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Impact of short-term overfeeding and caloric restriction on gut permeability, gut microbiota composition, and metabolic function in humans

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Abbrevations

ACS	Acetyl-CoA-Synthetase
ALT	Alanine transaminase
APC	Allophycocyanin
AST	Aspartate aminotransferase
BMI	Body mass index
CD	Cluster of differentiation
cDNA	complementary deoxyribonucleic acid
CFU	Colony-forming units
CI	Confidence intervall
CV	Coefficient of variation
DES	Desferroxamine
DETC	Diethyldithocarbamate
DNA	Deoxyribonucleic acid
EDTA	Ethylenediamine tetraacetic acid
ELISA	Enzyme-linked immunosorbent assay
EN	Energy percent
EPR	Electron paramagnetic resonance spectroscopy
FITC	Fluorescein
FSC	Forward scatter
γGT	γ-Glytamyltransferase
HCL	Hydrochloride acid
HDL	High-density lipoprotein

HEC	Hyperinsulinemic euglycemic clamp
HFD	High-fat diet
HLA-DR	Human leucocyte antigen- antigen D related
HMW adiponectin	High-molecular weight adiponectin
HOMA-IR	Homeostasis model assessment-insulin resistance
hsCRP	High-sensitivity C-reactive protein
IgA	Immunglobulin-A
IL-10	Interleukin-10
Interferon-y	IFN-y
IPO8	Importin-8
JAM	Junctional adhesion molecule
JNK	c-Jun N-terminal kinase
iNKT	invariant natural killer t cells
Kcal	Kilocalorie
kDa	Kilo Dalton
Kg	Kilogram
KHB	Krebs-HEPES buffer
LBP	Lipopolysaccharide-binding protein
LDL	Low-density lipoprotein
LiHe	Lithium heparin
LPS	Lipopolysaccharide
M cells	Microfold cells
MCP-1	Monocyte chemoattractant protein-1

MEHA	3-Methyl-N-Ethyl-N-(ß-Hydroxyethyl)-Anilin
MET	Metabolic equivalent
MFI	Mean fluorescence intensity
MLCK	Myosin-light-chain-kinase
Mr	Molecular masses
MRI	Magnetic resonance imaging
MetS	Metabolic syndrome
NEFA	Non-esterified fatty acids
NF-κB	Nuclear factor 'kappa-light-chain-enhancer' of activated B cells
NK cells	Natural killer cells
NKT cells	Natural killer T cells
NMDS	Nonparametric multiple dimensional scaling
NSAID	Nonsteroidal anti-inflammatory drugs
OD	Optical density
OGTT	Oral glucose tolerance test
OTU	Operational taxonomic units
PBMC	Peripheral blood mononuclear cells
PD	Peroxidase
PE	Phycoerythrin
PEG	Polyethylene glycol
PPIA	Peptidylprolyl isomerase A
qPCR	Quantitative polymerase chain reaction

RANTES	Regulated on Activation Normal T cell Expressed and Secreted
RMR	Resting metabolic rate
ROS	Reactive oxygen species
RNA	Ribonucleic acid
rRNA	Ribosomal ribonucleic acid
RYGB	Roux-en-Y gastric bypass
S6k	S6 kinase
SAT	Subcutaneous adipose tissue
SCFA	Short-chain fatty acids
SSC	Side scatter
$\mathbf{T}_{reg}$ cells	Regulatory T cells
TG	Triglyceride
Th cells	T- helper cells
TLR	Toll-like receptor
Tm	Temperature
TMB	3,3',5,5'-tetramethylbenzidine
TNF-α	Tumor necrosis factor α
TSH	Thyroid-stimulating hormone
VAT	Visceral adipose tissue
VLCD	Very low caloric diet
WHO	World Health Organization
WHR	Waist- to- Hip ratio
ZO	zonula occludens

Summary

A Western diet is characterised by high-energy density and excessive consumption of fat. This energy overload is complemented by the lack of physical activity thereby causing obesity, associated with low-grade inflammation. This chronic subclinical inflammatory state is considered to pave the way for insulin resistance. The reasons for this low-grade inflammation are yet poorly understood, but accumulating evidence suggests that obesity is associated with an impaired intestinal barrier function. Furthermore, obesity is closely linked to changes in microbiota profiles in the sense of an up- or downregulation of specific bacterial taxa. It is supposed that a high-fat diet (HFD) can cause increased gut permeability, but the underlying mechanisms and the contribution of an altered intestinal microbiota remains unclear. As a consequence of the disrupted gut barrier, an increased influx of bacteria-derived antigens and metabolites such as lipopolysaccharides into the systemic circulation leads to an activation of the immune system. In contrast, weight reduction improves metabolic disturbances and thereby decreases the inflammatory load. Therefore, the purpose of this study was to examine the effect of caloric restriction and subsequent weight reduction on gut permeability in obese women. The impact on inflammatory markers and fecal microbiota was also investigated. Furthermore, the aim of the present thesis was to assess whether acute overfeeding for seven days is sufficient to provoke significant changes in insulin resistance, gut barrier dysfunction, and changes in the microbiota profiles.

To investigate the effects of lifestyle modification on the disease-related parameters mentioned before, two studies were developed. In the first study 20 obese women were assigned a standardised very low caloric diet (VLCD, 800 kcal/day) for 28 days with an additional allowance of 200g/day of vegetables. The second study with 24 lean healthy young men imitated a high-fat diet lifestyle providing 1,000 kcal above their individual energy expenditure for seven days. In both groups, the gut permeability (by a sugar and polyethylene glycol absorption test, plasma zonulin measurement), gut microbiota profiles (high-throughput 16S ribosomal RNA gene sequencing), glucose metabolism (by oral glucose tolerance test, hyperinsulinemic euglycemic clamp) and markers of low-grade inflammation (such as high-sensitivity C-reactive protein) were assessed before and after intervention, as well as two weeks after intervention. Additionally, in the caloric restriction study, the examination was completed by magnetic resonance imaging (MRI) and abdominal needle biopsy before and after 28 days of formula diet.

28 days of caloric restriction induced a mean weight loss of 6.9 ± 1.9 kg accompanied by reductions in HOMA-index, fasting plasma glucose and insulin levels, plasma leptin levels, and leptin gene expression in subcutaneous adipose tissue (SAT). In contrast, plasma high-molecular weight adiponectin (HMW adiponectin) was significantly increased after VLCD. Plasma levels of high-sensitivity C-reactive protein (hsCRP) and lipopolysaccharide-binding protein (LBP) were significantly decreased after 28 days of VLCD. Using three different methods, paracellular gut permeability was decreased after VLCD. These changes in clinical parameters were not associated with major changes in dominant bacterial communities in feces.

Seven days of highly standardised overnutrition resulted in weight gain in healthy young men. But, although participants gained 0.9 ± 0.6 kg body weight, overnutrition was not associated with a significant change in insulin sensitivity (M-value and glucose disposal). In addition, there was no significant change in inflammatory markers. Urine excretion of four sugars and polyethylene glycols arguing that gut permeability seems unchanged. Likewise, plasma levels of zonulin as a marker of paracellular gut permeability did not change significantly after seven days of overfeeding, either. Moreover, overfeeding was not associated with consistent changes in gut microbiota profiles, but in a subgroup of 6 individuals marked alterations were observed.

It can be concluded that caloric restriction resulted in improved gut barrier integrity and an overall reduced systemic inflammatory status in obese women, independent of the microbiota profile. Whereas, short-term overfeeding with a high-fat diet does not significantly impair insulin sensitivity and gut permeability in normal-weight healthy men, and that changes in dominant communities of fecal bacteria are individual specific.

Zusammenfassung

Eine westliche Ernährungsweise zeichnet sich durch eine hohe Energiedichte und einem hohen Konsum von Fett aus. Die hohe Energiezufuhr und der ausgeprägter Bewegungsmangel verursachen die Entstehung von Adipositas, was mit einer chronischen Entzündung in Verbindung gebracht wird. Dieser Entzündungszustand trägt zur Insulinresistenz bei. Die Gründe für diesen Entzündungsprozess sind noch nicht gut verstanden, jedoch gibt es Hinweise, dass Adipositas mit Veränderungen der Darmpermeabilität einhergeht. Neue Studien weisen darauf hin, dass Adipositas eng mit der Hoch- und Herunterregulation bestimmter Bakterienstämme assoziiert ist. Daraus wurde abgeleitet, dass eine Änderung der Darmpermeabilität einen Beitrag zur systemischen Entzündung leisten könnte. Dementsprechend führt eine gestörte Darmbarriere zu einer gesteigerten Aufnahme von bakteriellen Komponenten, wie Lipopolysacchariden und anderen komplexen Antigenen in den Körperkreislauf und aktiviert dadurch das Immunsystem. Es wird angenommen, dass eine erhöhte Durchlässigkeit des Darms durch eine Hochfettdiät verursacht werden könnte. Im Gegenzug dazu verbessert eine Gewichtsreduktion Adipositas-assoziierte Stoffwechselstörungen und verringert dadurch die inflammatorische Last. Das Ziel dieser Arbeit war es zu untersuchen, ob eine kalorische Restriktion eine Verbesserung der Insulinresistenz, der Darmpermeabilität und der Zusammensetzung der Bakterien hervorruft. Darüber hinaus war es das Ziel der vorliegenden Arbeit zu prüfen, ob eine kurzfristige Überernährung über 7 Tage ausreicht, um eine signifikante Änderung in der Insulinresistenz, der Darmbarrierefunktion und der Zusammensetzung des Mikrobioms zu provozieren.

Um die Auswirkungen von Veränderungen der Lebensgewohnheiten zu untersuchen, wurden zwei Studien entwickelt. In der einen Studie stellen 20 adipöse Frauen ihre Ernährung auf eine vierwöchige Formuladiät mit einer täglichen Kalorienzufuhr von 800 kcal für 28 Tage um. In der zweiten Studie erhalten 24 stoffwechselgesunde, normalgewichtige, junge Männer über sieben Tage eine überkalorische Ernährung mit einem Energieüberschuss von 1000 kcal/Tag über Ihrem Energieverbrauch. In beiden Studien wird die Darmpermeabilität (Vier-Zucker-Test, Polyethylenglykol-Test und Zonulin im Plasma), die Zusammensetzung der Mikrobiota (High-Throughput-16S ribosomale RNA-Gen-Sequenzierung), der Glukosestoffwechsel (oraler Glukosetoleranztest, hyperinsulinämische euglykämiche Clamp) und Entzündungsmarker (wie hochsensitives C-reaktives Protein) vor der Intervention und nach der Intervention gemessen. In der kalorischen Restriktionsstudie wurde eine Fettgewebsbiopsie durch Nadelaspiration vor und 28 Tage nach der Formuladiät durchgeführt.

Nach 28 Tagen kalorischer Restriktion hatten die Probandinnen im Mittel einen Gewichtsverlust von 6,9 ± 1,9 kg. Die kalorische Restriktion induzierte eine Verbesserung des HOMA-IR (Homöostase Modell Bewertung-Insulinresistenz), der Nüchternplasmaglukose und des Nüchterninsulins, Plasma-Leptin und Leptin-Genexpression in subkutanem Fettgewebe. Das HMW- Adiponektin im Plasma war nach der kalorischen Restriktion signifikant erhöht. Die Konzentration von hsCRP und des Lipopolysaccharid-bindenden Proteins im Plasma verringerte

sich nach 28 tägiger kalorischer Restriktion signifikant. Unter Verwendung der drei verschiedenen Methoden hat sich die parazelluläre Darmpermeabilität nach 28 Tagen verringert. Eine signifikante Änderung im Mikrobiotaprofil im Stuhl konnte nicht festgestellt werden.

Eine siebentägige Hochfettdiät führte zu einer Gewichtszunahme bei den jungen gesunden Männern. Obwohl die Teilnehmer 0,9 ± 0,6 kg Körpergewicht zugenommen haben, hat sich die Insulinempfindlichkeit der hyperinsulinämischen euglykämischen Clamp-Technik nach der Hochfettdiät nicht signifikant verändert. Zudem konnte keine signifikante Änderung der Entzündungswerte festgestellt werden. Der Zuckerresorptionstest, der Polyethylenglykol-Test und die Plasma-Zonulin-Werte änderten sich nach der siebentägigen Hochfettdiät ebenfalls nicht signifikant. Schließlich konnte auch eine signifikante Veränderung des Mikrobiotaprofils nach der Hochfettdiät nur bei 6 Probanden festgestellt werden.

Aus den erhobenen Daten lässt sich schließen, dass eine kalorische Restriktion zu einer Verbesserung der Darmbarriere und zu einem reduzierteren Entzündungsstatus bei adipösen Frauen führt, unabhängig von Veränderungen des Mikrobiotaprofils. Dagegen scheint eine siebentägige Hochfettdiät nicht zu einer erhöhten Insulinresistenz und Darmpermeabilität beizutragen. Die Veränderung der Mikrobiota scheint bei den jungen Männern nach der Hochfettdiät individuell unterschiedlich zu sein.

1 Introduction and aim of the thesis

Obesity is characterised by excessive fat accumulation defined as body mass index (BMI) of over 30 kg/m². Obesity is a powerful contributor to cardiovascular diseases and type 2 diabetes. In 2014, the world health organization (WHO) published data, showing that 12.9 % of the world population suffers from obesity. 26.8 % of the American population and 23 % of the Europeans are obese. A lower prevalence of obesity with 5 % in both sexes was reported for South-East Asia. Comparing the prevalence of obesity in all WHO regions between genders, the frequency of obesity in women is higher than in men (WHO, 2014). Alarming data were recently published in February 2016 regarding the prevalence of overweight or obesity in children. According to this report 41 million of children younger than 5 years are overweight or obese (Nishtar et al., 2016), which is of special relevance considering that childhood obesity is associated with the development of obesity-related diseases (Lobstein and Jackson-Leach, 2006). Obesity leads to chronic low-grade inflammation and might be associated with metabolic disorders, such as type 2-diabetes (Hotamisligil, 2006), while gut microbiota composition and gut permeability are considered as intestinal factors contributing to low-grade inflammation (Cox et al., 2015).

Based on the fact that obesity is considered to be associated with impaired gut permeability, altered gut microbiota composition and a chronic low-grade inflammatory state, this PhD thesis aims to

- assess the impact of a caloric restriction on gut permeability, microbiota profiles, low-grade inflammation and glucose homeostasis in humans.

- examine the effect of a short-term high-fat overfeeding on gut permeability, microbiota profiles, low-grade inflammation and glucose metabolism in humans.

The overall aim of this study was to reveal new insight on how (1) acute overfeeding and (2) caloric restriction followed by moderate weight loss impact on gut barrier function, gut microbiota profiles and obesity-related inflammatory and metabolic parameters.

2 Background

2.1 Obesity- induced inflammation

Long-term hypercaloric diet combined with a lack of exercise leads to obesity and low-grade inflammation (Wellen and Hotamisligil, 2005) (Shoelson, 2006). Low-grade inflammation is defined as a state, when plasma levels of cytokines and acute phase proteins are two- to three-fold increased (Petersen and Pedersen, 2005). Chronic low-grade inflammation in obesity is linked with metabolic dysfunction leading to cardiovascular diseases and type 2-diabetes (Hotamisligil, 2006).

A factor that significantly impacts low-grade inflammation is the number and activity of peripheral mononuclear cells (PBMC). PBMCs are a heterogeneous cell population including numerous leukocyte subsets. Leucocytes can be grouped in myeloid cells and lymphocytes (Kondo et al., 2003):

Myeloid cells can be divided in macrophages, dendritic cells, mast cells, neutrophils, basophils, and eosinophils. Myeloid cells play an important role in innate and adaptive immunity (e.g. antigen presentation) (Lee and Lee, 2014). Considering obesity-related inflammation, macrophages are a key mediator, since they are able to secrete numerous cytokines (such as TNF-α) and cause recruitment of other immune cells (Arango Duque and Descoteaux, 2014).

Lymphocytes can be categorized in T cells, natural killer T cells (NKT), and B cells (Kondo et al., 2003): T cells develop in bone marrow and maturate in the thymus. The heterogeneous T cell population can be divided into T helper cells (CD4) and cytotoxic T cells (CD8). CD4 can be subdivided in T helper type 1 (Th), Th1, Th2, Th17 and regulatory T cells (T_{regs}). Th1 cells exert a proinflammatory function by secreting Interferon γ (IFN-γ), which mediates the release of Tumor necrosis factor α (TNF-α) from macrophages. Th2 cells promote the humoral immune response by activating B cells which is an important component of inflammation (Winer et al., 2009). Th17 cells are associated with dendritic cells in obese individuals leading to obesity-associated insulin resistance (Bertola et al., 2012) (Chen et al., 2014). In addition to Th2 cells, T_{regs} play a beneficial key role in the suppression of inflammation (Josefowicz et al., 2012). In detail, Feuerer et al. assessed that Interleukin 10 (IL-10), produced by T_{regs}, mediates the suppression of a proinflammatory cytokine response *in vitro* (Feuerer et al., 2009). The findings of Tiemessen et al. revealed new functions of $CD4^+$ $CD25^+$ $FoxP3^+$ T_{regs}. The research group concluded that $CD4^+$ $CD25^+$ $FoxP3^+$ T_{regs} activate monocytes/macrophages by a cytokine independent pathway (Tiemessen et al., 2007). In addition to Th1 cells, cytotoxic T cells (CD8) produce cytokines (e.g. IFN-γ) that play a proinflammatory role. Nishimura et al. assessed that activity of CD8 T cells is highly linked with adipose tissue inflammation and systemic insulin resistance (Nishimura et al., 2009). Another lymphocyte subset comprise natural killer T cells (NKT). It is a heterogeneous group of T cells. The best-known subgroup of NKT cells are invariant natural killer T cells (iNKT). iNKT cells are able to produce a variety of cytokines resulting in the

production and release of obesity-related inflammatory markers such as IFNα, IL-4, IL-2, IL-10, TNF-α (Matsuda et al., 2008). Moreover, iNKT cells play a protective role in the development of obesity by mediating cytokine production towards lower levels (Lynch et al., 2012). B cells, which are completely developing in the bone marrow, are the main producer of antibodies, and play an important role in antigen presentation and cytokine secretion (Lee and Lee, 2014) (Shaikh et al., 2015). In addition to the before mentioned functions, B cells contribute to metabolic dysfunction in obesity. Diet-induced obesity leads to an infiltration of B cells in visceral adipose tissue (VAT) resulting in obesity-related inflammation by activating $CD8^+$ and Th1 cells (Winer et al., 2011).

2.1.1 Inflammation mediated by adipose tissue

Investigating adipose tissue as a source of chronic inflammation contains many aspects for understanding the link between obesity and metabolic disorders. In 1993, it was demonstrated that the cytokine TNF-α is secreted from adipose tissue and triggers insulin resistance (Hotamisligil et al., 1993). Over the following years, it was generally accepted that adipose tissue is not only a energy storage organ. Abdominal SAT plays a major role in the gene expression of proinflammatory factors in obesity (Spoto et al., 2014). Adipose tissue impacts on metabolism by secreting pro- and anti-inflammatory cytokines. A dysregulation of adipokines contributes to the pathogenesis of metabolic disorders, such as type 2 diabetes (Hotamisligil, 2006). The following proinflammatory proteins were secreted and are upregulated in obesity: The expression of TNF-α is elevated in obese individuals (Kern et al., 2001). TNF-α promotes obesity-linked metabolic diseases by activating toll-like receptor-4 (TLR-4) resulting in the suppression of the insulin signaling cascade. In obese individuals, increased leptin levels in plasma (Maffei et al., 1995) and in subcutaneous adipose (Kouidhi et al., 2010) tissue were detected. Leptin mediates its effects via the Janus kinase, signal transducer and activator of transcription (JAK-STAT) pathway, extracellular signal-regulated kinase (ERK)-1/2, phosphatidylinositol-3-kinase (PI3K), and AMP-activated protein kinase (AMPK) (Paz-Filho et al., 2012). Monocyte chemoattractant protein-1 (MCP-1) level is elevated in obesity (Kim et al., 2006). MCP-1 induce macrophage recruitment and inflammation in adipose tissue (Weisberg et al., 2006) (Kanda et al., 2006). Regulated on activation normal T cell expressed and secreted (RANTES) takes part in the obesity-driven inflammatory process by mediating T-cell chemotaxis in adipose tissue (Wu et al., 2007). Obese individuals (Wu et al., 2007) and patients with type 2 diabetes (Herder et al., 2005) show higher levels of RANTES and chemerin is expressed in adipose tissue (Roh et al., 2007). Furthermore, chemerin levels are increased in morbidly obese individuals and are linked to insulin resistance (Sell et al., 2010) (Lee et al., 2013).

One example of a anti-inflammatory cytokine is adiponectin, which has an insulin-sensitizing effect. The beneficial effect is mediated by the activation of adenosine monophosphate dependent kinase (AMPK) and PPAR-α in the skeletal muscle and liver resulting in increased glucose uptake (Yamauchi et al., 2002). Obese people exhibit lower levels of adiponectin compared to their lean counterparts (Ouchi et al., 2011).

As already mentioned, proinflammatory cytokines such as TNF-α mediate the activation of the NF-κB pathway. When inactive, the nuclear factor is located in the cytoplasma and inactivates κB proteins (IKK). The inactive NF-κB/IKK complex can be induced upon stimulation by e.g. TNF-α or LPS (Oeckinghaus and Ghosh, 2009). Activation of IKKα and IKKβ results in phosphorylation of inhibitor of kappa B (IκBα) (Häcker and Karin, 2006). Thereafter, when IκBα is degraded and thereby unmasks NF-κB, it can be translocated to the nucleus and bind to its respective DNA-binding site (Perkins, 2007) (Kumar et al., 2004), where it triggers gene expression of proinflammatory mediators (Gregor and Hotamisligil, 2011). Alternatively, the NF-κB signaling pathway can be activated by toll-like receptor 4 (TLR4) (Beutler, 2009). TLR4 recognizes LPS and free fatty acids (Shi et al., 2006) (Song et al., 2006) thereby activating NF-κB. In addition to the kinase IKK pathway, the C-jun N-terminal kinase (JNK) pathway can be activated by TNF-α and free fatty acids and subsequently promotes low-grade inflammation (Hirosumi et al., 2002) (Solinas and Karin, 2010).

Adipocyte hypertrophy and the associated dysregulated adipokine secretion profile aggravate the proinflammatory situation. The secretion of proinflammatory adipokines was increased in enlarged adipocytes compared to small cells. (Skurk et al., 2007). Other studies confirmed these data. Couillard et al. assessed a link between adipose cell hypertrophy and elevated plasma leptin levels (Couillard et al., 2000). TNF-α levels in the subcutaneous and VAT were also associated with adipocyte volume (Winkler et al., 2003). Furthermore, also an association between type 2 diabetes and adipocyte hypertrophy could be demonstrated (Henninger et al., 2014). The link between fat cell size and adipokine secretion was investigated by Varaday et al.. This group assessed visceral and subcutaneous fat cell size before and after weight loss. They demonstrated that already a moderate weight loss of 5% led to a decline of fat cell size. Moreover, a decreased fat cell size was linked with increased adiponectin levels and decreased leptin (Varady et al., 2009).

In addition to fat cell size and the amount of abdominal adipose tissue, fat distribution in the body is a significant factor that determines the risk for metabolic diseases. To measure different depots various, anthropometric variables were established: A waist-to-hip ratio > 0.85 in women and > 1.0 in men are first indicators of this unfavourable metabolic fat distribution (Ohlson et al., 1985). Numerous studies have demonstrated that an android fat distribution, meaning the adipose tissue around the trunk and upper body, is highly correlated with metabolic disturbances (Kissebah et al., 1982) (Fox et al., 2007). Android fat distribution includes visceral and abdominal subcutaneous fat depots. The visceral fat is lipolytically more active (Hajer et al., 2008) and secretes a higher amount of proinflammatory cytokines than subcutaneous fat (Fain et al., 2004). Furthermore, SAT is linked to insulin resistance (Kelley et al., 2000). In contrast to android fat distribution, gluteo-femoral body fat (gynoid type) is associated with a reduced risk for diabetes and cardiovascular disease (Manolopoulos et al., 2010).

Adipose tissue consists of different cell types, such as mature adipocytes, preadipocytes, endothelial cells, and immune cells (Olefsky and Glass, 2010). Immune cells in adipose tissue

play an important role in obesity associated inflammation. In 2003, the first studies described an infiltration of macrophages into adipose tissue (Weisberg et al., 2003) (Xu et al., 2003) (Bornstein et al., 2000). Adipose tissue in obesity seems to have more "classically activated" M1-macrophages than anti-inflammatory "alternatively activated" M2-macrophages (Lee et al., 2006). M1-macrophages secrete proinflammatory cytokines, such as TNF-α and IL-6 and promote insulin resistance. In contrast, M2-macrophages that occur in adipose tissue are characterised by anti-inflammatory cytokines, such as IL-10 (Lee et al., 2006). In addition, Wu et al. have assessed an elevated accumulation of T cells and macrophages in adipose tissue in obesity. Moreover, this research group demonstrated an upregulation of the T cell specific chemokine RANTES in obese humans (Wu et al., 2007). Also the relationship between cell size and hypoxia was investigated (Skurk et al., 2009). In 2006, MCP-1 expression in adipose tissue was assessed to be involved in macrophage infiltration into adipose tissue in mice (Kanda et al., 2006) (Weisberg et al., 2006). In humans, it was shown that obese participants have elevated MCP-1 concentrations in serum in contrast to their lean counterparts (Kim et al., 2006). Experimental evidence clearly indicates that the inflammatory load can be modulated by weight loss. Weight reduction improves metabolic disturbances and ameliorates the parameters of obesity – relevant inflammation mentioned above (Cancello et al., 2005b) (Cottam et al., 2004) (Clément et al., 2004b). Also T-lymphocyte infiltration seems to be relevant in the development of insulin resistance during obesity. In a mouse model of obesity-mediated insulin resistance, five weeks of high-fat diet resulted in insulin resistance and elevated T- lymphocyte infiltration in VAT. After ten weeks of HFD, recruitment of macrophages was shown suggesting that T-lymphocytes mediate the inflammatory process in adipose tissue (Kintscher et al., 2008).

In conclusion, an increased amount of adipose tissue mass is a significant contributor to proinflammatory cytokines, such as leptin and TNF-α both compromising insulin sensitivity. Anti-inflammatory proteins such as adiponectin were discovered as potential insulin sensitizers and are considered as beneficial factors in glucose homeostasis (Weyer et al., 2001).

2.1.2 Obesity - relevant inflammation triggered by the gut

Nowadays, also the gastrointestinal tract is described as another potential source of inflammation relevant to obesity. Studies have shown that obesity is linked with up- or downregulation of specific bacteria of the intestinal microbiota (Turnbaugh et al., 2009). For example high-fat feeding is associated with lower numbers of *Bifidobacterium spp.* in the caecal content in mice. The reduction in *Bifidobacterium spp*. is linked to increased plasma lipopolysaccharides (LPS) levels (Cani et al., 2007). LPS are components of the outer cell membrane of Gram-negative bacteria and are key mediators of inflammatory processes induced by bacteria. There are different routes for LPS entering the circulation: At first, LPS can translocate paracellularly. An impaired gut barrier can thereby contribute to an increased translocation of LPS (Creely et al., 2007) (Cani et al., 2008). Additionally, LPS was shown to be transferred by chylomicrons (Ghoshal et al., 2009) (Laugerette et al., 2011). LPS appearance in the blood is termed "endotoxemia" and is able to activate proinflammatory cascades involving

the following procedure: in the blood, LPS is bound to its specific liposaccharide-binding protein (LBP). Afterwards, this LPS/LBP complex binds to glycophosphatidylinositol-linked receptor cluster of differentiation 14 (CD14). CD14 is found either on the plasma membrane of e.g. macrophages (mCD14) or in the plasma in its soluble form (sCD14). The complex of LBS-LBP-CD14 binds to TLR4 and initiates an inflammatory process via induction of NFκB. Finally, proinflammatory cytokines, such as IL-6 or TNF-α are expressed and mediate a low-grade inflammatory process. The role of intestinal microbiota in inflammation was shown by the group of Ding (Ding et al., 2010). Ding et al. studied HFD with conventionally grown specific-pathogen free and germ-free mice. HFD induced body weight gain and inflammation in conventionally raised specific-pathogen free, but not in germ-free mice. This data showed that microbiota seems to be a main factor for development of intestinal inflammation in diet-induced obesity. Regarding HFD and LPS concentration, numerous studies have reported that fat intake causes an increased circulating LPS concentration: Erridge et al. showed that LPS levels were increased postprandially after a high-fat bolus (Erridge et al., 2007). Ghanim et al. demonstrated in humans, that a high-fat diet leads to postprandial inflammation by increased TLR4 abundance in mononuclear cells (Ghanim et al., 2009). Laugerette et al. also showed increased LPS concentrations induced by 8 week overfeeding (+ 760 kcal) intervention. One month of a Western-style diet leads to a 71 % increase in plasma levels of endotoxins (Pendyala et al., 2012). Furthermore, the group of Amar has shown that energy intake is positively linked to LPS concentration (Amar et al., 2008).

2.2 Obesity and the gut microbiota profile

The next sections provide background information about the gut microbiota and highlight its importance in obesity-related inflammatory processes.

2.2.1 The gastrointestinal tract and the composition of the human gut microbiome

In general, colonization density changes in the different sections of the gastrointestinal tract. The stomach and duodenum harbors about 10^3 colony-forming units (cfu) per gram content due to the acidic milieu in the stomach as well as bile and pancreatic secretion. In the following parts of the gastrointestinal tract, bacteria counts successively increase along the small intestine (approximately 10^4 cfu per gram in the jejunum and 10^7 cfu per gram in the ileum). Finally, the large intestine is highly populated by anaerobes and the amount of bacteria increases to 10^{12} cfu per gram of fecal content. In contrast to the microbial density, the transit time is fast in the upper gut and slows down in the colon, which enables the microorganisms to ferment available substances presented with the diet (Angelakis et al., 2012) (Sommer and Backhed, 2013).

Looking at gut microbiota composition in detail, microbiota composition depends on age, geographical and cultural factors, and diet: several studies demonstrated that first microbial exposure occurs already during pregnancy. Those studies have measured prenatal microbial exposure and bacterial deoxyribonucleic acid (DNA) was detected in umbilical cord blood (Jiménez et al., 2005) and placenta (Satokari et al., 2009). Vaginally born infants are exposed to bacteria of the maternal fecal and vaginal microbes, such as *Bifidobacterium, Bacteroidetes, Prevotella* and *Lactobacillus spp*. In contrast, infants born by Caesarean section are initially colonized by skin bacteria and bacteria from the hospitals's environment (Dominguez-Bello et al., 2010) (Penders et al., 2006). During the first days of life, the intestine is colonized by *Escherichia coli* and *Enterococcus sp*. (Bourlioux et al., 2003) (Jiménez et al., 2008). After some days the intestine harbors strictly anaerobic species, such as *Bifidobacterium, Clostridium*, and *Bacteroides* (Matamoros et al., 2013). Besides other factors, the diet has a considerable impact on the individual assembly of the intestinal microbiota. Children that are breastfed have an higher amount of fecal *Bifidobacterium spp.* than formula-fed infants (Rinne et al., 2005) (Fallani et al., 2011). This is because oligosaccharides stimulate the enrichment of "beneficial" bacteria such as *Bifidobacteria* (Marcobal et al., 2010). During the first three years of life children show a lower diversity and a higher inter-individual variability of the gut microbiota compared to adults (Yatsunenko et al., 2012) (Palmer et al., 2007). However, due to efficient colonization of the infants' gut the microbiota reaches a high degree of similarity to the average adult microbiome already after 12 months of life (Palmer et al., 2007). In principal, the human gut microbiota is composed of two phyla: Gram-positive *Firmicutes* (64%) and Gram-negative *Bacteroidetes* (23%), followed by the less abundant *Proteobacteria* (8%), and *Actinobacteria* (3%) (Eckburg et al., 2005). In elderly, an increased number of *Bacteroidetes* and a decline of *Bifidobacteria* was seen (Zwielehner et al., 2009). In addition to age, the environmental differences due to geographic characteristics affect microbiota composition. The abundance of *Bifidobacteria* is higher in Italians than in French, Germans or Swedes (Mueller et al., 2006). Moreover, African children harbor more *Actinobacteria* and *Bacteroidetes* and less *Firmicutes* and *Proteobacteria* than European children (Filippo et al., 2010). In 2011, Arumugam et al. proposed the existence of three enterotypes classifying the gut microbiomes from healthy Western Europeans and Japanese: *Bacteroides* (phylum *Bacteroidetes*), *Prevotella* (phyulum *Bacteroidetes*), *Ruminococcus* (phylum *Firmicutes*) (Arumugam et al., 2011). These enterotypes seem to be also linked to long-term diet, such as the enterotype *Bacteroidetes* was linked to meat consumption in a Western diet (Wu et al., 2011). Short-term dietary intervention studies indicate that rather short exposure times of a few days may be sufficient to induce changes of the microbiome (Wu et al., 2011) (David et al., 2014).

2.2.2 Function of the gut

The microbiota plays an important role in several metabolic aspects. One important function is the fermentation of complex polysaccharides, which strictly depends on the presence of microorganisms and represents advantages for both human and bacteria. While humans are not able to digest all kinds of fibers and other indigestible sugars, they receive short chain fatty acids

(SCFA) from the fermentation of these indigestible food components. Bacteria in the colon ferment complex carbohydrates to SCFA like butyrate, acetate, and propionate providing beneficial effects on gut and systemic health (Cummings and Macfarlane, 1991). Acetate and propionate enter the circulation and are used by the host for gluconeogenesis and lipogenesis (Wolever et al., 1991) (Schwiertz et al., 2010). Additionally, butyrate provides energy for the cells of the colonic mucosa and serve as growth substrate to intestinal bacteria (Macfarlane and Macfarlane, 2003). Germ-free mouse studies have shown that the lack of the intestinal microbiota leads to a decreased energy harvest (Bäckhed et al., 2004). Additionally, SCFA seem to mediate inflammation (Tolhurst et al., 2012) and to affect energy balance (Jandhyala et al., 2015) by binding to the G-protein-coupled receptors G-protein coupled receptor 41 of immune cells (Samuel et al., 2008). In addition to fermentation, gut bacteria deconjugate primary bile acids, which in turn are further metabolized to secondary bile acids. Secondary bile acids improve energy expenditure in muscle and increase the secretion of glucagon-like peptide 1 from enteroendocrine L-cells resulting in improved insulin secretion (Thomas et al., 2009). Furthermore, bacteria also synthesize vitamins, e.g. vitamin K (Ramakrishna, 2013). Noteworthy is the role of the gut microbiota in the development of the immune system. Bacteria take part in the development of gut-associated lymphoid tissues, differentiation of B and T lymphocytes, immunoglobulins and follicle structure (Kabat et al., 2014).

2.2.3 Modification of the microbiota profile

In section 2.2.1, it was described that the gut microbiota composition is influenced by age. Additional factors, e.g. pre- and probiotics, antibiotic medication, and diseases can also modulate the assembly of the gut microbiota composition. In 2009, Turnbaugh et al. showed that the composition of the intestinal microbiota is relatively similar within a family in contrast to non-relatives (Turnbaugh et al., 2008). Furthermore, the comparison of humans from different countries and ethnicities revealed different level of bacterial diversity (Mueller et al., 2006) (Fallani et al., 2011) (Filippo et al., 2010). Some evidence exists that BMI is a major covariate of microbiome variation as analysed by amplicon sequencing and that obesity is associated with changes in intestinal microbiota composition (Falony et al., 2016) (Zhang et al., 2009) (Schwiertz et al., 2010). Probiotics and prebiotics as well as the use of antibiotics resulted in a significant decline of bacterial diversity (Bartosch et al., 2004). Finally, further studies indicated that the gut microbiota is altered in distinct diseases. Regarding type 2 diabetes, it was shown that the abundance of *Firmicutes* and *Clostridia* was reduced compared to a non-diabetic control group (Larsen et al., 2010a).

It is still a matter of debate, whether the gut microbiota of obese individuals can be identified by distinct characteristics that are significantly associated with BMI and different to the microbiota of non-obese participants. On the one hand, researchers have found a significant reduction of *Bacteroidetes* in obese participants (Armougom et al., 2009) (Ley et al., 2005). In contrast to these publications, Schwiertz and his colleagues assessed a significant increase in the abundance of *Bacteroidetes* (Schwiertz et al., 2010). Additionally, Duncan and his colleagues

demonstrated no significant differences between lean and obese individuals regarding *Bacteroidetes* (Duncan et al., 2008). In 2008, Duncan et al. performed a weight loss study, in which the weight loss in obese participants was accompanied by a modification of the gut microbiome (Duncan et al., 2008). An alteration of the gut microbiota by diet was also reported in other studies (Zhang et al., 2009) (Furet et al., 2010) (Santacruz et al., 2009) (Ley et al., 2006) (Simões et al., 2014). In addition to the fluctuating abundance of bacterial species, the level of bacterial richness seems to be associated with dysmetabolism and inflammation (Cotillard et al., 2013b). Regarding the obesity-associated gut microbiome, Jumpertz et al. assessed the effect of an increased nutrition load on energy harvest in obese and lean individuals. They demonstrated that lean individuals had an increased energy harvest of 150 kcal compared to their obese counterparts (Jumpertz et al., 2011), which confirmed the findings by Turnbaugh et al. that the microbiota of obese individuals had an elevated nutrient absorption (Turnbaugh et al., 2006)

Table 1 and **Table 2** summarise existing human studies dealing with gut microbiota composition in obesity and after weight loss.

Table 1: Overview of existing literature dealing with gut microbiota composition in obesity in human

Author	Year	Results
Ley et al.	2006	Significantly reduced level of *Bacteroidetes* in obese participants.
Duncan et al.	2008	No differences in *Bacteroidetes* between obese and lean participants.
Zhang et al.	2009	*Bacteroidetes, Actinobacteria, Firmicutes and Proteobacteria* are increased in obese compared with controls.
Turnbaugh et al.	2009	Lower proportion of *Bacteroidetes* and increased *Actinobacteria* in obese individuals compared with their lean counterparts.
Armougom et al.	2009	*Lactobacillus species* increased and *Bacteroidetes* decreased in obese group.
Schwiertz et al.	2010	Significant increase in *Bacteroidetes* in obese participants.
Furet et al.	2010	Decreased Bacteroidetes in obesity.
Verdam et al.	2013	Obese individuals showed reduced bacterial diversity, decreased *Bacteroidetes/Firmicutes* ratio, and increased abundance of *Proteobacteria.*

Table 2: Overview of the existing literature dealing with gut microbiota composition after weight loss in humans

Author	Year	Weight loss strategy	Results after weight loss
Ley et al.	2006	Restricted fat or carbohydrate intake	Increased proportions of *Bacteroidetes* after weight loss.
Duncan et al.	2008	4 weeks of weight loss	Abundance of *Firmicutes* decreased in obese participants.
Nadal et al.	2009	10 weeks of calorie-restricted diet	Low-weight-loss group: no significant alterations were assessed; high-weight-loss group: exhibit a decline of proportions of *Firmicutes* and elevated proportions of *Bacteroidetes*.
Santacruz et al.	2009	Diet	*Firmicutes* to *Bacteroidetes* ratio declined after weight loss.
Furet et al.	2010	RYGB	Amount of *Bacteroidetes* were increased, number of *Firmicutes* and *Actinobacteria* decreased.
Russel et al.	2011	4 weeks high protein, low-carbohydrate diet	The relative abundance of *Bacteriodetes* was decreased. Decrease in *Collinsella spp*., *E. rectale*, and *Roseburia spp.*
Walker et al.	2011	Carbohydrate weight loss diet over 10 weeks	Decrease in *Collinsella* spp.,*E. rectale*, and *Roseburia* spp.
Simoes et al.	2014	6 weeks of high protein, low carbohydrate VLCD	Number of *Bifidobacteria* and Lactobacillus were decreased.
Cotillard et al.	2013	6-week energy-restricted high-protein diet followed by a 6-week weight –maintenance diet	Significant increase of abdundance of most gene clusters
Remely et al.	2015	4 months	*Firmicutes/Bacteroidetes* ratio was significantly decreased during intervention; Lactobacilli and *Faecalibacterium prausnitzii* was increased.
Haro et al.	2016	1 year low-fat, high-complex carbohydrate	*Prevotella* were increased, *Roseburia* were decreased.

RYGB, Roux-en-Y Gastric Bypass; VLCD, very-low caloric diet

2.3 Obesity and gut permeability

Recent studies have suggested that increased gut permeability (Teixeira al., 2012) and LPS translocation may also play an important role (Creely et al., 2007) and thereby activates the immune system of the host (Moreira et al., 2012).

2.3.1 Development of the intestinal gut barrier

The development of the intestinal gut barrier is already initiated during pregnancy. In the first trimester epithelial cells, villi, crypts, goblet and enteroendocrine cells are already developed in the small intestine (Montgomery et al., 1999). At this time of life, the fetal intestinal mucosa is permeable for macromolecules. In the first weeks after birth a gut integrity is making progress

(Maheshwari and Zemlin, 2006). In addition to the observation at the cellular level, the maturation of the intestinal barrier is also recognizable by looking at the elongation of the gastrointestinal tract with a length of about 3 m at birth (Neu, 2007) and a length of about 7 m as an adult (Kraus, 1993).

2.3.2 Structure of intestinal gut barrier

Anderson et al. categorized four different barriers types of barriers in the mature gut barrier (Anderson et al., 2012).

The physical barrier consists of enterocytes, goblet cells, Paneth cells, and enteroendocrine cells (Anderson et al., 2012). The enterocytes with their tight junctions form a selective barrier which protects the host from penetration of noxious luminal molecules and enable nutrients and water to pass through (Arrieta et al., 2006). Goblet cells secrete mucus and trefoil peptides. Paneth cells are located at the crypt and secrete lysozymes, antimicrobials and defensins (van der Flier et al., 2009). Enteroendocrine cells are specialized cells in the gastrointestinal tract to secrete secretin, glucagon-like peptide 1, serotonin, gastrin (Schonhoff et al., 2004).

The chemical barrier is formed by the intestinal mucus layer. This mucus layer promotes homeostasis of the mucosal surfaces and supports cell regeneration (Kindon et al., 1995). In the intestine and stomach the glycosylated gel-forming mucin, MUC2, shields the epithelium and the Peyer's patches from bacteria (Johansson et al., 2008) (Pelaseyed et al., 2014). A thick mucus layer above the intestinal epithelium protects the enterocytes from colonization. In the large intestine the mucus is structured in two layers. The outer layer is colonized by bacteria and the inner layer is associated with the epithelium and free of bacteria (Johansson et al., 2008). Under normal conditions bacteria are not able to penetrate the mucus layer and to adhere to the underlying epithelium (Deplancke and Gaskins, 2001).

The immunological barrier consists of secretory immunoglobulin A (IgA), microfold cells (M cells), and dendritic cells. The IgA-antigen complex interacts with the apical surface of M cells. M cells play a substantial role in the specific mucosal immune response in the Peyer's patches. In this context, phagocytosis and transcytosis of luminal antigens is a substantial feature performed by antigen presenting cells, such as dendritic cells and T cells. The mechanism is associated with secretion of IFN-γ and activation of macrophages secreting TNF-α. IL-10 is also released and has a feedback effect to terminate the Th1 response (Anderson et al., 2012). In general, the immune response is organised by the gut-associated lymphoid tissue. Peyers' patches belong to the gut-associated lymphoid tissues and are located throughout the epithelium or lamina propria (MacDonald, 2003).

The fourth barrier consists of microbes and their products. Bacteria ferment non-digestible products and produce SCFA (acetate, propionate und butyrate), which were shown to promote colonic health (HAMER et al., 2008). In addition, butyrate is the main source of energy for colonic epithelial cells. Furthermore, particular species of the microflora such as *Bifidobacteria*

secrete antimicrobial substances and promote the protection of the intestinal barrier (Liévin et al., 2000) (Fukuda et al., 2011).

The illustration (**Figure 1**) adapted from Cox et al. (Cox et al., 2015) shows a schematic view of the intestinal barrier.

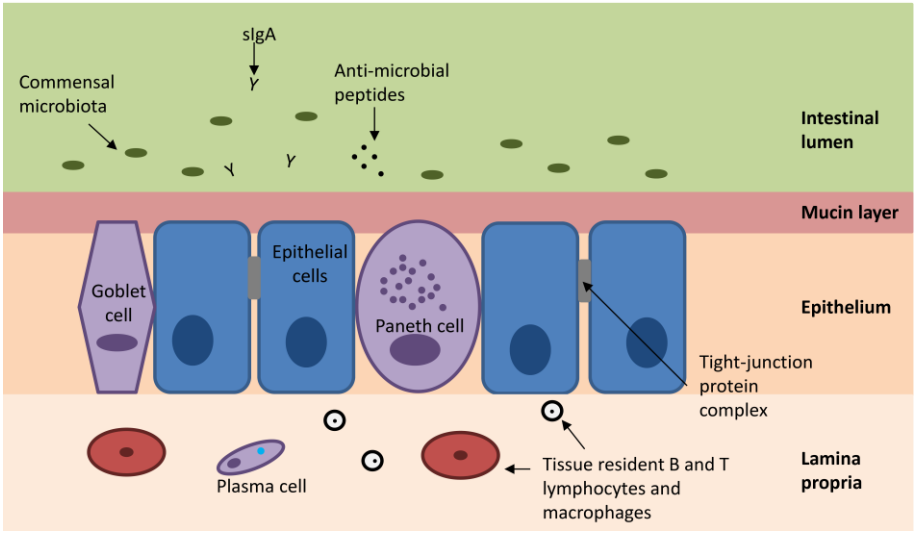


Figure 1: Intestinal barrier (Adapted from Cox et al. 2015). sIgA, secretory immunoglobulin A. The structure of the intestinal barrier is described in detail in text above.

2.3.3 Gut permeability – a functional feature of the intestinal barrier

In the small intestine, monosaccharides, vitamins, electrolytes, and water are absorbed. Thereby, the surface area enlarged by circular folds, villi and microvilli enables the absorption of nutrients. In the large intestine the villi are nearly absent and consequently the surface area is reduced (Clevers, 2013). For transport reasons, the intestinal barrier is a semi-permeable barrier and allows translocation of nutrients, electrolytes and water. In contrast, it shields the translocation of microorganisms from the luminal environment into the circulation (Groschwitz and Hogan, 2009). In general, the intestinal permeability enables two different pathways through the epithelium: transcellular and paracellular transport. The transcellular route requires a selective transport through epithelial cells for factors, such as amino acids or sugars. The paracellular route is regulated by protein complexes between enterocytes including tight junctions, adherent junctions, and desmosomes (**Figure 2**) (Ulluwishewa et al., 2011): Tight junctions are positioned subapically to the brush boarder, connect neighboring epithelial cells, consist of a multiprotein complex, and inhibit the translocation of bacterial antigens such as LPS. The tight junctions include transmembrane proteins (e.g. occludin, claudin, junctional adhesion molecule (JAMs)), junctional complex proteins (e.g. zonula occludens (zo) and cell cytoskeleton structures e.g. microfilaments). Claudins and occludin are one of the most important transmembrane proteins, which are associated with the apical perijunctional actomyosin ring

through intermediate proteins ZO-1, -2, -3. These proteins are part of the membrane-associated guanylate kinase superfamily (Arrieta et al., 2006). ZO-1 interconnects the tight junctions and the cytoskeleton by interacting with ZO-2, occludin, and F-actin (Fanning et al., 1998). The rearrangement of the paracellular organisation can be initiated by extracellular stimuli, which enable the secretion of mucosal zonulin. Zonulin activates the zonula occludens toxin receptor. This binding favors the activation of protein kinase c and myosin light chain kinase (MLCK). Phosphorylation of MLCK results in a rearrangement of cytoskeletal elements. Finally, actinomyosin contraction is performed triggering increased paracellular permeability (Fasano, 2000). The family of claudin-proteins forms paracellular pores and selects ions and small non-ionic molecules. Occludin, a 65 kDa protein (Furuse et al., 1993), interacts directly with claudins and actin. (Furuse et al., 1993). Moreover, JAM-A belongs to the transmembrane proteins and exerts regulatory functions of the epithelial barrier (Ulluwishewa et al., 2011).

The adherens junction is located directly below the tight junctions and is composed of cadherins, a family of transmembrane proteins. E-cadherin (also known as cadherin-1) interacts directly with ß-catenin. ß-catenin and the actinomyosin complex is combined by α-catenin which control the actin construction. The perijunctional actomyosin ring is linked with tight junctions and adherens junctions (Fanning et al., 2012).

Desmosomes are protein complexes consisting of desmosomal cadherins, called desmogleins and desmocollins, and desmoplakin (Kowalczyk and Green, 2013). Moreover, desmosome are linked to kreatin filaments (Turner, 2009). The main function of the desmosome is to mediate cell-cell contact and takes part in cell proliferation and cell differentiation (Kowalczyk and Green, 2013).

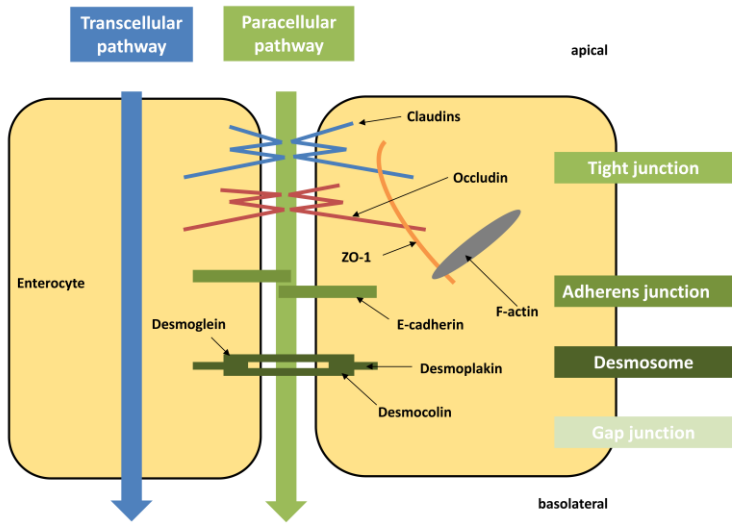


Figure 2: Scheme of the subcellular mechanisms determining intestinal permeability (Adapted from Suzuki 2013) (Suzuki, 2013). The illustration is described in detail in the text above. ZO, zonula occludens

2.3.4 Impaired gut permeability and disease

Intestinal permeability has a significant impact on the course of various diseases. An influx of numerous luminal noxious molecules can be best prevented by a intact gut barrier. If the intestinal barrier is disrupted, pathogens, toxins as well as luminal antigens are able to cross the epithelium and resulting in disturbed mucosal homeostasis and triggering intestinal inflammation. Many factors can influence intestinal permeability. Pathogens such as *Enteropathogenic E. coli* (Yuhan et al., 1997) and the protozoa *Giardia* (Scott et al., 2002) have the ability to modulate intestinal permeability. Further, cytokines can lead to altered paracellular translocation. In this respect, two prominent proinflammatory cytokines are IFN-γ and TNF-α. IFN-γ is secreted by lymphocytes and by antigen presenting cells (dendritic cells, monocytes) and causes an impaired intestinal tight junction barrier (Madara and Stafford, 1989) (Watson et al., 2005). TNF-α is a key regulator of the inflammatory response. Numerous studies have demonstrated that TNF-α causes a disruption of the intestinal tight junction barrier and increased tight junction permeability (Ye et al., 2006). The inhibition of several cytokines (such as transforming growth factor-ß induces protective effects against intestinal mucosal damage and the development of intestinal inflammation (Al-Sadi et al., 2009). Patients under nonsteroidal anti-inflammatory drug (NSAID) treatment suffer from impaired gut permeability (Sigthorsson et al., 1998) (Kerckhoffs et al., 2010). Lifestyle and dietary factors can contribute to impaired gut permeability as well. Excessive consumption of alcohol alters the intestinal permeability (Bode and Bode, 1997) (Robinson et al., 1981) (Parlesak et al., 2000). Moreover, gliadin is a powerful trigger to activate zonulin signalling including phosphorylation of MLCK and disassembling intercellular tight junctions (Fasano, 2000) (Ukabam and Cooper, 1984) resulting in an increased gut permeability. Also, an impaired intestinal permeability is associated with coeliac disease (Fasano et al., 2000) (Heyman et al., 2012), cancer cachexia (Klein et al., 2013), inflammatory bowel diseases (Crohn's disease (Hollander, 1986) (Zeissig et al., 2007) (Haas et al., 2009), and ulcerative colitis (Schmitz et al., 1999) (Almer et al., 1993), pancreatitis, irritable bowel syndrome (Marshall et al., 2004) (Del Valle-Pinero et al., 2013), and non-alcoholic fatty liver disease (Miele et al., 2009).

2.3.5 Impact of obesity on gut permeability

There is growing evidence that increased intestinal permeability leads to an increased influx of luminal LPS which is associated with low-grade inflammation. This low-grade inflammation was linked to metabolic diseases, such as type 1 (Sapone et al., 2006) and type 2 diabetes (Horton et al., 2014). Numerous studies presented data about how gut permeability is affected by different diseases investigated in the small intestine (section 1.4.2), but less is known about its role in obese humans. Moreover, the impact of caloric restriction on gut barrier function is not completely understood. In the following tables, the literature regarding gut permeability in obesity (**Table 3**) and weight loss (**Table 4**) is summarised.

Table 3: Published data on gut permeability and obesity in human

Author	Year	Results
Brignardello et al.	2010	Obese individuals with systemic low-grade inflammation do not show evidence of colonic inflammation or gut barrier alteration.
Gummersson et al.	2011	Positive correlation between intestinal permeability and visceral adiposity and liver fat content in healthy women.
Texeira et al.	2012	Increased paracellular permeability; positive correlation with anthropometric measures and metabolic variables.
Verdam et al.	2013	Increased gastroduodenal permeability in obesity.

Table 4: Published data on gut permeability after weight loss

Author	Year	Results
Xiao et al.	2014	Lactulose/Mannitol ratio as a measure for gut permeability in the small intestine and plasma endotoxin levels were decreased after weight loss of 5.79 ± 4.64 kg
Savossi Rocha et al.	2014	Roux-en-Y Gastric bypass (RYGB) led to a decreased mannitol excretion rate, but returned to pre-operative values at 6 months after surgery
Damms-Machado	2016	Obese patients had an significantly increased gut permeability. After weight reduction gut permeability were in normal range

3 Study intervention

To investigate the effects of overfeeding and caloric restriction with respect to gut permeability, gut microbiota profiles and obesity-related inflammatory and metabolic parameters, two studies with complementary questions and readouts were designed.

3.1 Caloric restriction

3.1.1 Ethics statement

The study protocol was reviewed and approved by the ethics committee of the Faculty of Medicine of the Technical University of Munich, Germany (approval no. 5499/12). The study was registered in the German Clinical Trial Register (DRKS00006210). Written informed consent was obtained from all participants before study inclusion. The guidelines of the International Conference on Harmonization of Good Clinical Practice and the declaration of Helsinki (in the revised version of Seoul, South Korea 2008) was basis of the study.

3.1.2 Study design

The human intervention study was an uncontrolled, single-arm multicentre study. The study centres were located at the Technical University of Munich, Campus D in Munich, Germany and in Freising, Germany. **Figure 3** summarises the study design of this prospective trial. In total, the study duration was seven weeks and was structured into three time periods. During the first period participants were instructed to maintain their usual eating habits. Then, participants underwent a caloric restriction protocol consuming 800 kcal/day for 28 days. The dietary intervention included a formula diet which was kindly provided by Nutrition Santé SAS (Revel, France). The formula diet included beverages, creams, soups and bars in different flavours (e.g. vanilla, chocolate) but with identical macronutrient composition. An additional intake of 200 g of vegetables (raw or cooked without fat) was allowed (**Figure 4**). During the caloric restriction period, participants were invited for weekly routine examinations including measurement of weight, hip and waist circumference, body composition, pulse rate, and blood pressure. During these visits, formula products were handed out for the forthcoming week. Additionally, to support motivation of the participants, they received a telephone call each week. Finally, during the two weeks following intervention, participants were instructed to follow a balanced diet of 1,800 kcal/day. Before, immediately after, and two weeks after the formula diet intervention, clinical and biochemical parameters, gut permeability, and fecal microbiota profiles were assessed. Magnetic resonance imaging (MRI) of subcutaneous and visceral fat depots was carried out before and after the 4 weeks of hypocaloric intervention. Likewise, a needle aspiration of periumbilical SAT was performed before and after the caloric restriction.

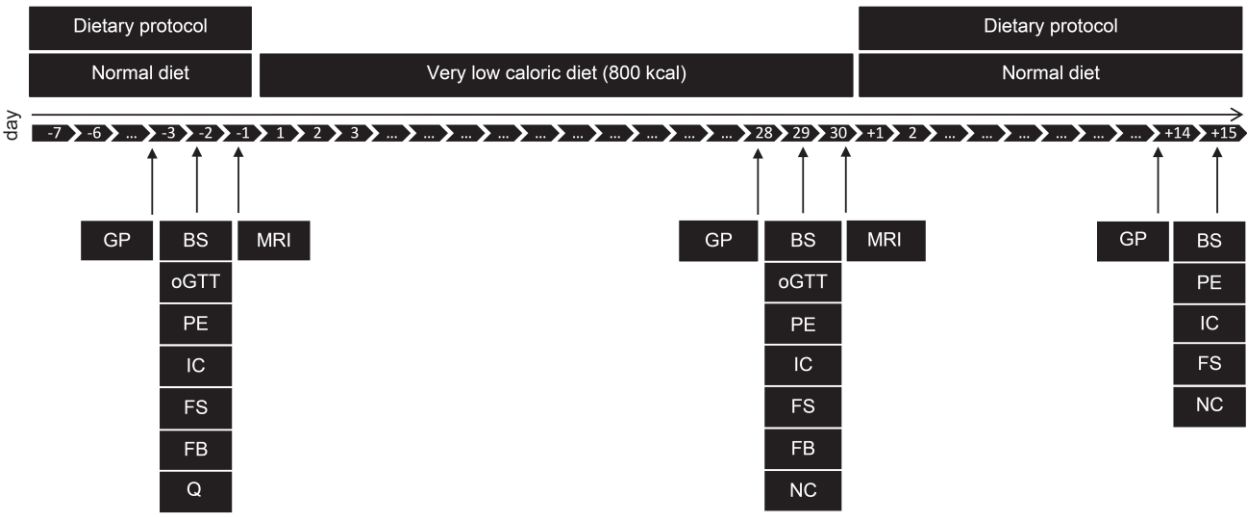


Figure 3: Study design of the study "Caloric restriction". The scheme gives an overview of the timeline and the different examinations performed. BS, blood sample; FB, fat biopsy; FS, fecal sample; GP, gut permeability; IC, indirect calorimetry; MRI, magnetic resonance imaging; NC, nutritional counseling; PE, physical examination; oGTT, oral glucose tolerance test; Q, questionnaire

Figure 4: Caloric restriction protocol including formula diet (800 kcal) with additional 200 g of vegetables. Pack shots were kindly provided from Modifast®

3.1.3 Study population

Twenty female participants with a BMI of ≥ 30 kg/m² were recruited on a voluntary basis in October 2013 via advertisements in newspapers (Appendix, Study document 1) in the area of Munich, Germany. The participants' eligibility was assessed with a detailed screening questionnaire (Appendix, Study document 2). Volunteers were assigned to the study only after checking the inclusion and exclusion criteria (**Table 5**), after being informed on the purpose and possible risks of the study, and after giving written informed consent (Appendix, Study document 3).

Table 5: Inclusion and exclusion criteria of the "Caloric restriction" study

Inclusion criteria	Exclusion criteria
Age above 18 years	Acute infections
Women	Severe disease (e.g. cancer)
Body mass index ≥ 30 kg/m²	Diabetes mellitus
Non-smoker	Treatment with oral anticoagulants or other antithrombotic medication with anti-inflammatory properties (e.g. NSAID)
Caucasian origin	Intestinal surgery in the last three months
Written informed consent	Sucrose intolerance, lactose intolerance, sucrose intolerance
	Inflammatory intestinal diseases

3.1.4 Data and sample collection

All data and samples were pseudonymized with a 6 digit code. For confidentiality reasons, a participant identification list was kept in the study centre in a locked cupboard. The data were documented in case report forms (Appendix, Study document 4) and saved in EXCEL (version 2010).

3.2 Overnutrition

3.2.1 Ethic statement

The study protocol was approved by the ethical committee of the Faculty of Medicine of the Technical University of Munich, Germany (approval no. 5499/12). The study was registered in the German Clinical Trial Register (DRKS00006211). Written informed consent was obtained from all participants before study inclusion. The study followed the guidelines of the International Conference on Harmonization of Good Clinical Practice and the declaration of Helsinki (in the revised version of Seoul, South Korea 2008).

3.2.2 Study design

The human study was an uncontrolled intervention study and was performed in the Core Facility for Human Studies of the ZIEL - Institute for Food and Health at the Technical University of Munich, Germany. **Figure 5** summarises the design and the course of this trial. The duration of the study was four weeks, divided into three time periods. During the first study week, participants were informed to keep their usual eating habits. Thereafter, volunteers were subjected to the 7d-overfeeding programme. Based on the individual resting metabolic rate (RMR), total energy requirement for each participant was calculated by multiplying the RMR with a physical activity level (PAL) factor of 1.5 to achieve the individual total energy expenditure. A surplus of 1,000 kcal/day was provided by adding whipping cream, selected due to its high content of saturated fatty acids. The dietary intervention was carried out with standardised frozen prepared meals (Bofrost, Straelen, Germany). Also other components of the

nutrition were bought from the same manufacturer. The macronutrient composition of the nutrition consisted of 48 energy percent (EN%) from fat, 34 EN% from carbohydrates and 18 EN% from protein. During the first and third study period, participants were asked to record their dietary intake, after having obtained detailed instruction for a standardised documentation.

Study participants were asked not to exercise throughout the study. Furthermore, participants were told to minimise physical activity, which was monitored by accelerometry (ActiGraph GT3X+, Pensacola, FL, USA). The volunteers were asked to wear the accelerometer on their dominant hip for the entire four weeks of the study. An example of one intervention day can be found in **Figure 6**. To ascertain compliance, participants received breakfast, lunch and dinner in the Human Study Centre of the ZIEL Institute for Food and Health. Participants were monitored to completely consume all energy-enriched meals provided. In addition, participants were allowed to drink only mineral or tap water or coffee without milk during the whole week of the intervention. Finally, during the subsequent two weeks, participants were asked to return to their usual eating habits.

Anthropometric and clinical characteristics were assessed and insulin sensitivity, gut permeability and microbiota composition were measured before, immediately after intervention, and 14 days after intervention. Insulin sensitivity was assessed using the hyperinsulinemic euglycemic clamp technique at the time points indicated.

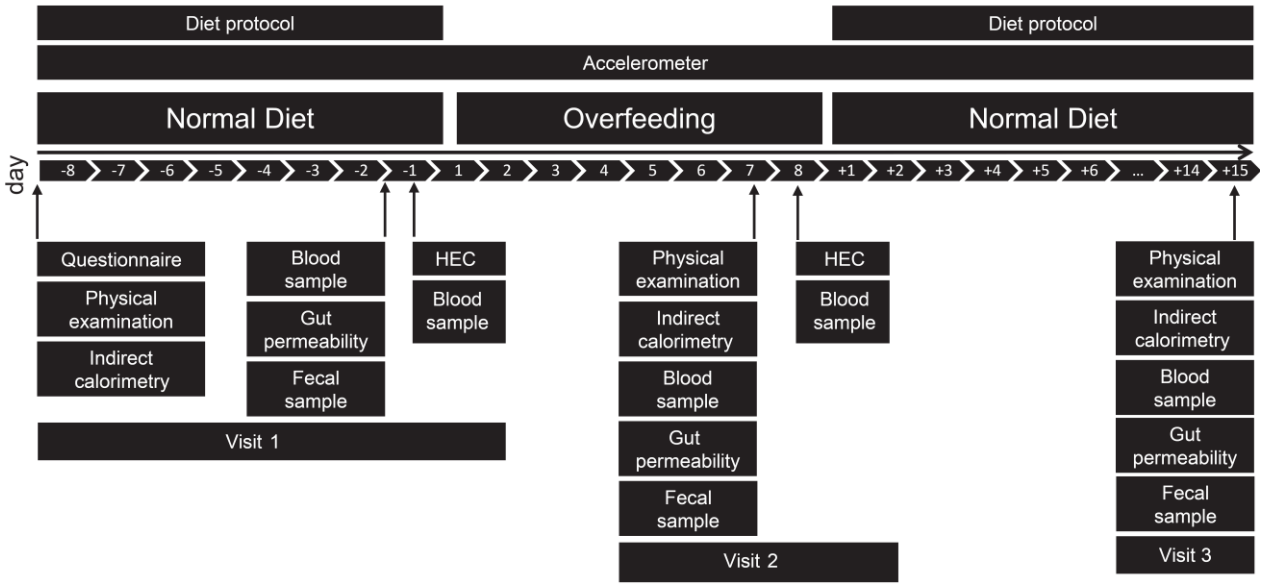


Figure 5: Design of the "Overnutrition" study. The scheme gives an overview of the timeline and the different examinations. HEC, hyperinsulinemic- euglycemic clamp

Breakfast
313g chicken breast
276g cottage cheese
100g buns
50g wholemeal bread
40g butter
40g jam
80g tomato
100g cucumber
100g whipping cream
100ml orange juice

Lunch
500g „Mettbällchen mit Kohlrabigemüse“
100g cheese cake
100g whipping cream
100ml orange juice

Dinner
420 g „Hähnchen-Schnitzel Madagaskar“
50g walnuts
125g apple cake
141g whipping cream
100ml orange juice
287ml coke

Figure 6: Exemplary menu of one day with 4,252 kcal.

3.2.3 Study population

Twenty-five young, healthy, non-smoking, normal-weight men with a BMI below 25 kg/m² and age between 19 and 31 years were recruited on a voluntary basis between 2013 and 2014 at the campus of the Technical University of Munich in Freising, Germany. The recruitment strategy was to disseminate study-flyers and call for participants by advertising on the *campus Weihenstephan* (Appendix, Study document 5) at the Technical University of Munich, Munich, Freising, Germany. Initially, the volunteers passed a detailed screening (Appendix, Study document 6) including checking inclusion and exclusion criteria (**Table 6**). Before including participants into the study, it was assessed whether participants understood the purpose and procedure of the study (Appendix, Study document 7). One volunteer dropped out due to hypoglycaemia during the clamp.

Table 6: Inclusion and exclusion criteria of the "Overnutrition" study

Inclusion criteria	Exclusion criteria
Age above 18 years	Acute infections
Men	Severe disease (e.g. cancer)
Body mass index ≤ 25 kg/m²	Diabetes mellitus
Non-smoker	Treatment with oral anticoagulants or antithrombotic anti-inflammatory drugs (e.g. NSAID)
Caucasian origin	Intestinal surgery in the last three months
Written informed consent	Sucrose intolerance, lactose intolerance, sucrose intolerance
	Inflammatory intestinal diseases

3.2.4 Data and sample collection

All data and samples were pseudonymized with 6 digit code. For confidentiality reasons, the participant identification list was kept in the study centre in a locked cupboard. The data were documented in case report forms (Appendix, Study document 8) and saved in EXCEL (version 2010).

4 Material and methods

Further details about material and methods are listed in the study documents in the appendix.

4.1 Clinical examination and technical measurements

4.1.1 General participants' information

The participants received a questionnaire including questions regarding medical history, lifestyle, dietary habits, and education. The questionnaire is documented in the appendix (Appendix, Study document 9).

Moreover, study participants were instructed to record their food consumption during the whole study period (Appendix, Study document 10). Energy content and macronutrient composition of the diet were calculated using the OptiDiet Plus software (Version 5.1.2.046, GOE mbH, Linden, Germany) .

Participants from the "Caloric restriction" study received an AS 50 activity sensor (Beurer, Ulm, Germany) for monitoring their physical activity for the entire duration of the study. The individual step length was calculated by dividing a defined distance (20 m) by the amount of steps needed. Participants were instructed to wear the activity sensor as described above for the whole study period (day and night). The activity sensor included a 3-level user reward system. The primary aim of the AS 50 activity sensor was to motivate participants to do physical activity. The steps were analysed by using the Easyfit 2.3 software.

In the "Overnutrition" study participants were instructed to minimise physical activity, which was monitored by accelerometry (ActiGraph GT3X+, Pensacola, FL, USA). Participants were asked to wear the accelerometer on dominant hip daylong during the study. Data from all three axes (vertical, horizontal and perpendicular axis) were recorded at 1-second epoch times and a 100 Hz data sample frequency. Participants received a log diary including an instruction sheet (Appendix, Study document 11) to document wear times and non-wearing times (e.g. having a shower). Physical activity was analysed using the ActiLife software (Version 6.8.2, Pensacola, FL, USA).

4.1.2 Phenotyping

Anthropometric measurements

All anthropometric and clinical parameters were measured in a standardised manner for both studies between 8 and 9 am in the morning following an overnight fast. Participants were instructed to avoid physical activity the day before the examination. Body height (cm) was measured using a stadiometer (Seca, Hamburg, Germany). Body weight and body composition

were measured using the TANITA Body Composition Analyser Type BC-418 MA III (Amsterdam Netherlands). Measurements were performed barefoot, without outdoor clothing i.e. coats, jackets, heavy outerwear, pocket, keys and an empty urinary bladder. BMI was calculated by dividing weight in kg by height in meter squared (kg/m²). Waist was measured with a soft tape midway between the lowest rib and the iliac crest. Hip circumference was measured at the widest part of the gluteal region.

Magnetic resonance imaging

MRI (PHILIPS 3.0T Ingenia Kernspintomograph, Hamburg, Germany) was applied to monitor liver fat content as well as VAT and SAT of participants before and after intervention. The examination was performed by Dr. Dimitrios Karampinos at the *Klinikum rechts der Isar,* Munich, Germany. Therefore, participants were positioned within an MRI scanner which forms a strong magnetic field around the area to be imaged. The test took 60 minutes and was previously described in detail by Cordes et al. (Cordes et al., 2015).

Cardiovascular tests

Systolic and diastolic blood pressure and pulse rate were assessed in a sitting position after five minutes resting time. Participants in the "Caloric restriction" study were measured using Omron M8 comfort (Mannheim, Germany). Systolic and diastolic blood pressure of participants in the "Overnutrition" study were assessed using blood pressure meter (Maxi Stabil 3, WelchAllyn GmbH &Co.KG (Hechingen, Germany) .

Resting metabolic rate

RMR measurement was performed using a canopy hood (COSMED Quark RMR, Fridolfing, Germany). After the warm-up period of at least 30 minutes, both components, the turbine and the gas sensors were calibrated. Before starting the measurement, participants remained recumbent for at least 10 minutes in a silent room. Data acquisition was performed during the following 15 minutes. All participants were monitored during the indirect calorimetry procedure. It was ensured that participants did not freeze or move during the measurements.

Oral glucose tolerance test

The oral glucose tolerance test was performed in the "Caloric restriction" study only. The oral glucose tolerance test (OGTT) began between 8 am and 9 am following a 12-hour overnight fast. After taking a baseline blood sample through a venous line in the fasting state, volunteers received 75 g glucose in a volume of 300 ml (AccuCheck®-O.G.T., Roche, Mannheim, Germany). After 30, 60 and 120 minutes blood was drawn and glucose levels were determined (HemoCue Glucose 201^{+}, plasma-calibrated, Ängelholm, Sweden).

Hyperinsulinemic euglycemic clamp (HEC)

Insulin sensitivity in the "Overnutrition" study was measured by the hyperinsulinemic euglycemic clamp technique. After an overnight fast, two peripheral catheters (Vasofix® Braunüle®, 21G, Braun, Melsungen, Germany) were placed for infusion for glucose/insulin (right arm) and blood sampling (left arm). To obtain arterialised blood electric blankets (60 °C) were used. The blood sampling catheter was kept open with a slow, controlled infusion of 0.9 % sodium chloride solution (Braun, Germany). The setting of a HEC situation is shown in **Figure 7**. Initially, glucose infusion (20 %, Braun, Germany) was started at 4 mg/kg/body weight*min followed by an insulin bolus (60 mU/m²surface area*min) for five minutes to suppress endogenous glucose production. After the priming dose, insulin (Aventis, Straßbourg, France) was infused at a constant rate (40 mU/m² surface area*min) for at least 150-180 minutes. Blood glucose infusion was adjusted on the basis of a continuous analysis of blood glucose levels (HaemoCue Glucose 201^{+}, plasma-calibrated, Ängelholm, Sweden) at intervals of 5 min during the clamp. Steady-state was defined as a stable blood glucose level of 80 mg/dl ± 5 after a clamp time of 150 minutes. Glucose infusion rate during the final 15 min of the clamp was used to calculate insulin sensitivity. Subsequently, insulin infusion was discontinued but glucose was given for another 30 minutes. The clamp measurements were performed directly before and after the HFD under the same conditions. The results of the clamps were analysed in a blinded manner by PD Dr. med. Andreas Lechner (Diabetes Research Group, Ludwig-Maximilians Universität, Munich, Germany).

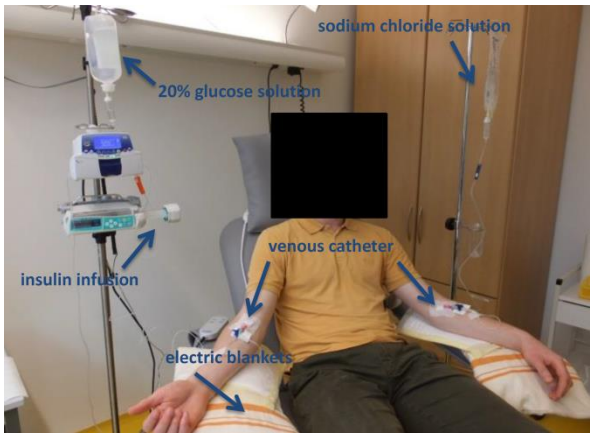


Figure 7: Exemplary representation of a hyperinsulinemic euglycemic clamp.

4.1.3 Gut permeability

Gut barrier function was assessed by means of different non-invasive tests. First, the intestinal permeability was measured via a validated sugar absorption test and a test using polyethylene glycol particles (PEG). Both tests were performed in parallel. The principle is to measure urine excretion of orally administrated substances of different molecular masses. The tests were done

directly before intervention, after intervention, and two weeks after intervention. Data are presented as percentage of ingested sugars and PEGs that were discovered in the urine, referred to as % urine recovery. Participants were guided through the tests by a detailed instruction sheet (Appendix, Study document 12). Finally, the gut permeability marker zonulin was measured in blood via ELISA, too. Detailed description of these tests is given in the sections below.

Sugar absorption test

To check for gut permeability, urine excretion of four different sugars was assessed. A high appearance of sugars in urine would indicate impairment of barrier function in different segments of the gut. Sucrose is used to analyse the gastroduodenal permeability. Under normal circumstances, sucrose is hydrolysed by the duodenal enzyme sucrose-isomaltase. Appearance of the disaccharide in the urine indicates gastric epithelium leakage (Meddings et al., 1993) (Sutherland et al., 1994). Lactulose and Mannitol are validated test substances for monitoring gut permeability in the small intestine. Mannitol, a water-soluble, 182.2 Da sugar-alcohol, cannot be metabolized and is considered to serve as a marker of transcellular permeation proportional to small bowel absorptive capacity. A decreased surface area diminishes uptake of urinary excretion of a mannitol. Lactulose is a tight junction marker, consisting of D-galactose and fructose, with a molecular weight of 342.3 Da. This disaccharide reflects the transcellular permeability and is characterised as a non-degradable and unmetabolizable substance. Both substances can be degraded by the bacterial flora and thus do not provide any information on colonic permeability (Travis and Menzies, 1992) (Bjarnason et al., 1995). Sucralose, a chlorinated derivative of sucrose with a molecular weight of 397.6 Da, is resistant against bacterial enzymes and is therefore well suited for the investigation of colonic permeability. As a result of impaired intestinal barrier integrity sucralose crosses the intestinal barrier into the circulation and becomes detectable in urine (Meddings and Gibbons, 1998).

Participants received a sugar test solution following an overnight fast and after collecting a baseline urine sample. The 100 ml sugar test solution containing mannitol (5 g), lactulose (10 g), sucrose (20 g) and six tablets with sucralose (333.33 mg/tablet). Participants were instructed to collect their whole urine at certain time-defined intervals (0-5 h, 5-26 h). Urine was sampled in containers with sodium acid (0.002 g) as a preservative. During the first five hours participants collected their complete urine. Sugars in the first five collection hours reflect the small intestine permeability. During the following 21 hours again the complete urine was sampled. Urine samples reflect the permeability of the colon. Samples were stored at -20 °C until analysis. The sugars were quantified by high-performance liquid chromatography with pulsed electrochemical detection (chromatography module: 250, Dionex, Idstein, Germany). The sugar absorption test was performed as described in detail by Norman et al. (Norman et al., 2012). Data were expressed as percentage of ingested sugars, referred to as % urine recovery.

Polyethylene glycol test

To analyse gastro-intestinal leakage we also used a PEG (Merck Darmstadt, Germany) containing test solution. Participants received 100 ml test solution containing 1 mg of M_r 400 (PEG_6-PEG_{13}; mass range: 285-678 Da), 200 mg of M_r 1500 (PEG_{20}–PEG_{45}; mass range: 899–2000 Da), 4 g of Mr 3000 (PEG_{51}-PEG_{90}; mass range: 2264-3982 Da) und 4 g of Mr 4000 (PEG_{75}-PEG_{115}: mass range: 3322-5084 Da). After collecting a baseline urine sample, the PEG test solution was ingested by the study participants and the urine was sampled during the following 24 hours. PEG excretion was analysed by liquid chromatography-mass spectrometry as described by Lichtenegger and Rychlik (Lichtenegger and Rychlik, 2015). Data were expressed as percentage of ingested PEGS, referred to as % urine recovery.

Zonulin

In addition to different urinary excretions reflecting different parts of the gut, a protein named zonulin was measured in blood by ELISA. In 2000 Fasano (Fasano et al., 2000) and his research group published a new potential marker of intestinal permeability: zonulin. The eukaryotic protein with a molecular mass of 47 kDa is secreted by the intestinal mucosa. Zonulin interacts with specific surface receptors and the protein kinase C-α-mediated actin polymerization. The polymerization causes movements of the filaments of actin and the subsequent displacement of proteins from junctional complex. This signalling results in loosening of intestinal tight junctions.

4.2 Blood and tissue collection

Blood sampling

Before, immediately after, and two weeks after intervention, blood samples were drawn in the fasting state between 8.30 am and 9.00 am. Routine parameters (**Table 7**) were analysed at the certified laboratory Synlab Labordienstleistungen München. Additionally, plasma (EDTA KE monovettes, Nümbrecht, Sarstedt) was collected and centrifuged (Centrifuge 5702 R, Eppendorf AF, Hamburg, Germany) at 2,500 *g* for 10 min at room temperature. Serum (Serum, Nümbrecht, Sarstedt) was collected, left for 20 minutes to allow clotting, and was finally centrifuged (2,500 *g* for 10 min at 20 °C). Plasma and serum were aliquoted and stored at -80 °C for later measurement of selected biochemical parameters. For PBMC isolation two lithium-heparine monovettes (LiHe monovettes, Nümbrecht, Sarstedt) were taken. All aliquots were stored at -80 °C untill analysis. The standard operation procedure of blood sample processing is documented elsewhere.

Table 7: Routine clinical chemistry analysed by Synlab, Munich, Germany

Visit Parameter	Material (Monovettes, Sarstedt)	before intervention	after intervention	14d after intervention
Complete blood count	EDTA KE plasma	√	√	√
Coagulation	Citrat	√	√	√
Fasting blood sugar	Natrium-Flourid	√	√	√
TSH basal	Serum	√		
Uric acid		√	√	√
Liver enzymes [a)]		√	√	√
Electrolytes [b)]		√	√	√
Lipid profile [c)]		√	√	√
Kidney function [d)]		√	√	√

TSH, thyroid stimulating hormone
a) Liver enzymes (AST, aspartate aminotransferase; ALT, alanine transaminase; γ –GT, glutamyltransferase)
b) Electrolytes (sodium, calcium)
c) Lipid profile (total cholesterol, LDL-cholesterol, HDL-cholesterol, triglycerides)
d) Kidney function (creatinine, glomerular filtration rate)

Adipose tissue

Abdominal SAT specimens were obtained by needle aspiration before and after the intervention with the formula diet. After thorough disinfection, a region lateral from the umbilicus was anesthetized with 1% lidocaine and the biopsy needle (Strauss-cannula; 2.0 x 43 mm; 14G) was inserted into the superficial fat layer. Then, 4 ml of 0.9 % sodium chloride solution (NaCl, 0.9%, Braun, Melsungen, Germany) was injected (**Figure 8A**). Aspirated adipose tissue was mixed with 0.9 % NaCl-Heparin (50 IE/ml) (**Figure 8B**). The fat sample was filtered and was subsequently washed with 0.1 % Krebs-Ringer-buffer (**Figure 8C**).

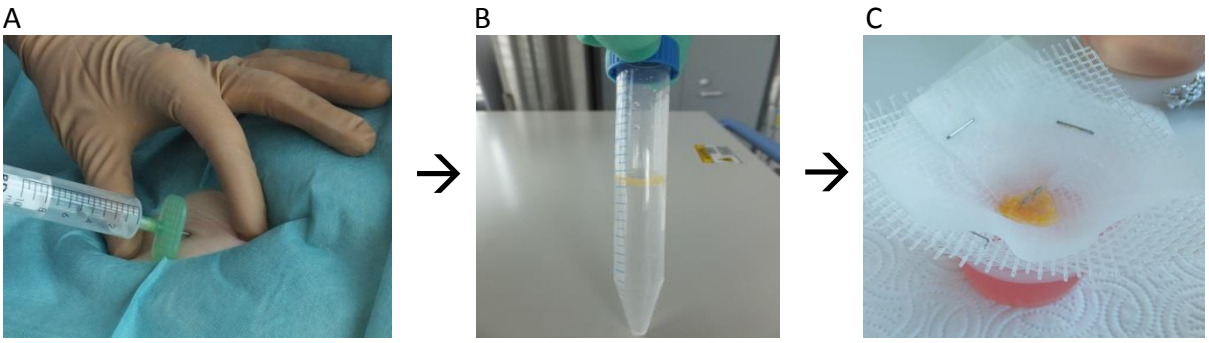


Figure 8: The different steps of an abdominal needle aspiration. A) The needle was inserted below skin. B) Aspirated adipose tissue was mixed with NaCl-Heparin. C) Adipose tissue was washed with 0.1 % Krebs-Ringer- Buffer.

For ribonucleic acid (RNA) processing, fat tissue was aliquoted into tubes containing sterilized zirkonia-glas-beads (Carl Roth, Karlsruhe, Germany), RLT-buffer (RNeasy Mini Kit, Qiagen, Hilden, Germany) and 1 % (v/v) ß-mercaptoethanol (#M3148, Sigma-Aldrich, St. Louis, Missouri, USA) and was immediately frozen and stored at -80 °C. For fat histology, fat tissue was fixed in 4 % buffered formalin (pH 7.4) for 24 h and finally embedded in paraffin. Until analysis the paraffin-embedded blocks were stored at room temperature. Determination of adipocyte size was done by using open source cellprofiler® image analysis software (http://www.cellprofiler.org/).

Fecal samples

Participants of the "Caloric restriction" study collected their stool directly in sterile plastic containers (1,000 ml; VWR International, Munich, Germany). Participants were carefully instructed to avoid stool contamination with water, urine or other materials (e.g. toilet paper). Moreover, participants were then asked (Appendix, Study document 13) to collect one plastic spoon at one location of the fecal material into a stool collection tube containing 8 ml DNA stabilization buffer (Stratec Moluclar GmbH, Berlin, Germany). Afterwards, feces sample were immediately frozen at -18 °C until the next visit. Participants transported the frozen fecal samples by using cooling aggregate to the lab. Finally, collection tubes were immediately stored at -80 °C.

Participants of the "Overnutrition" study collected one complete bowl movement directly in sterile plastic containers (1,000 ml; VWR International, Munich, Germany). Participants were instructed to avoid stool contamination with water, urine or other materials (e.g. toilet paper). Within one hour after collecting, participants were asked to bring their containers to the HSC in Freising, Germany (Appendix, Study document 14). The stool was immediately aliquoted into ten separate tubes with 100-200 mg stool each. Afterwards, the stool was immediately stored at -80 °C.

4.3 Laboratory analyses

4.3.1 Enzyme-linked immunosorbent assays

Leptin, chemerin, hsCRP, RANTES, MCP-1, HMW adiponectin, and LBP (all: R&D, Wiesbaden, Germany), insulin (Dako, Glostrup, Denmark), and zonulin (Immundiagnostik AG, Bensheim, Germany) were assayed in plasma using commercially available ELISAs. Fecal calprotectin was measured by ELISA (CALPROLAB™ Calprotectin ELISA (HRP), FROST Diagnostika GmbH, Otterstadt, Germany). All ELISAs were performed as described by the manufacturers. The ELISA is a well-known technique to determine and quantify biological molecules.

The principle of a Sandwich ELISA is shown in **Figure 9**. At first, primary (capture) antibody was coated in a 96-well microplate overnight. Afterwards, plasma containing the respective antigen is added and is recognised with high specificity by the capture antibody. In the next step, the biotinylated secondary (detection) antibody binds to the target analyte. Next, streptavidin with the conjugated horseradish-peroxidase is added which catalyses the colour reaction. Finally, a substrate solution which is a mixture of 3,3',5,5'-tetramethylbenzidine (TMB) and hydrogen peroxide (H_2O_2) undergoes a colorimetric change by HRP. The reaction is terminated after adding 1 molar sulphuric acid. The resulting absorbance was measured at 450 nm using a Tecan infinite M2000 reader (Tecan GmbH, Crailsheim, Germany). The optical imprecisions of the system were corrected by the reference wavelength of 540 nm. Finally, the optical density was proportional to the amount of detected target analyte in the sample. The calculation of the analyte concentration was done by constructing a standard curve. The standard curve was based on known concentrations of the analyte across a range of concentrations near the expected unknown concentrations. The concentration of unknown samples is determined by interpolation.

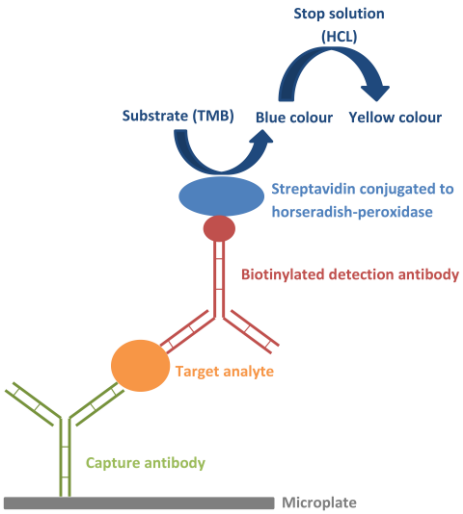


Figure 9: The principle of a Sandwich ELISA (description is shown in text above). HCL, hydrochlorid acid; TMB, 3,3',5,5'-tetramethylbenzidine

In addition to participants' plasma, also a blank (reagent diluent), control plasma (Precinorm U, Roche, Mannheim, Germany), and standards were included in the analysis. The blank is used to determine any variations of the plate itself. Therefore, the OD value of the blank was subtracted from each OD value of the samples on the plate. The control plasma was used for repeated measurements to monitor the inter-assay coefficient and the validity of the test. The standards are important to calculate a standard curve to assess the values of each participant. The concentration of each participant was assessed by interpolation of the regression curve formula in a form of quadratic equation using GraphPad Prism (Version 5). If there were any dilutions used, respective dilution factors were included in the calculations.

All ELISAs were done using a fully automated ELISA processor (ELISA STAR, Hamilton Bonaduz AG, Bonaduz, Switzerland). For the measurement, only original tips were used (1,000 µl high volume CO-RE Tips and 300 µl Standard Volume CO-RE Tips). Because of saving blood samples and due to the high precision of the ELISA STAR only single determinations, except the standards, were measured. In case of duplicate measurements (samples for standard curve) the mean value, standard deviation and coefficient of variation (CV [%] = standard deviation/mean value) were determined. The intra-assay CV of every analyte of both studies is listed in the following table (**Table 8**). The biochemical molecules hsCRP, leptin, HMW adiponectin, RANTES, MCP-1, chemerin, zonulin, and LBP were measured in both studies. Each analyte of each study could be measured on one plate.

Table 8: Intra-assay CV of each analyte of both studies

	Caloric restriction		Overnutrition	
Target analytes	**Number of Duplicates**	**Coefficient of variation**	**Number of Duplicates**	**Coefficient of variation**
hsCRP	10	6 %	10	6 %
Leptin	9	4 %	10	7 %
HMW adiponectin	9	3 %	10	2 %
RANTES	10	4 %	10	4 %
MCP-1	2	3 %	9	4 %
Chemerin	10	6 %	9	7 %
Zonulin	7	1 %	7	4 %
LBP	10	2 %	9	2 %
Insulin plate 1	-	-	6	1 %
Insulin plate 2	6	2 %	6	2 %
Insulin plate 3	6	2 %	-	-
Insulin plate 4	6	2 %	-	-
Insulin plate 5	6	6 %	6	6 %
Calprotectin plate 1	11	4 %	-	-
Calprotectin plate 2	11	3 %	-	-
Calprotectin plate 3	-	-	10	3 %
Calprotectin plate 4	3	1 %	3	1 %

HMW, high-molecular-weight adiponectin; hsCRP, high-sensitivity C-reactive protein; MCP-1, monocyte chemoattractant protein-1; LBP, lipopolysaccharide-binding protein; RANTES, regulated on activation, normal T-cell expressed and secreted

4.3.2 Acyl-CoA synthetase-Acyl-CoA oxidase-method

Nonesterified fatty acids (NEFA) concentration in plasma were measured by using a reagent enzymatic colorimetric method (Wako Chemicals GmbH, Neuss, Germany). The enzymatic reaction was carried out by 3-Methyl-N-Ethyl-N-(ß-Hydroxyethyl)-Anilin (MEHA) as a violet

colour agent. In general, NEFAs are transformed by Acyl-CoA synthase (ACS). The product Acyl-Co reacts with Acyl-CoA oxidase (ACOD) to 2.3-trans-Enoyl-CoA. Peroxidase (POD) changes hydrogen peroxide to a blue purple pigment. The NEFA concentration is obtained by measuring absorbance of the blue purple colour (**Figure 10**).

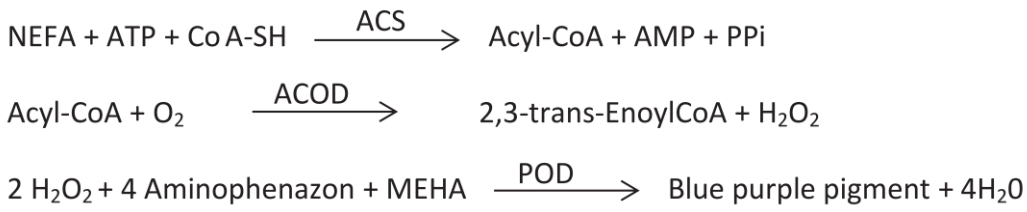


Figure 10: The principle of the ACS-ACOD-Method. ACOD, acyl-CoA oxidase; AMP, adenosinmonophoshphat; ATP, adenosintriphosphate; MEHA, 3-Methyl-N-Ethyl-N-(ß-Hydroxyethyl)-Anilin; NEFA, non-esterified fatty acids; POD, peroxidase

4.3.3 Gene expression analysis

DNA isolation, RNA extraction and cDNA synthesis

Firstly, DNA was isolated from abdominal SAT using the DNeasy Blood & Tissue Kit (Qiagen, Hilden, Germany). In the next step, total RNA from adipose tissue was isolated using the RNeasy Mini kit (Qiagen, Hilden, Germany) following the manufacturer's instruction. The quantity and quality of the isolated RNA was analysed spectrophotometrically (Tecan Infinite M200 with the NanoQuant16 Plate™, Männedorf, Switzerland). The absorbance was measured at 260 nm and 280 nm. Considering the fact, that the concentration is proportional to its photometric absorbance, the quantity of RNA can be determined. For assessing the purity of the nucleic acids, the ratio of the absorbance at the wavelengths of 260 nm and 280 nm was checked. In the samples, the 260:280 ratio was very close to 2.0 for RNA. It is an indication for high RNA purity and integrity. A complementary deoxyribonucleic acid (cDNA) synthesis kit (Applied Biosystems, Darmstadt, Germany) was used to transcribe RNA into cDNA with an input of 100 ng total RNA per sample, followed by an dilution (1:10) with ultrapure PCR water.

qPCR analysis

The expression of specific target genes was assessed by using quantitative polymerase chain reaction (qPCR). The primer sequences were selected and the specificity of the gene was proven by the online resource: http://www.ncbi.nlm.nih.gov/tools/primer-blast/. In **Table 9,** the primer pairs (Eurofins MWG Synthesis GmbH, Ebersberg, Germany) are shown:

Table 9: Primer paires used for qPCR

Primer	Forward	Reverse	Tm [°C]
Adiponectin	5'-ggtgagaagggtgagaaagga-3'	3'-acactgaatgctgagcggta-5'	62,6
Leptin	5'-tccaagatgacaccaaaaccc-3'	3'-acgtgaagaagatcccggag-5'	61,2
CD68	5'-gctacatggcggtggagtacaa-3'	3'-atgatgagaggcagcaagatgg-5'	61,2
MCP-1	5'-gcaatcaatgccccagtc-3'	3'-ggtggtccatggaatccga-5'	57,4
IPO8	5'-cggattatagtctctgaccatgtg-3'	3'-tgtgtcaccatgttcttcagg-5'	60
PPIA	5'-tggttcccagtttttcatc -3'	3'-cgagttgtccacagtcagc-5'	60

CD68, cluster of differentiation; IPO8, importin 8; MCP-1, monocyte chemoattractant protein-1; PPIA, peptidylprolyl isomerase A; qPCR, quantitative polymerase chain reaction; Tm, melting temperature

qPCR was performed by the Mastercycler® ep realplex (Eppendorf, Hamburg, Germany). Target and housekeeping genes were amplified by SYBR Green (Thermo Fisher Scientific, Darmstadt, Germany). IPO8 and PPIA were used for normalization (Dankel et al., 2014) (McCulloch et al., 2015). Each reaction sample contained 2.4 µl RNAse free water, 0.3 µl of forward and reverse primer (0.3 µM end concentration), 5.0 µl SYBR Green, and 2 µl diluted cDNA. Mastercycler conditions were as follows: 95°C hot start to activate the polymerase for 10 min, followed by 40 cycles of specific annealing temperature of each target gene for 1 min. Each sample was run in duplicate, and additional controls without cDNA. Controls were included in each set of experiment. The calculation of relative expression was performed by using the relative expression software tool REST© (http://rest.gene-quantification.info/) (Pfaffl et al., 2002).

4.3.4 High-throughput 16S ribosomal RNA gene amplicon sequencing

Samples were processed as described previously (Lagkouvardos et al., 2015). Briefly, cells were lysed by bead-beating and heat-treatment and the metagenomic DNA was purified using gDNA columns (Macherey-Nagel, Düren, Germany). Concentrations and purity were tested using the NanoDrop® system (Thermo Scientific, Waltham, Massachusetts, USA). The V4 region of 16S rRNA genes was amplified (25 cycles) from 24 ng DNA using primers 519F (5' CAG CMG CCG CGG TAA TWC) and 785R (5' GAC TAC HVG GGT ATC TAA TCC) (Klindworth et al., 2013). After purification (AMPure XP system, Beckmann Coulter Biomedical GMBH, Munich, Germany) and pooling in an equimolar amount, the 16S rRNA gene amplicon libraries were sequenced in paired-end modus (PE175) using a MiSeq system (Illumina, Inc., San Diego, California, USA) following the manufacturer's instructions.

Raw read files were processed based on the UPARSE approach (Edgar, 2013) using IMNGS (www.imngs.org) (Lagkouvardos et al., 2016). Sequences were tested for the presence of chimeras using UCHIME (Edgar, 2010) and operational taxonomic units (OTU) were clustered at a threshold of 97 % sequence similarity. To avoid analysis of spurious OTUs, only those with a

relative abundance >0.5 % total sequences in at least one sample were kept. SILVA (SILVA Incremental Aligner version 1.2.11) (Quast et al.) and RDP classifier (set 15; 80% confidence) (Wang et al., 2007) were used to assign taxonomic classification to the OTUs representative sequences. Specific OTUs with differential abundances between groups were further identified using EzTaxon (http://www.ezbiocloud.net/). A species-level identification was reported for these OTUs under the condition that the 16S rRNA gene sequence of only one cultured strain in the database returned a similarity >97 %. If several hits above this conservative species-level threshold were obtained or if the closest relative was >95 %, OTUs identity was reported as unknown species within the corresponding genus. Phylogenetic relationships were examined using the generalized UniFrac procedure (Chen et al., 2012). Shannon-effective counts were determined to estimate diversity within samples (*alpha*-diversity) as described by Jost et al. (Jost, 2007)

4.3.5 Determination of fat cell size

Fat cells were fixed in paraffin (Surgipath Paraplast®, Sigma-Aldrich Chemie GmbH, USA) for 24 hours after sampling. Afterwards, 5 µm thick sections were obtained using a rotary microtome (Leica RM2255, Leica Biosystems Nussloch GmbH, Nussloch, Germany). The sections were stained with Hematoxylin & Eosin. Digital photographs of the Hematoxylin & Eosin stained slides were generated with fluorescence microscopy (Leica DMI 4000B, Wetzlar, Germany) using a Texas red filter cube. Additionally, images of the adipocyte sections were acquired. Pictures were obtained with a scale bar attached. This allows calculating the scaling factor from pixels to µm later. Determination of adipocyte size was done by using open source cellprofiler® image analysis software (http://www.cellprofiler.org/). The adipocyte area was exported in pixels and was converted into area in µm² by multiplying area with a scaling factor (**Figure 11A**). The diameter was determined in two steps. Firstly, the area in pixels was converted to diameter in pixels (**Figure 11B**). Afterwards, diameter in µm was calculated by a scaling factor and diameter in pixel (**Figure 11C**). Before intervention, 319 ± 89 adipocytes and after intervention 270 ± 104 adipocytes per participant were analysed.

$$A) \quad Area\ (\mu m^2) = Area\ (pixel)\ x\ scaling\ factor^2$$

$$B) \quad Diameter\ (pixel) = \sqrt{\frac{4x\ Area\ (pixel)}{\pi}}$$

$$C) \quad Diameter\ (\mu m) = Diameter\ (pixel)\ x\ scaling\ factor$$

Figure 11: Determination of adipocyte size

4.3.6 Peripheral Blood Mononuclear Cells

The PBMCs were isolated via Ficoll gradient density centrifugation. This method enabled an effective isolation of this cell type. First, the heparinised blood was diluted with sterile PBS (Phosphate buffered saline, Biochrom, Berlin, Germany) at a ratio 1:2 (**Figure 12A**). Afterwards,

PBS mixed blood was layered on the top of the Ficoll (Biochrom, Berlin, Germany) solution (**Figure 12B/C**). Finally, the tube was spun (400 *g*, 25 min, accelleration 1, break 0). Because of the different density of the Ficoll solution (1.077 g/ml), red blood cells (>1.077 g/ml), and lymphocytes (<1.077 g/ml), different layers could be identified after centrifugation (**Figure 12D**). The lymphocytes were harvested (**Figure 12E**) and washed with PBS by centrifugation. The supernatant were aspirated and the PBMCs were counted by countess™ automated cell counter (Invitrogen, Carlsbad, CA USA). After counting the PBMCs, the PBMCs were splitt for measuring RNA and ROS and for doing flow cytometry analysis.

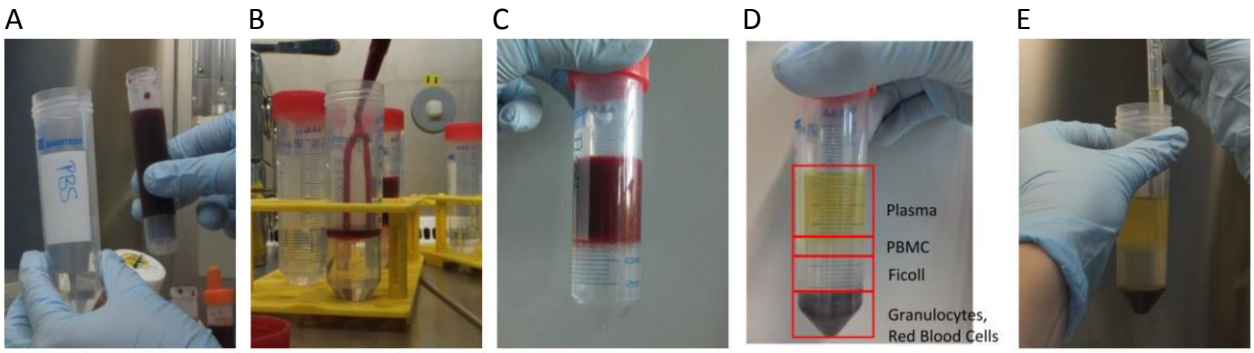


Figure 12: PBMC isolation via Ficoll gradient density centrifugation. A) PBS and blood was mixed. B/C) PBS-Blood mixture was overlayed on the top of the Ficoll. D) Identification of the PBMC layer. E) Harvest PBMCs. PBMC, peripheral blood mononuclear cells.

4.3.7 Flow cytometry

The surface and intracellular markers were stained with different fluorescence labelled antibodies. Thereby, the different cell populations could be characterised.

Staining

Frozen PBMCs were thawed and resuspended in a mixture containing RPMI (Roswell Park Memorial Institute, Gibco, Karlsruhe Germany) and 10 % heat inactivated FBS (Fetal Bovine Serum Gold, PAA Laboratories, Pasching, Austria). Afterwards, the medium was rinsed away and surface antibodies were added. After 20 minutes of incubation at 4 °C, the cells were mixed with FACS buffer (PBS with 2 % heat inactivated FCS Gold). Nonspecifically binding antibodies were washed away. In the next step, a fixating buffer was added for 10 minutes to stabilize the antigen-antibody binding. To enable intracellular staining, the plasma membrane had to be permeabilised. Cells were incubated for 25 minutes with Perm Buffer III (BD Bioscience, Heidelberg, Germany). Washing steps followed and finally the intracellular markers were added. This step was followed by 25 minutes of incubation on ice in the dark. Before measurement cells were washed and resuspended in FACS buffer.

Measurement

The flow cytometry is able to analyse multiple characteristics of single cells. This method is used for immunophenotyping. In general, flow cytometry consists of three different components: fluidics system, optics system and electronics system. Firstly, the fluorescently labelled cells in the sample were carried to the laser beam in a fluid system for interrogation. Cells were forced into the centre of the stream forming a single file by the principle of hydrodynamic focusing. When cells pass through the laser intercept, they scatter light. Forward- scattered light is proportional to cell-surface area or size. Side-scattered light is proportional to cell granularity. The scattered light is sorted in several wavelength packages by mirrors and filters in the optics system. Each fluorophore got a specific absorption spectrum and can be identified by special detectors. The light signals are converted to electronical signals. Data acquisition was done by a computer connected to the flow cytometer. The data were analysed by the software FACSDiva 6.0 (BD Biosciences, USA).

Settings

According to the laser and filter setting of the BD LSR II flow cytometer at the Comprehensive Pneumology Centre Munich, Germany (see **Table 10**) and our immunophenotyping setting the following combinations were chosen. The fluorochromes were selected carefully and the primary aim was to minimise spectral overlapping. Moreover, the following voltages of each fluorochrome were chosen (see **Table 11**).

Combination 1: CD3 CD4 CD8 CD25 FoxP3
Combination 2: CD14 CD16 CD19 HLA-DR
Combination 3: CD3 CD4 CD8 CD14 CD19 S6

Table 10: Laser and filter setting of BD LSR II

Laser	Filter	Fluorochrome
Blue (488 nm)	A 780/60	PE-Cy7
	B 695/40	PE-Cy5
	C 575/26	PE
	D 530/30	FITC
	E 488/10	SSC
Violet (405 nm)	B 450/50	Horizon V450
Red (633 nm)	B 660/20	APC, Alexa Fluor 647

APC, allphycocyanin; FITC, fluorescein; SSC, side scatter; PE, phycoerythrin

Table 11: Voltage settings of each fluorochrome

Fluorochrome	Combination 1	Combination 2	Combination 3
	CD3 CD4 CD8 CD25 FoxP3	CD14 CD16 CD19 HLA-DR	CD3 CD4 CD8 CD14 CD19 S6
FSC	420	420	420
SSC	198	198	198
FITC	453	453	453
PE	470		470
Pe-Cy7	600	650	600
Pe-Cy5		650	
APC	613		613
Alexa Fluor 700	600		600
V450		500	600

APC, allophycocyanin; FITC, fluorescein; FSC, forward scatter; SSC, side scatter; PE, phycoerythrin

Flourochrome Combination

According to the FACS settings, antibody-fluorochrome combinations were chosen. BD provided a fluorescence spectrum viewer which enabled to illustrate the emission spectra of all selected fluorochrome in one chart. Additionally filter settings from the LSR II at the CPC could be included in the programme. The Spectrum Viewer enabled to check the selected fluorochromes on overlapping to minimise spectral overlapping for each antibody- fluorochrome combination. The following **figures 13-15** depict the BD Spectrum Viewer for the antibody-fluorochrome combinations which were chosen for analysis. (https://www.bdbiosciences.com/br/research/multicolor/spectrum_viewer/index.jsp)

Combination 1: CD3 (PE-Cy7), CD4 (APC), CD8 (Alexa Fluor 700), CD25 (FITC), FoxP3 (PE)

Combination 2: CD14 (V450), CD16 (PE-Cy7), CD19 (FITC), HLA-DR (PE-Cy5)

Combination 3: CD3 (Pe-Cy7), CD4 (APC), CD8 (Alexa Flour), CD14 (V450), CD19 (FITC), S6 (PE)

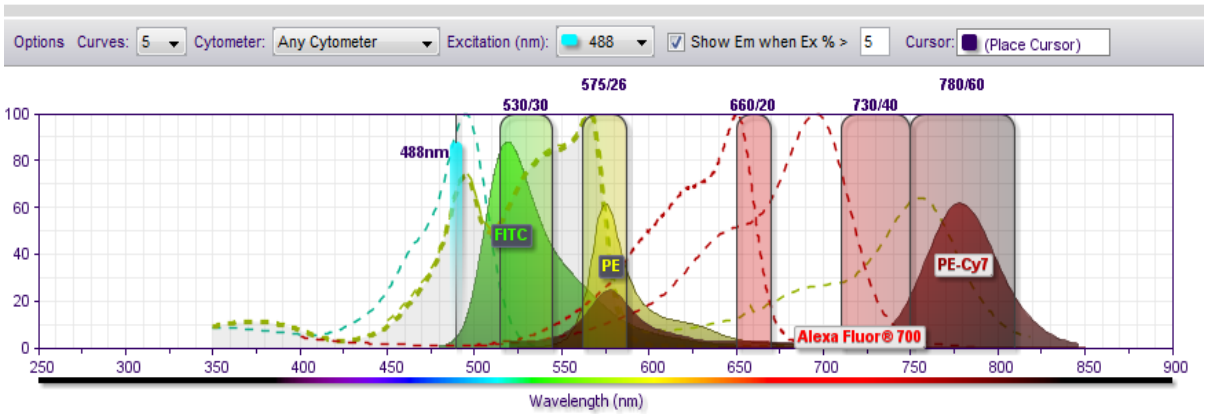


Figure 13: Fluorochrome illustration combination 1. The figure shows the wavelength and emission spectra of FITC, PE, Alexa Fluor 700, Pe-Cy7 and APC in dependence on the selected filter, illustrated by BD Spectrum Viewer

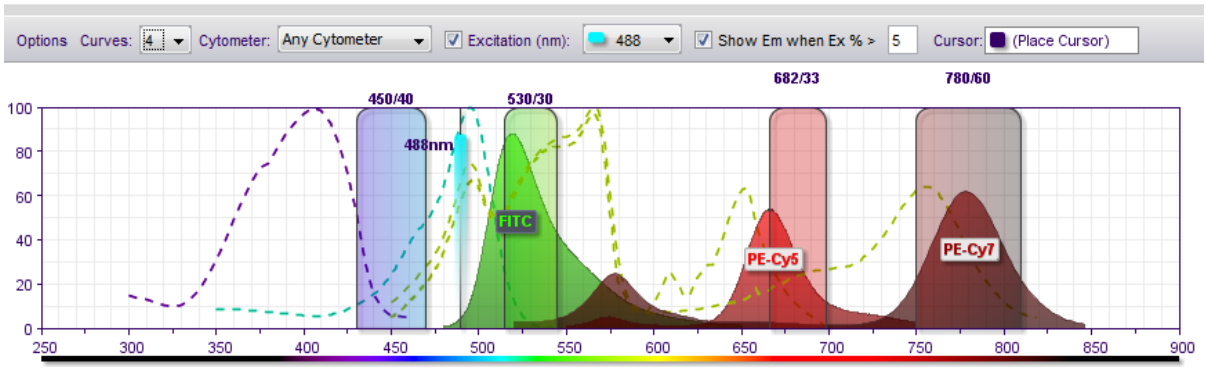


Figure 14: Fluorochrome illustration combination 2. The figure shows the wavelength and emission spectra of V 450, FITC, Pe-Cy5 and Pe-Cy7 in dependence on the selected filter, illustrated by BD Spectrum Viewer

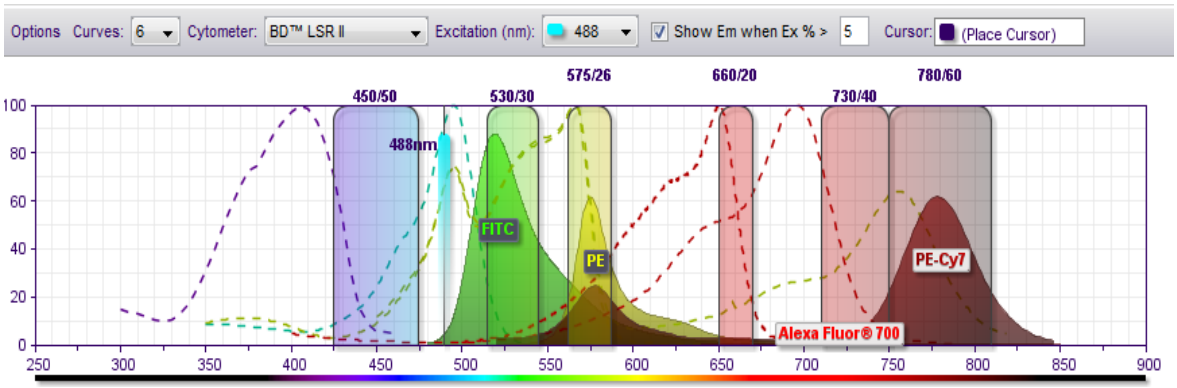


Figure 15: Fluorochrome illustration combination 3 .The figure shows the wavelength and emission spectra of V 450, FITC, PE, APC, Alexa Fluor 700 and Pe-Cy7 in dependence on the selected filter, illustrated by BD Spectrum Viewer

Compensation and Isotype Controls

Before starting the measurements, compensation controls were used to correct spectral overlapping. Compensation is always necessary when multicolor analyses are performed. For each fluorochrome PBMC were stained and measured by flow cytometry. Finally, marked compensations were taken into consideration for the following measurements. Additionally, isotype antibodies were used to detect unspecific binding of an antibody.

Gating strategy

As shown in the following **figures 16-18**, each fluorochrome combination had its specific gating strategy. Therefore, isotypes were used to separate the unspecific binding from the specific binding. The gating for the isotypes were firstly set and were not changed for the gating of the specific binding cells. The following gates show the results of extra- and intracellular staining:

Combination 1: The gating strategy of combination 1 is shown in **figure 16**. Firstly, PBMCs were separated in lymphocytes and monocytes in a FSC/SSC dot plot **(A)**. Secondly, all lymphocytes were selected in a histogram to ascertain CD3 positive cells **(B)**. Afterwards, based on the CD3 positive cells, CD4 and CD8 positive cells were determined in a dot plot **(C)**. In the next step, CD25 positive cells are illustrated in the CD4/CD25 dot plot **(D).** T_{regs} (FoxP3 positive cells) were determined in the CD4/FoxP3 dot plot **(E)**. To detect the number of all T_{regs}, a histogram was generated **(F).** The population hierarchy is shown in chart (**G)**.

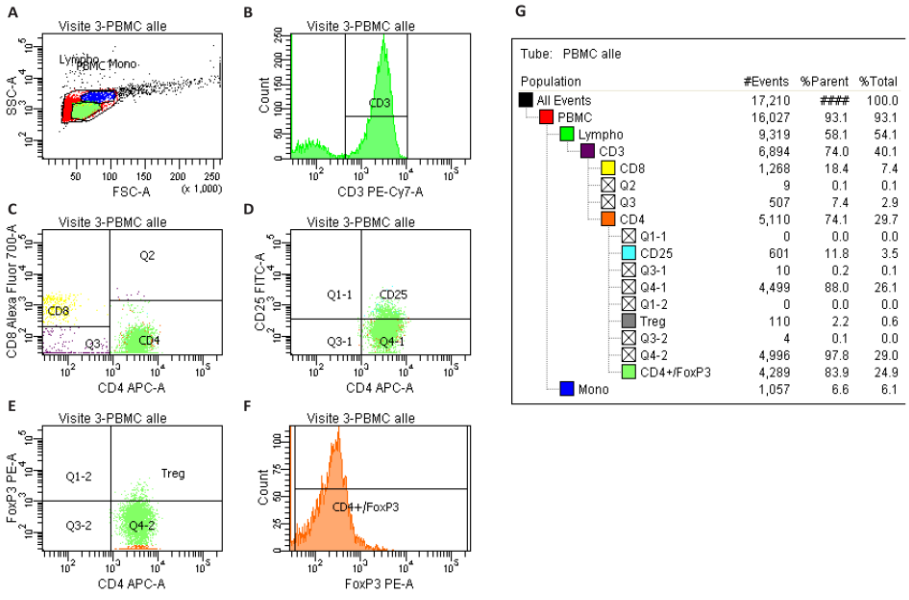


Figure 16: Gating strategy CD3/CD4/CD8/CD25/FoxP3

Combination 2: The analysis of the monocytes and B cells was conducted within combination 2. The gating strategy of combination one is shown in **figure 17**. Firstly, PBMCs were separated in lymphocytes and monocytes in a FSC/SSC dot plot **(A)**. Secondly, CD19 positive cells were selected from all lymphocytes in a histogram **(B)**. Afterwards, CD14 positive cells were determined in a histogram **(C)**. CD14 positive HLA-DR cells were selected in a histogram **(D)**. To exclude NK cells based on their lack of HLA-DR expression, CD14 was plotted against HLA-DR **(E)**. Afterwards, all monocytes subtypes were differentiated in a CD14/CD16 plot. The cells are then categorized into classical, non-classical and intermediate monocytes **(F)**. The population hierarchy is shown in chart (**G**).

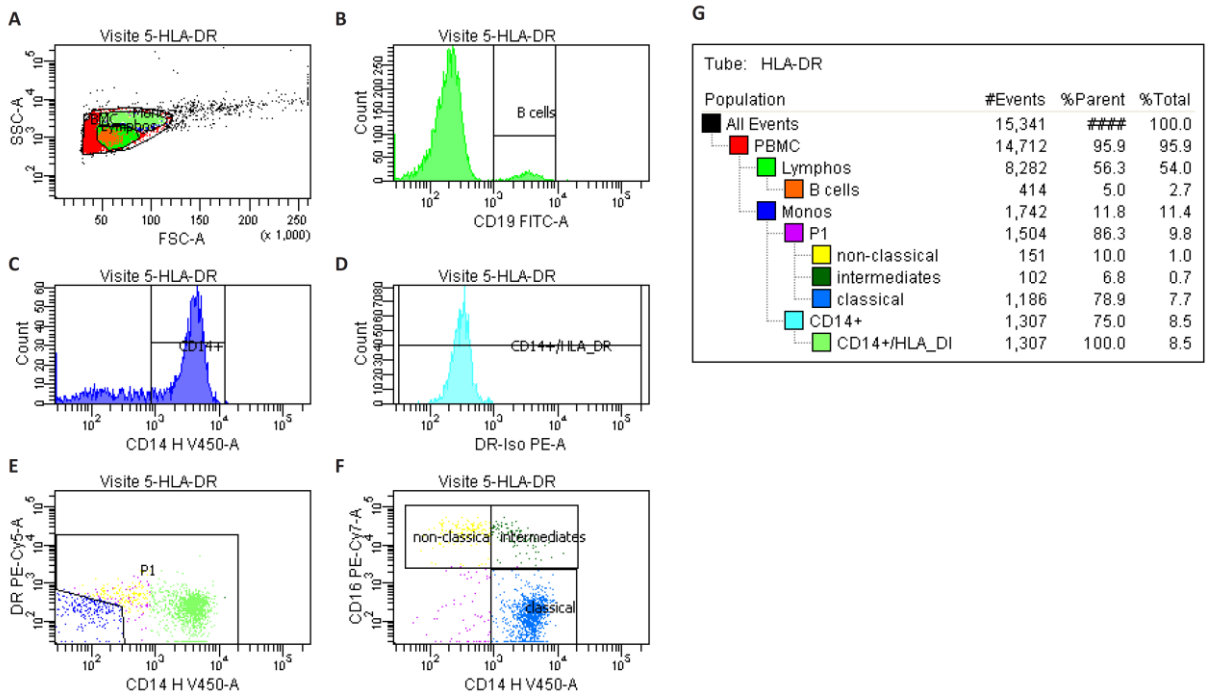
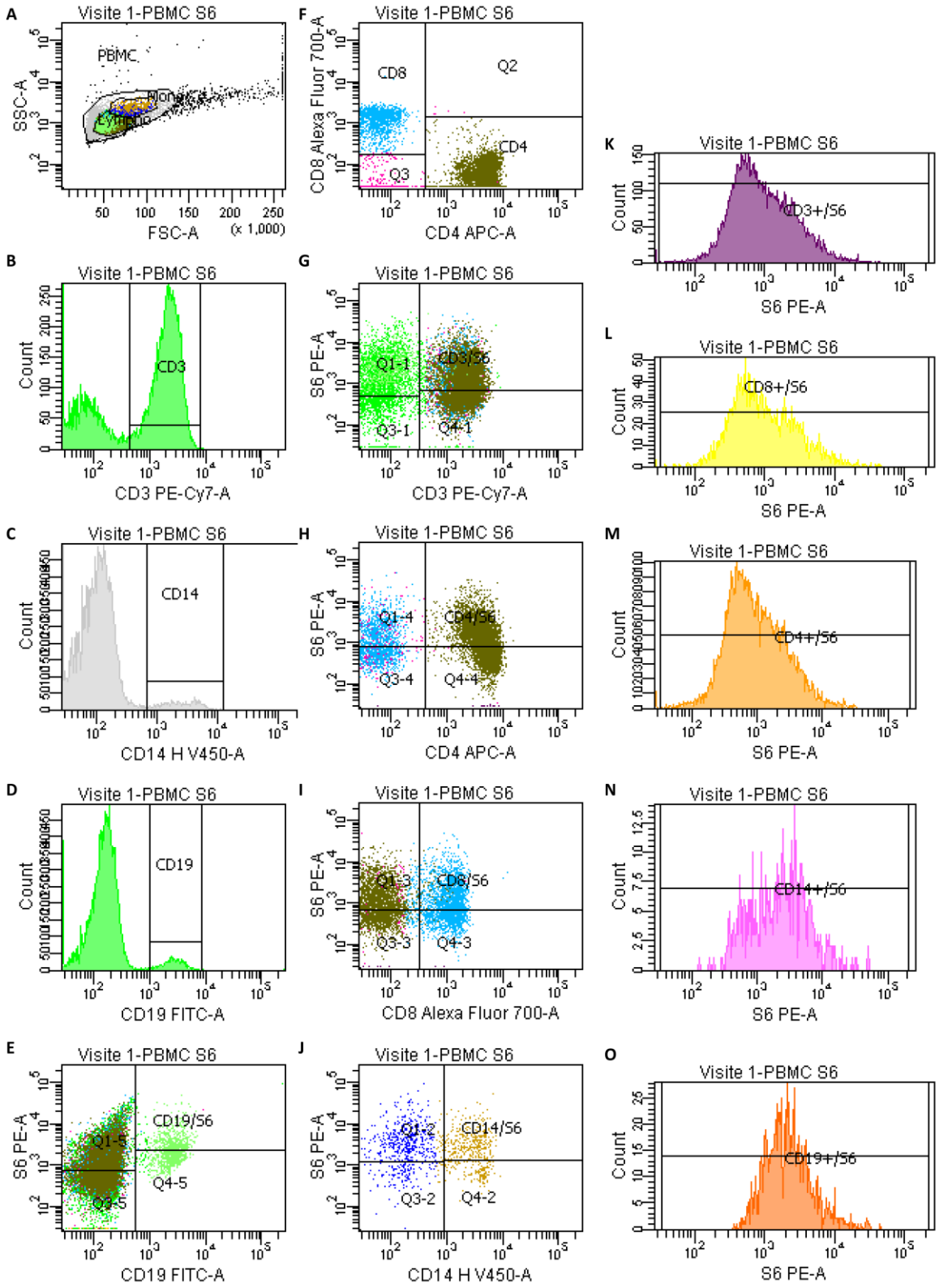


Figure 17: Gating strategy CD19/CD14/CD16/HLA-DR

Combination 3: The third combination aimed to determine the intracellular S6 levels in different immune cell subpopulations. The gating strategy is shown in **figure 18**. Firstly, PBMCs were separated in lymphocytes and monocytes in a FSC/SSC dot plot **(A).** Secondly, CD3 positive cells **(B)**, CD 14 positive cells **(C)**, and CD19 positives cell **(D)**, were plotted in a histogram. Thirdly, CD4 and CD8 positive cells were determined within the CD3 positive population in a dot plot **(F)**. Afterwards, each immune cell population was gated against S6: CD19+/S6 **(E),** CD3+/S6 **(G),** CD4+/S6 **(H),** CD8+/S6 **(I),** CD14+/S6 **(J)**. Positive S6 cells are selected and plotted in a histogram **(K, L, M, N, O).** Plot **(P)** shows the population hierarchy.


A
Visite 1-PBMC S6
PBMC
Monocytes
Lymphocytes
SSC-A
FSC-A
(x 1,000)
B
Visite 1-PBMC S6
CD3
Count
CD3 PE-Cy7-A
C
Visite 1-PBMC S6
CD14
Count
CD14 H V450-A
D
Visite 1-PBMC S6
CD19
Count
CD19 FITC-A
E
Visite 1-PBMC S6
CD19/S6
Q1-5
Q4-5
Q3-5
S6 PE-A
CD19 FITC-A
F
Visite 1-PBMC S6
CD8
Q2
CD4
Q3
CD8 Alexa Fluor 700-A
CD4 APC-A
G
Visite 1-PBMC S6
CD3/S6
Q1-1
Q3-1
Q4-1
S6 PE-A
CD3 PE-Cy7-A
H
Visite 1-PBMC S6
CD4/S6
Q1-4
Q3-4
Q4-4
S6 PE-A
CD4 APC-A
I
Visite 1-PBMC S6
CD8/S6
Q1-3
Q3-3
Q4-3
S6 PE-A
CD8 Alexa Fluor 700-A
J
Visite 1-PBMC S6
CD14/S6
Q1-2
Q3-2
Q4-2
S6 PE-A
CD14 H V450-A
K
Visite 1-PBMC S6
CD3+/S6
Count
S6 PE-A
L
Visite 1-PBMC S6
CD8+/S6
Count
S6 PE-A
M
Visite 1-PBMC S6
CD4+/S6
Count
S6 PE-A
N
Visite 1-PBMC S6
CD14+/S6
Count
S6 PE-A
O
Visite 1-PBMC S6
CD19+/S6
Count
S6 PE-A

P

Tube: PBMC S6

Population	#Events	%Parent	%Total
All Events	24,555	####	100.0
PBMC	23,251	94.7	94.7
Lympho	15,225	65.5	62.0
CD3	8,805	57.8	35.9
CD8	2,420	27.5	9.9
Q1-3	66	2.7	0.3
CD8/S6	1,291	53.3	5.3
Q3-3	57	2.4	0.2
Q4-3	1,006	41.6	4.1
CD8+/S6	2,417	99.9	9.8
Q2	5	0.1	0.0
Q3	674	7.7	2.7
CD4	5,706	64.8	23.2
Q1-4	0	0.0	0.0
CD4/S6	2,829	49.6	11.5
Q3-4	0	0.0	0.0
Q4-4	2,877	50.4	11.7
CD4+/S6	5,693	99.8	23.2
Q1-1	0	0.0	0.0
CD3/S6	4,889	55.5	19.9
Q3-1	0	0.0	0.0
Q4-1	3,916	44.5	15.9
CD3+/S6	8,787	99.8	35.8
CD19	944	6.2	3.8
Q1-5	0	0.0	0.0
CD19/S6	405	42.9	1.6
Q3-5	0	0.0	0.0
Q4-5	539	57.1	2.2
CD19+/S6	944	100.0	3.8
Mono	1,100	4.7	4.5
CD14	507	46.1	2.1
Q1-2	18	3.6	0.1
CD14/S6	329	64.9	1.3
Q3-2	8	1.6	0.0
Q4-2	152	30.0	0.6
CD14+/S6	507	100.0	2.1

Figure 18: Gating strategy CD3/CD4/CD8/CD14/CD19/S6

Analysis of flow cytometry data

All samples were treated according to standard operation procedure by using the same machine (LSR II) and constant experimental conditions. We made sure that the samples contained the exact same number of cells and stained with the exact same concentration of antibody. Furthermore, as already mentioned, compensation and isotype controls were included. Analyses were done by using % of positive cells and Mean Flourescent Intensity (MFI) of positive cells to compare level of expression of a given receptor.

4.3.8 Electron Paramagnetic Resonance

Electron Paramagnetic Resonance spectroscopy (EPR) is a quantitative method detecting reactive oxygen species (ROS) with unpaired electrons (e.g. superoxide, hydroxyl radicals). EPR

is based on the absorption of electromagnetic radiation which is specific for each radical. As the absorption is proportional to the amount of a radical, the concentration of radicals in the sample can be calculated. In both studies EPR was used to detect superoxide radicals in plasma and PBMCs. Due to the short half-life time of superoxide radicals, these substrates had to be stabilized by a reaction which spin-trap solution (CMH, 1-hydroxy-3-methoxycarbonyl-2,2,5,5-tetramethylpyrrolidine, Noxygen Science Transfer & Diagnostics GmbH, Elzach, Germany) generating in a proportional matter a radical with a much higher half life time which makes it suitable for detection in the EPR. In plasma, in addition to spin trap, desferroxamine (DES, Sigma-Aldrich, St. Louis, Missouri, USA) for buffering iron ions and dietyldithiocarbamate (DETC, Sigma-Aldrich, St. Louis, Missouri, USA) for buffering iron and copper ions were added. Treatment with these chelating agents was necessary to remove traces of iron and copper ions from the reaction mix which otherwise could react with the spintrap and therefore could interfere with the measurement. A control with Krebs-Hepes buffer (KHB) instead of plasma with additional DES, DETC and spin trap was measured. Regarding PBMCs, the cells were isolated as described in section 3.3.6. Firstly, PBMCs were resuspended in KHB, DES, DETC and spin trap. Secondly, the mixture was slightly inverted and incubated for 20 min on ice and for 10 minutes on dry ice. Finally, it was stored at -80 °C. The control consisted of KHB, spin trap, DES, and DETC.

4.4 Statistical methods

Data were analysed in the R programming environment (Version 3.1.2; 2014-10-31 “Pumkin Helmet”). Anthropometric and metabolic data are presented as mean ± standard deviation. *P*-values <0.05 were regarded as statistically significant. The Shapiro-Wilk test was performed to test of normality. According to the distribution paired Student’s test or Wilcoxon-signed rank test were applied to assess mean differences before and after intervention. By using generalised least squares (*nlme* package, version 3.1-22) we included measurements in the same model to compare all three time points. Linear regression analysis and Spearman correlation coefficients were determined for the relationships. Mann-Whitney U test was used to compare the baseline values of the immune cell subpopulation at baseline on two different days. For the analysis of cell size of adipose tissue, 200 cells were selected randomly from histological section of each participants.

Rhea (v1.0.1-5) was used for analysis of fecal microbiota (Lagkouvardos et al., 2017). Detailed description of all tests and analyses performed is provided online (https://lagkouvardos.github.io/Rhea/). The effect of intervention on OTUs and taxonomic counts was tested using Friedman Rank Test for the analysis of a nonparametric randomized block design. Missing values were handled by using Skillings-Mack test. Wilcoxon Signed Rank Sum Test for matched pairs was applied for pairwise comparisons.. The Benjamini-Hochberg method was used for adjustment after multiple testing. For *beta*-diversity analysis, generalized UniFrac distances were calculated using the package GUniFrac (Chen et al., 2012).

5 Results

The results of the two studies are shown separately.

5.1 Caloric restriction

Twenty women with a mean age of 46.8 ± 11.5 years were included in this analysis and no participant fulfilled dropout criteria. Among the particpants, we identified 8 individuals with the metabolic syndrome (MetS) (Appendix, Supplemental Table 1) and seven participants were postmenopausal. Additional information on the participants including their history and lifestyle habits are shown in the Supplemental Table 2.

5.1.1 Dietary behaviour and physical activity

Participants were asked to keep a formula diet with an energy content of 800 kcal/day with additional 200 g of vegetables. Before and after intervention participants were instructed to record their food consumption. Compared to the original dietary habits, energy intake was decreased by about 800 kcal to 809 ± 32.7 kcal/day ($p < 0.0001$). Fat intake decreased from 69.3 ± 36.8 g/d to 20.3 ± 1.6 ($p < 0.001$). Protein intake decreased by 49.1 ± 36.5 g/day. Carbohydrates were reduced by 80 ± 66.3 g/day. During the following two weeks after formula diet participants returned to their usual dietary habits. Consequently, energy intake increased again **(Table 12)**.

In addition, participants received an activity sensor. On average, the 20 obese women walked 4.5 ± 1.3 km/day or 6,880 ± 1,962 steps/day.

Table 12: Macronutrient composition over time

	Mean ± Standard deviation (n=20)			p - value	
	before VLCD	during VLCD	14d after VLCD		
	t1	t2	t3	t1-t2	t2-t3
Energy intake (kcal/d)	1,698.1 ± 592.7	809.1 ± 32.7	1,281.9 ± 594.9	< 0.0001	< 0.0001
Fat intake (g/d)	69.3 ± 36.8	20.3 ± 1.6	54.6 ± 272.8	< 0.001	< 0.0001
Protein intake (g/d)	70.1 ± 22.8	52.8 ± 1.8	64.4 ± 18.4	< 0.01	< 0.01
Carbohydrate intake (g/d)	183.9 ± 65.4	103.5 ± 4.4	124.5 ± 14.5	< 0.0001	< 0.01

VLCD, very low caloric diet

5.1.2 Body composition

Physical and biochemical characteristics of the participants before intervention, immediately after the hypocaloric intervention and 14 days after intervention are shown in **Table 13**.

Twenty-eight days of caloric restriction resulted in a significant decrease in body weight (-6.9 ± 1.9 kg) corresponding to 7.2 ± 1.6 % of their initial body weight. This corresponded to a decrease in BMI (-2.5 ± 0.7 kg/m²), lean mass (-2.7 ± 1.6 kg), and body fat mass (-4.2 ± 1.6 kg). In addition, waist circumference (-5.7 ± 5.5 cm) and hip circumference (-4.5 ± 3.7 cm) were significantly decreased. Due to the same proportional decline of waist and hip circumference, the waist-to-hip ratio remained stable during the whole study. During the subsequent two weeks after the intervention phase, participants regained 250 ± 1.5 g ($p = 0.47$). The BMI, total fat mass ($p = 0.38$), waist circumference ($p = 0.44$), hip circumference ($p = 0.64$) did not change significantly during this post-study period. However, lean ($p < 0.01$) and the fat mass ($p = 0.02$) increased significantly during the last two weeks. The detailed decrease of body weight, waist and hip circumference during the four weeks is shown in **Table 14**. A linear regression model revealed at negative association between weight loss and baseline weight. The higher the baseline weight was, the higher the weight loss ($p < 0.01$, $r^2 = 0.39$) (**Figure 19**). Moreover, the association between waist circumference and total fat mass was significant as well ($p < 0.001$, $r^2 = 0.52$) (**Figure 20A/B**). As shown already in the publication of Cordes et al. (Cordes et al., 2015), a substantial decline of SAT (8.5 ± 4.4 %), VAT (15.1 ± 8.7 %), and liver fat fraction (40.3 ± 23.5) was also observed after the 4 weeks intervention phase in our investigation. Furthermore, waist circumference was significantly associated with SAT before VLCD (**Figure 21A**, p < 0-001, $r^2 = 0.52$) and after VLCD (**Figure 21B**, $p < 0.01$, $r^2 = 0.43$). Surprisingly, VAT (**Table 15**) and SAT (**Table 16**) were not significantly associated with metabolic factors such as fasting blood glucose, blood pressure and triglycerides.

Table 13: Body composition before, after and 14d after caloric restriction

	Mean ± Standard deviation (n=20)			p - value	
	before VLCD	**after VLCD**	**14d after VLCD**		
	t1	**t2**	**t3**	**t1-t2**	**t2-t3**
Weight (kg)	95.1 ± 13.4	88.2 ± 12.3	88.5 ± 12.6	< 0.0001	0.48
Body mass index (kg/m²)	34.9 ± 3.8	32.5 ± 3.5	32.6 ± 3.8	< 0.0001	0.38
Waist circumference (cm)	106.9 ± 10.6	101.2 ± 9.4	100.5 ± 9.3	< 0.001	0.44
Hip circumference (cm)	118.5 ± 12.6	113.9 ± 10.8	114.4 ± 11.5	< 0.001	0.64
WHR	0.9 ± 0.04	0.89 ± 0.06	0.88 ± 0.04	0.17	0.26
Lean Mass (kg)	52.7 ± 5.7	50.0 ± 5.5	50.9 ± 5.3	< 0.0001	< 0.01
Fat Mass (kg)	42.5 ± 8.8	38.2 ± 7.9	37.6 ± 8.4	< 0.0001	0.02
Liver Fat Fraction (%)	10.3 ± 8.3	5.5 ± 5.1		< 0.001	
SAT (cm³)	2,459 ± 800	2,248 ± 739		< 0.001	
VAT (cm³)	918 ± 561	780 ± 238		< 0.001	
Nonadipose tissue (cm³)	33.5 ± 6.8	35.6 ± 7.1		< 0.001	

SAT, subcutaneous adipose tissue; VAT; visceral adipose tissue; VLCD, very low caloric diet;;WHR,waist-to-hip ratio; Non-adipose tissue = mostly water

Table 14: Routine examination during 28 days of VLCD

	Mean ± Standard deviation (n=19)				
	before VLCD	**week 1**	**week 2**	**week 3**	**after VLCD**
Weight (kg)	95.1 ± 13.4	92.7 ± 13.2	91.2 ± 13.1	88.3 ± 10.7	88.2 ± 12.3
Pulse rate (HF/min)	72.4 ± 10.7	75.8 ± 12.3	73.2 ± 12.7	75.3 ± 14.7	69.0 ± 12.6
Blood pressure systolic (mmHg)	123.8 ± 11.5	119.6 ± 10.9	115.1 ± 12.7	110.9 ± 9.9	124.8 ± 42.4
Blood pressure diastolic (mmHg)	83.1 ± 7.4	81.27 ± 5.1	77.8 ± 5.9	76.9 ± 5.2	77.5 ± 9.2

HF, heart frequency; VLCD, very low caloric diet

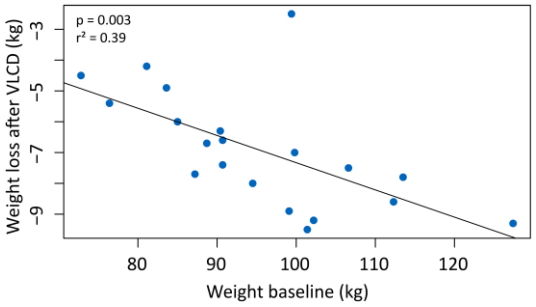


Figure 19: Association between weight loss and baseline body weight

A

B

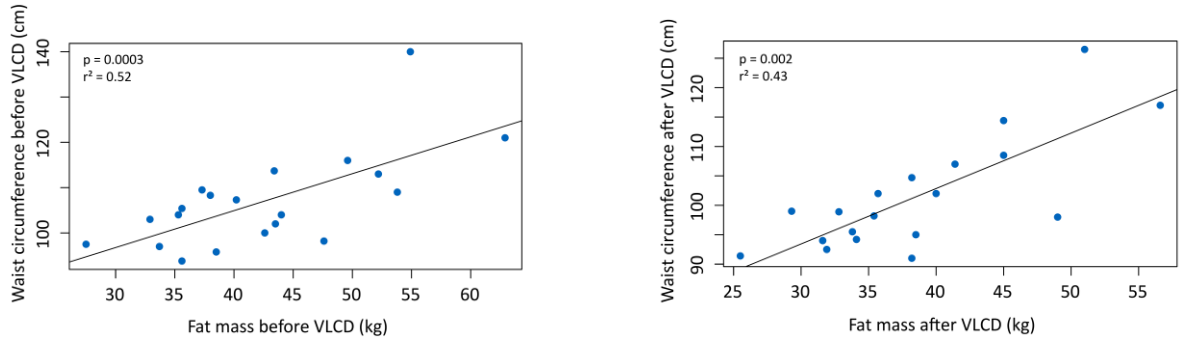


Figure 20: Association between waist circumference and total fat mass A) before intervention and B) after intervention

A B

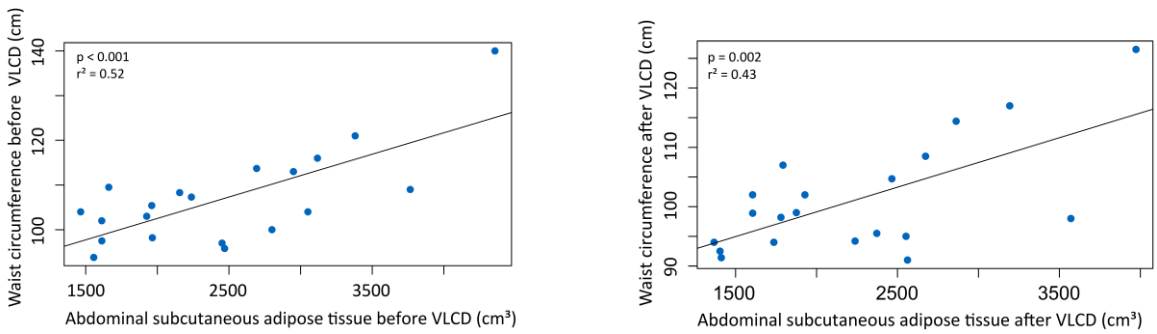


Figure 21: Association between waist circumference and abdominal subcutaneous adipose tissue A) before intervention and B) after intervention

Table 15: Association between metabolic factors and SAT before and after VLCD

	before VLCD		after VLCD	
	p-value	r^2	p-value	r^2
Fasting glucose/SAT	0.515	0.023	0.597	0.016
Systolic blood pressure/SAT	0.19	0.09	0.27	0.07
Diastolic blood pressure/SAT	0.856	0.001	0.727	0.007
Triglyceride/SAT	0.213	0.085	0.63	0.013

SAT, subcutaneous adipose tissue; VLCD, very low caloric diet

Table 16: Association between metabolic risk factors and VAT mass before and after VLCD.

	before VLCD		after VLCD	
	p-value	r^2	p-value	r^2
Fasting glucose/VAT	0.277	0.065	0.035	0.224
Systolic blood pressure/VAT	0.719	0.007	0.229	0.079
Diastolic blood pressure/VAT	0.253	0.072	0.351	0.049
Triglyceride/VAT	0.052	0.19	0.044	0.207

VAT, visceral adipose tissue; VLCD, very low caloric diet

5.1.3 Metabolic consequences of caloric restriction

Resting metabolic rate

The RMR and respiratory quotient did not change significantly during the four weeks of the intervention. As additional information, RMR was measured two weeks after intervention. The

RMR and respiratory quotient were significantly increased following the last two weeks (**Table 17**). As shown in **figure 22**, the higher the initial body weight, the higher the RMR. Moreover, the impact of weight loss on RMR was analysed. Regression analysis did not reveal significant evidence of an association (95% CI -10.36 (-46.36,25.64), p = 0.56).

Grouping the RMR in participants with and without MetS, it could be demonstrated that RMR in participants with MetS did not significantly alter during the whole study. In contrast, RMR in participants without MetS decreased significantly after 28 days of VLCD (p = 0.03) and increased during the following two weeks again (p < 0.01). Interestingly, the baseline RMR were not significantly different between participants with MetS and without MetS (p = 0.07). Regarding RMR in postmenopausal participants no differences were observed.

Table 17: Resting metabolic rate before, after and 14 days after intervention

		Mean ± Standard deviation				
	N	**before VLCD**	**after VLCD**	**14d after VLCD**	**p - value**	
		t1	**t2**	**t3**	**t1-t2**	**t2-t3**
RMR (kcal)	20	1,675.5 ± 29.4	1,598.0 ± 168.6	1,687.3 ± 170.6	0.07	< 0.001
Respiratory quotient	20	0.73 ± 0.12	0.68 ± 0.05	0.72 ± 0.05	0.06	< 0.001
RMR (kcal)/ Participants with MetS	08	1,797.12 ± 207.45	1,735.38 ± 140.66	1,767.50 ± 108.57	0.52	0.36
RMR (kcal)/ Participants without MetS	12	1,594.42 ± 199.57	1,506.42 ± 128.47	1,633.75 ± 145.58	0.03	<0.01
RMR (kcal)/ Postmenopausal participants	07	1,575.29 ± 250.20	1,486.43 ± 167.63	1,561.14 ± 200.23	0.19	0.07
RMR (kcal)/ No postmenopausal participants	14	1,729.46 ± 207.45	1,658.08 ± 140.66	1,755.15 ± 108.57	0.22	0.02

MetS, metabolic syndrome; RMR, resting metabolic rate

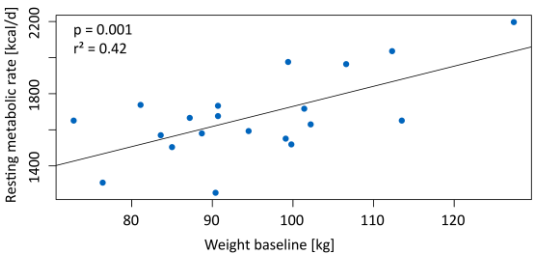


Figure 22: Resting metabolic rate dependent on initial body weight

Blood pressure and pulse rate

Systolic and diastolic blood pressure decreased 28 days after caloric restriction compared to baseline. During the last two weeks, pulse rate and blood pressure did not change significantly (**Table 18**).

Table 18: Results of the functional test before, after and 14 days of intervention.

	Mean ± Standard deviation (n=20)			p - value	
	before VLCD	after VLCD	14d after VLCD		
	t1	t2	t3	t1-t2	t2-t3
Pulse rate (HF/min)	72.4 ± 10.7	69.0 ± 12.6	70.8 ± 9.7	0.1	0.36
Blood pressure systolic (mmHg)	123.8 ± 11.5	124.7 ± 42.4	118.9 ± 11.6	0.02	0.58
Blood pressure diastolic (mmHg)	83.1 ± 7.4	77.5 ± 9.2	79.4 ± 7.6	< 0.001	0.18

VLCD, very low caloric diet; HF, heart frequency

Metabolism and inflammation

All metabolic data are shown in **Table 19**. Regarding the analysis of liver enzymes, AST and ALT did not change significantly during the study period. γGT significantly decreased during the 28 days of caloric restriction. After VLCD, γGT returned to its baseline value. The creatine level increased by 0.02 ± 0.1 mg/dl after VLCD and returned to the baseline status during the subsequent two weeks. Uric acid significantly decreased ($p = 0.01$) during the VLCD and increased again during the last two weeks. Electrolytes like sodium and potassium were stable during the whole study. Total cholesterol (-28.6 ± 22.9 mg/dl), HDL-cholesterol (-7.6 ± 5.6 mg/dl), LDL-cholesterol (-18.9 ± 18.7 mg/dl), and triglycerides (-23.5 ± 46.2 mg/dl) decreased significantly following the VLCD. During the subsequent two weeks, these three parameters of the lipid profile increased significantly ($p < 0.001$ each) again. In addition, we observed a significant ($p = 0.03$) increase in NEFA during VLCD. During the subsequent two weeks, NEFA decreased significantly ($p < 0.0001$). HMW adiponectin was increased and leptin was reduced after 28 days of caloric restriction. The chemokines RANTES and MCP-1 did not change significantly during the whole study. Chemerin showed a significant decrease after caloric restriction (-2.8 ± 17.0 ng/ml). After the intervention, chemerin increased significantly ($p = 0.003$) again. The hypocaloric diet induced a decrease of the inflammation marker hsCRP ($p < 0.001$) and significantly returned during the subsequent two weeks. LBP levels, a marker of gram-negative bacterial infection, significantly decreased ($p < 0.01$) and significantly increased again during the last two weeks ($p < 0.0001$). The detection of fecal calprotectin did not change significantly during the diet ($p = 0.79$). During the last two weeks, calprotectin decreased significantly ($p = 0.0.2$).

In addition to the measurements of inflammation markers in plasma, gene expression in SAT was examined for different inflammation markers. qPCR analysis (**Table 20**) demonstrated a significant down-regulation of leptin by a mean factor of 0.45 (standard error range is 0.18 -

1.07) after VLCD in comparison to the baseline status (p = 0.001). However, adiponectin, MCP-1, and CD68 were not significantly different after the hypocaloric intervention.

Table 19: Metabolic profile during the three study time points

	Mean ± Standard deviation (n=20)			p - value	
	before VLCD	after VLCD	14d after VLCD		
	t1	t2	t3	t1-t2	t2-t3
AST (U/l)	17.4 ± 4.7	19.1 ± 5.7	21.1 ± 10.0	0.14	0.43
ALT (U/l)	20.0 ± 5.4	20.89 ± 6.7	24.9 ± 12.4	0.59	0.18
γGT (U/l)	23.7 ± 19.1	14.6 ± 9.8	23.6 ± 27.3	< 0.01	0.04
Creatinine (mg/dl)	0.7 ± 0.2	0.8 ± 0.2	0.7 ± 0.2	0.34	0.03
Uric acid (mg/dl)	5.9 ± 1.5	5.4 ± 1.6	5.8 ± 1.6	0.01	0.09
Sodium (mmol/ml)	140.9 ± 2.2	142.1 ± 2.1	142.9 ± 1.9	0.05	0.11
Potassium (mmol/l)	4.4 ± 0.3	4.4 ± 0.2	4.3 ± 0.4	0.66	0.69
Calcium (mmol/l)	2.3 ± 0.1	2.4 ± 0.1	2.3 ± 0.1	< 0.0001	0.02
Total cholesterol (mg/dl)	191.1 ± 39.1	162.5 ± 29.5	185.5 ± 32.9	< 0.001	< 0.0001
HDL-cholesterol (mg/dl)	51.6 ± 9.3	44.0 ± 9.2	50.8 ± 9.8	< 0.0001	< 0.001
LDL-cholesterol (mg/dl)	118.5 ± 37.2	99.6 ± 26.1	110.4 ± 30.6	< 0.001	< 0.001
Triglycerides (mg/dl)	121.5 ± 64.2	98.1 ± 48.1	110.8 ± 63.2	0.05	0.15
LDL/HDL	2.4 ± 0.8	2.3 ± 0.7	2.2 ± 0.7	0.68	0.33
NEFA (mmol/l)	0.6 ± 0.2	0.7 ± 0.2	0.5 ± 0.2	0.03	< 0.0001
Leptin (ng/ml)	43.8 ± 25.2	20.9 ± 16.1	25.9 ± 20.3	< 0.0001	0.04
HMW adiponectin (mg/ml)	0.4 ± 0.2	0.5 ± 0.3	0.5 ± 0.3	< 0.01	0.12
MCP-1 (pg/ml)	82.1 ± 32.6	84.5 ± 23.4	89.1 ± 25.5	0.49	0.4
RANTES (ng/ml)	47.6 ± 19.3	45.6 ± 22.6	39.9 ± 24.2	0.28	0.16
Chemerin (ng/ml)	77.6 ± 25.7	64.8 ± 20.2	75.7 ± 21.1	< 0.01	< 0.01
hsCRP (mg/dl)	0.3 ± 0.4	0.2 ± 0.2	0.3 ± 0.3	< 0.01	0.01
LBP (mg/dl)	2.7 ± 0.3	2.6 ± 0.4	2.8 ± 0.4	< 0.01	< 0.0001
Calprotectin (mg/kg)	21.4 ± 27.9	17.2 ± 17.6	7.2 ± 6.7	0.79	0.02

ALT, alanine transaminase; γGT, γ-glutamyltransferase; AST, aspartate aminotransferase; HDL, high-density cholesterol; HMW, high-molecular weight; hsCRP, high-sensitivity C-reactive protein; LBP, lipopolysaccharide binding protein; LDL, low-density cholesterol; MCP-1, monocyte chemoattractant protein-1; NEFA, non-esterified fatty acids; RANTES, regulated on activation normal T cell expressed and secreted; VLCD, very low caloric diet

Table 20: Gene expression in SAT compared to baseline status.

Gene	p - value	regulation
	t1-t2	
Leptin	< 0.0001	down
Adiponectin	0.912	no
CD68	0.178	no
MCP-1	0.612	no

Reference genes were PPIA and IPO8. CD68, cluster of differentiation; IPO 8, importin 8; MCP-1, monocyte chemoattractant protein-1; PPIA, peptidylprolyl isomerase A; SAT; subcutaneous adipose tissue

Glucose metabolism

Regarding markers of glucose metabolism, fasting glucose (-6.8 ± 7.9 mg/dl), plasma insulin (-2.9 ± 4.0 µU/ml), and Homeostasis model assessment-insulin resistance (HOMA-IR) index (-0.8 ± 0.9) were significantly lowered ($p < 0.001$ each) after VLCD compared to baseline values. During the last two weeks of the study (follow-up after intervention), plasma glucose significantly increased ($p = 0.04$), whereas plasma insulin levels and HOMA-IR did not change significantly. Post-glucose concentration during the glucose tolerance test did not change significantly ($p = 0.16$) (**Table 21**).

Table 21: Glucose and insulin concentrations in blood during the whole study

		Mean ± Standard deviation			p - value	
		before VLCD	after VLCD	14d after VLCD		
	N	t1	t2	t3	t1-t2	t2-t3
Fasting blood glucose (mg/dl)	20	87.3 ± 11.8	80.4 ± 7.1	84.1 ± 11.1	< 0.001	0.04
120 min blood glucose (mg/dl)	18	117.7 ± 43.7	123.8 ± 24.1		0.16	
Fasting insulin level (µU/ml)	20	8.9 ± 6.2	5.9 ± 2.7	8.3 ± 8.8	< 0.0001	0.11
HOMA-IR	20	2.1 ± 1.5	1.3 ± 0.7	1.8 ± 1.8	< 0.0001	0.12

HOMA-IR, homeostasis model assessment of insulin resistance; VLCD, very low caloric diet; HOMA-IR was calculated by the following formula: HOMA-IR=insulin (µU/ml) x glucose (mmol/l)/22.5 (Matthews et al., 1985)

Reactive oxygen species

The ROS production rate both in plasma and PBMC was not altered during the VLCD and the subsequent two weeks (**Table 22**).

Table 22: ROS production rate in plasma and PBMC over time

		Mean ± Standard deviation			p - value	
		before VLCD	after VLCD	14d after VLCD		
	N	t1	t2	t3	t1-t2	t2-t3
ROS production rate in plasma (µM superoxide /µg protein/10min Plasma)	20	0.0089 ± 0.004	0.0089 ± 0.002	0.0084 ± 0.002	0.3	0.4
ROS production rate in PBMC (µM superoxide /µg protein/10min PBMC)	17	0.0074 ± 0.005	0.0094 ± 0.007	0.0073± 0.002	0.4	0.2

ROS, reactive oxygen species; VLCD, very low caloric diet

PBMC – immune cell subpopulations

The effect of the 4-week VLCD on immune cell subpopulations was assessed by flow cytometry (**Table. 23**). The first combination of antibodies was chosen to focus on the status of regulatory T cells (T_{regs}) including FoxP3, which is a marker specific for natural T_{reg} cells. Within this immune cell subpopulation, no alterations over time could be detected. In the second combination of antibodies, the percentage of different monocyte subpopulations including classical monocytes ($CD14^{++}CD16^{-}$), intermediate monocytes ($CD14^{++}CD16^{+}$), and non-classical monocytes ($CD14^{+}CD16^{++}$) were assessed before and after, and 14 days after intervention. The percentage distribution among monocytes did not change following caloric restriction. In the third combination of antibodies, the MFI of S6 kinase (S6k) in the different immune cell subpopulations, including $CD3^{+}$, $CD4^{+}$, $CD8^{+}$, $CD14^{+}$, $CD19^{+}$ were examined. The MFI of S6 within the different immune cell subpopulations did not show changes following 28 days of caloric restriction, whereas the MFI of S6 in CD8+ ($p = 0.01$), $CD14^{+}$ ($p = 0.02$), and CD19+ cells ($p = 0.002$) were significantly decreased during the 14 days after intervention. Moreover, percentages of $CD3^{+}$ and $CD19^{+}$ of PBMCs were calculated during the three intervention periods: VLCD seemed to have no impact of T cell population and B cell population.

Table 23: Changes in immune cells over time

		Mean ± Standard deviation			p - value	
		before VLCD	after VLCD	14d after VLCD		
	N	t1	t2	t3	t1-t2	t2-t3
Combination 1						
$CD25^+$ (% of CD4+)	11	9.9 ± 5.3	11.9 ± 5.25	12.8 ± 6.9	0.09	0.42
T_{reg} (% of CD4+)	11	18.8 ± 12.8	25.5 ± 19.3	19.1 ± 14.2	0.06	0.08
$FoxP3^+$ (MFI)	10	483.2 ± 292.1	557.4 ± 543.4	439.3 ± 296.9	0.68	0.23
Combination 2						
$CD14^{++}CD16^-$ (% of monocytes)	12	84.3 ± 8.6	86.3 ± 9.9	86.9 ± 10.2	0.18	0.62
$CD14^{++}CD16^+$ (%of monocytes)	12	9.1 ± 4.8	8.7 ± 7.5	6.1 ± 3.1	0.47	0.27
$CD14^+CD16^{++}$ (% of monocytes)	12	5.9 ± 5.5	5.3 ± 4.4	4.3 ± 3.8	0.36	0.14
Combination 3						
$CD3^+$/S6 (MFI)	11	580.0 ± 510.8	573.6 ± 479.7	375.4 ± 577.9	0.96	0.05
$CD4^+$/S6 (MFI)	11	574.6 ± 526.5	579.5 ± 493.7	371.0± 248.5	0.97	0.05
$CD8^+$/S6 (MFI)	12	581.3 ± 489.9	606.8 ± 430.4	398.9 ± 238.0	0.86	0.01
$CD14^+$/S6 (MFI)	12	1,005.7 ± 812.8	1,144.8 ± 969.3	729.36 ± 423.3	0.58	0.02
$CD19^+$/S6 (MFI)	10	874.9 ± 592.1	799.6 ± 470.0	505.9 ± 272.7	0.95	0.002
$CD3^+$ (% of PBMC)	11	106.3 ± 127.9	135.9 ± 226.9	105.9 ± 127.0	0.46	0.33
$CD19^+$ (% of PBMC)	9	39.2 ± 107.0	92,3 ± 275.7	51,3 ± 130.18	0.35	0.31

CD , cluster of differentiation; FoxP3, forkhead-Box-Protein P3; MFI; mean fluorescence intensity; T_{reg}, T regulatory cells; VLCD, very low caloric diet

5.1.4 Abdominal subcutaneous adipose tissue

From a subgroup of participants fat cell size from para-umbilical SAT biopsies was measured before and after VLCD. Adipocyte size before the VLCD was 70.5 ± 7.7 µm (n=8) (**Figure 23A**) and decreased to 67.3 ± 7.1 µm (n=8), although not significantly (p = 0.38) after caloric restriction (**Figure 23B**). The mean adipocyte surface area before VLCD was 4,478 ± 997 μm^2 and after VLCD 4,020 ± 773 μm^2 (n = 8, p = 0.31). Moreover, there was no significant association between adipocyte size, adipocyte surface area and gene expression in SAT.

A B

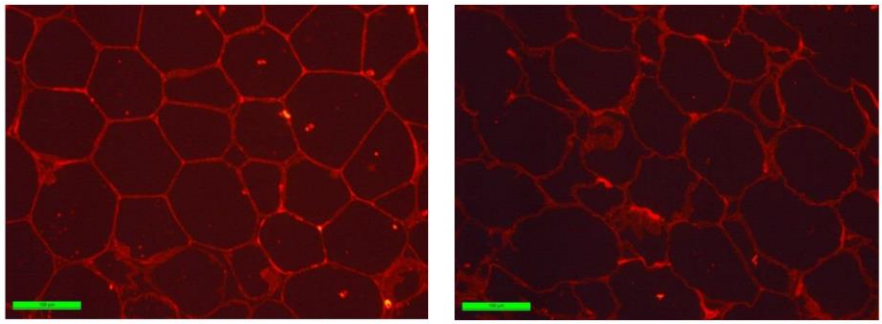


Figure 23: Adipocytes from abdominal needle aspiration before (A) and after (B) intervention. Red fluorescent images of Hematoxylin and eosin stained adipose tissue sections. Bar represents = 100µm

Table 24: Association between gene expression (leptin, MCP-1, adiponectin, CD68) and adipocyte size

	before VLCD		after VLCD	
	p-value	convidence intervall	p-value	convidence intervall
Leptin/ diameter	0.89	-0.25(-4.3,3,8)	0.94	-0.07(-2.5,2,4)
MCP-1/ diameter	0.18	2.0(-1.3,5.3)	0.61	-0.6(-3.1,1.9)
Adiponectin/ diameter	0.79	-0.5(-4.9,3.9)	0.89	0.2(-2.8,3.1)
CD68/ diameter	0.24	-2.3(-6.5,1.9)	0.71	-0.4(-3.1,2.2)

MCP-1, monocyte chemoattractant protein-1; CD68, cluster of differentiation 68

Table 25: Association between gene expression (leptin, MCP-1, adiponectin, CD68) and adipocyte surface area

	before VLCD		after VLCD	
	p-value	convidence intervall	p-value	convidence intervall
Leptin/ Area	0.87	-36.8(-555.1,481.55)	0.85	-21.1(-289.0,246.9)
MCP-1/ Area	0.16	274.2 (-138.5,686.9)	0.56	-69.9(-344.4,204.6)
Adiponectin/ Area	0.84	-50.9(-624.2,522.3)	0.97	5.9(-313.6,325.3)
CD68/ Area	0.25	-291.2(-845.7,263.2)	0.65	-56-7(-342.8,229.5)

MCP-1, monocyte chemoattractant protein-1; CD68, cluster of differentiation 68

5.1.5 Gut permeability

Table 26 summarises results acquired by different approaches reflecting gut leakiness. In brief, paracellular gut permeability markers, including lactulose, PEG 1500 (PEG_{25}, PEG_{30}, PEG_{35}, and PEG_{40}), and zonulin were consistently decreased after caloric restriction.

Gut permeability was first measured using a mixture of four different sugars. Sucrose, used as a proxy for gastroduodenal permeability, significantly decreased after caloric restriction ($p = 0.012$). Two weeks after intervention, sucrose increased significantly again ($p = 0.006$). In contrast, mannitol, a marker for intestinal permeability, did not change significantly during the 28 days of caloric restriction ($p = 0.162$). However, after intervention, its percental urinary recovery was significantly increased ($p < 0.001$). Lactulose, used as a surrogate to monitor tight junction fitness significantly decreased during intervention ($p = 0.01$), while it relapsed afterwards ($p < 0.01$). Also, sucralose, used as colonic permeability marker, significantly decreased during VLCD ($p = 0.018$) and increased again after caloric restriction ($p = 0.019$).

In addition to sugar translocation, we used the translocation of PEGs as an additional measure for gut permeability. The mixture of the different molecular weights of PEG enabled size-dependent assessment of permeability properties of the mucosa. Translocation of PEG M_r 400 was significantly decreased during VLCD (**Table 26**). After intervention, these low molecular weight PEGs returned again and reached baseline levels. All larger homologues of different chain lengths (PEG_{25}, PEG_{30}, PEG_{35}, and PEG_{40}) significantly decreased after caloric restriction ($p < 0.01$ for each measurement), and remained stable during the subsequent two weeks. Similarly, PEG_{70} and PEG_{80} significantly decreased during 28 days of caloric restriction and remained stable thereafter (**Table 26**).

Finally, zonulin was used as a third marker of paracellular gut integrity (Fasano et al., 2000). Plasma concentration at baseline declined significantly after the 4-week hypocaloric intervention ($p < 0.01$). After the subsequent two weeks, zonulin levels returned to baseline values again ($p < 0.01$, **Table 26**).

Table 26: Assessment of gut permeability

		Mean ± Standard deviation			p - value	
		before VLCD	after VLCD	14d after VLCD		
	N	t1	t2	t3	t1-t2	t2-t3
Zonulin (ng/ml)	20	58.4 ± 21.7	47.45 ± 11.88	54.34 ± 14.75	< 0.01	< 0.001
Sucrose (urine recovery in %)	18	0.17 ± 0.12	0.10 ± 0.06	0.73 ± 0.94	0.01	< 0.01
Mannitol (urine recovery in %)	18	12.35 ± 5.38	9.30 ± 4.99	14.66 ± 9.16	0.17	< 0.001
Lactulose (urine recovery in %)	18	0.26 ± 0.18	0.15 ± 0.10	0.32 ± 0.32	0.01	< 0.01
L/M ratio	18	0.02 ± 0.01	0.02 ± 0.01	5.34 ± 23.81	0.18	0.5
Sucralose (urine recovery in %)	18	1.09 ± 1.52	0.26 ± 0.48	1.30 ± 1.79	0.02	0.02
PEG_9 (urine recovery in %)	20	16.8 ± 10.34	12.4 ± 7.29	16.6 ± 12.7	0.03	0.15
PEG_{11} (urine recovery in %)	20	16.5 ± 15.15	7.6 ± 5.57	17.3 ± 19.5	0.01	0.02
PEG_{13} (urine recovery in %)	20	4.2 ± 3.59	2.14 ± 1.25	4.2 ± 4.1	< 0.01	0.02
PEG_{25} (urine recovery in %)	20	0.38 ± 0.33	0.17 ± 0.15	0.30 ± 0.28	< 0.001	0.06
PEG_{30} (urine recovery in %)	20	0.22 ± 0.17	0.11 ± 0.08	0.15 ± 0.12	< 0.001	0.16
PEG_{35} (urine recovery in %)	20	0.20 ± 0.18	0.08 ± 0.09	0.15 ± 0.20	< 0.001	0.18
PEG_{40} (urine recovery in %)	20	0.24 ± 0.24	0.10 ± 0.11	0.16 ± 0.19	< 0.01	0.24
PEG_{70} (urine recovery in %)	20	0.1 ± 0.08	0.04 ± 0.08	0.04 ± 0.06	0.02	0.84
PEG_{80} (urine recovery in %)	16	0.04 ± 0.05	0.02 ± 0.04	0.02 ± 0.04	0.09	0.43

VLCD, very low caloric diet; L/M, lactulose/mannitol; PEG, polyethylene glycol

Special attention was given in the next step of analysis to the amount of weight loss. The regression analysis showed that weight loss did not have substantial impact on levels of gut permeability markers. Thus, it cannot be concluded that the extent of weight loss influenced the extent of the decrease of permeability (**Table 27**).

Table 27: Impact of weight change on gut permeability

	p - value	convidence intervall
Zonulin (ng/ml)	0.1	1.552 (-0.52, 3.62)
Mannitol (urine recovery in %)	0.6	0.447 (-1.16, 2.05)
Lactulose (urine recovery in %)	0.6	-0.008 (-0.04, 0.02)
PEG_9 (urine recovery in %)	0.2	0.973 (-0.58, 2.52)
PEG_{11} (urine recovery in %)	0.3	0.637 (-0.62, 1.89)
PEG_{13} (urine recovery in %)	0.6	0.076 (-0.197, 0.35)
PEG_{25} (urine recovery in %)	0.3	0.012 (-0.01, 0.03)
PEG_{30} (urine recovery in %)	0.5	0.004 (-0.01, 0.02)
PEG_{35} (urine recovery in %)	0.3	0.007 (-0.01, 0.02)
PEG_{40} (urine recovery in %)	0.4	0.006 (-0.01, 0.02)
PEG_{70} (urine recovery in %)	0.5	0.006 (0.01, 0.03)
PEG_{80} (urine recovery in %)	0.4	0.005(-0.01, 0.02)

PEG, polyethylene glycol

Another point of interest was to investigate the impact of the paracellular gut permeability change on inflammation markers such as LBP. Zonulin, lactulose and PEG_{25} (as a representative of the PEG mixture with a molecular mass (range) of Mr 1500 Da) were chosen as marker for paracellular gut permeability. Regression analysis showed that there was no significant association between the diminished gut permeability markers and reduced inflammation marker (**Table 28**). Finally, there was no association between baseline BMI and different markers for paracellular gut permeability (**Table 29**).

Table 28: Association between the change of different gut permeability marker on LBP level 28 days after of VLCD

	p - value	convidence intervall
Δ Zonulin (ng/ml)	0.19	0.004(-0.002,0.01)
Δ Lactulose (urine recovery in %)	0.33	-0.27(-0.86,0.31)
Δ PEG_{25} (urine recovery in %)	0.30	0.23(-0.23,0.71)

PEG, polyethylene glycol

Table 29: Association between different baseline BMI and gut permeability markers

	p - value	convidence intervall
Δ Zonulin (ng/ml)	0.91	0.15(-2.65,2.96)
Δ Lactulose (urine recovery in %)	0.50	-0.007(0.03,0.02)
Δ PEG_{25} (urine recovery in %)	0.70	-0.007 (0.05,0.03)

PEG, polyethylene glycol

5.1.6 Gut microbiota profiles

After quality- and chimera-check, a total of 815,773 sequences clustering in 235 OTUs were analysed. Caloric restriction did not affect *alpha*-diversity (**Figure 24 A**). *Beta*-diversity analysis revealed marked inter-individual differences and no significantly distinct clustering according to time points (**Figure 24 B**). These data suggest that caloric restriction did not trigger consistent shifts in the overall phylogenetic makeup of fecal bacterial populations; microbiota profiles remained individual-specific throughout the study (Appendix, Supplemental Figure 1). Furthermore, the phylogenetic distance between different time points did not change significantly ($p = 0.33$) (Appendix, Supplemental Figure 2). Concerning microbiota composition, *Firmicutes* and *Bacteroidetes* were the two major phyla, followed by *Actinobacteria*, *Proteobacteria*, and *Verrucomicrobia* (Appendix, Supplemental Figure 3). Moreover, the Firmicutes/Bacteroidetes ratio did not alter after 28 days of caloric restriction ($p = 0.5$). The relative abundance of members of the phylum of *Proteobacteria* was significantly decreased after caloric restriction. This decrease did not hold up after correction for multiple testing. No taxonomic groups within this phylum (e.g. *Enterobacteriaceae*) showed significant differences

(**Figure 24 C**). Of the 235 OTUs detected, five showed significant differences in their relative abundances during intervention as per explorative analysis (before correction for multiple testing) (**Figure 24 D**). Three OTUs belonged to the family *Lachnospiraceae* within the phylum *Firmicutes*. OTU 8 (*Anaerostipes hadrus,* 100% sequence identity) and OTU 10 (*Blautia* sp.*,* several hits >97% sequence identity) showed higher relative abundance after VLCD and returned to baseline values after two weeks. Relative abundances of OTU 1 (*Agathobacter rectalis* – formerly *Eubacterium rectale*, 100%) decreased after caloric restriction and throughout the end of the study. Two additional OTUs outside the *Lachnospiraceae* were characterized by intervention-related increase in their relative abundances: OTU 18 (*Ruminococcus faecis*, 100%) within the *Ruminococcaceae* and OTU 3 (*Bifidodbacterium sp.,* several hits >97% sequence identity) within the family *Bifidobacteriaceae* (phylum *Actinobacteria*).

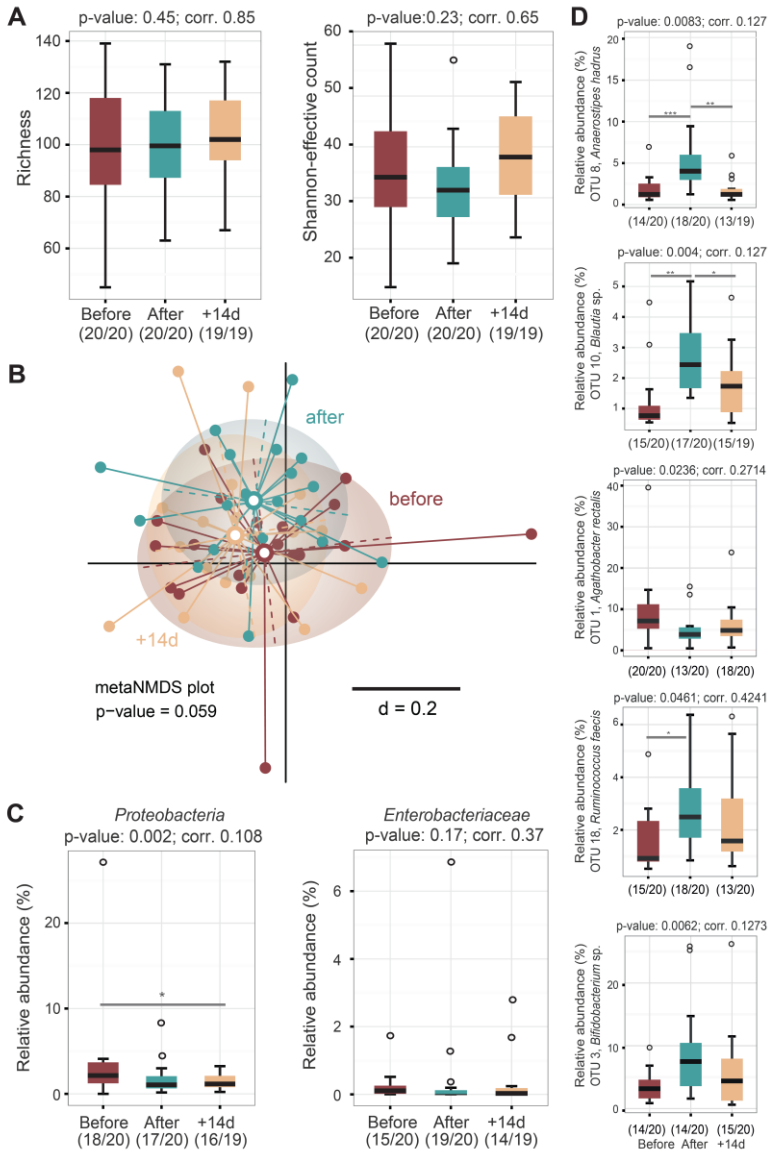


Figure 24: Fecal microbiota analysis by 16S rRNA gene amplicon analysis. (A) Diversity within samples (*alpha*-diversity) was estimated by species richness and Shannon-effective counts. (B) metaNMDS plot of phylogenetic distances based on generalised UniFrac (*beta*-diversity). (C) Occurrence of members of the phylum *Proteobacteria* and the family *Enterobacteriaceae*, including significance before and after Benjamini-Hochberg adjustment. (D) Relative abundances of the five dominant OTUs showing significance over time. NMDS plot, nonparametric multidimensional scaling plot; OTU, operational taxonomy units; VLCD, very low caloric diet; ; ***,$p < 0.001$; **, $p < 0.01$; *$p < 0.5$

5.1.7 Comparison of routine, anthropometric, clinical chemistry and metabolic parameters at baseline with parameters 14 days after VLCD

As additional information, we assessed different markers two weeks after caloric restriction (**Table 30**). Comparing anthropometric, clinical chemistry and metabolic values at baseline with 14 days after caloric restriction, the following parameters showed significant changes: energy intake, body composition parameter, HMW adiponectin and leptin concentrations in plasma. Looking at these parameters in detail, energy intake was significantly decreased compared to baseline values ($p < 0.01$). Measures of body composition were significantly lower than at baseline, as well. HMW adiponectin plasma concentrations were higher than baseline values ($p < 0.001$). Leptin was significantly decreased compared to the baseline status ($p < 0.001$). (All values are depicted in Appendix, Supplemental Table 3 and Table 4).

Table 30: Comparison of values at baseline and 14 days after VLCD

	p value
Parameter	**t1-t3**
Dietary behaviour and physical activity	
Energy intake (kcal/d)	< 0.01
Fat intake (g/d)	0.15
Protein intake (g/d)	0.34
Carbohydrate intake (g/d)	< 0.001
Body composition	
Weight (kg)	< 0.001
BMI (kg/m²)	< 0.001
Waist circumference (cm)	< 0.001
Hip circumference (cm)	< 0.001
WHR	0.03
Lean Mass (kg)	< 0.001
Fat Mass (kg)	< 0.001
RMR, blood pressure and pulse rate	
Pulse rate (HF/min)	0.42
Blood pressure systolic (mmHg)	0.07
Blood pressure diastolic (mmHg)	< 0.01
RMR (kcal)	0.79

	p value
Parameter	**t1-t3**
Clinical chemistry, metabolic and inflammation markers	
AST (U/l)	0.12
ALT (U/l)	0.07
GGT (U/l)	0.96
Creatinine (mg/dl)	0.66
Uric acid (mg/dl)	0.46
Sodium (mmol/ml)	< 0.01
Potassium (mmol/l)	0.57
Calcium (mmol/l)	0.04
Total cholesterol (mg/dl)	0.31
HDL-cholesterol (mg/dl)	0.64
LDL-cholesterol (mg/dl)	0.1
Triglycerides (mg/dl)	0.41
LDL/HDL	0.22
NEFA (mmol/l)	0.02
hsCRP (mg/dl)	0.57
HMW Aaiponectin (mg/ml)	< 0.001
Leptin (ng/ml)	< 0.001
RANTES (ng/ml)	0.05
MCP-1 (pg/ml)	0.29
Chemerin (ng/ml)	0.62
LBP (mg/dl)	0.19
Calprotectin (mg/kg)	0.04

	p value
Parameter	**t1-t3**
Glucose metabolism	
Fasting blood glucose (mg/dl)	0.02
Fasting insulin level (µU/ml)	0.52
HOMA-IR	0.06
ROS	
ROS production	0.58
ROS production rate	0.97
Gut permeability markers	
Zonulin (ng/ml)	0.19
Sucrose (urine recovery in %)	0.02
Mannitol (urine recovery in %)	0.34
Lactulose (urine recovery in %)	0.31
L/M ratio	0.24
Sucralose (urine recovery in %)	0.63
PEG_9 (urine recovery in %)	0.96
PEG_{11} (urine recovery in %)	0.87
PEG_{13} (urine recovery in %)	0.98
PEG_{25} (urine recovery in %)	0.41
PEG_{30} (urine recovery in %)	0.15
PEG_{35} (urine recovery in %)	0.34
PEG_{40} (urine recovery in %)	0.22
PEG_{70} (urine recovery in %)	0.01
PEG_{80} (urine recovery in %)	0.69

ALT, alanine transaminase; γGT, γ-glutamyltransferase; AST, aspartate aminotransferase; BMI, body mass index; HDL, high-density cholesterol; HMW, high-molecular weight; HOMA-IR, homeostasis model assessment of insulin resistance; hsCRP, high-sensitivity C-reactive protein; LBP, lipopolysaccharide binding protein; LDL, low-density cholesterol; L/M, lactulose/mannitol ratio; MCP-1, monocyte chemoattractant protein-1; NEFA, non-esterified fatty acids; PEG, polyethylene glycol; RANTES, regulated on activation normal T cell expressed and secreted; RMR, resting metabolic rate; ROS, reactive oxygen species; VLCD, very low caloric diet; WHR, waist-to-hip ratio

5.2 Short-term overfeeding

Baseline characteristics of the volunteers are presented in **Table 31**. The 24 participants were young healthy men with a mean age of 23.0 ± 2.8 years and mean BMI of 23.0 ± 2.1 kg/m². Additional information of participants including history and lifestyle is shown in the supplement (Appendix, Supplemental Table 5).

5.2.1 Dietary behaviour and physical activity

The analysis of dietary protocols revealed an average energy intake of 2,731 ± 708 kcal/day 7 days before HFD. The calculated caloric intake for the overfeeding period was 3,926 ± 340 kcal/day on average. During the overfeeding period, fat intake was increased by 100.3 ± 30.6 g/day including an increased intake of saturated fatty acids by 66.1 ± 14.7 g/day. During the two weeks following the high-caloric interventio, mean energy intake returned to 2,589 ± 628.3 again (kcal/day). Macronutrient intake over time is summarised in **Table 31**.

During the study, the physical activity level was monitored using ActiGraph system. Participants were instructed to avoid physical activity such as running, cycling, and swimming. To test for adherence to this advice, physical activity was monitored with an ActiGraph® throughout the study. According to the data, participants had an average daily metabolic equivalent of task (MET) value of 1.37 ± 0.09 at baseline, while they had an average value of 1.35 ± 0.08 during and 1.38 ± 0.10 after the high-caloric intervention. MET values can be classified in different physical activity intensity levels such as light (< 3.0 MET), moderate (3.0 - 5.9 MET), vigorous (6.0 - 8.9 MET) and very vigorous (> 8.9 MET) (Freedson et al., 1998). As requested, participants spent their time at light intensity level.

Table 31: Macronutrient composition during the study

	Mean ± Standard deviation (n=24)			p - value	
	before HFD	after HFD	14d after HFD		
	t1	t2	t3	t1-t2	t2-t3
Energy intake (kcal/d)	2,730.8 ± 707.9	3,926.2 ± 339.9	2,589.1 ± 628.3	<0.0001	<0.0001
Fat intake (g/d)	108.4 ± 34.7	208.7 ± 17.9	100.6 ± 32.9	<0.0001	<0.0001
Protein intake (g/d)	100.0 ± 24.9	176.1 ± 15.3	95.4 ± 25.8	<0.0001	<0.0001
Carbohydrate intake (g/d)	303.3 ± 109.7	332.5 ± 28.9	280.4 ± 73.2	0.01	<0.001

HFD, high-fat diet

5.2.2 Body composition

As shown in **Table 32**, the 24 participants gained 938 ± 615 g body weight during the 7 days of HFD. This significant weight gain ($p < 0.0001$) was associated with a significant increase in body fat mass ($p = 0.03$). During the two weeks after overfeeding body weight and body fat did not

change significantly. Although not significant, waist and hip circumference increased by 0.7 ± 1.7 cm and 0.6 ± 1.9 cm, respectively. Other anthropometric data did not show significantly during the post-intervention period.

Table 32: Anthropometric characterization and body composition of the participants

	Mean ± Standard deviation (n=24)			p - value	
	before HFD	after HFD	14d after HFD		
	t1	t2	t3	t1-t2	t2-t3
Weight (kg)	76.6 ± 10.3	77.6 ± 10.23	77.3 ± 10.4	< 0.0001	0.08
Body mass index (kg/m²)	23.0 ± 2.1	23.3 ± 2.1	23.2 ± 2.1	< 0.0001	0.08
Waist circumference (cm)	83.5 ± 5.9	84.2 ± 5.9	83.7 ± 6.1	0.06	0.27
Hip circumference (cm)	87.8 ± 5.5	88.4 ± 5.1	87.8 ± 4.9	0.14	0.05
Lean Mass (kg)	66.9 ± 8.0	67.3 ± 7.8	67.1 ±8.0	0.08	0.31
Fat Mass (kg)	9.8 ± 3.9	10.3 ± 3.8	10.2 ± 3.9	0.03	0.69

HFD, high-fat diet

5.2.3 Metabolic consequences of short-term overfeeding

Resting metabolic rate and cardiovascular function

RMR, respiratory quotient, pulse rate and blood pressure data did not differ between the different study periods (**Table 33**). Regression analysis of weight gain and RMR did not show a significant dependency (95% CI 62.4 (-79.9,204.8), (p = 0.37)).

Table 33: Summarised results of the functional tests

	Mean ± Standard deviation (n=24)			p - value	
	before HFD	after HFD	14d after HFD		
	t1	t2	t3	t1-t2	t2-t3
RMR (kcal)	1,958.9 ± 232.6	2,010.7 ± 289.1	1,962.5 ± 290.9	0.21	0.1
RMR (kcal)/ Weight gain less than 1kg	1,898.2 ± 209.8	1,907.0 ± 229.8	1,862.9 ± 236.3	0.8	0.1
RMR (kcal)/ Weight gain more than 1kg	2,044.0 ± 246.6	2,155.8 ± 311.7	2,102.0 ± 314.1	0.18	0.3
Respiratory quotient	0.72 ± 0.1	0.73 ± 0.1	0.73 ± 0.1	0.68	0.67
Pulse (HF/min)	62.2 ± 7.6	62.6 ± 6.9	64.9 ± 7.5	0.81	0.05
Blood pressure systolic (mmHg)	115.7 ± 10.9	110.6 ± 9.2	111.3 ± 8.9	< 0.01	0.71
Blood pressure diastolic (mmHg)	78.6 ± 6.9	77.9 ± 5.2	75.3 ± 4.8	0.5	0.03

HF, heart frequency; HFD, high-fat diet; RMR, resting metabolic rate

Metabolism and inflammation

All metabolic data are shown in **Table 34**. Regarding the liver profile, AST and ALT were not altered significantly during the whole study period, but γGT decreased significantly during overfeeding. After HFD, the value returned to its baseline value. Creatinine levels increased by 0.02 ± 0.1 mg/dl after HFD and returned to the baseline status during the subsequent weeks. Uric acid decreased significantly ($p = 0.01$) during the HFDand increased again during the last two weeks. Expectedly, electrolytes like sodium and potassium remained stable during the whole study.

The short-term overfeeding resulted in significantly elevated total cholesterol ($p < 0.001$), LDL-cholesterol ($p < 0.001$), but also HDL-cholesterol levels increased (each $p < 0.0001$). These three parameters returned to baseline levels 14 days after finishing the HFD. In contrast, the triglyceride and NEFA levels declined significantly ($p < 0.0001$) during overfeeding and increased again under the normal diet. The proinflammatory adipokine leptin did not change during the three study periods. Surprisingly, HMW adiponectin rose significantly during the intervention ($p < 0.0001$) and decreased again during the following two weeks ($p = 0.001$). The proinflammatory adipokine leptin also did not change during the three study time periods. MCP-1 did not vary during the HFD, but significantly rose in the third study phase ($p = 0.008$). RANTES showed a significantly decrease during intervention ($p < 0.001$), but remained unchanged during the subsequent two weeks after intervention. The hypercaloric diet did not trigger significant changes in inflammatory markers such as hsCRP and LBP. Furthermore, fecal calprotectin was not affected by the dietary intervention but declined significantly during the subsequent 14 days ($p = 0.02$).

Table 34: Metabolic parameters during the study

	Mean ± Standard deviation (n=24)			p - value	
	before HFD	after HFD	14d after HFD		
	t1	t2	t3	t1-t2	t2-t3
AST (U/l)	20.9 ± 5.7	19.3 ± 5.5	20.9 ± 10.1	0.06	0.31
ALT (U/l)	24.1 ± 5.9	27.3 ± 9.4	25.9 ± 17.2	0.08	0.68
γGT (U/l)	21.0 ± 8.6	19.1 ± 7.1	19.2 ± 8.2	0.02	0.97
Creatinine (mg/dl)	0.9 ± 0.1	0.9 ± 0.1	0.9 ± 0.1	0.02	<0.01
Uric acid (mg/dl)	6.1 ± 0.9	6.5 ± 0.8	6.3 ± 1.1	<0.01	0.09
Sodium (mmol/ml)	142.2 ± 2.4	141.5 ± 1.9	141.8 ± 1.6	0.19	0.49
Potassium (mmol/l)	3.9 ± 0.4	4.1 ± 0.3	4.1 ± 0.4	0.1	0.95
Calcium (mmol/l)	2.4 ± 0.1	2.4 ± 0.1	2.4 ± 0.1	0.51	0.44
Total cholesterol (mg/dl)	166.8 ± 27.9	183.8 ± 31.1	167.5 ± 32.8	<0.001	<0.0001
HDL-cholesterol (mg/dl)	54.1 ± 10.1	61.6 ± 10.6	54.3 ± 11.3	<0.0001	<0.0001
LDL-cholesterol (mg/dl)	95.8 ± 27.3	109.6 ± 29.6	98.3 ± 29.5	<0.001	<0.001
Triglycerides (mg/dl)	91.0 ± 30.2	64.4 ± 21.8	78.6 ± 45.2	<0.0001	0.06
LDL/HDL	1.9 ± 0.7	1.9 ± 0.7	1.9 ± 0.7	0.91	0.64

NEFA (mmol/l)	0.4 ± 0.2	0.2 ± 0.1	0.3 ± 0.3	<0.0001	0.01
Leptin (ng/ml)	1.3 ± 0.7	1.5 ± 0.9	1.4 ± 0.8	0.06	0.36
HMW adiponectin (mg/ml)	0.34 ± 0.21	0.45 ± 0.23	0.35 ± 0.24	<0.0001	<0.01
MCP-1 (pg/ml)	80.8 ± 14.3	79.6 ± 14.9	87.9 ± 16.4	0.89	<0.01
RANTES (ng/ml)	26.9 ± 14.9	17.3 ± 7.7	16.3 ± 8.7	<0.001	0.58
Chemerin (ng/ml)	37.7 ± 10.6	37.0 ± 8.6	35.9 ± 7.9	0.73	0.52
hsCRP (mg/dl)	0.03 ± 0.04	0.03 ± 0.02	0.05 ± 0.05	0.94	0.13
LBP (mg/dl)	0.65 ± 0.30	0.59 1 ± 0.17	0.58 ± 0.21	0.46	0.84
Calprotectin (mg/kg)	22.9 ± 20.6	30.0 ± 27.4	15.2 ± 15.4	0.46	0.03

ALT, alanine transaminase; AST, aspartate aminotransferase; γGT, γ-glutamyltransferase; HDL, high-density cholesterol; HFD, high-fat diet; HMW; high-molecular weight; HOMA-IR, homeostasis model assessment of insulin resistance; hsCRP, high-sensitivity C-reactive protein; LBP, lipopolysaccharide binding protein; LDL, low-density cholesterol; MCP-1, monocyte chemoattractant protein-1; NEFA, nonesterified fatty acids; RANTES, regulated on activation normal T cell expressed and secreted

ROS

The ROS production rate in PBMC was not significantly altered during seven days of HFD and during the subsequent two weeks. In contrast, the ROS production rate in plasma was significantly decreased after seven days of short-term high fat diet. During the subsequent two weeks, the ROS production rate significantly relapsed (**Table 35**).

Table 35: ROS production rate in plasma and PBMC

		Mean ± Standard deviation			p - value	
		before HFD	**after HFD**	**14d after HFD**		
	N	**t1**	**t2**	**t3**	**t1-t2**	**t2-t3**
ROS production rate in plasma (µM superoxide /µg protein/10min Plasma)	24	0.0098 ± 0.005	0.0081 ± 0.003	0.009 ± 0.003	0.01	0.02
ROS production rate in PBMC (µM superoxide /µg protein/10min PBMC)	18	0.0059 ± 0.050	0.0049 ± 0.003	0.0042 ± 0.002	0.4	0.4

HFD, high-fat diet; PBMC, peripheral blood mononuclear cells; ROS, reactive oxygen species

PBMC – immune cell subpopulations

The effect of a HFD on immune cell subpopulations was assessed by flow cytometry (**Table 36**). The first combination of antibodies was chosen to focus on the status of T_{regs} including FoxP3, which is a marker specific for natural T_{reg} cells. Within this immune cell subpopulation, no alterations over time could be detected. In the second combination of antibodies, the percentage of different monocyte subpopulations including classical monocytes ($CD14^{++}CD16^{-}$),

intermediate monocytes ($CD14^{++}CD16^{+}$), and non-classical monocytes ($CD14^{+}CD16^{++}$) were assessed before and after, and 14 days after intervention. Within monocytes the classical and non-classical monocytes significantly increased after HFD, whereas the percentage of intermediate monocytes significantly decreased. In the third combination of antibodies, the MFI of S6k in the different immune cell subpopulations, including $CD3^{+}$, $CD4^{+}$, $CD8^{+}$, $CD14^{+}$, $CD19^{+}$, were examined. The MFI did not show changes during the whole study. The percentage of $CD3^{+}$ cells in PBMCs was significantly elevated after seven days of HFD. The subpopulation of $CD19^{+}$ cells was not altered due to the dietary intervention (**Table 37**).

Table 36: Immune cell subpopulation over time

		Mean ± Standard deviation			p - value	
		before HFD	after HFD	14d after HFD		
	N	t1	t2	t3	t1-t2	t2-t3
Combination 1						
$CD25^{+}$ (% of CD4+)	13	8.2 ± 3.4	10.5 ± 4.7	10.6 ± 5.1	0.09	0.59
T_{reg} (%of CD4+)	13	10.5 ± 9.9	8.1 ± 6.4	11.8 ± 17.9	0.06	0.41
FoxP3 (MFI)	10	163.1 ± 110.6	250.3 ± 431.4	153.7 ± 116.9	0.76	0.38
Combination 2						
$CD14^{++}CD16^{-}$ (% of monocytes)	19	85.8 ± 9.2	86.8 ± 18.9	85.7 ± 19.7	< 0.01	0.58
$CD14^{++}CD16^{+}$ (% of monocytes)	19	9.2 ± 12.9	5.4 ± 3.3	5.5 ± 2.5	< 0.01	0.98
$CD14^{+}CD16^{++}$ (% of monocytes)	19	5.9 ± 3.9	7.8 ± 16.9	9.6 ± 18.7	< 0.01	0.16
Combination 3						
$CD3^{+}$/S6 (MFI)	16	2,844.7 ± 1956.8	2,769.3 ± 2056.4	3,217.8 ± 2169.1	0.97	0.87
$CD4^{+}$/S6 (MFI)	16	2,864.9 ± 2171.9	2,793.2 ± 1956.9	3,208.4 ± 2037.2	0.73	0.92
$CD8^{+}$/S6 (MFI)	16	2,737.2 ± 2519.2	3,119.3 ± 3369.3	3,507.8 ± 2985.5	0.21	0.24
$CD14^{+}$/S6 (MFI)	15	9,957.6 ± 2142.0	4,871.4 ± 3471.1	5,759.3 ± 4023.9	0.33	0.23
$CD19^{+}$/S6 (MFI)	16	3,670.2 ± 2564.3	3,864.4 ± 3164.2	4,017.6 ± 2698.9	0.70	0.78
$CD3^{+}$ (% of PBMC)	16	62.5 ± 13.1	73.5 ± 7.8	68.3 ± 16.33	< 0.001.	0.10
$CD19^{+}$ (% of PBMC)	16	8.9 ± 15.5	9.2 ± 16.2	5.9 ± 1.8	0.55	0.94

CD, cluster of differentiation; HFD, high-fat diet; MFI, mean fluorescence intensity; PBMC, peripheral blood mononuclear cells

PBMC – immune cell subpopulations during hyperinsulinemic euglycemic clamp

Blood was drawn before starting the hyperinsulinemic euglycemic clamp and during the steady-state period **(Table 37)**. Within monocytes, the percentage of classical monocytes, intermediate monocytes, and non-classical monocytes did not change in the clamp situation before and after hyperinsulinaemia. Furthermore, the MFI of S6k in the different immune cell subpopulations kept stable. Finally, the $CD3^{+}$ and $CD19^{+}$ percentage of PBMC did not change

Table 37: Immune cell subpopulation during hyperinsulinemic euglycemic clamp before and after HFD

	Mean ± Standard deviation (N=10)					
	before HFD		after HFD		p-value	
	t1 (basal)	t1 (steady-state)	t2 (basal)	t2 (steady-state)	basal	steady-state
Combination 2						
$CD14^{++}CD16^{-}$ (% of monocytes)	89.9± 4.9	90.5± 5.6	91.7± 2.2	90.7± 2.2	0.31	0.91
$CD14^{++}CD16^{+}$ (%of monocytes)	6.8± 4.5	6.2± 5.2	4.6± 1.6	4.9± 1.2	0.12	0.43
$CD14^{+}CD16^{++}$ (% of monocytes)	3.3± 2.4	3.3± 2.5	3.7±2.2	4.3± 2.2	0.69	0.36
Combination 3						
$CD3^{+}$/S6 (MFI)	2,422.2 ±	2,149.1 ±	2,547.5 ±	2,959.3±	0.74	0.26
$CD4^{+}$/S6 (MFI)	2,477.5±	2,214.9±	2,576.6±	2,228.4±	0.79	0.92
$CD8^{+}$/S6 (MFI)	2,434.0±	2,190.7±	2,615.4±	3,475.8±	0.63	0.36
$CD14^{+}$/S6 (MFI)	5,071.8±	3,883.8±	5,189.9±	4,481.1±	0.92	0.52
$CD19^{+}$/S6(MFI)	3,612.5±	2,887.4±	3,143.9±	10,428.9±	0.79	0.52
$CD3^{+}$ (% of PBMC)	68.7±	70.0±	72.1±	73.3±	0.14	0.68
$CD19^{+}$ (% of PBMC)	10.4±	5.7±	5.3±	6.3±	0.49	0.26

CD, cluster of differentiation; HFD, high-fat diet; MFI, mean fluorescence intensity

The second and third combination was measured in the fasting state before and after intervention. Moreover, these two combinations were analysed in the fasting state in the hyperinsulinemic euglycemic clamp before and after intervention. By using Mann-Whitney U test, these two measurement days were compared. As shown in the following two **tables 38** and **39**, the variability of these two combinations was not significantly different between the two days.

Table 38: Comparison of the different monocyte types between the two following days.

	p-value
classical monocytes before intervention	0.18
classical monocytes after intervention	0.68
intermediate monocytes before intervention	0.92
intermediate monocytes after intervention	0.67
nonclassical monocytes before intervention	0.07
nonclasscial monocytes after intervention	0.50

Table 39: Comparison of S6k in the different immune cell subpopulations between two following days

	p value
CD3 before intervention	0.33
CD3 after intervention	0.99
CD4 before intervention	0.62
CD4 after intervention	0.88
CD8 before intervention	0.87
CD8 after intervention	0.95
CD14 before intervention	0.62
CD14 after intervention	0.90
CD19 before intervention	0.26
CD19 after intervention	0.57

CD, cluster of differentiation

Glucose metabolism

Fasting plasma glucose levels decreased significantly during the seven days of HFD ($p = 0.03$) and were stable during the subsequent two weeks. Fasting insulin levels and HOMA-Index remained constant during the whole study time period. The mean M-value measured by the HEC was 3.6 ± 1.7 mmol/min before overfeeding and remained unchanged after overfeeding (3.7 ± 1.9 mmol/min, $p = 0.072$). Glucose disposal rate did not change significantly either (197.7 ± 89.6 ml/h and 204.9 ± 104.6 ml/h before and after intervention, respectively; $p = 0.73$). There was also no effect of the HFD on measures of insulin sensitivity, when M-values were normalised for body weight and fat-free mass (**Table 40**). Interestingly, fasting plasma glucose concentrations from participants gaining more than 1 kg significantly decreased after the HFD ($p = 0.03$) (**Table 42**). However, comparing clamp results of both groups, there were no significant differences in the readings **(Table 41/42)**. Of note, from the 24 clamps only 17 could be validated. Four participants did not achieve steady-state after 180 minutes. One participants was excluded because of phlebitis after the initial glucose infusion. Two more participants were excluded after blinded evaluation because no steady-state was achieved.

Table 40: Glucose and insulin concentration before, after and 14d after HFD

		Mean ± Standard deviation			p - value	
	N	before HFD	after HFD	14d after HFD		
		t1	t2	t3	t1-t2	t2-t3
Fasting blood glucose (mg/dl)	24	81.9 ± 6.5	79.2 ± 5.9	80.8 ± 4.5	0.03	0.2
Fasting insulin levels (µU/ml)	24	4.1 ± 0.9	4.3 ± 1.2	3.9 ± 0.7	0.44	0.14
HOMA-IR	24	0.8 ± 0.2	0.8 ± 0.3	0.8 ± 0.2	0.79	0.28
Clamp						
M-value (mmol/min)	17	3.6 ± 1.7	3.7 ± 1.9		0.72	
Glucose disposal (ml/h)	17	197.7 ± 89.6	204.9 ± 104.6		0.73	

HFD, high-fat diet; HOMA-IR. homeostasis model assessment of insulin resistance; HOMA-IR was calculated by the following formula: HOMA-IR=insulin (µU/ml) x glucose (mmol/l)/22.5 (Matthews et al., 1985)

Table 41: Glucose and insulin concentration before, after and 14d after HFD in participants gaining less than 1kg

	Mean ± Standard deviation (N=14)			p - value	
	before HFD	after HFD	14d after HFD		
	t1	t2	t3	t1-t2	t2-t3
Fasting blood glucose (mg/dl)	80.4 ± 7.4	80.6 ± 5.3	79.8 ± 3.6	0.9	0.6
Fasting insulin levels (µU/ml)	3.9 ± 0.9	3.8 ± 0.8	3.8 ± 0.7	0.5	0.9
HOMA-IR	0.8 ± 0.2	0.8 ± 0.2	0.8 ± 0.1	0.5	0.9
Clamp					
M-value (mmol/min)	3.1 ± 1.7	3.6 ± 1.9		0.4	
Glucose disposal (ml/h)	172 ± 92.9	195.6 ± 102.9		0.4	

HFD, high-fat diet; HOMA-IR. homeostasis model assessment of insulin resistance; HOMA-IR was calculated by the following formula: HOMA-IR=insulin (µU/ml) x glucose (mmol/l)/22.5 (Matthews et al., 1985)

Table 42: Glucose and insulin concentrations before, after and 14d after HFD in participants gaining more than 1kg

	Mean ± Standard deviation (N=10)			p - value	
	before HFD	after HFD	14d after HFD		
	t1	t2	t3	t1-t2	t2-t3
Fasting blood glucose (mg/dl)	84.0 ± 4.4	77.6 ± 6.5	82.1 ± 5.4	0.002	0.04
Fasting insulin levels (µU/ml)	4.2 ± 0.9	4.9 ± 1.4	4.1 ± 0.7	0.14	0.04
HOMA-IR	0.9 ± 0.2	0.9 ± 0.3	0.8 ± 0.2	0.4	0.2
Clamp					
M-value (mmol/min)	4.2 ± 1.5	3.9 ± 2.1		0.7	
Glucose disposal (ml/h)	226.6 ± 81.8	215.4 ± 112.7		0.7	

HFD, high-fat diet; HOMA-IR. homeostasis model assessment of insulin resistance; HOMA-IR was calculated by the following formula: HOMA-IR=insulin (µU/ml) x glucose (mmol/l)/22.5 (Matthews et al., 1985)

5.2.4 Gut permeability

Table 43 summarises the results obtained using different approaches for the measurement of gut permeability. The mean appearance of the four sugars in urine did not change during the 7 days of high-fat high-calorie diet. Regarding the PEG approach, the percentage of urinary excretion of PEG25 decreased significantly (p = 0.03), while excretion of the other PEG molecular sizes was unchanged. Surprisingly, the excretion of larger PEG molecules (PEG_{70}, PEG_{80}) was significantly decreased after seven days of HFD and remained stable during the following two weeks after intervention. The gut permeability marker zonulin remained unchanged during the whole study period. Taken together, with the exception of smaller PEG molecules short-term overfeeding did not demonstrate alterations of paracellular permeability. Additionally, with linear regression analysis a possible association between weight gain and gut permeability markers after 7 days of HFD were assessed. As described in **Table 44**, there is no evidence of any significant association.

Table 43: Overview of the different gut permeability markers

		Mean ± Standard deviation			p - value	
	N	before HFD	after HFD	14d after HFD		
		t1	t2	t3	t1-t2	t2-t3
Zonulin (ng/ml)	24	49.04 ± 13.66	46.45 ± 12.44	45.21 ± 14.27	0.11	0.57
Sucrose (urine recovery in %)	24	0.14 ± 0.05	0.09 ± 0.05	0.11 ± 1.02	0.7	0.32
Sucralose (urine recovery in %)	24	0.46 ± 0.47	0.39 ± 0.6	0.75 ± 1.02	0.66	0.14
Mannitol (urine recovery in %)	24	14.35 ± 5.37	15.02 ± 5.12	15.16 ± 4.19	0.66	0.89
Lactulose (urine recovery in %)	24	0.23 ± 0.09	0.24 ± 0.09	0.23 ± 0.08	0.6	0.77
L/M ratio	24	0.02 ± 0.01	0.02 ± 0.01	0.02 ± 0.01	0.99	0.47
PEG_9 (urine recovery in %)	24	13.77 ± 7.09	10.95 ± 6.51	12.13 ± 6.51	0.02	0.55
PEG_{11} (urine recovery in %)	24	12.77 ± 9.13	8.81 ± 6.92	10.31 ± 8.26	0.07	0.52
PEG_{13} (urine recovery in %)	24	2.95 ± 1.57	2.26 ± 1.52	2.83 ± 1.69	0.01	0.21
PEG_{25} (urine recovery in %)	24	0.30 ± 0.17	0.21 ± 0.12	0.26 ± 0.19	0.03	0.2
PEG_{30} (urine recovery in %)	24	0.19 ± 0.12	0.14 ± 0.09	0.15 ± 0.12	0.05	0.64
PEG_{35} (urine recovery in %)	24	0.20 ± 0.18	0.14 ± 0.12	0.12 ± 0.12	0.11	0.69
PEG_{40} (urine recovery in %)	24	0.21 ± 0.17	0.16 ± 0.13	0.13 ± 0.13	0.12	0.47
PEG_{70} (urine recovery in %)	24	0.11 ± 0.10	0.04 ± 0.05	0.04 ± 0.07	0.01	1.00
PEG_{80} (urine recovery in %)	21	0.09 ± 0.09	0.02 ± 0.03	0.04 ± 0.05	<0.01	0.47

HF, high-fat diet; L/M, lactulose/mannitol; PEG, polyethylene glycol

Table 44: Impact of weight gain on gut permeability markers

	p - value	95 % convidence intervall
Zonulin (ng/ml)	0.92	0.24(-4.79,5.28)
Mannitol (urine recovery in %)	0.29	-1.91(-5.63,1.82)
Lactulose (urine recovery in %)	0.67	-0.01(-0.08,0.05)
PEG_9 (urine recovery in %)	0.56	-1.03(-4.64,2.57)
PEG_{11} (urine recovery in %)	0.53	-1.51(-6.51,3.48)
PEG_{13} (urine recovery in %)	0.67	-0.15(-0.89,0.59)
PEG_{25} (urine recovery in %)	0.58	-0.02(-0.11,0.07)
PEG_{30} (urine recovery in %)	0.42	-0.02(-0.09,0.04)
PEG_{35} (urine recovery in %)	0.37	-0.04(-0.12,0.05)
PEG_{40} (urine recovery in %)	0.62	-0.02(-0.11,0.07)
PEG_{70} (urine recovery in %)	0.69	-0.01(-0.04,0.03)
PEG_{80} (urine recovery in %)	0.31	-0.01(-0.03,0.01)

PEG, polyethylene glycol

5.2.5 Gut microbiota profiles

After quality- and chimera-check, a total of approximately 860,000 sequences (12,468 ± 2,262 per sample) clustering in 276 OTUs were analysed. The short-term HFD did not affect *alpha*-diversity (**Figure 25 A**). *Beta*-diversity analysis revealed marked inter-individual differences and no distinct and significant clustering according to time points (**Figure 25 B**). The phylogenetic distance between different time points did not change significantly (p = 0.33) (Appendix, Supplemental Figure 4). In spite of these inter-individuals differences (Appendix, Supplemental Figure 5), explorative analysis of taxonomic groups across all individual revealed a decreased relative abundance of sequences classified within the family *Bacteroidaceae*, and an increase in those classified within the order *Betaproteobacteria* associated with overfeeding (**Figure 25 C**). In general, *Firmicutes* and *Bacteroidetes* were the two major phyla, followed by *Actinobacteria*, *Proteobacteria*, and *Verrucomicrobia* (Appendix, Supplemental Figure 6). Of note, 6 individuals were characterised by drastic shifts (>40 % dissimilarity) in gut microbial diversity after HFD (**Figure 25 D**). Explorative analysis of taxa for these individuals revealed lower relative abundance of sequences within the phylum *Bacteroidaceae* and higher relative abundance of 3 moleuclar species: *Blautia wexlerae* (OTU 2), *Coprococcus comes* (OTU 21), and *Alistipes* sp. (OTU 93) . (**Figure 25 E**). Altogether, the data suggest that short-term HFD did not trigger consistent and substantial rearrangements of fecal bacterial populations in this cohort of healthy male subjects. Moreover, there were no major differences between participants gaining more or less than 1 kg (Appendix, Supplemental Figure 5).

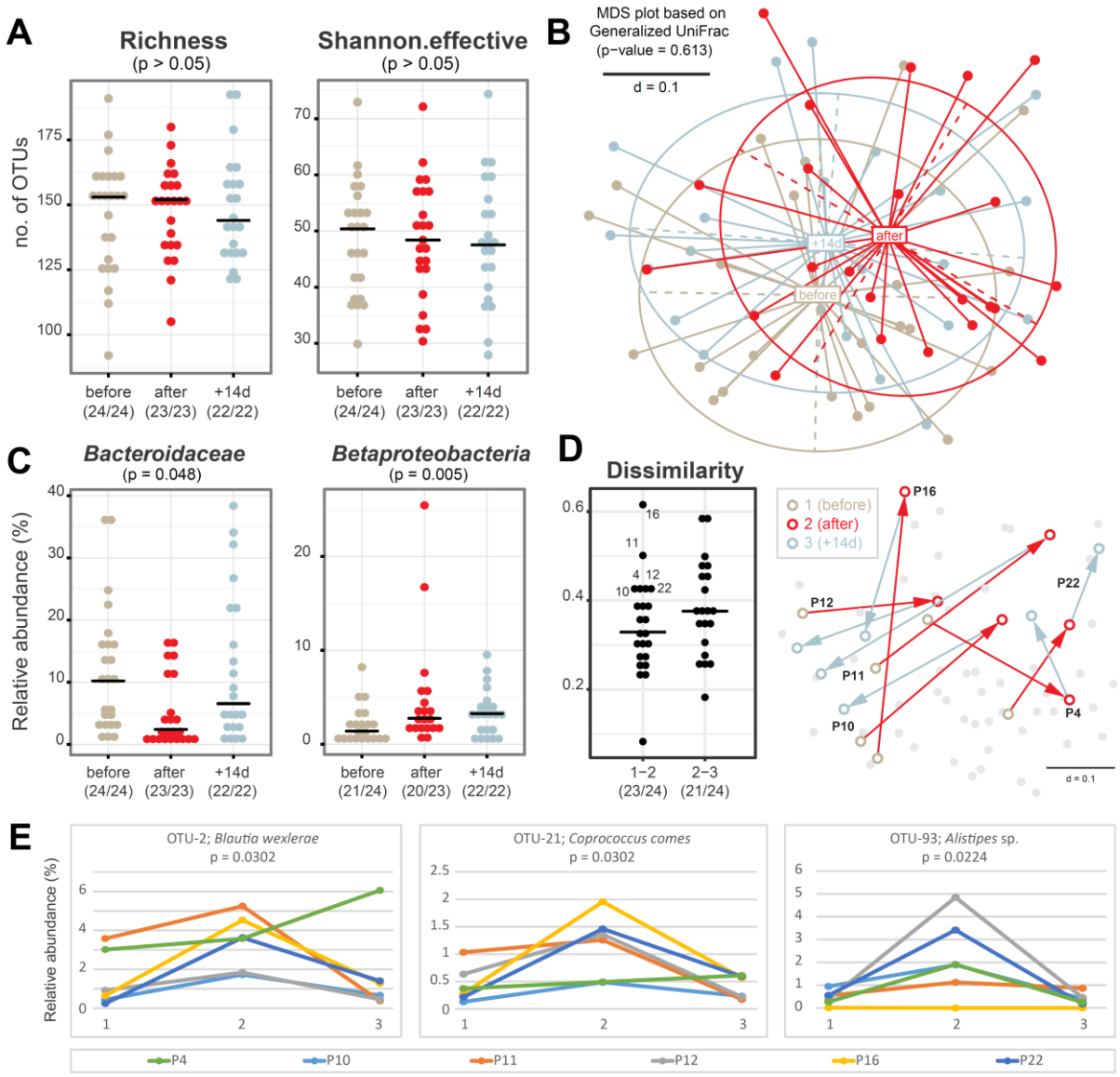


Figure 25: Fecal microbiota analysis by 16S rRNA gene amplicon analysis. (A) Diversity within samples (alpha-diversity) was estimated by species richness and Shannon-effective counts. (B) Multidimensional scaling plot of phylogenetic distances based on generalized UniFrac (beta-diversity). C) Bacterial taxonomic groups altered by the intervention. D) Overtime analysis of phylogenetic profiles revealed 6 individuals with marked shifts after overfeeding. E) Individuals in D were characterised by significant changes in the relative abundances of specific taxa. NMDS plot, nonparametric multidimensional scaling plot; OTU, operational taxonomy unit, VLCD, very low caloric diet

5.2.6 Comparison of routine, anthropometric, clinical chemistry and metabolic parameters at baseline with parameters 14 days after HFD

As additional information, we assessed the different markers two weeks after HFD. Comparing anthropometric, clinical chemistry and metabolic parameters at baseline with 14 days after HFD, the following parameters showed significant changes: body weight, RANTES and PEG with high molecular weight were significantly altered (**Table 45**). Body weight increased from 76.6 ± 10.3 to 77.3 ± 10.4 kg. RANTES decreased from 27.0 ± 15.00 to 16.3 ± 8.7 ng/ml. Finally, the PEGs were significantly decreased compared to the baseline values. (All parameters were presented in Appendix, Supplemental Table 6 and Table 7).

Table 45: Comparison of baseline values with values 14d after HFD

	p-value t1-t3
Energy intake (kcal/d)	0.25
Fat intake (g/d)	0.15
Protein intake (g/d)	0.24
Body composition	
Weight (kg)	< 0.001
Body mass index (kg/m²)	< 0.001
Waist circumference (cm)	0.65
Hip circumference (cm)	0.91
Lean Mass (kg)	0.38
Fat Mass (kg)	0.01
RMR, blood pressure, pulse rate	
RMR (kcal)	0.94
Pulse (HF/min)	0.09
Blood pressure systolic (mmHg)	0.01
Blood pressure diastolic (mmHg)	0.03

	p-value t1-t3
Clinical chemistry, metabolic and inflammation markers	
AST (U/l)	0.98
ALT (U/l)	0.63
GGT (U/l)	0.1
Creatinine (mg/dl)	0.98
Uric acid (mg/dl)	0.23
Sodium (mmol/ml)	0.36
Potassium (mmol/l)	0.11
Calcium (mmol/l)	0.24
Total cholesterol (mg/dl)	0.85
HDL-cholesterol (mg/dl)	0.66
LDL-cholesterol (mg/dl)	0.41
Triglycerides (mg/dl)	0.21
NEFA (mmol/l)	0.13
hsCRP (mg/dl)	0.26
HMW Adiponectin (mg/ml)	0.58
Leptin (ng/ml)	0.3
RANTES (ng/ml)	< 0.001
MCP-1 (pg/ml)	0.08
Chemerin (ng/ml)	0.33
LBP (mg/dl)	0.15
Calprotectin (mg/kg)	0.07

	p-value t1-t3
ROS	
ROS production rate	0.67
ROS production rate]	0.35
Glucose metabolism	
Fasting blood glucose (mg/dl)	0.35
Fasting insulin levels (µU/ml)	0.47
Gut permeability markers	
Zonulin (ng/ml)	0.05
Sucrose (urine recovery in %)	0.71
Sucralose (urine recovery in %)	0.17
Mannitol (urine recovery in %)	0.55
Lactulose (urine recovery in %)	0.91
L/M ratio	0.23
PEG_9 (urine recovery in %)	0.37
PEG_{11} (urine recovery in %)	0.32
PEG_{13} (urine recovery in %)	0.79
PEG_{25} (urine recovery in %)	0.27
PEG_{30} (urine recovery in %)	0.13
PEG_{35} (urine recovery in %)	0.02
PEG_{40} (urine recovery in %)	0.01
PEG_{70} (urine recovery in %)	< 0.01
PEG_{80} (urine recovery in %)	< 0.01

ALT, alanine transaminase; γGT, γ-glutamyltransferase; AST, aspartate aminotransferase; BMI, body mass index; HDL, high-density cholesterol; HFD, high-fat diet; HMW, high-molecular weight; HOMA-IR, homeostasis model assessment of insulin resistance; hsCRP, high-sensitivity C-reactive protein; LBP, lipopolysaccharide binding protein; LDL, low-density cholesterol; L/M, lactulose/mannitol ratio; MCP-1, monocyte chemoattractant protein-1; NEFA, non-esterified fatty acids; PEG, polyethylene glycol; RANTES, regulated on activation normal T cell eExpressed and secreted; RMR, resting metabolic rate; ROS, reactive oxygen species; WHR, waist-to-hip ratio

6 Discussion

6.1 Caloric restriction

Purpose of this study was to assess the effect of a standardised 4-week caloric restriction on gut permeability, inflammatory markers, and the gut microbiota profiles in obese women.

6.1.1 Formula diet and weight loss

Formula diets are a well-known and a common method for loosing weight (Larsen et al., 2010b) (Edholm et al., 2011) (Harder et al., 2004) (Gripeteg et al., 2010). In the present study, participants received the Modifast® diet providing 800 kcal of energy/day. The formula diet consisted of 22.7 EN% from fat, 26.5 EN% from protein, and 50.8 EN% from carbohydrates (Appendix, Supplemental Table 8). Compared to the recommendation of the German Society of Nutrition which includes 30.0 EN% fat, 15.0 EN% protein, and 55.0 EN% carbohydrates, the dietary pattern comprises a low-energy and a high-protein content. By using this formula diet, participants received a highly-standardised diet during the 28 days. The formula diet was easy to prepare for the participants to promote a high compliance rate.

During the last two weeks of the study following the intervention programme, participants tried to follow the recommendations of our nutritional counselor and documented their dietary habits again. The dietary behaviour of the participants before and after intervention was assessed by using dietary protocols. Based on their documentation, macronutrient intake was calculated with OptiDiet®. Before the intervention, calculation of the energy intake was on average 1,698.1 ± 592.7 kcal/day. During the last two weeks, the energy intake was on average 1,281.9 ± 594.9 kcal/day. Interestingly, comparing the baseline macronutrient intake before intervention and macronutrient intake during the last two weeks, energy intake and carbohydrate intake was significantly decreased, whereas fat intake and protein intake returned to their baseline levels. The German Nutrition Society recommends an average energy intake of 1,800 kcal/day for women with normal physical activity level.

Our analysis of the dietary records of the obese participants showed an energy intake below the official recommendations. Obviously, there is a high discrepancy between the documented energy intake and the *de facto* energy intake of obese participants. Although dietary protocols, 24h-recalls or other food records are common methods to assess dietary intake it is well accepted that systematic errors occur during documentation causing bias in the results and their interpretation. One problem in this context is underreporting which is more prevalent in obese people (Braam et al., 1998), people at higher age (Johansson et al., 2001), and in women (Heitmann and Lissner, 1995) (Novotny et al., 2003). Additionally, the process from writing a dietary record to the calculation of nutritional values includes many sources of errors: First, participants have to weigh or to quantify all of their foods and beverages by using common household measures. Misreporting is characterised as inaccurate reporting of foods (Poslusna et

al., 2009) and can be made if a participant documents that he/she ate bread with cheese without any information on weights, fat content or type of bread and cheese. Secondly, not all food items recorded by the participants are listed in the respective database, OptiDiet® in our case. Therefore, similar food products had to be chosen for analysis while the nutrient content can vary substantially. We tried to minimise these limitations by handing out detailed information.

6.1.2 Weight loss improves metabolic features

As expected, in the current study, treatment with a VLCD providing 800 kcal/day resulted in a substantial weight reduction. Participants lost on average 6.9 kg during the 28 days of VLCD, which was on average 7.2 ± 1.6 % below their initial body weight. The course of weight loss over time was assessed during weekly routine examinations. The recorded weight loss was in line with the findings of Clément and colleagues in 2004, who also included a VLCD with 800 kcal for 28 days (Clément et al., 2004a). Also worth noting is the fact that a higher weight loss could be detected in participants with higher initial body weight. Again, this finding is in accordance with previous studies (Webster and Garrow, 1989) (Hansen et al., 2001) (Handjieva-Darlenska et al., 2010). One reason could be that obese participants had higher RMR (Hoffmans et al., 1979). Participants with a higher RMR had a greater energy efficiency than participants with lower RMR (Handjieva-Darlenska et al., 2010). In addition to a decline of body weight, the weight loss resulted in a decline of waist and hip circumference, lean mass, and total fat mass which was also shown before (Verhoef et al., 2013) (Gu et al., 2013) (Pendyala et al., 2011). Weight loss was followed by significantly decreased lean mass and fat mass. Furthermore, in the present study the regression analysis showed a link between waist circumference and total body fat mass, especially abdominal SAT. In the recent literature, waist circumference was shown as a predictor for SAT (Janssen et al., 2002). Furthermore, SAT, VAT, and the liver fat fraction declined significantly in our study. The findings are in agreement with previous studies, as already discussed in the publication of Cordes et al. (Cordes et al., 2015). In the Framingham Heart Study with 3001 participants, an association between metabolic risk factors such as blood pressure, fasting plasma glucose, triglycerides etc. and SAT and VAT was identified (Fox et al., 2007). In our study, we could not show this association. One reason could be that the small sample size had not the adequate power.

As additional information, we studied the participants after returning to a balanced weight-maintaining diet for two consecutive weeks. Participants gained approx. 300 g of body weight which could also be explained by physiologic body weight fluctuations. Body weight, waist circumference and hip circumference remained stable during this post-intervention period. Lean mass significantly increased and fat mass decreased during the subsequent two weeks after intervention. Finally, comparing baseline body composition values from the first visit with the body composition values from the last visit, it can be stated that the body composition was significantly improved. Therefore, it can be hypothesised, that participants followed the instructions of the dietary counsellor.

Another outcome was the measurement of blood pressure and heart rate, because obesity increases the risk of developing hypertension (Mertens and Van Gaal, L F, 2000). The positive effect of moderate weight loss on blood pressure was observed in several studies (Parker et al., 2002) (Clifton et al., 2009) (Gu et al., 2013). In the present study, the obese participants had an average blood pressure of 123 ± 83 mmHg and an average pulse rate of 72 HF/min, both at baseline. These parameters were in a normal range and remained stable during the whole study period.

6.1.3 Impact of VLCD on energy metabolism

With an average RMR of 1,675 kcal/day, participants presented comparable values to the published literature (Johannsen et al., 2008). Handjieva-Darlenska et al. showed that the higher the initial body weight, the higher the RMR is (Handjieva-Darlenska et al., 2010). In our study, we could also demonstrate that the initial RMR correlated with the initial body weight. Surprisingly, 28 days of caloric restriction did not result in an alteration of the RMR, whereas it significantly increased during the subsequent two weeks after VLCD. Grouping participants with MetS (n = 8) and without MetS (n = 12) according their RMR value, those with MetS did not show any change in RMR during the whole study. In contrast, RMR readings of participants without MetS were significantly decreased following caloric restriction which is in line with other studies investigating a similar study population and a comparable diet (Hill et al., 1987) (Clément et al., 2004a). During the last two weeks of the study, RMR of participants without MetS significantly increased again and return to their baseline status. In literature it is described that participants with MetS had a lower RMR that participants without MetS (Buscemi et al., 2007) (Soares et al., 2011). In the current study, we could not confirm this finding. The small sample size of the two groups could be one reason for this effect. Moreover, we could not show differences in the RMR between pre- and postmenopausal women over all time points of the study. One reason could be the inter-individual variations in RMR during weight loss described in Sénéchal et al. (Sénéchal et al., 2010). Moreover, the age of participants, as a determinant of RMR, varied from 24 years to 63 years, which could enhance the inter-individual variations in RMR. In addition to the determinant age influencing RMR, the RMR can be influenced by sex, race, fat free mass, fat, and the brain (Javed et al., 2010). But, sex and race can be neglected because only Caucasian women were included into the study. The lean mass is a key determinant of RMR (Korth et al., 2007) and was significantly decreased after VLCD. So, it was expected that the RMR decreased as well (Ferraro et al., 1992). But this effect could be due to the already mentioned high inter-individual variation.

In addition to the dietary behaviour and RMR, we were interested in the physical activity of our participants. Although this parameter is not a primary readout of the study, all participants received an activity sensor for motivational reasons. Participants had an average of 4.5 ± 1.3 km/day or 6,880 ± 1,962 steps/day. If participants reach 10,000 steps/day, activity sensor show a smiley emoticons. On average participants did not reach the 10,000 steps/day, but participants told me to be more motivated to go for a walk by using activity sensor.

6.1.4 The inflammatory status improved during hypocaloric intervention

Over the four week period, the VLCD resulted in a significant improvement of the lipid profile. The effect of caloric restriction on lipids were in accordance with another study with a similar study population (Clément et al., 2004a). In obesity, NEFA levels in plasma are elevated and have been linked with insulin resistance, risk of cardiovascular disease, atherosclerosis, and hypertension (Boden, 2008). In the present study, the acchieved weight loss did not result in decreasing NEFA concentrations in plasma. Other studies with a similar degree in weight loss by 7 % (Xydakis et al., 2004) or 10 % from the initial body weight (Maraki et al., 2011) did not show alterations of NEFA levels either, and were therefore in line with the current study.

With respect to inflammation, hsCRP should always be considered. It is an acute phase reactant secreted mainly by hepatocytes (Pepys and Hirschfield, 2003). HsCRP levels are elevated in cardiovascular diseases (Heilbronn and Clifton, 2002), but also in obese and overweight people (Visser et al., 1999). In the present study, the plasma concentrations of the inflammation marker hsCRP were decreased following VLCD, which is in agreement with literature (Bassuk et al., 2004). Therefore, a reversal of elevated hsCRP concentrations towards normal levels is not surprising, as it is predominantly produced by the liver and indicates an improvement of systemic inflammation in response to weight loss. To complement the inflammatory state we also measured LBP levels before, immediately after and 14 days after overfeeding. LBP as circulating LPS-binding protein, was used as a biomarker of intestinal bacterial translocation, since LPS has only a short half-life and is hard to detect (Novitsky, 1998). The impact of LBP was measured in humans and higher levels of LBP were detected in obesity (Moreno-Navarrete et al., 2011). Baseline concentrations of LBP in the presented study were similar to other studies (Sun et al., 2010). The caloric restriction resulted in a decline of LBP, indicating an improvement of the gut permeability. Interestingly, an increase of LBP and paracellular gut permeability in the last two weeks could be detected. Furthermore, the detection of calprotectin levels in fecal samples, as a neutrophilic intestinal inflammation marker in the gut, which is widely used as non-invasive marker for inflammatory bowel diseases, was included (Däbritz et al., 2014). Recent publications demonstrated elevated calprotectin concentrations in obese individuals (Mortensen et al., 2009) (Pedersen et al., 2014). In the present study, fecal calprotectin was measured, which was comparable to concentrations from lean people. Also Gøebel et al. did not detect significant differences in the levels of fecal calprotectin between lean and obese subjects. The authors hypothesised that their test might not be sensitive enough. Fecal calprotectin was measured by using ELISA methodology (Gøbel et al., 2012). This could also be an explanation for our findings. In contrast, Mortensen et al. demonstrated increased plasma calprotectin in obese subjects by using ELISA technique, as well. But, the research group used another kit (Hycult biotechnology,Uden, NL) which is more sensitive (Mortensen et al., 2009).

As additional information, 14 days after the dietary intervention, the lipid profile and inflammation markers were measured again. At that time point, all markers returned to baseline values before the study at visit 1. The data suggest that most changes observed under

the VLCD disappeared, indicating that these changes were mainly due to the acute and marked caloric restriction rather than to the moderate decrease in body weight, as participants did not gain substantial weight during the two weeks of follow-up.

6.1.5 Adipose tissue and its adipokines and chemokines

Despite the significant weight reduction, no decline in fat cell size of SAT was observed. Verhoef et al. showed that a 10 % weight loss mediated a significant decline in adipocyte size (Verhoef et al., 2013). As this association is very much accepted by the scientific community, this result was ascribed to the low number of histological sections (n = 8). The low number of histological sections was explained by the low amount of fat tissue obtaining from abdominal subcutaneous adipose tissue biopsy. However, leptin mRNA levels from the puncture material showed that its expression was significantly reduced. This decrease might indicate a relief of the obesity-associated inflammatory load, although other parameters like adiponectin, RANTES, MCP-1 and CD68 were not different before and after intervention. Regarding adiponectin, RANTES, MCP-1 and CD68, the small sample size could be one reason why the VLCD had no significant impact on these parameters, which is in contrast to published data. The expression of adiponectin in SAT was increased after gastric bypass surgery (Savu et al., 2009) and a 12 weeks lifestyle intervention including walking (Moghadasi et al., 2013). Furthermore, RANTES expression in SAT was also decreased after surgery-induced weight loss (Montecucco et al., 2015). Moreover, CD68 expression in SAT was reduced after six months of dietary intervention (Kovacikova et al., 2011). Cancello et al. confirmed the decline of both macrophages and MCP-1 expression in SAT after bypass surgery (Cancello et al., 2005a) indicating only greater weight loss led to a significant downregulation of RANTES, CD68, and MCP-1 expression.

Plasma levels of leptin follow its expression in fat tissue and decreased. HMW adiponectin levels in plasma increased after the intervention phase. The status of leptin and HMW adiponectin remained stable during the last two weeks. Finally, the leptin levels were significantly lower 14 days after intervention compared to the baseline status. HMW adiponectin levels were also significantly higher 14 days after intervention compared to the baseline status. The present study confirmed the positive impact of caloric restriction on leptin and HMW adiponectin and was in line with previous studies (Varady et al., 2009) (Yang et al., 2001). In addition, Abbenhardt et al. showed that the combination of weight loss and moderate-to-vigorous intensity aerobic exercise promoted an improvement of adipokine levels (Abbenhardt et al., 2013). In summary, lower plasma levels of leptin and increased plasma levels of adiponectin with its vasoprotective and antidiabetic effects have a beneficial effects on chronic diseases such as type 2 diabetes (Mather et al., 2008). Finally, chemerin expressed by liver and adipose tissue was measured because of its link to obesity, MetS (Li et al., 2014) (Bozaoglu et al., 2007) (Lehrke et al., 2009) and insulin resistance (Weigert et al., 2010). In the present study, no association between obesity and MetS- associated- parameters and chemerin were identified (Appendix, Supplemental Table 9), which might be explained by the small sample size. In literature, decreased levels of chemerin were shown after bariatric surgery (Ress et al., 2010),

12 weeks of lifestyle modification (Kim et al., 2014), and 8 weeks of weight loss (Lee et al., 2013). In this study, we could show that a moderate weight loss mediates a decline of the chemoattractant protein chemerin.

In addition to adipokines, several chemokines in plasma were investigated. The proinflammatory cytokine MCP-1 is mainly secreted by macrophages and is a key determinant in the regulation of macrophage infiltration and is linked with type 2 diabetes (Nomura et al., 2000) (Sartipy and Loskutoff, 2003) and atherosclerosis (Deo et al., 2004). As shown in previous studies, a weight reduction leads to a decline of MCP-1 in plasma and can improve the inflammatory status (Christiansen et al., 2005) (Bruun et al., 2006). However, we were not able to detect any improvements in plasma levels of MCP-1 after 28 days of caloric restriction, which might be explained by the relatively slight weight loss of approximately 7 % (Forsythe et al., 2008) , because a higher weight loss was shown to cause reductions in MCP-1. Christiansen et al. observed a weight loss of 12 % and had a decline of MCP-1 of 20% (Christiansen et al., 2005). Bruun et al. showed that the level of MPC-1 in plasma decreased after 15 weeks of weight loss (Bruun et al., 2006). Another relevant chemokine in this context is RANTES. RANTES is expressed by mature adipocytes and its release is depending on the size of adipocytes (Skurk et al., 2009). Levels of this chemokine are elevated in obese humans and are associated with inflammatory conditions. RANTES seems to be associated with T-cell and macrophage recruitment into the VAT (Wu et al., 2007). Moreover, higher RANTES concentrations were measured in patients with type 2 diabetes (Herder et al., 2005). Surprisingly, RANTES concentrations remained stable during the entire study which might be explained by the small sample size. Dalmas et al. showed in his publication that obese participants had significantly decreased RANTES concentrations in plasma at 3, 4, 12 months after a RYGB (Dalmas et al., 2011). It can be hypothesised that the sample size of our study was to small or the weight loss was not sufficient to mediate a decline of RANTES concentration.

6.1.6 Effect of caloric restriction on immune cell subpopulation of PBMCs

The subpopulation of PBMCs plays an important role in inflammation and obesity-associated diseases. Monocytes are a heterogeneous population of PBMCs producing cytokines such as TNF and mediating to inflammatory cascades (Frankenberger et al., 1996). According to nomenclature, the following three types of monocytes were shown: $CD14^{++}CD16^{-}$ (classical monocytes), $CD14^{++}CD16^{+}$ (intermediate monocytes), $CD14^{+}CD16^{++}$(non-classical monocytes) (Ziegler-Heitbrock et al., 2010). A former nomenclature classified two types of monocytes: $CD16^{+}$ monocytes and $CD16^{-}$ monocytes (Passlick et al., 1989). In the present study, we showed 84.3 ± 8.6 % of classical monocyte before VLCD. Abundance of classical monocytes were in the normal, healthy range, which is comparable to values found in literature like for participants with an BMI of 25 ± 4 kg/m² (Rogacev et al., 2010). We were not able to demonstrate an alteration of the different monocyte subpopulations after 28 days of caloric restriction. In contrast to that, Poitou et al. showed that $CD14^{++}CD16^{+}$ and $CD14^{+}CD16^{+}$ were increased in obese subjects and were declined following hypocaloric diet and RYGB. It should be mentioned,

that the baseline BMI in this study before gastric surgery was 49.1 ± 1.4 kg/m² and the samples size was higher (n = 38), which could be a reason for this discrepancy between the studies (Poitou et al., 2011).

$CD4^+FoxP3^+T_{reg}$ cells express anti-inflammatory cytokines, such as IL-10 (Feuerer et al., 2009). In our study, the percentage of T_{reg} cells within $CD4^+$ were assessed after caloric restriction and no alteration was detected. Regarding the status of T_{reg} cell in other humans studies, Van der Weerd et al. demonstrated in morbid obese participants an increased number of $CD4^+CD25^+FoxP3$ T_{reg} cells (van der Weerd et al., 2012). In contrast Wagner et al. showed that T_{reg} cells were reduced in obese compared to lean participants (Wagner et al., 2013). However, T_{reg} population in lean participants and patients with anorexia nervosa seem to be similar in both groups (Paszthy et al., 2007). Regarding T_{reg} cells, there are conflicting data and more studies with a higher sample size are needed to better understand the role of T_{regs} in obesity and after weight loss in human.

The percentage of $CD3^+$ or $CD19^+$ within PBMCs was not altered due to changes in caloric intake. Pekkarinen et al. showed a decrease of $CD3^{+,}$ $CD4^+$, and $CD8^+$ after 14.4 weeks (mean duration) of VLCD and 15% of the initial weight in obese participants (Pekkarinen and Mustajoki, 1997). There was no alteration detected in B cells after bariatric surgery (Merhi et al., 2009). In addition to the subpopulations, we measured intracellular S6k, which is a downstream target of the insulin signaling pathway (Um et al., 2006). It was measured before and after caloric restriction in the different immune cell subpopulations (CD3/CD4/CD8/CD14/CD19). Again, the caloric restriction did not result in any alterations of the MFI of S6k in different immune cell subpopulations. While caloric restriction activated the S6k in the brain of C57BL/6 mice (Ma et al., 2015), no data on clinical studies in the context of weight loss is available, suggesting that more studies are needed on the role of S6 in different immune cell subpopulations.

6.1.7 Effect of caloric restriction on oxidative stress

As mentioned before, inflammation plays a key role in the development of diabetes. In addition to proinflammatory adipokines, chemokines and bacterial factors, also oxidative stress seems to be involved in the development of hyperlycemia and insulin resistance (Dandona et al., 2004). Mohanty et al. demonstrated that glucose intake (Mohanty et al., 2000), fat and protein intake (Mohanty et al., 2002) leads to increased ROS production. Furthermore, elevated ROS levels were detected in obesity (Furukawa et al., 2004). Dandona furthermore stated that ROS produced by mononuclear cells and polymorphonuclear cells were decreased after 4 weeks of caloric restriction (Dandona et al., 2001). In the presented study, neither in plasma nor in PBMCs, ROS changed following the 28 days of caloric restriction. The different results could be due to different measurement techniques: In our study ROS was measured by using EPR spectroscopy. The research group of Danodana analysed the release of superoxide radical by using chemiluminescence (Dandona et al., 2004). Moreover, ROS can be influenced by factors

such as copper and iron. Therefore, ROS levels are difficult to detect accurately in biological specimen (Dikalov et al., 2011).

6.1.8 Glucose and insulin status before and after VLCD

Obesity is linked with disturbances in the glucose-insulin homeostasis leading to type 2 diabetes (Wellen and Hotamisligil, 2005). Regarding the respective baseline status in our study, average fasting glucose concentrations in plasma were in the normal range. Moreover, results of the oGTT showed that plasma glucose levels after 120 min were also in a healthy range. The oral glucose tolerance tests after VLCD showed lower glucose levels in plasma compared to the study start. In addition to fasting glucose concentrations in plasma, fasting insulin levels were assessed and exhibited significant improvements after 28 days of VLCD which underlines the data available in published studies (Clément et al., 2004a) (Parker et al., 2002) (Gu et al., 2013). The beneficial impact of weight loss on glucose metabolism can be highlighted in one participant: Before VLCD, this participant had a baseline glucose concentration of 122 mg/dl and the glucose level after 120 min (oGTT) was 241 mg/dl. After intervention, this participant lost 6 kg body weight and had a baseline glucose level of 87 mg/dl and the 120 min glucose level was at 118 mg/dl. In this case, weight loss led to a decreased inflammation status such as hsCRP resulting in improved glucose levels.

6.1.9 Markers of paracellular gut permeability decreased during VLCD

The primary question was to find out whether gut permeability, which is reported to be compromised in obesity (Teixeira et al., 2012), can be positively modified by caloric restriction. In the present study gut permeability parameters in obese women were comparable with lean counterparts (control groups) from other studies (Haas et al., 2009) (Norman et al., 2012). Nevertheless, our findings based on a variety of different methods indicate that a standardised caloric restriction resulting in moderate weight loss significantly improved gut permeability, in particular paracellular translocation. These data are consistent with results of a recent Chinese study reporting better epithelial integrity after a 9-week diet consisting of traditional Chinese foods and prebiotics (1,000-1,600kcal/day) (Xiao et al., 2014b). Moreover, a study including obese participants undergoing a 52 week weight-reduction programme demonstrated an improvement of paracellular gut permeability after weight reduction, as well (Damms-Machado et al., 2016). However, our study extends this observation by providing a more comprehensive dataset using a variety of methods. It is interesting to note that an improved gut barrier was observed for sucrose, which reflects gastroduodenal permeability and sucralose, which is taken up in the colon. Likewise, decreases in the translocation of PEG particles of various sizes reflecting paracellular permeability were measured in urine. Zonulin, a physiological modulator of intercellular tight junctions (Fasano, 2000) (Fasano, 2011), was also found to decrease during caloric restriction, but rapidly returned to baseline levels after returning to a normal diet. In conclusion, caloric restriction induced uniform and consistent changes towards a more tight gut barrier. To be able to between dissect effects elicited by caloric restriction or by weight loss, we

reinvited the participants into the study center after returning to a weight-maintaining diet for two consecutive weeks. Moreover, there was no association between weight change during the VLCD period and markers of gut permeability. The data suggest that most positive changes observed under the VLCD disappeared, indicating that these changes were mainly due to the marked caloric restriction rather than to the moderate decrease in body weight, as participants did not gain substantial weight during the two weeks of follow-up.

The present study demonstrated that LBP and paracellular gut permeability marker were significantly improved following 28 days of caloric restriction. As a consequence, the impact of the paracellular gut permeability change on inflammation marker such as LBP were analysed. We could not find an association between improved paracellular gut permeability and LBP. Explanations could be due to the small sample size or that LPS is mainly translocated via chylomicrons into the circulation.

Finally, Moreno-Navarrete showed that zonulin increased with BMI (Moreno-Navarrete et al., 2012). In our study, we used the same ELISA kit detecting circulating zonulin. However, we were not able to detect an association between zonulin and BMI. The research group around Moreno-Navarrete included 123 men with BMI under 30 kg/m². It therefore remains speculative if our sample size or gender differences might account for this discrepancy.

6.1.10 No major alterations in the gut microbiota profiles

With respect to fecal microbiota profiles, the overall bacterial phylogenetic makeup was not substantially affected by caloric restriction in a consistent manner across al individuals. Specifically, we did not observe changes in *alpha*-diversity, in contrast to the drop in richness reported in association with detrimental dietary habits and metabolic health (Le Chatelier et al., 2013) (Cotillard et al., 2013a). Regarding gut microbiota composition, an “obese phenotype” of the gut microbiota composition is characterised by an increased ratio of *Firmicutes/Bacteroidetes*. In our study, before and after intervention the ratio of *Firmicutes/Bacteroidetes* did not alter significantly. Ley et al. demonstrated that weight loss can be a strategy to shift this ration towards a lower abundance of *Firmicutes* and a higher abundance of Bacteroidetes (Ley et al., 2006), which was also shown by several other groups (Santacruz et al., 2009) (Remely et al., 2015) (Xiao et al., 2014b). Decreased relative abundances of *Proteobacteria* were observed, which altogether confirms other reports on their occurrence in individuals with impaired metabolic health (Shin et al., 2015) (Xiao et al., 2014a). Relative abundance of the species *Anaerostipes hadrus* was increased after 28 days of caloric restriction and returned to baseline values after intervention. Although data in the present study are descriptive and exploratory, and no cause-to-effect relationship can be established, *A. hadrus* was described as a butyrate-producing bacterium (Allen-Vercoe et al., 2012) (Kant et al., 2015). Butyrate is usually regarded to be beneficial for the gastrointestinal tract function with respect to epithelial proliferation (Kripke et al., 1989) and improvement of barrier function of the gastrointestinal epithelium (Peng et al., 2009). In contrast, median relative abundances of

another butyrate-producing species, *Agathobacter rectalis*, were decreased from ca. 7 to 4% after caloric restriction. Additional studies including targeted metabolite measurement will be needed to clarify the impact of caloric restriction on butyrate production in the gut. The intervention was also associated with increased occurrence of one OTU in each the *Ruminococcus* and *Bifidobacterium* genus, which both include degraders of complex dietary and host-derived polysaccharides (Baer et al., 2014). Santacruz et al. also showed an increase in qPCR counts of *Bifidobacterium* spp. after weight loss (-6,9 kg) in obese adolescents following 10 weeks of caloric restriction (Santacruz et al., 2009).

To sum up, a four-week dietary restriction resulted in significant weight loss without major effects on gut microbiota profiles. In addition to factors such as age, culture, and lifestyle, diet plays an important role on health. In the current study, we focused on the impact of moderate weight loss on gut microbiota profiles. Recent studies showed that short exposure time may be sufficient to modify the gut microbotia profiles (Wu et al., 2011) (David et al., 2014). In our study, we could confirm this assumption regarding improvement of several butuyrat producing bacteria.

6.2 Short-term overfeeding

The study was performed to investigate the effect of acute overfeeding on gut barrier function and microbiota composition. Therefore, the impact of a hypercaloric diet including both high-fat and high-caloric (HFD) (similar to the "Western diet") on different aspects: inflammation, glucose metabolism, gut permeability, and gut microbiota profiles were assessed.

6.2.1 Dietary behaviour and physical activity before and after hypercaloric diet

In the present study, we aimed for an excess energy intake of 1,000 kcal above the calculated total energy expenditure of the study participants. A macronutrient composition of 48 EN% from fat, 18 EN% of protein and 34 EN% of carbohydrate was targeted. Before and after the overfeeding intervention, participants recorded their daily dietary behaviour for 7 days. 7 days before the high-fat, high-caloric intervention the average energy intake of 2,730 ± 707.9 kcal/day and the macronutrient composition of 38 EN% of fat, 15 EN% of protein and 47 EN% of carbohydrates was matching the recommendations of the German Nutrition Society for lean healthy young men. During the two weeks after the HFD all participants were asked to return to their usual dietary behaviour. Compared to the initial energy intake, participants had a similar energy intake and macronutrient composition during this post-intervention period. During the whole study, the participants were asked to minimise their physical exercise. The analysis of the accelerometers confirmed that the participants minimised physical activity. We aimed to achieve an excess energy intake during 7 days and wanted to prevent that participants increase their physical activity and thereby change energy expenditure.

6.2.2 Impact of short-term overnutrition on the anthropometric phenotype

The highly-standardised HFD resulted in a significant increase of body weight. Hereby, the weight gain of about 900 g was in the expected range. The initial waist (Boden et al., 2015) and hip circumference and the total body fat (Cahill et al., 2013) (Boon et al., 2015) (Wadden et al., 2012) was in accordance to other studies in healthy young men. As revealed by our bioimpendance analysis measurements, an excess intake of 1,000 kcal/day had a substantial impact on fat mass but not on fat free mass, which confirms data from Boden et al. (Boden et al., 2015). In contrast, the weight gain or gained body fat mass, respectively, did not induce a significant increase in waist and/or hip circumference. Therefore, it could be hypothesised that the abdominal SAT did not expand during the short term overfeeding for one week. Moreover, also lean mass was not significantly altered during the whole study. During the last two weeks participants lost about 300 g of body weight again. Consequently, the waist circumference, hip circumference, and fat mass were not significantly altered during the subsequent two weeks after intervention.

6.2.3 Effect of short-term overfeeding on energy metabolism

In the present study the RMR did not increase significantly after 7 days of positive energy surplus, even not after grouping the participants according to their weight gain. Dirlewanger et al. investigated 3 days of overfeeding with dietary fat in ten healthy lean women and was not able to find significant changes in RMR after overfeeding (Dirlewanger et al., 2000b). Other studies showed a significant increase of the RMR after either a higher energy intake or longer duration of overfeeding. Harris et al. showed that after 8 weeks of overfeeding (1,000 kcal/day) the RMR rapidly increased by 5.2% only in the first two weeks. Afterwards, the increase remained stable (Harris et al., 2006). Looking at the study in more detail RMR values increased from 1,693 ± 155 kcal/day to 1,711 ± 201 kcal/day during the first week. During the second week the RMR increased to 1,781 ± 172 kcal/day. It seemed that the increase of RMR was mainly due to the increase in the second week. Furthermore, an excessive energy intake with 6,000 kcal/day (approximately 2,5 time of the usual energy intake) following 7 days resulted in a marked increase of RMR to 1,719 ± 127 kcal/day (Boden et al., 2015). Ravussin et al. demonstrated a significant increase of the RMR after 9 days of overfeeding (60% above their energy requirement) (Ravussin et al., 1985). The diet, including energy and fat content, seemed to stimulate respiration. In addition, alterations in the RMR were shown to be closely linked to age, race, sex, lean mass and fat mass (Ferraro et al., 1992) (Javed et al., 2010). In the current study, only male Caucasians aged between 19 and 31 years were included and, therefore, influences of age, race and sex can be excluded. The lean mass, as an predominant determinant of the RMR (Ferraro et al., 1992), was not significantly altered during the whole study. The body fat mass increased significantly after 7 days of HFD, but did not influence the RMR in a significant manner. It can be hypothesised that the RMR was not altered because of the marginal alterations of the lean mass during the study, although various other influencing factors are known.

The pulse rate remained stable during the whole study. Surprisingly, the systolic blood pressure was significantly decreased after 7 days of HFD, which is in contrast to previous studies. Jakulj et al. reported that a single fat meal led to an increase of systolic blood pressure (Jakulj et al., 2007). In contrast to saturated fat content in meals, a diet rich in unsaturated fat can decrease systolic blood pressure (Appel et al., 2005). During the last two weeks, the systolic blood pressure was not significantly altered. The diastolic blood pressure was stable during the HFD and decreased during the subsequent two weeks.

6.2.4 High-fat feeding does not result in an inflammatory burst

In the present study, the high-fat, high-caloric diet, including 48 EN% of predominantly saturated-fatty acids, resulted in an increase of total cholesterol and LDL-cholesterol. The results are in line with previous studies of 7-day overfeeding including a 70% caloric surplus (Wadden et al., 2012) and 5 days of a HFD (Boon et al., 2015). Moreover, in the present study triglycerides levels decreased and HDL-cholesterol levels increased after 7 days of HFD. Brøns et

al. investigated the same lipid parameters and explained this alterations with the fatty acid composition of the diet (Brøns et al., 2009). Additionally, in the present study NEFA levels were decreased after overfeeding, which confirms previously published studies (Cornier et al., 2006) (Brøns et al., 2009). Brøns et al. speculated, that the decreased amount of NEFAs is caused by the suppression of lipolysis in adipose tissue (Brøns et al., 2009). In the current study, we were not able to demonstrate significantly increased fasting insulin levels, but the average insulin levels were only marginally higher after HFD than before. During the subsequent weeks, the lipid profiles returned to the initial levels, suggesting that short-term HFD potently influences the lipid profile.

Leptin is released almost exclusively by adipose tissue (Zhang et al., 1994). In the present study, the leptin levels in plasma were not altered after 7 days of standardised HFD as well as during the subsequent two weeks. This finding is in contrast to previous studies, where leptin was increased after overfeeding (Hagobian et al., 2008) (Alligier et al., 2012). An experiment conducted in 1996 tested the effect of acute and chronic overfeeding on circulating leptin levels. The researchers showed that acute overfeeding led to a moderate increase of leptin. A 10% weight change triggered an increase of leptin levels (Kolaczynski et al., 1996). However, not only the duration of overfeeding and amount of energy surplus plays an important role in the alteration of leptin concentrations in plasma. Dirlewanger et al. investigated the effect of 3 days of carbohydrate overfeeding and fat overfeeding on plasma leptin levels in ten healthy lean females. They showed that fat overfeeding did not result in an increase of plasma leptin concentrations (Dirlewanger et al., 2000a). In the present study, the stable concentrations of leptin in plasma could lead to the conclusion that a weight gain of about 900 g body weight did not mediate a significant increase in fat cell size, which leaves leptin unaffected. In addition to leptin, HMW adiponectin increased significantly during the overfeeding intervention and decreased again during the following 2 weeks. A similar finding was reported by Brøns et al. (Brøns et al., 2009) after 5 days of high-fat diet (HFD) (60 % fat, 50 % overfeeding). Other studies also detected elevated adiponectin levels after 3 days (Heilbronn et al., 2013) and 7 days of overfeeding (Cahill et al., 2013). It was speculated that adiponectin may have an attenuating effect on insulin resistance during the development of obesity (Cahill et al., 2013) which could also be the case under the experimental conditions used in the present study.

Chemokines play an important role in obesity-related inflammation. MCP-1 is mainly produced by monocytes and is secreted in increased amounts in the case of inflammation. In the present study, MCP-1 did not change during 7 days of HFD. Surprisingly, during the last two weeks MCP-1 level increased significantly. Tam et al. showed that 28 days of overfeeding with 1,250 kcal/day did not result in an increase of MPC-1 levels (Tam et al., 2010). In addition to MCP-1, RANTES mediates the recruitment of monocytes into adipose tissue (Meurer et al., 1993). Surprisingly, in the present study RANTES was significantly decreased after 7 days of HFD and levels did not change following the last two weeks. This chemokine is mainly secreted by omental and SAT dependent on adipocyte size and BMI. We checked the technical aspect and literature regarding this finding and could not explain the decline of RANTES following HFD. As

an additional adipokine, chemerin was assessed because of its association with obesity and the MetS (Bozaoglu et al., 2007). The chemoattractant protein chemerin remained stable during the whole study. In the literature, only scarce data exist for chemerin and the association to overfeeding. In a mouse study (DIO C57B1/6J; 42 days old) it was shown that chemerin increased after HFD (12 weeks, ad libitum, 59% fat, 22% carbohydrate, and 19% protein) (Lloyd et al., 2015). The release of chemerin, therefore, seemed to be promoted by HFD, suggesting that in the current study the duration of HFD was too short to expect changes. Despite the weight gain in an expected range, the chemokines under investigation did not show a clear direction towards an increased inflammatory response in association with a HFD.

Other inflammatory parameters could not confirm an elevated inflammatory status, as well. hsCRP is mainly produced in the liver, influenced by IL-6, and is rapidly released during inflammation (Pepys and Hirschfield, 2003). The baseline status of this acute-phase reactant was within the healthy range and not altered during the whole study. In summary, MCP-1, chemerin, and hsCRP did not change following 7 days of a high-fat high-caloric diet. The impression that the duration of intervention plays an important role is in line with another overfeeding study in healthy, young lean study participants (Chen et al., 2016).

We measured LBP levels before, immediately after and 14 days after overfeeding. This circulating LPS-binding protein, was used as a biomarker of intestinal bacterial translocation, since LPS has only a short half-life and is therefore hardly detectable (Novitksy, 1998). Recent studies showed a positive correlation between caloric intake and plasma LPS concentrations (Amar et al., 2008). Moreover, Pendyala et al. demonstrated that 4 weeks of a Western-style diet (2,209 kcal, 40% fat of total calories, 20% saturated fat of total calories) resulted in increased plasma endotoxin levels. (Pendyala et al., 2012). In our study, there was no hint for an increase of LBP concentrations arguing that short-term overfeeding does not affect gut permeability or bacterial translocation (Ghoshal et al., 2009). This statement is also supported by the results of our measurements of paracellular gut permeability markers, including zonulin, lactulose and PEG 1500 which remained unchanged following seven days of high-fat overfeeding (see below section 6.2.7). Furthermore, calprotectin was measured in fecal samples as a marker of intestinal inflammation. The baseline concentration of fecal calprotectin was similar to other studies (Gøbel et al., 2012), where a fecal concentration under 50 mg/kg was classified as normal (Jahnsen et al., 2009). Fecal calprotectin was not affected by the dietary intervention. The decline of calprotectin concentrations after HFD can be interpreted as a decline of intestinal inflammation.

Surprisingly, in the present study, the ROS production in plasma was significantly decreased after 7 days of HFD. In contrast, ROS production in PBMCs was not altered during the whole study. Previous studies showed postprandial effects of lipid and protein intakes on polymorphonuclear leukocytes and mononuclear cells (Mohanty et al., 2002). Patel et al. described that lean subjects had increased oxidative stress in mononuclear cells two hours after a high-fat, high-carbohydrate meal returning to baseline levels afterwards (Patel et al., 2007). Less is known about the impact of short-term overfeeding on ROS production in plasma and

PBMCs as measured by electron paramagnetic resonance. In this context, more studies are needed to unravel the role of ROS in different compartments in response to dietary changes.

6.2.5 Immune cell subpopulation after seven days of HFD

It was shown that mononuclear cells are activated by nutritional challenges (van Oostrom et al., 2004) (Gower et al., 2011) (Ehlers et al., 2014). In obesity, T cell activation plays an important role in immune adaption and mediation of inflammation (Gerriets and MacIver). We measured T_{regs} and did not recognise any changes after HFD. There is little evidence in the literature that T_{regs} are influenced by HFD, respectively by overfeeding in humans. In mice, HFD led to a decrease in hepatic T_{regs} due to apoptosis (Ma et al., 2007) (Issazadeh-Navikas et al., 2012). It can be speculated that the effect of 7 days of overfeeding was too weak to result in a significant activation of T cells. Moreover, in our study, T cell populations were characterised before and after intervention by (1) measurements performed within the whole blood sampling procedure and (2) in the fasting state of the HEC (before and after intervention). In this first measurement setting, $CD3^+$ positive cells (percentage of PBMCs) were significantly increased in the blood of individuals after HFD. In the second measurement setting, we did not detect significant changes of $CD3^+$ cells (percentage of PBMCs). The same measurements were done in the same participants at the other day. All PBMC-related analyses were performed in a similar way and by the same person. To show the variability of monocytes and T cells ($CD3^+$, $CD4^+$, $CD8^+$), and B cells on two following days, the measurements were compared by using Mann-Whitney U test and we could not detect any significant changes during the two settings. One explanation could be a high inter-individual variability of the amount of PBMCs. However, the second measurement setting confirmed a previous study performed by Tam et al., which showed that 28 days of overfeeding (+1,250 kcal/day, 45% fat) did not result in an alteration of $CD3^+$, $CD4^+$, and $CD8^+$ (Tam et al., 2010).

S6k is a downstream target of the mTOR pathway (Hay and Sonenberg, 2004). It also plays an important role in the insulin signalling pathway by phosphorylation of IRS1 und IRS2 mediating the inhibition of insulin signalling (Blandino-Rosano et al., 2012). Ehlers et al. showed increased postprandial of S6k phosphorylation in MNC (Ehlers et al., 2014). Based on this fact, we investigated the impact of short-time overfeeding on S6k in different immune cell subpopulations before and after the intervention and during the clamp. In the present study, 7 days of HFD did not result in an alteration of MFI of S6 in different immune cell subpopulations. It can be speculated that the downstream target of the mTor pathway is not activated. The combination was also measured in the clamp situation before and after intervention and no significant changes were measured. This finding is in accordance with the stable insulin and glucose concentrations over time.

The postprandial state after lipid challenges was characterised as a proinflammatory situation with activation of monocytes (van Oostrom et al., 2004) (Gower et al., 2011). In the present study, we investigated the activation of monocytes following short-term overfeeding to assess

whether the proinflammatory situation can be detected 24h after challenge. Here, the percentage of classical and non-classical monocytes was increased after intervention, whereas the percentage of intermediate monocytes was decreased. The intermediate monocytes seem to have surface markers at levels between the classical and nonclassical monocyte subsets (Ziegler-Heitbrock et al., 2010) (Wong et al., 2011). In the clamp setup, there were no differences before and after intervention. In our study, the impact of HFD on monocytes were not consistent and we could not confirm the recent findings.

6.2.6 Short term overfeeding does not cause impaired insulin sensitivity

Insulin sensitivity remained unchanged after the high-caloric high-fat intervention, even after normalising for body weight and fat free mass. Only a few human studies have looked at the effect of short-term high-fat overfeeding on insulin sensitivity as measured by HEC. Brøns et al. investigated a high-fat high-caloric diet including an energy surplus of 50 % above the regular diet for 5 days. The high-fat high-caloric diet (60 % fat, 32.5 % carbohydrate and 7.5 % proteins) resulted in increased fasting glucose and insulin concentrations due to an increased hepatic glucose production, but there was no effect on insulin-mediated glucose uptake or the M-value during a clamp test (Brøns et al., 2009). Likewise, Adochio et al. assessed the effect of a high-fat high-caloric diet (50 % fat, 30 % carbohydrate) on insulin sensitivity and did not observe any change in whole-body insulin sensitivity expressed as glucose disposal rate and M-value (Adochio et al., 2009). Furthermore, Chen et al. studied the impact of 3 days of a high-fat overfeeding diet (+1,250 kcal/day, 45% fat) on insulin sensitivity and did not demonstrate alterations in insulin sensitivity (Chen et al., 2016). The results of these three studies are thus in line with our findings, but in contrast to other human overfeeding studies using surrogate markers such as HOMA-IR (Sun et al., 2007) (Otten et al., 2014). Hence, based on HEC as the gold standard for assessment of whole-body insulin sensitivity, there is some consistency that short-term overfeeding up to 7 days does not result in impaired insulin sensitivity in healthy subjects.

Fasting blood glucose, fasting insulin levels, HOMA-IR were stable during the whole study. After grouping into categories loosing more or less than 1 kg, participants' fasting blood glucose was significantly decreased in participants gaining more than 1 kg. The other parameters (fasting insulin, HOMA-IR, M-value, glucose disposal) were kept stable during the whole study in both groups. This finding was surprising and cannot be explained by the inflammatory state of a HFD leading to insulin resistance.

6.2.7 Seven days of overfeeding have no major effects on gut permeability

In our study, there was no hint for an increase of LBP concentrations arguing that short-term overfeeding does not affect gut leakiness or bacterial translocation (Ghoshal et al., 2009). This statement is also supported by the results of our measurements of paracellular gut permeability markers, including PEG 1500 (except PEG_{25}), lactulose, and zonulin which remained unchanged

following 7 days of high-fat overfeeding. As additional information, urinary excretion of the different gut permeability markers was not linked with weight gain. The urinary excretion of PEG_{25} was significantly decreased after short-term overfeeding and is in contrast to the other homologues of PEG_{30}, PEG_{35}, and PEG_{40}. PEG_{25} is one of several homologues and part of PEG 1500 and, therefore, this discrepancy cannot be explained. PEG is a fat-soluble, nontoxic, non-degradable, and unmetabolisable substance which is absorbed in the gut lumen (Chadwick et al., 1977), and entirely excreted by the kidneys (Bjarnason et al., 1995). In addition to PEG 1500, PEG 400 was measured before, after and two weeks after intervention. PEG 400 was significantly decreased following the intervention. One explanation could be that the baseline results of PEG 400 were contaminated by several bodycare products (e.g. body lotion, face cream) known to contain PEG 400. Finally, the percentage of PEG 3000/PEG 4000 excretion was significantly decreased after 7 days of HFD. This finding cannot be explained neither physiologically nor chemically. In summary, the data from the PEG absorption test do not provide evidence for impaired gut permeability after 7 days of high-fat diet. PEG-based permeability provides only information regarding the small intestine.

We extended the assessment of gut permeability from the gastroduodenal tract up to the colon by using a combined sugar absorption test. The four sugar absorption test reflects both paracellular and transcellular pathways and revealed no significant changes regarding paracellular and transcellular absorption following short-term overfeeding. In humans, the effects of a high-fat diet on gut permeability is poorly understood and few data are available. In contrast, animal studies have shown that HFD increases intestinal permeability (Cani et al., 2008) (Kim et al., 2012). The unphysiological diet consisted of 60 % (Kim et al., 2012) up to 75% fat of total energy intake (Cani et al., 2008). Other groups were not able to reproduce these results (Kless et al., 2015). In mice, it is also interesting to note that the housing conditions may play an important role in the link between HFD and gut barrier function (Müller et al., 2016).

6.2.8 Changes in dominand communities of fecal bacteria are individual specific after HFD

Finally, fecal microbiota profiles were measured before, immediately after and two weeks following overfeeding. Marked inter-individual differences (Appendix, Supplemental Figure 5) were observed and there was no uniform response to the defined intervention, but the shifts in the phylogenetic makeup of fecal bacteria were substantial in some individuals. Richness and Shannon effective counts were not affected by overfeeding and only two taxonomic groups (*Bacteroidaceae* and *Betaproteobacteria*) showed altered relative abundance of sequences across all individuals. In addition to inter-individual differences, the duration of intervention may have been too short. although there are studies indicating that rather short exposure times of a few days may be sufficient to induce changes of the microbiome (David et al.,2014) (Wu et al., 2011) (O'Keefe et al., 2015). In further consideration, in these studies the dietary interventions were more drastic than in the present trial: exclusively plant- or animal-based diets or drastic shifts in fiber and fat content (e.g. from 14 to 55 g/d and 16 to 52%,

respectively). Moreover, the amount of fat intake (48 EN%) was too low to modulate the gut microbiota composition. Finally, one explanation for these contrary results could be the different methods used for microbiota analysis (16S sequencing, fluorescent in situ hybridization, Metagenomics, 16S 454 Pyrosequencing, 16S qPCR) and the selection of the study population (different obesity grades) included in the studies.

7 Strengths and Limitations of the studies

The strength of both studies was the high degree of standardisation. The caloric restriction was performed by using a formula diet from Modifast® and to ensure adherence, all participants were called once a week to go through the sampling processing and dietary pattern. Furthermore, the participants were invited for routine examinations and asked several questions regarding the handling with additional information sheets provided. In general, the participants followed the instructions and showed an excellent compliance and only one participant dropped out of the study. The overfeeding study was performed in a specialised study unit and participants had to eat their meals under supervision. The dietary intervention was carried out with standardised frozen convenience products (Bofrost, Straelen, Germany). Also other components of the diet were bought from the same manufacturer.

In addition, both studies presented here performed an extensive array of different methods to characterise the impact of dietary interventions on several related parameters: the body composition was assessed by a comprehensive anthropometric measurements, bioimpedance analysis and MRI ("Caloric restriction" study). The fat distribution was assessed by MRI ("Caloric restriction" study). The metabolic function was assessed by oGTT ("Caloric restriction" study) or hyperinsulinemic euglycemic clamp ("Overfeeding" study) being the gold standard method in this field. The RMR was measured by indirect calorimetry and was not calculated. Moreover, glucose and insulin levels were measured in the fasting state and after oGTT. The inflammation status was investigated by using plasma and SAT biosamples and by different measurements in the gut. The results were further substantiated by data related to the gut microbiota composition. In addition, changes of gut permeability were studied using a variety of different methods (sugar absorption test, PEG aborption test, and plasma zonulin).

Finally, a strength of the current study was that two dietary intervention studies were performed with supplementary questions and overlapping readouts (inflammation, insulin sensitivity, gut permeability, gut microbiota profiles). But, it would be preferred to recruit individuals of the same gender. It remains speculative whether a larger sample size would have given more significant results. One limitation of the study was that we were not able to assess the energy loss with the feces by using bomb calorimetry. The impact of gut microbiota on moderate weight gain and moderate weight loss would be interesting in this context and may be included in future studies.

8 Conclusion and perspectives

The overall aim of this study was to reveal new insight on how overfeeding and caloric restriction followed by moderate weight loss affect gut barrier function, the composition of the intestinal microbiota and obesity-related inflammatory and metabolic parameters. Therefore, two dietary intervention studies were performed with additional questions and overlapping readouts (inflammation, insulin sensitivity, gut permeability, gut microbiota profiles).

Figure 26 summarises the results of the two studies. The first study focusing on a four-week dietary restriction resulted in significant weight loss, improved gut barrier integrity and reduced systemic inflammation in obese women without major effects on fecal microbiota profiles. The second study dealt with short-term high-fat high-caloric overfeeding. Our findings suggest that short-term overfeeding with a high-fat diet does not significantly impair insulin sensitivity, gut permeability, and gut microbiota profiles in normal-weight, healthy men. The two studies are different regarding gender and age of the study population.

Regarding the readouts such as inflammation, insulin sensitivity, and gut permeability, we could demonstrate only minor effects with a short-term high-fat diet, whereas a caloric restriction programme with a moderate weight loss of about 7 kg seemed to have beneficial effects. Our findings of the “Overfeeding” study suggested that short-term overfeeding with a HFD does not significantly impair insulin sensitivity, gut permeability, and gut microbiota composition in normal-weight men. A longer exposure time may be needed to show an impairment of these functions in a clinical setting. In the “Caloric restriction” study, it can be speculated that caloric restriction leads to a decline of the secretion of pro-inflammatory adipokines and that less LPS is translocated due to the improved gut permeability.

Based on the positive effects of moderate weight loss caused by caloric restriction, the effect of bariatric surgery (Laparoscopic Sleeve Gastrectomy, LSG) on intestinal permeability and the inflammatory status in the fat tissue is worth to investigated. Recent study assessed the effect of RYGB on gut permeability (Savassi-Rocha et al., 2014). Moreover, at present, there is rather limited knowledge about the changes of gut permeability across the different stages of life. As demonstrated by Nicoletti in his review, several changes occur in the intestinal epithelial barrier due to aging making it an interesting research target (Nicoletti, 2015).

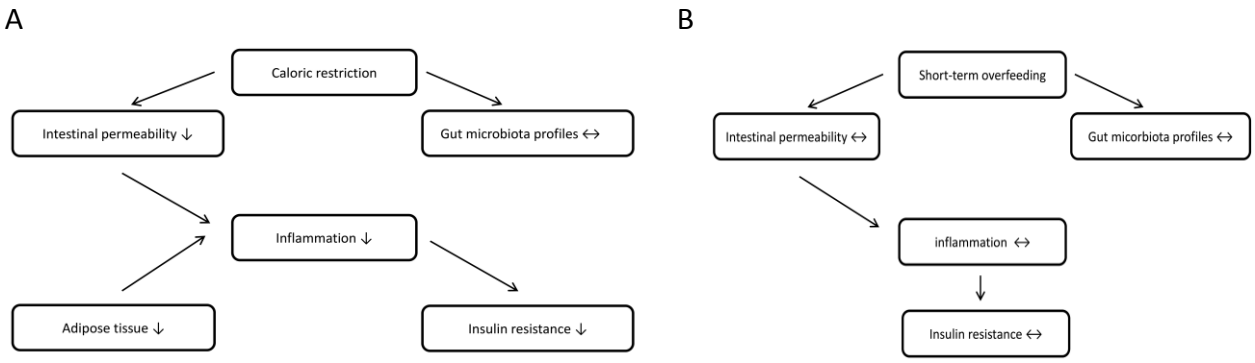


Figure 26: Summary of the results of the A) impact of caloric restriction and B) short-term overfeeding on gut permeability, gut microbiota profiles and glucose metabolism.
↓ = Decrease/Improvement; ↔ = No significant alteration

Publications

Ott B., Skurk T., Hastreiter L., Lagkouvardos I., Fischer S., Büttner J., Kellerer T., Clavel T., Rychlik M., Haller D., Hauner H.: Caloric restriction improves gut permeability and low-grade inflammation in obese women. *Submitted*

Ott B., Skurk T., Clavel T., Büttner J., Lichtenegger M., Lagkouvardos I., Fischer S., Lechner A., Rychlik M., Haller D., Hauner H.: Impact of short-term high-fat overfeeding on gut permeability, fecal bacteria composition and glucose metabolism in young healthy men. *Submitted*

Ott B., Norman K., Büttner J., Skurk T., Hauner H.: Impact of short-term high-fat overnutrition on glucose metabolism and gut permeability. Diabetologie und Stoffwechsel. 2016 April; 11 (S01)

Shen J., BaumT., Cordes C., **Ott B**., Skurk T. Kooijman H., Rummeny EJ., Hauner H., Karampinos DC.: Automatic segmentation of abdominal organs and adipose tissue compartments in water-fat MRI: Application to weight-loss in obesity. Eur J Radiol. 2016 Sep; 85(9):1613-21

Cordes C., Dieckmeyer M., **Ott B.**, Shen J., Ruschke S., Settles M., Eichhorn C., Bauer JS., Kooijman H., Rummeny EJ., Skurk T., Baum T., Hauner H., Karampinos DC.: MR-detected changes in liver fat, abdominal fat, and vertebral bone marrow fat after a four-week calorie restriction in obese women. J Magn Reson Imaging. 2015 Nov; 42(5):1272-80

Talks

Ott B., Norman K., Hastreiter L., Skurk T., Hauner H.: Impact of caloric restriction on gut barrier integrity in obese women. ECO 2015 Prague

Poster

Ott B., Norman K., Büttner J., Skurk T., Hauner H.: Impact of short-term high-fat overnutrition on glucose metabolism and gut permeability in healthy men. DDG 2016, Berlin

Cordes C., Dieckmeyer M., **Ott B.**, Shen J., Ruschke S., Settles M., Eichhorn C., Bauer JS., Kooijman H., Rummeny EJ., Skurk T., Baum T., Hauner H., Karampinos DC. Bone marrow fat behaves differently from abdominal fat, liver fat and serum lipids after a four-week calorie restriction in obese women. ISMRM conference Toronto 2015

Grants

Danone Institute “Nutrition for Health”-Travel grant for “22nd European Congress of Obesity” in Prague.

Appendix

8.1 Study document

Study document 1: Press release, “Caloric restriction” study

Am rechts der Isar

Übergewichtige Frauen für Studie gesucht

AZ, 09.10.2013 15:40 Uhr

Für die Studie werden Frauen gesucht, die einen Body-Mass-Index zwischen 30 und 42 haben. Foto: Fotolia

Eine Studie der TU erforscht den Zusammenhang zwischen Übergewicht und Stoffwechselstörungen. Dafür werden Teilnehmerinnen gesucht, die auch bei einer Diät begleitet werden.

Haidhausen - Übergewicht und Adipositas (Fettleibigkeit) zählen zu den modernen Volkskrankheiten: Immer häufiger sind vor allem Jüngere betroffen. Mit zunehmendem Körpergewicht und Körperfettanteil steigt auch das Risiko für adipositas-bedingte Stoffwechselstörungen wie Insulinresistenz, gestörte Glukosetoleranz und Typ-2-Diabetes. Genetische Faktoren beeinflussen wahrscheinlich die Anfälligkeit und die Schwere dieser Störungen.

Das erforscht die Studie

Neue Forschungsergebnisse weisen darauf hin, dass bei Personen mit Übergewicht und Adipositas die Darmflora im Dickdarm und die Darmbarrierefunktion verändert sind. Die Darmwand ist undicht. Dadurch steigt das Risiko, dass auch schädliche Stoffe in den Körper eindringen.

Diese Veränderungen stehen im Verdacht, Entzündungsprozesse zu fördern und darüber die Entwicklung von Insulinresistenz, gestörter Glukosetoleranz und Typ-2-Diabetes zu begünstigen.

In der Studie soll zunächst geklärt werden, ob und wie die Darmflora und die Darmbarriere bei übergewichtigen Personen verändert sind und welchen Einfluss die Ernährung – speziell eine eingeschränkte Kalorienzufuhr – hat.

Darüber hinaus untersuchen die Wissenschaftler, ob und wie sich Veränderungen im Darm auf den Stoffwechsel auswirken.

Ziel der Studie

Ziel ist, aufzuklären, welche Rolle Darmflora und Darmbarriere bei der Entstehung adipositas-bedingter Stoffwechselstörungen spielen. Die so gewonnenen Erkenntnisse können genutzt werden, um gezielt neue ernährungstherapeutische Maßnahmen zur Vorbeugung und Behandlung adipositas-bedingter Stoffwechselerkrankungen zu entwickeln.

Gesucht werden

Frauen im Alter zwischen 18 und 65 Jahren mit einem BMI von 30 bis 42.

So läuft die Studie ab

Die Studie läuft über acht Wochen, vier Wochen davon sollen die Teilnehmerinnen ihre Ernährung auf eine kalorienreduzierte Kost mit circa 800 Kalorien pro Tag (Formula-Diät) umstellen. Studienteilnehmerinnen erhalten eine umfassende ärztliche Betreuung sowie Ernährungsberatung mit ausführlicher Diagnostik und insgesamt sechs Untersuchungen.

Zwei Termine finden am Klinikum rechts der Isar statt. Die anderen Untersuchungen können wahlweise am Lehrstuhl für Ernährungsmedizin am Georg-Brauchle-Ring in München oder in Freising-Weihenstephan durchgeführt werden. Die Teilnehmerinnen erhalten eine Aufwandsentschädigung.

Anmeldung und Infos

Erna Stephanie Jobst, Telefon: 08161 71-2398, Email: erna.jobst (at) tum.de

Study document 2: Screening questionnaire, “Caloric restriction” study

Einfluss einer kalorischen Restriktion auf die Darmbarrierefunktion bei adipösen Personen

Screening $_{S0}$

Datum: __ __ / __ __ / __ __ __ __ Uhrzeit: |__||__|:|__||__| Uhr

Nachname:
Vorname:
Telefonnummer:
Email:
S01.1 Geburtsdatum: S01.2 Alter >18 Jahre:
S02.0 Größe:
S03.1 Gewicht: S03.2 BMI >30:

Teilnehmer |__||__||__||__|

	JA	NEIN
S1 Teilnahme an anderen Studien in den letzten drei Monaten		
S2 Drogen-/Alkoholprobleme		
S3 Rauchen		
S4 Erkrankungen Krebs; schwere kardiovaskuläre Erkrankung ; Lebererkrankung (GOT, GPT > 3-fach der Norm); Nierenerkrankung (Kreatinin > 1,2 mg/dl; **gastrointestinale**, neurologische, rheumatische Erkrankungen, Zöliakie; **akute Infektionen**		
S5 Gallensteine		
S6 Stoffwechselerkrankung , DIABETES		
S7 Chronische Erkrankungen		
S8 Autoimmunerkrankungen		
S9 Allergien: Lactuloseintoleranz, Sucraloseintoleranz, Saccaroseintoleranz, andere Nahrungsmittelintoleranzen, Kreuzallergien, **andere Allergien** z.B. Heuschnupfen		
S10 Frische Operation keine Darm-Operation, keine Coloskopie (nicht in den letzten zwei Wochen)		
S11 Prothesen, Herzschrittmacher, Herzklappen, Herzschirmchen, Clips im Hirn oder Rückenmarkbereich, Gefäßstens, Glenkprothese, Metallsplitter, Osteosynthesematerial, Neurostimulator, Hörhilfe, Cochlea-Implantat, Piercing, Schmuckimplantate, Tätowierungen, Permanen Make-ups		

Teilnehmer |__||__||__||__|

	JA	NEIN
S12 Bluttransfusion in den letzten 2 Monaten		
S13 Psychische Probleme wie Depressionen		
S14 Angst vor engen Räumen		
S15 Regelmäßige Medikamente Cortikosteroide, Glucocorticoide, Antihistamine, Antibiotika, NSAIDs, Immunsuppresiva, Laxantien, Anti-Durchfall-Medikamente, Aspirin, Schmerzstillende Medikamente, Schilddrüsenhormon-Präparate, Antikoagulantien/Antithrombotika (ausgenommen ASS), Orlistat		

Study document 3: Participant information and informed of consent, "Caloric restriction" study

Technische Universität München

Teilnehmerinformation

Studie: Einfluss einer kalorischen Restriktion auf die Darmbarrierefunktion bei adipösen Personen

Sehr geehrte Studienteilnehmerinnen,

Sie sind eingeladen, an der oben genannten Studie teilzunehmen. Nachfolgend sind die wichtigsten Informationen zu Hintergrund, Zielsetzung und Ablauf der Studie zusammengefasst. Bitte lesen Sie sich die Teilnehmerinformation sorgfältig und in Ruhe durch, bevor Sie Ihre Entscheidung zur Teilnahme treffen.

1. Hintergrund und Ziel der Studie

Übergewicht und Adipositas (Fettleibigkeit) zählen heute zu den modernen Volkskrankheiten. Vor allem jüngere Personen sind immer häufiger betroffen. Überhöhte Energiezufuhr bei gleichzeitig ungünstiger Zusammensetzung der Ernährung und ausgeprägte Bewegungsarmut kennzeichnen unsere moderne Lebensweise. Sie gilt als entscheidende Ursache für die steigenden Zahlen übergewichtiger und fettleibiger Personen.

Mit dem Körpergewicht und Körperfettanteil steigt auch das Risiko für Adipositas- bedingte Stoffwechselstörungen wie Insulinresistenz, gestörte Glukosetoleranz und schließlich Typ-2-Diabetes. Genetische Faktoren beeinflussen wahrscheinlich zusätzlich die individuelle Anfälligkeit und die Schwere dieser Störungen.

Neue Forschungsergebnisse darauf hin, dass bei Personen mit Übergewicht und Adipositas die Darmflora im Dickdarm und die Darmbarrierefunktion verändert sind. Die Darmwand ist gewissermaßen „undicht". Dadurch steigt das Risiko, dass vermehrt auch schädliche Stoffe in den Körper eindringen. Diese Veränderungen stehen in Verdacht, Entzündungsprozesse im Körper zu fördern und darüber die Entwicklung von Insulinresistenz, gestörter Glukosetoleranz und Typ-2-Diabetes zu begünstigen.

In dieser Studie soll zunächst geklärt werden, ob und wie bei übergewichtigen Personen die Darmflora und die Darmbarriere verändert sind und welchen Einfluss die Ernährung, speziell eine eingeschränkte Kalorienzufuhr, hat. Darüber hinaus soll untersucht werden, ob und wie sich Veränderungen von Darmflora und Darmbarriere auf den Stoffwechsel auswirken. Ziel ist es, aufzuklären, welche Rolle Darmflora und Darmbarriere bei der Entstehung adipositas-bedingter Stoffwechselstörungen spielen.

Die so gewonnenen Erkenntnisse können genutzt werden, um gezielt neue ernährungstherapeutische Maßnahmen zur Vorbeugung und Behandlung adipositas-bedingter Stoffwechselerkrankungen zu entwickeln.

2. Studienablauf

Die Gesamtdauer Ihrer Teilnahme beträgt 7 Wochen. Für 4 Wochen dieses Zeitraums werden Sie gebeten Ihre Ernährung auf eine kalorienreduzierte Kost mit ca. 800 kcal/Tag umzustellen. Dazu erhalten Sie eine spezielle Fertigkost mit einer Auswahl an sättigenden Drinks, Cremes, Suppen und Riegeln in verschiedenen Geschmacksrichtungen. Vier Produkte ersetzen alle Mahlzeiten eines Tages, wobei Sie frei über die Produktkombination entscheiden können. Die Lebensmittel sowie eine ausführliche Erläuterung des Kostplanes erhalten Sie von Ihren Studienbetreuern.

Im Rahmen der Studie nehmen Sie an 4 Untersuchungsterminen im Studienzentrum Uptown München Campus D oder Freising-Weihenstephan teil. Zwei Termine finden zusätzlich am Klinikum rechts der Isar, München statt. Während der vierwöchigen Reduktionsphase sind drei Routineuntersuchungen geplant.

Zu allen Untersuchungsterminen sollten Sie **nüchtern** erscheinen, d.h. die letzte Mahlzeit sollte 10-12 Stunden zurückliegen. Wassertrinken ist vor den Untersuchungen erlaubt. Medikamente dürfen nach Absprache eingenommen werden. In den **48 Stunden** bevor Sie den Untersuchungstermin haben, sind Bier, Wein, Schnaps oder sonstige alkoholische Getränke **nicht erlaubt**! Durch einen Blutalkoholtest wird dies überprüft und führt u.U. zum Ausschluss aus der Studie.

Am Tag vor der Untersuchung und am Untersuchungstag sollten keine körperlich anstrengenden Tätigkeiten verrichtet werden (z.B. nicht mit dem Rad zu den Untersuchungen kommen).

Eingangsuntersuchungen (V_1)

Zu Beginn der Studie werden an zwei Terminen folgende Untersuchungen durchgeführt. Zudem erhalten Sie Ihre Lebensmittel sowie eine ausführliche Erläuterung des Kostplanes für die 4-wöchige kalorienreduzierte Ernährung.

Anamnese

Sie erhalten Fragebögen zu Themen wie medizinischer Vorgeschichte, möglicher familiärer Vorbelastung, regelmäßig eingenommene Medikamente, Lebensstil und Soziodemographie. Bei den Teilnehmerinnen wird ein Schwangerschaftstest durchgeführt.

Körperliche Untersuchung

Im Rahmen der körperlichen Untersuchung werden Größe, Gewicht, Taillen- und Hüftumfang bestimmt. Zusätzlich werden Blutdruck und Puls gemessen.

Bioimpendanzanalyse (Körperfettmessung)

Die Bioimpendanzanalyse (BIA) dient der Messung der Körperzusammensetzung (Muskel-, Fett-, Wasseranteil) mittels einer speziellen Körperanalysewaage. Die Untersuchung dauert etwa 5 Minuten.

Indirekte Kalorimetrie

Die indirekte Kalorimetrie ist eine Messung des Energieverbrauchs („Grundumsatz“). Die Messung erfolgt im Ruhe-Nüchtern-Zustand im Liegen unter einer Atemhaube. Die gesamte Untersuchung dauert ca. 30 Minuten. Eine Gefährdung besteht bei dieser Untersuchung nicht.

Blutabnahme

Insgesamt werden Ihnen 8 Röhrchen Blut abgenommen. Die Gesamtmenge des abgenommenen Blutes beträgt ca. 47ml. Die Blutabnahme dient zur Bestimmung verschiedener Laborwerte z.B. Nüchternblutzucker, Nüchtern-insulin, Entzündungsmesswerten, Blutfetten, Leber- und Nierenfunktion und Endotoxin (Abbauprodukte von Bakterien) und der Gewinnung von genetischem Material .

Oraler Glukosetoleranztest

Der orale Glukosetoleranztest dient dazu, Störungen im Glukosestoffwechsel festzustellen. Nach Bestimmung des Nüchternblutzuckers bekommen Sie eine Glukoselösung von 75 g Glucose in 300 ml Wasser zu trinken. Anschließend wird über 2 Stunden die Veränderung Ihres Blutzuckerspiegels gemessen. Dazu werden Blutproben über eine Venenverweilkanüle nach 30, 60, 120 Minuten entnommen (ca. 5 ml pro Abnahme).

Fettgewebsbiopsie

Bei dieser Untersuchung werden seitlich des Bauchnabels mit einer Nadel ca. 0,5 g Fett aus dem Unterhautfettgewebe abgesaugt. Der Eingriff wird unter örtlicher Betäubung durchgeführt und ist für Sie nahezu schmerzfrei. An der Einstichstelle kann es zu einem Bluterguss („blauer Fleck“) kommen, der sich üblicherweise innerhalb von 1 bis 2 Wochen folgenlos zurückbildet. In sehr seltenen Fällen kann es zu einer Infektion an der Einstichstelle kommen.

Magnetresonanztomographie (MRT) und Leberspektroskopie

Diese kombinierte Untersuchung dient zur Bestimmung des Körperfetts und der Körperfettverteilung sowie des Leberfettgehalts. Bei diesem Verfahren werden die Weichteilgewebe des Körpers (Muskeln, Haut, Fett, innere Organe, usw.) durch elektromagnetische Resonanzsignale bildlich dargestellt. Die Signale werden in einem sehr starken Magnetfeld erzeugt. Die Untersuchung dauert ca. 45 bis 60 Minuten und ist völlig ungefährlich und schmerzfrei für Sie. Ein ausführliches Informationsblatt zur Vorbereitung der Untersuchung erhalten sie von Ihren Studienbetreuern.

Messung der intestinalen Permeabilität

Mit Hilfe dieser Untersuchung wird die Durchlässigkeit der Darmschleimhaut bestimmt bzw. die Funktion der Darmwand gemessen. Dazu werden folgende zwei Tests (Zuckerresorptionstest und Polyethylenglykol-Test) miteinander kombiniert:

Nach nächtlichem Fastenzustand benötigen wir eine Urinprobe von Ihnen. Danach bekommen Sie eine Zuckerlösung zum Trinken und 6 „Zuckerkapseln“ zum Einnehmen. Nach Testbeginn wird über 5 Stunden Urin gesammelt. Danach trinken Sie eine Lösung, in der

verschiedene Polyethylenglykole gelöst sind. Anschließend wird nochmals über 21 Stunden Urin gesammelt.

Eine ausführliche Anleitung zum Sammelurin und die nötigen Utensilien bekommen Sie bei der Visite.

Stuhlprobe

Zur ausführlichen Untersuchung der Darmflora soll eine Stuhlprobe abgegeben werden. Eine genaue Anleitung und die benötigten Utensilien für zu Hause erhalten Sie von Ihren Studienbetreuern bei den jeweils vorausgehenden Visiten.

Ernährungsprotokoll

Sie werden gebeten über 7 Tage Ihre Ernährung nach der Eingangsuntersuchung zu dokumentieren. Die benötigten Unterlagen erhalten Sie von Ihren Studienbetreuern.

Aktivitätssensor AS 50

Um Ihre körperliche Aktivität zu messen, werden Sie gebeten, während der Studienphase einen Aktivitätssensor AS 50 von der Firma Beurer zu tragen.

Untersuchung nach der vierwöchigen Formuladiät (V_2)

Nach den vier Wochen mit kalorienreduzierter Kost erfolgt Visite 2. Es wird das Untersuchungsprogramm von Visite 1 wiederholt. Es werden ebenfalls zwei Untersuchungstermine angesetzt. Darüber hinaus erhalten Sie eine Ernährungsberatung zu einer Normalkost für die nachfolgenden zwei Wochen. Bitte bringen Sie dazu Ihr Ernährungsprotokoll mit.

Nach der vierwöchigen Formuladiät wird folgendes Untersuchungsprogramm wiederholt:
- Blutabnahme
- Körperliche Untersuchung mit Größe, Gewicht, Taillen- und Hüftumfang, Messung von Blutdruck und Puls
- BIA
- Indirekte Kalorimetrie
- Fettgewebsbiopsie
- Magnetresonanztomographie
- Oraler Glukosetoleranztest
- Messung der Permeabilität
- Stuhlprobe
- Ernährungsprotokoll (14 Tage lang nach der vierwöchigen Formuladiät)

Abschlussuntersuchung (V_3)

Bei der abschließenden Visite 3 wird folgendes Untersuchungsprogramm wiederholt:
- Blutabnahme
- Körperliche Untersuchung mit Größe, Gewicht, Taillen- und Hüftumfang, Messung von Blutdruck und Puls

- BIA
- Indirekte Kalorimetrie
- Messung der Permeabilität
- Stuhlprobe
- optional Ernährungsberatung

3. Nutzen einer Studienteilnahme

Mit Ihrer Studienteilnahme unterstützen Sie den Erkenntnisgewinn zu den Ursachen adipositas-bedingter Stoffwechselerkrankungen. Insbesondere die Rolle von Darmflora und Darmbarriere sowie deren Beeinflussung durch die Begrenzung der Kalorienzufuhr kann aufgeklärt werden. Darauf aufbauend können neuartige Ernährungsprogramme zur gezielten Stärkung der Darmbarriere entwickelt werden. Damit ergäben sich neue Möglichkeiten zur wirksamen Vorbeugung schwerwiegender Folgeerkrankungen oder zur Linderung bereits bestehender Beschwerden bei Personen mit Adipositas.

Bei Einhaltung des Kostplanes ist durch die Begrenzung der Kalorienzufuhr mit einer Gewichtsreduktion zu rechnen, die aber individuell unterschiedlich ausfallen kann. Im Rahmen Ihrer Studienteilnahme haben Sie die Möglichkeit an einem umfangreichen medizinischen Untersuchungsprogramm teilzunehmen. Die erhaltenen Ergebnisse werden Ihnen mitgeteilt und erläutert. Bitte beachten Sie, dass dies keine therapeutischen Maßnahmen mit einschließt. Auf Wunsch wird gerne der Hausarzt informiert.

4. Risiken einer Studienteilnahme

Alle Untersuchungen werden von erfahrenen Studienärzten und geschultem Personal durchgeführt. Bei den Untersuchungen handelt es sich um standardisierte Methoden, die schon lange Anwendung finden. Die Risiken der Blutabnahme gehen nicht über die üblichen Risiken bei Blutabnahmen hinaus. Das Legen der Venenverweilkanüle bzw. die Blutabnahme verursacht einen kurzen Schmerz. Es kann auch ein Hämatom (Bluterguss, „blauer Fleck“) entstehen. In seltenen Fällen kann eine Nervenläsion an der Einstichstelle, eine Venenentzündung oder eine Infektion auftreten.

Die zwei Testlösungen zur Messung der Darmfunktion mittels PEG-Test und Zuckerresorptionstest (Laktulose, Mannitol, Saccharose, Sucralose) können in seltenen Fällen (da niedrige Mengen) nach Einnahme Bauchschmerzen, Durchfälle und Blähungen verursachen. Diese klingen nach Ende des Testes rasch ab.

Die Fettgewebsbiopsie ist ein minimal-invasiver Eingriff unter örtlicher Betäubung. Die Nadelbiopsie ist fast schmerzfrei. Für die Betäubung wird ein gut verträgliches Medikament (Lidocain) verwendet. In sehr seltenen Fällen kann eine allergische Reaktion auftreten. An der Entnahmestelle kann es zur Bildung eines Blutergusses („blauer Fleck“) oder zu Verhärtungen kommen. In sehr seltenen Fällen kann es zu einer Infektion der Einstichstelle kommen.

Die Magnetresonanztherapie ist eine ungefährliche und schmerzfreie Untersuchung. Es erfolgt keine Kontrastmittelgabe. In einigen Fällen wurde über Unwohlsein aufgrund der Enge der „Röhre“ berichtet.

Sollten Sie während der Untersuchungen Schmerzen verspüren oder sich unwohl fühlen, teilen Sie dies bitte umgehend dem Studienarzt mit.

Nutzung und Aufbewahrung von Bioproben und DNA

Die im Rahmen Ihrer Studienteilnahme entnommenen Blut- und Gewebeproben dienen der eingehenden Untersuchung des Stoffwechsels, von Entzündungsprozessen im Körper und möglichen ursächlichen Stoffen. Die gesammelten Urin- und Stuhlproben dienen der Überprüfung der Funktion der Darmwand und der Darmflora. Durch die regelmäßige Kontrolle dieser Werte, kann beurteilt werden ob und wie die begrenzte Kalorienzufuhr Darmflora und Darmbarriere beeinflusst und wie sich dies auf den Stoffwechsel auswirkt.

Im Rahmen der Untersuchungen sind zukünftige Analysen für weitere medizinisch bedeutsame Fragestellungen vorgesehen. Da die meisten dieser Fragestellungen sich erst aus dem Wissensstand der nächsten Jahre ergeben werden, können sie derzeit noch nicht in allen Einzelheiten benannt werden. Wir planen daher, Ihre Bioproben (Blut, Gewebe) für die nächsten Jahre aufzubewahren und – auch ohne Sie erneut zu kontaktieren – für weitere Forschungsfragen zu verwenden. Dies erfolgt nur nach vorheriger positiver Bewertung durch die Ethikkommission.

5. Aufwandsentschädigung/Kostenerstattung

Bei erfolgreicher Teilnahme erhalten Sie eine Aufwandsentschädigung in Höhe von 300 €. Die anfallenden Fahrtkosten werden Ihnen nach Vorlage der entsprechenden Nachweise (Bahn-, Parktickets) erstattet.

6. Verwendung der Studienergebnisse und Datenschutz

Im Rahmen ihrer Studienteilnahme werden von Ihnen medizinische Befunde und persönliche Informationen erhoben und in der Studieneinheit in Ihrer persönlichen Akte niedergeschrieben und elektronisch gespeichert. Alle erhobenen Daten werden entsprechend den Bestimmungen des Bundesdatenschutzgesetzes und der ärztlichen Schweigepflicht streng vertraulich behandelt. Die Weitergabe und Auswertung der Daten/Biomaterialien erfolgt ohne Angabe von Namen oder Initialen (d.h. pseudonymisiert); es wird nur ein Nummern- und/oder Buchstabencode verwendet. Die erhobenen Daten können in einem Computer gespeichert und verarbeitet werden.

Ihre Einwilligung zur Teilnahme an dieser klinischen Studie schließt die Aufzeichnung Ihrer im Rahmen dieser klinischen Studie erhobenen Krankheitsdaten durch beteiligte Ärzte ein, sowie die Weitergabe dieser Daten an den Leiter der klinischen Prüfung, Herrn Univ.-Prof. Dr. med. Hans Hauner, einschließlich dessen Vertreter und Auftragnehmer sowie die zuständigen Überwachungsbehörden und Ethikkommissionen.

Sie haben das Recht, jegliche Informationen, die sich auf Ihre Teilnahme an dieser Studie beziehen, einzusehen. Dies betrifft Informationen über Ihre allgemeine Gesundheit, jegliche aufgetretenen Nebenwirkungen und alle Testergebnisse, die während dieser Studie gesammelt worden sind. Die Ergebnisse der genetischen Analyse dienen ausschließlich zu Forschungszwecken. Deshalb werden Ergebnisse dieser Forschung insgesamt und individuelle Befunde weder Ihnen noch Ihrem Arzt oder Versicherungen zugänglich gemacht.

7. Rücktritt/Abbruch

Ihre Studienteilnahme ist freiwillig. Im Studienverlauf haben Sie das Recht, Ihre Zustimmung zur Teilnahme jederzeit und ohne Angabe von Gründen zu widerrufen, ohne dass sich daraus persönliche Nachteile für Sie ergeben. Sollten Sie sich bereits nach dem Lesen dieses Aufklärungsbogens gegen eine Teilnahme entscheiden, teilen Sie uns dies bitte kurz mit.

Sollte eine weitere Teilnahme am Forschungsvorhaben ärztlich nicht mehr vertretbar sein, kann der Prüfarzt/Leiter der klinischen Prüfung (LKP) Ihre Teilnahme auch vorzeitig beenden, ohne dass Sie auf diese Entscheidung Einfluss haben.

Im Falle des Widerrufs/Abbruchs können Sie entscheiden, ob Ihre Blut-/und Gewebeproben vernichtet werden sollen oder in pseudonymisierter Form für weitere wissenschaftliche Zwecke verwendet werden dürfen. Bereits erhobene Daten verbleiben auch nach Vernichtung der Probe in der Studie, soweit der Personenbezug nicht mehr besteht.

8. Recht auf Information

Sie haben das Recht vor, während und nach Ende der Studie jederzeit Fragen über die Studie zu stellen.

Werden aus Ihren entnommenen Bioproben (Blut, Gewebe) wider Erwarten medizinische Erkenntnisse gewonnen, die für die Erhaltung oder Wiederherstellung Ihrer eigenen Gesundheit von erheblicher Bedeutung sind, so wird Ihr Hausarzt darüber informiert, damit er mit Ihnen das weitere Vorgehen besprechen kann.

Wenn Sie eine Information Ihres Hausarztes nicht wünschen, können Sie dies in der Einverständniserklärung zum Ausdruck bringen.

Für alle weiteren Fragen im Zusammenhang mit dieser Studie stehen Ihnen der Studienarzt und seine Mitarbeiter gerne zur Verfügung. Auch Fragen, die Ihre Rechte als Teilnehmer dieser klinischen Studie betreffen, werden Ihnen gerne beantwortet.

Bei Rückfragen können Sie sich gerne an das Studienteam wenden.

Kontakt

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http://www.em-tum.de/

Einverständniserklärung

Studie: Einfluss einer kalorischen Restriktion auf die Darmbarrierefunktion bei adipösen Personen

Hiermit gebe ich mein freiwilliges Einverständnis zur Teilnahme an der klinischen Studie gemäß der vorstehenden Teilnehmerinformation.

✓ Ich bin bereit, freiwillig an dem unter Punkt 2 beschriebenen Untersuchungsprogramme teilzunehmen.
❒ Ja ❒ Nein

✓ Ich bin einverstanden, dass meine Bioproben (Blut, Gewebe) einschließlich DNA für die Untersuchung der unter Punkt 1 beschriebenen Fragestellungen verwendet werden.
❒ Ja ❒ Nein

✓ Mit der Erhebung und Aufzeichnung der wissenschaftlichen Daten in pseudonymisierter Form, d.h. nur unter Angabe der Teilnehmernummer, bin ich einverstanden.
❒ Ja ❒ Nein

✓ Ich bin einverstanden, dass meine Bioproben (Blut, Gewebe) einschließlich DNA für die zukünftige Forschung aufbewahrt und verwendet werden.
❒ Ja ❒ Ja, aber nur nach vorheriger Kontaktaufnahme ❒ Nein

Durch meine Unterschrift bestätige ich, dass ich die Teilnehmerinformation gelesen und verstanden habe. Ich hatte Gelegenheit Fragen zu stellen, die mir zufriedenstellend beantwortet wurden.

Ich weiß, dass meine Teilnahme freiwillig ist und ich meine Einwilligung jederzeit mündlich oder schriftlich ohne Angabe von Gründen widerrufen und die Vernichtung meiner Blut-/Gewebeproben fordern kann, ohne dass mir daraus irgendwelche Nachteile entstehen.

Über mögliche gesundheitliche Risiken (siehe Punkt 4) wurde ich aufgeklärt. Die Studie findet unter ärztlicher Überwachung statt. Es werden keine therapeutischen Maßnahmen durchgeführt. Für meine Teilnahme erhalte ich eine Aufwandsentschädigung.

Werden aus meinen entnommenen Bioproben (Blut, Gewebe) wider Erwarten medizinische Erkenntnisse gewonnen, die für die Erhaltung oder Wiederherstellung meiner eigenen Gesundheit von erheblicher Bedeutung sind, wird mein Hausarzt darüber informiert, damit er mit mir das weitere Vorgehen besprechen kann.

❒ Ich möchte **nicht**, dass mein Hausarzt über Befunde im vorstehenden Sinn informiert wird.

Ich erkläre mich damit einverstanden, dass im Rahmen dieser klinischen Studie personenbezogene Daten, insbesondere Angaben über meine Gesundheit, über mich erhoben und in Papierform sowie auf elektronischen Datenträgern aufgezeichnet werden. Soweit erforderlich, dürfen die erhobenen Daten ohne Namensnennung und Geburtsdatum (pseudonymisiert) weitergegeben werden an:

a) den Auftraggeber der Studie (BMBF, TU München) zur wissenschaftlichen Auswertung.
b) die zuständige(n) Überwachungsbehörde(n) (Landesamt/-ämter) oder Bezirksregierung(en) und Ethikkommissionen(en).

Außerdem erkläre ich mich damit einverstanden, dass autorisierte und zur Verschwiegenheit verpflichtete Beauftragte des Auftraggebers der zuständigen inländischen und ausländischen Überwachungs- und Zulassungsbehörden in meine beim Studienarzt vorhandenen personenbezogenen Daten, insbesondere meine Gesundheitsdaten, Einsicht nehmen, soweit dies für die Überprüfung der ordnungsgemäßen Durchführung des Forschungsvorhabens notwendig ist. Für diese Maßnahme entbinde ich den Studienarzt von der ärztlichen Schweigepflicht.

Die Einwilligung zur Erhebung und Verarbeitung der Angaben über meine Gesundheit ist unwiderruflich. Ich bin bereits darüber aufgeklärt worden, dass ich jederzeit die Teilnahme an der klinischen Studie beenden kann. Im Falle dieses Widerrufs erkläre ich mich damit einverstanden, dass die bis zu diesem Zeitpunkt gespeicherten Daten ohne Namensnennung weiterhin verwendet werden dürfen, soweit dies erforderlich ist.

Ich erkläre mich damit einverstanden, dass meine Daten nach Beendigung oder Abbruch der klinischen Studie mindestens 15 Jahre aufbewahrt werden. Danach werden meine personenbezogenen Daten gelöscht, soweit nicht gesetzliche, satzungsmäßige oder vertragliche Aufbewahrungsfristen entgegenstehen.

Ich hatte ausreichend Zeit mich zu entscheiden. Zum Zeitpunkt der Unterzeichnung der Einverständniserklärung habe ich keine weiteren Fragen. Mir ist bekannt, dass ich jederzeit, sowohl während als auch nach der klinischen Studie, Fragen stellen kann.

Eine Kopie der Teilnehmerinformation und eine Einverständniserklärung habe ich erhalten.

München/Freising-Weihenstephan,

Name des aufklärenden Arztes	Unterschrift
Name der Studienteilnehmerin	Unterschrift

Study document 4: Case Report Form, “Caloric restriction” study

Case Report Form

Teilstudie 1

|__||__||__||__|

Liste der verwendeten Abkürzungen

A	Anamnese
AK	Anthropometrische und klinische Parameter
BE	Blutabnahme
BIA	Bioimpendanzanalyse
BIC	Bank Identifier Code
BLZ	Bankleitzahl
BMBF	Bundeministerium für Bildung und Forschung
BW	Body Weight
CT	Euglykämisch-hyperinsulinämische Clamp-Technik
BMI	Body Mass Index
EB	Ernährungsberatung
EDTA	Ethylendiamintetraacetat
EP	Ernährungsprotokoll
FEE	Fat-free mass
FZ	Fäzesprobe
IBAN	International Bank Account Number
IK	Indirekte Kalorimetrie
IP	Intestinale Permeabilität
M	Glukosestoffwechsel
M_r	relatives Molekulargewicht
MRT	Magnetresonanztomographie
oGTT	Oraler Glukosetoleranz-Test
PEG	Polyethylenglycose
REE	resting energy expenditure
S	Screening
V	Visite

Visite 1 (V_1)

Datum: __ __ / __ __ / __ __ __ __ Uhrzeit: |__||__| : |__||__| Uhr

Indirekte Kalorimetrie $_{IK1}$

IK101 Indirekte Kalorimetrie erfolgreich durchgeführt? ❒ Ja ❒ Nein

IK102 Wenn nein, bitte begründen: _____

IK103 Ergebnisse nachfolgend angefügt? ❒ Ja ❒ Nein

IK104 Grundumsatz

IK104.1 Soll |__||__||__||__| kcal

IK104.2 IST |__||__||__||__| kcal

IK105 Fett |__||__|,|__| %

IK106 Kohlenhydrate |__||__|,|__| %

IK107 Protein |__||__|,|__| %

Anthropometrische und klinische Parameter $_{AK0}$

AK101 Ruhe-Puls (HF/min):

AK101.1 Messung 1: |__||__||__|

AK101.2 Messung 2: |__||__||__|

AK101.3 Messung 3: |__||__||__|

AK101.4 Mittelwert (AK108.1-108.3): |__||__||__|

AK102 Blutdruck im Ruhezustand (systolisch/diastolisch in mmHg):

AK102.1 Messung dominanter Arm |__||__||__|/|__||__||__|

AK102.2 Messung nicht dominanter Arm

AK102.2.1	Messung 1:	|__||__||__|/|__||__||__|
AK102.2.2	Messung 2:	|__||__||__|/|__||__||__|
AK102.2.3	Messung 3:	|__||__||__|/|__||__||__|
AK102.2.4	Mittelwert (AK102.2.1-102.2.3):	|__||__||__|/|__||__||__|

Erläuterungen: AK101 Ruhe-Puls - Pulsfrequenz in Schlägen pro Minute im Sitzen (HF)
AK102.2 Blutdruck - Messung im Sitzen am nicht dominanten Arm und in Ruhe (nach 5 min) (Empfehlung der Deutschen Hochdruckliga 2012)

AK103 Körpergröße:	|__||__||__|,|__| cm
AK104 Körpergewicht:	|__||__||__|,|__| kg
AK105 Body Mass Index (BMI):	|__||__|,|__| kg/m²
AK106 Taillenumfang:	|__||__||__|,|__| cm
AK107 Hüftumfang:	|__||__||__|,|__| cm
AK108 Taille-Hüft-Quotient:	|__|,|__||__||__|
AK109BIA_Messung:	
AK110.1 Fett (%):	|__||__|,|__| %
AK110.2 Fettmasse	|__||__|,|__| kg
AK110.3 Fettfreie Masse	|__||__|,|__| kg
AK010.4 Körperwasser	|__||__|,|__| kg

Erläuterungen: AK103 ohne Schuhe und in kg; Für Standard-Kleidung (ohne Schuhe) wird 1kg subtrahiert
AK109 BIA _Messungen (Tanita Waage) :
AK103-10 Ist die zweite Ziffer nach dem Komma eine 0,1,2,3,4, wird abgerundet
Ist die zweite Ziffer nach dem Komma eine 5,6,7,8,9, wird aufgerundet

Blutabnahme BA1

BA101 Patient nüchtern? ❒ Ja ❒ Nein

BA102 Wenn nein, neuer Termin: __ __ / __ __ / __ __ __ __

BA103 Blutabnahme erfolgreich durchgeführt? ❒ Ja ❒ Nein

BA104 Wenn nein, bitte begründen: _____

BA105 Uhrzeit: |__||__|:|__||__| Uhr

BA106 Besonderheiten (z.B. Entzündung der Einstichstelle, mehrere Einstiche zur Blutentnahme waren nötig)

❒ Ja ❒ Nein

BA107 Wenn ja, welche? _____

BA108 Blut abgenommen durch: _____ (Name) _____ (Unterschrift)

BA109 Analysecodierung: |_____|

BA110 Citrat (2,5 ml)	❒ k.A.	❒ 1x	
BA111 EDTA (2,7 ml)	❒ k.A.	❒ 1x	
BA112 Natrium-Fluorid (2,7 ml)	❒ k.A.	❒ 1x	
BA113 Serum (9,0 ml)	❒ k.A.	❒ 1x	
BA114 Lithium-Heparin (9,0 ml)	❒ k.A.	❒ 1x	❒ 2x
BA115 EDTA (9,0 ml)	❒ k.A.	❒ 1x	
BA115 Serum(2,7ml)	❒ k.A.	❒ 1x	

Teilnehmer |__||__||__||__|

Oraler Glukosetoleranztest oGT1

oGT101 Messung durchgeführt von: _____ _____
Name Unterschrift

Messung (Minuten)	Uhrzeit	Glukose mg/dl	Insulin mg/dl
0		oGT102	oGT106
30		oGT103	oGT107
60		oGT104	oGT108
120		oGT105	oGT109

oGT110Besonderheiten? ❒ Ja ❒ Nein

oGT111 Wenn ja, welche? _____

Fettbiopsie FB1

FB101 Biopsie erfolgreich durchgeführt?

❒ Ja am: __ __ / __ __ / __ __ __ __ ❒ Nein

FB102 Wenn nein, bitte begründen: _____

FB103 Besonderheiten (z.B. Entzündung der Einstichstelle)

❒ Ja ❒ Nein

FB104 Wenn ja, welche? _____

Magnetresonanztomographie MT1

MT101 MRT erfolgreich durchgeführt?

❒ Ja am: __ __ / __ __ / __ __ __ __ ❒ Nein

MT102 Wenn nein, bitte begründen: _____

Teilnehmer |__||__||__||__|

MT103 Ergebnisse nachfolgend angefügt?

❒ Ja ❒Nein

MT104 Besonderheiten

❒ Ja ❒ Nein

MT105 Wenn ja, welche? _____

MT106 Ergebnisse nachfolgend angefügt? ❒ Ja ❒ Nein

Ernährungsprotokoll EP1

EP101 Erste Ernährungsprotokoll mit Anleitung ausgegeben?

❒ Ja am: __ __ / __ __ / __ __ __ __ ❒ Nein

EP102 Wenn nein, bitte begründen: _____

Aktivitätssensor AS1

AS101 Aktivitätssensor ausgegeben?

❒ Ja am: __ __ / __ __ / __ __ __ __ ❒ Nein

AS102 Wenn nein, bitte begründen: _____

Fäzesprobe FZ1

FZ101 Utensilien für die erste Fäzesprobe ausgegeben?

❑ Stuhlbehältnis

❑ Stuhlröhrchen mit DNA Stabilisator

❒ Ja am: __ __ / __ __ / __ __ __ __ ❒ Nein

FZ102 Wenn nein, bitte begründen: _____

FZ103 Analysecodierung: |_____|

FZ104 Erste Fäzesprobe zurück erhalten? ❒ Ja ❒ Nein

FZ105 Wenn nein, bitte begründen: _____

FZ106 Stuhlröhrchen befüllt
Datum: __ __ / __ __ / __ __ __ __ Uhrzeit: |__||__| : |__||__| Uhr

FZ107 Stuhlröhrchen zum Studienzentrum gebracht
Datum: __ __ / __ __ / __ __ __ __ Uhrzeit: |__||__| : |__||__| Uhr

Intestinale Darmpermeabilität IP1

IP101 Utensilien für den ersten PEG-Test/ Zuckerresorptionstest ausgegeben?

- ❒ 1x250ml Behältnis
- ❒ 3x 3l Behältnisse
- ❒ Zuckerlösung mit Kapseln
- ❒ 100ml PEG-Lösung
- ❒ 6x10ml Urinmonovetten

❒ Ja am: __ __ / __ __ / __ __ __ __ ❒ Nein

IP102 Wenn nein, bitte begründen: _____

IP103 Sechs Urinmonovetten erhalten? ❒ Ja ❒ Nein

IP104 Wenn nein, bitte begründen: _____

IP105 Urinvolumen:

IP105.1 0h- Sammelvolumen (A) |__||__||__||__| ml

IP105.2 5h- Sammelvolumen (B) |__||__||__||__| ml

IP105.3 21h- Sammelvolumen (C) |__||__||__||__| ml

PEG

IP106 Erste Analysecodierung

IP106.1 A-Vorharn-PEG-Analysecodierung: |_____|

IP106.2 B-5h-Sammelurin-PEG-Analysecodierung: |_____|

IP106.3 C-21h-Sammelurin-PEG-Analysecodierung: |_____|

IP107 PEG-Konzentration 400M_r |__||__|,|__| % im Urin

IP108 PEG-Konzentration 1500M_r |__||__|,|__| % im Urin

IP109 PEG-Konzentration 3000M_r |__||__|,|__| % im Urin

IP110PEG-Konzentration 4000M_r |__||__|,|__| % im Urin

IP111Messung erfolgreich durchgeführt? ❒ Ja ❒ Nein

IP112 Wenn nein, bitte begründen: _____

Zuckerresorptionstest

IP113 Erste Analysecodierung

IP113.1 A-Vorharn-Zucker-Analysecodierung: |_____|

IP113.2 B-5h-Sammelurin- Zucker-Analysecodierung: |_____|

IP113.3 C-21h-Sammelurin-ZuckerAnalysecodierung: |_____|

IP114 Laktulose: |__||__|,|__||__||__| %LAC

IP115 Mannitol: |__||__|,|__||__||__| %LAC

IP116 Permeabilitätsindex (%LAC/%MAN): |__||__|,|__||__||__|

IP117 Saccarose |__||__|,|__||__||__| %

IP118 Sucralose |__||__|,|__| |__||__|%

Erläuterungen: IP107-19 Ist die zweite Ziffer nach dem Komma eine 0,1,2,3,4, wird abgerundet
Ist die zweite Ziffer nach dem Komma eine 5,6,7,8,9, wird aufgerundet

Teilnehmer |__||__||__||__|

Untersuchungsbogen während Restriktionsphase (1. Woche)

Anthropometrische und klinische Parameter AK2

AK203 Körpergröße: |__||__||__|,|__| cm

AK204 Körpergewicht: |__||__||__|,|__| kg

AK205 Body Mass Index (BMI): |__||__|,|__| kg/m²

AK206 Taillenumfang: |__||__||__|,|__| cm

AK207 Hüftumfang: |__||__||__|,|__| cm

AK208 Taille-Hüft-Quotient: |__|,|__||__||__|

AK209 BIA_Messung:

AK210.1 Fett (%): |__||__|,|__| %

AK210.2 Fettmasse |__||__|,|__| kg

AK210.3 Fettfreie Masse |__||__|,|__| kg

AK210.4 Körperwasser |__||__|,|__| kg

Erläuterungen: AK203 ohne Schuhe und in kg; Für Standard-Kleidung (ohne Schuhe) wird 1kg subtrahiert
AK209 BIA _Messungen (Tanita Waage) :
AK203-10 Ist die zweite Ziffer nach dem Komma eine 0,1,2,3,4, wird abgerundet
Ist die zweite Ziffer nach dem Komma eine 5,6,7,8,9, wird aufgerundet

AK201 Ruhe-Puls (HF/min):

AK201.1 Messung 1: |__||__||__|

AK201.2 Messung 2: |__||__||__|

AK201.3 Messung 3: |__||__||__|

AK201.4 Mittelwert (AK208.1-208.3): |__||__||__|

AK202 Blutdruck im Ruhezustand (systolisch/diastolisch in mmHg):

AK202.1 Messung dominanter Arm |__||__||__|/|__||__||__|

Teilnehmer |__||__||__||__|

AK202.2 Messung nicht dominanter Arm

AK202.2.1 Messung 1: |__||__||__|/|__||__||__|

AK202.2.2 Messung 2: |__||__||__|/|__||__||__|

AK202.2.3 Messung 3: |__||__||__|/|__||__||__|

AK202.2.4 Mittelwert (AK202.2.1-202.2.3): |__||__||__|/|__||__||__|

Erläuterungen: AK201 Ruhe-Puls - Pulsfrequenz in Schlägen pro Minute im Sitzen (HF)
AK202.2 Blutdruck - Messung im Sitzen am nicht dominanten Arm und in Ruhe (nach 5 min) (Empfehlung der Deutschen Hochdruckliga 2012)

Befindlichkeit B2

B21.0. Haben Sie in den letzten 7 Tagen unter folgenden Symptomen gelitten?

- ❑ Müdigkeit
- ❑ verminderte Leistungsfähigkeit
- ❑ Nervosität
- ❑ Frieren
- ❑ Kälteintoleranz

B22.0. Haben Sie regelmäßigen Stuhlgang?

- ❑ täglich
- ❑ ≥ 3x in der Woche
- ❑ ≤3x in der Woche
- ❑ unregelmäßig

B23.0 Haben Sie in den letzten 7 Tagen an Durchfall gelitten?

- ❑ nein
- ❑ ≤ 3x Durchfall in den letzten 7 Tagen
- ❑ ≥ 3x Durchfall in den letzten 7 Tagen

B24.1 Haben Sie Ihre Menstruation regelmäßig?

- ❑ ja
- ❑ nein
- ❑ nicht mehr
- ❑ _____

B24.2. Wann hat ihr letzter Menstruationszyklus begonnen?

_____

B25. Leiden Sie in den letzten 7 Tage unter folgenden Beschwerden?

- ❑ Ohrensausen
- ❑ Schwindel
- ❑ Bewusstseinsstörungen
- ❑ andere_____

Untersuchungsbogen während Restriktionsphase (2. Woche)

Anthropometrische und klinische Parameter AK3

AK303 Körpergröße: |__||__||__|,|__| cm

AK304 Körpergewicht: |__||__||__|,|__| kg

AK305 Body Mass Index (BMI): |__||__|,|__| kg/m²

AK306 Taillenumfang: |__||__||__|,|__| cm

AK307 Hüftumfang: |__||__||__|,|__| cm

AK308 Taille-Hüft-Quotient: |__|,|__||__||__|

AK309 BIA_Messung:

AK310.1 Fett (%): |__||__|,|__| %

AK310.2 Fettmasse |__||__|,|__| kg

AK310.3 Fettfreie Masse |__||__|,|__| kg

AK310.4 Körperwasser |__||__|,|__| kg

Erläuterungen: AK303 ohne Schuhe und in kg; Für Standard-Kleidung (ohne Schuhe) wird 1kg subtrahiert
AK309 BIA _Messungen (Tanita Waage) :
AK303-10 Ist die zweite Ziffer nach dem Komma eine 0,1,2,3,4, wird abgerundet
Ist die zweite Ziffer nach dem Komma eine 5,6,7,8,9, wird aufgerundet

AK301 Ruhe-Puls (HF/min):

AK301.1 Messung 1: |__||__||__|

AK301.2 Messung 2: |__||__||__|

AK301.3 Messung 3: |__||__||__|

AK301.4 Mittelwert (AK308.1-308.3): |__||__||__|

AK302 Blutdruck im Ruhezustand (systolisch/diastolisch in mmHg):

AK302.1 Messung dominanter Arm |__||__||__|/|__||__||__|

AK302.2 Messung nicht dominanter Arm

AK302.2.1	Messung 1:	|__||__||__|/|__||__||__|
AK302.2.2	Messung 2:	|__||__||__|/|__||__||__|
AK302.2.3	Messung 3:	|__||__||__|/|__||__||__|
AK302.2.4	Mittelwert (AK302.2.1-302.2.3):	|__||__||__|/|__||__||__|

Erläuterungen: AK301 Ruhe-Puls - Pulsfrequenz in Schlägen pro Minute im Sitzen (HF)
AK302.2 Blutdruck - Messung im Sitzen am nicht dominanten Arm und in Ruhe (nach 5 min) (Empfehlung der Deutschen Hochdruckliga 2012)

Befindlichkeit B3

B31.0. Haben Sie in den letzten 7 Tagen unter folgenden Symptomen gelitten?

- ❑ Müdigkeit
- ❑ verminderte Leistungsfähigkeit
- ❑ Nervosität
- ❑ Frieren
- ❑ Kälteintoleranz

B32.0. Haben Sie regelmäßigen Stuhlgang?

- ❑ täglich
- ❑ ≥ 3x in der Woche
- ❑ ≤3x in der Woche
- ❑ unregelmäßig

B33.0. Haben Sie in den letzten 7 Tagen an Durchfall gelitten?

- ❑ nein
- ❑ ≤ 3x Durchfall in den letzten 7 Tagen
- ❑ ≥ 3x Durchfall in den letzten 7 Tagen

B34.4. Leiden Sie in den letzten 7 Tage unter folgenden Beschwerden?

- ❑ Ohrensausen
- ❑ Schwindel
- ❑ Bewusstseinsstörungen
- ❑ andere_____

Untersuchungsbogen während Restriktionsphase (3. Woche)

Anthropometrische und klinische Parameter AK4

AK403 Körpergröße: |__||__||__|,|__| cm

AK404 Körpergewicht: |__||__||__|,|__| kg

AK405 Body Mass Index (BMI): |__||__|,|__| kg/m²

AK406 Taillenumfang: |__||__||__|,|__| cm

AK407 Hüftumfang: |__||__||__|,|__| cm

AK408 Taille-Hüft-Quotient: |__|,|__||__||__|

AK409 BIA_Messung:

AK410.1 Fett (%): |__||__|,|__| %

AK410.2 Fettmasse |__||__|,|__| kg

AK410.3 Fettfreie Masse |__||__|,|__| kg

AK410.4 Körperwasser |__||__|,|__| kg

Erläuterungen: AK403 ohne Schuhe und in kg; Für Standard-Kleidung (ohne Schuhe) wird 1kg subtrahiert
AK409 BIA _Messungen (Tanita Waage) :
AK403-10 Ist die zweite Ziffer nach dem Komma eine 0,1,2,3,4, wird abgerundet
Ist die zweite Ziffer nach dem Komma eine 5,6,7,8,9, wird aufgerundet

AK401 Ruhe-Puls (HF/min):

AK401.1 Messung 1: |__||__||__|

AK401.2 Messung 2: |__||__||__|

AK401.3 Messung 3: |__||__||__|

AK401.4 Mittelwert (AK408.1-408.3): |__||__||__|

AK402 Blutdruck im Ruhezustand (systolisch/diastolisch in mmHg):

AK402.1 Messung dominanter Arm |__||__||__|/|__||__||__|

AK402.2 Messung nicht dominanter Arm

AK402.2.1 Messung 1: |__||__||__|/|__||__||__|

AK402.2.2 Messung 2: |__||__||__|/|__||__||__|

AK402.2.3 Messung 3: |__||__||__|/|__||__||__|

AK402.2.4 Mittelwert (AK402.2.1-402.2.3): |__||__||__|/|__||__||__|

Erläuterungen: AK401 Ruhe-Puls - Pulsfrequenz in Schlägen pro Minute im Sitzen (HF)
AK402.2 Blutdruck - Messung im Sitzen am nicht dominanten Arm und in Ruhe (nach 5 min) (Empfehlung der Deutschen Hochdruckliga 2012)

Befindlichkeit B4

B41.0. Haben Sie in den letzten 7 Tagen unter folgenden Symptomen gelitten?

- ❑ Müdigkeit
- ❑ verminderte Leistungsfähigkeit
- ❑ Nervosität
- ❑ Frieren
- ❑ Kälteintoleranz

B42.0. Haben Sie regelmäßigen Stuhlgang?

- ❑ täglich
- ❑ ≥ 3x in der Woche
- ❑ ≤3x in der Woche
- ❑ unregelmäßig

B43.0. Haben Sie in den letzten 7 Tagen an Durchfall gelitten?

- ❑ nein
- ❑ ≤ 3x Durchfall in den letzten 7 Tagen
- ❑ ≥ 3x Durchfall in den letzten 7 Tagen

B44.4. Leiden Sie in den letzten 7 Tage unter folgenden Beschwerden?

- ❑ Ohrensausen
- ❑ Schwindel
- ❑ Bewusstseinsstörungen
- ❑ andere_____

Untersuchungsbogen während Restriktionsphase (4. Woche)

Befindlichkeit B5

B51.0. Haben Sie in den letzten 7 Tagen unter folgenden Symptomen gelitten?

- ❑ Müdigkeit
- ❑ verminderte Leistungsfähigkeit
- ❑ Nervosität
- ❑ Frieren
- ❑ Kälteintoleranz

B52.0. Haben Sie regelmäßigen Stuhlgang?

- ❑ täglich
- ❑ ≥ 3x in der Woche
- ❑ ≤3x in der Woche
- ❑ unregelmäßig

B53.0. Haben Sie in den letzten 7 Tagen an Durchfall gelitten?

- ❑ nein
- ❑ ≤ 3x Durchfall in den letzten 7 Tagen
- ❑ ≥ 3x Durchfall in den letzten 7 Tagen

B54.1. Hat sich Ihr Menstruationszyklus verändert?

- ❑ ja
- ❑ nein
- ❑ _____

B55 Leiden Sie in den letzten 7 Tage unter folgenden Beschwerden?

- ❑ Ohrensausen
- ❑ Schwindel
- ❑ Bewusstseinsstörungen
- ❑ andere_____

Visite 2 (V_2)

Datum: __ __ / __ __ / __ __ __ __ Uhrzeit: |__||__| : |__||__| Uhr

Indirekte Kalorimetrie IK6

IK611 Indirekte Kalorimetrie erfolgreich durchgeführt? ❒ Ja ❒ Nein

IK612 Wenn nein, bitte begründen: _____

IK613 Ergebnisse nachfolgend angefügt? ❒ Ja ❒ Nein

IK604 Grundumsatz

IK604.1 Soll |__||__||__||__| kcal

IK604.2 IST |__||__||__||__| kcal

IK605 Fett |__||__|,|__| %

IK606 Kohlenhydrate |__||__|,|__| %

IK607 Protein |__||__|,|__| %

Anthropometrische und klinische Parameter AK6

AK601 Ruhe-Puls (HF/min):

AK601.1 Messung 1: |__||__||__|

AK601.2 Messung 2: |__||__||__|

AK601.3 Messung 3: |__||__||__|

AK601.4 Mittelwert (AK608.1-608.3): |__||__||__|

AK602 Blutdruck im Ruhezustand (systolisch/diastolisch in mmHg):

AK602.1 Messung dominanter Arm |__||__||__|/|__||__||__|

AK602.2 Messung nicht dominanter Arm

AK602.2.1	Messung 1:	|__|__|__|/|__|__|__|
AK602.2.2	Messung 2:	|__|__|__|/|__|__|__|
AK602.2.3	Messung 3:	|__|__|__|/|__|__|__|
AK602.2.4	Mittelwert (AK602.2.1-602.2.3):	|__|__|__|/|__|__|__|

Erläuterungen: AK601 Ruhe-Puls - Pulsfrequenz in Schlägen pro Minute im Sitzen (HF)
AK602.2 Blutdruck - Messung im Sitzen am nicht dominanten Arm und in Ruhe (nach 5 min) (Empfehlung der Deutschen Hochdruckliga 2012)

AK603 Körpergröße:	|__|__|__|,|__| cm
AK604 Körpergewicht:	|__|__|__|,|__| kg
AK605 Body Mass Index (BMI):	|__|__|,|__| kg/m²
AK606 Taillenumfang:	|__|__|__|,|__| cm
AK607 Hüftumfang:	|__|__|__|,|__| cm
AK608 Taille-Hüft-Quotient:	|__|,|__|__|__|
AK609BIA_Messung:	
AK610.1 Fett (%):	|__|__|,|__| %
AK610.2 Fettmasse	|__|__|,|__| kg
AK610.3 Fettfreie Masse	|__|__|,|__| kg
AK610.4 Körperwasser	|__|__|,|__| kg

Erläuterungen: AK603 ohne Schuhe und in kg; Für Standard-Kleidung (ohne Schuhe) wird 1kg subtrahiert
AK609 BIA _Messungen (Tanita Waage) :
AK603-10 Ist die zweite Ziffer nach dem Komma eine 0,1,2,3,4, wird abgerundet
Ist die zweite Ziffer nach dem Komma eine 5,6,7,8,9, wird aufgerundet

Blutabnahme BA2

BA201 Patient nüchtern? ❒ Ja ❒ Nein

BA202 Wenn nein, neuer Termin: __ __ / __ __ / __ __ __ __

BA203 Blutabnahme erfolgreich durchgeführt? ❒ Ja ❒ Nein

BA204 Wenn nein, bitte begründen: _____

BA205 Uhrzeit: |__||__|:|__||__| Uhr

BA206 Besonderheiten (z.B. Entzündung der Einstichstelle, mehrere Einstiche zur Blutentnahme waren nötig)

❒ Ja ❒ Nein

BA207 Wenn ja, welche? _____

BA208 Blut abgenommen durch: _____ (Name) _____ (Unterschrift)

BA209 Analysecodierung: |_____|

BA210 Citrat (2,5 ml)	❒ k.A.	❒ 1x	
BA211 EDTA (2,7 ml)	❒ k.A.	❒ 1x	
BA212 Natrium-Fluorid (2,7 ml)	❒ k.A.	❒ 1x	
BA213 Serum (9,0 ml)	❒ k.A.	❒ 1x	
BA214 Lithium-Heparin (9,0 ml)	❒ k.A.	❒ 1x	❒ 2x
BA215 EDTA (9,0 ml)	❒ k.A.	❒ 1x	
BA215 Serum(2,7ml)	❒ k.A.	❒ 1x	

Teilnehmer |__||__||__||__|

Oraler Glukosetoleranztest oGT2

oGT201 Messung durchgeführt von: _____ _____
Name Unterschrift

Messung (Minuten)	Uhrzeit	Glukose mg/dl	Insulin mg/dl
0		oGT202	oGT206
30		oGT203	oGT207
60		oGT204	oGT208
120		oGT205	oGT209

oGT210Besonderheiten? ❒ Ja ❒ Nein

oGT211 Wenn ja, welche? _____

Fettbiopsie FB2

FB201 Biopsie erfolgreich durchgeführt?

❒ Ja am: __ __ / __ __ / __ __ __ __ ❒ Nein

FB202 Wenn nein, bitte begründen: _____

FB203 Besonderheiten (z.B. Entzündung der Einstichstelle)

❒ Ja ❒ Nein

FB204 Wenn ja, welche? _____

Magnetresonanztomographie MT2

MT201 MRT erfolgreich durchgeführt?

❒ Ja am: __ __ / __ __ / __ __ __ __ ❒ Nein

MT202 Wenn nein, bitte begründen: _____

Teilnehmer |__||__||__||__|

MT203 Ergebnisse nachfolgend angefügt?

❒ Ja ❒Nein

MT204 Besonderheiten

❒ Ja ❒ Nein

MT205 Wenn ja, welche? _____

MT206 Ergebnisse nachfolgend angefügt? ❒ Ja ❒ Nein

Ernährungsberatung EB2

EB201 Ernährungsberatung durchgeführt? ❒ Ja ❒ Nein

EB202 Wenn nein, bitte begründen: _____

Ernährungsprotokoll EP2

EP201 Zweite Ernährungsprotokoll mit Anleitung ausgegeben?

❒ Ja am: __ __ / __ __ / __ __ __ __ ❒ Nein

EP202 Wenn nein, bitte begründen: _____

Fäzesprobe FZ2

FZ201 Utensilien für die zweite Fäzesprobe ausgegeben?

❒ Stuhlbehältnis

❒ Stuhlröhrchen mit DNA Stabilisator

❒ Ja am: __ __ / __ __ / __ __ __ __ ❒ Nein

FZ202 Wenn nein, bitte begründen: _____

FZ203 Analysecodierung: |_____|

FZ204 Zweite Fäzesprobe zurück erhalten? ❒ Ja ❒ Nein

FZ205 Wenn nein, bitte begründen: _____

FZ206 Stuhlröhrchen befüllt
Datum: __ __ / __ __ / __ __ __ __ Uhrzeit: |__||__| : |__||__| Uhr

FZ207Stuhlröhrchen zum Studienzentrum gebracht
Datum: __ __ / __ __ / __ __ __ __ Uhrzeit: |__||__| : |__||__| Uhr

Intestinale Darmpermeabilität IP2

IP201 Utensilien für den zweiten PEG-Test/ Zuckerresorptionstest ausgegeben?

- ❒ 1x250ml Behältnis
- ❒ 3x 3l Behältnisse
- ❒ Zuckerlösung mit Kapseln
- ❒ 100ml PEG-Lösung
- ❒ 6x10ml Urinmonovetten
- ❒ Ja am: __ __ / __ __ / __ __ __ __ ❒ Nein

IP202 Wenn nein, bitte begründen: _____

IP203 Sechs Urinmonovetten erhalten? ❒ Ja ❒ Nein

IP204 Wenn nein, bitte begründen: _____

IP205 Urinvolumen:

IP205.1 0h- Sammelvolumen (A) |__||__||__||__| ml

IP205.2 5h- Sammelvolumen (B) |__||__||__||__| ml

IP205.3 21h- Sammelvolumen (C) |__||__||__||__| ml

PEG

IP206 Zweite Analysecodierung

IP206.1 A-Vorharn-PEG-Analysecodierung: |_____|

IP206.2 B-5h-Sammelurin-PEG-Analysecodierung: |_____|

IP206.3 C-21h-Sammelurin-PEG-Analysecodierung: |_____|

IP206 PEG-Konzentration 400M_r |__||__|,|__| % im Urin

IP207 PEG-Konzentration 1500M_r |__||__|,|__| % im Urin

IP208 PEG-Konzentration 3000M_r |__||__|,|__| % im Urin

IP209 PEG-Konzentration 4000M_r |__||__|,|__| % im Urin

IP210 Messung erfolgreich durchgeführt? ❒ Ja ❒ Nein

IP211 Wenn nein, bitte begründen: _____

Zuckerresorptionstest

IP212 Zweite Analysecodierung

IP213.1 A-Vorharn-Zucker-Analysecodierung: |_____|

IP213.2 B-5h-Sammelurin- Zucker-Analysecodierung: |_____|

IP213.3 C-21h-Sammelurin-ZuckerAnalysecodierung: |_____|

IP214 Laktulose: |__||__|,|__||__||__| %LAC

IP215 Mannitol: |__||__|,|__||__||__| %LAC

IP216 Permeabilitätsindex (%LAC/%MAN): |__||__|,|__||__||__|

IP217 Saccarose |__||__|,|__||__||__| %

IP218 Sucralose |__||__|,|__| |__||__|%

Erläuterungen: IP206-18 Ist die zweite Ziffer nach dem Komma eine 0,1,2,3,4, wird abgerundet
Ist die zweite Ziffer nach dem Komma eine 5,6,7,8,9, wird aufgerundet

Teilnehmer |__||__||__||__|

Visite 3 (V_3)

Datum: __ __ / __ __ / __ __ __ __ Uhrzeit: |__||__| : |__||__| Uhr

Indirekte Kalorimetrie IK7

IK711 Indirekte Kalorimetrie erfolgreich durchgeführt? ❒ Ja ❒ Nein

IK712 Wenn nein, bitte begründen: _____

IK713 Ergebnisse nachfolgend angefügt? ❒ Ja ❒ Nein

IK704 Grundumsatz

IK704.1 Soll |__||__||__||__| kcal

IK704.2 IST |__||__||__||__| kcal

IK705 Fett |__||__|,|__| %

IK706 Kohlenhydrate |__||__|,|__| %

IK707 Protein |__||__|,|__| %

Anthropometrische und klinische Parameter AK7

AK701 Ruhe-Puls (HF/min):

AK701.1 Messung 1: |__||__||__|

AK701.2 Messung 2: |__||__||__|

AK701.3 Messung 3: |__||__||__|

AK701.4 Mittelwert (AK708.1-708.3): |__||__||__|

AK702 Blutdruck im Ruhezustand (systolisch/diastolisch in mmHg):

AK702.1 Messung dominanter Arm |__||__||__|/|__||__||__|

AK702.2 Messung nicht dominanter Arm

AK702.2.1 Messung 1: |__||__||__|/|__||__||__|

AK702.2.2 Messung 2: |__||__||__|/|__||__||__|

AK702.2.3 Messung 3: |__||__||__|/|__||__||__|

AK702.2.4 Mittelwert (AK602.2.1-602.2.3): |__||__||__|/|__||__||__|

Erläuterungen: AK701 Ruhe-Puls - Pulsfrequenz in Schlägen pro Minute im Sitzen (HF)
AK702.2 Blutdruck - Messung im Sitzen am nicht dominanten Arm und in Ruhe (nach 5 min) (Empfehlung der Deutschen Hochdruckliga 2012)

AK703 Körpergröße: |__||__||__|,|__| cm

AK704 Körpergewicht: |__||__||__|,|__| kg

AK705 Body Mass Index (BMI): |__||__|,|__| kg/m²

AK706 Taillenumfang: |__||__||__|,|__| cm

AK707 Hüftumfang: |__||__||__|,|__| cm

AK708 Taille-Hüft-Quotient: |__|,|__||__||__|

AK709BIA_Messung:

AK710.1 Fett (%): |__||__|,|__| %

AK710.2 Fettmasse |__||__|,|__| kg

AK710.3 Fettfreie Masse |__||__|,|__| kg

AK710.4 Körperwasser |__||__|,|__| kg

Erläuterungen: AK703 ohne Schuhe und in kg; Für Standard-Kleidung (ohne Schuhe) wird 1kg subtrahieren

AK709 BIA _Messungen (Tanita Waage) :

AK703-10 Ist die zweite Ziffer nach dem Komma eine 0,1,2,3,4, wird abgerundet
Ist die zweite Ziffer nach dem Komma eine 5,6,7,8,9, wird aufgerundet

Blutabnahme BA3

BA301 Patient nüchtern? ❒ Ja ❒ Nein

BA302 Wenn nein, neuer Termin: __ __ / __ __ / __ __ __ __

BA303 Blutabnahme erfolgreich durchgeführt? ❒ Ja ❒ Nein

BA304 Wenn nein, bitte begründen: _____

BA305 Uhrzeit: |__||__|:|__||__| Uhr

BA306 Besonderheiten (z.B. Entzündung der Einstichstelle, mehrere Einstiche zur Blutentnahme waren nötig)

❒ Ja ❒ Nein

BA307 Wenn ja, welche? _____

BA308 Blut abgenommen durch: _____ (Name) _____ (Unterschrift)

BA309 Analysecodierung: |_____|

BA310 Citrat (2,5 ml)	❒ k.A.	❒ 1x	
BA311 EDTA (2,7 ml)	❒ k.A.	❒ 1x	
BA312 Natrium-Fluorid (2,7 ml)	❒ k.A.	❒ 1x	
BA313 Serum (9,0 ml)	❒ k.A.	❒ 1x	
BA314 Lithium-Heparin (9,0 ml)	❒ k.A.	❒ 1x	❒ 2x
BA315 EDTA (9,0 ml)	❒ k.A.	❒ 1x	
BA315 Serum(2,7ml)	❒ k.A.	❒ 1x	

Ernährungsprotokoll EP3

EP301 Zweite Ernährungsprotokoll mit Anleitung erhalten?

❒ Ja am: __ __ / __ __ / __ __ __ __ ❒ Nein

EP202 Wenn nein, bitte begründen: _____

Ernährungsberatung EB2

EB201 Ernährungsberatung durchgeführt? ❒ Ja ❒ Nein

EB202 Wenn nein, bitte begründen: _____

Fäzesprobe FZ3

FZ301 Utensilien für die dritte Fäzesprobe ausgegeben?

❑ Stuhlbehältnis

❑ Stuhlröhrchen mit DNA Stabilisator

❒ Ja am: __ __ / __ __ / __ __ __ __ ❒ Nein

FZ302 Wenn nein, bitte begründen: _____

FZ303 Analysecodierung: |_____|

FZ304 Dritte Fäzesprobe zurück erhalten? ❒ Ja ❒ Nein

FZ305 Wenn nein, bitte begründen: _____

FZ306 Stuhlröhrchen befüllt
Datum: __ __ / __ __ / __ __ __ __ Uhrzeit: |__||__| : |__||__| Uhr

FZ307 Stuhlröhrchen zum Studienzentrum gebracht
Datum: __ __ / __ __ / __ __ __ __ Uhrzeit: |__||__| : |__||__| Uhr

Teilnehmer |__||__||__||__|

Intestinale Darmpermeabilität IP3

IP301 Utensilien für den zweiten PEG-Test/ Zuckerresorptionstest ausgegeben?

- ❑ 1x250ml Behältnis
- ❑ 3x 3l Behältnisse
- ❑ Zuckerlösung mit Kapseln
- ❑ 100ml PEG-Lösung
- ❑ 6x10ml Urinmonovetten
- ❒ Ja am: __ __ / __ __ / __ __ __ __ ❒ Nein

IP302 Wenn nein, bitte begründen: _____

IP303 Sechs Urinmonovetten erhalten? ❒ Ja ❒ Nein

IP304 Wenn nein, bitte begründen: _____

IP305 Urinvolumen:

IP305.1 0h- Sammelvolumen (A) |__||__||__||__| ml

IP305.2 5h- Sammelvolumen (B) |__||__||__||__| ml

IP305.3 21h- Sammelvolumen (C) |__||__||__||__| ml

PEG

IP306 Zweite Analysecodierung

IP306.1 A-Vorharn-PEG-Analysecodierung: |_____|

IP306.2 B-5h-Sammelurin-PEG-Analysecodierung: |_____|

IP306.3 C-21h-Sammelurin-PEG-Analysecodierung: |_____|

IP306 PEG-Konzentration 400M_r |__||__|,|__| % im Urin

IP307 PEG-Konzentration 1500M_r |__||__|,|__| % im Urin

IP308 PEG-Konzentration 3000M_r |__||__|,|__| % im Urin

IP309 PEG-Konzentration 4000M_r |__||__|,|__| % im Urin

IP310 Messung erfolgreich durchgeführt? ❒ Ja ❒ Nein

IP311 Wenn nein, bitte begründen: _____

Teilnehmer |__||__||__||__|

Zuckerresorptionstest

IP312 Zweite Analysecodierung

IP313.1 A-Vorharn-Zucker-Analysecodierung: |_____|

IP313.2 B-5h-Sammelurin- Zucker-Analysecodierung: |_____|

IP313.3 C-21h-Sammelurin-ZuckerAnalysecodierung: |_____|

IP314 Laktulose: |__||__|,|__||__||__| %LAC

IP315 Mannitol: |__||__|,|__||__||__| %LAC

IP316 Permeabilitätsindex (%LAC/%MAN): |__||__|,|__||__||__|

IP317 Saccarose |__||__|,|__||__||__| %

IP318 Sucralose |__||__|,|__| |__||__|%

Erläuterungen: IP206-18 Ist die zweite Ziffer nach dem Komma eine 0,1,2,3,4, wird abgerundet
Ist die zweite Ziffer nach dem Komma eine 5,6,7,8,9, wird aufgerundet

Study document 5: Flyer, “Overnutrition” Study

Else Kröner-Fresenius-Zentrum für Ernährungsmedizin
Klinikum rechts der Isar
der Technischen Universität München
Direktor: Univ.-Prof. Dr. med. Hans Hauner

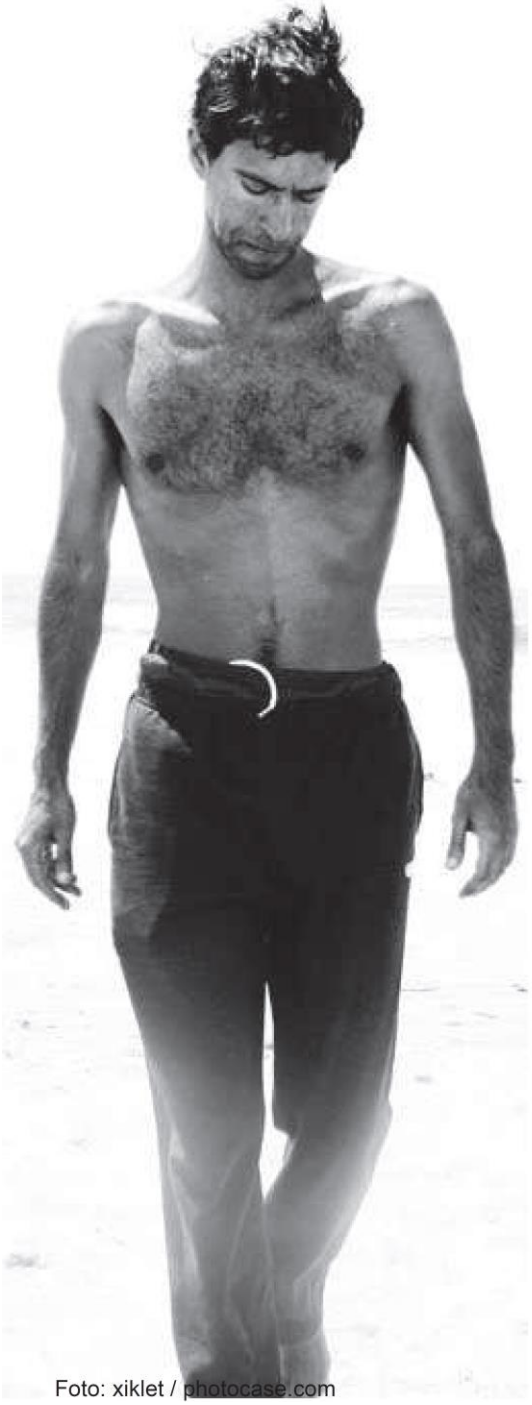

Foto: xiklet / photocase.com

männlich ?

über 18 ?

Nichtraucher ?

BMI < 25 ?

dann suchen wir Sie

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08161 / 712398 oder 712384
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Sie erhalten eine angemessene
Aufwandsentschädigung

Screening $_{S0}$

Datum: __ __ / __ __ / __ __ __ __ Uhrzeit: |__||__|:|__||__| Uhr

Nachname:
Vorname:
Telefonnummer:
Email:
S01.1 Geburtsdatum: S01.2 Alter >18 Jahre:
S02. Größe:
S03.1 Gewicht: S03.2 BMI <25:

	JA	NEIN
S1 Erkrankungen Krebs ; schwere kardiovaskuläre Erkrankung ; Lebererkrankung (GOT, GPT > 3-fach der Norm) ; Nierenerkrankung (Kreatinin > 1,2 mg/dl ; **gastrointestinale**, neurologische, rheumatische Erkrankungen, Zöliakie ; akute Infektionen		
S2 Stoffwechselerkrankung , DIABETES		
S3 Chronische Erkrankungen		
S4 Autoimmunerkrankungen		
S5 Allergien: Lactuloseintoleranz, Sucralos-eintoleranz, Saccaroseintoleranz, andere Nah-rungsmittelintoleranzen, Kreuzallergien, **ande-re Allergien** z.B. Heuschnupfen		
S6 Frische Operation keine Darm-Operation, keine Coloskopie (nicht in den letzten zwei Wochen)		
S7 Prothesen, Herzschrittmacher		
S8 Bluterkrankungen		
S9 Drogen-/Alkoholprobleme		
S11 Rauchen		
S12 Psychische Probleme wie Depressionen		

Study document 6: Screening questionnaire, "Overnutrition" Study

Study document 7: Participant information and informed of consent, “Overnutrition” Study

Teilnehmerinformation

Studie: Effekt einer definierten Überernährung auf die Darmbarrierefunktion bei stoffwechselgesunden Teilnehmern.

Sehr geehrter Studienteilnehmer,

Sie sind eingeladen, an der oben genannten Studie teilzunehmen. Nachfolgend sind die wichtigsten Informationen zu Hintergrund, Zielsetzung und Ablauf der Studie zusammengefasst. Bitte lesen Sie sich die Teilnehmerinformation sorgfältig und in Ruhe durch, bevor Sie Ihre Entscheidung zur Teilnahme treffen.

1. Hintergrund und Ziel der Studie

Übergewicht und Fettleibigkeit (Adipositas) zählen heute zu den modernen Volkskrankheiten. Eine überhöhte Energiezufuhr bei einer gleichzeitig ungünstigen Zusammensetzung der Ernährung und einer ausgeprägten Bewegungsarmut kennzeichnen unsere moderne Lebensweise, die als entscheidende Ursache für die steigenden Zahlen stark übergewichtiger Personen gilt. Mit dem Körpergewicht und Körperfettanteil steigt auch das Risiko für Adipositas-bedingte Stoffwechselstörungen und chronische Folgeerkrankungen.

Neue Forschungsergebnisse weisen nun darauf hin, dass bei Personen mit Übergewicht und Adipositas auch die Darmflora des Dickdarms und die Darmbarrierefunktion verändert sind. Die Darmwand ist gewissermaßen „undicht“. Dadurch steigt das Risiko, dass vermehrt schädliche Stoffe - insbesondere bakterielle Abbauprodukte - in den Körper eindringen. Diese Veränderungen stehen in Verdacht, Entzündungsprozesse im Körper zu fördern und so die Entwicklung von Insulinresistenz, gestörter Glukosetoleranz und schließlich Typ-2- Diabetes zu begünstigen.

In der oben genannten Studie soll aufgeklärt werden, welchen Einfluss die „moderne“ hochkalorische und fettreiche Ernährung auf Darmflora und Darmbarriere hat und wie sich eine mögliche Beeinträchtigung der Darmgesundheit auf die Regulation des Stoffwechsels auswirkt.

Ziel ist die Entwicklung neuartiger ernährungstherapeutischer Methoden, die auf den Schutz und die Stärkung der Darmbarriere abgestimmt werden. Dies soll zur Steigerung der Wirksamkeit von Maßnahmen zur Vorbeugung und Behandlung der Begleiterkrankungen der Adipositas beitragen.

2. Studienablauf

Die Gesamtdauer Ihrer Teilnahme beträgt 4 Wochen. Für 1 Woche dieses Zeitraums werden Sie gebeten Ihre Ernährung auf eine Kost mit zusätzlich 1000 kcal/Tag umzustellen. Eine ausführliche Erläuterung des Kostplanes erhalten Sie von Ihren Studienbetreuern.

Im Rahmen der Studie nehmen Sie an **6 Untersuchungsterminen** im Studienzentrum Freising-Weihenstephan teil.

Zu allen Untersuchungsterminen sollten Sie **nüchtern** erscheinen, d.h. die letzte Mahlzeit sollte 10-12 Stunden zurückliegen. Wassertrinken ist vor den Untersuchungen erlaubt. Auch Medikamente dürfen mit Wasser eingenommen werden.

In den **48 Stunden** bevor Sie den Untersuchungstermin haben, sind Bier, Wein, Schnaps oder sonstige alkoholische Getränke **nicht erlaubt**! Durch einen Blutalkoholtest wird dies überprüft und u.U. die Aufwandsentschädigung einbehalten.

Am Morgen der Untersuchung sollten keine körperlich anstrengenden Tätigkeiten verrichtet werden (z.B. nicht mit dem Rad zu den Untersuchungen kommen).

Visite 0 (Eingangsuntersuchung)

Bei der Eingangsuntersuchung erhalten Sie Fragebögen zu Themen wie medizinischer Vorgeschichte, möglicher familiärer Vorbelastung, regelmäßig eingenommene Medikamente, Lebensstil und Soziodemographie.

Im Rahmen der körperlichen Untersuchung werden Größe, Gewicht, Taillen- und Hüftumfang bestimmt. Zusätzlich werden Blutdruck und Puls gemessen.

Bioimpendanzanalyse (Körperfettmessung)

Die Bioimpendanzanalyse (BIA) dient der Messung der Körperzusammensetzung (Muskel-, Fett-, Wasseranteil) mittels einer speziellen Körperanalysewaage. Die BIA ist völlig ungefährlich und schmerzfrei für Sie. Die Untersuchung dauert etwa 5 Minuten.

Indirekte Kalorimetrie

Die indirekte Kalorimetrie ist eine Messung des Energieverbrauchs („Grundumsatz“). Die Messung erfolgt im Ruhe-Nüchtern-Zustand im Liegen unter einer Atemhaube. Die gesamte Untersuchung dauert ca. 1 Stunde. Eine Gefährdung besteht bei dieser Untersuchung nicht.

Sie werden des Weiteren gebeten, Ihre Ernährung im Ernährungsprotokoll zu dokumentieren und einen Bewegungsmessgerät (ActiGraph GT3X+) zu tragen. Die benötigten Unterlagen erhalten Sie von Ihren Studienbetreuern. Die Ergebnisse der indirekten Kalorimetrie, des Bewegungsmessgerätes und der Ernährungsprotokolle ermöglichen eine Abschätzung Ihres Gesamtenergieverbrauchs. Darauf basierend erstellen wir Ihren persönlichen Kostplan.

Darüber hinaus erhalten Sie eine ausführliche Anleitung und die nötigen Utensilien zur Sammlung einer Stuhlprobe:

Stuhlprobe

Zur ausführlichen Untersuchung der Darmflora soll eine Probe von 1 bis 2 g Stuhl abgegeben werden. Eine genaue Anleitung und die benötigten Utensilien für zu Hause erhalten Sie von Ihren Studienbetreuern bei den jeweils vorausgehenden Visiten.

Visite 1

Eine Wochen nach der Eingangsuntersuchung findet Visite 1 statt. Neben der Abgabe Ihrer ersten Stuhlprobe und des Ernährungsprotokolls sind folgende Untersuchungen vorgesehen:

Blutabnahme

Insgesamt werden Ihnen 6 Röhrchen Blut abgenommen. Die Gesamtmenge des abgenommenen Blutes beträgt ca. 62ml. Die Blutabnahme dient der Gewinnung von genetischem Material und zur Bestimmung verschiedener Laborwerte z.B. Nüchternblutzucker, Nüchterninsulin, Entzündungsmesswerten, Blutfetten, Leber- und Nierenfunktion und Endotoxin (Abbauprodukte von Bakterien).

Messung der intestinalen Permeabilität

Mit Hilfe dieser Untersuchung wird die Durchlässigkeit der Darmschleimhaut bestimmt bzw. die Funktion der Darmwand gemessen. Dazu werden folgende zwei Tests gemacht:

Zuckerresorptionstest und Polyethylenglykol-Test (PEG-Test): Nach nächtlichem Fastenzustand benötigen wir eine Urinprobe von Ihnen. Danach bekommen Sie eine Zuckerlösung zum Trinken und 6 „Zuckerkapseln“ zum Einnehmen. Nach Testbeginn wird über 5 Stunden Urin gesammelt. Danach trinken Sie eine Lösung, in der verschiedene Polyethylenglykole gelöst sind. Anschließend wird nochmals über 21 Stunden Urin gesammelt.

Eine ausführliche Anleitung zum Sammelurin und die nötigen Utensilien bekommen Sie bei der Visite.

Euglykämisch-hyperinsulinämische Clamp-Technik

Nach einer nächtlichen Fastenzeit von 12 Stunden wird der Test durchgeführt. Bei dieser Technik bekommen Sie kontinuierlich Insulin intravenös zugeführt. Je nach Bedarf wird gleichzeitig Glukose intravenös zugeführt. So ist es möglich, den Blutzucker einzustellen und über einen bestimmten Zeitraum beizubehalten. Durch diese Methodik wird die Insulinsensitivität bestimmt.

Visite 2

Nach einer Woche mit überkalorischer Kost findet Visite 2 statt. Neben der Abgabe der zweiten Stuhlprobe wird folgendes Untersuchungsprogramm wiederholt:
- Blutabnahme
- Körperliche Untersuchung mit Größe, Gewicht, Taillen- und Hüftumfang, Messung von Blutdruck und Puls, BIA und indirekte Kalorimetrie
- PEG-Test
- Zuckerresorptionstest
- Euglykämisch-hyperinsulinämische Clamp-Technik

Visite 3

Bei der abschließenden Visite 3 wird die dritte Stuhlprobe abgegeben und folgende Messungen werden wiederholt:
- Blutabnahme
- Körperliche Untersuchung mit Größe, Gewicht, Taillen- und Hüftumfang, Messung von Blutdruck und Puls, BIA und indirekte Kalorimetrie
- PEG-Test
- Zuckerresorptionstest

Bei Fragen oder Problemen zu den Untersuchungen steht Ihnen Ihre Studienbetreuung sehr gerne zur Seite.

3. Nutzen einer Studienteilnahme

Mit Ihrer Studienteilnahme unterstützen Sie den Erkenntnisgewinn zu den Ursachen Adipositas-bedingten Stoffwechselstörungen. Die erzielten Ergebnisse ermöglichen ein besseres Verständnis darüber, wie sich eine kalorien- und fettreiche Ernährung auf die Darmflora und Darmbarriere auswirkt und welche Folgen dies für die Stoffwechselregulation hat. Darauf aufbauend können neuartige Ernährungsprogramme zur gezielten Stärkung der Darmbarriere entwickelt werden. Damit ergäben sich neue Möglichkeiten zur wirksamen Vorbeugung schwerwiegender Folgeerkrankungen oder zur Linderung bereits bestehender Beschwerden bei Personen mit Adipositas.

Aus den genetischen Analysen können Sie persönlich keinen unmittelbaren Nutzen oder Vorteil für Ihre Gesundheit erwarten. Im Rahmen Ihrer Studienteilnahme haben Sie die Möglichkeit, an einem umfangreichen medizinischen Untersuchungsprogramm teilzunehmen. Die erhaltenen Ergebnisse werden Ihnen mitgeteilt und erläutert. Bitte beachten Sie, dass dies keine therapeutischen Maßnahmen mit einschließt. Auf Wunsch wird gerne der Hausarzt informiert.

4. Risiken einer Studienteilnahme

Alle Untersuchungen werden von erfahrenen Studienärzten und geschultem Personal durchgeführt. Bei den Untersuchungen handelt es sich um standardisierte Methoden, die schon lange Anwendung finden. Die Risiken der Blutabnahme gehen nicht über die üblichen Risiken bei Blutabnahmen hinaus. Das Legen der Venenverweilkanüle bzw. die Blutabnahme verursacht einen kurzen Schmerz. Es kann auch ein Hämatom (Bluterguss, „blauer Fleck“) entstehen. In seltenen Fällen kann eine Nervenläsion an der Einstichstelle, eine Venenentzündung oder eine Infektion auftreten.

Die zwei Testlösungen zur Messung der Darmfunktion mittels PEG-Test und Zuckerresorptionstest (Laktulose, Mannitol, Saccarose, Sucralose) können in seltenen Fällen (da niedrige Mengen) nach Einnahme Bauchschmerzen, Durchfälle und Blähungen verursachen. Diese klingen nach Ende des Testes rasch ab.

Bei der euglykämisch-hyperinsulinämische Clamp-Technik kann es während der Untersuchung zur Unterzuckerung, Schwindel und Übelkeit kommen. Während der Glukoseinfusion kann ein Brennen entstehen. Zudem können während der Blutentnahme die oben genannten Risiken auftreten.

Das Bewegungsmessgerät (ActiGraph GT3X+) misst das Bewegungsverhalten, indem er Beschleunigungen aufzeichnet und diese wiederum in Signale umwandelt, welche dann aufgezeichnet werden. Das Tragen des ActiGraph GT3X+ ist mit keinerlei gesundheitlichen Risiken für Sie verbunden.

Sollten Sie während der Untersuchungen Schmerzen verspüren oder sich unwohl fühlen, teilen Sie dies bitte umgehend dem Studienarzt mit.

5. Nutzung und Aufbewahrung von Bioproben und DNA

Die im Rahmen Ihrer Studienteilnahme entnommenen Blutproben werden zur eingehenden Untersuchung des Stoffwechsels, von Entzündungsprozessen im Körper und möglichen ursächlichen Stoffen verwendet. Die gesammelten Urin- und Stuhlproben dienen der Überprüfung der Darmwandfunktion und der Darmflora. Mit der regelmäßigen Kontrolle dieser Werte im Studienverlauf erfolgt die Beurteilung, ob und wie die kalorien- und fettreiche Ernährung Darmflora und Darmbarriere beeinflusst und wie sich dies auf den Stoffwechsel auswirkt.

Die Blutproben dienen darüber hinaus der Gewinnung von genetischem Material (DNA), um den genetischen Einfluss auf den individuellen Energieverbrauch („Grundumsatz“) näher zu untersuchen.

Im Rahmen der Untersuchungen sind zukünftige Analysen für weitere medizinisch bedeutsame Fragestellungen vorgesehen. Da die meisten dieser Fragestellungen sich erst aus dem Wissensstand der nächsten Jahre ergeben werden, können sie derzeit noch nicht in allen Einzelheiten benannt werden. Wir planen daher, Ihre Bioproben (einschließlich DNA) für die nächsten Jahre aufzubewahren und – auch ohne Sie erneut zu kontaktieren – für weitere Forschungsfragen zu verwenden. Dies erfolgt nur nach vorheriger positiver Bewertung durch die Ethikkommission.

6. Aufwandsentschädigung/Kostenerstattung

Bei erfolgreicher Teilnahme erhalten Sie eine Aufwandsentschädigung in Höhe von 500 €. Die anfallenden Fahrtkosten werden Ihnen nach Vorlage der entsprechenden Nachweise (Bahn-, Parktickets) erstattet.

7. Verwendung der Studienergebnisse und Datenschutz

Im Rahmen ihrer Studienteilnahme werden von Ihnen medizinische Befunde und persönliche Informationen erhoben und in der Studieneinheit in Ihrer persönlichen Akte niedergeschrieben und elektronisch gespeichert. Alle erhobenen Daten werden entsprechend den Bestimmungen des Bundesdatenschutzgesetzes und der ärztlichen Schweigepflicht streng vertraulich behandelt. Die Weitergabe und Auswertung der Daten/Biomaterialien erfolgt ohne Angabe von Namen oder Initialen (d.h. pseudonymisiert); es wird nur ein Nummern- und/oder Buchstabencode verwendet. Die erhobenen Daten können in einem Computer gespeichert und verarbeitet werden.

Ihre Einwilligung zur Teilnahme an dieser klinischen Studie schließt die Aufzeichnung Ihrer im Rahmen dieser klinischen Studie erhobenen Krankheitsdaten durch beteiligte Ärzte ein, sowie die Weitergabe dieser Daten an den Leiter der klinischen Prüfung, Herrn Univ.-Prof. Dr. med. Hans Hauner, einschließlich dessen Vertreter und Auftragnehmer sowie die zuständigen Überwachungsbehörden und Ethikkommissionen.

Sie haben das Recht, jegliche Informationen, die sich auf Ihre Teilnahme an dieser Studie beziehen, einzusehen. Dies betrifft Informationen über Ihre allgemeine Gesundheit, jegliche aufgetretenen Nebenwirkungen und alle Testergebnisse, die während dieser Studie gesammelt worden sind. Die Ergebnisse der genetischen Analyse dienen ausschließlich zu Forschungszwecken. Deshalb werden Ergebnisse dieser Forschung insgesamt und individuelle Befunde weder Ihnen noch Ihrem Arzt oder Versicherungen zugänglich gemacht.

8. Rücktritt/Abbruch

Ihre Studienteilnahme ist freiwillig. Im Studienverlauf haben Sie das Recht, Ihre Zustimmung zur Teilnahme jederzeit und ohne Angabe von Gründen zu widerrufen, ohne dass sich daraus persönliche Nachteile für Sie ergeben. Sollten Sie sich bereits nach dem Lesen dieses Aufklärungsbogens gegen eine Teilnahme entscheiden, teilen Sie uns dies bitte kurz mit.

Sollte eine weitere Teilnahme am Forschungsvorhaben ärztlich nicht mehr vertretbar sein, kann der Prüfarzt/Leiter der klinischen Prüfung (LKP) Ihre Teilnahme auch vorzeitig beenden, ohne dass Sie auf diese Entscheidung Einfluss haben.

Im Falle des Widerrufs/Abbruchs können Sie entscheiden, ob Ihre Blut-/und Gewebeproben vernichtet werden sollen oder in pseudonymisierter Form für weitere wissenschaftliche Zwecke verwendet werden dürfen. Bereits erhobene Daten verbleiben auch nach Vernichtung der Probe in der Studie, soweit der Personenbezug nicht mehr besteht.

9. Recht auf Information

Sie haben das Recht vor, während und nach Ende der Studie jederzeit Fragen über die Studie zu stellen.

Werden aus Ihren entnommenen Bioproben (Blut, Gewebe) wider Erwarten medizinische Erkenntnisse gewonnen, die für die Erhaltung oder Wiederherstellung Ihrer eigenen Gesundheit von erheblicher Bedeutung sind, so wird Ihr Hausarzt darüber informiert, damit er mit Ihnen das weitere Vorgehen besprechen kann.

Wenn Sie eine Information Ihres Hausarztes nicht wünschen, können Sie dies in der Einverständniserklärung zum Ausdruck bringen.

Für alle weiteren Fragen im Zusammenhang mit dieser Studie stehen Ihnen der Studienarzt und seine Mitarbeiter gerne zur Verfügung. Auch Fragen, die Ihre Rechte als Teilnehmer dieser klinischen Studie betreffen, werden Ihnen gerne beantwortet.

Bei Rückfragen können Sie sich gerne an das Studienteam wenden.

Kontakt

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mailto: beate.ott@tum.de
http://www.em-tum.de/

Einverständniserklärung

Studie: Effekt einer definierten Überernährung auf die Darmbarrierefunktion bei stoffwechselgesunden Teilnehmern.

Hiermit gebe ich mein freiwilliges Einverständnis zur Teilnahme an der klinischen Studie gemäß der vorstehenden Teilnehmerinformation.

✓ Ich bin bereit, freiwillig an dem unter Punkt 2 beschriebene Untersuchungsprogramme teilzunehmen.
❒ Ja ❒ Nein

✓ Ich bin einverstanden, dass meine Bioproben (Blut, Gewebe) einschließlich DNA für die Untersuchung der unter Punkt 1 beschriebenen Fragestellungen verwendet werden.
❒ Ja ❒ Nein

✓ Mit der Erhebung und Aufzeichnung der wissenschaftlichen Daten in pseudonymisierter Form, d.h. nur unter Angabe der Teilnehmernummer, bin ich einverstanden.
❒ Ja ❒ Nein

✓ Ich bin einverstanden, dass meine Bioproben (Blut, Gewebe) einschließlich DNA für die zukünftige Forschung aufbewahrt und verwendet werden.
❒ Ja ❒ Ja, aber nur nach vorheriger Kontaktaufnahme ❒ Nein

Durch meine Unterschrift bestätige ich, dass ich die Teilnehmerinformation gelesen und verstanden habe. Ich hatte Gelegenheit Fragen zu stellen, die mir zufriedenstellend beantwortet wurden.

Ich weiß, dass meine Teilnahme freiwillig ist und ich meine Einwilligung jederzeit mündlich oder schriftlich ohne Angabe von Gründen widerrufen und die Vernichtung meiner Blut-/Gewebeproben fordern kann, ohne dass mir daraus irgendwelche Nachteile entstehen.

Über mögliche gesundheitliche Risiken (siehe Punkt 4) wurde ich aufgeklärt. Die Studie findet unter ärztlicher Überwachung statt. Es werden keine therapeutischen Maßnahmen durchgeführt. Für meine Teilnahme erhalte ich eine Aufwandsentschädigung.

Werden aus meinen entnommenen Bioproben (Blut, Gewebe) wider Erwarten medizinische Erkenntnisse gewonnen, die für die Erhaltung oder Wiederherstellung meiner eigenen Gesundheit von erheblicher Bedeutung sind, wird mein Hausarzt darüber informiert, damit er mit mir das weitere Vorgehen besprechen kann.

❒ Ich möchte **nicht**, dass mein Hausarzt über Befunde im vorstehenden Sinn informiert wird.

Ich erkläre mich damit einverstanden, dass im Rahmen dieser klinischen Studie personenbezogene Daten, insbesondere Angaben über meine Gesundheit, über mich erhoben und in Papierform sowie auf elektronischen Datenträgern aufgezeichnet werden. Soweit erforderlich, dürfen die erhobenen Daten ohne Namensnennung und Geburtsdatum (pseudonymisiert) weitergegeben werden an:

a) den Auftraggeber der Studie (BMBF, TU München, Nestlé Research Center) zur wissenschaftlichen Auswertung.
b) die zuständige(n) Überwachungsbehörde(n) (Landesamt/-ämter) oder Bezirksregierung(en) und Ethikkommissionen(en).

Außerdem erkläre ich mich damit einverstanden, dass autorisierte und zur Verschwiegenheit verpflichtete Beauftragte des Auftraggebers der zuständigen inländischen und ausländischen Überwachungs- und Zulassungsbehörden in meine beim Studienarzt vorhandenen personenbezogenen Daten, insbesondere meine Gesundheitsdaten, Einsicht nehmen, soweit dies für die Überprüfung der ordnungsgemäßen Durchführung des Forschungsvorhabens notwendig ist. Für diese Maßnahme entbinde ich den Studienarzt von der ärztlichen Schweigepflicht.

Die Einwilligung zur Erhebung und Verarbeitung der Angaben über meine Gesundheit ist unwiderruflich. Ich bin bereits darüber aufgeklärt worden, dass ich jederzeit die Teilnahme an der klinischen Studie beenden kann. Im Falle dieses Widerrufs erkläre ich mich damit einverstanden, dass die bis zu diesem Zeitpunkt gespeicherten Daten ohne Namensnennung weiterhin verwendet werden dürfen, soweit dies erforderlich ist.

Ich erkläre mich damit einverstanden, dass meine Daten nach Beendigung oder Abbruch der klinischen Studie mindestens 15 Jahre aufbewahrt werden. Danach werden meine personenbezogenen Daten gelöscht, soweit nicht gesetzliche, satzungsmäßige oder vertragliche Aufbewahrungsfristen entgegenstehen.

Ich hatte ausreichend Zeit mich zu entscheiden. Zum Zeitpunkt der Unterzeichnung der Einverständniserklärung habe ich keine weiteren Fragen. Mir ist bekannt, dass ich jederzeit, sowohl während als auch nach der klinischen Studie, Fragen stellen kann.

Eine Kopie der Teilnehmerinformation und eine Einverständniserklärung habe ich erhalten.

München/Freising-Weihenstephan,

Name des aufklärenden Arztes	Unterschrift
Name der/s Studienteilnehmerin/s	Unterschrift

Study document 8: Case Report Form, “Overnutrition” Study

Case Report Form

Teilstudie 2

|__||__||__||__|

Liste der verwendeten Abkürzungen

A	Anamnese
AC	Akzelerometer
AK	Anthropometrische und klinische Parameter
BE	Blutabnahme
BIA	Bioimpendanzanalyse
BIC	Bank Identifier Code
BLZ	Bankleitzahl
BMBF	Bundeministerium für Bildung und Forschung
BW	Body Weight
CT	Euglykämisch-hyperinsulinämische Clamp-Technik
BMI	Body Mass Index
EB	Ernährungsberatung
EDTA	Ethylendiamintetraacetat
EP	Ernährungsprotokoll
FEE	Fat-free mass
FZ	Fäzesprobe
IBAN	International Bank Account Number
IK	Indirekte Kalorimetrie
IP	Intestinale Permeabilität
M	Glukosestoffwechsel
M_r	relatives Molekulargewicht
PEG	Polyethylenglycose
REE	resting energy expenditure
S	Screening
V	Visite

Visite 0 (V_0)

Datum: __ __ / __ __ / __ __ __ __ Uhrzeit: |__||__| : |__||__| Uhr

Anamnese $_{A0}$

A001 Gesundheitszustand/Medikation erfasst? ❒ Ja ❒ Nein

A002 Wenn nein, bitte begründen: _____

Indirekte Kalorimetrie $_{IK0}$

IK001 Indirekte Kalorimetrie erfolgreich durchgeführt? ❒ Ja ❒ Nein

IK002 Wenn nein, bitte begründen: _____

IK003 Ergebnisse nachfolgend angefügt? ❒ Ja ❒ Nein

IK004 Grundumsatz

IK004.1 Soll |__||__||__||__| kcal

IK004.2 IST |__||__||__||__| kcal

IK005 Fett |__||__|,|__| %

IK006 Kohlenhydrate |__||__|,|__| %

IK007 Protein |__||__|,|__| %

Anthropometrische und klinische Parameter $_{AK0}$

AK001 Ruhe-Puls (HF/min):

AK001.1 Messung 1: |__||__||__|

AK001.2 Messung 2: |__||__||__|

AK001.3 Messung 3: |__||__||__|

AK001.4 Mittelwert (AK008.1-008.3): |__||__||__|

AK002 Blutdruck im Ruhezustand (systolisch/diastolisch in mmHg):

AK002.1 Messung dominanter Arm |__||__||__|/|__||__||__|

AK002.2 Messung nicht dominanter Arm

AK002.2.1 Messung 1: |__||__||__|/|__||__||__|

AK002.2.2 Messung 2: |__||__||__|/|__||__||__|

AK002.2.3 Messung 3: |__||__||__|/|__||__||__|

AK002.2.4 Mittelwert (AK002.2.1-002.2.3): |__||__||__|/|__||__||__|

Erläuterungen: AK001 Ruhe-Puls - Pulsfrequenz in Schlägen pro Minute im Sitzen (HF)
AK002.2 Blutdruck - Messung im Sitzen am nicht dominanten Arm und in Ruhe (nach 5 min) (Empfehlung der Deutschen Hochdruckliga 2012)

AK003 Körpergröße: |__||__||__|,|__| cm

AK004 Körpergewicht: |__||__||__|,|__| kg

AK005 Body Mass Index (BMI): |__||__|,|__| kg/m²

AK006 Taillenumfang: |__||__||__|,|__| cm

AK007 Hüftumfang: |__||__||__|,|__| cm

AK008 Taille-Hüft-Quotient: |__|,|__||__||__|

AK009BIA_Messung:

AK010.1 Fett (%): |__||__|,|__| %

AK010.2 Fettmasse |__||__|,|__| kg

AK010.3 Fettfreie Masse |__||__|,|__| kg

AK010.4 Körperwasser |__||__|,|__| kg

Erläuterungen: AK003 ohne Schuhe und in kg; Für Standard-Kleidung (ohne Schuhe) wird 1kg subtrahiert
AK009 BIA _Messungen (Tanita Waage) :
AK003-10 Ist die zweite Ziffer nach dem Komma eine 0,1,2,3,4, wird abgerundet
Ist die zweite Ziffer nach dem Komma eine 5,6,7,8,9, wird aufgerundet

Ernährungsprotokoll EP0

EP001 Erste Ernährungsprotokoll mit Anleitung ausgegeben?

❒ Ja am: __ __ / __ __ / __ __ __ __

❒ Nein

EP002 Wenn nein, bitte begründen: _____

AktiGraph AC0

AC501 Actigraph mit Anleitung ausgegeben?

❒ Ja am: __ __ / __ __ / __ __ __ __

❒ Nein

AC502 Wenn nein, bitte begründen: _____

AC503 Seriennummer: _____

Fäzesprobe FZ0

FZ001 Utensilien für die erste Fäzesprobe ausgegeben?

❒ Ja am: __ __ / __ __ / __ __ __ __

❒ Nein

FZ002 Wenn nein, bitte begründen: _____

FZ003 Analysecodierung: |_____|

Intestinale Darmpermeabilität $_{IP0}$

IP001 Utensilien für den ersten PEG-Test/ Zuckerresorptionstest ausgegeben?

- ❑ 2x250ml Behältnis
- ❑ 3x 1,5l Behältnisse
- ❑ Zuckerlösung mit Kapseln
- ❑ 100ml PEG-Lösung
- ❑ Tasche zum Transportieren

❒ Ja am: __ __ / __ __ / __ __ __ __

❒ Nein

IP002 Wenn nein, bitte begründen: _____

Kontaktaufnahme $_{KA0}$

KA001 Der Studienteilnehmer wurde per Telefon oder Email kontaktiert?

❒ Ja am: __ __ / __ __ / __ __ __ __ ❒ Nein

KA002. Fragen zu folgenden Themen konnten geklärt werden?

- ❑ Ernährungsprotokoll
- ❑ AktiGraph
- ❑ Sammelurin
- ❑ Fäzesproben
- ❑ nächsten Untersuchungen (Blutentnahme, Clamp)

Teilnehmer |__||__||__||__|

Visite 1 (V_1)

Datum: __ __ / __ __ / __ __ __ __ Uhrzeit: |__||__| : |__||__| Uhr

Ernährungsprotokoll $_{EP1}$

EP101 Erste Ernährungsprotokoll zurückerhalten am: __ __ / __ __ / __ __ __ __

EP102 Durchschnittliche tägliche Energieaufnahme: |__||__||__||__|kcal

Erläuterungen: EP102 Durchschnittliche tägliche Energieaufnahme
Ist die erste Ziffer nach dem Komma eine 0,1,2,3,4, wird abgerundet
Ist die erste Ziffer nach dem Komma eine 5,6,7,8,9, wird aufgerundet

Fäzesprobe $_{FZ1}$

FZ101 Erste Fäzesprobe abgegeben? ❒ Ja ❒ Nein

FZ102 Wenn nein, bitte begründen: _____

FZ103 Erste Analysecodierung: |_____|

FZ104 Erste Fäzesprobe
Datum: __ __ / __ __ / __ __ __ __ Uhrzeit: |__||__| : |__||__| Uhr

FZ105 Erste Fäzesprobe zum Studienzentrum gebracht
Datum: __ __ / __ __ / __ __ __ __ Uhrzeit: |__||__| : |__||__| Uhr

FZ106 Utensilien für die zweite Probe ausgegeben? ❒ Ja ❒ Nein

FZ107 Wenn nein, bitte begründen: _____

FZ108 Zweite Analysecodierung: |_____|

Intestinale Darmpermeabilität IP1

IP101 Vier Behältnisse für Sammelurin erhalten? ❒ Ja ❒ Nein

IP102 Wenn nein, bitte begründen: _____

IP103 Urinvolumen:

IP103.1 0h- Sammelvolumen (A) |__||__||__||__| ml

IP103.2 5h- Sammelvolumen (B) |__||__||__||__| ml

IP103.3 21h- Sammelvolumen (C) |__||__||__||__| ml

IP103.3 29h- Sammelvolumen (D) |__||__||__||__| ml

PEG

IP104 Erste Analysecodierung PEG-Vorharn: |_____|

IP105 Erste Analysecodierung PEG-5h-Urin: |_____|

IP106 Erste Analysecodierung PEG-24h-Urin: |_____|

IP107 Urinvolumen:

IP107.1 0h- Sammelvolumen (A) |__||__||__||__| ml

IP107.2 5h- Sammelvolumen (B) |__||__||__||__| ml

IP107.3 24h- Sammelvolumen (C+D) |__||__||__||__| ml

IP107.4 Gesamturinvolumen |__||__||__||__| ml

IP108 PEG-Konzentration 400M_r |__||__|,|__| % im Urin

IP109 PEG-Konzentration 1500M_r |__||__|,|__| % im Urin

IP110 PEG-Konzentration 3000M_r |__||__|,|__| % im Urin

IP111 PEG-Konzentration 4000M_r |__||__|,|__| % im Urin

IP112Messung erfolgreich durchgeführt? ❒ Ja ❒ Nein

IP113 Wenn nein, bitte begründen: _____

Zuckerresorptionstest

IP114 Erste Analysecodierung

IP114.1 Vorharn-Analysecodierung: |_____|

IP114.2 5h-Sammelurin-Analysecodierung: |_____|

IP114.3 21h-Sammelurin-Analysecodierung: |_____|

IP115 Urinvolumen:

IP115.1 0h- Sammelvolumen (A) |__||__||__||__| ml

IP115.2 5h- Sammelvolumen (B) |__||__||__||__| ml

IP115.3 21h- Sammelvolumen (C) |__||__||__||__| ml

IP115.4 Gesamturinvolumen |__||__||__||__| ml

IP116 Laktulose |__||__|,|__||__||__| %LAC

IP117 Mannitol |__||__|,|__||__||__| %MAN

IP118 Permeabilitätsindex (%LAC/%MAN): |__||__|,|__||__||__|

IP119 Saccarose |__||__|,|__||__||__| %

IP120 Sucralose |__||__|,|__| |__||__|%

IP121 Utensilien für den zweiten PEG-Test/ Zuckerresorptionstest ausgegeben?

- ❒ 2x250ml Behältnis
- ❒ 3x 1,5l Behältnisse
- ❒ Zuckerlösung mit Kapseln
- ❒ 100ml PEG-Lösung
- ❒ Tasche zum Transportieren

❒ Ja am: __ __ / __ __ / __ __ __ __

❒ Nein

Wenn nein, bitte begründen: _____

Erläuterungen: IP1016-20 Ist die zweite Ziffer nach dem Komma eine 0,1,2,3,4, wird abgerundet
Ist die zweite Ziffer nach dem Komma eine 5,6,7,8,9, wird aufgerundet

Teilnehmer |__||__||__||__|

Blutabnahme $_{BA1}$

BA101 Patient nüchtern? ❒ Ja ❒ Nein

BA102 Wenn nein, neuer Termin: __ __ / __ __ / __ __ __ __

BA103 Blutabnahme erfolgreich durchgeführt? ❒ Ja ❒ Nein

BA104 Wenn nein, bitte begründen: _____

BA105 Uhrzeit: |__||__|:|__||__| Uhr

BA106 Besonderheiten (z.B. Entzündung der Einstichstelle, mehrere Einstiche zur Blutentnahme waren nötig)

❒ Ja ❒ Nein

BA107 Wenn ja, welche? _____

BA108 Blut abgenommen durch: _____ (Name) _____ (Unterschrift)

BA109 Analysecodierung: |_____|

BA110 Citrat (2,5 ml)	❒ k.A.	❒ 1x	
BA111 EDTA (2,7 ml)	❒ k.A.	❒ 1x	
BA112 Natrium-Fluorid (2,7 ml)	❒ k.A.	❒ 1x	
BA113 Serum (9,0 ml)	❒ k.A.	❒ 1x	
BA114 Lithium-Heparin (9,0 ml)	❒ k.A.	❒ 1x	❒ 2x
BA115 EDTA (9,0 ml)	❒ k.A.	❒ 1x	

Teilnehmer |__||__||__||__|

Visite 2 (V_2)

Datum: __ __ / __ __ / __ __ __ __ Uhrzeit: |__||__| : |__||__| Uhr

Blutabnahme $_{BA2}$

BA201 Patient nüchtern? ❒ Ja ❒ Nein

BA202 Wenn nein, neuer Termin: __ __ / __ __ / __ __ __ __

BA203 Blutabnahme erfolgreich durchgeführt? ❒ Ja ❒ Nein

BA204 Wenn nein, bitte begründen: _____

BA205 Uhrzeit: |__||__|:|__||__| Uhr

BA206 Besonderheiten (z.B. Entzündung der Einstichstelle, mehrere Einstiche zur Blutentnahme waren nötig)

❒ Ja ❒ Nein

BA207 Wenn ja, welche? _____

BA208 Blut abgenommen durch: _____ (Name) _____ (Unterschrift)

VOR Clamp

BA209 EDTA (4,9 ml)	❒ k.A.	❒ 1x	
BA210 Lithium-Heparin (9,0 ml)	❒ k.A.	❒ 1x	❒ 2x

Am Ende des steady-state

BA210 EDTA (4,9 ml)	❒ k.A.	❒ 1x	
BA211 Lithium-Heparin (9,0 ml)	❒ k.A.	❒ 1x	❒ 2x

Euglykämisch-hyperinsulinämische Clamp-Technik CT2

CT201 Messung erfolgreich durchgeführt? ❒ Ja ❒ Nein

CT202 Wenn nein, bitte begründen:_____

CT203 Nüchternglucosespiegel |__||__| mg/dl

CT204 Infusion G20 Plateau |__||__||__| ml/h

CT205 Insulin Plateau |__||__|,|__| pmol/l

CT206 M-Wert |__|,|__||__| mmol/min

CT208 M_{KG} |__||__|,|__| $\mu mol \cdot min^{-1} \cdot kg^{-1}$

CT209 M_{ffKG} |__||__|,|__| $\mu mol \cdot min^{-1} \cdot kg^{-1}$

CT211 M/I |__||__|,|__| $mmol \cdot l/nmol \cdot min$

CT212 M_{KG}/I |__||__|,|__| $\mu mol \cdot l/nmol \cdot min \cdot kg$

CT213 M_{ffKG}/I |__||__|,|__| $\mu mol \cdot l/nmol \cdot min \cdot kg$

Erläuterungen: CT203-13 Ist die zweite Ziffer nach dem Komma eine 0,1,2,3,4, wird abgerundet
Ist die zweite Ziffer nach dem Komma eine 5,6,7,8,9, wird aufgerundet

Kontaktaufnahme KA2

KA201 Der Studienteilnehmer wurde per Telefon oder Email kontaktiert?

❒ Ja am: __ __ / __ __ / __ __ __ __ ❒ Nein

KA202. Befindlichkeiten während der Intervention?

- ❒ Übelkeit
- ❒ Erbrechen
- ❒ Durchfälle
- ❒ keine Auffälligkeiten

KA203. Fragen zu folgenden Themen konnten geklärt werden?

- ❒ Ernährungsprotokoll
- ❒ AktiGraph
- ❒ Sammelurin
- ❒ Fäzesproben
- ❒ nächsten Untersuchungen (Blutentnahme, Clamp)

Teilnehmer |__||__||__||__|

Visite 3 (V_3)

Datum: __ __ / __ __ / __ __ __ __ Uhrzeit: |__||__| : |__||__| Uhr

Fäzesprobe $_{FZ3}$

FZ301 Zweite Fäzesprobe zurückgegeben? ❒ Ja ❒ Nein

FZ302 Wenn nein, bitte begründen: _____

FZ303 Zweite Analysecodierung: |_____|

FZ304 Fäzesprobe
Datum: __ __ / __ __ / __ __ __ __ Uhrzeit: |__||__| : |__||__| Uhr

FZ205 Fäzesprobe zum Studienzentrum gebracht
Datum: __ __ / __ __ / __ __ __ __ Uhrzeit: |__||__| : |__||__| Uhr

FZ306 Utensilien für die dritte Probe ausgegeben? ❒ Ja ❒ Nein

FZ307 Wenn nein, bitte begründen: _____

FZ308 Dritte Analysecodierung: |_____|

Intestinale Darmpermeabilität $_{IP3}$

IP301 Vier Behältnisse für Sammelurin erhalten? ❒ Ja ❒ Nein

IP302 Wenn nein, bitte begründen: _____

IP303 Urinvolumen:

IP303.1 0h- Sammelvolumen (A) |__||__||__||__| ml

IP303.2 5h- Sammelvolumen (B) |__||__||__||__| ml

IP303.3 21h- Sammelvolumen (C) |__||__||__||__| ml

IP303.3 29h- Sammelvolumen (D) |__||__||__||__| ml

PEG

IP304 Erste Analysecodierung PEG-Vorharn: |_____|

IP305 Erste Analysecodierung PEG-5h-Urin: |_____|

IP306 Erste Analysecodierung PEG-24h-Urin: |_____|

IP307 Urinvolumen:

IP307.1 0h- Sammelvolumen (A) |__||__||__||__| ml

IP307.2 5h- Sammelvolumen (B) |__||__||__||__| ml

IP307.3 24h- Sammelvolumen (C+D) |__||__||__||__| ml

IP307.4 Gesamturinvolumen |__||__||__||__| ml

IP308 PEG-Konzentration 400M_r |__||__|,|__| % im Urin

IP309 PEG-Konzentration 1500M_r |__||__|,|__| % im Urin

IP310 PEG-Konzentration 3000M_r |__||__|,|__| % im Urin

IP311 PEG-Konzentration 4000M_r |__||__|,|__| % im Urin

IP312Messung erfolgreich durchgeführt? ❒ Ja ❒ Nein

IP313 Wenn nein, bitte begründen: _____

Zuckerresorptionstest

IP314 Erste Analysecodierung

IP314.1 A-Vorharn-Analysecodierung: |_____|

IP314.2 B-5h-Sammelurin-Analysecodierung: |_____|

IP314.3 C-21h-Sammelurin-Analysecodierung: |_____|

IP315 Urinvolumen:

IP315.1 0h- Sammelvolumen (A) |__||__||__||__| ml

IP315.2 5h- Sammelvolumen (B) |__||__||__||__| ml

IP315. 21h- Sammelvolumen (C) |__||__||__||__| ml

IP315.4 Gesamturinvolumen |__||__||__||__| ml

IP316 Laktulose: |__||__|,|__||__||__| %LAC

IP317 Mannitol: |__||__|,|__||__||__| %LAC

IP318 Permeabilitätsindex (%LAC/%MAN): |__||__|,|__||__||__|

IP319 Saccarose |__||__|,|__||__||__| %

IP320 Sucralose |__||__|,|__| |__||__|%

IP321 Utensilien für den zweiten PEG-Test/ Zuckerresorptionstest ausgegeben?

- ❑ 2x250ml Behältnis
- ❑ 3x 1,5l Behältnisse
- ❑ Zuckerlösung mit Kapseln
- ❑ 100ml PEG-Lösung
- ❑ Tasche zum Transportieren

❒ Ja am: __ __ / __ __ / __ __ __ __

❒ Nein

Wenn nein, bitte begründen: _____

Erläuterungen: IP3016-20 Ist die zweite Ziffer nach dem Komma eine 0,1,2,3,4, wird abgerundet
Ist die zweite Ziffer nach dem Komma eine 5,6,7,8,9, wird aufgerundet

Ernährungsprotokoll EP3

EP301 Zweite Ernährungsprotokoll mit Anleitung ausgegeben?

❒ Ja am: __ __ / __ __ / __ __ __ __

❒ Nein

Wenn nein, bitte begründen: _____

Indirekte Kalorimetrie IK3

IK301 Indirekte Kalorimetrie erfolgreich durchgeführt? ❒ Ja ❒ Nein

IK302 Wenn nein, bitte begründen: _____

IK303 Ergebnisse nachfolgend angefügt? ❒ Ja ❒ Nein

IK304 Grundumsatz

IK304.1 Soll |__||__||__||__| kcal

IK304.2 IST |__||__||__||__| kcal

IK305 Fett |__||__|,|__| %

IK306 Kohlenhydrate |__||__|,|__| %

IK307 Protein |__||__|,|__| %

Anthropometrische und klinische Parameter AK3

AK301 Ruhe-Puls (HF/min):

AK301.1 Messung 1: |__||__||__|

AK301.2 Messung 2: |__||__||__|

AK301.3 Messung 3: |__||__||__|

AK301.4 Mittelwert (AK308.1-308.3): |__||__||__|

AK302 Blutdruck im Ruhezustand (systolisch/diastolisch in mmHg):

AK302.1 Messung dominanter Arm |__||__||__|/|__||__||__|

AK302.2 Messung nicht dominanter Arm

AK302.2.1 Messung 1: |__||__||__|/|__||__||__|

AK302.2.2 Messung 2: |__||__||__|/|__||__||__|

AK302.2.3 Messung 3: |__||__||__|/|__||__||__|

AK302.2.4 Mittelwert (AK309.2.1-302.2.3): |__||__||__|/|__||__||__|

Erläuterungen: AK301 Ruhe-Puls - Pulsfrequenz in Schlägen pro Minute im Sitzen (HF)
AK302.2 Blutdruck - Messung im Sitzen am nicht dominanten Arm und in Ruhe (nach 5 min) (Empfehlung der Deutschen Hochdruckliga 2012)

AK303 Körpergröße:	|__|__|__|,|__| cm
AK304 Körpergewicht:	|__|__|__|,|__| kg
AK305 Body Mass Index (BMI):	|__|__|,|__| kg/m²
AK306 Taillenumfang:	|__|__|__|,|__| cm
AK307 Hüftumfang:	|__|__|__|,|__| cm
AK308 Taille-Hüft-Quotient:	|__|,|__|__|__|
AK309 BIA_Messung:	
AK310.1 Fett (%):	|__|__|,|__| %
AK310.2 Fettmasse	|__|__|,|__| kg
AK310.3 Fettfreie Masse	|__|__|,|__| kg
AK310.4 Körperwasser	|__|__|,|__| kg

Erläuterungen: AK303 ohne Schuhe und in kg; Für Standard-Kleidung (ohne Schuhe) wird 1kg subtrahiert
AK309 BIA _Messungen (Tanita Waage) :
AK303-10 Ist die zweite Ziffer nach dem Komma eine 0,1,2,3,4, wird abgerundet
Ist die zweite Ziffer nach dem Komma eine 5,6,7,8,9, wird aufgerundet

Blutabnahme BA3

BA301 Patient nüchtern? ❒ Ja ❒ Nein

BA302 Wenn nein, neuer Termin: __ __ / __ __ / __ __ __ __

BA303 Blutabnahme erfolgreich durchgeführt? ❒ Ja ❒ Nein

BA304 Wenn nein, bitte begründen: _____

BA305 Uhrzeit: |__||__|:|__||__| Uhr

BA306 Besonderheiten (z.B. Entzündung der Einstichstelle, mehrere Einstiche zur Blutentnahme waren nötig)

❒ Ja ❒ Nein

BA307 Wenn ja, welche? _____

BA308 Blut abgenommen durch: _____ (Name) _____ (Unterschrift)

BA309 Analysecodierung: |_____|

BA310 Citrat (2,5 ml)	❒ k.A.	❒ 1x	
BA311 EDTA (2,7 ml)	❒ k.A.	❒ 1x	
BA312 Natrium-Fluorid (2,7 ml)	❒ k.A.	❒ 1x	
BA313 Serum (9,0 ml)	❒ k.A.	❒ 1x	
BA314 Lithium-Heparin (9,0 ml)	❒ k.A.	❒ 1x	❒ 2x
BA315 EDTA (9,0 ml)	❒ k.A.	❒ 1x	

Teilnehmer |__||__||__||__|

Visite 4 (V_4)

Datum: __ __ / __ __ / __ __ __ __ Uhrzeit: |__||__| : |__||__| Uhr

Blutabnahme BA4

BA401 Patient nüchtern? ❒ Ja ❒ Nein

BA402 Wenn nein, neuer Termin: __ __ / __ __ / __ __ __ __

BA403 Blutabnahme erfolgreich durchgeführt? ❒ Ja ❒ Nein

BA404 Wenn nein, bitte begründen: _____

BA405 Uhrzeit: |__||__|:|__||__| Uhr

BA406 Besonderheiten (z.B. Entzündung der Einstichstelle, mehrere Einstiche zur Blutentnahme waren nötig)

❒ Ja ❒ Nein

BA407 Wenn ja, welche? _____

BA408 Blut abgenommen durch: _____ (Name) _____ (Unterschrift)

VOR Clamp

BA409 EDTA (4,9 ml) ❒ k.A. ❒ 1x

BA410 Lithium-Heparin (9,0 ml) ❒ k.A. ❒ 1x ❒ 2x

Am Ende des steady-state

BA410 EDTA (4,9 ml) ❒ k.A. ❒ 1x

BA411 Lithium-Heparin (9,0 ml) ❒ k.A. ❒ 1x ❒ 2x

Teilnehmer |__||__||__||__|

Euglykämisch-hyperinsulinämische Clamp-Technik CT4

CT401 Messung erfolgreich durchgeführt? ❒ Ja ❒ Nein

CT402 Wenn nein, bitte begründen: _____

CT403 Nüchternglucosespiegel |__||__| mg/dl

CT404 Infusion G20 Plateau |__|__||__| ml/h

CT405 Insulin Plateau |__||__|,|__| pmol/l

CT406 M-Wert |__|,|__||__| $\mu mol \cdot min^{-1} \cdot kg^{-1}$

CT408 M_{KG} |__||__|,|__| $\mu mol \cdot min^{-1} \cdot kg^{-1}$

CT409 M_{ffKG} |__||__|,|__| $\mu mol \cdot min^{-1} \cdot kg^{-1}$

CT411 M/I |__||__|,|__| $mmol \cdot l/nmol \cdot min$

CT412 M_{KG}/I |__||__|,|__| $\mu mol \cdot l/nmol \cdot min \cdot kg$

CT413 M_{ffKG}/I |__||__|,|__| $\mu mol \cdot l/nmol \cdot min \cdot kg$

Erläuterungen: CT403-13 Ist die zweite Ziffer nach dem Komma eine 0,1,2,3,4, wird abgerundet
Ist die zweite Ziffer nach dem Komma eine 5,6,7,8,9, wird aufgerundet

Kontaktaufnahme KA4

KA401 Der Studienteilnehmer wurde per Telefon oder Email kontaktiert?

❒ Ja am: __ __ / __ __ / __ __ __ __ ❒ Nein

KA402. Fragen zu folgenden Themen konnten geklärt werden?

- ❒ Ernährungsprotokoll
- ❒ AktiGraph
- ❒ Sammelurin
- ❒ Fäzesproben
- ❒ nächsten Untersuchungen (Blutentnahme, Clamp)

Teilnehmer |__||__||__||__|

Visite 5 (V_5)

Datum: __ __ / __ __ / __ __ __ __ Uhrzeit: |__||__| : |__||__| Uhr

Fäzesprobe FZ5

FZ501 Dritte Fäzesprobe zurückgegeben? ❒ Ja ❒ Nein

FZ502 Wenn nein, bitte begründen: _____

FZ503 Dritte Analysecodierung: |_____|

FZ504 Fäzesprobe
Datum: __ __ / __ __ / __ __ __ __ Uhrzeit: |__||__| : |__||__| Uhr

FZ505 Fäzesprobe zum Studienzentrum gebracht
Datum: __ __ / __ __ / __ __ __ __ Uhrzeit: |__||__| : |__||__| Uhr

Intestinale Darmpermeabilität IP5

IP501 Vier Behältnisse für Sammelurin erhalten? ❒ Ja ❒ Nein

IP502 Wenn nein, bitte begründen: _____

IP503 Urinvolumen:

IP503.1 0h- Sammelvolumen (A) |__||__||__||__| ml

IP503.2 5h- Sammelvolumen (B) |__||__||__||__| ml

IP503.3 21h- Sammelvolumen (C) |__||__||__||__| ml

IP503.3 29h- Sammelvolumen (D) |__||__||__||__| ml

PEG

IP504 Erste Analysecodierung PEG-Vorharn: |_____|

IP505 Erste Analysecodierung PEG-5h-Urin: |_____|

IP506 Erste Analysecodierung PEG-24h-Urin: |_____|

IP507 Urinvolumen:

IP507.1 0h- Sammelvolumen (A) |__||__||__||__| ml

IP507.2 5h- Sammelvolumen (B) |__||__||__||__| ml

IP507.3 24h- Sammelvolumen (C+D) |__||__||__||__| ml

IP507.4 Gesamturinvolumen |__||__||__||__| ml

IP508 PEG-Konzentration 400M_r |__||__|,|__| % im Urin

IP509 PEG-Konzentration 1500M_r |__||__|,|__| % im Urin

IP510 PEG-Konzentration 3000M_r |__||__|,|__| % im Urin

IP511 PEG-Konzentration 4000M_r |__||__|,|__| % im Urin

IP512Messung erfolgreich durchgeführt? ❒ Ja ❒ Nein

IP513 Wenn nein, bitte begründen: _____

Zuckerresorptionstest

IP514 Erste Analysecodierung

IP514.1 A-Vorharn-Analysecodierung: |_____|

IP514.2 B-5h-Sammelurin-Analysecodierung: |_____|

IP514.3 C-21h-Sammelurin-Analysecodierung: |_____|

IP515 Urinvolumen:

IP515.1 0h- Sammelvolumen (A) |__||__||__||__| ml

IP515.2 5h- Sammelvolumen (B) |__||__||__||__| ml

IP515.3 21h- Sammelvolumen (C) |__||__||__||__| ml

IP515.4 Gesamturinvolumen |__||__||__||__| ml

IP516 Laktulose |__||__|,|__||__||__| %LAC

IP517 Mannitol |__||__|,|__||__||__| %MAN

IP518 Permeabilitätsindex (%LAC/%MAN): |__||__|,|__||__||__|

IP519 Saccarose |__||__|,|__||__||__| %

IP520 Sucralose |__||__|,|__| |__||__|%

IP521 Utensilien für den zweiten PEG-Test/ Zuckerresorptionstest ausgegeben?

- ❑ 2x250ml Behältnis
- ❑ 3x 1,5l Behältnisse
- ❑ Zuckerlösung mit Kapseln
- ❑ 100ml PEG-Lösung
- ❑ Tasche zum Transportieren

❒ Ja am: __ __ / __ __ / __ __ __ __

❒ Nein

Wenn nein, bitte begründen: _____

Erläuterungen: IP5016-20 Ist die zweite Ziffer nach dem Komma eine 0,1,2,3,4, wird abgerundet
Ist die zweite Ziffer nach dem Komma eine 5,6,7,8,9, wird aufgerundet

Ernährungsprotokoll EP5

EP501 Erste Ernährungsprotokoll zurückerhalten am: __ __ / __ __ / __ __ __ __

EP502 Durchschnittliche tägliche Energieaufnahme: |__||__||__||__|kcal

Erläuterungen: EP502 Durchschnittliche tägliche Energieaufnahme
Ist die erste Ziffer nach dem Komma eine 0,1,2,3,4, wird abgerundet
Ist die erste Ziffer nach dem Komma eine 5,6,7,8,9, wird aufgerundet

AktiGraph AC5

AC501 Actigraph zurückerhalten

❒ Ja am: __ __ / __ __ / __ __ __ __

❒ Nein

AC502 Wenn nein, bitte begründen_____

Seriennummer: _____

Indirekte Kalorimetrie IK5

IK501 Indirekte Kalorimetrie erfolgreich durchgeführt? ❒ Ja ❒ Nein

IK502 Wenn nein, bitte begründen: _____

IK503 Ergebnisse nachfolgend angefügt? ❒ Ja ❒ Nein

IK504 Grundumsatz

IK504.1 Soll |__||__||__||__| kcal

IK504.2 IST |__||__||__||__| kcal

IK505 Fett |__||__|,|__| %

IK506 Kohlenhydrate |__||__|,|__| %

IK507 Protein |__||__|,|__| %

Anthropometrische und klinische Parameter AK5

AK501 Ruhe-Puls (HF/min):

AK501.1 Messung 1: |__||__||__|

AK501.2 Messung 2: |__||__||__|

AK501.3 Messung 3: |__||__||__|

AK501.4 Mittelwert (AK508.1-508.3): |__||__||__|

AK502 Blutdruck im Ruhezustand (systolisch/diastolisch in mmHg):

AK502.1 Messung dominanter Arm |__||__||__|/|__||__||__|

AK502.2 Messung nicht dominanter Arm

AK502.2.1 Messung 1: |__||__||__|/|__||__||__|

AK502.2.2 Messung 2: |__||__||__|/|__||__||__|

AK502.2.3 Messung 3: |__||__||__|/|__||__||__|

AK502.2.4 Mittelwert (AK509.2.1-509.2.3): |__||__||__|/|__||__||__|

Erläuterungen: AK501 Ruhe-Puls - Pulsfrequenz in Schlägen pro Minute im Sitzen (HF)
AK502.2 Blutdruck - Messung im Sitzen am nicht dominanten Arm und in Ruhe (nach 5 min) (Empfehlung der Deutschen Hochdruckliga 2012)

AK503 Körpergröße: |__||__||__|,|__| cm

AK504 Körpergewicht: |__||__||__|,|__| kg

AK505 Body Mass Index (BMI): |__||__|,|__| kg/m²

AK506 Taillenumfang: |__||__||__|,|__| cm

AK507 Hüftumfang: |__||__||__|,|__| cm

AK508 Taille-Hüft-Quotient: |__|,|__||__||__|

AK509BIA_Messung:

AK510.1 Fett (%): |__||__|,|__| %

AK510.2 Fettmasse |__||__|,|__| kg

AK510.3 Fettfreie Masse |__||__|,|__| kg

AK510.4 Körperwasser |__||__|,|__| kg

Erläuterungen: AK503 ohne Schuhe und in kg; Für Standard-Kleidung (ohne Schuhe) wird 1kg subtrahiert
AK509 BIA _Messungen (Tanita Waage) :
AK503-10 Ist die zweite Ziffer nach dem Komma eine 0,1,2,3,4, wird abgerundet
Ist die zweite Ziffer nach dem Komma eine 5,6,7,8,9, wird aufgerundet

Blutabnahme BA5

BA501 Patient nüchtern? ❒ Ja ❒ Nein

BA502 Wenn nein, neuer Termin: __ __ / __ __ / __ __ __ __

BA503 Blutabnahme erfolgreich durchgeführt? ❒ Ja ❒ Nein

BA504 Wenn nein, bitte begründen: _____

BA505 Uhrzeit: |__||__|:|__||__| Uhr

BA506 Besonderheiten (z.B. Entzündung der Einstichstelle, mehrere Einstiche zur Blutentnahme waren nötig)

❒ Ja ❒ Nein

BA507 Wenn ja, welche? _____

BA508 Blut abgenommen durch: _____ (Name) _____ (Unterschrift)

BA509 Analysecodierung: |_____|

BA510 Citrat (2,5 ml)	❒ k.A.	❒ 1x	
BA511 EDTA (2,7 ml)	❒ k.A.	❒ 1x	
BA512 Natrium-Fluorid (2,7 ml)	❒ k.A.	❒ 1x	
BA513 Serum (9,0 ml)	❒ k.A.	❒ 1x	
BA514 Lithium-Heparin (9,0 ml)	❒ k.A.	❒ 1x	❒ 2x
BA515 EDTA (9,0 ml)	❒ k.A.	❒ 1x	

Teilnehmer |__||__||__||__|

Visite 1 (V_1)

Datum: __ __ / __ __ / __ __ __ __ Uhrzeit: |__||__| : |__||__| Uhr

Rekrutierung R10

R10. Wie sind Sie auf die Humanstudie aufmerksam geworden?

- ❑ Kontaktaufnahme von vorherigen Studien
- ❑ Pressemitteilung, Zeitung
- ❑ andere_____

Kontaktaufnahme K11

K11. Ich bin damit einverstanden, dass mich das Studienteam per Telefon oder Email während der Studie kontaktiert.

❑ Nein ❑ Ja

Anmerkungen:

_____

Teilnehmer |__||__||__||__|

Familienanamnese A13

A13.1. Hatten oder haben Sie Blutsverwandte mit Fettstoffwechselstörungen (z.B. erhöhtes Cholesterin)?

❑ Nein ❑ Ja

Wenn ja, nennen Sie bitte alle Personen (z.B. Mutter, Vater, Oma etc.) sowie das Lebensalter beim Eintritt der Erkrankung:

_____

A13.2. Hatten oder haben Sie Blutsverwandte mit Diabetes mellitus Typ 2?

❑ Nein ❑ Ja

Wenn ja, nennen Sie bitte alle Personen (z.B. Mutter, Vater, Oma etc.) sowie das Lebensalter beim Eintritt der Erkrankung:

_____

A13.3. Hatten oder haben Sie Blutsverwandte mit Adipositas (krankhaftes Übergewicht mit einem BMI>30kg/m^2)?

❑ Nein ❑ Ja

Wenn ja, nennen Sie bitte alle Personen (z.B. Mutter, Vater, Oma etc.) sowie das Lebensalter beim Eintritt der Erkrankung:

_____

Lebensstil A14

A14.1. Rauchen Sie?

- ❑ Nein ❑ Exraucher seit ____Jahren
- ❑ Ja Wenn ja,

  - seit wie vielen Jahren rauchen Sie? _____
  - wieviele Zigaretten rauchen Sie pro Tag? _____

A14.2. Wieviel Alkohol konsumieren Sie im Durchschnitt pro Woche?

- ❑ Ich trinke keinen Alkohol
- ❑ < 5 Gläser *
- ❑ ≥ 5 Gläser *

* 1 Glas ≈ 500 ml Bier, 250 ml Wein

A14.3. Wieviel Bohnenkaffee konsumieren Sie im Durchschnitt pro Tag?

- ❑ Ich trinke keinen Kaffee
- ❑ < 3 Tassen *
- ❑ ≥ 3 Tassen *

* 1 Tasse ≈ 200ml

A14.4. Worauf achten Sie bei Ihrer Ernährung?

- ❑ Fettarm
- ❑ Fleischarm
- ❑ Vegetarisch
- ❑ andere_____

A14.5. Nehmen Sie Nahrungsergänzungsmittel, Eiweißpulver und/oder Schlankheitspräparate zu sich?

❑ Nein ❑ Ja

Wenn ja, welche?_____

A14.6. Wie ernähren Sie sich?

A14.6.1 Gewöhnlich esse ich Fleischwaren oder Wurst etc.:
❑ < 3 x/Woche ❑ ≥ 3 x/Woche

A14.6.2 Ich esse Obstportionen, Gemüse bzw. Salatportionen:
❑ nicht jeden Tag ❑ täglich ❑ mehrmals täglich

A14.6.3 Ich esse Vollkornprodukte:
❑ nie ❑ mehrmals pro Woche ❑ täglich

A14.6.4 Ich esse Süßigkeiten, Marmelade, Honig usw.:
❑ nie ❑ mehrmals pro Woche ❑ täglich

A14.7. Hat sich Ihr Gewicht im letzten Jahr verändert?

❑ Nein, mein Gewicht war stabil (+/- 3kg)

❑ Ja, ich habe abgenommen ❑ Ja, ich habe zugenommen

Bitte nennen, Sie:

- Ausmaß der Gewichtsabnahme/-zunahme (in Kilogramm): _____
- Zeitraum der Gewichtsabnahme/-zunahme: _____ –_____
- Grund der Gewichtsabnahme/-zunahme (Diät, Krankheit, Stress etc.): _____

A14.8. Treiben Sie regelmäßig Sport?

❑ Nein ❑ Ja

Wenn ja, bitte nennen Sie die Sportarten und die Stunden/Woche:

_____

_____

Sozialanamnese A15

A15.1. Welchen Schulabschluss habe Sie?

❑ Hauptschule ❑ Abitur

❑ Realschule ❑ Hochschulstudium

A15.2. Welchen Beruf üben Sie aus? (Frage nur in Teilstudie 1)

_____

A15.3. Welchen Belastungen Sind Sie am Arbeitsplatz ausgesetzt (mehrere Antworten sind möglich)?

❑ Keinen, ich bin mit meiner Tätigkeit sehr zufrieden

❑ schwere körperliche Arbeit

❑ Schichtarbeit

❑ Zeitdruck, Akkordarbeit

❑ Staub, Gase, Geruch, Chemikalien

❑ Lärm, Hitze, Kälte,

❑ ständiger Leistungsdruck, Stress

❑ einseitige Arbeitshaltung (ständiges Sitzen, ständiges Stehen etc.)

❑ Mehrfachbelastungen (Nebenberufe, Haushalt etc.)

❑ Monotonie, Langeweile, Unterforderung

❑ drohenden Arbeitsplatzverlust

❑ andere:_____

Ethnische Herkunft A16

❑ Weiß/Europäisch

❑ Schwarz

❑ Asiatisch, Orientalisch

❑ andere_____

Ich habe den Fragebogen nach bestem Wissen und Gewissen ausgefüllt.

_____ _____

Name Unterschrift

Teilnehmer |__||__||__||__|

Anleitung zum Ernährungsprotokoll

Sehr geehrter Studienteilnehmer,

das Ernährungsprotokoll soll Ihre individuellen Ernährungsgewohnheiten erfassen. Bitte beachten Sie daher folgende Hinweise:

- Füllen Sie das Ernährungsprotokoll möglichst genau und ehrlich aus
- Bitte ändern Sie während der Protokollführung Ihre Ernährungsgewohnheiten nicht.
- Bitte notieren Sie **alle** Lebensmittel und Getränke
- Tragen Sie auch Lebensmittel ein, die Sie im Restaurant, Mensa oder als Zwischenmahlzeit bzw. Snacks (z.B. Bonbons, ein Apfel) zu sich nehmen.
- Bitte notieren Sie auch Vitamin- und Mineralstoffpräparate.

In die ***erste Spalte*** tragen Sie bitte ein, **wann und wo** Sie die Mahlzeit zu sich genommen haben (z. B. zu Hause, in der Mensa, im Restaurant, unterwegs…).

In die ***zweite Spalte*** tragen Sie bitte genau ein, **wie viel** Sie von einem Lebensmittel verzehrt haben.

- Dies geschieht in „haushaltsüblichen Maßen“, z.B. geben Sie die Portionen in „Scheiben“, „Esslöffeln“ oder „Tassen“ bzw. „Bechern“ an.
- Versuchen Sie diese Mengenangaben so genau wie möglich zu machen (z.B. kleine, mittlere, große Portionen oder 200ml, 250ml Tasse/Becher/Glas oder dünn, dick bestrichenes Brot mit Konfitüre . . .)
- Wenn Angaben auf der Verpackung zu finden sind (z. B. bei Joghurt oder Fertiggerichten), ist es sinnvoll, diese anzugeben.
- Gerichte – wenn möglich – nach einzelnen Zutaten aufschlüsseln.

Die ***dritte Spalte*** soll die **genaue Bezeichnung des Lebensmittels** enthalten, z. B.:

- „Edamer, 40 % F.i.Tr.“ anstatt „Käse“,
- „Roggenmischbrot“ anstatt „Brot“ oder
- „Apfel, Boskop, geschält“ anstatt „Apfel“.
- Wenn möglich, geben Sie bitte auch immer den Marken- oder Sortennamen an.

Teilnehmer |__||__||__||__|

In der ***vierten*** Spalte tragen Sie bitte die **Zubereitungsart** des Essens ein. Die Zubereitungsart sollte möglichst genau beschrieben werden:

- bei Fleisch und Fisch: „paniert“ oder „gebraten in Rapsöl“
- bei Gemüse “in Olivenöl angebraten“ oder „gedünstet“

Angabe des Fettgehalts:

- Bei Milch- und Milchprodukten (Joghurt, Käse, Milch, usw.) in % Fett (z. B. Milch, 1,5 % Fett) oder % Fettgehalt i. Tr. (z. B. Camembert 60 % i. Tr.)
- Bei Fleisch- und Wurstwaren Einteilung in fett, mager, fettreduziert (z. B. „Schweineschinken mager, gekocht“ oder „Speck, durchwachsen“)

Angabe des Brat-, Salat- und Aufstrichfettes bezüglich Sorte und Menge:

- z. B. Butter, Sonnenblumenmargarine, Diätmargarine (Handelsnamen angeben)
- oder: Rapsöl, Olivenöl, Leinöl (Handelsnamen angeben)

Zubereitungsart:

- gekocht, gebraten, frittiert, gegrillt, gedünstet, gedämpft, überbacken, paniert, gebunden…

Nähere Beschreibung des Lebensmittels (Art, Sorte, Markenname usw.):

- Fleisch: von welchem Tier (Rind, Schwein, Huhn) und Stück (Brust, Filet, usw.)
- Wurst & Käse: z.B. Salami, Fleischwurst, Schinken (gekocht oder geräuchert) oder Camembert, Gouda, Mozzarella, Feta, Appenzeller, usw.
- Soße/Suppe: Bratensoße gebunden, Holländische Soße, Essig-Öl-Salatsoße, Joghurtdressing, Suppe mit Fleischeinlage, Cremesuppe, usw.
- Kuchen: Hefekuchen, Streuselkuchen, Sahnetorte, Obstkuchen (Mürbteig, Biskuit)
- Getränke: Unterscheidung von Saft und Nektar, Markennamen angeben, z.B. bei Bier

Angabe über die verwendeten Lebensmittel: frisch, Tiefkühlkost oder Konserven

- Zum Beispiel bei Kompott oder Gemüse

Sollten Fragen oder Unsicherheiten auftreten, stehen wir Ihnen jederzeit gern zur Verfügung!

Beate Ott, Dipl.-Ern. Wiss.
Tel: 08161/71-2352
mailto: beate.ott@tum.de

Teilnehmer |__||__||_1_||_2_|

Ernährungsprotokoll 1. Tag

Wochentag: Dienstag Datum: 01/10/ 2013

Frühstück / Zwischenmahlzeit:

Zeit / Ort	Menge	Lebensmittel/Getränk	Zubereitung
06:30 Uhr, zu Hause	100 g	Basismüsli	
	1	Apfel „Jona Gold“, klein	ungeschält
	1	Banane klein	
	150 ml	Milch, 1,5% Fett	
	200 ml	Kaffee schwarz	
	50 ml	Milch, 1,5% Fett	
	200 ml	Mineralwasser	

Mittagessen / Zwischenmahlzeit:

Zeit / Ort	Menge	Lebensmittel/Getränk	Zubereitung
10:00 Uhr, zu Hause	150 g	Joghurt, Kirsche	
	400 ml	Mineralwasser	
12:30 Uhr, zu Hause	2 Teller	Kürbissuppe	
	1	Laugengebäck	
	400 ml	Mineralwasser	

Abendessen / Spätmahlzeit:

Zeit / Ort	Menge	Lebensmittel/Getränk	Zubereitung
18:00 Uhr zu Hause	1 Scheibe	Roggenvollkornbrot	
	10 g	Butter	
	1 Scheibe	Gouda, 40% Fett	
	1	Möhre	
	2	Tomaten	
	400 ml	Mineralwasser	

Study document 11: Manual of the ActiGraph GT3X+ and Log sheet

Teilnehmer |__||__||__||__|

Anleitung zum Tragen des ActiGraph GT3X+

Sehr geehrter Studienteilnehmer,

im Studienzentrum wurde Ihnen ein Bewegungsmessgerät angelegt. Bitte tragen Sie dieses Messgerät für die nächsten 4 Wochen.

Für das Tragen des **ActiGraph GT3X+** beachten Sie bitte folgende Hinweise:

- Gehen Sie allen Ihren gewohnten Aktivitäten wie gewöhnlich nach.
- Der Sensor den ganzen Tag getragen werden – auch nachts.
- Der Sensor soll immer auf der rechten Hüfte liegen, **mit der schwarzen Verschluss-kappe nach oben**.
- Der Sensor kann ober- oder unterhalb der Kleidung getragen werden.
- Der Sensor ist wasserfest und kann während des Duschens getragen werden.
- Nehmen Sie den Sensor bitte ab, wenn Sie länger als 30 Minuten schwimmen gehen.
- Nehmen Sie den Sensor zum Tauchen, für Saunabesuche oder bei Sportarten mit hohem Körperkontakt (Kampfsportarten, Rugby, usw.) bitte ab.
- Bitte schrauben Sie die schwarze Verschlusskappe nicht auf.
- Bitte lassen Sie den Sensor nicht aus großer Höhe fallen.
- Falls Sie den Sensor abnehmen, so tragen Sie bitte den Zeitpunkt, den Grund für die Abnahme und den Zeitpunkt des Wiederanlegens im Tragetagebuch ein. Eine **genaue Dokumentation** ist für die anschließende Auswertung sehr wichtig!
- Bitte geben Sie den Sensor an niemanden weiter.
- Die Batterie des Sensors hält maximal 7 Tage. Bitte lassen Sie daher bei jedem Besuch des Studienzentrums die Batterie überprüfen und den Bewegungssensor gegebenenfalls aufgeladen.

Wenn Störungen am Gerät auftreten oder Sie Fragen zum Sensor haben, so können Sie sich jederzeit im Studienzentrum melden.

Sollten Fragen oder Unsicherheiten auftreten, stehen wir Ihnen jederzeit gern zur Verfügung!

Beate Ott, Dipl.-Ern. Wiss.
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mailto: beate.ott@tum.de

ActiGraph GT3X+ - Logbuch

Um ein optimales Ergebnis des Sensors zu erzielen, versuchen Sie bitte den ActiGraph GT3X+ mindestens 7 Tage lang zu tragen. Zudem sollten notiert werden, sobald der Sensor für mehr als 5 Minuten abgenommen wurde. Wie im Beispiel beschrieben, schreiben Sie den Zeitpunkt mit Begründung auf, wann Sie den Sensor abgenommen haben und wieder angelegt haben.

Datum	24.04.2013.	
Tag	Mittwoch	
	Aus	**An**
	07:00	15:00
Grund	Schwimmen	

Datum														
Tag														
	Aus	**An**	**Aus**	**An**	**Aus**	**An**	**Aus**	**An**	**Aus**	**An**	**Aus**	**An**	**Aus**	**An**
Grund														
Grund														
Grund														
Grund														
Grund														

Wird von den Mitarbeitern im Studienzentrum ausgefüllt:

Studienteilnehmer _____ Mitarbeiter _____

Seriennummer _____
Datum der Initialisierung _____
Gültige Tage _____

Teilnehmer |__||__||__||__|

GEFÖRDERT VOM
Bundesministerium für Bildung und Forschung

Technische Universität München

EKFZ

Anleitung zum Sammeln von Urin zur Bestimmung der intestinalen Permeabilität

Sehr geehrter Studienteilnehmer,

bitte beachten Sie folgende Hinweise:

- Einige Zahnpasten und Nahrungsergänzungsmittel enthalten Polyethylenglykole bzw. Polyoxyethylen, welche die Genauigkeit der Analysen stören könnten. Daher bitten wir Sie, Ihre Zahnpasta und Ihre Nahrungsergänzungsmitteln auf Polyethylenglykol (PEG) oder Polyoxyethylen (POE) zu überprüfen. Wenn PEG oder POE enthalten ist, bitten wir Sie einen geeigneten Ersatz zu nehmen. Bei Fragen oder Unklarheiten können Sie gern das Studienteam fragen

- In den **48 Stunden** bevor Sie den Test ausführen, sind Bier, Wein, Schnaps oder sonstige alkoholische Getränke **nicht erlaubt**!!!

- Am Vorabend des Tests ist die letzte Mahlzeit **bis spätestens 18 Uhr** erlaubt, danach dürfen nur noch Leitungswasser bzw. Mineralwasser getrunken werden.

- In den **letzten 24 Stunden** vor dem Test möglichst auf Medikamente verzichten:
  Nicht erlaubt sind: Antibiotika (Absprache!!), Diuretika, Abführmittel, Rheumamittel, Aspirin

- Bitte notieren Sie, ob Sie in der Woche vor dem Test alkoholische Getränke zu sich genommen haben (Bier, Wein, Spirituosen)

  ❑ nein, ich habe keine alkoholischen Getränke in der Woche vor dem Test zu mir genommen

  ❑ ja, ich habe alkoholische Getränke in der Woche vor dem Test zu mir genommen

  Datum: __ . __ . __ . was und wie viel _____

  Datum: __ . __ . __ . was und wie viel _____

  Datum: __ . __ . __ . was und wie viel _____

  Datum: __ . __ . __ . was und wie viel _____

  Datum: __ . __ . __ . was und wie viel _____

Materialien

Zu allen drei Visiten V_1, V_2, V_3 bekommen Sie

- eine Testlösung mit den Zuckern Laktulose, Mannitol und Saccharose zum Trinken, sowie sechs Kapseln mit je 333,33mg Sucralose zum Einnehmen.
- eine Testlösung mit PEG
- 1 kleine Plastikflasche A zum Sammeln des ersten Harn nach dem Aufstehen
- 3 große Plastikflaschen B und 2xC zum Sammeln allen weiteren Harns bis Testende
- 6 Urin-Monovetten zum Entnehmen des Harns
- Hygienehandschuhe
- verschließbarer Kunststoffbeutel

WARNUNG: Die Sammelflaschen enthalten als Konservierungsmittel Natrium-Azid, daher für Kinder unerreichbar aufbewahren!!

Ausführung des 26-h-Sammelurins:

1. Nach dem Aufstehen sammeln Sie den ersten Harn in **Flasche A** und verschließen Sie das Gefäß.
   - ➔ **Notieren** Sie sich die **Urinmenge** der Flasche A
   - ➔ **DANACH entnehmen Sie 2x10 ml Harn von Gefäß A mittels den 2 Monovetten** (siehe Anleitung)
   - ➔ **Lagern** Sie die beschrifteten Monovetten im verschließbaren Kunststoffbeutel in der Tiefkühltruhe

2. Dann trinken Sie innerhalb von maximal 10 Minuten den Inhalt der Flasche mit der **Zuckerlösung** aus und nehmen bitte die 6 Kapseln zusammen mit der Testlösung ein. Der Urin wird nun in **Flasche B** gesammelt.

   Hinweise für die nächsten 2 Stunden:
   - **NICHTS** trinken und **NICHTS** essen, auch **KEINEN** Kaugummi!
   - die Zähne **NICHT** putzen
   - **KEINE** Medikamente einnehmen
   - den gesamten Harn in der großen **Plastikflasche B** sammeln!
   - Sammelurin bitte **im Kühlschrank** senkrecht aufbewahren!

3. **2 Stunden später:** 2 Stunden nach Trinken der Zuckertestlösung und Einnahme der 6 Kapseln müssen Sie Leitungswasser bzw. Mineralwasser trinken, **mindestens** jedoch ¼ Liter bis zu einem Liter.

Hinweis für die nächsten 3 Stunden:

- **NICHTS** trinken und **NICHTS** essen, auch **KEINEN** Kaugummi!
- die Zähne **NICHT** putzen
- **KEINE** Medikamente einnehmen
- den gesamten Harn in der großen Plastikflasche B sammeln! Bitte im Kühlschrank aufbewahren!

4. **3 Stunden später bzw. insgesamt 5 Stunden** nach Trinken der **Zuckertestlösung** und Einnahme der 6 Kapseln
   - ➔ sammeln Sie **den letzten Harn in die Flasche B**
   - ➔ **DANACH notieren** Sie sich die **Urinmenge** der Flasche B
   - ➔ **DANACH entnehmen Sie 2x10 ml Harn von Gefäß B mittels den 2 Monovetten** (siehe Anleitung)
   - ➔ **Lagern** Sie die beschrifteten Monovetten im verschließbaren Kunststoffbeutel in der Tiefkühltruhe
   - ➔ Trinken Sie bitte die **zweite Testlösung**, in der **PEG** enthalten ist.
   - ➔ Die Testlösungs-Flaschen und das Gefäß für die Kapseln können Sie wegwerfen.
   - ➔ Nun dürfen Sie essen und trinken und Ihre Medikamente einnehmen!

5. Während der **darauffolgenden 21 Stunden** sammeln Sie allen anfallenden Harn in **Gefäß C**. Verschließen Sie Gefäß C gut und stellen es ebenfalls in den Kühlschrank. Falls ein Gefäß C nicht reicht, so verwenden Sie bitte das zweite Gefäß C.

6. Nach den 21h Stunden sammeln Sie den letzten Harn in **Gefäß C.**
   - ➔ **Notieren** Sie sich die **Urinmenge** der Flasche C
   - ➔ **DANACH entnehmen Sie 2x 10 ml Harn von Gefäß C mittels den 2 Monovetten** (siehe Anleitung)
   - ➔ **Lagern** Sie die beschrifteten Monovetten im verschließbaren Kunststoffbeutel in der Tiefkühltruhe

7. Bringen Sie den verschließbaren Kunststoffbeutel mit den beschrifteten, tiefgekühlten Monovetten und das ausgefüllte Blatt *„Anleitung zum Sammeln von Urin“* bei Ihrem nächsten Termin zum Studienzentrum.

Sollten Fragen oder Unsicherheiten auftreten, stehen wir Ihnen jederzeit gern zur Verfügung!

Beate Ott, Dipl.-Ern. Wiss.
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Anleitung zum Sammeln von Urin im Überblick

Harnsammelschema

Sammelphase	Gefäß	Sammel-Stunden	Uhrzeit	Sammelurinmenge
Morgen-Harn	A	0		
Haupt-Harn	B	5		
Haupt-Harn	C	21		

Anleitung für das Abfüllen der Harne

- Während des Sammelns von Urin bewahren Sie bitte den Sammelurin **im Kühlschrank** senkrecht auf.
- Bitte entleeren Sie am Ende der Sammelphase nochmals Ihre Blase.
- Bitte notieren Sie sich das Harnvolumina des Sammelgefäßes auf der Monovette und im Harnsammelschema (siehe oben)

  ➔ **Ohne Harnvolumina ist keine quantitative Analyse möglich**

- **Mischen** Sie das Gefäß vor Entnehmen des Harns
- Versuchen Sie den Harn von der Mitte des Gefäßes mittels Monovette zu entnehmen

  ➔ Wenn zwei C-Gefäße verwendet wurden, dann bitte den Harn aus beiden C-Gefäßen in einen sauberen Eimer zusammenschütten und mit einem sauberen Gegenstand gründlich mischen. Aus diesem Harn-Gemisch befüllen Sie die Monovetten

- **Bitte beschriften** Sie die gelben Harn-Röhrchen mit NAME, FRAKTION (A, B,C) und Sammelurinmenge
- Am Ende haben sie von Gefäß A, B, C je **zwei** gelbe Harn-Röhrchen, welche mit Name, Fraktion und Sammelurinmenge beschriftet sind.
- GESCHAFFT - Bitte lagern Sie die Röhrchen im verschließbaren Kunststoffbeutel im Tiefkühlfach

VIELEN DANK !!!!!!!

Study document 13: Manual of stool collection procedure, “Caloric restriction” study

Teilnehmer |__||__||__||__|

GEFÖRDERT VOM
Bundesministerium für Bildung und Forschung

Technische Universität München

EKFZ

Anleitung zum Sammeln von Stuhlproben zur Bestimmung der Darmflora

Sehr geehrter Studienteilnehmer,

die Qualität der Ergebnisse hängt maßgeblich von der sachgerechten Gewinnung des Untersuchungsmaterials ab, deshalb beachten Sie folgende Hinweise:

- **Beschriften** Sie das **Stuhlröhrchen** und das Sammelbehältnis mit ihren persönlichen Geburtsdatum
  ➔ Achtung das Stuhlröhrchen enthält eine Flüssigkeit (DNA-Stabilisator). Achten Sie darauf, dass die Flüssigkeit **nicht verschüttet** wird.

- Der Stuhl darf **nicht mit Wasser, Urin, Blut bzw. anderen Materialien (z.B. Klopapier)** in Berührung kommen. Daher schlagen wir Ihnen vor, dass Sie erst Ihren Urin lassen und danach das Behältnis mit Stuhl befüllen.

- Bitte füllen Sie Ihren **Stuhl direkt** in das Behältnis

- Entnehmen Sie nach dem Stuhlgang mit dem in den Deckel integrierten Löffel von mehreren Stellen eine Probe. Wenn der Stuhl hart ist, so versuchen Sie mehrere Stücken zu entnehmen. Schrauben Sie dann den Deckel (samt Löffel) wieder auf das Röhrchen und verschließen Sie es fest.

- Schütteln Sie das geschlossene Röhrchen bis der Inhalt gut gemixt ist.

- Bitte lagern Sie das Probenröhrchen bis zur Abgabe im Tiefkühlfach.

- Bringen Sie bitte das Probenröhrchen zur nächsten Untersuchung mit.

- Bitte notieren Sie, wann Sie das Probenröhrchen gefüllt haben
  Datum: __ __ / __ __ / __ __ __ __ Uhrzeit: |__||__| : |__||__| Uhr

- Bitte notieren Sie, wann Sie das Probenröhrchen zum Studienzentrum gebracht haben.
  Datum: __ __ / __ __ / __ __ __ __ Uhrzeit: |__||__| : |__||__| Uhr

Sollten Fragen oder Unsicherheiten auftreten, stehen wir Ihnen jederzeit gern zur Verfügung!

Beate Ott, Dipl.-Ern. Wiss., Tel: 08161/71-2352, mailto: beate.ott@tum.de

Study document 14: Manual of stool collection procedure, “Overnutrition” study

Teilnehmer |__||__||__||__|

GEFÖRDERT VOM
Bundesministerium für Bildung und Forschung

Technische Universität München

EKFZ ERNÄHRUNGSMEDIZIN

Anleitung zum Sammeln von Stuhlproben zur Bestimmung der Darmflora

Sehr geehrter Studienteilnehmer,

bitte bringen Sie zu allen drei Visiten V_1, V_2, V_3 Stuhlproben mit. Von der Studienbetreuung bekommen Sie für dafür vorgesehene gekennzeichnete Behältnisse.

Bitte füllen Sie die Behältnisse mit Ihrem Stuhl und dokumentieren die Zeit

- am letzten Tag der einwöchigen Normalkostphase
  Datum: __ __ / __ __ / __ __ __ __ Uhrzeit: |__||__| : |__||__| Uhr
- am letzten Tag der überkalorischen Kost
  Datum: __ __ / __ __ / __ __ __ __ Uhrzeit: |__||__| : |__||__| Uhr
- am letzten Tag der zweiwöchigen Normalkostphase
  Datum: __ __ / __ __ / __ __ __ __ Uhrzeit: |__||__| : |__||__| Uhr

<u>Hinweise</u>

- Bitte füllen Sie Ihren **Stuhl direkt** in das Behältnis
- Bringen Sie bitte das Behältnis mit Ihrem gesammelten Stuhl **innerhalb** von **einer Stunde** zum Studienzentrum.
- Sie können auch im Studienzentrum Ihren Stuhl sammeln.
- Der Stuhl sollte **nicht mit Urin bzw. anderen Materialien (z.B. Klopapier)** in Berührung kommen. Daher schlagen wir Ihnen vor, dass Sie erst Ihren Urin lassen und danach das Behältnis mit Stuhl befüllen.
- Der Stuhl kann **bis 18:00 Uhr** im Studienzentrum abgegeben werden

Sollten Fragen oder Unsicherheiten auftreten, stehen wir Ihnen jederzeit gern zur Verfügung!

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8.2 Supplemental

Supplemental Table 1: Caloric restriction - Metabolic syndrome

ID	Waist circumference [cm]	Fasting blood glucose [mg/dl]	Triglycerides [mg/dl]	HDL-cholesterol [mg/dl]	Blood pressure systolic [mmHg]	Blood pressure diastolic [mmHg]	3 of 5 criteria fulfilled
41	113.7	85	134	48	122	90	**MetS**
42	108.3	80	83	60	113	90	
44	105.4	89	139	49	128	81	
45	104	68	46	54	119	66	
46	98.2	91	276	34	130	86	**MetS**
47	100	92	157	63	138	84	
48	97	122	193	61	141	94	**MetS**
49	116	84	117	56	118	86	
50	104	84	55	76	119	78	
51	93.8	71	103	42	118	75	
52	102	107	283	38	110	73	**MetS**
53	95.8	94	86	51	108	78	
54	97.5	84	106	54	120	95	
55	107.3	88	91	50	115	88	
56	103	86	75	48	144	89	**MetS**
57	109.5	96	124	52	110	76	
58	121	84	74	48	130	86	**MetS**
59	109	77	91	44	115	78	
60	113	84	118	48	139	83	**MetS**
61	140	88	79	55	139	86	**MetS**

HDL, high-density lipoprotein; MetS, metabolic syndrome

Supplemental Table 2: Caloric restriction - Anamnesis and Lifestyle

	Participants (n=20)
Anamnesis of Relations (n (%))	
Relations with Dyslipida	5 (25%)
Relations with Diabetes	7 (35%)
Relations with Obesity	6 (30%)
Lifestyle (n (%))	
Alcohol consumption per week	
no alcohol	
< 5 cups (200 ml)	9 (45%)
≥ 5 cups (200 ml)	1 (5%)
Coffee consumption per day	
no coffee	4 (20%)
< 3 cups (200 ml)	12 (60%)
≥ 3 cups (200 ml)	3 (15%)
Diet	
low-fat	8 (40%)
less meat	1 (5%)
vegetarian	2 (5%)
other	10 (50%)
Dietary Supplements	7 (35%)
Meat consumption	
< 3x/week	7 (35%)
≥ 3x/week	13 (65%)
Fruit consumption	
not every day	2 (5%)
daily	
several times daily	4 (20%)
Whole-grain products	
never/infrequently	6 (30%)
several times weekly	10 (50%)
daily	4 (20%)
Sweets	
never/infrequently	4 (20%)
several times weekly	7 (35%)
daily	9 (45%)
Weight management	
stable during the last year (± 3kg)	2 (5%)
weight gain	4 (20%)
weight lost	14 (70%)
Physical activity (n (%))	11 (55%)
Graduation	
General secondary school	2 (5%)
Intermediate secondary school	9 (45%)
High school/Grammar school	8 (40%)
University degree	1 (5%)

Supplemental Table 3: Caloric restriction – Parameter overview over time

	Mean±SD		
	before VLCD	**after VLCD**	**14d after VLCD**
Dietary behaviour and physical activity			
Energy intake (kcal/d)	1698.06±592.73	809.05±32.65	1281.94±594.89
Fat intake (g/d)	69.3±36.76	20.30±1.56	54.63±272.83
Protein intake (g/d)	70.1±22.80	52.83±1.81	64.36±18.38
Carbohydrate intake (g/d)	183.99±65.37	103.45±4.44	124.50±14.53
Body composition			
Weight (kg)	95.14±13.37	88.24±12.28	88.48±12.61
Body mass index (kg/m²)	34.92±3.83	32.47±3.53	32.59±3.75
Waist circumference (cm)	106.92±10.60	101.19±9.44	100.50±9.32
Hip circumference (cm)	118.47±12.63	113.96±10.77	114.42±11.46
WHR	0.9±0.04	0.89±0.06	0.88±0.04
Lean Mass (kg)	52.70±5.65	50.03±5.45	50.90±5.27
Fat Mass (kg)	42.45±8.83	38.23±7.85	37.60±8.35
RMR, blood pressure and pulse rate			
Pulse rate (HF/min)	72.40±10.73	69.00±12.63	70.75±9.65
Blood pressure systolic (mmHg)	123.80±11.46	124.75±42.40	118.95±11.62
Blood pressure diastolic (mmHg)	83.10±7.40	77.45±9.24	79.40±7.56
RMR (kcal)	1675.50±29.44	1598.00±168.60	1687.25±170.64
Inflammation marker			
AST (U/l)	17.40±4.71	19.14±5.68	21.05±10.01
ALT (U/l)	20.00±5.36	20.85±6.67	24.90±12.35
GGT (U/l)	23.70±19.04	14.60±9.75	23.55±27.28
Creatinine (mg/dl)	0.73±0.17	0.76±0.18	0.72±0.17
Uric acid (mg/dl)	5.91±1.54	5.41±1.61	5.80±1.61
Sodium (mmol/ml)	140.85±2.23	142.05±2.12	142.90±1.92
Potassium (mmol/l)	4.41±0.32	4.37±0.23	4.34±0.38
Calcium (mmol/l)	2.26±0.11	2.36±0.08	2.31±0.08
Total cholesterol (mg/dl)	191.05±39.16	162.45±29.45	185.50±32.97
HDL-cholesterol (mg/dl)	51.55±9.27	44.00±9.22	50.80±9.84
LDL-cholesterol (mg/dl)	118.50±37.19	99.55±26.10	110.40±30.63
Triglycerides (mg/dl)	121.50±64.22	98.05±48.12	110.80±63.23
LDL/HDL	2.36±0.78	2.33±0.69	2.24±0.70
NEFA (mmol/l)	0.62±0.23	0.72±0.22	0.48±0.21

	Mean±SD		
	before VLCD	**after VLCD**	**14d after VLCD**
hsCRP (mg/dl)	0.31±0.37	0.17±0.16	0.27±0.26
HMW Adiponectin (mg/ml)	0.41±0.22	0.47±0.27	0.49±0.28
Leptin (ng/ml)	43.76±25.21	20.86±16.06	25.97±20.27
RANTES (ng/ml)	47.59±19.27	45.59±22.63	39.86±24.14
MCP-1 (pg/ml)	82.09±32.62	84.50±23.41	89.11±25.48
Chemerin (ng/ml)	77.62±25.69	64.81±20.22	75.67±21.11
LBP (mg/dl)	2.73±0.33	2.58±0.35	2.8±0.35
Calprotectin (mg/kg)	21.36±27.93	17.15±17.58	7.15±6.65
Glucose metabolism			
Fasting blood glucose (mg/dl)	87.25±11.84	80.35±7.06	84.05±11.05
Fasting insulin level (µU/ml)	8.88±6.22	5.90±2.68	8.32±8.77
HOMA-IR	2.05±1.52	1.29±0.68	1.77±1.82
ROS			
ROS production rate in plasma (µM superoxide /µg protein/10min Plasma)	0.0089 ± 0.004	0.0089 ± 0.002	0.0084 ± 0.002
ROS production rate in PBMC (µM superoxide /µg protein/10min PBMC)	0.0074 ± 0.005	0.0093 ± 0.007	0.0074± 0.002
Gut permeability marker			
Zonulin (ng/ml)	58.40±21.72	47.45±11.88	54.34±14.75
Sucrose (urine recovery in %)	0.17±0.12	0.10±0.06	0.73±0.94
Mannitol (urine recovery in %)	12.35±5.38	9.30±4.99	14.66±9.16
Lactulose (urine recovery in %)	0.26±0.18	0.15±0.10	0.32±0.32
Sucralose (urine recovery in %)	1.09±1.52	0.26±0.48	1.30±1.79
PEG_9 (urine recovery in %)	16.81±10.34	12.35±7.29	16.63±12.70
PEG_{11} (urine recovery in %)	16.48±15.15	7.63±5.57	17.33±19.54
PEG_{13} (urine recovery in %)	4.21±3.59	2.14±1.25	4.23±4.11
PEG_{25} (urine recovery in %)	0.38±0.33	0.17±0.15	0.30±0.28
PEG_{30} (urine recovery in %)	0.22±0.17	0.11±0.08	0.15±0.12
PEG_{35} (urine recovery in %)	0.20±0.18	0.08±0.09	0.15±0.20
PEG_{40} (urine recovery in %)	0.24±0.24	0.10±0.11	0.16±0.19
PEG_{70} (urine recovery in %)	0.09±0.08	0.04±0.08	0.04±0.06
PEG_{80} (urine recovery in %)	0.04±0.05	0.02±0.04	0.02±0.04

ALT, alanine transaminase; γGT, γ-glutamyltransferase; AST, aspartate aminotransferase; BMI, body mass index; HDL, high-density cholesterol; HMW, high-molecular weight; HOMA-IR, homeostasis model assessment of insulin resistance; hsCRP, high-sensitivity C-reactive protein; LBP, lipopolysaccharide binding protein; LDL, low-density cholesterol; L/M, lactulose/mannitol ratio; MCP-1, monocyte chemoattractant protein-1; NEFA, non-esterified fatty acids; PEG, polyethylene glycol; RANTES, regulated on activation normal T cell expressed and secreted; RMR, resting metabolic rate; ROS, reactive oxygen species; VLCD, very low caloric diet; WHR, waist-to-hip ratio

Supplemental Table 4: Caloric restriction – Significance niveaus over time

	p - value		
	t1-t2	**t2-t3**	**t1-t3**
Dietary behaviour and physical activity			
Energy intake (kcal/d)	< 0.0001	< 0.0001	< 0.01
Fat intake (g/d)	< 0.001	< 0.0001	0.15
Protein intake (g/d)	< 0.01	< 0.01	0.34
Carbohydrate intake (g/d)	< 0.0001	< 0.01	< 0.001
Body composition			
Weight (kg)	< 0.0001	0.48	< 0.0001
Body mass index (kg/m²)	< 0.0001	0.38	< 0.0001
Waist circumference (cm)	< 0.001	0.44	< 0.0001
Hip circumference (cm)	< 0.001	0.64	< 0.0001
WHR	0.17	0.26	0.03
Lean Mass (kg)	< 0.0001	< 0.01	< 0.0001
Fat Mass (kg)	< 0.0001	0.02	< 0.0001
RMR, blood pressure and pulse rate			
Pulse rate (HF/min)	0.1	0.36	0.42
Blood pressure systolic (mmHg)	0.02	0.58	0.07
Blood pressure diastolic (mmHg)	< 0.001	0.18	< 0.01
Resting metabolic rate (kcal)	0.07	< 0.001	0.79
Inflammation marker			
AST (U/l)	0.14	0.43	0.12
ALT (U/l)	0.59	0.18	0.07
GGT (U/l)	< 0.01	0.04	0.96
Creatinine (mg/dl)	0.34	0.03	0.66
Uric acid (mg/dl)	0.01	0.09	0.46
Sodium (mmol/ml)	0.05	0.11	< 0.01
Potassium (mmol/l)	0.66	0.69	0.57
Calcium (mmol/l)	< 0.0001	0.02	0.04
Total cholesterol (mg/dl)	< 0.001	< 0.0001	0.31
HDL-cholesterol (mg/dl)	< 0.0001	< 0.001	0.64
LDL-cholesterol (mg/dl)	< 0.001	< 0.001	0.1
Triglycerides (mg/dl)	0.05	0.15	0.41
LDL/HDL	0.68	0.33	0.22
NEFA (mmol/l)	0.03	< 0.0001	0.02
	p - value		

	t1-t2	t2-t3	t1-t3
hsCRP (mg/dl)	< 0.01	0.01	0.57
HMW Adiponectin (mg/ml)	< 0.01	0.12	< 0.001
Leptin (ng/ml)	< 0.0001	0.04	< 0.0001
RANTES (ng/ml)	0.28	0.16	0.05
MCP-1 (pg/ml)	0.49	0.4	0.29
Chemerin (ng/ml)	< 0.01	< 0.01	0.62
LBP (mg/dl)	< 0.01	< 0.0001	0.19
Calprotectin (mg/kg)	0.79	0.02	0.04
Glucose metabolism			
Fasting blood glucose (mg/dl)	< 0.001	0.04	0.02
Fasting insulin level (µU/ml)	< 0.0001	0.11	0.52
HOMA-IR	< 0.0001	0.12	0.06
ROS			
ROS production rate in plasma (µM superoxide /µg protein/10min Plasma)	0.3	0.4	0.58
ROS production rate in PBMC (µM superoxide /µg protein/10min PBMC)	0.4	0.2	0.97
Gut permeability marker			
Zonulin (ng/ml)	< 0.01	< 0.001	0.19
Sucrose (urine recovery in %)	0.01	< 0.01	0.02
Mannitol (urine recovery in %)	0.17	< 0.001	0.34
Lactulose (urine recovery in %)	0.01	< 0.01	0.31
L/M ratio	0.18	0.5	0.24
Sucralose (urine recovery in %)	0.02	0.02	0.63
PEG_9 (urine recovery in %)	0.03	0.15	0.96
PEG_{11} (urine recovery in %)	0.01	0.02	0.87
PEG_{13} (urine recovery in %)	< 0.01	0.02	0.98
PEG_{25} (urine recovery in %)	< 0.001	0.06	0.41
PEG_{30} (urine recovery in %)	< 0.001	0.16	0.15
PEG_{35} (urine recovery in %)	< 0.001	0.18	0.34
PEG_{40} (urine recovery in %)	< 0.01	0.24	0.22
PEG_{70} (urine recovery in %)	0.02	0.84	0.01
PEG_{80} (urine recovery in %)	0.09	0.43	0.69

ALT, alanine transaminase; γGT, γ-glutamyltransferase; AST, aspartate aminotransferase; BMI, body mass index; HDL, high-density cholesterol; HMW, high-molecular weight; HOMA-IR, homeostasis model assessment of insulin resistance; hsCRP, high-sensitivity C-reactive protein; LBP, lipopolysaccharide binding protein; LDL, low-density cholesterol; L/M, lactulose/mannitol ratio; MCP-1, monocyte chemoattractant protein-1; NEFA, non-esterified fatty acids; PEG, polyethylene glycol; RANTES, regulated on activation normal T cell expressed and secreted; RMR,

resting metabolic rate; ROS, reactive oxygen species; VLCD, very low caloric diet; WHR, waist-to-hip ratio

Supplemental Table 5: Overfeeding - Anamnesis and Lifestyle

	Participants (n=24)
Anamnese of Relations (n (%))	
Relations with Dyslipida	6(25%)
Relations with Diabetes	3(13%)
Relations with Obesity	4(17%)
Lifestyle (n (%))	
Alcohol consumption per week	
no alcohol	0(0%)
< 5 cups (200 ml)	19(79%)
≥ 5 cups (200 ml)	5(21%)
Coffee consumption per day	
no coffee	7(29%)
< 3 cups (200 ml)	17(71%)
≥ 3 cups (200 ml)	0(0%)
Diet	
low-fat	0(0%)
less meat	2(8%)
vegetarian	2(8%)
other	20(83%)
Dietary Supplements	2(8%)
Meat consumption	
< 3x/week	5(21%)
≥ 3x/week	19(79%)
Fruit consumption	
not every day	5(21%)
daily	16(67%)
several times daily	3(13%)
Whole-grain products	
never/infrequently	4(17%)
several times weekly	16(67%)
daily	4(17%)
Sweets	
never/infrequently	4(17%)
several times weekly	13(54%)
daily	7(29%)
Weight management	
stable during the last year (± 3kg)	2(8%)
weight gain	21(88%)
weight lost	1(4%)
Physical activity (n (%))	20
Graduation	
General secondary school	0 (0%)
Intermediate secondary school	0 (0%)
High school/Grammar school	18 (75%)
University degree	6 (25%)

Supplemental Table 6: Overfeeding - Parameter overview over time

	Mean ± Standard deviation (n=24)		
	before HFD	**after HFD**	**14d after HFD**
Energy intake (kcal/d)	2,730.8 ± 707.9	3,926.2 ± 339.9	2,589.1 ± 628.3
Fat intake (g/d)	108.4 ± 34.7	208.7 ± 17.9	100.6 ± 32.9
Protein intake (g/d)	100.0 ± 24.9	176.1 ± 15.3	95.4 ± 25.8
Body composition			
Weight (kg)	76.6 ± 10.3	77.6 ± 10.23	77.3 ± 10.4
Body mass index (kg/m²)	23.0 ± 2.1	23.3 ± 2.1	23.2 ± 2.1
Waist circumference (cm)	83.5 ± 5.9	84.2 ± 5.9	83.7 ± 6.1
Hip circumference (cm)	87.8 ± 5.5	88.4 ± 5.1	87.8 ± 4.9
Lean Mass (kg)	66.9 ± 8.0	67.3 ± 7.8	67.1 ±8.0
Fat Mass (kg)	9.8 ± 3.9	10.3 ± 3.8	10.2 ± 3.9
RMR, blood pressure, pulse rate			
Resting metabolic rate (kcal)	1,958.9 ± 232.6	2,010.7 ± 289.1	1,962.5 ± 290.9
Respiratory quotient	0.72 ± 0.1	0.73 ± 0.1	0.73 ± 0.1
Pulse (HF/min)	62.2 ± 7.6	62.6 ± 6.9	64.9 ± 7.5
Blood pressure systolic (mmHg)	115.7 ± 10.9	110.6 ± 9.2	111.3 ± 8.9
Blood pressure diastolic (mmHg)	78.6 ± 6.9	77.9 ± 5.2	75.3 ± 4.8
Inflammation marker			
AST (U/l)	20.9 ± 5.7	19.3 ± 5.5	20.9 ± 10.1
ALT (U/l)	24.1 ± 5.9	27.3 ± 9.4	25.9 ± 17.2
GGT (U/l)	21.0 ± 8.6	19.1 ± 7.1	19.2 ± 8.2
Creatinine (mg/dl)	0.9 ± 0.1	0.9 ± 0.1	0.9 ± 0.1
Uric acid (mg/dl)	6.1 ± 0.9	6.5 ± 0.8	6.3 ± 1.1
Sodium (mmol/ml)	142.2 ± 2.4	141.5 ± 1.9	141.8 ± 1.6
Potassium (mmol/l)	3.9 ± 0.4	4.1 ± 0.3	4.1 ± 0.4
Calcium (mmol/l)	2.4 ± 0.1	2.4 ± 0.1	2.4 ± 0.1
Total cholesterol (mg/dl)	166.8 ± 27.9	183.8 ± 31.1	167.5 ± 32.8
HDL-cholesterol (mg/dl)	54.1 ± 10.1	61.6 ± 10.6	54.3 ± 11.3
LDL-cholesterol (mg/dl)	95.8 ± 27.3	109.6 ± 29.6	98.3 ± 29.5
Triglycerides (mg/dl)	91.0 ± 30.2	64.4 ± 21.8	78.6 ± 45.2
LDL/HDL	1.9 ± 0.7	1.9 ± 0.7	1.9 ± 0.7
NEFA (mmol/l)	0.4 ± 0.2	0.2 ± 0.1	0.3 ± 0.3

Mean ± Standard deviation (n=24)			
	before HFD	**after HFD**	**14d after HFD**
hsCRP (mg/dl)	0.03 ± 0.04	0.03 ± 0.02	0.05 ± 0.05
HMW Adiponectin (mg/ml)	0.34 ± 0.21	0.45 ± 0.23	0.35 ± 0.24
Leptin (ng/ml)	1.3 ± 0.7	1.5 ± 0.9	1.4 ± 0.8
RANTES (ng/ml)	26.9 ± 14.9	17.3 ± 7.7	16.3 ± 8.7
MCP-1 (pg/ml)	80.8 ± 14.3	79.6 ± 14.9	87.9 ± 16.4
Chemerin (ng/ml)	37.7 ± 10.6	37.0 ± 8.6	35.9 ± 7.9
LBP (mg/dl)	0.65 ± 0.30	0.59 1 ± 0.17	0.58 ± 0.21
Calprotectin (mg/kg)	22.9 ± 20.6	30.0 ± 27.4	15.2 ± 15.4
ROS			
ROS production rate in plasma [µM superoxide /µg protein/10min Plasma]	0.0098 ± 0.005	0.0081 ± 0.003	0.009 ± 0.003
ROS production rate in PBMC [µM superoxide /µg protein/10min PBMC]	0.0059 ± 0.050	0.0049 ± 0.003	0.0042 ± 0.002
Glucose metabolism			
Fasting blood glucose (mg/dl)	81.9 ± 6.5	79.2 ± 5.9	80.8 ± 4.5
Fasting insulin levels (µU/ml)	4.1 ± 0.9	4.3 ± 1.2	3.9 ± 0.7
Gut permeability marker			
Zonulin (ng/ml)	49.04 ± 13.66	46.45 ± 12.44	45.21 ± 14.27
Sucrose (urine recovery in %)	0.14 ± 0.05	0.09 ± 0.05	0.11 ± 1.02
Sucralose (urine recovery in %)	0.46 ± 0.47	0.39 ± 0.6	0.75 ± 1.02
Mannitol (urine recovery in %)	14.35 ± 5.37	15.02 ± 5.12	15.16 ± 4.19
Lactulose (urine recovery in %)	0.23 ± 0.09	0.24 ± 0.09	0.23 ± 0.08
L/M ratio	0.02 ± 0.01	0.02 ± 0.01	0.02 ± 0.01
PEG_9 (urine recovery in %)	13.77 ± 7.09	10.95 ± 6.51	12.13 ± 6.51
PEG_{11} (urine recovery in %)	12.77 ± 9.13	8.81 ± 6.92	10.31 ± 8.26
PEG_{13} (urine recovery in %)	2.95 ± 1.57	2.26 ± 1.52	2.83 ± 1.69
PEG_{25} (urine recovery in %)	0.30 ± 0.17	0.21 ± 0.12	0.26 ± 0.19
PEG_{30} (urine recovery in %)	0.19 ± 0.12	0.14 ± 0.09	0.15 ± 0.12
PEG_{35} (urine recovery in %)	0.20 ± 0.18	0.14 ± 0.12	0.12 ± 0.12
PEG_{40} (urine recovery in %)	0.21 ± 0.17	0.16 ± 0.13	0.13 ± 0.13
PEG_{70} (urine recovery in %)	0.11 ± 0.10	0.04 ± 0.05	0.04 ± 0.07
PEG_{80} (urine recovery in %)	0.09 ± 0.09	0.02 ± 0.03	0.04 ± 0.05

ALT, alanine transaminase; γGT, γ-glutamyltransferase; AST, aspartate aminotransferase; BMI, body mass index; HDL, high-density cholesterol; HFD, high-fat diet; HMW, high-molecular weight; HOMA-IR, homeostasis model assessment of insulin resistance; hsCRP, high-sensitivity C-reactive protein; LBP, lipopolysaccharide binding protein; LDL, low-density cholesterol; L/M, lactulose/mannitol ratio; MCP-1, monocyte chemoattractant protein-1; NEFA, non-esterified fatty acids; PEG, polyethylene glycol; RANTES, regulated on activation normal T cell expressed and secreted; RMR, resting metabolic rate; ROS, reactive oxygen species; WHR, waist-to-hip ratio

Supplemental Table 7: Overfeeding – Significance niveau over time

	Mean ± Standard deviation (n=24)		
	before HFD	**after HFD**	**14d after HFD**
Energy intake (kcal/d)	2,730.8 ± 707.9	3,926.2 ± 339.9	2,589.1 ± 628.3
Fat intake (g/d)	108.4 ± 34.7	208.7 ± 17.9	100.6 ± 32.9
Protein intake (g/d)	100.0 ± 24.9	176.1 ± 15.3	95.4 ± 25.8
Body composition			
Weight (kg)	76.6 ± 10.3	77.6 ± 10.23	77.3 ± 10.4
Body mass index (kg/m²)	23.0 ± 2.1	23.3 ± 2.1	23.2 ± 2.1
Waist circumference (cm)	83.5 ± 5.9	84.2 ± 5.9	83.7 ± 6.1
Hip circumference (cm)	87.8 ± 5.5	88.4 ± 5.1	87.8 ± 4.9
Lean Mass (kg)	66.9 ± 8.0	67.3 ± 7.8	67.1 ±8.0
Fat Mass (kg)	9.8 ± 3.9	10.3 ± 3.8	10.2 ± 3.9
RMR, blood pressure, pulse rate			
Resting metabolic rate (kcal)	1,958.9 ± 232.6	2,010.7 ± 289.1	1,962.5 ± 290.9
Respiratory quotient	0.72 ± 0.1	0.73 ± 0.1	0.73 ± 0.1
Pulse (HF/min)	62.2 ± 7.6	62.6 ± 6.9	64.9 ± 7.5
Blood pressure systolic (mmHg)	115.7 ± 10.9	110.6 ± 9.2	111.3 ± 8.9
Blood pressure diastolic (mmHg)	78.6 ± 6.9	77.9 ± 5.2	75.3 ± 4.8
Inflammation marker			
AST (U/l)	20.9 ± 5.7	19.3 ± 5.5	20.9 ± 10.1
ALT (U/l)	24.1 ± 5.9	27.3 ± 9.4	25.9 ± 17.2
GGT (U/l)	21.0 ± 8.6	19.1 ± 7.1	19.2 ± 8.2
Creatinine (mg/dl)	0.9 ± 0.1	0.9 ± 0.1	0.9 ± 0.1
Uric acid (mg/dl)	6.1 ± 0.9	6.5 ± 0.8	6.3 ± 1.1
Sodium (mmol/ml)	142.2 ± 2.4	141.5 ± 1.9	141.8 ± 1.6
Potassium (mmol/l)	3.9 ± 0.4	4.1 ± 0.3	4.1 ± 0.4
Calcium (mmol/l)	2.4 ± 0.1	2.4 ± 0.1	2.4 ± 0.1
Total cholesterol (mg/dl)	166.8 ± 27.9	183.8 ± 31.1	167.5 ± 32.8
HDL-cholesterol (mg/dl)	54.1 ± 10.1	61.6 ± 10.6	54.3 ± 11.3
LDL-cholesterol (mg/dl)	95.8 ± 27.3	109.6 ± 29.6	98.3 ± 29.5
Triglycerides (mg/dl)	91.0 ± 30.2	64.4 ± 21.8	78.6 ± 45.2
LDL/HDL	1.9 ± 0.7	1.9 ± 0.7	1.9 ± 0.7
NEFA (mmol/l)	0.4 ± 0.2	0.2 ± 0.1	0.3 ± 0.3

Mean ± Standard deviation (n=24)			
	before HFD	**after HFD**	**14d after HFD**
hsCRP (mg/dl)	0.03 ± 0.04	0.03 ± 0.02	0.05 ± 0.05
HMW Adiponectin (mg/ml)	0.34 ± 0.21	0.45 ± 0.23	0.35 ± 0.24
Leptin (ng/ml)	1.3 ± 0.7	1.5 ± 0.9	1.4 ± 0.8
RANTES (ng/ml)	26.9 ± 14.9	17.3 ± 7.7	16.3 ± 8.7
MCP-1 (pg/ml)	80.8 ± 14.3	79.6 ± 14.9	87.9 ± 16.4
Chemerin (ng/ml)	37.7 ± 10.6	37.0 ± 8.6	35.9 ± 7.9
LBP (mg/dl)	0.65 ± 0.30	0.59 1 ± 0.17	0.58 ± 0.21
Calprotectin (mg/kg)	22.9 ± 20.6	30.0 ± 27.4	15.2 ± 15.4
ROS			
ROS production rate in plasma [µM superoxide /µg protein/10min Plasma]	0.0098 ± 0.005	0.0081 ± 0.003	0.009 ± 0.003
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Glucose metabolism			
Fasting blood glucose (mg/dl)	81.9 ± 6.5	79.2 ± 5.9	80.8 ± 4.5
Fasting insulin levels (µU/ml)	4.1 ± 0.9	4.3 ± 1.2	3.9 ± 0.7
Gut permeability marker			
Zonulin (ng/ml)	49.04 ± 13.66	46.45 ± 12.44	45.21 ± 14.27
Sucrose (urine recovery in %)	0.14 ± 0.05	0.09 ± 0.05	0.11 ± 1.02
Sucralose (urine recovery in %)	0.46 ± 0.47	0.39 ± 0.6	0.75 ± 1.02
Mannitol (urine recovery in %)	14.35 ± 5.37	15.02 ± 5.12	15.16 ± 4.19
Lactulose (urine recovery in %)	0.23 ± 0.09	0.24 ± 0.09	0.23 ± 0.08
L/M ratio	0.02 ± 0.01	0.02 ± 0.01	0.02 ± 0.01
PEG_9 (urine recovery in %)	13.77 ± 7.09	10.95 ± 6.51	12.13 ± 6.51
PEG_{11} (urine recovery in %)	12.77 ± 9.13	8.81 ± 6.92	10.31 ± 8.26
PEG_{13} (urine recovery in %)	2.95 ± 1.57	2.26 ± 1.52	2.83 ± 1.69
PEG_{25} (urine recovery in %)	0.30 ± 0.17	0.21 ± 0.12	0.26 ± 0.19
PEG_{30} (urine recovery in %)	0.19 ± 0.12	0.14 ± 0.09	0.15 ± 0.12
PEG_{35} (urine recovery in %)	0.20 ± 0.18	0.14 ± 0.12	0.12 ± 0.12
PEG_{40} (urine recovery in %)	0.21 ± 0.17	0.16 ± 0.13	0.13 ± 0.13
PEG_{70} (urine recovery in %)	0.11 ± 0.10	0.04 ± 0.05	0.04 ± 0.07
PEG_{80} (urine recovery in %)	0.09 ± 0.09	0.02 ± 0.03	0.04 ± 0.05

ALT, alanine transaminase; γGT, γ-glutamyltransferase; AST, aspartate aminotransferase; BMI, body mass index; HDL, high-density cholesterol; HFD, high-fat diet; HMW, high-molecular weight; HOMA-IR, homeostasis model assessment of insulin resistance; hsCRP, high-sensitivity C-reactive protein; LBP, lipopolysaccharide binding protein; LDL, low-density cholesterol; L/M, lactulose/mannitol ratio; MCP-1, monocyte chemoattractant protein-1; NEFA, non-esterified fatty acids; PEG, polyethylene glycol; RANTES, regulated on activation normal T cell expressed and secreted; RMR, resting metabolic rate; ROS, reactive oxygen species; WHR, waist-to-hip ratio

Supplemental Table 8: Caloric restriction – Example of macronutrient content (formula diet) for one day

	1 Bar	1 Drink	1 Soup	1 Creme	Summe	Summe (kcal)	%
Energy content	118.5 kcal	216 kcal	202 kcal	218 kcal	754.5 kcal	754.5	100.0
Protein	7.75 g	13.5 g	15 g	13.7 g	49.95 g	199.8	26.5
Carbohydrates	14.45 g	29.7 g	24.8 g	27 g	95.95 g	383.8	50.9
Fat	3.25 g	4.8 g	4.8 g	6.2 g	19.05 g	171.45	22.7

Supplemental Table 9: Caloric restriction - Chemerin and Metabolic syndrome

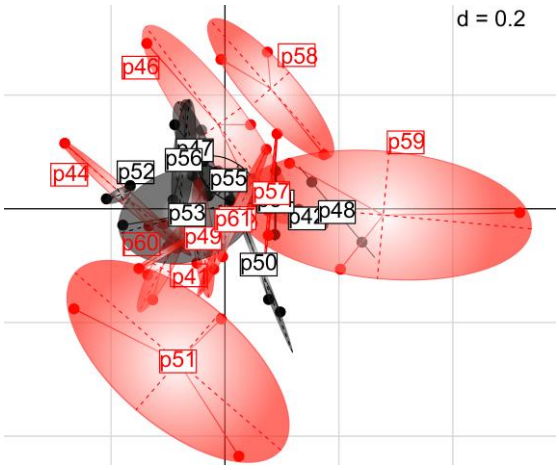
Association between chemerin and obesity and metabolic syndrome parameters

Baseline chemerin values	p-value	r^2
Weight (kg)	0.44	0.03
BMI (kg/m²)	0.23	0.08
Waist-to hip ratio	0.76	0.01
Fat mass (kg)	0.25	0.07
HDL-cholesterol (mg/dl)	0.68	<0.01
LDL-cholesterol (mg/dl)	0.86	<0.01
Triglycerides (mg/dl)	0.26	0.07

BMI; body mass index; HDL, high-density cholesterol; LDL, low-density cholesterol

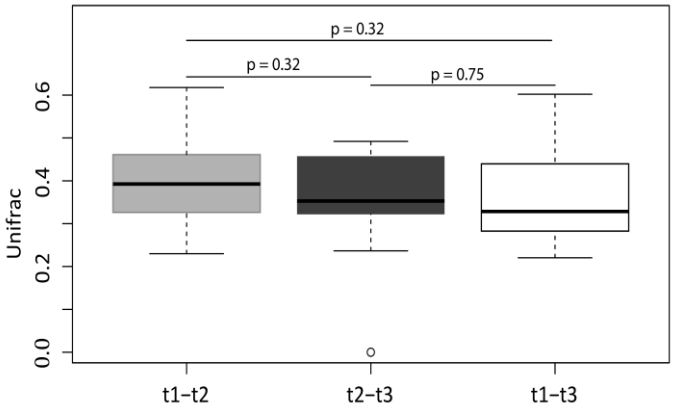
Supplemental Figure 1: Caloric restriction – Individual bacterial profiles highlighted by weight loss.

(red = weight loss more than 7 kg, ID = 41, 44, 45, 46, 49, 51, 57, 58, 59, 60, 61;
black = weight loss more less than 7 kg, ID = 42, 47, 48, 50, 52, 53, 54, 55, 56)



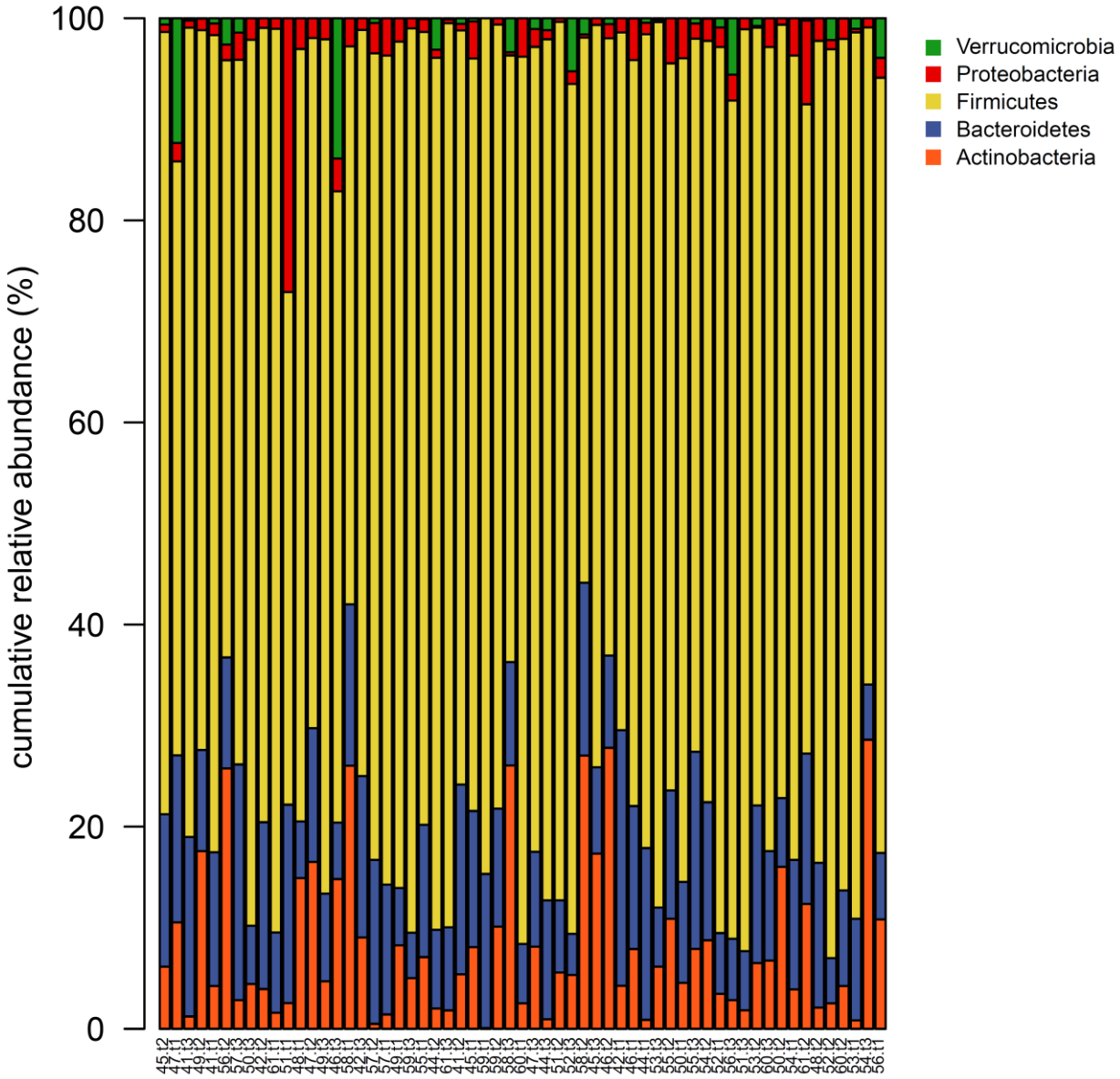
P, participant

Supplemental Figure 2: Caloric restriction – Phylogenetic distance between time points



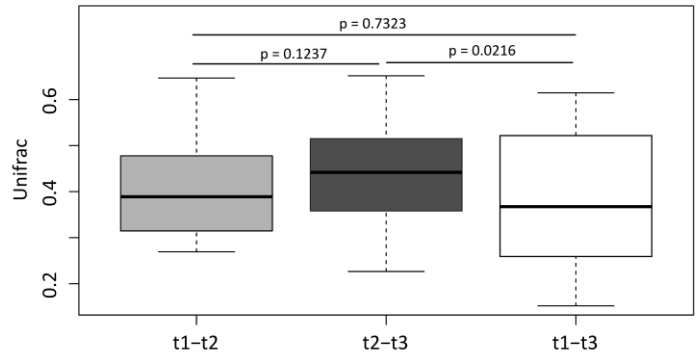
t1, before intervention; t2, after intervention; t3, 14 days after intervention

Supplemental Figure 3: Caloric restriction – Bacterial families in the fecal microbiota. Bacterial families in the fecal microbiota. One bar represents one study participant at different time points.



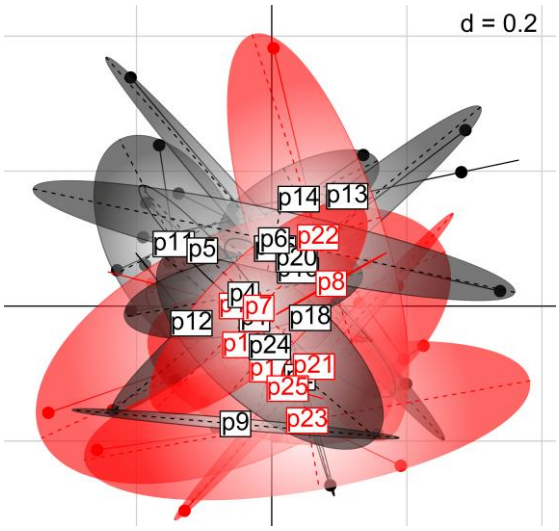
T1, before intervention; t2, after intervention; t3, 14 days after intervention

Supplemental Figure 4: Overfeeeding – Phylogenetic distance between time points



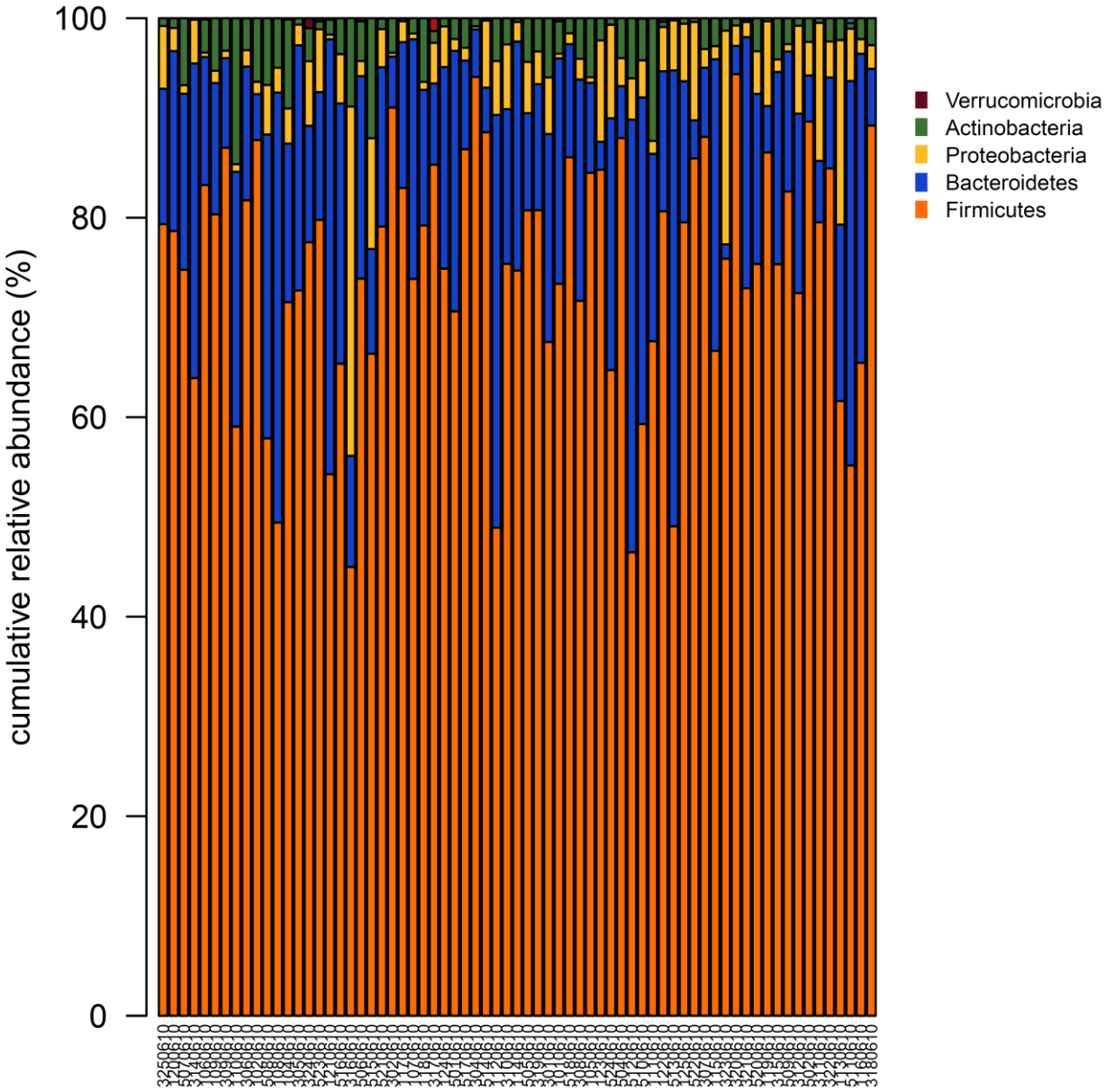
t1, before intervention; t2, after intervention; t3, 14 days after intervention

Supplemental Figure 5: Overfeeding – Individual bacterial profile highlighted by weight gain.
(red = weight gain more than 1 kg, ID = 9, 10, 12, 15, 16, 17, 19, 22, 23, 25;
black = weight loss more less than 7 kg, ID = 1, 2, 4, 5, 6, 7, 8, 11, 13, 14, 18, 20, 21, 24)



P, participant

Supplemental Figure 6: Overfeeding – Bacterial families in the fecal microbiota. Bacterial families in the fecal microbiota. One bar represents one study participant at different time points.



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