnehmprogramme auf breiter Ebene anzubieten. Diesbezüglich haben wir die Wirksamkeit eines kommerziellen Programms mit den üblichen hausärztlichen Empfehlungen zur Gewichtsreduktion verglichen.

Methodik: In einer einjährigen, multi-zentrischen, internationalen, randomisierten klinischen Studie wurden 772 Teilnehmer zwei verschiedenen Interventionen zugelost. In der Hausarztgruppe blieb die Intervention zur Gewichtsreduktion den Hausärzten überlassen („standard care“, nach länderspezifischen Leitlinien), in der kommerziellen Gruppe (Weight Watchers (WW)) wurde den Patienten die regelmäßige kostenlose Teilnahme an lokalen WW-Treffen ermöglicht. Das Körpergewicht und andere klinische und metabolische Parameter wurden nach 0, 2, 4, 6, 9 und 12 Monaten bestimmt. Wir untersuchten die Hypothese in wieweit die Serumkonzentration der inflammatorischen Chemokine MCP-1, IP-10, RANTES und PGRN bei Gewichtsreduktion sinken. Weiterhin wollten wir klären, ob es zu einem Anstieg des anti-inflammatorischen Adiponectins und seiner HMW Isoform kommt. Dafür haben wir 103 Blutproben aus einer Sub-Gruppe der deutschen Kohorte mittels Immuno-Assays analysiert.

Ergebnis: Nach einem Jahr hatten die Teilnehmer des kommerziellen Abnehmprogramms signifikant mehr Gewicht verloren als bei der hausärztlichen Beratung (in Deutschland: -5.15 *vs.* 2.67 kg; $p = 0.001$; $n = 201$; alle Länder: -6.65 *vs* -3.26 kg; $p < 0.001$; $n = 444$). Bezüglich der gemessenen Adipokine/Chemokine lag kein Unterschied zwischen den beiden Interventionen in der untersuchten Sub-Gruppe vor, so dass diese für die weitere Analyse kombiniert wurden. Eine Gewichtsabnahme von -1 % war mit einer signifikanten Reduktion der MCP-1 Serumkonzentration (-0,7 %, p=0,045) und einem Anstieg des gesamt- und HMW Adiponectins (+0,6 and 0,7 %, p = 0,010 and 0,027, entsprechend) assoziiert. Weiterhin kam

es zu einer signifikanten Verbesserung der Glukose (-0,6 %, p = 0,001), Insulin (-2,4 %, p = 0,003), HOMA-IR (-2,9 %, p = 0,001), Triglyceride (-1,4 %, p = 0,002), Gesamt- und LDL-Cholesterol (-0,5 und -0,8 %, p = 0,001 und < 0,001, entsprechend) Werte, sowie zu einer Absenkung des Blutdrucks (-0,3 %, p = 0,038) und des CRP (-4,5 %, p = 0,029). Eine Einteilung der Teilnehmer in Kategorien hinsichtlich des Abnahmeerfolges (< 5 %, 5-10 % und > 10 % des Anfangsgewichts) zeigte, dass schon eine Gewichtsabnahme von 5-10% zu einer Verringerung des MCP-1- und zu einem Anstieg des Gesamt- und HMW Adiponectins führt, die negativ mit HOMA-IR (r = -0,26 and -0,38, entsprechend) und den Triglyceriden (r = -0,28 and -0,25, ensprechend) und positiv mit HDL-Cholesterol (r = 0,50 and 0,48, entsprechend) korreliert waren. MCP-1 korreliert positiv mit IP-10 und RANTES (r = 0,25 and 0,33, entsprechend).

Schlussfolgerung. Diese Daten zeigen, dass schon eine moderate Gewichtsabnahme von 5-10 % zu einer Verbesserung der systemischen Adiponectin (ca. +0.9 µg/ml) und MCP-1 (ca. -10.8 pg/ml) Spiegel führt, wobei die Gewichtsreduktion keinen signifikanten Einfluss auf die Serumkonzentration von IP-10, RANTES und PGRN zeigt. Die Verbesserung der oben genannten metabolischen Risikofaktoren bei Gewichtsabnahme könnte zu einer Reduzierung des späteren Risikos für metabolische Erkrankungen beitragen.

Abbreviations

AdipoR	adiponectin receptor
AP1	activator protein-1
ATF6	activating transcription factor 6
BIA	bioimpedance analysis
BMI	body mass index
CI	confidence interval
CRP	C-reactive protein
CP	commerical programme
CV	coefficient of variation
d	day
DNA	deoxyribonucleic acid
DXA	X-ray absorptiometry
ELISA	enzyme-linked immunosorbent assay
ER	endoplasmic reticulum
FFA	free fatty acids
g	gram
GGT	glutamyltransferase
GOT	glutamic-oxaloacetic transaminase
GP	general practitioner
GPT	glutamic-pyruvate transaminase
HbA1c	glycosylated hemoglobin
HDL	high-density lipoprotein
HMW	high molecular weight (adiponectin)
HOMA-IR	homeostasis model assessment-insulin resistance
hsCRP	high sensitive c-reactive protein

IGT	impaired glucose tolerance
IKK	inhibitory kappa B kinase
IL	interleukin
IP-10	Interferon-induced protein 10
IR	insuin resistance
IRE1	inositol-requiring kinase 1
IRS	insulin receptor substrate
JNK	c-Jun N-terminal kinase
kcal	kilocalorie
kDa	kilo Dalton
kg	kilogram
LDL	low-density lipoprotein
LPL	liprprotein lipase
MCP-1	monocyte chemoattractant protein-1
mg/l	milligram per liter
µg	microgram
mmol/l	millimole per liter
mRNA	messenger ribonucleic acid
NF-κB	nuclear factor kappa B
ng	nanogram
NGT	normal glucose tolerant
OGTT	oral glucose tolerance test
PAI-1	plasminogen activator inhibitor-1
PERK	PKR-like endoplasmatic reticulum kinase
PGRN	progranulin
RANTES	regulated upon activation normal T-cell expressed and secreted
RCT	randomized controlled trial

RBP4	retinol-binding protein 4
SC	standard care
SD	standard deviation
SEM	standard error of the mean
T2DM	type-2 diabetes mellitus
TG	triglyceride
TGF	transforming growth factor
Th1/Th2	helper T-cell (type 1/type 2)
TLR	Toll-like receptor
TNF	tumor necrosis factor
Treg	regulatory T-cell
TSH	thyroid-stimulating hormone
UPR	unfolded protein response
VEGF	vascular endothelial growth factor
WW	Weight Watchers

1 Introduction

Like an epidemic threat, the prevalence of obesity is increasing at an alarming rate. The total number of obese human beings worldwide has doubled since 1980 (World Health Organisation 2012). The main issue is that obesity is accompanied by serious medical consequences. Overweight and obese adult subjects are at increased risk of early mortality (Fontaine et al. 2003) and of medical conditions like type 2 diabetes (T2DM), dyslipidemia and hypertension, all of which are acknowledged risk factors for developing cardiovascular diseases (Hauner 2005). Recent epidemiological data show that obesity accounts for up to 20 % of all cancer deaths, which makes it, after smoking, the second main risk factor for cancer mortality (Calle et al. 2003). Furthermore, obesity is linked to detrimental psychosocial factors associated with the development of diseases, *i.e.*, obese people are characterized by lower quality of life, social isolation and depression (Wadden and Phelan 2002; Kushner and Foster 2000). Prevention of overweight and obesity would help lowering the risk of these chronic diseases and reducing the obesity-associated costs of approximately 13 billion euros per year in Germany (Knoll and Hauner 2008).

The following sections in the introduction will provide background information relevant to the topic of interest of the present PhD thesis, namely the association between body weight loss and inflammatory molecules by (i) defining obesity (p 12), (ii) introducing principles underlying weight loss programs (p 14), (iii) characterizing parameters of inflammation in obesity (p 17), and (iv) stating the objectives of the work (p 34).

1.1 Definition of Obesity

The body-mass-index (BMI) defines the relation of the body mass (body weight) to body size and is expressed in kilogram per square meters (kg/m^2). The BMI is only an indicator of obesity and associated risks because it does not account for sex, body shape and body mass composition (fat and muscle mass). According to the classification by the World Health Organisation (www.who.int/mediacentre/factsheets/fs311/en/), the BMI discriminates between underweight (BMI < 18.5 kg/m^2), normal weight (18.5 kg/m^2 ≤ BMI < 25 kg/m^2), overweight (BMI ≥ 25 kg/m^2) and obese (BMI ≥ 30.0 kg/m^2) subjects. Among German individuals aged ≥ 18 years, 37.4 % are overweight and 20.8 % are obese (Deutsche

Adipositas-Gesellschaft 2008). The prevalence of obesity is increasing constantly and the rise in the proportion of obese children and adolescents is of particular concern (Lobstein and Frelut 2003; Kurth and Schaffrath Rosario 2007).

The etiology of obesity is multifactorial. The genetic and epigenetic predisposition for obesity interacts with changes in environmental, lifestyle and emotional factors (Galgani and Ravussin 2008). Nowadays, obesogenic environments favor deleterious habits including higher caloric consumption through increased portion sizes, high fat and sugar intake, and meals outside from home. Additionally, most citizens in Westernized countries enjoy a lifestyle with reduced daily energy expenditure. The complex signaling networks in the central nervous system that control energy balance by modulating signals from adipose tissue, liver, muscle and gastrointestinal tract have evolved over thousands of years to save energy and are not well suited for coping with these obesogenic environments (Galgani and Ravussin 2008; Woods et al. 1998; Woods and D'Alessio 2008). Counteracting body weight gain requires changes in lifestyle habits which are not easy to maintain in the long-term. Dietary restraint and physical activity are the two most important factors favoring low risk of developing obesity **(Figure 1)** (Hill and Peters 1998).

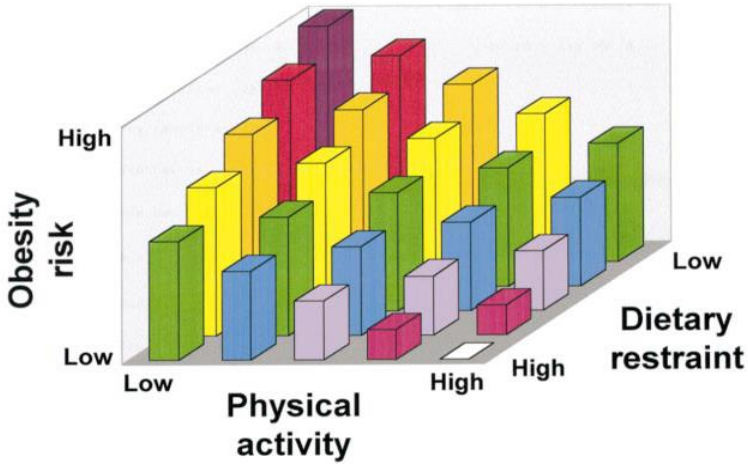


Figure 1: Hypothetical risk for obesity is dependent on physical activity and dietary restraint (Hill and Peters 1998)

1.2 Lifestyle Intervention Programs for Weight Loss

The commonly accepted recommendation for treatment of overweight and obesity is a combination of nutrition, exercise and social support (Hauner et al. 2007). In addition, surgical treatment is often required for subjects with a BMI > 40 kg/m^2. The first step is always to put emphasis on body weight reduction, yet body weight maintenance is the major long-term challenge. The following three sections present rationales for intervention programs focused on changes in nutrition and physical activity to support people who are in need of reducing body weight.

1.2.1 Impact of Diet

Diets that reduce overall caloric intake can induce weight loss independently of macronutrient composition (Freedman et al. 2001). Nevertheless, it is still a matter of debate whether low-fat or low-carbohydrate diets should be favoured. Since fat is energy dense (9 kcal/g) and is widely consumed in developed countries where obesity rates are high, it has been blamed for the increase in adiposity. In a review including six randomized controlled trials (RCT), the effect of low-fat diets compared to other weight reducing diets was investigated (Pirozzo et al. 2003). The overall weight loss was moderate (-2 to -4 kg) after 12 to 18 months follow-up and the low-fat diet was as effective as the other ones. In the last ten years, carbohydrate-restricted diets became also popular following the traditional Atkins concept based on very low carbohydrate intakes (< 20 g/d). A systematic review revealed insufficient evidence to give recommendations for or against low-carbohydrate diets (Bravata et al. 2003). In this review, weight loss was associated with decreased caloric intake and longer diet duration but not with reduced carbohydrate content. A meta-analysis of RCT also assessed the effects of low-fat *vs.* low-carbohydrate diets on weight loss and cardiovascular risk factors (Nordmann et al. 2006). The weighted mean reduction in body weight after six months of intervention was significantly higher with the low-carbohydrate diet than with the low-fat diet (mean difference -3 kg) but the difference was marginal after one year (mean difference -1.0 kg). When taking blood lipids into account, the low-carbohydrate diet was characterized by more favourable outcomes with respect to triglycerides (mean difference -22.1 mg/dl) and high-density-lipoprotein (HDL)-cholesterol (mean difference 4.6 mg/dl) after six months whereas total- and low-density-lipoprotein (LDL)-cholesterol levels changed more favourably in the low-fat diet (mean difference 8.9 mg/dl and 5.4 mg/dl, respectively). As a matter of fact, in the “Leitlinie Prävention und Therapie der Adipositas 2007”, low-

carbohydrate diets are criticized with regard to long-term effects because food selection is limited and LDL- and total cholesterol levels are not influenced positively (Hauner et al. 2007). The guideline of the German society for nutrition regarding carbohydrate intake based on evidence from available studies in children, adolescents and adults indicates that carbohydrate intake is not associated with the risk of obesity (Hauner et al. 2012). Moreover, the quality of carbohydrates should be taken into account as an increase in dietary fiber intake is associated with a reduced risk for obesity in adults, whereas a higher consumption of sugar-sweetened beverages is accompanied by an increased obesity risk (Hauner et al. 2012).

1.2.2 Impact of Physical Activity

Physical activity supports body weight loss and maintenance by favouring a negative energy balance (Jakicic 2009). Some studies indicate a modest effect of physical activity alone on body weight reduction (-3 % of initial body weight) compared to dietary intervention (Wing et al. 1998; Hagan et al. 1986). Wing et al. assessed the effect of a controlled diet (maximum calorie intake of 1 000 kcal/d during the first 8 weeks with a gradual increase to 1 500 kcal up to 16 weeks) compared to exercise intervention (1 500 kcal/week of moderate activity) alone or in combination in 154 obese subjects. After one year, participants in the diet and diet-plus-exercise group lost significantly more body weight compared to exercise alone (-5.5 kg and -7.4 kg *vs.* -0.4 kg, respectively). However, even a modest reduction in body weight can improve cardio-metabolic risk factors (Wing et al. 1998). Moreover, physical activity may be an important factor for maintenance of reduced body weight. In individuals who achieved a weight loss of >10 % of initial body weight that was maintained after 24 months, the physical activity level was high (4h/week above baseline levels) (Jakicic et al. 2008). The observation of 42 twin pairs discordant for leisure physical activity also revealed that weight gain in the active group was 5.4 kg less than in the inactive group over a period of 30 years (Waller et al. 2008).

1.2.3 Comparative Effects of Lifestyle Programs

In accordance with the aforementioned issue on the search for most effective diets favouring sustained weight loss, different lifestyle programs have been set up.

One study compared the degree of weight loss in 160 men and women randomly assigned to either the Atkins diet [very low in carbohydrate (20 g/d)], Zone [40 % calories from carbohydrate, 30 % from protein, 30 % from fat], WeightWatchers (WW) diet [balanced diet with 60 % carbohydrate, 15 % protein and 25 % fat] or Ornish diet [vegetarian and very high in carbohydrate with 10 % fat] (Dansinger et al. 2005). All diets resulted in modest yet significant weight loss after one year (Atkins -2.1 ± 4.8 kg; Zone-3.2 ± 6.0 kg; WW -3.0 ± 4.9 kg; Ornish -3.3 ± 7.3 kg). There was no significant difference between the different diets (p = 0.40). In another trial with 311 women, the Atkins group completed the intervention with increased weight loss after one year compared to the Zone, LEARN (Lifestyle, Exercise, Attitudes, Relationships, and Nutrition) and Ornish diet (Atkins, -4.4 kg, 95 % CI [-6.3 to -3.1 kg]; Zone, -1.6 kg, [-2.8 to -0.4 kg]; LEARN, -2.6 kg, [-3.8 to -1.3 kg]; Ornish, -2.2 kg, [-3.6 to -0.8 kg]) (Gardner et al. 2007).

The M.O.B.I.L.I.S. program is an interdisciplinary program that aims at modifying exercise levels and nutrition (Berg et al. 2008). In 454 obese subjects, lifestyle changes after M.O.B.I.L.I.S resulted in a mean weight reduction of 6.4 ± 7.5 kg over 12 months. Another multifactorial intervention strategy is proposed by the WW program, the efficacy of which has been proven in one multicentric RCT in the United States (Heshka et al. 2003). In this trial, the WW program resulted in modest, but significantly greater weight loss than the self-help group after one year (-4.3 ± 6.0 kg *vs.* -1.3 ± 6.1 kg). In the Look AHEAD trial (5 145 overweight subjects with T2DM), an intensive lifestyle intervention (diet modification, physical activity and group behavioural programmes) resulted in greater weight loss (-8.6 % of initial body weight) compared to a standard programme according to recommendations by the American Diabetes Association and characterized by a weight loss of -0.7 % of initial body weight after one year (Pi-Sunyer et al. 2007).This was also accompanied by improved diabetes control and cardiovascular disease risk factors (blood pressure, triglycerides (TG), HDL). In the Finnish Diabetes Prevention Study 522 overweight (mean BMI 31 kg/m^2) were randomly assigned to a lifestyle intervention (detailed individualized counselling, regular meetings) or to a control group (general verbal and written information, no individual tailoring, only one annual meeting) (Lindström et al. 2003a). After one and two years, subjects in the intensive lifestyle intervention lost significantly more weight (-4.2 and -3.5 kg, respectively) compared to the control group (-0.8 kg at both time points).

Regardless of the type of intervention, a high level of adherence to a diet is associated with weight loss, and adherence is influenced by personal preferences and habits (Dansinger et al. 2005; Heshka et al. 2003; Del Corral et al. 2011). These facts point out the need for different lifestyle programmes and associated dietary interventions so that individuals can easily integrate the most appropriate programme in their daily life. As briefly mentioned above, the rationale for setting up prevention and intervention programmes against obesity lies in the need to limit collateral financial and social costs due to the rise in associated chronic inflammatory and metabolic disorders.

1.3 Adipose Tissue as an Endocrine Organ: Linking Obesity to Inflammation

In recent years, it has become clear that obesity is a state of chronic low-grade inflammation. Elevated circulating concentrations of inflammatory markers are observed in obese subjects when compared to lean individuals (Sell and Eckel 2009) and are discussed as links between obesity and metabolic consequences like T2DM, cardiovascular diseases and the metabolic syndrome (Hauner 2005). The occurrence and accumulation of macrophages and T-cells in adipose tissues of obese subjects has been proposed as a cause for elevated pro-inflammatory molecules in obesity (Heilbronn and Campbell 2008). The following sections aim at providing detailed information on molecules, molecular mechanisms and immune cell types involved in inflammatory reactions related to obesity.

White adipose tissue is considered as an endocrine organ secreting a variety of biologically active factors referred to as adipokines (Trayhurn and Wood 2004). Adipokines include factors involved in energy homeostasis, glucose and lipid metabolism (leptin, adiponectin, lipoproteinlipase (LPL), retinol-binding protein 4 (RBP4) and free fatty acids (FFA)), growth factors (transforming growth factor-beta (TGF-β), vascular endothelial growth factor (VEGF)), proteins involved in vascular homeostasis (plasminogen activator inhibitor-1 (PAI-1)) or regulating blood pressure (angiotensinogen), as well as inflammatory mediators (tumor necrosis factor (TNF), interleukins (IL-6, IL-8), monocyte chemoattractant protein-1 (MCP-1)) (Trayhurn and Wood 2004; Waki and Tontonoz 2007) (**Figure 2**). Dysregulation of adipokine production seems to be an important mechanism by which adipose tissue contributes to the pathogenesis of metabolic disorders.

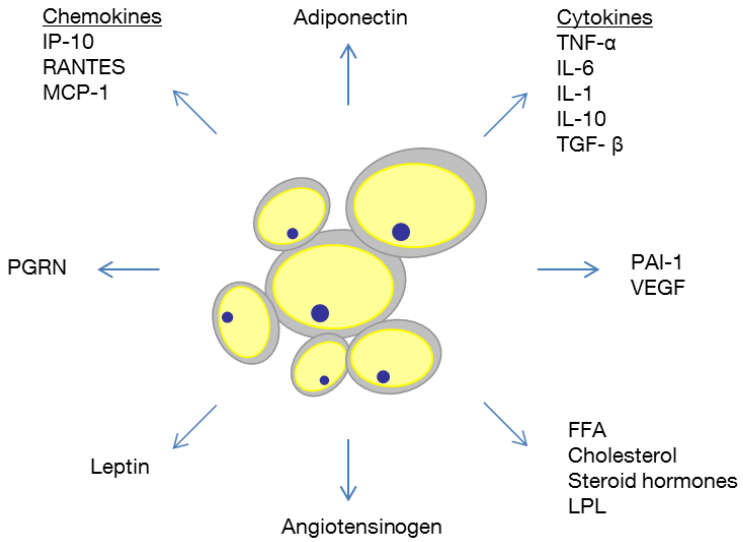


Figure 2: Secretory products by adipose tissue. IP-10, Interferon-inducible protein-10 kDa; MCP-1, Monocyte chemoattractant protein-1; RANTES, Regulated upon Activation normal T-cell expressed and secreted; IL, interleukin; LPL, lipoproteinlipase; FFA, free fatty acids; TNF, tumor necrosis factor; TGF, transforming growth factor; PAI, plasminogen activator inhibitor; VEGF, vascular endothelial growth factor; PGRN, progranulin.

Inflammation can be triggered by nutrients and metabolic surplus: adipocytes begin to get hypertrophic, which leads to a shift towards a dominance of pro-inflammatory adipokine secretion (Skurk et al. 2007) and further cellular dysfunction, like an increase in FFA levels. Plasma FFA are chronically elevated in human obesity (Jensen et al. 1989; Opie and Walfish 1963) and were found to be potent inducers of chemokines in adipocytes (Jiao et al. 2009). FFA bind to innate immune receptors such as Toll-like receptor-4 (TLR-4), the expression of which is increased in the adipose tissue and skeletal muscle of T2DM individuals (**Figure 3**) (Reyna et al. 2008). TLR are mostly expressed on haematopietic cells (precursor of immune cells) and epithelial cells but also on neurons and adipocytes, where the expression pattern is dependent on the state of differentiation (Song et al. 2006; Schäffler and Schölmerich 2010). TLR-4 activation can result in higher expression and secretion of pro-inflammatory adipokines like the cytokine TNF and the chemokine MCP-1 (Vitseva et al. 2008; Kopp et al. 2010). TLR-4-deficient mice are protected from lipid-induced inflammatory changes in adipose tissue and from insulin resistance (Shi et al. 2006). TLR-2-deficient mice seem to be partially protected from diet-induced obesity compared to wild-type animals resulting also in improved insulin resistance (IR) (Himes and Smith 2010). In contrast, TLR-5-deficient mice on normal diet develop hyperlipidemia, hypertension, IR, and increased adiposity correlating

with altered composition of the gut microbiota (Vijay-Kumar et al. 2010). Altogether, the role of TLR in energy homeostasis and metabolic disorders is dependent on receptor types and expression patterns.

TLR activation is linked to two most important pro-inflammatory pathways, JNK and NF-κB (Vitseva et al. 2008; Kopp et al. 2010). JNK has been directly linked to IR, the metabolic syndrome and T2DM as it phosphorylates the insulin receptor substrate-1 (IRS-1) at the inhibitory site Ser-307, thereby suppressing insulin signaling cascades (**Figure 3**) (Weston and Davis 2007). Attenuated insulin signaling is associated with decreased glucose uptake in adipocytes (Song et al. 2006). In obese mice, JNK was found to be activated while a genetically-modified mouse model deficient in JNK1 was protected from diet-induced IR and diabetes (Hirosumi et al. 2002). Upon activation, NF-κB is released from binding to inhibitory proteins (IκB) and translocates to the nucleus to bind specific deoxyribonucleic acid (DNA) sequences leading to the transcription of pro-inflammatory cytokines, *e.g.*, TNF (Lawrence 2009; Hayden and Ghosh 2012). *In vivo* administration of salsalate, an inhibitor of NF-κB, has been found to reduce glycemia, IR and inflammatory parameters in obese adults (Fleischman et al. 2008).

Another molecular network reacting to metabolic overload and linked to inflammatory pathways is the endoplasmatic reticulum (ER) stress (Karalis et al. 2009). The ER is primarily responsible for protein synthesis, lipid droplet formation and cholesterol homeostasis (de Ferranti and Mozaffarian 2008). Each nascent protein must be properly folded to become functional, a process that is supervised by chaperone proteins in the ER. The accumulation of unfolded or misfolded proteins, due for instance to nutrient overload, triggers a signaling cascade known as the unfolded protein response (UPR) to rescue cellular functions. The UPR is mediated by three gatekeeper molecules that are activated during ER stress: PKR-like ER kinase (PERK), activating transcription factor 6 (ATF6) and inositol-requiring enzyme 1 (IRE1). The UPR is also linked to the activation of two inflammatory pathways, JNK-activator protein-1 (AP1) and inhibitory kappa B kinase (IKK)/NF-κB, able to disturb insulin action (see above) and induce the production of pro-inflammatory adipokines (**Figure 3**) (Lawrence 2009). Adipocytes have been shown to up-regulate expression of the chemokines regulated upon activation normal T-cell expressed and secreted (RANTES) and MCP-1 under ER stress (Zeyda et al. 2010). In obese and insulin resistant ob/ob mice, prevention of ER stress by treatment with a chemical chaperone (phenyl butyric acid or taurine-conjugated ursodeoxycholic acid) markedly improved glucose tolerance and IR (Ozcan et al. 2006).

Thus ER stress and the UPR seem to be key molecular mechanisms underlying the inflammatory state in obesity and metabolic disorders.

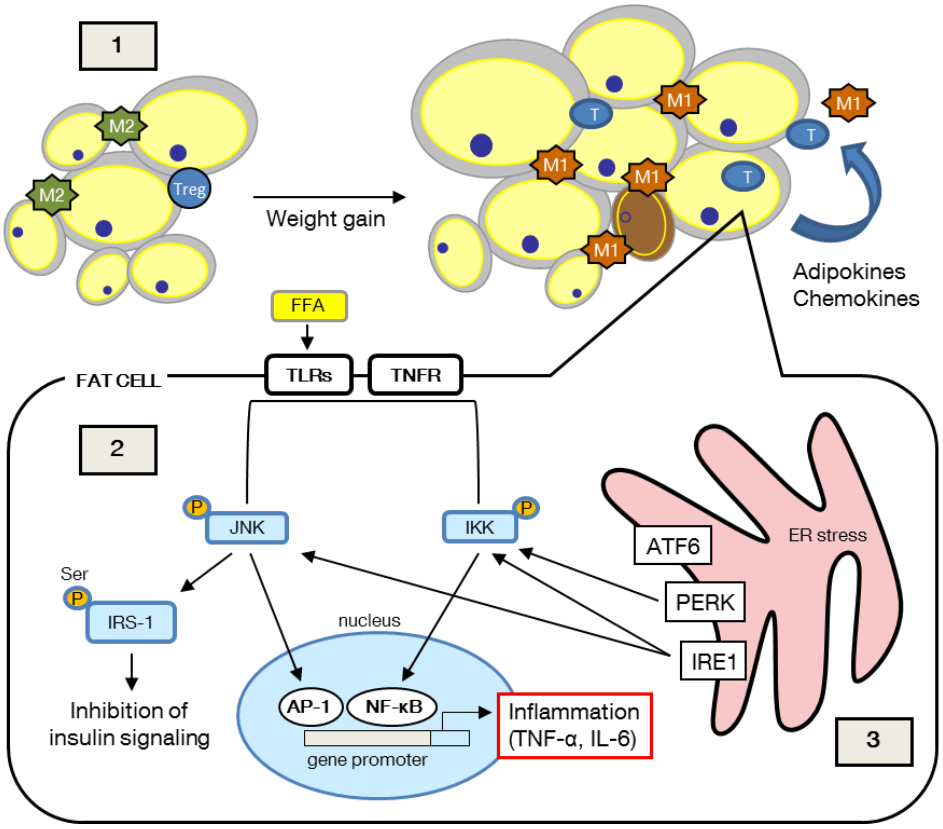


Figure 3: Mechanisms underlying obesity-induced inflammation in adipose tissue **1:** Weight gain is associated with expansion of adipose tissue, *i.e.*, an increase in both the number (hyperplasia) and size (hypertrophy) of fat cells (adipocytes). Hypertrophic adipocytes secret higher amounts of chemokines, which drive infiltration of circulating leukocytes. Hence, weight gain is characterized by the presence of a substantial number of activated macrophages (M1) and T-cells (T) in adipose tissue, which are involved in adipocyte necrosis (brown cell in the picture). These immune cells are able to produce more inflammatory mediators such as tumor necrosis factor (TNF), contributing to the state of chronic low-grade inflammation in obesity via signaling through the TNF receptor (TNFR). **2:** Inflammation can also be triggered by nutrients, *e.g.*, free fatty acids (FFA). FFA can bind to Toll-like receptor-4 (TLR-4), resulting in pro-inflammatory responses via c-Jun N-terminal kinase (JNK) and nuclear factor kappa B (NF-κB) signaling cascades. JNK activation triggers also phosphorylation of the insulin receptor substrate (IRS-1) at the inhibitoring site Ser-307, thereby suppressing insulin signaling cascades. **3:** Furthermore, stress of the endoplasmatic reticulum (ER), *i.e.*, activation of three adaptative signaling pathways via IRE1 (inositol-requiring enzyme 1), PERK (PKR-like ER kinase) and ATF6 (activating transcription factor 6) after accumulation of un- or misfolded proteins in the ER, can be triggered by metabolic surplus. These signaling cascades are known as the unfolded protein response (UPR), which is linked to inflammatory responses via JNK- and NF-κB-dependent mechanisms.

As proposed above, cellular dysfunctions like chronic activation of the NF-κB signaling and the UPR can affect adipocytes, thereby contributing to the development of a pro-inflammatory phenotype. However, other cell types play a major role in the chronic inflammatory state underlying obesity, namely immune cells infiltrating adipose tissues.

1.4 Infiltration of Immune Cells into Adipose Tissue

Adipose tissue contains mature adipocytes, preadipocytes, endothelial cells, fibroblasts and immune cells, *e.g.* macrophages (Rutkowski et al. 2009). Adipose tissue expansion in form of hyperplasia (increased number of cells) or hypertrophy (increased cell size) leads to hypoxia, adipocyte cell death, enhanced chemokine expression and dysregulation of fatty acid fluxes (Sun et al. 2011). These consequences require macrophages to create a permissive environment for remodeling processes like removal of cell debris and vascularization of the extracellular matrix. In the obese state, adipose tissue is markedly infiltrated by immune cells, including neutrophils, T-cells and macrophages, the latter being numerically and functionally dominant (**Figure 3**) (Weisberg et al. 2003; Kintscher et al. 2008; Lolmède et al. 2011).

1.4.1 Macrophages

Macrophages that reside in adipose tissue display remarkable heterogeneity in their activities (Gordon and Taylor 2005). In obesity, a phenotypic shift from an anti-inflammatory "alternatively activated" M2 form to a more pro-inflammatory "classically activated" M1 form has been observed (Lumeng et al. 2007). In lean individuals, resident macrophages are polarized towards the M2 state generated after exposure to cytokines (IL-4, IL-13) from T-helper 2-cells (Th2-cells) and to adiponectin resulting in high levels of anti-inflammatory IL-10 and arginase, an inducible nitric oxide synthase (iNOS) activity blocker (Lumeng et al. 2007; Odegaard and Chawla 2011). The obesity-driven shift into an M1 phenotype with enhanced production of pro-inflammmatory TNF, IL-6, and IL-12 (Lumeng et al. 2007) is due to the migration of inflammatory monocytes from the circulation, and not to the conversion of resident M2 macrophages (Lumeng et al. 2008). Monocytes that express the chemokine receptor 2 (CCR2) are preferentially attracted to adipose tissue and differentiate to pro-inflammatory macrophages (Odegaard and Chawla 2011). CCR2 is the receptor for MCP-1, an adipokine secreted by hypertrophic adipocytes (see section 1.4.3.2). Macrophage

activation can be triggered on the one hand directly by dietary saturated fatty acids via TLR-2 and TLR-4 (Suganami et al. 2007) and on the other hand by chemotactic factors secreted by adipocytes, *e.g.*, MCP-1 (Chawla et al. 2011), Th1 cytokines (interferon-gamma (IFN-γ)) or by bacterial products (lipopolysaccharide (LPS)) (Lumeng et al. 2007).

In humans, macrophage infiltration correlates with adipocyte size and BMI (Weisberg et al. 2003). Moreover, a reduced number of macrophages in subcutaneous white adipose tissue and increased expression of the anti-inflammatory cytokine IL-10 supporting a phenotypic shift towards M2 have been observed in subjects after surgery-induced weight loss (Cancello et al. 2005). With respect to central obesity, there is a preferential infiltration of macrophages into omental *vs*. subcutaneous fat (Cancello et al. 2006). Furthermore, increased macrophage infiltration is associated with hepatic lesions, systemic arterial dysfunctions and IR in obese subjects (Cancello et al. 2006; Apovian et al. 2008).

1.4.2 T-cells

Recent studies have reported that T-lymphocytes (T-cells) infiltration in adipose tissue is a primary event in inflammation and the development of IR (Wu et al. 2007; Kintscher et al. 2008). In humans, there is a positive correlation between T-cell number in adipose tissue and the degree of adiposity, with greater accumulation in visceral than in subcutaneous fat depots (Duffaut et al. 2009). Chemokines, *e.g.*, RANTES and interferon-inducible protein-10 (IP-10), produced by adipose tissue are key regulators of T-cell attraction via binding to specific receptors, *e.g.*, CCR5 (**Figure 4**) (Wu et al. 2007; Krinninger et al. 2011). While Th1-cells secrete pro-inflammatory cytokines like IFN-γ, which attracts additional monocytes into the adipose tissue, Th2-cells produce anti-inflammatory IL-4 and IL-13 (Chawla et al. 2011). In lean mice, both subtypes of T-cells are abundant in adipose tissue. In contrast, there is a shift towards higher Th1-cell numbers in diet-induced obesity but no change in Th2-cell numbers (Strissel et al. 2010). Regulatory T-cells (Treg) that express high levels of anti-inflammatory IL-10 are also enriched in visceral adipose tissue in lean mice (Feuerer et al. 2009). In humans, greater visceral tissue depots correlate with reduced number of Treg (Deiuliis et al. 2011).

The physiological reason underlying the abundance of T-cells in adipose tissue from obese individuals is that adipocytes which reach a maximal triglyceride storage capacity may undergo necrotic cell death (Strissel et al. 2007). Infiltrating M1 macrophages contribute to

the clearance of cell debris and to remodeling of the extracellular matrix, which allows differentiation of new adipocytes. Under chronically increased nutrient intake, more adipocytes undergo necrosis, given rise to an antigenic pool that can favor stimulation of adaptive immune cells in major histocompatibility complex (MHC) class I- and II-dependent manners. In mice, blockage of cluster of differentiation (CD) 40, a co-stimulatory molecule involved in antigen presentation, could prevent T-cell infiltration into the adipose tissue and normalized metabolic changes associated with obesity (Poggi et al. 2011). *In vitro*, activated T-cells were shown to inhibit preadipocyte-to-adipocyte differentiation (adipogenesis) and thus reduced triglyceride accumulation. This may lead to increased ectopic triglyceride deposition in liver and skeletal muscles and cause IR in these organs (Wu et al. 2007).

As briefly mentioned above, the attraction of immune cells, mainly macrophages and T-cells, to adipose tissue during inflammatory processes underlying obesity is under the control of chemotactic cytokines referred to as chemokines. These molecules are introduced in the following section.

1.4.3 Adipokines, Chemokines and Chemokine Receptors: Triggers for the Attraction of Immune Cells into Adipose Tissue

Chemokines are low molecular weight proteins (8 to 10 kDa) involved in leukocyte recruitment (Luster and Ravetch 1987). They are subdivided into families depending on the nature of cysteine residues in the mature form of proteins. For the CXC chemokines, *e.g.*, interferon-inducible protein 10 (IP-10), cysteine residues are separated by a single amino acid (CXC). For the CC chemokine, *e.g.*, regulated upon activation normal T-cell expressed and secreted (RANTES) and monocyte chemoattractant protein (MCP-1), the cysteine residues are adjacent (CC). Chemokines can be secreted by several cell types given the appropriate stimulus, *e.g.*, IFNγ for IP-10 secretion (Herder et al. 2007a) or TNF for MCP-1 secretion by adipocytes (Fasshauer et al. 2004). Under inflammation, chemokines are detected in many organs like the skin, brain, kidneys, joints, blood vessels, gastrointestinal tract and adipose tissue (Luster 1998; Herder et al. 2007a; Madani et al. 2009). Each chemokine interacts with one or more receptors and thereby affects one or more cell types (**Figure 4**).

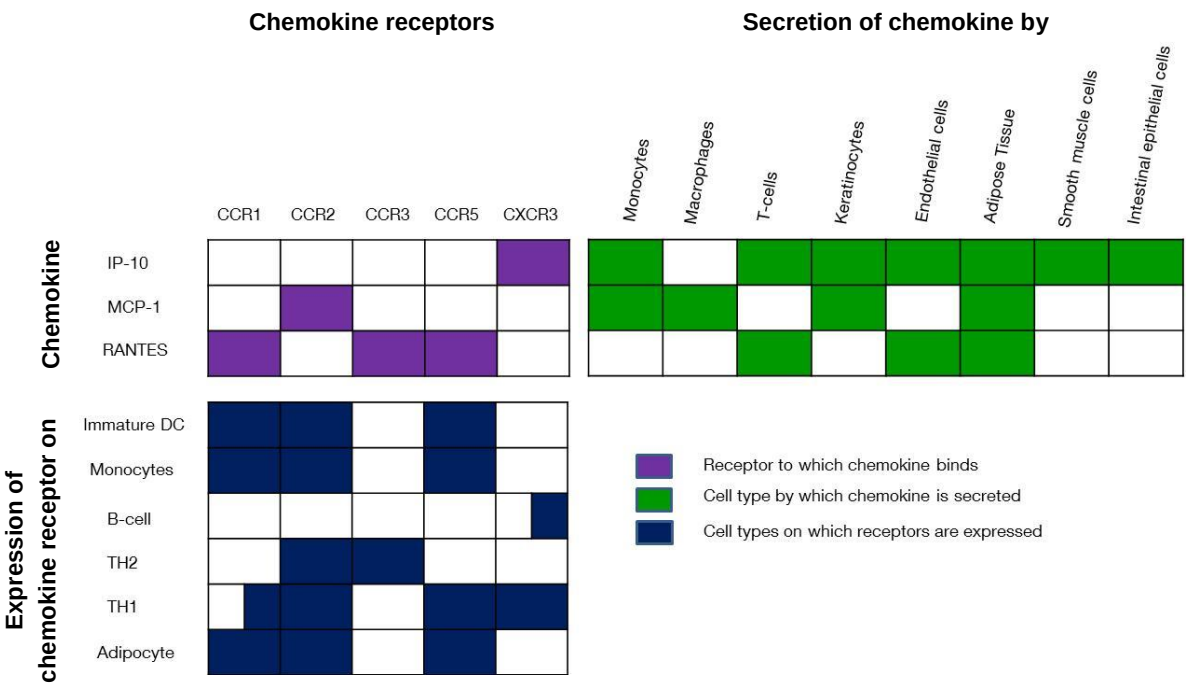


Figure 4: Human chemokine/receptor specificity, expression profile and secretion. The boxes are color-codeded as follows: purple indicates to which receptor type the chemokine of interest binds (*e.g.*, IP-10 binds to receptor type CXCR3); green indicates by which cell types the chemokine of interest is secreted (*e.g.*, RANTES is secreted by endothelial and T-cells as well as adipose tissue); dark blue indicates on which cell types chemokine receptors are expressed (*e.g.*, CCR3 is expressed on Th2-type cells). Half-filled boxes indicate that the given chemokine receptor is expressed only on a subset of target cells. Abbreviations: CCR, C-C motif receptor; CXCR, C-X-C motif receptor; IP-10, Interferon-inducible protein-10 kDa; MCP-1, Monocyte chemoattractant protein-1; RANTES, Regulated upon Activation normal T-cell expressed and secreted; DC, dendritic cells; Th, T helper-cell; B-cell, B lymphocyte; T-cell, T lymphocytes

In obesity, there is upregulation of several CC chemokines and their respective receptor in adipose tissue (Huber et al. 2008). These receptors are members of the G-protein-coupled receptors, which signal through the second messenger cyclic adenosine-mono-phosphat (cAMP) or phospholipase C after adenylate cyclase activation and eventually give rise to inositol triphosphate (IP3) and calcium release. Second messengers also activate further pathways that affect cell metabolism, motility, gene expression and cell division. Depending on the nature of the receptor, its G proteins and targeted downstream signals, cell activation by chemokines leads to a variety of effects in different cell types. Thus, chemokines act in a broad manner and regulate lymphoid trafficking, lymphoid organ development, wound healing, Th1/Th2-cell development, angiogenesis, metastasis, cell recruitment, and inflammation (Rossi and Zlotnik 2000). The attraction of immune cells to sites of acute infection or inflammation is a beneficial process that becomes however detrimental in the case of chronic inflammation. Because chemokines act in concert with

many other cytokines and control the infiltration of inflammatory cells, they are potential target molecules for therapeutic interventions. The following paragraphs provide information on adipokines and chemokines of relevance to the present work.

1.4.3.1 Interferon-inducible Protein 10 kDa (IP-10)

The CXC chemokine IP-10 (CXCL10) was one of the first chemokines identified (Luster 1985). It binds and activates the receptor CXCR3 located on the surface of leukocytes. CXCR3 was found to be expressed at high levels on helper Th1 cells but at low levels on Th2 cells (Sallusto et al. 1998). IP-10 is induced by the typical Th1 cytokine IFN-γ, which hints at an amplification loop involving Th1 cells and chemokine-secreting immune, endothelial and epithelial cells resulting in the attraction of more CXCR3-expressing Th1 cells (Lacotte et al. 2009).

IP-10 is produced by numerous different cell types, including endothelial, smooth muscle, respiratory and intestinal epithelial cells, keratinocytes and leukocytes (Thalmann and Meier 2007). It has also been demonstrated that mature human adipocytes express and secrete IP-10 and that expression can be induced by IFN-γ (Herder et al. 2007a). Recently, data from our group showed that IP-10 is up-regulated in visceral *vs.* subcutaneous adipose tissue in obese mice (Krinninger et al. 2011). This supports the hypothesis that visceral fat depots mainly contribute to the inflammatory state in obesity. In the 3T3-L1 cell line and in primary human adipocytes, the NF-κB pathway plays a central role in IP-10 regulation. Furthermore, addition of an IP-10-neutralizing antibody to adipocyte culture supernatants could inhibit T-cell migration (Krinninger et al. 2011), implying that IP-10 plays a functional role in modulating levels of T-cells in adipose tissues. Another finding linking obesity and chronic inflammation arises from the observation that leptin can induce the expression and secretion of IP-10 in monocytes (Meier et al. 2003). Since leptin is an adipocyte-derived hormone, one can speculate that hyperleptinemia in obesity and cardiovascular disease is linked to increased IP-10 secretion by monocytes (Meier et al. 2003).

Although human adipocytes secrete IP-10, the association between IP-10 release and donor BMI is not straightforward (Herder et al. 2007a). In a study focused on chemokine measurement in samples from the MONICA/KORA cohort, there was only a modest correlation ($r = 0.125$, $p < 0.001$) between serum levels of IP-10 and the BMI of participants (Herder et al. 2006a). The significant correlation between IP-10 levels and incidence of T2DM was lost after adjustment for clinical and metabolic parameters, such as BMI. In a

cross-over study in adolescent subjects, higher IP-10 levels were associated with higher BMI (regression coefficient 0.02, $p < 0.001$) but only a weak correlation with fasting insulin levels was observed (regression coefficient 0.148, $p < 0.05$) (Herder et al. 2007b).

1.4.3.2 Monocyte chemoattractant Protein-1 (MCP-1)

MCP-1 (CCL2) can be secreted by fibroblasts, endothelial cells, vascular smooth muscle cells, monocytes, T-cells and adipocytes after induction by oxidative stress, cytokines (TNF and IL-6) or growth factors (Deshmane et al. 2009; Sartipy and Loskutoff 2003; Christiansen et al. 2005). The MCP-1 receptor, CCR2, is expressed on several cell types, including adipocytes (Rollins 1997; Gerhardt et al. 2001). CCR2 can respond to several ligands, *e.g.*, MCP-1, CCL 7, 12 and 13 but it is the only receptor known for MCP-1 (Charo and Ransohoff 2006). In obese subjects, expression of MCP-1 and CCR2 was higher in adipose tissue compared to lean individuals and the expression correlated with the macrophage marker CD68 (Huber et al. 2008; Bruun et al. 2005).

The essential role of MCP-1 and its receptor CCR2 has been demonstrated by experimental studies in knockout mice. Macrophage accumulation in adipose tissue induced by high fat feeding occurs in wild-type mice but not in MCP-1 homozygous knockout mice (Kanda et al. 2006). Furthermore, CCR2 deficiency reduces macrophage number and inflammatory profile in adipose tissue, increases adiponectin expression, and improves systemic glucose homeostasis and insulin sensitivity (Weisberg et al. 2006). A chronic increase in circulating levels of MCP-1 in mice induces macrophage infiltration into adipose tissue, increases hepatic triacylglycerol content and induces IR (Kamei et al. 2006; Tateya et al. 2010). In addition, MCP-1 was shown to reduce insulin-stimulated glucose uptake by adipocytes, pointing at its functional role in the development of IR (Sartipy and Loskutoff 2003). In diabetic patients, CCR2 expression on monocytes was found to be elevated (Mine et al. 2006). However, molecular mechanisms underlying the role of MCP-1 in diabetes are not yet fully understood. MCP-1 has been proposed to trigger ER stress, which has been associated with disturbances in insulin-dependent signaling pathways and the development of obesity and T2DM (Oezcan et al. 2004; Kolattukudy and Niu 2012).

In humans, serum MCP-1 levels were found to be significantly higher in obese subjects and positively correlated with BMI, waist circumference, homeostasis model assessment-IR (HOMA-IR, insulin resistance index) and c-reactive protein (CRP, classical acute phase

protein). Furthermore, MCP-1 levels correlated negatively with HDL-cholesterol (Kim C.S. 2006). In a large population-based cohort, plasma MCP-1 correlated with risk factors for atherosclerosis (*e.g.*, smoking, hypertension, hypercholesterolemia, diabetes) (Deo et al. 2004). Endothelial expression of MCP-1 is thought to initiate sub-endothelial migration of monocytes to atherosclerotic lesions where they differentiate into foam cells after uptake of oxidized LDL-cholesterol (Nelken et al. 1991). Oxidized LDL by itself is able to induce MCP-1 production by vascular and smooth muscle cells, suggesting that MCP-1 may be a molecular link between oxidized lipoproteins and foam cell recruitment. These observations suggest that MCP-1 is implicated in vascular dysfunctions caused by obesity.

1.4.3.3 Regulated upon Activation normal T-cell expressed and secreted (RANTES)

The chemokine RANTES (CCL5) predominantly mediates chemotaxis of T-cells, but also monocytes (Ding et al. 2000; Keophiphath et al. 2010). It is mainly secreted by endothelial, smooth muscle and activated T-cells, but also by macrophages and adipocytes (Thalmann and Meier 2007; Skurk et al. 2009). As in the case of MCP-1, RANTES expression can be induced by TNF in human adipocytes (Wu et al. 2007). RANTES is able to bind to different receptors, *e.g.*, CCR1, 3, 4 and 5 (Appay and Rowland-Jones 2001). Whereas CCR1 and CCR5 are predominantly expressed on monocytes and Th1 cells, CCR3 is mainly expressed on Th2 cells. Other non-immune cells like vascular smooth muscle cells (Schecter et al. 2000) and adipocytes (Gerhardt et al. 2001) also carry CCR5, the gene expression of which together with CCR1 and CCR3 seems to be higher in adipose tissue from obese patients compared to lean subjects (Huber et al. 2008).

The release of RANTES is dependent on adipocyte size in omental tissue and correlates positively with donor BMI (Skurk et al. 2009). Gene expression in adipose tissue and serum concentrations of RANTES seem to be higher in obesity and are associated with increased macrophage infiltration and systemic inflammatory markers like TNF and IL-6 (Huber et al. 2008; Keophiphath et al. 2010). This indicates that RANTES is likely to play an important role in obesity-driven inflammatory processes. Importantly, RANTES expression in white adipose tissue correlates with markers of the pro-inflammatory phenotype of macrophages (M1), which are likely the major cellular source of RANTES (Keophiphath et al. 2010). Blocking of RANTES reduces T-cell chemotaxis induced by media from adipose

tissue in obese mice (Wu et al. 2007). There is also a strong correlation between RANTES and CD3 (T-cell marker) as well as CD11b (macrophage marker) mRNA expression in human visceral adipose tissue, which hints at the involvement of RANTES in T-cell and macrophage recruitment (Wu et al. 2007).

RANTES production by fibroblasts, platelets and macrophages is a feature of atherosclerosis (Eriksson 2004) and contributes to transendothelial migration of monocytes and T-cells in atherosclerotic lesions via the receptors CCR1 and CCR5 (Zernecke et al. 2008). Blockade of signaling pathways using a RANTES antagonist (Met-RANTES) reduces the progression of atherosclerosis and inhibits leukocyte infiltration into lesions in a hypercholesterolemic mouse model (Veillard 2004). Systemic levels of RANTES were shown to be higher in patients with T2DM and impaired glucose tolerance (IGT) (Herder et al. 2005). This may represent rather a consequence than a cause of hyperglycemia since neither genotypes nor serum levels were associated with T2DM incidence (Herder et al. 2008).

1.4.3.4 Progranulin (PGRN)

PGRN is a secreted precursor glycoprotein consisting of a signal sequence and several granulin-like domains composed of repeats of 12 cysteine motifs (de Muynck and van Damme 2011). PGRN is often expressed during tissue remodeling when cells are dividing and actively migrating (Bateman and Bennett 2009). Hence, it plays a critical role in early embryogenesis (Desmarais et al. 2008), wound healing/tissue repair (He et al. 2003), inflammation (Zhu et al. 2002), and tumorigenesis (Ong and Bateman 2003). Mutations in the PGRN gene cause frontotemporal dementia (van Deerlin et al. 2010) suggesting that PGRN can protect neurons from premature death (Bateman and Bennett 2009).

The full-length PGRN protein can be proteolytically cleaved into small subunits called granulins (GRN) by metalloproteinases, proteinase 3 and neutrophil-related elastase (Kessenbrock et al. 2008). While PGRN has anti-inflammatory properties via inhibition of TNF-induced activation of neutrophils, some of the cleaved GRN promote inflammation by eliciting secretion of IL-8 and TNF (Zhu et al. 2002). The balance between PGRN and the smaller GRN peptides is maintained by the secretory leukocyte protease inhibitor (SLPI), which is released by neutrophils after inflammatory stimuli (via TNF for instance), binds to PGRN and protects it against cleavage (Zhu et al. 2002). Although it is known that various PGRN isoforms have different biological activities, it is still unclear whether effects are

mediated by several specific receptors or by one receptor interacting with different binding partners. It has been recently demonstrated that PGRN can bind to TNF receptors (TNFR), thereby disturbing TNF-TNFR interactions and preventing inflammatory arthritis in mice (Tang et al. 2011).

Very recently, PGRN was proposed to promote IR and obesity via IL-6-dependent pathways in mice fed a high-fat diet (Matsubara et al. 2012). PGRN was detected in macrophages and in the cytoplasm of adipocytes and levels were increased in adipose tissues from mice fed the high-fat diet. IL-6 expression in adipose tissue is known to contribute to chronic inflammation (Senn et al. 2002; Shoelson et al. 2007) and increased concentrations can be detected in obese diabetic subjects (Kern et al. 2001). In human atherosclerotic plaque**,** the expression of PGRN was found to reduce inflammation possibly due to its ability to suppress MCP-1-induced chemotaxis of monocytes, while degradation into GRN enhanced the inflammatory status (Kojima et al. 2009). In a human cross-sectional study, PGRN serum levels were higher in individuals with T2DM compared to NGT (normal glucose tolerant) and obese subjects with predominant visceral fat distribution (Youn et al. 2009). In addition, circulating PGRN correlated with BMI, CRP, glycosylated hemoglobin (HbA1c), total cholesterol and with macrophage accumulation in omental adipose tissue. This suggests that PGRN may be a novel marker of chronic inflammation in obesity and T2DM, which reflects macrophage infiltration into adipose tissue.

1.4.3.5 Adiponectin and its High Molecular Weight (HMW) Isoform

Adiponectin is a 30 kDa adipocyte-derived protein consisting of four domains: an N-terminal signal peptide, a variable domain, a collagenous domain and a globular domain that binds to receptors (Yamauchi et al. 2003). During secretion, adiponectin is modified extensively at the post-translational level, including glycosylation and hydroxylation of different prolin and lysine residues (Richards et al. 2006). These post-translational modifications influence the functional properties of adiponectin by enhancing its ability to form multimers (Richards et al. 2006; Wang 2006). The monomeric form of adiponectin has not yet been detected under native conditions (Wang et al. 2008). In the blood, adiponectin is present in trimeric (low molecular weight, LMW), hexameric (medium molecular weight, MMW) and oligomeric (high molecular weight, HMW) complexes (Kadowaki 2005). A globular cleavage product of full length adiponectin, likely generated by monocyte-derived elastase, has also been detected in human plasma (Fruebis et al. 2001; Waki et al. 2004), even though its presence is still

controversial (Fang and Sweeney 2006). Globular adiponectin was shown to strongly activate NF-κB, thereby inducing the expression of pro-inflammatory genes (Tomizawa et al. 2008). Impaired multimerization of adiponectin is associated with T2DM, which indicates that multimeric forms are crucial for biological activity (Waki et al. 2003). Higher HMW levels, but not total adiponectin, were correlated with improvement of metabolic indices (increased insulin sensitivity, high basal lipid oxidation, higher HDL level) (Lara-Castro et al. 2006; Fujimatsu et al. 2009). Taken together, HMW deficiency may be an important factor that links obesity to medical complications.

Two isoforms of the adiponectin receptor have been detected so far, AdipoR1 and AdipoR2. In humans, both receptors are ubiquitously expressed in body tissues (Buechler et al. 2010), with particularly high levels in skeletal muscle (Civitarese et al. 2004). Conversely, in mice, AdipoR1 is predominantly expressed in skeletal muscle and AdipoR2 mostly in the liver (Kadowaki 2005). Obesity is characterized by decreased expression of both receptors resulting in reduced adiponectin sensitivity, which might lead to IR (Nannipieri et al. 2007). In non-diabetic Mexican Americans, insulin sensitivity and the expression of both receptors were lower in subjects with a family history of T2DM (Civitarese et al. 2004). In diabetic patients, AdipoR1/2 expression seems to be decreased in skeletal muscle (Debard et al. 2004). In contrast, in insulin-resistant obese subjects, receptor expression increased in the liver (Felder et al. 2010), which may partly reflect compensatory mechanisms triggered by lower adiponectin concentrations.

Blood concentration of adiponectin was found to correlate negatively with BMI (Arita et al. 1999). Circulating levels are decreased in obesity (Arita et al. 1999; Torigoe et al. 2007) and inversely correlate with IR (Hotta et al. 2000; Weyer et al. 2001; Steffes et al. 2004). Furthermore, adiponectin mediates anti-atherogenic effects by preventing macrophage transformation into foam cells (Ouchi et al. 2001). Hypoadiponectemia is associated with coronary artery disease (Kumada et al. 2002) and low plasma levels correlate with atherosclerotic risk factors, such as high LDL-cholesterol and triglyceride levels (Kazumi et al. 2004). The impact of adiponectin on molecular mechanisms underlying inflammatory responses is still controversial. Adiponectin interfers with NF-κB signaling, but the HMW form seems to be less active when compared to the globular form (Tomizawa et al. 2008). Incubation of inflamed adipocytes with adiponectin resulted in a lower expression of MCP-1 and IL-6 and NF-κB activity was reduced by 50 % (Zoico et al. 2009). Because adiponectin receptors are also expressed on immune cells (Alberti et al. 2007; Weigert et al. 2008; Pang

and Narendran 2008), adiponectin can also influence the network of infiltrating monocytes/macrophages into adipose tissue in obesity. The abundance of CCR2 and CCR5 on the surface of primary human monocytes was found to be decreased after incubation with adiponectin (Neumeier et al. 2011). *In vitro*, adiponectin was also able to prime human monocytes into the anti-inflammatory phenotype (M2 macrophages) (Lovren et al. 2010).

The insulin-sensitizing action of adiponectin is mediated primarily by activation of AMP-activated protein kinase (AMPK) and peroxisome proliferator activator receptor α (PPARα), resulting in fatty acid oxidation and glucose uptake in liver and muscle cells (Yamauchi et al. 2002). Recently, a protein called adaptor protein, phosphotyrosine interaction, PH domain and leucin zipper containing 1 (APPL1) was shown to interact with the cytoplasmic domain of AdipoR1 (Buechler et al. 2010; Tian et al. 2012). APPL1 levels were increased in T2DM and obesity and decreased with weight loss (Holmes et al. 2011), which is suggestive of compensatory mechanisms for lower adiponectin levels in the obese state.

1.5 Aim of the study

To manage the current rate of overweight and obesity, there is a real need to find methods of treatment that are effective in the long-term. A recurrent problem in weight loss programmes is that compliance decreases overtime. For this purpose, the Weight Watchers (WW) Global Efficacy Trial has been set up.

The primary aim of the WW trial was to investigate whether referral to a commercial programme by general practitioners (GP) is a better strategy for effective weight loss success than standard care alone.

Moreover, it is nowadays proven that white adipose tissue is an endocrine organ secreting a variety of biologically active factors called adipokines. Adipokines can act in a pro-inflammatory manner leading to a state of constant low-grade inflammation thought to be a key event in the pathogenesis of chronic disorders linked to obesity. Some adipokines, referred to as chemokines, function as chemottractants of immune cells, which in turn infiltrate into adipose tissue and maintain a pro-inflammatory status. During weight loss, the migration of immune cells into adipose tissue is reversed.

The second aim of our study was to assess whether weight loss leads to changes in circulating blood levels of pro- and anti-inflammatory adipokines/chemokines.

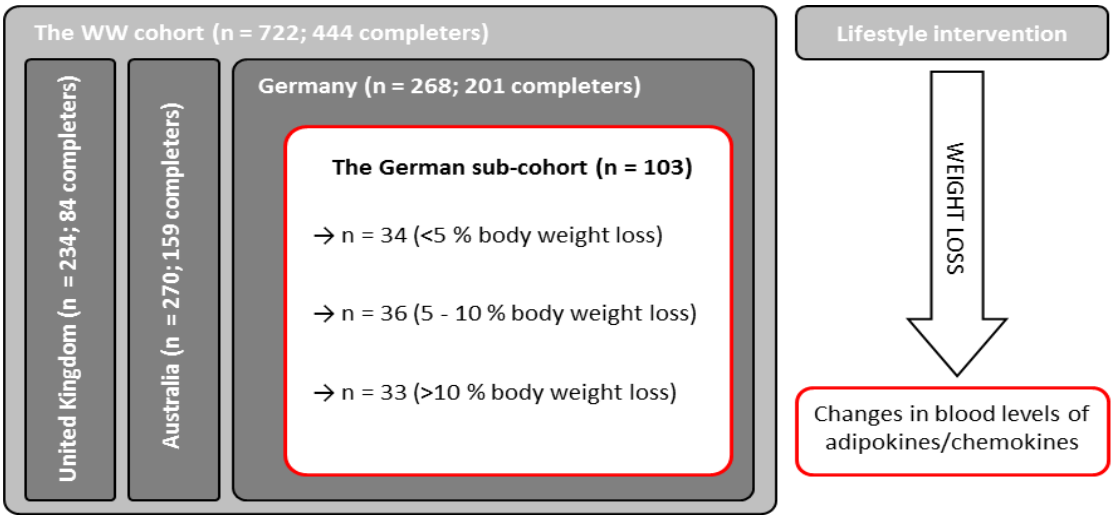


Figure 5: Schematic view of the study cohort and the hypothesis underlying the present work. The WW study was carried out in cooperation between three study centers in the UK, Australia and Germany. In Germany, a number of 103 participants (the German sub-cohort) was selected on the basis of body weight loss categories in order to test the hypotehsis that blood concentrations of factors secreted by adipose tissue (adipokines/chemokines) vary during weight loss induced by a lifestyle intervention.

2 Materials and Methods

2.1 Design of the Study

The “Weight Watchers (WW) Global Efficacy Trial” was a multi-centre randomised controlled trial sponsored by WW (Weight Watchers International, Inc, New York) and coordinated by the Medical Research Concil (MRC) in Cambridge (UK). The primary purpose was to examine the effectiveness of the WW programme for weight loss compared to current standard care, as per national guidelines, in three countries: the UK (MRC, Dr. S. Jebb), Australia (Boden Institute of Obesity, Prof. I. Caterson) and Germany (Else Kroener-Fresenius-Centre for Nutritional Medicine (EKFZ), Prof. H. Hauner). The study protocol, including all documents for participants, was approved by the ethics review committee in charge in each country (in Germany, by the ethics committee of the Technische Universität München). The study was performed in accordance with the ethical principles in the Declaration of Helsinki 2000 and applicable regulatory requirements. All participants gave their written informed consent prior to starting the study. The data obtained during the study were stored in a computer database and the subjects were identified by initials and subject numbers only. The study details have already been published (Jebb et al. 2011). A total of 772 participants were recruited across the three countries.

Throughout the rest of the manuscript, detailed information related to the study design is given only for the German cohort, since samples from this cohort were used to study systemic inflammatory markers. The study flow chart for the first year of intervention is shown in **Figure 6.** The detailed schedule of study procedures is listed in **Appendix C**. In Germany, 268 participants who were deemed eligible for randomisation (see criteria below in Chapter 2.2) after an initial medical screening (including blood lipid profile, medication history) were allocated to a treatment group: Commercial Programme or Standard Care (CP or SC). Random group allocation (considering gender and T2DM status) was done automatically upon entry of the participants’ details in the study database. After Visit A, all participants attended five visits (month 2, 4, 6, 9, 12) at their general practitioners (GP) for a health check and weight control. The follow-up visits over an additional period of twelve months (month 18 and 24) for the sake of weight measurement were optional for study participants. During this follow-up period, participants were free to choose a method to control their body weight.

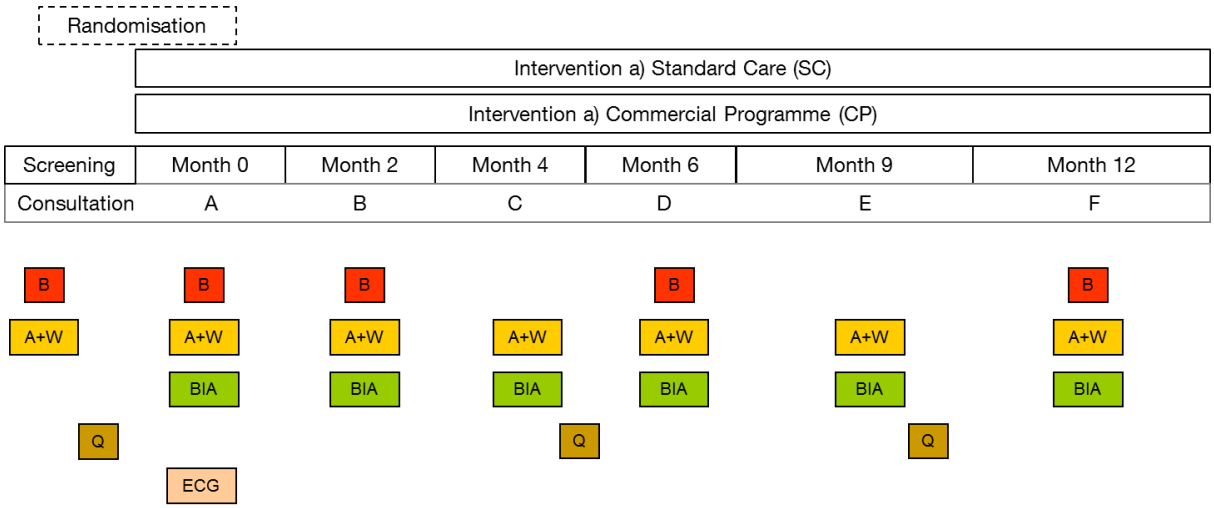


Figure 6: Study flow chart for the first year of intervention showing a summary of outcome measurements. Abbreviations: B, blood sample; A+W, health check-up and weight control; BIA, bioimpedance analysis (measurement of body composition); Q, questionnaires (dietary intake, physical activity); ECG, electrocardiogram.

2.2 Inclusion and Exclusion Criteria

The recruitment of participants took place at multiple GP offices in Munich during regular visits. GPs assessed the eligibility of patients using screening questionnaires. Patients gave their informed consent to participate in a weight loss program and, if allocated to the CP group, to attend weekly meetings for the time of the study. Inclusion and exclusion criteria are listed in **Table 1** and **2.**

Table 1. Inclusion criteria for the study

Inclusion criteria	
Gender	male or female
Age	≥ 18 years
BMI	27 – 35 kg/m^2
Answer YES for at least one of the risk factors	- family history of T2DM - stable T2DM; not treated with insulin - previous gestational diabetes mellitus - impaired glucose tolerance/fasting glycaemia - mild-moderate dyslipidemia or corresponding treatment - treatment for hypertension - central adiposity (waist circumference >88 cm in women or >102 cm in men) - polycystic ovarial syndrome/infertility without cause other than overweight - osteoarthritis in lower limbs - abdominal hernia

Table 2. Exclusion criteria for the study

Exclusion criteria	
Factors that might affect weight	- weight loss of >5 kg over the previous 3 months - history of clinically-diagnosed eating disorder - orthopaedic limitations preventing participation in regular physical activity - untreated thyroid disease or >1 change in thyroid medication over the last 6 months - medication with known effects on appetite or weight - use of oral steroids - chronic/inflammatory gastrointestinal disorders (irritable bowel syndrome was acceptable) - previous surgical procedure for weight loss - major surgery within the previous 3 months - pregnancy or lactation

Exclusion criteria (continued)	
Co-existing disease	- insulin-treated diabetes mellitus - HbA1c (define) >9.0 % - diagnosis of T2DM in the previous 6 months - heart problems within the previous 3 months (*e.g.* angina, myocardial infarction, stroke) or implanted cardiac defibrillator or pacemaker - uncontrolled hypertension (>160/95 mmHg) - start of a new prescription medication within the last 3 months - change in dosage of a prescription medication within the last month - history or presence of cancer (completely resected basal or squamous cell carcinoma acceptable if treatment completed more than 6 months prior to enrolment)
Additional excluded medication	- weight-loss medications - other drugs for weight reduction including herbal preparations - neuroleptics - prolonged use of laxatives - gastrointestinal prokinetic drugs - antidepressants/psychotropic medications with appetite effects - participation in another trial up to 30 days before enrolment

2.3 Intervention program: Commercial Programme or Standard Care

Participants randomized to the CP group followed the standard WW programme offered by local meetings. Furthermore, they had free access to WW “eSource”, which is an internet platform for users to chart their weight loss, to obtain weight loss tips and recipes and to communicate with other companions. Participants randomized to the GP group sought advice for weight loss from their GP. The intervention programmes are described in detail in **Appendix A and B**. Both programmes were free of charge for the participants and their health insurances. For each bioimpedance (BIA) measurement, a participant received 15 EUR. The quarterly practise fee was refunded if a participant finished the study successfully. The same applied to the follow-up period. The GP office received 250 EUR per participant who fulfilled visit A and 250 EUR per participant who completed the study (visit F). For the follow-up period, the GP received additional 25 EUR per patient and visit.

2.4 Study procedures

Body height (cm) was measured using a stadiometer (standard GP equipment) with an accuracy of 0.5 cm. Body weight (kg) was assessed using GP regular scales to the nearest of 0.1 kg. If not available, scales were provided to GP (HD 327 S, Tanita Europe B.V., Hoofddorp, The Netherlands). BMI (kg/m^2) was calculated from the recorded weight and height using the following equation: $weight/height^2$. Waist circumference (cm) was measured using a non-stretchable measuring tape midway between the top of the iliac crest and the most inferior part of the rib cage. Bioimpedance analysis (BIA) was done using a BC-418 Body Composition Analyser (Tanita Europe B.V., Hoofddorp, The Netherlands). Participants were barefoot and dressed in light clothing. Blood pressure (mmHg) and radial pulse rate (beats per minute) were measured under standardised conditions. A 12-lead ECG was recorded using GP's standard conditions.

2.5 Sample Collection and Standard Laboratory Testing

Blood sampling took place at the GPs and samples were transported by a collector to be analysed at the "Synlab Labordienstleistungen München". Fasting blood samples (max. 20 ml) were collected for analysis of the biochemical parameters listed in **Table 3.** The methods used for measurement of the biochemical parameters at Synlab are listed in **Appendix D.** In addition, one ethylendiaminetetraacetic acid (EDTA)- and one serum-tube were collected for investigation of genetic variants and measurement of adipokine and chemokine concentrations, respectively. The extra serum samples were kept at room temperature for a maximum of 4 h before aliquoting and immediate storage at -80 °C.

Table 3. Blood parameters measured at each visit.

Month	-1	0	2	6	12
Visit	Screening Visit	Baseline Visit A	Visit B	Visit D	Visit F
Fasting glucose [a]	+	+	+	+	+
Fastin insulin [a]	+	+		+	+
Full lipid profile [b]	+	+	+	+	+
hsCRP		+		+	+
HbA1c [c]	+	+		(+)	(+)
Liver function [d]		+			
Kidney function test [e]		+			
TSH	+				
Adipokines / chemokines		**+**		**+**	**+**
DNA collection		+			

[a] Insulin resistance was estimated using the homeostatic model assessment (HOMA) calculated as follows: (fasting glucose [mmol/l] x fasting insulin [mmol/l]) / 22.5; [b] Total cholesterol, HDL, LDL; [c] If a patient was diabetic, HbA1c was also determined at visit D and F; [d] alkaline phosphatase, glutamic-oxaloacetic transaminase (GOT), glutamic-pyruvate transaminase (GPT), glutamyltransferase (GGT), bilirubin; [e] serum creatinine; Abbr. TSH, Thyroid-stimulating hormone

2.6 Serum Adipokine and Chemokine Measurement

Serum concentrations of the adipokine adiponectin, both total and high molecular weight forms (HMW), PGRN as well as the chemokines MCP-1, IP-10, and RANTES were measured. Samples were analyzed using sandwich Enzyme-Linked Immunosorbent Assay (ELISA) kits validated for human serum, following the manufacturer's instructions (ALPCO Diagnostics cat. no. 47-ADPHU-E01 for adiponectin, AdipoGen cat. no. AG-45A-0018PP-KI01 for progranulin, R&D Systems cat. no. SIP100, SCP00 and SRN00B for IP-10, MCP-1 and RANTES, respectively). The principle of a sandwich ELISA is depicted in **Figure 7**. To assess whether storage of the serum samples at room temperature for 4 h altered concentration of the adipokines of interest, extra samples from 4 to 6 healthy lean female adults were measured immediately or 4 h after sampling. For all adipkoines, there was no significant difference in serum concentrations (total Adiponectin: 6.18 ± 1.56 µg/ml *vs.* 5.77 ± 1.11 µg/ml, p = 0.88; HMW: 2.99 ± 1.31 µg/ml *vs.* 2.79 ± 1.05 µg/ml, p = 0.38; IP-10: 147.55 ± 34.47 *vs.* 156.57 ± 35.41 pg/ml, p = 0.31; MCP-1: 303.00 ± 107.88 *vs.* 314.95 ± 98.20 pg/ml, p = 0.13; RANTES: 47.10 ± 9.44 *vs.* 41.08 ± 10.45 pg/ml, p = 0.31; PGRN: 157.72 ± 31.25 *vs.* 171.31 ± 30.03 pg/ml, p = 0.13).

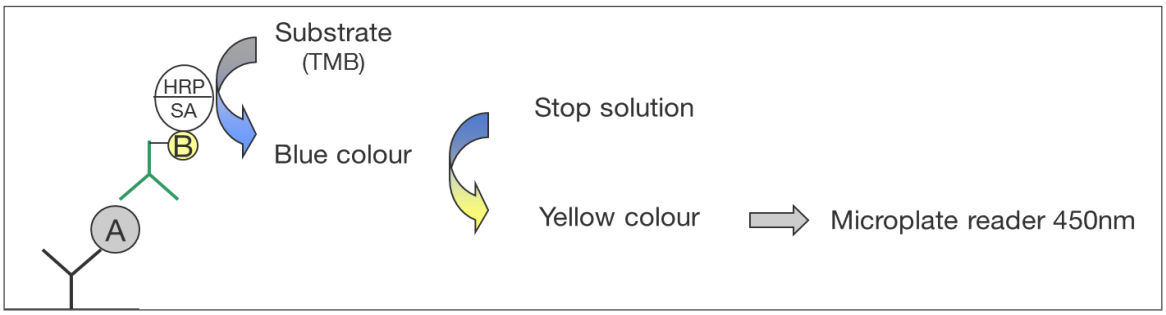


Figure 7. Principle of a Sandwich ELISA.
The technique relies on the use of two antibodies binding to distinct specific epitopes of the target protein, which enhances the specificity of detection. In between all steps of the assay, samples are washed to remove excess of solutions and prevent unspecific signals. During samples incubation, the target protein binds to the specific capture antibodies. After binding of the secondary (detection) antibody, which is biotinylated, the concentration of analyte is determined by colorimetry. Thereby, the enzyme horseradish peroxidase coupled to streptavidin binds to the biotinylated secondary antibodies. The substrate tetramethylbenzidine (TMB) mixed with hydrogen peroxide (H_2O_2) is then oxidized by protons generated by the peroxidase/H_2O_2 reaction resulting in a colour change proportional to the amount of peroxidase, *i.e.*, analyte, contained in each well. Colour change is detected by photometric measurement of the optical density (OD) at 450 nm and using 570 nm as reference wavelength to correct for optical imperfections. Abbreviations: A, analyte; B, biotinylated antibody; HRP-SA, horseradish peroxidase coupled with streptavidin.

To determine absolute adipokine/chemokine levels, standard samples of known concentrations as well as blank samples (diluent solution free of target proteins) were pipetted on each ELISA plate. After photometric reading using the Tecan infinite M200 reader (Tecan GmbH, Crailsheim, Germany), the OD value of the blank was subtracted from each sample OD value to adjust for background signals. A standard curve was constructed by plotting the known standard concentrations (y-axis) against corresponding absorbance values (x-axis). The analyte concentration in each study sample was calculated by interpolation of the regression curve formula in a form of quadratic equation ($y = ax^2 + bx + c$) using GraphPad Prism (Version 4). To obtain final concentrations of the target protein in the plasma samples, calculated values were adjusted according to dilution factors. Plasma samples were diluted 1:5 151, 1:2, 1:1, 1:100 and 1:200 for Adiponectin (multimeric), MCP-1, IP-10, RANTES and PGRN, respectively, so that OD values lie in the linear range of standard curves for accurate determination. To look for the fit of the standard curve, r^2 was used as sole parameter. The more r^2 is closer to 1.0, the better is the linear relationship between x and y. The r^2 values obtained in the present study testified to high

quality standard curves (**Table 4**). Each plasma sample was measured in duplicate for IP-10, MCP-1, RANTES and PGRN. The mean value, standard deviation (sd) and coefficient of variation (CV [%] = sd/mean) of each duplicate measurment were calculated. If the CV was over the 10 % threshold, measurement of the corresponding sample was repeated. Concentrations in samples measured on different microtiter plates were corrected using an internal standard sample (i.e. aliquots of one single serum sample from one healthy male donor pippeted on all plates). Total and HMW adiponectin were determined in a single measurement, yet the standard curve and the internal standard sample were measured in duplicate. The coefficient of variation (CV test $serum_{mean}$ = 2 %) clearly demonstrated that data were reliable. For the different ELISA systems, we assessed the inter-assay CV via repeated measure of total adiponectin, HMW, IP-10, MCP-1, RANTES and PGRN using the internal standard sample. Mean concentrations were 5.4 µg/ml, 2.6 µg/ml, 73 pg/ml, 347 pg/ml, 48 ng/ml, and 200 ng/ml, respectively. The results obtained testify to acceptable to good reproducibility, i.e., CV values were below the 15 % threshold (**Table 4)**.

Table 4. Standard curve fitness and inter-assay coefficients of variation for the ELISA systems used to determine serum adipokine and chemokine concentrations

	Adiponectin total	Adiponectin HMW	PGRN	MCP-1	RANTES	IP-10
r^2 *	0.9995 ± 0.0005	0.9995 ± 0.0005	0.9958 ± 0.0036	0.9985 ± 0.0018	0.9978 ± 0.0015	0.9961 ± 0.0034
Inter-assay CV **	8 %	9 %	12 %	6 %	14 %	14 %

* This parameter reflect the fit of the standard curve. The more r^2 is nearing to 1.0, the better is the linear relationship between x and y. Data are shown as means ± sd; ** The coefficient of variation reflects the variability of the assay, *i.e.*, it corresponds to the percentage of variations between different measurements on different plates due to technical bias.

2.7 Statistical Analysis

This section refers only to the analysis of the German data. Unless otherwise stated, all data were expressed as mean ± sd (95 % confidence interval of the mean) or median (interquartile range). Boxplots with median and quartiles were used to illustrate distribution of absolute and relative data. Outliers and extreme values were displayed as circles and asterisks, respectively. They were defined as values distant from Q1 (25^{th} percentile) for lower outliers or from Q3 (75^{th} percentile) for upper outliers by more than 1.5 and 3 IQR (interquartile range = 75^{th}-25^{th} percentile), respectively. All tests were done using the software SPSS version 19.0. For all tests, the alpha-risk was 0.05. Differences in weight loss and metabolic parameters between the two intervention groups were analyzed using a generalized mixed model adjusted for baseline values. The Chi-Square-test was used to compare categorical data, *i.e.* proportions of patients in the different weight loss categories (<5 %, 5-10 % and >10 % weight loss). With respect to the measurement of adipokines/chemokines within the German sub-cohort, no power analysis was done and multiple testing was not considered due to the exploratory nature of analysis. Non-parametric tests were used, which were considered to be more conservative since there is no need for proof of assumptions regarding data distribution (Altman 1991). In order to assess variations in adipokine levels solely according to weight loss, data obtained with participants from the two intervention groups were pooled. Before pooling data, a linear mixed model adjusting for age, baseline BMI and interaction with time was estimated to ensure that there was no difference between the two intervention groups at baseline and no significant time-dependent changes in the German sub-cohort. A linear mixed model adjusted for age for the period from baseline to visit D (6 months) and from visit D to visit F (12 months) was estimated to check whether weight and fat mass change was associated with changes in metabolic markers adipokine/chemokine levels. The effect of weight loss on adipokine levels was further characterized by grouping participants according to weight loss categories (<5 %, 5-10 % and >10 % weight loss). The Wilcoxon-test was used for comparing concentrations at baseline vs. post-intervention (12 months) in each weight loss category. The Kruskal-Wallis-test was used for comparing data between weight loss categories. Spearman's correlation coefficients were calculated to quantify the bivariate relationship between adipokine/chemokine concentrations and anthropometric as well as metabolic variables (*e.g.*, triglycerides, insulin, etc…).

3 Results

3.1 Characteristics of the German Study Cohort

In Germany, 268 subjects (42 males and 226 females) were randomised and 201 of them (75 %) completed the study (89 in the CP and 112 in the SC group after 12 months). The study characteristics of all German participants who completed the study are given in **Table 5**. Demographic and clinical outcomes of participants did not differ significantly between the treatment groups ($p > 0.05$; Student t-test or Mann-Whitney-U-test).

Table 5. Baseline characteristics of German completers (n = 201).[a]

Parameter	Commercial programme (CP) (n = 89)	Standard care (SC) (n = 112)
Women	79 (89 %)	93 (83 %)
Age [years]	48.0 ± 12.9	49.7 ± 13.0
Weight [kg]	87.4 ± 11.2	87.3 ± 11.0
Height [m]	1.68 ± 0.1	1.68 ± 0.1
BMI [kg/m^2]	30.9 ± 2.4	30.9 ± 2.8
Fat mass [kg]	33.8 ± 5.9	33.2 ± 6.9
Waist circumference [cm]	97.9 ± 8.9	99.9 ± 9.7
Systolic blood pressure [mmHg]	120.8 ± 16.0	122.0 ± 15.0
Diastolic blood pressure [mmHg]	75.7 ± 10.8	78.9 ± 9.3 80.0 (110.6 to 132.5)[a]
Glucose [mmol/l]	5.1 (4.6 to 5.4)[a]	5.0 (4.6 to 5.5)[a]
Insulin [mmol/l]	50.0 (33.0 to 68.8)[a]	48.0 (34.0 to 71.5)[a]
HOMA-IR	1.5 (1.0 to 2.3)[a]	1.5 (0.9 to 2.3)[a]
Triglycerides [mmol/l]	1.4 ± 0.8	1.6 ± 0.8 1.4 (1.1 to 1.9)[a]
Total cholesterol [mmol/l]	5.4 ± 1.1	5.4 ± 1.0
LDL-Cholesterol [mmol/l]	3.3 ± 0.9	3.3 ± 0.8
HDL-Cholesterol [mmol/l]	1.4 ± 0.4	1.4 ± 0.4

[a] Data are shown as means ± sd or medians (IQR) for non-parametric data.

After 12 months of intervention, participants following the CP lost significantly more body weight compared to participants getting SC support (**Table 6**). This significant greater weight loss was accompanied by larger reductions in waist circumference and fat mass. Additionally, blood levels of insulin and LDL-cholesterol significantly improved in the CP compared to the SC group while there was no difference in blood pressure, glucose, HOMA-IR, triglyceride, HDL- and cholesterol levels. The study results for the 201 completers are shown in **Table 6**.

Table 6. Changes (Δ-values) in clinical outcomes and biomarkers of cardiovascular disease risk between baseline and 12 months of intervention adjusted for baseline observation.[a]

Parameters	n	Commercial programme (CP)	Standard Care (SC)	Adjusted difference (95% CI)*	p-value
Weight [kg]	201	-5.15 ± 6.79	-2.67 ± 4.75	-2.36 (-3.73 to -0.98)	**0.001**
BMI [kg/m^2]	201	-1.82 ± 2.37	-0.96 ± 1.67	-0.83 (-1.31 to -0.35)	**0.001**
Waist circumference [cm]	190	-5.61 ± 7.53	-3.48 ± 5.75	-1.98 (-3.58 to -0.39)	**0.02**
Fat mass [kg]	151	-3.71 ± 5.38	-1.45 ± 3.69	-2.32 (-3.59 to -1.04)	**<0.001**
Systolic blood pressure [mmHg]	200	-1.27 ± 14.36	-0.44 ± 15.05	-1.69 (-4.66 to 1.27)	0.26
Diastolic blood pressure [mmHg]	200	-0.05 ± 9.70	-1.42 ± 10.19	0.62 (-1.47 to 2.72)	0.56
Glucose [mmol/l]	197	-0.09 ± 0.56 0.00 (-0.40 to 0.18)[b]	-0.03 ± 0.71 0.00 (-0.30 to 0.30)[b]	-0.08 (-0.21 to 0.05)	0.23
Insulin [pmol/l]	196	-10.77 ± 27.85 -11.0 (-26.75 to 1.0)[b]	-2.08 ± 30.20 -0.5 (-15.27 to [illegible][b]	-6.50 (-12.78 to -0.23)	**0.04**
HOMA-IR	196	-0.81 ± 4.09 -0.30 (-0.84 to 0.06)[b]	-0.11 ±1.21 -0.01 (-0.56 to 0.38)[b]	-0.32 (-0.67 to 0.04)	0.08
Triglycerides [mmol/l]	197	0.04 ± 0.60 0.05 (-0.22 to 0.31)[b]	-0.05 ± 0.76 -0.02 (-0.36 to 0.19)[b]	0.09 (-0.08 to 0.27)	0.29
Cholesterol [mmol/l]	198	0.09 ± 0.84	0.19 ± 0.64	-0.13 (-0.30 to 0.03)	0.12
LDL cholesterol [mmol/l]	197	-0.02 ± 0.65	0.12 ± 0.56	-0.17 (-0.30 to -0.04)	**0.02**
HDL cholesterol [mmol/l]	197	0.17 ±0.25	0.10 ± 0.22	0.02 (-0.03 to 0.07)	0.47

[a] Data are shown as means ± sd and in medians (IQR) if data were non-normally distributed; [b] The adjusted difference is presented with 95% confidence interval (CI).

The Chi-Square-test revealed a significant difference between the two treatment groups regarding the proportion of participants (%) losing <5 %, 5-10 % and >10 % initial body weight after 6 and 12 months (p = 0.002 and 0.03, respectively). After 12 months, the proportion of participants who lost at least 10 % of their initial body weight was higher in the CP group compared to the SC group (27 % *vs.* 12 %) (**Table 7**). There was no difference in the number of participants who lost 5 to 10 % initial body weight (19 % in both intervention groups). A proportion of 31 % in the WW and 45 % in the SC group lost less than 5 % initial body weight, while there was no difference concerning weight gain (22 % in the CP and 25 % in the SC group).

Table 7. Number of participants (%) in the German cohort who lost <5 %, 5 to 10 % or >10 % of initial body weight or who gained weight over the study period (6 and 12 months).[a]

Weight categories	Visit D (6 months)		Visit F (12 months)	
	CP n = 89	SC n = 112	CP n = 89	SC n = 112
Weight gain, n (%)	9 (10)	25 (22)	20 (22)	28 (25)
Weight loss <5 % of initial body weight, n (%)	30 (34)	50 (45)	28 (31)	50 (45)
Weight loss 5-10 % of initial body weight, n (%)	34 (38)	31 (28)	17 (19)	21 (19)
Weight loss >10 % of initial body weight, n (%)	16 (18)	6 (5)	24 (27)	13 (12)

[a] Data are shown in number of participants (%). n = 201 completers (172 females). The Chi-Square-test was used to assess significance of overall difference in proportions between the CP and SC group after 6 and 12 months (p = 0.002 and 0.03, respectively).

3.2 Body Weight Maintenance after 24 months

The follow-up visits over an additional period of twelve months (month 18 and 24) to assess weight control were optional for study participants. During this follow-up period, participants were free to choose a method to control their body weight. Body weight was measured at the GP offices. From the 201 completers of the German cohort (75 % of randomised participants), body weight at 18 and 24 months were available for 123 (46 %) and 113 (42 %) participants, respectively (**Table 8**). These 123 participants are referred to as follow-up completers, *i.e.*, participants for whom measured body weight data was available at baseline and after 12 and 24 months.

Table 8. Number of participants with available follow-up data

Follow-up data available	Commercial Programme	Standard Care	Total
Completers at 12 months, n (%)	89 (33 %)	112 (42 %)	201 (75 %)
Measured weight at 18 months, n (%)	59 (22 %)	64 (24 %)	123 (46 %)
Measured weight at 24 months, n (%)	53 (20 %)	60 (22 %)	113 (42 %)

Body weight was still significantly reduced after 24 months compared to baseline in the CP group (p = 0.02) but not in the SC group (p = 0.11). The estimates for weight loss differences between the intervention groups suggest that weight loss is greater for participants in the CP arm including the follow-up period. However this difference is only statistically significant in the LOCF analysis. Weight regain was observed in both treatment groups and was not significantly different between the two groups (adjusted difference -3.12 (-7.2 to 1.0), p = 0.13) in the completers analysis (**Table 9 and Figure 8).** The weight maintenance data for the whole study cohort are available in **Appendix E** (Holzapfel et al. in preparation).

Table 9. Weight change (kg) between baseline and 24 months adjusted for baseline values and weight regain between 12 and 24 months

Body weight	N	Commercial Programme	Standard Care	Adjusted difference (95 % CI)	p-value
LOCF * Baseline to 24 months	201	-3.6 ±7.3	-2.1 ± 5.6	-2.0 (-3.6 to -0.4)	**0.02**
12 to 24 months	201	1.6 ± 4.0	0.6 ± 3.3	-2.0 (-3.6 to -0.4)	**0.02**
Follow-up completers Baseline to 24 months	113	-5.1 ± 7.9	-2.5 ± 7.4	-3.2 (-6.8 to 0.4)	0.08
12 to 24 months	113	2.0 ± 3.0	0.8 ± 5.2	-3.1 (-7.2 to 1.0)	0.13

* Due to missing body weight data during the follow-up period, two different approaches were used: the “last observation carried forward (LOCF)” and the completers analysis.

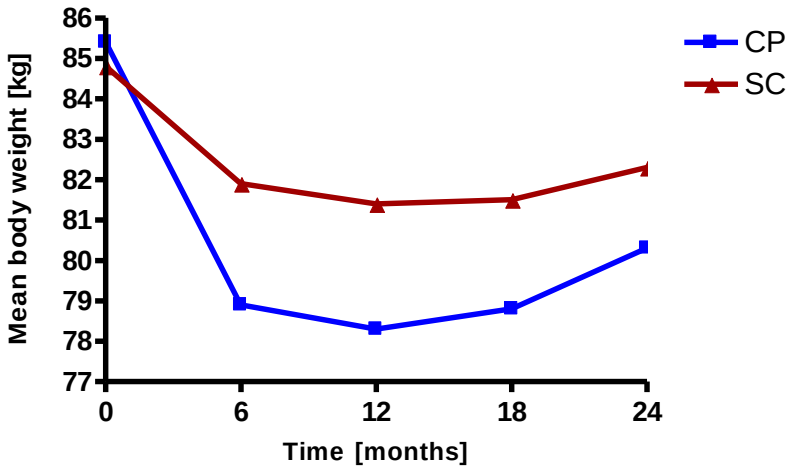


Figure 8. Body weight change for follow-up completers (n = 113) during intervention (month 0 to 12) and follow-up (month 12 to 24). Abbreviations: CP, commercial programme; SC, standard care

3.3 Characteristics of the German sub-cohort

To study the effect of weight change on serum levels of pro- and anti-inflammatory adipokines/chemokines, samples from German female participants were selected who lost >10 % (n = 33; 20 in the CP and 13 in the SC group) and 5 to 10 % (n = 36; 17 in the CP and 19 in the SC group) of initial body weight between baseline and the final visit after 12 months. As control, we selected participants who lost <5 % of initial body weight (n = 34; 15 in the CP and 19 in the SC group) (**Figure 5**). Male samples were excluded from analysis because the number of male participants completing the study was low (14 %). The study characteristics of this German sub-cohort (n = 103) according to treatment group and combined are shown in **Table 10**. There was no significant difference in study characteristics at baseline between the two intervention groups in this German sub-cohort.

Table 10. Baseline characteristics of the German sub-cohort (n = 103).[a]

	Commercial Programme (CP)		Standard Care (SC)		Combined		
Parameter	n	Mean ± sd	n	Mean ± sd	n	Mean ± sd	p-value
Age [years]	52	46.5 ± 12.2	51	48.6 ± 12.5	103	47.5 ± 12.3	0.38
Weight [kg]	52	85.2 ± 8.9	51	84.4 ± 9.6	103	84.8 ± 9.2	0.65
BMI [kg/m^2]	52	30.6 ± 2.4	51	30.5 ± 3.0	103	30.6 ± 2.7	0.74
Systolic blood pressure [mmHg]	52	119.6 ± 15.0	51	119.2 ± 14.9	103	119.4 ± 14.8	0.92
Diastolic blood pressure[mmHg]	52	74.7 ± 10.2	51	79.0 ± 9.4	103	76.8 ± 10.0	0.06
Waist circumference [cm]	51	96.1 ± 8.7	49	96.2 ± 7.8	100	96.1 ± 8.2	0.92
Fat mass [kg]	44	34.4 ± 5.8	40	33.1 ± 5.8	84	33.7 ± 5.8	0.31
Glucose [mmol/l]	52	4.8 (4.6 to 5.2) [b]	51	4.8 (4.6 to 5.2) [b]	103	4.8 (4.6 to 5.2) [b]	0.84[b]
Insulin [pmol/l]	52	53.5 (35.5 to 68.8) [b]	51	45.0 (29.0 to 61.0) [b]	103	48.0 (32.0 to 65.0) [b]	0.09[b]
HOMA-IR	52	1.5 (1.0 to 2.2) [b]	51	1.3 (0.8 to 1.9) [b]	103	1.4 (0.9 to 2.1) [b]	0.11[b]
Triglycerides [mmol/l]	52	1.2 (0.8 to 1.5) [b]	51	1.2 (1.0 to 1.6) [b]	103	1.2 (0.9 to 1.6) [b]	0.43[b]
Total cholesterol [mmol/l]	52	5.5 ± 1.0	51	5.3 ± 1.0	103	5.4 ± 1.0	0.35
HDL cholesterol [mmol/l]	52	1.5 ± 0.4	51	1.5 ± 0.4	103	1.5 ± 0.4	0.61
LDL cholesterol [mmol/l]	52	3.4 ± 0.8	51	3.2 ± 0.7	103	3.3 ± 0.8	0.15
CRP [mg/l]	52	0.022 (0.014 to 0.039) [b]	51	0.022 (0.013 to 0.054) [b]	103	0.022 (0.014 to 0.043) [b]	0.44[b]
Total adiponectin [µg/ml]	52	5.7 ± 2.3	51	5.7 ± 2.4	102	5.7 ± 2.3	0.95
HMW adiponectin [µg/ml]	51	3.0 ± 1.6	51	2.9 ± 1.5	102	2.9 ± 1.5	0.73
MCP-1 [pg/ml]	51	330.0 ± 161.5	51	357.9 ± 149.6	102	344.0 ± 155.6	0.32
IP-10 [pg/ml]	52	100.9 (82.0 to 131.9) [b]	50	107.5 (69.7 to 142.9) [b]	102	102.6 (76.9 to 135.2) [b]	0.91[b]
RANTES [ng/ml]	51	31.3 ± 14.7	51	31.7 ± 11.4	102	31.5 ± 13.1	0.88
PGRN [ng/ml]	52	129.0 ± 54.3	51	127.9 ± 33.0	103	128.4 ±44.8	0.89

[a] Data are shown as means ± sd or medians (IQR) for non-normally distributed data; [b] P-values were determined by Students t-test for normally distributed data and by Mann-Whitney-U-test for non-parametric data.

Prior to combining samples from the CP and SC group in order to analyze the effect of body weight loss independently of the intervention type, a linear mixed model, adjusting for baseline values, BMI and interaction of time by group, was used to ensure that there was no difference in the markers of inflammation measured (total and HMW adiponectin, MCP-1, IP-10, RANTES, PGRN) between the two groups over the study period. The results given in **Table 11** show that values did not differ significantly between treatment groups, which speaks in favour of pooling data from the CP and SC group and further analyze the effect of weight change on adipokine levels.

Table 11. Blood adipokine/chemokine levels over time according to treatment group.[a]

Parameter	Time point	Adipokine levels		p-value (interaction group*time)
		Intervention group		
		CP	SC	
Total adiponectin [µg/ml]	D (6 months)	6.0 ± 0.2 (5.7 to 6.3)	5.9 ± 0.1 (5.6 to 6.1)	0.845
	F (12 months)	6.4 ± 0.2 (6.0 to 6.7)	6.1 ± 0.1 (5.9 to 6.4)	
HMW adiponectin [µg/ml]	D (6 months)	3.1 ± 0.1 (2.9 to 3.4)	3.1 ± 0.1 (2.9 to 3.3)	0.645
	F (12 months)	3.4 ± 0.1 (3.1 to 3.6)	3.3 ± 0.1 (3.1 to 3.5)	
IP-10 [pg/ml]	D (6 months)	122.6 ± 7.5 (107.6 to 137.5)	110.4 ± 14.1 (82.3 to 138.5)	0.261
	F (12 months)	113.0 ± 7.4 (98.2 to 127.8)	131.6 ± 14.3 (103.3 to 160.0)	
MCP-1 [pg/ml]	D (6 months)	314.0 ± 13.5 (287.0 to 340.9)	312.8 ± 11.4 (290.1 to 335.6)	0.371
	F (12 months)	298.0 ± 13.3 (271.4 to 324.6)	319.0 ± 11.3 (296.5 to 341.5)	
RANTES [ng/ml]	D (6 months)	31.4 ± 1.8 (28.0 to 34.9)	36.0 ± 1.9 (32.3 to 39.8)	0.143
	F (12 months)	32.4 ± 1.7 (28.5 to 35.8)	32.4 ± 1.9 (28.7 to 36.2)	
PGRN [ng/ml]	D (6 months)	124.4 ± 4.5 (115.6 to 133.3)	127.8 ± 5.1 (117.6 to 138.0)	0.716
	F (12 months)	121.4 ± 4.4 (112.6 to 130.2)	126.9 ± 5.1 (116.7 to 137.1)	

[a] Data are shown as means ± SEM (95 % CI).

3.4 Changes in Weight, Fat mass and Blood Adipokine/Chemokine Levels during Intervention

In the German sub-cohort (n = 103), body weight decreased significantly by -6.6 % after 6 months and by -7.0 % after 12 months when compared to baseline values **(Table 12 and Figure 9).** A similar decrease was observed for fat mass with a significant reduction after 6 and 12 months (-11 % at both time points). Total adiponectin increased significantly by +3.6 % during the first 6 months and further increased up to +9.4 % after 12 months. HMW adiponectin levels also increased after 6 and 12 months (+5.9 % and +12.4 %). MCP-1 levels decreased significantly after 6 and 12 months (-4.6 and -6.9 %). In contrast, there was no significant change in IP-10, RANTES and PGRN levels. The unspecific inflammatory marker CRP decreased by -17 % and -24 % after 6 and 12 months, respectively.

Table 12. Percent changes in body weight, fat mass and adipokine/chemokine levels after 6 and 12 months when compared to baseline.[a]

		Visit D (after 6 months)	Baseline to Visit D		Visit F (after 12 months)	Baseline to Visit F	Visit D to Visit F
	n	**Median (IQR)**	**p-value**	**n**	**Median (IQR)**	**p-value**	**p-value**
Δ Weight (%)	103	-6.6 (-2.5 to -9.6)	**<0.001**	103	-7.0 (-12.5 to -1.3)	**<0.001**	0.378
Δ Fat mass (%)	80	-11.2 (-19.9 to -3.3)	**<0.001**	83	-11.7 (-23.0 to -2.3)	**<0.001**	0.655
Δ Total adiponectin (%)	99	+3.6 (-7.1 to +13.8)	**0.035**	102	+9.4 (-0.9 to +18.8)	**<0.001**	**0.009**
Δ HMW adiponectin (%)	99	+5.9 (-7.5 to +23.2)	**0.003**	102	+12.4 (-1.8 to +26.0)	**<0.001**	**0.003**
Δ MCP-1 (%)	98	-4.6 (-23.1 to +10.6)	**0.042**	102	-6.9 (-20.5 to +13.8)	**0.009**	0.891
Δ IP-10 (%)	101	+2.1 (-22.0 to +22.8)	0.931	101	-4.6 (-20.3 to +12.3)	0.097	0.468
Δ RANTES (%)	101	+3.9 (-18.9 to +37.2)	0.180	102	+4.3 (-23.2 to +47.7)	0.565	0.379
Δ PGRN (%)	102	+2.1 (-10.1 to 13.1)	0.930	102	-4.5 (-17.1 to +11.9)	0.059	0.064
Δ CRP (%)	102	-17.0 (-47.4 to 37.4)	0.073	103	-24.0 (-59.1 to 8.3)	**<0.001**	**0.039**

[a] Data are shown as medians (IQR). P-values were determined by Wilcoxon-test for changes from baseline to visit D and F and from visit D to F. The data that were used to calculate Δ-values between visits are shown in Table 10 above (combined baseline values) and in **Appendix F** (raw values at all study visits).

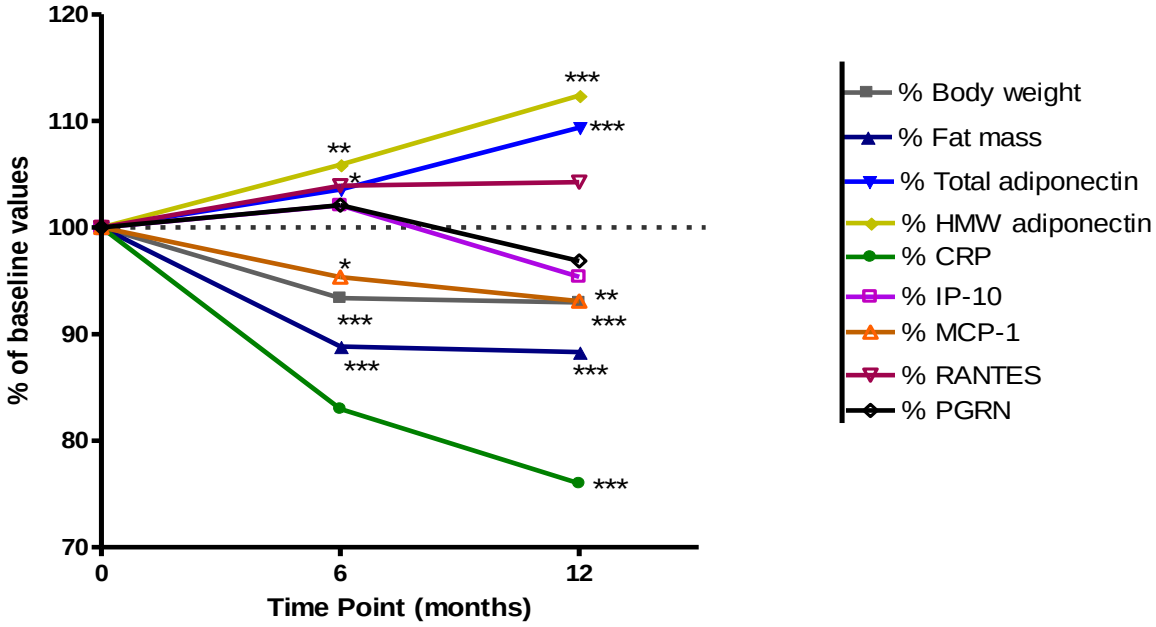


Figure 9. Relative changes in weight, fat mass, total and HMW adiponectin, CRP, IP-10, MCP-1, RANTES and PGRN during the study period. Data are shown as medians of relative changes compared with baseline values. The number of participants was 98 to 102, depending on parameters. For fat mass n = 80 to 83 because not all participants came for bioimpedance analysis. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$ (Wilcoxon-test).

3.5 Effect of Changes in Body Weight and Fat Mass on Metabolic Markers

We performed a multiple linear regression analysis adjusted for age on the data from baseline to visit D (6 months) and from visit D to visit F (12 months) in order to test whether weight change was associated with changes in metabolic markers as well as changes in adipokine/chemokine levels.

A weight loss of -1 % (approximately -0.8 kg) was significantly associated with changes in glucose (-0.6 %; -0.03 mmol/l), insulin (-2.4 %; -1.15 pmol/l), HOMA-IR (-3 %) triglycerides (-1.4 %; -0.02 mmol/l), total cholesterol (-0.5 %; -0.03 mmol/l), LDL-cholesterol (-0.9 %; -0.03 mmol/l), systolic and diastolic blood pressure (-0.3 %; -0.36 mmHg for systolic and -0.24 mmHg for diastolic), CRP (-4.5 %; -0.001 mg/l), total and HMW adiponectin (+0.6 % and +0.7 %; 0.03 and 0.02 µg/ml, respectively) and MCP-1 (-0.7 %; -2.12 pg/ml) **(Table 13).** No significant associations were observed between weight change and HDL-cholesterol, IP-10, RANTES and PGRN.

A decrease in fat mass by -1 % explained a significant reduction in insulin (-0.7 %), HOMA-IR (-0.7 %), total cholesterol (-0.2 %), LDL-cholesterol (-0.3 %) and systolic blood pressure (-0.2 %) and an increase in total adiponectin (+0.2 %) and PGRN (+0.4 %). No significant associations were observed between fat mass changes and glucose, triglycerides, HDL-cholesterol, diastolic blood pressure, CRP, HMW adiponectin, MCP-1, IP-10, and RANTES **(Table 13)**.

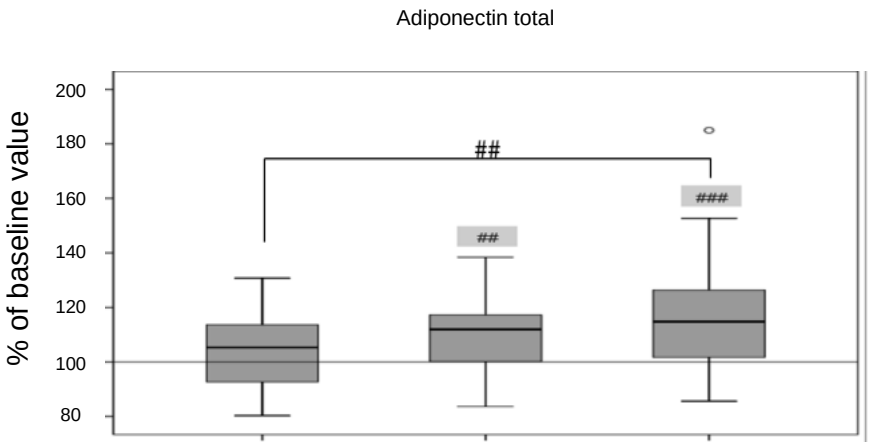
Table 13. Effect of a 1 %-decrease in body and fat mass on metabolic markers and adipokines/chemokines.

	Effect of 1% decrease in body mass		Effect of 1 % decrease in fat mass	
Parameter	**Estimate ± SEM (95% CI)**	**p-value**	**Estimate ± SEM (95% CI)**	**p-value**
Glucose	-0.57 ± 0.16 (-0.25 to -0.89)	**0.001**	-0.09 ± 0.05 (+0.01 to -0.20)	0.081
Insulin	-2.36 ± 0.77 (-0.84 to -3.89)	**0.003**	-0.71 ± 0.35 (-0.03 to -1.40	**0.042**
HOMA-IR	-2.94 ± 0.84 (-1.28 to -4.59)	**0.001**	-0.74 ± 0.37 (-0.004 to -1.48)	**0.049**
Triglycerides	-1.44 ± 0.45 (-0.55 to -2.34)	**0.002**	-0.34 ± 0.21 (+0.08 to -0.77)	0.114
Total cholesterol	-0.54 ± 0.16 (-0.23 to -0.85)	**0.001**	-0.18 ± 0.07 (-0.04 to -0.32)	**0.013**
HDL-cholesterol	+0.28 ± 0.19 (+0.65 to -0.09)	0.143	+0.07 ± 0.08 (+0.22 to -0.09)	0.394
LDL-cholesterol	-0.87 ± 0.22 (-0.44 to -1.30)	**0.000**	-0.30 ± 0.09 (-0.12 to -0.49)	**0.002**
Systolic blood pressure	-0.31 ± 0.15 (-0.02 to -0.60)	**0.038**	-0.15 ± 0.07 (-0.02 to -0.28)	**0.026**
Diastolic blood pressure	-0.34 ± 0.16 (-0.02 to -0.67)	**0.038**	-0.08 ± 0.07 (+0.05 to -0.22)	0.227
CRP	-4.48 ± 2.03 (-0.47 to -8.48)	**0.029**	-1.52 ± 0.80 (+0.07 to -3.10)	0.061
Total adiponectin	+0.58 ± 0.22 (+1.01 to +0.14)	**0.010**	+0.22 ± 0.10 (+0.42 to +0.02)	**0.031**
HMW adiponectin	+0.73 ± 0.32 (+1.37 to +0.09)	**0.027**	+0.22 ± 0.13 (+0.48 to -0.03)	0.082
MCP-1	-0.74 ± 0.36 (-0.02 to -1.45)	**0.045**	-0.04 ± 0.17 (+0.29 to -0.38)	0.800
IP-10	+0.11 ± 0.91 (+1.91 to -1.70)	0.908	+0.32 ± 0.38 (+1.08 to-0.44)	0.402
RANTES	+0.02 ± 0.65 (+1.30 to -1.26)	0.972	0.19 ± 0.28 (-0.37 to 0.75)	0.503
PGRN	+0.19 ± 0.41 (+1.0 to -0.61)	0.638	+0.42 ± 0.16 (+0.74 to +0.09)	**0.012**

[a] Data are shown as Estimate (%) ± SEM (95% CI) according to a linear mixed model including weight and fat mass changes, adjusted for age (n = 103).

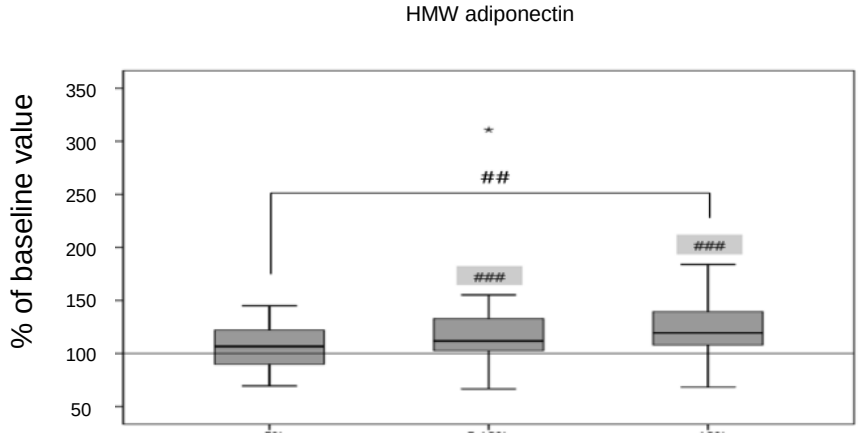
3.6 Change of Adipokine/Chemokine Levels according to the Degree of Weight Loss

To further investigate the effect of body weight loss on inflammatory markers, we stratified the population into weight loss groups, including weight loss < 5 % of initial body weight (n = 34), 5 to 10 % (n = 36), and >10 % (n = 33) after 12 months. The mean weight loss in the >10 % category was -15.6 % (-13.5 kg), -7.3 % (-6.0 kg) in the 5-10 % category and -0.2 % (-0.2 kg) in the <5% category. First of all, there was no difference in study characteristics at baseline between the three weight loss groups, as tested using the Kruskal-Wallis-test (**Appendix G**). The relative increase in both total and HMW adiponectin serum levels was significantly higher when comparing a body weight loss >10 % *vs.* <5 % **(Figure 10 and 11).** Moreover, only participants with a weight loss of 5-10 % and >10 % were characterized by significantly increased total- (+11.9 % for 5-10 % and +14.8 % for >10 % weight loss) and HMW adiponectin levels (+11.7 % for 5-10 % and 19.4 % for >10 % weight loss) compared to baseline values.



Weight loss group Median (IQR)	<5 %	5-10 %	>10 %
Baseline [µg/ml]	5.0 (4.0 to 6.2)	5.3 (4.1 to 7.1)	5.8 (4.2 to 8.2)
12 months [µg/ml]	5.2 (3.9 to 6.2)	6.2 (4.4 to 7.9)	6.9 (5.0 to 9.2)
% change	+5.3 (-7.6 to 13.8)	+11.9 (-0.2 to 17.4)	+14.8 (1.5 to 27.8)
p-value	0.092	**0.002**	**<0.001**

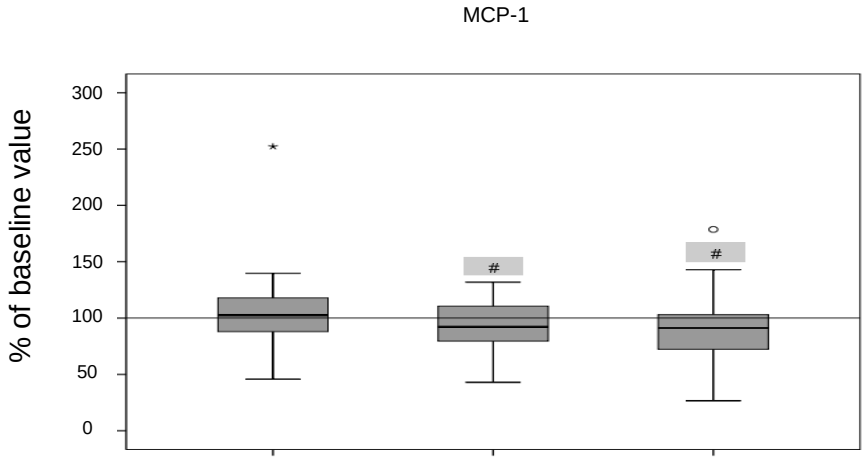
Figure 10 (above, page 55). Effect of weight loss on relative changes in total adiponectin. The sub-cohort of participants was divided into three groups according to the degree of weight loss from baseline to 12 months (< 5 % initial body weight, n = 34; 5 to 10 %, n = 36; >10 %, n = 33). Data are shown as box plots where the upper and lower lines represent the 25th and 75th percentiles and the middle line represents the median. The lower and upper whiskers show the 5th and 95th percentiles. Outliers are marked with stars (*) and extreme outliers with circles (o). The exact values in the table are shown as median (IQR). # $p < 0.05$, ## $p < 0.01$ and ### $p < 0.001$. Kruskal-Wallis-test for comparison between the weight loss groups; Wilcoxon-test for in-group differences from baseline (100% line) to 12 months.



Weight loss group Median (IQR)	<5 %	5-10 %	>10 %
Baseline [µg/ml]	2.5 (1.8 to 3.1)	2.6 (1.7 to 3.8)	3.0 (2.1 to 4.7)
12 months [µg/ml]	2.4 (1.8 to 3.2)	3.1 (2.0 to 3.8)	3.9 (2.6 to 5.2)
% change	+6.7 (-10.1 to 22.3)	+11.7 (2.6 to 34.5)	+19.4 (7.9 to 39.9)
p-value	0.130	**<0.001**	**<0.001**

Figure 11. Effect of weight loss on relative changes in blood concentrations of HMW adiponectin according to weight loss group. Data are presented as in Figure 10.

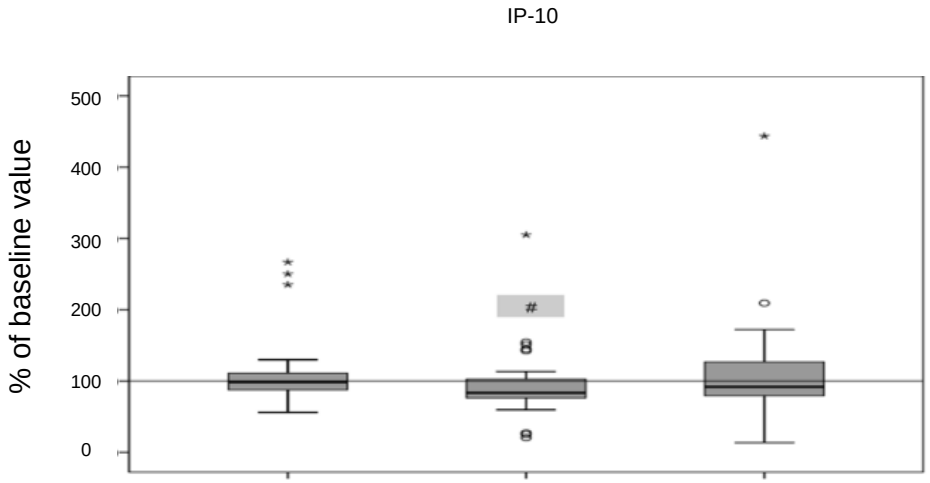
Relative **MCP-1** levels decreased significantly in subjects losing between 5 and 10 % as well as more than 10 % of initial body weight after 1 year (-7.8 and -8.8 %, respectively) (**Figure 12**). The relative increase during the study did not differ between the three weight loss groups (p = 0.127, Kruskal-Wallis-test). Pairwise comparison revealed a tendency towards a more pronounced decrease in relative MCP-1 levels in subjects from the highest weight loss group (>10 %) when compared with the lowest weight loss group (<5 %) (p = 0.06, Mann-Whitney-U-test).



Weight loss group Median (IQR)	<5 %	5-10 %	>10 %
Baseline [pg/ml]	278.1 (212.3 to 400.2)	309.7 (252.8 to 414.0)	299.7 (268.4 to 435.3)
12 months [pg/ml]	295.5 (230.7 to 363.8)	298.9 (212.2 to 356.3)	286.6 (240.7 to 394.6)
% change	+2.6 (-13.1 to 18.1)	-7.8 (-20.5 to 11.8)	-8.8 (-29.1 to 4.8)
p-value	0.966	**0.036**	**0.023**

Figure 12. Effect of weight loss on relative changes in blood concentrations of MCP-1 according to weight loss group. Data are presented as in Figure 10.

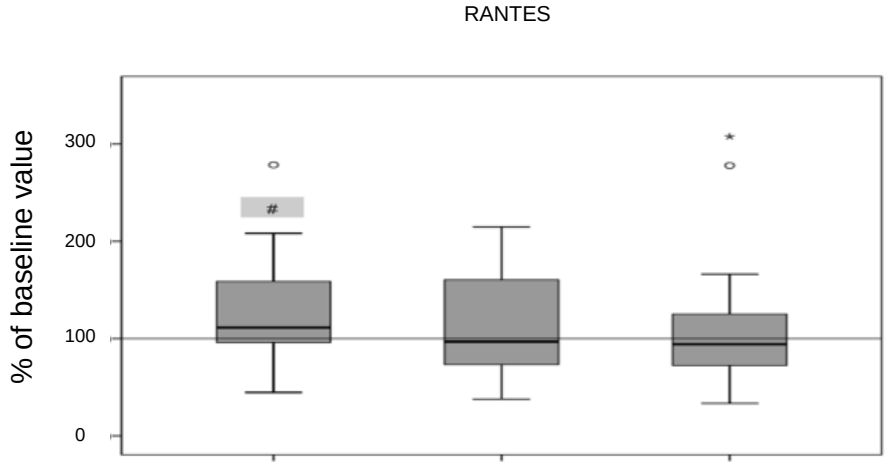
A significant decrease in relative **IP-10** levels was found in subjects losing 5 to 10 % of initial body weight (-16.4 %, p = 0.035). In contrast, the decrease was not significant in the group of participants who lost more than >10 % body weight (-8.0 %, p = 0.845) (**Figure 13**). The serum levels of IP-10 showed a high variability between the subjects and there were several outliers. Relative changes in blood concentrations of IP-10 did not differ significantly between the three weight loss groups after 1 year (p = 0.127, Kruskal-Wallis-test).



Weight loss group Median (IQR)	<5 %	5-10 %	>10 %
Baseline [pg/ml]	114.8 (80.6 to 139.8)	110.6 (77.6 to 150.0)	92.2 (74.3 to 132.1)
12 months [pg/ml]	107.7 (66.8 to 160.2)	93.1 (62.2 to 130.5)	89.1 (74.6 to 127.8)
% change	-1.5 (-12.9 to 11.8)	-16.4 (-23.7 to 2.8)	-8.0 (-21.2 to 27.9)
p-value	0.638	**0.035**	0.845

Figure 13. Effect of weight loss on relative changes in blood concentrations of IP-10 according to weight loss group. Data are presented as in Figure 10.

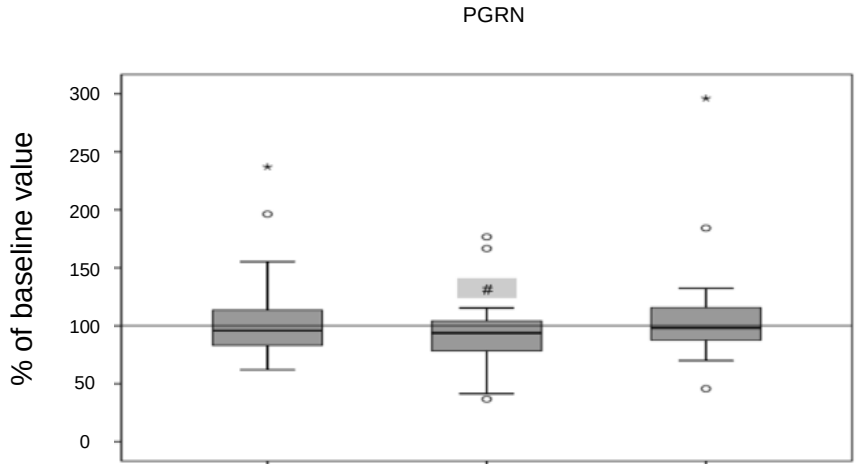
Relative blood levels of **RANTES** increased significantly by 11.3 % in participants who lost <5 % of initial body weight after 1 year **(Figure 14)**. The relative levels did not differ significantly between the weight loss groups (p = 0.13, Kruskal-Wallis-test). There was a tendency towards a more pronounced decrease in relative RANTES levels in subjects from the highest weight loss group (>10 %) when compared with the lowest weight loss group (<5 %) (p = 0.05, Mann-Whitney-U-test).



Weight loss group Median (IQR)	<5 %	5-10 %	>10 %
Baseline [ng/ml]	29.5 (23.4 to 36.6)	27.7 (21.2 to 39.1)	34.2 (25.5 to 42.9)
12 months [ng/ml]	32.1 (23.1 to 46.0)	29.2 (21.7 to 39.4)	30.5 (21.7 to 38.3)
% change	+11.3 (-5.1 to 60.2)	-3.2 (-27.7 to 60.3)	-5.7 (-29.1 to 26.3)
p-value	**0.046**	0.937	0.262

Figure 14. Effect of weight loss on relative changes in blood concentrations of RANTES according to weight loss group. Data are presented as in Figure 10.

The relative increase of **PGRN** did not differ significantly between the three weight loss groups after 1 year (p = 0.51, Kruskal-Wallis-test). Nevertheless, we observed a slight decrease (-6.3 %) in blood concentration of PGRN in subjects who lost between 5 and 10 % initial body weight when compared to baseline **(Figure 15)**.



Weight loss group Median (IQR)	<5 %	5-10 %	>10 %
Baseline [ng/ml]	125.7 (94.3 to 143.3)	118.7 (95.9 to 157.0)	123.7 (100.0 to162.0)
12 months [ng/ml]	114.9 (96.8 to 140.1)	114.8 (84.0 to 139.6)	124.8 (98.8 to 158.7)
% change	-4.2 (-17.6 to 15.5)	-6.3 (-23.1 to 4.2)	-1.9 (-13.1 to 16.3)
p-value	0.447	**0.044**	0.809

Figure 15. Effect of the level of weight loss on relative changes in Progranulin according to weight loss group. Data are presented as described in Figure 10.

3.7 Change in Blood Lipids, Glucose, Insulin, and HOMA-IR according to the Degree of Weight Loss

Relative changes in lipids, glucose, insulin and HOMA-IR after 12 months of intervention according to the three different weight loss groups are shown in **Table 14** (data after 6 months are available in **Appendix H**). After 12 months, weight and fat mass decreased significantly in subjects who lost 5-10 % (-7.1 % and -14.0 %, respectively) and >10 %

(-14.5 % and -27.2 %, respectively) of initial body weight. Waist circumference decreased in all groups yet this decrease was more pronounced in the highest weight loss group. Systolic blood pressure slightly increased in the group who did not loose weight significantly but decreased together with the diastolic blood pressure only in subjects losing >10 % of initial weight. Fasting glucose, insulin and HOMA-IR decreased significantly only in subjects who lost more than 10 % of initial body weight (-4.0 %, -47.8 % and -47.7 %, respectively). Triglycerides and total cholesterol levels decreased significantly after 12 months in the group of subjects who lost more than 10 % initial body weight when compared to baseline (-16.9 % and -5.7 %, respectively). LDL-cholesterol increased significantly by +8.3 % in the <5 % weight loss group, but decreased significantly by -9 % in the >10 % weight loss group. Surprisingly, HDL-cholesterol increased in all groups yet this increase was most pronounced in the highest weight loss group (+14.9 %).

Table 14. Relative changes in metabolic parameters (%) from baseline to the final visit after 12 months according to weight loss.[a]

	Group	Baseline Median (IQR)	After 12 months Median (IQR)	% change Median (IQR)	p-value
Weight [kg]	< 5%	85.1 (79.5 to 90.3)	84.7 (78.8 to 91.3)	-0.2 (-1.3 to 1.1)	0.460
	5-10%	82.1 (75.0 to 89.3)	75.6 (69.6 to 82.9)	-7.1 (-8.6 to -6.3)	**<0.001**
	> 10%	85.6 (79.5 to 93.3)	72.0 (65.4 to 80.4)	-14.5 (-17.0 to -12.5)	**<0.001**
Fat mass [kg]	< 5%	32.6 (29.9 to 37.7)	33.7 (28.5 to 38.8)	+1.1 (-4.9 to 4.6)	0.805
	5-10%	31.6 (28.0 to 38.2)	27.5 (24.4 to 35.1)	-14.0 (-16.8 to -9)	**<0.001**
	> 10%	34.3 (30.2 to 39.0)	25.4 (21.0 to 28.4)	-27.2 (-32.2 to -23.1)	**<0.001**
Waist circumference [cm]	< 5%	97.5 (90.3 to 101.8)	94.0 (89.4 to 98.0)	-3.0 (-5.3 to 0.7)	**0.004**
	5-10%	96.0 (89.8 to 101.0)	88.0 (82.5 to 97.0)	-8.0 (-11.3 to -4.7)	**<0.001**
	> 10%	93.0 (89.8 to 101.5)	80.0 (75.0 to 90.0)	-11.8 (-17.2 to -7.4)	**<0.001**
Systolic blood pressure [mmHg]	< 5%	119.0 (104.4 to 133.1)	120.0 (110.0 to 135.0)	4.2 (-2.7 to 11.0)	**0.043**
	5-10%	120.0 (110.0 to 130.0)	116.0 (101.3 to 126.9)	-2.2 (-12.4 to 7.9)	0.263
	> 10%	120.0 (111.3 to 130.0)	115.5 (100.0 to 125.5)	-3.1 (-11.3 to 0.00)	**0.028**

Table 14. (continued)

	Group	Baseline Median (IQR)	After 12 months Median (IQR)	% change Median (IQR)	p-value
Diastolic blood pressure [mmHg	< 5%	80.0 (70.0 to 82.5)	80.0 (74.4 to 82.6)	0.00 (-6.4 to 11.2)	0.455
	5-10%	76.3 (66.3 to 80.5)	77.5 (70.0 to 80.0)	0.00 (-9.0 to 5.3)	0.946
	> 10%	80.0 (75.8 to 82.8)	72.5 (67.5 to 80.8)	-7.7 (-12.5 to 0.00)	**0.004**
Glucose [mmol/l]	< 5%	4.9 (4.7 to 5.4)	5.0 (4.7 to 5.5)	0.0 (-4.9 to 8.5)	0.773
	5-10%	4.8 (4.6 to 5.0)	4.8 (4.5 to 5.3)	0.0 (-4.4 to 6.6)	0.724
	> 10%	4.9 (4.5 to 5.3)	4.7 (4.3 to 4.9)	-4.0 (-12.8 to 1.2)	**0.005**
Insulin [pmol/l]	< 5%	53.0 (32.3 to 70.0)	42.0 (31.5 to 74.0)	-13.1 (-26.9 to 13.1)	0.312
	5-10%	44.5 (31.3 to 62.8)	39.5 (24.3 to 51.3)	-8.3 (-37.0 to 8.2)	0.193
	> 10%	50.0 (33.0 to 65.0)	26.0 (17.0 to 44.5)	-47.8 (-58.5 to -13.5)	**<0.001**
HOMA-IR	< 5%	1.7 (0.9 to 2.3)	1.5 (0.9 to 2.3)	-12.8 (-31.2 to 17.3)	0.278
	5-10%	1.3 (0.9 to 1.9)	1.2 (0.7 to 1.9)	-7.1 (-38.5 to 21.0)	0.282
	> 10%	1.4 (0.9 to 2.1)	0.7 (0.5 to 1.2)	-47.7 (-63.7 to -14.6)	**<0.001**
Triglycerides [mmol/l]	< 5%	1.3 (1.0 to 1.7)	1.5 (1.0 to 1.8)	9.3 (-10.2 to 54.6)	0.114
	5-10%	1.2 (0.9 to 1.4)	1.2 (1.0 to 1.5)	-2.2 (-21.5 to 25.0)	0.922
	> 10%	1.1 (0.9 to 1.8)	1.1 (0.8 to 1.4)	-16.9 (-32.5 to 6.7)	**0.016**
Total cholesterol [mmol/l]	< 5%	5.2 (4.4 to 6.2)	5.5 (4.7 to 6.4)	+4.9 (-5.6 to 15.2)	0.054
	5-10%	5.3 (4.9 to 6.0)	5.6 (5.0 to 6.1)	+4.5 (-5.2 to 12.1)	0.080
	> 10%	5.4 (4.7 to 5.9)	5.3 (4.6 to 5.7)	-5.7 (-13.0 to 1.4)	**0.020**
LDL-cholesterol [mmol/l]	< 5%	3.1 (2.6 to 3.9)	3.4 (2.8 to 4.2)	+8.3 (-7.7 to 20.0)	**0.023**
	5-10%	3.3 (2.9 to 3.9)	3.5 (2.8 to 3.8)	+2.2 (-10.4 to 15.1)	0.387
	> 10%	3.3 (2.9 to 3.7)	2.9 (2.5 to 3.7)	-9.0 (-19.1 to 2.4)	**0.004**
HDL-cholesterol [mmol/l]	< 5%	1.4 (1.2 to 1.7)	1.5 (1.2 to 1.8)	+4.0 (-2.7 to 13.5)	**0.014**
	5-10%	1.4 (1.2 to 1.8)	1.6 (1.3 to 1.9)	+13.0 (3.7 to 22.9)	**<0.001**
	> 10%	1.5 (1.3 to 1.8)	1.6 (1.4 to 2.0)	+14.9 (-3.5 to 23.7)	**0.007**

[a] Relative changes are shown as medians (IQR). P-values for changes from baseline to visit F (after 12 months) within one group were determined by the Wilcoxon-test. N = 34, 36 and 33 for the <5 %, 5 to 10 % and >10 % group, respectively. Fat mass was only measured in 84-88 participants (n = 30-32 for the <5 %, n = 28-30 for the 5-10 % and n = 26 for the >10 % weight loss group).

3.8 Correlation between Adipokine/Chemokine Levels and Indices of Obesity as well as Metabolic Markers before and after Weight Loss

Using Spearman correlation, the correlation of measured adipokine/chemokine concentrations in serum with metabolic markers and with each other before and after weight loss was tested (**Table 15**). Before and after weight loss (12 months), total and HMW adiponectin correlated negatively with glucose and insulin levels as well as with HOMA-IR and TG, while a positive correlation with HDL-cholesterol levels was found. The two adiponectin isoforms correlated highly to each other. IP-10 levels correlated positively with MCP-1 and PGRN but only after weight loss. MCP-1 and RANTES correlated positively with baseline glucose levels. Furthermore, MCP-1 correlated positively with RANTES before and after weight loss. Baseline PGRN and IP-10 levels correlated positively.

Table 15. Spearman correlation coefficients between inflammatory markers and anthropometric as well as metabolic parameters before and after weight loss.[a]

	Before weight loss						After weight loss (12 months)					
	Total adiponectin	HMW adiponectin	MCP-1	IP-10	RANTES	PGRN	Total adiponectin	HMW adiponectin	MCP-1	IP-10	RANTES	PGRN
Body Weight	-0.04	-0.16	0.01	0.16	0.18	-0.03	**-0.23**	**-0.30**	-0.12	**0.21**	0.06	0.04
	(0.69)	(0.88	(0.90)	(0.12)	(0.08)	(0.77)	**(0.02)**	**(0.002)**	(0.24)	**(0.04)**	(0.55)	(0.70)
Fat mass	-0.02	0.001	0.11	0.08	**0.29**	0.02	**-0.36**	**-0.44**	-0.12	0.10	0.03	0.05
	(0.84)	(0.99)	(0.31)	(0.47)	**(0.008)**	(0.83)	**(0.001)**	**(<0.001)**	(0.27)	(0.38)	(0.79)	(0.67)
Waist circumference	-0.16	-0.18	**0.21**	0.03	**0.33**	0.08	**-0.33**	**-0.38**	0.07	**0.23**	0.17	0.06
	(0.11)	(0.08)	**(0.04)**	(0.75)	**(0.001)**	(0.42)	**(0.001)**	**(<0.001)**	(0.47)	**(0.02)**	(0.09)	(0.56)
Glucose	**-0.27**	**-0.27**	**0.26**	0.10	**0.20**	0.09	**-0.21**	**-0.21**	0.16	**0.25**	0.14	0.05
	(0.007)	**(0.005)**	**(0.009)**	(0.24)	**(0.045)**	(0.36)	**(0.04)**	**(0.03)**	(0.12)	**(0.01)**	(0.17)	(0.65)
Insulin	**-0.25**	**-0.27**	0.15	0.11	0.11	0.13	**-0.35**	**-0.34**	0.04	0.17	**0.20**	-0.05
	(0.01)	**(0.005)**	(0.12)	(0.27)	(0.27)	(0.21)	**(<0.001)**	**(0.001)**	(0.69)	(0.10)	**(0.04)**	(0.61)

Table 15. (continued)

	Before weight loss						After weight loss (12 months)					
	Total adiponectin	HMW adiponectin	MCP-1	IP-10	RANTES	PGRN	Total adiponectin	HMW adiponectin	MCP-1	IP-10	RANTES	PGRN
HOMA-IR	**-0.26**	**-0.29**	0.19	0.11	0.14	0.13	**-0.38**	**-0.36**	0.09	0.20	**0.20**	-0.02
	(0.008)	**(0.003)**	(0.05)	(0.26)	(0.16)	(0.19)	**(<0.001)**	**(<0.001)**	(0.39)	(0.05)	**(0.04)**	(0.82)
TG	**-0.28**	**-0.25**	0.13	0.08	0.02	0.12	**-0.37**	**-0.32**	0.06	0.19	0.09	0.16
	(0.004)	**(0.01)**	(0.20)	(0.42)	(0.87)	(0.24)	**(<0.001)**	**(0.001)**	(0.54)	(0.06)	(0.35)	(0.11)
Total Cholesterol	**0.25**	**0.26**	**0.21**	0.03	-0.006	0.16	0.06	0.003	0.17	0.05	0.09	0.15
	(0.01)	**(0.009)**	**(0.04)**	(0.75)	(0.95)	(0.12)	(0.54)	(0.98)	(0.08)	(0.60)	(0.36)	(0.12)
LDL-cholesterol	0.11	0.12	**0.22**	0.10	-0.04	0.10	-0.04	-0.10	0.18	0.06	0.12	0.13
	(0.29)	(0.24)	**(0.03)**	(0.30)	(0.68)	(0.33)	(0.72)	(0.34)	(0.07)	(0.53)	(0.22)	(0.20)
HDL-cholesterol	**0.50**	**0.48**	0.09	-0.11	0.02	0.07	**0.39**	**0.35**	-0.03	-0.09	-0.12	0.00
	(<0.001)	**(<0.001)**	(0.39)	(0.29)	(0.81)	(0.48)	**(<0.001)**	**(<0.001)**	(0.78)	(0.37)	(0.23)	(1.00)
Total adiponectin	-	**0.96**	0.08	-0.16	0.05	0.001	-	**0.93**	0.001	-0.12	-0.19	0.10
		(<0.001)	(0.45)	(0.11)	(0.66)	(0.93)		**(<0.001)**	(0.99)	(0.23)	(0.06)	(0.34)

	Before weight loss						After weight loss (12 months)					
	Total adiponectin	HMW adiponectin	MCP-1	IP-10	RANTES	PGRN	Total adiponectin	HMW adiponectin	MCP-1	IP-10	RANTES	PGRN
HMW adiponectin	**0.96** **(<0.001)**	-	0.08 (0.43)	-0.12 (0.24)	0.04 (0.66)	0.001 (0.95)	**0.93** **(<0.001)**	-	-0.02 (0.85)	-0.16 (0.12)	-0.17 (0.09)	0.06 (0.58)
MCP-1	0.08 (0.45)	0.08 (0.43)	-	0.19 (0.06)	**0.33** **(0.001)**	**0.25** **(0.01)**	0.001 (0.99)	-0.02 (0.85)	-	**0.25** **(0.01)**	**0.30** **(0.002)**	**0.21** **(0.03)**
IP-10	-0.16 (0.11)	-0.12 (0.24)	0.19 (0.06)	-	-0.04 (0.72)	**0.25** **(0.01)**	-0.12 (0.23)	-0.16 (0.12)	**0.25** **(0.01)**	-	0.16 (0.11)	0.18 (0.07)
RANTES	0.05 (0.66)	0.04 (0.66)	**0.33** **(0.001)**	-0.04 (0.72)	-	-0.00 (0.98)	-0.19 (0.06)	-0.17 (0.09)	**0.30** **(0.002)**	0.16 (0.11)	-	-0.08 (0.41)
PGRN	0.001 (0.93)	0.001 (0.95)	**0.25** **(0.01)**	**0.25** **(0.01)**	-0.00 (0.98)	-	0.10 (0.34)	0.06 (0.58)	**0.21** **(0.03)**	0.18 (0.07)	-0.08 (0.41)	-

[a] Spearman correlation, bivariate (2-sided significance). Significant associations appear in bold letters. Coefficients highlighted in grey were non-significant after controlling for fat mass via partial correlation.

4 Discussion

In the framework of the present thesis, it was investigated whether referral to a commercial programme (CP) by general practitioners (GP) is an effective option for weight management. Moreover, we analyzed the association between the extent of weight loss and changes in metabolic parameters, *e.g.* blood lipid, glucose and insulin levels as well as circulating concentrations of adipokines/chemokines. In the following sections, the main results are discussed in the context of the existing literature as well as future perspectives.

4.1 Weight Loss Efficacy and Changes in Clinical Blood Parameters

4.1.1 Commercial Programme vs. Standard Care

In the present clinical study, the CP resulted in a significantly greater weight loss compared to SC in Germany (-5.15 ± 6.79 *vs.* -2.65 ± 4.75, n = 112 *vs.* 89) and in all countries (-6.65 (SEM 0.43) *vs.* -3.26 (SEM 0.33) kg, n = 230 *vs.* 214). Moreover, the proportion of participants losing ≥5 % and ≥10 % of initial body weight was significantly higher in the CP compared to SC. In the completer analysis, weight loss was higher in the UK and Australia when compared to Germany (Jebb et al. 2011). This could be explained by significant differences in the attrition rate, *i.e.*, the proportion of participants who did not complete the 12-month assessment was 64 % in the UK, 41 % in Australia and only 25 % in Germany. In Germany, we indeed put effort in encouraging participants to stay on track after starting the weight loss programme in order to minimize the drop-out rate. However, this probably increased the chance of having persons who were not intrinsically motivated anymore to follow the programme in order to lose weight.

Overall, the results of our intervention trial are in agreement with a previous study published in 2003 involving 423 participants in the USA, which revealed a significant greater weight loss after one year in the commercial intervention group when compared to a self-help programme (-5.0 (SEM 0.5) *vs.* -1.4 (SEM 0.5) kg (Heshka et al. 2003). Holzapfel and Hauner accurately listed several life-style based human studies focusing on weight loss with a duration of at least 1 year (Holzapfel and Hauner 2011). Overall, a weight loss between 2 and 4 kg can be expected after an intervention period of 1 year. Several studies analyzing lifestyle intervention programmes for weight loss have been set up in the last years and show results comparable to ours (**Table 16**). All these studies were performed in many different settings and comparison is thus difficult due to the heterogeneity in cohorts, *e.g.*, number,

age, origin and health status of enrolled participants, time period studied, and anthropometric parameters measured. Thus, the output of our clinical trial (2 to 5 kg) is in the range of already published studies.

Table 16. Overview of lifestyle intervention programmes for weight loss

Subjects	mean BMI [kg/m^2]	Programme	Weight loss	Reference
5,145 adults (40 % men) with T2DM LOOK AHEAD Trial	35 – 36	1) Lifestyle intervention (nutrition / physical activity) with group sessions 2) Control group: diabetes support and education	After 1 year: 1) -8.7 ± 6.9 % 2) -0.7 ± 4.8 %	(Pi-Sunyer et al. 2007)
			After 4 years: 1) -6.15 % (CI:-6.39 to -5.91) 2) -0.88 %(CI: -1.12 to -0.64)	(Wadden et al. 2011)
213 adults	35	1) Maintenance-tailored therapy 2) Standard behavior therapy	1) 8.3 ± 8.9 kg 2) 9.3 ± 8.8 kg After 18 months	(Jeffery et al. 2009)
740 adults Lighten Up Study	~34	1) Weight Watchers 2) Slimming World 3) Rosemary Conley 4) Size down 5) General practice 6) Pharmacy 7) Choice 8) Comparator (minimal intervention)	1) 3.46 kg (CI: 2.1 to 4.8) 2) 1.89 (CI: 0.9 to 2.9) 3) 2.12 (CI: 0.9 to 3.4) 4) 2.45 (CI: 1.3 to 3.6) 5) 0.83 (CI: 0.4 to 2.0) 6) 0.66 (CI: 0.4 to 1.7) 7) 2.15 (CI: 0.9 to 3.4) 8) 1.08 (CI: 0.1 to 2.1) BOCF analysis after 1 year	(Jolly et al. 2011)
309 adults (42 % men)	32	Commercial web-based programme 1) Standard version 2) Enhanced version 3) Control	1) -2.1 ± 3.3 kg 2) -3.0 ± 4.1 kg 3) +0.4 ± 2.3 kg Intention-to-treat analysis after 3 months	(Collins et al. 2012)

Abbreviations: BOCF, baseline observation carried forward; CI, confidence interval

One major challenge faced by weight loss strategies in obesity is to maintain body weight loss in the long-term. In the study by Heshka et al. mentioned above, body weight was still significantly lower by 3 kg when compared to baseline values in the CP one year after the end of the intervention. Our follow-up data go along this line, since we observed a significant reduction in body weight by -5.1 kg in the CP and a non-significant reduction by -2.5 kg in the SC group still one year after the end of the intervention (24 months) when compared to baseline (data for the entire WW cohort are available in **Appendix E**) (Holzapfel et al. in preparation). However, participants in both intervention groups gained weight from month 12 to 24, and weight regain was significantly higher in the CP only in the more conservative LOCF analysis (n = 201). Lowe et al. analyzed weight loss maintenance after 1, 2 and 5 years following successful completion of the same CP in 699 participants (Lowe et al. 2008). The proportion of participants who maintained a weight loss of at least 5 % of initial body weight was 80 %, 71 % and 50 %, respectively. Although only the most successful persons agreed to take part in these follow-up studies (Lowe et al. 2008; Holzapfel et al. in preparation), the results provide further evidence that weight loss maintenance after completing a CP is feasible, even though success rates rapidly drop overtime. However, long-term data in weight loss trials are scarce due to high drop-out rates from 10 to 80 % (Farley et al. 2003). The ability of persons to stay on track when starting a weight loss programme is influenced by a variety of factors, yet psychological situation and emotional behavior are keys to adherence to the programme (Grossi et al. 2006). The best way to increase adherence is to support re-establishment of social network, feelings of community adherence and self-esteem. Effective communication between participants and intervention team is also crucial. In 35 studies based on dietary counseling for weight loss, the frequency of counseling was shown to be positively predictive for weight loss (Finkler et al. 2012). This supports the findings by Heshka et al. showing that weight loss success was associated with higher self-reported attendance at meetings proposed by the CP (Heshka et al. 2003).

Most general practitioners, at least in Germany, do not take sufficient time and do not feel enough financial support in order to effectively advice patients for weight loss. The results of our study, conducted as in the sense of a partnership model between a CP and general practitioners, clearly emphasize the efficacy of referring patients to a commercial programme that provides regular weighing, nutritional and physical activity advices, motivation and group support. Such a programme can be used as basis for a clinically relevant intervention targeting overweight and obese people coordinated by general practitioners.

In summary, many intervention strategies for losing weight show efficacy in the short-term, yet multi-factorial programmes favoring social networks are most effective in the long term. The importance of such pogrammes targeting obese people to lose weight, even little weight yet over longer time periods, is highlighted by studies showing substantial improvement of clinical parameters of relevance to cardiovascular diseases and chronic metabolic disorders after intervention (Dattilo and Kris-Etherton 1992; Tuomilehto et al. 2001; Lindström et al. 2003b).

4.1.2 Weight Loss and Clinical Blood Parameters

Moderate reductions in body weight (5 to 10 % reduction of initial body weight) have been associated with metabolic benefits, like improvement in fasting glycaemia, blood pressure and plasma lipid profile (Wing et al. 1998; Goldstein 1992; Vidal 2002). This is in accordance with this study in which subjects in the CP had greater improvements in insulin and LDL-cholesterol levels compared to SC. There were no significant differences in other cardiovascular risk factors, e.g. TG, total cholesterol and blood pressure between the two groups. This could be due to the fact that the study population has been overweight to moderately obese (BMI 27-35kg/m^2) with limited severity of comorbidities (Jebb et al. 2011).

The analysis of our German sub-cohort including 103 individuals based on a linear regression model revealed that weight loss by -1 % (ca. 0.8 kg) was associated with significant improvement in glucose and insulin levels as well as a decrease in the insulin resistance index HOMA. Nonetheless, when separating the sub-cohort into weight loss categories (<5 %, 5 to 10 %, >10 %) only participants losing >10 % of initial weight show a significant reduction in relative levels of glucose, insulin, and HOMA-IR compared to their respective baseline levels. These effects associated with weight loss may be indicators of reduced risk to develop diabetes in the long-term. The US Diabetes Prevention Program Research Group published the 10-year follow-up data of a randomized clinical trial (Knowler et al. 2009). A total of 2 766 overweight adults with impaired glucose tolerance were allocated to placebo, metformin treatment (antidiabetic agent) or intensive lifestyle intervention group for a period of one year. Although body weight was regained in the intensive lifestyle intervention group, it was still reduced by -2 kg compared to baseline after 10 years. Diabetes incidence was reduced by 34 % in the lifestyle intervention group and by 18 % in the metformin group compared to placebo, showing that lifestyle intervention can be highly effective in preventing, or at least delaying, the onset of T2DM. The preventive

character of weight loss with respect to the development of T2DM has been highlighted by additional studies in high risk subjects (Wing et al. 1998; Tuomilehto et al. 2001; Mason et al. 2011).

In addition, the linear mixed model revealed that weight loss is associated with a decrease in factors associated with cardiovascular risk: TG, total and LDL-cholesterol. There was however no significant association with HDL-cholesterol. These data are in line with a meta-analysis of the effects of weight reduction on blood lipids (Dattilo and Kris-Etherton 1992). The authors reported a decrease in serum total cholesterol by -0.05 mmol/l, in LDL-cholesterol by -0.02 mmol/l and in triglycerides by -0.015 mmol/l per kg weight loss (changes in this study were: -0.03, -0.03 and -0.02 mmol/l, respectively, per 1 % (~0.8 kg) weight loss). When separating the German sub-cohort into weight loss categories, only participants losing >10 % of initial weight were characterized by a significant reduction in the relative levels of TG, total and LDL-cholesterol. In contrast, HDL increased in all three weight loss categories, but the increase was more pronounced in the >5 % and >10 % group when compared to the <5 % group. One possible reason for the increase in HDL in the <5 % weight loss group might be, in addition to weight loss, that dietary factors influence serum lipoprotein levels. Thus, in spite of the limited body weight loss, participants may have substantially changed their dietary habits. It has been shown for instance that a Mediterranean diet rich in olive oil or nuts can improve lipid profile compared to a low-fat diet (Estruch et al. 2006; Serra-Majem et al. 2006). Scientific evidence has also been accumulating on the beneficial effects of diets with relatively high content of mono-unsaturated fatty acids (MUFA, in olive oil) on cardiovascular risk factors, obesity and diabetes (Kris-Etherton et al. 1999; Martínez-González and Sánchez-Villegas 2004). Of note, in addition to the use of virgin olive oil and nuts, the typical Mediterranean diet includes less red meat, more fruits and vegetables than the typical Western diet. To explain the discrepancy in higher HDL levels in all weight loss groups, it would be interesting to look at correlations between the intake of different fatty acids and serum HDL levels. However, the use of dietary protocols is limited for that purpose in this study since participants did not necessarily report the types of oil they consume (*e.g.*, olive, sunflower or rapseed oil).

Finally, in the German sub-cohort, weight loss was associated with a decrease in systolic and diastolic blood pressure. The influence of weight loss on blood pressure was summarized in a meta-analysis of randomized controlled trials (Neter et al. 2003). The output of this analysis was a reduction in systolic and diastolic blood pressure by ca. -1 mmHg per kg weight loss. This reduction was more pronounced when including subjects taking antihypertensive medication in the analysis. This larger effect compared to the results in this study (-0.36 mmHg for systolic and -0.24 mmHg for diastolic blood pressure per 1 % (ca. 0.8 kg) weight loss) can be explained by another meta-analysis (Ebrahim and Smith 1998), in which blood pressure response was higher in hypertensive compared to normotensive patients. The median values for systolic and diastolic blood pressure in the German sub-cohort were in the normal range (120/80 mmHg) and only 28 persons were on a hypertensive medication. In the whole study sample, measured changes in blood pressure were small, although a quarter were receiving antihypertensive medication which could have masked any improvements (Jebb et al. 2011). Like in the German sub-cohort, the remainder had normal blood pressure and these subjects are less likely to reduce blood pressure during weight loss (Ebrahim and Smith 1998; Hauner et al. 2004).

4.2 Weight Loss and Inflammatory Markers

Due to the fact that weight loss is associated with improvement of metabolic parameters (*e.g.*, blood pressure, insulin resistance), it is reasonable to ask the question to what extent excessive adipose tissue expansion in obesity might trigger metabolic imbalance via for instance increased secretion of pro-inflammatory and decreased secretion of anti-inflammatory signaling molecules.

4.2.1 Influence of Weight Loss on Serum Levels of Total and HMW Adiponectin

There is nowadays good evidence that obese people are characterized by lower serum concentrations of total and HMW adiponectin compared to lean subjects (**Table 17**).

Table 17: Circulating total and HMW adiponectin in obesity and weight loss

Factor	Effect of obesity	Reference	Effect of weight loss	Reference
Total adiponectin	↓	(Arita et al. 1999)	↑	(Butner et al. 2010)
	↓	(Matsubara et al. 2002b)	↑	(Coughlin et al. 2007)
			↑	**This study**
HMW adiponectin	↓	(Kaser et al. 2008)	↑	(Linscheid et al. 2008)
	↓	(Araki et al. 2006)	↑	**This study**

The median concentration of adiponectin that was measured at the beginning of the study in the blood obtained from the 103 overweight participants in the German sub-cohort (5.1 µg/ml for total and 2.5 µg/ml for HMW adiponectin) are in line with concentrations usually reported in the literature (2 to 20 µg/ml) (Arita et al. 1999; Swarbrick et al. 2006; Bobbert et al. 2005; Engl et al. 2007; Kopp et al. 2005). This range of concentrations could be dependent upon the different methods or immunoassays used for quantification (native electrophoresis, ELISA, multiplex) (Madsen et al. 2008).

In this study, after 12 months of intervention, a median weight loss of -7 % (ca. 6 kg) resulted in a significant 9.4 %-increase in total adiponectin (median range: 5.1 - 5.9 µg/ml) and a 12.4 %-increase in HMW adiponectin (median range: 2.5 - 3.0 µg/ml). These data were confirmed using a linear mixed model, which revealed that a loss of -1 % initial body weight explains an increase of approximately 0.6 % in total and 0.7 % in HMW adiponectin. Furthermore, when grouping subjects according to the degree of weight loss, concentrations of adiponectin increased significantly only in participants losing 5 to 10 % body weight (-6 kg; total adiponectin +11.9 %, HMW adiponectin +11.7 %) and >10 % (-14 kg; total adiponectin +14.8 %, HMW adiponectin +19.4 %) compared to respective baseline levels. These differences are in agreement with a study by Varady et al. reporting an increase in adiponectin levels by 20 % in 13 severely obese women (mean BMI of 50 kg/m^2) losing between 5 and 10 % initial body weight (~10 kg) (Varady et al. 2009). However, another study reported that a minimum weight loss of 10 % (ca. 12 kg) was required to elevate

adiponectin levels significantly in obese individuals (mean BMI of 37 kg/m^2) (Madsen et al. 2008). A possible explanation for this discrepancy may be the different grade of obesity (grade III in the Varady's *vs.* grade II obesity in the Madsen's study). However, this hypothesis is refuted by this data showing changes in levels of adiponectin even in grade I obese subjects (mean BMI of 30 kg/m^2). In one additional study, researchers also observed an elevation in adiponectin during weight loss in subjects with a BMI below 30 kg/m^2 (Lang et al. 2011). Thus, the metabolic state of overweight participants may be a better explanation for the differences seen in adiponectin levels after weight loss. In the study by Madsen et al., elevated glucose and insulin levels (6.6 mmol/l and 15.2 IU/l, respectively) suggest that these participants were more insulin resistant than participants in our study (median glucose and insulin levels of 4.8 mmol/l and 48 pmol/l, respectively). This hypothesis is supported by *in vitro* and *in vivo* studies demonstrating that insulin can inhibit secretion of adiponectin by adipocytes (Fasshauer et al. 2002). In 60 non-diabetic subjects stratified by degree of obesity and insulin resistance, Abassi et al. found that insulin-resistant subjects had lower adiponectin levels independently of obesity compared to insulin-sensitive subjects (obese and non-obese) (Abbasi et al. 2004). Interestingly, the same authors also found that weight loss did not influence adiponectin levels whereas treatment with rosiglitazone (an insulin sensitizer) led to a 3-fold increase in adiponectin levels in insulin-resistant individuals (Abbasi et al. 2006). Finally, Kopp et al. reported a more pronounced increase in adiponectin during weight loss in normal-glucose tolerant *vs.* impaired glucose tolerant and T2DM participants (Kopp et al. 2005).

Different adiponectin oligomers have been discovered and the HMW isoform is proposed to be the most biologically active form to mediate beneficial metabolic effects (Lara-Castro et al. 2006; Araki et al. 2006; Pajvani et al. 2004). Genetic data also support the hypothesis that HMW serves as the most active form, since patients with a mutation in the adiponectin gene that specifically decreases HMW levels exhibit diabetes (Waki et al. 2003). Moreover, circulating levels of adiponectin isoforms are highly heritable (Menzaghi et al. 2010). Therefore, an increasing number of studies have been focusing on oligomer distribution of adiponectin during weight loss. The data of this study have demonstrated that increased HMW levels were significantly associated with weight loss, which is in line with other studies (Salani et al. 2006; Linscheid et al. 2008; Swarbrick et al. 2006). Whether the observed increase in total adiponetin was only due to an increase in HMW cannot be answered, since we did not measure other isoforms of adiponectin (MMW, LMW). Kaser et al. have shown that reduced total adiponectin levels in obese people are due to reduced

concentrations of HMW (Kaser et al. 2008) and an increase in plasma adiponectin levels was attributable to the HMW complex (Linscheid et al. 2008).

The anti-diabetic effect of adiponectin can be explained by its ability to inhibit hepatic glucose production (Berg et al. 2001; Combs et al. 2001) and to stimulate glucose uptake and fatty acid oxidation in muscles via AMPK activation after binding to AdipoR (Yamauchi et al. 2002). As described above, low adiponectin levels are usually detected in insulin-resistant subjects (Abbasi et al. 2004). Our data show that total and HMW adiponectin inversely correlated with HOMA-IR before and after weight loss (total: r = -0.26 and -0.38; HMW: -0.29 and -0.36, respectively). These data were still significant after controlling for fat mass and are in line with other studies from the literature (Hotta et al. 2000; Andreasson et al. 2012; Kazumi et al. 2004; Steffes et al. 2004). In a cross-sectional study by Matsubara et al., total adiponectin levels were inversely correlated with HOMA-IR independently of the grade of obesity (Matsubara et al. 2003). In Pima Indians, it has been shown that high adiponectin levels (5.3 *vs.* 4.3 µg/ml) are associated with high insulin-sensitivity and that subjects were thereby less likely to develop T2DM (Lindsay et al. 2002). Data from the Diabetes Prevention Program suggest that baseline adiponectin level is a strong predictor of incident diabetes (Mather et al. 2008). In subjects at increased risk of developing diabetes (IGT, obesity) following a lifestyle intervention, an increase in total adiponectin by ca. 1 µg/ml was associated with a 16 %-reduction in the progression of T2DM. However, it is still a matter of debate whether hypoadiponectemia is the cause or the consequence of impaired insulin action.

Also in this study, total and HMW adiponectin correlated positively with HDL-cholesterol levels before and after weight loss (total: r = +0.50 and +0.39; HMW: r = +0.48 and +0.35, respectively) and negatively with TG (total: r = -0.28 and -0.27; HMW: r = -0.25 and -0.32, respectively), even after controlling for fat mass. This association between higher adiponectin levels and reduced cardiovascular risk factors are in agreement with the literature (Matsubara et al. 2002a; Zietz et al. 2003; Kazumi et al. 2004; Fujimatsu et al. 2009; Andreasson et al. 2012). In one analysis by the Health Professionals Follow-up Study including diabetic subjects, higher adiponectin levels were positively correlated with HDL (r = 0.42) and negatively correlated with TG (r = -0.38) and the correlations were not appreciably altered after controlling for lifestyle, medical conditions and obesity-related variables (Schulze et al. 2004). Bobbert et al. also found a strong correlation between HDL and HMW which, together with free fatty acids, predicted about 60 % of the change in HDL

during weight loss intervention (Bobbert et al. 2005). Adiponectin has been proposed to play an inhibitory role in the development of atherosclerosis by influencing endothelial cells via stimulation of NO production and reduction of adhesion molecule expression (Chen et al. 2003). It also inhibits proliferation of smooth muscle cells (Wang et al. 2005) and suppresses macrophage-to-foam cell transformation by inhibiting the expression of class A scavenger receptors (Ouchi et al. 2001). Furthermore, in macrophages, adiponectin blocks the production of pro-inflammatory cytokines (Tilg and Moschen 2006) and it regulates macrophage polarization towards an anti-inflammatory M2 phenotype (Lovren et al. 2010; Ohashi et al. 2010). Through its inhibitory effect on the production of IL-6 and MCP-1 by adipocytes and by reducing the expression of the chemokine receptors CCR2 and CCR5 on monocytes, it could also be involved in controlling obesity-induced macrophage infiltration and subsequent production of chemokines in adipose tissues (Zoico et al. 2009; Neumeier et al. 2011). In the German sub-cohort, there were no correlations between total or HMW adiponectin and circulating chemokine levels, including MCP-1.

4.2.2 Influence of Weight Loss on Chemokine Serum Levels: MCP-1, IP-10 and RANTES

Obese subjects are known to have higher systemic MCP-1, IP-10 and RANTES levels than lean individuals (**Table 18**).

Table 18. Circulating MCP-1, IP-10 and RANTES in obesity and weight loss

Factor	Effect of obesity	Reference	Effect of weight loss	Reference
MCP-1	↑	(Christiansen et al. 2005)	↓	(Christiansen et al. 2005)
	↑	(Kim C.S. 2006)	↓	**This study**
IP-10	↑	(Herder et al. 2007a)	→	(Dalmas et al. 2011)
	↑	(Dalmas et al. 2011)	→	(Wong et al. 2008)
			→	**This study**
RANTES	↑	(Dalmas et al. 2011)	→	**This study**
	↑	(Keophiphath et al. 2010)		

A baseline median MCP-1 concentration of 302.9 pg/ml in the German sub-cohort of 103 subjects was measured. Using ELISA-based approaches too, other groups reported values between 180 and 580 pg/ml in obese people within different BMI categories ranging from overweight to severely obese (Kim C.S. 2006; Christiansen et al. 2005; Schernthaner et al. 2006; Chacón et al. 2007). In this study, after 12 months of intervention, a median weight loss of -7 % (~6 kg) resulted in a significant 6.9 %-decrease in MCP-1 levels (from 309.7 to 298.6 pg/ml). These data were confirmed using a linear mixed model, which revealed that a loss of -1 % of initial body weight explains a decrease of approximately -0.7 % in MCP-1 serum levels. Furthermore, when grouping subjects according to the degree of weight loss, concentrations significantly decreased only in participants losing 5 to 10 % initial body weight (-6 kg; MCP-1 -7.8 %, -10.8 pg/ml) or >10 % (-14 kg; MCP-1 -8.8 %, -13.1 pg/ml) compared to respective baseline levels. Schernthaner et al. found that massive weight loss by 35 kg resulted in a 47 %-decrease in MCP-1 levels in morbidly obese people (mean BMI 45 kg/m^2) after bariatric surgery (Schernthaner et al. 2006). In another study, a weight loss by only 12 % led to a significant reduction in circulating MCP-1 by 20 % in severely obese people (mean BMI 51 kg/m^2) (Christiansen et al. 2005).

MCP-1 release by adipose tissue was shown to be higher in obese (1.0 µg protein/µg DNA) compared to lean subjects (0.2 µg protein/µg DNA) and in visceral compared to subcutaneous adipose tissue, but both differences disappeared after adjusting for the number of resident macrophages (Bruun et al. 2005). Indeed, although isolated adipocytes can produce MCP-1, it is mainly the stromal-vascular fraction of adipose tissue, especially resident macrophages, that accounts for MCP-1 release (Bruun et al. 2005; Christiansen et al. 2005). MCP-1 production by adipocytes has been shown to be less than 12 % of that by non-fat cells (Fain 2006). Cancello et al. showed that drastic weight loss led to a reduction in both macrophage number and MCP-1 gene expression in subcutaneous adipose tissue (Cancello et al. 2005). In contrast, Bruun et al. did not find a reduction in MCP-1 mRNA levels in subcutaneous abdominal fat tissue during weight loss (~ -18 kg) in 27 severe obese patients (BMI 46) after 15 weeks of intervention, although mRNA levels of macrophage specific markers (CD68 and CD14) were significantly reduced by more than 40 % and plasma levels of MCP-1 also decreased significantly (Bruun et al. 2006). In this study, changes in fat mass were not associated with changes in MCP-1 levels. Since fat tissue biopsies could not be sampled, it is not possible to determine whether macrophage number might be responsible for blood MCP-1 levels.

MCP-1 is proposed to be involved in the pathogenesis of atherosclerosis via recruitment of monocytes/macrophages into the vascular wall during the pathogenesis of vascular lesion formation in atherosclerosis (Gosling et al. 1999). In a large population-based sample of 3 499 subjects in the Dallas Heart Study, plasma MCP-1 levels were positively associated with traditional risk factors for atherosclerosis (*e.g.*, hypercholesterolemia) (Deo et al. 2004). Also, Kim et al. found a negative correlation between MCP-1 and HDL, a major predictor of decreased cardiovascular disease risks (Kim 2006). Data from the prospective MONICA/KORA cohort in 2 358 subjects indicated that elevated systemic levels of MCP-1, IL-8 and IP-10 precede incident coronary heart disease but do not represent independent risk factors (Herder et al. 2006b). In spite of these associations reported in the literature, there were no significant associations between serum MCP-1 levels and HDL-cholesterol before and after weight loss in this study. The positive correlation that observed between MCP-1 and total as well as LDL-cholesterol at baseline disappeared after controlling for fat mass and reached only borderline significance after weight loss ($p = 0.08$ and 0.07, respectively).

In contrast to the aforementioned results with respect to factors usually associated with cardiovascular diseases, baseline MCP-1 levels significantly correlated with glucose levels ($r = 0.26$) and a borderline significant correlation with HOMA-IR ($r = 0.19$; $p = 0.05$) was found. MCP-1 is thought to play a role in insulin resistance as it inhibits insulin-mediated glucose uptake by approximately 30 % in differentiated 3T3-L1 adipocytes (Sartipy and Loskutoff 2003). Furthermore, mouse models overexpressing MCP-1 in adipose tissue develop insulin resistance and are characterized by macrophage infiltration into adipose tissue and liver steatosis, which occurs without changes in body weight (Kanda et al. 2006). Clinical data also provide good evidence that elevated serum MCP-1 levels are associated with insulin resistance, e.g. diabetic patients display higher serum MCP-1 levels (Piemonti et al. 2003; Simeoni et al. 2004). Treatment with the insulin-sensitizer pioglitazone has been shown to reduce expression of MCP-1 and CD68 (macrophage marker) in adipose tissue with a parallel increase in insulin sensitivity by 60 % in IGT subjects (Di Gregorio et al. 2005) while in women with polycystic ovary syndrome MCP-1 levels were not affected during treatment (Glintborg et al. 2009). Of note, MCP-1 expression and secretion by 3T3-L1 adipocytes is inhibited by adiponectin (Zoico et al. 2009), which increases during treatment with insulin-sensitizers (Abbasi et al. 2006). In our study, however, we did not find a negative correlation between MCP-1 and adiponectin levels.

Data from the MONICA/KORA study also indicated that high MCP-1 levels contribute to diabetes risk independently of classical risk factors like age, BMI, smoking status, or history of diabetes in the family (Herder et al. 2006a). This study revealed that elevated MCP-1 and IP-10 levels reaching a certain threshold (205.8 and 221.7 pg/ml, respectively) had a combined effect on T2DM risk with a hazard ratio of 1.5. In the German sub-cohort, MCP-1 and IP-10 correlated significantly after weight loss over the 12 months of intervention (r = 0.25). The correlation between IP-10 and glucose levels as well as HOMA-IR after weight loss was no more significant after controlling for fat mass. Similarly, Herder et al. reported that IP-10 levels and T2DM risk did not correlate after adjusting for age and BMI in 526 individuals with and 1 695 individuals without incident T2DM (Herder et al. 2006a). In another analysis of the KORA Augsburg Survey (S4), IP-10 levels in IGT and T2DM subjects were not higher than in normal glucose tolerant subjects (310.2 and 291.8 *vs.* 281.0 pg/ml, respectively) (Herder et al. 2005).

IP-10 serum concentrations were shown to be higher in severely obese (mean BMI 48.2 kg/m^2, n = 33) compared to lean women (mean BMI 21.5 kg/m^2, n = 14) (1077 *vs.* 328 pg/ml) (Dalmas et al. 2011). However, these concentrations seem to be really high, as it was reported that IP-10 concentrations in human serum are in the range of 20 to 400 pg/ml (Devaraj and Jialal 2009). In this study, serum IP-10 levels ranged between 24 and 880 pg/ml. Using a linear mixed model, it was found that weight loss is not significantly associated with changes in IP-10 levels. When grouping subjects according to the degree of weight loss, concentrations decreased significantly only in participants losing 5 to 10 % initial body weight (-6 kg; IP-10 -16.4%, from 110.6 to 93.1 pg/ml) compared to respective baseline levels but not in the group who lost more than 10 %, although these two groups did not differ at baseline regarding metabolic parameters like glucose or lipid levels. Of note, a high inter-individual variability in IP-10 levels as well as a relatively high inter-assay variance (coefficient of variation for IP-10 was 15 %) were observed. Similar results were published by Wong et al. in the WOMAN study, a randomized controlled trial investigating the effect of activity and nutrition on cardiovascular risk factors in 290 overweight women. The authors reported a large unexplained variability in cytokine levels within and between subjects, probably due to genetic-environmental associations (Wong et al. 2008). Because of the high inter-individual variability in IP-10 levels, this cohort was probably too small to detect a potential influence of weight loss on systemic concentrations. This is in line with the aforementioned study by Dalmas et al. investigating 51 severely obese women (mean BMI 49.8 kg/m^2) undergoing gastric bypass surgery. IP-10 serum levels did not change after

12 months during massive weight loss (mean BMI 36.4 kg/m^2) (Dalmas et al. 2011). Also in the WOMAN study, weight loss by 8.5 kg did not result in a significant reduction in serum IP-10 levels (Wong et al. 2008).

In the German sub-cohort of this study, MCP-1 concentrations also correlated significantly with RANTES at baseline (r = 0.33) and after weight loss (r = 0.30). Although RANTES levels before weight loss correlated significantly with fat mass (r = 0.29) and waist circumference (r = 0.33), our linear mixed model did not reveal any significant effect of weight loss on serum RANTES concentrations during the intervention. This is in contradiction to a few studies reporting increased RANTES expression in white adipose tissue and higher serum levels in obese patients (Huber et al. 2008; Dalmas et al. 2011; Keophiphath et al. 2010). The values that we measured (approximately 30 ng/ml) are in the range of values reported by others in the literature using immunoassays (Keophiphath et al. 2010; Madani et al. 2009), while values between 2 and 16 ng/ml have been reported using multiplex-assays (Huber et al. 2008; Dalmas et al. 2011). With respect to weight loss, Dalmas et al. showed that RANTES levels decreased by 60 % (from 16.8 to 6.6 ng/ml) during the first 3 months after gastric bypass and increased again to nearly baseline levels after 6 months despite further decrease in fat mass (Dalmas et al. 2011). This study included severely obese subjects with a baseline BMI of 49.8 kg/m^2 when compared to the overweight subjects in our study (BMI 30.0 kg/m^2). Moreover, surgery is prone to influence measurement of pro-inflammatory markers already in short-term. Altogether, it is likely that weight change cannot solely explain changes in systemic RANTES levels. Moreover, Madani et al. found that the release of RANTES by human adipose tissue *in vivo* was lower than circulating levels, suggesting that white adipose tissue is able to produce RANTES but its contribution to systemic levels may not be substantial (Madani et al. 2009). It is also known that RANTES is primarily released by nonfat cells and the release *in vitro* was unaffected by extreme adiposity (fat mass of 65 kg compared to 29 kg) (Fain et al. 2008; Fain et al. 2009).

Results from the KORA Survey indicate that RANTES may be involved in the development of T2DM independently of metabolic syndrome-related risk factors (BMI, age, sex, hypertension, LDL- and HDL-cholesterol) (Herder et al. 2005). In the latter study, levels were significantly elevated in individuals with IGT and T2DM compared to normoglycemic control subjects (25.96, 28.29 and 19.93 ng/ml, respectively). Results from the Finnish Diabetes Prevention Study indicated that progression to T2DM was significantly higher in subjects in the group with the highest RANTES concentrations with a calculated hazard ratio

of 2.6 (Herder 2006). On the other hand, a study analyzing the relationship between *RANTES* gene variants related to decreased RANTES serum levels did not reveal associations with incident diabetes (Herder et al. 2008). In the German sub-cohort, baseline RANTES was significantly correlated to fasting glucose levels before (r = 0.20) but not after weight loss. The correlation to HOMA-IR was not significant anymore after controlling for fat mass. No correlations with other cardiovascular risk factors like triglycerides, LDL or HDL levels were observed. These results are in line with the controversial implication of RANTES in cardiovascular disease risk factors (Koh et al. 2009; Herder et al. 2011). Of note, Oran et al. have currently criticized available immunoassays for RANTES measurement because they are not appropriate for detection of specific isoforms also existing endogenously (Oran et al. 2010). The development of a mass spectrometric immunoassay revealed 19 variants in different cohorts of human beings (T2DM, heart failure, cancer), suggesting that RANTES variants should be monitored in order to assess RANTES chemokine functions more accurately in the context of diseases.

4.2.3 Influence of Weight Loss on Serum PGRN Levels

A recent cross-sectional human study including 209 subjects reported higher serum PGRN concentrations in obese subjects with predominant visceral fat accumulation than in lean subjects (240 *vs.* 140 ng/ml) (**Table 19**).

Table 19: Circulating PGRN in obesity and weight loss

Factor	Effect of obesity	Reference	Effect of weight loss	Reference
PGRN	↑	(Youn et al. 2009)	↓	(Blüher et al. 2012)
			→	**This study**

The authors of the aforementioned study by Youn et al. also found a significant 1.4-fold increase in PGRN levels in T2DM *vs.* NGT subjects and proposed that CRP is a strong predictor of PGRN levels. Thereby, it has been hypothesized that PGRN is a novel inflammatory marker reflecting omental adipose tissue macrophage infiltration. In our study, after 12 months of intervention, a median weight loss of -7 % (~6 kg) resulted in a trend towards lower PGRN levels by -4.5 % (from 123.7 to 117.9 ng/ml, p = 0.06). Based on statistical analysis using a linear mixed model, it was found that weight loss was not

significantly associated with changes in PGRN. However, a decrease in fat mass by -1 % (-0.3 kg) was associated with an increase in PGRN levels by 0.4 % (0.5 ng/ml). When grouping subjects according to the degree of weight loss, a borderline significant decrease in PGRN concentrations only in participants losing 5 to 10 % (-6 kg; PGRN -6.3 %, -3.9 ng/ml) compared to baseline levels was observed. So far, there is only one published study reporting a decrease in PGRN levels after weight loss (Blüher et al. 2012). In this 2-year Dietary Intervention RCT (DIRECT), 322 participants were allocated to a low-fat, Mediterranean or a low-carbohydrate diet. At baseline, there was no difference in PGRN levels between the BMI groups (27.1 to 35.8 kg/m^2) and the mean blood concentration of PGRN was 260 ng/ml (*vs.* 123.7 ng/ml in our study). A rapid weight loss phase in the first 6 months of intervention (mean weight loss of 5.5 %) was followed by a weight maintenance/regain phase up to month 24. PGRN displayed a continuous decrease (-80 % from baseline values) throughout the 24 months irrespective of partial weight regain. Taking this result into account, changes in systemic PGRN concentration do not reflect body weight dynamics and may rather be linked to reduced food intake or changes in quality of diet. However, to the best of my knowledge, there is not data in the literature on the effect of specific dietary components on PGRN expression.

One other confounding factor that may influence PGRN levels is the metabolic status of participants. In 60 severely obese men and women (mean BMI 45 kg/m^2), PGRN levels were 1.5-fold higher in insulin-resistant compared to insulin-sensitive subjects (261 *vs.* 176 ng/ml) (Klöting et al. 2010). A study by Tönjes et al. also suggested that PGRN levels are dependent on the metabolic state: normal glucose-tolerant subjects had significantly lower levels of PGRN compared to impaired fasting glucose, IGT and T2DM subjects (138.8 *vs.* 155.4, 178.2 and 192.2 ng/ml, respectively) (Tönjes et al. 2010). Nevertheless, in the German sub-cohort, there was no significant correlation between PGRN levels fasting glucose and insulin levels as well as the insulin resistance index HOMA. Of note, the German sub-cohort included only four subjects with acknowledged disease and taking anti-diabetic medication (n = 4), which may explain why no association between PGRN and the metabolic status of patients was found.

Taken together, blood measurement of PGRN in the context of obesity and weight loss remains an open issue. First, it has been shown only recently that PGRN is an adipokine secreted by adipose tissue in mice upon TNF induction thereby mediating high fat diet-induced insulin resistance through the production of IL-6 in adipose tissue (Matsubara et al.

2012). Nonetheless, PGRN is constitutively expressed in a number of epithelial cells, specific neurons in the brain and also by immune cells (Daniel et al. 2000). Thus, better determination of the origin of PGRN in adipose tissues may be a prerequisite for interpreting blood measurements. Second, PGRN occurs as full-length protein and as shorter GRN peptides with different biological functions. In contrast to full-length PGRN, the granulin peptides (GRN) can increase the expression of the pro-inflammatory cytokines IL-8 and TNF (Zhu et al. 2002; Kojima et al. 2009). A tight control of the conversion of PGRN to the smaller GRN appears to be pivotal. The secretory leukocyte protease inhibitor (SLPI) binds PGRN and prevents proteolysis by neutrophil proteases which are released during inflammation (Bateman and Bennett 2009). Furthermore HDL/apolipoprotein A-I binds to macrophage-derived PGRN and suppresses its conversion into GRN suggesting this complex formation to be responsible for the anti-inflammatory effects of HDL on macrophages (Okura et al. 2010). In human atherosclerotic plaques, PGRN suppressed MCP-1 induced chemotaxis of monocytes (Kojima et al. 2009). Therefore, PGRN could be an inhibitor of the progression of atherosclerosis. Thus, future studies may want to include measurements of these various forms of PGRN and the so far unknown receptors in order to refine interpretation.

4.3 Strengths and Limitations of the Study

This study was conducted as a randomized controlled trial in an international setting including three countries. It is one of the first studies involving General Practitioners in an intensive commercial lifestyle intervention. We analyzed a large study population with 772 participants over an intervention period of 12 months with an additional follow-up period of another 12 months. This time represents a daily life situation as the participants controlled body weight on their own and we did not intervene with giving any recommendations for weight loss or maintenance. Including self-reported body weight, we still had data from 341 participants (77 % of those completing the intervention period) after 24 months. For measurements of anthropometric data and blood profiles, standardized methods were used and the ELISA systems for analysis of adipokines/chemokines were validated for human serum. However, the study has following limitations: 1) we did not perform a multivariate analysis so far. It is known that physical activity also influences weight loss success and different dietary factors might also partly be involved; 2) adipokine/chemokine concentrations were only determined in a German sub-group of participants and it would be important to analyze samples from the other countries to increase the power of analysis; 3) fat mass was

also measured only in a sub-group of participants using the BIA device by Tanita BC 418, which tends to underestimate percentage body fat mass compared to dual enery X-ray absorptiometry (DXA); 4) finally, blood withdrawal in this study was not standardized, *i.e.*, blood was sampled by the respective GP taking part in the study and thereafter transported after a maximum laps of time of 4 h at room temperature to the laboratory. Although we tested the effect of a 4-h-long storage of blood samples at room temperature on all pro-inflammatory molecules measured (adiponectin, MCP-1, IP-10, RANTES and PGRN) and we found no significant difference, we cannot exclude that a substantial amount of chemokines of interest were degraded. However, this limitation would apply to all samples independent of group allocation and visit.

5 Conclusion

Data from our large scale clinical trial demonstrate that referral to a commercial lifestyle intervention programme by general practitioners that provides advice about diet, physical activity, motivation and feelings of community and social network is a useful approach for weight management in overweight and obese people. Thus, future strategies to effectively improve body weight loss in overweight and obese subjects could rely on tripartite interaction between general practitioners (acting as contact person of trust and providing medical support), commercial suppliers (providing efficient and diversified platforms and tools dedicated to lifestyle improvement) and health insurance systems (for financial support, at least in early phases to put people on track). It is nonetheless important to remember that the present study was focused on weight loss in overweight and moderate obese subjects. The proportion of overweight people in Germany has not changed since 1998 whereas the prevalence of obesity increased from 19 to 23 % in men and from 23 to 24 % in women ("Robert Koch Institut" and "Bundesministerium für Gesundheit", press statement, June 2012). Concerning severely obese people, additional strategies such as surgery (*e.g.* gastric bypass) or pharmacological treatment (*e.g.* phentermine/topiramat) are indicated beyond the use of behavioral approaches (Brolin 2002; Hauner et al. 2004).

The rising prevalence of obesity puts pressure on health care resources devoted to the management of obesity-related disorders, mainly cardiovascular diseases, diabetes and cancer (Wang et al. 2011). The combined medical costs for the treatment of these preventable disorders have been calculated to increase by EUR 38-52 billion/y in the USA and by approximately EUR 2.5 billion/y in the UK by 2030 (Wang et al. 2011). Also in Germany, the health care system recognized obesity to be associated with rising costs reaching EUR 13 billion/y (Knoll and Hauner 2008). This is exemplified by the fact that obesity (as defined by a BMI ≥30 kg/m^2) has been listed up in the "morbiditätsorientierter Risikostrukturausgleich" for the year 2013, thereby offering in the future more possibilities for health insurances to deal with obese patients (Bundesversicherungsamt March 2012). In a cross-sectional analysis including 3 003 Germans, the majority of participants (60 %) was willing to take part in obesity preventive programmes and would even accept to pay related costs at least partially (87 %) (Sikorski et al. 2012). Policies to promote healthy body weight and greater funding of effective prevention programmes are crucial because they could lead to economic benefits via reduction of medical costs associated with obesity. These policies

require initiatives from many parties such as governments, industries and media which play a predominant role in the overall environment favoring obesity (Gortmaker et al. 2011).

Obesity is assumed to be a state of low-grade inflammation. Among the four pro-inflammatory molecules measured in the present study (MCP-1, IP-10, PGRN and RANTES), body weight loss was associated with decreased MCP-1 levels whereas the anti-inflammatory adipokine adiponectin increased with weight loss. Although the decrease in MCP-1 levels correlated with improvement of blood glucose levels and the insulin resistance index HOMA, its clinical relevance with respect to the development of metabolic disorders in the long-term basis is unclear. Indeed, clinical relevance is dependent on a multitude of interacting parameters, is a matter of decades and is thus very time-consuming and expensive to assess in the context of human studies. Therefore, it would be important to follow-up our cohort regularly in a long-term basis to assess whether chemokine levels are associated with increased risks of developing metabolic disorders (*e.g.*, T2DM, cardiovascular disease) and to determine relevant chemokine thresholds. In future studies, it would be also important to assess the origin of pro-inflammatory molecules via collection of adipose tissue biopsies and determine whether such molecules act in an endocrine-like fashion or more locally on specific target tissues or cell types.

One new paradigm is that the systemic inflammatory status in human subjects is not solely dependent upon body weight or fat mass. It has been repeatedly observed that certain obese patients are metabolically healthy, *i.e.*, they do not display clinical signs of metabolic diseases like insulin resistance, hypertension or dyslipidemia, at least on a short- to mid-term basis. Thus, beyond the sole objective of losing weight, the quality of nutrition plays a role with respect to the inflammatory status independently of overweight. It has been shown that a diet rich in saturated fatty acids induces a pro-inflammatory gene expression profile in adipose tissue whereas consumption of a MUFA diet is associated with anti-inflammatory effects. A Mediterranean diet (high in MUFA) supplemented with virgin olive oil or nuts has beneficial effects on cardiovascular risk factors compared with a low-fat diet (Estruch et al. 2006) and has been confirmed to be protective against overall mortality and incidences of chronic diseases in a meta-analysis (Sofi et al. 2010). These relationships should also be considered in the context of treating obesity and resulting inflammatory and metabolic disorders.

Original publications

Stoll J, Holzapfel C, Hauner H et al. (2012) Differential effect of weight loss on serum chemokine levels in human subjects, *in preparation*

Holzapfel C, Cresswell L, Ahern AL, Eberhard M, Aston L, Fuller N, Simpson A, Stoll J, Mander AP, Caterson ID, Jebb SA, Hauner H (2012) Can weight loss be maintained? Results and challenges in the follow-up of weight management interventions in primary care, *in preparation*

Jebb SA, Ahern AL, Olson AD, Aston LM, Holzapfel C, Stoll J, Amann-Gassner U, Simpson AE, Fuller NR, Pearson S, Lau NS, Mander AP, Hauner H and Caterson ID (2011) Primary care referral to a commercial provider for weight loss treatment versus standard care: a randomised controlled trial. *Lancet* 378:1485-92.

Stoll J and Hauner H (2009) Gibt es Lebensmittel und Nahrungsergänzungsmittel mit gewichtssenkender Wirkung? Adipositas 3: 82-87

Appendices

Appendix A: The Weight Watchers (WW) programme

The WW program is based on four basic modules **(Figure A-1**): nutrition, physical activity, behaviour, and WW meetings. This is the basis to support long-time weight loss success.

Figure A-1: The four modules of the WW concept

The nutritional module of the WW program consists of a balanced diet with 55 to 60 % of total energy from carbohydrates, 20 to 30 % from fat, and 15 to 20 % from proteins. It is based on an individual calculation of *POINTS* taking into account age, sex, weight, height, and daily energy expenditure (**Figure A-2**). The *POINTS* value of each food reflects the energy and macronutrient content and the calculation is based on an unknown formula. Participants can individually select the food in the range of *POINTS* they are allowed to eat.

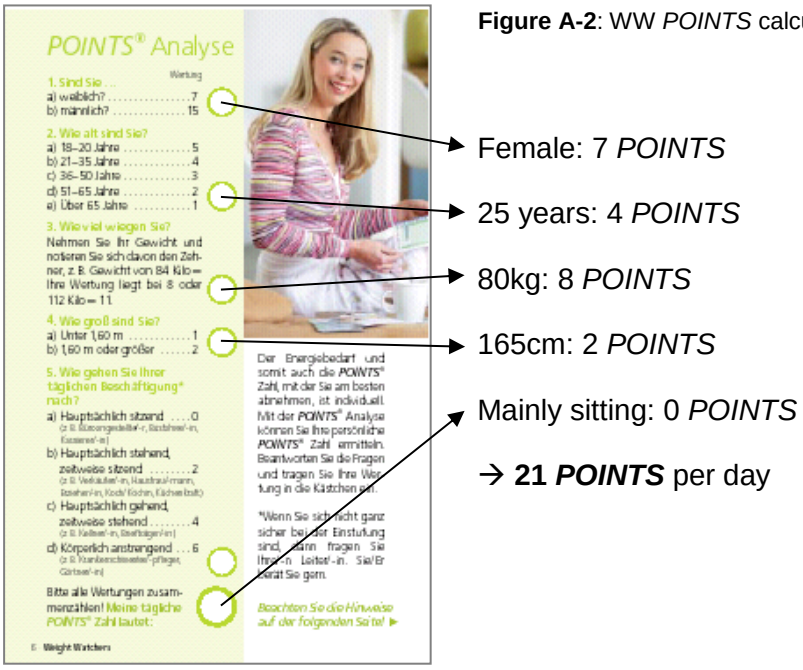


Figure A-2: WW *POINTS* calculation

Each food is characterized by a *POINTS* value. Fruits and vegetables have a *POINTS* value of zero. Eighteen food items (*e.g.*, whole grain noodles, potatoes, low-fat fish), fall into the category *SuperFlex* (**Figure A-3**). In general, these items are healthy foods with a low energy-density within their category and with a satiable effect. They have a fixed *POINTS* value, which is independent of portion size. Thereby, participants can eat for a fixed *POINTS* value from *SuperFlex* foods until they are satisfied, *i.e.*, they can choose to calculate the meal on a portion level or a SuperFlex basis. For example, the SuperFlex portion of pasta has 4 POINTS, independently of portion size. However, points for side items like chicken and oil have to be added **(Figure A-3)**.

Figure A-3: Example of *POINTS* values (left panel). Picture of 18 *SuperFlex* food items with an example for *POINTS* calculation on the *SuperFlex* basis (right panel).

Furthermore, in order to avoid “unhealthy” behaviour (*e.g.*, eating only fruits during the day to save *POINTS* and enjoy unhealthy food in the evening), participants must follow six fit rules: (1) five servings of fruits and vegetables per day, (2) adequate drinking, (3) “good” lipids, (4) adequate calcium intake, (5) variety of food, (6) physical activity.

The new WW program, including an update of *POINTS* calculation for food items and participants, started in Germany in November 2009. However, this program was not used in the present study, since the last patient finished intervention in January 2010.

Appendix B: Weight loss advice provided by the General Practitioner (GP)

The GP care was not defined in detail. Every GP could give his own advice regarding weight loss and healthy lifestyle. They were not allowed to offer any other specific weight loss program (*e.g.*, metabolic balance) or to prescribe any medication to induce weight loss. The study team provided information material for a healthy balanced diet, like the “Zehn Regeln” from “Deutsche Gesellschaft für Ernährung, (DGE)” and a weight loss brochure “Aktiv Abnehmen” from the “Zentrale Marketing-Gesellschaft der deutschen Agrarwirtschaft mbH (CMA)”. Additionally, a list of websites was handed out (www.aid.de, www.ernaehrung.de).

Appendix C: Schedule of the study procedures

Screening visit: Every potentially suitable participant was invited by GP to take part in the study. The study procedure was discussed and a preliminary screening questionnaire was completed. If the inclusion criteria were met, a brief medical history was filled out. The information sheet and one original of the signed participant consent form were handed out by the GP. To determine the participant’s eligibility, the following parameters were recorded: height, weight, BMI, waist circumference, blood pressure, radial pulse rate, blood samples (glucose, insulin, full lipid profile, HbA1c, TSH) and concomitant medications. Once participants were deemed eligible for randomisation, they were allocated to a treatment group and were contacted to make an appointment for the baseline visit A. The participants got a pedometer (WW^{TM}, WeightWatchers GmbH Düsseldorf, Germany) as well as a diet- and activity diary. They were asked to record their dietary intake for four days and number of steps per day for seven days prior to the baseline visit A.

Visit A (month 0: Baseline): Participants were informed, to which group they had been allocated and, if appropriate, received vouchers for free access to WW meetings at the WW location of their choice and free use of the internet portal “eSource”. The GP group received weight loss advice from their GP. All participants were asked to attend the first WW or GP session within two weeks after the baseline visit. Following measurements were done at baseline: height, weight, BMI, waist circumference, BIA, blood pressure, radial pulse rate, ECG, laboratory tests (fasting glucose and insulin, lipid profile, hsCRP, HbA1c, liver function test, kidney function test, DNA and serum collection). Changes in concomitant medications were recorded. The completed four day diet diary, the seven day pedometer record and the

three questionnaires were collected. Additionally, participants were asked about their ethnicity (patient and the four grandparents).

Visit B (month 2): Following parameters were recorded: weight, BMI, waist circumference, BIA, blood pressure, radial pulse rate, laboratory testing (fasting glucose, lipid profile, HbA1c (for patients with T2DM or impaired glucose tolerance), changes in concomitant medication and review of compliance diary.

Visit C (month 4) and E (month 9): Following parameters were recorded: weight, BMI, waist circumference, BIA, blood pressure, radial pulse rate, record of changes to concomitant medication, and review of compliance diary. The documents for the four day diet diary, the seven day pedometer sheet and the three questionnaires were handed out.

Visit D (month 6) and F (month 12): Following parameters were recorded: weight, BMI, waist circumference, BIA, blood pressure, radial pulse rate, laboratory tests (fasting glucose and insulin, lipid profile, hsCRP, HbA1c (for patients with T2DM or impaired glucose tolerance), and serum collection). Changes in concomitant medications were recorded. The completed four day diet diary, the seven day pedometer record and the three questionnaires were collected. In addition, the change of smoking habits was documented and a questionnaire concerning the satisfaction with the intervention study was asked.

Follow-up (visit G (month 18) and visit H (month 24): The following procedures were arranged at these visits: weight, BMI, waist circumference, BIA, blood pressure, and radial pulse rate. Any changes of concomitant medication were recorded. The four day diet diary, the seven day pedometer sheet and the three questionnaires were handed out. The participants were asked to send the completed data per post to the study team. Additionally, the participant was asked for his method of weight control used within the prior six months.

Appendix D: Measurements at Synlab

Table D-1: Blood parameters measured at “Medizinisches Versorgungszentrum München”

Parameter	Tube	Method	Company
Glucose	Natrium-Fluorid (NaF)	Photometriy (hexokinase)	Modular DPE, Roche Diagnostics GmbH, Mannheim, Germany
Insulin	Serum	Electrochemiluminescence immunoassay (ECLIA)	Immulite 2000, Siemens Healthcare Diagnostics GmbH, Eschborn, Germany
HbA1c	EDTA	Turbidimetric immunoassay Tina-quant	Integra 800, Roche Diagnostics GmbH, Mannheim, Germany
Total cholesterol	Serum	Photometry (CHOD-PAP)	Modular DPE, Roche Diagnostics GmbH, Mannheim, Germany
Triglycerides (TG), High-, low-density lipoprotein (HDL, LDL)	Serum	Enzymatic photometry	Modular DPE, Roche Diagnostics GmbH, Mannheim, Germany
Total prote in	Serum	Biuret	Modular DPE, Roche Diagnostics GmbH, Mannheim, Germany
High-sensitivity C reactive protein (hsCRP)	Serum	Turbidimetrie	Integra 800, Roche Diagnostics GmbH, Mannheim, Germany
Bilirubin	Serum	Photometry (DPD reagens)	Modular DPE, Roche Diagnostics GmbH, Mannheim, Germany

Table D-1 (continued)

Alkaline phosphatase, Glutamic-oxaloacetic transaminase (GOT), Glutamic-pyruvate transaminase (GPT), γ-glutamyltransferase (GGT)	Serum	Photometry: IFCC, 37°C	Modular DPE, Roche Diagnostics GmbH, Mannheim, Germany
Thyreotropin (TSH)	Serum	Electrochemiluminescence immunoassay (ECLIA)	Modular DPE, Roche Diagnosxtics GmbH, Mannheim, Germany
Creatinine	Serum	Jaffé	Modular DPE, Roche Diagnostics GmbH, Mannheim, Germany

Appendix E: The International WW-Study Cohort

Characteristics of the International WW Study Cohort

The baseline characteristics of all 772 randomized subjects are shown in **Table E-1**. Demographic and clinical outcomes of participants did not differ significantly between the treatment groups, adjusted for country site (UK, Australia, Germany). No country-by-treatment interactions were identified ($p > 0.10$), which allows for pooled analysis for further findings. Sex-specific differences were not considered because only 13 % men were included (Jebb et al. 2011).

Table E-1. Baseline characteristics of participants from all three study centres according to treatment group.[a]

Parameter	Commercial programme (CP) (n = 377)	Standard care (SC) (n = 395)
Women	330 (88 %)	338 (86 %)
Age [years]	46.5 ± 13.5	48.2 ± 12.2
Weight [kg]	86.9 ± 11.6	86.5 ± 11.25
Height [m]	1.66 ± 0.1	1.66 ± 0.1
BMI [kg/m^2]	31.5 ± 2.6	31.3 ± 2.6
Fat mass [kg]	33.3 ± 7.0	32.9 ± 7.4
Waist circumference [cm]	100 ± 9.2	99.9 ± 9.3
Systolic blood pressure [mmHg]	124.7 ± 17.1	124.2 ± 14.7
Diastolic blood pressure [mmHg]	78.2 ± 9.8	79.1 ± 9.0
T2DM	24 (6 %)	27 (7 %)

[a] Data are shown as means ± sd or numbers of participants (%).

After 12 months of intervention, 444 participants completed the study (230 in the CP and 214 in SC group). The overall study results are shown in **Table E-2**. A significant greater weight loss in participants following the CP was accompanied by larger reductions in waist circumference and fat mass. Additionally, blood levels of insulin, LDL-and HDL-cholesterol significantly improved in the CP- compared to the SC group while there was no difference in glucose, triglyceride and cholesterol levels. These observations did not differ between the three countries.

Table E-2. Changes (Δ-values) in clinical outcomes and biomarkers of cardiovascular disease risk between baseline and 12 months of intervention according to treatment group and adjusted for baseline observation and country.[a]

Parameters	n	Commercial programme (CP)	Standard Care (SC)	Adjusted difference (95% CI)*	p-value
Weight [kg]	444	-6.65 (0.43)	-3.26 (0.33)	-3.16 (-4.23 to -2.11)	**<0.001**
Waist circumference [cm]	429	-6.86 (0.50)	-4.34 (0.43)	-2.36 (-3.65 to -1.08)	**<0.001**
Fat mass [kg]	397	-5.36 (0.38)	-2.54 (0.30)	-2.52 (-3.45 to -1.60)	**<0.001**
Systolic blood pressure [mmHg]	441	-3.38 (0.92)	-1.77 (0.96)	-1.46 (-3.82 to 0.89)	0.220
Diastolic blood pressure [mmHg]	441	-2.34 (0.59)	-1.42 (0.65)	-1.40 (-2.95 to 0.15)	0.080
Insulin [pmol/l]	423	-6.15 (1.44)	-0.84 (1.67)	-5.74 (-9.86 to -1.61)	**0.007**
Glucose [mmol/l]	428	-0.10 (0.03)	-0.02 (0.05)	-0.09 (-0.19 to 0.01)	0.080
HbA1c [%]	248	-0.18 (0.03)	-0.16 (0.03)	0.00 (-0.08 to 0.07)	0.960
Triglycerides [mmol/l]	429	-0.09 (0.04)	-0.10 (0.05)	-0.01 (-0.12 to 0.09)	0.800
Cholesterol [mmol/l]	430	0.01 (0.05)	0.13 (0.05)	-0.11 (-0.24 to 0.02)	0.090
LDL cholesterol [mmol/l]	428	-0.02 (0.04)	0.11 (0.04)	-0.13 (-0.24 to -0.02)	**0.022**
HDL cholesterol [mmol/l]	428	0.12 (0.02)	0.07 (0.02)	0.05 (0.01 to 0.09)	**0.020**

[a] Data are shown as means (SEM). The adjusted difference is presented with 95% confidence interval (CI).

Body Weight Maintenance

From the 444 completers of the entire study cohort (58 %), measured body weight at 24 months was available for 203 participants (26 %). Additionally, self-reported weight was available for 138 participants (18 %). Results are shown in (**Figure E-1**) (Holzapfel et al., in preparation). Body weight was still significantly reduced after 24 months compared to baseline in both groups ($p < 0.001$, completers analysis). Weight regain was observed in both groups, yet it was significantly greater in the CP *vs.* SC group ($p<0.001$) (adjusted difference 1.97 kg). There was no significant difference in body weight between the two groups after 24 months.

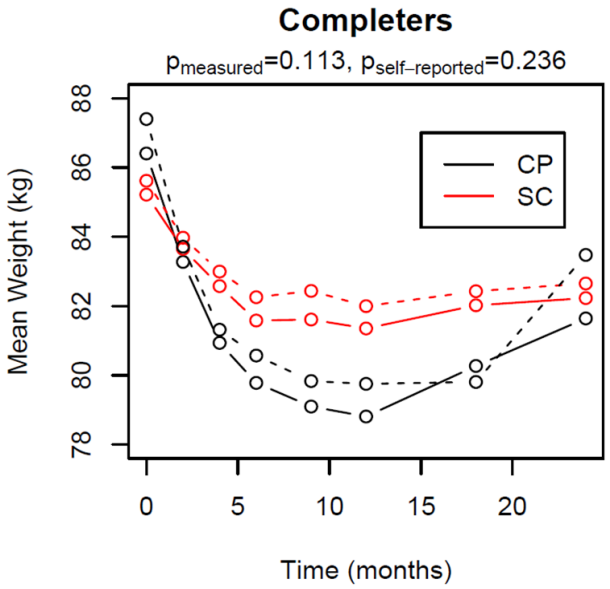


Figure E-1: Body weight change for completer participants (n = 444) randomised to a Commercial Programme (CP) and Standard Care (SC) during the entire study period, i.e., intervention (month 0 to 12) and follow-up (month 12 to 24) (Holzapfel et al., in preparation). Dashed lines correspond to self-reported weight data. P-values indicate that differences between CP and SC after 24 months were not significant.

Appendix F: Raw values of the German Sub-Cohort

Table F-1. Raw values of the German sub-cohort over the time of study duration (12 months).[a]

Parameter	n	Visit A (Baseline) Median (IQR)	Visit D (6 months) Median (IQR)	Visit F (12 months) Median (IQR)
Age [years]	103	46.0 (39.0 to 57.0)	-	-
Weight [kg]	103	83.6 (78.1 to 91.0)	77.4 (72.5 to 83.1)	78.1 (70.0 to 85.1)
BMI [kg/m^2]	103	30.2 (28.2 to 32.8)	28.0 (26.3 to 31.0)	28.2 (25.7 to 30.6)
Systolic [mmHg]	103	120.0 (110.0 to 130.0)	119.0 (110.0 to 127.5)	120.0 (105.0 to 130.0)
Diastolic [mmHg]	103	80.0 (70.0 to 82.5)	77.5 (70.0 to 80.0)	80.0 (70.0 to 80.0)
Waist circumference [cm]	100	95.3 (90.0 to 101.0)	91.0 (84.0 to 97.0)	89.8 (80.5 to 97.0)
Fat mass [kg]	84	32.2 (30.0 to 38.2)	29.3 (25.4 to 34.0)	28.3 (24.9 to 34.6)
Trunk Fat mass [kg]	84	16.8 (14.6 to 19.8)	14.9 (12.4 to 17.8)	14.4 (12.3 to 17.6)
Glucose [mmol/l]	103	4.8 (4.6 to 5.2)	4.7 (4.4 to 5.0)	4.8 (4.5 to 5.1)
Insulin [pmol/l]	103	48.0 (32.0 to 65.0)	32.0 (23.8 to 48.5)	35.0 (23.0 to 56.0)
HOMA-IR	103	1.4 (0.9 to 2.1)	0.9 (0.7 to 1.4)	1.1 (0.7 to 1.8)
Triglycerides [mmol/l]	103	1.2 (0.9 to 1.6)	1.1 (0.9 to 1.4)	1.2 (0.9 to 1.7)
Total cholesterol [mmol/l]	103	5.4 (4.7 to 6.0)	5.3 (4.6 to 5.9)	5.4 (4.7 to 6.1)
HDL cholesterol [mmol/l]	103	1.4 (1.2 to 1.8)	1.5 (1.2 to 1.8)	1.6 (1.3 to 1.9)
LDL cholesterol [mmol/l]	103	3.3 (2.7 to 3.8)	3.2 (2.6 to 3.7)	3.3 (2.7 to 3.8)
CRP [mg/l]	103	0.022 (0.014 to 0.043)	0.021 (0.011 to 0.038)	0.016 (0.009 to 0.036)
Adiponectin total [µg/ml]	102	5.1 (4.1 to 6.9)	5.5 (4.4 to 7.3)	5.9 (4.5 to 7.8)
HMW Adiponectin [µg/ml]	102	2.5 (1.8 to 3.7)	2.8 (2.0 to 4.0)	3.0 (2.1 to 4.4)
IP-10 [pg/ml]	102	102.6 (76.9 to 135.2)	98.2 (78.5 to 138.2)	95.1 (68.7 to 133.0)
MCP-1 [pg/ml]	102	302.9 (245.6 to 405.0)	297.6 (234.3 to 375.7)	298.6 (233.7 to 361.5)
RANTES [ng/ml]	102	30.8 (22.8 to 40.0)	30.2 (22.0 to 42.9)	31.7 (22.8 to 40.8)
PGRN [ng/ml]	103	123.7 (95.7 to 151.5)	124.5 (97.8 to 148.0)	117.7 (95.0 to 145.0)

[a] Data are shown as medians (IQR)

Appendix G: Baseline characteristics of the German Sub-Cohort according to weight loss category

Table G-1. Baseline characteristics of the German sub-cohort according to weight loss group.[a]

Weight loss group	<5 % (n = 34)	5-10 % (n = 36)	>10 % (n = 33)	p-value
Parameter	Median (IQR)	Median (IQR)	Median (IQR)	
Age [years]	46.0 (39.25 to 55.0)	50.5 (41.0 to 62.3)	42.0 (36.0 to 52.0)	0.087
Body weight [kg]	85.1 (79.5 to 90.3)	82.1 (75.0 to 89.3)	85.6 (79.5 to 93.3)	0.248
BMI [kg/m^2]	29.9 (27.9 to 32.5)	30.4 (27.8 to 33.3)	30.4 (28.5 to 32.3)	0.818
Fat mass [kg]	32.6 (29.9 to 37.7)	31.6 (28.0 to 38.2)	34.3 (30.2 to 39.0)	0.470
Waist circumference (cm]	85.1 (79.5 to 90.3)	82.1 (75.0 to 89.3)	85.6 (79.5 to 93.3)	0.766
Systolic [mmHg]	119.0 (104.4 to 133.1)	120.0 (110.0 to 130.0)	120.0 (111.3 to 130.0)	0.882
Diastolic [mmHg]	80.0 (70.0 to 82.5)	76.3 (66.3 to 80.5)	80.0 (75.8 to 82.8)	0.188
Glucose [mmol/l]	4.9 (4.7 to 5.4)	4.8 (4.6 to 5.0)	4.9 (4.5 to 5.3)	0.510
Insulin [pmol/l]	53.0 (32.3 to 70.0)	44.5 (31.3 to 62.8)	50.0 (33.0 to 65.0)	0.502
HOMA-IR	1.6 (0.9 to 2.3)	1.3 (0.9 to 1.9)	1.4 (0.9 to 2.1)	0.447
Triglycerides [mmol/l]	1.3 (1.0 to 1.7)	1.2 (0.9 to 1.4)	1.1 (0.9 to 1.8)	0.655
Total cholesterol [mmol/l]	5.2 (4.4 to 6.2)	5.3 (4.9 to 6.0)	5.4 (4.7 to 5.9)	0.880
HDL cholesterol [mmol/l]	1.4 (1.2 to 1.7)	1.4 (1.2 to 1.8)	1.5 (1.3 to 1.8)	0.617
LDL cholesterol [mmol/l]	3.1 (2.6 to 3.9)	3.3 (2.9 to 3.9)	3.3 (2.9 to 3.7)	0.823
CRP [mg/l]	0.03 (0.01 to 0.06)	0.02 (0.01 to 0.04)	0.02 (0.02 to 0.03)	0.256
Adiponectin total [µg/ml]	5.0 (4.0 to 6.2)	5.3 (4.1 to 7.1)	5.8 (4.2 to 8.2)	0.260
HMW Adiponectin [µg/ml]	2.5 (1.8 to 3.1)	2.6 (1.7 to 3.8)	3.0 (2.1 to 4.7)	0.137
IP-10 [pg/ml]	114.8 (80.6 to 139.8)	110.6 (77.6 to 150.0)	92.2 (74.3 to 132.1)	0.537
MCP-1 [pg/ml]	278.1 (212.3 to 400.2)	309.7 (252.8 to 414.0)	299.7 (268.4 to 435.3)	0.258
RANTES [ng/ml]	29.5 (23.4 to 36.6)	27.7 (21.2 to 39.1)	34.2 (25.5 to 42.9)	0.438
PGRN [ng/ml]	125.7 (94.3 to 143.3)	118.7 (95.9 to 157.0)	123.7 (100.0 to 162.0)	0.617

[a] Data are presented as medians (IQR). P-values for group differences were determined by Kruskal-Wallis-test. There were no significant differences between the weight loss groups (<5 %, 5-10 %, >10 %) at baseline ($p > 0.05$).

Appendix H: Changes in Metabolic Markers after 6 months according to the Degree of Weight Loss

Relative changes in lipids, glucose, insulin and HOMA-IR after 6 months of intervention according to the three different weight loss groups are shown in **Table H-1**. Weight, fat mass and waist circumference decreased significantly in all groups yet this decrease was more pronounced in the highest weight loss group. Systolic blood pressure slightly increased in the group who lost <5 % of initial weight whereas diastolic blood pressure decreased significantly only in subjects losing >10 % of initial weight. Fastin glucose decreased only significantly in subjects losing >10 % in the first 6 months in contrast to insulin and HOMA-IR which decreased significantly in all groups yet the decrease was more pronounced in subjects losing >10 % of initital weight. Triglyceride, total- and LDL-cholesterol levels decreased significantly after 6 months in the group of subjects who lost more than 10 % of initial body weight when compared to baseline. HDL-cholesterol increased significantly in the first 6 months in participants who lost 5-10 % of initial body weight.

Table H-1. Relative changes in metabolic parameters (%) from baseline to the visit after 6 months according to weight loss.[a]

	Group	Baseline Median (IQR)	After 6 months Median (IQR)	% change Median (IQR)	p-value
Weight [kg]	< 5%	85.1 (79.5 to 90.3)	80.9 (77.1 to 89.5)	-1.8 (-4.1 to -0.4)	**<0.001**
	5-10%	82.1 (75.0 to 89.3)	76.4 (70.3 to 82.4)	-7.2 (-9.4 to -5.6)	**<0.001**
	> 10%	85.6 (79.5 to 93.3)	75.7 (67.6 to 81.3)	-10.6 (-15.4 to -8.2)	**<0.001**
Fat mass [kg]	< 5%	32.6 (29.9 to 37.7)	32.2 (28.1 to 38.4)	-2.2 (-5.6 to 1.1)	**0.04**
	5-10%	31.6 (28.0 to 38.2)	28.7 (25.0 to 34.0)	-12.4 (-17.9 to -6.3)	**<0.001**
	> 10%	34.3 (30.2 to 39.0)	27.6 (21.5 to 30.6)	-23.2 (-30.8 to -13.2)	**<0.001**
Waist circumference [cm]	< 5%	97.5 (90.3 to 101.8)	94.0 (87.0 to 100.5)	-3.1 (-6.8 to 0.5)	**0.005**
	5-10%	96.0 (89.8 to 101.0)	91.0 (81.5 to 95.8)	-7.4 (-10.4 to -3.2)	**<0.001**
	> 10%	93.0 (89.8 to 101.5)	86.0 (79.0 to 95.5)	-8.2(-12.3 to -5.5)	**<0.001**
Systolic blood pressure [mmHg]	< 5%	119.0 (104.4 to 133.1)	126.3 (110.0 to 134.3)	+5.0 (-6.9 to 13.6)	0.111
	5-10%	120.0 (110.0 to 130.0)	110.0 (103.7 to 120.0)	-6.1 (-9.1 to 0.6)	0.061
	> 10%	120.0 (111.3 to 130.0)	115.0 (110.0 to 125.8)	-3.8 (-8.7 to 1.3)	0.123

Table H-1 (continued)

	Group	Baseline Median (IQR)	After 6 months Median (IQR)	% change Median (IQR)	p-value
Diastolic blood pressure [mmHg	< 5%	80.0 (70.0 to 82.5)	80.0 (72.1 to 82.5)	0.0 (-7.7 to 9.6)	0.392
	5-10%	76.3 (66.3 to 80.5)	73.8 (60.0 to 80.0)	0.0 (-13.7 to 11.6)	0.289
	> 10%	80.0 (75.8 to 82.8)	77.5 (70.0 to 82.0)	-3.2 (-12.5 to 0.0)	**0.023**
Glucose [mmol/l]	< 5%	4.9 (4.7 to 5.4)	4.9 (4.4 to 5.3)	-2.0 (-6.6 to 4.3)	0.202
	5-10%	4.8 (4.6 to 5.0)	4.7 (4.4 to 5.0)	-2.2 (-6.1 to 2.2)	0.104
	> 10%	4.9 (4.5 to 5.3)	4.5 (4.4 to 4.8)	-8.3 (-13.0 to -1.0)	**<0.001**
Insulin [pmol/l]	< 5%	53.0 (32.3 to 70.0)	38.0 (27.8 to 65.0)	-21.9 (-39.5 to 0.3)	**0.001**
	5-10%	44.5 (31.3 to 62.8)	31.0 (25.0 to 42.0)	-23.8 (-44.4 to 0.0)	**0.001**
	> 10%	50.0 (33.0 to 65.0)	29.0 (16.0 to 40.0)	-40.0 (-57.9 to -16.9)	**<0.001**
HOMA-IR	< 5%	1.7 (0.9 to 2.3)	1.1 (0.7 to 1.9)	-25.5 (-39.1 to 2.2)	**0.001**
	5-10%	1.3 (0.9 to 1.9)	0.9 (0.7 to 1.2)	-25.5 (-42.6 to -1.8)	**0.002**
	> 10%	1.4 (0.9 to 2.1)	0.8 (0.4 to 1.2)	-44.7 (-62.5 to -24.8)	**<0.001**
Triglycerides [mmol/l]	< 5%	1.3 (1.0 to 1.7)	1.3 (1.0 to 1.7)	+15.2 (-12.5 to 39.0)	0.118
	5-10%	1.2 (0.9 to 1.4)	1.1 (0.9 to 1.4)	-4.0 (-19.4 to 16.4)	0.486
	> 10%	1.1 (0.9 to 1.8)	1.0 (0.9 to 1.3)	-17.9 (-31.3 to 2.7)	**0.003**
Total cholesterol [mmol/l]	< 5%	5.2 (4.4 to 6.2)	5.3 (4.6 to 6.0)	+5.0 (-9.4 to 12.0)	0.765
	5-10%	5.3 (4.9 to 6.0)	5.4 (4.8 to 6.2)	+2.5 (-5.7 to 6.8)	0.302
	> 10%	5.4 (4.7 to 5.9)	5.0 (4.4 to 5.7)	-6.5 (-17.2 to 1.4)	**0.01**
LDL-cholesterol [mmol/l]	< 5%	3.1 (2.6 to 3.9)	3.1 (2.5 to 3.6)	-1.2 (-16.7 to 12.0)	0.256
	5-10%	3.3 (2.9 to 3.9)	3.4 (3.1 to 3.9)	0.0 (-5.5 to 11.1)	0.309
	> 10%	3.3 (2.9 to 3.7)	3.2 (2.5 to 3.5)	-9.5 (-19.5 to 1.9)	**0.014**
HDL-cholesterol [mmol/l]	< 5%	1.4 (1.2 to 1.7)	1.4 (1.2 to 1.6)	-0.7 (-9.4 to 11.6)	0.789
	5-10%	1.4 (1.2 to 1.8)	1.5 (1.2 to 1.8)	+4.5 (0.0 to 11.8)	**0.009**
	> 10%	1.5 (1.3 to 1.8)	1.5 (1.3 to 1.9)	+3.2 (-8.5 to 13.4)	0.544

[a] Relative changes are shown as medians (IQR). P-values for changes from baseline to visit D (after 6 months) within one group were determined by the Wilcoxon-test. N = 34, 36 and 33 for the <5 %, 5 to 10 % and >10 % group, respectively. Fat mass was only measured in 84-88 participants (n = 30-32 for the <5 %, n = 28-30 for the 5-10 % and n = 26 for the >10 % weight loss group).

Cited Literature

Abbasi, F.; Chang, S.-A.; Chu, J. W.; Ciaraldi, T. P.; Lamendola, C.; McLaughlin, T. et al. (2006): Improvements in insulin resistance with weight loss, in contrast to rosiglitazone, are not associated with changes in plasma adiponectin or adiponectin multimeric complexes. In: *Am. J. Physiol. Regul. Integr. Comp. Physiol.* 290 (1), R139-44.

Abbasi, F.; Chu, J. W.; Lamendola, C.; McLaughlin, T.; Hayden, J.; Reaven, G. M.; Reaven, P. D. (2004): Discrimination between obesity and insulin resistance in the relationship with adiponectin. In: *Diabetes* 53 (3), 585–590.

Alberti, L.; Gilardini, L.; Girola, A.; Moro, M.; Cavagnini, F.; Invitti, C. (2007): Adiponectin receptors gene expression in lymphocytes of obese and anorexic patients. In: *Diabetes Obes Metab* 9 (3), 344–349.

Altman, Douglas G. (1991): Practical statistics for medical research. 1. Aufl. London ;, New York: Chapman and Hall.

Andreasson, A. Nixon; Undén, A.-L.; Elofsson, S.; Brismar, K. (2012): Leptin and adiponectin: Distribution and associations with cardiovascular risk factors in men and women of the general population. In: *Am. J. Hum. Biol.*, n/a.

Apovian, C. M.; Bigornia, S.; Mott, M.; Meyers, M. R.; Ulloor, J.; Gagua, M. et al. (2008): Adipose macrophage infiltration is associated with insulin resistance and vascular endothelial dysfunction in obese subjects. In: *Arterioscler. Thromb. Vasc. Biol* 28 (9), 1654–1659.

Appay, V.; Rowland-Jones, S. L. (2001): RANTES: a versatile and controversial chemokine. PII: S1471-4906(00)01812-3. TRENDS in Immunology (2), zuletzt aktualisiert am 25.01.2001, zuletzt geprüft am 09.07.2010.

Araki, S.; Dobashi, K.; Kubo, K.; Asayama, K.; Shirahata, A. (2006): High molecular weight, rather than total, adiponectin levels better reflect metabolic abnormalities associated with childhood obesity. In: *J. Clin. Endocrinol. Metab.* 91 (12), 5113–5116.

Arita, Y.; Kihara, S.; Ouchi, N.; Takahashi, M.; Maeda, K.; Miyagawa, J. et al. (1999): Paradoxical decrease of an adipose-specific protein, adiponectin, in obesity. In: *Biochem. Biophys. Res. Commun.* 257 (1), 79–83.

Bateman, A.; Bennett, H. P. J. (2009): The granulin gene family: from cancer to dementia. In: *Bioessays* 31 (11), 1245–1254.

Berg, A. H.; Combs, T. P.; Du, X.; Brownlee, M.; Scherer, P. E. (2001): The adipocyte-secreted protein Acrp30 enhances hepatic insulin action. In: *Nat. Med.* 7 (8), 947–953.

Berg, A.; Berg, A.; Frey, I.; König, D.; Predel, H.-G. (2008): Exercise Based Lifestyle Intervention in Obese Adults. M.O.B.I.L.I.S. In: *Deutsches Ärzteblatt International* (105(11):), 197–203.

Blüher, M.; Rudich, A.; Klöting, N.; Golan, R.; Henkin, Y.; Rubin, E. et al. (2012): Two Patterns of Adipokine and Other Biomarker Dynamics in a Long-Term Weight Loss Intervention. In: *Diabetes Care* 35 (342-349).

Bobbert, T.; Rochlitz, H.; Wegewitz, U.; Akpulat, S.; Mai, K.; Weickert, M. O. et al. (2005): Changes of adiponectin oligomer composition by moderate weight reduction. In: *Diabetes* 54 (9), 2712–2719.

Bravata, D. M.; Sanders, L.; Huang, J.; Krumholz, H. M.; Olkin, I.; Gardner, C. D.; Bravata, D. M. (2003): Efficacy and safety of low-carbohydrate diets: a systematic review. In: *JAMA* 289 (14), 1837–1850.

Brolin, R. E. (2002): Bariatric surgery and long-term control of morbid obesity. In: *JAMA* 288 (22), 2793–2796.

Bruun, J. M.; Helge, J.; Richelsen, B.; Stallknecht, B. (2006): Diet and exercise reduce low-grade inflammation and macrophage infiltration in adipose tissue but not in skeletal muscle in severely obese subjects. In: *AJP: Endocrinology and Metabolism* 290 (5), E961–E967.

Bruun, J. M.; Lihn A.S.; Pedersen S.B.; Richelsen, B. (2005): Monocyte Chemoattractant Protein-1 Release Is Higher in Visceral than Subcutaneous Human Adipose Tissue (AT): Implication of Macrophages Resident in the AT. In: *Journal of Clinical Endocrinology & Metabolism* 90 (4), 2282–2289.

Buechler, C.; Wanninger, J.; Neumeier, M. (2010): Adiponectin receptor binding proteins – recent advances in elucidating adiponectin signalling pathways. In: *FEBS Letters* 584 (20), 4280–4286.

Butner, K. L.; Nickols-Richardson, S. M.; Clark, S. F.; Ramp, W. K.; Herbert, W. G. (2010): A Review of Weight Loss Following Roux-en-Y Gastric Bypass vs Restrictive Bariatric Surgery: Impact on Adiponectin and Insulin (5), zuletzt geprüft am 29.05.2012.

Calle, E. E.; Rodriguez, C.; Walker-Thurmond, K.; Thun, M. J. (2003): Overweight, obesity, and mortality from cancer in a prospectively studied cohort of U.S. adults. In: *N. Engl. J. Med.* 348 (17), 1625–1638.

Cancello, R.; Henegar, C.; Viguerie, N.; Taleb, S.; Poitou, C.; Rouault, C. et al. (2005): Reduction of macrophage infiltration and chemoattractant gene expression changes in white adipose tissue of morbidly obese subjects after surgery-induced weight loss. In: *Diabetes* 54 (8), 2277–2286.

Cancello, R.; Tordjman, J.; Poitou, C.; Guilhem, G.; Bouillot, J.; Hugol, D. et al. (2006): Increased Infiltration of Macrophages in Omental Adipose Tissue Is Associated With Marked Hepatic Lesions in Morbid Human Obesity. In: *Diabetes* 55 (6), 1554–1561.

Chacón, M. R.; Fernández-Real, J. Manuel; Richart, C.; Megía, A.; Gómez, J. Manuel; Miranda, M. et al. (2007): Monocyte chemoattractant protein-1 in obesity and type 2 diabetes. Insulin sensitivity study. In: *Obesity (Silver Spring)* 15 (3), 664–672.

Charo, I. F.; Ransohoff, R. M. (2006): The many roles of chemokines and chemokine receptors in inflammation. In: *N. Engl. J. Med.* 354 (6), 610–621.

Chawla, A.; Nguyen, K. D.; Goh, Y. P. Sharon (2011): Macrophage-mediated inflammation in metabolic disease. In: *Nat Rev Immunol* 11 (11), 738–749.

Chen, H.; Montagnani, M.; Funahashi, T.; Shimomura, I.; Quon, M. J. (2003): Adiponectin stimulates production of nitric oxide in vascular endothelial cells. In: *J. Biol. Chem.* 278 (45), 45021–45026.

Christiansen, T.; Richelsen, B.; Bruun, J. M. (2005): Monocyte chemoattractant protein-1 is produced in isolated adipocytes, associated with adiposity and reduced after weight loss in morbid obese subjects. In: *Int J Obes Relat Metab Disord* 29 (1), 146–150.

Civitarese, A. E.; Jenkinson, C. P.; Richardson, D.; Bajaj, M.; Cusi, K.; Kashyap, S. et al. (2004): Adiponectin receptors gene expression and insulin sensitivity in non-diabetic Mexican Americans with or without a family history of Type 2 diabetes. In: *Diabetologia* 47 (5), 816–820.

Collins, C. E.; Morgan, P. J.; Jones, P.; Fletcher, K.; Martin, J.; Aguiar, E. J. et al. (2012): A 12-week commercial web-based weight-loss program for overweight and obese adults: randomized controlled trial comparing basic versus enhanced features. In: *J. Med. Internet Res.* 14 (2), e57.

Combs, T. P.; Berg, A. H.; Obici, S.; Scherer, P. E.; Rossetti, L. (2001): Endogenous glucose production is inhibited by the adipose-derived protein Acrp30. In: *J. Clin. Invest.* 108 (12), 1875–1881.

Coughlin, C. C.; Finck, B. N.; Eagon, J. Christopher; Halpin, V. J.; Magkos, F.; Mohammed, B. Selma; Klein, S. (2007): Effect of marked weight loss on adiponectin gene expression and plasma concentrations. In: *Obesity (Silver Spring)* 15 (3), 640–645.

Dalmas, E.; Rouault, C.; Abdennour, M.; Rovere, C.; Rizkalla, S.; Bar-Hen, A.; Nahon, J.-L. (2011): Variations in circulating inflammatory factors are related to changes in calorie and carbohydrate intakes early in the course of surgery-induced weight reduction. In: *Am J Clin Nutr* 94, 450–458.

Daniel, R.; He, Z.; Carmichael, K. P.; Halper, J.; Bateman, A. (2000): Cellular localization of gene expression for progranulin. In: *J. Histochem. Cytochem.* 48 (7), 999–1009.

Dansinger, M.; Gleason, J.; Griffith, J.; Selker, H.; Schaefer, E. (2005): Comparison Atkins, Ornish, WW, Zone. In: *JAMA* 293, 43–53.

Dattilo, A. M.; Kris-Etherton, P. M. (1992): Effects of weight reduction on blood lipids and lipoproteins: a meta-analysis. In: *Am. J. Clin. Nutr* 56, 320–328.

Debard, C.; Laville, M.; Berbe, V.; Loizon, E.; Guillet, C.; Morio-Liondore, B. et al. (2004): Expression of key genes of fatty acid oxidation, including adiponectin receptors, in skeletal muscle of Type 2 diabetic patients. In: *Diabetologia* 47 (5), 917–925.

Deiuliis, J.; Shah, Z.; Shah, N.; Needleman, B.; Mikami, D.; Narula, V. et al. (2011): Visceral adipose inflammation in obesity is associated with critical alterations in tregulatory cell numbers. In: *PLoS ONE* 6 (1), e16376.

Del Corral, P.; Bryan, D. R.; Garvey, W. Timothy; Gower, B. A.; Hunter, G. R. (2011): Dietary adherence during weight loss predicts weight regain. In: *Obesity (Silver Spring)* 19 (6), 1177–1181.

Deo, R.; Khera, A.; McGuire, D. K.; Murphy, S. A.; P. Meo Neto, J. de; Morrow, D. A.; Lemos, J. A. de (2004): Association among plasma levels of monocyte chemoattractant protein-1, traditional cardiovascular risk factors, and subclinical atherosclerosis. In: *Journal of the American College of Cardiology* 44 (9), 1812–1818.

Deshmane, S. L.; Kremlev, S.; Amini, S.; Sawaya, B. E. (2009): Monocyte chemoattractant protein-1 (MCP-1): an overview. In: *J. Interferon Cytokine Res.* 29 (6), 313–326.

Desmarais, J. A.; Cao, M.; Bateman, A.; Murphy, B. D. (2008): Spatiotemporal expression pattern of progranulin in embryo implantation and placenta formation suggests a role in cell proliferation, remodeling, and angiogenesis. In: *Reproduction* 136 (2), 247–257.

Deutsche Adipositas-Gesellschaft (Hg.) (2008): Die Nationale Verzehrsstudie II (NVS II). Online verfügbar unter http://www.adipositas-gesellschaft.de/index.php?id=40.

Devaraj, S.; Jialal, I. (2009): Increased secretion of IP-10 from monocytes under hyperglycemia is via the TLR2 and TLR4 pathway. In: *Cytokine* 47 (1), 6–10.

Di Gregorio, G. B.; Yao-Borengasser, A.; Rasouli, N.; Varma, V.; Lu, T.; Miles, L. M. et al. (2005): Expression of CD68 and macrophage chemoattractant protein-1 genes in human

adipose and muscle tissues: association with cytokine expression, insulin resistance, and reduction by pioglitazone. In: *Diabetes* 54 (8), 2305–2313.

Ding, Z.; Xiong, K.; Issekutz, T. B. (2000): Regulation of chemokine-induced transendothelial migration of T lymphocytes by endothelial activation: differential effects on naive and memory T cells. In: *J. Leukoc. Biol.* 67 (6), 825–833.

Duffaut, C.; Zakaroff-Girard, A.; Bourlier, V.; Decaunes, P.; Maumus, M.; Chiotasso, P. et al. (2009): Interplay between human adipocytes and T lymphocytes in obesity: CCL20 as an adipochemokine and T lymphocytes as lipogenic modulators. In: *Arterioscler. Thromb. Vasc. Biol.* 29 (10), 1608–1614.

Ebrahim, S.; Smith, G. (1998): Lowering blood pressure: a systematic review of sustained effects of non-pharmacological interventions. In: *J Public Health Med* 20 (4), 441–448.

Engl, J.; Bobbert, T.; Ciardi, C.; Laimer, M.; Tatarczyk, T.; Kaser, S. et al. (2007): Effects of pronounced weight loss on adiponectin oligomer composition and metabolic parameters. In: *Obesity (Silver Spring)* 15 (5), 1172–1178.

Eriksson, E. E. (2004): Mechanisms of leukocyte recruitment to atherosclerotic lesions: future prospects. In: *Curr. Opin. Lipidol.* 15 (5), 553–558.

Estruch, R.; Martínez-González, M. Angel; Corella, D.; Salas-Salvadó, J.; Ruiz-Gutiérrez, V.; Covas, M. Isabel et al. (2006): Effects of a Mediterranean-style diet on cardiovascular risk factors: a randomized trial. In: *Ann. Intern. Med.* 145 (1), 1–11.

Fain, J. N. (2006): Release of interleukins and other inflammatory cytokines by human adipose tissue is enhanced in obesity and primarily due to the nonfat cells. In: *Vitam. Horm.* 74, 443–477.

Fain, J. N.; Buehrer, B.; Bahouth, S. W.; Tichansky, D. S.; Madan, A. K. (2008): Comparison of messenger RNA distribution for 60 proteins in fat cells vs the nonfat cells of human omental adipose tissue. In: *Metabolism* 57 (7), 1005–1015.

Fain, J. N.; Tagele, B. M.; Cheema, P.; Madan, A. K.; Tichansky, D. S. (2009): Release of 12 Adipokines by Adipose Tissue, Nonfat Cells, and Fat Cells From Obese Women. In: *Obesity* 18 (5), 890–896.

Fang, X.; Sweeney, G. (2006): Mechanisms regulating energy metabolism by adiponectin in obesity and diabetes. In: *Biochem. Soc. Trans* 34 (Pt 5), 798–801.

Farley, R. L.; Wade, T. D.; Birchmore, L. (2003): Factors influencing attendance at cardiac rehabilitation among coronary heart disease patients. In: *Eur J Cardiovasc Nurs* 2 (3), 205–212.

Fasshauer, M.; Klein, J.; Kralisch, S.; Klier, M.; Lossner, U.; Bluher, M.; Paschke, R. (2004): Monocyte chemoattractant protein 1 expression is stimulated by growth hormone and interleukin-6 in 3T3-L1 adipocytes. In: *Biochem. Biophys. Res. Commun* 317 (2), 598–604.

Fasshauer, M.; Klein, J.; Neumann, S.; Eszlinger, M.; Paschke, R. (2002): Hormonal regulation of adiponectin gene expression in 3T3-L1 adipocytes. In: *Biochem. Biophys. Res. Commun.* 290 (3), 1084–1089.

Felder, T. K.; Hahne, P.; Soyal, S. M.; Miller, K.; Höffinger, H.; Oberkofler, H. et al. (2010): Hepatic adiponectin receptors (ADIPOR) 1 and 2 mRNA and their relation to insulin resistance in obese humans. In: *Int J Obes Relat Metab Disord* 34 (5), 846–851.

Ferranti, S. de; Mozaffarian, D. (2008): The perfect storm: obesity, adipocyte dysfunction, and metabolic consequences. In: *Clin. Chem* 54 (6), 945–955.

Feuerer, M.; Herrero, L.; Cipolletta, D.; Naaz, A.; Wong, J.; Nayer, A. et al. (2009): Lean, but not obese, fat is enriched for a unique population of regulatory T cells that affect metabolic parameters. In: *Nat. Med.* 15 (8), 930–939.

Finkler, E.; Heymsfield, S. B.; St-Onge, M.-P. (2012): Rate of Weight Loss Can Be Predicted by Patient Characteristics and Intervention Strategies. In: *Journal of the Academy of Nutrition and Dietetics* 112 (1), 75–80.

Fleischman, A.; Shoelson, S. E.; Bernier, R.; Goldfine, A. B. (2008): Salsalate improves glycemia and inflammatory parameters in obese young adults. In: *Diabetes Care* 31 (2), 289–294.

Fontaine, K. R.; Redden, D. T.; Wang, C.; Westfall, A. O.; Allison, D. B. (2003): Years of life lost due to obesity. In: *JAMA* 289 (2), 187–193.

Freedman; King, J.; Kennedy, E. (2001): Popular diets: a scientific review. In: *Obesity Research* 9 (S1).

Fruebis, J.; Tsao, T. S.; Javorschi, S.; Ebbets-Reed, D.; Erickson, M. R.; Yen, F. T. et al. (2001): Proteolytic cleavage product of 30-kDa adipocyte complement-related protein increases fatty acid oxidation in muscle and causes weight loss in mice. In: *Proc. Natl. Acad. Sci. U.S.A.* 98 (4), 2005–2010.

Fujimatsu, D.; Kotooka, N.; Inoue, T.; Nishiyama, M.; Node, K. (2009): Association between high molecular weight adiponectin levels and metabolic parameters. In: *J. Atheroscler. Thromb.* 16 (5), 553–559.

Galgani, J.; Ravussin, E. (2008): Energy metabolism, fuel selection and body weight regulation. In: *Int J Obes (Lond)* 32 Suppl 7, S109-19.

Gardner, C.; Kiazand, A.; Alhassan, S.; Kim, S.; Stafford, R.; Balise, R. et al. (2007): Comparison of the Atkins, Zone, Ornish, and LEARN Diets for Change in Weight and Related Risk Factors Among Overweight Premenopausal Women The A TO Z Weight Loss Study: A Randomized Trial. In: *JAMA* (297), 969–977.

Gerhardt, C. C.; Romero, I. A.; Cancello, R.; Camoin, L.; Strosberg, A. D. (2001): Chemokines control fat accumulation and leptin secretion by cultured human adipocytes. In: *Mol. Cell. Endocrinol.* 175 (1-2), 81–92.

Glintborg, D.; Andersen, M.; Richelsen, B.; Bruun, J. M. (2009): Plasma monocyte chemoattractant protein-1 (MCP-1) and macrophage inflammatory protein-1alpha are increased in patients with polycystic ovary syndrome (PCOS) and associated with adiposity, but unaffected by pioglitazone treatment. In: *Clin. Endocrinol. (Oxf)* 71 (5), 652–658.

Goldstein, D. J. (1992): Beneficial health effects of modest weight loss. In: *Int J Obes Relat Metab Disord* 16 (6), 397–415.

Gordon, S.; Taylor, P. R. (2005): Monocyte and macrophage heterogeneity. In: *Nat. Rev. Immunol* 5 (12), 953–964.

Gortmaker, S. L.; Swinburn, B. A.; Levy, D.; Carter, R.; Mabry, P. L.; Finegood, D. T. et al. (2011): Changing the future of obesity: science, policy, and action. In: *Lancet* 378 (9793), 838–847.

Gosling, J.; Slaymaker, S.; Gu, L.; Tseng, S.; Zlot, C.; Young, S. et al. (1999): MCP-1 deficiency reduces susceptibility to atherosclerosis in mice that overexpress human apolipoprotein B. In: *J Clin Invest* 103, 773–778.

Grossi, E.; Dalle Grave, R.; Mannucci, E.; Molinari, E.; Compare, A.; Cuzzolaro, M.; Marchesini, G. (2006): Complexity of attrition in the treatment of obesity: clues from a structured telephone interview. In: *Int J Obes Relat Metab Disord* 30 (7), 1132–1137.

Hagan, R. D.; Upton, S. J.; Wong, L.; Whittam, J. (1986): The effects of aerobic conditioning and/or caloric restriction in overweight men and women. In: *Med Sci Sports Exerc* 18 (1), 87–94.

Hauner, H.; Bechthold, A.; Boeing, H.; Brönstrup, A.; Buyken, A.; Leschik-Bonnet, E. et al. (2012): Evidence-Based Guideline of the German Nutrition Society: Carbohydrate Intake and Prevention of Nutrition-Related Diseases. In: *Ann Nutr Metab* 60 (s1), 1–58.

Hauner, H.; Buchholz, G.; Hamann, A.; HusemannB.; Koletzko, B.; Liebermeister, H. et al. (2007): Evidenzbasierte Leitlinie Prävention und Therapie der Adipositas 2007. Hg. v. Deutsche Adipositas-Gesellschaft, Deutsche Gesellschaft für Ernährung Deutsche Diabetes-Gesellschaft und Deutsche Gesellschaft für Ernährungsmedizin.

Hauner, H. (2005): Obesity and its Co-Morbidities. *In* Obesity and Metabolic Disorders, Antel, J.; Finer, N.; Heal, D.; Krause, G. (eds), IOS press, Oxford, pp. 15-23.

Hauner, H.; Meier, M.; Wendland, G.; Kurscheid, T.; Lauterbach, K. (2004): Weight reduction by sibutramine in obese subjects in primary care medicine: the SAT Study. In: *Exp. Clin. Endocrinol. Diabetes* 112 (4), 201–207.

Hayden, M. S.; Ghosh, S. (2012): NF- B, the first quarter-century: remarkable progress and outstanding questions. In: *Genes & Development* 26 (3), 203–234.

He, Z.; Ong, C. H. P.; Halper, J.; Bateman, A. (2003): Progranulin is a mediator of the wound response. In: *Nat. Med.* 9 (2), 225–229.

Heilbronn, L.; Campbell, L. (2008): Adipose tissue macrophages, low grade inflammation and insulin resistance in human obesity. In: *Curr. Pharm. Des.* 14 (12), 1225–1230.

Herder, C. (2006): Systemic Immune Mediators and Lifestyle Changes in the Prevention of Type 2 Diabetes: Results From the Finnish Diabetes Prevention Study. In: *Diabetes* 55 (8), 2340–2346.

Herder, C.; Baumert, J.; Thorand, B.; Koenig, W.; Jager, W. de; Meisinger, C. et al. (2006a): Chemokines as risk factors for type 2 diabetes: results from the MONICA/KORA Augsburg study, 1984-2002. In: *Diabetologia* 49 (5), 921–929.

Herder, C.; Baumert, J.; Thorand, B.; Martin, S.; Löwel, H.; Kolb, H.; Koenig, W. (2006b): Chemokines and Incident Coronary Heart Disease: Results From the MONICA/KORA Augsburg Case-Cohort Study, 1984-2002. In: *Arteriosclerosis, Thrombosis, and Vascular Biology* 26 (9), 2147–2152.

Herder, C.; Haastert, B.; Müller-Scholze, S.; Koenig, W.; Thorand, B.; Holle, R. et al. (2005): Association of systemic chemokine concentrations with impaired glucose tolerance and type 2 diabetes: results from the Cooperative Health Research in the Region of Augsburg Survey S4 (KORA S4). In: *Diabetes* 54 Suppl 2, S11-7.

Herder, C.; Hauner, H.; Kempf, K.; Kolb, H.; Skurk, T. (2007a): Constitutive and regulated expression and secretion of interferon-gamma-inducible protein 10 (IP-10/CXCL10) in human adipocytes. In: *Int J Obes (Lond)* 31 (3), 403–410.

Herder, C.; Illig, T.; Baumert, J.; Müller, M.; Klopp, N.; Khuseyinova, N. et al. (2008): RANTES/CCL5 gene polymorphisms, serum concentrations, and incident type 2 diabetes: results from the MONICA/KORA Augsburg case-cohort study, 1984-2002. In: *Eur. J. Endocrinol.* 158 (5), R1-5.

Herder, C.; Peeters, W.; Illig, T.; Baumert, J.; Kleijn, D. P. V. de; Moll, F. L. et al. (2011): RANTES/CCL5 and risk for coronary events: results from the MONICA/KORA Augsburg case-cohort, Athero-Express and CARDIoGRAM studies. In: *PLoS ONE* 6 (12), e25734.

Herder, C.; Schneitler, S.; Rathmann, W.; Haastert, B.; Schneitler, H.; Winkler, H. et al. (2007b): Low-Grade Inflammation, Obesity, and Insulin Resistance in Adolescents. In: *Journal of Clinical Endocrinology & Metabolism* 92 (12), 4569–4574.

Heshka, S.; Stafford, J.; Atkinson, R.; Greenway, F.; Hill, J.; Phinney, S. et al. (2003): Weight Loss with self-help compared with a structured commercial program. In: *JAMA* (289), 1792–1798.

Hill, J. O.; Peters, J. (1998): Environmental Contributions to the Obesity Epidemic. In: *Science* 280 (5368), 1371–1374.

Himes, R. W.; Smith, C. Wayne (2010): Tlr2 is critical for diet-induced metabolic syndrome in a murine model. In: *FASEB J.* 24 (3), 731–739.

Hirosumi, J.; Tuncman, G.; Chang, L.; Görgün, C. Z.; Uysal, K. Teoman; Maeda, K. et al. (2002): A central role for JNK in obesity and insulin resistance. In: *Nature* 420 (6913), 333–336.

Holmes, R. M.; Yi, Z.; Filippis, E. de; Berria, R.; Shahani, S.; Sathyanarayana, P. et al. (2011): Increased abundance of the adaptor protein containing pleckstrin homology domain, phosphotyrosine binding domain and leucine zipper motif (APPL1) in patients with obesity and type 2 diabetes: evidence for altered adiponectin signalling. In: *Diabetologia* 54 (8), 2122–2131.

Holzapfel, C.; Hauner, H. (2011): Gewichtserhaltung nach Gewichtsreduktion - wie der Körper sein Gewicht verteidigt. In: *Dtsch. Med. Wochenschr.* 136 (3), 89–94.

Hotta, K.; Funahashi, T.; Arita, Y.; Takahashi, M.; Matsuda, M.; Okamoto, Y. et al. (2000): Plasma Concentrations of a Novel, Adipose-Specific Protein, Adiponectin, in Type 2 Diabetic Patients. In: *Arterioscler Thromb Vasc Biol (Arteriosclerosis, Thrombosis, and Vascular Biology)* 20 (6), 1595–1599.

Huber, J.; Kiefer, F. W.; Zeyda, M.; Ludvik, B.; Silberhumer, G. R.; Prager, G. et al. (2008): CC Chemokine and CC Chemokine Receptor Profiles in Visceral and Subcutaneous Adipose Tissue Are Altered in Human Obesity. In: *Journal of Clinical Endocrinology & Metabolism* 93 (8), 3215–3221.

Jakicic, J. (2009): The effect of physical activity on body weight. In: *Obesity (Silver Spring)* 17 Suppl 3, S34-8.

Jakicic, J.; Marcus, B.; Lang, W.; Janney, C. (2008): Effect of exercise on 24-month weight loss maintenance in overweight women. In: *Arch Intern Med* 168 (14), 1550-9; discussion 1559-60.

Jebb, S. A.; Ahern, A. L.; Olson, A. D.; Aston, L. M.; Holzapfel, C.; Stoll, J. et al. (2011): Primary care referral to a commercial provider for weight loss treatment versus standard care: a randomised controlled trial. In: *Lancet* 378 (9801), 1485–1492.

Jeffery, R. W.; Levy, R. L.; Langer, S. L.; Welsh, E. M.; Flood, A. P.; Jaeb, M. A. et al. (2009): A comparison of maintenance-tailored therapy (MTT) and standard behavior therapy (SBT) for the treatment of obesity. In: *Prev Med* 49 (5), 384–389.

Jensen, M. D.; Haymond, M. W.; Rizza, R. A.; Cryer, P. E.; Miles, J. M. (1989): Influence of body fat distribution on free fatty acid metabolism in obesity. In: *J. Clin. Invest* 83 (4), 1168–1173.

Jiao, P.; Chen, Q.; Shah, S.; Du, J.; Tao, B.; Tzameli, I. et al. (2009): Obesity-related upregulation of monocyte chemotactic factors in adipocytes: involvement of nuclear factor-kappaB and c-Jun NH2-terminal kinase pathways. In: *Diabetes* 58 (1), 104–115.

Jolly, K.; Lewis, A.; Beach, J.; Denley, J.; Adab, P.; Deeks, J. et al. (2011): Comparison of range of commercial or primary care led weight reduction programmes with minimal intervention control for weight loss in obesity: Lighten Up randomised controlled trial. In: *BMJ* 343, d6500.

Kadowaki, T. (2005): Adiponectin and Adiponectin Receptors. In: *Endocrine Reviews* 26 (3), 439–451.

Kamei, N.; Tobe, K.; Suzuki, R.; Ohsugi, M.; Watanabe, T.; Kubota, N. et al. (2006): Overexpression of monocyte chemoattractant protein-1 in adipose tissues causes macrophage recruitment and insulin resistance. In: *J. Biol. Chem.* 281 (36), 26602–26614.

Kanda, H.; Tateya, S.; Tamori, Y.; Kotani, K.; Hiasa, K.-i.; Kitazawa, R. et al. (2006): MCP-1 contributes to macrophage infiltration into adipose tissue, insulin resistance, and hepatic steatosis in obesity. In: *J. Clin. Invest.* 116 (6), 1494–1505.

Karalis, K. P.; Giannogonas, P.; Kodela, E.; Koutmani, Y.; Zoumakis, M.; Teli, T. (2009): Mechanisms of obesity and related pathology: linking immune responses to metabolic stress. In: *FEBS J.* 276 (20), 5747–5754.

Kaser, S.; Tatarczyk, T.; Stadlmayr, A.; Ciardi, C.; Ress, C.; Tschoner, A. et al. (2008): Effect of obesity and insulin sensitivity on adiponectin isoform distribution. In: *Eur. J. Clin. Invest.* 38 (11), 827–834.

Kazumi, T.; Kawaguchi, A.; Hirano, T.; Yoshino, G. (2004): Serum adiponectin is associated with high-density lipoprotein cholesterol, triglycerides, and low-density lipoprotein particle size in young healthy men. In: *Metab. Clin. Exp.* 53 (5), 589–593.

Keophiphath, M.; Rouault, C.; Divoux, A.; Clément, K.; Lacasa, D. (2010): CCL5 promotes macrophage recruitment and survival in human adipose tissue. In: *Arterioscler. Thromb. Vasc. Biol.* 30 (1), 39–45.

Kern, P. A.; Ranganathan, S.; Li, C.; Wood, L.; Ranganathan, G. (2001): Adipose tissue tumor necrosis factor and interleukin-6 expression in human obesity and insulin resistance. In: *Am. J. Physiol. Endocrinol. Metab.* 280 (5), E745-51.

Kessenbrock, K.; Fröhlich, L.; Sixt, M.; Lämmermann, T.; Pfister, H.; Bateman, A. et al. (2008): Proteinase 3 and neutrophil elastase enhance inflammation in mice by inactivating antiinflammatory progranulin. In: *J. Clin. Invest.* 118 (7), 2438–2447.

Kim C.S., P. H.S. T. Kawada Kim J.H. D. Lim NE Hubbard B-S Kwon KL Erickson (2006): Circulating levels of MCP-1 and IL-8 are elevated in human obese subjects and associated with obesity related parameters. In: *Int J Obes Relat Metab Disord* 30, 1347–1355.

Kintscher, U.; Hartge, M.; Hess, K.; Foryst-Ludwig, A.; Clemenz, M.; Wabitsch, M. et al. (2008): T-lymphocyte infiltration in visceral adipose tissue: a primary event in adipose tissue inflammation and the development of obesity-mediated insulin resistance. In: *Arterioscler. Thromb. Vasc. Biol.* 28 (7), 1304–1310.

Klöting, N.; Fasshauer, M.; Dietrich, A.; Kovacs, P.; Schön, M. R.; Kern, M. et al. (2010): Insulin-sensitive obesity. In: *Am. J. Physiol. Endocrinol. Metab.* 299 (3), E506-15.

Knoll, K.; Hauner, H. (2008): Kosten der Adipositas in der Bundesrepublik Deutschland - Eine aktuelle Krankheitskostenstudie Adipositas. In: *Adipositas* 2 (4), 204–210.

Knowler, W.; Fowler, S.; Hamman, R.; Christophi, C.; Hoffman, H.; Brenneman, A. et al. (2009): 10-year follow-up of diabetes incidence and weight loss in the Diabetes Prevention Program Outcomes Study 374 (9702), 1677–1686.

Koh, S. Jeong; Kim, J. Young; Hyun, Y. Jung; Park, S. Hyun; Chae, J. Sook; Park, S. et al. (2009): Association of serum RANTES concentrations with established cardiovascular risk markers in middle-aged subjects (1), zuletzt geprüft am 08.05.2012.

Kojima, Y.; Ono, K.; Inoue, K.; Takagi, Y.; Kikuta, K.-i.; Nishimura, M. et al. (2009): Progranulin expression in advanced human atherosclerotic plaque. In: *Atherosclerosis* 206 (1), 102–108.

Kolattukudy, P. E.; Niu, J. (2012): Inflammation, Endoplasmic Reticulum Stress, Autophagy, and the Monocyte Chemoattractant Protein-1/CCR2 Pathway. In: *Circulation Research* 110 (1), 174–189.

Kopp, A.; Buechler, C.; Bala, M.; Neumeier, M.; Scholmerich, J.; Schaffler, A. (2010): Toll-Like Receptor Ligands Cause Proinflammatory and Prodiabetic Activation of Adipocytes via Phosphorylation of Extracellular Signal-Regulated Kinase and c-Jun N-Terminal Kinase But Not Interferon Regulatory Factor-3. In: *Endocrinology* 151 (3), 1097–1108.

Kopp, H.-P.; Krzyzanowska, K.; Möhlig, M.; Spranger, J.; Pfeiffer, A. F. H.; Schernthaner, G. (2005): Effects of marked weight loss on plasma levels of adiponectin, markers of chronic subclinical inflammation and insulin resistance in morbidly obese women. In: *Int J Obes Relat Metab Disord* 29 (7), 766–771.

Krinninger, P.; Brunner, C.; Ruiz, P. A.; Schneider, E.; Marx, N.; Foryst-Ludwig, A. et al. (2011): Role of the adipocyte-specific NF-κB activity in the regulation of IP-10 and T cell migration. In: *Am. J. Physiol. Endocrinol. Metab* 300 (2), E304-11.

Kris-Etherton, P. M.; Pearson, T.; Wan, Y.; Hargrove, R.; Moriarty, K.; Fishell, V.; Etherton, T. (1999): High–monounsaturated fatty acid diets lower both plasma cholesterol and triacylglycerol concentrations. In: *Am J Clin Nutr* 70 (6), 1009–1015.

Kumada, M.; Kihara, S.; Sumitsuji, S.; Kawamoto, T.; Matsumoto, S.; Ouchi, N. et al. (2002): Association of Hypoadiponectinemia With Coronary Artery Disease in Men. In: *Arterioscler Thromb Vasc Biol (Arteriosclerosis, Thrombosis, and Vascular Biology)* 23 (1), 85–89.

Kurth, B.-M.; Schaffrath Rosario, A. (2007): Die Verbreitung von Ubergewicht und Adipositas bei Kindern und Jugendlichen in Deutschland. Ergebnisse des bundesweiten Kinder- und Jugendgesundheitssurveys (KiGGS). In: *Bundesgesundheitsblatt Gesundheitsforschung Gesundheitsschutz* 50 (5-6), 736–743.

Kushner, R. F.; Foster, G. D. (2000): Obesity and quality of life. In: *Nutrition* 16 (10), 947–952.

Lacotte, S.; Brun, S.; Muller, S.; Dumortier, H. (2009): CXCR3, inflammation, and autoimmune diseases. In: *Ann. N. Y. Acad. Sci.* 1173, 310–317.

Lang, H.-F.; Chou, C.-Y.; Sheu, W. Huey-Herng; Lin, J.-Y. (2011): Weight loss increased serum adiponectin but decreased lipid levels in obese subjects whose body mass index was lower than 30 kg/m². In: *Nutr Res* 31 (5), 378–386.

Lara-Castro, C.; Luo, N.; Wallace, P.; Klein, R. L.; Garvey, W. Timothy (2006): Adiponectin multimeric complexes and the metabolic syndrome trait cluster. In: *Diabetes* 55 (1), 249–259.

Lawrence, T. (2009): The nuclear factor NF-kappaB pathway in inflammation. In: *Cold Spring Harb Perspect Biol* 1 (6), a001651.

Lindsay, R. S.; Funahashi, T.; Hanson, R. L.; Matsuzawa, Y.; Tanaka, S.; Tataranni, P. Antonio et al. (2002): Adiponectin and development of type 2 diabetes in the Pima Indian population. In: *Lancet* 360 (9326), 57–58.

Lindström, J.; Eriksson, J. G.; Valle, T. T.; Aunola, S.; Cepaitis, Z.; Hakumäki, M. et al. (2003a): Prevention of diabetes mellitus in subjects with impaired glucose tolerance in the Finnish Diabetes Prevention Study: results from a randomized clinical trial. In: *J. Am. Soc. Nephrol.* 14 (7 Suppl 2), S108-13.

Lindström, J.; Louheranta, A.; Mannelin, M.; Rastas, M.; Salminen, V.; Eriksson, J. et al. (2003b): The Finnish Diabetes Prevention Study (DPS): Lifestyle intervention and 3-year results on diet and physical activity. In: *Diabetes Care* 26 (12), 3230–3236.

Linscheid, P.; Christ-Crain, M.; Stoeckli, R.; Reusch, C. E.; Lutz, T. A.; Müller, B.; Keller, U. (2008): Increase in high molecular weight adiponectin by bariatric surgery-induced weight loss. In: *Diabetes Obes Metab* 10 (12), 1266–1270.

Lobstein, T.; Frelut, M. L. (2003): Prevalence of overweight among children in Europe. In: *Obes Rev* 4 (4), 195–200.

Lolmède, K.; Duffaut, C.; Zakaroff-Girard, A.; Bouloumié, A. (2011): Immune cells in adipose tissue: Key players in metabolic disorders. In: *Diabetes Metab* 37 (4), 283–290.

Lovren, F.; Pan, Y.; Quan, A.; Szmitko, P. E.; Singh, K. K.; Shukla, P. C. et al. (2010): Adiponectin primes human monocytes into alternative anti-inflammatory M2 macrophages. In: *Am. J. Physiol. Heart Circ. Physiol* 299 (3), H656-63.

Lowe, M. R.; Kral, T. V. E.; Miller-Kovach, K. (2008): Weight-loss maintenance 1, 2 and 5 years after successful completion of a weight-loss programme. In: *BJN* 99 (04).

Lumeng, C. N.; Bodzin, J. L.; Saltiel, A. R. (2007): Obesity induces a phenotypic switch in adipose tissue macrophage polarization. In: *J Clin Invest* (117), 175–184.

Lumeng, C. N.; DelProposto, J. B.; Westcott, D. J.; Saltiel, A. R. (2008): Phenotypic switching of adipose tissue macrophages with obesity is generated by spatiotemporal differences in macrophage subtypes. In: *Diabetes* 57 (12), 3239–3246.

Luster, A. D. (1998): Chemokines--chemotactic cytokines that mediate inflammation. In: *N. Engl. J. Med.* 338 (7), 436–445.

Luster, A.; Ravetch, J. (1987): Biochemical characterization of a y interferon-inducible protein (IP-10). In: *J. Exp. Med.* (166), 1084–1097.

Madani, R.; Karastergiou, K.; Ogston, N. C.; Miheisi, N.; Bhome, R.; Haloob, N. et al. (2009): RANTES release by human adipose tissue in vivo and evidence for depot-specific differences. In: *Am. J. Physiol. Endocrinol. Metab.* 296 (6), E1262-8.

Madsen, E. L.; Rissanen, A.; Bruun, J. M.; Skogstrand, K.; Tonstad, S.; Hougaard, D. M.; Richelsen, B. (2008): Weight loss larger than 10% is needed for general improvement of levels of circulating adiponectin and markers of inflammation in obese subjects: a 3-year weight loss study. In: *Eur. J. Endocrinol.* 158 (2), 179–187.

Martínez-González, M. Angel; Sánchez-Villegas, A. (2004): The emerging role of Mediterranean diets in cardiovascular epidemiology: monounsaturated fats, olive oil, red wine or the whole pattern? In: *Eur. J. Epidemiol.* 19 (1), 9–13.

Mason, C.; Foster-Schubert, K. E.; Imayama, I.; Kong, A.; Xiao, L.; Bain, C. et al. (2011): Dietary Weight Loss and Exercise Effects on Insulin Resistance in Postmenopausal Women. In: *American Journal of Preventive Medicine* 41 (4), 366–375.

Mather, K. J.; Funahashi, T.; Matsuzawa, Y.; Edelstein, S.; Bray, G. A.; Kahn, S. E. et al. (2008): Adiponectin, change in adiponectin, and progression to diabetes in the Diabetes Prevention Program. In: *Diabetes* 57 (4), 980–986.

Matsubara, M.; Katayose, S.; Maruoka, S. (2003): Decreased plasma adiponectin concentrations in nondiabetic women with elevated homeostasis model assessment ratios. In: *Eur. J. Endocrinol.* 148 (3), 343–350.

Matsubara, M.; Maruoka, S.; Katayose, S. (2002a): Decreased plasma adiponectin concentrations in women with dyslipidemia. In: *J. Clin. Endocrinol. Metab.* 87 (6), 2764–2769.

Matsubara, M.; Maruoka, S.; Katayose, S. (2002b): Inverse relationship between plasma adiponectin and leptin concentrations in normal-weight and obese women. In: *Eur. J. Endocrinol.* 147 (2), 173–180.

Matsubara, T.; Mita, A.; Minami, K.; Hosooka, T.; Kitazawa, S.; Takahashi, K. et al. (2012): PGRN is a Key Adipokine Mediating High Fat Diet-Induced Insulin Resistance and Obesity through IL-6 in Adipose Tissue. In: *Cell Metab* 15 (1), 38–50.

Meier, C. A.; Chicheportiche, R.; Dreyer, M.; Dayer, J.-M. (2003): IP-10, but not RANTES, is upregulated by leptin in monocytic cells. In: *Cytokine* 21 (1), 43–47.

Menzaghi, C.; Salvemini, L.; Paroni, G.; Bonis, C. de; Mangiacotti, D.; Fini, G. et al. (2010): Circulating high molecular weight adiponectin isoform is heritable and shares a common genetic background with insulin resistance in nondiabetic White Caucasians from Italy: evidence from a family-based study. In: *Journal of Internal Medicine* 267 (3), 287–294.

Mine, S.; Okada, Y.; Tanikawa, T.; Kawahara, C.; Tabata, T.; Tanaka, Y. (2006): Increased expression levels of monocyte CCR2 and monocyte chemoattractant protein-1 in patients with diabetes mellitus. In: *Biochemical and Biophysical Research Communications* 344 (3), 780–785.

Muynck, L. de; van Damme, P. (2011): Cellular Effects of Progranulin in Health and Disease. In: *J Mol Neurosci* 45 (3), 549–560.

Nannipieri, M.; Bonotti, A.; Anselmino, M.; Cecchetti, F.; Madec, S.; Mancini, E. et al. (2007): Pattern of expression of adiponectin receptors in human adipose tissue depots and its relation to the metabolic state. In: *International Journal of Obesity* 31, 1843–1848.

Nelken, N.; Coughlin, S.; Gordon, D.; Wilcox, J. (1991): Monocyte Chemoattractant Protein-1 in Human Atheromatous Plaques. In: *J. Clin. Invest* 88, 1121–1127.

Neter, J. E.; Stam, B.; Kok, F.; Grobbee, D.; Geleijnse, J. (2003): Influence of Weight Reduction on Blood Pressure: A Meta-Analysis of Randomized Controlled Trials. In: *Hypertension* 42 (5), 878–884.

Neumeier, M.; Bauer, S.; Brühl, H.; Eisinger, K.; Kopp, A.; Abke, S. et al. (2011): Adiponectin stimulates release of CCL2, -3, -4 and -5 while the surface abundance of CCR2 and -5 is simultaneously reduced in primary human monocytes. In: *Cytokine* 56 (3), 573–580.

Nordmann, A.; Nordmann, A.; Briel, M.; Keller, U.; Yancy, W.; Brehn, B.; Bucher, H. (2006): Effects of Low-Carbohydrate vs Low-Fat Diets on Weight Loss and Cardiovascular Risk Factors. In: *Arch Intern Med.* (166), 285–293.

Odegaard, J. I.; Chawla, A. (2011): Alternative Macrophage Activation and Metabolism. In: *Annu. Rev. Pathol. Mech. Dis* 6 (1), 275–297.

Oezcan, U.; Cao, Q.; Yilmaz, E.; Lee, A.-H.; Iwakoshi, N.; Oezdelen, E. et al. (2004): Endoplasmic Reticulum Stress Links Obesity, Insulin Action, and Type 2 Diabetes. In: *Science* 306 (5695), 457–461.

Ohashi, K.; Parker, J. L.; Ouchi, N.; Higuchi, A.; Vita, J. A.; Gokce, N. et al. (2010): Adiponectin promotes macrophage polarization toward an anti-inflammatory phenotype. In: *J. Biol. Chem.* 285 (9), 6153–6160.

Okura, H.; Yamashita, S.; Ohama, T.; Saga, A.; Yamamoto-Kakuta, A.; Hamada, Y. et al. (2010): HDL/apolipoprotein A-I binds to macrophage-derived progranulin and suppresses its conversion into proinflammatory granulins. In: *J. Atheroscler. Thromb.* 17 (6), 568–577.

Ong, C. H.P.; Bateman, A. (2003): Progranulin (granulin-epithelin precursor, PC-cell derived growth factor, acrogranin) in proliferation and tumorigenesis. In: *Histol Histopathol* 18 (4), 1275–1288.

OPIE, L. H.; WALFISH, P. G. (1963): Plasma free fatty acid concentrations in obesity. In: *N. Engl. J. Med* 268, 757–760.

Oran, P. E.; Sherma, N. D.; Borges, C. R.; Jarvis, J. W.; Nelson, R. W. (2010): Intrapersonal and Populational Heterogeneity of the Chemokine RANTES. In: *Clinical Chemistry* 56 (9), 1432–1441.

Ouchi, N.; Kihara, S.; Arita, Y.; Nishida, M.; Matsuyama, A.; Okamoto, Y. et al. (2001): Adipocyte-Derived Plasma Protein, Adiponectin, Suppresses Lipid Accumulation and Class A Scavenger Receptor Expression in Human Monocyte-Derived Macrophages. In: *Circulation* 103, 1057–1063.

Ozcan, U.; Yilmaz, E.; Ozcan, L.; Furuhashi, M.; Vaillancourt, E.; Smith, R. O. et al. (2006): Chemical chaperones reduce ER stress and restore glucose homeostasis in a mouse model of type 2 diabetes. In: *Science* 313 (5790), 1137–1140.

Pajvani, U. B.; Hawkins, M.; Combs, T. P.; Rajala, M. W.; Doebber, T.; Berger, J. P. et al. (2004): Complex distribution, not absolute amount of adiponectin, correlates with thiazolidinedione-mediated improvement in insulin sensitivity. In: *J. Biol. Chem.* 279 (13), 12152–12162.

Pang, T. T.L; Narendran, P. (2008): The Distribution of Adiponectin Receptors on Human Peripheral Blood Mononuclear Cells. In: *Annals of the New York Academy of Sciences* 1150 (1), 143–145.

Piemonti, L.; Calori, G.; Mercalli, A.; Lattuada, G.; Monti, P.; Garancini, M. Paola et al. (2003): Fasting plasma leptin, tumor necrosis factor-alpha receptor 2, and monocyte chemoattracting protein 1 concentration in a population of glucose-tolerant and glucose-intolerant women: impact on cardiovascular mortality. In: *Diabetes Care* 26 (10), 2883–2889.

Pirozzo, S.; Summerbel, l. C.; Cameron, C.; Glasziou, P. (2003): Should we recommend low-fat diets for obesity? In: *Obesity Reviews* 4, 83–90.

Pi-Sunyer, X.; Blackburn, G.; Brancati, F. L.; Bray, G. A.; Bright, R.; Clark, J. M. et al. (2007): Reduction in weight and cardiovascular disease risk factors in individuals with type 2 diabetes: one-year results of the look AHEAD trial. In: *Diabetes Care* 30 (6), 1374–1383.

Poggi, M.; Engel, D.; Christ, A.; Beckers, L.; Wijnands, E.; Boon, L. et al. (2011): CD40L Deficiency Ameliorates Adipose Tissue Inflammation and Metabolic Manifestations of Obesity in Mice. In: *Arteriosclerosis, Thrombosis, and Vascular Biology* 31 (10), 2251–2260.

Reyna, S. M.; Ghosh, S.; Tantiwong, P.; Meka, C. S. Reddy; Eagan, P.; Jenkinson, C. P. et al. (2008): Elevated toll-like receptor 4 expression and signaling in muscle from insulin-resistant subjects. In: *Diabetes* 57 (10), 2595–2602.

Richards, A. A.; Stephens, T.; Charlton, H. K.; Jones, A.; Macdonald, G. A.; Prins, J. B.; Whitehead, J. P. (2006): Adiponectin multimerization is dependent on conserved lysines in the collagenous domain: evidence for regulation of multimerization by alterations in posttranslational modifications. In: *Mol. Endocrinol.* 20 (7), 1673–1687.

Rollins, B. J. (1997): Chemokines. In: *Blood* 90 (3), 909–928.

Rossi, D.; and Zlotnik, A. (2000): The Biology of Chemokines and their Receptors. In: *Annu Rev Immunol* (18), 217–242.

Rutkowski, J. M.; Davis, K. E.; Scherer, P. E. (2009): Mechanisms of obesity and related pathologies: the macro- and microcirculation of adipose tissue. In: *FEBS J.* 276 (20), 5738–5746.

Salani, B.; Briatore, L.; Andraghetti, G.; Adami, G. Franco; Maggi, D.; Cordera, R. (2006): High-molecular weight adiponectin isoforms increase after biliopancreatic diversion in obese subjects. In: *Obesity (Silver Spring)* 14 (9), 1511–1514.

Sallusto, F.; Lanzavecchia, A.; Mackay, C. R. (1998): Chemokines and chemokine receptors in T-cell priming and Th1/Th2-mediated responses. In: *Immunol. Today* 19 (12), 568–574.

Sartipy, P.; Loskutoff, D. J. (2003): Monocyte chemoattractant protein 1 in obesity and insulin resistance. In: *Proc. Natl. Acad. Sci. U.S.A.* 100 (12), 7265–7270.

Schäffler, A.; Schölmerich, J. (2010): Innate immunity and adipose tissue biology. In: *Trends Immunol.* 31 (6), 228–235.

Schecter, A.; Calderonc, T.; Bermana, A.; McManusc, C.; Fallon, J.; Rossikhina, M. et al. (2000): Human Vascular Smooth Muscle Cells Possess Functional CCR5. In: *Journal of Biological Chemistry* 275 (8), 5466–5471.

Schernthaner, G.-H.; Kopp, H.-P.; Kriwanek, S.; Krzyzanowska, K.; Satler, M.; Koppensteiner, R.; Schernthaner, G. (2006): Effect of Massive Weight Loss induced by Bariatric Surgery on Serum Levels of Interleukin-18 and Monocyte-Chemoattractant-Protein-1 in Morbid Obesity. In: *OBES SURG* 16 (6), 709–715.

Schulze, M. B.; Rimm, E. B.; Shai, I.; Rifai, N.; Hu, F. B. (2004): Relationship between adiponectin and glycemic control, blood lipids, and inflammatory markers in men with type 2 diabetes. In: *Diabetes Care* 27 (7), 1680–1687.

Sell, H.; Eckel, J. (2009): Chemotactic cytokines, obesity and type 2 diabetes: in vivo and in vitro evidence for a possible causal correlation? In: *Proc. Nutr. Soc* 68 (04), 378.

Senn, J. J.; Klover, P. J.; Nowak, I. A.; Mooney, R. A. (2002): Interleukin-6 induces cellular insulin resistance in hepatocytes. In: *Diabetes* 51 (12), 3391–3399.

Serra-Majem, L.; Roman, B.; Estruch, R. (2006): Scientific evidence of interventions using the Mediterranean diet: a systematic review. In: *Nutr. Rev.* 64 (2 Pt 2), S27-47.

Shi, H.; Kokoeva, M. V.; Inouye, K.; Tzameli, I.; Yin, H.; Flier, J. S. (2006): TLR4 links innate immunity and fatty acid-induced insulin resistance. In: *J. Clin. Invest.* 116 (11), 3015–3025.

Shoelson, S. E.; Herrero, L.; Naaz, A. (2007): Obesity, inflammation, and insulin resistance. In: *Gastroenterology* 132 (6), 2169–2180.

Sikorski, C.; Luppa, M.; Schomerus, G.; Werner, P.; König, H.-H.; Riedel-Heller, S. G.; Gorlova, O. Y. (2012): Public Attitudes towards Prevention of Obesity. In: *PLoS ONE* 7 (6), e39325.

Simeoni, E.; Hoffmann, M. M.; Winkelmann, B. R.; Ruiz, J.; Fleury, S.; Boehm, B. O. et al. (2004): Association between the A-2518G polymorphism in the monocyte chemoattractant protein-1 gene and insulin resistance and Type 2 diabetes mellitus. In: *Diabetologia* 47 (9), 1574–1580.

Skurk, T.; Alberti-Huber, C.; Herder, C.; Hauner, H. (2007): Relationship between adipocyte size and adipokine expression and secretion. In: *J. Clin. Endocrinol. Metab.* 92 (3), 1023–1033.

Skurk, T.; Mack, I.; Kempf, K.; Kolb, H.; Hauner, H.; Herder, C. (2009): Expression and secretion of RANTES (CCL5) in human adipocytes in response to immunological stimuli and hypoxia. In: *Horm. Metab. Res.* 41 (3), 183–189.

Sofi, F.; Abbate, R.; Gensini, G.; Casini, A. (2010): Accruing evidence on benefits of adherence to the Mediterranean diet on health: an updated systematic review and meta-analysis. In: *Am J Clin Nutr* 92, 1189–1196.

Song, M.; Kim, K.; Yoon, J.; Kim, J. (2006): Activation of Toll-like receptor 4 is associated with insulin resistance in adipocytes. In: *Biochem Biophys Res Commun* 346 (3), 739–745.

Steffes, M. W.; Gross, M. D.; Schreiner, P. J.; Yu, X.; Hilner, J. E.; Gingerich, R.; Jacobs, D. R. (2004): Serum adiponectin in young adults--interactions with central adiposity, circulating levels of glucose, and insulin resistance: the CARDIA study. In: *Ann Epidemiol* 14 (7), 492–498.

Strissel, K. J.; Defuria, J.; Shaul, M. E.; Bennett, G.; Greenberg, A. S.; Obin, M. S. (2010): T-cell recruitment and Th1 polarization in adipose tissue during diet-induced obesity in C57BL/6 mice. In: *Obesity (Silver Spring)* 18 (10), 1918–1925.

Strissel, K. J.; Stancheva, Z.; Miyoshi, H.; Perfield, J. W.; Defuria, J.; Jick, Z. et al. (2007): Adipocyte death, adipose tissue remodeling, and obesity complications. In: *Diabetes* 56 (12), 2910–2918.

Suganami, T.; Tanimoto-Koyama, K.; Nishida, J.; Itoh, M.; Yuan, X.; Mizuarai, S. et al. (2007): Role of the Toll-like receptor 4/NF-kappaB pathway in saturated fatty acid-induced inflammatory changes in the interaction between adipocytes and macrophages. In: *Arterioscler. Thromb. Vasc. Biol* 27 (1), 84–91.

Sun, K.; Kusminski, C.; Scherer, P. (2011): Adipose tissue remodeling and obesity. In: *J Clin Invest* 121 (6), 2094–2101.

Swarbrick, M. M.; Austrheim-Smith, I. T.; Stanhope, K. L.; van Loan, M. D.; Ali, M. R.; Wolfe, B. M.; Havel, P. J. (2006): Circulating concentrations of high-molecular-weight adiponectin are increased following Roux-en-Y gastric bypass surgery. In: *Diabetologia* 49 (11), 2552–2558.

Tang, W.; Lu, Y.; Tian, Q.-Y.; Zhang, Y.; Guo, F.-J.; Liu, G.-Y. et al. (2011): The growth factor progranulin binds to TNF receptors and is therapeutic against inflammatory arthritis in mice. In: *Science* 332 (6028), 478–484.

Tateya, S.; Tamori, Y.; Kawaguchi, T.; Kanda, H.; Kasuga, M. (2010): An increase in the circulating concentration of monocyte chemoattractant protein-1 elicits systemic insulin resistance irrespective of adipose tissue inflammation in mice. In: *Endocrinology* 151 (3), 971–979.

Thalmann, S.; Meier, C. A. (2007): Local adipose tissue depots as cardiovascular risk factors. In: *Cardiovasc. Res* 75 (4), 690–701.

Tian, L.; Luo, N.; Zhu, X.; Chung, B.-H.; Garvey, W. Timothy; Fu, Y. (2012): Adiponectin-AdipoR1/2-APPL1 signaling axis suppresses human foam cell formation: Differential ability of AdipoR1 and AdipoR2 to regulate inflammatory cytokine responses. In: *Atherosclerosis* 221 (1), 66–75.

Tilg, H.; Moschen, A. R. (2006): Adipocytokines: mediators linking adipose tissue, inflammation and immunity. In: *Nat. Rev. Immunol.* 6 (10), 772–783.

Tomizawa, A.; Hattori, Y.; Kasai, K.; Nakano, Y. (2008): Adiponectin induces NF-kappaB activation that leads to suppression of cytokine-induced NF-kappaB activation in vascular endothelial cells: globular adiponectin vs. high molecular weight adiponectin. In: *Diabetes & vascular disease research : official journal of the International Society of Diabetes and Vascular Disease* 5 (2), 123–127.

Tönjes, A.; Fasshauer, M.; Kratzsch, J.; Stumvoll, M.; Blüher, M. (2010): Adipokine pattern in subjects with impaired fasting glucose and impaired glucose tolerance in comparison to normal glucose tolerance and diabetes. In: *PLoS ONE* 5 (11), e13911.

Torigoe, M.; Matsui, H.; Ogawa, Y.; Murakami, H.; Murakami, R.; Cheng, X. Wu et al. (2007): Impact of the high-molecular-weight form of adiponectin on endothelial function in healthy young men. In: *Clin. Endocrinol. (Oxf)* 67 (2), 276–281.

Trayhurn, P.; Wood, I. S (2004): Adipokines: inflammation and the pleiotropic role of white adipose tissue. In: *Br. J. Nutr* 92 (3), 347–355.

Tuomilehto, J.; Lindström, J.; Eriksson, J. G.; Valle, T. T.; Hämäläinen, H.; Ilanne-Parikka, P. et al. (2001): Prevention of type 2 diabetes mellitus by changes in lifestyle among subjects with impaired glucose tolerance. In: *N. Engl. J. Med.* 344 (18), 1343–1350.

van Deerlin, V. M.; Sleiman, P. M. A.; Martinez-Lage, M.; Chen-Plotkin, A.; Wang, L.-S.; Graff-Radford, N. R. et al. (2010): Common variants at 7p21 are associated with frontotemporal lobar degeneration with TDP-43 inclusions. In: *Nat. Genet.* 42 (3), 234–239.

Varady, K. A.; Tussing, L.; Bhutani, S.; Braunschweig, C. L. (2009): Degree of weight loss required to improve adipokine concentrations and decrease fat cell size in severely obese women. In: *Metab. Clin. Exp.* 58 (8), 1096–1101.

Veillard, N. R. (2004): Antagonism of RANTES Receptors Reduces Atherosclerotic Plaque Formation in Mice. In: *Circulation Research* 94 (2), 253–261.

Vidal, J. (2002): Benefits of weight loss. In: *Int J Obes (Lond)* (26, Suppl 4,), S25-S28.

Vijay-Kumar, M.; Aitken, J. D.; Carvalho, F. A.; Cullender, T. C.; Mwangi, S.; Srinivasan, S. et al. (2010): Metabolic Syndrome and Altered Gut Microbiota in Mice Lacking Toll-Like Receptor 5 (5975), zuletzt geprüft am 11.04.2012.

Vitseva, O. I.; Tanriverdi, K.; Tchkonia, T. T.; Kirkland, J. L.; McDonnell, M. E.; Apovian, C. M. et al. (2008): Inducible Toll-like receptor and NF-kappaB regulatory pathway expression in human adipose tissue. In: *Obesity (Silver Spring)* 16 (5), 932–937.

Wadden, T. A.; Phelan, S. (2002): Assessment of quality of life in obese individuals. In: *Obes. Res.* 10 Suppl 1, 50S-57S.

Wadden, T. A.; Neiberg, R. H.; Wing, R. R.; Clark, J. M.; Delahanty, L. M.; Hill, J. O. et al. (2011): Four-year weight losses in the Look AHEAD study: factors associated with long-term success. In: *Obesity (Silver Spring)* 19 (10), 1987–1998.

Waki, H.; Tontonoz, P. (2007): Endocrine Functions of Adipose Tissue. In: *Annu. Rev. Pathol. Mech. Dis* 2 (1), 31–56.

Waki, H.; Yamauchi, T.; Kamon, J.; Ito, Y.; Uchida, S.; Kita, S. et al. (2003): Impaired Multimerization of Human Adiponectin Mutants Associated with Diabetes: Molecular Structure And Multimer Formation Of Adiponectin. In: *Journal of Biological Chemistry* 278 (41), 40352–40363.

Waki, H.; Yamauchi, T.; Kamon, J.; Kita, S.; Ito, Y.; Hada, Y. et al. (2004): Generation of Globular Fragment of Adiponectin by Leukocyte Elastase Secreted by Monocytic Cell Line THP-1. In: *Endocrinology* 146 (2), 790–796.

Waller, K.; Kaprio, J.; Kujala, U. (2008): Associations between long-term physical activity, waist circumference and weight gain: a 30-year longitudinal twin study. In: *Int J Obes (Lond)* (32), 353–361.

Wang, Y. (2006): Post-translational Modifications of the Four Conserved Lysine Residues within the Collagenous Domain of Adiponectin Are Required for the Formation of Its High Molecular Weight Oligomeric Complex. In: *Journal of Biological Chemistry* 281 (24), 16391–16400.

Wang, Y. Claire; McPherson, K.; Marsh, T.; Gortmaker, S. L.; Brown, M. (2011): Health and economic burden of the projected obesity trends in the USA and the UK. In: *Lancet* 378 (9793), 815–825.

Wang, Y.; Lam, K. S. L.; Xu, J. Yu; Lu, G.; Xu, L. Yi; Cooper, G. J. S.; Xu, A. (2005): Adiponectin inhibits cell proliferation by interacting with several growth factors in an oligomerization-dependent manner. In: *J. Biol. Chem.* 280 (18), 18341–18347.

Wang, Y.; Lam, K. S. L.; Yau, M.-h.; Xu, A. (2008): Post-translational modifications of adiponectin: mechanisms and functional implications. In: *Biochem. J.* 409 (3), 623–633.

Weigert, J.; Neumeier, M.; Wanninger, J.; Wurm, S.; Kopp, A.; Schober, F. et al. (2008): Reduced response to adiponectin and lower abundance of adiponectin receptor proteins in type 2 diabetic monocytes. In: *FEBS Lett.* 582 (12), 1777–1782.

Weisberg, S. P.; Hunter, D.; Huber, R.; Lemieux, J.; Slaymaker, S. (2006): CCR2 modulates inflammatory and metabolic effects of high-fat feeding. In: *J Clin Invest* 116 (1), 115–124.

Weisberg, S. P.; McCann, D.; Desai, M.; Rosenbaum, M.; Leibel, R. L.; Ferrante, A. W., JR (2003): Obesity is associated with macrophage infiltration in adipose tissue. In: *J Clin Invest* 112 (12), 1796–1808.

Weston, C. R.; Davis, R. J. (2007): The JNK signal transduction pathway. In: *Current Opinion in Cell Biology* 19 (2), 142–149.

Weyer, C.; Funahashi, T.; Tanaka, S.; Hotta, K.; Matsuzawa, Y.; Pratley, R. E.; Tataranni, P. A. (2001): Hypoadiponectinemia in Obesity and Type 2 Diabetes: Close Association with Insulin Resistance and Hyperinsulinemia. In: *J Clin Endokrinol Metab (The Journal of Clinical Endocrinology & Metabolism)* 86, 1930–1935.

Wing, R. R.; Venditti, E.; Jakicic, J. M.; Polley, B. A.; Lang, W. (1998): Lifestyle intervention in overweight individuals with a family history of diabetes. In: *Diabetes Care* 21 (3), 350–359.

Wong, E.; Freiberg, M.; Tracy, R.; Kuller, L. (2008): Epidemiology of Cytokines: The Women On the Move through Activity and Nutrition (WOMAN) Study. In: *American Journal of Epidemiology* 168 (4), 443–453.

Woods, S. C.; D'Alessio, D. A. (2008): Central control of body weight and appetite. In: *J. Clin. Endocrinol. Metab* 93 (11 Suppl 1), S37-50.

Woods, S. C.; Seeley, R.; Porte, D.; Schwarz, M. (1998): Signals That Regulate Food Intake and Energy Homeostasis. In: *Science* 280 (5368), 1378–1383.

World Health Organisation (Hg.) (2012): Obesity and overweight (Fact sheet N°311). Online verfügbar unter http://www.who.int/mediacentre/factsheets/fs311/en/index.html.

Wu, H.; Ghosh, S.; Perrard, X. Dai; Feng, L.; Garcia, G. E.; Perrard, J. L. et al. (2007): T-cell accumulation and regulated on activation, normal T cell expressed and secreted upregulation in adipose tissue in obesity. In: *Circulation* 115 (8), 1029–1038.

Yamauchi, T.; Kamon, J.; Ito, Y.; Tsuchida, A.; Yokomizo, T.; Kita, S. et al. (2003): Cloning of adiponectin receptors that mediate antidiabetic metabolic effects. In: *Nature* 423 (6941), 762–769.

Yamauchi, T.; Kamon, J.; Minokoshi, Y.; Ito, Y.; Waki, H.; Uchida, S. et al. (2002): Adiponectin stimulates glucose utilization and fatty-acid oxidation by activating AMP-activated protein kinase. In: *Nat. Med.* 8 (11), 1288–1295.

Youn, B.-S.; Bang, S.-I.; Klöting, N.; Park, J. Woo; Lee, N.; Oh, J.-E. et al. (2009): Serum progranulin concentrations may be associated with macrophage infiltration into omental adipose tissue. In: *Diabetes* 58 (3), 627–636.

Zernecke, A.; Shagdarsuren, E.; Weber, C. (2008): Chemokines in Atherosclerosis: An Update. In: *Arteriosclerosis, Thrombosis, and Vascular Biology* 28 (11), 1897–1908.

Zeyda, M.; Gollinger, K.; Kriehuber, E.; Kiefer, F. W.; Neuhofer, A.; Stulnig, T. M. (2010): Newly identified adipose tissue macrophage populations in obesity with distinct chemokine and chemokine receptor expression. In: *Int J Obes (Lond)* 34 (12), 1684–1694.

Zhu, J.; Nathan, C.; Jin, W.; Sim, D.; Ashcroft, G.; Wahl, S. et al. (2002): Conversion of Proepithelin to Epithelins: Roles of SLPI and Elastase in Host Defense and Wound Repair. In: *Cell biochemistry and biophysics* 111, 867–878.

Zietz, B.; Herfarth, H.; Paul, G.; Ehling, A.; Müller-Ladner, U.; Schölmerich, J.; Schäffler, A. (2003): Adiponectin represents an independent cardiovascular risk factor predicting serum HDL-cholesterol levels in type 2 diabetes. In: *FEBS Lett.* 545 (2-3), 103–104.

Zoico, E.; Garbin, U.; Olioso, D.; Mazzali, G.; Fratta Pasini, A. Maria; Di Francesco, V. et al. (2009): The effects of adiponectin on interleukin-6 and MCP-1 secretion in lipopolysaccharide-treated 3T3-L1 adipocytes: role of the NF-kappaB pathway. In: *Int. J. Mol. Med.* 24 (6), 847–851.