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Impact of obesity and laparoscopic sleeve gastrectomy on gut permeability

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Table of contents

Summary V
Zusammenfassung VII
Abbreviations X
1. Introduction 1
1.1 Obesity - a major health problem 1
1.2 Bariatric surgery – a modern option to treat morbid obesity 2
1.3 Obesity and associated inflammation 3
1.3.1 Adipose tissue derived inflammation 3
1.3.2 Gut derived inflammation 5
1.4 Obesity and gut permeability 6
1.4.1 Structure of the intestinal barrier 6
1.4.2 Development and maturation of the intestinal barrier 10
1.4.3 Impairment of barrier function and associated diseases 10
1.4.4 Gut permeability in obesity 11
1.4.5 Gut permeability and bariatric surgery 12
1.5 Obesity and gut microbiota 13
1.5.1 Development and composition of the gut microbiota 13
1.5.2 Specific function of the gut microbiota and influence on the host 14
1.5.3 Impacts on gut microbiota composition 15
1.5.4 Gut microbiota in obesity 16
1.5.5 Gut microbiota and bariatric surgery 17
1.6 Adipose tissue and bioactive lipid mediators 18
1.6.1 FAHFAs – a new class of bioactive lipids 19
1.6.2 Metabolic effects of FAHFAs 20
2. Aim 22
3. Study interventions 23
3.1 Ethics statement 23
3.2 Approval and registration 23
3.3 Study design 23
3.4 Study population 24
3.5 Data and sample collection 25
4. Methods 26
4.1 General patient information 26
4.2 Dietary behaviour 26

4.3 Anthropometric measurements 26
4.4 Gut permeability 27
4.4.1 Sugar absorption test 27
4.4.2 Zonulin 28
4.5 Blood samples 29
4.6 Faecal samples 30
4.7 Laboratory measurements 31
4.7.1 Enzyme-linked immunosorbent assay (ELISA) 31
4.7.2 ACS-ACOD-method for the quantification of free fatty acids 31
4.7.3 High-throughput 16S rRNA gene sequencing analysis 32
4.8 Establishment of a LC-MS method for the analysis of FAHFAs 33
4.8.1 FAHFA extraction 33
4.8.2 LC-MS analysis of FAHFAs 35
4.8.3 MRM3 Method 39
4.9 Human Study cohorts for FAHFA analysis 43
4.9.1 Anthropometric measurements 44
4.9.2 Blood samples and biochemical analyses 44
4.10 Statistical analysis 44
5. Results 46
5.1 Bariatric surgery and intestinal permeability 46
5.1.1 Anthropometric measurements 46
5.1.2 Dietary behaviour 48
5.1.3 Glucose metabolism 51
5.1.4 Metabolic and inflammatory parameters 52
5.1.5 Gut permeability after LSG 56
5.1.6 Gut permeability in dependence of BMI 60
5.1.7 Gut microbiota composition 62
5.2 FAHFAs 68
5.2.1 Establishment of a LC-MS method for the quantification of FAHFAs in human serum samples 68
5.2.2 FAHFA concentrations after sleeve gastrectomy 68
5.2.3 FAHFA concentrations in dependence of type 2 diabetes 71
5.2.4 FAHFA concentrations in dependence of age and sex 75
5.2.5 FAHFA concentrations in dependence of specific diets 77
6. Discussion 82
6.1 LSG induced energy restriction and weight loss 82

6.2 Effect of LSG on metabolic and inflammatory parameters ... 85
6.3 LSG induced significant changes in gut permeability ... 87
6.4 The effect of PPI use and LSG on gut microbiota ... 92
6.5 FAHFA levels under different physiological and pathophysiological conditions ... 97
7. Conclusion and outlook ... 101
8. Supplemental data ... 103
9. References ... 116
List of tables ... 142
List of figures ... 144
Publications and presentations ... 146
Funding ... 147
Acknowledgements ... 148
Appendix ... 149

Summary

Obesity is associated with a systemic, low-grade inflammation. This inflammation can arise directly from the increased adipose tissue mass and an altered secretory profile of hypertrophic fat cells. In addition, pro-inflammatory stimuli in obesity can also arise from the gut. Studies in rodents have claimed that diet-induced obesity and associated diseases such as diabetes are linked to alterations in gut microbiota composition and diversity as well as to disturbed gut barrier function. This results in an elevated translocation of bacterial products such as lipopolysaccharide (LPS) into the circulation, where inflammatory processes are triggered.

The present study aimed to investigate gut permeability and gut microbiota composition in severely obese patients compared to non-obese controls as well as their changes 6 months after bariatric surgery-induced weight loss. Anthropometric data, metabolic and inflammatory parameters, dietary protocols as well as gut permeability and gut microbiota composition were analysed in 17 morbidly obese patients (BMI ≥ 40 kg/m^2) undergoing laparoscopic sleeve gastrectomy (LSG) as well as 17 age- and sex-matched non-obese controls (BMI < 30 kg/m^2). Total caloric intake and diet composition of the obese patients before surgery was quite similar to the non-obese controls, while 6 months after surgery total caloric intake was drastically reduced. At this point, the obese patients had reduced their body weight by 41.0 (33.7; 46.5) kg. Weight loss was accompanied by improvements in metabolic and inflammatory parameters. Gut permeability, assessed with a 4-probe sugar test, did not differ between obese patients and non-obese controls. Moreover, in an extended cohort of obese women no differences in gut permeability were detected between different classes of obesity. LSG, however, led to a significant decrease in gastroduodenal as well as small intestinal permeability and an increase in colonic permeability, while LBP levels were not affected. Gut microbiota analysis showed that alpha-diversity was decreased in patients with obesity compared to non-obese controls and increased after surgery. Differences were noted for the main phyla of gut microbiota of obese patients compared to non-obese controls. After surgery, there was a tendency towards the composition of non-obese controls, but changes were in most cases minor and not significant. Moreover, the effect of bariatric surgery on microbiota composition was quite variable among individuals with some patients showing a more stable microbiota than others.

From the results of the present study it can be concluded that obesity is not associated with alterations in gut permeability. LSG, however, has a considerable impact on gut permeability with distinct changes of gastroduodenal, small intestinal and colonic permeability. Moreover,

LSG can affect gut microbiota composition, but changes are highly heterogeneous among individuals and no clear association with gut permeability can be seen.

The second part of this thesis focussed on a recently discovered group of fatty acids, the FAHFAs (fatty acid esters of hydroxy fatty acids). As previous studies revealed anti-inflammatory and anti-diabetic effects of FAHFAs, they might also be involved in obesity-associated metabolic and inflammatory disturbances. Yet, the human serum levels of FAHFAs under different physiological and pathophysiological conditions have hardly been studied so far. In the present thesis, different FAHFA isomers were quantitatively analysed in serum samples from several human cohorts to investigate possible relations of endogenous FAHFA levels with factors such as BMI, weight loss, diabetic status, age, sex, and diet.

In the cohort of obese subjects undergoing LSG, no changes in FAHFA levels were observed at six months after surgery. However, serum levels of most of the analysed FAHFAs were lower in obese than in non-obese subjects. As obesity is often associated with insulin resistance and FAHFAs have been credited with anti-diabetic effects, FAHFA levels were also investigated in diabetic compared to non-diabetic subjects in two other independent cohorts. In both cohorts no differences in FAHFA levels were observed. Next, FAHFA levels were investigated in dependence of sex and age. While there was no difference between sexes for all FAHFAs analysed, differences were found for several FAHFAs between different age groups. As a novel and interesting finding, the present study could show that dietary overfeeding in lean young men significantly increased FAHFA levels. Moreover, subjects on a vegetarian or vegan diet had substantially lower levels of FAHFAs compared to omnivores.

To conclude, the present study showed that FAHFA serum levels are strongly regulated by diet. Moreover, BMI and age seem to be related to FAHFA levels, while there is no detectable association with sex and diabetic status. Future research is needed to better clarify the physiological role of FAHFAs in humans.

Zusammenfassung

Adipositas ist mit einer chronischen, systemischen Entzündung assoziiert. Diese kann durch die vermehrte Fettgewebsmasse und das veränderte sekretorische Profil der hypertrophen Fettzellen hervorgerufen werden. Darüber hinaus können pro-inflammatorische Reize auch aus dem Darm kommen. Untersuchungen an Nagern haben gezeigt, dass ernährungsbedingte Adipositas und assoziierte Erkrankungen wie Diabetes mit Veränderungen in der Zusammensetzung und Diversität der Darmmikrobiota als auch mit einer gestörten Darmbarrierefunktion verknüpft sind. Beides kann zu einem erhöhten Übertritt von bakteriellen Produkten wie Lipopolysaccharid (LPS) in den Blutkreislauf führen, wodurch Entzündungsprozesse getriggert werden.

Ziel der vorliegenden Studie war es, die Darmbarrierefunktion und die Zusammensetzung der Darmmikrobiota von adipösen Patienten vor und sechs Monate nach einer bariatrischen Operation zu untersuchen sowie im Vergleich zu nicht-adipösen Kontrollpersonen. Die Studie wurde mit 17 morbid adipösen Personen (BMI ≥ 40 kg/m^2) durchgeführt, die sich einer laparoskopischen Schlauchmagenoperation unterzogen, sowie mit 17 nicht-adipösen Kontrollpersonen (BMI < 30 kg/m^2), die bezogen auf Alter und Geschlecht den übergewichtigen Patienten entsprachen. Es wurden anthropometrische Daten und Ernährungsprotokolle erhoben, metabolische und inflammatorische Parameter gemessen sowie die Darmbarrierefunktion und die Darmmikrobiota untersucht. Die Gesamtenergiezufuhr sowie die Zusammensetzung der aufgenommenen Nahrung waren vergleichbar zwischen adipösen Patienten vor der Operation und nicht-adipösen Kontrollen. Sechs Monate nach der Operation war die Gesamtenergiezufuhr jedoch deutlich verringert. Zu diesem Zeitpunkt hatten die adipösen Patienten 41,0 (33,7; 46,5) kg Körpergewicht verloren. Der Gewichtsverlust war verbunden mit Verbesserungen der metabolischen und inflammatorischen Parameter. Die Darmbarrierefunktion wurde mit einem Vier-Zucker-Test gemessen und war nicht unterschiedlich zwischen adipösen und nicht-adipösen Personen. Darüber hinaus zeigten sich auch in einer erweiterten Kohorte von Frauen mit Adipositas keine signifikanten Unterschiede in der Darmbarrierefunktion zwischen den verschiedenen Schweregraden der Adipositas. Eine Schlauchmagenoperation führte allerdings zu einer Abnahme der gastroduodenalen Permeabilität sowie der Dünndarmpermeabilität und zu einer Erhöhung der Permeabilität des Kolons. Die LBP-Spiegel hingegen blieben unverändert. Die Analyse der Darmmikrobiota zeigte, dass im Vergleich zu nicht-adipösen Kontrollen die alpha-Diversität bei den adipösen Patienten verringert war, diese nach der Operation aber zunahm. Es zeigten sich auch Unterschiede in den vorherrschenden Phyla zwischen adipösen und nicht-adipösen Personen. Nach der Operation zeigte sich eine Tendenz hin zur Darmmikrobiota von nicht-adipösen Personen. Allerdings waren diese

Veränderungen in den meisten Fällen gering und nicht signifikant. Der Effekt der bariatrischen Operation auf die Zusammensetzung der Darmmikrobiota war sehr unterschiedlich zwischen den Individuen, wobei einige Patienten eine stabilere Mikrobiota aufwiesen als andere.

Aus den Ergebnissen der vorliegenden Studie lässt sich schließen, dass Adipositas nicht mit einer Veränderung der Darmbarrrierefunktion assoziiert ist, während eine Schlauchmagenoperation einen deutlichen Effekt auf die Darmbarrierefunktion ausübt. Die Auswirkungen auf die Permeabilität der einzelnen Darmbereiche ist hierbei unterschiedlich. Darüber hinaus kann eine Schlauchmagenoperation Effekte auf die Zusammensetzung der Darmmikrobiota ausüben, wobei die Veränderungen individuell sehr verschieden sein können und sich keine klare Assoziation mit der Darmbarrierefunktion erkennen lässt.

Der zweite Teil der Dissertation beschäftigt sich mit einer vor Kurzem entdeckten Gruppe an Fettsäuren, den FAHFAs (fatty acid esters of hydroxy fatty acids). Da Studien einen anti-inflammatorischen und anti-diabetischen Effekt der FAHFAs zeigten, könnten diese Fettsäuren auch an Adipositas-assoziierten metabolischen und inflammatorischen Störungen beteiligt sein. Jedoch gibt es bisher kaum humane Daten zu den Serumspiegeln der FAHFAs unter verschiedenen physiologischen und pathophysiologischen Bedingungen. Ziel der vorliegenden Arbeit war es daher, in unterschiedlichen humanen Kohorten verschiedene FAHFA-Isomere in Serumproben quantitativ zu bestimmen und mögliche Zusammenhänge endogener FAHFA-Spiegel mit Faktoren wie BMI, Gewichtsverlust, diabetischem Status, Alter, Geschlecht und Ernährung zu untersuchen.

In der Kohorte der adipösen Patienten, die sich einer Schlauchmagenoperation unterzogen, wurden sechs Monate nach der Operation keine Veränderungen der FAHFA-Spiegel gemessen. Jedoch waren die Gehalte der meisten FAHFAs in den adipösen Patienten verringert im Vergleich zu den nicht-adipösen Kontrollen. Adipositas geht häufig mit Insulinresistenz einher und den FAHFAs werden positive anti-diabetische Effekte zugeschrieben. Daher wurden die FAHFA-Gehalte zwischen Personen mit und ohne Diabetes in zwei verschiedenen Kohorten verglichen. Es konnten in beiden Kohorten keine Unterschiede zwischen Diabetikern und Nicht-Diabetikern festgestellt werden. Als nächstes wurden FAHFA-Gehalte in Abhängigkeit von Alter und Geschlecht untersucht. Während es keinen Unterschied zwischen den Geschlechtern gab, zeigten sich einzelne Unterschiede zwischen den verschiedenen Altersgruppen. Eine neue und interessante Erkenntnis der vorliegenden Arbeit war, dass Überernährung bei normalgewichtigen jungen Männern die FAHFA-Gehalte signifikant erhöht. Des Weiteren zeigen Vegetarier oder Veganer geringere FAHFA-Gehalte als Omnivore.

Zusammenfassend zeigt die vorliegende Arbeit, dass die FAHFA-Gehalte im Serum stark von der Ernährung beeinflusst werden. Außerdem zeigen sich Assoziationen der FAHFA-Gehalte mit Alter und BMI, jedoch nicht mit Geschlecht oder diabetischem Status. Die physiologische Rolle der FAHFAs muss in zukünftigen Studien weiter aufgeklärt werden.

Abbreviations

ACOD	Acyl-CoA oxidase
ACS	Acyl-CoA synthetase
AGB	Adjustable gastric banding
ALAT	Alanine transaminase
ASAT	Aspartate transaminase
BMI	Body mass index
BPD/DS	Biliopancreatic diversion with duodenal switch
CD14	Cluster of differentiation 14
ChREBP	Carbohydrate-responsive element-binding protein
CRP	C-reactive protein
DHA	Docosahexaenoic acid
DNA	Deoxyribonucleic acid
EDTA	Ethylenediaminetetraacetic acid
ELISA	Enzyme-linked immunosorbent assay
EWL	Excessive weight loss
FAHFA	Fatty acid esters of hydroxy-fatty acid
gDNA	Genomic deoxyribonucleic acid
GFR	Glomerular filtration rate
GLP-1	Glucagon-like peptide-1
Glut4	Glucose transporter type 4
GPCR	G protein-coupled receptor
GPR40	G protein-coupled receptor 40
GPR120	G protein-coupled receptor 120
γ-GT	γ-Glutamyltransferase
HDL	High-density lipoprotein
HFD	High-fat diet
HOMA-IR	Homeostatic model assessment – insulin resistance
HPLC	High-performance liquid chromatography
IBD	Inflammatory bowel disease
IFN-γ	Interferon gamma
IKK-β	Inhibitor of nuclear factor kappa-B kinase subunit beta
IL-6	Interleukin 6
IL-1β	Interleukin 1 beta
IL-10	Interleukin 10
IQR	Inter quartile range

IRS-1	Insulin receptor substrate 1
JNK	c-Jun N-terminal kinase
Kcal	Kilocalorie
kDa	Kilodalton
LBP	Lipopolysaccharide-binding protein
LC	Liquid chromatography
LDL	Low-density lipoprotein
LPS	Lipopolysaccharide
LSG	Laparoscopic sleeve gastrectomy
MCH	Mean corpuscular haemoglobin
MCHC	Mean corpuscular haemoglobin concentration
MCP-1	Monocyte chemoattractant protein 1
MCV	Mean corpuscular volume
MGB	Mini gastric bypass
MRM	Multiple reaction monitoring
MS	Mass spectrometry
NAFDL	Non-alcoholic fatty liver disease
NEFAs	Non-esterified fatty acids
NFκB	Nuclear factor kappa-light-chain-enhancer of activated B cells
Nrf2	Nuclear factor-like 2
NSAID	Non-steroidal anti-inflammatory drug
OAHSA	Oleic acid hydroxy-stearic acid
OD	Optical density
OTU	Operational taxonomic unit
PAHPA	Palmitic acid hydroxy-palmitic acid
PAHSA	Palmitic acid hydroxy-stearic acid
PEG	Polyethylene glycol
PPI	Proton pump inhibitor
RDW	Red blood cell distribution width
RNA	Ribonucleic acid
ROS	Reactive oxygen species
rRNA	Ribosomal ribonucleic acid
RYGB	Roux-en-Y gastric bypass
SAHSA	Stearic acid hydroxy-stearic acid
SCFAs	Short-chain fatty acids
SG	Sleeve gastrectomy
sIgA	Secretory immunoglobulin A

SPE	Solid phase extraction
TG	Triglycerides
TLR	Toll-like receptor
TMB	3,3',5,5'-Tetramethylbenzidine
TNF-α	Tumour necrosis factor alpha
TSH	Thyroid-stimulating hormone
TWL	Total weight loss
UPLC	Ultra performance liquid chromatography
WHO	World Health Organization

1. Introduction

1.1 Obesity - a major health problem

Obesity is defined as an excessive accumulation of body fat that may present a health threat (World Health Organization, 2020). By using the Body mass index (BMI) obesity and overweight in adults can be classified in a simple way (Table 1). Obesity can be categorised into class I to III. Furthermore, the term “super-obesity” is used occasionally in the context of literature about bariatric surgery. There is no definition of super-obesity by the World Health Organisation (WHO) and the term is not uniformly defined, but often used for patients with BMI ≥ 50 or ≥ 60 kg/m^2. Obesity develops, when energy intake is exceeding energy expenditure in the long-term. Increased food consumption, mainly by energy-dense foods with a high content of fats and sugar, together with a sedentary lifestyle foster the development of obesity (World Health Organization, 2020). Nevertheless, also genetic factors involved in appetite regulation and energy expenditure can contribute to the disease (Albuquerque et al., 2015). The effect sizes of identified genetic loci, however, are small, and in most cases obesity is a complex and multifactorial disease.

Table 1: Weight classification by BMI according to the WHO (World Health Organization, 2000)

BMI (kg/m^2)	Classification of weight status
< 18.5	Underweight
18.5–24.9	Normal weight
≥ 25.0 25.0–29.9	Overweight Pre-obesity
≥ 30.0 30.0–34.9 35.0–39.9 ≥ 40.0	Obesity Obesity class I Obesity class II Obesity class III

The WHO reported that, according to the weight classification by BMI, in 2016, 39% of adults worldwide were overweight and 13% were obese. Thus, obesity has nearly tripled since 1975 and the prevalence is further increasing (World Health Organization, 2020). Overweight and obesity are major risk factors for a variety of chronic non-infectious diseases, including type 2 diabetes mellitus, cardiovascular disease and cancer (Field et al., 2001; Basen-Engquist and Chang, 2011), thereby increasing overall mortality risk (Borrell and Samuel, 2014). In addition to an increased morbidity and mortality risk, obese persons often suffer from restricted mobility, loss of quality of life and psychological problems (Forhan and Gill, 2013; Taylor et al., 2013).

1.2 Bariatric surgery – a modern option to treat morbid obesity

The interdisciplinary German guideline for the prevention and therapy of obesity states that treatment of obesity should aim to reduce body weight and associated disorders together with an overall improvement in quality of life of the patient (Hauner et al., 2014). In addition, a stable weight should be reached in the long run. Thereby, bariatric surgery is commonly the last treatment option in case a basic therapy (including nutrition, exercise and behavioural therapy) as well as weight reduction programmes have not been successful. Indications for bariatric surgery are: 1) obesity grade III, 2) obesity grade II with substantial comorbidities, or 3) obesity grade I with associated type 2 diabetes (specific cases) (Hauner et al., 2014). The most common bariatric procedures are Roux-en-Y gastric bypass (RYGB) and sleeve gastrectomy (SG), followed by adjustable gastric banding (AGB), mini gastric bypass (MGB) and biliopancreatic diversion with duodenal switch (BPD/DS) (Angrisani et al., 2017). SG and AGB are restrictive procedures, i.e. the stomach size is reduced and patients can eat less and experience early satiety (Nguyen and Varela, 2017). In SG, a partial gastrectomy is performed and a small tubular pouch remains with drastically reduced volume (Kehagias et al., 2016). RYGB, MGB and BPD/DS have a restrictive as well as a malabsorptive component, because part of the intestine is bypassed by the food and absorption of nutrients is hampered (Nguyen and Varela, 2017). In addition to reduced stomach capacity and block of nutrient absorption also hormonal changes can contribute to weight loss after bariatric surgery. Levels of Peptide YY (PYY) and Glucagon-like peptide-1 (GLP-1) have been found to be increased after surgery, while the “hunger hormone” ghrelin is decreased (Karamanakos et al., 2008; Yousseif et al., 2014).

As reported by Angrisani and colleagues, SG has experienced the greatest rise in the last years and has outnumbered RYGB as the most common bariatric surgery procedure. In 2014, 45.9% of bariatric surgeries worldwide were performed as SG, followed by RYGB (39.6%) and AGB (7.4%) (Angrisani et al., 2017). SG was originally performed as a first step operation in super-obese patients or in patients with high surgical risk before performing more complex operations like RYGB (Cottam et al., 2006; Tucker et al., 2008). However, as SG alone results in adequate weight loss, it has evolved as an autonomous procedure (Kehagias et al., 2016). Different recent reviews and meta-analyses reported weight loss after SG as similar or slightly less effective compared to RYGB (Malin and Kashyap, 2015; Li et al., 2016; Golzarand et al., 2017; Kang and Le, 2017; Osland et al., 2017). Furthermore, SG represents a shorter and easier operational technique compared to RYGB, and postoperative nutritional deficiencies are less prominent (Kehagias et al., 2016).

Bariatric surgery not only dramatically reduces body weight of the patients, but also initiates improvements of associated disorders. It is therefore often referred to as “metabolic surgery”.

Type 2 diabetes for example can be ameliorated or completely resolved in 86.6% of patients (Buchwald et al., 2009). These metabolic improvements often precede the actual weight loss and may be related to changes in hormone secretion (Basso et al., 2011).

1.3 Obesity and associated inflammation

A systemic and subclinical state of inflammation is a characteristic of obesity and type 2 diabetes. Circulating levels of a variety of pro-inflammatory cytokines and acute phase proteins are elevated in obese compared to lean subjects together with a decrease in anti-inflammatory cytokines. There are no diagnostic criteria for the definition of this obesity-associated low-grade inflammation; however, it differs from classical inflammation as it is low-grade, chronic and with locally induced pro-inflammatory mediators. (Shoelson et al., 2007; Calder et al., 2011; Gregor and Hotamisligil, 2011)

The chronic inflammation is a major driver of obesity-related metabolic dysfunction. Excess fat accumulation in the adipose tissue as well as other organs leads to increased non-esterified fatty acids (NEFAs) in the circulation and dysregulation of inflammatory molecules that are important molecular players in the development of insulin resistance, type 2 diabetes, non-alcoholic fatty liver disease (NAFLD) and cardiovascular events. (Jung and Choi, 2014)

The inflammatory status of obese subjects can be reduced by diet, exercise and weight loss (Nicklas et al., 2005). Accordingly, also bariatric surgery can reverse obesity-associated macrophage infiltration and alterations in cytokine profiles and leads to overall improvements in the systemic inflammatory profile (Rao, 2012; Labrecque et al., 2017), which may contribute to the clear improvements in insulin sensitivity.

1.3.1 Adipose tissue derived inflammation

The pro-inflammatory status in obesity may arise directly from the adipose tissue. Today, we know that the functions of adipose tissue for the organism exceed by far pure energy storage. Next to fat storing cells, adipose tissue contains pre-adipocytes, endothelial cells, fibroblasts and immune cells such as leukocytes and macrophages (Wozniak et al., 2009). As an endocrine organ it secretes various cytokines, chemokines, acute phase proteins, hormones, growth factors and components of the alternative complement system (Calder et al., 2011). By secreting and receiving those factors the adipose tissue modulates energy homeostasis, adipocyte differentiation, insulin sensitivity, bone metabolism, endocrine and reproductive functions, inflammation, and immunity (Shoelson et al., 2007).

Leptin and adiponectin are the most prominent adipokines, i.e. cytokines produced by the adipose tissue. Leptin has inflammatory properties (Wozniak et al., 2009), and it can also act on the hypothalamus to suppress appetite function and achieve energy homeostasis (Inui, 1999). In hypertrophic fat cells in obesity, leptin expression is increased (Jernås et al., 2006; Skurk et al., 2007); nevertheless, the suppression of hunger is decreased, because of leptin resistance in obesity (Sáinz et al., 2015). Adiponectin is an anti-inflammatory adipokine, whose expression is decreased in obesity (Arita et al., 1999). It can also act on energy homeostasis in the hypothalamus and has beneficial influence on vascular function and glucose homeostasis (Wozniak et al., 2009).

In obesity, shape and composition of the cells in adipose tissue is altered together with their secretion pattern, which drives systemic inflammation and pathogenesis of metabolic disorders (Booth et al., 2016). Hypertrophy of adipocytes, which accompanies obesity, determines adipokine secretion and induces a shift towards a more pro-inflammatory profile (Skurk et al., 2007). In addition to adipocytes, the adipose tissue-resident immune cells contribute to the secretion of inflammatory molecules from adipose tissue and closely interact with adipocytes (Booth et al., 2016). In obesity, macrophage infiltration of adipose tissue can be observed (Weisberg et al., 2003; Xu et al., 2003), which is induced by the production and release of monocyte chemoattractant protein 1 (MCP-1) by adipocytes (Kanda et al., 2006; Ito et al., 2008).

While under normal-weight conditions adipose tissue-resident macrophages express the M2 (anti-inflammatory) phenotype, diet-induced obesity shifts macrophages into a M1 (pro-inflammatory) phenotype (Lumeng et al., 2007). In addition to macrophage infiltration, an increase in mast cells and natural killer T (NKT) cells, as well as activation of natural killer (NK) cells seems to be involved in adipose tissue inflammation (Liu et al., 2009; Ohmura et al., 2010; Wensveen et al., 2015), while immunosuppressive regulatory T (Treg) cells are decreased (Feuerer et al., 2009).

Cinti and colleagues observed that macrophages accumulate at sites of adipocyte death. As the frequency of adipocyte death increases with adipocyte size, it can be suggested that one of their functions is to clean up cell debris (Cinti et al., 2005). In addition, macrophages can directly influence adipocyte function. Experiments with co-culture of adipocytes and macrophages or with macrophage-conditioned media showed diminished differentiation of adipocytes (Constant et al., 2006; Lacasa et al., 2007), induction of a pro-inflammatory expression pattern (Suganami et al., 2005), increased lipolysis and reduced insulin responsiveness (Permana et al., 2006). These effects are most likely caused by macrophage secreted factors like tumour necrosis factor alpha (TNF-α) and interleukin 6 (IL-6) (Lagathu et al., 2003; Suganami et al., 2005).

TNF-α and IL-6, together with other cytokines secreted by immune cells and adipocytes, are main mediators of obesity-associated insulin resistance. They contribute to insulin resistance via the IKK-β/NFκB (inhibitor of nuclear factor kappa-B kinase subunit beta / nuclear factor kappa-light-chain-enhancer of activated B cells) and JNK (c-Jun N-terminal kinase) pathways in adipocytes, hepatocytes and associated macrophages. The kinases IKK-β and JNK both can phosphorylate the Ser 307 residue of insulin receptor substrate 1 (IRS-1). This prevents IRS-1 phosphorylation by the insulin receptor and its downstream signalling cascade. In addition, IKK-β activation leads to the translocation of NFκB into the nucleus and subsequent induced expression of inflammatory mediators, which can further contribute to insulin resistance. (Shoelson et al., 2007; Calder et al., 2011)

Next to IKK-β and JNK kinases, the protein kinase R (PKR) is another important kinase in the mediation of inflammation and insulin resistance (Nakamura et al., 2010). The three kinases are activated by downstream signalling not only of cytokines, but also reactive oxygen species (ROS), endoplasmic reticulum stress, ceramides and Toll-like receptors (TLRs), which are activated for example by lipopolysaccharide (LPS) and free fatty acids (Shoelson et al., 2007).

1.3.2 Gut derived inflammation

Besides the adipose tissue, the gut has been identified as another source of pro-inflammatory stimuli in obesity.

The microbiota composition as well as the species and gene richness can have considerable impact on host inflammatory processes (Boulangé et al., 2016). In individuals with obesity, the microbiota composition is altered together with a decrease in bacterial richness compared to lean individuals (Turnbaugh et al., 2009; Verdam et al., 2013). Individuals with a low bacterial richness have been characterised by a more pronounced pro-inflammatory phenotype compared to individuals with a high bacterial richness (Le Chatelier et al., 2013). The importance of gut microbiota for inflammatory processes in obesity can also be seen in mice studies. Ding and colleagues could show that a high-fat diet (HFD) in conventionally grown mice induces inflammation prior to obesity and insulin resistance; however these effects were not present in germ-free mice (Ding et al., 2010). Furthermore, antibiotic treatment of obese mice has proven that the disturbance of obesity-associated gut microbiota can reverse its effect on endotoxemia, inflammation, body weight and insulin resistance (Cani et al., 2008).

Microbial-derived products and metabolites are inducers of this bacterial-mediated inflammation. Short-chain fatty acids (SCFAs), for instance, can communicate with colonic endothelial cells and leukocytes via G protein-coupled receptors (GPCRs) and inhibition of

histone deacetylase (HDAC) to modulate inflammatory processes (Vinolo et al., 2011). While SCFAs mainly sustain anti-inflammatory actions (Vinolo et al., 2011), LPS is a major component of bacterial-derived pro-inflammatory signals (Boulangé et al., 2016). LPS is part of the outer membrane of gram-negative bacteria. It can pass the intestinal barrier and enter the circulation on different routes. First, it can use the paracellular route of migration, which is facilitated in case of impaired barrier function (Brun et al., 2007). Second, it can be incorporated into chylomicrons and transported together with dietary lipids (Ghoshal et al., 2009). A HFD can therefore increase translocation of LPS (Cani et al., 2007a; Erridge et al., 2007). In the blood, LPS is bound by lipopolysaccharide-binding protein (LBP) and this complex can then be further bound to cluster of differentiation 14 (CD14), a pattern recognition receptor on the surface of monocytes. CD14 forms a complex with Toll-like receptor 4 (TLR4) and an intracellular signalling cascade is activated, which includes the translocation of NFκB into the nucleus and subsequent transcription of inflammatory genes (Boulangé et al., 2016). The presence of endotoxins in the circulation is called "endotoxemia", and levels 2-3 fold elevated to the normal state have been referred to as "metabolic endotoxemia", which has been described as frequent feature in obesity and type 2 diabetes (Creely et al., 2007; Trøseid et al., 2013; Jayashree et al., 2014).

Next to the microbiota, nutrients from the gut can influence inflammatory processes. Following ingestion of a meal, induction of a pro-inflammatory state and oxidative stress has been described, whereby especially postprandial hyperglycaemia and saturated fatty acids foster this effect, while unsaturated fats, fibres, vegetables and anti-oxidative vitamins seem to be protective (Calder et al., 2011). The stimulation of an inflammatory response by nutrients may take place via activation of certain receptors and transcription factors (Dhindsa et al., 2004; Shi et al., 2006) and increase in oxidative stress (Mohanty et al., 2002). A general postprandial endotoxemia has been described in mice (Cani et al., 2007a) and humans (Erridge et al., 2007), especially after high-fat meals. Facilitated transport of LPS with dietary lipids, as described above, contributes to this effect.

1.4 Obesity and gut permeability

An impairment of the intestinal barrier function has been associated with obesity and related diseases. It may contribute to an increased translocation of bacterial products, like LPS, into the circulation and trigger a pro-inflammatory response. (Teixeira et al., 2012a)

1.4.1 Structure of the intestinal barrier

The intestinal barrier is a protective component of the gut that separates the external milieu (gut lumen) and the internal milieu of the host. It has to fulfil an important task: on the one hand, it has to protect the internal milieu from invasion of bacteria and bacterial products and

toxins and avert the loss of water and electrolytes; while on the other hand, it has to allow targeted absorption of nutrients and selected exchange of molecules across this barrier. The term "intestinal permeability" describes the functional feature of the intestinal barrier that can be measured with various tests. (Bischoff et al., 2014)

According to Anderson and colleagues, the intestinal barrier can be divided into four different components: the physical, the chemical, the immunological and the microbial barrier (Anderson et al., 2012). Those four barrier components are presented in Figure 1 and will be explained in the following. Other researchers, however, define just two components of the gut barrier: the physical and the immunological barrier (Bischoff et al., 2014).

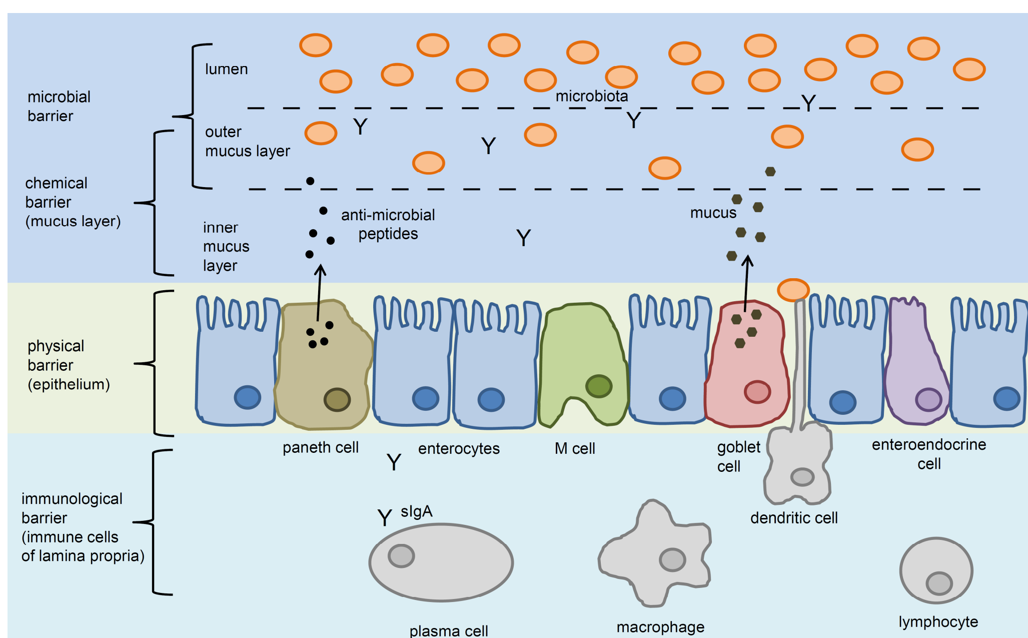

Figure 1: Schematic representation of the four components of the intestinal barrier (adapted from Anderson et al., 2012; Cox et al., 2015)

The physical barrier is composed of the epithelial cell line with intercellular junctional complexes in between. Together, they form the first line of barrier for external bacteria and pro-inflammatory molecules and allow selected passage of electrolytes and nutrients (Anderson et al., 2012). More than 80% of the epithelium is made up of enterocytes, and the rest consists of enteroendocrine cells, goblet cells and Paneth cells (van der Flier and Clevers, 2009). The cells are connected by tight junctions, gap junctions, adherens junctions and desmosomes (Figure 2). Adherens junctions and desmosomes are important for cell-cell adhesion and gap junctions play a role for intercellular communication (Anderson et al., 2012). As summarised in the publication of Suzuki, the paracellular permeability is regulated by the tight junctions. Tight junctions are multiprotein complexes at the apical end of the

lateral membrane of intestinal epithelial cells. They contain transmembrane as well as cytosolic proteins and are anchored to the actin cytoskeleton. The paracellular permeability can thereby be regulated via contractions of the cytoskeleton. The tight junction dynamics can be regulated and affected by various stimuli, like cytokines, pathogens or nutrients (Suzuki, 2013). An endogenous physiological modulator of the tight junctions is zonulin, which is released by enterocytes and increases intestinal permeability (Fasano, 2000; Wang et al., 2000).

The chemical barrier is represented by the mucus layer on top of the epithelial cells and presents a diffusion barrier for molecules and microbes (Anderson et al., 2012). The main components of the mucus layer are mucins, heavily glycosylated proteins that are secreted by goblet cells. Mucins can be classified as transmembrane mucins, which are on the surface of the enterocytes (glycocalyx), and gel-forming mucins, mainly MUC2, which form large polymers (Johansson et al., 2011). Expression, composition and secretion of the mucins are accessible for regulation through intestinal microbes and the activation of host immune cells (Deplancke and Gaskins, 2001).

The immunological barrier consists of the immune cells of the lamina propria and antimicrobial peptides and cytokines that are secreted into the lumen (Anderson et al., 2012). Secretory immunoglobulin A (sIgA) is the first defence mechanism for the protection of the intestinal epithelium (Mantis et al., 2011). It blocks adherence of toxins and pathogens to the intestinal epithelium, entraps them in the mucus and transports them to Peyer's patch M cells, where they are targeted to underlying dendritic cells in the lamina propria with further T-cell activation (Mantis et al., 2011). Interferon γ (IFN-γ) from T-cells further activates macrophages to produce TNF-α. The TH1 response is limited by a feedback mechanism through interleukin 10 (IL-10) from antigen-presenting cells (Anderson et al., 2012). In this process, sIgA may function in mucosal immunity and intestinal homeostasis by actively reducing the capacity of certain antigens to evoke severe pro-inflammatory responses following uptake via M cells (Mantis et al., 2011). The host intestinal immune system owns the tremendous ability to tolerate commensal bacteria and dietary antigens and preserve intestinal homeostasis while remaining responsive to pathogen infection (Bibiloni and Schiffrin, 2010).

The microbial barrier is the last of the four barriers and consists of commensal microbes and their products. They can limit the colonisation of pathogens by competition for adherence to epithelial membranes, production of antimicrobial molecules, induction of host immune responses and stimulation of mucin production (Anderson et al., 2012). In addition, they produce SCFAs which provide positive features for the host. Butyrate is an important energy source for intestinal epithelial cells, stimulates their proliferation and differentiation (Plöger et

al., 2012), increases colonic motility (Hurst et al., 2014) and may also have direct effects on tight junctions (Peng et al., 2009; Wang et al., 2012).

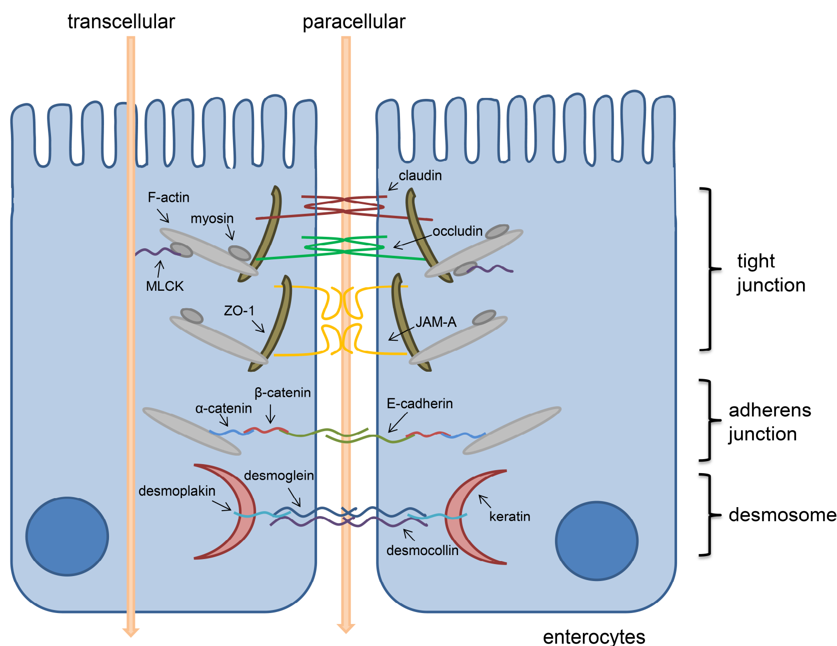

Figure 2: Schematic representation of the molecular structure of different junctions between intestinal epithelial cells (adapted from Turner, 2009; Suzuki, 2013)

Intestinal epithelial cells are connected through tight junctions, adherens junctions, desmosomes and gap junctions. Tight junctions are multiprotein complexes located at the apical end of the lateral membrane of the epithelial cells. They consist of transmembrane as well as cytosolic proteins. Occludin, claudins, junctional adhesion molecule (JAM) and tricellulin are transmembrane proteins of tight junctions and form the selective barrier in the paracellular pathway. Their intracellular part is connected to cytoplasmic scaffold proteins, e.g. zonula occludens proteins (ZO), which link the transmembrane proteins to the actin cytoskeleton. The paracellular permeability can therefore be regulated by the cytoskeleton. Phosphorylation of myosin light chain (MLC) by myosin light chain kinase (MLCK) or Rho-associated kinase (ROCK) leads to contractions of the actomyosin ring and thereby opens the paracellular passage. Adherens junctions and desmosomes ensure a tight bond between neighbouring epithelial cells. Together with gap junctions they also play a role in intercellular communication. (Suzuki, 2013)

There are several ways for molecules to pass the intestinal epithelial barrier: 1) The paracellular route through the tight junctions, 2) the transcellular passive diffusion, 3) endocytosis, followed by transcytosis and exocytosis and 4) active carrier-mediated absorption. Each transport mechanism depends on the structure of the membrane (composition, charge, and thickness), the physicochemical properties of the solute (molecular size, molecular volume, stereochemistry, charge, chemical stability, solubility and partition into membranes) and its interaction with the media (solvent). Additional physiological factors such as gastric emptying, gastrointestinal motility, intestinal pH, blood flow, lymph flow, pathological state, drug interactions, nutrition, and mucus dissolution are

important when assessing absorption of molecules. (Bjarnason et al., 1995; Le Ferrec et al., 2001)

1.4.2 Development and maturation of the intestinal barrier

The development of the physical intestinal barrier already starts in utero. The basic structure is formed at the end of the first trimester, and by the end of the 22nd week the foetal absorptive epithelial cells resemble those of adults (Montgomery et al., 1999). In the early postnatal period, the intestinal barrier is still permeable to macromolecules and the “gut closure” occurs during the first days of live (Vukavić, 1984). This process is supported by hormones and growth factors in human milk which stimulate growth and maturation of the intestinal epithelium (Cummins and Thompson, 2002). Accordingly, during the first weeks of life, breast fed infants show decreased intestinal permeability in comparison to formula fed infants (Catassi et al., 1995; Taylor et al., 2009). The colonisation of the gastrointestinal tract with microbes and the formation of the microbial barrier, which already may start in utero and mainly takes place in the first weeks to years of life, is a further important step in the development of the intestinal barrier (Greenhalgh et al., 2016). The proper maturation of the intestinal barrier in newborns is of high importance, as a “leaky gut” predisposes the infant to infectious, inflammatory, and allergic diseases (Maheshwari and Zemlin, 2009).

1.4.3 Impairment of barrier function and associated diseases

Disruption of the intestinal barrier leads to an increased permeability followed by the elevated transmigration of bacteria and noxious agents into the lamina propria, where they induce a local immune reaction or enter the circulation and cause systemic inflammation (Bischoff et al., 2014; König et al., 2016). A variety of different gastrointestinal and also extra-intestinal diseases with an inflammatory component have been associated with an increased permeability. In inflammatory bowel diseases (IBD) intestinal barrier dysfunction is a main feature of the disease and precedes clinical manifestation (May et al., 1993); however, it is most likely not the sole driver to cause the disease. In critically ill patients in an intensive care unit, intestinal barrier disruption may be causative associated with the appearance of multiple organ failure (Doig et al., 1998). Patients with celiac disease exhibit alteration in tight junction structure (Schulzke et al., 1998; Montalto et al., 2002) and increased intestinal permeability (Bjarnason et al., 1983; van Elburg et al., 1993; Duerksen et al., 2005). These alterations may be caused by gliadin itself (Sander et al., 2005). Other diseases associated with intestinal barrier dysfunction are liver diseases (Lee et al., 2008; Miele et al., 2009), psychological disorders (Kelly et al., 2015), Parkinson’s disease (Forsyth et al., 2011) as well as obesity (Teixeira et al., 2012b) and obesity-associated disorders like diabetes (Horton et al., 2014).

As the integrity of the intestinal barrier is of high importance, it is interesting to look at the factors that can regulate or disturb barrier function. Many bacteria can interact with the intestinal barrier. This can be achieved via secreted peptides and toxins (Fasano et al., 1995; Ewaschuk et al., 2008), extracellular vesicles (Chelakkot et al., 2018), SCFAs (Peng et al., 2007; Peng et al., 2009; Fukuda et al., 2011) or cellular structural components (Cario et al., 2007). While commensal microbes are important for an intact barrier and probiotics can help to protect and restore barrier function (Rao and Samak, 2013), many pathogens also interact with the intestinal barrier to promote inflammatory responses (Barreau and Hugot, 2014). Besides bacteria, also nutrients (Amasheh et al., 2009), drugs like alcohol (Wang et al., 2014) and nonsteroidal anti-inflammatory drugs (NSAIDs) (Bjarnason and Takeuchi, 2009), and exercise (Pals et al., 1997) can influence intestinal permeability.

1.4.4 Gut permeability in obesity

The hypothesis that increased intestinal permeability is a major feature of obesity and associated disorders like type 2 diabetes, NAFLD and cardiovascular disease came from mouse studies. A systemic low-grade inflammatory status is a characteristic of those diseases. This inflammation may arise from an increased translocation of gut microbiota-derived LPS into the circulation, and LPS itself can also increase fasted glucose and insulin levels as well as adipose tissue mass (Cani et al., 2007a). The increased translocation of endotoxin in rodents is provoked by a HFD, which decreases intestinal barrier function and leads to an altered composition of the gut microbiota (Cani et al., 2007b; Cani et al., 2008; Lam et al., 2012). Intestinal permeability may therefore be an important trigger for obesity-associated inflammation and alterations in glucose metabolism. Human data measuring permeability in obesity, however, are quite inconsistent. The most relevant studies are listed in Table 2. The role of intestinal permeability in disease pathogenesis is still unclear.

Table 2: Literature summary of the most relevant studies on gut permeability in obesity (human data)

Authors	Findings
Wilbrink et al., 2020	• Gastroduodenal permeability was increased in morbidly obese subjects compared to lean controls • Small intestinal and colonic permeability did not differ
Genser et al., 2018	• Small intestinal permeability was unaltered in obese compared to lean subjects • Impairments in tight junctions in jejunal samples of obese patients • LBP and zonulin in serum were increased in obese patients
Pedersen et al., 2018	• No difference in small intestinal as well as colonic permeability between lean and obese patients with and without diabetes
Verdam et al., 2013	• Small and large intestinal permeability were not related to BMI and microbiota composition • Higher gastroduodenal permeability in obesity
Teixeira et al., 2012b	• Increased paracellular permeability in obesity • Parameters of intestinal permeability were correlated with anthropometric and metabolic variables
Gummesson et al., 2011	• Positive association of intestinal permeability with visceral adiposity and fat content of the liver, but not with subcutaneous or total body fat
Brignardello et al., 2010	• Small intestinal as well as colonic permeability were not altered in obese subjects in comparison to lean subjects

1.4.5 Gut permeability and bariatric surgery

Although an increased permeability in human obesity could not consistently be proven, research over the last years could show that dietary interventions to reduce body weight of obese individuals are able to decrease intestinal permeability (Xiao et al., 2014; Damms-Machado et al., 2017; Ott et al., 2017). The impact of excessive weight loss following bariatric surgery on intestinal permeability in humans has barely been studied so far (Table 3). Savassi-Rocha and colleagues could show, that RYGB led to a decrease in transcellular intestinal permeability at 1 month after surgery, which increased again at 6 months after surgery (Savassi-Rocha et al., 2014). The effect of the less invasive SG has been studied in mice, showing that SG decreases intestinal permeability in these animals (Blanchard et al., 2017). Only very recently, the group of Wilbrink et al. published a study on gut permeability after sleeve gastrectomy. They found in their study cohort that the small intestinal permeability increased after surgery, while colonic permeability was not significantly altered. Gastroduodenal permeability decreased slightly, but not significantly, after surgery to values not different to controls (Wilbrink et al., 2020).

Table 3: Literature summary of gut permeability after bariatric surgery (human data)

Authors	Findings
Savassi-Rocha et al., 2014	• RYGB led to a decreased intestinal permeability (mannitol excretion), which returned to pre-operative values at 6 months after surgery • Subgroup of patients exhibited considerable increase in small intestinal permeability (lactulose excretion)
Casselbrant et al., 2015	• Measurements of protein expression of tight junctions and Ussing chamber measurements were performed with jejunal mucosal samples from RYGB surgery and 6-8 months post-surgery samples • Changes indicate decreased paracellular permeability after RYGB
Wilbrink et al., 2020	• Small intestinal permeability increased after sleeve gastrectomy • Colonic permeability was unaltered • Gastroduodenal permeability did decrease to values not different to controls; the decrease, however, was not significant

1.5 Obesity and gut microbiota

The composition and function of the gut microbiota has a major impact on the host, and alterations of this sensitive ecological system have been associated, among many others, with obesity and related diseases.

1.5.1 Development and composition of the gut microbiota

The human gut is a habitat for a vast and diverse microbial community that contains cells from all three domains of life: Bacteria, Eukaryota and Archaea. In addition, also viruses, mainly phages, are present. The dominating bacterial phyla in the gut are *Bacteroidetes* and *Firmicutes*, followed by *Actinobacteria*, *Proteobacteria* and *Verrucomicrobia* and the archaeal phylum *Euryarchaeota*. Typical representatives for *Bacteroidetes* are the genera *Bacteroides*, *Prevotella* and *Xylanibacter*, and *Firmicutes* is represented by *Ruminiococcus*, *Lactobacillus* and *Clostriudium*. As most studies investigating gut microbiota use faecal or colonic samples, the composition studied mainly represents the microbiota in the colon, while less is known about the upper parts of the gastrointestinal tract. (Barlow et al., 2015)

The first contact to microbes and colonisation of the gastrointestinal tract already starts *in utero*. The intrauterine environment has been considered sterile for a long time. However, studies with human placental tissue revealed a complex and unique placental microbiome that may have metabolic and immunological impact on the foetus (Aagaard et al., 2014). During the process of birth, the newborn is then exposed to a variety of maternal and environmental microbes. A vaginal delivery exposes the child to the vaginal and faecal microbiota of the mother, dominated by *Lactobacillus*, *Prevotella*, or *Sneathia spp.*, while on the contrary, a child born by Caesarean section has initial contact to bacteria of the skin and

bacteria of clinical environment resulting in a microbial profile that is dominated by *Staphylococcus*, *Corynebacterium*, and *Propionibacterium spp*. (Dominguez-Bello et al., 2010). Further on, the type of feeding (breast versus bottle fed) has an influence on infant microbiota (Guaraldi and Salvatori, 2012; Fan et al., 2014). During the first years of life the infant microbiota is relatively variegated and volatile and changes with the introduction of new foods into the diet (Koenig et al., 2011). By the age of 2–5 years, microbiota profiles have reached a lower inter-individual variance and a higher intra-individual diversity, which increases stability of the bacterial community, and during adulthood, the microbiota is quite stable, until it becomes less stable and less diverse again in the elderly (Greenhalgh et al., 2016). Despite high inter-individual variance and genetic, environmental and dietary influences, Arumugam and colleagues postulated the existence of three different robust microbial clusters that are not specific for nations or continents and not dictated by age, sex or BMI. These so called enterotypes show specific species composition and metabolic function and might represent different stable symbiotic states of host-microbe interaction. Enterotype 1 is characterized by high levels of *Bacteroides*, enterotype 2 by high levels of *Prevotella* and low levels of *Bacteroides*, and type 3 has a relative high abundance of *Ruminococcus* (Arumugam et al., 2011). However, the concept of enterotypes has also been met with controversy (Knights et al., 2014; Gorvitovskaia et al., 2016).

16S rRNA sequencing methods today offer a good tool for in-depth analysis of large sample sets. However, it has to be kept in mind that faecal samples, which are generally used for determination of gut microbiota, do not necessarily represent the situation in the gastrointestinal tract. The distribution of microbes in the gut varies along the gastrointestinal tract, from the stomach with acidic environment down to the duodenum, intestine and finally the colon, where the highest content of microbes is present (O'Hara and Shanahan, 2006). In addition, there are differences in the composition of the microbiota of the mucosal surface and the lumen (Eckburg et al., 2005). Moreover, the functional genetic profile of the microbiota may be of more informative value and cannot be directly derived from the composition on higher taxonomic levels. With data from the Human Microbiome Project (HMP) it was shown that metabolic pathways are stable within healthy populations despite high variance on the phyla level (Huttenhower et al., 2012).

1.5.2 Specific function of the gut microbiota and influence on the host

The gut microbiota has important functions for the whole organism and therefore provides valuable contribution to human health. It is a quite stable community that protects the host against pathogens, for example via production of inhibitory molecules, nutrient competition or the stimulation of local immune cell responses (Ubeda et al., 2017). In addition, it contributes to nutrient and energy extraction from our diet. Complex carbohydrates and polysaccharides

that cannot be degraded enzymatically are fermented by the bacteria in the colon, providing energy for the microbes and generating end products like SCFAs of which acetate, butyrate and propionate are the most abundant ones (Tremaroli and Bäckhed, 2012). Butyrate is an important energy source for colonic epithelial cells and acetate and propionate for peripheral tissues (Bergman, 1990). Together, they provide about 10% of daily energy to the host (Bergman, 1990). The amount and types of SCFAs that are produced is dependent on dietary intake and microbiota composition, which can therefore define energy harvest (Tremaroli and Bäckhed, 2012). SCFAs can also serve as bioactive lipid mediators and thereby influence fatty acid (Ge et al., 2008) and glucose metabolism (Sakakibara et al., 2006), inflammatory processes (Vinolo et al., 2011), vasodilatation (Mortensen et al., 1990), gut motility (Soret et al., 2010) and wound healing (Topçu et al., 2002). Next to fibre degradation, certain microorganisms make valuable contribution to vitamin K supply by synthesis of vitamin K2 (LeBlanc et al., 2013). Gut microorganisms also influence the bile acid pool of the host by deconjugation of primary bile acids and further metabolisation to secondary bile acids (Tremaroli and Bäckhed, 2012). Those can serve as signalling molecules and stimulate GLP-1 secretion via TGR5 receptor leading to enhanced glucose tolerance (Thomas et al., 2009). Another important function of gut microbiota is its influence on immune function. Microbiota can influence both innate and adaptive immune responses and the conditioning of the immune system in early life is of major importance for immune response in later life (Belkaid and Hand, 2014). As mentioned above, the microbiota may also alter gut permeability via interaction with diet and host mechanisms, thereby increasing inflammatory responses of the host.

To conclude, gut microbiota is an important contributor to human health, and disruptions of microbiota composition can play a role in various disease pathologies. Though, in most cases it is difficult to assign disease pathologies to specific microbes, while it is rather a general shift in the delicate equilibrium that exerts influence on the host.

1.5.3 Impacts on gut microbiota composition

Gut microbiota composition is influenced by a variety of different factors. Next to age, which has already been mentioned above, genetic and environmental factors, nutrition, pre- and probiotics as well as drugs can shape gut microbiota composition.

Genetic and environmental influences on microbiota have been studied in twin studies. The study of twins and their families by Turnbaugh et al. revealed that family members exhibit greater similarity in their microbial profile than non-related persons. Interestingly, there was no difference in the similarity between monozygotic and dizygotic twins and twin pairs were just slightly more similar than mother and child pairs, indicating an influence of both genetic

as well as environmental factors. The similarity in microbial communities was also reflected in similar metabolic profiles (Turnbaugh et al., 2009).

In addition, the comparison of genetically different populations has revealed differences in microbial profiles. One study, for example, found significant differences in the microbiota of Columbians, Europeans and Americans (Escobar et al., 2014). Another study investigated people from the US, Malawi and Venezuela, and could also show significant differences of gut microbiota with pronounced separation of the US population (Yatsunenko et al., 2012). These human populations, however, do not only differ in genetic profiles, but also in environmental factors, diet, exercise, sanitation and drug intake. These factors may to a certain extent explain differences on a population level. In a study by Filippo and colleagues, comparing children from rural African regions with children from Europe, Africans exhibited higher abundance of *Bacteroidetes* and lower abundance of *Firmicutes* as well as higher levels of SCFAs than children from Italy. The phylum *Bacteroidetes* included the genera *Prevotella* and *Xylanibacter*, which can perform cellulose and xylan hydrolysis. The microbiota of African children seems therefore adapted to the extraction of energy from a rural African diet rich in fibres (Filippo et al., 2010).

Thus, long-term dietary habits shape our gut microbiota, which adapts to dietary contents of specific nutrients like fibre or fat (Wu et al., 2011). Changes in dietary habits can induce alterations of the microbial composition (Ley et al., 2006; Wu et al., 2011), although these alterations are relatively small compared to inter-individual differences. The intake of pre- and probiotics or their combination (synbiotics) can also modulate human gut microbiota. This is of special interest for the prevention or alleviation of certain inflammatory and infectious diseases. Implementation of pre- and probiotics is discussed in formula fed infants (Vandenplas et al., 2015) as well as elderly people (Rondanelli et al., 2015) to make them less vulnerable to gastrointestinal disturbances or diseases associated with gut microbiota. In addition, the use of pre- and probiotics can have positive effects for the prevention and treatment of gastrointestinal diseases, like diarrhoea (Guarino et al., 2015) and IBD (Shen et al., 2014).

1.5.4 Gut microbiota in obesity

Disruption of the normal balance of the microbiota communities, so-called dysbiosis, has been associated with a variety of different disease pathologies. Growing evidence links obesity and the metabolic syndrome to an alteration in gut microbial composition (Barlow et al., 2015). The importance of microbiota for energy extraction and obesity is shown by the comparison of conventionally raised mice with germ-free mice, as shown by a study of Bäckhed and colleagues. Despite higher food intake, germ-free mice exhibit less body weight

and are protected from diet-induced obesity. After intestinal microbiota transplantation from conventional mice, however, they increase body fat and develop insulin resistance (Bäckhed et al., 2004). The microbiota from obese donors thereby results in greater rise in body fat than from lean donors (Turnbaugh et al., 2006), while transplantation of microbiota from RYGB-operated mice to germ-free mice promotes reduced fat deposition (Tremaroli et al., 2015). In mice, the obese microbiota was described by a substantial decrease in the abundance of *Bacteroidetes* and an increase in *Firmicutes* (Ley et al., 2005). Human studies could partly support these findings; however, study results are more heterogeneous and also the results of systematic reviews and meta-analyses are not consistent concerning the association of *Bacteroidetes/Firmicutes* ratio and obesity (Walters et al., 2014; Sze and Schloss, 2016; Castaner et al., 2018; Crovesy et al., 2020). This may be caused by variations in population, diet and environment. Moreover, it suggests that the interplay between gut microbiota and obesity is more complex and abundance of the main phyla alone is not conclusive.

The obese microbiome has been associated with higher capacity for energy extraction from food (Bäckhed et al., 2004), lower butyrate production (Brahe et al., 2013), metabolic endotoxemia and inflammatory processes (Cani et al., 2007b; Cani et al., 2008) and reduced diversity (Verdam et al., 2013). Furthermore, higher abundance of methanogenic *Archaea* has been described in obesity (Armougom et al., 2009; Zhang et al., 2009). The methanogens use H_2 and formate, by-products of bacterial fibre degradation, to reduce CO_2 into methane (Bauchop and Mountfort, 1981), thereby relieving accumulation of H_2 and allowing increased production of SCFAs (Samuel and Gordon, 2006). Elevated methane and H_2 in breath test have also been associated with higher BMI and body fat (Mathur et al., 2013).

1.5.5 Gut microbiota and bariatric surgery

The effect of weight loss on human microbiota composition has been studied in a number of different studies. Diet induced weight loss was accompanied by a modification of gut microbiota in most of the studies (Ley et al., 2006; Duncan et al., 2008; Santacruz et al., 2009; Simões et al., 2014), yet some studies reported only minor effects on the gut microbiota composition (Ott et al., 2017; Alemán et al., 2018). In comparison to calorie-restricted diet, bariatric surgery leads to a more pronounced weight loss together with a rapid improvement in metabolic parameters and the inflammatory status. These systemic changes often occur already days to weeks after surgery, before marked weight loss is observed (Jørgensen et al., 2012). A shift in microbiota composition may play a role in these processes and correlations between alterations of the microbiota after bariatric surgery and anthropometric, inflammatory and metabolic parameters have been made in human studies

(Furet et al., 2010; Graessler et al., 2013; Medina et al., 2017). *F. prausnitzii* for example, was found to increase after surgery and to correlate negatively with inflammatory parameters (Furet et al., 2010). Another study by Graessler and colleagues, however, could not confirm this association and reported a tendency to a decrease of *F. prausnitzii* and a positive correlation with blood glucose after RYGB (Graessler et al., 2013). Both studies though, reported an increase in the *Bacteroidetes/Firmicutes* ratio after surgery-induced weight loss. In general, most studies report an increase in Bacteroidetes and/or a decrease in Firmicutes after bariatric surgery (Luijten et al., 2019). However, there are still conflicting results, especially for SG, including an increase on the *Bacteroidetes/Firmicutes* ratio (Damms-Machado et al., 2015), no effect (Campisciano et al., 2018), as well as a decrease of that ratio (Medina et al., 2017). Most studies that directly compare the effect of RYGB and SG found surgery-specific alterations of the composition and functions of the gut microbiota (Medina et al., 2017; Murphy et al., 2017), and in general alterations are more pronounced after RYGB (Luijten et al., 2019). As RYGB is a very invasive technique and alters the whole anatomy of the gastrointestinal tract, changes in microbiota composition may be partly dependent on that effect. In summary, despite a rising number of studies, our understanding of the effects of bariatric surgery on gut microbiota and the underlying mechanisms is still very limited.

1.6 Adipose tissue and bioactive lipid mediators

Adipose tissue is an important organ for the storage of a surplus of energy in the form of lipids and the provision of the same in times of energy demand. Moreover, it is an endocrine organ, secreting cytokines and bioactive lipids, thereby regulating whole body metabolic homeostasis (Booth et al., 2016). Bioactive lipid mediators are lipids, that do not only serve as energy source, but, through binding to specific GPCRs, also transmit signals to target cells and are involved in a variety of different physiological processes (Lopategi et al., 2016). In obesity, the excessive accumulation of fat in adipose tissue causes functional and secretory dysregulation (Booth et al., 2016). Dysregulation of lipid mediators have been associated with diseases such as inflammation, atherosclerosis, cancer, and metabolic syndrome (Murakami, 2011). The majority of inflammatory lipid mediators (eicosanoids) are pro-inflammatory and elevated circulating lipids are generally considered to be associated with inflammation and insulin resistance (Tripathy et al., 2003; Glass and Olefsky, 2012). Nevertheless, some fatty acids, such as omega-3 fatty acids, show beneficial metabolic effects (Kalupahana et al., 2011).

1.6.1 FAHFAs – a new class of bioactive lipids

Recently, a new class of metabolically favourable fatty acids has been discovered, the fatty acid esters of hydroxy fatty acids (FAHFAs) (Yore et al., 2014). These fatty acids consist of a fatty acid and a hydroxy fatty acid which are joined by an ester bond. Initially, 16 family members of the FAHFAs were detected, consisting of 4 different fatty acids and their corresponding hydroxy fatty acids (Figure 3) (Yore et al., 2014). Until then, further FAHFAs have been described containing different fatty acids including also long chain unsaturated fatty acids like linoleic acid and docosahexaenoic acid (DHA) (Ma et al., 2015; Kuda et al., 2016; Hancock et al., 2018; Hu et al., 2018; Zhu et al., 2018). In theory, every combination of a fatty acid and a hydroxy fatty acid is conceivable. For every member of the FAHFA group, different regioisomers exist, depending on the position of the hydroxy group of the hydroxy fatty acid. Moreover, FAHFAs can be present as different stereoisomers. Stereochemistry is important for bioactive lipid function, and also biosynthesis as well as degradation of FAHFAs have been shown to be stereo-selective (Nelson et al., 2017). Due to the high diversity of the FAHFA lipids and complexity of their structure, the detection of these lipids is challenging.

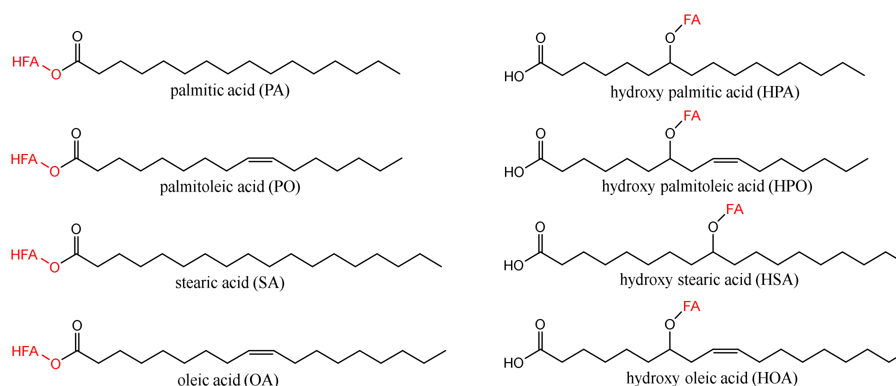

Figure 3: Structure of different FAHFAs

Structure of the four different fatty acids and four hydroxy-fatty acids that build up the 16 FAHFA family members discovered by Yore et al., 2014 (Adapted from Yore et al., 2014)

FAHFAs have been found in different foods from plant as well as animal sources (Yore et al., 2014; Zhu et al., 2018; Liberati-Čizmek et al., 2019) and dietary FAHFAs are most likely enzymatically digested (Kolar et al., 2016; Parsons et al., 2016). However up to date there is little knowledge concerning FAHFA metabolism upon oral intake.

In the original publication by Yore et al., FAHFAs have been detected in blood and in a variety of different tissues, with the highest levels in brown and white adipose tissue and liver. Yet, the regulation of serum and tissue FAHFA levels do not seem to be based on dietary intake, and endogenous levels of different isomers do not correlate with those in the diet (Yore et al., 2014). Endogenous synthesis in the adipocyte is therefore the major

regulator of human FAHFA levels (Yore et al., 2014; Kuda et al., 2016). Moreover, FAHFA-containing TGs may serve as a storage form for FAHFAs in adipose tissue and incorporation and release of FAHFAs from these TGs may contribute to regulation of FAHFA levels (Tan et al., 2019). The endogenous synthesis of FAHFAs takes place most likely via esterification of a hydroxy fatty acid with a saturated acyl-CoA (Yore et al., 2014; Kuda et al., 2016). The fatty acid can originate from de-novo lipogenesis in the adipocyte and the hydroxy fatty acid from membrane phospholipid hydroperoxides (Kuda et al., 2018). Lipolysis in adipocytes is induced in a glucose-dependent manner by the transcription factor carbohydrate-responsive element-binding protein (ChREBP), which seems to be one main regulator of FAHFA synthesis (Yore et al., 2014). Moreover, the Nrf2-regulated antioxidant system is involved in the biosynthesis of FAHFAs, as Nuclear factor-like 2 (Nrf2) can limit de-novo lipogenesis and Nrf2 target genes controlling redox status are involved in the remodelling of membrane phospholipid hydroperoxides and provision of hydroxy fatty acids (Kuda et al., 2018).

1.6.2 Metabolic effects of FAHFAs

The FAHFAs have first been described in a mouse model of adipose-selective overexpression of Glut4, called AG4OX mouse model. AG4OX mice have increased basal and insulin-stimulated glucose uptake in adipocytes despite increased body weight and increased body fat mass (Shepherd et al., 1993). Yore and colleagues could show that adipose tissue selective overexpression of Glut4 and the subsequent enhanced adipose tissue lipogenesis driven by ChREBP promote the synthesis of metabolically favourable fatty acids, the FAHFAs. Those are produced from newly synthesised long-chain fatty acids that are coupled via esterification to hydroxy fatty acids. AG4OX mice therefore have elevated FAHFA levels in white and brown adipose tissue as well as in serum (Yore et al., 2014).

It has been known before that insulin resistance is associated with decreased expression of Glut4 and ChREBP in adipose tissue (Carvalho et al., 2001; Herman et al., 2012). Consistent with these observations, Yore and colleagues could show that palmitic acid hydroxy-stearic acids (PAHSAs) are reduced in serum and adipose tissue of obese, diabetic mice and insulin-resistant humans. Oral administration of PAHSAs could restore the levels in obese, diabetic mice, lower basal glycaemia and ameliorate insulin sensitivity. The mechanisms of these anti-diabetic effects of PAHSAs could be revealed in *in vitro* experiments: First, insulin secretion is enhanced through both direct effects on the β-cells of the pancreas as well as through stimulation of GLP-1 secretion in intestinal enteroendocrine cells. Second, insulin-stimulated glucose transport into the adipocyte is increased via promotion of Glut4 translocation to the membrane (Yore et al., 2014) (Figure 4).

In addition to their anti-diabetic effects, anti-inflammatory effects of PAHSAs have been described. PAHSAs can attenuate the LPS-induced activation and subsequent cytokine secretion of bone-marrow-derived dendritic cells (BMDC) as well as further T-cell activation (Yore et al., 2014; Lee et al., 2016). Furthermore, PAHSAs diminish the secretion of the inflammatory cytokines interleukin 1 beta (IL-1β) and TNF-α in macrophages (Yore et al., 2014). Through these anti-inflammatory processes, orally induced PAHSAs can also prevent against chemically induced colitis (Lee et al., 2016).

The anti-inflammatory as well as the anti-diabetic effects of FAHFAs are most likely mediated through the G protein-coupled receptor 120 and 40 (GPR120, GPR40) and possibly other unidentified receptors (Yore et al., 2014; Syed et al., 2018). The enhanced interest in the role of the newly identified FAHFAs has produced research in different fields, publishing effects of FAHFAs for example also in protection against type 1 diabetes (Syed et al., 2019), diabetes-mellitus related cognitive impairment (Wen et al., 2020), induction of browning of white adipose tissue (Wang et al., 2018) and mediation of positive effects of cold exposure (Paluchova et al., 2020) and exercise training (Brezinova et al., 2020). Due to their favourite metabolic effects and endogenous origin, FAHFAs may provide important insights into metabolic and inflammatory disease pathologies and possibly inherit a high potential for new treatment strategies.

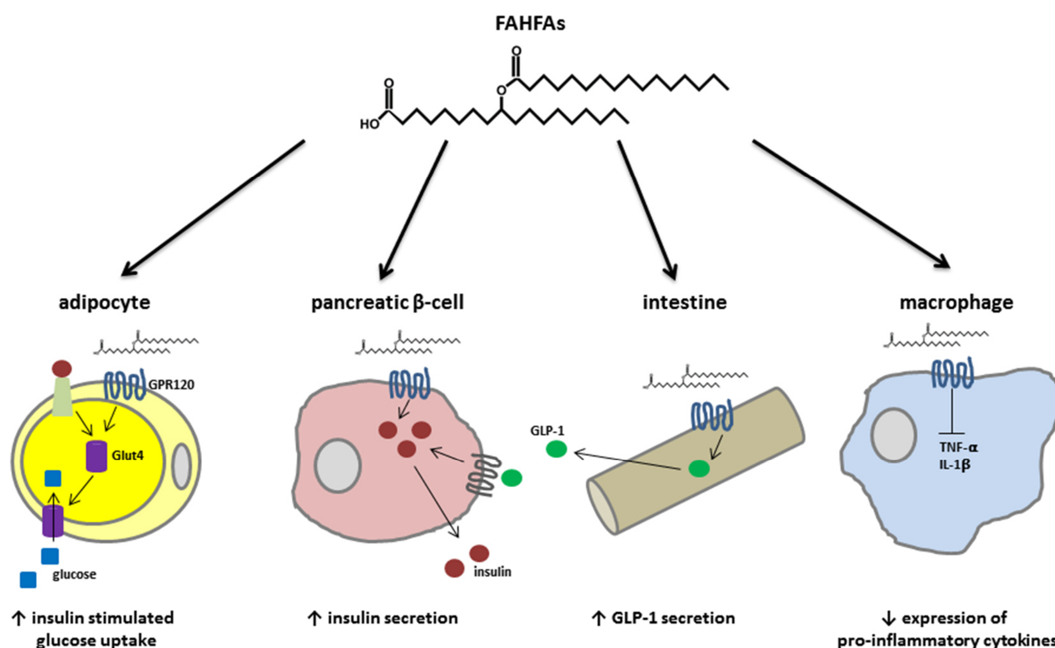

Figure 4: Anti-diabetic and anti-inflammatory effects of FAHFAs (Adapted from Muoio and Newgard, 2014)

2. Aim

The gut barrier is a huge mucosal surface that separates the host internal milieu from the intestinal lumen and gut microorganisms. It has the important task to protect the host from possible pathogenic factors while it also represents a crucial point of communication and interaction of the host with external and microbial signals. Microbiota-derived products and metabolites can directly influence gut barrier function and gut permeability. The gut permeability is tightly connected with inflammation as increases in gut permeability lead to elevated translocation of microbial products, like LPS, from the gut into the circulation, where LPS causes inflammatory responses of the host and may also trigger insulin resistance. Therefore, gut permeability is regarded as an important determinant in diseases with inflammatory components, including obesity and type 2 diabetes. Bariatric surgery is an effective tool to rapidly improve obesity-associated metabolic and inflammatory disturbances; yet it is not clear, if changes in gut permeability are involved in these improvements.

Therefore, the aim of the present study was to investigate gut permeability in morbidly obese patients before as well as after considerable weight loss induced by bariatric surgery in comparison to non-obese subjects. The investigated bariatric surgery procedure was SG, as the effect of SG on intestinal permeability has barely been studied so far and SG, as opposed to RYGB, is devoid of invasive anatomical changes in the intestine. To further investigate the influence of BMI, gut permeability was evaluated in different grades of obesity. Next to gut permeability, also inflammatory and metabolic parameters as well as the composition of the microbiota were studied to explore possible associations of these parameters with gut permeability.

An additional aim of the study was to determine the levels of several FAHFAs in serum of obese patients before and after bariatric surgery in comparison to non-obese subjects. This group of fatty acids has potent anti-inflammatory and anti-diabetic effects and may be involved in obesity-associated metabolic and inflammatory disturbances. As data on endogenous FAHFA levels under different physiological and pathophysiological conditions are very limited, FAHFAs were also measured in further study cohorts to investigate possible influences of BMI, age, sex, diabetic status and diet.

3. Study interventions

3.1 Ethics statement

The declaration of Helsinki (in the revised version of Fortaleza, Brazil 2013) and the guidelines of the International Conference on Harmonization (ICH) of Good Clinical Practice (GCP) were basis of the human studies where appropriate. All participants gave written informed consent before they were included in the study.

3.2 Approval and registration

The study protocol was reviewed and approved by the Ethics Committee of the Faculty of Medicine at the Technical University of Munich (approval no. 387/14s). The study was registered in the German Clinical Trial Register (DRKS00009008).

For the comparison of gut permeability within different BMI groups, data from a former study were used (Ott et al., 2017). This study was funded by the BMBF (Federal Ministry of Education and Research, grant no. 0315674) and registered in the German Clinical Trial Register (DRKS00006210). 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).

3.3 Study design

This human study was a non-randomised, observational study. It was a multicentre study carried out at the Human Study Centre of the Else Kröner-Fresenius Centre of Nutritional Medicine in Freising-Weihenstephan, Germany, and at three surgical clinics: Chirurgische Klinik München-Bogenhausen, Chirurgische Klinik I Krankenhaus Landshut-Achdorf, Kreiskrankenhaus Schrobenhausen. The study design is depicted in Figure 5. Obese patients undergoing laparoscopic sleeve gastrectomy (LSG) were recruited in cooperation with the three clinics. In the weeks before surgery, patients recorded their food intake in a 7-day dietary protocol, took faecal samples and performed a 4-probe sugar test to assess gut permeability. On the day before surgery, when patients came to the clinic for inpatient admission, they got anthropometric measurements. During surgery, blood samples as well as fat tissue samples from the visceral and subcutaneous fat depot were taken. Six months after the surgical procedure, the study programme was repeated (V2 = Visit 2). The range for the V2 visit of the study participants was between 25 and 39 weeks (5.8–9.0 months) after surgery.

For control subjects (BMI 18.5–30 kg/m²) either patients with an abdominal surgery performed in one of the two cooperating clinics were recruited or subjects without any

surgical procedure. Control subjects underwent the same study programme as the obese patients: they recorded their food intake in a 7-day dietary protocol, got anthropometric measurements, collected faecal samples, performed the 4-probe sugar gut permeability test and provided fasting blood. From patients undergoing abdominal surgery, also visceral and subcutaneous fat tissue samples were taken.

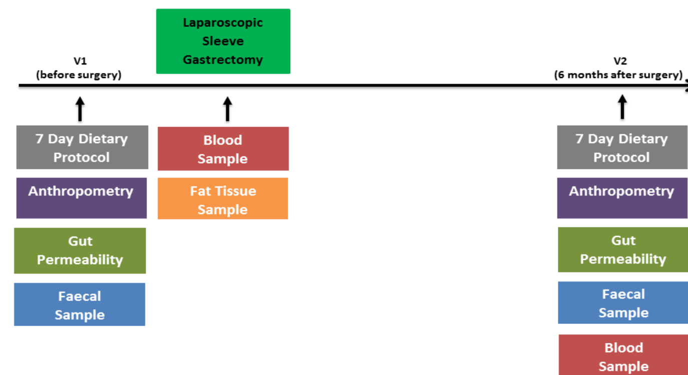

Figure 5: Study design for obese patients undergoing LSG

Documentation of food intake, anthropometric measurements, gut permeability measurements and faecal sample collection were performed before surgery of obese study participants. During surgery blood and fat tissue samples were taken. Measurements before surgery and samples collected during surgery correspond to Visit 1 (V1). Six months after surgery, the study programme was repeated (Visit 2 = V2).

3.4 Study population

Patients at the clinics were mainly recruited by direct information through the surgeons and dieticians at the clinics as well as with flyers (Appendix). Recruitment of control patients without surgery was carried out by distributing flyers and a call for participants (Appendix) among the staff at the campus Weihenstephan at the Technical University of Munich. Subjects who were interested in the study were screened according to our in- and exclusion criteria (Table 4). Subjects were informed in detail about the aims of the study, the study procedure, risks and rewards, expense allowance, rescission, utilization of study results and data protection before they gave written informed consent (Appendix).

Twenty-five obese patients undergoing LSG at one of the three cooperating hospitals were initially enrolled in the study. Two study patients did not complete the visit 6 months after surgery. Six study patients had to be excluded because of intake of antibiotics, NSAIDs or corticosteroids during the study period. Therefore, 17 obese patients undergoing LSG remained in the final analysis.

As controls, 23 subjects were initially recruited. One subject did not complete the study. Analyses are performed with 17 obese study patients who completed the study and with 17 controls who were matched in case of age and sex to the obese patients.

Table 4: Inclusion and exclusion criteria for the study

Inclusion Criteria	Exclusion Criteria
Male and female	Severe disease (e.g. cancer)
Age 18–65 years	Intolerance to lactulose, sucrose, sucralose, fructose
BMI ≥ 40 kg/m² (patients with obesity undergoing laparoscopic sleeve gastrectomy) BMI 18.5–30 kg/m² (controls)	Regular intake of antibiotics, laxative agents, immunosuppressive drugs, diarrhoea medication
Written informed consent	Acute infections
	Drug- or alcohol abuse
	Antibiotic intake within the last 2 months
	Insulin-dependent diabetes mellitus

3.5 Data and sample collection

A six digital code was used to encrypt all samples and data collected during the study. Written informed consent of the patients as well as all data sheets and the identification list were stored in a lockable cabinet. Pseudonymised data were recorded in a case report file (CRF) (Appendix) and transferred into an excel sheet (Excel version 2010).

4. Methods

4.1 General patient information

At each visit study participants received a questionnaire to gather information about the overall health status, medication, life style and anamnesis (Appendix). Additionally, for patients undergoing surgery, the case history was received from the clinic.

4.2 Dietary behaviour

Study participants recorded their diet in a 7-day dietary protocol (Appendix). Rarely, in case it was not possible for the study participants to write a dietary protocol because of lacking time until surgery, a recently conducted dietary protocol from the participant was used. Food items from the dietary protocols were entered into OptiDiet Plus (version 5.1.2.046, GOE mbH, Linden, Germany), which is based on data from the German Bundeslebensmittelschlüssel (BLS). The dietary protocol data were analysed by the programme.

4.3 Anthropometric measurements

Participants were instructed to avoid sports the day before anthropometric measurements. Measurements were performed without shoes, without outdoor clothing such as jackets and coats, and accessories such as bags and keys. 1 kg of measured body weight was subtracted to account for residual clothing. Height was assessed using a stadiometer and body weight and composition with a calibrated standard scale (Seca, Hamburg, Germany). BMI (kg/m^2) was calculated by dividing body weight by height in meter squared. Waist circumference was measured with a soft tape mid-way between the lowest rib margin and the iliac crest. Hip circumference was measured with a soft tape at the widest part of the gluteal region. The waist-to-hip ratio is calculated by dividing the waist circumference (cm) by the hip circumference (cm).

For the quantification of weight loss after surgery different parameters were used (Deitel and Greenstein, 2003) which were calculated as follows:

Percent excess weight loss (%EWL) = [(pre-operative body weight (kg) − follow-up body weight (kg)) / (pre-operative body weight (kg) − ideal body weight (kg))] × 100

Ideal body weight (kg) = 25 kg/m^2 × $(\text{height (m)})^2$

Percent total weight loss (%TWL) = [(pre-operative body weight (kg) − follow-up body weight (kg)) / pre-operative body weight (kg)] × 100

4.4 Gut permeability

4.4.1 Sugar absorption test

The integrity of the gut barrier was assessed by a validated 4-probe sugar test, which has been performed and described in detail before (Norman et al., 2012; Ott et al., 2017). This test is based on the assumption that larger oligosaccharides would not pass the gut barrier in a healthy situation. However, in case of barrier integrity loss, increased molecule permeation can occur mainly through the paracellular route. These molecules then enter the blood circulation and are detectable in urine after renal excretion. By combination of four different sugars the permeability of different sections of the gastrointestinal tract and different routes of passage (paracellular and transcellular) can be studied.

Sucrose was used as a marker of gastroduodenal permeability (Meddings et al., 1993; Sutherland et al., 1994). This sugar has a molecular mass of 342.3 Da and normally passes the stomach and duodenum without being absorbed. Brush-border enzymes in the intestine (sucrase-isomaltase) degrade the disaccharide into the two monosaccharides glucose and fructose, which can then be absorbed. The appearance of sucrose molecules in the urine indicates a barrier loss of the oesophagus, stomach or duodenum. For the evaluation of small intestinal permeability, lactulose and mannitol were used. Both sugars are not hydrolysed in the human intestine and are not actively absorbed. They are fermented by bacteria as they reach the colon. Lactulose is a synthetic disaccharide composed of D-galactose and fructose with a molecular weight of 342.3 Da. Due to its size it is assumed that it only passes the barrier via the paracellular way through tight junctions of epithelial crypts. Lactulose therefore functions as a tight junction marker. In contrast, mannitol can be used to assess small intestinal permeability of the transcellular route. This small monosaccharidic alcohol, with a molecular weight of only 182.2 Da, most likely passes the barrier transcellularly at the villus tips. Decreased urinary recovery indicates villus atrophy or diminished surface area of the intestine (Strobel et al., 1984; Ukabam and Cooper, 1984). The last of the four sugars used to determine gut permeability was sucralose. Sucralose has a molecular weight of 397.6 Da and is an artificial sweetener produced by chlorination of sucrose. Sucralose is not metabolised by the microbiota in the colon and therefore can be used for the assessment of large intestinal/colonic permeability (Meddings and Gibbons, 1998; Anderson et al., 2004). Sucralose enters the circulation via the paracellular route in case of barrier loss.

Study participants received detailed instructions for the test in both oral and written form (Appendix). They were instructed not to drink alcohol and not to take NSAIDs during the test day as well as during the two days before. Proton pump medication was also discontinued on the test day and the day before, in agreement with the physician. Smokers were told not to

smoke during the morning and the first sampling period of the test. After an overnight fast, patients collected their first morning urine as a baseline urine sample. Next, they ingested a 100 ml solution of lactulose (10 g), mannitol (5 g) and sucrose (20 g) dissolved in water, and swallowed six pills containing sucralose (2 g in total). In the following 26 hours after ingestion of the sugars, participants were instructed to collect their whole urine in two different time intervals. Urine of the first five hours was collected for the assessment of gastroduodenal and intestinal permeability, i.e. quantification of lactulose, mannitol and sucrose. During this five-hour time interval, participants were not allowed to eat or drink with the exception of drinking 0.25 to 1.0 L of water at two hours after ingestion of the sugars. In addition, intake of medication was not allowed during that time interval. Urine of the next 21 hours was sampled in a new container and reflected colonic permeability, i.e. sucralose excretion was determined. 0.002 g of sodium acid (Sigma Aldrich, Steinheim, Germany) was added as preservative in each container for urine sampling. At the end of each sampling period, participants noted the total volume of the urine fraction and took two aliquots of urine (Urine Monovette® 10 ml, 10.252, Sarstedt, Nümbrecht, Germany) which were frozen immediately at -18°C. Frozen samples were taken to the study centre with cooling packs to avoid thawing. Samples were stored at -20 °C until analysis.

The analysis of sugars in urine was performed as described by Norman and colleagues (Norman et al., 2012). First, protein content was removed by the use of sulphosalicylic acid. Next, amberlite-mixed bed-3 resin was used to desalt the urine. Sugars were separated with meso-erythritol and turanose as internal standards. Analysis and quantification was performed by high-performance liquid chromatography (HPLC) with pulsed electrochemical detection (chromatography module: 250, Dionex, Idstein, Germany). A 40 mm Carbopac PA-1 column (Dionex; eluent 150 mmol NaOH) was used and the flow rate was set to 1 ml/min. Results are presented as percentage recovery of the ingested dose.

For the comparison of gut permeability in different groups of BMI, we included data from a former study, which was performed within the same laboratories and by identical methods (Ott et al., 2017). Only women, that did fulfil the inclusion criteria for our study and were non-smokers, were included in the analysis.

4.4.2 Zonulin

Next to the four sugar test, zonulin concentration was measured in plasma as an additional permeability marker. Zonulin has been identified in 2000 as the eukaryotic analogue to the zonula occludens toxin of *Vibrio cholera* (Fasano et al., 2000). Zonulin regulates tight junction function in the intestine and this effect is mediated by an intracellular cascade resulting in protein kinase C (PKC)-dependent polymerisation of actin filaments and the

following disassembly of tight junctions and increase in paracellular permeability (Fasano, 2011). Circulating zonulin can be used as a marker for intestinal permeability. In this study, zonulin concentration in plasma was determined by ELISA (Immundiagnostik AG, Bensheim, Germany).

4.5 Blood samples

Blood from obese patients was drawn during surgery by the anaesthetists. At 6 months after surgery, blood was drawn again in a fasting state. One EDTA (ethylenediaminetetraacetic acid) Monovette (S-Monovette® 9 ml K3E, 02.1066, Sarstedt, Nümbrecht, Germany) and one serum monovette (S-Monovette® 7.5 ml Z-Gel, 01.1602, Sarstedt, Nümbrecht, Germany) were collected. The EDTA monovette was centrifuged immediately at 2,500 g for 10 min at room temperature and plasma was aliquoted and stored at -80 °C. The serum monovette was allowed to clot for 30 min at room temperature and subsequently centrifuged at 2,500 g for 10 min at room temperature. Serum was aliquoted and stored at -80 °C. In addition, laboratory analyses of the last blood sampling before surgery and blood sampling from follow-up care of obese patients were received from the clinics.

From control patients without surgery, blood was drawn in the morning in a fasting state. One EDTA Monovette (S-Monovette® 9 ml K3E, 02.1066, Sarstedt, Nümbrecht, Germany) and one serum monovette (S-Monovette® 7.5 ml Z-Gel, 01.1602, Sarstedt, Nümbrecht, Germany) were collected und handled in accordance with blood of obese patients. In addition, routine parameters (Table 5) were analysed at a certified laboratory (Synlab, Munich, Germany).

Blood glucose levels of patients with obesity were received from laboratory analyses of the clinics. Blood glucose from patients with missing values and from all control patients were measured in frozen serum samples at the Synlab laboratory (Munich, Germany).

Table 5: Routine parameters analysed in blood of study participants

Material (Monovettes Sarstedt)	Parameters	
EDTA	Blood count	Haemoglobin Erythrocytes MCH MCV Haematocrit MCHC RDW (Ery) Thrombocytes Leucocytes
Serum	Liver enzymes	ASAT ALAT γ-GT
	Function of liver and bile tracts	Bilirubin
	Lipid profile	Cholesterol Triglycerides HDL LDL
	Electrolytes	Sodium Potassium Calcium
	Kidney function	Creatinine GFR (MDRD) GFR (CKD-EPI)
	Uric acid levels	Uric acid
	Thyroid function	TSH

ALAT, alanine transaminase; ASAT, aspartate transaminase; γ-GT, γ-glutamyltransferase; GFR, glomerular filtration rate; HDL, high-density lipoprotein; LDL, low-density lipoprotein; MCH, mean corpuscular haemoglobin; MCHC, mean corpuscular haemoglobin concentration; MCV, mean corpuscular volume; RDW, red blood cell distribution width; TSH, thyroid-stimulating hormone.

4.6 Faecal samples

Study patients collected the stool samples at home. They received instructions for correct sampling both orally and in written form (Appendix). They were instructed to directly collect the stool sample into a sterile plastic container (1,000 ml, VWR International, Munich, Germany) and to avoid any contamination with toilet paper, water, urine or blood. Participants then collected one spoon of faecal sample with the Stool Collection Tubes containing Stool deoxyribonucleic acid (DNA) Stabiliser (Stratec, Berlin, Germany). This tube was vigorously shaken by hand and stored at room temperature until it was delivered to the study centre. Upon delivery, the stool tube was vortexed and frozen at -80 °C. Two additional faeces tubes (Sarstedt, Nümbrecht, Germany) were filled by the study patients from the same stool sample with one spoon of faecal sample each. These tubes were frozen immediately by the study patients and delivered with cooling packs to the study centre,

where they were stored at -80 °C. The faecal samples were delivered within a maximum of four days after sampling.

4.7 Laboratory measurements

4.7.1 Enzyme-linked immunosorbent assay (ELISA)

Insulin, LBP and C-reactive protein (CRP) concentrations were measured in plasma using commercially available Enzyme-linked immunosorbent assay (ELISA) kits (Insulin: DRG Diagnostics, Marburg, Germany; LBP and CRP: R&D, Wiesbaden, Germany). The ELISAs were performed in accordance with the manufacturer's protocol. The sandwich ELISA technique works with two different antibodies. The first antibody (capture antibody) is coated onto a 96-well plate. The sample is added to the plate and the proteins of interest can bind to the specific capture antibody. After incubation, the sample is removed and the plates are washed. Then, the biotinylated second antibody (detection antibody) is added and can bind to the antigen at a different epitope than the capture antibody. Excess of antibody is removed by washing. Next, the streptavidin-conjugated enzyme horseradish peroxidase is added, which can activate the chromogen 3,3',5,5'-tetramethylbenzidine (TMB) in presence of H_2O_2 into a blue coloured agent. Sulfuric acid stops the reaction and turns TMB yellow. The colour can be read at a microplate reader (Tecan Infinite M200, Crailsheim, Germany) at 450 nm and the optical density is proportional to the amount of antigen in the sample. A correction wavelength of 540 nm was used and subtracted from the measurement wavelength. A series of standards with known concentrations was measured with every plate and the resulting standard curve was used to interpolate unknown concentrations of the samples in GraphPad Prism (GraphPad Software, version 5, San Diego California, USA). A blank sample (reagent diluent) was measured with every plate and the blank optical density (OD) value subtracted from the sample OD values. A control sample was used to take into account variations between plates.

4.7.2 ACS-ACOD-method for the quantification of free fatty acids

The concentration of non-esterified fatty acids (NEFAs) bound to albumin in serum was determined by the ACS-ACOD Method with the use of a commercially available kit (Wako Chemicals GmbH, Neuss, Germany). NEFAs in the sample are converted by the action of Acyl-CoA synthetase (ACS) to AcylCoA, which is further oxidised to 2,3-trans-Enoyl-CoA by the enzyme Acyl-CoA oxidase (ACOD). The resulting hydrogen peroxide in the presence of peroxidase (POD) undergoes quantitative oxidation condensation with 4-aminoantipyrine and 3-methyl-N-ethyl-N-(β-hydroxyethyl)-aniline (MEHA) to form a blue purple pigment (Figure 6). By measuring absorbance of this pigment the concentration of NEFAs in the sample can be determined.

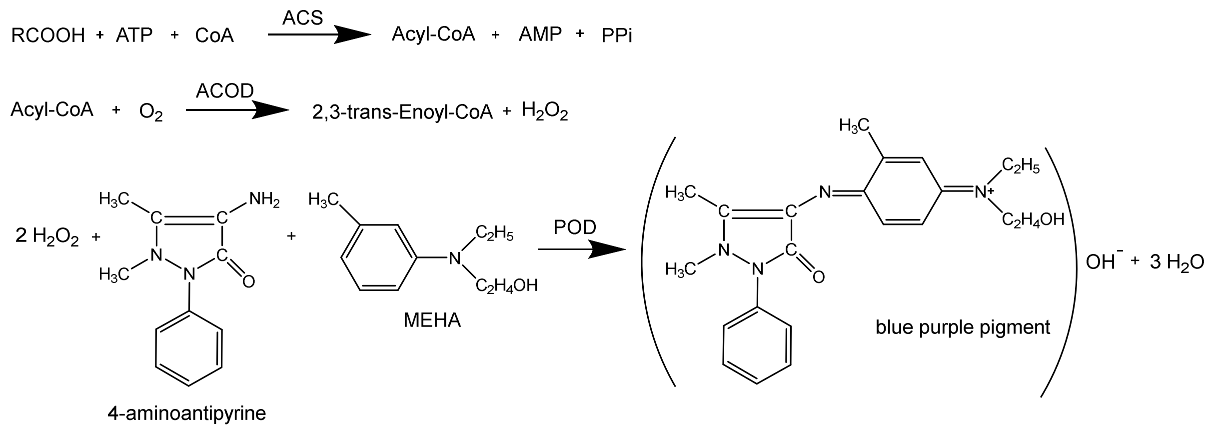

Figure 6: Reactions of the ACS-ACOD Method

ACOD, acyl-CoA oxidase; ACS, acyl-CoA synthetase; AMP, adenosine monophosphate; ATP, adenosine 5-triphosphate disodium salt; MEHA, 3-methyl-N-ethyl-N-(β-hydroxyethyl)-aniline; POD, peroxidase.

4.7.3 High-throughput 16S rRNA gene sequencing analysis

Faecal samples were processed as described previously (Lagkouvardos et al., 2015). Briefly, microbial cells were lysed by beat-beating followed by heat treatment (95 °C, 5 min). After centrifugation, RNAse was added to the supernatant. Then, metagenomic DNA was purified by the use of genomic deoxyribonucleic acid (gDNA) columns (Macherey-Nagel, Düren, Germany). For concentration measurements and purity control the NanoDrop® system (Thermo Scientific Waltham, Massachusetts, USA) was used.

A total amount of 24 ng DNA was used and the V3/V4 region of 16S ribosomal RNA (rRNA) genes was amplified (25 cycles) using the bacteria-specific primers 341F and 785R (Klindworth et al., 2013) and following a two-step procedure to limit amplification bias (Berry et al., 2011). 16S rRNA gene amplicons were purified (AMPure XP system, Beckmann Coulter Biomedical GmbH), pooled in an equimolar amount, and finally sequenced in paired-end modus (PE275) using a MiSeq system (Illumina, Inc., San Diego, Kalifornien, USA) following the manufacturer's instructions and a final DNA concentration of 10 pM and 15% (v/v) PhiX standard library.

Raw sequence reads were processed using IMNGS (https://www.imngs.org/) (Lagkouvardos et al., 2016) with a pipeline based on UPARSE (Edgar, 2013). Parameters were: barcode mismatches allowed: 1; expected errors allowed: 3; 5'-end trim: 3; 3'-end trim: 3 nt; trim score: 3 nt; min. sequence length: 300 bp; max. sequence length: 600 bp. Operational taxonomic units (OTUs) were clustered at a threshold of 97% sequence similarity. To exclude artificial OTUs, only those with a relative abundance > 0.25% of total sequences in at least one sample were analysed further. For each OTU, the final taxonomy was assigned using the most detailed taxonomic classification among SILVA (Quast et al., 2013) and RDP

(Wang et al., 2007). OTUs with different abundances between the groups were further identified using EzTaxon (http://www.ezbiocloud.net/). Downstream analyses of composition and diversity were performed using the Rhea pipeline, a set of R scripts that allows standardized analysis of OTU tables, including normalization steps, alpha- and beta-diversity analysis, taxonomic composition, statistical comparisons, and calculation of correlations (Lagkouvardos et al., 2017). The Rhea scripts and details of the analysis can be found online (https://lagkouvardos.github.io/Rhea/). The final analysis (without patients taking proton pump inhibitors (PPIs)) delivered 497,737 quality- and chimera-checked reads (20,739 ± 6,344 per sample), which clustered in 394 OTUs (168 ± 41 OTUs per sample). To account for differences in sequence depth, normalisation of OTUs was performed via simple division to their sample size and successive multiplication by the size of the smaller sample. Generalized UniFrac distances were calculated for analysis of beta-diversity (Chen et al., 2012). Assessment of diversity within samples (alpha-diversity) was performed on the basis of species richness and Shannon and Simpson effective diversity (Jost, 2007). The effect of LSG on taxonomic counts and OTUs was tested between obese patients before as well as after surgery with the Paired Wilcoxon Signed Rank Sum Test. Missing values were removed from the analysis. The comparison of obese patients before as well as after surgery with controls was performed by Kruskall Wallis Rank Sum Test and pairwise comparison by Wilcoxon Rank Sum Test. When needed, p-values were corrected for multiple comparisons according to the Benjamini-Hochberg method.

4.8 Establishment of a LC-MS method for the analysis of FAHFAs

4.8.1 FAHFA extraction

FAHFA extraction from human serum samples was performed according to an adapted version of the protocol by Zhang and colleagues (Zhang et al., 2016). During all steps of sample processing, plastic pipette tips and plastic vials were avoided. A syringe-Pasteur pipette setup was used for pipetting of small sample volumes (Figure 7) and glass serological pipettes for bigger volumes. All glass vials were rinsed before use with a mixture of 1:1 (vol/vol) chloroform:methanol to reduce fatty acid contamination.

Citric acid buffer, pH 3.6

Component	Manufacturer	Final concentration
Sodium citrate tribasic dihydrate	Merck, Darmstadt, Germany	100 mM
Sodium chloride	Sigma-Aldrich, Steinheim, Germany	1 M

Samples from the different groups of one cohort were randomly assigned to FAHFA extraction batches, and one blank sample (H_2O) was included in each batch. The first step of sample processing was the extraction of lipids from serum samples. Therefore, 900 µl of serum sample was used and samples were spiked with 1 ng of isotope labelled internal standard (10 µl of 0.1 µg/ml $^{13}C_4$-9-PAHSA in methanol) to correct for loss of analyte during extraction. Afterwards, 2 ml of citric acid buffer, 3 ml of methanol (Merck, Darmstadt, Germany) and 6 ml of chloroform (Merck, Darmstadt, Germany) were added consecutively and samples were shaken vigorously for 30 sec and vortexed for 15 sec. Samples were centrifuged at 2,200 g for 12 min at 4 °C and the chloroform layer was transferred into a new sample vial. To remove possible residual aqueous contamination, the transferring step was repeated once. The samples were then dried under a gentle stream of N_2 and were tightly stored at -80 °C.

The second step was the FAHFA enrichment via solid phase extraction (SPE). All solvents used were of chromatographic grade (Merck, Darmstadt, Germany). SPE columns (HyperSep silica SPE column, 500 mg bed weight, 6 ml column volume; Thermo Scientific, Darmstadt, Germany) were washed with 8 ml of 95:5 (vol/vol) hexane:ethyl acetate and 8 ml of ethyl acetate. Afterwards, columns were conditioned with 15 ml of hexane. Samples, which were reconstituted in 200 µl of chloroform, were loaded onto the column. Subsequently, sample vials were washed with 100 µl of chloroform and the wash was added to the column. Neutral lipids were eluted with 15.5 ml of 95:5 (vol/vol) hexane:ethylacetate and FAHFAs were eluted with 15.5 ml of ethyl acetate. Both fractions were dried under a gentle stream of N_2. The dried FAHFA fraction was reconstituted in 1 ml of chloroform, vortexed for 15 sec and transferred into a new sample vial. To ensure complete transfer of the lipids, this step was repeated twice. After evaporation of chloroform under a gentle stream of N_2, the dried FAHFA fraction was again reconstituted in 200 µl of chloroform, vortexed for 15 sec and dried again under N_2. The dried samples were stored at -80 °C until analysis.

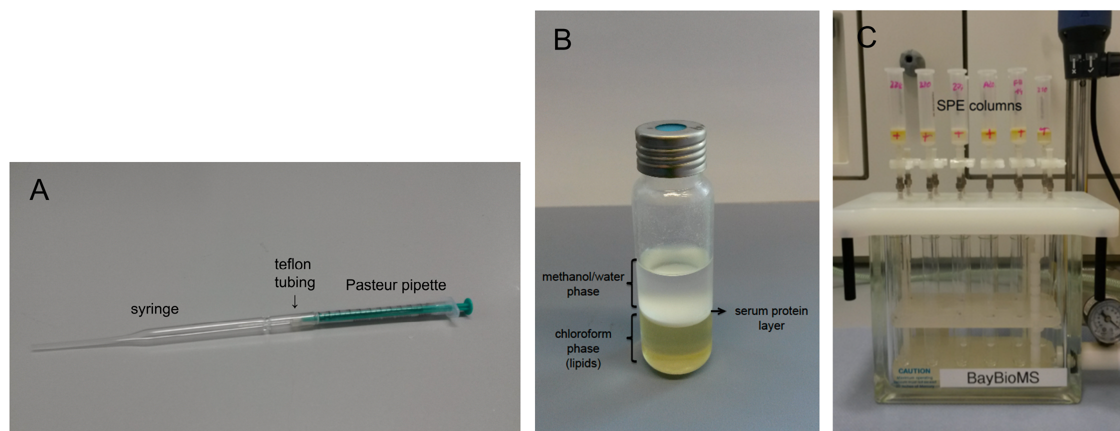

Figure 7: FAHFA extraction from serum samples

A) Syringe Pasteur pipette setup. B) Lipid extraction with phase separation. C) SPE manifold setup.

4.8.2 LC-MS analysis of FAHFAs

The chromatographic conditions are based on the work of Yore et al. (Yore et al., 2014) and Zhang et al. (Zhang et al., 2016). For liquid chromatography-mass spectrometry (LC-MS) analysis, samples were reconstituted in 100 µl of methanol. Chromatographic separation was performed with a Nexera ultra performance liquid chromatography (UPLC) equipped with a DGU-20A5R degassing unit, a LC-30AD pump, a SIL-30AC autosampler unit and a CTO-30A column oven (Shimadzu, Duisburg, Germany). A Kinetex XB-C18 100 Å column (100 x 2.1 mm) with 1.7 µm particle size was used (Phenomenex, Aschaffenburg, Germany). Injection volume was 10 µl and flow rate was 400 µl/min at 40 °C. The gradient program used to separate FAHFAs is shown in Table 6. With this method palmitic acid hydroxy-stearic acids (PAHSAs), oleic acid hydroxy-stearic acids (OAHSAs), stearic acid hydroxy-stearic acids (SAHSAs), and palmitic acid hydroxy-palmitic acids (PAHPAs) could be analysed. A chromatographic profile of the FAHFA standards is presented in Figure 8A and an exemplary chromatogram of one serum sample is presented in Figure 8B. The separation of PAHSA regioisomers was not sufficient for separate analysis. Therefore, they were analysed together, reported as tPAHSAs.

Table 6: HPLC gradient program

Time (min)	A (%)	B (%)
0	10	90
2	10	90
17	0	100
24	0	100
25	10	90
30	10	90

Solvent A: 5 mM ammonium acetate, pH 5.
Solvent B: acetonitrile / isopropyl alcohol / 5 mM ammonium acetate (55/40/5).

After the first run of samples (obese patients before and after LSG in comparison to non-obese controls), the HPLC method was modified to gain an improved separation of PAHSA regioisomers. The new HPLC method was an adaption to the protocol published in a recent paper (Kolar et al., 2018) and was used for all other study cohorts. The column used was an Acquity UPLC BEH C18 column (100 x 2.1 mm) with 1.7 µm particle size (Waters, Milford, MA, USA). 10 µl of sample were injected and flow rate was 300 µl/min at 40 °C. The running buffer used to separate FAHFAs was 93:7 methanol/water with 5 mM ammonium acetate and 0.03% ammonium hydroxide (v/v) under isocratic conditions.

For the quantitation of FAHFAs, UPLC was coupled to a QTRAP 6500 mass spectrometer with IonDrive Turbo V source and the software Analyst Version 1.6.2. (AB Sciex, Darmstadt). Negative electrospray ionization mode was used and further MS-settings are shown in Table 7.

A calibration curve of a mixture containing all FAHFA standards was used for quantitation. Seven different concentrations of FAHFAs in methanol, ranging from 0 to 100 ng/ml, were used for the calibration curve. The following FAHFA standards were used: 5-PAHSA, 9-PAHSA, 10-PAHSA, 12-PAHSA, 13-PAHSA, 9-SAHSA, 9-OAHSA, and 9-PAHPA (Biomol, Hamburg, Germany). A chromatographic profile of the FAHFA standards is presented in Figure 9A and an exemplary chromatogram of one serum sample is presented in Figure 9B. For each of the FAHFA standards, two multiple reaction monitoring (MRM) transitions were measured. The first one was used as quantifier and a second one as qualifier. The MRM transitions and the optimised collision energy (CE) and cell exit potential (CXP) are listed in Table 8. To distinguish between the different FAHFAs, the specific molecular mass of the mother ion, the specific MRM transitions as well as the retention time were utilised. Only those FAHFAs were quantified, whose retention time and ion ratios of the quantifying and qualifying MRM transition fitted the corresponding standard. By controlling the ratios of the two MRM transitions, we tried to exclude matrix effects and by using the $^{13}C_4$-9-PAHSA internal standard, we corrected for errors during extraction. In the case of 5-PAHSA, the ratios of the MRM transitions did not always match that of the corresponding standard. Therefore, 5-PAHSA was excluded from the analysis. The peaks of 12- and 13-PAHSA could not be separated with our method. Therefore, 12- and 13-PAHSA were analysed together. The expression tPAHSAs stands for the total sum of PAHSAs analysed (9-, 10-, 12/13-PAHSA) and the expression tFAHFAs stands for the total sum of FAHFAs analysed (PAHSAs, 9-OAHSA, 9-SAHSA, 9-PAHPA).

Table 7: MS parameters

Parameter	Setting
Ion spray voltage	−4500 V
Source temperature	450 °C
Curtain gas	35 psi
Ion source gas 1	55 psi
Ion source gas 2	65 psi
Collison gas	nitrogen
Collision activated dissociation (CAD)	−2
Declustering potential (DP)	−40 V
Entrance potential (EP)	−10 V

Table 8: Compound-specific MS parameters for FAHFA determination

FAHFA Compound		Q_1 (m/z)	Q_3 (m/z)	Dwell Time (msec)	Collision Energy (CE)	Cell Exit Potential (CXP)
$^{13}C_4$-PAHSA		541.5	259.1	100	−48	−15
PAHSA	Quantifier $[M-H]^-$ Qualifier $[M-H]^-$	537.5 537.5	255.1 281.2	100 100	−48 −38	−10 −1
OAHSA	Quantifier $[M-H]^-$ Qualifier $[M-H]^-$	563.3 563.3	281.0 299.2	100 100	−38 −38	−31 −15
SAHSA	Quantifier $[M-H]^-$ Qualifier $[M-H]^-$	565.3 565.3	282.8 281.1	100 100	−42 −38	−27 −29
PAHPA	Quantifier $[M-H]^-$ Qualifier $[M-H]^-$	509.3 509.3	255.1 253.1	100 100	−36 −34	−27 −25

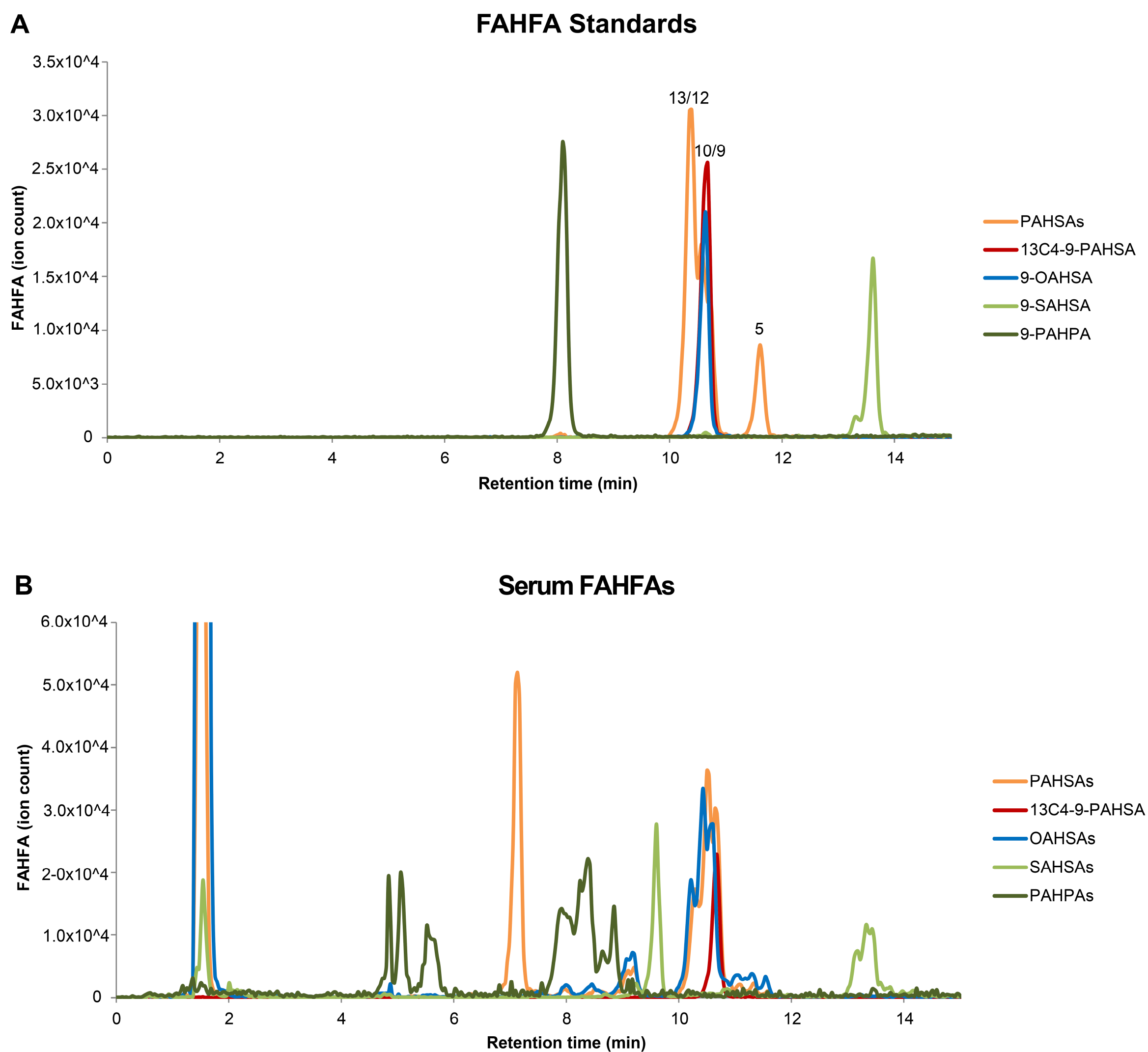

Figure 8: Chromatographic profile of all FAHFA standards (A) and of one exemplary serum sample (B) - first method

The LC-MS method enabled the separation of 5-, 9/10-, and 12/13-PAHSA as well as 9-OAHSA, 9-SAHSA and 9-PAHPA. $^{13}C_4$-9-PAHSA was used as internal standard.

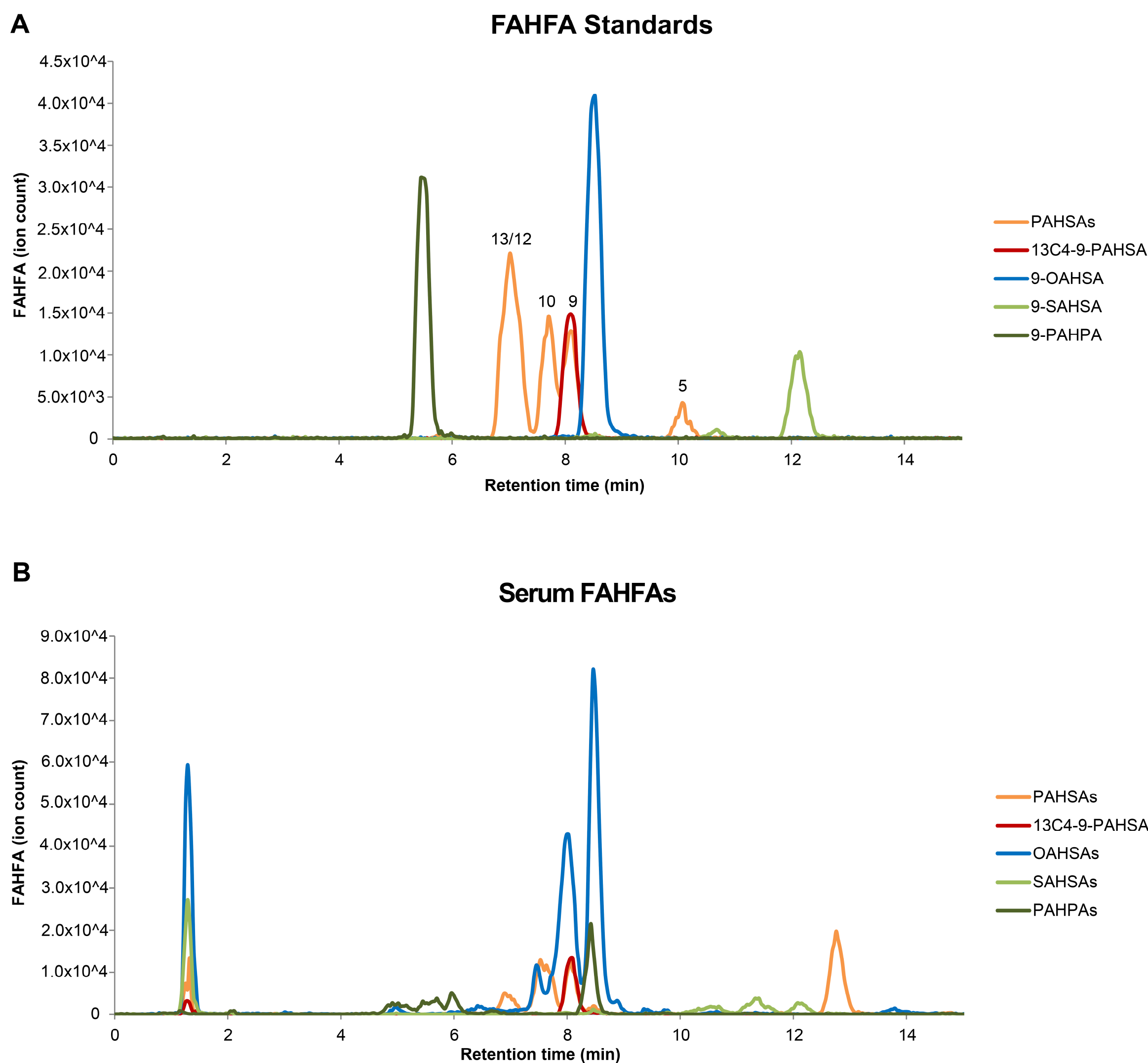

Figure 9: Chromatographic profile of all FAHFA standards (A) and of one exemplary serum sample (B) - improved method

The LC-MS method enabled the separation of 5-, 9-, 10-, and 12/13-PAHSA as well as 9-OAHSA, 9-SAHSA and 9-PAHPA. $^{13}C_4$-9-PAHSA was used as internal standard.

4.8.3 MRM3 Method

Multiple Reaction Monitoring Cube (MRM3) was performed for the different FAHFAs. Each single FAHFA standard at a concentration of 10 µg/ml in methanol:H_2O (1:1) (vol/vol) was used to optimise MRM parameters. The final settings can be found in Table 9. MRM3 enables not only the distinction between different FAHFA isomers, but also between different PAHSA regioisomers, as the branching position on the hydroxy-fatty-acid-backbone can be determined. For example, in a first step, 9-PAHSA is ionised to $[M-H]^-$ ion with a *m/z* ratio of 537.5. Fragmentation of this mother ion produces three different daughter ions: 9-hydroxy-stearic acid (*m/z* 299.3), palmitic acid (*m/z* 255.2) and octadecenoic acid (*m/z* 281.3). A further fragmentation of the hydroxy-stearic acid results in two fragments that are specific for

the position of the hydroxy-group. In the case of 9-hydroxy-stearic acid, the following fragments are produced: *m/z* 127.1 and *m/z* 155.1. Table 10 presents the 1st and 2nd precursor ions for fragmentation of the different FAHFAs and the respective collision energy. MS3 spectra of the different FAHFAs are shown in Figure 10 together with their specific fragmentation. For the analysis of human serum samples MRM3 could not be used for quantification, as the concentrations of the single FAHFAs in the samples were too low for reliable MS3 spectra. However, MRM3 was performed for selected probes with high FAHFA content to validate qualification of different FAHFAs.

Table 9: MRM3 method - fixed parameters

Parameter	Setting
Resolution Q1	Unit
Resolution Q3	LIT
Scan rate	10 000 Da/s
Settling time	0 msec
MR pause	1.5 msec
Q0 trapping	No
LIT fill time	100 msec
Q3 entry barrier	8 V
Excitation time	25 msec
Step size	0.12 Da
Mass range	90-215 Da
AF2	0.14
AF3	m/z dependent standard parameters given by Analyst algorithm
EXB	m/z dependent standard parameters given by Analyst algorithm
CES	1.5 V

Table 10: MRM3 method - Precursor ions and collision energy

FAHFA Compound	1st Precursor	2nd Precursor	Collision Energy (CE)
PAHSA	537.5	299.3	-40
OAHSA	563.3	299.3	-38
SAHSA	565.5	299.1	-38
PAHPA	509.3	271.2	-34

A

9-PAHPA

Chemical Formula: $C_{32}H_{61}O_4^-$
Exact Mass: 509.3

9-Hydroxy-palmitic acid
Chemical Formula: $C_{16}H_{31}O_3^-$
Exact Mass: 271.2

Palmitic acid
Chemical Formula: $C_{16}H_{31}O_2^-$
Exact Mass: 255.2

Hexadecenoic acid
Chemical Formula: $C_{16}H_{29}O_2^-$
Exact Mass: 253.2

Chemical Formula: $C_8H_{15}O^-$
Exact Mass: 127.1

Chemical Formula: $C_8H_{15}O^-$
Exact Mass: 127.1

9-PAHPA

B

9-SAHSA

Chemical Formula: $C_{36}H_{69}O_4^-$
Exact Mass: 565.3

9-Hydroxy-stearic acid
Chemical Formula: $C_{18}H_{35}O_3^-$
Exact Mass: 299.3

Stearic acid
Chemical Formula: $C_{18}H_{35}O_2^-$
Exact Mass: 283.3

Octadecenoic acid
Chemical Formula: $C_{18}H_{33}O_2^-$
Exact Mass: 281.2

Chemical Formula: $C_8H_{15}O^-$
Exact Mass: 127.1

Chemical Formula: $C_{10}H_{19}O^-$
Exact Mass: 155.1

9-SAHSA

C

9-OAHSA

Chemical Formula: $C_{36}H_{67}O_4^-$
Exact Mass: 563.5

9-Hydroxy-stearic acid
Chemical Formula: $C_{18}H_{35}O_3^-$
Exact Mass: 299.3

Oleic acid
Chemical Formula: $C_{18}H_{33}O_2^-$
Exact Mass: 281.3

Octadecenoic acid
Chemical Formula: $C_{18}H_{33}O_2^-$
Exact Mass: 281.3

Chemical Formula: $C_8H_{15}O^-$
Exact Mass: 127.1

Chemical Formula: $C_{10}H_{19}O^-$
Exact Mass: 155.1

9-OAHSA

D

9-PAHSA

Chemical Formula: $C_{34}H_{65}O_4^-$
Exact Mass: 537.5

9-Hydroxy-stearic acid
Chemical Formula: $C_{18}H_{35}O_3^-$
Exact Mass: 299.3

Palmitic acid
Chemical Formula: $C_{16}H_{31}O_2^-$
Exact Mass: 255.2

Octadecenoic acid
Chemical Formula: $C_{18}H_{33}O_2^-$
Exact Mass: 281.3

Chemical Formula: $C_8H_{15}O^-$
Exact Mass: 127.1

Chemical Formula: $C_{10}H_{19}O^-$
Exact Mass: 155.1

9-PAHSA

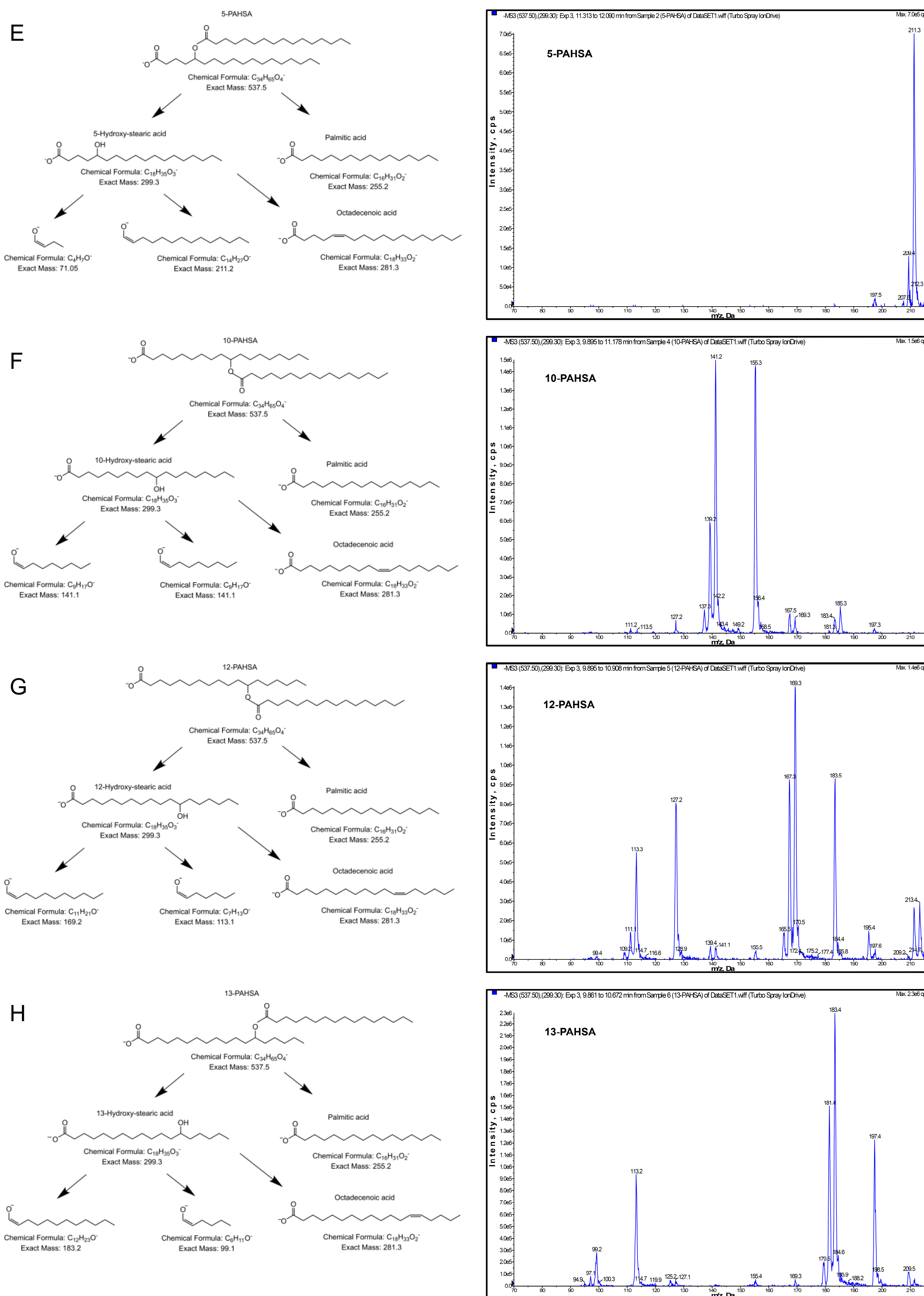

Figure 10: MRM3 method – specific fragmentation scheme and MS3 spectra for different FAHFAs

MS3 spectrum and MS3 fragmentation scheme of 9-PAHPA (A), 9-SAHSA (B), 9-OAHSA (C), 9-PAHSA (D), 5-PAHSA (E), 10-PAHSA (F), 12-PAHSA (G) and 13-PAHSA (H).

4.9 Human Study cohorts for FAHFA analysis

Serum samples were collected within the framework of different clinical studies conducted in the same study unit (Human Study Center, Freising, Germany). Written informed content was obtained from all participants before their inclusion in the study.

The cohort of obese patients undergoing bariatric surgery together with non-obese controls originated from the study on gut permeability as described in the first part of this thesis (Kellerer et al., 2019). The n number differs due to in- or exclusion of patients with medications that might influence the respective analysis.

For the comparison of obese patients with and without diabetes, samples were provided from the German Obesity Biomaterial Bank (GOBB). The samples were collected during bariatric surgery. Subjects were classified as obese diabetic with BMI ≥ 40 kg/m^2 and HbA1c ≥ 6.5% and as obese non-diabetic with BMI ≥ 40 kg/m^2 and HbA1c < 5.7%. Both groups consisted of each 5 males and 5 females. None of the patients did regularly take insulin or oral antidiabetic medication. Values for blood glucose, HbA1c and CRP were collected from the lab reports of the clinic.

To investigate the influence of age, three different age cohorts were used which were recruited and characterized in the course of the *enable* cluster (DRKS00009797). This cohort study investigated, inter alia, anthropometrics, body composition, energy metabolism and nutrition in four defined phases of life including children (3–5 years), adolescents (18–25 years), middle-aged adults (40–65 years), and elderly people (75–85 years). The *enable* study participants were otherwise healthy and without any acute severe diseases. For the analysis of FAHFAs each 10 females and 10 males were used from the group of adolescents, middle-aged adults and elderly people (n=60 in total).

The influence of nutrition was evaluated in vegetarians/vegans (n=10) and in omnivores (n=5) from the JPI-project FOODBALL (Brouwer-Brolsma et al., 2017) (DRKS00010133) as well as in omnivores from the study on gut permeability (n=4) of which 2 subjects were undergoing laparoscopic abdominal surgery. Omnivores were a cross-sectional group of healthy, lean (BMI < 25 kg/m^2) volunteers that habitually followed a normal mixed diet including meat and fish (3 females, 6 males) and vegetarians/vegans were a group of vegetarian or vegan volunteers practicing a vegan diet for at least seven days (7 females, 3 males).

The group of men undergoing one week overfeeding intervention originated from a recently published study cohort (Ott et al., 2018) (DRKS00006211). In this overfeeding study lean (BMI < 27 kg/m^2), healthy, young men were provided with a surplus of 1000 kcal above their

calculated energy demand based on their measured resting metabolic rate. Saturated fatty acids from whipping cream were thereby the main source of the additional kcals.

Anthropometric and biochemical analyses were performed as described in the respective study protocol and in brief as follows.

4.9.1 Anthropometric measurements

Anthropometric measurements were made without shoes, outdoor clothing such as jackets and coats, and accessories such as bags and keys. These measurements were corrected by one kg to account for residual clothing. In the *enable*, FOODBALL and overnutrition study, measurements were performed in underwear without correction. Height was determined using a stadiometer and body weight with a calibrated standard scale (Seca, Hamburg, Germany). BMI (kg/m^2) was calculated by dividing body weight (kg) by height in meter squared (m^2).

4.9.2 Blood samples and biochemical analyses

Blood samples were drawn in a fasting state. In patients with severe obesity, blood samples were taken during bariatric surgery in the cooperating clinic. Blood was drawn into a serum monovette (S-Monovette® 7.5 ml Z-Gel, 01.1602, Sarstedt, Nümbrecht, Germany), allowed to clot for 30 min at room temperature and subsequently centrifuged at 2,500 g for 10 min at room temperature (FOODBALL project: centrifugation at 4 °C). Serum was aliquoted and stored at -80 °C until analysis. Non-esterified fatty acids (NEFAs) were measured in plasma using a commercial test kit (Wako Chemicals GmbH, Neuss, Germany). Blood glucose was determined either by SynLab (Munich, Germany) or in frozen blood plasma with Hemocue (HemoCue AB, Ängelholm, Sweden). Blood glucose of the patients with obesity was recorded from the lab reports of the respective clinic. Insulin in plasma was either quantified by Synlab (Munich, Germany) or by commercially available ELISA kits (DRG Diagnostics, Marburg, Germany; DAKO, Glostrup, Denmark). HOMA-IR was calculated according to Matthews (Matthews et al., 1985): HOMA-IR = insulin (µU/mL) × glucose (mmol/L) / 22.5.

4.10 Statistical analysis

Statistical analyses and graphical outputs are performed with GraphPad Prism 5 (GraphPad Software, San Diego California, USA) and the R programming environment (R studio, Version 3.3.2). Boxplots are defined as follows: the bottom and the top of the box are defined by the 25th and 75th percentile and the centre by the median. The whiskers reach until the lowest data point still within 1.5 IQR (inter quartile range) of the lower quartile, and the highest data point still within 1.5 IQR of the upper quartile. Outliers are indicated by dots.

Data are presented as median (25th percentile; 75th percentile), unless otherwise stated.

Data were checked for normal distribution using Shapiro-Wilk normality test. If all groups were normally distributed, parametric tests were used. Otherwise, the non-parametric alternative was applied. For the comparison of obese patients before and after surgery (LSG V1, LSG V2) the paired *t*-test / Wilcoxon Matched-Pairs test was used. For the comparison of obese patients with non-obese controls the unpaired *t*-test / Mann-Whitney test was used. For the comparison of obese diabetic and obese non-diabetic patients unpaired *t*-test / Mann-Whitney test was used. For the comparison of gut barrier function in dependence of BMI, Kruskal–Wallis and Dunn's multiple comparison tests were employed. P-values < 0.05 are regarded as statistically significant. N.s. = not significant ($p \geq 0.05$). Asterisks indicate significance levels as follows: *$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$.

Sample size calculation for gut permeability was based on recently published data (Ott et al., 2017) using *t*-test assessing differences before and after the intervention for lactulose and zonulin. Regarding lactulose a study with an effect size of 1.1 will require a total sample number of 11. An effect size of 0.9 for zonulin revealed a subject number of 8. These numbers would give a power of 80% and an association at 5%. Power calculations were performed with the R-package pwr.

For the analysis of faecal microbiota the programme Rhea was used (Lagkouvardos et al., 2017) and analysis was performed as described in section 4.7.3.

For FAHFA analysis, nonparametric tests were used due to small sample size and non-Gaussian distribution of most of the FAHFA data. The Wilcoxon matched-pairs test was used to compare the same group at two different time points. Mann-Whitney test was used to compare two independent groups. Differences in the three different age groups were detected by the Kruskal-Wallis test and Dunn’s post test. Correlation analysis was performed by Spearman correlation. For multiple linear regression analysis all data were $\log_{10}$ transformed.

Images showing chemical structures, e.g. FAHFAs, were drawn with ChemDraw software (ChemDraw Professional 16.0., PerkinElmer Inc.).

5. Results

5.1 Bariatric surgery and intestinal permeability

In the following, the results of the human study investigating intestinal permeability in dependence of weight and weight loss after bariatric surgery are presented. Parts of the results of this human study have previously been published (Kellerer et al., 2019). The figures and tables containing data that have already been published in this publication are marked with an asterisk (*). If only parts of a table / figure were published in this publication, the corresponding table rows / parts of the figure are marked with an asterisk (*). Also parts of the corresponding text in the methods, results and discussion section have been published in this publication.

Twenty-five obese subjects undergoing LSG were initially recruited. Two study patients did not complete the visit 6 months after surgery. Six study patients had to be excluded because of intake of antibiotics, NSAIDs or corticosteroids during the study period. As controls, 23 subjects were initially recruited. One patient did not complete the study. Analyses are performed with 17 obese study patients who completed the study and with 17 control subjects who were matched in case of age and sex to the obese patients. Three of the 17 subjects in each group were male, while the rest were female. The mean age (± SD) of obese study patients was 41.8 ± 9.1 years and the mean age of control subjects was 41.7 ± 9.7 years. Additional information about the study patients, including lifestyle, is presented in the Supplemental Table 1.

5.1.1 Anthropometric measurements

Anthropometric parameters of study participants are presented in Table 11. The obese study patients undergoing LSG could reduce their body weight by 41.0 (33.7; 46.5) kg. This corresponds to a total weight loss (%TWL) of 26.0% (22.3%; 30.3%) and 50.9% (40.4%; 64.8%) excessive weight loss (%EWL). Within the 6 months after surgery, the BMI of the patients declined by 13.8 (11.8; 15.7) kg/m^2. Both waist as well as hip circumference decreased significantly. As the decrease in waist (-30.3 (-38.1; -22.0) cm) was more prominent than the decrease in hip circumference (-20.2 (-27.6; -15.8) cm) the waist-to-hip ratio also declined significantly (-0.07 (-0.11; -0.02)). The obese patients had a significantly greater waist-to-hip ratio compared to the non-obese controls, but this difference was not significant any more after surgery. Waist and hip circumference, however, were still significantly higher compared to the controls. The body composition of non-obese controls and of part of the obese patients after surgery was measured by bioelectrical impedance analysis. Data can be found in Supplemental Table 2.

Table 11*: Anthropometric data of study participants

	n	Median (25th percentile; 75th percentile)			p-value		
		LSG V1	LSG V2	Controls	LSG V1 – LSG V2	LSG V1 - Controls	LSG V2 - Controls
Body weight (kg)	17	152.0 (133.9; 175.7)	111.8 (95.2; 136.7)	62.7 (56.8; 73.6)	*** <0.001	*** <0.001	*** <0.001
Body mass index (kg/m²)	17	52.5 (47.0; 56.8)	39.1 (32.6; 44.0)	21.5 (19.6; 23.3)	*** <0.001	*** <0.001	*** <0.001
Waist circumference (cm)	14	144 (129; 156)	109 (103; 128)	74 (69; 80)	*** <0.001	*** < 0.001	*** <0.001
Hip circumference (cm)	14	152 (139; 172)	135 (116; 148)	95 (86; 98)	*** <0.001	*** < 0.001	*** <0.001
Waist-to-hip ratio	14	0.87 (0.82; 1.04)	0.83 (0.78; 0.92)	0.81 (0.74; 0.86)	** 0.007	** 0.006	n.s. 0.460

Depending on the distribution of the data paired *t*-test or Wilcoxon matched-pairs test was used to compare obese patient before and after surgery (LSG V1 and LSG V2) and unpaired *t*-test or Mann-Whitney test to compare obese patients with non-obese controls. n.s. = not significant, ** $p < 0.01$, and *** $p < 0.001$.

Weight loss (kg) at 6 months after surgery was neither dependent on initial body weight nor on initial BMI (Table 12). Though, Pearson correlation revealed a significant negative association between the initial body weight and the %EWL (Figure 11). The higher the initial body weight was, the lower was the %EWL. The coefficient of determination of the corresponding linear regression was $R^2 = 0.041$.

Table 12: Association of initial body weight and BMI with weight loss parameters

	r	p-value	
Weight loss / Body weight V1	-0.279	n.s.	0.278
%TWL / Body weight V1	-0.351	n.s.	0.167
%EWL / Body weight V1	-0.641	**	0.006
Weight loss / BMI V1	-0.466	n.s.	0.059
%TWL / BMI V1	-0.046	n.s.	0.861
%EWL / BMI V1	-0.450	n.s.	0.070

Pearson correlation was used for correlation analysis. n = 17. n.s. = not significant, ** $p < 0.01$.

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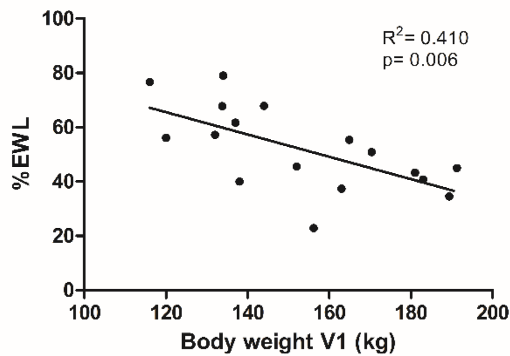

Figure 11: Association between percentage excessive weight loss (%EWL) and initial body weight of patients undergoing sleeve gastrectomy

Linear regression was performed. %EWL was plotted against pre-operative body weight. n = 17.

5.1.2 Dietary behaviour

The study participants reported their diet in a 7-day dietary protocol. Six patients were regularly taking dietary supplements before surgery (one patient taking multivitamins) and nine patients after surgery (five patients taking multivitamins). From the control group, two subjects were regularly taking dietary supplementation (one patient taking multivitamins). A list of the supplements and their nutrient composition can be found in the supplemental data (Supplemental Table 3).

According to the dietary protocols, the median total caloric intake of the obese patients before surgery was 1826 kcal/day and was significantly reduced by 51.7% six months after LSG to 882 kcal/day (Figure 12, Table 13). However, when regarding the macronutrient composition of the diet, the percentage portion of carbohydrates, fat and protein did not change significantly (Table 13, Figure 12). The total amount of all nutrients, micronutrients and vitamins was reduced after surgery, and this reduction was significant in most cases (Table 13). The median total caloric intake of the obese patients before surgery was not significantly different to the controls. Also the dietary composition was comparable except for significantly lower water and alcohol intake and higher protein intake in the obese group (%kcal) (Table 13).

Table 13: Dietary intake of total calories, water and different nutrients

	Median (25th percentile; 75th percentile)			p-value		
	LSG V1	LSG V2	Controls	LSG V1 - LSG V2	LSG V1 - Controls	LSG V2 - Controls
Total caloric intake / day (kcal/day)	1826 (1450; 2117)	882 (788; 1087)	1979 (1726; 2370)	*** <0.001	n.s. 0.136	*** <0.001
Fat intake (g/day)	67.7 (56.9; 84.2)	37.0 (29.4; 41.7)	76.5 (68.3; 104.0)	*** <0.001	n.s. 0.063	*** <0.001
Fat intake (%kcal)	34.8 (31.6; 37.3)	34.1 (31.0; 39.1)	36.6 (34.0; 41.2)	n.s. 0.704	n.s. 0.210	n.s. 0.530
Carbohydrate intake (g/day)	207.6 (168.2; 226.3)	101.9 (74.0; 122.3)	218.2 (191.6; 255.8)	*** <0.001	n.s. 0.191	*** <0.001
Carbohydrate intake (%kcal)	46.5 (41.4; 49.2)	43.2 (39.6; 48.2)	44.0 (41.3; 49.8)	n.s. 0.185	n.s. 0.744	n.s. 0.558
Protein intake (g/day)	79.5 (65.7; 96.4)	38.2 (34.4; 60.2)	69.6 (64.2; 82.7)	*** <0.001	n.s. 0.389	*** <0.001
Protein intake (%kcal)	17.5 (16.6; 19.9)	18.7 (16.4; 24.3)	16.1 (13.4; 17.2)	n.s. 0.126	** 0.002	*** <0.001
Fibre intake (g/day)	19.5 (14.3; 22.7)	8.2 (6.1; 10.5)	20.5 (17.3; 24.7)	*** <0.001	n.s. 0.285	*** <0.001
Water intake (l/day)	2.48 (1.84; 2.89)	1.71 (1.49; 2.11)	3.12 (2.21; 3.52)	* 0.025	* 0.035	*** <0.001
Sucrose intake (g/day)	35.7 (21.2; 47.6)	19.4 (17.7; 29.6)	42.5 (31.5; 50.7)	** 0.006	n.s. 0.130	*** <0.001
Alcohol intake (g/day)	0.2 (0.0; 0.9)	0.0 (0.0; 0.2)	3.2 (0.8; 8.6)	* 0.025	*** <0.001	*** <0.001
Saturated fatty acids (g/day)	28.5 (22.4; 32.4)	14.5 (12.9; 17.9)	31.5 (27.2; 49.8)	*** <0.001	n.s. 0.073	*** <0.001
n3 – Fatty acids (g/day)	1.4 (0.9; 2.0)	0.7 (0.5; 1.1)	1.4 (1.0; 2.2)	** 0.004	n.s. 0.582	*** <0.001
n6 – Fatty acids (g/day)	9.7 (8.3; 14.9)	4.3 (3.7; 5.8)	11.6 (9.2; 16.1)	*** <0.001	n.s. 0.256	*** <0.001
Cholesterol intake (mg/day)	251 (212; 416)	158 (112; 219)	259 (222; 340)	** 0.001	n.s. 0.904	*** <0.001
Sodium (g/day)	2.5 (1.9; 3.4)	1.2 (1.0; 1.8)	2.3 (2.0; 2.8)	*** <0.001	n.s. 0.740	*** <0.001
Potassium (g/day)	2.8 (2.1; 3.3)	1.5 (1.3; 1.9)	2.8 (2.4; 3.2)	*** <0.001	n.s. 0.674	*** <0.001
Calcium (mg/day)	813 (548; 890)	577 (423; 1252)	837 (741; 1231)	n.s. 0.6360	n.s. 0.215	n.s. 0.098
Magnesium (mg/day)	302 (239; 336)	199 (164; 273)	317 (278; 422)	** 0.002	n.s. 0.318	*** <0.001
Phosphorus (mg/day)	1190 (972; 1481)	710 (547; 1057)	1221 (1095; 1520)	*** <0.001	n.s. 0.718	*** <0.001
Iron (mg/day)	11.3 (9.0; 13.3)	5.2 (4.5; 8.6)	9.9 (9.2; 12.8)	** 0.002	n.s. 1.000	** 0.001

	Median (25th percentile; 75th percentile)			p-value		
	LSG V1	**LSG V2**	**Controls**	**LSG V1 - LSG V2**	**LSG V1 - Controls**	**LSG V2 - Controls**
Zinc (mg/day)	9.8 (7.9; 11.5)	6.7 (4.3; 10.5)	9.6 (8.5; 12.8)	* 0.020	n.s. 0.679	** 0.004
Copper (mg/day)	1.6 (1.3; 2.0)	1.0 (0.9; 1.3)	1.7 (1.5; 2.0)	** 0.004	n.s. 0.513	*** <0.001
Manganese (mg/day)	4.0 (3.1; 5.1)	2.2 (1.7; 3.1)	5.4 (3.8; 6.8)	** 0.005	n.s. 0.098	*** <0.001
Iodine (µg/day)	94 (62; 111)	78 (48; 148)	101 (80; 128)	n.s. 0.855	n.s. 0.483	n.s. 0.720
Retinol equivalent (mg/day)	0.9 (0.7; 1.9)	0.6 (0.5; 1.2)	1.3 (1.0; 1.8)	* 0.037	n.s. 0.139	** 0.005
Vitamin A (Retinol) (mg/day)	0.3 (0.2; 0.6)	0.2 (0.2; 0.5)	0.4 (0.3; 0.6)	n.s. 0.218	n.s. 0.428	** 0.007
Vitamin D (Calciferol) (µg/day)	2.6 (1.1; 19.3)	1.6 (1.1; 18.6)	2.7 (1.4; 4.6)	n.s. 0.938	n.s. 0.877	n.s. 0.523
Vitamin E (Tocopherol) (mg/day)	12.3 (6.7; 16.9)	7.3 (4.9; 13.8)	13.5 (10.7; 16.7)	* 0.042	n.s. 0.558	** 0.008
Vitamin K (µg/day)	97.6 (53.4; 150.0)	48.1 (25.0; 103.3)	140.9 (74.2; 192)	n.s. 0.098	n.s. 0.148	** 0.003
Vitamin B1 (Thiamine) (mg/day)	1.2 (1.0; 1.3)	0.6 (0.5; 1.7)	1.1 (0.9; 1.5)	n.s. 0.088	n.s. 0.667	* 0.0496
Vitamin B2 (Riboflavin) (mg/day)	1.4 (0.9; 1.8)	0.9 (0.7; 2.4)	1.3 (1.1; 1.7)	n.s. 0.218	n.s. 0.973	n.s. 0.270
Niacin equivalent (mg/day)	30.1 (27.1; 38.7)	16.5 (13.7; 24.0)	28.6 (25.9; 32.8)	*** <0.001	n.s. 0.558	** 0.001
Pantothenic acid (mg/day)	4.4 (3.1; 5.6)	2.9 (1.9; 7.0)	3.9 (3.5; 5.2)	n.s. 0.320	n.s. 0.836	n.s. 0.158
Vitamin B6 (Pyridoxine) (mg/day)	1.6 (1.2; 1.9)	0.8 (0.7; 2.1)	1.5 (1.4; 1.7)	n.s. 0.098	n.s. 0.931	* 0.025
Biotin (µg/day)	45.6 (28.1; 54.6)	31.6 (20.0; 72.1)	44.9 (31.6; 51.1)	n.s. 1.000	n.s. 0.918	n.s. 0.256
Folic acid (µg/day)	220 (159; 313)	167 (95; 338)	254 (207; 292)	n.s. 0.080	n.s. 0.318	n.s. 0.091
Vitamin B12 (Cobalamin) (µg/day)	4.6 (3.4; 5.8)	4.0 (1.9; 7.7)	4.3 (3.2; 5.2)	n.s. 0.449	n.s. 0.617	n.s. 0.931
Vitamin C (Ascorbic acid) (mg/day)	91.6 (63.7; 140.6)	65.1 (25.4; 126.7)	128.8 (79.8; 156.7)	n.s. 0.130	n.s. 0.168	* 0.011

Data were analysed from 7-day dietary protocols with the use of OptiDiet Plus. Depending on the distribution of the data paired *t*-test or Wilcoxon matched-pairs test was used to compare obese patient before and after surgery (LSG V1 and LSG V2) and unpaired *t*-test or Mann-Whitney test to compare obese patients with non-obese controls. n = 17 per group. n.s. = not significant, * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$.

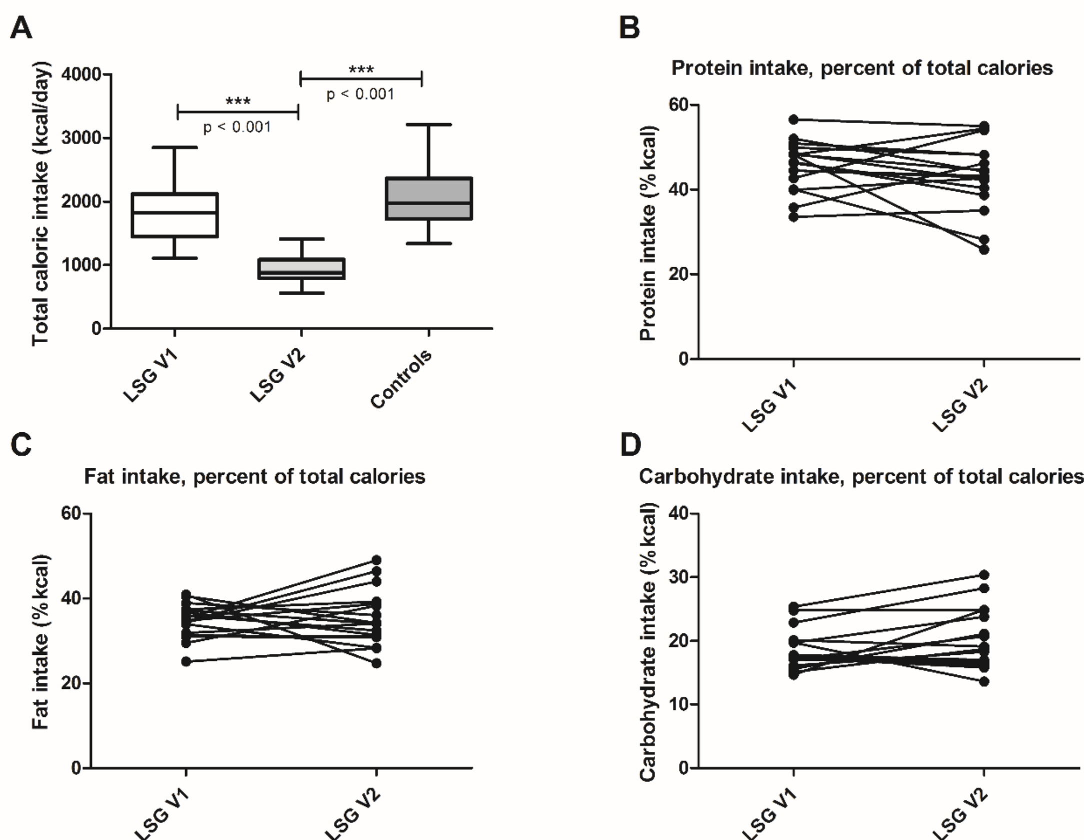

Figure 12*: Caloric intake before and after sleeve gastrectomy

A) Total caloric energy intake per day.

B) Percentage energy intake of calories from the different macronutrients (carbohydrates, fat, protein).

Depending on distribution of the data paired *t*-test or Wilcoxon matched-pairs test was used to compare obese patient before and after surgery (LSG V1 and LSG V2) and unpaired *t*-test or Mann-Whitney test to compare obese patients with non-obese controls. n = 17 per group. *** $p < 0.001$.

5.1.3 Glucose metabolism

According to the anamnesis, six obese patients had a diagnosed diabetes mellitus type 2 before undergoing LSG. Diabetic patients were treated by metformin or nutritional therapy. None of them injected insulin. Regarding controls, no one had previously diagnosed diabetes, and blood glucose levels measured during the study were within the normal range, with the exception of two subjects who had fasting values above 100 mg/dl (101 and 106 mg/dl), which indicates impaired fasting glucose. In patients with obesity fasting blood glucose and fasting insulin were all higher compared to lean participants and dropped after surgery (Table 14). Insulin was still significantly elevated compared to controls 6 months after surgery. Hb1Ac values dropped significantly from 5.4% to 5.3% after surgery (Table 14).

All of the diabetic patients undergoing bariatric surgery could stop diabetes medication and returned to normal blood glucose values. From the patients undergoing surgery, the patients

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with diagnosed diabetes mellitus type 2 had significantly higher blood glucose and insulin values compared to the non-diabetic patients, while HbA1c was not significantly different (Table 15).

Table 14*: Laboratory values of glucose metabolism in study participants

		Median (25th percentile; 75th percentile)			p-value		
	n	LSG V1	LSG V2	Controls	LSG V1 – LSG V2	LSG V1 – Controls	LSG V2 – Controls
Fasting blood glucose (mg/dl)*	15	91 (80; 101)	79 (69; 89)	89 (82; 94)	** 0.002	n.s. 0.442	* 0.036
Fasting insulin (µU/ml)*	15	51.9 (27.4; 70.5)	31.2 (16.5; 42.0)	12.4 (8.9; 19.9)	* 0.015	*** <0.001	** 0.009
HbA1c (%)	11	5.4 (5.3; 5.7)	5.3 (5.1; 5.4)	NA	* 0.013	NA	NA

Wilcoxon matched-pairs test was used to compare obese patient before and after surgery (LSG V1 and LSG V2) and Mann-Whitney test to compare obese patients with non-obese controls. n.s. = not significant, * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$.

Table 15: Comparison of laboratory values of glucose metabolism between obese patients with and without diabetes mellitus type 2

		Median (25th percentile; 75th percentile)		p-value
	n (non-diabetic/ diabetic)	LSG V1 non-diabetic	LSG V1 diabetic	
Fasting blood glucose (mg/dl)	9/6	82 (78; 99)	99 (91; 115)	n.s. 0.070
Fasting insulin (µU/ml)	9/6	31.1 (26.9; 55.4)	69.6 (47.1; 74.7)	* 0.031
HbA1c (%)	8/4	5.4 (5.3; 5.6)	5.8 (5.4; 6.6)	n.s. 0.338

Mann-Whitney test was used to compare the groups. n.s. = not significant, * $p < 0.05$.

5.1.4 Metabolic and inflammatory parameters

To gain an overview of overall health status of the study participants, laboratory routine parameters were collected and are listed in Table 16. In comparison to non-obese subjects, patients with obesity exhibited a more unfavourable blood lipid profile (higher triglycerides, lower high-density lipoprotein (HDL), higher LDL/HDL ratio), higher γ-glutamyltransferase (γ-GT) values, higher amount of leucocytes, and higher levels of thyroid-stimulating hormone

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(TSH), as well as lower mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) and lower bilirubin. Most of those parameters changed in the direction of the non-obese controls after surgery.

Table 16: Metabolic profile of study participants

		Median (25th percentile; 75th percentile)			p-value		
	n	LSG V1	LSG V2	Controls	LSG V1 - LSG V2	LSG V1 - Controls	LSG V2 - Controls
ASAT (U/l)	10	27 (18; 41)	16 (15; 23)	21 (18; 23)	* 0.018	n.s. 0.240	n.s. 0.087
ALAT (U/l)	17	27 (14; 42)	17 (13; 28)	19 (17; 25)	n.s. 0.074	n.s. 0.407	n.s. 0.398
γ-GT (U/l)	16	30 (21; 41)	16 (10; 21)	13 (9; 19)	*** <0.001	*** <0.001	n.s. 0.428
Creatinine (mg/dl)	16	0.72 (0.64; 0.80)	0.70 (0.60; 0.80)	0.77 (0.69; 0.81)	n.s. 0.245	n.s. 0.355	n.s. 0.065
Uric acid (mg/dl)	7	5.9 (3.9; 8.7)	5.4 (3.9; 6.7)	4.8 (4.2; 4.9)	n.s. 0.256	n.s. 0.077	n.s. 0.218
Bilirubin (mg/dl)	13	0.38 (0.24; 0.60)	0.59 (0.49; 0.80)	0.58 (0.46; 0.63)	** 0.003	* 0.031	n.s. 0.644
Sodium (mmol/l)	15	140 (139; 143)	142 (141; 143)	140 (139; 142)	* 0.047	n.s. 1.000	n.s. 0.066
Potassium (mmol/l)	16	4.3 (4.2; 4.5)	4.0 (3.8; 4.4)	4.3 (4.2; 4.5)	n.s. 0.058	n.s. 0.730	* 0.023
Calcium (mmol/l)	11	2.38 (2.34; 2.45)	2.35 (2.31; 2.42)	2.31 (2.31; 2.36)	n.s. 0.838	n.s. 0.138	n.s. 0.337
Triglycerides (mg/dl)	11	178 (108; 216)	96 (75; 156)	66 (42; 126)	* 0.018	** 0.004	n.s. 0.093
Total cholesterol (mg/dl)	11	195 (166; 224)	194 (155; 223)	196 (155; 246)	n.s. 0.588	n.s. 0.583	n.s. 0.468
LDL (mg/dl)	11	111 (90; 149)	126 (86; 145)	134 (89; 144)	n.s. 0.736	n.s. 0.807	n.s. 0.950
HDL (mg/dl)	11	47 (38; 52)	50 (44; 56)	74 (61; 84)	n.s. 0.298	*** <0.001	*** <0.001
LDL/HDL	11	2.37 (2.10; 3.20)	2.28 (1.91; 3.10)	1.56 (1.17; 2.36)	n.s. 0.493	* 0.014	* 0.029
Thrombocytes (G/l)	14	285 (251; 345)	278 (234; 301)	247 (213; 286)	n.s. 0.114	n.s. 0.212	n.s. 0.822
Leucocytes (G/l)	14	8.3 (6.8; 9.6)	6.2 (5.4; 7.6)	5.1 (4.0; 7.1)	** 0.001	*** <0.001	n.s. 0.128
Erythrocytes (T/l)	14	4.8 (4.7; 5.2)	4.5 (4.3; 4.9)	4.7 (4.5; 4.9)	** 0.007	n.s. 0.210	n.s. 0.525
Haemoglobin (g/dl)	14	14.2 (13.3; 15.2)	13.2 (12.9; 15.1)	14.4 (13.7; 14.6)	* 0.013	n.s. 0.947	n.s. 0.181

		Median (25th percentile; 75th percentile)			p-value		
	n	LSG V1	LSG V2	Controls	LSG V1 - LSG V2	LSG V1 - Controls	LSG V2 - Controls
Haematocrit (%)	14	43.4 (41.4; 47.0)	40.6 (38.7; 44.4)	40.8 (39.6; 43.0)	* 0.012	n.s. 0.069	n.s. 0.936
MCH (pg/Ery)	14	28.6 (28.0; 30.2)	30.0 (28.8; 30.3)	30.6 (29.5; 31.0)	n.s. 0.235	* 0.042	n.s. 0.129
MCV (fl)	14	89.6 (86.0; 91.9)	89.9 (85.9; 92.0)	87.4 (85.1; 91.1)	n.s. 0.687	n.s. 0.367	n.s. 0.632
MCHC (g/dl)	14	32.8 (32.2; 33.7)	33.0 (32.0; 34.0)	34.6 (34.0; 35.3)	n.s. 0.812	*** <0.001	*** <0.001
TSH (µU/ml)	13	1.9 (1.4; 2.7)	2.6 (1.1; 2.7)	1.3 (0.7; 1.4)	n.s. 0.685	* 0.012	n.s. 0.058

Depending on the distribution of the data paired *t*-test or Wilcoxon matched-pairs test was used to compare obese patient before and after surgery (LSG V1 and LSG V2) and unpaired *t*-test or Mann-Whitney test to compare obese patients with non-obese controls. n.s. = not significant, * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$.

In addition to the routine parameters, which were analysed in an external laboratory or provided by the clinic, levels of CRP and LBP, markers of systemic and gut-derived inflammation, were analysed by ELISA, as well as levels of NEFAs, which were measured by a commercial test kit. The level of NEFAs and CRP were markedly elevated in subjects with obesity compared to non-obese controls (NEFAs: 1.0 vs. 0.3 mmol/l; CRP: 0.56 vs. 0.03 mg/dl). After bariatric surgery, levels were significantly reduced (NEFAs: 0.64 mg/dl; CRP: 0.35 mg/dl), however, still higher than those of controls (Figure 13A and13C). LBP was also significantly increased in obese patients before surgery in comparison to non-obese controls (0.56 vs. 0.43 mg/dl), but did not significantly decrease afterwards (Figure 13B). No significant differences in the levels of NEFAs, CRP and LBP were found between obese patients with and without diabetes (Table 17). In obese patients, CRP levels correlated positively and strongly with glucose levels (rho = 0.739; $p < 0.001$; Supplemental Table 4). This was the only significant correlation between inflammatory markers and markers of glucose metabolism in the obese patients and no correlation was found in the non-obese subjects (Supplemental Table 5).

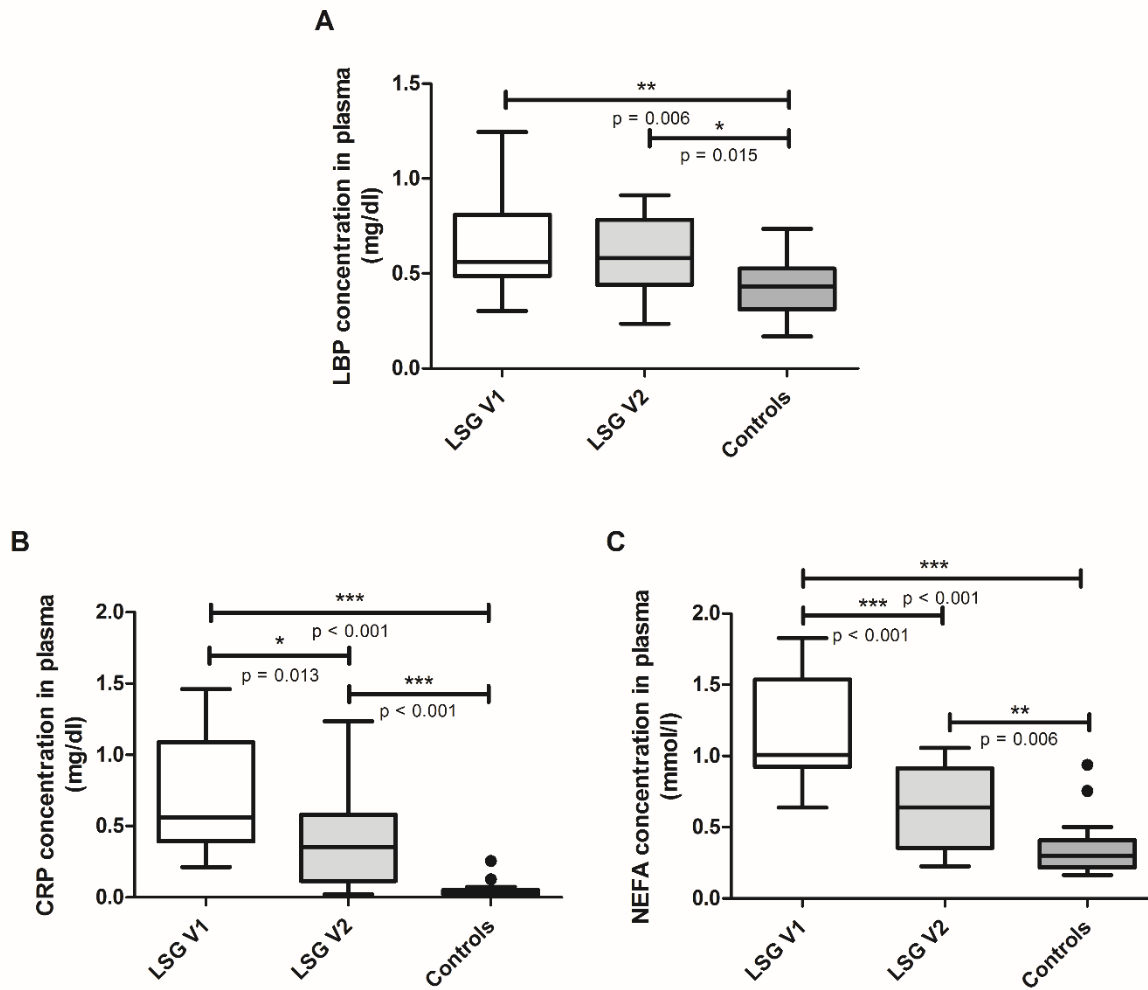

Figure 13*: Plasma concentration of CRP (A), LBP (B), and NEFAs (C)

Depending on the distribution of the data paired *t*-test or Wilcoxon matched-pairs test was used to compare obese patient before and after surgery (LSG V1 and LSG V2) and unpaired *t*-test or Mann-Whitney test to compare obese patients with non-obese controls. n = 15 per group. * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$.

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Table 17: Comparison of CRP, LBP and NEFA levels between obese patients with and without diabetes mellitus type 2

		Median (25th percentile; 75th percentile)		p-value
	n (non-diabetic/ diabetic)	LSG V1 non-diabetic	LSG V1 diabetic	
CRP concentration in plasma (mg/dl)	9/6	0.5 (0.3; 1.0)	0.7 (0.5; 1.4)	n.s. 0.207
LBP concentration in plasma (mg/dl)	9/6	0.6 (0.5; 0.8)	0.6 (0.4; 1.1)	n.s. 0.953
NEFA concentration in plasma (mmol/l)	9/6	1.0 (0.7; 1.2)	1.2 (0.9; 1.7)	n.s. 0.440

Mann-Whitney test was used to compare the groups. n.s. = not significant.

5.1.5 Gut permeability after LSG

A standardised 4-probe sugar test (Norman et al., 2012) was used to assess permeability within different sections of the gastrointestinal tract (Figure 14A-E). Lactulose is a marker of the paracellular route of small intestinal permeability. Urinary recovery of lactulose was decreased in patients with obesity after LSG compared to the status before LSG (0.19 vs. 0.31 %urine recovery; p = 0.008). Moreover, urinary recovery of mannitol, a marker of the transcellular route in the small intestine, was decreased as well (8.7 vs. 12.4 %urine recovery; p = 0.012). The lactulose/mannitol ratio, often used as a small intestinal permeability index, was not altered by surgery-induced weight loss. Urinary recovery of sucrose was decreased after surgery (0.08 vs. 0.18 %urine recovery; p = 0.012), indicating decreased gastroduodenal permeability. On the contrary, excretion of sucralose increased (1.24 vs. 0.40 %urine recovery; p = 0.002), stating an increased permeability of the colon. When comparing non-obese and obese patients before surgery, no significant difference was found for any of the four sugars (Figure 14A-E).

In addition to the 4-probe sugar test, the concentration of zonulin, a protein regulating tight junction function in the small intestine, was measured in plasma (Figure 14F). The concentration of zonulin did not change after LSG. There was no difference between the zonulin concentration of obese patients before surgery and non-obese controls, but obese patients after surgery had slightly lower zonulin levels compared to non-obese controls.

Markers of gut permeability were also compared between obese patients with and without diabetes mellitus type 2 and no significant differences were found between those two groups (Table 18).

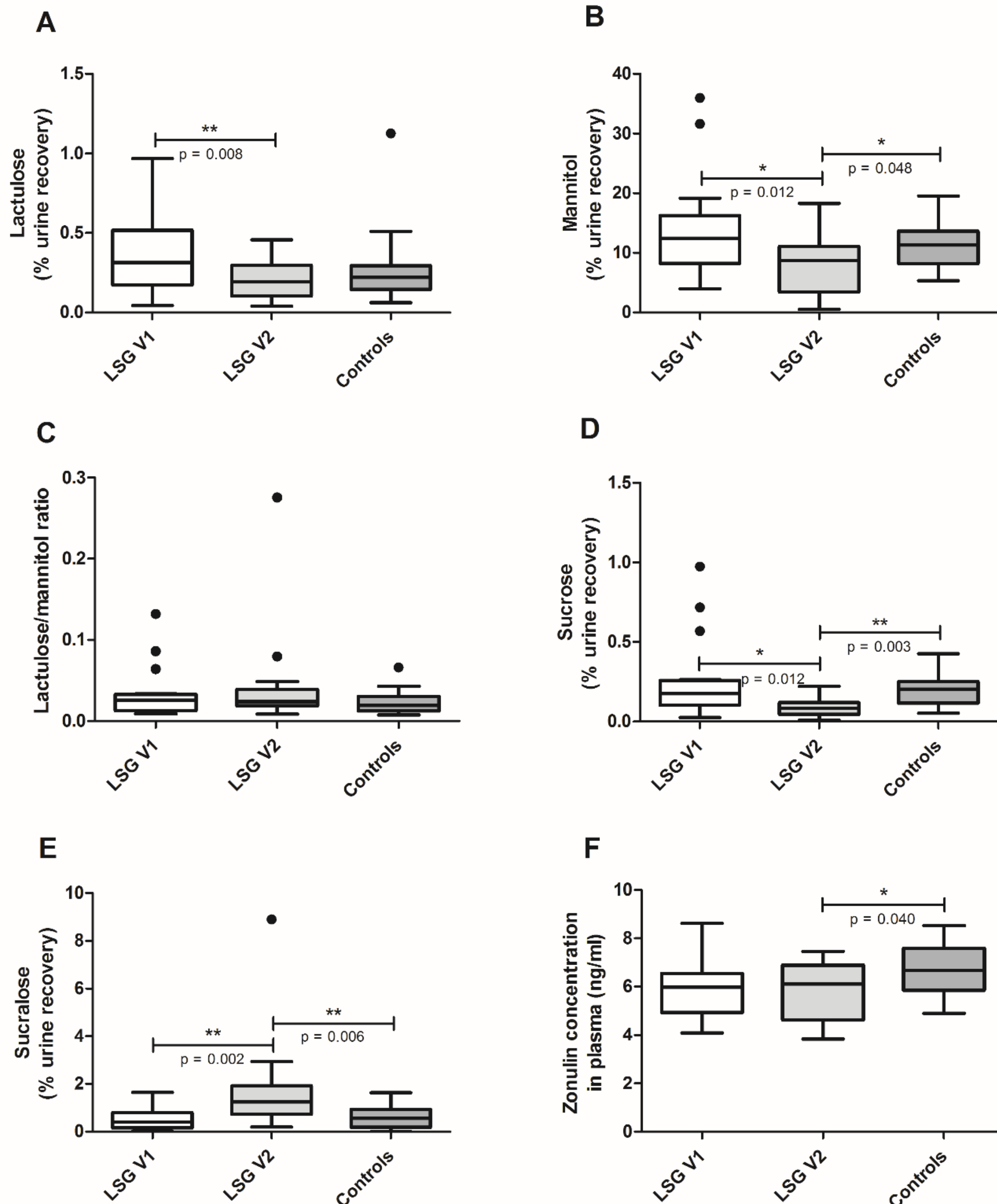

Figure 14*: Gut permeability of study participants

Gut permeability was assessed using a 4-probe sugar test. Lactulose (A) and mannitol (B) represent markers of intestinal permeability. The lactulose / mannitol ratio (C) represents a permeability index of small intestinal permeability. Sucrose (D) reflects gastroduodenal permeability and sucralose (E) colonic permeability. Zonulin concentration in blood (F) is a marker for the tight junctions.

Depending on distribution of the data paired *t*-test or Wilcoxon matched-pairs test was used to compare obese patient before and after surgery (LSG V1 and LSG V2) and unpaired *t*-test or Mann-Whitney test to compare obese patients with non-obese controls. A-D: n = 16, E: n = 15, F: n = 17. * $p < 0.05$, ** $p < 0.01$.

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Kellerer, T., Brandl, B., Büttner, J. et al. Impact of Laparoscopic Sleeve Gastrectomy on Gut Permeability in Morbidly Obese Subjects. OBES SURG 29, 2132–2143 (2019). https://doi.org/10.1007/s11695-019-03815-6, Springer Nature (2019)

Table 18: Comparison of gut permeability markers between obese patients with and without diabetes mellitus type 2

		Median (25th percentile; 75th percentile)		p-value
	n (non-diabetic/ diabetic)	**LSG V1 non-diabetic**	**LSG V2 diabetic**	
LM (% urine recovery)	10/6	0.02 (0.01; 0.03)	0.03 (0.01; 0.07)	n.s. 0.704
Lactulose (% urine recovery)	10/6	0.36 (0.18; 0.49)	0.27 (0.17; 0.84)	n.s. 0.958
Mannitol (% urine recovery)	10/6	13.22 (7.07; 20.45)	11.83 (9.59; 14.47)	n.s. 0.793
Sucrose (% urine recovery)	10/6	0.18 (0.10; 0.24)	0.17 (0.10; 0.67)	n.s. 0.793
Sucralose (% urine recovery)	9/6	0.25 (0.12; 0.72)	0.76 (0.21; 1.61)	n.s. 0.272
Zonulin concentration in plasma (ng/ml)	11/6	5.99 (5.26; 6.48)	5.55 (4.43; 7.60)	n.s. 0.725

Mann-Whitney test was used to compare the groups. n.s. = not significant.

Another aspect of the analysis was to investigate, whether the change in body weight upon LSG influenced the change in gut permeability. Correlation analysis showed no significant correlation between body weight loss and the change in different markers of gut permeability (Table 19), except for a significant positive correlation between change in gastroduodenal permeability (Δ sucrose) and %EWL. This correlation, however, was mainly dependent on two individuals with the highest change in sucrose excretion (Figure 15). The linear regression model was not significant for this association. Thus, overall, the extent of weight loss does not seem to have an impact on the extent of change in gut permeability.

Table 19: Correlation of weight loss with changes in gut permeability in obese patients undergoing LSG

	rho	p-value	
Δ Lactulose (% urine recovery) / Δ Body weight	0.006	n.s.	0.987
Δ Mannitol (% urine recovery) / Δ Body weight	0.309	n.s.	0.244
Δ Sucrose (% urine recovery) / Δ Body weight	-0.121	n.s.	0.656
Δ Sucralose (% urine recovery) / Δ Body weight	-0.089	n.s.	0.753
Δ Zonulin / Δ Body weight	0.031	n.s.	0.907
Δ Lactulose (% urine recovery) / Δ BMI	-0.053	n.s.	0.848
Δ Mannitol (% urine recovery) / Δ BMI	0.265	n.s.	0.321
Δ Sucrose (% urine recovery) / Δ BMI	-0.197	n.s.	0.463
Δ Sucralose (% urine recovery) / Δ BMI	-0.039	n.s.	0.893
Δ Zonulin / Δ BMI	0.197	n.s.	0.448
Δ Lactulose (% urine recovery) / %TWL	-0.091	n.s.	0.738
Δ Mannitol (% urine recovery) / %TWL	0.444	n.s.	0.087
Δ Sucrose (% urine recovery) / %TWL	-0.406	n.s.	0.120
Δ Sucralose (% urine recovery) / %TWL	-0.257	n.s.	0.354
Δ Zonulin / %TWL	0.002	n.s.	0.993
Δ Lactulose (% urine recovery) / %EWL	0.179	n.s.	0.505
Δ Mannitol (% urine recovery) / %EWL	-0.388	n.s.	0.138
Δ Sucrose (% urine recovery) / %EWL	0.526	*	0.038
Δ Sucralose (% urine recovery) / %EWL	0.311	n.s.	0.259
Δ Zonulin / %EWL	0.098	n.s.	0.708

Spearman rank correlation was used for correlation analysis. n = 15-17. n.s. = not significant, * $p < 0.05$.

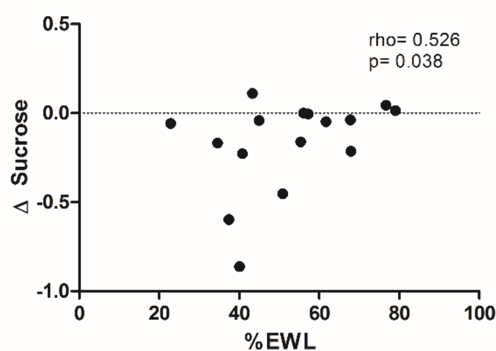

Figure 15: Association between the change in sucrose urinary recovery (Δ sucrose) with the percentage excessive weight loss (%EWL) of patients undergoing sleeve gastrectomy

Spearman correlation was performed. The change in sucrose urinary recovery (Δ sucrose = % urine recovery of sucrose at V2 - % urine recovery of sucrose at V1) was plotted against %EWL. n = 16.

5.1.6 Gut permeability in dependence of BMI

As no difference in gut permeability between the non-obese and obese study group could be detected, a further aim was to investigate the influence of BMI on gut permeability. Therefore, participants from a previous weight loss study (Ott et al., 2017) were included. In this study, gut permeability was assessed with the same methods. The cohorts from the current study as well as from the previous weight loss study were combined and participants were assigned to one of the following groups: non-obese controls (BMI < 28 kg/m^2), obesity class I (BMI 30–34.9 kg/m^2), obesity class II (BMI 35–39.9 kg/m^2), and obesity class III (BMI ≥ 40 kg/m^2). As men represented a minority and were not present in all of the groups, only female participants were used for analysis. The study characteristics are presented in Table 20. The median age was comparable between the groups ($p > 0.05$).

Table 20*: Characteristics of female study participants in different BMI groups

	Median (25th percentile; 75th percentile)			
	Controls	**Obesity class I**	**Obesity class II**	**Obesity class III**
n*	15	11	7	13
Age*	45 (32; 52)	47 (38; 59)	41 (33; 54)	40 (32; 50)
Body weight (kg)*	62.1 (56.3; 73.2)	87.2 (81.1; 90.7)	101.4 (99.4; 112.3)	134.0 (123.7; 154.1)
Body mass index (kg/m²)*	21.5 (19.6; 23.5)	33.1 (31.3; 34.0)	39.1 (35.1; 39.3)	48.5 (44.6; 53.3)
Total caloric intake / day (kcal/day)	1964 (1715; 2213)	1780 (1340; 1980)	1690 (1368; 1773)	1826 (1469; 2142)
Carbohydrate intake (g/day)	218.2 (172.5; 238.3)	200.0 (141.0; 248.0)	180.7 (128.6; 198.3)	202.5 (167.4; 226.4)
Fat intake (g/day)	72.5 (69.4; 97.0)	64.3 (44.4; 88.5)	68.3 (19.0; 76.6)	73.5 (57.2; 91.9)
Protein intake (g/day)	67.3 (60.9; 83.5)	64.6 (62.0; 90,.5)	66.0 (53.7; 94.7)	79.5 (60.6; 90.3)
Fibre intake (g/day)	21.1 (18.8; 26.0)	17.0 (14.8; 22.0)	18.0 (14.2; 18.8)	19.5 (13.5; 21.1)

When comparing gut permeability assessed by the four different sugars, no significant differences between the different groups of BMI could be detected (Figure 16A-E). The only significant result was detected for zonulin concentration, which was increased in obesity class I compared to controls (8.0 vs. 7.0 ng/ml) and decreased again at higher BMI classes (6.3 ng/ml in obesity class III) (Figure 16F). Levels of LBP were elevated in obesity compared to the controls, yet this was only significant for obesity class I (0.65 vs. 0.43 mg/dl, $p = 0.0098$) (Figure 16B). CRP was also increased in obesity, with a high variation in CRP level in the morbidly obese subjects (Figure 16A).

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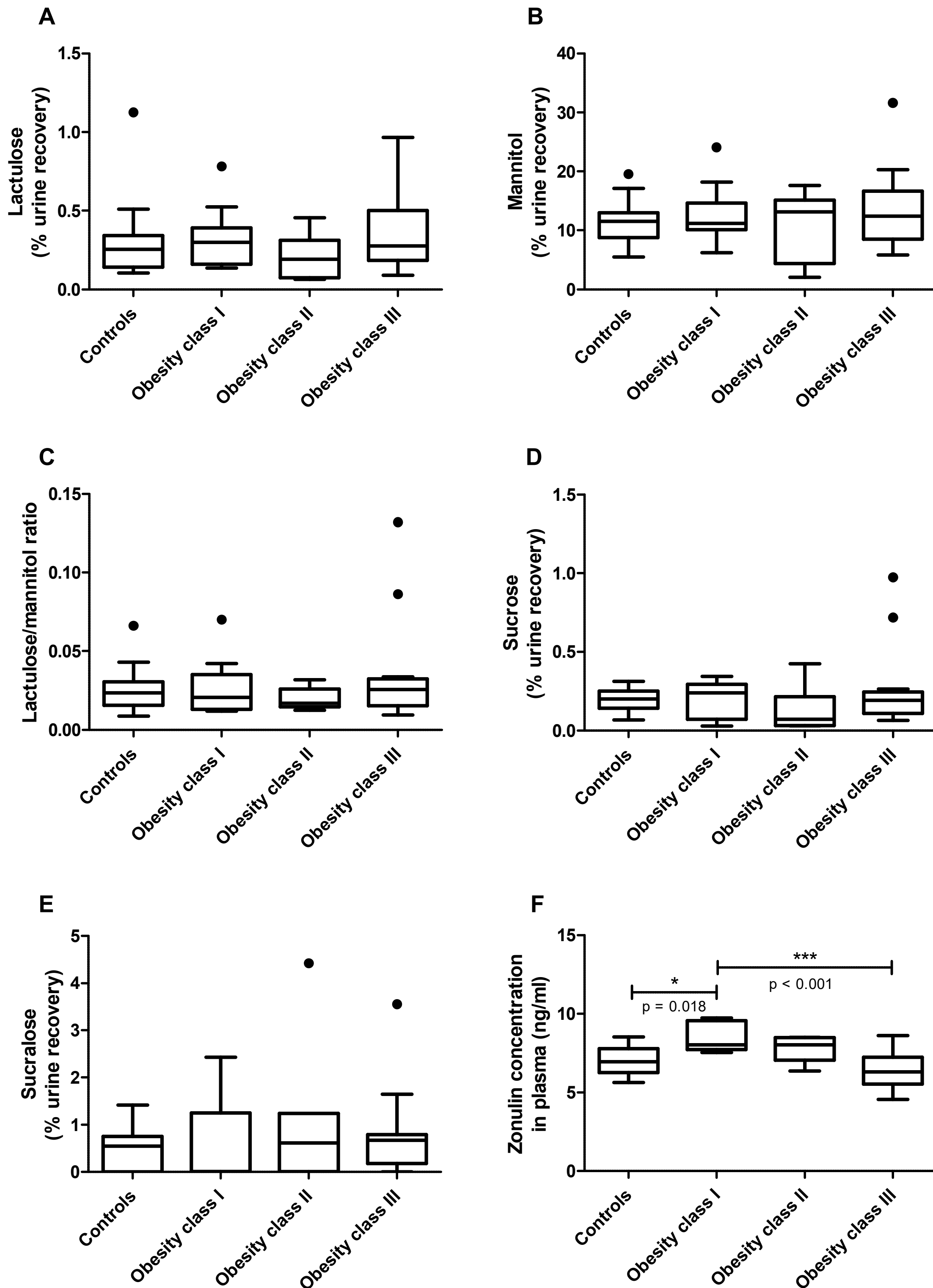

Figure 16*: Markers of gut permeability in dependence of BMI

Gut permeability was measured with a 4-probe sugar test in female study participants in different BMI groups. Lactulose (A) and mannitol (B) represent markers of intestinal permeability. The lactulose/mannitol ratio (C) represents a small intestinal permeability index. Sucrose (D) reflects gastroduodenal and sucralose (E) colonic permeability. Zonulin concentration in blood (F) is a marker of tight junctions. P-values were determined using Kruskal–Wallis test and Dunn's multiple comparison test. n = 7–15 per group. * $p < 0.05$, *** $p < 0.001$.

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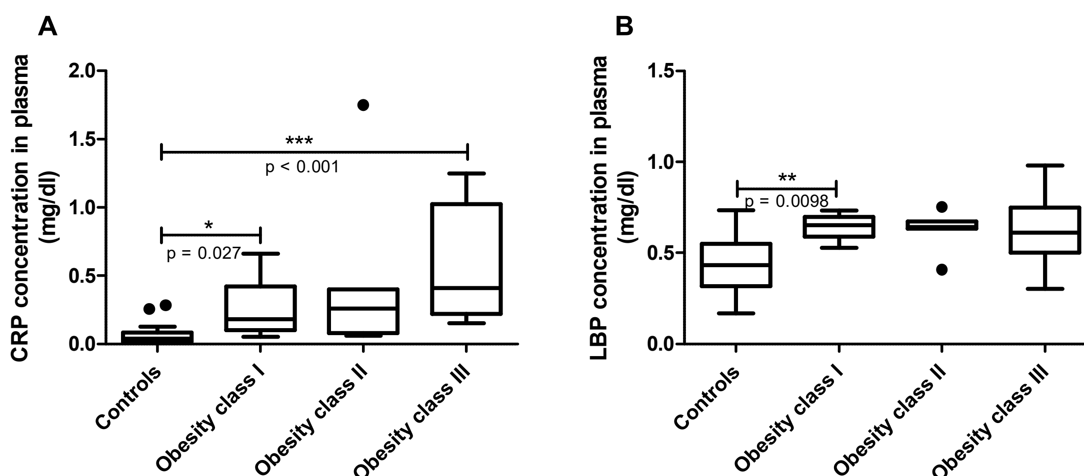

Figure 17*: LBP (A) and CRP (B) levels in dependence of BMI

LBP and CRP levels were measured in plasma of female study participants in different BMI groups. P-values were determined using Kruskal–Wallis test and Dunn's multiple comparison test. n = 7–15 per group. * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$.

5.1.7 Gut microbiota composition

Stool samples of study participants were analysed for gut microbiota composition. Five patients with obesity were regularly taking proton pump inhibitor (PPI) medication before surgery and seven patients after surgery, while none of the controls took PPIs. It is known that PPIs have an effect on gut microbiota. Therefore, in a first analysis, the samples were analysed in dependence of PPI medication. Figure 18B shows beta-diversity of patients with and without PPI medication before as well as after the surgery and of non-obese controls. As expected, there was a significant difference between the groups. Pairwise comparison of the groups (Supplemental Figure 1) revealed that the difference in microbiota composition between patients with and without PPI medication did not significantly differ before as well as after surgery. However, when compared with the non-obese control group, patients without PPI intake showed significant difference to the control group, while patients with PPI medication did not. Similar results were found for alpha-diversity, estimated by richness and Shannon-effective count, as well as the main phyla *Bacteroidetes* and *Firmicutes* (Figure 18A, 18C). There were significant differences between obese patients with and without PPI medication, especially before surgery, and patients with PPI medication were more similar to controls than patients without PPI medication. Overall, differences between groups were detected for two phyla, six classes, seven orders, seven families, and 16 genera.

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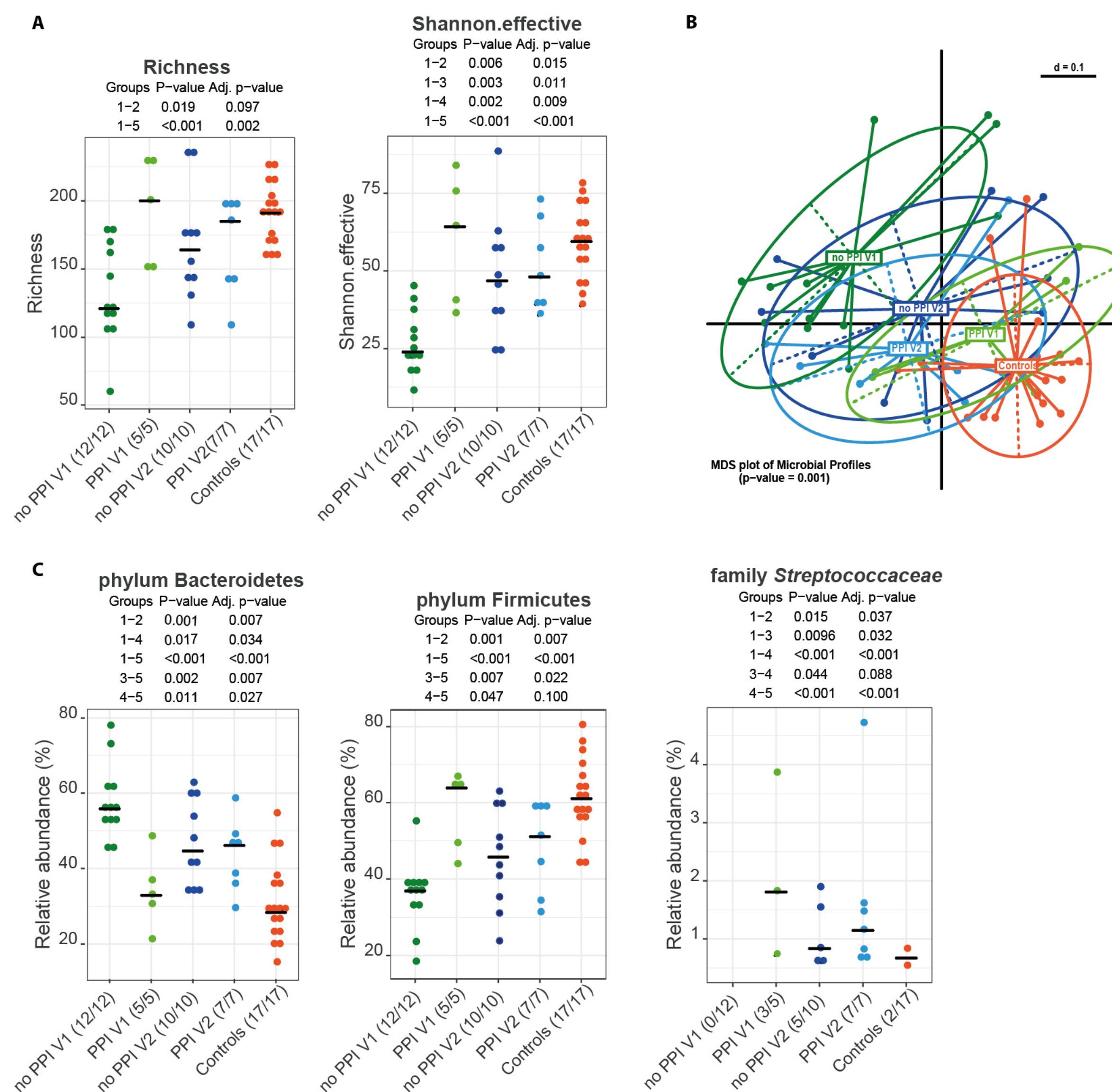

Figure 18*: Faecal microbiota analysis of study participants in dependence of PPI medication

A) Diversity within samples (alpha-diversity) estimated by richness and Shannon-effective counts. Numbers in brackets below the x-axis indicate prevalence (number of analysed samples / number of total samples). B)* Multidimensional scaling (MDS) plot of phylogenetic distances (beta-diversity). C) Relative abundance of the main phyla *Bacteroidetes* and *Firmicutes* and of the family *Streptococcaceae* within the different groups.

Controls: non-obese control subjects; PPI V1: obese patients with PPI medication before surgery; no PPI V1: obese patients without PPI medication before surgery; PPI V2: obese patients with PPI medication 6 months after surgery; no PPI V2: obese patients without PPI medication 6 months after surgery.

Due to these observed differences, subjects which regularly took PPI medication before or after surgery were excluded from the analysis. Therefore, the final analysis was performed with 8 patients with obesity as well as their matched controls.

The analysis delivered 497,737 quality- and chimera-checked reads (20,739 ± 6,344 per sample) which clustered in 394 operational taxonomic units (OTUs).

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Alpha-diversity of the microbiota was different between subjects with and without obesity. Richness as well as Shannon effective number of species both were lower in patients with obesity compared to non-obese controls and increased after the surgery (Figure 19D).

Subjects showed high inter-individual variance in beta-diversity. There was a significant difference ($p = 0.004$) between the three groups, and there seemed to be a shift of the microbiota composition of obese patients after surgery towards the composition of controls (Figure 19A). Pairwise comparison, however, showed no significant differences (Supplemental Figure 3). When combining obese subjects before and after surgery, the obese group differed significantly from the non-obese group (Figure 19B).

When regarding the phylogram, four groups could be detected based on the similarity of the samples, with the control samples clustering together mainly in one group (Figure 19C). The patients P1-05, P1-20, P1-21 and P1-22 changed their microbiota composition after sleeve gastrectomy in the direction of the controls. The other four patients with obesity did not show major changes, whereas patient P1-18 exhibited a microbiota which was quite similar to the controls even before the surgery.

When performing de-novo clustering into three groups based on the similarity of samples, one of the groups (group 3) solely consisted of controls. After deletion of the controls and editing of the samples according to their group, two separate clusters remained, which were not dependent on the time point of sampling (before vs. after LSG) (Supplemental Figure 4). Most of the patients remained in their cluster after surgery, with only two patients (P1-12 and P1-22) that changed clusters.

A further intention was to look for significant differences in the relative abundance of taxonomic groups between the three study groups. Overall, there were differences detected for two phyla, three classes, four orders, five families and eight genera. The most abundant phyla in the samples were *Firmicutes* and *Bacteroidetes,* followed by *Proteobacteria* and *Actinobacteria* (Supplemental Figure 5). Bacteria from the phylum *Bacteroidetes* were more abundant in patients with obesity, while bacteria from the phylum *Firmicutes* were less abundant. The abundance of both phyla tended towards the control group after surgery (Figure 19E).

Within the phylum *Bacteroidetes*, the class *Bacteroidia* and the order *Bacteroidales* showed significantly higher abundance in the obese group compared to the non-obese group and a tendency to decrease after surgery. The abundance of *Bacteroidales* almost completely represented the abundance of the whole phylum *Bacteroidetes* in the subjects. Difference was also found for the family *Prevotellaceae*, which was present in six out of eight patients

with obesity, but only in one single non-obese subject. *Rikenellaceae* significantly increased in abundance after the surgery.

Within the phylum *Firmicutes*, there was an overall higher abundance of *Clostridia* in controls compared to obese individuals. This was also seen for the order *Clostridiales*, which almost completely represented the phylum Firmicutes, as well as the family *Ruminococcaceae* and several genera within the family *Ruminococcaceae* (*Ruminococcaceae NK4A214*, *Ruminococcaceae UCG-005*, *Ruminococcaceae UCG-014*, *Subdoligranulum*). Besides, two genera within the family *Lachnospiraceae* (*Lachnospira*, *Anaerostipes*) were significantly different between the groups. The genera *Ruminococcaceae NK5A214* and *Anaerostipes* were, besides *Rikenellaceae*, the only taxonomic groups, that showed significant differences between obese patients before and after surgery. *Ruminococcaceae NK4A214* was only present in samples of patients after surgery, while *Aanaerostipes* disappeared after surgery. The *Selenomonadales* and *Acidaminococcaceae* as well as the whole class of *Negativicutes* showed higher abundance in the obese group compared to the controls.

No differences were found for the phylum *Proteobacteria*. However, when comparing obese and non-obese subjects, the relative abundance of *Betaproteobacteriales* and *Burkholderiaceae* from the phylum of *Proteobacteria* was higher in the obese group, while LSG did not lead to marked changes.

Among the 394 OTUs analysed, nine showed significant differences between groups. Eight of them belonged to the class *Clostridia* within the phylum *Firmicutes*, while one belonged to *Negativicutes* (OTU 15: *Phasolactobacerium faecium*, 99.12% sequence similarity). Six of the OTUs were found in the family of *Ruminococcacea* and showed higher abundance in the non-obese controls compared to the patients with obesity (OTU 5: *Subdolligranulum genus*, 98.38% sequence similarity; OTU 40: *Oscillibacter*, 98.61% sequence similarity; OTU 41 *Ruminococcus genus*, 98.84% sequence similarity; OTU 124: *JN713389_g*, 98.61% sequence similarity; OTU 765: *Faecalibacterium*, 97.91% sequence similarity). OTU 37 belonged to the family *Christenellacea* (*AB239481_g*, 98.85% sequence similarity) and was only present in controls. OTU44 belonged to the family *Lachnospiraceae* (*Anaerostipes hadrus*, 99.07% sequence similarity) and is the only one that showed difference before and after surgery: it was present in five patients before surgery but did not occur in any patient after surgery.

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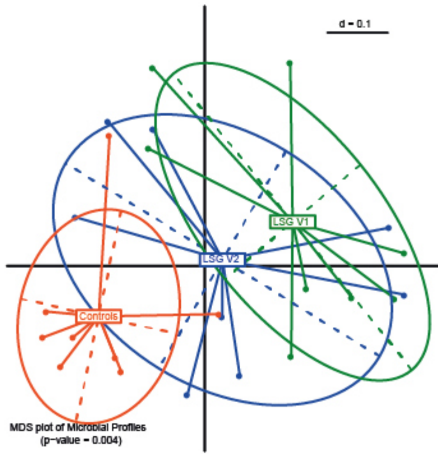

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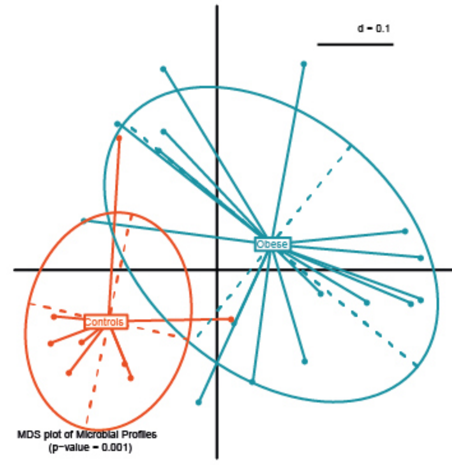

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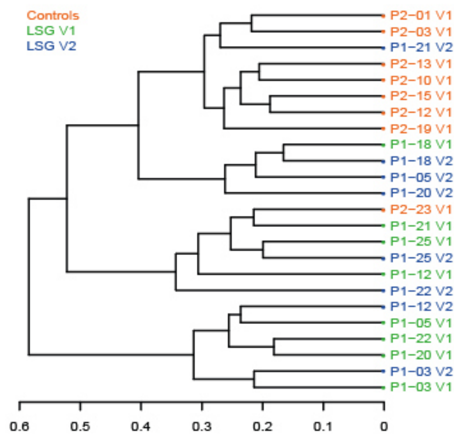

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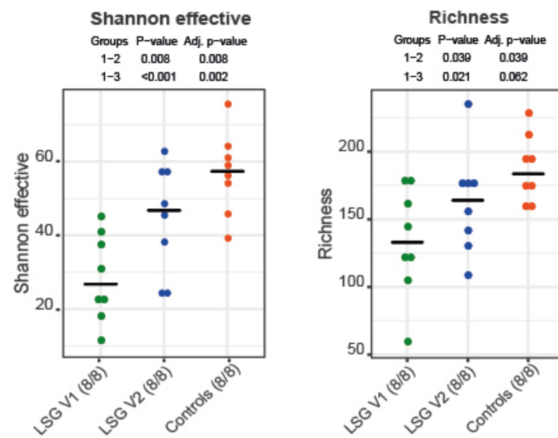

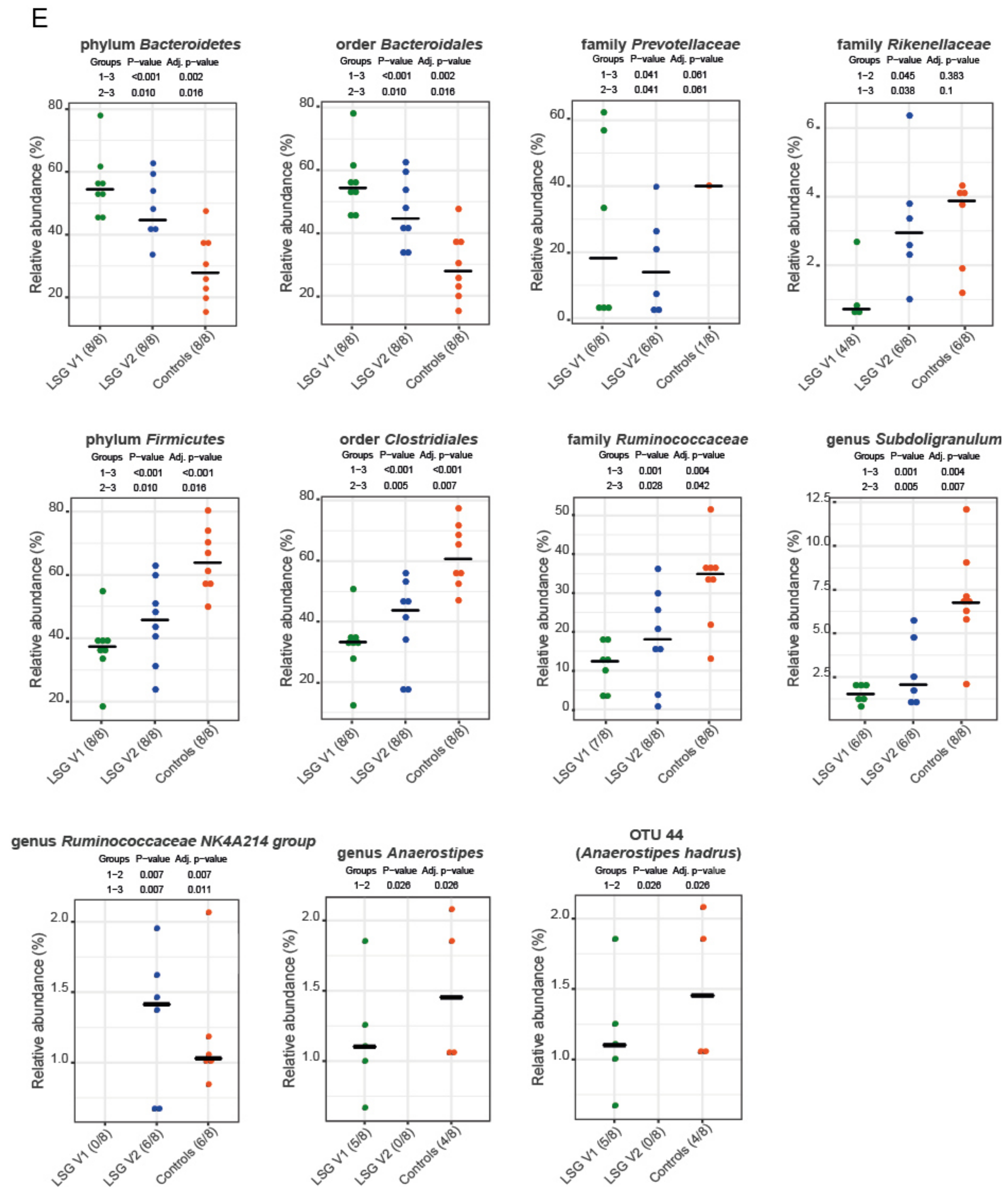

Figure 19*: Faecal microbiota analysis of study participants (n = 8 per group)

A)* Multidimensional scaling (MDS) plot of phylogenetic distances (beta-diversity) depicting the groups LSG V1, LSG V2 and Controls. B) Multidimensional scaling (MDS) plot of phylogenetic distances (beta-diversity) depicting the groups Obese (consisting of LSG V1 and LSG V2) and Controls. C)* Phylogram showing the hierarchical clustering of samples. D)* Diversity within samples (alpha-diversity). Numbers in brackets below the x-axis indicate prevalence (number of analysed samples / number of total samples). E)* Bacterial taxonomic groups with significant differences between groups.

Controls: non-obese control subjects; LSG V1: obese patients before surgery; LSG V2: obese patients after surgery; Obese: LSG V1 and LSG V2.

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5.2 FAHFAs

So far, the results of the present study showed that weight loss by bariatric surgery is accompanied by marked metabolic and functional changes. A further aim was to investigate the role of body weight and bariatric surgery on the levels of a selected group of fatty acids credited with positive metabolic effects, the FAHFAs.

Parts of the results of the following FAHFA analyses are included in a manuscript in submission (Kellerer et al., in submission). The figures and tables containing data that are included in that manuscript are marked with two asterisks (**). Also parts of the corresponding text in the methods, results and discussion section are included in that manuscript.

5.2.1 Establishment of a LC-MS method for the quantification of FAHFAs in human serum samples

In cooperation with the Bavarian Center for Biomolecular Mass Spectrometry (BayBioMS), an LC-MS method for the quantitative analysis of human serum-derived FAHFAs in the nano-molar range was established at our university. The final method allowed the separation and quantitation of 9-, 10-, and 12/13-PAHSA as well as 9-OAHSA, 9-SAHSA and 9-PAHPA. Detailed description of the method can be found in Methods.

5.2.2 FAHFA concentrations after sleeve gastrectomy

The effect of BMI and weight loss on FAHFA levels was analysed in our study cohort of morbidly obese subjects (BMI ≥ 40 kg/m^2) before and 6 months after LSG together with age- and sex-matched non-obese controls (BMI < 30 kg/m^2). The n number between the FAHFA analysis and the previous experiments differs according to in- or exclusion of participants with medications that might have influenced the respective analysis. Characteristics of the participants included in the FAHFA analysis can be found in Table 21. FAHFA levels of the samples were in the low nano-molar range. Inter-individual variation was high, especially in the non-obese control group. Significant differences were found between non-obese and obese subjects, with higher levels in the non-obese controls, with the exception of 9-PAHPA (Figure 20). At 6 months after surgery, the median weight loss of obese patients was 39.5 (30.7; 44.7) kg. Together with weight loss, metabolic parameters of the patients improved, as determined by a decrease in fasting glucose and insulin, NEFA levels, and CRP concentration (Table 21). The levels of 9-OAHSA were significantly increased at 6 months after surgery, while all other FAHFAs were not altered (Figure 20).

Table 21: Characteristics of morbidly obese patients undergoing bariatric surgery and non-obese controls used for FAHFA analysis**

		Median (25th percentile; 75th percentile)			p-value		
	n	LSG V1	LSG V2	Controls	LSG V1 – LSG V2	LSG V1 – Controls	LSG V2 – Controls
Age	18	46 (35; 50)	-	47 (35; 51)	-	n.s. 0.958	-
Body weight (kg)	18	136.0 (119.8; 157.9)	109.5 (93.9; 136.6)	63.3 (56.2; 73.4)	*** <0.001	*** <0.001	*** <0.001
Body mass index (kg/m²)	18	52.6 (46.2; 60.4)	39.3 (31.4; 45.0)	21.6 (19.6; 32.6)	*** <0.001	*** <0.001	*** <0.001
Fasting blood glucose (mg/dl)	18	95 (82; 104)	83 (74; 90)	89 (84; 94)	** 0.001	n.s. 0.268	n.s. 0.074
Fasting insulin (mg/dl)	18	38.3 (26.9; 69.2)	26.0 (16.4; 39.2)	11.5 (9.5; 18.0)	* 0.013	*** <0.001	** 0.002
CRP (mg/dl)	18	0.55 (0.39; 0.99)	0.30 (0.13; 0.56)	0.03 (0.02; 0.06)	** 0.008	*** <0.001	*** <0.001
NEFA (mmol/l)	18	1.01 (0.92; 1.54)	0.54 (0.35; 0.91)	0.32 (0.25; 0.41)	*** <0.001	*** <0.001	** 0.006
Total caloric intake / day (kcal/day)	18	1747 (1407; 2080)	881 (757; 1060)	1990 (1793; 2305)	*** <0.001	* 0.021	*** <0.001
Fat intake (g/day)	18	66 (56; 86)	37 (30; 40)	79 (70; 98)	*** <0.001	n.s. 0.071	*** <0.001
Fat intake (%kcal)	18	35 (33; 39)	34 (31; 43)	36 (34; 41)	n.s. 0.931	n.s. 0.438	n.s. 0.548

Depending on the distribution of the data Wilcoxon matched-pairs test or paired *t*-test was used to compare obese patients before and after surgery (LSG V1 and LSG V2) and Mann-Whitney test or unpaired *t*-test to compare obese patients with non-obese controls. n.s. = not significant; * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$.

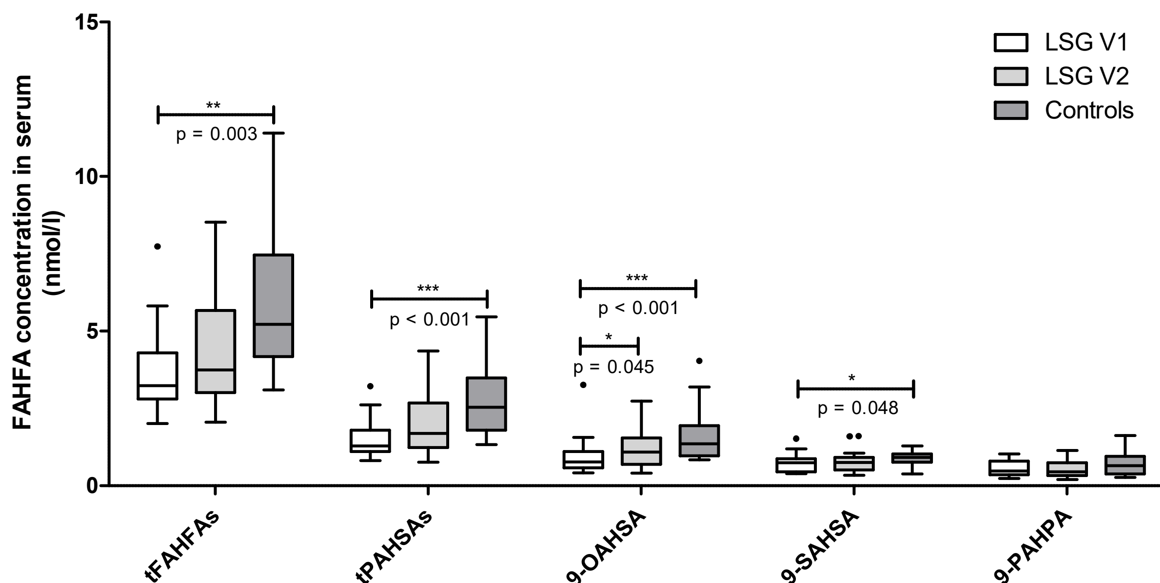


Figure 20: FAHFA concentration in morbidly obese individuals before (LSG V1) and 6 months after laparoscopic sleeve gastrectomy (LSG V2) as well as in non-obese controls**

Wilcoxon matched-pairs test was used to compare obese patient before and after surgery (LS V1 and LSG V2) and Mann-Whitney test to compare obese patients with non-obese controls. n = 18 per group. * p < 0.05, ** p < 0.01, and *** p < 0.001.

When analysing the change in tFAHFA and tPAHSA levels in obese patients, Spearman rank correlation revealed no significant correlation with the change in anthropometric, metabolic or dietary factors; solely the change in tPAHSAs was positively correlated with the alteration of NEFA levels (rho = 0.534, p = 0.024). However, this association was influenced mainly by one outlier (Supplemental Figure 6).

Furthermore, the role of anthropometric, metabolic and dietary factors on tFAHFA and tPAHSA levels at baseline was investigated. When obese patients before surgery and non-obese controls were analysed together, significant correlations were seen for tPAHSAs with BMI (rho = -0.398, p = 0.017), levels of NEFAs (rho = -0.652, p < 0.001), CRP (rho = -0.547, p < 0.001) and insulin (rho = -0.365, p = 0.029) as well as for tFAHFAs with BMI (rho = -0.361, p = 0.031), NEFAs (rho = -0.618, p < 0.001) and CRP (rho = -0.457, p = 0.006) (see also Supplemental Figure 7). When combining these factors in a multiple linear regression model adjusted for obesity status, none of those factors had significant impact, except for NEFAs (Table 22). It should be mentioned, that CRP correlated with NEFAs (rho = 0.755, p < 0.001) and insulin (rho = 0.558, p < 0.001); and NEFAs also correlated with insulin (rho = 0.521, p = 0.001). When CPR and insulin were removed from the model, NEFAs still had a significant impact on tFAHFAs as well as tPAHSAs (Table 23). Additionally, the non-obese and obese groups were also analysed separately, to exclude the influence of BMI. In

this analysis no significant correlations were noted between tFAHFAs or tPAHSAs and CRP, insulin or NEFAs.

Table 22: Multiple regression analysis: FAHFA levels and metabolic factors adjusted for obesity status

FAHFA	Metabolic factors included as independent variables	Coefficient	p-value	
tPAHSAs	CRP	-0.018	n.s.	0.837
	NEFAs	-0.346	n.s.	0.056
	Insulin	0.060	n.s.	0.592
	Factor BMI	-0.153	n.s.	0.641
tFAHFAs	CRP	0.006	n.s.	0.936
	NEFAs	-0.344	*	0.038
	Insulin	0.072	n.s.	0.484
	Factor BMI	-0.133	n.s.	0.658

A multiple regression analysis for FAHFA levels and metabolic factors adjusted for obesity status was performed: lm(log(FAHFA) ~ log(CRP) + log(NEFAs) + log(Insulin) + factor(BMI)). Factor BMI is 1 for obese subjects and 0 for non-obese controls. Multiple adjusted R^2 for the tPAHSAs model is 0.351 and for the tFAHFAs model 0.309. Estimated coefficients and p-values for the included metabolic factors are given. Asterisks indicate if the corresponding coefficient is not equal to zero. n = 36. n.s. = not significant; * $p < 0.05$.

Table 23: Multiple regression analysis: FAHFA levels and NEFAs adjusted for obesity status

FAHFA	Metabolic factors included as independent variables	Coefficient	p-value	
tPAHSAs	NEFAs	-0.359	*	0.035
	Factor BMI	-0.127	n.s.	0.581
tFAHFAs	NEFAs	-0.344	*	0.028
	Factor BMI	-0.040	n.s.	0.851

A multiple regression analysis for FAHFA levels and NEFAs adjusted for obesity status was performed: lm(log(FAHFA) ~ log(NEFA) + factor(BMI)). Factor BMI is 1 for obese subjects and 0 for non-obese controls. Multiple adjusted R^2 for the tPAHSAs model is 0.384 and for the tFAHFAs model 0.340. Estimated coefficients for the included metabolic factors are given. Asterisks indicate if the corresponding coefficient is not equal to zero. n = 36. n.s. = not significant; * $p < 0.05$.

5.2.3 FAHFA concentrations in dependence of type 2 diabetes

It was previously reported that PAHSA levels are reduced in insulin-resistant subjects and correlate with insulin sensitivity. Therefore, it was an aim to measured FAHFA levels in dependence of diabetes. FAHFAs were analysed in a group of diabetic obese individuals as well as and non-diabetic obese individuals from the GOBB cohort. Both groups were similar in age, body weight, BMI and CRP levels and consisted each of 5 females and 5 males (Table 24). Obese diabetic patients had significantly higher blood glucose levels as well as

higher HbA1c. Analysis of FAHFAs showed no significant differences in FAHFA levels between obese diabetic and obese non-diabetic patients (Figure 21). When diabetic and non-diabetic subjects were combined, correlation analysis revealed positive correlation between body weight and tPAHSAs as well as tFAHFAs (rho = 0.666, p = 0.001; rho = 0.632, p = 0.003), and between BMI and tPAHSAs (rho = 0.451, p = 0.047).

Table 24: Characteristics of obese diabetic and non-diabetic individuals of the GOBB cohort**

		Median (25th percentile; 75th percentile)		p-value
	n (diabetic/ non-diabetic)	**Obese diabetic**	**Obese non-diabetic**	**Diabetic - non-diabetic**
Age	10/10	44 (36; 47)	42 (35; 45)	n.s. 0.668
Body weight (kg)	10/10	159.5 (138.8; 171.1)	145.5 (122.7; 184.4)	n.s. 0.517
Body mass index (kg/m²)	10/10	53.6 (47.3; 57.3)	46.7 (42.2; 59.0)	n.s. 0.264
Fasting blood glucose (mg/dl)	10/10	124 (96; 223)	82 (73; 97)	** 0.005
HbA1c (%)	10/10	7.1 (6.7; 9.1)	5.2 (5.1; 5.3)	*** <0.001
CRP (mg/dl)	10/8	1.64 (0.89; 3.00)	1.35 (0.60; 2.80)	n.s. 0.762

Depending on the distribution of the data Mann-Whitney test or unpaired *t*-test was used to compare the groups. n.s. = not significant; ** $p < 0.01$, *** $p < 0.001$.

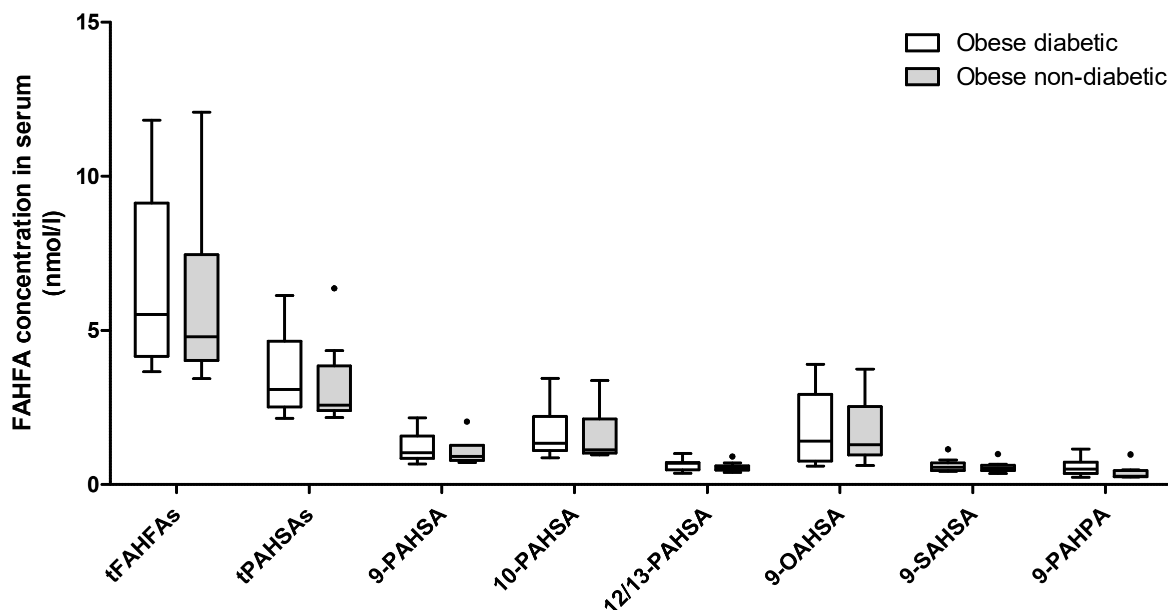


Figure 21: FAHFA concentration in obese individuals with and without type 2 diabetes of the GOBB cohort**

Mann-Whitney test was used to compare the groups. n = 10 per group.

Additionally, FAHFA levels of diabetic and non-diabetic individuals were analysed in a second cohort (*enable* cohort). Subjects all were healthy adults between 40 and 65 years. Patients assigned to the diabetic group had newly diagnosed diabetes due to elevated fasting glucose levels (≥ 126 mg/dl) and/or a 2h blood glucose value of ≥ 200 mg/dl in the oral glucose tolerance test (OGTT) of the *enable* programme. This group of newly diagnosed diabetic patients (n = 8) was compared with the non-diabetic adults showing normal fasting and 2h blood glucose levels (n = 20). The diabetic group had significantly higher fasting glucose and insulin levels and additionally higher body weight and BMI (Table 25). There were no significant differences in the analysed FAHFA levels between diabetic and non-diabetic adult individuals (Figure 22). No significant correlations could be detected between tFAHFA or tPAHSA levels and anthropometric or metabolic parameters, when diabetic and non-diabetics were analysed together.

Table 25: Characteristics of adult diabetic and non-diabetic individuals of the *enable* cohort**

		Median (25th percentile; 75th percentile)		p-value
	n (diabetic/ non-diabetic)	**Diabetic**	**Non-diabetic**	**Diabetic – Non-diabetic**
Age	8/20	55 (49; 64)	56 (59; 58)	n.s. 0.607
Body weight (kg)	8/20	98.4 (95.9; 108.9)	66.3 (54.2; 75.0)	*** <0.001
Body mass index (kg/m²)	8/20	31.9 (30.7, 33.4)	21.8 (20.6; 23.1)	*** <0.001
Fasting blood glucose (mg/dl)	8/20	118 (93; 126)	78 (72; 82)	*** <0.001
Fasting insulin (µU/ml)	8/20	10.9 (7.8; 14.7)	2.0 (2.0; 2.9)	*** <0.001
HOMA-IR	8/20	3.00 (1.90; 4.35)	0.39 (0.36; 0.58)	*** <0.001
CRP (mg/dl)	8/20	0.32 (0.29; 0.43)	0.06 (0.04; 0.12)	** 0.001

Depending on the distribution of the data Mann-Whitney test or unpaired *t*-test was used to compare the groups. n.s. = not significant; ** $p < 0.01$, *** $p < 0.001$.

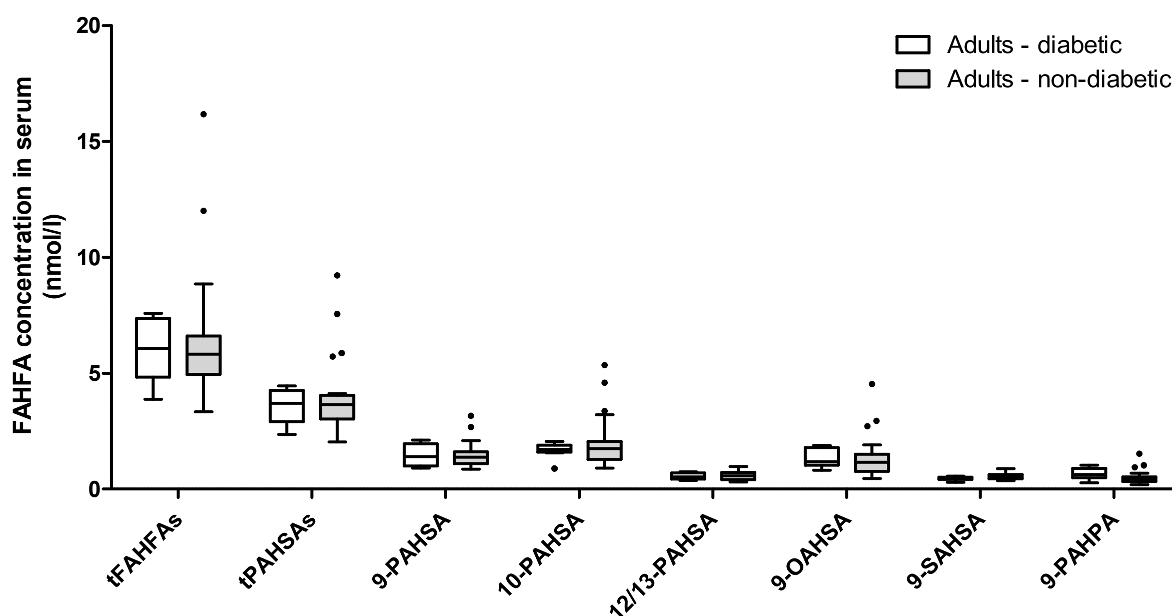


Figure 22: FAHFA concentration in individuals with and without type 2 diabetes of the *enable* cohort**

Mann-Whitney test was used to compare the groups. Adults – diabetic: n = 8; Adults – non-diabetic: n = 20.

5.2.4 FAHFA concentrations in dependence of age and sex

FAHFA concentrations were also measured in a group of healthy, non-diabetic individuals enrolled in the *enable* study cohort to investigate the association of FAHFA levels with age and sex. Adolescents and adults were all normal weight (BMI 18.5–25 kg/m^2), while a slightly higher BMI for elderly people was accepted (up to 30 kg/m^2) due to the natural increase in BMI with higher age. The group of elderly also showed slightly, but significantly increased fasting glucose levels and HOMA-IR compared to the younger groups (Table 26). However, levels were still in the normal range (Gayoso-Diz et al., 2013; Müller-Wieland et al., 2016), except for the HOMA-IR value of one adult and one elderly person (5.16 and 3.09, respectively). Regarding FAHFA analysis, the adult group showed significantly increased levels of 9-PAHPA, 9-PAHSA and 10-PAHSA as well as of tPAHSAs and tFAHFAs compared with the adolescents (Figure 23). In the elderly subjects the levels of those FAHFAs were not significantly different any more from the levels of the adolescents, except for 9-PAHPA, which remained still elevated (Figure 23). To investigate the influence of sex on FAHFA levels, the total of 30 females and 30 males from all age groups were compared and no differences were detected for FAHFA levels (Figure 24). The same was true for the separate analysis of females and males in each of the three different age groups (data not show).

When all three different age groups were analysed together, there were no significant correlations between levels of tFAHFAs or tPAHSAs and anthropometric or metabolic variables. Separate analysis of the adult group showed significant negative correlations of BMI with tFAHFAs (rho = -0.672, $p = 0.001$) and tPAHSAs (rho = -0.504, $p = 0.023$), respectively (Supplemental Figure 8). A subsequent multiple linear regression analysis adjusted for age was performed and revealed an independent effect of BMI on tFAHFA as well as tPAHSA levels (Table 27).

Table 26: Characteristics of individuals from three different age groups of the *enable* cohort**

		Median (25th percentile; 75th percentile)			p-value			
	n	**Adol.**	**Adults**	**Elderly**	**Analysis of variance**	**Adol. – Adults**	**Adol. – Elderly**	**Adults – Elderly**
Age	20/20/20	22 (21; 23)	56 (59; 58)	77 (75; 80)	*** <0.001	*** <0.001	*** <0.001	*** <0.001
Body weight (kg)	20/20/20	66.2 (58.9; 74.4)	66.3 (54.2; 75.0)	69.2 (61.9; 78.4)	n.s. 0.328	-	-	-
Body mass index (kg/m²)	20/20/20	20.4 (19.9; 21.3)	21.8 (20.6; 23.1)	24.9 (22.8; 26.9)	*** < 0.001	n.s. 0.152	*** <0.001	*** <0.001
Fasting blood glucose (mg/dl)	20/20/20	77 (73; 81)	78 (72; 82)	87 (81; 89)	*** <0.001	n.s. 0.919	*** <0.001	*** <0.001
Fasting insulin (µU/ml)	20/20/20	2.0 (2.0; 3.2)	2.0 (2.0; 2.9)	2.9 (2.0, 6.1)	n.s. 0.067	-	-	-
HOMA-IR	20/20/20	0.39 (0.37; 0.58)	0.39 (0.36; 0.58)	0.62 (0.42; 1.32)	** 0.007	n.s. 1.0	* 0.027	* 0.013
CRP (mg/dl)	20/20/20	0.04 (0.02; 0.06)	0.06 (0.04; 0.12)	0.13 (0.08; 0.19)	** 0.004	n.s. 0.449	** 0.003	n.s. 0.176

Adol. = Adolescents: 18–25 years; Adults: 40–65 years; Elderly: 75–85 years. Depending on the distribution of the data Kruskal-Wallis test with Dunn's post test or one-way ANOVA and Tukey post test were used to compare the groups. n.s. = not significant; * p < 0.05, ** p < 0.01, and *** p < 0.001.

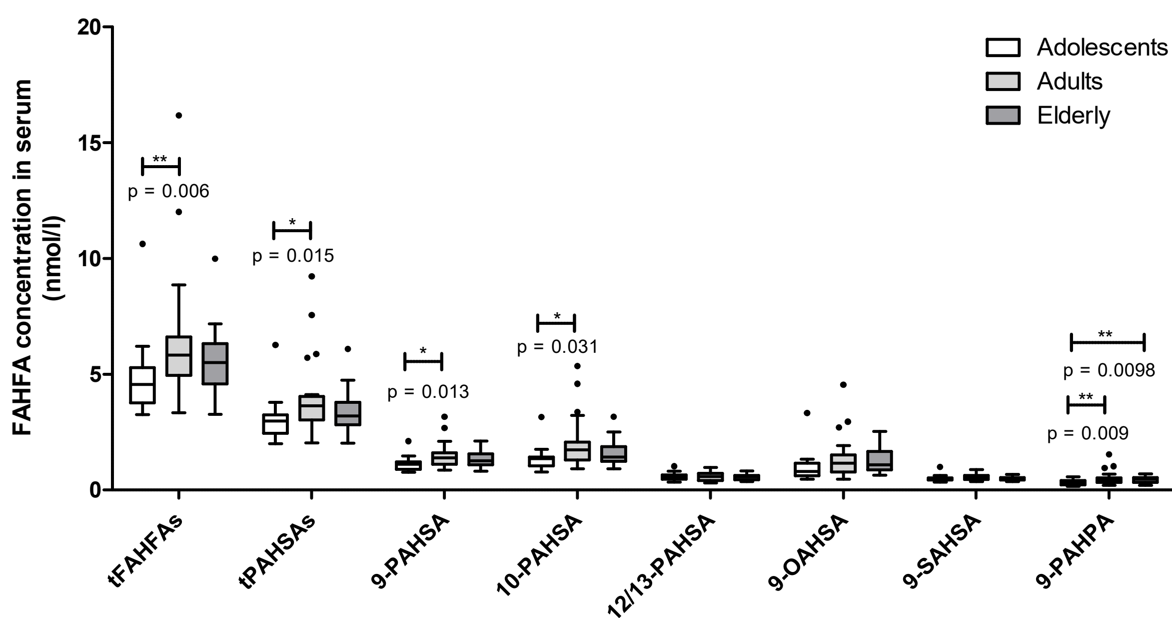


Figure 23: FAHFA concentrations in different age groups of the *enable* cohort**

Adolescents: 18–25 years; Adults: 40–65 years; Elderly: 75–85 years. Kruskal-Wallis test with Dunn's post test was used to compare the groups. n = 20 per group. * p < 0.05, ** p < 0.01.

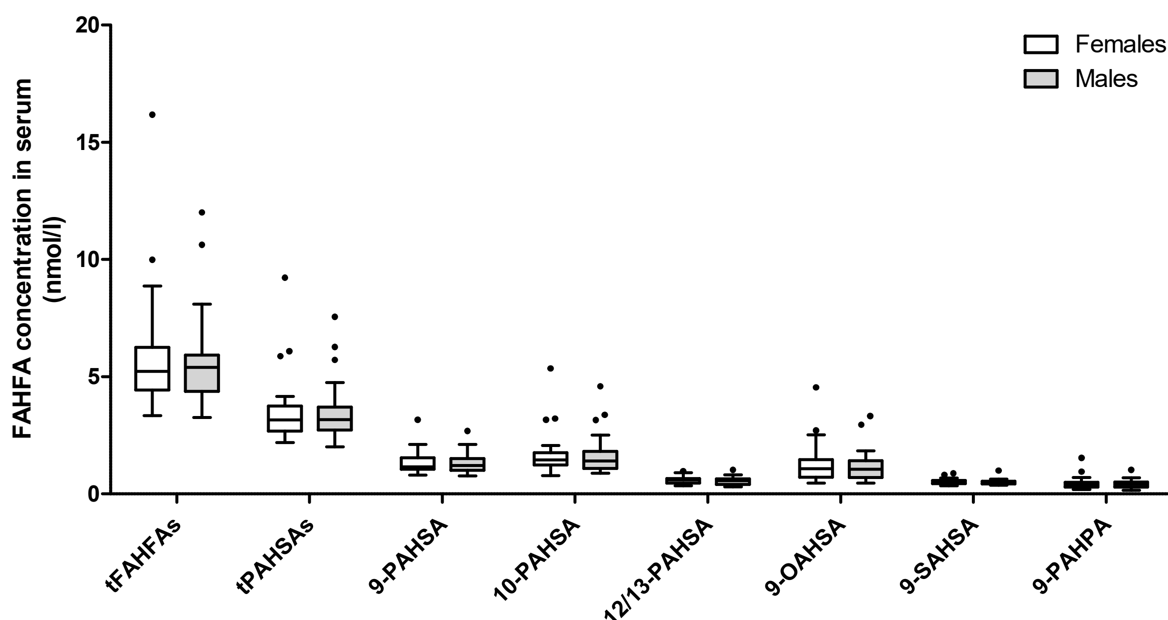


Figure 24: FAHFA concentration in dependence of sex (*enable* cohort)**

Mann-Whitney test was used to compare females and males. n = 30 per group.

Table 27: Multiple regression analysis: FAHFA levels and BMI adjusted for age

FAHFA	Coefficient BMI	Adjusted R^2	p-value	
tPAHSAs	-1.067	0.154	*	0.024
tFAHFAs	-1.057	0.155	*	0.031

A multiple regression analysis for FAHFA levels and BMI adjusted for age was performed: lm(log(FAHFA) ~ log(BMI) + factor(age)). Factor age = adolescents, adults, elderly. Estimated coefficients for BMI and adjusted R^2 of the model are given. Asterisks indicate if the corresponding coefficient is not equal to zero. n = 60. * $p < 0.05$.

5.2.5 FAHFA concentrations in dependence of specific diets

Last, it was of interest to investigate the association of systemic FAHFA levels with diet. Therefore, FAHFA levels of omnivores that habitually followed a normal mixed diet including meat and fish (3 females, 6 males) were compared with those of a group of subjects practicing a vegetarian or vegan diet (7 females, 3 males). Both groups slightly differed in age, but body weight, BMI and fasting glucose levels were similar (Table 28). The vegetarian group exhibited significantly lower levels of all different FAHFAs analysed compared to the omnivore group, with the exception of 9-SAHSA and 12/13-PAHSA (Figure 25).

Table 28: Characteristics of vegetarians and omnivores**

		Median (25th percentile; 75th percentile)		p-value
	n (vegetarians/ omnivores)	Vegetarians	Omnivores	Vegetarians - Omnivores
Age	10/9	22 (20; 23)	28 (26; 36)	** 0.002
Body weight (kg)	10/9	63.8 (56.4; 76.3)	81.3 (56.8; 84.1)	n.s. 0.206
Body mass index (kg/m²)	10/9	21.6 (19.8; 22.6)	23.0 (20.4; 24.9)	n.s. 0.159
Fasting blood glucose (mg/dl)	10/9	88 (86; 90)	92 (89; 100)	n.s. 0.061

Depending on the distribution of the data Mann-Whitney test or unpaired *t*-test was used to compare the groups. n.s. = not significant; ** p < 0.01.

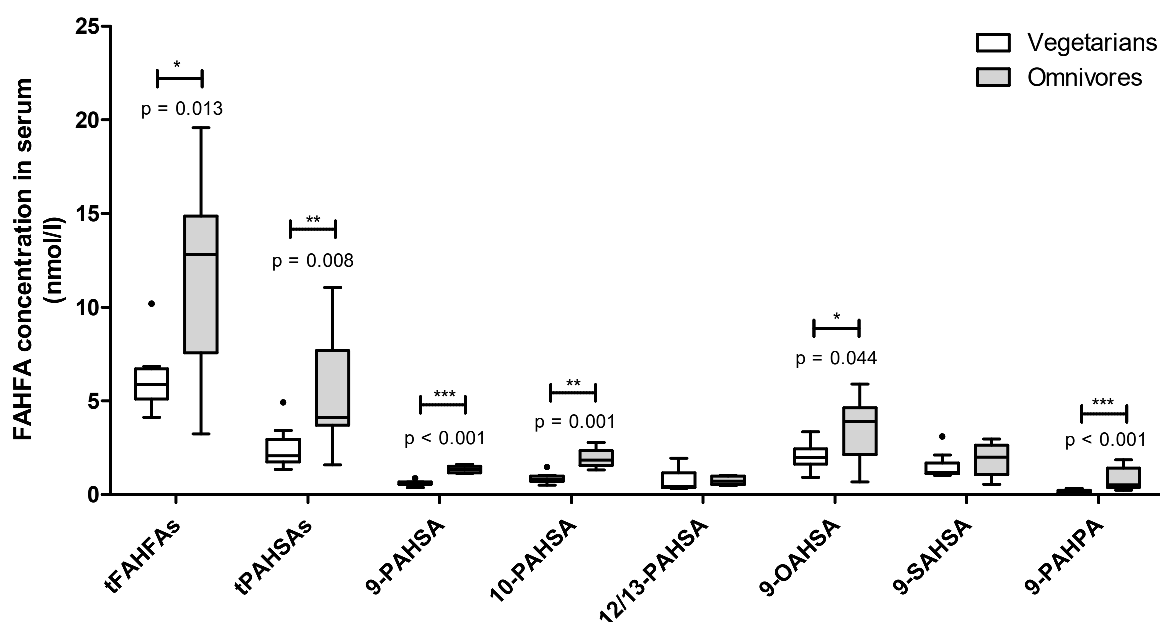


Figure 25: FAHFA concentration in individuals following vegetarian diet (vegetarians) in comparison to individuals with normal diet (omnivores)**

Mann-Whitney test was used to compare the groups. Vegetarians: n = 10. Omnivores: n = 9 for for tFAHFAs, tPAHSAs, 9-OAHSA, 9-SAHSA and 9-PAHPA, and n = 5 for 9-PAHSA, 10-PAHSA and 12/13-PAHSA. * p < 0.05, ** p < 0.01, and *** p < 0.001.

To further explore the effect of diet on FAHFA levels, FAHFAs were analysed in a group of lean (BMI < 27 kg/m^2), healthy, young men undergoing overfeeding for one week (Ott et al., 2018). The median weight gain of participants was 1.2 (0.6; 1.4) kg of body weight during the period of overfeeding. Fasting blood glucose levels and levels of free fatty acids (NEFAs)

were decreased after the intervention, while fasting insulin and CRP levels were unaltered (Table 29). The levels of FAHFAs in fasted serum were increased after one week of overfeeding (Figure 26), which was highly significant for all different FAHFAs analysed. Spearman correlation analysis revealed no significant correlation at baseline between anthropometric, metabolic or dietary parameters and tPAHSA or tFAHFA levels, except for CRP and FAHFAs (rho = -0.560; $p = 0.0499$). Furthermore, no significant correlation was seen for an increase in tFAHFA or tPAHSA levels and changes of anthropometric, metabolic or dietary parameters.

Table 29: Characteristics of young men undergoing short-term overfeeding**

	n	Median (25th percentile; 75th percentile) T1	T2	p-value T1 – T2
Age	15	22 (21; 24)	-	-
Body weight (kg)	15	79.6 (66.8; 82.8)	79.6 (68.1; 84.2)	*** <0.001
Body mass index (kg/m²)	15	23.3 (21.3; 24.9)	23.5 (21.7; 25.2)	*** <0.001
Fasting blood glucose (mg/dl)	15	85 (78; 87)	80 (71; 84)	* 0.037
Fasting insulin (µU/ml)	15	4.3 (4.0; 5.0)	4.5 (3.9; 5.2)	n.s. 0.265
HOMA-IR	15	0.9 (0.8; 1.1)	0.9 (0.8; 1.0)	n.s. 0.574
CRP (mg/dl)	13	0.02 (0.01; 0.03)	0.04 (0.01; 0.04)	n.s. 0.946
NEFA (mmol/l)	15	0.37 (0.28; 0.54)	0.17 (0.12; 0.23)	*** <0.001
Total caloric intake / day (kcal/day)	15	2570 (2330; 3490)	4015 (3910; 4252)	*** <0.001
Fat intake (g/day)	15	112 (80; 131)	214 (209; 227)	*** <0.001
Fat intake (%kcal)	15	35 (32; 39)	48 (48; 48)	*** <0.001
SFA intake (g)	15	50 (32; 58)	114 (112; 117)	*** <0.001
SFA intake (%)	15	15 (14; 17)	25 (25; 26)	*** <0.001
Protein intake (g)	15	96 (80; 123)	181 (176; 191)	*** <0.001
Protein intake (%kcal)	15	14 (13; 15)	18 (18; 18)	** 0.001
Carbohydrate intake (g)	15	312 (250; 380)	341 (332; 361)	n.s. 0.169
Carbohydrate intake (%kcal)	15	44 (42; 49)	34 (34; 34)	*** <0.001

Depending on the distribution of the data Wilcoxon matched-pairs test or paired *t*-test was used to compare participants before (T1) and after (T2) intervention. Intervention consisted of a 7 day overfeeding with a surplus of 1000 kcal/day. n.s. = not significant; * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$.

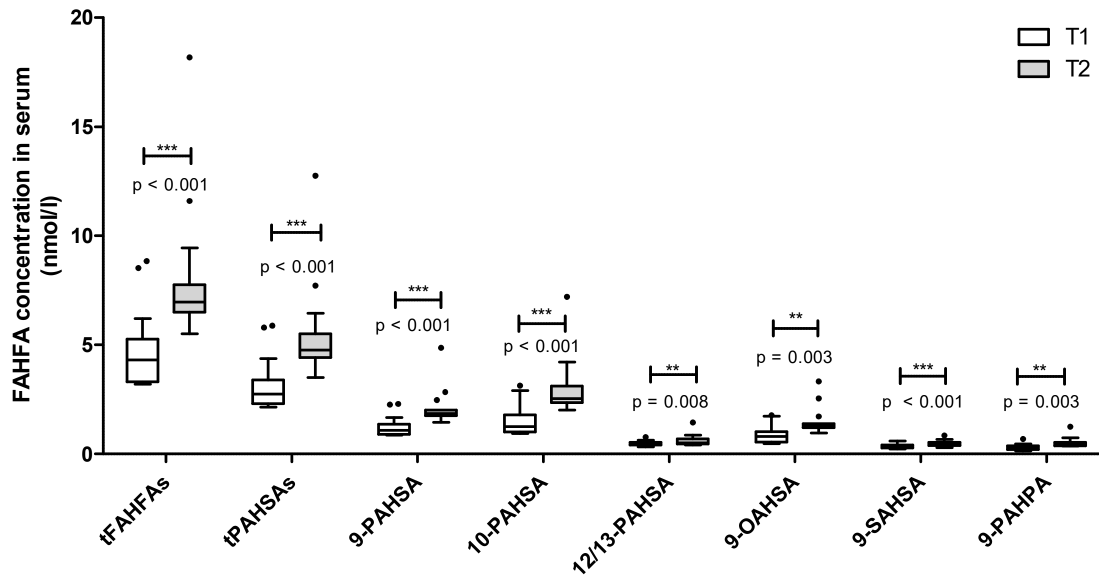


Figure 26: FAHFA concentration in individuals undergoing short-term overfeeding**

Wilcoxon matched-pairs test was used to compare participants before (T1) and after (T2) intervention. Intervention consisted of a 7 day overfeeding with a surplus of 1000 kcal/day. n = 15. ** p < 0.01, *** p < 0.001.

6. Discussion

The present study was performed with the aim to investigate complications of severe obesity. Especially, it was interesting to analyse whether gut permeability is altered in patients with obesity compared to non-obese controls and if sleeve gastrectomy can modulate permeability. The effect of sleeve gastrectomy on the microbiota composition as well as inflammatory and metabolic markers was an additional objective of the study.

6.1 LSG induced energy restriction and weight loss

The patients included in this study all had a BMI ≥ 40 kg/m² (obesity stage III) with or without comorbidities. They therefore fulfilled the indication for bariatric surgery, if conservative therapies are exhausted (Hauner et al., 2014). Patients underwent LSG, whereby about 90% of the stomach volume was removed, which created a stomach pouch with a residual volume of approximately 50-150 ml. The median weight loss within the first half a year after surgery was 40.2 kg. This corresponds to 50.9% excessive weight loss. In the studies summarised in a recent meta-analysis, the mean %EWL 6 months after LSG varied between 43.9% and 67.8% (Ye et al., 2017). Weight loss is often achieved rapidly within the first year, while some studies report moderate weight regain after 2 to 6 years, assessed by decreasing %EWL (Himpens et al., 2010; Deitel et al., 2011; D'Hondt et al., 2011). Long-term follow up studies for sleeve gastrectomy are still rare. One recent review reported excessive weight loss between 56.4% and 62.5% within 5 to 11 years after surgery (Juodeikis and Brimas, 2017).

Absolute weight loss during the first 6 months after surgery was neither dependent on initial body weight nor on BMI. However, initial body weight as well as initial BMI showed a significant negative correlation with %EWL. This indicates that all patients experienced similar absolute weight loss independent of initial body weight and therefore the %EWL was lower in patients with higher initial weight. Within the first 6 months after surgery, weight loss is very fast and most likely the maximal weight loss state is not reached yet. Therefore, those associations should be revised 12 to 48 months after surgery, to see if the maximal %EWL is dependent on initial body weight and if it takes longer for patients with higher initial body weight to reach the status of maximal %EWL. Livhits and colleagues reviewed the existing literature with follow up times ranging from 6 to 144 months and found suggestive evidence for a negative association between initial BMI or super-obesity and weight loss, especially when reported in relative terms as EWL, after bariatric surgery (Livhits et al., 2012).

Besides weight loss, patients also reduced their waist as well as hip circumferences after surgery. As the decrease in waist circumference was higher than that of the hip, the waist-to-hip ratio decreased as well. Increased waist circumference (> 94 cm for males, < 80 cm for

females) as well as increased waist-to-hip ratio (≥ 0.90 cm for males, ≥ 0.85 cm for females) indicate abdominal adiposity and an elevated risk of metabolic complications (World Health Organization, 2011). LSG could therefore decrease the patients risk for comorbidities; however, none of the patients reached the above mentioned cut-off points defined by the WHO by 6 months post-operative.

The main reason for weight loss after sleeve gastrectomy is the reduced stomach size and the resulting decreased food intake and early satiety. The surgeons in our cooperating clinics used bougie sizes of 36 and 42 Fr (1 French (Fr) equals ⅓ mm), which are common sizes (Kehagias et al., 2016), and created sleeves with a remaining gastric volume of approximately 50–150 ml. Next to the restriction in stomach size, also accelerated gastric emptying and transit time can occur, that may diminish energy absorption from the diet (Melissas et al., 2008; Shah et al., 2010). Furthermore, hormonal changes after sleeve gastrectomy contribute to appetite regulation and weight loss (Kehagias et al., 2016). In the weeks after surgery, a nutritional therapy has to be implemented with stepwise progression to normal diet (Mechanick et al., 2013). One of our cooperating hospitals follows a standardised nutritional plan with prescribing a predominantly liquid diet during the first two weeks post-surgery, followed by two further weeks of pureed food. Afterwards, a balanced light normal diet is built up, whereby it is important to have small portion sizes and to pay attention to adequate liquid intake (Hüttl et al., 2016).

According to dietary protocols, the obese study patients had an extremely reduced energy intake of 882 kcal/day (median) at 6 months after surgery compared to 1826 kcal/day before surgery. Bioelectrical impedance analysis (BIA) of body composition was measured in a subgroup of patients after surgery, which revealed a calculated basal metabolic rate of 1956 kcal, which equates to 3130 kcal total energy expenditure using a PAL level of 1.6 (Supplemental Table 2). As physical activity was not measured in the present study, an average PAL level of 1.6 was used, defining mostly sedentary activity with temporary standing and walking (Deutsche Gesellschaft für Ernährung, Österreichische Gesellschaft für Ernährung, Schweizerische Gesellschaft für Ernährung, 2019). Energy demand therefore by far exceeded energy intake and patients were still in a state of catabolism and proceeding weight loss. The total amount of all macronutrients, micronutrients and vitamins in the diet was reduced after surgery. The percentage of macronutrient intake, however, was not significantly altered, suggesting that dietary habits did not change much. The recommendation of the DGE (Deutsche Gesellschaft für Ernährung e.V.) that carbohydrates should represent more than 50 energy percent of the diet was not reached by the study patients. Thus, the percentage of fats and protein was slightly higher than recommended (recommendation: protein: <15 energy%, fats: 30 energy%) (Deutsche Gesellschaft für

Ernährung, Österreichische Gesellschaft für Ernährung, Schweizerische Gesellschaft für Ernährung, 2019).

The median intake of all vitamins, except Niacin and Biotin, were below the recommendations, although nine patients took regular dietary supplements after surgery. Nutritional deficits are a well described complication after bariatric surgery, whereby patients undergoing malabsorptive and combined surgery techniques, such as RYGB, are more affected than those with restrictive SG (Gehrer et al., 2010). Deficiencies are most frequent for vitamin B12, iron, vitamin D and folic acid (Davies et al., 2007). Factors that can contribute to vitamin deficiencies after SG are caloric restriction, nausea and emesis, increased gastrointestinal transit time and resection of the gastric fundus (Davies et al., 2007; Snyder-Marlow et al., 2010). Through the resection of the fundus, the number of parietal cells is reduced which produce the intrinsic factor for the absorption of cobalamin. Furthermore, also the secretion of gastric acid is diminished, which is important for vitamin B1 as well as iron metabolism and absorption (Davies et al., 2007; Bal et al., 2012). Proton pump inhibitors additionally reduce gastric acid secretion.

Dietary intake of patients with obesity shortly before surgery was quite similar to the controls. Even total energy intake was not different between groups (1826 vs. 1979 kcal/day), which may not have been expected. Though, it has to be considered that obese patients recorded their diet shortly before surgery. All patients have had nutritional counselling and were encouraged to aim for a slight weight reduction already before surgery to prove motivation as well as to reduce liver volume and enhance metabolism, which facilitates the surgical procedure and reduces the risk of complications. Furthermore, underreporting is a well-described effect for self-reported dietary intake, with greater prevalence in people with overweight and obesity, as well as elderly people and females (Braam et al., 1998; Johansson et al., 2001; Gemming et al., 2014). As study patients were matched for age and sex, the difference in BMI may account for higher underreporting in patients with obesity versus controls. Moreover, estimation of portion sizes may differ between lean and obese subjects. Finally, the transfer of the data from the dietary record to the OptiDiet programme can contain sources of error, for example if special food items are not listed in the database and similar products have to be selected. It was tried to minimize those effects by detailed information for the participants and the use of standardization lists for food items and portion sizes. The composition of the diet, i.e. the content of macro- and micronutrients as well as vitamins was also very similar between obese patients before surgery and non-obese controls. The higher intake of protein in percent of the total energy intake in the obese patients may be due to the consumption of protein drinks by some of the participants and the general recommendation of protein rich diets for achieving weight loss.

Regarding the non-obese controls, total energy expenditure was calculated according to bioelectrical impedance analysis, using a PAL level of 1.6. This calculated total energy expenditure (2247 kcal) was higher than the reported dietary intake (1979 kcal/day). The above mentioned factors as well as an overestimation of the physical activity level could contribute to this discrepancy.

6.2 Effect of LSG on metabolic and inflammatory parameters

In our study cohort, overall metabolic status was impaired in the obese group compared to the control group. Regarding liver enzymes, γ-GT was significantly elevated in patients with obesity. Liver enzymes in blood are enhanced in case of liver damage and disease. NAFLD is a common comorbidity in obesity, with steatosis being the hallmark of this disease (Fabbrini et al., 2010). The prevalence of NAFDL increases with increasing BMI and can be prevalent in more than 90% of patients with morbid obesity undergoing bariatric surgery (summarised by Chalasani et al., 2012). Fatty acid accumulation and inflammation of the liver are closely associated with insulin resistance and cardiovascular disease (Fabbrini et al., 2010; Gaggini et al., 2013). The decrease in circulating liver enzymes after bariatric surgery, also seen in our study cohort, reflects the effect of weight loss on the reduction in chronic liver damage.

Dyslipidemia and an increase in NEFAs are further consequences of NAFLD and adiposity. In obesity, release of free fatty acids from adipose tissue is increased and ectopic fat storage in liver, muscle and other tissue occurs; moreover, in the pathogenesis of NAFDL and insulin resistance, de novo lipogenesis in liver is elevated and fatty acids are esterified into triglycerides (TG) and either stored in the liver or secreted as very low-density lipoprotein (VLDL) (Jung and Choi, 2014). In accordance with this, TG and NEFAs were elevated in our obese study patients, while HDL was decreased. Interestingly, LDL was not significantly different between non-obese and obese subjects. After surgery, the concentration of TGs decreased significantly, which has been seen in most studies on bariatric surgery so far (Heffron et al., 2016). Levels of NEFAs also decreased after surgery, as observed before (Thomas et al., 2016; Farey et al., 2017). This may be due to a decrease in adipose tissue mass as well as a decrease in insulin-resistance, as insulin has anti-lipolytic effects and inhibits the release of NEFAs from adipose tissue (Bierman et al., 1957).

Erythrocytes decreased after surgery in our study cohort together with a decrease in hemoglobin concentration. A shortage of iron, folic acid or vitamin B12, which are essential for the formation of erythrocytes, may be responsible. Dietary protocols revealed a significant decrease in iron uptake and intake was clearly below the recommendations (men: 10 mg/day, menstruating women 15 mg/day) (Deutsche Gesellschaft für Ernährung,

Österreichische Gesellschaft für Ernährung, Schweizerische Gesellschaft für Ernährung, 2019). The increase in bilirubin after surgery can further hint to an elevated erythrocyte breakdown. Leucocytes were significantly elevated in obesity and also thrombocytes were slightly increased, however, not reaching statistical significance. Systemic low-grade inflammation, as commonly observed in obesity, can explain these findings (Zaldivar et al., 2006; Samocha-Bonet et al., 2008). Both leucocytes as well as thrombocytes decreased after bariatric surgery, which is in accordance with former studies (Iannelli et al., 2013; Johansson et al., 2013), and possibly reflect a decrease in systemic inflammation.

LBP was measured as a marker for gut-derived inflammation. LPS is a key factor in the inflammatory process of obesity. Elevated concentrations in the circulation are due to high fat diets, an increase in gut permeability or alterations in the microbiota profile (Tuomi and Logomarsino, 2016). As LPS has a short half-life and its accurate quantification in blood samples is difficult (Novitsky, 1998), LBP was used as a surrogate marker. It was suggested that LPS is a sufficient trigger for the onset of obesity, insulin resistance and type 2 diabetes (Cani et al., 2007a). Moreover, LPS and LBP levels were reported to be associated with insulin resistance (Moreno-Navarrete et al., 2012; Jayashree et al., 2014). It was therefore also investigated, if LBP levels differed between diabetic and non-diabetic obese subjects before surgery. In the present study cohort, there was no effect of diabetes on LBP levels. However, as some of the diabetic patients were taking metformin before surgery, which might have an effect on LBP levels (Moreno-Navarrete et al., 2012), it might not be possible to draw reliable conclusions about LBP levels in dependence on diabetes. After surgery, LBP levels did not significantly decrease. This may point towards unaltered gut permeability and gut microbiota composition at 6 months after surgery. Other studies have recorded reduced LPS and LBP levels after bariatric surgery (Tuomi and Logomarsino, 2016). However, it can take up to one year until this decrease becomes significant (van Dielen et al., 2004).

Systemic inflammation, measured by CRP levels, was increased in obesity. Inflammation plays an important role in the development of diabetes and CRP has been postulated to be a predictor of an elevated diabetes risk (Hu et al., 2004); yet it was not able to see any difference in CRP levels between obese diabetic and non-diabetic subjects. Just as for LBP, a possible association of diabetes with CRP might have been masked by the metformin intake of diabetic patients, as metformin can lower CRP levels (Carter et al., 2005). After bariatric surgery, CRP was significantly decreased. Selvin and colleagues showed that weight loss per se, irrespective of the method of weight loss (i.e. life style change, dieting, exercise or bariatric surgery), decreases CRP levels, and the magnitude of weight loss correlates with the decline in CRP (Selvin et al., 2007). As LBP levels were unaltered, metabolic endotoxemia does not seem to be responsible for the decrease in CRP. Therefore,

other factors, such as a reduction of adipose tissue with a decrease of macrophage infiltration and inflammatory cytokine production, may have contributed to the overall decline in the inflammatory load (Labrecque et al., 2017).

Before surgery, as expected, patients with obesity had significantly elevated fasting glucose and insulin levels, and levels were higher in obese diabetic compared to non-diabetic patients. At six months after surgery, there was an improvement in glucose metabolism, seen by a reduction in fasting glucose and insulin. The reduction in systemic inflammation and free fatty acids may have contributed to this. Moreover, alterations in gastrointestinal hormones (Basso et al., 2011; Jacobsen et al., 2012), the enforced hypocaloric diet after surgery (Henry et al., 1985) and improved ß-cell function (Kashyap et al., 2010) could contribute to these improvements. Patients with diagnosed type 2 diabetes showed improvement or even resolution of the disease. For obese patients with diabetes, it has been shown that bariatric surgery is more effective than medical therapy alone to improve or even resolve diabetes in the long term (Schauer et al., 2017). Overall, our study patients showed improvements in metabolic and inflammatory markers, proving that this type of bariatric surgery is a metabolic surgery.

It has to be mentioned that blood glucose levels and routine laboratory parameters of patients with obesity were received from laboratory reports of the clinics. Therefore, there were not the same clear standardised fasting conditions as for samples from the control subjects, and there hence may be some bias. Insulin and inflammatory parameters of patients with obesity were measured from blood drawn during surgery. Non-diabetic obese patients received dexamethasone as anti-emetic agent during surgery, which increases glucose levels and has anti-inflammatory and immunosuppressive properties. Therefore, it cannot be excluded that there was a confounding effect of dexamethasone as well as the surgical procedure per se on insulin and inflammatory parameters of obese patients at V1.

6.3 LSG induced significant changes in gut permeability

For assessment of gut permeability a standardized 4-probe sugar test (Norman et al., 2012) was used. Next to the sugar test, also ^{51}Cr-EDTA and polyethylene glycols (PEGs) can be used for *in vivo* determination of gut permeability. In a previous study of our group, the sugar test was used in parallel to the PEG test (Ott et al., 2017). While the sugar test provided information on the localisation of altered barrier function, the PEG test provided size-dependent assessment of gut permeability. Both tests revealed similar outcome (Ott et al., 2017). However, the analysis of PEGs was challenging with high background levels. Moreover, the parallel performance of both tests increased complexity and was challenging for study participants. In contrast to the study by Ott et al., the participants of the present

study performed the permeability test at home. We therefore tried to minimize complexity of the test to avoid errors during test performance. Thus, we decided to solely use the sugar test in combination with zonulin levels in blood to determine gut permeability in the present study.

The present study could show that gastroduodenal as well as small intestinal permeability in patients with obesity were decreased 6 months after LSG. Regarding small intestinal permeability, the decrease in permeability was seen for the paracellular as well as for the transcellular route of permeation. As both routes of permeation were changed in the same direction, the L/M ratio was not altered. It is generally assumed, that the urinary recovery rate of mannitol is proportional to the intestinal mucosal area and therefore remains stable, while the urinary recovery of lactulose is dependent on the tight junction regulation (Gaggiotti et al., 1995; Sigalet et al., 2000; Savassi-Rocha et al., 2014). As the intestine is not affected by LSG, the mannitol excretion rate is assumed to be stable. However, an accelerated gastric emptying and an increase in intestinal peristalsis have been described after LSG (Shah et al., 2010; Melissas et al., 2013), which, at least in part, could contribute to the observed decrease in urinary recovery of lactulose as well as mannitol. Yet, recent studies on gut permeability after caloric restriction and associated weight loss also reported a decrease in gastroduodenal as well as small intestinal permeability (Xiao et al., 2014; Damms-Machado et al., 2017; Ott et al., 2017), which would support the assumption that our findings are not solely dependent on changes in peristalsis, but also on caloric restriction and weight loss.

Gastroduodenal permeability was also decreased after surgery. This could hint to a reduction in inflammation and ulcerative lesions in the mucosa of the esophagus, stomach and duodenum (Sutherland et al., 1994). However, as gastroduodenal permeability was not elevated in obese patients before surgery, this explanation is not likely. Alternatively, a reduction of gastric surface area due to gastrectomy could result in a reduction of urinary excretion of sucrose. Moreover, caloric restriction per se can decrease gastroduodenal permeability, as described in a recent study (Ott et al., 2017).

In contrast to gastroduodenal and intestinal permeability, the permeability of the colon was increased after surgery. Therefore, permeability of the different sections of the gastrointestinal tract seems to be regulated by different mechanism. As weight loss induced by caloric restriction decreases colonic permeability (Ott et al., 2017), other mechanisms apart from weigh loss have to be responsible for the alteration in colonic permeability after LSG, and those still need to be elucidated.

In addition to the sugar test, plasma zonulin concentration has been measured in the study. This protein interacts with tight junctions, thereby increasing paracellular intestinal

permeability (Fasano et al., 1995). Despite a decrease in paracellular permeability, the concentration of zonulin was not altered 6 months after LSG. This result indicates that other factors, such as nutrients, may be involved in the regulation of intestinal paracellular permeability, as measured by lactulose excretion. Several studies so far have demonstrated the potential influence of nutrients on gut barrier function and translocation of LPS (Osanai et al., 2007; Bergheim et al., 2008; Suzuki and Hara, 2010).

Moreover, it is important to note that recently there have been concerns regarding the preciseness of the widely used zonulin ELISA kit which was also used in the present study. After completion of the data, a study was published that claimed that the kit in question does not detect zonulin (pre-haptoglobin 2), but rather a variety of other proteins that are structurally and possibly functionally related to zonulin, such as members of the mannose-associated serine protease family (Scheffler et al., 2018). The impreciseness of the kit could provide an explanation for the discrepancy between decreased paracellular intestinal permeability and unaltered zonulin levels in the present study.

The effect of bariatric surgery on gut permeability has barely been studied so far in other studies. In a human study by Savassi-Rocha and colleagues, RYGB surgery led to a decrease in urinary excretion of mannitol at 1 month after surgery, but values returned to preoperative levels at 6 months after the procedure (Savassi-Rocha et al., 2014). Gastroduodenal and colonic permeability have not been investigated. The results from this study are most likely due to the surgery specific shortening of the intestine and subsequent intestinal adaptions (Savassi-Rocha et al., 2014). In comparison to RYGB, sleeve gastrectomy is a less invasive procedure that reduces stomach size, but does not directly affect the intestine. Therefore, it is likely that the observed alterations in gut permeability 6 months after LSG are primarily dependent on weight loss and caloric restriction rather than on the surgical procedure itself. Yet, as mentioned before, modifications in gastric emptying and small bowel transit time may be contributing factors. The results of our present study are largely supported by a recent study by Blanchard and colleagues that investigated the effect of sleeve gastrectomy on gut permeability in diet-induced obese mice. In these mice, the paracellular and transcellular jejunal permeability decreased after sleeve gastrectomy, whereas colonic permeability was increased (Blanchard et al., 2017). Just recently, however, the group of Wilbrink and colleagues published the results of another human study on gut permeability after LSG (Wilbrink et al., 2020). Part of that study had previously been published as a conference abstract (Ovink-Wilbrink et al., 2014). This group reported a slight decrease in gastroduodenal permeability in obese patients 6 months after LSG to levels not significantly different to lean controls. The decrease, however, was not significant. Small intestinal permeability, assessed by lactulose/rhamnose ratio, was increased at 6 months

after surgery, and colonic permeability was unaltered. The authors concluded from their results, that disaccharides (paracellular route of transport) are not affected, while monosaccharides (transcellular route of transport) are reduced after SG (Wilbrink et al., 2020). This conclusion cannot be supported by our study, as we found differences in urinary secretion of monosaccharides as well as disaccharides after surgery. We do not have a clear explanation for the differences in the results of our study and the study by Wilbrink et al., as study designs were quite similar; however, in part different sugars were used for assessment of permeability.

In our study participants, the change in gut permeability was not associated with weight loss parameters. Therefore, the change in gut permeability seems to be independent of the excess of weight loss, and caloric restriction may be more important for alterations in gut permeability. As reported from the dietary protocols, the total caloric intake 6 months after surgery was still significantly decreased in comparison to the pre-surgery state. The same conclusions have been made by a recent study that showed that most effects of caloric restriction and associated weight loss on gut permeability disappeared in the follow-up period with normal diet, although participants did not gain substantial weight (Ott et al., 2017).

The decrease in gut permeability also did not correlate with the reduction in overall inflammation, measured by CRP, or with alterations in LBP levels. Interestingly, LBP levels did not decrease after surgery, although intestinal permeability was decreased. However, as colonic permeability in contrast to intestinal permeability increased after surgery, there was no overall decrease in paracellular permeability that could restrain the translocation of LPS. Moreover, LPS can use other routes of translocation into the circulation, for example via chylomicron incorporation (Ghoshal et al., 2009).

Gut permeability was also compared between obese diabetic and non-diabetic patients, as permeability has been shown to be increased in both type 1 as well as type 2 diabetes mellitus (Mooradian et al., 1986; Damci et al., 2003; Secondulfo et al., 2004; Bosi et al., 2006; Sapone et al., 2006; Horton et al., 2014; Sato et al., 2014). While in type 1 diabetes increased intestinal permeability precedes the clinical manifestation of the disease and is likely to contribute to the pathogenesis (Bosi et al., 2006; Sapone et al., 2006), its role in the progression of type 2 diabetes is not clearly defined yet. In our cohort of obese patients, we could not see any differences in gut permeability in the diabetic patients, which might be due to the small study groups. Moreover, as in most other human studies, part of our diabetic patients was taking metformin as anti-diabetic agent. The effect of metformin on intestinal permeability is not known yet. While there is evidence from rodent studies, that metformin protects against the loss of tight junction proteins and subsequent elevation of plasma endotoxin levels in high-fructose diet and high-fat diet conditions (Moreno-Navarrete et al.,

2012; Spruss et al., 2012), another study could not find a significant effect of metformin on intestinal permeability and systemic endotoxin levels (Shin et al., 2014). Human clinical data regarding the effect of metformin on intestinal permeability are still missing.

Furthermore, other drugs might interact with mechanisms regulating intestinal permeability. PPIs can increase gastro-duodenal permeability (Mullin et al., 2008) and NSAIDs gastro-duodenal as well as small intestinal permeability (Bjarnason et al., 1986; Lambert et al., 2012). We tried to control for possible influences of medication in the present study. Patients with regular NSAID intake were excluded. Moreover, participants were not allowed to take PPIs and NSAIDs for at least 48 hours before the permeability test as well as during the test. No differences in the permeability were detected between PPI users and non-users. Moreover, permeability of obese patients was similar to non-obese controls before surgery. Therefore, the efforts to exclude possible influences of medication in measurement of gut permeability in the present study seemed to be effective and an effect of medication on gut permeability is not likely in our cohort.

Interestingly, we could not detect any difference in gut permeability between obese patients before surgery and non-obese subjects. Gut permeability in obesity has been contrarily discussed so far. While some studies observed an elevated small intestinal permeability in obesity (Gummesson et al., 2011; Teixeira et al., 2012b), others did not (Brignardello et al., 2010; Verdam et al., 2013). We therefore wanted to further investigate gut permeability in obesity and compared different classes of obesity. We did not find any differences in gut permeability between the various classes of obesity as well as between non-obese and obese patients. Solely the concentration of zonulin in plasma was elevated in obesity class I and decreased again at higher BMI values. This increase in zonulin was not reflected by an increase in small intestinal paracellular permeability. As mentioned before, the possible impreciseness of the zonulin kit might make the reliability of these data questionable (Scheffler et al., 2018). We therefore cannot conclude any association of BMI and gut permeability. It is possible that not BMI itself, but rather diet, especially high-fat diets, and associated changes in gut microbiota influences gut permeability (Cani et al., 2007b; Cani et al., 2008). As these factors may differ between study cohorts, this can provide an explanation for the conflicting results between the studies. In the present study, non-obese and obese individuals showed similar dietary intake and composition, which supports this hypothesis. Interestingly, a recent study did also not see differences in small intestinal permeability assessed by L/M ratio in obese compared to lean subjects in a fasted state, but impairments were found in tight junctions in jejunal samples of obese patients and these impairments of the barrier were exacerbated under lipid challenge *ex vivo* (Genser et al., 2018). It is therefore conceivable that lipid challenges can contribute to barrier dysfunctions in obesity.

Despite unaltered gut permeability, the levels of LBP were increased in obesity, suggesting increased levels of systemic endotoxin. Apart from an increase in gut permeability, LPS can enter the circulation through incorporation into chylomicrons (Ghoshal et al., 2009). The increased levels of LBP can contribute to an increased overall inflammation, measured by CRP, but other factors, such as an increased adipose tissue mass and its pro-inflammatory secretion pattern may also explain this surge in inflammation (Skurk et al., 2007). The different classes of obesity showed similar median levels of LBP and CRP, yet not all were statistically significantly different to the non-obese control group, which may be due to small sample size in the subgroups of obesity.

Overall, we cannot confirm impairment of gut permeability in obesity. However, weight loss induced by LSG resulted in modification of gut permeability in morbidly obese patients. Recent studies with modification of the diet and caloric restriction, associated with moderate weight loss, could demonstrate a reduction of gastroduodenal, small intestinal and colonic permeability (Xiao et al., 2014; Ott et al., 2017). Furthermore, obese patients with steatosis showed reduction of intestinal permeability after a weight-reduction programme (Damms-Machado et al., 2017). Together, these results suggest that body weight per se does not influence gut barrier function, whereas interventions such as caloric restriction and consecutive weight loss can initiate distinct improvements in permeability.

6.4 The effect of PPI use and LSG on gut microbiota

Alterations in gut permeability have been associated with changes in gut microbiota composition (Cani et al., 2007b; Cani et al., 2008; Cani et al., 2009). This finding, however, could not be confirmed in humans (Verdam et al., 2013). In this study, the aim was to compare gut microbiota composition in non-obese and obese patients as well as before and after bariatric surgery and to investigate possible associations with gut permeability.

Gut microbiota was analysed in all study participants. Part of the patients with obesity was taking proton pump inhibitors to treat gastroesophageal reflux disease (GERD) before as well as after surgery. The risk of GERD is increased in obesity (Hampel et al., 2005) and PPI medication is common. Around 30% of patients undergoing bariatric surgery are taking regularly PPIs before surgery (Sheppard et al., 2015). Some bariatric surgery procedures can improve GERD symptoms by decreasing body weight and altering the anatomy of the gastrointestinal tract (Sise and Friedenberg, 2008). Sleeve gastrectomy, however, is more likely to increase GERD risk as well as intensity, but the reasons for that are not clearly defined yet (Sheppard et al., 2015).

PPIs block acid transport in the proton pumps of parietal cells in the stomach. The resulting decrease in pH is limited to the stomach and the proximal duodenum, while most of the small

intestine and the colon is unaffected (Freedberg et al., 2014). Nevertheless, PPIs can influence bacterial composition in the whole gastrointestinal tract and therefore the faecal microbiota composition (Freedberg et al., 2014; Hojo et al., 2018; Reveles et al., 2018).

In the present study, study participants did not take PPIs for at least 48 hours before the intestinal permeability test, but as expected, for microbiota composition, effects of regular PPI intake cannot be reversed within this short time. We could confirm differences in microbiota composition due to PPI intake. This was especially true for obese patients before surgery, which showed significant differences in alpha- as well as beta-diversity and relative abundance of the main taxa. Interestingly, alpha-diversity, which has been reported to be unaffected (Reveles et al., 2018; Takagi et al., 2018) or decreased (Imhann et al., 2016; Jackson et al., 2016) by PPIs, was higher in PPI users in the present study. This may be an effect of high variability between samples as well as small sample size.

Beta-diversity was different in obese patients with and without PPI intake and was reflected in differences within single taxonomic groups. Nearly all studies on PPIs so far revealed an increase in members of the family *Streptococcaceae* (Imhann et al., 2016; Jackson et al., 2016; Hojo et al., 2018; Reveles et al., 2018; Takagi et al., 2018), which could be confirmed with the present data. Streptococcus bacteria are commensal members of the microbiome in the oral cavity (Dewhirst et al., 2010). A decrease in gastric pH, which normally functions as a barrier against ingested microbes, could lead to increased translocation of oral bacteria into the deeper gastrointestinal tract and may explain the increased abundance of *Streptococcus* (Imhann et al., 2016; Hojo et al., 2018). Furthermore, we found differences for several other taxa. Overall, the composition of the gut microbiota of our patients was considerably altered by the intake of PPIs. Therefore PPI use is a confounding factor in studying microbiota in obesity and bariatric surgery. However, this has not been considered in most studies so far. In the present study we thus decided to exclude PPI users from the final analysis, to reveal surgery-induced differences independent of PPI intake.

The presence of diabetes mellitus and therapeutic treatment with metformin may also have influences on gut microbiota composition and function (Forslund et al., 2015; Wu et al., 2017). In the present study, two patients in the final microbiota analysis were taking metformin. We did not see essential differences in the gut microbiota of these patients compared to those of untreated obese patients and exclusion of metformin-treated patients did not change the main findings of the analysis. Therefore, the effect of bariatric surgery is likely to have stronger impact on the gut microbiota than anti-diabetic medication, as also concluded by a former study (Kikuchi et al., 2018). We thus decided to keep those patients in the final analysis.

When comparing non-obese and obese subjects, alpha-diversity was lower in the obese patients. Previous studies reported similar results (Turnbaugh et al., 2009; Verdam et al., 2013) and low bacterial richness has generally been associated with weight gain and metabolic disturbances such as insulin resistance and inflammation (Le Chatelier et al., 2013). Furthermore, non-obese and obese individuals clustered together and showed differences in their microbial composition. Most strikingly, the relative abundance of *Bacteroidetes* was enhanced in obesity, whereas *Firmicutes* were more abundant in the control group. This finding is in contrast to the proposed hypothesis of an increased *Firmicutes*/*Bacteroidetes* ratio in obesity (Ley et al., 2005). Conflicting data to this common hypothesis have already been published before (Collado et al., 2008; Schwiertz et al., 2010). Though, in humans, factors such as sample cohort, diet, exercise, comorbidities, medication and environment may have a greater impact on microbiota composition as obesity per se and the postulation of a specific type of microbiota being a biomarker for obesity may not yet be possible. Moreover, specific functional profiles of the microbes together with microbial derived products, like SCFAs, may differ more significantly in obesity and weight loss (Schwiertz et al., 2010; Graessler et al., 2013; Damms-Machado et al., 2015) and have to be investigated further.

However, the induction of the enterotype concept in 2011 can lead to the conclusion that comparative microbiota analysis between human study groups so far may be not conclusive, as far as the enterotype of the subjects has not been investigated (Arumugam et al., 2011). The concept of human enterotypes states that each human individual fits into one of three clusters of well-balanced, defined, and stable microbial communities and these defined communities are thereby not dependent on factors such as nationality, age, or BMI (Arumugam et al., 2011; Costea et al., 2018). As most investigations conducted so far did not select study groups on the basis of enterotypes, the comparisons of taxonomic differences are not conclusive. Indeed, in our study cohort, it seems that patients with obesity and non-obese controls derived from different enterotypes. While controls resembled enterotype 3 with a high abundance of *Firmicutes*, especially *Ruminococcus*, patients with obesity are more likely to have originated from enterotype 1 with a low richness and a high abundance of *Bacteroidetes* (Arumugam et al., 2011). Characteristic of enterotype 2 would be an enrichment of Prevotella (Arumugam et al., 2011). We therefore cannot draw reliable conclusions by comparing the gut microbiota of our non-obese and obese study cohort.

When investigating the effects of weight loss after LSG on the gut microbiota, there was an increase in alpha-diversity after surgery. A previous study attained similar results after SG in comparison with a more pronounced increase in bacterial richness after RYGB (Murphy et al., 2017). Taxonomic changes tended towards the composition of our non-obese study

group, yet were in most cases minor and not significant. Regarding the main phyla, *Bacteroidetes* tended to decrease, while there was a trend to increased abundance of *Firmicutes*. Similar outcome was observed in the microbiome of obese patients after SG in a recent study (Medina et al., 2017), while other studies noticed an opposite trend (Damms-Machado et al., 2015; Sanmiguel et al., 2017; Kikuchi et al., 2018). Significant alteration after surgery in our study was observed for the family *Rikenellaceae*, which increased in abundance. Furthermore, the genus *Ruminococcaceae NK4A124*, which was not present in any patient before surgery, came up afterwards, and the whole family of *Ruminococcaceae* tended to increase after surgery. Most members of *Ruminococcaceae* are able to degrade complex plant polysaccharides (Biddle et al., 2013). Fibre intake, however, which would support growth of fibre degrading bacteria, was drastically decreased after surgery. However, an increased peristalsis and accelerated gastric emptying after SG (Shah et al., 2010; Melissas et al., 2013) could elevate passage of undigested nutrients to the colon and contribute to adaptation of microbiota. *Anaerostipes hadru*s disappeared in obese patients after surgery. A reduction in *Anaerostipes* abundance has been reported after RYGB (Graessler et al., 2013; Aron-Wisnewsky et al., 2018) and bilio-intestinal bypass (Patrone et al., 2016) and has been associated with changes in anthropometric parameters (Aron-Wisnewsky et al., 2018). A general decline in butyrate producing bacteria, such as *Anaerostipes hadrus*, has also been described after LSG (Damms-Machado et al., 2015), whereby this did not lead to changes in faecal SCFAs. The changes in microbial metabolism and resulting metabolites, such as SCFAs, are potentially more important for microbiota-host interactions and improvements after bariatric surgery and should be investigated in more detail in future studies.

It is particularly noteworthy, that LSG had a varying impact on the gut microbiota composition of individual patients in our cohort. While some underwent considerable shifts in the direction of the controls or changed between the two observed clusters after surgery, others remained quite stable. High inter-individual differences in microbial changes after surgery have also been observed in a former study (Aron-Wisnewsky et al., 2018). In our study, this shift in microbiota seemed to be independent of changes in weight, diet, metabolic parameters or gut permeability, i.e. no differences in changes of these parameters could be seen in patients that experienced considerable shifts in their microbiota and those who did not. Yet, our sample sizes were too small to perform statistical tests. Therefore, the factors triggering microbiota changes after LSG are still unclear. Other studies, which investigated microbiota after SG so far, have also not yielded consistent results regarding microbiota composition and diversity (Patil et al., 2012; Damms-Machado et al., 2015; Tremaroli et al., 2015; Liu et al., 2017; Medina et al., 2017; Murphy et al., 2017; Sanmiguel et al., 2017; Campisciano et al., 2018; Kikuchi et al., 2018; Paganelli et al., 2019; Palmisano et al., 2019). It is possible

that this is also due to differences in enterotype status between study cohorts. Enterotypes may vary in their resilience as well as their recovery after interventions such as weight loss. Moreover, all of these studies, including our own research, have been performed with small samples sizes and different analytical techniques. Comorbidities and medication of patients with obesity can further increase variability. In the future, larger study cohorts that are stratified according to the enterotype status of the patients and controls are needed.

An association of gut permeability and microbiota composition has been previously reported in mouse studies (Cani et al., 2007b; Cani et al., 2007a; Cani et al., 2008; Cani et al., 2009) and even single species, like *Akkermansia muciniphila*, have been found to be potentially involved in barrier defects observed in obesity (Everard et al., 2013). In our human study, we could not observe such an association. Non-obese and obese patients before surgery had similar gut permeability, yet differences in microbial composition of the gut. This is similar to two further human studies, which noted altered microbiota composition in obese compared to non-obese subjects, but no alteration in intestinal permeability and no association of intestinal or colonic permeability with gut microbiota (Brignardello et al., 2010; Verdam et al., 2013). One study in diabetic patients, however, reported that high colonic permeability is associated with altered abundance of some microbial taxa, e.g. *Enterobacteriales*, in diabetic and non-diabetic patients (Pedersen et al., 2018). Overall, the relation of gut permeability and gut microbiome in humans seems to be more complex compared to the situation in rodents and needs further research in human cohorts.

6.5 FAHFA levels under different physiological and pathophysiological conditions

Thus far, the present study showed that weight loss induced by LSG leads to marked improvements in glucose metabolism and in the inflammatory status of patients. The decrease in adipose tissue mass and remodelling of adipose tissue is one main driver for these processes and the function of lipids and adipokines in these processes has extensively been studied (Booth et al., 2016; Magkos et al., 2016). We further were interested to investigate the role of a special class of lipids, the FAHFAs, in this context of surgery induced weight loss. FAHFAs have been attributed with potent anti-inflammatory and anti-diabetic effects, which could be involved in the overall improvements after weight loss. However, research about FAHFAs is still in its infancy, as this class of lipids has been discovered just a few years ago.

As a first step, we established at our university a method for extraction and HPLC-MS/MS based detection and quantification of FAHFAs from human serum samples. By the use of previously published protocols (Yore et al., 2014; Zhang et al., 2016; Kolar et al., 2018) and their adaption and optimisation, we were able to quantitatively analyse 9-OAHSA, 9-SAHSA, 9-PAHPA as well as PAHSAs, separated into 9-, 10-, and 12/13-PAHSA. Similar to previously published data (Yore et al., 2014; Zhang et al., 2016; Kolar et al., 2018), the peaks of 12- and 13-PAHSA could not be separated by our method and were therefore quantified together. We detected peaks for 5-PAHSA; however, the ratios of the MRM transitions did not always match that of the corresponding standard. This was probably due to an overlay of 5-PAHSA with a ceramide contaminant (Zhang et al., 2016; Kolar et al., 2018). We thus excluded 5-PAHSA from our analysis. The levels of FAHFAs detected in human serum samples were in the low nano-molar range. They were thus slightly lower, yet comparable to the levels in human serum analysed by Yore and colleagues (Yore et al., 2014) and higher compared to those analyzed by Zhu and colleagues (Zhu et al., 2017). Differences in the analytical procedures and sensitivity of the respective methods may be responsible for these slight differences. However, although this might limit the comparability to the other studies it does not interfere with the interpretation of our well defined sample sets.

In our study population of non-obese subjects and obese patients undergoing LSG, we investigated the effect of BMI and excessive weight loss on serum FAHFA levels. The n number slightly differed in comparison to the above analysis of gut permeability, as for the FAHFA measurements we did not exclude patients with special medication or antibiotic treatment during the study period. As a first result we could see, that the morbidly obese patients showed decreased FAHFA levels in blood compared to the non-obese control subjects. Furthermore, a decrease in body weight at 6 months after bariatric surgery did not

significantly alter serum FAHFA levels. A longer period of follow-up would be of interest to see if FAHFA levels remain reduced or if they just slowly increase over time. As blood samples of patients with obesity were taken during surgery, we cannot exclude a possible confounding effect of surgery. However, we could confirm an effect of BMI in another cohort of healthy adults aged 18–85 years, where BMI, independent of age, had significant influence on FAHFA levels. In mice, it has been shown, that high-fat diet-induced obesity results in decreased 5- and 12/13-PAHSA levels, while other PAHSAs were not significantly altered (Yore et al., 2014). Moreover, in these mice PAHSA levels in adipose tissue were differently affected than those in serum. In humans, lower levels of palmitoleic-acid-hydroxy-palmitic-acid (POHPA) have been reported in overweight compared to normal weight subjects, but no differences were seen for other groups of FAHFAs in this study cohort (López-Bascón et al., 2016). Another group of researchers reported reduced levels of PAHSAs in breast milk of obese compared to lean mothers (Brezinova et al., 2018). This result could indicate lower levels also in adipose tissue and serum; however, this has not been analysed in the study. Therefore, our own and previous studies show that BMI has impact on FAHFA levels, and this impact seems to be tissue-specific and isomer-specific.

PAHSAs have been credited with potent anti-diabetic effects. The administration of PAHSAs to mice resulted in improved insulin sensitivity and glucose tolerance (Yore et al., 2014; Syed et al., 2018). These effects are obtained by enhanced insulin secretion, induced directly at the beta-cells via GPR40 as well as by enhanced GLP-1 secretion in the gut (Yore et al., 2014; Bandak et al., 2018; Syed et al., 2018). Moreover, insulin-stimulated glucose uptake in the adipocyte is promoted via binding to the receptor GPR120 (Yore et al., 2014). The reduced levels of PAHSAs in obesity could represent one mechanism, by which obesity contributes to insulin resistance. We therefore next investigated FAHFA levels in diabetic compared to non-diabetic individuals. We could not detect any significant differences between diabetic and non-diabetic subjects in two independent cohorts. Moreover, no correlations between fasting glucose, fasting insulin or HOMA-IR and tFAHFAs or tPAHSAs were seen in the enable cohort of normalweight to obese subjects. But, in the morbidly obese cohort (GOBB), a positive correlation of FAHFAs and BMI was reported. As this cohort consists solely of very obese patients, the positive correlation with BMI does not necessarily contradict the finding of lower FAHFA levels in obese compared to lean subjects in our previous cohort. A possible explanation may be the high variance in the FAHFA concentrations and increasing variance with higher BMI in this data set. A larger cohort may thus be needed to validate these findings.

Also, reduced levels of PAHSAs in insulin-resistant compared to insulin-sensitive subjects have been reported previously (Yore et al., 2014). However, both groups differed in BMI,

which may have caused this result, as we observed differences in dependence on BMI but not diabetic status. It is also interesting to note that we found lower levels of FAHFAs in subjects maintaining a vegetarian/vegan diet compared to omnivores, despite the appraisal of vegetarian diets being protective for the development of diabetes (Olfert et al., 2018). Moreover, the group of young men undergoing overfeeding for one week showed significantly increased FAHFA levels after the intervention, but unaltered insulin sensitivity measured by the euglycemic hyperinsulinemic clamp technique (Ott et al., 2018). It is therefore questionable, if reduced FAHFA levels represent an independent risk factor for the development of diabetes.

Next to BMI and diabetic status, we were interested in other factors that possibly are associated with serum FAHFA levels. We therefore investigated if FAHFA levels differed between sexes and between different groups of age. While sex did not seem to be related to FAHFA levels, we found higher levels of most FAHFAs in adults compared to adolescents. When comparing adolescents and elderly subjects, FAHFA levels were not different any more, except for 9-PAHPA. This observation is quite interesting, however needs further investigation. Changes in the regulation of metabolic pathways with age or differences in dietary habits which we could not analyse in this study could provide possible explanations for these results and should be further investigated.

Finally, we investigated FAHFA levels in dependence of diet. As a novel finding, we described a considerable influence of the dietary background on serum FAHFA levels in healthy human subjects. Individuals following a vegetarian or vegan diet showed significantly lower serum levels of FAHFAs compared to omnivores. In a second study cohort, we analysed FAHFAs in a group of young men before and after one week of defined overfeeding. There was a considerable increase in levels of all FAHFAs analysed.

To date, only little is known about the origin of FAHFAs in the circulation. Although FAHFAs have been found in different foods (Yore et al., 2014; Zhu et al., 2018; Liberati-Čizmek et al., 2019), their uptake from dietary sources has yet to be shown. Interestingly, animal products, such as beef and egg, seem to contain more FAHFAs compared to plant products (Yore et al., 2014), which would explain our finding of higher FAHFA levels in omnivores compared to vegetarians. However, FAHFAs can also be synthesized endogenously in adipose tissue via esterification of hydroxyl-fatty acids with fatty acids. This has been confirmed at least in mice where dietary supplementation with the non-endogenous hydroxy fatty acid 9-hydroxy heptadecanoic acid (9-HHA) was used to detect the corresponding FAHFA 9-palmitic-acid-hydroxy-heptadecanoic-acid (9-PAHHA) in blood serum (Yore et al., 2014). Furthermore, it was assumed, that levels of FAHFAs in serum and adipose tissue mainly reflect endogenous synthesis rather than dietary intake, as isomer distribution in the diet differs from isomer

distribution in serum and tissue (Yore et al., 2014). Moreover, FAHFA levels in serum and adipocytes may be regulated via synthesis and breakdown of FAHFA-TGs in adipocytes (Tan et al., 2019). Therefore, it can be assumed that the higher concentrations of FAHFAs in serum from omnivores in comparison to vegetarians do not predominantly result from higher intakes of FAHFAs with the diet. The fat content and fatty acid composition of an ovo-lacto-vegetarian or vegan diet, however, differs from that of a diet containing fish and meat, as a vegetarian or vegan diet results in lower intake of total fatty acids and saturated fatty acids compared to non-vegetarians (Mann et al., 2006; Fernandes Dourado et al., 2011). The differences in amount and type of fatty acids that are taken up and deposited in adipocytes may influence the endogenous synthesis of FAHFAs. The results of the overfeeding study may be in agreement with this. The intake of a surplus of 1000 kcal, mainly provided by whipping cream, containing high amounts of saturated fatty acids, resulted in a marked increase in endogenous FAHFA levels. The FAHFAs analysed also contain mainly saturated fatty acids (palmitic and stearic acid) and only 9-OAHSA contains a mono-unsaturated fatty acid (oleic acid). It can be speculated that the levels of other FAHFAs, containing unsaturated fatty acids, or FAHFAs derived from PUFAs (Kuda et al., 2016) may not have been increased after the overfeeding period. However, correlation analysis by Spearman rank correlation revealed no significant correlation of the increase in tPAHSAs or tFAHFAs and changes in dietary parameters, such as total energy intake, total fat intake, and saturated fat intake. Therefore, endogenous FAHFA production does not seem to be directly correlated to the intake of dietary fats or energy. Future studies still need to clearly identify the origin of circulating FAHFAs with regard to dietary intake or endogenous production.

In summary, the present study provides important data for getting an overview of serum FAHFA levels under different physiological and pathophysiological conditions. Especially the influence of diet, as seen with the overnutrition of SFAs, is an important new finding. For the comparison of different groups the small sample sizes may partially yet be a limitation of the present study. In combination with large variability in FAHFA contents this may reduce the power to detect differences. Also confounding is a general issue in observational studies, even if we tried to match groups for various factors. Further research will be needed to confirm the results of the present study.

7. Conclusion and outlook

The present study provided valuable data to the barely studied field of gut permeability in obesity as well as after bariatric surgery. Using a 4-sugar test, the permeability of different sections of the gastrointestinal tract was analysed. It was shown that gut permeability is not altered at different stages of obesity compared to non-obese controls. However, when analysing obese patients before and after LSG, a considerable effect on gut permeability with distinct changes of gastroduodenal, small intestinal and colonic permeability could be detected. It cannot be distinguished if the effect is driven by caloric restriction or the surgical procedure per se. However, as previous studies revealed similar effects of non-surgical weight reduction and caloric restriction on gut permeability, it seems likely that the alterations in gut permeability after LSG are also mainly dependent on these factors. For future studies it would be interesting to analyse the effect of different restrictive as well as malabsorptive bariatric surgery procedures on gut permeability to see if the effects are surgery-dependent.

A strength of the present study is that gut microbiota composition was analysed together with intestinal permeability. In patients undergoing LSG there was an increase in alpha-diversity after surgery and taxonomic changes tended towards the composition of the non-obese controls, yet were in most cases minor and not significant. As reported in previous studies, the effect of bariatric surgery on gut microbiota composition was quite heterogeneous with some patients showing a more stable microbiota than others. This study thus did not show a clear association between gut microbiota composition and gut permeability in obese patients after LSG. Obese patients showed lower alpha-diversity and compositional differences compared to non-obese controls, with a higher abundance of *Bacteroidetes* in the obese group and a higher abundance of *Firmicutes* in the control group. As it can be speculated that obese and non-obese subjects derived from different enterotypes, future studies should be stratified to the enterotype status of the study participants.

The second part of the thesis provided an overview of serum FAHFA levels in different human cohorts and identified possible factors related to them. BMI could be identified as one factor, as obese patients had significantly lower FAHFA levels than non-obese controls. However, no difference could be detected between diabetic and non-diabetic subjects. Thus, the influence of FAHFAs on insulin-resistance and diabetes in humans is still unclear and has to be further investigated. The present study could also show that FAHFA levels do not differ between sexes, while differences related to age were detected. As a novel and interesting finding, it could be shown that the FAHFA levels of subjects on a vegetarian or vegan diet are substantially lower than those of omnivores. Moreover, differences were detected before and after dietary overfeeding. This points at a profound influence of specific dietary factors on endogenous FAHFA levels.

To sum up, the present data contribute to the understanding of this relatively new group of metabolically active fatty acids, as the research about FAHFAs, especially in humans, is just at the beginning. Future research is needed to further investigate the origin and regulation of circulating FAHFA levels, for example by tracking FAHFAs and fatty acids of nutritional origin.

8. Supplemental data

Supplemental Table 1: Ethnical origin and lifestyle criteria of study participants

	LSG V1	LSG V2	Controls
Ethnical Origin			
White/Caucasian	17 (100%)	17 (100%)	17 (100%)
Africans	0 (0%)	0 (0%)	0 (0%)
Asians	0 (0%)	0 (0%)	0 (0%)
Others	0 (0%)	0 (0%)	0 (0%)
Sports	3 x NA		1x NA
Never	1 (6%)	0 (0%)	0 (0%)
Seldom and irregular	7 (41%)	3 (18%)	4 (24%)
Once a week	6 (35%)	2 (12%)	2 (12%)
Several times a week	0 (0%)	12 (71%)	9 (53%)
Every day	0 (0%)	0 (0%)	1 (6%)
Smoking			
Non-smoker (never smoked)	10 (59%)	10 (59%)	10 (59%)
Non-smoker (former smoker)	3 (18%)	2 (12%)	6 (35%)
Smoker (< 10 cigarettes/day)	0 (0%)	3 (18%)	1 (6%)
Smoker (> 10 cigarettes/day)	4 (24%)	2 (12%)	0 (0%)
Alcohol consumption	3 x NA		
Never/seldom	12 (71%)	14 (82%)	5 (29%)
< ½ a bottle of bear or ⅛ l of wine a day	2 (12%)	3 (18%)	11 (65%)
1 bottle of beer or ¼ l of wine a day	0 (0%)	0 (0%)	1 (6%)
> 1 bottle of beer or ¼ l of wine a day	0 (0%)	0 (0%)	0 (0%)
Dietary supplements			
Brewer’s yeast	0 (0%)	0 (0%)	1 (6%)
Biotin	0 (0%)	1 (6%)	0 (0%)
Calcium	1 (6%)	3 (18%)	0 (0%)
Folic acid	1 (6%)	2 (12%)	0 (0%)
Iron	0 (0%)	1 (6%)	0 (0%)
Magnesium	2 (12%)	1 (6%)	1 (6%)
Vitamin B12	2 (12%)	1 (6%)	0 (0%)
Vitamin D3	4 (24%)	4 (24%)	0 (0%)
Vitamin E	1 (6%)	0 (0%)	0 (0%)
Multivitamin preparations	1 (6%)	5 (29%)	1 (6%)

Data were assessed by questionnaire. n = 17 per group.

Supplemental Table 2: Body composition measured by bioelectrical impedance analysis in obese patients after surgery (LSG V2) and non-obese controls

		Median (25th percentile; 75th percentile)		p-value
	n (LSG V2/ Controls)	LSG V2	Controls	LSG V2 - Controls
Fat mass (kg)	10/17	53.8 (33.2; 62.9)	15.8 (14.0; 23.6)	*** <0.001
Fat mass (%)	10/17	44.0 (40.4; 51.8)	27.3 (23.8; 34.5)	*** <0.001
Lean mass (kg)	10/17	57.5 (48.8; 73.9)	46.3 (40.8; 53.3)	** 0.004
Lean mass (%)	10/17	56.0 (48.2; 59.6)	72.8 (65.5; 76.2)	*** <0.001
Basal metabolic rate (kcal/day)	10/17	1956 (1526; 2323)	1404 (1311; 1518)	*** <0.001
Total energy expenditure (kcal/day) (with PAL = 1.6)	10/17	3130 (2441; 3717)	2247 (2097; 2428)	*** <0.001
Visceral fat (l)	10/17	2.83 (1.61; 6.30)	0.63 (0.23; 1.17)	*** <0.001

Depending on the distribution of the data unpaired *t*-test or Mann-Whitney test was used for comparison of the two groups. ** $p < 0.01$, *** $p < 0.001$.

Supplemental Table 3: Dietary supplements and their constituents

Dietary supplement	Ingredients	Amount per daily dose
Bierhefe Plus, altapharma (3 tablets, daily dose)	Biotin	150 µg
	Folic acid	200 µg
	Niacin equivalent	16 mg
	Pantothenic acid	6 mg
	Vitamin B1 (Thiamine)	1.1 mg
	Vitamin B2 (Riboflavin)	1.4 mg
	Vitamin B6 (Pyridoxine)	1.4 mg
	Vitamin B12 (Cobalamin)	2.5 µg
	Zinc	2 mg
Biotin Plus, Abtei Kieselerde (2 tablets, daily dose)	Biotin	350 µg
	Calcium	120 mg
	Copper	1 mg
	Folic acid	200 µg
	Magnesium	60 mg
	Pantothenic acid	6 mg
	Vitamin B2 (Riboflavin)	1.4 mg
	Vitamin C (Ascorbic acid)	40 mg
	Zinc	5 mg
Calcium + Vitamin D3, Multinorm (1 tablet, daily dose)	Calcium	400 mg
	Vitamin D (Calciferol)	2.5 µg
Gelenk Aktiv Tonikum, Salus (30 ml, daily dose)	Carbohydrates	11 g
	Manganese	0.3 mg
	Sodium	0.01 g
	Vitamin C (Ascorbic acid)	80 mg
Langzeit Vitamine A-Z Depot, Doppelherz	Biotin	300 µg
	Calcium	137 mg
	Copper	0.9 mg
	Folic acid	450 µg
	Iodine	100 µg
	Iron	2.1 mg
	Magnesium	56.3 mg
	Niacin equivalent	18 mg
	Pantothenic acid	12 mg
	Phosphorus	105 mg
	Retinol equivalent	0.4 mg
	Vitamin B1 (Thiamine)	3.5 mg
	Vitamin B2 (Riboflavin)	4 mg
	Vitamin B6 (Pyridoxine)	5 mg
	Vitamin B12 (Cobalamin)	2.5 µg
	Vitamin C (Ascorbic acid)	150 mg
	Vitamin D (Calciferol)	5 µg
	Vitamin E (Tocopherol)	10 mg
	Vitamin K	20 µg
	Zinc	5 mg
Langzeit Vitamine A-Z, Multinorm (1 tablet, daily dose)	Biotin	150 µg
	Calcium	199 mg
	Folic acid	200 µg
	Iodide	100 µg
	Magnesium	100 mg
	Niacin equivalent	17 mg
	Pantothenic acid	6 mg
	Phosphorus	155 mg
	Retinol equivalent	0.4 mg
	Vitamin B1 (Thiamine)	1.4 mg
	Vitamin B2 (Riboflavin)	1.6 mg
	Vitamin B6 (Pyridoxine)	2 mg
	Vitamin B12 (Cobalamin)	1 µg
	Vitamin C (Ascorbic acid)	60 mg
	Vitamin D (Calciferol)	5 µg
	Vitamin E (Tocopherol)	10 mg
	Vitamin K	30 µg
	Zinc	2.25 mg

Dietary supplement	Ingredients	Amount per daily dose
Langzeit Vitamine A-Z, Generation 50+, Multinorm	Biotin	50 µg
	Calcium	160 mg
	Folic acid	300 µg
	Iodide	75 µg
	Magnesium	140 mg
	Niacin equivalent	16 mg
	Pantothenic acid	6 mg
	Phosphorus	124 mg
	Retinol equivalent	0.4 mg
	Vitamin B1 (Thiamine)	1.65 mg
	Vitamin B2 (Riboflavin)	2.1 mg
	Vitamin B6 (Pyridoxine)	2.1 mg
	Vitamin B12 (Cobalamin)	3.75 µg
	Vitamin C (Ascorbic acid)	120 mg
	Vitamin D (Calciferol)	5 µg
	Vitamin E (Tocopherol)	12 mg
	Vitamin K	75 µg
	Zinc	2 mg
Lutschtabletten frisch & fruchtig, Centrum	Biotin	50 µg
	Folic acid	200 µg
	Iron	4.5 mg
	Magnesium	40 mg
	Manganese	0.5 mg
	Niacin equivalent	5 mg
	Pantothenic acid	2.5 mg
	Retinol equivalent	0.33 mg
	Vitamin B1 (Thiamine)	0.5 mg
	Vitamin B2 (Riboflavin)	0.5 mg
	Vitamin B6 (Pyridoxine)	0.5 mg
	Vitamin B12 (Cobalamin)	1 µg
	Vitamin C (Ascorbic acid)	50 mg
	Vitamin D (Calciferol)	3 µg
	Vitamin E (Tocopherol)	5 mg
	Vitamin K	10 µg
	Zinc	2.8 mg
Nutrition Tropfen, Multibionta (16 drops, daily dose)	Niacin equivalent	8.0 mg
	Pantothenic acid	2.7 mg
	Retinoläquivalent	0.8 mg
	Vitamin B1 (Thiamine)	1.3 mg
	Vitamin B2 (Riboflavin)	1.2 mg
	Vitamin B6 (Pyridoxine)	1.2 mg
	Vitamin C (Ascorbic acid)	67 mg
	Vitamin D (Calciferol)	6.7 µg
	Vitamin E (Tocopherol)	6.7 mg
Sport Basis, Ortho Therapia (1 capsule, daily dose)	Calcium	168 mg
	Folic acid	400 µg
	Magnesium	79 mg
	Manganese	1.0 mg
	Niacin equivalent	32 mg
	Pantothenic acid	12 mg
	Potassium	300 mg
	Retinol equivalent	0.12 mg
	Vitamin B1 (Thiamine)	2.2 mg
	Vitamin B2 (Riboflavin)	2.8 mg
	Vitamin B6 (Pyridoxine)	2.8 mg
	Vitamin B12 (Cobalamin)	5 µg
	Vitamin C (Ascorbic acid)	40 mg
	Vitamin D (Calciferol)	10 µg
	Zinc	1.5 mg
Orthomol Immun Trinkfläschchen (1 vial á 20 ml, daily dose)	Biotin	165 µg
	Copper	0.5 mg
	Folic acid	800 µg
	Iron	8 mg
	Manganese	2 mg
	Niacin equivalent	60 mg
	Pantothenic acid	18 mg

Dietary supplement	Ingredients	Amount per daily dose
	Retinol equivalent Vitamin B1 (Thiamine) Vitamin B2 (Riboflavin) Vitamin B6 (Pyridoxine) Vitamin B12 (Cobalamin) Vitamin C (Ascorbic acid) Vitamin D (Calciferol) Vitamin E (Tocopherol) Vitamin K Zinc	0.45 mg 25 mg 25 mg 20 mg 6 µg 950 mg 5 µg 150 mg 60 µg 10 mg
Vitamine A-Z, Centrum	Biotin Calcium Copper Folic acid Iodide Iron Magnesium Manganese Niacin equivalent Pantothenic acid Phosphorus Retinol equivalent Vitamin B1 (Thiamine) Vitamin B2 (Riboflavin) Vitamin B6 (Pyridoxine) Vitamin B12 (Cobalamin) Vitamin C (Ascorbic acid) Vitamin D (Calciferol) Vitamin E (Tocopherol) Vitamin K Zinc	62.5 µg 162 mg 0.5 mg 200 µg 100 µg 5 mg 100 mg 2 mg 20 mg 7.5 mg 125 mg 0.8 mg 1.4 mg 1.75 mg 2 mg 2.5 µg 100 mg 5.0 µg 15 mg 30 µg 5 mg

Supplemental Table 4: Correlation of inflammatory parameters with parameters of glucose metabolism in obese patients before LSG

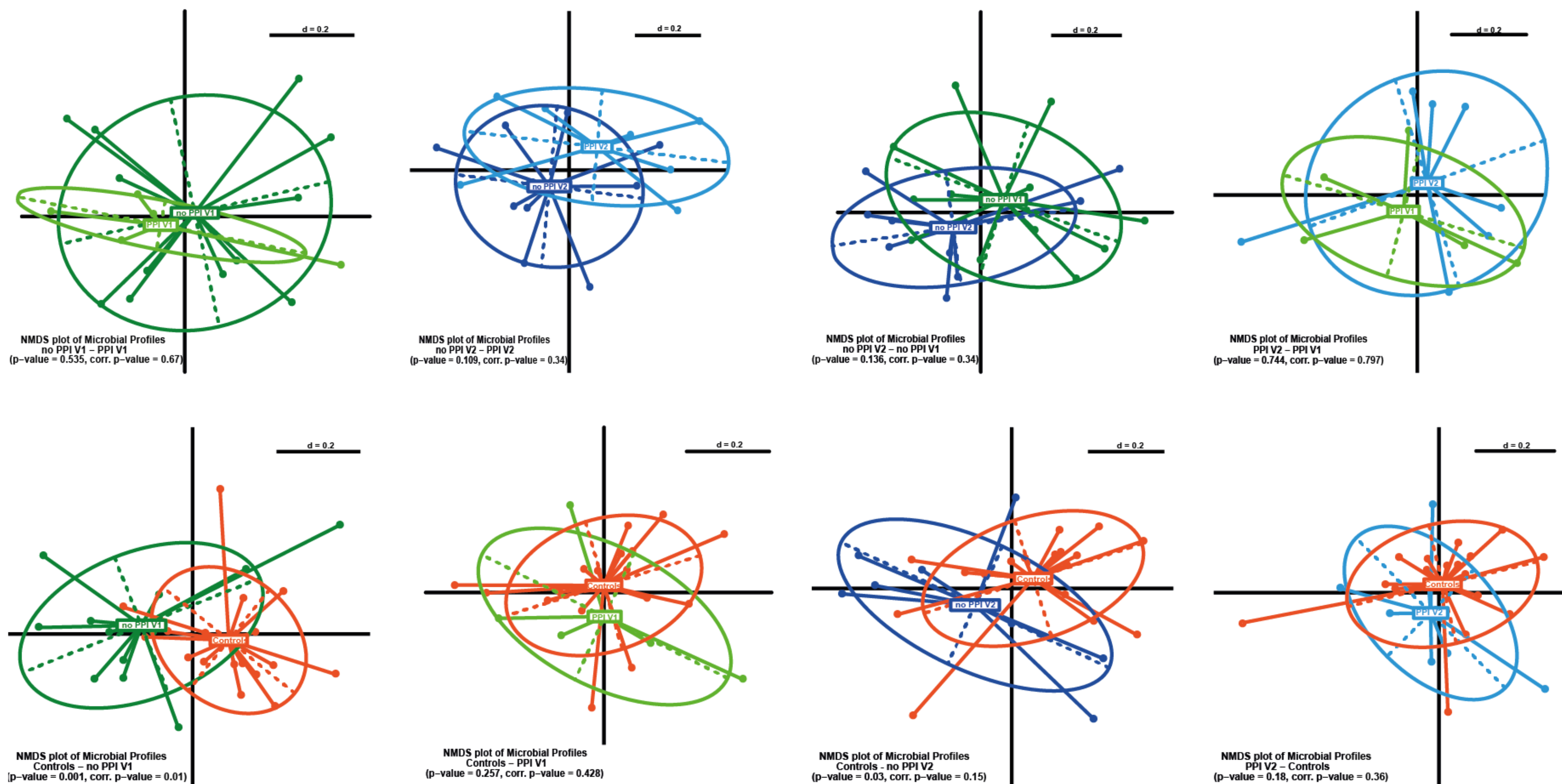
	rho	p-value	
LBP / Glucose	0.321	n.s.	0.209
LBP / Insulin	-0.069	n.s.	0.795
CRP / Glucose	0.739	***	< 0.001
CRP / Insulin	0.461	n.s.	0.065
NEFA / Glucose	0.226	n.s.	0.383
NEFA / Insulin	0.135	n.s.	0.605

Spearman rank correlation was used for correlation analysis. n = 15. n.s. = not significant; *** p < 0.001.

Supplemental Table 5: Correlation of inflammatory parameters with parameters of glucose metabolism in non-obese controls

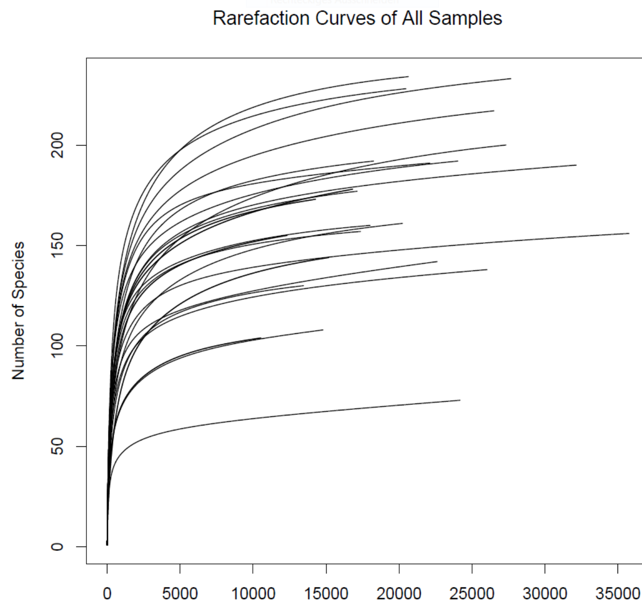
	rho	p-value	
LBP / Glucose	0.198	n.s.	0.447
LBP / Insulin	0.275	n.s.	0.285
CRP / Glucose	-0.163	n.s.	0.531
CRP / Insulin	-0.022	n.s.	0.936
NEFA / Glucose	-0.184	n.s.	0.479
NEFA / Insulin	-0.108	n.s.	0.680

Spearman rank correlation was used for correlation analysis. n = 15. n.s. = not significant.



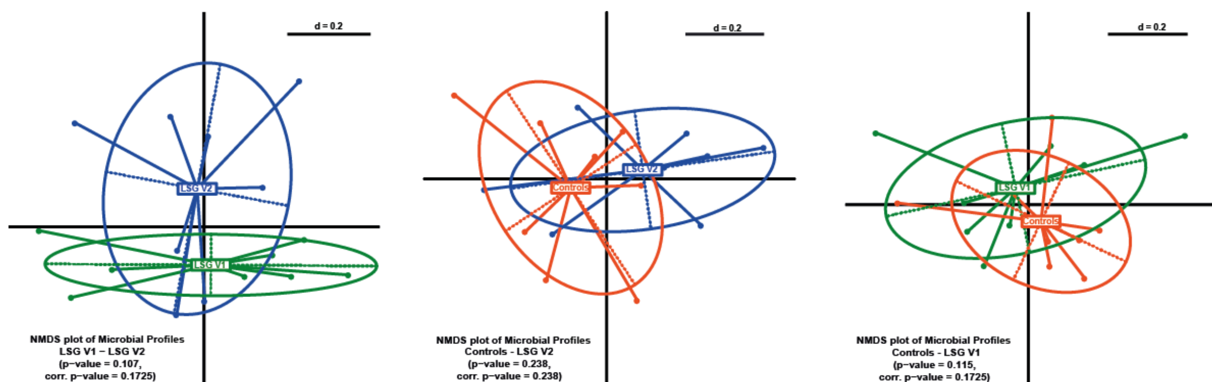
Supplemental Figure 1: Non-metric multidimensional scaling (NMDS) plots of phylogenetic distances (beta-diversity) as pairwise comparison between groups – analysis with participants taking PPIs

Controls: non-obese control subjects, n = 17; PPI V1: obese patients with PPI medication before surgery, n =5; no PPI V1: obese patients without PPI medication before surgery, n = 12; PPI V2: obese patients with PPI medication 6 months after surgery, n = 7; no PPI V2: obese patients without PPI medication 6 months after surgery, n = 10.



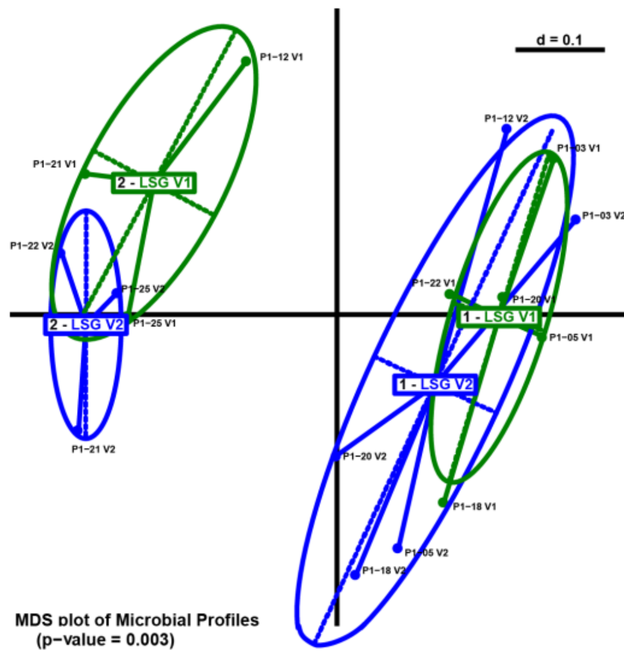
Supplemental Figure 2: Rarefaction curves of all samples analysed

Rarefaction curves are depicting the number of species at different sequencing depths for each sample. The curves indicate that all samples reached saturation approximately between 11.000 and 35.000 sequences. n = 24 (8 per group).



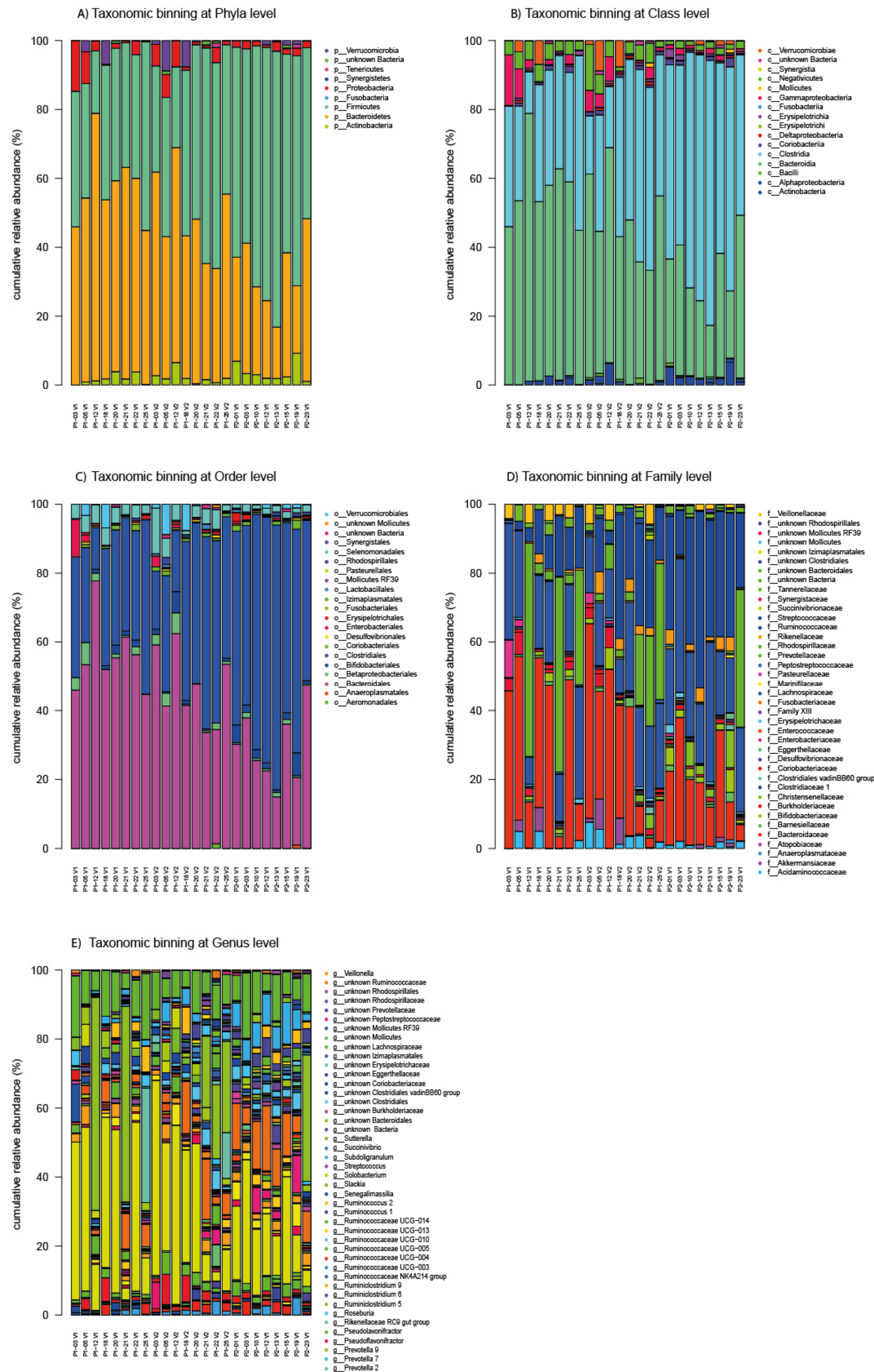
Supplemental Figure 3: Non-metric multidimensional scaling (NMDS) plots of phylogenetic distances (beta-diversity) as pairwise comparison between groups – analysis without participants taking PPIs

Controls: non-obese control subjects; LSG V1: obese patients before surgery; LSG V2: obese patients after surgery. n = 8 per group.



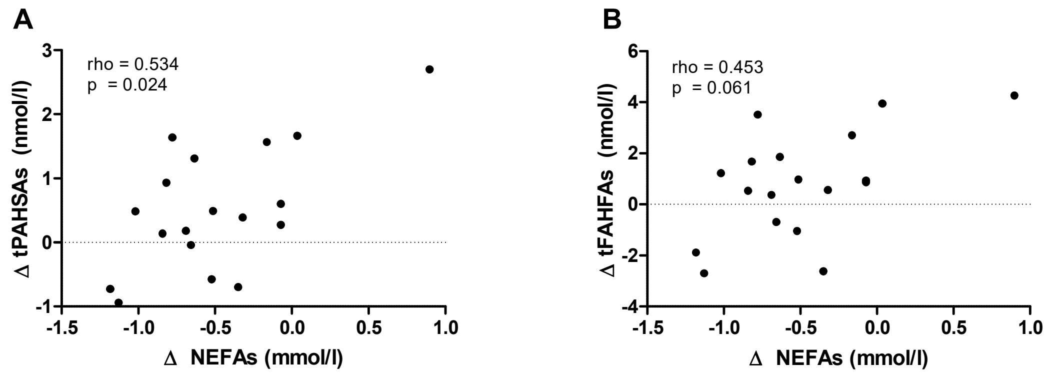
Supplemental Figure 4: Multidimensional scaling (MDS) plots of phylogenetic distances (beta-diversity) after de-novo clustering of samples of obese patients

LSG V1: obese patients before surgery; LSG V2: obese patients after surgery. n = 8 per group.



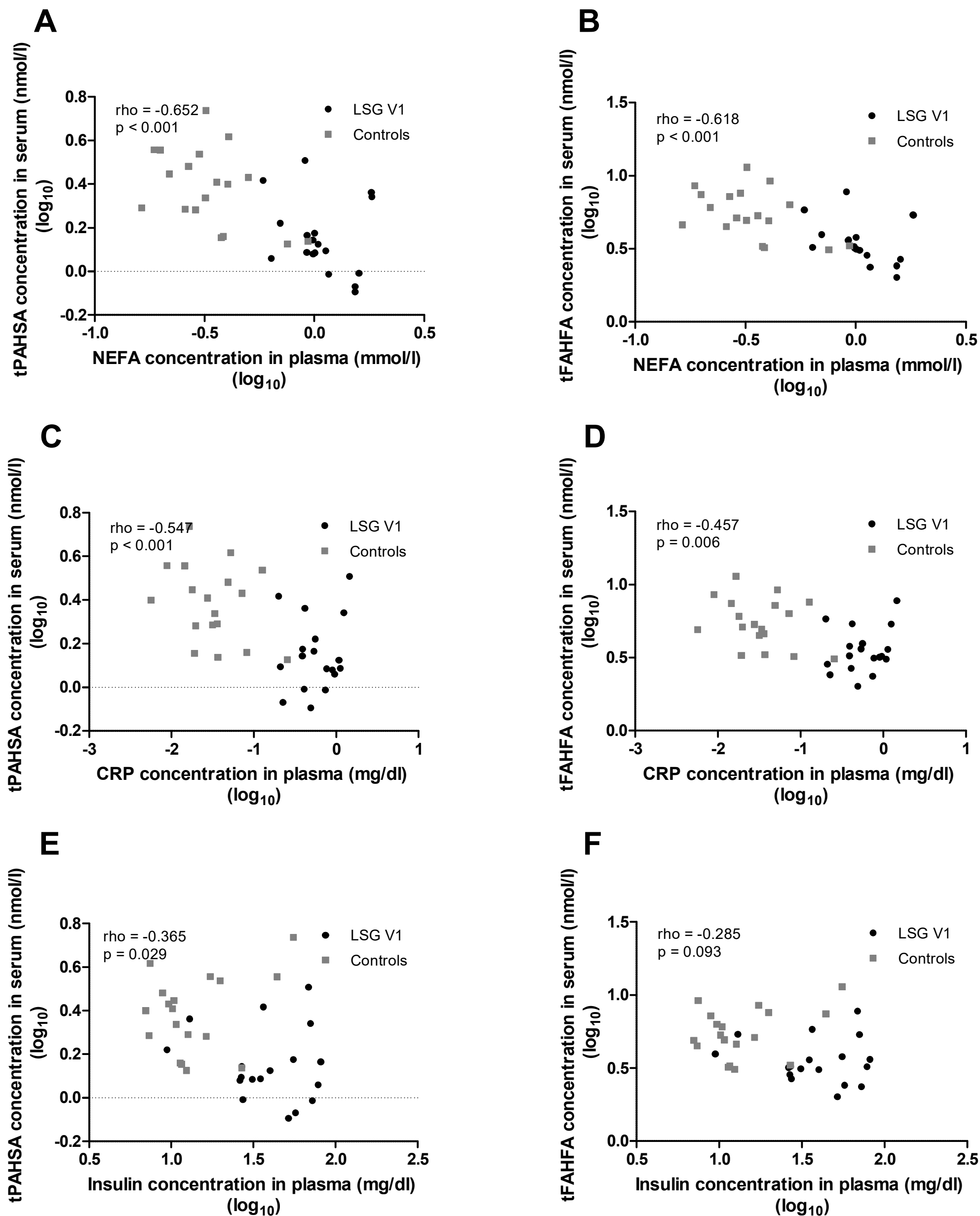
Supplemental Figure 5: Taxonomic composition of the samples at phylum level (A), class level (B), order level (C), family level (C) and genus level (E)

Taxonomic binning is performed by summing up the relative sequence abundance of all analysed OTUs that share the same assignment at a given level. Each bar represents one study participant at a certain time point. n = 8 per group.



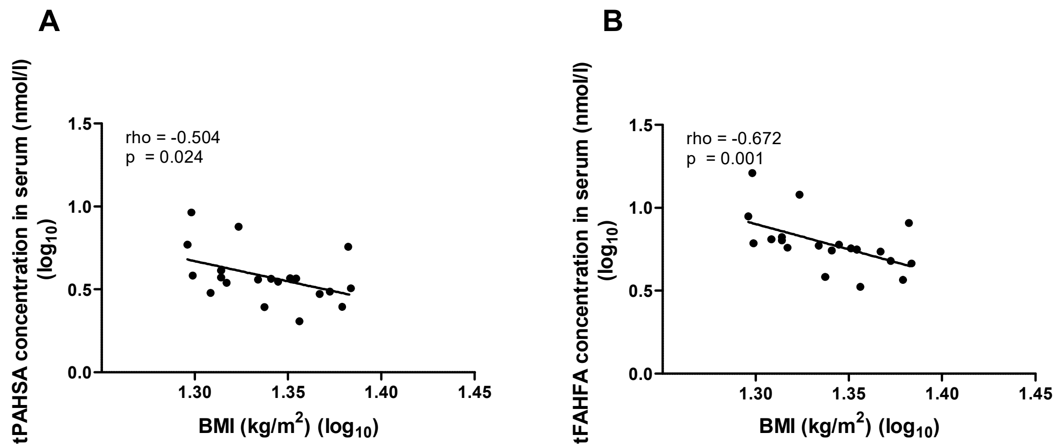
Supplemental Figure 6: Association between change in tPAHSA levels (A) or change in tFAHFA levels (B) with change in NEFA levels of patients undergoing LSG

Spearman rank correlation was used for correlation analysis. Change in total PAHSA (Δ tPAHSAs = tPAHSAs at V2 – tPAHSAs at V1) or total FAHFA levels (Δ tFAHFAs = tFAHFAs at V2 – tFAHFAs at V1) was plotted against change in NEFA levels (Δ NEFAs = NEFAs at V2 – NEFAs at V1). n = 18.



Supplemental Figure 7: Correlation of tFAHFAs and tPAHSAs with metabolic factors

Spearman rank correlation was performed for the combined group of obese patient before surgery (LSG V1) and non-obese controls (Controls). The concentration of tFAHFAs ($\log_{10}$) or tPAHSAs ($\log_{10}$) was plotted against the concentration of insulin, CRP or NEFAs ($\log_{10}$). n = 18 per group.



Supplemental Figure 8: Association between tPAHSA levels (A) or tFAHFA levels (B) with BMI of subjects from the adult cohort

Spearman rank correlation was used for correlation analysis. Total PAHSA or total FAHFA concentration in serum (log_{10}) was plotted against BMI (log_{10}). n = 20.

9. References

Aagaard, K., Ma, J., Antony, K.M., Ganu, R., Petrosino, J., and Versalovic, J. (2014). The placenta harbors a unique microbiome. Science translational medicine *6*, 237ra65.

Albuquerque, D., Stice, E., Rodríguez-López, R., Manco, L., and Nóbrega, C. (2015). Current review of genetics of human obesity: from molecular mechanisms to an evolutionary perspective. Molecular genetics and genomics : MGG *290*, 1191-1221.

Alemán, J.O., Bokulich, N.A., Swann, J.R., Walker, J.M., Rosa, J.C. De, Battaglia, T., Costabile, A., Pechlivanis, A., Liang, Y., and Breslow, J.L., et al. (2018). Fecal microbiota and bile acid interactions with systemic and adipose tissue metabolism in diet-induced weight loss of obese postmenopausal women. Journal of translational medicine *16*, 244.

Amasheh, M., Andres, S., Amasheh, S., Fromm, M., and Schulzke, J.-D. (2009). Barrier effects of nutritional factors. Annals of the New York Academy of Sciences *1165*, 267-273.

Anderson, A.D.G., Jain, P.K., Fleming, S., Poon, P., Mitchell, C.J., and MacFie, J. (2004). Evaluation of a triple sugar test of colonic permeability in humans. Acta physiologica Scandinavica *182*, 171-177.

Anderson, R., Dalziel, J., Gopal, P., Bassett, S., Ellis, A., and Roy, N. (2012). The Role of Intestinal Barrier Function in Early Life in the Development of Colitis. In Colitis, M. Fukata, ed. (Rijeka: InTech), Ch. 1.

Angrisani, L., Santonicola, A., Iovino, P., Vitiello, A., Zundel, N., Buchwald, H., and Scopinaro, N. (2017). Bariatric Surgery and Endoluminal Procedures. IFSO Worldwide Survey 2014. Obesity surgery *27*, 2279-2289.

Arita, Y., Kihara, S., Ouchi, N., Takahashi, M., Maeda, K., Miyagawa, J.-i., Hotta, K., Shimomura, I., Nakamura, T., and Miyaoka, K., et al. (1999). Paradoxical Decrease of an Adipose-Specific Protein, Adiponectin, in Obesity. Biochemical and biophysical research communications *257*, 79-83.

Armougom, F., Henry, M., Vialettes, B., Raccah, D., and Raoult, D. (2009). Monitoring bacterial community of human gut microbiota reveals an increase in Lactobacillus in obese patients and Methanogens in anorexic patients. PloS one *4*, e7125.

Aron-Wisnewsky, J., Prifti, E., Belda, E., Ichou, F., Kayser, B.D., Dao, M.C., Verger, E.O., Hedjazi, L., Bouillot, J.-L., and Chevallier, J.-M., et al. (2018). Major microbiota dysbiosis in severe obesity: fate after bariatric surgery. Gut *68*, 70-82. Epub ahead of print: doi:10.1136/gutjnl-2018-316103

Arumugam, M., Raes, J., Pelletier, E., Le Paslier, D., Yamada, T., Mende, D.R., Fernandes, G.R., Tap, J., Bruls, T., and Batto, J.-M., et al. (2011). Enterotypes of the human gut microbiome. Nature *473*, 174-180.

Bäckhed, F., Ding, H., Wang, T., Hooper, L.V., Koh, G.Y., Nagy, A., Semenkovich, C.F., and Gordon, J.I. (2004). The gut microbiota as an environmental factor that regulates fat storage. Proceedings of the National Academy of Sciences of the United States of America *101*, 15718-15723.

Bal, B.S., Finelli, F.C., Shope, T.R., and Koch, T.R. (2012). Nutritional deficiencies after bariatric surgery. Nature reviews. Endocrinology *8*, 544-556.

Bandak, B., Yi, L., and Roper, M.G. (2018). Microfluidic-enabled quantitative measurements of insulin release dynamics from single islets of Langerhans in response to 5-palmitic acid hydroxy stearic acid. Lab on a chip *18*, 2873-2882.

Barlow, G.M., Yu, A., and Mathur, R. (2015). Role of the Gut Microbiome in Obesity and Diabetes Mellitus. Nutrition in clinical practice : official publication of the American Society for Parenteral and Enteral Nutrition *30*, 787-797.

Barreau, F., and Hugot, J.P. (2014). Intestinal barrier dysfunction triggered by invasive bacteria. Current opinion in microbiology *17*, 91-98.

Basen-Engquist, K., and Chang, M. (2011). Obesity and cancer risk: recent review and evidence. Current oncology reports *13*, 71-76.

Basso, N., Capoccia, D., Rizzello, M., Abbatini, F., Mariani, P., Maglio, C., Coccia, F., Borgonuovo, G., Luca, M.L. de, and Asprino, R., et al. (2011). First-phase insulin secretion, insulin sensitivity, ghrelin, GLP-1, and PYY changes 72 h after sleeve gastrectomy in obese diabetic patients: the gastric hypothesis. Surgical endoscopy *25*, 3540-3550.

Bauchop, T., and Mountfort, D.O. (1981). Cellulose fermentation by a rumen anaerobic fungus in both the absence and the presence of rumen methanogens. Applied and environmental microbiology *42*, 1103-1110.

Belkaid, Y., and Hand, T.W. (2014). Role of the microbiota in immunity and inflammation. Cell *157*, 121-141.

Bergheim, I., Weber, S., Vos, M., Krämer, S., Volynets, V., Kaserouni, S., McClain, C.J., and Bischoff, S.C. (2008). Antibiotics protect against fructose-induced hepatic lipid accumulation in mice: Role of endotoxin. Journal of hepatology *48*, 983-992.

Bergman, E.N. (1990). Energy contributions of volatile fatty acids from the gastrointestinal tract in various species. Physiological reviews *70*, 567-590.

Berry, D., Ben Mahfoudh, K., Wagner, M., and Loy, A. (2011). Barcoded primers used in multiplex amplicon pyrosequencing bias amplification. Applied and environmental microbiology *77*, 7846-7849.

Bibiloni, R., and Schiffrin, E.J. (2010). Intestinal Host-Microbe Interactions under Physiological and Pathological Conditions. International journal of inflammation *2010*, Article ID 386956, 8 pages.

Biddle, A., Stewart, L., Blanchard, J., and Leschine, S. (2013). Untangling the Genetic Basis of Fibrolytic Specialization by Lachnospiraceae and Ruminococcaceae in Diverse Gut Communities. Diversity *5*, 627-640.

Bierman, E.L., Schwartz, I.L., and Dole, V.P. (1957). Action of insulin on release of fatty acids from tissue stores. The American journal of physiology *191*, 359-362.

Bischoff, S.C., Barbara, G., Buurman, W., Ockhuizen, T., Schulzke, J.-D., Serino, M., Tilg, H., Watson, A., and Wells, J.M. (2014). Intestinal permeability-a new target for disease prevention and therapy. BMC gastroenterology *14*, 189.

Bjarnason, I., MacPherson, A., and Hollander, D. (1995). Intestinal permeability: An overview. Gastroenterology *108*, 1566-1581.

Bjarnason, I., Peters, T.J., and Veall, N. (1983). A persistent defect in intestinal permeability in coeliac disease demonstrated by a 51Cr-labelled EDTA absorption test. Lancet (London, England) *321*, 323-325.

Bjarnason, I., and Takeuchi, K. (2009). Intestinal permeability in the pathogenesis of NSAID-induced enteropathy. Journal of gastroenterology *44 Suppl 19*, 23-29.

Bjarnason, I., Williams, P., Smethurst, P., Peters, T.J., and Levi, A.J. (1986). Effect of non-steroidal anti-inflammatory drugs and prostaglandins on the permeability of the human small intestine. Gut *27*, 1292-1297.

Blanchard, C., Moreau, F., Chevalier, J., Ayer, A., Garcon, D., Arnaud, L., Pais de Barros, J.-P., Gautier, T., Neunlist, M., and Cariou, B., et al. (2017). Sleeve Gastrectomy Alters Intestinal Permeability in Diet-Induced Obese Mice. Obesity surgery *27*, 2590-2598.

Booth, A., Magnuson, A., Fouts, J., and Foster, M.T. (2016). Adipose tissue: an endocrine organ playing a role in metabolic regulation. Hormone molecular biology and clinical investigation *26*, 25-42.

Borrell, L.N., and Samuel, L. (2014). Body mass index categories and mortality risk in US adults: the effect of overweight and obesity on advancing death. American journal of public health *104*, 512-519.

Bosi, E., Molteni, L., Radaelli, M.G., Folini, L., Fermo, I., Bazzigaluppi, E., Piemonti, L., Pastore, M.R., and Paroni, R. (2006). Increased intestinal permeability precedes clinical onset of type 1 diabetes. Diabetologia *49*, 2824-2827.

Boulangé, C.L., Neves, A.L., Chilloux, J., Nicholson, J.K., and Dumas, M.-E. (2016). Impact of the gut microbiota on inflammation, obesity, and metabolic disease. Genome medicine *8*, 42.

Braam, L.A.J.L.M., Ocke, M.C., Bueno-de-Mesquita, H.B., and Seidell, J.C. (1998). Determinants of Obesity-related Underreporting of Energy Intake. American Journal of Epidemiology *147*, 1081-1086.

Brahe, L.K., Astrup, A., and Larsen, L.H. (2013). Is butyrate the link between diet, intestinal microbiota and obesity-related metabolic diseases? Obesity reviews : an official journal of the International Association for the Study of Obesity *14*, 950-959.

Brezinova, M., Cajka, T., Oseeva, M., Stepan, M., Dadova, K., Rossmeislova, L., Matous, M., Siklova, M., Rossmeisl, M., and Kuda, O. (2020). Exercise training induces insulin-sensitizing PAHSAs in adipose tissue of elderly women. Biochimica et biophysica acta. Molecular and cell biology of lipids *1865*, 158576.

Brezinova, M., Kuda, O., Hansikova, J., Rombaldova, M., Balas, L., Bardova, K., Durand, T., Rossmeisl, M., Cerna, M., and Stranak, Z., et al. (2018). Levels of palmitic acid ester of hydroxystearic acid (PAHSA) are reduced in the breast milk of obese mothers. Biochimica et biophysica acta. Molecular and cell biology of lipids *1863*, 126-131.

Brignardello, J., Morales, P., Diaz, E., Romero, J., Brunser, O., and Gotteland, M. (2010). Pilot study: Alterations of intestinal microbiota in obese humans are not associated with colonic inflammation or disturbances of barrier function. Alimentary pharmacology & therapeutics *32*, 1307-1314.

Brouwer-Brolsma, E.M., Brennan, L., Drevon, C.A., van Kranen, H., Manach, C., Dragsted, L.O., Roche, H.M., Andres-Lacueva, C., Bakker, S.J.L., and Bouwman, J., et al. (2017).

Combining traditional dietary assessment methods with novel metabolomics techniques: present efforts by the Food Biomarker Alliance. The Proceedings of the Nutrition Society *76*, 619-627.

Brun, P., Castagliuolo, I., Di Leo, V., Buda, A., Pinzani, M., Palù, G., and Martines, D. (2007). Increased intestinal permeability in obese mice: new evidence in the pathogenesis of nonalcoholic steatohepatitis. American journal of physiology. Gastrointestinal and liver physiology *292*, G518-525.

Buchwald, H., Estok, R., Fahrbach, K., Banel, D., Jensen, M.D., Pories, W.J., Bantle, J.P., and Sledge, I. (2009). Weight and type 2 diabetes after bariatric surgery: systematic review and meta-analysis. The American journal of medicine *122*, 248-256.e5.

Calder, P.C., Ahluwalia, N., Brouns, F., Buetler, T., Clement, K., Cunningham, K., Esposito, K., Jönsson, L.S., Kolb, H., and Lansink, M., et al. (2011). Dietary factors and low-grade inflammation in relation to overweight and obesity. The British journal of nutrition *106 Suppl 3*, S5-78.

Campisciano, G., Palmisano, S., Cason, C., Giuricin, M., Silvestri, M., Guerra, M., Macor, D., Manzini, N. de, Crocé, L.S., and Comar, M. (2018). Gut microbiota characterisation in obese patients before and after bariatric surgery. Beneficial microbes *9*, 367-373.

Cani, P.D., Amar, J., Iglesias, M.A., Poggi, M., Knauf, C., Bastelica, D., Neyrinck, A.M., Fava, F., Tuohy, K.M., and Chabo, C., et al. (2007a). Metabolic endotoxemia initiates obesity and insulin resistance. Diabetes *56*, 1761-1772.

Cani, P.D., Bibiloni, R., Knauf, C., Waget, A., Neyrinck, A.M., Delzenne, N.M., and Burcelin, R. (2008). Changes in gut microbiota control metabolic endotoxemia-induced inflammation in high-fat diet-induced obesity and diabetes in mice. Diabetes *57*, 1470-1481.

Cani, P.D., Neyrinck, A.M., Fava, F., Knauf, C., Burcelin, R.G., Tuohy, K.M., Gibson, G.R., and Delzenne, N.M. (2007b). Selective increases of bifidobacteria in gut microflora improve high-fat-diet-induced diabetes in mice through a mechanism associated with endotoxaemia. Diabetologia *50*, 2374-2383.

Cani, P.D., Possemiers, S., van de Wiele, T., Guiot, Y., Everard, A., Rottier, O., Geurts, L., Naslain, D., Neyrinck, A., and Lambert, D.M., et al. (2009). Changes in gut microbiota control inflammation in obese mice through a mechanism involving GLP-2-driven improvement of gut permeability. Gut *58*, 1091-1103.

Cario, E., Gerken, G., and Podolsky, D.K. (2007). Toll-like receptor 2 controls mucosal inflammation by regulating epithelial barrier function. Gastroenterology *132*, 1359-1374.

Carter, A.M., Bennett, C.E., Bostock, J.A., and Grant, P.J. (2005). Metformin reduces C-reactive protein but not complement factor C3 in overweight patients with Type 2 diabetes mellitus. Diabetic Medicine *22*, 1282-1284.

Carvalho, E., Jansson, P.-A., Nagaev, I., Wenthzel, A.-M., and Smith, U. (2001). Insulin resistance with low cellular IRS-1 expression is also associated with low GLUT4 expression and impaired insulin-stimulated glucose transport. The FASEB Journal *15*, 1101-1103.

Casselbrant, A., Elias, E., Fändriks, L., and Wallenius, V. (2015). Expression of tight-junction proteins in human proximal small intestinal mucosa before and after Roux-en-Y gastric bypass surgery. Surgery for obesity and related diseases : official journal of the American Society for Bariatric Surgery *11*, 45-53.

Castaner, O., Goday, A., Park, Y.-M., Lee, S.-H., Magkos, F., Shiow, S.-A.T.E., and Schröder, H. (2018). The Gut Microbiome Profile in Obesity: A Systematic Review. International journal of endocrinology *2018*, 4095789.

Catassi, C., Bonucci, A., Coppa, G.V., Carlucci, A., and Giorgi, P.L. (1995). Intestinal permeability changes during the first month: effect of natural versus artificial feeding. Journal of pediatric gastroenterology and nutrition *21*, 383-386.

Chalasani, N., Younossi, Z., Lavine, J.E., Diehl, A.M., Brunt, E.M., Cusi, K., Charlton, M., and Sanyal, A.J. (2012). The diagnosis and management of non-alcoholic fatty liver disease: Practice Guideline by the American Association for the Study of Liver Diseases, American College of Gastroenterology, and the American Gastroenterological Association. Hepatology (Baltimore, Md.) *55*, 2005-2023.

Chelakkot, C., Choi, Y., Kim, D.-K., Park, H.T., Ghim, J., Kwon, Y., Jeon, J., Kim, M.-S., Jee, Y.-K., and Gho, Y.S., et al. (2018). Akkermansia muciniphila-derived extracellular vesicles influence gut permeability through the regulation of tight junctions. Experimental & molecular medicine *50*, e450.

Chen, J., Bittinger, K., Charlson, E.S., Hoffmann, C., Lewis, J., Wu, G.D., Collman, R.G., Bushman, F.D., and Li, H. (2012). Associating microbiome composition with environmental covariates using generalized UniFrac distances. Bioinformatics (Oxford, England) *28*, 2106-2113.

Cinti, S., Mitchell, G., Barbatelli, G., Murano, I., Ceresi, E., Faloia, E., Wang, S., Fortier, M., Greenberg, A.S., and Obin, M.S. (2005). Adipocyte death defines macrophage localization and function in adipose tissue of obese mice and humans. Journal of lipid research *46*, 2347-2355.

Collado, M.C., Isolauri, E., Laitinen, K., and Salminen, S. (2008). Distinct composition of gut microbiota during pregnancy in overweight and normal-weight women. The American journal of clinical nutrition *88*, 894-899.

Constant, V.A., Gagnon, A., Landry, A., and Sorisky, A. (2006). Macrophage-conditioned medium inhibits the differentiation of 3T3-L1 and human abdominal preadipocytes. Diabetologia *49*, 1402-1411.

Costea, P.I., Hildebrand, F., Arumugam, M., Bäckhed, F., Blaser, M.J., Bushman, F.D., Vos, W.M. de, Ehrlich, S.D., Fraser, C.M., and Hattori, M., et al. (2018). Enterotypes in the landscape of gut microbial community composition. Nature microbiology *3*, 8-16.

Cottam, D., Qureshi, F.G., Mattar, S.G., Sharma, S., Holover, S., Bonanomi, G., Ramanathan, R., and Schauer, P. (2006). Laparoscopic sleeve gastrectomy as an initial weight-loss procedure for high-risk patients with morbid obesity. Surgical endoscopy *20*, 859-863.

Cox, A.J., West, N.P., and Cripps, A.W. (2015). Obesity, inflammation, and the gut microbiota. The Lancet Diabetes & Endocrinology *3*, 207-215.

Creely, S.J., McTernan, P.G., Kusminski, C.M., Fisher, f.M., Da Silva, N.F., Khanolkar, M., Evans, M., Harte, A.L., and Kumar, S. (2007). Lipopolysaccharide activates an innate immune system response in human adipose tissue in obesity and type 2 diabetes. American journal of physiology. Endocrinology and metabolism *292*, E740-E747.

Crovesy, L., Masterson, D., and Rosado, E.L. (2020). Profile of the gut microbiota of adults with obesity: a systemic review. European journal of clinical nutrition. doi: 10.1038/s41430-020-0607-6

Cummins, A.G., and Thompson, F.M. (2002). Effect of breast milk and weaning on epithelial growth of the small intestine in humans. Gut *51*, 748-754.

Damci, T., Nuhoglu, I., Devranoglu, G., Osar, Z., Demir, M., and Ilkova, H. (2003). Increased intestinal permeability as a cause of fluctuating postprandial blood glucose levels in Type 1 diabetic patients. European journal of clinical investigation *33*, 397-401.

Damms-Machado, A., Louis, S., Schnitzer, A., Volynets, V., Rings, A., Basrai, M., and Bischoff, S.C. (2017). Gut permeability is related to body weight, fatty liver disease, and insulin resistance in obese individuals undergoing weight reduction. The American journal of clinical nutrition *105*, 127-135.

Damms-Machado, A., Mitra, S., Schollenberger, A.E., Kramer, K.M., Meile, T., Königsrainer, A., Huson, D.H., and Bischoff, S.C. (2015). Effects of surgical and dietary weight loss therapy for obesity on gut microbiota composition and nutrient absorption. BioMed research international *2015*, 806248.

Davies, D.J., Baxter, J.M., and Baxter, J.N. (2007). Nutritional deficiencies after bariatric surgery. Obesity surgery *17*, 1150-1158.

Deitel, M., Gagner, M., Erickson, A.L., and Crosby, R.D. (2011). Third International Summit. Current status of sleeve gastrectomy. Surgery for obesity and related diseases : official journal of the American Society for Bariatric Surgery *7*, 749-759.

Deitel, M., and Greenstein, R.J. (2003). Recommendations for reporting weight loss. Obesity surgery *13*, 159-160.

Deplancke, B., and Gaskins, H.R. (2001). Microbial modulation of innate defense: goblet cells and the intestinal mucus layer. The American journal of clinical nutrition *73*, 1131S-1141S.

Deutsche Gesellschaft für Ernährung, Österreichische Gesellschaft für Ernährung, Schweizerische Gesellschaft für Ernährung (Hrsg.) (2019). Referenzwerte für die Nährstoffzufuhr. Bonn, 2. Auflage, 5. aktualisierte Ausgabe.

Dewhirst, F.E., Chen, T., Izard, J., Paster, B.J., Tanner, A.C.R., Yu, W.-H., Lakshmanan, A., and Wade, W.G. (2010). The human oral microbiome. Journal of bacteriology *192*, 5002-5017.

Dhindsa, S., Tripathy, D., Mohanty, P., Ghanim, H., Syed, T., Aljada, A., and Dandona, P. (2004). Differential effects of glucose and alcohol on reactive oxygen species generation and intranuclear nuclear factor-κB in mononuclear cells. Metabolism *53*, 330-334.

D'Hondt, M., Vanneste, S., Pottel, H., Devriendt, D., van Rooy, F., and Vansteenkiste, F. (2011). Laparoscopic sleeve gastrectomy as a single-stage procedure for the treatment of morbid obesity and the resulting quality of life, resolution of comorbidities, food tolerance, and 6-year weight loss. Surgical endoscopy *25*, 2498-2504.

Ding, S., Chi, M.M., Scull, B.P., Rigby, R., Schwerbrock, N.M.J., Magness, S., Jobin, C., and Lund, P.K. (2010). High-fat diet: bacteria interactions promote intestinal inflammation which precedes and correlates with obesity and insulin resistance in mouse. PloS one *5*, e12191.

Doig, C.J., Sutherland, L.R., Sandham, J.D., Fick, G.H., Verhoef, M., and Meddings, J.B. (1998). Increased intestinal permeability is associated with the development of multiple organ dysfunction syndrome in critically ill ICU patients. American journal of respiratory and critical care medicine *158*, 444-451.

Dominguez-Bello, M.G., Costello, E.K., Contreras, M., Magris, M., Hidalgo, G., Fierer, N., and Knight, R. (2010). Delivery mode shapes the acquisition and structure of the initial microbiota across multiple body habitats in newborns. Proceedings of the National Academy of Sciences of the United States of America *107*, 11971-11975.

Duerksen, D.R., Wilhelm-Boyles, C., and Parry, D.M. (2005). Intestinal permeability in long-term follow-up of patients with celiac disease on a gluten-free diet. Digestive diseases and sciences *50*, 785-790.

Duncan, S.H., Lobley, G.E., Holtrop, G., Ince, J., Johnstone, A.M., Louis, P., and Flint, H.J. (2008). Human colonic microbiota associated with diet, obesity and weight loss. International journal of obesity (2005) *32*, 1720-1724.

Eckburg, P.B., Bik, E.M., Bernstein, C.N., Purdom, E., Dethlefsen, L., Sargent, M., Gill, S.R., Nelson, K.E., and Relman, D.A. (2005). Diversity of the human intestinal microbial flora. Science (New York, N.Y.) *308*, 1635-1638.

Edgar, R.C. (2013). UPARSE: highly accurate OTU sequences from microbial amplicon reads. Nature methods *10*, 996-998.

Erridge, C., Attina, T., Spickett, C.M., and Webb, D.J. (2007). A high-fat meal induces low-grade endotoxemia. Evidence of a novel mechanism of postprandial inflammation. The American journal of clinical nutrition *86*, 1286-1292.

Escobar, J.S., Klotz, B., Valdes, B.E., and Agudelo, G.M. (2014). The gut microbiota of Colombians differs from that of Americans, Europeans and Asians. BMC microbiology *14*, 311.

Everard, A., Belzer, C., Geurts, L., Ouwerkerk, J.P., Druart, C., Bindels, L.B., Guiot, Y., Derrien, M., Muccioli, G.G., and Delzenne, N.M., et al. (2013). Cross-talk between Akkermansia muciniphila and intestinal epithelium controls diet-induced obesity. Proceedings of the National Academy of Sciences of the United States of America *110*, 9066-9071.

Ewaschuk, J.B., Diaz, H., Meddings, L., Diederichs, B., Dmytrash, A., Backer, J., Looijer-van Langen, M., and Madsen, K.L. (2008). Secreted bioactive factors from Bifidobacterium infantis enhance epithelial cell barrier function. American journal of physiology. Gastrointestinal and liver physiology *295*, G1025-G1034.

Fabbrini, E., Sullivan, S., and Klein, S. (2010). Obesity and nonalcoholic fatty liver disease: biochemical, metabolic, and clinical implications. Hepatology (Baltimore, Md.) *51*, 679-689.

Fan, W., Huo, G., Li, X., Yang, L., and Duan, C. (2014). Impact of diet in shaping gut microbiota revealed by a comparative study in infants during the six months of life. Journal of microbiology and biotechnology *24*, 133-143.

Farey, J.E., Preda, T.C., Fisher, O.M., Levert-Mignon, A.J., Stewart, R.L., Karsten, E., Herbert, B.R., Swarbrick, M.M., and Lord, R.V. (2017). Effect of Laparoscopic Sleeve Gastrectomy on Fasting Gastrointestinal, Pancreatic, and Adipose-Derived Hormones and on Non-Esterified Fatty Acids. Obesity surgery *27*, 399-407.

Fasano, A. (2000). Regulation of intercellular tight junctions by zonula occludens toxin and its eukaryotic analogue zonulin. Annals of the New York Academy of Sciences *915*, 214-222.

Fasano, A. (2011). Zonulin and its regulation of intestinal barrier function: the biological door to inflammation, autoimmunity, and cancer. Physiological reviews *91*, 151-175.

Fasano, A., Fiorentini, C., Donelli, G., Uzzau, S., Kaper, J.B., Margaretten, K., Ding, X., Guandalini, S., Comstock, L., and Goldblum, S.E. (1995). Zonula occludens toxin modulates tight junctions through protein kinase C-dependent actin reorganization, in vitro. The Journal of clinical investigation *96*, 710-720.

Fasano, A., Not, T., Wang, W., Uzzau, S., Berti, I., Tommasini, A., and Goldblum, S.E. (2000). Zonulin, a newly discovered modulator of intestinal permeability, and its expression in coeliac disease. The Lancet *355*, 1518-1519.

Fernandes Dourado, K., Arruda Cámara E Siqueira Campos, F. de, and Sakugava Shinohara, N.K. (2011). Relation between dietary and circulating lipids in lacto-ovo vegetarians. Nutricion hospitalaria *26*, 959-964.

Feuerer, M., Herrero, L., Cipolletta, D., Naaz, A., Wong, J., Nayer, A., Lee, J., Goldfine, A.B., Benoist, C., and Shoelson, S., et al. (2009). Lean, but not obese, fat is enriched for a unique population of regulatory T cells that affect metabolic parameters. Nature medicine *15*, 930-939.

Field, A.E., Coakley, E.H., Must, A., Spadano, J.L., Laird, N., Dietz, W.H., Rimm, E., and Colditz, G.A. (2001). Impact of Overweight on the Risk of Developing Common Chronic Diseases During a 10-Year Period. Archives of internal medicine *161*, 1581-1586.

Filippo, C. de, Cavalieri, D., Di Paola, M., Ramazzotti, M., Poullet, J.B., Massart, S., Collini, S., Pieraccini, G., and Lionetti, P. (2010). Impact of diet in shaping gut microbiota revealed by a comparative study in children from Europe and rural Africa. Proceedings of the National Academy of Sciences of the United States of America *107*, 14691-14696.

Forhan, M., and Gill, S.V. (2013). Obesity, functional mobility and quality of life. Best practice & research. Clinical endocrinology & metabolism *27*, 129-137.

Forslund, K., Hildebrand, F., Nielsen, T., Falony, G., Le Chatelier, E., Sunagawa, S., Prifti, E., Vieira-Silva, S., Gudmundsdottir, V., and Pedersen, H.K., et al. (2015). Disentangling type 2 diabetes and metformin treatment signatures in the human gut microbiota. Nature *528*, 262-266.

Forsyth, C.B., Shannon, K.M., Kordower, J.H., Voigt, R.M., Shaikh, M., Jaglin, J.A., Estes, J.D., Dodiya, H.B., and Keshavarzian, A. (2011). Increased intestinal permeability correlates with sigmoid mucosa alpha-synuclein staining and endotoxin exposure markers in early Parkinson's disease. PloS one *6*, e28032.

Freedberg, D.E., Lebwohl, B., and Abrams, J.A. (2014). The impact of proton pump inhibitors on the human gastrointestinal microbiome. Clinics in laboratory medicine *34*, 771-785.

Fukuda, S., Toh, H., Hase, K., Oshima, K., Nakanishi, Y., Yoshimura, K., Tobe, T., Clarke, J.M., Topping, D.L., and Suzuki, T., et al. (2011). Bifidobacteria can protect from enteropathogenic infection through production of acetate. Nature *469*, 543-547.

Furet, J.-P., Kong, L.-C., Tap, J., Poitou, C., Basdevant, A., Bouillot, J.-L., Mariat, D., Corthier, G., Doré, J., and Henegar, C., et al. (2010). Differential adaptation of human gut

microbiota to bariatric surgery-induced weight loss. Links with metabolic and low-grade inflammation markers. Diabetes *59*, 3049-3057.

Gaggini, M., Morelli, M., Buzzigoli, E., DeFronzo, R.A., Bugianesi, E., and Gastaldelli, A. (2013). Non-alcoholic fatty liver disease (NAFLD) and its connection with insulin resistance, dyslipidemia, atherosclerosis and coronary heart disease. Nutrients *5*, 1544-1560.

Gaggiotti, G., Catassi, C., Sgattoni, C., Bonucci, A., Ricci, S., Spazzafumo, L., and Coppa, G.V. (1995). Modifications of Intestinal Permeability Test Induced by Biliopancreatic Diversion: Preliminary Results. Obesity surgery *5*, 424-426.

Gayoso-Diz, P., Otero-González, A., Rodriguez-Alvarez, M.X., Gude, F., García, F., Francisco, A. de, and Quintela, A.G. (2013). Insulin resistance (HOMA-IR) cut-off values and the metabolic syndrome in a general adult population: Effect of gender and age: EPIRCE cross-sectional study. BMC endocrine disorders *13*, 47.

Ge, H., Li, X., Weiszmann, J., Wang, P., Baribault, H., Chen, J.-L., Tian, H., and Li, Y. (2008). Activation of G protein-coupled receptor 43 in adipocytes leads to inhibition of lipolysis and suppression of plasma free fatty acids. Endocrinology *149*, 4519-4526.

Gehrer, S., Kern, B., Peters, T., Christoffel-Courtin, C., and Peterli, R. (2010). Fewer nutrient deficiencies after laparoscopic sleeve gastrectomy (LSG) than after laparoscopic Roux-Y-gastric bypass (LRYGB)-a prospective study. Obesity surgery *20*, 447-453.

Gemming, L., Jiang, Y., Swinburn, B., Utter, J., and Mhurchu, C.N. (2014). Under-reporting remains a key limitation of self-reported dietary intake: an analysis of the 2008/09 New Zealand Adult Nutrition Survey. European journal of clinical nutrition *68*, 259-264.

Genser, L., Aguanno, D., Soula, H.A., Dong, L., Trystram, L., Assmann, K., Salem, J.-E., Vaillant, J.-C., Oppert, J.-M., and Laugerette, F., et al. (2018). Increased jejunal permeability in human obesity is revealed by a lipid challenge and is linked to inflammation and type 2 diabetes. The Journal of pathology *246*, 217-230.

Ghoshal, S., Witta, J., Zhong, J., Villiers, W. de, and Eckhardt, E. (2009). Chylomicrons promote intestinal absorption of lipopolysaccharides. Journal of lipid research *50*, 90-97.

Glass, C.K., and Olefsky, J.M. (2012). Inflammation and lipid signaling in the etiology of insulin resistance. Cell metabolism *15*, 635-645.

Golzarand, M., Toolabi, K., and Farid, R. (2017). The bariatric surgery and weight losing. A meta-analysis in the long- and very long-term effects of laparoscopic adjustable gastric banding, laparoscopic Roux-en-Y gastric bypass and laparoscopic sleeve gastrectomy on weight loss in adults. Surgical endoscopy *31*, 4331-4345.

Gorvitovskaia, A., Holmes, S.P., and Huse, S.M. (2016). Interpreting Prevotella and Bacteroides as biomarkers of diet and lifestyle. Microbiome *4*, 15.

Graessler, J., Qin, Y., Zhong, H., Zhang, J., Licinio, J., Wong, M.-L., Xu, A., Chavakis, T., Bornstein, A.B., and Ehrhart-Bornstein, M., et al. (2013). Metagenomic sequencing of the human gut microbiome before and after bariatric surgery in obese patients with type 2 diabetes: correlation with inflammatory and metabolic parameters. The pharmacogenomics journal *13*, 514-522.

Greenhalgh, K., Meyer, K.M., Aagaard, K.M., and Wilmes, P. (2016). The human gut microbiome in health: establishment and resilience of microbiota over a lifetime. Environmental microbiology *18*, 2103-2116.

Gregor, M.F., and Hotamisligil, G.S. (2011). Inflammatory mechanisms in obesity. Annual review of immunology *29*, 415-445.

Guaraldi, F., and Salvatori, G. (2012). Effect of breast and formula feeding on gut microbiota shaping in newborns. Frontiers in cellular and infection microbiology *2*, 94.

Guarino, A., Guandalini, S., and Lo Vecchio, A. (2015). Probiotics for Prevention and Treatment of Diarrhea. Journal of clinical gastroenterology *49 Suppl 1*, S37-45.

Gummesson, A., Carlsson, L.M.S., Storlien, L.H., Bäckhed, F., Lundin, P., Löfgren, L., Stenlöf, K., Lam, Y.Y., Fagerberg, B., and Carlsson, B. (2011). Intestinal permeability is associated with visceral adiposity in healthy women. Obesity (Silver Spring, Md.) *19*, 2280-2282.

Hampel, H., Abraham, N.S., and El-Serag, H.B. (2005). Meta-analysis. Obesity and the risk for gastroesophageal reflux disease and its complications. Annals of internal medicine *143*, 199-211.

Hancock, S.E., Ailuri, R., Marshall, D.L., Brown, S.H.J., Saville, J.T., Narreddula, V.R., Boase, N.R., Poad, B.L.J., Trevitt, A.J., and Willcox, M.D.P., et al. (2018). Mass spectrometry-directed structure elucidation and total synthesis of ultra-long chain (O-acyl)-ω-hydroxy fatty acids. Journal of lipid research *59*, 1510-1518.

Hauner, H., Moss, A., Berg, A., Bischoff, S.C., Colombo-Benkmann, M., Ellrott, T., Heintze, C., Kanthak, U., Kunze, D., and Stefan, N., et al. (2014). Interdisziplinäre Leitlinie der Qualität S3 zur "Prävention und Therapie der Adipositas". Adipositas - Ursachen, Folgeerkrankungen, Therapie *08*, 179-221.

Heffron, S.P., Parikh, A., Volodarskiy, A., Ren-Fielding, C., Schwartzbard, A., Nicholson, J., and Bangalore, S. (2016). Changes in Lipid Profile of Obese Patients Following Contemporary Bariatric Surgery: A Meta-Analysis. The American journal of medicine *129*, 952-959.

Henry, R.R., Scheaffer, L., and Olefsky, J.M. (1985). Glycemic effects of intensive caloric restriction and isocaloric refeeding in noninsulin-dependent diabetes mellitus. The Journal of clinical endocrinology and metabolism *61*, 917-925.

Herman, M.A., Peroni, O.D., Villoria, J., Schön, M.R., Abumrad, N.A., Blüher, M., Klein, S., and Kahn, B.B. (2012). A novel ChREBP isoform in adipose tissue regulates systemic glucose metabolism. Nature *484*, 333-338.

Himpens, J., Dobbeleir, J., and Peeters, G. (2010). Long-term results of laparoscopic sleeve gastrectomy for obesity. Annals of surgery *252*, 319-324.

Hojo, M., Asahara, T., Nagahara, A., Takeda, T., Matsumoto, K., Ueyama, H., Matsumoto, K., Asaoka, D., Takahashi, T., and Nomoto, K., et al. (2018). Gut Microbiota Composition Before and After Use of Proton Pump Inhibitors. Digestive diseases and sciences *63*, 2940-2949.

Horton, F., Wright, J., Smith, L., Hinton, P.J., and Robertson, M.D. (2014). Increased intestinal permeability to oral chromium (51 Cr) -EDTA in human Type 2 diabetes. Diabetic medicine : a journal of the British Diabetic Association *31*, 559-563.

Hu, F.B., Meigs, J.B., Li, T.Y., Rifai, N., and Manson, J.E. (2004). Inflammatory Markers and Risk of Developing Type 2 Diabetes in Women. Diabetes *53*, 693-700.

Hu, T., Lin, M., Zhang, D., Li, M., and Zhang, J. (2018). A UPLC/MS/MS method for comprehensive profiling and quantification of fatty acid esters of hydroxy fatty acids in white adipose tissue. Analytical and bioanalytical chemistry *410*, 7415-7428.

Hurst, N.R., Kendig, D.M., Murthy, K.S., and Grider, J.R. (2014). The short chain fatty acids, butyrate and propionate, have differential effects on the motility of the guinea pig colon. Neurogastroenterology and motility : the official journal of the European Gastrointestinal Motility Society *26*, 1586-1596.

Huttenhower, C., Gevers, D., Knight, R., Abubucker, S., Badger, J.H., Chinwalla, A.T., Creasy, H.H., Earl, A.M., FitzGerald, M.G., and Fulton, R.S., et al. (2012). Structure, function and diversity of the healthy human microbiome. Nature *486*, 207-214.

Hüttl, T., Stauch, P., Wood, H., and Fruhmann, J. (2016). Bariatrische Chirurgie. Diabetologie und Stoffwechsel *11*, 11-30.

Iannelli, A., Anty, R., Schneck, A.S., Tran, A., Hébuterne, X., and Gugenheim, J. (2013). Evolution of low-grade systemic inflammation, insulin resistance, anthropometrics, resting energy expenditure and metabolic syndrome after bariatric surgery: A comparative study between gastric bypass and sleeve gastrectomy. Journal of visceral surgery *150*, 269-275.

Imhann, F., Bonder, M.J., Vila, A.V., Fu, J., Mujagic, Z., Vork, L., Tigchelaar, E.F., Jankipersadsing, S.A., Cenit, M.C., and Harmsen, H.J.M., et al. (2016). Proton pump inhibitors affect the gut microbiome. Gut *65*, 740-748.

Inui, A. (1999). Feeding and body-weight regulation by hypothalamic neuropeptides—mediation of the actions of leptin. Trends in Neurosciences *22*, 62-67.

Ito, A., Suganami, T., Yamauchi, A., Degawa-Yamauchi, M., Tanaka, M., Kouyama, R., Kobayashi, Y., Nitta, N., Yasuda, K., and Hirata, Y., et al. (2008). Role of CC chemokine receptor 2 in bone marrow cells in the recruitment of macrophages into obese adipose tissue. The Journal of biological chemistry *283*, 35715-35723.

Jackson, M.A., Goodrich, J.K., Maxan, M.-E., Freedberg, D.E., Abrams, J.A., Poole, A.C., Sutter, J.L., Welter, D., Ley, R.E., and Bell, J.T., et al. (2016). Proton pump inhibitors alter the composition of the gut microbiota. Gut *65*, 749-756.

Jacobsen, S.H., Olesen, S.C., Dirksen, C., Jørgensen, N.B., Bojsen-Møller, K.N., Kielgast, U., Worm, D., Almdal, T., Naver, L.S., and Hvolris, L.E., et al. (2012). Changes in gastrointestinal hormone responses, insulin sensitivity, and beta-cell function within 2 weeks after gastric bypass in non-diabetic subjects. Obesity surgery *22*, 1084-1096.

Jayashree, B., Bibin, Y.S., Prabhu, D., Shanthirani, C.S., Gokulakrishnan, K., Lakshmi, B.S., Mohan, V., and Balasubramanyam, M. (2014). Increased circulatory levels of lipopolysaccharide (LPS) and zonulin signify novel biomarkers of proinflammation in patients with type 2 diabetes. Molecular and cellular biochemistry *388*, 203-210.

Jernås, M., Palming, J., Sjöholm, K., Jennische, E., Svensson, P.-A., Gabrielsson, B.G., Levin, M., Sjögren, A., Rudemo, M., and Lystig, T.C., et al. (2006). Separation of human adipocytes by size: hypertrophic fat cells display distinct gene expression. FASEB journal : official publication of the Federation of American Societies for Experimental Biology *20*, 1540-1542.

Johansson, G., Wikman, Å., Åhrén, A.-M., Hallmans, G., and Johansson, I. (2001). Underreporting of energy intake in repeated 24-hour recalls related to gender, age, weight

status, day of interview, educational level, reported food intake, smoking habits and area of living. Public Health Nutrition *4*, 919-927.

Johansson, H.-E., Haenni, A., and Zethelius, B. (2013). Platelet counts and liver enzymes after bariatric surgery. Journal of obesity *2013*, 567984.

Johansson, M.E.V., Ambort, D., Pelaseyed, T., Schütte, A., Gustafsson, J.K., Ermund, A., Subramani, D.B., Holmén-Larsson, J.M., Thomsson, K.A., and Bergström, J.H., et al. (2011). Composition and functional role of the mucus layers in the intestine. Cellular and molecular life sciences : CMLS *68*, 3635-3641.

Jørgensen, N.B., Jacobsen, S.H., Dirksen, C., Bojsen-Møller, K.N., Naver, L., Hvolris, L., Clausen, T.R., Wulff, B.S., Worm, D., and Lindqvist Hansen, D., et al. (2012). Acute and long-term effects of Roux-en-Y gastric bypass on glucose metabolism in subjects with Type 2 diabetes and normal glucose tolerance. American journal of physiology. Endocrinology and metabolism *303*, E122-131.

Jost, L. (2007). Partitioning diversity into independent alpha and beta components. Ecology *88*, 2427-2439.

Jung, U.J., and Choi, M.-S. (2014). Obesity and its metabolic complications. The role of adipokines and the relationship between obesity, inflammation, insulin resistance, dyslipidemia and nonalcoholic fatty liver disease. International journal of molecular sciences *15*, 6184-6223.

Juodeikis, Ž., and Brimas, G. (2017). Long-term results after sleeve gastrectomy: A systematic review. Surgery for obesity and related diseases : official journal of the American Society for Bariatric Surgery *13*, 693-699.

Kalupahana, N.S., Claycombe, K.J., and Moustaid-Moussa, N. (2011). (n-3) Fatty acids alleviate adipose tissue inflammation and insulin resistance: mechanistic insights. Advances in nutrition (Bethesda, Md.) *2*, 304-316.

Kanda, H., Tateya, S., Tamori, Y., Kotani, K., Hiasa, K.-i., Kitazawa, R., Kitazawa, S., Miyachi, H., Maeda, S., and Egashira, K., et al. (2006). MCP-1 contributes to macrophage infiltration into adipose tissue, insulin resistance, and hepatic steatosis in obesity. The Journal of clinical investigation *116*, 1494-1505.

Kang, J.H., and Le, Q.A. (2017). Effectiveness of bariatric surgical procedures. A systematic review and network meta-analysis of randomized controlled trials. Medicine *96*, e8632.

Karamanakos, S.N., Vagenas, K., Kalfarentzos, F., and Alexandrides, T.K. (2008). Weight loss, appetite suppression, and changes in fasting and postprandial ghrelin and peptide-YY levels after Roux-en-Y gastric bypass and sleeve gastrectomy. A prospective, double blind study. Annals of surgery *247*, 401-407.

Kashyap, S.R., Daud, S., Kelly, K.R., Gastaldelli, A., Win, H., Brethauer, S., Kirwan, J.P., and Schauer, P.R. (2010). Acute effects of gastric bypass versus gastric restrictive surgery on β-cell function and insulinotropic hormones in severely obese patients with type 2 diabetes. International Journal Of Obesity *34*, 462-471.

Kehagias, I., Zygomalas, A., Karavias, D., and Karamanakos, S. (2016). Sleeve gastrectomy: have we finally found the holy grail of bariatric surgery? A review of the literature. European Review for Medical and Pharmacological Sciences *20*, 4930-4942.

Kellerer, T., Brandl, B., Büttner, J., Lagkouvardos, I., Hauner, H., and Skurk, T. (2019). Impact of Laparoscopic Sleeve Gastrectomy on Gut Permeability in Morbidly Obese Subjects. Obesity surgery *29*, 2132-2143. https://doi.org/10.1007/s11695-019-03815-6

Kellerer, T., Kleigrewe, K., Brandl, B., Hofmann, T., Hauner, H., Skurk, T. Fatty acid esters of hydroxy fatty acids (FAHFAs) are associated with diet, BMI and age. (Manuscript in submission).

Kelly, J.R., Kennedy, P.J., Cryan, J.F., Dinan, T.G., Clarke, G., and Hyland, N.P. (2015). Breaking down the barriers: the gut microbiome, intestinal permeability and stress-related psychiatric disorders. Frontiers in cellular neuroscience *9*, 392.

Kikuchi, R., Irie, J., Yamada-Goto, N., Kikkawa, E., Seki, Y., Kasama, K., and Itoh, H. (2018). The Impact of Laparoscopic Sleeve Gastrectomy with Duodenojejunal Bypass on Intestinal Microbiota Differs from that of Laparoscopic Sleeve Gastrectomy in Japanese Patients with Obesity. Clinical drug investigation *38*, 545-552.

Klindworth, A., Pruesse, E., Schweer, T., Peplies, J., Quast, C., Horn, M., and Glöckner, F.O. (2013). Evaluation of general 16S ribosomal RNA gene PCR primers for classical and next-generation sequencing-based diversity studies. Nucleic acids research *41*, e1.

Knights, D., Ward, T.L., McKinlay, C.E., Miller, H., Gonzalez, A., McDonald, D., and Knight, R. (2014). Rethinking "enterotypes". Cell host & microbe *16*, 433-437.

Koenig, J.E., Spor, A., Scalfone, N., Fricker, A.D., Stombaugh, J., Knight, R., Angenent, L.T., and Ley, R.E. (2011). Succession of microbial consortia in the developing infant gut microbiome. Proceedings of the National Academy of Sciences of the United States of America *108 Suppl 1*, 4578-4585.

Kolar, M.J., Kamat, S.S., Parsons, W.H., Homan, E.A., Maher, T., Peroni, O.D., Syed, I., Fjeld, K., Molven, A., and Kahn, B.B., et al. (2016). Branched Fatty Acid Esters of Hydroxy Fatty Acids Are Preferred Substrates of the MODY8 Protein Carboxyl Ester Lipase. Biochemistry *55*, 4636-4641.

Kolar, M.J., Nelson, A.T., Chang, T., Ertunc, M.E., Christy, M.P., Ohlsson, L., Härröd, M., Kahn, B.B., Siegel, D., and Saghatelian, A. (2018). Faster Protocol for Endogenous Fatty Acid Esters of Hydroxy Fatty Acid (FAHFA) Measurements. Analytical chemistry *90*, 5358-5365.

König, J., Wells, J., Cani, P.D., García-Ródenas, C.L., MacDonald, T., Mercenier, A., Whyte, J., Troost, F., and Brummer, R.-J. (2016). Human Intestinal Barrier Function in Health and Disease. Clinical and translational gastroenterology *7*, e196.

Kuda, O., Brezinova, M., Rombaldova, M., Slavikova, B., Posta, M., Beier, P., Janovska, P., Veleba, J., Kopecky, J., Jr., and Kudova, E., et al. (2016). Docosahexaenoic Acid-Derived Fatty Acid Esters of Hydroxy Fatty Acids (FAHFAs) With Anti-inflammatory Properties. Diabetes *65*, 2580-2590.

Kuda, O., Brezinova, M., Silhavy, J., Landa, V., Zidek, V., Dodia, C., Kreuchwig, F., Vrbacky, M., Balas, L., and Durand, T., et al. (2018). Nrf2-Mediated Antioxidant Defense and Peroxiredoxin 6 Are Linked to Biosynthesis of Palmitic Acid Ester of 9-Hydroxystearic Acid. Diabetes *67*, 1190-1199.

Labrecque, J., Laforest, S., Michaud, A., Biertho, L., and Tchernof, A. (2017). Impact of Bariatric Surgery on White Adipose Tissue Inflammation. Canadian journal of diabetes *41*, 407-417.

Lacasa, D., Taleb, S., Keophiphath, M., Miranville, A., and Clement, K. (2007). Macrophage-secreted factors impair human adipogenesis. Involvement of proinflammatory state in preadipocytes. Endocrinology *148*, 868-877.

Lagathu, C., Bastard, J.-P., Auclair, M., Maachi, M., Capeau, J., and Caron, M. (2003). Chronic interleukin-6 (IL-6) treatment increased IL-6 secretion and induced insulin resistance in adipocyte: prevention by rosiglitazone. Biochemical and biophysical research communications *311*, 372-379.

Lagkouvardos, I., Fischer, S., Kumar, N., and Clavel, T. (2017). Rhea: a transparent and modular R pipeline for microbial profiling based on 16S rRNA gene amplicons. PeerJ *5*, e2836.

Lagkouvardos, I., Joseph, D., Kapfhammer, M., Giritli, S., Horn, M., Haller, D., and Clavel, T. (2016). IMNGS: A comprehensive open resource of processed 16S rRNA microbial profiles for ecology and diversity studies. Scientific reports *6*, 33721.

Lagkouvardos, I., Kläring, K., Heinzmann, S.S., Platz, S., Scholz, B., Engel, K.-H., Schmitt-Kopplin, P., Haller, D., Rohn, S., and Skurk, T., et al. (2015). Gut metabolites and bacterial community networks during a pilot intervention study with flaxseeds in healthy adult men. Molecular nutrition & food research *59*, 1614-1628.

Lam, Y.Y., Ha, C.W.Y., Campbell, C.R., Mitchell, A.J., Dinudom, A., Oscarsson, J., Cook, D.I., Hunt, N.H., Caterson, I.D., and Holmes, A.J., et al. (2012). Increased gut permeability and microbiota change associate with mesenteric fat inflammation and metabolic dysfunction in diet-induced obese mice. PloS one *7*, e34233.

Lambert, G.P., Schmidt, A., Schwarzkopf, K., and Lanspa, S. (2012). Effect of aspirin dose on gastrointestinal permeability. International journal of sports medicine *33*, 421-425.

Le Chatelier, E., Nielsen, T., Qin, J., Prifti, E., Hildebrand, F., Falony, G., Almeida, M., Arumugam, M., Batto, J.-M., and Kennedy, S., et al. (2013). Richness of human gut microbiome correlates with metabolic markers. Nature *500*, 541-546.

Le Ferrec, E., Chesne, C., Artusson, P., Brayden, D., Fabre, G., Gires, P., Guillou, F., Rousset, M., Rubas, W., and Scarino, M.L. (2001). In vitro models of the intestinal barrier. The report and recommendations of ECVAM Workshop 46. European Centre for the Validation of Alternative methods. Alternatives to laboratory animals : ATLA *29*, 649-668.

LeBlanc, J.G., Milani, C., Giori, G.S. de, Sesma, F., van Sinderen, D., and Ventura, M. (2013). Bacteria as vitamin suppliers to their host: a gut microbiota perspective. Current opinion in biotechnology *24*, 160-168.

Lee, J., Moraes-Vieira, P.M., Castoldi, A., Aryal, P., Yee, E.U., Vickers, C., Parnas, O., Donaldson, C.J., Saghatelian, A., and Kahn, B.B. (2016). Branched Fatty Acid Esters of Hydroxy Fatty Acids (FAHFAs) Protect against Colitis by Regulating Gut Innate and Adaptive Immune Responses. The Journal of biological chemistry *291*, 22207-22217.

Lee, S., Son, S.-C., Han, M.-J., Kim, W.-J., Kim, S.-H., Kim, H.-R., Jeon, W.-K., Park, K.-H., and Shin, M.-G. (2008). Increased intestinal macromolecular permeability and urine nitrite

excretion associated with liver cirrhosis with ascites. World journal of gastroenterology *14*, 3884-3890.

Ley, R.E., Bäckhed, F., Turnbaugh, P., Lozupone, C.A., Knight, R.D., and Gordon, J.I. (2005). Obesity alters gut microbial ecology. Proceedings of the National Academy of Sciences of the United States of America *102*, 11070-11075.

Ley, R.E., Turnbaugh, P.J., Klein, S., and Gordon, J.I. (2006). Human gut microbes associated with obesity. Nature, brief communications *444*, 1022-1023.

Li, J., Lai, D., and Wu, D. (2016). Laparoscopic Roux-en-Y Gastric Bypass Versus Laparoscopic Sleeve Gastrectomy to Treat Morbid Obesity-Related Comorbidities: a Systematic Review and Meta-analysis. Obesity surgery *26*, 429-442.

Liberati-Čizmek, A.-M., Biluš, M., Brkić, A.L., Barić, I.C., Bakula, M., Hozić, A., and Cindrić, M. (2019). Analysis of Fatty Acid Esters of Hydroxyl Fatty Acid in Selected Plant Food. Plant foods for human nutrition (Dordrecht, Netherlands) *74*, 235-240.

Liu, J., Divoux, A., Sun, J., Zhang, J., Clément, K., Glickman, J.N., Sukhova, G.K., Wolters, P.J., Du, J., and Gorgun, C.Z., et al. (2009). Genetic deficiency and pharmacological stabilization of mast cells reduce diet-induced obesity and diabetes in mice. Nature medicine *15*, 940-945.

Liu, R., Hong, J., Xu, X., Feng, Q., Zhang, D., Gu, Y., Shi, J., Zhao, S., Liu, W., and Wang, X., et al. (2017). Gut microbiome and serum metabolome alterations in obesity and after weight-loss intervention. Nature medicine *23*, 859-868.

Livhits, M., Mercado, C., Yermilov, I., Parikh, J.A., Dutson, E., Mehran, A., Ko, C.Y., and Gibbons, M.M. (2012). Preoperative predictors of weight loss following bariatric surgery: systemic review. Obesity surgery *22*, 70-89.

Lopategi, A., López-Vicario, C., Alcaraz-Quiles, J., García-Alonso, V., Rius, B., Titos, E., and Clària, J. (2016). Role of bioactive lipid mediators in obese adipose tissue inflammation and endocrine dysfunction. Molecular and cellular endocrinology *419*, 44-59.

López-Bascón, M.A., Calderón-Santiago, M., and Priego-Capote, F. (2016). Confirmatory and quantitative analysis of fatty acid esters of hydroxy fatty acids in serum by solid phase extraction coupled to liquid chromatography tandem mass spectrometry. Analytica chimica acta *943*, 82-88.

Luijten, J.C.H.B.M., Vugts, G., Nieuwenhuijzen, G.A.P., and Luyer, M.D.P. (2019). The importance of the microbiome in bariatric surgery: a systemic review. Obesity Surgery *29*, 2338-2349.

Lumeng, C.N., Bodzin, J.L., and Saltiel, A.R. (2007). Obesity induces a phenotypic switch in adipose tissue macrophage polarization. The Journal of clinical investigation *117*, 175-184.

Ma, Y., Kind, T., Vaniya, A., Gennity, I., Fahrmann, J.F., and Fiehn, O. (2015). An in silico MS/MS library for automatic annotation of novel FAHFA lipids. Journal of cheminformatics *7*, 53.

Magkos, F., Fraterrigo, G., Yoshino, J., Luecking, C., Kirbach, K., Kelly, S.C., Las Fuentes, L. de, He, S., Okunade, A.L., and Patterson, B.W., et al. (2016). Effects of Moderate and Subsequent Progressive Weight Loss on Metabolic Function and Adipose Tissue Biology in Humans with Obesity. Cell metabolism *23*, 591-601.

Maheshwari, A., and Zemlin, M. (2009). Ontogeny of the intestinal immune system. Hematology Meeting Reports (formerly Haematologica Reports) *2*, 18-26.

Malin, S.K., and Kashyap, S.R. (2015). Differences in Weight Loss and Gut Hormones. Rouen-Y Gastric Bypass and Sleeve Gastrectomy Surgery. Current obesity reports *4*, 279-286.

Mann, N., Pirotta, Y., O'Connell, S., Li, D., Kelly, F., and Sinclair, A. (2006). Fatty acid composition of habitual omnivore and vegetarian diets. Lipids *41*, 637-646.

Mantis, N.J., Rol, N., and Corthésy, B. (2011). Secretory IgA's complex roles in immunity and mucosal homeostasis in the gut. Mucosal immunology *4*, 603-611.

Mathur, R., Amichai, M., Chua, K.S., Mirocha, J., Barlow, G.M., and Pimentel, M. (2013). Methane and hydrogen positivity on breath test is associated with greater body mass index and body fat. The Journal of clinical endocrinology and metabolism *98*, E698-E702.

Matthews, D.R., Hosker, J.P., Rudenski, A.S., Naylor, B.A., Treacher, D.F., and Turner, R.C. (1985). Homeostasis model assessment: Insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia *28*, 412-419.

May, G.R., Sutherland, L.R., and Meddings, J.B. (1993). Is small intestinal permeability really increased in relatives of patients with Crohn's disease? Gastroenterology *104*, 1627-1632.

Mechanick, J.I., Youdim, A., Jones, D.B., Garvey, W.T., Hurley, D.L., McMahon, M.M., Heinberg, L.J., Kushner, R., Adams, T.D., and Shikora, S., et al. (2013). Clinical practice guidelines for the perioperative nutritional, metabolic, and nonsurgical support of the bariatric surgery patient--2013 update: Cosponsored by American Association of Clinical Endocrinologists, The Obesity Society, and American Society for Metabolic & Bariatric Surgery. Obesity (Silver Spring, Md.) *21 Suppl 1*, S1-27.

Meddings, J.B., and Gibbons, I. (1998). Discrimination of site-specific alterations in gastrointestinal permeability in the rat. Gastroenterology *114*, 83-92.

Meddings, J.B., Sutherland, L.R., Byles, N.I., and Wallace, J.L. (1993). Sucrose: a novel permeability marker for gastroduodenal disease. Gastroenterology *104*, 1619-1626.

Medina, D.A., Pedreros, J.P., Turiel, D., Quezada, N., Pimentel, F., Escalona, A., and Garrido, D. (2017). Distinct patterns in the gut microbiota after surgical or medical therapy in obese patients. PeerJ *5*, e3443.

Melissas, J., Daskalakis, M., Koukouraki, S., Askoxylakis, I., Metaxari, M., Dimitriadis, E., Stathaki, M., and Papadakis, J.A. (2008). Sleeve Gastrectomy—A “Food Limiting” Operation. Obesity surgery *18*, 1251-1256.

Melissas, J., Leventi, A., Klinaki, I., Perisinakis, K., Koukouraki, S., Bree, E. de, and Karkavitsas, N. (2013). Alterations of global gastrointestinal motility after sleeve gastrectomy. A prospective study. Annals of surgery *258*, 976-982.

Miele, L., Valenza, V., La Torre, G., Montalto, M., Cammarota, G., Ricci, R., Mascianà, R., Forgione, A., Gabrieli, M.L., and Perotti, G., et al. (2009). Increased intestinal permeability and tight junction alterations in nonalcoholic fatty liver disease. Hepatology (Baltimore, Md.) *49*, 1877-1887.

Mohanty, P., Ghanim, H., Hamouda, W., Aljada, A., Garg, R., and Dandona, P. (2002). Both lipid and protein intakes stimulate increased generation of reactive oxygen species by

polymorphonuclear leukocytes and mononuclear cells. The American journal of clinical nutrition *75*, 767-772.

Montalto, M., Cuoco, L., Ricci, R., Maggiano, N., Vecchio, F.M., and Gasbarrini, G. (2002). Immunohistochemical analysis of ZO-1 in the duodenal mucosa of patients with untreated and treated celiac disease. Digestion *65*, 227-233.

Montgomery, R.K., Mulberg, A.E., and Grand, R.J. (1999). Development of the human gastrointestinal tract: twenty years of progress. Gastroenterology *116*, 702-731.

Mooradian, A.D., Morley, J.E., Levine, A.S., Prigge, W.F., and Gebhard, R.L. (1986). Abnormal intestinal permeability to sugars in diabetes mellitus. Diabetologia *29*, 221-224.

Moreno-Navarrete, J.M., Ortega, F., Serino, M., Luche, E., Waget, A., Pardo, G., Salvador, J., Ricart, W., Frühbeck, G., and Burcelin, R., et al. (2012). Circulating lipopolysaccharide-binding protein (LBP) as a marker of obesity-related insulin resistance. International journal of obesity (2005) *36*, 1442-1449.

Mortensen, F.V., Nielsen, H., Mulvany, M.J., and Hessov, I. (1990). Short chain fatty acids dilate isolated human colonic resistance arteries. Gut *31*, 1391-1394.

Müller-Wieland, D., Petermann, A., Nauck, M., Heinemann, L., Kerner, W., Müller, U., and Landgraf, R. (2016). Definition, Klassifikation und Diagnostik des Diabetes mellitus. Diabetologie und Stoffwechsel *11*, S78-S81.

Mullin, J.M., Valenzano, M.C., Whitby, M., Lurie, D., Schmidt, J.D., Jain, V., Tully, O., Kearney, K., Lazowick, D., and Mercogliano, G., et al. (2008). Esomeprazole induces upper gastrointestinal tract transmucosal permeability increase. Alimentary pharmacology & therapeutics *28*, 1317-1325.

Muoio, D.M., and Newgard, C.B. (2014). The good in fat. Nature *516*, 49-50.

Murakami, M. (2011). Lipid mediators in life science. Experimental animals *60*, 7-20.

Murphy, R., Tsai, P., Jüllig, M., Liu, A., Plank, L., and Booth, M. (2017). Differential changes in gut microbiota after gastric bypass and sleeve gastrectomy bariatric surgery vary according to diabetes remission. Obesity surgery *27*, 917-925.

Nakamura, T., Furuhashi, M., Li, P., Cao, H., Tuncman, G., Sonenberg, N., Gorgun, C.Z., and Hotamisligil, G.S. (2010). Double-stranded RNA-dependent protein kinase links pathogen sensing with stress and metabolic homeostasis. Cell *140*, 338-348.

Nelson, A.T., Kolar, M.J., Chu, Q., Syed, I., Kahn, B.B., Saghatelian, A., and Siegel, D. (2017). Stereochemistry of Endogenous Palmitic Acid Ester of 9-Hydroxystearic Acid and Relevance of Absolute Configuration to Regulation. Journal of the American Chemical Society *139*, 4943-4947.

Nguyen, N.T., and Varela, J.E. (2017). Bariatric surgery for obesity and metabolic disorders: state of the art. Nature reviews. Gastroenterology & hepatology *14*, 160-169.

Nicklas, B.J., You, T., and Pahor, M. (2005). Behavioural treatments for chronic systemic inflammation: effects of dietary weight loss and exercise training. CMAJ : Canadian Medical Association journal = journal de l'Association medicale canadienne *172*, 1199-1209.

Norman, K., Pirlich, M., Schulzke, J.-D., Smoliner, C., Lochs, H., Valentini, L., and Bühner, S. (2012). Increased intestinal permeability in malnourished patients with liver cirrhosis. European journal of clinical nutrition *66*, 1116-1119.

Novitsky, T.J. (1998). Limitations of the Limulus amebocyte lysate test in demonstrating circulating lipopolysaccharides. Annals of the New York Academy of Sciences *851*, 416-421.

O'Hara, A.M., and Shanahan, F. (2006). The gut flora as a forgotten organ. EMBO reports *7*, 688-693.

Ohmura, K., Ishimori, N., Ohmura, Y., Tokuhara, S., Nozawa, A., Horii, S., Andoh, Y., Fujii, S., Iwabuchi, K., and Onoé, K., et al. (2010). Natural killer T cells are involved in adipose tissues inflammation and glucose intolerance in diet-induced obese mice. Arteriosclerosis, thrombosis, and vascular biology *30*, 193-199.

Olfert, M.D., and Wattick, R.A. (2018). Vegetarian Diets and the Risk of Diabetes. Current diabetes reports *18*, 101.

Osanai, M., Nishikiori, N., Murata, M., Chiba, H., Kojima, T., and Sawada, N. (2007). Cellular retinoic acid bioavailability determines epithelial integrity. Role of retinoic acid receptor alpha agonists in colitis. Molecular pharmacology *71*, 250-258.

Osland, E., Yunus, R.M., Khan, S., Memon, B., and Memon, M.A. (2017). Weight Loss Outcomes in Laparoscopic Vertical Sleeve Gastrectomy (LVSG) Versus Laparoscopic Roux-en-Y Gastric Bypass (LRYGB) Procedures: A Meta-Analysis and Systematic Review of Randomized Controlled Trials. Surgical laparoscopy, endoscopy & percutaneous techniques *27*, 8-18.

Ott, B., Skurk, T., Hastreiter, L., Lagkouvardos, I., Fischer, S., Büttner, J., Kellerer, T., Clavel, T., Rychlik, M., and Haller, D., et al. (2017). Effect of caloric restriction on gut permeability, inflammation markers, and fecal microbiota in obese women. Scientific reports *7*, 11955.

Ott, B., Skurk, T., Lagkouvardos, L., Fischer, S., Büttner, J., Lichtenegger, M., Clavel, T., Lechner, A., Rychlik, M., and Haller, D., et al. (2018). Short-Term Overfeeding with Dairy Cream Does Not Modify Gut Permeability, the Fecal Microbiota, or Glucose Metabolism in Young Healthy Men. The Journal of nutrition *148*, 77-85.

Ovink-Wilbrink, J.A. Klein, Troost, F., Bernards, N., Mujagic, Z., Pijls, K., Bessems, B.A., Nienhuijs, S.W., Stronkhorst, A., and Masclee, A. (2014). Mo1773 Gastroduodenal Permeability Is Increased in Obesity but Normalizes After Sleeve Gastrectomy. Gastroenterology *146*, S-656.

Paganelli, F.L., Luyer, M., Hazelbag, C.M., Uh, H.-W., Rogers, M.R.C., Adriaans, D., Berbers, R.-M., Hendrickx, A.P.A., Viveen, M.C., and Groot, J.A., et al. (2019). Roux-Y Gastric Bypass and Sleeve Gastrectomy directly change gut microbiota composition independent of surgery type. Scientific reports *9*, 10979.

Palmisano, S., Campisciano, G., Silvestri, M., Guerra, M., Giuricin, M., Casagranda, B., Comar, M., and Manzini, N. de (2019). Changes in Gut Microbiota Composition after Bariatric Surgery: a New Balance to Decode. Journal of gastrointestinal surgery. doi: 10.1007/s11605-019-04321-x

Pals, K.L., Chang, R.T., Ryan, A.J., and Gisolfi, C.V. (1997). Effect of running intensity on intestinal permeability. Journal of applied physiology (Bethesda, Md. : 1985) *82*, 571-576.

Paluchova, V., Oseeva, M., Brezinova, M., Cajka, T., Bardova, K., Adamcova, K., Zacek, P., Brejchova, K., Balas, L., and Chodounska, H., et al. (2020). Lipokine 5-PAHSA is Regulated by Adipose Triglyceride Lipase and Primes Adipocytes for de novo Lipogenesis in Mice. Diabetes *69*, 300-312.

Parsons, W.H., Kolar, M.J., Kamat, S.S., Cognetta, A.B., Hulce, J.J., Saez, E., Kahn, B.B., Saghatelian, A., and Cravatt, B.F. (2016). AIG1 and ADTRP are atypical integral membrane hydrolases that degrade bioactive FAHFAs. Nature chemical biology *12*, 367-372.

Patil, D.P., Dhotre, D.P., Chavan, S.G., Sultan, A., Jain, D.S., Lanjekar, V.B., Gangawani, J., Shah, P.S., Todkar, J.S., and Shah, S., et al. (2012). Molecular analysis of gut microbiota in obesity among Indian individuals. Journal of biosciences *37*, 647-657.

Patrone, V., Vajana, E., Minuti, A., Callegari, M.L., Federico, A., Loguercio, C., Dallio, M., Tolone, S., Docimo, L., and Morelli, L. (2016). Postoperative Changes in Fecal Bacterial Communities and Fermentation Products in Obese Patients Undergoing Bilio-Intestinal Bypass. Frontiers in microbiology *7*, 200.

Pedersen, C., Ijaz, U.Z., Gallagher, E., Horton, F., Ellis, R.J., Jaiyeola, E., Duparc, T., Russell-Jones, D., Hinton, P., and Cani, P.D., et al. (2018). Fecal Enterobacteriales enrichment is associated with increased in vivo intestinal permeability in humans. Physiological reports *6*, e13649.

Peng, L., He, Z., Chen, W., Holzman, I.R., and Lin, J. (2007). Effects of butyrate on intestinal barrier function in a Caco-2 cell monolayer model of intestinal barrier. Pediatric Research *61*, 37-41.

Peng, L., Li, Z.-R., Green, R.S., Holzman, I.R., and Lin, J. (2009). Butyrate enhances the intestinal barrier by facilitating tight junction assembly via activation of AMP-activated protein kinase in Caco-2 cell monolayers. The Journal of nutrition *139*, 1619-1625.

Permana, P.A., Menge, C., and Reaven, P.D. (2006). Macrophage-secreted factors induce adipocyte inflammation and insulin resistance. Biochemical and biophysical research communications *341*, 507-514.

Plöger, S., Stumpff, F., Penner, G.B., Schulzke, J.-D., Gäbel, G., Martens, H., Shen, Z., Günzel, D., and Aschenbach, J.R. (2012). Microbial butyrate and its role for barrier function in the gastrointestinal tract. Annals of the New York Academy of Sciences *1258*, 52-59.

Quast, C., Pruesse, E., Yilmaz, P., Gerken, J., Schweer, T., Yarza, P., Peplies, J., and Glöckner, F.O. (2013). The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. Nucleic acids research *41*, D590-D596.

Rao, R.K., and Samak, G. (2013). Protection and Restitution of Gut Barrier by Probiotics. Nutritional and Clinical Implications. Current nutrition and food science *9*, 99-107.

Rao, S.R. (2012). Inflammatory markers and bariatric surgery: a meta-analysis. Inflammation research : official journal of the European Histamine Research Society … [et al.] *61*, 789-807.

Reveles, K.R., Ryan, C.N., Chan, L., Cosimi, R.A., and Haynes, W.L. (2018). Proton pump inhibitor use associated with changes in gut microbiota composition. Gut *67*, 1369-1370.

Rondanelli, M., Giacosa, A., Faliva, M.A., Perna, S., Allieri, F., and Castellazzi, A.M. (2015). Review on microbiota and effectiveness of probiotics use in older. World journal of clinical cases *3*, 156-162.

Sáinz, N., Barrenetxe, J., Moreno-Aliaga, M.J., and Martínez, J.A. (2015). Leptin resistance and diet-induced obesity: central and peripheral actions of leptin. Metabolism: clinical and experimental *64*, 35-46.

Sakakibara, S., Yamauchi, T., Oshima, Y., Tsukamoto, Y., and Kadowaki, T. (2006). Acetic acid activates hepatic AMPK and reduces hyperglycemia in diabetic KK-A(y) mice. Biochemical and biophysical research communications *344*, 597-604.

Samocha-Bonet, D., Justo, D., Rogowski, O., Saar, N., Abu-Abeid, S., Shenkerman, G., Shapira, I., Berliner, S., and Tomer, A. (2008). Platelet counts and platelet activation markers in obese subjects. Mediators of inflammation *2008*, 834153.

Samuel, B.S., and Gordon, J.I. (2006). A humanized gnotobiotic mouse model of host-archaeal-bacterial mutualism. Proceedings of the National Academy of Sciences of the United States of America *103*, 10011-10016.

Sander, G.R., Cummins, A.G., and Powell, B.C. (2005). Rapid disruption of intestinal barrier function by gliadin involves altered expression of apical junctional proteins. FEBS letters *579*, 4851-4855.

Sanmiguel, C.P., Jacobs, J., Gupta, A., Ju, T., Stains, J., Coveleskie, K., Lagishetty, V., Balioukova, A., Chen, Y., and Dutson, E., et al. (2017). Surgically Induced Changes in Gut Microbiome and Hedonic Eating as Related to Weight Loss: Preliminary Findings in Obese Women Undergoing Bariatric Surgery. Psychosomatic medicine *79*, 880-887.

Santacruz, A., Marcos, A., Wärnberg, J., Martí, A., Martin-Matillas, M., Campoy, C., Moreno, L.A., Veiga, O., Redondo-Figuero, C., and Garagorri, J.M., et al. (2009). Interplay between weight loss and gut microbiota composition in overweight adolescents. Obesity (Silver Spring, Md.) *17*, 1906-1915.

Sapone, A., Magistris, L. de, Pietzak, M., Clemente, M.G., Tripathi, A., Cucca, F., Lampis, R., Kryszak, D., Carteni, M., and Generoso, M., et al. (2006). Zonulin Upregulation Is Associated With Increased Gut Permeability in Subjects With Type 1 Diabetes and Their Relatives. Diabetes *55*, 1443-1449.

Sato, J., Kanazawa, A., Ikeda, F., Yoshihara, T., Goto, H., Abe, H., Komiya, K., Kawaguchi, M., Shimizu, T., and Ogihara, T., et al. (2014). Gut dysbiosis and detection of "live gut bacteria" in blood of Japanese patients with type 2 diabetes. Diabetes Care *37*, 2343-2350.

Savassi-Rocha, A.L., Diniz, M.T.C., Vilela, E.G., Diniz, M.d.F.H.S., Sanches, S.R.d.A., da Cunha, A.S., Ferrari, M.d.L.d.A., Torres, H.O.d.G., Maciente, B.A., and Ataliba, G.S., et al. (2014). Changes in intestinal permeability after Roux-en-Y gastric bypass. Obesity surgery *24*, 184-190.

Schauer, P.R., Bhatt, D.L., Kirwan, J.P., Wolski, K., Aminian, A., Brethauer, S.A., Navaneethan, S.D., Singh, R.P., Pothier, C.E., and Nissen, S.E., et al. (2017). Bariatric Surgery versus Intensive Medical Therapy for Diabetes - 5-Year Outcomes. The New England journal of medicine *376*, 641-651.

Scheffler, L., Crane, A., Heyne, H., Tönjes, A., Schleinitz, D., Ihling, C.H., Stumvoll, M., Freire, R., Fiorentino, M., and Fasano, A., et al. (2018). Widely Used Commercial ELISA Does Not Detect Precursor of Haptoglobin2, but Recognizes Properdin as a Potential Second Member of the Zonulin Family. Frontiers in endocrinology *9*, 22.

Schulzke, J.-D., Bentzel, C.J., Schulzke, I., Riecken, E.-O., and Fromm, M. (1998). Epithelial Tight Junction Structure in the Jejunum of Children with Acute and Treated Celiac Sprue. Pediatric Research *43*, 435 -441.

Schwiertz, A., Taras, D., Schäfer, K., Beijer, S., Bos, N.A., Donus, C., and Hardt, P.D. (2010). Microbiota and SCFA in lean and overweight healthy subjects. Obesity (Silver Spring, Md.) *18*, 190-195.

Secondulfo, M., Iafusco, D., Carratù, R., deMagistris, L., Sapone, A., Generoso, M., Mezzogiorno, A., Sasso, F.C., Cartenì, M., and Rosa, R. de, et al. (2004). Ultrastructural mucosal alterations and increased intestinal permeability in non-celiac, type I diabetic patients. Digestive and Liver Disease *36*, 35-45.

Selvin, E., Paynter, N.P., and Erlinger, T.P. (2007). The effect of weight loss on C-reactive protein. A systematic review. Archives of internal medicine *167*, 31-39.

Shah, S., Shah, P., Todkar, J., Gagner, M., Sonar, S., and Solav, S. (2010). Prospective controlled study of effect of laparoscopic sleeve gastrectomy on small bowel transit time and gastric emptying half-time in morbidly obese patients with type 2 diabetes mellitus. Surgery for obesity and related diseases : official journal of the American Society for Bariatric Surgery *6*, 152-157.

Shen, J., Zuo, Z.-X., and Mao, A.-P. (2014). Effect of probiotics on inducing remission and maintaining therapy in ulcerative colitis, Crohn's disease, and pouchitis: meta-analysis of randomized controlled trials. Inflammatory bowel diseases *20*, 21-35.

Shepherd, P.R., Gnudi, L., Tozzo, E., Yang, H., Leach, F., and Kahn, B.B. (1993). Adipose cell hyperplasia and enhanced glucose disposal in transgenic mice overexpressing GLUT4 selectively in adipose tissue. The Journal of biological chemistry *268*, 22243-22246.

Sheppard, C.E., Sadowski, D.C., Gara, C.J. de, Karmali, S., and Birch, D.W. (2015). Rates of reflux before and after laparoscopic sleeve gastrectomy for severe obesity. Obesity surgery *25*, 763-768.

Shi, H., Kokoeva, M.V., Inouye, K., Tzameli, I., Yin, H., and Flier, J.S. (2006). TLR4 links innate immunity and fatty acid-induced insulin resistance. The Journal of clinical investigation *116*, 3015-3025.

Shin, N.-R., Lee, J.-C., Lee, H.-Y., Kim, M.-S., Whon, T.W., Lee, M.-S., and Bae, J.-W. (2014). An increase in the Akkermansia spp. population induced by metformin treatment improves glucose homeostasis in diet-induced obese mice. Gut *63*, 727-735.

Shoelson, S.E., Herrero, L., and Naaz, A. (2007). Obesity, inflammation, and insulin resistance. Gastroenterology *132*, 2169-2180.

Sigalet, D.L., Martin, G.R., and Poole, A. (2000). Differential sugar absorption as a marker for adaptation in short bowel syndrome. Journal of pediatric surgery *35*, 661-664.

Simões, C.D., Maukonen, J., Scott, K.P., Virtanen, K.A., Pietiläinen, K.H., and Saarela, M. (2014). Impact of a very low-energy diet on the fecal microbiota of obese individuals. European journal of nutrition *53*, 1421-1429.

Sise, A., and Friedenberg, F.K. (2008). A comprehensive review of gastroesophageal reflux disease and obesity. Obesity reviews : an official journal of the International Association for the Study of Obesity *9*, 194-203.

Skurk, T., Alberti-Huber, C., Herder, C., and Hauner, H. (2007). Relationship between adipocyte size and adipokine expression and secretion. The Journal of clinical endocrinology and metabolism *92*, 1023-1033.

Snyder-Marlow, G., Taylor, D., and Lenhard, M.J. (2010). Nutrition care for patients undergoing laparoscopic sleeve gastrectomy for weight loss. Journal of the American Dietetic Association *110*, 600-607.

Soret, R., Chevalier, J., Coppet, P. de, Poupeau, G., Derkinderen, P., Segain, J.P., and Neunlist, M. (2010). Short-chain fatty acids regulate the enteric neurons and control gastrointestinal motility in rats. Gastroenterology *138*, 1772-1782.

Spruss, A., Kanuri, G., Stahl, C., Bischoff, S.C., and Bergheim, I. (2012). Metformin protects against the development of fructose-induced steatosis in mice: role of the intestinal barrier function. Laboratory investigation; a journal of technical methods and pathology *92*, 1020-1032.

Strobel, S., Brydon, W.G., and Ferguson, A. (1984). Cellobiose/mannitol sugar permeability test complements biopsy histopathology in clinical investigation of the jejunum. Gut *25*, 1241-1246.

Suganami, T., Nishida, J., and Ogawa, Y. (2005). A paracrine loop between adipocytes and macrophages aggravates inflammatory changes. Role of free fatty acids and tumor necrosis factor alpha. Arteriosclerosis, thrombosis, and vascular biology *25*, 2062-2068.

Sutherland, L.R., Verhoef, M., Wallace, J.L., van Rosendaal, G., Crutcher, R., and Meddings, J.B. (1994). A simple, non-invasive marker of gastric damage: sucrose permeability. Lancet (London, England) *343*, 998-1000.

Suzuki, T. (2013). Regulation of intestinal epithelial permeability by tight junctions. Cellular and molecular life sciences : CMLS *70*, 631-659.

Suzuki, T., and Hara, H. (2010). Dietary fat and bile juice, but not obesity, are responsible for the increase in small intestinal permeability induced through the suppression of tight junction protein expression in LETO and OLETF rats. Nutrition & metabolism *7*, 19.

Syed, I., Lee, J., Moraes-Vieira, P.M., Donaldson, C.J., Sontheimer, A., Aryal, P., Wellenstein, K., Kolar, M.J., Nelson, A.T., and Siegel, D., et al. (2018). Palmitic Acid Hydroxystearic Acids Activate GPR40, Which Is Involved in Their Beneficial Effects on Glucose Homeostasis. Cell metabolism *27*, 419-427.e4.

Syed, I., Rubin de Celis, M.F., Mohan, J.F., Moraes-Vieira, P.M., Vijayakumar, A., Nelson, A.T., Siegel, D., Saghatelian, A., Mathis, D., and Kahn, B.B. (2019). PAHSAs attenuate immune responses and promote β cell survival in autoimmune diabetic mice. The Journal of clinical investigation *129*, 3717-3731.

Sze, M.A., and Schloss, P.D. (2016). Looking for a Signal in the Noise: Revisiting Obesity and the Microbiome. mBio *7*.

Takagi, T., Naito, Y., Inoue, R., Kashiwagi, S., Uchiyama, K., Mizushima, K., Tsuchiya, S., Okayama, T., Dohi, O., and Yoshida, N., et al. (2018). The influence of long-term use of proton pump inhibitors on the gut microbiota: an age-sex-matched case-control study. Journal of clinical biochemistry and nutrition *62*, 100-105.

Tan, D., Ertunc, M.E., Konduri, S., Zhang, J., Pinto, A.M., Chu, Q., Kahn, B.B., Siegel, D., and Saghatelian, A. (2019). Discovery of FAHFA-Containing Triacylglycerols and Their Metabolic Regulation. Journal of the American Chemical Society *141*, 8798-8806.

Taylor, S.N., Basile, L.A., Ebeling, M., and Wagner, C.L. (2009). Intestinal permeability in preterm infants by feeding type: mother's milk versus formula. Breastfeeding medicine : the official journal of the Academy of Breastfeeding Medicine *4*, 11-15.

Taylor, V.H., Forhan, M., Vigod, S.N., McIntyre, R.S., and Morrison, K.M. (2013). The impact of obesity on quality of life. Best practice & research. Clinical endocrinology & metabolism *27*, 139-146.

Teixeira, T.F.S., Collado, M.C., Ferreira, C.L.L.F., Bressan, J., and Peluzio, M.d.C.G. (2012a). Potential mechanisms for the emerging link between obesity and increased intestinal permeability. Nutrition research (New York, N.Y.) *32*, 637-647.

Teixeira, T.F.S., Souza, N.C.S., Chiarello, P.G., Franceschini, S.C.C., Bressan, J., Ferreira, C.L.L.F., and Peluzio, M.d.C.G. (2012b). Intestinal permeability parameters in obese patients are correlated with metabolic syndrome risk factors. Clinical nutrition (Edinburgh, Scotland) *31*, 735-740.

Thomas, C., Gioiello, A., Noriega, L., Strehle, A., Oury, J., Rizzo, G., Macchiarulo, A., Yamamoto, H., Mataki, C., and Pruzanski, M., et al. (2009). TGR5-mediated bile acid sensing controls glucose homeostasis. Cell metabolism *10*, 167-177.

Thomas, F., Smith, G.C., Lu, J., Babor, R., Booth, M., Beban, G., Chase, J.G., and Murphy, R. (2016). Differential Acute Impacts of Sleeve Gastrectomy, Roux-en-Y Gastric Bypass Surgery and Matched Caloric Restriction Diet on Insulin Secretion, Insulin Effectiveness and Non-Esterified Fatty Acid Levels Among Patients with Type 2 Diabetes. Obesity surgery *26*, 1924-1931.

Topçu, Ö., Karadayı, K., Kuzu, A.M., Ulukent, S., Erkek, B., and Alaçayır, İ. (2002). Enteral and intraluminal short-chain fatty acids improves ischemic left colonic anastomotic healing in the rat. International journal of colorectal disease *17*, 171-176.

Tremaroli, V., and Bäckhed, F. (2012). Functional interactions between the gut microbiota and host metabolism. Nature *489*, 242-249.

Tremaroli, V., Karlsson, F., Werling, M., Ståhlman, M., Kovatcheva-Datchary, P., Olbers, T., Fändriks, L., Le Roux, C.W., Nielsen, J., and Bäckhed, F. (2015). Roux-en-Y Gastric Bypass and Vertical Banded Gastroplasty Induce Long-Term Changes on the Human Gut Microbiome Contributing to Fat Mass Regulation. Cell metabolism *22*, 228-238.

Tripathy, D., Mohanty, P., Dhindsa, S., Syed, T., Ghanim, H., Aljada, A., and Dandona, P. (2003). Elevation of Free Fatty Acids Induces Inflammation and Impairs Vascular Reactivity in Healthy Subjects. Diabetes *52*, 2882-2887.

Trøseid, M., Nestvold, T.K., Rudi, K., Thoresen, H., Nielsen, E.W., and Lappegård, K.T. (2013). Plasma lipopolysaccharide is closely associated with glycemic control and abdominal obesity. Evidence from bariatric surgery. Diabetes Care *36*, 3627-3632.

Tucker, O.N., Szomstein, S., and Rosenthal, R.J. (2008). Indications for sleeve gastrectomy as a primary procedure for weight loss in the morbidly obese. Journal of gastrointestinal surgery : official journal of the Society for Surgery of the Alimentary Tract *12*, 662-667.

Tuomi, K., and Logomarsino, J.V. (2016). Bacterial Lipopolysaccharide, Lipopolysaccharide-Binding Protein, and Other Inflammatory Markers in Obesity and After Bariatric Surgery. Metabolic syndrome and related disorders *14*, 279-288.

Turnbaugh, P.J., Hamady, M., Yatsunenko, T., Cantarel, B.L., Duncan, A., Ley, R.E., Sogin, M.L., Jones, W.J., Roe, B.A., and Affourtit, J.P., et al. (2009). A core gut microbiome in obese and lean twins. Nature *457*, 480-484.

Turnbaugh, P.J., Ley, R.E., Mahowald, M.A., Magrini, V., Mardis, E.R., and Gordon, J.I. (2006). An obesity-associated gut microbiome with increased capacity for energy harvest. Nature *444*, 1027-1031.

Turner, J.R. (2009). Intestinal mucosal barrier function in health and disease. Nature reviews. Immunology *9*, 799-809.

Ubeda, C., Djukovic, A., and Isaac, S. (2017). Roles of the intestinal microbiota in pathogen protection. Clinical & translational immunology *6*, e128.

Ukabam, S.O., and Cooper, B.T. (1984). Small intestinal permeability to mannitol, lactulose, and polyethylene glycol 400 in celiac disease. Digestive diseases and sciences *29*, 809-816.

van der Flier, L.G., and Clevers, H. (2009). Stem cells, self-renewal, and differentiation in the intestinal epithelium. Annual review of physiology *71*, 241-260.

van Dielen, F.M.H., Buurman, W.A., Hadfoune, M., Nijhuis, J., and Greve, J.W. (2004). Macrophage inhibitory factor, plasminogen activator inhibitor-1, other acute phase proteins, and inflammatory mediators normalize as a result of weight loss in morbidly obese subjects treated with gastric restrictive surgery. The Journal of clinical endocrinology and metabolism *89*, 4062-4068.

van Elburg, R.M., Uil, J.J., Mulder, C.J., and Heymans, H.S. (1993). Intestinal permeability in patients with coeliac disease and relatives of patients with coeliac disease. Gut *34*, 354-357.

Vandenplas, Y., Zakharova, I., and Dmitrieva, Y. (2015). Oligosaccharides in infant formula: more evidence to validate the role of prebiotics. The British journal of nutrition *113*, 1339-1344.

Verdam, F.J., Fuentes, S., Jonge, C. de, Zoetendal, E.G., Erbil, R., Greve, J.W., Buurman, W.A., Vos, W.M. de, and Rensen, S.S. (2013). Human intestinal microbiota composition is associated with local and systemic inflammation in obesity. Obesity (Silver Spring, Md.) *21*, E607-15.

Vinolo, M.A.R., Rodrigues, H.G., Nachbar, R.T., and Curi, R. (2011). Regulation of inflammation by short chain fatty acids. Nutrients *3*, 858-876.

Vukavić, T. (1984). Timing of the gut closure. Journal of pediatric gastroenterology and nutrition *3*, 700-703.

Walters, W.A., Xu, Z., and Knight, R. (2014). Meta-analyses of human gut microbes associated with obesity and IBD. FEBS letters *588*, 4223-4233.

Wang, H.-B., Wang, P.-Y., Wang, X., Wan, Y.-L., and Liu, Y.-C. (2012). Butyrate enhances intestinal epithelial barrier function via up-regulation of tight junction protein Claudin-1 transcription. Digestive diseases and sciences *57*, 3126-3135.

Wang, Q., Garrity, G.M., Tiedje, J.M., and Cole, J.R. (2007). Naive Bayesian classifier for rapid assignment of rRNA sequences into the new bacterial taxonomy. Applied and environmental microbiology *73*, 5261-5267.

Wang, W., Uzzau, S., Goldblum, S.E., and Fasano, A. (2000). Human zonulin, a potential modulator of intestinal tight junctions. Journal of cell science *113 Pt 24*, 4435-4440.

Wang, Y., Tong, J., Chang, B., Wang, B., Zhang, D., and Wang, B. (2014). Effects of alcohol on intestinal epithelial barrier permeability and expression of tight junction-associated proteins. Molecular medicine reports *9*, 2352-2356.

Wang, Y.-M., Liu, H.-X., and Fang, N.-Y. (2018). 9-PAHSA promotes browning of white fat via activating G-protein-coupled receptor 120 and inhibiting lipopolysaccharide / NF-kappa B pathway. Biochemical and biophysical research communications *506*, 153-160.

Weisberg, S.P., McCann, D., Desai, M., Rosenbaum, M., Leibel, R.L., and Ferrante, A.W. (2003). Obesity is associated with macrophage accumulation in adipose tissue. The Journal of clinical investigation *112*, 1796-1808.

Wen, X.-H., Guo, Q.-L., and Guo, J.-C. (2020). Effect of 9 - PAHSA on cognitive dysfunction in diabetic mice and its possible mechanism. Biochemical and biophysical research communications *524*, 525-532.

Wensveen, F.M., Jelenčić, V., Valentić, S., Šestan, M., Wensveen, T.T., Theurich, S., Glasner, A., Mendrila, D., Štimac, D., and Wunderlich, F.T., et al. (2015). NK cells link obesity-induced adipose stress to inflammation and insulin resistance. Nature immunology *16*, 376-385.

Wilbrink, J., Bernards, N., Mujagic, Z., van Avesaat, M., Pijls, K., Klaassen, T., van Eijk, H., Nienhuijs, S., Stronkhorst, A., and Wilms, E., et al. (2020). Intestinal barrier function in morbid obesity: results of a prospective study on the effect of sleeve gastrectomy. International journal of obesity (2005) *44*, 368-376.

World Health Organization (2000): Obesity. Preventing and managing the global epidemic. Published in: World Health Organization (Ed.): WHO Technical Report Series, Bd. 894.

World Health Organization (2011). Waist circumference and waist-hip ratio. Report of a WHO expert consultation, Geneva, 8-11 December 2008 (Geneva: World Health Organization).

World Health Organization (2020). Obesity and overweigth. Fact sheet. Updated 1. April 2020. https://www.who.int/news-room/fact-sheets/detail/obesity-and-overweight. 16.06.2020.

Wozniak, S.E., Gee, L.L., Wachtel, M.S., and Frezza, E.E. (2009). Adipose tissue: the new endocrine organ? A review article. Digestive diseases and sciences *54*, 1847-1856.

Wu, G.D., Chen, J., Hoffmann, C., Bittinger, K., Chen, Y.-Y., Keilbaugh, S.A., Bewtra, M., Knights, D., Walters, W.A., and Knight, R., et al. (2011). Linking long-term dietary patterns with gut microbial enterotypes. Science (New York, N.Y.) *334*, 105-108.

Wu, H., Esteve, E., Tremaroli, V., Khan, M.T., Caesar, R., Mannerås-Holm, L., Ståhlman, M., Olsson, L.M., Serino, M., and Planas-Fèlix, M., et al. (2017). Metformin alters the gut microbiome of individuals with treatment-naive type 2 diabetes, contributing to the therapeutic effects of the drug. Nature medicine *23*, 850-858.

Xiao, S., Fei, N., Pang, X., Shen, J., Wang, L., Zhang, B., Zhang, M., Zhang, X., Zhang, C., and Li, M., et al. (2014). A gut microbiota-targeted dietary intervention for amelioration of chronic inflammation underlying metabolic syndrome. FEMS microbiology ecology *87*, 357-367.

Xu, H., Barnes, G.T., Yang, Q., Tan, G., Yang, D., Chou, C.J., Sole, J., Nichols, A., Ross, J.S., and Tartaglia, L.A., et al. (2003). Chronic inflammation in fat plays a crucial role in the development of obesity-related insulin resistance. The Journal of clinical investigation *112*, 1821-1830.

Yatsunenko, T., Rey, F.E., Manary, M.J., Trehan, I., Dominguez-Bello, M.G., Contreras, M., Magris, M., Hidalgo, G., Baldassano, R.N., and Anokhin, A.P., et al. (2012). Human gut microbiome viewed across age and geography. Nature *486*, 222-227.

Ye, Q., Chen, Y., Zhan, X., Wang, Y., and Zhu, J. (2017). Comparison of Laparoscopic Sleeve Gastrectomy and Laparoscopic Greater Curvature Plication Regarding Efficacy and Safety: A Meta-Analysis. Obesity surgery *27*, 1358-1364.

Yore, M.M., Syed, I., Moraes-Vieira, P.M., Zhang, T., Herman, M.A., Homan, E.A., Patel, R.T., Lee, J., Chen, S., and Peroni, O.D., et al. (2014). Discovery of a class of endogenous mammalian lipids with anti-diabetic and anti-inflammatory effects. Cell *159*, 318-332.

Youssef, A., Emmanuel, J., Karra, E., Millet, Q., Elkalaawy, M., Jenkinson, A.D., Hashemi, M., Adamo, M., Finer, N., and Fiennes, A.G., et al. (2014). Differential effects of laparoscopic sleeve gastrectomy and laparoscopic gastric bypass on appetite, circulating acyl-ghrelin, peptide YY3-36 and active GLP-1 levels in non-diabetic humans. Obesity surgery *24*, 241-252.

Zaldivar, F., McMurray, R.G., Nemet, D., Galassetti, P., Mills, P.J., and Cooper, D.M. (2006). Body fat and circulating leukocytes in children. International journal of obesity (2005) *30*, 906-911.

Zhang, H., DiBaise, J.K., Zuccolo, A., Kudrna, D., Braidotti, M., Yu, Y., Parameswaran, P., Crowell, M.D., Wing, R., and Rittmann, B.E., et al. (2009). Human gut microbiota in obesity and after gastric bypass. Proceedings of the National Academy of Sciences of the United States of America *106*, 2365-2370.

Zhang, T., Chen, S., Syed, I., Ståhlman, M., Kolar, M.J., Homan, E.A., Chu, Q., Smith, U., Borén, J., and Kahn, B.B., et al. (2016). A LC-MS-based workflow for measurement of branched fatty acid esters of hydroxy fatty acids. Nature protocols *11*, 747-763.

Zhu, Q.-F., Yan, J.-W., Gao, Y., Zhang, J.-W., Yuan, B.-F., and Feng, Y.-Q. (2017). Highly sensitive determination of fatty acid esters of hydroxyl fatty acids by liquid chromatography-mass spectrometry. Journal of chromatography. B, Analytical technologies in the biomedical and life sciences *1061-1062*, 34-40.

Zhu, Q.-F., Yan, J.-W., Zhang, T.-Y., Xiao, H.-M., and Feng, Y.-Q. (2018). Comprehensive Screening and Identification of Fatty Acid Esters of Hydroxy Fatty Acids in Plant Tissues by Chemical Isotope Labeling-Assisted Liquid Chromatography-Mass Spectrometry. Analytical chemistry *90*, 10056-10063.

List of tables

Table 1: Weight classification by BMI according to the WHO (World Health Organization, 2000)....1
Table 2: Literature summary of the most relevant studies on gut permeability in obesity (human data)....12
Table 3: Literature summary of gut permeability after bariatric surgery (human data)....13
Table 4: Inclusion and exclusion criteria for the study....25
Table 5: Routine parameters analysed in blood of study participants....30
Table 6: HPLC gradient program....35
Table 7: MS parameters....37
Table 8: Compound-specific MS parameters for FAHFA determination....37
Table 9: MRM3 method - fixed parameters....40
Table 10: MRM3 method - Precursor ions and collision energy....40
Table 11*: Anthropometric data of study participants....47
Table 12: Association of initial body weight and BMI with weight loss parameters....47
Table 13: Dietary intake of total calories, water and different nutrients....49
Table 14*: Laboratory values of glucose metabolism in study participants....52
Table 15: Comparison of laboratory values of glucose metabolism between obese patients with and without diabetes mellitus type 2....52
Table 16: Metabolic profile of study participants....53
Table 17: Comparison of CRP, LBP and NEFA levels between obese patients with and without diabetes mellitus type 2....56
Table 18: Comparison of gut permeability markers between obese patients with and without diabetes mellitus type 2....58
Table 19: Correlation of weight loss with changes in gut permeability in obese patients undergoing LSG....59
Table 20*: Characteristics of female study participants in different BMI groups....60
Table 21**: Characteristics of morbidly obese patients undergoing bariatric surgery and non-obese controls used for FAHFA analysis....69
Table 22: Multiple regression analysis: FAHFA levels and metabolic factors adjusted for obesity status....71
Table 23: Multiple regression analysis: FAHFA levels and NEFAs adjusted for obesity status....71
Table 24**: Characteristics of obese diabetic and non-diabetic individuals of the GOBB cohort....72
Table 25**: Characteristics of adult diabetic and non-diabetic individuals of the *enable* cohort....74
Table 26**: Characteristics of individuals from three different age groups of the *enable* cohort....76
Table 27: Multiple regression analysis: FAHFA levels and BMI adjusted for age....77
Table 28**: Characteristics of vegetarians and omnivores....78
Table 29**: Characteristics of young men undergoing short-term overfeeding....80

Supplemental Table 1: Ethnical origin and lifestyle criteria of study participants....103
Supplemental Table 2: Body composition measured by bioelectrical impedance analysis in obese patients after surgery (LSG V2) and non-obese controls....104
Supplemental Table 3: Dietary supplements and their constituents....105

Supplemental Table 4: Correlation of inflammatory parameters with parameters of glucose metabolism in obese patients before LSG....108
Supplemental Table 5: Correlation of inflammatory parameters with parameters of glucose metabolism in non-obese controls....108

List of figures

Figure 1: Schematic representation of the four components of the intestinal barrier (adapted from Anderson et al., 2012; Cox et al., 2015)............7
Figure 2: Schematic representation of the molecular structure of different junctions between intestinal epithelial cells (adapted from Turner, 2009; Suzuki, 2013)............9
Figure 3: Structure of different FAHFAs............19
Figure 4: Anti-diabetic and anti-inflammatory effects of FAHFAs (Adapted from Muoio and Newgard, 2014)............21
Figure 5: Study design for obese patients undergoing LSG............24
Figure 6: Reactions of the ACS-ACOD Method............32
Figure 7: FAHFA extraction from serum samples............35
Figure 8: Chromatographic profile of all FAHFA standards (A) and of one exemplary serum sample (B) - first method............38
Figure 9: Chromatographic profile of all FAHFA standards (A) and of one exemplary serum sample (B) - improved method............39
Figure 10: MRM3 method – specific fragmentation scheme and MS3 spectra for different FAHFAs............42
Figure 11: Association between percentage excessive weight loss (%EWL) and initial body weight of patients undergoing sleeve gastrectomy............48
Figure 12*: Caloric intake before and after sleeve gastrectomy............51
Figure 13*: Plasma concentration of CRP (A), LBP (B), and NEFAs (C)............55
Figure 14*: Gut permeability of study participants............57
Figure 15: Association between the change in sucrose urinary recovery (Δ sucrose) with the percentage excessive weight loss (%EWL) of patients undergoing sleeve gastrectomy............59
Figure 16*: Markers of gut permeability in dependence of BMI............61
Figure 17*: LBP (A) and CRP (B) levels in dependence of BMI............62
Figure 18*: Faecal microbiota analysis of study participants in dependence of PPI medication............63
Figure 19*: Faecal microbiota analysis of study participants (n = 8 per group)............67
Figure 20**: FAHFA concentration in morbidly obese individuals before (LSG V1) and 6 months after laparoscopic sleeve gastrectomy (LSG V2) as well as in non-obese controls............70
Figure 21**: FAHFA concentration in obese individuals with and without type 2 diabetes of the GOBB cohort............73
Figure 22**: FAHFA concentration in individuals with and without type 2 diabetes of the *enable* cohort............74
Figure 23**: FAHFA concentrations in different age groups of the *enable* cohort............76
Figure 24**: FAHFA concentration in dependence of sex (*enable* cohort)............77
Figure 25**: FAHFA concentration in individuals following vegetarian diet (vegetarians) in comparison to individuals with normal diet (omnivores)............78
Figure 26**: FAHFA concentration in individuals undergoing short-term overfeeding............81

Supplemental Figure 1: Non-metric multidimensional scaling (NMDS) plots of phylogenetic distances (beta-diversity) as pairwise comparison between groups – analysis with participants taking PPIs............109
Supplemental Figure 2: Rarefaction curves of all samples analysed............110

Supplemental Figure 3: Non-metric multidimensional scaling (NMDS) plots of phylogenetic distances (beta-diversity) as pairwise comparison between groups – analysis without participants taking PPIs..110
Supplemental Figure 4: Multidimensional scaling (MDS) plots of phylogenetic distances (beta-diversity) after de-novo clustering of samples of obese patients 111
Supplemental Figure 5: Taxonomic composition of the samples at phylum level (A), class level (B), order level (C), family level (C) and genus level (E) 112
Supplemental Figure 6: Association between change in tPAHSA levels (A) or change in tFAHFA levels (B) with change in NEFA levels of patients undergoing LSG.......................113
Supplemental Figure 7: Correlation of tFAHFAs and tPAHSAs with metabolic factors 114
Supplemental Figure 8: Association between tPAHSA levels (A) or tFAHFA levels (B) with BMI of subjects from the adult cohort...115

Publications and presentations

Publications

- Ott, B., Skurk, T., Hastreiter, L., Lagkouvardos, I., Fischer, S., Büttner, J., Kellerer, T., Clavel, T., Rychlik, M., and Haller, D., et al. (2017). Effect of caloric restriction on gut permeability, inflammation markers, and fecal microbiota in obese women. Scientific reports *7*, 11955.
- Kellerer, T., Brandl, B., Büttner, J., Lagkouvardos, I., Hauner, H., and Skurk, T. (2019). Impact of Laparoscopic Sleeve Gastrectomy on Gut Permeability in Morbidly Obese Subjects. Obesity surgery *29*, 2132-2143.

Manuscript in submission

- Kellerer, T., Kleigrewe, K., Brandl, B., Hofmann, T., Hauner, H., Skurk, T. Fatty acid esters of hydroxy fatty acids (FAHFAs) are associated with diet, BMI and age

Poster presentations

- Kellerer, T., Hauner, H., Skurk, T.: Impact of sleeve gastrectomy on gut barrier function. DAG 2017, Potsdam, Germany.
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Appendix

Study documents

Flyer for recruitment of obese study participants

Haben wir Ihr Interesse an der Studie geweckt?

Dann sprechen Sie Ihren behandelnden Arzt an oder melden Sie sich bei uns, wir freuen uns auf Sie!

Gerne können Sie sich auch jederzeit mit Fragen an uns wenden.

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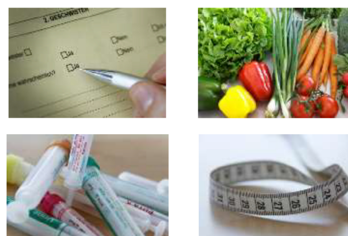
Lehrstuhl für Ernährungsmedizin

Technische Universität München

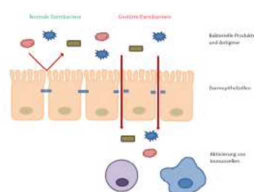
Prof. Dr. med. Hans Hauner

Studie zur Darmbarrierefunktion nach Schlauchmagen-Operation



Fotos: Stobrawe, Klinikum r.d.I.

Was wird untersucht?



Adaptiert von Suzuki, T. (2013). Regulation of intestinal epithelial permeability by tight junctions. Cell. Mol. Life Sci. 70, 631-659.

Neue Forschungsergebnisse deuten darauf hin, dass bei Personen mit Übergewicht die Zusammensetzung der Darmflora und die Darmbarrierefunktion verändert sind. Dies kann Entzündungen im Fettgewebe und die Entstehung von Krankheiten wie Diabetes beeinflussen.

In dieser Studie soll geklärt werden, ob eine Gewichtsabnahme nach Schlauchmagen-Operation einen positiven Einfluss auf die Darmbarriere und den Entzündungsstatus im Fettgewebe hat.

Wie wird das getestet?

- Einnahme einer Lösung mit verschiedenen Zuckern
- Sammeln des Urins während eines Tages
- Durchführung kann nach ausführlicher Erklärung und Bereitstellung der Materialien selbst zu Hause erfolgen, oder auf Wunsch in einem unserer Studienzentren in München und Freising

Wir suchen:

- Personen, zwischen 18 und 65 Jahren, welche sich einer Schlauchmachen-Operation unterziehen

Ablauf der Studie:

- Begleitend zu Ihrer Operation sowie der Vor- und Nachbetreuung in der Klinik
- Entnahme von Blut- und Fettgewebsproben während der Operation (kein zusätzlicher Eingriff)
- Auswertung von Daten zu Gewicht, Vorerkrankungen, Aktivität und Ernährung
- Abgabe einer Stuhlprobe
- Darmbarrieretest
- Fettgewebsbiopsie 6 Monate nach Operation (nicht zwingend erforderlich)

Aufwandsentschädigung:

- 2 x 50,- Euro für den Darmbarrieretest
- 100,- Euro für die Fettgewebsbiopsie
- Erstattung eventueller Fahrtkosten

Call for participants (control subjects)

Lehrstuhl für Ernährungsmedizin

Technische Universität München

Prof. Dr. med. Hans Hauner

24.03.2016

Teilnehmer gesucht

für

Studie zur Darmbarrierefunktion

Im Rahmen einer laufenden Studie, in welcher der Zusammenhang zwischen Gewicht, Darmflora und Darmbarriere untersucht wird, suchen wir noch **gesunde, normalgewichtige Teilnehmer/innen zwischen 25 und 60 Jahren**.

Die Studie beinhaltet:

- Ein Termin am Studienzentrum (ca. 1 Std.)
  - Aufklärung und Nüchtern-Blutabnahme
  - Erhebung von Daten zu Größe und Gewicht
- Zu Hause
  - Ein Test zur Messung der Darmbarrierefunktion
  - Eine Stuhlprobe
  - Ein 7-Tage Ernährungsprotokoll
  - Ein kurzer Fragebogen
- Probenabgabe

Weitere Informationen erhalten Sie bei Interesse bzw. Terminvereinbarung.

Aufwandsentschädigung: 50 Euro

Wo: Else Kröner-Fresenius-Zentrum für Ernährungsmedizin, Lehrstuhl für Ernährungsmedizin, Gregor-Mendel-Str. 2, 85354 Freising - Weihenstephan

Wann: Bitte um Terminvereinbarung

Zur Terminvereinbarung bzw. bei Fragen wenden Sie sich bitte an:
E-Mail: teresa.kellerer@tum.de
Teresa Kellerer, Tel.: 08161-712011

DFG Deutsche Forschungsgemeinschaft

Participant information and informed consent for obese study participants

Teilnehmerinformation und Einverständniserklärung

(bariatrische Operation)

Studie: Effekt einer Gewichtsabnahme nach bariatrischer Operation auf die Darmbarrierefunktion und den Entzündungsstatus.

Sehr geehrter Studienteilnehmer,

Sie sind eingeladen an der oben genannten Untersuchung zur DFG-Studie teilzunehmen. Im Folgenden möchten wir Sie über das Ziel und den Ablauf dieser Untersuchung informieren. Bevor Sie über Ihre Teilnahme entscheiden, bitten wir Sie, dieses Informationsschreiben sorgfältig zu lesen.

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.

Neue Forschungsergebnisse deuten darauf hin, dass bei Personen mit Übergewicht und Adipositas die Darmflora und die Darmbarrierefunktion verändert sind. Die Darmwand ist gewissermaßen „undicht“. Dadurch steigt das Risiko, dass vermehrt auch schädliche Stoffe, wie bakterielle Bestandteile, 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 adipösen Personen die Darmflora und die Darmbarriere verändert sind im Vergleich zu normal- leicht übergewichtigen Kontrollpersonen. Ein weiteres Ziel ist es, den Entzündungsstatus im Fettgewebe zu analysieren. Darüber hinaus soll untersucht werden, ob eine bariatrische Operation, im speziellen eine laparoskopische Sleeve Gastrektomie (Magenverkleinerung), und ein damit verbundener ausgeprägter Gewichtsverlust die Darmflora verändern und die Darmbarrierefunktion und den Entzündungsstatus möglicherweise positiv beeinflussen.

Seite 1 von 8

Teilnehmerinformation

2. Studienablauf

Die Studie verläuft begleitend zu Ihrer Operation inklusive der Vor- und Nachbetreuung durch die Klinik.

Während der Operation werden Ihnen zusätzlich zur routinemäßigen Blutabnahme der Klinik zwei Röhrchen Blut abgenommen. Des Weiteren wird am Ende der Operation je ein kleines Stück Unterhaut- und Eingeweidefettgewebe (jeweils etwa 2-5 g) entnommen. Für die Probenentnahmen ist kein zusätzlicher Eingriff erforderlich.

Eine weitere Blutabnahme ist im Rahmen der Nachuntersuchungen an der Klinik 6 Monate nach der Operation vorgesehen.

Messungen zu Größe und Gewicht werden an der Klink im Rahmen der Vor- und Nachsorge durchgeführt.

Den Test zur Messung der Darmbarrierefunktion sowie die Entnahme einer Stuhlprobe können Sie nach ausführlicher Aufklärung selbst zu Hause durchführen. Dies ist zum einen in dem Monat vor der Operation und zum anderen 6 Monate nach der Operation geplant. Sie erhalten dafür von unserem Studienteam ausführliche Informationen und alle benötigten Materialien. Der Test zur Messung der Darmbarrierefunktion dauert insgesamt 26 Stunden, in denen der Urin gesammelt wird. In den 48 Stunden bevor Sie den Test durchführen, sind Bier, Wein, Schnaps oder sonstige alkoholische Getränke nicht erlaubt. Am Vorabend des Tests ist die letzte Mahlzeit bis spätestens 18 Uhr erlaubt, danach bitte nur noch Leitungswasser bzw. Mineralwasser trinken. In den letzten 24 Stunden vor dem Test sollte möglichst auf Medikamente verzichtet werden. Die Einnahme von Medikamenten wird mit dem Studienteam abgesprochen. Am Tag vor der Untersuchung und am Untersuchungstag sollten keine körperlich anstrengenden Tätigkeiten verrichtet werden und kein Sport betrieben werden. Für diese Untersuchung erhalten Sie eine Aufwandsentschädigung von 2x 50,- Euro.

Zusätzlich bitten wir Sie 6 Monate nach Ihrer Operation eine Fettgewebsbiopsie durchzuführen zu lassen (optional). Dafür laden wir Sie an unser Studienzentrum in München oder Freising ein. Für diese Untersuchung erhalten Sie eine Aufwandsentschädigung von 100,- Euro sowie die Fahrtkosten erstattet.

Zeitliche Gliederung der Untersuchungen:

Präoperative Untersuchungen (V_1)

In dem Monat vor Ihrer Operation werden folgende Untersuchungen durchgeführt:

Anamnese, Ernährungsverhalten und körperliche Untersuchung

Im Rahmen der Vorbesprechungen an der Klinik werden Sie gebeten einen Fragebogen (Anamnesebogen Adipositas) auszufüllen zu Themen wie medizinischer Vorgeschichte, möglicher familiärer Vorbelastung, regelmäßig eingenommene Medikamente, Lebensstil und Soziodemographie. Zudem werden Sie gebeten Ihre Nahrungsaufnahme in Form eines 7-

Seite 2 von 8

Tage-Ernährungsprotokolls festzuhalten. Im Rahmen der körperlichen Untersuchung werden Größe, Gewicht, Taillen- und Hüftumfang bestimmt.

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.

Messung der intestinalen Permeabilität

Mit Hilfe eines Zuckerresorptionstests wird die Durchlässigkeit der Darmschleimhaut bestimmt bzw. die Funktion der Darmwand gemessen.

Nach nächtlichem Fastenzustand wird Urin gesammelt. Danach trinken Sie eine Zuckerlösung und nehmen 6 „Zuckerkapseln“ ein. Nach Testbeginn wird über 5 Stunden Urin gesammelt. Anschließend wird nochmals über 21 Stunden Urin gesammelt.

Eine ausführliche Anleitung zum Sammelurin und die nötigen Utensilien bekommen Sie von Ihrem Studienteam.

Probenentnahme während der Operation

Während der Operation erfolgt eine Entnahme von Blut- und Fettgewebsproben. Dafür ist kein zusätzlicher Eingriff notwendig und es besteht kein erhöhtes Komplikationsrisiko.

Blutabnahme

Es werden Ihnen 2 Röhrchen Blut abgenommen (jeweils ca. 9 ml). Die Blutabnahme dient zur Bestimmung verschiedener Laborwerte z.B. Nüchternblutzucker, Entzündungswerte, Blutfette, Leberfunktion, Endotoxin (Abbauprodukte von Bakterien).

Fettgewebsentnahme

Es wird Ihnen im Rahmen der Operation ein kleines Stück Unterhaut- und Eingeweidefett entnommen. Es werden jeweils ca. 2-5 g Fett entfernt.

Postoperative Untersuchungen (V_2)

Nach der Operation haben Sie Nachsorgetermine an der Klinik. In diesem Rahmen wird 6 Monate nach der Operation nochmals eine körperliche Untersuchung durchgeführt und es wird Ihnen Blut abgenommen. Die Stuhlprobe und den Test zur Messungen der Darmbarrierefunktion führen Sie 6 Monate nach der Operation selbstständig zu Hause durch.

Sechs Monate nach der Operation wird folgendes Untersuchungsprogramm wiederholt:

- Blutabnahme
- Anamnese, Ernährungsverhalten und körperliche Untersuchung
- Stuhlprobe
- Messung der Darmbarrierefunktion

Zusätzlich zu dem genannten Untersuchungsprogramm wird 6 Monate nach der Operation eine Fettgewebsbiopsie an einem unserer Studienzentren durchgeführt.

Fettgewebsbiopsie (optional)

Bei dieser Untersuchung werden seitlich des Bauchnabels mit einer Nadel ca. 0,5 - 3 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.

3. Nutzen einer Studienteilnahme

Mit Ihrer Studienteilnahme unterstützen Sie den Erkenntnisgewinn zur Veränderung der Darmflora und der Darmbarrierefunktion bei Adipositas sowie deren Einfluss auf den Entzündungsstatus im Fettgewebe und damit auf die Entstehung von Adipositas-bedingten Stoffwechselerkrankungen. Zudem kann geklärt werden, ob eine bariatrische Operation, ins Besondere eine Magenverkleinerung, und der damit verbundene ausgeprägte Gewichtsverlust die Darmflora und die Darmbarrierefunktion verändern und dadurch einen positiven Einfluss auf den Entzündungsstatus im Fettgewebe sowie die Insulinsensitivität ausüben. Darauf aufbauend kann der Nutzen einer bariatrischen Operation und der Einfluss der Darmflora neu bewertet werden.

Persönlich können Sie für Ihre Gesundheit keinen unmittelbaren Vorteil oder Nutzen aus der Spende Ihrer Proben und Daten erwarten. Die Ergebnisse sind ausschließlich zu Forschungszwecken bestimmt. Eine Rückmeldung von Ergebnissen aus der Untersuchung der Biomaterialien ist nicht vorgesehen.

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. Der Einstich der Nadel für 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 Testlösung zur Messung der Darmfunktion mittels Zuckerresorptions-Test (Laktulose, Mannitol, Saccharose, Sucralose) kann in seltenen Fällen nach Einnahme Bauchschmerzen, Durchfälle und/oder 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.

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

Aufgrund des geringen Risikos der Studienteilnahme besteht keine verschuldensunabhängige Versicherung.

5. Aufwandsentschädigung/Kostenerstattung

Bei erfolgreicher Teilnahme am Test zur Messung der Darmbarrierefunktion erhalten Sie eine Aufwandsentschädigung in Höhe von jeweils 50,- Euro. Für das Einverständnis zur Entnahme einer Fettbiopsie erhalten Sie einmalig 100,- Euro. 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 Studie, Herrn Univ.-Prof. Dr. med. Hans Hauner, einschließlich dessen Vertreter und Auftragnehmer sowie die zuständigen 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 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 Studienarzt 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 informieren wir Sie, damit Sie das weitere Vorgehen mit Ihrem Hausarzt absprechen können.

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

Teresa Kellerer, M.Sc. Ernährungswissenschaften
Else Kröner-Fresenius-Zentrum für Ernährungsmedizin
TU München, Lehrstuhl für Ernährungsmedizin
Gregor-Mendel-Str. 2
D - 85350 Freising-Weihenstephan
Tel: 08161/71-2011 oder -2001
mailto: teresa.kellerer@tum.de
http://www.em-tum.de/

Einverständniserklärung

Studie: Effekt einer Gewichtsabnahme nach bariatrischer Operation auf die Darmbarrierefunktion und den Entzündungsstatus.

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 Untersuchungsprogramm teilzunehmen.
❒ Ja ❒ Nein

✓ Ich bin einverstanden, dass meine Bioproben (Blut, Gewebe, Stuhl, Urin) 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

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, Stuhl, Urin) wider Erwarten medizinische Erkenntnisse gewonnen, die für die Erhaltung oder Wiederherstellung meiner eigenen Gesundheit von erheblicher Bedeutung sind, werde ich informiert, damit ich das weitere Vorgehen mit meinem Hausarzt besprechen kann.

Nach der Studie werden Ihre Biomaterialien vernichtet sofern Sie nicht einer Speicherung im Rahmen einer Biobank zugestimmt haben.

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 (TU München) zur wissenschaftlichen Auswertung,
b) die zuständige(n) Ethikkommissionen(en).

Außerdem erkläre ich mich damit einverstanden, dass autorisierte und zur Verschwiegenheit verpflichtete Beauftragte des Auftraggebers der zuständigen inländischen und ausländischen Überwachungsbehörden 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 10 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/Schrobenhausen/Landshut, den ___________

Name des aufklärenden Arztes — Unterschrift

München/Freising-Weihenstephan/Schrobenhausen/Landshut, den ___________

Name des Studienteilnehmers — Unterschrift

Participant information and informed consent for control patients with operation

Teilnehmerinformation und Einverständniserklärung

(Abdominale Operation)

Studie: Effekt einer Gewichtsabnahme nach bariatrischer Operation auf die Darmbarrierefunktion und den Entzündungsstatus.

Sehr geehrter Studienteilnehmer,

Sie sind eingeladen an der oben genannten Untersuchung zur DFG-Studie teilzunehmen. Im Folgenden möchten wir Sie über das Ziel und den Ablauf dieser Untersuchung informieren. Bevor Sie über Ihre Teilnahme entscheiden, bitten wir Sie, dieses Informationsschreiben sorgfältig zu lesen.

1. Hintergrund und Ziel der Studie

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Mit dem Körpergewicht und Körperfettanteil steigt auch das Risiko für Adipositas-bedingte Stoffwechselstörungen wie Insulinresistenz, gestörte Glukosetoleranz und schließlich Typ-2-Diabetes.

Neue Forschungsergebnisse deuten darauf hin, dass bei Personen mit Übergewicht und Adipositas die Darmflora und die Darmbarrierefunktion verändert sind. Die Darmwand ist gewissermaßen „undicht". Dadurch steigt das Risiko, dass vermehrt auch schädliche Stoffe, wie bakterielle Bestandteile, 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 adipösen Personen die Darmflora und die Darmbarriere verändert sind im Vergleich zu normal- bis mäßig übergewichtigen Kontrollpersonen. Ein weiteres Ziel ist es, den Entzündungsstatus im Fettgewebe zu analysieren. Darüber hinaus soll untersucht werden, ob eine bariatrische Operation, im speziellen eine laparoskopische Sleeve Gastrektomie (Magenverkleinerung), und ein damit verbundener ausgeprägter Gewichtsverlust die Darmflora verändern und die Darmbarrierefunktion und den Entzündungsstatus möglicherweise positiv beeinflussen.

2. Studienablauf

Die Studie verläuft begleitend zu Ihrer Operation inklusive der Vor- und Nachbetreuung durch die Klinik.

Während der Operation werden Ihnen zusätzlich zur routinemäßigen Blutabnahme der Klinik zwei Röhrchen Blut abgenommen. Des Weiteren wird am Ende der Operation je ein kleines Stück Unterhaut- und Eingeweidefettgewebe (jeweils etwa 2-5 g) entnommen. Für die Probenentnahmen ist kein zusätzlicher Eingriff erforderlich.

Messungen zu Größe und Gewicht werden an der Klink im Rahmen der Vorsorge durchgeführt.

Den Test zur Messung der Darmbarrierefunktion sowie die Entnahme einer Stuhlprobe können Sie nach ausführlicher Aufklärung selbst zu Hause durchführen. Dies ist in dem Monat vor der Operation geplant. Sie erhalten dafür von unserem Studienteam ausführliche Informationen und alle benötigten Materialien. Der Test zur Messung der Darmbarrierefunktion dauert insgesamt 26 Stunden, in denen der Urin gesammelt wird. In den 48 Stunden bevor Sie den Test durchführen, sind Bier, Wein, Schnaps oder sonstige alkoholische Getränke nicht erlaubt. Am Vorabend des Tests ist die letzte Mahlzeit bis spätestens 18 Uhr erlaubt, danach bitte nur noch Leitungswasser bzw. Mineralwasser trinken. In den letzten 24 Stunden vor dem Test sollte möglichst auf Medikamente verzichtet werden. Die Einnahme von Medikamenten wird mit dem Studienteam abgesprochen. Am Tag vor der Untersuchung und am Untersuchungstag sollten keine körperlich anstrengenden Tätigkeiten verrichtet werden und kein Sport betrieben werden. Für diese Untersuchung erhalten Sie eine Aufwandsentschädigung von 50,- Euro.

Zeitliche Gliederung der Untersuchungen:

<u>Präoperative Untersuchungen (V_1)</u>

In dem Monat vor Ihrer Operation werden folgende Untersuchungen durchgeführt:

Anamnese, Ernährungsverhalten und körperliche Untersuchung

Im Rahmen der Vorbesprechungen an der Klinik werden Sie gebeten einen Fragebogen auszufüllen zu Themen wie medizinischer Vorgeschichte, möglicher familiärer Vorbelastung, regelmäßig eingenommene Medikamente, Lebensstil und Soziodemographie. Zudem werden Sie gebeten Ihre Nahrungsaufnahme in einem 7-Tage-Ernährungsprotokoll festzuhalten. Im Rahmen der körperlichen Untersuchung werden Größe, Gewicht, Taillen- und Hüftumfang bestimmt.

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.

Messung der intestinalen Permeabilität

Mit Hilfe eines Zuckerresorptionstests wird die Durchlässigkeit der Darmschleimhaut bestimmt bzw. die Funktion der Darmwand gemessen.

Nach nächtlichem Fastenzustand wird Urin gesammelt. Danach trinken Sie eine Zuckerlösung und nehmen 6 „Zuckerkapseln" ein. Nach Testbeginn wird über 5 Stunden Urin gesammelt. Anschließend wird nochmals über 21 Stunden Urin gesammelt.

Eine ausführliche Anleitung zum Sammelurin und die nötigen Utensilien bekommen Sie von Ihrem Studienteam.

Probenentnahme während der Operation

Während der Operation erfolgt eine Entnahme von Blut- und Fettgewebsproben. Dafür ist kein zusätzlicher Eingriff notwendig und es besteht kein erhöhtes Komplikationsrisiko.

Blutabnahme

Es werden Ihnen 2 Röhrchen Blut abgenommen (jeweils ca. 9 ml). Die Blutabnahme dient zur Bestimmung verschiedener Laborwerte z.B. Nüchternblutzucker, Entzündungsmesswerte, Blutfette, Leberfunktion, Endotoxin (Abbauprodukte von Bakterien).

Fettgewebsentnahme

Es wird Ihnen im Rahmen der Operation ein kleines Stück Unterhaut- und Eingeweidefett entnommen. Es werden jeweils ca. 2-5 g Fett entfernt.

3. Nutzen einer Studienteilnahme

Mit Ihrer Studienteilnahme unterstützen Sie den Erkenntnisgewinn zur Veränderung der Darmflora und der Darmbarrierefunktion bei Adipositas und nach bariatrischer Operation im Vergleich zu normalgewichtigen Personen sowie deren Einfluss auf den Entzündungsstatus im Fettgewebe und damit auf die Entstehung von Adipositas-bedingten Stoffwechselerkrankungen.

Persönlich können Sie für Ihre Gesundheit keinen unmittelbaren Vorteil oder Nutzen aus der Spende Ihrer Proben und Daten erwarten. Die Ergebnisse sind ausschließlich zu Forschungszwecken bestimmt. Eine Rückmeldung von Ergebnissen aus der Untersuchung der Biomaterialien ist nicht vorgesehen.

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. Der Einstich der Nadel für 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 Testlösung zur Messung der Darmfunktion mittels Zuckerresorptions-Test (Laktulose, Mannitol, Saccharose, Sucralose) kann in seltenen Fällen nach Einnahme Bauchschmerzen, Durchfälle und/oder Blähungen verursachen. Diese klingen nach Ende des Testes rasch ab.

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

Aufgrund des geringen Risikos der Studienteilnahme besteht keine verschuldensunabhängige Versicherung.

5. Aufwandsentschädigung/Kostenerstattung

Bei erfolgreicher Teilnahme am Test zur Messung der Darmbarrierefunktion erhalten Sie eine Aufwandsentschädigung in Höhe von einmalig 50,- Euro. Eventuell anfallende 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 Studie, Herrn Univ.-Prof. Dr. med. Hans Hauner, einschließlich dessen Vertreter und Auftragnehmer sowie die zuständigen 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 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 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 informieren wir Sie, damit Sie das weitere Vorgehen mit Ihrem Hausarzt absprechen können.

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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Einverständniserklärung

Studie: Effekt einer Gewichtsabnahme nach bariatrischer Operation auf die Darmbarrierefunktion.

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 Untersuchungsprogramm teilzunehmen.
❒ Ja ❒ Nein

✓ Ich bin einverstanden, dass meine Bioproben (Blut, Gewebe, Stuhl, Urin) 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

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, Stuhl, Urin) wider Erwarten medizinische Erkenntnisse gewonnen, die für die Erhaltung oder Wiederherstellung meiner eigenen Gesundheit von erheblicher Bedeutung sind, werde ich informiert, damit ich das weitere Vorgehen mit meinem Hausarzt besprechen kann.

Nach der Studie werden Ihre Biomaterialien vernichtet sofern Sie nicht einer Speicherung im Rahmen einer Biobank zugestimmt haben.

Einverständniserklärung

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 (TU München) zur wissenschaftlichen Auswertung,
b) die zuständige(n) Ethikkommissionen(en).

Außerdem erkläre ich mich damit einverstanden, dass autorisierte und zur Verschwiegenheit verpflichtete Beauftragte des Auftraggebers der zuständigen inländischen und ausländischen Überwachungsbehörden 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 10 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/Schrobenhausen/Landshut, den _____________

Name des aufklärenden Arztes	Unterschrift

München/Freising-Weihenstephan/Schrobenhausen/Landshut, den _____________

Name des Studienteilnehmers	Unterschrift

Participant information and informed consent for control patients without operation

Teilnehmerinformation und Einverständniserklärung

(Kontrollpersonen)

Studie: Effekt einer Gewichtsabnahme nach bariatrischer Operation auf die Darmbarrierefunktion und den Entzündungsstatus.

Sehr geehrter Studienteilnehmer,

Sie sind eingeladen an der oben genannten Untersuchung zur DFG-Studie teilzunehmen. Im Folgenden möchten wir Sie über das Ziel und den Ablauf dieser Untersuchung informieren. Bevor Sie über Ihre Teilnahme entscheiden, bitten wir Sie, dieses Informationsschreiben sorgfältig zu lesen.

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.

Neue Forschungsergebnisse deuten darauf hin, dass bei Personen mit Übergewicht und Adipositas die Darmflora und die Darmbarrierefunktion verändert sind. Die Darmwand ist gewissermaßen „undicht“. Dadurch steigt das Risiko, dass vermehrt auch schädliche Stoffe, wie bakterielle Bestandteile, 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 adipösen Personen die Darmflora und die Darmbarriere verändert sind im Vergleich zu normal- bis mäßig übergewichtigen Kontrollpersonen. Ein weiteres Ziel ist es, den Entzündungsstatus im Fettgewebe zu analysieren. Darüber hinaus soll untersucht werden, ob eine bariatrische Operation, im speziellen eine laparoskopische Sleeve Gastrektomie (Magenverkleinerung), und ein damit verbundener ausgeprägter Gewichtsverlust die Darmflora verändern und die Darmbarrierefunktion und den Entzündungsstatus möglicherweise positiv beeinflussen.

2. Studienablauf

Die Studie wird an einem der identisch ausgestatteten Studienzentren (Uptown München Campus D & Wissenschaftszentrum Weihenstephan) des Else Kröner-Fresenius-Zentrums für Ernährungsmedizin durchgeführt.

Am Studienzentrum werden Körpergröße, Gewicht, Taillen- und Hüftumfang bestimmt, sowie eine Blutabnahme durchgeführt.

Den Test zur Messung der Darmbarrierefunktion sowie die Entnahme einer Stuhlprobe können Sie nach ausführlicher Aufklärung selbst zu Hause durchführen. Sie erhalten dafür von unserem Studienteam ausführliche Informationen und alle benötigten Materialien. Der Test zur Messung der Darmbarrierefunktion dauert insgesamt 26 Stunden, in denen der Urin gesammelt wird. In den 48 Stunden bevor Sie den Test durchführen, sind Bier, Wein, Schnaps oder sonstige alkoholische Getränke nicht erlaubt. Am Vorabend des Tests ist die letzte Mahlzeit bis spätestens 18 Uhr erlaubt, danach bitte nur noch Leitungswasser bzw. Mineralwasser trinken. In den letzten 24 Stunden vor dem Test sollte möglichst auf Medikamente verzichtet werden. Die Einnahme von Medikamenten wird mit dem Studienteam abgesprochen. Am Tag vor der Untersuchung und am Untersuchungstag sollten keine körperlich anstrengenden Tätigkeiten verrichtet werden und kein Sport betrieben werden. Für diese Untersuchung erhalten Sie eine Aufwandsentschädigung von 50,- Euro.

Untersuchungsprogramm

Es werden folgende Untersuchungen durchgeführt:

Anamnese, Ernährungsverhalten und körperliche Untersuchung

Sie werden gebeten einen Fragebogen auszufüllen zu Themen wie medizinischer Vorgeschichte, möglicher familiärer Vorbelastung, regelmäßig eingenommene Medikamente, Lebensstil und Soziodemographie. Zudem werden Sie gebeten Ihre Nahrungsaufnahme in einem 7-Tage-Ernährungsprotokoll festzuhalten. Im Rahmen der körperlichen Untersuchung werden Größe, Gewicht, Taillen- und Hüftumfang bestimmt.

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.

Messung der intestinalen Permeabilität

Mit Hilfe eines Zuckerresorptionstests wird die Durchlässigkeit der Darmschleimhaut bestimmt bzw. die Funktion der Darmwand gemessen.

Nach nächtlichem Fastenzustand wird Urin gesammelt. Danach trinken Sie eine Zuckerlösung und nehmen 6 „Zuckerkapseln“ ein. Nach Testbeginn wird über 5 Stunden Urin gesammelt. Anschließend wird nochmals über 21 Stunden Urin gesammelt.

Eine ausführliche Anleitung zum Sammelurin und die nötigen Utensilien bekommen Sie von Ihrem Studienteam.

Blutabnahme

Es werden Ihnen 2 Röhrchen Blut abgenommen (jeweils ca. 9 ml). Die Blutabnahme dient zur Bestimmung verschiedener Laborwerte z.B. Nüchternblutzucker, Entzündungsmesswerte, Blutfette, Leberfunktion, Endotoxin (Abbauprodukte von Bakterien).

3. Nutzen einer Studienteilnahme

Mit Ihrer Studienteilnahme unterstützen Sie den Erkenntnisgewinn zur Veränderung der Darmflora und der Darmbarrierefunktion bei Adipositas und nach bariatrischer Operation im Vergleich zu normalgewichtigen Personen sowie deren Einfluss auf den Entzündungsstatus im Fettgewebe und damit auf die Entstehung von Adipositas-bedingten Stoffwechselerkrankungen.

Persönlich können Sie für Ihre Gesundheit keinen unmittelbaren Vorteil oder Nutzen aus der Spende Ihrer Proben und Daten erwarten. Die Ergebnisse sind ausschließlich zu Forschungszwecken bestimmt. Eine Rückmeldung von Ergebnissen aus der Untersuchung der Biomaterialien ist nicht vorgesehen.

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. Der Einstich der Nadel für 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 Testlösung zur Messung der Darmfunktion mittels Zuckerresorptions-Test (Laktulose, Mannitol, Saccharose, Sucralose) kann in seltenen Fällen nach Einnahme Bauchschmerzen, Durchfälle und/oder Blähungen verursachen. Diese klingen nach Ende des Testes rasch ab.

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

Aufgrund des geringen Risikos der Studienteilnahme besteht keine verschuldensunabhängige Versicherung.

5. Aufwandsentschädigung/Kostenerstattung

Bei erfolgreicher Teilnahme am Test zur Messung der Darmbarrierefunktion erhalten Sie eine Aufwandsentschädigung in Höhe von einmalig 50,- Euro. Eventuell anfallende 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 Studie, Herrn Univ.-Prof. Dr. med. Hans Hauner, einschließlich dessen Vertreter und Auftragnehmer sowie die zuständigen 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 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 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, Urin, Stuhl) wider Erwarten medizinische Erkenntnisse gewonnen, die für die Erhaltung oder Wiederherstellung Ihrer eigenen Gesundheit von erheblicher Bedeutung sind, so informieren wir Sie, damit Sie das weitere Vorgehen mit Ihrem Hausarzt absprechen können.

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: Effekt einer Gewichtsabnahme nach bariatrischer Operation auf die Darmbarrierefunktion.

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 Untersuchungsprogramm teilzunehmen.
  ❒ Ja ❒ Nein
- ✓ Ich bin einverstanden, dass meine Bioproben (Blut, Stuhl, Urin) 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

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 Bioproben (Blut, Stuhl, Urin) 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, Stuhl, Urin) wider Erwarten medizinische Erkenntnisse gewonnen, die für die Erhaltung oder Wiederherstellung meiner eigenen Gesundheit von erheblicher Bedeutung sind, werde ich informiert, damit ich das weitere Vorgehen mit meinem Hausarzt besprechen kann.

Nach der Studie werden Ihre Biomaterialien vernichtet sofern Sie nicht einer Speicherung im Rahmen einer Biobank zugestimmt haben.

Einverständniserklärung

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 (TU München) zur wissenschaftlichen Auswertung,
b) die zuständige(n) Ethikkommissionen(en).

Außerdem erkläre ich mich damit einverstanden, dass autorisierte und zur Verschwiegenheit verpflichtete Beauftragte des Auftraggebers der zuständigen inländischen und ausländischen Überwachungsbehörden 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 10 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/Schrobenhausen/Landshut, den ____________

______________________	______________________
Name des aufklärenden Arztes	Unterschrift

München/Freising-Weihenstephan/Schrobenhausen/Landshut, den ____________

______________________	______________________
Name des Studienteilnehmers	Unterschrift

Seite 7 von 7

CRF for obese study participants

Case Report Form

Patienten mit bariatrischer Operation

Teilnehmernummer

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

Liste der verwendeten Abkürzungen

AP	Anthropometrische Parameter
BE	Befindlichkeit
BA	Blutabnahme
BIC	Bank Identifier Code
BLZ	Bankleitzahl
BMI	Body Mass Index
CH	Chirurg
EH	Ethnische Herkunft
EDTA	Ethylendiamintetraacetat
EP	Ernährungsprotokoll
FEV	Fettgewebeentnahme viszerales Fett
FES	Fettgewebeentnahme subkutanes Fett
FZ	Fäzesprobe
IBAN	International Bank Account Number
IP	Intestinale Permeabilität
KA	Kontaktaufnahme
M_r	relatives Molekulargewicht
PEG	Polyethylenglycose
S	Screening
V	Visite

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

Screening $_{S0}$

Daten von Krankenhaus bezogen

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

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

	JA	NEIN
S1 Drogen-/Alkoholprobleme		
S2 Erkrankungen Krebs; COPD; schwere kardiovaskuläre Erkrankung; Lebererkrankung (GOT, GPT > 3-fach der Norm); Nierenerkrankung (Kreatinin > 1,2 mg/dl; gastrointestinale, neurologische, rheumatische Erkrankungen; Zöliakie; akute Infektionen; Hypokaliämie; Autoimmunerkrankung; Malabsorptionssyndrom; autonome Neuropathie		
S3 Gallensteine		
S4 Stoffwechselerkrankungen S4.1 Diabetes S4.2 Insulin-pflichtig		
S5 Allergien: Lactuloseintoleranz, Sucraloseintoleranz, Saccaroseintoleranz, andere Nahrungsmittelintoleranzen, Kreuzallergien, **andere Allergien** z.B. Heuschnupfen		
S6 Frische Operation Darm-Operation, Koloskopie		
S7 Prothesen, Herzschrittmacher, Herzklappen, Herzschirmchen, Clips im Hirn oder Rückenmarkbereich, Gefäßstens, Gelenkprothese, Metallsplitter, Osteosynthesematerial, Neurostimulator		
S8 Psychische Probleme wie Depressionen		
S9 Regelmäßige Medikamente Antibiotika, Immunsuppresiva, Laxantien, Anti-Durchfall-Medikamente, andere		

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

Visite 1 (V_1) Präoperativ

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

Kontaktaufnahme $_{KA1}$

KA1.1 Ich bin damit einverstanden, dass mich das Studienteam per Telefon oder Email während der Studie kontaktiert.

- ❑ Nein (0)
- ❑ Ja (1)

Anmerkungen:

____

KA1.2 Haben Sie während der letzten drei Monate an anderen klinischen Studien teilgenommen?

- ❑ Nein (0)
- ❑ Ja (1)

Anmerkungen:

____

Ethnische Herkunft $_{EH1}$

EH1.1 Welche ethnische Herkunft haben Sie?

- ❑ Weiß/Europäisch (1)
- ❑ Schwarz (2)
- ❑ Asiatisch, Orientalisch (3)
- ❑ andere__________________________________ (4)

Seite 5 von 21

- vertraulich - 11.02.2015

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

Befindlichkeit $_{B1}$

BE1.1 Haben Sie in den letzten 7 Tagen unter folgenden Symptomen gelitten?

- ❑ Müdigkeit (1)
- ❑ verminderte Leistungsfähigkeit (2)
- ❑ Nervosität (3)
- ❑ Frieren (4)

BE1.2 Haben Sie regelmäßigen Stuhlgang?

- ❑ Täglich (1)
- ❑ ≥ 3x in der Woche (2)
- ❑ ≤3x in der Woche (3)
- ❑ unregelmäßig (4)

BE1.3 Haben Sie in den letzten 7 Tagen an Durchfall gelitten?

- ❑ Nein (0)
- ❑ ≤ 3x Durchfall in den letzten 7 Tagen (1)
- ❑ ≥ 3x Durchfall in den letzten 7 Tagen (2)

BE1.4 Haben Sie Ihre Menstruation regelmäßig?

- ❑ Ja (1)
- ❑ Nein (2)
- ❑ nicht mehr (3)
- ❑ ______________________________________ (4)

Seite 6 von 21

- vertraulich - 11.02.2015

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

BE1.5 Besteht die Möglichkeit einer Schwangerschaft?

❑ Ja (1)

❑ Nein (0)

Anthropometrische Parameter $_{AP1}$

Datum: __ __ / __ __ / __ __ __ __

AP1.1 Körpergröße: |__||__||__|,|__| cm

AP1.2 Körpergewicht: |__||__||__|,|__| kg

AP1.3 Body Mass Index (BMI): |__||__|,|__| kg/m²

AP1.4 Taillenumfang: |__||__||__|,|__| cm

AP1.5 Hüftumfang: |__||__||__|,|__| cm

AP1.6 Taille-Hüft-Quotient: |__|,|__||__||__|

AP1.7 Hat sich Ihr Gewicht in den letzten 3 Monaten verändert (um mehr als 2 kg)?

❑ Ja (1)
Wie? ____

❑ Nein (0)

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

Ernährungsprotokoll $_{EP1}$

EP1.1 Ernährungsprotokoll mit Anleitung ausgegeben?

❒ Ja, am: __ __ / __ __ / __ __ __ __ (1)

❒ Nein (0)

EP1.2 Wenn nein, bitte begründen: ________________________________

EP1.3 Ernährungsprotokoll ausgefüllt zurückerhalten?

❒ Ja, am: __ __ / __ __ / __ __ __ __ (1)

❒ Nein (0)

Fäzesprobe $_{FZ1}$

FZ1.1 Utensilien für die Fäzesprobe ausgegeben?

❒ Ja, am: __ __ / __ __ / __ __ __ __ (1)

❒ Stuhlbehältnis

❒ 3 Stuhlröhrchen

❒ Nein (0)

FZ1.2 Wenn nein, bitte begründen: ________________________________

FZ1.3 Analysecodierung: |____________________|

FZ1.4 Fäzesprobe zurück erhalten?

❒ Ja Anzahl ________ (1)

❒ Nein (0)

FZ1.5 Wenn nein, bitte begründen: ________________________________

FZ1.6 Stuhlröhrchen befüllt
Datum: __ __ / __ __ / __ __ __ __ Uhrzeit: |__||__| : |__||__| Uhr

FZ1.7 Stuhlröhrchen abgegeben
Datum: __ __ / __ __ / __ __ __ __ Uhrzeit: |__||__| : |__||__| Uhr

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

Intestinale Permeabilität $_{IP1}$

IP1.1 Utensilien für den PEG-Test/ Zuckerresorptionstest ausgegeben?

- ❑ 1x 500ml Behältnis
- ❑ 3x 3l Behältnisse
- ❑ Zuckerlösung mit Kapseln
- ❑ 100ml PEG-Lösung
- ❑ 6x10ml Urinmonovetten
- ❒ Ja, am: __ __ / __ __ / __ __ __ __ (1)
- ❒ Nein (0)

IP1.2 Wenn nein, bitte begründen: ____________________________________

IP1.3 Urinmonovetten zurück erhalten?

❒ Ja Anzahl ____________________ (1)

❒ Nein (0)

IP1.4 Urinmonovetten abgegeben

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

IP1.5 Wenn nein, bitte begründen: ____________________________________

IP1.6 Urinvolumen:

IP1.6.1 0h- Sammelvolumen (A) |__||__||__||__| ml

IP1.6.2 5h- Sammelvolumen (B) |__||__||__||__| ml

IP1.6.3 21h- Sammelvolumen (C) |__||__||__||__| ml

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

PEG

IP1.7 Erste Analysecodierung

IP1.7.1 A-Vorharn-PEG-Analysecodierung: |_______________|

IP1.7.2 B-5h-Sammelurin-PEG-Analysecodierung: |_______________|

IP1.7.3 C-21h-Sammelurin-PEG-Analysecodierung: |_______________|

IP1.8 Messung erfolgreich durchgeführt? ❒ Ja (1) ❒ Nein (0)

IP1.9 Wenn nein, bitte begründen: ____________________________________

Zuckerresorptionstest

IP1.10 Erste Analysecodierung

IP1.10.1 A-Vorharn-Zucker-Analysecodierung: |_______________|

IP1.10.2 B-5h-Sammelurin- Zucker-Analysecodierung: |_______________|

IP1.10.3 C-21h-Sammelurin-ZuckerAnalysecodierung: |_______________|

IP1.11 Messung erfolgreich durchgeführt? ❒ Ja (1) ❒ Nein (0)

IP1.12 Wenn nein, bitte begründen:____________________________________

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

Operation

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

Operation: ______________________________

Blutabnahme $_{BA1}$

BA1.1 Blutabnahme erfolgreich durchgeführt?

❐ Ja (1)

❐ Nein (0)

BA1.2 Wenn nein, bitte begründen: ______________________

BA1.3 Uhrzeit: |__||__|:|__||__| Uhr

BA1.4 Besonderheiten (z.B. Entzündung der Einstichstelle, mehrere Einstiche zur Blutentnahme waren nötig)

❐ Ja (1)

❐ Nein (0)

BA1.5 Wenn ja, welche? ______________________

BA1.6 Serum-Röhrchen

❐ Ja (1)

❐ Nein (0)

BA1.7 EDTA-Plasma-Röhrchen

❐ Ja (1)

❐ Nein (0)

Chirurg $_{CH1}$

CH1.1 Name Chirurg

____

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

Fettgewebeentnahme viszerales Fett $_{FEV1}$

FEV1.1 Entnahme von viszeralem Fett erfolgreich durchgeführt?

❐ Ja, um |__||__|:|__||__| Uhr (1)

❐ Nein (0)

FEV1.2 Wenn nein, bitte begründen: ______________________

FEV1.3 Besonderheiten

❐ Ja (1)

❐ Nein (0)

FEV1.4 Wenn ja, welche? ______________________

Fettgewebeentnahme subkutanes Fett $_{FES2}$

FES1.1 Entnahme von subkutanem Fett erfolgreich durchgeführt?

❐ Ja, um |__||__|:|__||__| Uhr (1)

❐ Nein (0)

FES1.2 Wenn nein, bitte begründen: ______________________

FES1.3 Besonderheiten

❐ Ja (1)

❐ Nein (0)

FES1.4 Wenn ja, welche? ______________________

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

Visite 2 (V_2) Postoperativ

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

Befindlichkeit $_{BE2}$

BE2.1 Haben Sie in den letzten 7 Tagen unter folgenden Symptomen gelitten?

- ❑ Müdigkeit (1)
- ❑ verminderte Leistungsfähigkeit (2)
- ❑ Nervosität (3)
- ❑ Frieren (4)

BE2.2 Haben Sie regelmäßigen Stuhlgang?

- ❑ Täglich (1)
- ❑ ≥ 3x in der Woche (2)
- ❑ ≤3x in der Woche (3)
- ❑ unregelmäßig (4)

BE2.3 Haben Sie in den letzten 7 Tagen an Durchfall gelitten?

- ❑ Nein (0)
- ❑ ≤ 3x Durchfall in den letzten 7 Tagen (1)
- ❑ ≥ 3x Durchfall in den letzten 7 Tagen (2)

BE2.4 Haben Sie Ihre Menstruation regelmäßig?

- ❑ Ja (1)
- ❑ Nein (2)
- ❑ nicht mehr (3)
- ❑ ____ (4)

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

BE2.5 Besteht die Möglichkeit einer Schwangerschaft?

- ❑ Ja (1)
- ❑ Nein (0)

Anthropometrische Parameter $_{AP2}$

Datum: __ __ / __ __ / __ __ __ __

AP2.1 Körpergröße:	|__||__||__|,|__| cm
AP2.2 Körpergewicht:	|__||__||__|,|__| kg
AP2.3 Body Mass Index (BMI):	|__||__|,|__| kg/m²
AP2.4 Taillenumfang:	|__||__||__|,|__| cm
AP2.5 Hüftumfang:	|__||__||__|,|__| cm
AP2.6 Taille-Hüft-Quotient:	|__|,|__||__||__|

Ernährungsprotokoll $_{EP2}$

EP2.1 Ernährungsprotokoll mit Anleitung ausgegeben?

- ❒ Ja, am: __ __ / __ __ / __ __ __ __ (1)
- ❒ Nein (0)

EP2.2 Wenn nein, bitte begründen: ________________________________

EP2.3 Ernährungsprotokoll ausgefüllt zurückerhalten?

- ❒ Ja, am: __ __ / __ __ / __ __ __ __ (1)
- ❒ Nein (0)

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

Fäzesprobe $_{FZ2}$

FZ2.1 Utensilien für die Fäzesprobe ausgegeben?

- ❑ Ja, am: __ __ / __ __ / __ __ __ __ (1)
- ❑ Stuhlbehältnis
- ❒ 3 Stuhlröhrchen
- ❒ Nein (0)

FZ2.2 Wenn nein, bitte begründen: ______________________________

FZ2.3 Analysecodierung: |______________________|

FZ2.4 Fäzesprobe zurück erhalten?

- ❒ Ja Anzahl ________ (1)
- ❒ Nein (0)

FZ2.5 Wenn nein, bitte begründen: ______________________________

FZ2.6 Stuhlröhrchen befüllt
Datum: __ __ / __ __ / __ __ __ __ Uhrzeit: |__||__| : |__||__| Uhr

FZ2.7 Stuhlröhrchen abgegeben
Datum: __ __ / __ __ / __ __ __ __ Uhrzeit: |__||__| : |__||__| Uhr

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

Intestinale Permeabilität $_{IP2}$

IP2.1 Utensilien für den PEG-Test/ Zuckerresorptionstest ausgegeben?

- ❑ 1x 500ml Behältnis
- ❑ 3x 3l Behältnisse
- ❑ Zuckerlösung mit Kapseln
- ❑ 100ml PEG-Lösung
- ❑ 6x10ml Urinmonovetten
- ❒ Ja, am: __ __ / __ __ / __ __ __ __ (1)
- ❒ Nein (0)

IP2.2 Wenn nein, bitte begründen: ______________________________

IP2.3 Urinmonovetten zurück erhalten?

- ❒ Ja Anzahl ____________________ (1)
- ❒ Nein (0)

IP2.4 Urinmonovetten abgegeben

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

IP2.5 Wenn nein, bitte begründen: ______________________________

IP2.6 Urinvolumen:

IP2.6.1 0h- Sammelvolumen (A) |__||__||__||__| ml

IP2.6.2 5h- Sammelvolumen (B) |__||__||__||__| ml

IP2.6.3 21h- Sammelvolumen (C) |__||__||__||__| ml

PEG

IP2.7 Erste Analysecodierung

IP2.7.1 A-Vorharn-PEG-Analysecodierung: |_______________|

IP2.7.2 B-5h-Sammelurin-PEG-Analysecodierung: |_______________|

IP2.7.3 C-21h-Sammelurin-PEG-Analysecodierung: |_______________|

IP2.8 Messung erfolgreich durchgeführt? ❒ Ja (1) ❒ Nein (0)

IP2.9 Wenn nein, bitte begründen: ____

Zuckerresorptionstest

IP2.10 Erste Analysecodierung

IP2.10.1 A-Vorharn-Zucker-Analysecodierung: |_______________|

IP2.10.2 B-5h-Sammelurin- Zucker-Analysecodierung: |_______________|

IP2.10.3 C-21h-Sammelurin-ZuckerAnalysecodierung: |_______________|

IP2.11 Messung erfolgreich durchgeführt? ❒ Ja (1) ❒ Nein (0)

IP2.12 Wenn nein, bitte begründen:____

Blutabnahme BA2

BA2.1 Blutabnahme erfolgreich durchgeführt?

❒ Ja am: __ __ / __ __ / __ __ __ __ (1)

❒ Nein (0)

BA2.2 Wenn nein, bitte begründen: ____

BA2.3 Uhrzeit: |__||__|:|__||__| Uhr

BA2.4 Besonderheiten (z.B. Entzündung der Einstichstelle, mehrere Einstiche zur Blutentnahme waren nötig)

❒ Ja (1)

❒ Nein (0)

BA2.5 Wenn ja, welche? ____

BA2.6 Serum-Röhrchen

❒ Ja (1)

❒ Nein (0)

BA2.7 EDTA-Plasma-Röhrchen

❒ Ja (1)

❒ Nein (0)

Fettbiopsie FB2

FB2.1 Biopsie erfolgreich durchgeführt?

❒ Ja am: __ __ / __ __ / __ __ __ __ (1)

❒ Nein (0)

FB2.2 Wenn nein, bitte begründen: ____

FB2.3 Besonderheiten (z.B. Entzündung der Einstichstelle)

❒ Ja (1)

❒ Nein (0)

FB2.4 Wenn ja, welche? ____

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

Aufwandsentschädigung

Name ______________________________

wohnhaft ______________________________

(Straße, PLZ, Ort)

hat an der **Studie „Effekt einer Gewichtsabnahme nach bariatrischer Operation auf die Darmbarrierefunktion und den Entzündungsstatus“** teilgenommen.

Die vereinbarte Aufwandsentschädigung von

□ 50,00 Euro für den ersten Darmbarrieretest,

□ 50,00 Euro für den zweiten Darmbarrieretest,

□ 100,00 Euro für die Fettbiopsie (6 Monate nach Operation),

□ sowie die Fahrtkosten von __________ EUR (0,30 EUR pro km)

sind auf die unten genannte Bankverbindung zu überweisen.

Auflistung Fahrtkosten:

von ______________________________

nach ______________________________

Anzahl Kilometer _______________

Kilometer x 0,30 Euro ______________

Wie oft ___________

Gesamtbetrag _______________

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

Konto-Inhaber: ______________________________

Konto-Nummer: ______________________________

BLZ: ______________________________

Kreditinstitut: ______________________________

BIC: ______________________________

IBAN: ______________________________

Bestätigung des/der Teilnehmers/in:

Mir ist bekannt, dass das Finanzamt von dieser Zahlung in Kenntnis gesetzt werden muss.

Ort, Datum ____________________ Unterschrift ____________________

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

Originaldaten wurden in CRF übertragen von

_____________________________ _____________________

Name Mitglied Studienteam Ort, Datum

CRF for controls

Case Report Form

Kontrollpersonen

Teilnehmernummer

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

Liste der verwendeten Abkürzungen

AP	Anthropometrische Parameter
BE	Befindlichkeit
BA	Blutabnahme
BIC	Bank Identifier Code
BLZ	Bankleitzahl
BMI	Body Mass Index
CH	Chirurg
EH	Ethnische Herkunft
EDTA	Ethylendiamintetraacetat
EP	Ernährungsprotokoll
FB	Fragebogen
FEV	Fettgewebeentnahme viszerales Fett
FES	Fettgewebeentnahme subkutanes Fett
FZ	Fäzesprobe
IBAN	International Bank Account Number
IP	Intestinale Permeabilität
KA	Kontaktaufnahme
M_r	relatives Molekulargewicht
PEG	Polyethylenglycole
S	Screening
V	Visite

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

Screening $_{S0}$

Daten von Krankenhaus bezogen

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

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

	JA	NEIN
S1 Drogen-/Alkoholprobleme		
S2 Erkrankungen Krebs; COPD; schwere kardiovaskuläre Erkrankung; Lebererkrankung (GOT, GPT > 3-fach der Norm); Nierenerkrankung (Kreatinin > 1,2 mg/dl); gastrointestinale, neurologische, rheumatische Erkrankungen; Zöliakie; akute Infektionen; Hypokaliämie; Autoimmunerkrankung; Malabsorptionssyndrom; autonome Neuropathie		
S3 Gallensteine		
S4 Stoffwechselerkrankungen S4.1 Diabetes S4.2 Insulin-pflichtig		
S5 Allergien: Lactuloseintoleranz, Sucraloseintoleranz, Saccaroseintoleranz, andere Nahrungsmittelintoleranzen, Kreuzallergien, andere Allergien z.B. Heuschnupfen		
S6 Frische Operation Darm-Operation, Coloskopie		
S7 Prothesen, Herzschrittmacher, Herzklappen, Herzschirmchen, Clips im Hirn oder Rückenmarkbereich, Gefäßstens, Glenkprothese, Metallsplitter, Osteosynthesematerial, Neurostimulator		
S8 Psychische Probleme wie Depressionen		
S9 Regelmäßige Medikamente Antibiotika, Immunsuppresiva, Laxantien, Anti-Durchfall-Medikamente, andere		

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

Visite 1 (V_1) Präoperativ

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

Kontaktaufnahme $_{KA1}$

KA1.1 Ich bin damit einverstanden, dass mich das Studienteam per Telefon oder Email während der Studie kontaktiert.

- ❑ Nein (0)
- ❑ Ja (1)

Anmerkungen:

____

KA1.2 Haben Sie während der letzten drei Monate an anderen klinischen Studien teilgenommen?

- ❑ Nein (0)
- ❑ Ja (1)

Anmerkungen:

____

Ethnische Herkunft $_{EH1}$

EH1.1 Welche ethnische Herkunft haben Sie?

- ❑ Weiß/Europäisch (1)
- ❑ Schwarz (2)
- ❑ Asiatisch, Orientalisch (3)
- ❑ andere__________________________________ (4)

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

Befindlichkeit $_{B1}$

BE1.1 Haben Sie in den letzten 7 Tagen unter folgenden Symptomen gelitten?

- ❑ Müdigkeit (1)
- ❑ verminderte Leistungsfähigkeit (2)
- ❑ Nervosität (3)
- ❑ Frieren (4)

BE1.2 Haben Sie regelmäßigen Stuhlgang?

- ❑ Täglich (1)
- ❑ ≥ 3x in der Woche (2)
- ❑ ≤3x in der Woche (3)
- ❑ unregelmäßig (4)

BE1.3 Haben Sie in den letzten 7 Tagen an Durchfall gelitten?

- ❑ Nein (0)
- ❑ ≤ 3x Durchfall in den letzten 7 Tagen (1)
- ❑ ≥ 3x Durchfall in den letzten 7 Tagen (2)

BE1.4 Haben Sie Ihre Menstruation regelmäßig?

- ❑ Ja (1)
- ❑ Nein (2)
- ❑ nicht mehr (3)
- ❑ ______________________________________ (4)

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

BE1.5 Besteht die Möglichkeit einer Schwangerschaft?

- ❑ Ja (1)
- ❑ Nein (0)

Anthropometrische Parameter AP1

Datum: __ __ / __ __ / __ __ __ __

AP1.1 Körpergröße:	|__||__||__|,|__| cm
AP1.2 Körpergewicht:	|__||__||__|,|__| kg
AP1.3 Body Mass Index (BMI):	|__||__|,|__| kg/m²
AP1.4 Taillenumfang:	|__||__||__|,|__| cm
AP1.5 Hüftumfang:	|__||__||__|,|__| cm
AP1.6 Taille-Hüft-Quotient:	|__|,|__||__||__|

AP1.7 Hat sich Ihr Gewicht in den letzten 3 Monaten verändert (um mehr als 2 kg)?

- ❑ Ja (1)
  Wie? ____
- ❑ Nein (0)

Fragebogen FB1

FB1.1 Fragebogen ausgegeben?

- ❒ Ja, am: __ __ / __ __ / __ __ __ __ (1)
- ❒ Nein (0)

FB1.2 Wenn nein, bitte begründen: ________________________________

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

FB1.3 Fragebogen ausgefüllt zurückerhalten?

- ❒ Ja, am: __ __ / __ __ / __ __ __ __ (1)
- ❒ Nein (0)

Ernährungsprotokoll EP1

EP1.1 Ernährungsprotokoll mit Anleitung ausgegeben?

- ❒ Ja, am: __ __ / __ __ / __ __ __ __ (1)
- ❒ Nein (0)

EP1.2 Wenn nein, bitte begründen: ________________________________

EP1.3 Ernährungsprotokoll ausgefüllt zurückerhalten?

- ❒ Ja, am: __ __ / __ __ / __ __ __ __ (1)
- ❒ Nein (0)

Fäzesprobe FZ1

FZ1.1 Utensilien für die Fäzesprobe ausgegeben?

- ❒ Ja, am: __ __ / __ __ / __ __ __ __ (1)
- ❒ Stuhlbehältnis
- ❒ 3 Stuhlröhrchen
- ❒ Nein (0)

FZ1.2 Wenn nein, bitte begründen: ________________________________

FZ1.3 Analysecodierung: |____________________|

FZ1.4 Fäzesprobe zurück erhalten?

- ❒ Ja Anzahl ________ (1)
- ❒ Nein (0)

FZ1.5 Wenn nein, bitte begründen: ________________________________

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

FZ1.6 Stuhlröhrchen befüllt
Datum: __ __ / __ __ / __ __ __ __ Uhrzeit: |__||__| : |__||__| Uhr

FZ1.7 Stuhlröhrchen abgegeben
Datum: __ __ / __ __ / __ __ __ __ Uhrzeit: |__||__| : |__||__| Uhr

Intestinale Permeabilität $_{IP1}$

IP1.1 Utensilien für den PEG-Test/ Zuckerresorptionstest ausgegeben?

- ❑ 1x 500ml Behältnis
- ❑ 3x 3l Behältnisse
- ❑ Zuckerlösung mit Kapseln
- ❑ 100ml PEG-Lösung
- ❑ 6x10ml Urinmonovetten
- ❐ Ja, am: __ __ / __ __ / __ __ __ __ (1)
- ❐ Nein (0)

IP1.2 Wenn nein, bitte begründen: ______________________________

IP1.3 Urinmonovetten zurück erhalten?

- ❐ Ja Anzahl ________________ (1)
- ❐ Nein (0)

IP1.4 Urinmonovetten abgegeben

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

IP1.5 Wenn nein, bitte begründen: ______________________________

IP1.6 Urinvolumen:

IP1.6.1 0h- Sammelvolumen (A) |__||__||__||__| ml

IP1.6.2 5h- Sammelvolumen (B) |__||__||__||__| ml

IP1.6.3 21h- Sammelvolumen (C) |__||__||__||__| ml

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

PEG

IP1.7 Erste Analysecodierung

IP1.7.1 A-Vorharn-PEG-Analysecodierung: |______________|

IP1.7.2 B-5h-Sammelurin-PEG-Analysecodierung: |______________|

IP1.7.3 C-21h-Sammelurin-PEG-Analysecodierung: |______________|

IP1.8 Messung erfolgreich durchgeführt? ❐ Ja (1) ❐ Nein (0)

IP1.9 Wenn nein, bitte begründen: ______________________________

Zuckerresorptionstest

IP1.10 Erste Analysecodierung

IP1.10.1 A-Vorharn-Zucker-Analysecodierung: |______________|

IP1.10.2 B-5h-Sammelurin- Zucker-Analysecodierung: |______________|

IP1.10.3 C-21h-Sammelurin-ZuckerAnalysecodierung: |______________|

IP1.11 Messung erfolgreich durchgeführt? ❐ Ja (1) ❐ Nein (0)

IP1.12 Wenn nein, bitte begründen:______________________________

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

Operation

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

Operation: ____

Blutabnahme $_{BA1}$

BA1.1 Blutabnahme erfolgreich durchgeführt?

❒ Ja (1)

❒ Nein (0)

BA1.2 Wenn nein, bitte begründen: ______________________________

BA1.3 Uhrzeit: |__||__|:|__||__| Uhr

BA1.4 Besonderheiten (z.B. Entzündung der Einstichstelle, mehrere Einstiche zur Blutentnahme waren nötig)

❒ Ja (1)

❒ Nein (0)

BA1.5 Wenn ja, welche? ______________________________

BA1.6 Serum-Röhrchen

❒ Ja (1)

❒ Nein (0)

BA1.7 EDTA-Plasma-Röhrchen

❒ Ja (1)

❒ Nein (0)

Chirurg $_{CH1}$

CH1.1 Name Chirurg

____

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

Fettgewebeentnahme viszerales Fett $_{FEV1}$

FEV1.1 Entnahme von viszeralem Fett erfolgreich durchgeführt?

❒ Ja, um |__||__|:|__||__| Uhr (1)

❒ Nein (0)

FEV1.2 Wenn nein, bitte begründen: ______________________________

FEV1.3 Besonderheiten

❒ Ja (1)

❒ Nein (0)

FEV1.4 Wenn ja, welche? ______________________________

Fettgewebeentnahme subkutanes Fett $_{FES2}$

FES1.1 Entnahme von subkutanem Fett erfolgreich durchgeführt?

❒ Ja, um |__||__|:|__||__| Uhr (1)

❒ Nein (0)

FES1.2 Wenn nein, bitte begründen: ______________________________

FES1.3 Besonderheiten

❒ Ja (1)

❒ Nein (0)

FES1.4 Wenn ja, welche? ______________________________

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

Aufwandsentschädigung

Name ____

wohnhaft ____

(Straße, PLZ, Ort)

hat an der **Studie „Effekt einer Gewichtsabnahme nach bariatrischer Operation auf die Darmbarrierefunktion und den Entzündungsstatus“** teilgenommen.

Die vereinbarte Aufwandsentschädigung von

□ 50,00 Euro für den Darmbarrieretest,

□ sowie die Fahrtkosten von __________ EUR (0,30 EUR pro km)

sind auf die unten genannte Bankverbindung zu überweisen.

Auflistung Fahrtkosten:

von ____________________________________

nach ____

Anzahl Kilometer _______________

Kilometer x 0,30 Euro ______________

Wie oft ___________

Gesamtbetrag _______________

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

Konto-Inhaber: ____

Konto-Nummer: ____

BLZ: ____

Kreditinstitut: ____

BIC: ____

IBAN: ____

<u>Bestätigung des/der Teilnehmers/in:</u>

Mir ist bekannt, dass das Finanzamt von dieser Zahlung in Kenntnis gesetzt werden muss.

______________________ __________________________

Ort, Datum Unterschrift

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

Originaldaten wurden in CRF übertragen von

________________________________ ________________________

Name Ort, Datum

Questionnaire for obese study participants at V1

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

GRK 1482

Patienten Fragebogen

Datum: ____/____/_______

Sehr geehrter Studienteilnehmer,

bitte füllen Sie den Fragebogen gewissenhaft und so genau wie möglich aus. Ihre Daten werden anonymisiert erfasst (ohne Verbindung zu persönlichen Daten wie Name, Geburtsdatum oder Adresse).

Ethnische Herkunft

- Welche ethnische Herkunft haben Sie?

❑ Weiß/Europäisch (1)
❑ Schwarz (2)
❑ Asiatisch, Orientalisch (3)
❑ andere____ (4)

Befindlichkeit

- Haben Sie in den letzten 7 Tagen unter folgenden Symptomen gelitten?

❑ Müdigkeit (1)
❑ verminderte Leistungsfähigkeit (2)
❑ Nervosität (3)
❑ Frieren (4)

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

- Haben Sie regelmäßigen Stuhlgang?

❑ Täglich (1)
❑ ≥ 3x in der Woche (2)
❑ ≤3x in der Woche (3)
❑ Unregelmäßig (4)

- Haben Sie in den letzten 7 Tagen an Durchfall gelitten?

❑ Nein (0)
❑ ≤ 3x Durchfall in den letzten 7 Tagen (1)
❑ ≥ 3x Durchfall in den letzten 7 Tagen (2)

- Haben Sie Ihre Menstruation regelmäßig?

❑ Ja (1)
❑ Nein (2)
❑ nicht mehr (3)
❑ ____ (4)

- Besteht die Möglichkeit einer Schwangerschaft?

❑ Ja (1)
❑ Nein (0)

Gewichtsverlauf

- Hat sich Ihr Gewicht in den letzten 3 Monaten verändert (um mehr als 2 kg)?

❑ Ja (1)
Wie? ____
❑ Nein (0)

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

Medikamente

- Welche Medikamente, Vitamine oder Nahrungsergänzungsmittel nehmen Sie regelmäßig zu sich? Bitte geben Sie den genauen Namen des Medikaments, sowie die Dosis und die Häufigkeit der Einnahme an (z.B. L-Thyroxin, morgens, 25 µg).

  ____
  ____
  ____
  ____
  ____

- Welche Medikamente, Vitamine oder Nahrungsergänzungsmittel nehmen Sie nur bei Bedarf zu sich?

  ____
  ____
  ____
  ____

Lebensstil

- Wie oft trinken Sie alkoholische Getränke?
  - ❑ Nie / selten (0)
  - ❑ Weniger als ½ Flasche Bier oder 1/8 l Wein am Tag (1)
  - ❑ 1 Flasche Bier oder ¼ l Wein am Tag (2)
  - ❑ Mehr als 1 Flasche Bier oder ¼ l Wein am Tag (Menge:______________) (3)

- Rauchen Sie?
  - ❑ Nie geraucht (0)
  - ❑ Nichtraucher – Seit _________ (Jahr) (1)
  - ❑ Ja, bis 10 Zigaretten am Tag (2)
  - ❑ Ja, mehr als 10 Zigaretten am Tag (3)

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

- Betreiben Sie Sport?
  - ❑ Nein (4)
  - ❑ Selten und unregelmäßig (3)
  - ❑ 1 mal pro Woche (2)
  - ❑ mehrmals pro Woche (1)
  - ❑ Täglich (0)

- Welche Sportarten betreiben Sie?
  - ❑ Schwimmen (a)
  - ❑ Spazierengehen, Walking (b)
  - ❑ Joggen (c)
  - ❑ Ballsport (z.B. Fußball, Basketball, Tennis…) (d)
  - ❑ Fitness, Kraftsport (e)
  - ❑ Sonstige:__________________ (f)

Sollten Fragen oder Unsicherheiten auftreten, stehen wir Ihnen jederzeit gern zur Verfügung!

Teresa Kellerer, M.Sc. Ernährungswissenschaften
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mailto: teresa.kellerer@tum.de

Questionnaire for obese study participants at V2

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

Patienten Fragebogen

Datum: ____/____/_______

Sehr geehrter Studienteilnehmer,

bitte füllen Sie den Fragebogen gewissenhaft und so genau wie möglich aus. Ihre Daten werden anonymisiert erfasst (ohne Verbindung zu persönlichen Daten wie Name, Geburtsdatum oder Adresse).

Befindlichkeit

- Haben Sie in den letzten 7 Tagen unter folgenden Symptomen gelitten?

❑ Müdigkeit (1)
❑ verminderte Leistungsfähigkeit (2)
❑ Nervosität (3)
❑ Frieren(4)

- Haben Sie regelmäßigen Stuhlgang?

❑ Täglich (1)
❑ ≥ 3x in der Woche (2)
❑ ≤3x in der Woche (3)
❑ Unregelmäßig (4)

Seite 1 von 4
12.02.2015

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

- Haben Sie in den letzten 7 Tagen an Durchfall gelitten?

❑ Nein (0)
❑ ≤ 3x Durchfall in den letzten 7 Tagen (1)
❑ ≥ 3x Durchfall in den letzten 7 Tagen (2)

- Haben Sie Ihre Menstruation regelmäßig?

❑ Ja (1)
❑ Nein (2)
❑ nicht mehr (3)
❑ ____________________________________ (4)

- Besteht die Möglichkeit einer Schwangerschaft?

❑ Ja (1)
❑ Nein (0)

Erkrankungen

- Sind bei Ihnen seit der Operation neue Erkrankungen aufgetreten?

____
____
____
____

- Haben sich bei Ihnen bestehende Krankheiten seit der Operation verbessert?

____
____
____
____

Seite 2 von 4
12.02.2015

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

Medikamente

- Welche Medikamente, Vitamine oder Nahrungsergänzungsmittel nehmen Sie regelmäßig zu sich? Bitte geben Sie den genauen Namen des Medikaments, sowie die Dosis und die Häufigkeit der Einnahme an (z.B. L-Thyroxin, morgens, 25 µg).

____
____
____
____
____

- Welche Medikamente, Vitamine oder Nahrungsergänzungsmittel nehmen Sie nur bei Bedarf zu sich?

____
____
____
____

Lebensstil

- Wie oft trinken Sie alkoholische Getränke?

❑ Nie / selten (0)
❑ Weniger als ½ Flasche Bier oder 1/8 l Wein am Tag (1)
❑ 1 Flasche Bier oder ¼ l Wein am Tag (2)
❑ Mehr als 1 Flasche Bier oder ¼ l Wein am Tag (Menge:_____________) (3)

- Rauchen Sie?

❑ Nie geraucht (0)
❑ Nichtraucher – Seit _________ (Jahr) (1)
❑ Ja, bis 10 Zigaretten am Tag (2)
❑ Ja, mehr als 10 Zigaretten am Tag (3)

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

- Betreiben Sie Sport?

❑ Nein (4)
❑ Selten und unregelmäßig (3)
❑ 1 mal pro Woche (2)
❑ mehrmals pro Woche (1)
❑ Täglich (0)

- Welche Sportarten betreiben Sie?

❑ Schwimmen (a)
❑ Spazierengehen, Walking (b)
❑ Joggen (c)
❑ Ballsport (z.B. Fußball, Basketball, Tennis…) (d)
❑ Fitness, Kraftsport (e)
❑ Sonstige:_________________ (f)

Sollten Fragen oder Unsicherheiten auftreten, stehen wir Ihnen jederzeit gern zur Verfügung!

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Questionnaire for controls

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

GRK 1482

Patienten Fragebogen

Datum: ____/____/_______

Sehr geehrter Studienteilnehmer,

bitte nehmen Sie sich ein paar Minuten Zeit und füllen Sie den Fragebogen gewissenhaft und so genau wie möglich aus. Ihre Daten werden anonymisiert erfasst (ohne Verbindung zu persönlichen Daten wie Name, Geburtsdatum oder Adresse).

Ethnische Herkunft

- Welche ethnische Herkunft haben Sie?

❑ Weiß/Europäisch (1)
❑ Schwarz (2)
❑ Asiatisch, Orientalisch (3)
❑ andere____ (4)

Befindlichkeit

- Haben Sie in den letzten 7 Tagen unter folgenden Symptomen gelitten?

❑ Müdigkeit (1)
❑ verminderte Leistungsfähigkeit (2)
❑ Nervosität (3)
❑ Frieren (4)

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

- Haben Sie regelmäßigen Stuhlgang?

❑ Täglich (1)
❑ ≥ 3x in der Woche (2)
❑ ≤3x in der Woche (3)
❑ unregelmäßig (4)

- Haben Sie in den letzten 7 Tagen an Durchfall gelitten?

❑ Nein (0)
❑ ≤ 3x Durchfall in den letzten 7 Tagen (1)
❑ ≥ 3x Durchfall in den letzten 7 Tagen (2)

- Haben Sie Ihre Menstruation regelmäßig?

❑ Ja (1)
❑ Nein (2)
❑ nicht mehr (3)
❑ ____ (4)

- Besteht die Möglichkeit einer Schwangerschaft?

❑ Ja (1)
❑ Nein (0)

Anthropometrie

- Hat sich Ihr Gewicht in den letzten 3 Monaten verändert (um mehr als 2 kg)?

❑ Ja (1)
Wie? ____
❑ Nein (0)

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

- Kommt Übergewicht bei Ihren Eltern vor?
  - ❑ Ja (2)
  - ❑ Nein (0)
  - ❑ Teilweise (1)
- Ist Ihr Kind / Sind Ihre Kinder übergewichtig?
  - ❑ Ja (2)
  - ❑ Nein (0)
  - ❑ Teilweise (1)

Erkrankungen

- Wurden bei Ihnen Gallensteine diagnostiziert?
  - ❑ Nein (0)
  - ❑ Ja Wann:____________ (1)
    Entfernt? ____________ (a/b)
- Leiden Sie an Bluthochdruck?
  - ❑ Nein (0)
  - ❑ Ja
    - ❑ Nicht medikamentös behandelt (1b)
    - ❑ Medikamentös behandelt (1a)
- Leiden Sie an Erkrankungen des Herzens (Verengung der Herzkranzgefäße, Herzmuskelschwäche, Herzrhythmusstörungen, etc.)?
  - ❑ Nein (0)
  - ❑ Ja _________________ (1)
    - ❑ Medikamentös behandelt/untersucht mit _________________ (a)
    - ❑ Nicht medikamentös behandelt (b)

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

- Haben Sie orthopädische Probleme?
  - ❑ Nein (0)
  - ❑ Ja
    - ❑ Nicht medikamentös behandelt (1b)
    - ❑ Medikamentös behandelt (1a)
- Wurden bei Ihnen hohe Blutfettwerte (Cholesterin, LDL, VLDL, TG) diagnostiziert?
  - ❑ Nein (0)
  - ❑ Ja
    - ❑ Nicht medikamentös behandelt (1b)
    - ❑ Medikamentös behandelt (1a)
- Leiden Sie an Diabetes?
  - ❑ Nein (0)
  - ❑ Ja
    - ❑ Typ 1 (1)
    - ❑ Typ 2 (2)
    - ❑ Nicht medikamentös behandelt (b)
    - ❑ Medikamentös behandelt seit _________ (a1)
    - ❑ mit Insulin behandelt seit ____________ (a2)
    - ❑ Blutzuckertagebuch wird geführt (a3)
    - ❑ Sonstiges ________________________ (a4)
- Haben Sie schon einmal einen Schlaganfall erlitten?
  - ❑ Nein (0)
  - ❑ Ja, wann ______________ (1)
- Wurden Sie schon einmal operiert?
  - ❑ Nein (0)
  - ❑ Ja (Wann, welche Operation?) (1)

    ____

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

____

____

- Sind bei Ihnen Allergien auf Nahrungsmittel, Medikamente, Sonstiges bekannt?
  - ❑ Nein (0)
  - ❑ Ja, folgende: (1)

____

____

____

____

Medikamente

- Welche Medikamente, Vitamine oder Nahrungsergänzungsmittel nehmen Sie regelmäßig zu sich? Bitte geben Sie den genauen Namen des Medikaments, sowie die Dosis und die Häufigkeit der Einnahme an (z.B. L-Thyroxin, morgens, 25 µg).

____

____

____

____

____

- Welche Medikamente, Vitamine oder Nahrungsergänzungsmittel nehmen Sie nur bei Bedarf zu sich?

____

____

____

____

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

Lebensstil

- Wie oft trinken Sie alkoholische Getränke?
  - ❑ Nie / selten (0)
  - ❑ Weniger als ½ Flasche Bier oder 1/8 l Wein am Tag (1)
  - ❑ 1 Flasche Bier oder ¼ l Wein am Tag (2)
  - ❑ Mehr als 1 Flasche Bier oder ¼ l Wein am Tag (Menge:______________) (3)
- Rauchen Sie?
  - ❑ Nie geraucht (0)
  - ❑ Nichtraucher – Seit _________ (Jahr) (1)
  - ❑ Ja, bis 10 Zigaretten am Tag (2)
  - ❑ Ja, mehr als 10 Zigaretten am Tag (3)
- Betreiben Sie Sport?
  - ❑ Nein (4)
  - ❑ Selten und unregelmäßig (3)
  - ❑ 1 mal pro Woche (2)
  - ❑ mehrmals pro Woche (1)
  - ❑ Täglich (0)
- Welche Sportarten betreiben Sie?
  - ❑ Schwimmen (a)
  - ❑ Spazierengehen, Walking (b)
  - ❑ Joggen (c)
  - ❑ Ballsport (z.B. Fußball, Basketball, Tennis…) (d)
  - ❑ Fitness, Kraftsport (e)
  - ❑ Sonstige:__________________ (f)

Sollten Fragen oder Unsicherheiten auftreten, stehen wir Ihnen jederzeit gern zur Verfügung!

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Dietary protocol and instruction

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

GRK 1482

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!

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

Ernährungsprotokoll 1. Tag

Wochentag:_________________ Datum: __ __ / __ __ / __ __ __ __

Frühstück / Zwischenmahlzeit:

Zeit / Ort	Menge	Lebensmittel/Getränk	Zubereitung

Mittagessen / Zwischenmahlzeit:

Zeit / Ort	Menge	Lebensmittel/Getränk	Zubereitung

Abendessen / Spätmahlzeit:

Zeit / Ort	Menge	Lebensmittel/Getränk	Zubereitung

Instruction manual for faecal sampling

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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 bitte folgende Hinweise:

- Sammeln Sie die Stuhlprobe 1 oder 2 Tage bevor Sie zur Operation in das Krankenhaus fahren und die Proben dort abgeben. Bitte Stuhlprobe nicht am Tag des Darmbarrieretests sammeln.

  ➔ Achtung: ein 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.

- Nehmen Sie ein **Stuhlröhrchen** und 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. Befüllen sie bitte alle 3 Röhrchen.

- Das **Röhrchen, welches die Flüssigkeit enthält** bitte nach dem Befüllen und Verschließen gut **schütteln** bis der Inhalt gut gemischt ist.

- Bitte **lagern** Sie die **Probenröhrchen ohne Flüssigkeit im Tiefkühlfach** und das **Röhrchen mit Flüssigkeit bei Raumtemperatur, geschützt vor Licht.**

- Bringen Sie bitte die Probenröhrchen in das Krankenhaus mit, **die tiefgekühlten in einer Kühlbox mit Kühlakkus**.

- Bitte notieren Sie, wann Sie die Probenröhrchen gefüllt haben
  Datum: __ __ / __ __ / __ __ __ __ Uhrzeit: |__||__| : |__||__| Uhr

- Bitte notieren Sie, wann Sie die Probenröhrchen abgegeben haben.
  Datum: __ __ / __ __ / __ __ __ __ Uhrzeit: |__||__| : |__||__| Uhr

04.02.2015

Sollten Fragen oder Unsicherheiten auftreten, stehen wir Ihnen jederzeit gern zur Verfügung!

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04.02.2015

Instruction manual for gut barrier test

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

Anleitung zur Durchführung des Darmbarrieretests

Sehr geehrter Studienteilnehmer,

bitte beachten Sie folgende Hinweise:

- 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 ______________________________

Bitte notieren Sie sich die Flüssigkeitszufuhr am Tag der Testdurchführung

Flüssigkeitszufuhr

Morgens	
Mittags	
Abends	

Materialien

Für den Darmbarrieretest bekommen Sie von Ihrem Studienteam folgende Materialien:

- eine Testlösung mit den Zuckern Laktulose, Mannitol und Saccharose zum Trinken, sowie sechs Kapseln mit Sucralose zum Einnehmen.
- 1 kleine Plastikflasche A zum Sammeln des ersten Urins nach dem Aufstehen
- 3 große Plastikflaschen (1xB und 2xC) zum Sammeln allen weiteren Urins bis Testende
- 6 Urin-Röhrchen zum Entnehmen des Urins
- 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 Urin in **Flasche A** und verschließen Sie die Flasche.
   ➔ **Notieren** Sie sich die **Urinmenge** der Flasche A.

   Sammelurinmenge Flasche A: _ _ _ _ ml

   ➔ **DANACH entnehmen Sie 2x10 ml Urin von Flasche A mittels der 2 Urin-Röhrchen** (siehe Anleitung).
   ➔ **Lagern** Sie die beschrifteten Urin-Röhrchen 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. Bitte schreiben Sie Datum und Uhrzeit auf, wann Sie die Zuckerlösung und die Kapseln eingenommen haben.

   Datum: _ _ / _ _ / _ _ _ _ Uhrzeit: _ _ : _ _ Uhr

   Hinweise für die nächsten 2 Stunden:
   - **NICHTS** trinken und **NICHTS** essen, auch **KEINEN** Kaugummi!
   - die Zähne **NICHT** putzen
   - **KEINE** Medikamente einnehmen
   - den gesamten Urin 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 Urin in der großen **Plastikflasche B** sammeln!
   - Sammelurin bitte **im Kühlschrank** senkrecht aufbewahren!

4. **3 Stunden später bzw. insgesamt 5 Stunden** nach Trinken der **Zuckertestlösung** und Einnahme der 6 Kapseln
   ➔ Sammeln Sie **den letzten Urin in die Flasche B.**
   ➔ **DANACH notieren** Sie sich die **Urinmenge** der Flasche B.

   Datum: _ _ / _ _ / _ _ _ _ Uhrzeit: _ _ : _ _ Uhr

   Sammelurinmenge Flasche B: _ _ _ _ ml

➔ **DANACH entnehmen Sie 2x10 ml Urin von Flasche B mittels der 2 Urin-Röhrchen** (siehe Anleitung).
➔ **Lagern** Sie die beschrifteten Urin-Röhrchen im verschließbaren Kunststoffbeutel in der Tiefkühltruhe.
➔ Nun dürfen Sie essen und trinken und Ihre Medikamente einnehmen!

5. Während der **darauffolgenden 21 Stunden** sammeln Sie allen anfallenden Urin in **Flasche C**. Verschließen Sie Flasche C gut und stellen sie ebenfalls in den Kühlschrank. Falls eine Flasche C nicht reicht, so verwenden Sie bitte die zweite Flasche C.

6. Nach den 21h Stunden sammeln Sie den letzten Urin in **Flasche C.**
   ➔ **Notieren** Sie sich die **Urinmenge** der Flasche C.

   Datum: _ _ / _ _ / _ _ _ _ Uhrzeit: _ _ : _ _ Uhr

   Sammelurinmenge Flasche C: _ _ _ _ ml

   ➔ **DANACH entnehmen Sie 2x 10 ml Urin von Flasche C mittels der 2 Urin-Röhrchen** (siehe Anleitung).
   ➔ **Lagern** Sie die beschrifteten Urin-röhrchen im verschließbaren Kunststoffbeutel in der Tiefkühltruhe.

7. Bringen Sie den verschließbaren Kunststoffbeutel mit den beschrifteten, tiefgekühlten Urin-Röhrchen und diese ausgefüllte Anleitung bei Ihrem nächsten Termin in der Klinik mit.

   Abgabe der Urinbehältnisse:

   Datum: _ _ / _ _ / _ _ _ _ Uhrzeit: _ _ : _ _ Uhr

   Haben Sie den ganzen Urin gesammelt:

   ❒ Ja ❒ Nein

   Sind Durchfälle während der ersten 5 Stunden nach Testbeginn aufgetreten?

   ❒ Ja ❒ Nein

   Hatten Sie Nebenwirkungen während des Testtages?

   ❒ Übelkeit
   ❒ Blähungen, Bauchschmerzen
   ❒ Durchfall
   ❒ Sonstiges ____________________

Sollten Fragen oder Unsicherheiten auftreten, stehen wir Ihnen jederzeit gern zur Verfügung!

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Anleitung zum Sammeln von Urin im Überblick

Urinsammelschema

Sammelphase	Flasche	Sammel-Stunden	Uhrzeit	Sammelurinmenge
Morgen-Urin	A	0		
Haupt-Urin	B	5		
Haupt-Urin	C	21		

Anleitung für das Abfüllen des Urins

- Während des Sammelns von Urin bewahren Sie bitte den Sammelurin **im Kühlschrank** senkrecht auf.
- Bitte entleeren Sie am Ende jeder Sammelphase nochmals Ihre Blase.
- Bitte notieren Sie sich das Urinvolumen der Sammelflasche auf den Urin-Röhrchen und im Urinsammelschema (siehe oben).

  ➔ **Ohne Urinvolumina ist keine quantitative Analyse möglich**

- **Mischen** Sie die Flasche vor Entnehmen des Urins!
- Versuchen Sie den Urin von der Mitte der Flasche mittels Urin-Röhrchen zu entnehmen.

  ➔ Wenn zwei C-Flaschen verwendet wurden, dann bitte den Urin aus beiden C-Flaschen in einen sauberen Eimer zusammenschütten und mit einem sauberen Gegenstand gründlich mischen. Aus diesem Urin-Gemisch befüllen Sie die Urin-Röhrchen.

- **Bitte beschriften** Sie die gelben Urin-Röhrchen mit Sammelurinmenge in ml.
- Am Ende haben sie von Flasche A, B, C je **zwei** gelbe Urin-Röhrchen, welche mit Fraktion und Sammelurinmenge beschriftet sind.
- GESCHAFFT - Bitte lagern Sie die Röhrchen im verschließbaren Kunststoffbeutel im Tiefkühlfach.

VIELEN DANK !!!!!!