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Human Dietary Interventions: Investigating the Interactions between Metabolism, Gut Microbiome, and Microbiome-Derived Metabolites for Optimized Health

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SUMMARY

Diet is a key factor in modulating the gut microbiome, which plays a pivotal role in human health. Tailored microbiome-targeted dietary strategies are thus a promising approach for the primary and secondary prevention of lifestyle-associated diseases linked to microbial dysbiosis. However, the underlying mechanisms are poorly understood. The objective of this thesis was therefore to investigate the interactions between metabolism, the gut microbiome, and microbiome-derived metabolites in the context of two dietary interventions to optimize human health.

To achieve this, two different oat interventions were conducted in a randomized controlled prospective parallel-design study in individuals with metabolic syndrome (*n* = 68). Participants were assigned either to a short-term, hypocaloric, high-dose oat diet (modified 'oat cure' according to Carl von Noorden: 3 x 100 g oats/day for 2 days) or a six-week, isocaloric, moderate oat diet (1 x 80 g oats/day for 6 weeks). These interventions were compared to an oat-free, macronutrient-adapted control diet or the participant's habitual Western diet. Blood and fecal samples were collected before, during and after each intervention period to determine the diet-induced effects on metabolism, gut microbiome composition and functional capacity, as well as metabolomic profiles, particularly ferulic and dihydroferulic acids ([DH]FA) in plasma, and to elucidate the underlying mechanisms using an integrative multi-omics analysis.

It was shown that a short-term, high-dose and, to a lesser extent, a six-week, moderate oat diet led to a significant increase in circulating (microbially produced) phenolic metabolites, including (DH)FA. Along with shifts in the gut microbiome and global metabolomic profile, these phenolic metabolites were identified as potential driving factors for the oat-induced reduction in serum total cholesterol and low-density lipoprotein cholesterol levels upon the short-term, high-dose oat diet. Moreover, a decrease in gut permeability, observed after the short-term oat diet compared to baseline, was associated with an increase in circulating short-chain fatty acids. The baseline gut microbiome composition was identified as a potential modulator of this response. The six-week, moderate oat diet stabilized metabolic markers and had attenuated effects on the gut microbiome and metabolomic profiles.

In conclusion, the findings of this thesis contribute significantly to the understanding of the complex host-diet-microbiome interaction, emphasizing its key role in human health. Microbiome-derived metabolites, in particular phenolic compounds, have been identified as important mediators in the interplay between gut microbes and the host, offering new therapeutic strategies for the treatment of lifestyle-associated diseases. This is of great potential since dietary strategies such as a short-term, high-dose oat-diet are a suitable and rapid approach to alleviate obesity-related lipid disorders and to reduce intestinal permeability. However, further intervention studies in different populations are needed to verify these results and address the challenge of inter-individual differences in the response to dietary interventions.

ZUSAMMENFASSUNG

Die Ernährung ist ein entscheidender Faktor zur Modulation des Darmmikrobioms, das eine zentrale Rolle für die humane Gesundheit spielt. Maßgeschneiderte, auf das Mikrobiom ausgerichtete Ernährungsstrategien gelten daher als vielversprechender Ansatz für die Primär- und Sekundärprävention lebensstilbedingter Erkrankungen, die mit einer mikrobiellen Dysbiose zusammenhängen. Die zugrunde liegenden Mechanismen sind bislang jedoch nur unzureichend verstanden. Ziel dieser Arbeit ist es daher, die Wechselwirkungen zwischen Stoffwechsel, Darmmikrobiom und mikrobiell produzierten Metaboliten im Kontext zwei verschiedener Ernährungsinterventionen zur Verbesserung der humanen Gesundheit zu untersuchen.

Um dies zu erreichen, wurden zwei verschiedene Haferinterventionen in einem randomisierten, kontrollierten, prospektiven Paralleldesign bei Personen mit metabolischem Syndrom (*n* = 68) durchgeführt. Die Teilnehmer wurden entweder einer kurzfristigen, hypokalorischen, hochdosierten Haferdiät (modifizierte „Haferkur“ nach Carl von Noorden: 3 x 100 g Hafer/Tag für 2 Tage) oder einer sechswöchigen, isokalorischen, moderaten Haferintervention (1 x 80 g Hafer/Tag für 6 Wochen) zugeteilt. Diese Interventionen wurden mit einer haferfreien Kontrolldiät, die an die Makronährstoffzufuhr der Haferdiät angepasst war, oder der habituellen westlichen Ernährung der Teilnehmer verglichen.

Vor, während und nach jedem Interventionszeitraum wurden Blut- und Stuhlproben entnommen, um die ernährungsbedingten Auswirkungen auf den Stoffwechsel, die Zusammensetzung und Funktionen des Darmmikrobioms sowie die Metaboliten-Profile, insbesondere Ferulasäure und Dihydroferulasäure ([DH]FA) im Plasma, zu bestimmen und die zugrunde liegenden Mechanismen mithilfe einer integrativen Multi-Omics-Analyse aufzuklären.

Es konnte gezeigt werden, dass eine kurzzeitige, hochdosierte und in geringerem Maße auch eine sechswöchige, moderate Haferdiät zu einem signifikanten Anstieg (mikrobiell produzierter) phenolischer Metabolite, einschließlich (DH)FA, im Blut führte. Zusammen mit Veränderungen im Darmmikrobiom und dem globalen Metaboliten-Profil wurden diese phenolischen Verbindungen als potenziell treibende Faktoren für die haferinduzierte Reduktion des Gesamt- und Low-Density-

Lipoprotein-Cholesterins im Serum in der kurzfristigen, hochdosierten Haferkur identifiziert. Darüber hinaus war eine Abnahme der Darmpermeabilität, beobachtet nach der kurzfristigen, hochdosierten Haferdiät im Vergleich zum Ausgangswert, mit einem Anstieg der kurzkettigen Fettsäuren im Blut verbunden. Das Darmmikrobiom zu Beginn der Intervention wurde als potenzieller Modulator dieser Reaktion identifiziert. Die sechswöchige, moderate Haferintervention stabilisierte die Stoffwechselmarker und hatte abgeschwächte Auswirkungen auf das Darmmikrobiom und die Metaboliten-Profile.

Zusammengefasst tragen die Ergebnisse dieser Arbeit wesentlich zum Verständnis der komplexen Interaktion zwischen Wirt, Ernährung und Mikrobiom bei und unterstreichen deren zentrale Rolle für die humane Gesundheit. Mikrobiell produzierte Metabolite, insbesondere phenolische Verbindungen, konnten als wichtige Mediatoren im Zusammenspiel zwischen Darmmikroben und Wirt identifiziert werden und bieten neue therapeutische Ansätze für die Behandlung von lebensstilbedingten Erkrankungen. Dies hat großes Potenzial, da diätetische Strategien wie eine kurzfristige, hochdosierte Haferkur einen geeigneten und schnellen Ansatz darstellen, um Adipositas-bedingte Fettstoffwechselstörungen zu lindern und die Darmpermeabilität zu verringern. Weitere Interventionsstudien in verschiedenen Bevölkerungsgruppen sind jedoch erforderlich, um diese Ergebnisse zu verifizieren und die Herausforderung inter-individueller Reaktionen auf Ernährungsinterventionen gezielt zu adressieren.

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- **Klümpen L**, Mantri A, Seel W, Stoffel-Wagner B, Coenen M, Schmid M, Weinhold L, Stehle P, Simon MC. Reduction of gut permeability induced by calorie-restricted oat diet is associated with short-chain fatty acid response. *Gut Microbes.* (*in revision*; manuscript-ID: 257773052)
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- Márquez Campos E, **Jakobs L**, Simon MC. Antidiabetic effects of flavan-3-ols and their microbial metabolites. *Nutrients*;12(6):1592 (2020). DOI: https://doi.org/10.3390/nu12061592

Contributions to Scientific Conferences (Abstracts)

- **Klümpen L,** Mantri A, Seel W, Stoffel-Wagner B, Coenen M, Schmid M, Weinhold L, Krawitz P, Stehle P, Simon MC. Oat-induced changes in serum zonulin are associated with plasma short-chain fatty acid responses in adults with metabolic syndrome. 61st Scientific Congress of the German Nutrition Society, 04. – 06.03.2024, Kassel. Talk. *Proc Germ Nutr Soc*;30:51 (2024)
- **Klümpen L,** Mantri A, Seel W, Stoffel-Wagner B, Coenen M, Nöthen M, Schmid M, Weinhold L, Krawitz P, Stehle P, Simon MC. A 2-day intensive oat intake beneficially alters the metabolism and gut microbiome in subjects with metabolic syndrome. 60th Scientific Congress of the German Nutrition Society, 15. – 17.03.2023, Bonn. Talk. *Proc Germ Nutr Soc*;29:10-11 (2023)
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LIST OF ABBREVIATIONS

ALT	alanine aminotransferase
ApoE	apolipoprotein E
AST	aspartate aminotransferase
ASVs	amplicon sequencing variants
AUC	area under the curve
AVAs	avenanthramides
Bca	bias corrected and accelerated bootstrapping method
BER	balanced error rate
BMI	body mass index
BP	blood pressure
CG	control group
CG^{6w}	six-week control group
CI	confidence interval
CLR	centered-log-ratio
COVID-19	coronavirus disease 2019
CVD	cardiovascular disease
DHFA	dihydroferulic acid
DIABLO	Data Integration Analysis for Biomarker discovery using Latent cOmponents
ESI	electrospray ionization
FA	ferulic acid
Faith-PD	Faith's phylogenetic diversity
FDR	false discovery rate
FFQ	food frequency questionnaire
FLI	fatty liver index
FXR	farnesoid X receptor
GFR	glomerular filtration rate
GI	gastrointestinal
GLP-1	glucagon-like peptide 1
GLUT4	glucose transporter type 4
GPCRs	G protein-coupled receptors
HDAC	histone deacetylase

HDL-C	high-density lipoprotein cholesterol
HOMA-β	homeostasis model assessment of β-cell function
HOMA-IR	homeostasis model assessment of insulin resistance
hsCRP	high-sensitivity C-reactive protein
IL-6	interleukin 6
IPAQ	International Physical Activity Questionnaire
ISI	insulin sensitivity index
KEGG	Kyoto encyclopedia of genes and genomes
KO	KEGG orthologous
LC-MS/MS	liquid chromatography-tandem mass spectrometry
LDL-C	low-density lipoprotein cholesterol
LinDA	linear models for differential abundance analysis
LMM	linear mixed model
LnR	linear regression model
LPS	lipopolysaccharides
MASLD	metabolic dysfunction-associated steatotic liver disease
MCTQ	Munich chronotype questionnaire
MetS	metabolic syndrome
MHCs	metabolically healthy controls
NEFAs	non-esterified fatty acids
OG	oat group
OG^{6w}	six-week oat group
OGIS	oral glucose insulin sensitivity index
OGTT	oral glucose tolerance test
PAL	physical activity level
PBMCs	peripheral blood mononuclear cells
PCG	physiological control group
PICRUSt2	phylogenetic investigation of communities by reconstruction of unobserved states 2
POG	physiological oat group
PLS	partial least square
QUICKI	quantitative insulin sensitivity check index
RCT	randomized controlled trial
REE	resting energy expenditure

SCFAs	short-chain fatty acids
SCG	starving control group
sGCCA	sparse generalized canonical correlation analysis
SOG	starving oat group
sPLS-DA	sparse partial least squares-discriminant analysis
TC	total cholesterol
TGR5	Takeda G protein-coupled membrane receptor 5
TJ	tight junction
TMAO	trimethylamine N-oxide
TNF-α	tumor necrosis factor alpha
TyG	triglyceride-glucose index
T2DM	type 2 Diabetes mellitus
UPLC-MS/MS	ultra-performance liquid chromatography-tandem mass spectrometry
V	visit
VA-NR	valeric acid non-responders
VA-R	valeric acid responders
WHO	World Health Organization
γ-GT	gamma-glutamyl transferase

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1 INTRODUCTION

1.1 The Gut Microbiome and its Impact on Host Health

The gut microbiota is a highly complex and dynamic ecosystem comprising hundreds of bacterial species[1] and also archaea[2], eukaryotes[3] and viruses[4]. The gut microbiome, the collection of the genomes of all microbes, expands the human genome by a multiple with up to 500 times more genes encoded.[5] In recent decades, considerable efforts have been made to elucidate the composition and functional capacity of the human gut microbiome.[6,7] Bacillota (synonym: Firmicutes) and Bacteroidota (synonym: Bacteroidetes) have been identified as the predominant phyla, followed by Actinobacteria, Proteobacteria, Fusobacteria and Verrucomicrobia.[8] However, the annotation and consequently the biological capabilities of many bacterial taxa present in the gut remain poorly defined.[6,7]

A growing body of evidence indicates that the composition and function of the gut microbiome is influenced by both host genetic[9] and environmental factors[10,11], starting in the prenatal phase[12]. Consequently, the gut microbiome is highly diverse among individuals.[7] Data suggests that environmental factors (e.g., diet, lifestyle, medication) are responsible for more than 20% of the inter-individual variation in the gut microbiome, while host genetics account for no more than 2%.[13]

It is well established that the gut microbiome plays a pivotal role in human health and is sometimes even considered a metabolic ‘organ’, as it is involved in the physiological functioning of the host through various mechanisms.[14,15] The gut microbiome contributes to the metabolization of nutrients[16] and xenobiotics[17] producing a variety of metabolites[18], including vitamins such as B_{12} and K[19], and is able to regulate the gut barrier[20] and endocrine functions[21], the energy homeostasis[22], the immune system[23] and even neurological signaling[24]. An imbalance of the gut microbiome, known as dysbiosis, is associated with various lifestyle-associated metabolic disorders such as obesity[25,26], insulin resistance[27,28] and cardiovascular disease (CVD)[29,30], which have been increasing worldwide for decades posing a major public heath challenge[31,32]. However, due to large inter-individual variations, there is no consensus yet on the definition of a “healthy” or “dysbiotic” microbiome.[33,34] In addition, in humans it is still difficult to determine whether microbial dysbiosis is the cause or the consequence of a disease.[35] It is

assumed that microbial diversity, equilibrium, stability, resilience and functional redundancy are important characteristics of a “healthy” microbiome, which are achieved through the interactions of microorganisms, including mutualism, commensalism and cross-feeding, but also amensalism and competition.[36–38] Conversely, an imbalance of the gut microbiome is often associated with (1) low microbial diversity, (2) depletion of bacteria considered beneficial (e.g., *Bifidobacterium*), (3) increase in opportunistic pathogens, (4) lack of compositional stability over time, and (5) unfavorable changes in microbial functions (e.g., decrease in short-chain fatty acid (SCFA) production).[39–41]

1.2 The Role of Microbial Metabolites in Host-Microbiome Interactions

Microbially produced metabolites are one of the best-known mechanisms through which the gut microbiota influences host metabolism. These metabolites reflect the potential function of the gut microbiome and are ascribed an important mediating role in the host–microbiome relationship.[42] Numerous microbiome-derived metabolites are absorbed from the gut and enter the systemic circulation and host tissues[43], thereby enabling the communication between the gut microbes and the immune and endocrine system, the metabolism, the brain and other host functions[44] (**Figure 1-1**). It has been shown that the gut microbiome explains a large proportion of the inter-individual variation in the plasma metabolome[18], even more than host genetics[45].

Short-chain fatty acids, predominantly butyrate, propionate, and acetate, which are produced by microbial fermentation of complex carbohydrates and proteins[46], are among the best-studied microbiome-derived metabolites.[47,48] It is well established that SCFAs provide numerous health benefits by serving as an energy source for enterocytes (especially butyrate)[49] and by acting as signaling molecules through various pathways, including activation of G protein-coupled receptors (GPCRs)[50] and inhibition of histone deacetylase (HDAC)[51]. In this manner, SCFAs influence several physiological processes of the host, including intestinal motility[52], secretory activity[53], gut barrier integrity[54], and mucosal and systemic immunity[55]. In addition, SCFAs are involved in the regulation of lipid metabolism[56,57], glucose homeostasis and insulin sensitivity[58,59], blood pressure (BP)[60] and appetite[61,62].

Other well-known microbially produced metabolites include secondary bile acids and trimethylamine N-oxide (TMAO). Secondary bile acids result from the microbial transformation of bile acids synthesized from cholesterol in the liver and have been shown to act as signaling molecules through the farnesoid X receptor (FXR) and the Takeda G protein-coupled membrane receptor 5 (TGR5), thereby influencing diverse host functions including glucose, lipid and immune homeostasis.[63] Trimethylamine N-oxide is produced by the microbial degradation of dietary phosphatidylcholine and attributed a particularly detrimental role in cardiometabolic health.[64]

More recently described microbiome-derived metabolites include indole, imidazole, and phenolic metabolites. Indoles are derived from the fermentation of aromatic amino acids (especially tryptophan)[65] and are associated with positive effects on human health, such as a lower risk of low-grade inflammation and type 2 diabetes mellitus (T2DM)[66,67]. In contrast, imidazoles, produced by the fermentation of histidine, have been linked to metabolic disorders such as insulin resistance[68,69] and heart failure[70].

Phenolic metabolites such as phenolic acids are produced during the microbial transformation of complex dietary phenolic compounds[71] or are released during the microbial degradation of plant cell walls, as they are mainly present in bound form[72]. Research suggests that microbial metabolization of these compounds includes various processes such as ring cleavage, deglycosylation, demethylation, and dihydroxylation.[73] The gut microbiome is considered to contribute significantly to the inter-individual differences in the bioavailability and metabolization of phenolic compounds.[74] However, the underlying mechanisms of the microbial degradation of phenolic compounds are still largely unknown.[75] In addition, little is known about the effects of microbial-derived phenolic metabolites on host health. It is assumed that the degradation products are more bioavailable and biologically active than their precursors.[76] Thus, microbial metabolites are considered to be the actual active form through which these compounds exert their potential health benefits[77], including their anti-oxidant, anti-inflammatory, antidiabetic and neuroprotective properties[78-81]. However, further research is needed to fully explore the health-promoting potential of microbially produced phenolic metabolites.

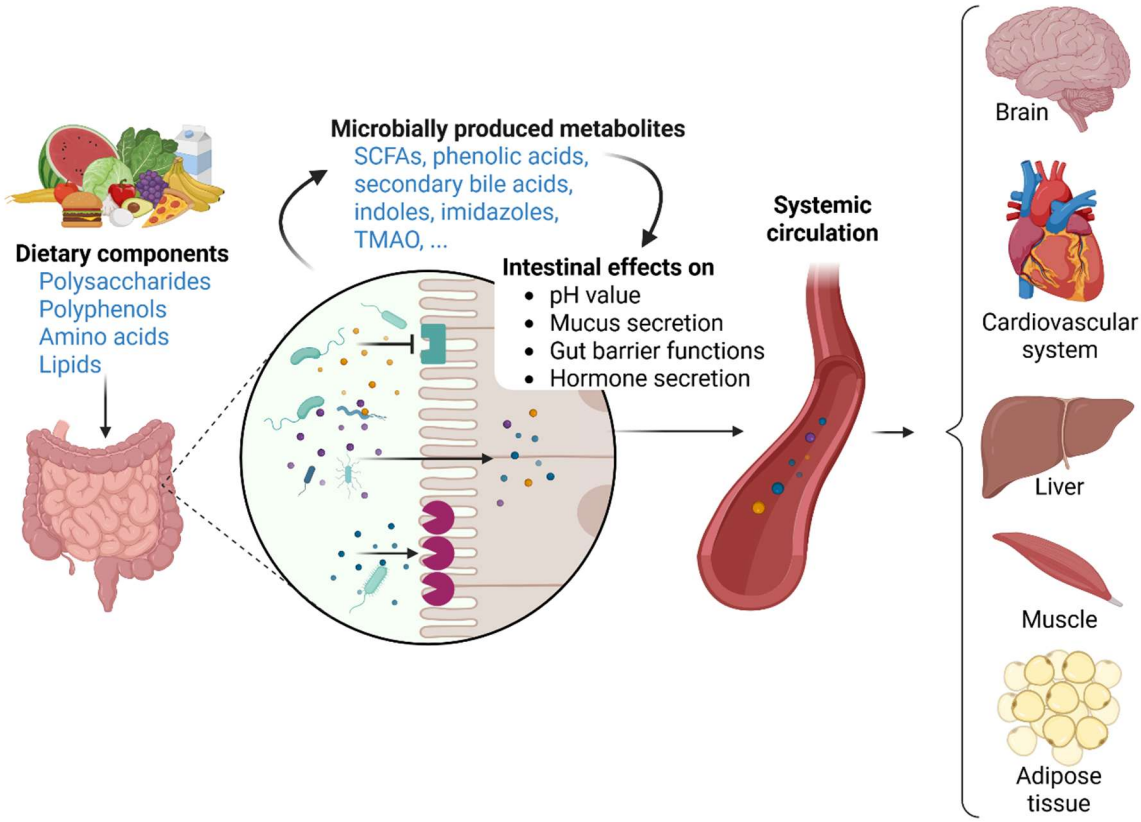

Figure 1-1: Microbially produced metabolites enable the communication between gut microbes and organs as well as tissues of the host

Interactions between gut microbes and dietary compounds such as polysaccharides and polyphenols (shown in blue) result in the production of several microbial metabolites (shown in blue), including SCFAs and phenolic acids. These metabolites are involved in the physiological functioning of the host by regulating the intestinal pH value, gut barrier functions, mucus and hormone secretion (shown in black) and by entering the systemic circulation, thus affecting distant organs and tissues.

Modified according to Schroeder and Bäckhed[44], Fan and Pedersen[82], Cox *et al.*[83], and Dong and Mayer[84]. Figure was created with BioRender.com. Abbreviations: SCFAs, short-chain fatty acids; TMAO, trimethylamine N-oxide.

1.3 Modulating the Gut Microbiome by Dietary Interventions

With increasing knowledge of the importance of the gut microbiome for host health, modulation of the gut microbiome aiming to maintain or restore microbial homeostasis in the gut is a potential strategy for the prevention and treatment of metabolic disorders. Numerous microbiome-targeted interventions have been developed including fecal microbiota transplants, pro-, pre- and postbiotics as well as change of diet.[85] Among these strategies, dietary modifications are considered a particularly promising approach, as individual dietary intake has been identified as one of the most important factors in shaping gut microbiota composition and its functional capacity.[86,87] Long-term dietary habits have been associated with microbial enterotypes that seem to remain rather stable over time.[88–90] For example, the *Bacteroides* enterotype is associated with a habitual diet rich in protein and animal fats, while the *Prevotella* enterotype is linked to a regular intake of complex carbohydrates.[88] Additionally, major changes in diet have been shown to induce rapid shifts in microbial composition within 24–48 hours.[91]

Among various food components, dietary fiber has been shown to be a key factor in maintaining and restoring microbial homeostasis, influencing host health.[92,93] Dietary fiber comprises a variety of indigestible carbohydrate polymers that differ significantly in chemical composition and physicochemical properties, affecting their impact on host physiology[94] and the gut microbiome[95]. In particular, fermentable fibers such as inulin, oligofructose and β-glucan are ascribed a decisive role in modulating the gut microbiota composition and function, as they provide an energy source for microbes.[96] It has been shown that a high-fiber diet selectively promoted the growth of certain putatively health-promoting bacteria such as *Bifidobacterium* and *Lactobacillus* in people with T2DM.[97] These bacteria did not share taxonomic similarity but encoded similar functions, namely the capability to produce SCFAs, which correlated with the metabolic improvements.[97] Substrates that are selectively metabolized by the microbes providing health benefits are defined as prebiotics.[98] In addition to selectively promoted growth based on the corresponding microbial enzymatic capacity or cross-feeding[99,100], the consumption of dietary fiber has also been associated with inhibited growth of certain taxa. The abundance of *Bacteroides* species has been observed to decrease after the intake of dietary fibers despite their fermenting capability[101], most likely due to their low tolerance to the acidic

conditions generated by SCFAs[102]. Lowering the pH value in the gut has also been demonstrated to inhibit the growth of pH-sensitive opportunistic pathogens.[103,104] Interestingly, changes in the colonic pH value not only affect the bacterial composition, but also gene expression and enzymatic activity, thus directly influencing metabolite production.[105] Moreover, health-promoting bacteria have been shown to inhibit the adhesion of certain pathogenic bacteria to intestinal epithelial cells[106] through competitive binding to receptors[107] or stimulation of host factors such as mucin production[108], thereby potentially contributing to the fiber-related reduction of pathogens. Furthermore, fiber-induced changes in the gut transit time may contribute to alterations in microbial composition and function[109,110], highlighting the link between gut physiology and microbiome characteristics[111].

More recently, dietary phenols have also been identified as potential microbiome-modulating compounds. They are able to inhibit the growth of opportunistic pathogens[112,113] by acidifying the microbial plasma membrane interface or by disrupting membrane transport[114,115]. In addition, they tolerate[113,116] or even stimulate[112,117] the growth of beneficial bacteria such as *Lactobacillus* and *Bifidobacterium* species by serving as an energy source. For instance, various *Bifidobacterium* and *Lactobacillus* species have been shown to possess feruloyl esterases[118,119], which are capable of hydrolyzing the ester linkage between hydroxycinnamic acid and plant-derived sugar polymers present in whole grains[120]. However, the evidence of the microbiome-modulating properties of phenolic compounds is still limited, particularly from human studies.[98,121]

1.4 Dietary Impact on Metabolism

It is widely accepted that diet plays a central role in the pathogenesis of lifestyle-associated metabolic disorders such as obesity, insulin resistance and CVD and is therefore a key factor in tackling these diseases.[122,123] According to data from the Global Burden of Disease Study from 1990 to 2017, improving diet could potentially prevent one in five deaths worldwide.[124] In particular, insufficient intake of dietary fiber is considered one of the main causes of the increasing prevalence of metabolic disorders.[125,126] In addition, inadequate fiber consumption has been associated with adverse effects on the gut microbiome such as a loss in diversity.[127,128] Whole-plant foods such as whole grains, which are an important

source of naturally occurring dietary fibers[129] as well as phenolic compounds[130], have been associated with multiple health benefits, including a lower risk of metabolic disorders such as T2DM and obesity[131]. In contrast, a low intake of whole grains has been identified as one of the leading dietary risk factors for mortality.[124] An intervention study investigating the impact of whole grains on metabolism and the gut microbiome in healthy individuals revealed that the improvements in glucose metabolism and inflammatory status coincided with diet-induced alterations in microbial ecology[101], indicating that the health benefits of whole grains may be mediated by their prebiotic properties[132]. Increased consumption of whole grains may therefore be an effective microbiome-targeted dietary strategy for improving host health, particularly for people consuming a 'Western diet'. This dietary pattern is characterized by a high intake of fat, refined carbohydrates, and animal as well as processed foods and a low intake of whole grains, fruits and vegetables, resulting in an inadequate supply of fiber, vitamins, antioxidants and phytochemicals such as phenols.[133] Consequently, the 'Western diet' is associated with negative effects on metabolism and the gut microbiome[134,135], which is also reflected in the metabolomic profile in blood[136]. It is widely acknowledged that the spread of Western dietary habits is a major factor contributing to the global increase in lifestyle-associated metabolic disorders.[134] Switching to a health-promoting diet by increasing the consumption of whole plant-based foods such as whole grains is therefore considered as a sustainable and cost-effective prevention strategy.[137] However, the mechanisms underlying the health benefits of whole grain products are not yet completely understood.[138] Further studies, especially well-designed human randomized controlled trials (RCT), are therefore needed to fully explore the interactions between whole grains, metabolism, the gut microbiome and microbiome-derived metabolites to optimize human health.

1.5 Whole Grain Oats as a Potential Microbiome-Targeted Dietary Strategy for Metabolic Syndrome

The metabolic syndrome (MetS) is described as a cluster of abnormalities including central obesity, hypertension, atherogenic dyslipidemia and dysglycemia[139], which is associated with an increased risk of T2DM[140,141] and CVD[142,143]. The prevalence of MetS has increased dramatically in recent decades and is considered a major

public health challenge in both developed and developing countries. It is estimated that up to 31 % of the world's population is affected[144], making cost-effective and easy-to-implement treatment strategies such as nutritional approaches essential.

It is well established that MetS is a multifactorial condition related to genetic[145] and environmental factors, including dietary habits[146], as well as psychological[147] and socioeconomic factors[148]. The complexity of MetS is further enhanced by the interaction of intrinsic and extrinsic factors that influence the predisposition to MetS.[149] While there is a consensus that both genetic and acquired factors are involved, the question of whether the individual components of MetS represent different pathologies or manifestations of a common pathogenic mechanism remains controversial.[150] Visceral obesity has been demonstrated to be a primary trigger for most pathways leading to MetS by initiating a cascade of systemic inflammation-related processes that can lead to whole body insulin resistance and dyslipidemia.[151–155] However, in humans, inflammation as the driver of insulin resistance should be regarded as a hypothesis rather than a proven fact.[156] More recently, the gut microbiome has been identified as an important factor contributing to the etiology of MetS.[157,158] Possible pathways include the involvement in energy homeostasis, the modulation of gut barrier functions and inflammatory signaling pathways, as well as interferences with the immune and renin-angiotensin systems.[158–160] Modulation of the gut microbiome is therefore considered as a key component of therapeutic interventions targeting MetS and associated comorbidities.

International guidelines recommend lifestyle modifications, such as changes in diet, to manage MetS and delay the manifestation of diabetes and CVD.[139] Among dietary modifications, whole grain oats have recently attracted considerable interest as a promising treatment approach due to their valuable nutritional profile.[161] Positive effects of oats on glucose metabolism were first revealed by Carl von Noorden, a German diabetologist, who developed a special oat cure for the treatment of diabetes at the beginning of the 20th century.[162] A few years later, de Groot *et al*. observed that oats also improve lipid metabolism, especially by reducing circulating cholesterol levels.[163] These health effects of oats have since been confirmed by several studies in individuals with various health conditions.[164–168] More recently, the consumption of oats has also been associated with positive effects on weight

control[167], satiety[169,170], BP[171], and inflammatory status[172,173], all of which are related to MetS; however, the body of evidence is limited. In addition, the underlying mechanisms for the health effects induced by oats are not yet fully understood and comprise various potential pathways (**Figure 1-2**).

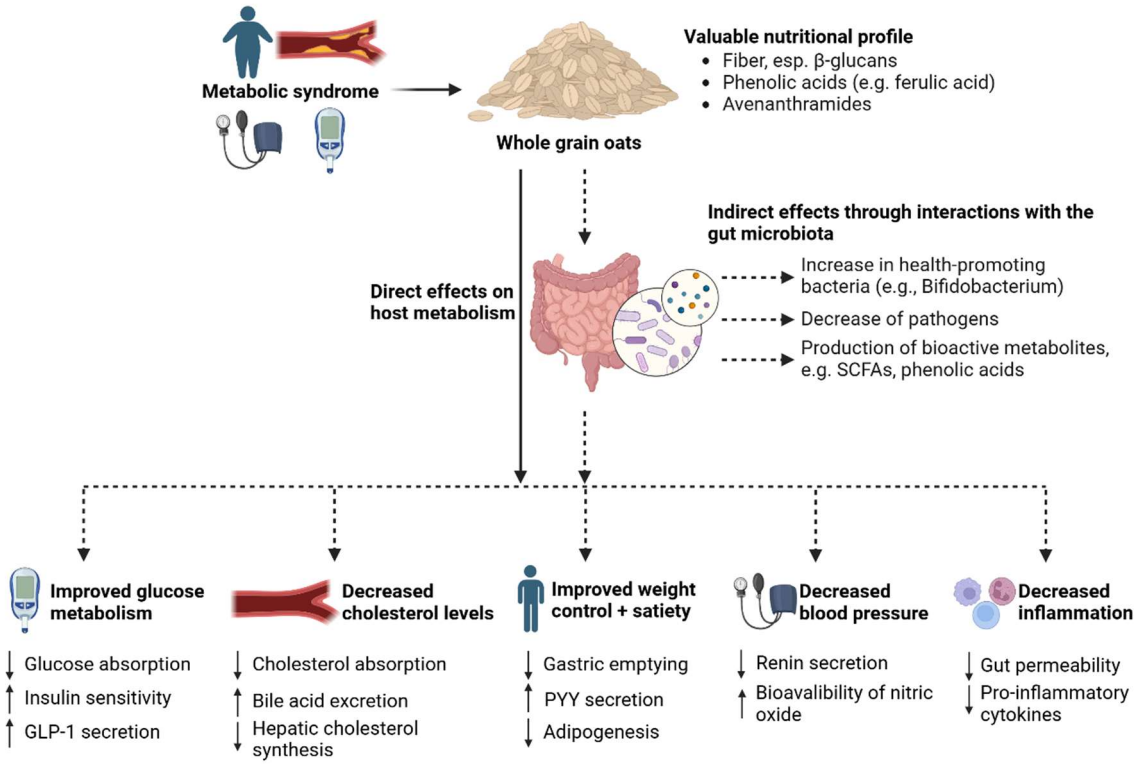

Figure 1-2: Whole grain oats as a potential microbiome-targeted dietary strategy for metabolic syndrome

Due to their valuable nutritional profile characterized by a high fiber content and several phenolic compounds, whole grain oats are considered a potential dietary strategy to address metabolic syndrome, a cluster of various abnormalities including central obesity, hypertension, atherogenic dyslipidemia and dysglycemia. It is assumed that the health effects of oats are on the one hand directly attributable to the oat components and on the other hand are mediated by interactions with the gut microbiome. While the direct effects of oats, especially of oat β-glucans, on metabolism have been extensively analyzed (solid line), the interactions between oats and the gut microbiome and their effects on metabolism are poorly understood (dashed line). This figure presents some key mechanisms but does not claim to be exhaustive.

Figure created with BioRender.com. Abbreviations: GLP-1, glucagon-like peptide 1; PYY, peptide YY; SCFAs, short-chain fatty acids.

Initially, the positive effects of oats on host metabolism were attributed to the excellent swelling properties of the soluble non-starch polysaccharide (1 → 3) (1 → 4) β-D-glucan, which is a key component of oats (4.5 g/100 g).[174] These swelling properties have been linked to an increase in viscosity of the meal bolus, which is associated with a delay in gastric emptying and in the degradation and absorption of nutrients.[175–177] This, in turn, has been shown to increase satiety perception and decrease energy intake as well as circulating glucose and cholesterol levels.[175,178,179] In addition, β-glucans have been shown to interact with bile acids in the small intestine, which results in bile acids being removed from the enterohepatic circulation. This causes an increase in hepatic synthesis of bile acids from cholesterol, which finally leads to a reduction in circulating cholesterol concentrations.[180,181]

Other bioactive compounds of oats such as phenols may also be important factors contributing to the health effects. Oats contain a variety of phenolic compounds including hydroxycinnamic acids (e.g., ferulic, sinapic, caffeic, *p*-coumaric, and *o*-coumaric acids), hydroxybenzoic acids (e.g., syringic, vanillic, *p*-hydroxybenzoic acid, protocatechuic, 4-hydroxybenzaldehyde, and gallic acids) and oat-specific avenanthramides (AVAs).[182–184] The majority of these phenols are bound to fiber components.[185] The total concentration of phenolic acids and AVAs in 100 g of commercial oat products range from 39.4–62.63 mg and 2.75–5 mg, respectively.[183] Interestingly, *in vitro* and animal studies have shown that phenols have various health effects due to their lipid- and glucose-lowering[186–189], hypotensive[190,191], anti-inflammatory[186,187,192], and body weight-regulating[186,193] properties. However, convincing data based on human studies is largely missing. In addition, effects of isolated phenols have often been analyzed, thus no conclusions can be drawn about the combined effects of oat phenols in their entirety.

More recently, the ability of oats to modulate microbial composition and function has been suggested as an underlying mechanism for the health effects of oats.[194] *In vitro* and animal studies have demonstrated that oats lead to an increase in the abundance of beneficial bacteria such as *Lactobacillus* and *Bifidobacterium* and promoted the production of SCFAs[195–208]. As signaling molecules, SCFAs have been shown to (1) improve glucose metabolism by stimulating the secretion of gut hormones and incretins such as glucagon-like peptide 1 (GLP-1)[53], enhancing

insulin sensitivity[58], and increasing the glucose uptake in muscle cells via the glucose transporter type 4 (GLUT4)[209], (2) lower circulating cholesterol levels by reducing intestinal cholesterol absorption via the Niemann-Pick C1-like 1 transmembrane protein[56] and by suppressing hepatic cholesterol synthesis[210], (3) improve satiety and weight control by influencing central appetite regulation[61] and the release of appetite-regulating hormones such as PYY[62], (4) regulate BP by modulating renin secretion[211], and (5) decrease systemic inflammation by strengthening gut barrier integrity[212] and influencing inflammatory responses[213]. However, research on the effects of oat consumption on the gut microbiome is still in its infancy. Convincing data from human studies is largely lacking, and the limited results available are ambiguous. An increase in *Bifidobacterium* and *Lactobacillus* was observed in subjects with an increased risk of cardio-metabolic disease consuming 45 g of wholegrain oats daily for six weeks, which is consistent with the results obtained from *in vitro* and animal studies, but no shift in fecal SCFA levels was observed.[214] An increase in *Bifidobacterium* was also found in individuals consuming oats as the sole carbohydrate-stable food for one week[215] and in patients with essential hypertension consuming 30 g oat bran daily for three months[216]; however, SCFAs concentrations were not measured. In contrast, another RCT did not observe an increase in *Lactobacillus* and *Bifidobacterium* after the consumption of 80 g oats for 45 days in individuals with mild hypercholesterolemia despite distinct changes in microbial composition, and no change in plasma SCFA levels was detected.[217] Similarly, the consumption of 60 g oatmeal porridge daily for one week did not change fecal SCFAs levels in healthy participants.[218] In contrast, an increase in fecal SCFA levels was observed in healthy volunteers who consumed 40 g of β-glucan-enriched oat bran per day for 12 weeks.[219]

To date, the prebiotic potential of oats has mainly been attributed to β-glucans, while other bioactive compounds such as phenols were not considered. An animal study, however, has shown that the intake of a mixture of oat phenolic compounds for 8 weeks led to distinct shifts in microbial composition in mice, characterized by an increase in microbial diversity and beneficial bacteria and a decrease in potentially harmful bacteria.[186] In line with this, ferulic acid (FA), the main phenolic acid in oats[183], has been shown to beneficially modulate microbial composition and functional capacity by increasing SCFA-producing bacteria and decreasing

opportunistic pathogens.[220] Thus, oat phenols might play a key role in the modulation of the gut microbiome and the related optimization of host metabolism. However, the interactions between gut microbes and phenols as well as the health implications of microbially produced phenolic metabolites are poorly understood. In addition, only a limited number of studies have focused on the interactive effects of oats on metabolism and the gut microbiome. Further research is therefore needed to fully elucidate the interactions between oats, metabolism and the gut microbiome and to uncover the potential of oats as an effective microbiome-targeted dietary strategy for MetS.

2 OBJECTIVES

The overall objective of the research presented in this thesis was to investigate the interactions between metabolism, the gut microbiome, and microbiome-derived metabolites in the context of two different dietary interventions to optimize human health. To achieve this objective, specific aims are defined:

Investigating the effects of a short-term, high-dose oat diet (a modified version of the original oat cure by Carl von Noorden[162]) and a six-week, moderate oat diet compared to corresponding control diets in individuals with MetS (RCT) on:

i) the plasma concentration of (microbially produced) phenolic compounds, in particular (dihydro)ferulic acid ([DH]FA).
ii) the fasting and postprandial glucose and lipid metabolism as well as kidney and liver functions.
iii) the gut microbiome and global metabolomic profiles, exploring the interaction between oats, metabolism, the gut microbiome and microbiome-derived metabolites.
iv) the gut permeability and inflammatory status, examining the relationship between changes in metabolism, plasma SCFA concentrations and the gut microbiome (subgroup analysis).

3 METHODS

3.1 Ethics Approval and Participants' Consent

This study complied with all relevant ethical regulations and was conducted in accordance with the principles of the Declaration of Helsinki and its subsequent amendments, approved by the ethics committee of the Medical Faculty, University of Bonn (Approval Number: 212/20), and prospectively registered at the German Clinical Trials Register (http://www.drks.de) under identifier DRKS00022169. Written informed consent was obtained from all the participants.

3.2 Study Design

This study comprised two 1:1 randomized, controlled, prospective dietary interventions, each with a parallel design, conducted between September 2020 and July 2022 at the Department of Nutrition and Microbiota, University of Bonn, Germany. The short-term intervention study was completed on schedule, while the six-week intervention study was delayed due to the COVID-19 pandemic.

Potential subjects completed an initial telephone pre-screening followed by an in-person screening visit which included anthropometric measurements and a detailed blood analysis to confirm eligibility as well as a detailed explanation of all study procedures and requirements. In the short-term intervention study, eligible participants were invited for two clinical visits, the first before (V1) and the second directly after the two-day intervention period (V2), followed by three clinic visits at two-week intervals during a six-week follow-up period (V3 – V5) to determine potential long-term effects of the oat diet (**Figure 3-1a**). During the six-weeks, intervention study, eligible participants were invited to four clinical visits at two-weeks intervals with the first before (V1) and the last after the six-week intervention period (V4) (**Figure 3-1b**).

After V1, participants were randomly assigned to the experimental group (short-term oat group [OG] or six-week oat group [OG^{6w}]) or the control group (short-term control group [CG] or six-week control group [CG^{6w}]) using computer-generated randomization tables in a block format with variable block length generated by a researcher not clinically involved in the study. These tables were concealed from the

researchers and participants until the interventions were assigned by study personnel not involved in final data analysis. Blinding after allocation to the interventions was not feasible.

At V1 and the clinical visit directly after each intervention period (V2 or V4), anthropometric data, fecal samples as well as fasting and postprandial blood samples, taken during an oral glucose tolerance test (OGTT), were collected. Office BP and resting energy expenditure (REE) were measured using a semiautomatic BP measurement device and indirect calorimetry, respectively. In addition, an in-depth nutritional and lifestyle assessment was conducted at V1. At the clinical visits during the six-week intervention study (V2+V3), anthropometric and BP measurements were performed and fasting blood and fecal samples were collected. These also took place at the clinical visits during the follow-up period of the short-term intervention study (V3 – V5). In addition, a fecal sample was collected after the first intervention day of the short-term intervention. Interim contact with the study coordinator (via telephone or e-mail) was made available to all participants on each intervention day.

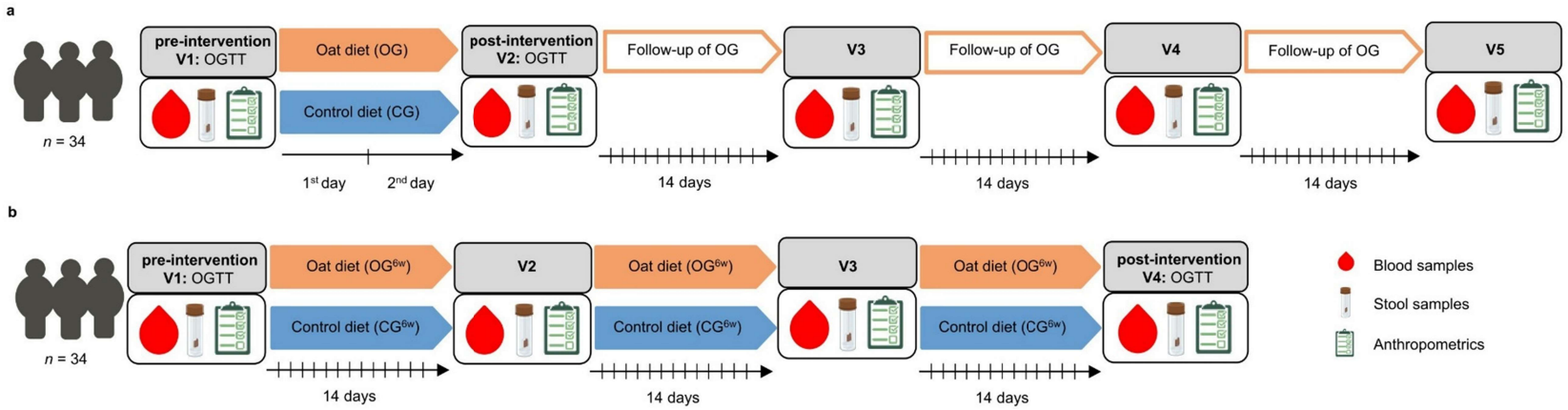

Figure 3-1: Study scheme

(**a**) The short-term intervention study included two clinical visits, before (V1) and after the two-day intervention period (V2), followed by three clinical visits during a six-week follow-up period within the oat group (OG; V3-V5). On V1 and V2, blood samples were taken during a 3 h oral glucose tolerance test (OGTT), fecal samples were collected and detailed anthropometric measurements and lifestyle assessments were performed. Additionally, fecal samples were collected after the first intervention day. During the follow-up period (V3 – V5), fasting blood and fecal samples were collected and detailed anthropometric measurements were performed. (**b**) The six-week intervention study included four clinical visits in two-week intervals with the first before (V1) and the last after the six-weeks intervention period (V4). On V1 and V4, blood samples were taken during a 3 h oral glucose tolerance test (OGTT), fecal samples were collected and detailed anthropometric measurements and lifestyle assessments were performed. At the visits during the intervention period (V2, V3), fasting blood samples, fecal samples and anthropometric data were collected.

Abbreviations: CG, control group; CG^{6w}, six-week control group; OG, oat group; OG^{6w}, six-week oat group; OGTT, oral glucose tolerance test; V, visit.

3.3 Participants

Participants were recruited through newspaper advertisements, flyers, and social media. The study subjects included female and male adults with overweight or obesity (body mass index [BMI] 27 - 39.9 kg/m^2) and MetS aged 45 - 70 years. MetS was primarily diagnosed based on the global consensus definition of the International Diabetes Federation[221] as central obesity (waist circumference ≥ 94 cm in men and ≥ 80 cm in women for Europeans) coupled with two of the following five criteria: (1) elevated BP (≥120 mmHg systolic and/or ≥80 mmHg diastolic)[222]; (2) elevated fasting serum triglycerides (≥150 mg/dl); (3) decreased fasting serum high-density lipoprotein cholesterol (HDL-C) (<40 mg/dl for men and <50 mg/dl for women); (4) elevated fasting plasma glucose (≥ 100 mg/dl); (5) indication of insulin resistance (HOMA-IR index > 2.5) (adapted to the World Health Organization (WHO) definition[223]). The latter criterion was added after the initial study registration in order to attach more importance to impaired glucose metabolism as a characteristic of MetS and to counteract recruitment difficulties due to the COVID-19 pandemic. In addition, the subjects' habitual diet corresponded to a Western dietary pattern[133] without regular oat consumption, defined as less than once a week for at least the last three months. Exclusion criteria were as follows: (1) diagnosed Diabetes mellitus, chronic kidney, liver, inflammatory, or digestive tract diseases, non-medicated thyroid diseases, past cardiovascular events, acute illnesses or recent surgeries; (2) chronic use of medication that influences glucose metabolism; (3) antibiotics treatment within three months prior to study inclusion; (4) pregnancy or lactation; (5) intolerance to oat; (6) history of smoking or alcoholism; (7) chronic intake of dietary-supplements; (8) vegetarian or vegan diet; (9) planned lifestyle changes. For the explicit research question regarding gut permeability and inflammatory status (‘aim iv’), the use of proton pump inhibitors, the presence of acute inflammatory and infectious diseases, as well as the receipt of vaccinations during study participation were defined as additional exclusion criteria.[224–226]

3.4 Dietary Interventions

During the short-term intervention period, the participants followed either a two-day, high-dose oat diet or a macronutrient-adapted control diet, both hypocaloric and high in fiber (1100-1200 kcal/d; carbohydrates: 67 energy% (E%), of which fiber > 15%; protein: 15 E%; fat: 17 E%), depending on their allocation (OG vs. CG). Participants in the OG consumed three oat meals per day instead of their habitual diet. Each meal consisted of 100 g rolled oat flakes (Demeterhof Schwab GmbH & Co. KG, Windsbach, Germany), providing 10 g of fiber, and has been consumed as porridge prepared with water. Fruits restricted to apples, pears, and berries and vegetables restricted to spinach and leeks were used as additives and included in the meal plans of the CG in the same amount. No salt, sugar, or sweeteners were added. Participants in the CG consumed two meals per day comprising bread and raw vegetables (breakfast and dinner) and one warm meal per day (lunch) instead of their habitual diet. There was a time interval of 4 hours between meals during which the participants were requested to consume only unsweetened beverages. For standardization, the participants received a recommendation for dinner the evening before the start of the intervention (carbohydrate-rich bread meal with raw vegetables). During the six-week follow-up period, participants of the OG adhered to their habitual diet prior study participation without consuming oats.

During the six-week dietary intervention, participants in the OG^{6w} replaced one meal per day with an oat meal consisting of 80 g oat flakes–in accordance with the health claims[227,228]–and providing 8 g of fiber, while maintaining their habitual diet under isocaloric conditions. For meal preparation, the participants received a collection of recipes that included classic porridges, overnight oats, smoothies, and baked goods and was adapted to the daily calorie requirements of each participant, measured by indirect calorimetry. The participants in the CG^{6w} maintained their habitual dietary pattern unchanged and did not consume any oats.

In both intervention periods, the meals were prepared by the subjects at home using detailed instructions. Participants were instructed to avoid any oat products–other than the oat flakes provided–for at least two weeks before and during the study. The nutrient composition of all meals was calculated using the computer-based nutrient calculation program EBISpro, based on the German nutrient database Bundeslebensmittelschlüssel, version 2016 (Max Rubner-Institut, Karlsruhe, Germany).

3.5 Compliance

Adherence to the treatment protocol was assessed based on three independent criteria: (1) the plasma concentration of the oat-specific biomarker AVAs[229,230] determined using liquid chromatography with tandem mass spectrometry (LC-MS/MS) by Metabolon Inc. (Morrisville) based on a method previously described[230]; (2) the number of (un)emptied returned oat packs; and (3) detailed checklists completed by the participants assigned to the oat diets (OG or OG^{6w}) or the control diet of the short-term intervention (CG) recording precise information on the selection and preparation of the respective test meals.

3.6 Assessment of Gastrointestinal (GI) Tolerability and Well-Being

The GI tolerability and well-being of the participants were assessed on the first (V1) and last intervention day (V2 or V4) using a standardized questionnaire to record the frequency of specific GI symptoms (abdominal pain, flatulence, diarrhea, and nausea) and well-being (headache, uneasiness, dizziness, concentration disorders, exhaustion, and other complaints), adapted according to Wolever *et al.*[231]. In brief, the frequency of each symptom was reported on a 4-point scale, with '0' denoting 'at no time', '1' denoting 'once a day', '2' denoting 'several times a day' and '3' denoting 'all day'. Separate scores for GI tolerability and well-being were calculated as the sum of each individual symptom score, with a range of 0-12 (GI tolerability) or 0–18 (well-being) representing complete and poor tolerance or well-being, respectively.

During the six-week intervention, GI tolerability and well-being were additionally recorded on a weekly basis. Here, the frequency of each symptom was reported on a 5-point scale, with '0' denoting 'at no time', '1.5' denoting 'once or twice a week', '3.5' denoting 'three to four times a week', '5.5' denoting 'five to six times a week', and '7' denoting 'every day of the week'. Separate scores for GI tolerability and well-being were calculated as the sum of each individual symptom score over the entire intervention period, with a range of 0-168 (GI tolerability) or 0-252 (well-being) representing complete and poor tolerance/well-being, respectively.

3.7 Anthropometrics and Blood Pressure

Anthropometric measurements were performed following a previously published standard operative procedure.[232] Briefly, body weight was measured in light clothing with an empty bladder, using electronic column scales with an accuracy of up to 100 g (seca scale 704, seca GmbH and Co. KG, Hamburg, Germany). Body height was determined to the nearest of 0.1 cm using a stadiometer (seca scale 704, seca GmbH and Co. KG, Hamburg, Germany). The BMI was calculated using the following formula: BMI = weight [kg]/(height [m])^2. Waist circumference was measured midway between the lowest rib and iliac crest at maximal exhalation to the nearest of 0.1 cm in duplicates. Body composition (fat mass and fat-free mass) was determined by air-displacement plethysmography using a BOD-POD body composition system (Cosmed, Firdolfingen, Germany).

Office BP and heart rate were measured twice in a seated position using a semiautomatic BP measurement device (Boso Carat Professional, Bosch + Sohn GmbH and Co. KG, Juningen, Germany) under standardized conditions in accordance with European and American guidelines.[233,234]

3.8 Nutrition and Lifestyle Assessments

Self-reported data on participant characteristics, including sex, age, disease, use of medications and dietary supplements, habitual diet, physical activity level (PAL), sleeping behavior, and socioeconomic status were collected by questionnaire. The habitual diet was characterized using a Food Frequency Questionnaire (FFQ) over 12 months. The PAL (7-day total metabolic equivalent of task score) was assessed at baseline (V1) using the short version of the validated International Physical Activity Questionnaire (IPAQ).[235,236] For the evaluation of the sleeping behavior (circadian rhythm), the validated Munich Chronotype Questionnaire (MCTQ) was used.[237,238] Socio-economic status was obtained using a questionnaire based on the parent questionnaire to measure socioeconomic status in the study on the health of children and adolescents in Germany (KiGGS) Wave 2.[239]

3.9 Indirect Calorimetry

For determination of whole-body REE and substrate oxidation via measurement of carbon dioxide production and oxygen consumption, indirect calorimetry was performed using Quark-RMR® (Cosmed, Fridolfing, Germany) with a coefficient of variation of < 10%.[240] Resting energy expenditure was multiplied by 1.5 (PAL) to calculate the total energy expenditure.

3.10 Collection of Blood and Stool Samples

Fasting venous blood samples were collected between 8:00 a.m. and 10:00 a.m. after a 12 h overnight fast. Postprandial blood samples were collected during a 3 h OGTT. All samples were taken under standardized conditions using tubes containing EDTA, fluoride, or a coagulation activator (S-Monovette, Sarstedt, Germany). Plasma and serum supernatants were obtained by centrifugation at 1700 × g for 15 min at 8 °C after complete coagulation (serum) and immediately frozen in cryovials at −80 °C until further analysis. Fecal samples were collected according to a standard operation procedure[241] before each clinical visit and immediately stored at −80 °C until further analysis.

3.11 Oral Glucose Tolerance Test

The OGTT was performed after an overnight fast with 75 g glucose and 300 ml water (Dextrose O.G-T.; Roche Diagnostics, GmbH, Mannheim, Germany). Venous blood samples were collected via a venous catheter before ingestion and at 15, 30, 45, 60, 120, and 180 min postprandially to analyze the time course of plasma glucose, serum insulin, serum triglyceride and serum non-esterified fatty acid (NEFA) concentrations.

Various indices were calculated to assess the diet-induced effects on glucose control and insulin resistance. Insulin sensitivity was evaluated using the oral glucose insulin sensitivity index (OGIS)[242,243] and the insulin sensitivity index (ISI), which provide a measurement of insulin-mediated glucose clearance[244]. The function of β-cells was assessed using the insulinogenic index[242,243], disposition index[245–247] and the homeostasis model assessment of β-cell function (HOMA-β)[248].

In addition, insulin resistance was evaluated using the quantitative insulin sensitivity check index (QUICKI)[249,250] and the triglyceride-glucose index (TyG)[251,252] based on fasting concentrations. Moreover, the area under the curve (AUC) of the glucose and insulin concentration over 180 minutes was used to measure differences in glucose tolerance upon intervention. The AUCs of the NEFA and TG concentration were calculated in the same way over 120 minutes. Furthermore, the presence of metabolic dysfunction-associated steatotic liver disease (MASLD) was estimated using the fatty liver index (FLI ≥ 60).[253,254]

3.12 Blood Sample Analysis

3.12.1 Routine Laboratory Analyses

Routine laboratory analyses of serum samples included fasting and postprandial triglycerides and insulin levels, fasting total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), HDL-C, and high-sensitivity C-reactive protein (hsCRP) levels, as well as markers of liver function (fasting gamma-glutamyl transferase [γ-GT], alanine aminotransferase [ALT], aspartate aminotransferase [AST], lipase, and bilirubin) and kidney function (fasting creatinine, glomerular filtration rate [GFR], urea, and uric acid). Routine laboratory analyses of plasma samples included fasting and postprandial glucose levels, fasting HbA1c, and hematology parameters. All parameters were measured in a certified medical laboratory (Central Laboratory of the Institute of Clinical Chemistry and Clinical Pharmacology at the University Hospital Bonn, Germany) within 4 h of blood sampling under standardized conditions using the Roche/Hitachi Cobas c system (Roche Diagnostics, Mannheim, Germany). Methods specifications are available online (https://www.ukbonn.de/ikckp/zentrallabor/ leistungsverzeichnis/). The HOMA index was calculated as follows: HOMA-IR = [insulin (mU/L) × glucose (mg/dL)] ÷ 405.[248] Insulin resistance was assumed if the HOMA index was > 2.5.[255]

3.12.2 Analysis of Non-Esterified Fatty Acids

Fasting and postprandial NEFA concentrations in serum were analyzed before and after each intervention period using an *in vitro* enzymatic colorimetric method assay (NEFA-HR(2), Wako Diagnostics, Mountain View, CA, USA) following the manufacturer's instructions and the recommended quality control procedure (inter-assay and intra-assay variability: 5.4% and 4.8%) at the Institute of Nutrition and Food Sciences, University Bonn, Germany, as previously described.[256]

3.12.3 Analysis of Zonulin and Lipopolysaccharides

To assess gut permeability, serum zonulin and LPS levels were determined. Zonulin concentration was measured in duplicate using an enzyme-linked immunosorbent assay (ELISA; antibodies-online GmbH, Aachen, Germany), following the manufacturer's protocol, with an additional dilution step (1:10). Lipopolysaccharide concentration was analyzed using an ELISA kit (Cusabio Technology LLC, Houston, TX, USA) following the manufacturer's protocol. Both measurements were performed at the Institute of Nutrition and Food Sciences, University of Bonn, Germany. The inter- and intra-assay variations were 10% and 10% for zonulin, and 10% and 8% for LPS, respectively.

3.12.4 Assessment of Inflammatory Status

To assess the inflammatory status of the participants before and after each intervention period, serum hsCRP levels were measured according to routine laboratory analysis (see above). In addition, interleukin 6 (IL-6) concentration in plasma before and after the short-term intervention period was analyzed in duplicate using an ELISA kit (Bio-Techne Ltd., Abingdon, UK) following the manufacturer's instructions at the Institute of Nutrition and Food Sciences, University Bonn, Germany. The inter- and intra-assay variations were 7% and 5%, respectively.

3.13 *In vitro* Experiments

3.13.1 Cell Culture Experiments

In addition to the conducted RCTs, *in vitro* cell culture experiments were performed to investigate whether phenolic compounds mediate the beneficial effect of oats on metabolism, particularly cholesterol metabolism. Briefly, blood was collected from a subset of the participants with MetS (*n* = 4; 65 ± 9 y, 29.3 ± 2.2 kg/m²) and from metabolically healthy controls (MHCs) (*n* = 4; 33 ± 11 y, 25.1 ± 4.9 kg/m²) to isolate peripheral blood mononuclear cells (PBMCs). After pre-processing, PBMCs were exposed to medium with or without 10 µM DHFA (Sigma-Aldrich, #17803-5G) that additionally contained 20 µg/mL alkyne cholesterol. In addition, HuH7 cells (JCRB0403) were treated with the same medium with or without 10 µM DHFA after 48 h of cultivation in growth medium. The medium was additionally supplemented either with 20 µg/mL alkyne cholesterol or 5 mM 1,2-$^{13}C_2$ sodium acetate (CIL, #CLM-440-1) and 100 µM alkyne fatty acid 182. After 24 h of incubation, the samples were subjected to lipid extraction and dissolved lipids were analyzed by mass spectrometry. A detailed description is provided in the **Appendix**.

3.13.2 Fecal Batch Culture Fermentation of in vitro Digested Oats

To evaluate whether the metabolism of the microbiome leads to the production of specific oat-derived metabolites, particularly phenolic compounds, an anaerobic *in vitro* fecal batch culture experiment was conducted. In brief, aliquots of homogenized baseline stool samples from four representative participants of the short-term intervention study were used to inoculate sterile anoxic basal medium either with or without *in vitro* digested oats in a starving group (anoxic basal buffer; starving oat group [SOG] vs. starving control group [SCG]) or a physiological group (Brain Heart Infusion medium; physiological oat group [POG] vs. physiological control group [PCG]). Samples for global metabolomic profiling including phenolic compounds, SCFAs and microbiome analysis were collected directly after inoculation, after 6 h, 24 h, and 36 h of incubation time. A detailed description is provided in the **Appendix**.

3.14 Targeted Plasma Metabolomic Profiles

3.14.1 Plasma Dihydroferulic and Ferulic Acids

Plasma DHFA concentration and FA values (peak areas) before and after each intervention period were generated by Metabolon Inc. (Morrisville) using LC-MS/MS according to Metabolon Method TAM223 ("LC-MS/MS quantitation of dihydroferulic acid and three avenanthramide compounds in human plasma") based on a previously described method.[257] Briefly, plasma samples were spiked with stable labeled internal standards and then incubated with glucuronidase and sulfatase enzymes to convert conjugated metabolites to their free form. After incubation, the samples were subjected to protein precipitation with acetonitrile. Next, the samples were centrifuged, and the supernatant dried down under nitrogen and reconstituted with water containing formic acid. The samples were mixed and injected onto an Agilent 1290/AB Sciex QTRAP 7500 LC MS/MS system equipped with a BEH C18 reversed phase UHPLC column. The flow rate was 625 µL/min. The mass spectrometer was operated in negative mode using electrospray ionization (ESI). The peak areas of the respective analyte product ions were measured against the peak area of the corresponding internal standard product ions (e.g., DHFA, AVAs). Quantitation was performed using a linear regression with a 1/x weighting generated from fortified calibration standards prepared immediately prior to each run. LC-MS/MS raw data were collected and processed using AB SCIEX software Analyst 1.6.3 and processed using SCIEX OS-MQ software v1.7. Data reduction was performed using Microsoft Excel for Office 365 v.16.[258] Details on quality control sample performance is provided in **Table 3-1**. Ferulic acid was not a validated analyte in this assay and has no quality control.

Table 3-1: Quality control (QC) sample performance for AVAs and DHFA

Analyte name	QC Low		QC Medium		QC High	
	Calculated concentration (ng/mL)	Accuracy (%)	Calculated concentration (ng/mL)	Accuracy (%)	Calculated concentration (ng/mL)	Accuracy (%)
AVA A	0.652	102	1.47	112	7.30	105
AVA B	0.548	95.2	1.29	100	6.31	93.6
AVA C	0.626	82.0	1.49	91.3	7.24	89.1
DHFA	12.5	97.6	54.5	107	74.9	106

Abbreviations: AVA, avenanthramide; DHFA, dihydroferulic acid.

3.14.2 Plasma Short-Chain Fatty Acids

Plasma acetic acid (C2), propionic acid (C3), isobutyric acid (C4), butyric acid (C4), 2-methylbutyric acid (C5), isovaleric acid (C5), valeric acid (C5), and hexanoic acid (caproic acid, C6) were quantified in a targeted multiplex panel by LC-MS/MS using a quantitative lipids platform according to Metabolon Method TAM148 ("LC-MS/MS Method for the quantitation of short chain fatty acid (C2 to C6) in human plasma and serum"; Metabolon Inc., Morrisville, NC, USA) as previously described.[259] Quality control samples met the acceptance criteria for all analytes at all levels (**Table 3-2**).

Table 3-2: Quality control (QC) sample performance for SCFAs

Analyte name	QC Low		QC Medium		QC High	
	Average (ng/mL)	Accuracy (%)	Average (ng/mL)	Accuracy (%)	Average (ng/mL)	Accuracy (%)
Acetic acid	4200	97.7	29423	92.0	72231	2.5
Propionic acid	384	101.0	3488	95.3	7809	101.0
Butyric acid	89.3	91.5	723	86.6	1861	94.0
Isobutyric acid	88	92.6	831	91.8	1978	97.0
2-Methylbutyric acid	83.2	93.7	811	91.4	1984	95.6
Isovaleric acid	84.3	102.0	741	93.7	1935	95.0
Valeric acid	72.4	85.8	767	85.2	1871	87.0
Hexanoic acid	163	96.0	1542	91.3	3976	98.6

Abbreviation: SCFAs, short-chain fatty acids.

3.15 Global Metabolomic Profiling

For both fasting plasma and fecal samples, non-targeted global metabolomic profiles before and after each intervention period were generated by Metabolon Inc. (Research Triangle) using ultra-high-performance liquid chromatography coupled with tandem high-resolution mass spectrometry (UPLC-MS/MS), as previously described.[260,261] The metabolomic dataset of both intervention studies comprised a total of 1149 and 1082 metabolites in plasma and feces, respectively. In the supernatant of the batch culture experiment a total of 995 metabolites was identified. This includes amino acids, peptides, carbohydrates, energy intermediates, lipids, nucleotides, cofactors and vitamins, xenobiotics, and partially characterized molecules of both endogenous and an established microbial origin.[262] After removing drug-associated metabolites, 1128 plasma metabolites and 1054 fecal metabolites (applies to both interventions) were included in the analyses.

3.16 Gut Microbiome Sample Processing

Total genomic DNA was extracted from 120 mg fecal material or from pelleted material from 700 µl cell suspension using ZR BashingBead lysis tubes (0.1 and 0.5 mm, Zymo Research, Freiburg, Germany) in combination with the Chemagic DNA stool kit (Perkin Elmer, Rodgau, Germany) following the manufacturer's instructions.[263–265] After the addition of lysis buffer, mechanical lysis was performed using a Precellys 24 tissue homogeniser (Bertin Instruments, Frankfurt am Main, Germany). After extraction, the DNA was stored at –20 °C until further analysis. High-throughput 16S rRNA amplicon sequencing of the fecal microbiome was performed at Life & Brain GmbH (Bonn, Germany). Briefly, the V3/V4 region of the 16S rRNA gene was amplified using the Bakt_341F and Bakt_805R primer combination. Details of the library preparation have been described previously.[266] The final pool was quantified using the Qubit dsDNA HS assay kit (Thermo Fisher Scientific, Waltham, MA, USA), and the fragment size was checked using a D1000 ScreenTape (Agilent, Satna Clara, CA, USA).

Sequencing was performed on an Illumina MiSeq system using the MiSeq reagent kit v3 with 2 × 300 cycles. Clustering was performed at 8 pM with a 20% spike-in of PhiX. Demultiplexing was performed using the MiSeq system. The 16S rRNA

sequencing data were processed using QIIME 2 version 2021.4.[267] Sequence quality control and denoising were performed using DADA2.[268] The QC step included the filtering of PhiX reads and chimeric sequences. After denoising, sequences were classified using the SILVA databases to identify amplicon sequencing variants (ASVs) for sequences with > 99% sequence similarity.

3.17 Gut Microbiome Analysis

Diversity analyses were performed in QIIME 2 based on a rarefied table with a sampling depth of 28,767 sequences (short-term intervention) and 21,876 sequences (six-week intervention), respectively, and were used for subsequent statistical analysis. The alpha diversity metrics included Shannon entropy, Pielou's evenness, and Faith's phylogenetic diversity (Faith-PD). A linear mixed model (LMM) was applied to assess the differences between the two diet groups in each study. This statistical method was used to address the natural complexity of microbiome data. The LMM was adjusted for confounding factors and used the corresponding control group (CG or CG^{6w}) and the baseline value as reference ($LMM^{adj.}$). The Person-ID was used as random effect to account for repeating measurements and a person-specific offset. The model was defined as: alpha diversity index ~ time + group + time*group + age + sex + BMI + (1|Person-ID), with the predictor of interest 'time*group'. Beta diversity was calculated based on the Jaccard distance, Bray-Curtis dissimilarity, and UniFrac distance matrices. To determine the differences between the diet groups in each study, a linear regression analysis adjusted for confounding factors was applied, using the control group of each study (CG or CG^{6w}) as the reference ($LnR^{adj.}$). As the distance metric for beta diversity is relative to the offset (baseline), the random effect was not applied. The model was defined as: beta diversity matrix ~ group + age + sex + BMI, with the predictor of interest 'group'.

To identify differences between the diet groups of the RCTs at feature level, linear models for differential abundance analysis (LinDA)[269], an established state-of-the-art method for analyzing microbiome data, was applied. The control group of each study (CG or CG^{6w}) and the baseline value were used as reference and the Person-ID was used as random effect. The model was defined as: taxon ~ time + group + time*group + age + sex + BMI + (1IPerson-ID), with the covariate of interest

'time*group'. The analyses were performed at genus level with a relative abundance threshold of 0.01%. Data were normalized using a centered-log-ratio (CLR) transformation with an offset of 1 prior to all analyses. Twenty-eight participants from the short-term intervention (*n* = 4 dropouts [3 female, 1 male] due to an incomplete data set) and all 34 participants from the six-week intervention were included in the respective analysis.

Phylogenetic Investigation of Communities by Reconstruction of Unobserved States 2 (PICRUSt2; version 2.5.1)[270] was used to predict the functional potential of the fecal microbiota. Such functional prediction tools play a valuable role in facilitating preliminary hypothesis testing based on amplicon sequences; however, this method has inherent limitations in accurately reproducing functional potential. It should be acknowledged that the prediction model based on 16S or shotgun sequencing data does not confirm whether functional capacity is activated at the mRNA or protein level. Given the prohibitive cost of shotgun sequencing, functional prediction tools serve as a valid guide for subsequent studies of actual functional capacity. Predicted gene products in each bacterium were annotated using the Kyoto Encyclopedia of Genes and Genomes (KEGG) database and classified into KEGG orthologous (KO) groups[271], resulting in the identification of 6,616 KO (short-term intervention: *n* = 28) and 6,424 KO (six-week intervention: *n* = 34) across all samples. The ko2kegg_abundance function of the ggpicrust2 package[272] was used for this task. In accordance with LinDA, a LMM was applied to the CLR-transformed count data with the control group of each study (CG or CG^{6w}) and the baseline value as reference and the Person-ID as random effect ($LMM^{adj.}$). The model was defined as: pathway ~ time*group + age + sex + BMI + (1|Person ID), with the covariate of interest 'time*group'. The analyses were performed with a relative abundance threshold of 0.01%.

3.18 Sparse Partial Least Squares-Discriminant Analysis

Sparse partial least squares-discriminant analysis (sPLS-DA) was performed (i) to identify key features that differentiate the two study groups in each intervention in terms of diet-induced changes in clinical markers, microbial composition, microbial functional potential, and global metabolomic profiles in plasma and feces, and (ii) to identify differences in baseline microbial composition between responders

and non-responders based on outcomes in the OG as part of the subgroup analysis. sPLS-DA, implemented in the *mixOmics* R package version 6.22.0, enables the selection of the most discriminative features in the data to classify the samples by projecting the data into a lower dimensional space in a supervised manner.[273,274] This approach is optimal if the number of features is very high compared to the sample size, as in this present work. The performance of the sPLS-DA was assessed on the balanced error rate (BER) and AUC. The results were visualized using two-component sample plots and loadings.

3.18.1 Data Preprocessing

For the targeted plasma metabolomic profiles, analyte concentrations that fell below the limit of quantitation–in case of DHFA, this means above the peak area of the background DHFA peak in the blank samples–or concentrations above the limit of quantitation were extrapolated. Missing values in the global metabolomics profiles were imputed with the minimum observed value for each compound.

Metabolic data were transformed with the natural logarithm prior to the analysis, except for the data of the subgroup analysis (zonulin, LPS, hsCRP, IL-6, SCFAs), for which no transformation was done. The DHFA and FA data obtained through targeted metabolomic profiling as well as the data obtained through global metabolomic profiling were transformed with the natural logarithm prior to the analysis. SCFA data as part of the subgroup analysis remained untransformed. Microbiome data were normalized by using CLR transformation with an offset of 1. The analysis was performed at genus level with a relative abundance threshold of 0.01%. Participants without post-intervention microbiome data were excluded (short-term intervention: $n = 4$).

To account for the repeated-measures design of both intervention studies, the logarithmic fold change (log fold change) was used as the input to the models, except for the metabolic data of the subgroup analysis (zonulin, LPS, hsCRP, IL-6, SCFAs) for which the absolute change (pre-intervention value subtracted from the post-intervention value) was applied, based on Lee *et al*.[275].

3.18.2 Procedure

The number of features and PLS components was chosen via stratified 5-fold cross-validation with 25 random repeats or via 10-fold cross-validation repeated 50 times (subgroup analysis), according to the best-balanced classification error rate (BER). The models were calibrated using the least absolute shrinkage and selection operator (LASSO)[276] to minimize the number of features per component while maximizing class discrimination. To identify distinctive baseline features between responders and non-responders as part of the subgroup analysis, the sPLS-DA model was built using LASSO and leave-one-out cross-validation. Due to an unequal sex distribution between responders and non-responders, the 'removeBatchEffect' function was used from the limma package[277] to remove the confounding effect of sex while preserving the effect of the study group. Detailed information on the workflow is provided in the tutorials of *mixOmics* (http://mixomics.org/case-studies/).

3.19 Integrative Multi-Omics Analysis

To determine the relationship between diet-induced changes in clinical markers, gut microbiota, and targeted and global metabolomic profiles, Data Integration Analysis for Biomarker discovery using Latent cOmponents (DIABLO) was applied for each study separately. This integrative multi-omics method extends sparse generalized canonical correlation analysis (sGCCA)[278] to the sPLS-DA framework and aims to identify common information across different types of data while distinguishing between different phenotypes[279], in this study between the diet groups of each intervention trial.

In total, three different DIABLO evaluations ("models") were performed. For model 1, clinical marker, metabolomic profiles including targeted plasma (DHFA, FA), global plasma and global fecal profiles, and microbial composition at genus level were used as inputs (short-term intervention: model 1.1; six-week intervention: model 1.2). Model 2 considered the functional potential of the microbiota instead of compositional data (short-term intervention: model 2.1; six-week intervention: model 2.2). Model 3 was performed as part of the subgroup analysis and included the markers of gut permeability and inflammatory status, plasma SCFAs, and

microbial composition at genus level (short-term intervention: model 3.1; six-week intervention: model 3.2). To account for the repeated-measure design of the intervention studies, the log fold change of the preprocessed data or the absolute change (subgroup analysis) was used as input to the models (see **Chapter 3.18**). The performance of the final models was assessed on the BER and AUC. The results were visualized using Circos plots (correlation cutoff: $r = \pm 0.7$ or $r = \pm 0.6$ [subgroup analysis]).

An initial partial least square (PLS) analysis between the five data sets of model 1 showed correlations in the range of 0.66 to 0.96 (model 1.1: short-term intervention) and 0.61 to 0.93 (model 1.2: six-week intervention), therefore a square matrix filled with 0.75 and diagonal set to zeros was used as an input to the design parameter of the DIABLO model. The correlations for model 2, which included KEGG pathways instead of bacterial genera, ranged from 0.63 to 0.96 (model 2.1: short-term intervention) and from 0.35 to 0.93 (model 2.2: six-week intervention). Thus, the same input was used for the design parameters of this DIABLO model. Similarly, the models 3.1 and 3.2 were built with a design matrix of 0.75, as initial pairwise PLS comparisons between the data sets showed correlations ranging 0.65 – 0.80 (model 3.1: short-term intervention) and 0.47–0.80 (model 3.2: six-week intervention). Tuning of the DIABLO model was done similar to the tuning of the sPLS-DA model as described in **Chapter 3.18.2**. Detailed information on the workflow can be found in the tutorials of *mixOmics* (http://mixomics.org/mixdiablo/diablo-tcga-case-study/).

3.20 Partial Least Square Regression Model

To indicate the predictive value of changes in the gut microbiome and metabolomic profile in modulating host metabolism, particularly cholesterol levels, PLS regression models were applied.[280] The models were implemented using the *mixOmics* R package version 4.2.2. The analyses were performed without sparse mode, i.e. all predictor variables of interest identified in the previous DIABLO models ($n = 9$ phenolic compounds, $n = 2$ bacterial, genera, $n = 1$ microbial pathway; cutoff $r = \pm 0.7$) were included in the models without variable selection. R^2 and Q^2 total (cross-validated R^2), calculated by leave-one-out cross-validation, were used to assess the fit and predictability of the model, respectively.[280] The log fold change of

the features of interest was used as the input for the predictor and response variables (see **Chapter 3.18**).

As part of the subgroup analysis, the regression mode of PLS was applied, using the shift in microbiome composition to explain ("predict") the changes in the blood parameters (zonulin, LPS, hsCRP, IL-6, and SCFAs) in both oat groups. The model was built with LASSO[276] and leave-one-out cross-validation. The log fold change of the preprocessed microbiome data and the absolute change of the blood parameters (see **Chapter 3.18**) were used as the input for these models

3.21 Statistics and Sample Size Calculation

Statistical analyses were performed using SPSS (version 29.0; IBM Crop., Chicago, IL, USA), R (version 3.6.2; Boston, MA, USA) and Python (version 3.10). Figures were created using R and GraphPad Prism (version 10; GraphPad Inc., San Diego, CA, USA).

For all analyses, a two- tailed significance level was set at $P < 0.05$. For metabolic data analysis, family-wise error rate correction was applied via the block-wise Bonferroni-Holm method[281] to correct for multiple testing ($P^{adj.} < 0.05$). This stringent multiple comparison method is particularly suitable for hypothesis-driven analyses as in the present case. For microbiome and global metabolomic data with a high noise-to-signal ratio, false discovery rate (FDR) correction based on the Benjamini/Hochberg method[282] was applied ($q < 0.05$), which is frequently used for high-throughput data.

Prior to the analysis, the distribution of the data was visualized individually. Additionally, Shapiro-Wilk tests were performed to test for normality of the data and determine the appropriate approach for further analysis. Variables were analyzed either on their original scale or logarithmic transformed to approximate a normal distribution. Postprandial results were described as the AUC for the 3 h OGTT, calculated using the trapezoidal method and only complete data sets.

To assess whether the data provided evidence of the superiority of the oat diets over the respective control in terms of the primary outcome and the metabolic and metabolomic outcomes, linear regression models (LnR) were applied including the baseline value and the confounding factors BMI, age, and sex ($LnR^{adj.}$) as

covariates. The control group of each study (CG or CG^{6w}) was used as reference. The model was defined as: “post-intervention_value ~ baseline_value + group + age + sex + BMI”. Beta estimates of intervention (β) with 95% confidence interval (CI) were reported to assess the magnitude of the effect size. In addition, a LMM adjusted for BMI, age, and sex ($LMM^{adj.}$) was used to assess differences in clinical markers across all four time points of the six-week intervention study. The control group of each study (CG or CG^{6w}) and the baseline value were used as reference, the Person-ID was used as random effect to account for repeating measurements. The model was defined as: clinical marker ~ time*group + age + sex + BMI + (1IPerson-ID), with the covariate of interest ‘time*group’. Residuals obtained from the models were inspected for normality to control for the fit of the statistical model. If the residuals were not normally distributed, log transformation was applied.

In case of the subgroup analysis, LnR models were fitted to evaluate whether the oat diets were superior to the corresponding control diets in terms of intestinal permeability, inflammation, and plasma SCFA response. The models were adjusted for (i) the baseline response value and (ii) additionally for sex, and the respective control group was selected as the reference group. Beta estimates of the intervention (β) with 95% CI, obtained by bias corrected and accelerated (BCa) bootstrapping method with 1,000 BCa-samples, were used to evaluate the magnitude of the effect size.

To determine the changes over time within each study group of the RCTs (pre- vs. post-intervention), a paired Student’s *t*-test (or Wilcoxon rank test) was performed depending on the data distribution. In addition, to account for the changes within the OG including the follow-up period, a LMM adjusted for BMI, age, and sex ($LMM^{adj.}$) was build using the baseline value as reference and the Person-ID as random effect.

Pairwise correlations between diet-induced altered clinical markers and changes in microbiome and metabolomic profiles were assessed using Pearson's and Spearman's rank correlation coefficient. Only variables with at least five data points were included, using the log fold change for analysis. Pairwise correlations were primarily intended to support the DIABLO results; therefore, no FDR correction was applied. In the case of the subgroup analysis, pairwise correlations between diet-induced altered clinical markers and changes SCFAs were assessed using Spearman's rank correlation coefficient.

Sample size calculation

Sample size (*n* = 17 participants per group in each study) was calculated based on data from a previous intervention study that successfully assessed the effect of whole grain intake on blood DHFA concentration[283], expecting a 2-fold change in the plasma DHFA concentration between the two diet groups in each intervention study using a two-sided *t*-test at a 5% significance level and with 95% power.

In case of the subgroup analysis, a recent study investigated the effects of GI nutrition therapy on serum zonulin and IL-6 levels and found large effect sizes for the differences between the nutrition and control groups after the intervention (Cohen's d >1.6).[284] Assuming an effect size of Cohen's d = 1.6 for each of the four outcomes (zonulin, LPS, hsCRP, and IL-6) and type I error probability $\alpha = 0.0125$ (0.05/4, Bonferroni corrected for multiple testing), 22 participants were estimated to provide 80% power with independent-samples two-sided *t*-tests.

4 RESULTS

4.1 Baseline Characteristics

In the short-term intervention, 32 of 34 participants were considered for the statistical analysis (OG: *n* = 17, CG: *n* = 15; **Figure 4-1a**) which included 15 males and 17 females aged 58.9 ± 7.4 years with a BMI of 32.0 ± 3.3 kg/m². Two male participants withdrew from the study for personal reasons. All participants had central obesity (100%) and at least two further MetS traits including increased BP (100%), impaired glucose metabolism (75%) and dyslipidemia (63%). Detailed information on the participants' baseline characteristics is provided in **Table 4-1**.

In the six-week intervention, all 34 participants, which included 15 males and 19 females aged 59.7 ± 7.6 years with a BMI of 31.6 ± 3.2 kg/m², completed the study and were considered for the statistical analysis (**Figure 4-1b**). According to the inclusion criteria, all participants had central obesity (100%) and at least two further MetS traits at the screening visit. Thus, at baseline, 97% of the subjects had elevated BP, 76% impaired glucose metabolism, and 59% dyslipidemia. Detailed information on the participants' baseline characteristics is provided in **Table 4-2**.

The habitual dietary behavior of the participants corresponded to a Western dietary pattern according to the inclusion criteria (**Table 4-3**).

Adherence to the study diets and tolerability were recorded, showing a compliancy rate of 99% (short-term intervention) and 89% (six-week intervention) and overall good tolerability with no severe side effects related to the diets.

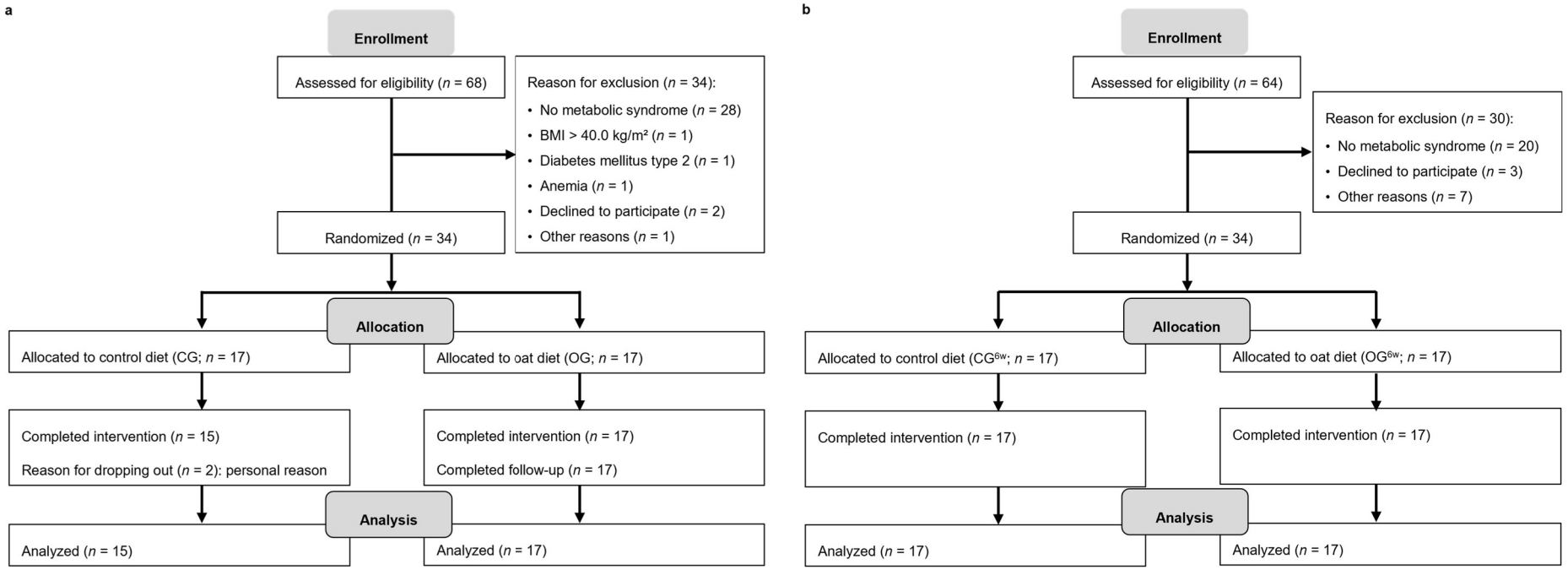

Figure 4-1: Participants flow diagram

a Participants flow diagram of the short-term intervention according CONSORT flow diagram. Out of 68 expressions of interest, 34 participants met the criteria of MetS per definition and were randomly assigned 1:1 to the study groups. A total of 32 participants completed the study with two dropouts (both males) in CG for personal reasons and were included in the final analysis. **b** Participants flow diagram of the six-week intervention according CONSORT flow diagram. Out of 64 expressions of interest, 34 participants met the criteria of MetS per definition and were randomly assigned 1:1 to the study groups. All participants completed the study and were included in the final analysis.

Table 4-1: Metabolic baseline characteristics of the participants in the short-term intervention

	Total (*n* = 32)	CG (*n* = 15)	OG (*n* = 17)	Δ	*P*
General information					
Age, years	60.0 (14.0)	61.0 (9.0)	60.0 (13.0)	-	0.766[1]
Sex, %male	47	60	35	-	0.287[2]
MET score[a]	1,413 (1,242)	1,025 (1,078)	1,605 (846)	-	0.173[1]
MSFsc[b]	3.9 ± 0.9	3.5 ± 0.8	4.2 ± 0.8	-0.6 ± 0.3	0.079
Anthropometrics					
Weight, kg	94.1 ± 12.2	97.3 ± 12.8	91.2 ± 11.2	6.1 ± 4.2	0.158
BMI, kg/m²	32.0 ± 3.3	32.8 ± 3.6	31.3 ± 2.9	1.5 ± 1.2	0.211
WC, cm	106.6 ± 9.4	111.0 ± 8.8	102.8 ± 8.3	8.1 ± 3.0	**0.012***
WHtR	0.62 ± 0.05	0.65 ± 0.06	0.60 ± 0.09	0.04 ± 0.02	**0.020***
Body fat, %	38.9 (10.6)	36.6 (10.7)	40.1 (10.9)	-	0.906
REE[c], kcal/d	1,834 ± 287	1,937 ± 309	1,738 ± 235	199 ± 98	0.052
SBP, mmHg	143.3 ± 15.0	148.5 ± 18.3	138.7 ± 9.7	9.8 ± 5.3	0.077
DBP, mmHg	93.9 ± 12.1	93.9 ± 16.2	93.8 ± 7.2	0.1 ± 4.5	0.987
Pulse, beats/min	62.3 (11.8)	63.0 (16.3)	62.0 (8.5)	-	0.439
Lipid metabolism					
TC, mg/dL	223.1 ± 51.2	205.3 ± 30.6	238.8 ± 60.8	-33.5 ± 16.7	0.056
LDL-C, mg/dL	153.4 ± 44.1	135.9 ± 28.6	168.9 ± 50.1	-33.1 ± 14.2	**0.028***
HDL-C, mg/dL	50.3 ± 9.8	48.9 ± 7.9	51.5 ± 11.3	2.6 ± 3.5	0.463
TG, mg/dL	152.5 (65)	153.0 (97.0)	152.0 (49.0)	-	0.972
NEFAs, mmol/L	0.69 (0.38)	0.67 (0.54)	0.70 (0.29)	-	0.943
Lipase, U/L	35.6 ± 9.1	35.6 ± 8.3	35.6 ± 10.1	0.0 ± 3.3	0.997
Bilirubin, mg/dL	0.58 (0.26)	0.58 (0.18)	0.51 (0.41)	-	0.544
γ-GT, U/L	30.0 (42.5)	38.0 (68.0)	29.0 (26.0)	-	0.356
ALT, U/L	31.3 ± 13.2	38.0 ± 15.2	25.5 ± 7.7	12.5 ± 4.2	**0.005****
AST, U/L	25.0 (13.8)	30.0 (13.0)	19.0 (9.5)	-	**0.031***
Glucose metabolism					
Glucose, mg/dL	98.7 ± 10.0	99.5 ± 10.4	98.0 ± 9.8	1.5 ± 3.6	0.685
Insulin, mU/L	15.8 (11.9)	18.4 (10.6)	14.1 (11.7)	-	0.356
HOMA-IR	3.9 (3.1)	4.1 (2.3)	3.3 (3.7)	-	0.348
HbA1c, %	5.6 ± 0.3	5.5 ± 0.2	5.5 ± 0.3	0.0 ± 0.1	0.950
hsCRP, mg/dL	2.22 (2.95)	1.8 (3.4)	2.3 (2.9)	-	0.463
Kidney function					
Creatinine, mg/dL	0.82 (0.25)	0.84 (0.19)	0.80 (0.26)	-	0.999
GFR, mL/min	86.5 ± 13.9	88.2 ± 9.7	85.0 ± 16.9	3.2 ± 4.8	0.513
Urea, mg/dL	30.3 (12.0)	30.7 (13.7)	29.8 (11.5)	-	0.843
Uric acid, mg/dL	6.1 ± 1.3	6.3 ± 1.2	5.9 ± 1.4	0.4 ± 0.5	0.396

Table 4-1: Metabolic baseline characteristics of the participants in the short-term intervention

Data are presented as mean ± SD or median (IQR) for continuous variables depending on data distribution, and as frequencies for count data. Mean differences (Δ) ± differences for standard error refer to unpaired Student's *t*-test with original data. *P*-values refer to unpaired Student's *t*-test performed with original or log-transformed data or Mann-Whitney-U test ([1]) depending on data distribution, or Fisher exact test ([2]). [a]*n* = 31 (CG: *n* = 14, OG: *n* = 17). [b]*n* = 25 (CG: *n* = 10, OG: *n* = 15). [c]*n* = 31 (CG: *n* = 15, OG: *n* = 16).

Abbreviations: ALT, alanine transaminase; AST, aspartate transaminase; BMI, body mass index; DBP, diastolic blood pressure; CG, control group; GFR, glomerular filtration rate; HbA1c, glycated hemoglobin; HDL-C, high-density lipoprotein cholesterol; HOMA-IR, homeostasis model assessment-estimated insulin resistance; hsCRP, high-sensitivity C-reactive protein; LDL-C, low-density lipoprotein cholesterol; MET, metabolic equivalent; MSFsc, corrected mid sleep on free days (chronotype); NEFAs, non-esterified fatty acids; OG, oat group; REE, resting energy expenditure; SBP, systolic blood pressure; TC, total cholesterol; TG, triglycerides; WC, waist circumference; WHtR, waist-to-height ratio; γ-GT; gamma-glutamyl transferase.

Table 4-2: Metabolic baseline characteristics of the participants in the six-week intervention

	Total (*n* = 34)	CG^{6w} (*n* = 17)	OG^{6w} (*n* = 17)	Δ	*P*
General information					
Age, years	59.7 ± 7.6	60.7 ± 7.2	58.7 ± 8.0	2.1 ± 2.6	0.436
Sex, %male	44	47	41	-	1.000[2]
MET score	1,519 (1,779)	1,544 (1,269)	1,038 (2,091)	-	0.350
MSFsc[a]	3.8 (1.3)	4.1 (0.9)	3.4 (0.9)	-	0.067[1]
Anthropometrics					
Weight, kg	94.2 ± 14.2	94.4 ± 16.6	94.0 ± 11.8	0.3 ± 4.9	0.941
BMI, kg/m²	31.6 ± 3.2	31.4 ± 3.6	31.9 ± 2.8	-0.4 ± 1.1	0.691
WC, cm	106.4 (8.4)	105.8 (7.5)	108.0 (9.0)	-	0.394[1]
WHtR	0.62 ± 0.04	0.62 ± 0.04	0.62 ± 0.04	0.00 ± 0.01	0.737
Body fat[b], %	41.5 ± 7.7	40.4 ± 8.5	42.4 ± 7.0	-2.0 ± 2.7	0.466
REE, kcal/d	1807.5 (520.0)	1,799.0 (534.0)	1,816.0 (525.5)	-	0.936[1]
SBP, mmHg	136.1 ± 12.1	137.2 ± 11.5	134.9 ± 12.8	2.3 ± 4.2	0.582
DBP, mmHg	87.3 ± 9.6	86.5 ± 10.0	88.0 ± 9.4	-1.5 ± 3.3	0.654
Pulse, beats/min	63.0 ± 9.8	62.5 ± 10.0	63.4 ± 10.0	-0.9 ± 3.4	0.792
Lipid metabolism					
TC, mg/dL	211.8 ± 44.1	212.4 ± 42.6	211.2 ± 46.7	1.2 ± 15.3	0.936
LDL-C, mg/dL	139.1 ± 37.5	138.5 ± 37.9	139.7 ± 38.2	-1.2 ± 13.0	0.929
HDL-C, mg/dL	49.0 (20.8)	54.5 ± 21.5	50.2 ± 12.4	4.4 ± 6.0	0.476
TG, mg/dL	137.0 (69.0)	147.0 (88.5)	135.0 (56.5)	-	0.746
NEFAs, mmol/L	0.57(0.30)	0.57 (0.32)	0.59 (0.37)	-	0.783
Lipase, U/L	31.0 (15.5)	30.0 (15.0)	31.0 (16.0)	-	0.724
Bilirubin, mg/dL	0.50 (0.36)	0.54 (0.40)	0.49 (0.33)	-	0.634[1]
γ-GT, U/L	21.5 (17.5)	21.9 ± 9.7	26.1 ± 13.6	-4.1 ± 4.1	0.318
ALT, U/L	24.0 (14.0)	17.0 (13.0)	25.0 (25.5)	-	0.019*
AST, U/L	25.0 (10.0)	23.0 (10.5)	25.0 (10.0)	-	0.369
Glucose metabolism					
Glucose, mg/dL	97.5 (13.5)	94.3 ± 5.8	105.1 ± 16.3	-10.8 ± 4.2	0.018*
Insulin, mU/L	14.4 (8.8)	14.0 (9.3)	14.9 (7.5)	-	0.608
HOMA-IR	3.6 (2.3)	3.1 (2.1)	3.9 (2.4)	-	0.324
HbA1c, %	5.6 (0.3)	5.6 (0.5)	5.6 (0.6)	-	0.245[1]
hsCRP, mg/dL	2.0 (3.5)	1.9 (2.5)	2.6 (4.2)	-	0.838
Kidney function					
Creatinine, mg/dL	0.86 ± 0.15	0.89 ± 0.16	0.82 ± 0.13	0.07 ± 0.05	0.167
GFR, mL/min	84.5 ± 10.2	81.2 ± 10.0	87.8 ± 9.6	-6.52 ± 3.36	0.061
Urea, mg/dL	29.7 ± 7.0	28.9 ± 7.4	30.5 ± 6.6	-1.61 ± 2.4	0.509
Uric acid, mg/dL	5.8 ± 1.4	5.8 ± 1.6	5.7 ± 1.1	0.15 ± 0.47	0.749

Table 4-2: Metabolic baseline characteristics of the participants in the six-week intervention

Data are presented as mean ± SD or median (IQR) for continuous variables depending on data distribution, and as frequencies for count data. Mean differences (∆) ± differences for standard error refer to unpaired Student's *t*-test with original data. *P*-values refer to unpaired Student's *t*-test performed with original or log-transformed data or Mann-Whitney-U test ([1]) depending on data distribution, or Fisher exact test ([2]). [a]*n* = 30 (CG^{6w}: *n* = 15, OG^{6w}: *n* = 15). [b]*n* = 33 (CG^{6w}: *n* = 16, OG^{6w}: *n* = 17).

Abbreviations: ALT, alanine transaminase; AST, aspartate transaminase; BMI, body mass index; CG^{6w}, six-week control group; DBP, diastolic blood pressure; GFR, glomerular filtration rate; HbA1c, glycated hemoglobin; HDL-C, high-density lipoprotein cholesterol; HOMA-IR, homeostasis model assessment-estimated insulin resistance; hsCRP, high-sensitivity C-reactive protein; LDL-C, low-density lipoprotein cholesterol; MET, metabolic equivalent; MSFsc, corrected mid sleep on free days (chronotype); NEFAs, non-esterified fatty acids; OG^{6w}, six-week oat group; REE, resting energy expenditure; SBP, systolic blood pressure; TC, total cholesterol; TG, triglycerides; WC, waist circumference; WHtR, waist-to-height ratio; γ-GT; gamma-glutamyl transferase.

Table 4-3: Habitual diet of the participants

	Short-term intervention					Six-week intervention				
	Total (n = 32)	**CG (n = 15)**	**OG (n = 17)**	**Δ**	***P***	**Total (*n* = 34)**	**CG^{6w} (*n* = 17)**	**OG^{6w} (*n* = 17)**	**Δ**	***P***
Total energy, kcal/d	2,050 (905)	2,099 (1,549)	1,964 (769)	0.05 ± 0.05	0.357	2,318.8 ± 689.7	2,311.8 ± 631.5	2,325.9 ± 763.1	-14.0 ± 240.2	0.954
Total protein, g/d	77.2 (34.22)	90.6 ± 34.2	76.8 ± 18.9	13.7 ± 9.6	0.163	83.0 ± 22.0	82.9 ± 22.5	83.2 ± 22.2	-0.3 ± 7.7	0.965
Total fat, g/d	102.9 ± 32.1	110.2 ± 37.9	96.5 ± 25.5	13.7 ± 11.3	0.235	109.9 ± 33.1	108.2 ± 29.7	111.7 ± 37.0	-3.5 ± 11.5	0.764
Total CH, g/d	176.0 (112.2)	207.6 (131.3)	172.1 (82.7)		0.478	211.5 (100.0)	208.0 (96.6)	214.9 (102.6)	-0.00 ± 0.05	0.928
Total fiber, g/d	18.8 (8.1)	18.8 (10.1)	18.8 (6.6)	0.05 ± 0.05	0.289	19.6 (9.7)	19.4 ± 5.3	21.3 ± 7.8	-1.9 ± 2.3	0.416
Energy%										
Protein	15.3 ± 2.4	15.4 ± 2.4	15.2 ± 2.5	0.2 ± 0.9	0.796	14.9 ± 1.7	14.7 ± 1.5	15.0 ± 1.9	-0.2 ± 0.6	0.681
Fat	42.4 (5.0)	43.4 (3.0)	42.2 (6.9)		0.628	43.3 (6.3)	42.3 (4.6)	44.0 (6.6)	-0.01 ± 0.02	0.509
CH	37.7 ± 5.6	37.7 ± 6.9	37.7 ± 4.2	0.0 ± 2.0	0.990	38.2 ± 5.5	37.7 ± 4.6	38.7 ± 6.3	-1.0 ± 1.9	0.602

Habitual diet of the subjects was assessed with food frequency questionnaire (FFQ). Data are presented as mean ± SD or median (IQR) depending on data distribution. Mean differences (Δ) ± differences for standard errors refer to unpaired Student's *t*-test performed with original or log-transformed data depending on data distribution. *P*-values refer to unpaired Student's *t*-test with original or log-transformed data and Mann-Whitney-U test depending on data distribution.

Abbreviations: CG, control group; CG^{6w}, six-week control group; CH, carbohydrates; OG, oat group; OG^{6w}, six-week out group.

4.2 Plasma Dihydroferulic and Ferulic acids

According to the primary hypothesis, significant higher plasma levels of FA (0.64 [0.26, 1.02] (β estimate [95% CI]), *P* = 0.002, $LnR^{adj.}$; **Figure 4-2a**) and DHFA (1.23 [0.44, 2.01], *P* = 0.003; **Figure 4-2b**) were observed in OG compared to CG after the two-day intervention period. Consistent with this, an increase in plasma FA level was observed in OG^{6w} compared with CG^{6w} after the six-week intervention period (0.55 [0.21, 0.89], *P* = 0.003; **Figure 4-2c**); however, no difference in DHFA level was found (0.63 [-0.12, 1.37], *P* = 0.096; **Figure 4-2d**). These results therefore suggest that both the short-term, high-dose and the six-week, moderate oat diet have a systemic effect. This effect is more pronounced and potentially stronger if related to the gut microbiota as shown in the short-term intervention due to the increase in DHFA, a major microbial metabolite of FA[285].

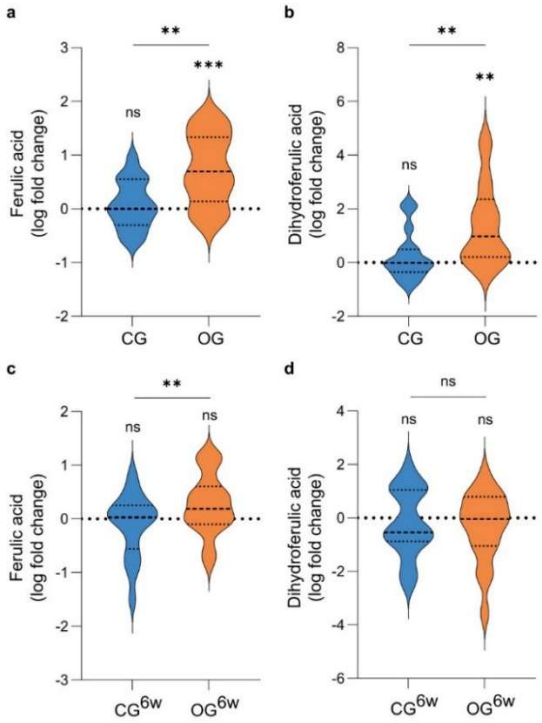

Figure 4-2: Oat diets increased plasma (dihydro)ferulic acid levels

Diet-induced changes in (**a**) plasma ferulic acid and (**b**) plasma dihydroferulic acid levels in OG (*n* = 17) and CG (*n* = 15). Diet-induced changes in (**c**) plasma ferulic acid and (**d**) plasma dihydroferulic acid levels in OG^{6w} (*n* = 17) and CG^{6w} (*n* = 17). (**a**–**d**): Violin plots show the log fold change (dashed center line: median; dotted line: upper and lower quartiles). $^{**}P < 0.01$, $^{*}P < 0.05$, ns $P > 0.05$. Within-group differences were analyzed using paired Student's *t*-test. Between-group differences were analyzed using linear regression models adjusted for baseline concentration, age, sex, BMI ($LnR^{adj.}$). Abbreviations: CG, control group; CG^{6w}, six-week control group; OG, oat group; OG^{6w}, six-week oat group.

4.3 Modulation of Metabolism

Investigating the diet-induced modulation of the metabolism using sPLS-DA, a significant difference between OG and CG was observed after the two-day intervention period (AUC = 0.88 (P = 1.99 x 10^{-4}), BER = 0.154; **Figure 4-3a**), with LDL-C, TC, and diastolic BP being the most important distinguishing features (**Figure 4-3b**). A significant reduction in LDL-C (-16.26 [-23.60, -10.32] mg/dL, $P^{adj.}$ = 0.011 (Bonferroni-Holm)) and TC (-15.61 [-24.17, -7.80] mg/dL, $P^{adj.}$ = 0.02) levels in OG compared with CG were also identified using $LnR^{adj.}$ (**Figure 4-3b,c**). Since cholesterol levels tended to remain below baseline during the six-week, oat-free follow-up period, persistent effects on lipid metabolism might be assumed (**Figure 4-3d**). Thus, the results indicate clearly that a high-dose oat diet improves lipid metabolism by decreasing serum TC and LDL-C levels, even after two days, which is consistent with the known cholesterol-lowering effect of oats[286]. In addition, beneficial effects on anthropometrics, glucose metabolism, as well as on liver and kidney functions were observed within each diet group (**Table 4-4**, **Table 4-5**, **Figure 4-4**). These effects are attributable to the diet-related calorie restriction[287-289] and confirm the participants' adherence to the study diets.

In the six-week intervention study, sPLS-DA revealed no differences in the diet-induced modulation of metabolism between OG^{6w} and CG^{6w} (AUC = 0.58 (P = 0.399), BER = 0.412; **Figure 4-3e**,**f**). Participants' metabolic status, including TC and LDL-C levels (**Figure 4-3g**,**h**), remained largely stable (**Table 4-6**, **Table 4-7**, **Figure 4-5**). This indicates that the consumption of a single oatmeal daily, integrated into a Western diet under isocaloric conditions, has a rather mild effect on metabolism. Thus, along the known positive effects of oats on lipid metabolism[286] observed in OG, the six-week, moderate oat diet under isocaloric conditions stabilized metabolic markers.

Details on the structure of all sPLS-DA models performed, including the number of selected features per component, are shown in **Table 4-8**.

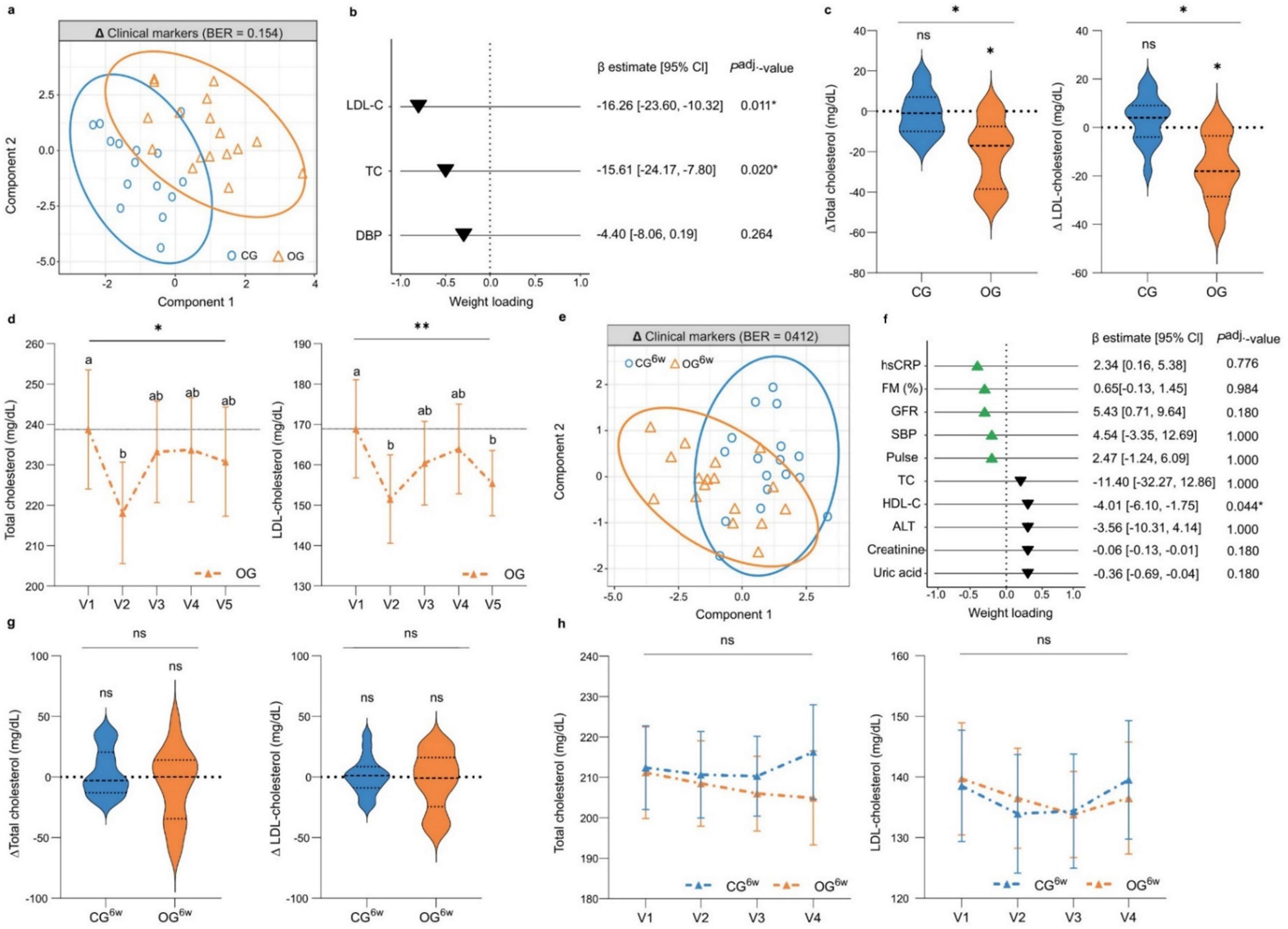

Figure 4-3: Oat diet reduced serum cholesterol levels

Figure 4-3: Oat diet reduced serum cholesterol levels

(**a**) Two-component sample plot of the sPLS-DA on metabolic changes in OG and CG. (**b**) Weight loadings of selected clinical markers in component 1 (green or black triangle: increase or decrease in OG vs. CG). Coefficient and 95% CI were calculated using linear regression models adjusted for baseline concentration, age, sex, BMI ($LnR^{adj.}$). (**c**) Violin plots showing absolute change in total cholesterol and LDL-cholesterol (dashed center line: median; dotted line: upper and lower quartiles). Within-group differences were analyzed using paired Student's *t*-test. Between-group differences were analyzed using linear regression models adjusted for baseline concentration, age, sex, BMI ($LnR^{adj.}$). *$P^{adj.}$ < 0.05, ns $P^{adj.}$ > 0.05. (**d**) Longitudinal course of total cholesterol and LDL-cholesterol levels during the six-week follow-up period. Data are presented as means ± standard error of the mean (SEM). Differences over time were analyzed using LMM adjusted for age, sex, BMI ($LMM^{adj.}$). Different letters indicate a significant difference between the time points. **$P^{adj.}$ < 0.01, *$P^{adj.}$ < 0.05. (**e**) Two-component sample plot of the sPLS-DA on metabolic changes in OG^{6w} and CG^{6w}. (**f**) Weight loadings of the top 10 selected clinical markers in component 1 (green or black triangle: increase or decrease in OG^{6w} vs. CG^{6w}). Coefficient and 95% CI were calculated using linear regression models adjusted for baseline concentration, age, sex, BMI ($LnR^{adj.}$). (**g**) Violin plots showing absolute change in total cholesterol and LDL-cholesterol (dashed center line: median; dotted line: upper and lower quartiles). Within-group differences were analyzed using paired Student's *t*-test. Between-group differences were analyzed using linear regression models adjusted for baseline concentration, age, sex, BMI ($LnR^{adj.}$). ns $P^{adj.}$ > 0.05. (**h**) Longitudinal course of total cholesterol and LDL-cholesterol levels during the six-week intervention period. Data are presented as means ± standard error of the mean (SEM). Between-groups differences were analyzed using LMM adjusted for age, sex, BMI ($LMM^{adj.}$) with the interaction term visit*group. (**a**–**e**) OG: *n* = 17, CG: *n* = 15. (**e**–**h**) OG^{6w}: *n* = 17, CG^{6w}: *n* = 17.

Abbreviations: BER, balanced error rate; CG, control group; CG^{6w}, six-week control group; LDL-C, low-density lipoprotein cholesterol; OG, oat group; OG^{6w}, six-week oat group.

Table 4-4: Fasting clinical markers before and after the short-term intervention

		Within-group comparison[1]					Between-group comparison[2]				
		Before (V1)	After (V2)	Δ	*P*	$P^{adj.3}$		β	95 % CI	*P*	$P^{adj.3}$
Anthropometrics											
BW, kg	CG	97.30 ± 3.31	95.62 ± 3.12	-1.68 ± 0.34	**<0.001*** **	**0.008** **	M1	-0.54	-1.21, -0.08	0.117	0.51
	OG	91.17 ± 2.71	89.23 ± 2.64	-1.94 ± 0.16	**<0.001*** **	**0.008** **	M2	-0.54	-1.32, 0.19	0.155	0.588
BMI, kg/m²	CG	32.78 ± 0.93	32.22 ± 0.89	-0.56 ± 0.11	**<0.001*** **	**0.008** **	M1	-0.18	-0.39, 0.02	0.102	0.51
	OG	31.30 ± 0.71	30.63 ± 0.69	-0.67 ± 0.05	**<0.001*** **	**0.008** **	M2	-0.19	-0.44, 0.02	0.098	0.588
WC, cm	CG	110.96 ± 2.27	110.47 ± 2.07	-0.49 ± 0.43	0.273	>0.999	M1	-1.03	-2.10, -0.06	0.075	0.483
	OG	102.84 ± 2.02	101.94 ± 1.96	-0.90 ± 0.40	**0.042***	0.156	M2	-1.12	-2.47, -0.13	0.130	0.588
WHtR	CG	0.63 (0.09)	0.64 (0.09)	-0.00 (0.01)	0.269	>0.999	M1	-0.01	-0.01, 0.00	0.120	0.51
	OG	0.59 (0.06)	0.57 (0.07)	-0.01 (0.01)	**0.039***	0.156	M2	-0.01	-0.02, -0.00	0.110	0.588
Body fat, %	CG	39.29 ± 1.45	39.37 ± 1.44	0.09 ± 0.17	0.618	>0.999	M1	-0.02	-0.60, 0.54	0.957	>0.999
	OG	39.44 ± 1.75	39.51 ± 1.86	0.1 ± 0.2	0.753	0.753	M2	0.13	-0.48, 0.74	0.663	>0.999
REE, kcal/d	CG	1896 ± 74	1870 ± 72	-26 ± 35	0.478	>0.999	M1	-26.25	-114.72, 56.74	0.508	>0.999
	OG	1738 ± 59	1713 ± 50	-25 ± 22	0.277	0.554	M2	-21.25	-128.60, 117.99	0.585	>0.999
SBP, mmHg	CG	148.52 ± 4.72	142.47 ± 5.14	-6.06 ± 2.17	**0.014***	0.084	M1	-5.85	-12.81, 0.41	0.069	0.483
	OG	138.71 ± 2.35	127.44 ± 2.47	-11.26 ± 1.77	**<0.001*** **	**0.008** **	M2	-7.16	-14.74, 1.29	0.068	0.476
DBP, mmHg	CG	93.90 ± 4.19	90.87 ± 3.80	-3.03 ± 1.17	**0.021***	0.105	M1	-4.84	-8.29, -1.13	**0.009** **	0.072
	OG	93.82 ± 1.74	85.96 ± 2.09	-7.86 ± 1.35	**<0.001*** **	**0.008** **	M2	-4.40	-8.06, 0.19	**0.033***	0.264

		Before (V1)	After (V2)	Δ	*P*	$P^{adj.3}$		β	95 % CI	*P*	$P^{adj.3}$
Lipid metabolism											
TC, mg/dL	CG	205.27 ± 7.89	204.00 ± 6.87	-1.27 ± 2.78	0.655	>0.999	M1	-13.49	-20.94, -6.21	**0.002****	**0.022***
	OG	238.76 ± 14.74	218.12 ± 12.55	-20.65 ± 3.99	**<0.001*****	**0.011***	M2	-15.61	-24.17, -7.80	**0.002****	**0.020***
LDL-C, mg/dL	CG	135.87 ± 7.38	138.93 ± 6.27	3.07 ± 2.53	0.245	>0.999	M1	-15.65	-24.08, -8.63	**0.002****	**0.022***
	OG	168.94 ± 12.15	151.53 ± 10.95	-17.41 ± 3.32	**<0.001*****	**0.011***	M2	-16.26	-23.60, -10.32	**<0.001*****	**0.011***
HDL-C, mg/dL	CG	48.87 ± 2.04	46.60 ± 1.93	-2.27 ± 0.66	**0.004****	**0.040***	M1	-2.08	-4.44, 0.01	0.130	>0.999
	OG	51.47 ± 2.75	46.88 ± 2.75	-4.59 ± 1.15	**0.001****	**0.011***	M2	-2.99	-5.06, -1.53	**0.036***	0.324
TG, mg/dL	CG[a]	153.00 (97.00)	123.00 (85.00)	-11.00 (58.00)	0.137	0.959	M1	4.29	-24.66, 36.01	0.798	>0.999
	OG[a]	152.00 (49.00)	141.00 (100.00)	-1.00 (47.00)	0.336	0.912	M2	-0.66	-31.29, 37.02	0.971	>0.999
NEFAs, mmol/L	CG	0.674 (0.540)	0.864 (0.270)	0.224 (0.510)	0.156	0.959	M1	-0.11	-0.29, 0.06	0.201	>0.999
	OG[a]	0.704 (0.290)	0.742 (0.260)	0.053 (0.350)	0.298	0.912	M2	-0.14	-0.33, 0.06	0.154	>0.999
Lipase, U/L	CG[a]	35.00 (17.00)	31.00 (11.00)	-2.00 (9.00)	0.429	>0.999	M1	-1.94	-7.12, 3.08	0.480	>0.999
	OG[a]	34.00 (12.00)	31.00 (10.00)	-3.00 (7.00)	**0.029***	0.145	M2	-1.37	-7.25, 4.96	0.651	>0.999
Bilirubin, mg/dL	CG	0.58 (0.18)	0.77 (0.43)	0.18 (0.34)	**<0.001*****	**0.011****	M1	0.04	-0.09, 0.18	0.549	>0.999
	OG	0.51 (0.41)	0.79 (0.70)	0.23 (0.31)	**<0.001*****	**0.011****	M2	0.03	-0.12, 0.16	0.662	>0.999
γ-GT, U/L	CG[a]	38.0 (68.0)	36.0 (54.0)	-2.0 (8.0)	0.331	>0.999	M1	-0.78	-3.37, 2.13	0.939	>0.999
	OG[a]	29.0 (26.0)	27.0 (28.0)	-2.0 (2.0)	**0.017***	0.102	M2	-0.55	-3.54, 3.24	0.815	>0.999
ALT, U/L	CG[a]	35.0 (18.0)	32.0 (20.0)	0.0 (6.0)	0.848	>0.999	M1	0.46	-4.23, 5.53	0.663	>0.999
	OG	27.0 (12.0)	25.0 (16.0)	1.0 (8.0)	0.247	0.912	M2	-0.10	-4.82, 4.46	0.471	>0.999

		Before (V1)	After (V2)	Δ	*P*	$P^{adj.3}$		β	95 % CI	*P*	$P^{adj.3}$
AST, U/L	CG	30.0 (13.0)	30.0 (15.0)	2.0 (11.0)	0.064	0.512	M1	-3.12	-7.32, 1.46	0.159	>0.999
	OG[a]	19.0 (9.5)	22.0 (9.0)	2.0 (8.0)	0.228	0.912	M2	-3.68	-8.35, 1.36	0.117	0.936
FLI	CG[a]	89.39 (17.88)	85.33 (14.26)	-1.61 (5.02)	**0.047***	0.423	M1	-1.97	-5.25, 1.09	0.229	>0.999
	OG	78.35 (32.65)	75.84 (42.49)	-2.47 (6.61)	**0.006****	**0.042***	M2	-1.78	-4.84, 1.34	0.236	>0.999
Glucose metabolism											
Glucose, mg/dL	CG	99.47 ± 2.69	97.67 ± 2.94	-1.80 ± 2.25	0.438	0.876	M1	-2.96	-8.14, 2.31	0.214	>0.999
	OG	98.00 ± 2.39	93.59 ± 2.16	-4.41 ± 1.23	**0.002****	**0.01***	M2	-3.53	-9.33, 2.57	0.223	>0.999
Insulin, mU/L	CG	18.40 (10.60)	11.90 (9.30)	-4.00 (9.80)	**0.006****	**0.036***	M1	0.17	-2.77, 2.50	0.898	>0.999
	OG[a]	14.10 (11.65)	12.10 (10.25)	-3.60 (7.30)	**<0.001*****	**0.008****	M2	-0.11	-3.59, 2.21	0.942	>0.999
HOMA-IR	CG[a]	4.07 (2.29)	3.09 (3.10)	-0.94 (2.75)	**0.004****	**0.032***	M1	-0.08	-0.91, 0.70	0.820	>0.999
	OG	3.31 (3.74)	2.92 (2.65)	-0.69 (1.84)	**0.001*****	**0.008****	M2	-0.18	-1.13, 0.61	0.703	>0.999
HbA1c, %	CG	5.60 (0.30)	5.50 (0.40)	-0.10 (0.30)	0.199	0.597	M1	0.01	-0.10, 0.11	0.854	>0.999
	OG	5.50 (0.50)	5.40 (0.50)	-0.10 (0.25)	0.071	0.276	M2	0.03	-0.07, 0.12	0.515	>0.999
hsCRP, mg/dL	CG[a]	1.81 (3.43)	2.19 (2.45)	0.31 (0.99)	0.498	0.876	M1	-1.15	-3.10, 0.57	0.978	>0.999
	OG[a]	2.29 (2.86)	2.00 (3.45)	-0.40 (1.10)	0.175	0.276	M2	-1.14	-3.46, 0.97	0.920	>0.999
TyG	CG	8.93 ± 0.11	8.81 ± 0.12	-0.12 ± 0.08	0.148	0.592	M1	0.01	-0.23, 0.25	0.969	>0.999
	OG	8.91 ± 0.08	8.80 ± 0.10	-0.12 ± 0.07	0.134	0.276	M2	-0.02	-0.24, 0.27	0.824	>0.999
HOMA-β	CG	193.66 (175.31)	129.82 (135.04)	-26.72 (109.40)	**0.029***	0.145	M1	14.12	-16.29, 39.35	0.358	>0.999
	OG[a]	158.63 (66.10)	137.14 (77.61)	-20.27 (67.80)	0.069	0.276	M2	16.61	-17.46, 40.60	0.347	>0.999

		Before (V1)	After (V2)	Δ	*P*	$P^{adj.3}$		β	95 % CI	*P*	$P^{adj.3}$
QUICKI	CG	0.311 ± 0.005	0.325 ± 0.006	0.014 ± 0.004	**0.004****	**0.032***	M1	0.00	-0.01, 0.01	0.943	>0.999
	OG	0.319 ± 0.007	0.335 ± 0.008	0.015 ± 0.00	**<0.001*****	**0.008****	M2	0.00	-0.01, 0.01	0.868	>0.999
Kidney function											
Creatinine, mg/dL	CG	0.84 (0.19)	0.80 (0.20)	0.02 (0.14)	0.364	0.597	M1	-0.04	-0.10, 0.03	0.294	0.759
	OG[a]	0.80 (0.26)	0.82 (0.29)	-0.01 (0.11)	0.640	>.0.999	M2	-0.03	-0.09, 0.04	0.399	0.968
GFR, mL/min	CG	88.19 ± 2.51	85.21 ± 2.86	-2.98 ± 2.21	0.199	0.597	M1	3.00	-2.19, 7.56	0.253	0.759
	OG	85.01 ± 4.09	85.80 ± 3.48	0.79 ± 1.85	0.678	>.0.999	M2	2.97	-3.13, 8.67	0.319	0.968
Urea, mg/dL	CG[a]	30.70 (13.70)	25.10 (9.30)	-5.0 (9.0)	**0.008****	**0.032***	M1	-2.90	-6.51, 0.56	0.170	0.68
	OG[a]	29.80 (11.50)	22.10 (5.90)	-8.5 (10.4)	**<0.001*****	**0.004****	M2	-1.82	-5.52, 1.70	0.382	0.968
Uric acid, mg/dL	CG	6.27 ± 0.30	6.47 ± 0.33	0.21 ± 0.18	0.258	0.597	M1	0.24	-0.24, 0.81	0.378	0.759
	OG	5.88 ± 0.33	6.35 ± 0.36	0.47 ± 0.18	**0.021***	0.063	M2	0.31	-0.21, 0.75	0.242	0.968

Data are shown as mean ± SEM or median (IQR) depending on data distribution. n = 32 (REE: n = 30). Absolute change (Δ) calculated as pre-intervention value subtracted from post-intervention value. [1]*P*-values refer to paired Student's *t*-test or Wilcoxon test depending on data distribution. In case log-transformed data were used for the paired Student's *t*-test, the intervention group is labelled with a superscript a. [2]*P*-value refers to linear regression model with the control group (CG) as the reference adjusted for pre-intervention value (M1) and additionally adjusted for BMI, age and sex (M2: $LnRa^{dj.}$). [3]$P^{adj.}$ is corrected for multiple testing via the Bonferroni-Holm method.

Abbreviations: ALT, alanine transaminase; AST, aspartate transaminase; BMI, body mass index; CG, control group; DBP, diastolic blood pressure; FLI, fatty liver index; GFR, glomerular filtration rate; HbA1c, glycated hemoglobin; HDL-C, high-density lipoprotein cholesterol; HOMA-IR, homeostasis model assessment-estimated insulin resistance; HOMA-β, homeostasis model assessment of beta-cell function; hsCRP, high-sensitivity C-reactive protein; LDL-C, low-density lipoprotein cholesterol; NEFAs, non-esterified fatty acids; OG, oat group; QUICKI, quantitative insulin sensitivity check index; REE, resting energy expenditure; SBP, systolic blood pressure; TC, total cholesterol; TG, triglycerides; TyG, triglyceride-glucose index; V, visit; WC, waist circumference; WHtR, waist-to-height ratio; γ-GT; gamma-glutamyl transferase.

Table 4-5: Postprandial clinical markers before and after the short-term intervention

		Within-group comparison[1]					Between-group comparison[2]				
		Before (V1)	**After (V2)**	**Δ**	***P***	***P*$^{adj.3}$**		**β**	**95 % CI**	***P***	***P*$^{adj.3}$**
AUC											
TG, mg * dL^{-1} * min	CG[a]	17,471.50 (11,759.50)	14,280.00 (8927.25)	-1282.00 (4255.75)	0.051	0.204	M1	205.15	-2,733.92, 3,456.27	0.898	>0.999
	OG	18,780.00 (5303.00)	15,870.00 (11,347.50)	-795.00 (4969.00)	0.214	0.404	M2	-27.02	-2,950.27, 4,466.93	0.988	>0.999
NEFAs, mmol* L^{-1} * min	CG[a]	37.17 (30.00)	52.79 (22.03)	7.72 (23.57)	0.404	0.808	M1	-1.44	-11.74, 11.68	0.811	>0.999
	OG	38.64 (12.32)	45.47 (13.89)	5.69 (12.49)	**0.003****	**0.012***	M2	-0.52	-10.79, 13.73	0.937	>0.999
Glucose, mg * dL^{-1} * min	CG	24,692.4 ± 1,450.1	22,633.6 ± 680.8	-2,595.1 ± 1,117.4	0.072	0.216	M1	-771.27	-2,352.45, 810.56	0.352	>0.999
	OG	23,636.4 ± 1,527.2	21,223.5 ± 1,244.1	-2,412.9 ± 575.5	**<0.001*****	**0.008****	M2	-1098.71	-2,362.08, 447.92	0.139	>0.999
Insulin, mU * L^{-1} * min	CG[a]	17,546.0 (11,521.0)	14,963.0 (9,775.0)	-3,035.0 (6,247.5)	**0.012***	0.066	M1	-1478.48	-3,615.23, 854.73	0.219	>0.999
	OG	18,087.0 (13,002.0)	11,959.0 (9,298.5)	-4,112.0 (5,003.0)	**<0.001*****	**0.008****	M2	-1792.79	-4,054.50, 602.56	0.168	>0.999
Indices											
Disposition index	CG[a]	2.11 (2.52)	2.83 (4.24)	0.71 (1.49)	**<0.001*****	**0.008****	M1	0.00	-0.78, 0.55	0.895	>0.999
	OG	2.78 (4.46)	4.29 (4.59)	0.64 (1.30)	0.068	0.204	M2	0.17	-0.58, 0.74	0.785	>0.999
Insulinogenic index	CG[a]	1.27 (1.94)	1.33 (2.53)	0.15 (0.72)	0.578	0.808	M1	-0.28	-0.72, 0.09	0.586	>0.999
	OG[a]	1.15 (1.10)	1.00 (0.72)	0.01 (0.45)	0.202	0.404	M2	-0.22	-0.73, 0.19	0.885	>0.999
OGIS	CG	339.02 ± 21.10	379.38 ± 16.91	40.36 ± 13.56	**0.011***	0.066	M1	16.80	-13.79, 48.89	0.303	>0.999
	OG	379.43 ± 18.59	426.40 ± 19.66	46.96 ± 11.67	**<0.001*****	**0.008****	M2	21.99	-6.63, 46.59	0.190	>0.999
Insulin sensitivity index	CG	1.96 (0.78)	2.29 (1.71)	0.47 (1.06)	**0.009****	0.063	M1	0.27	-0.22, 0.83	0.605	>0.999
	OG	2.65 (2.82)	3.27 (3.43)	0.62 (1.20)	**<0.001*****	**0.008****	M2	0.32	-0.26, 0.92	0.597	>0.999

Data are shown as mean ± SEM or median (IQR) depending on data distribution. CG: *n* = 14 (OGIS and insulin sensitivity index: *n* = 13), OG: *n* = 17 (AUC NEFAs: *n* = 16). Absolute change (∆) calculated as pre-intervention value subtracted from post-intervention value. [1]*P*-values refer to paired Student's *t*-test. In case log-transformed data were used, the intervention group is labelled with a superscript a. [2]*P*-values refer to linear regression model with the control group (CG) as the reference adjusted for pre-intervention value (M1) and additionally adjusted for BMI, age and sex (M2: $LnR^{adj.}$). [3]$P^{adj.}$ is corrected for multiple testing via the Bonferroni-Holm method.

Abbreviations: AUC, area under the cure; CG, control group; NEFAs, non-esterified fatty acids; OG, oat group; OGIS, oral glucose insulin sensitivity index; TG, triglycerides; V, visit.

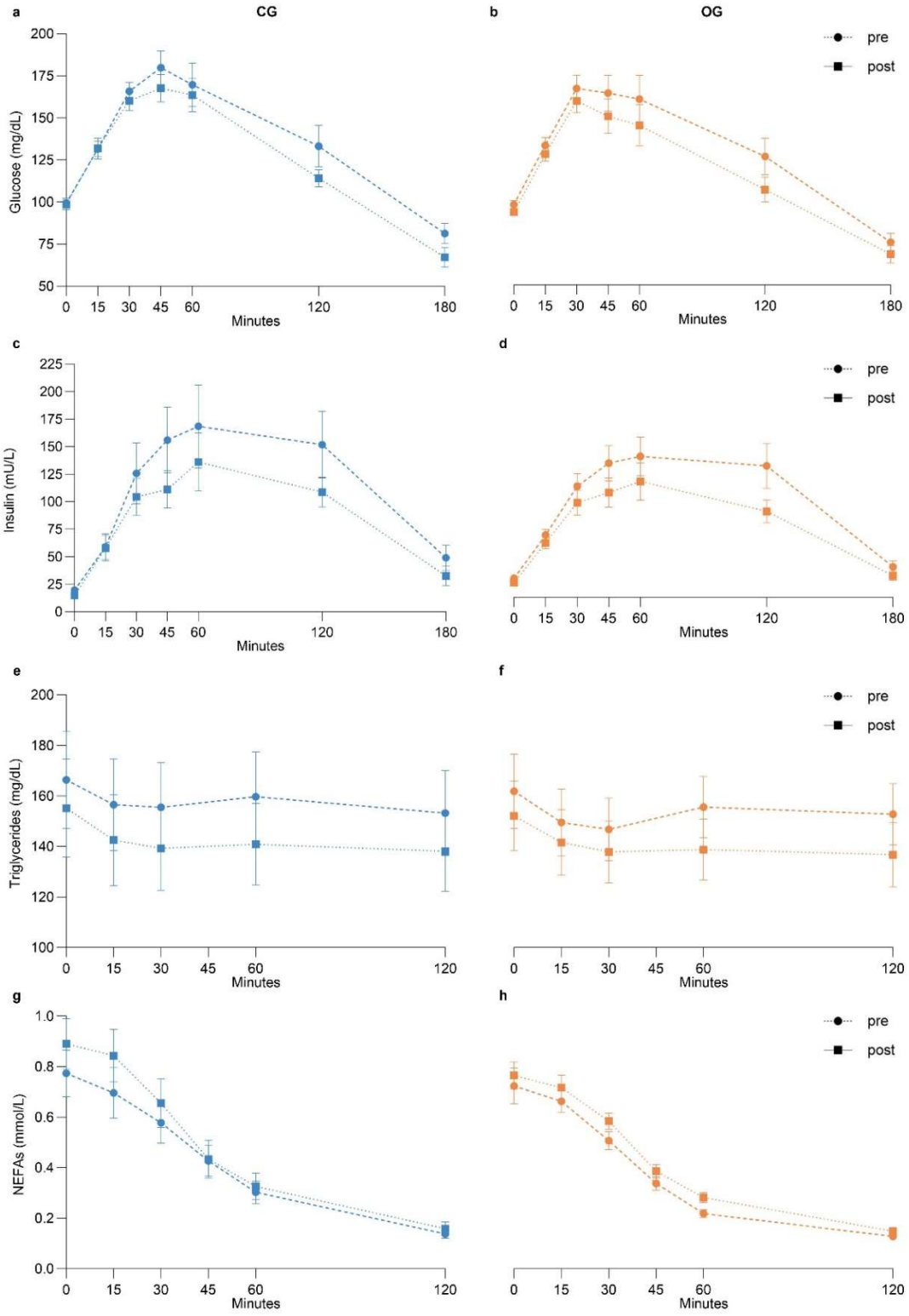

Figure 4-4: Diet-induced changes in postprandial metabolism during the short-term intervention

Figure 4-4: Diet-induced changes in postprandial metabolism during the short-term intervention

Changes within the CG and OG in (**a** + **b**) plasma glucose, (**c** + **d**) serum insulin, (**e** + **f**) serum triglycerides, and (**g** + **h**) serum NEFA concentration during the oral glucose tolerance test (OGTT). Data are shown as mean ± SEM. The circle markers show the measurements before the two-day intervention period (pre), square markers show the measurements after the intervention (post); lines are linearly interpolated between subsequent data. Blue lines refer to the CG, orange lines refer to the OG. (**a** – **f**): *n* = 31 (OG: *n* = 17, CG: *n* = 14), (**g** + **h**): *n* = 30 (OG: *n* = 16, CG: *n* = 14).

Abbreviations: CG, control group; OG, oat group; NEFAs, non-esterified fatty acids.

Table 4-6: Fasting clinical markers before and after the six-week intervention

		Within-group comparison[1]							Between-groups (V1, V4)[2]					Between-groups (V1-V4)[3]		
		Before (V1)	After 2 weeks (V2)	After 4 weeks (V3)	After 6 weeks (V4)	Δ	*P*	$P^{adj.4}$		β	95 % CI	*P*	$P^{adj.4}$		*P*	$P^{adj.4}$
Anthropometrics																
BW, kg	CG^{6w}	93.0 (20.0)	90.5 (18.3)	90.3 (17.7)	94.3 (17.5)	0.10 (1.65)	0.845	>0.999	M1	0.16	-0.51, 0.84	0.627	>0.999	M1	0.770	>0.999
	OG^{6w}	94.0 (19.3)	93.6 (20.8)	94.6 (17.4)	93.8 (19.0)	0.20 (1.73)	0.389	>0.999	M2	0.10	-0.62, 0.93	0.771	>0.999	M2	0.133	0.789
BMI, kg/m²	CG^{6w}	31.42 ± 0.87	31.13 ± 0.87	31.16 ± 0.88	31.44 ± 0.85	0.02 ± 0.08	0.770	>0.999	M1	0.08	-0.14, 0.29	0.482	>0.999	M1	0.840	>0.999
	OG^{6w}	31.86 ± 0.67	31.69 ± 0.66	32.08 ± 0.66	31.95 ± 0.65	0.09 ± 0.08	0.313	>0.999	M2	0.06	0.17, 0.26	0.611	>0.999	M2	0.841	>0.999
WC, cm	CG^{6w}	105.75 (7.50)	105.95 (8.09)	105.63 (10.04)	106.60 (7.50)	0.75 (3.50)	0.586	>0.999	M1	-0.55	-2.07, 0.87	0.484	>0.999	M1	0.609	>0.999
	OG^{6w}	108.00 (8.97)	107.83 (9.60)	108.33 (9.44)	107.75 (9.35)	0.50 (3.50)	0.752	>0.999	M2	-0.60	-2.21, 0.90	0.483	>0.999	M2	0.627	>0.999
WHtR	CG^{6w}	0.62 ± 0.01	0.62 ± 0.01	0.62 ± 0.01	0.62 ± 0.01	0.00 ± 0.00	0.529	>0.999	M1	0.00	-0.01, 0.01	0.546	>0.999	M1	0.661	>0.999
	OG^{6w}	0.62 ± 0.01	0.63 ± 0.01	0.63 ± 0.01	0.62 ± 0.01	0.00 ± 0.00	0.737	>0.999	M2	0.00	-0.01, 0.01	0.483	>0.999	M2	0.673	>0.999
Body fat[a], %	CG^{6w}	40.44 ± 2.14	-	-	39.79 ± 2.16	-0.66 ± 0.27	**0.028***	0.224	M1	0.63	-0.05, 1.27	0.095	0.760	M1	-	-
	OG^{6w}	42.44 ± 1.69	-	-	42.41 ± 1.69	-0.03 ± 0.28	0.917	>0.999	M2	0.65	-0.13, 1.45	0.123	0.984	M2	-	-
REE[a,b], kcal/d	CG^{6w}	1,833 (520)	-	-	1,814 (467)	26 (272)	0.441	>0.999	M1	21.43	-57.97, 102.93	0.640	>0.999	M1	-	-
	$OG^{6w(a)}$	1,623 (573)	-	-	1,780 (428)	-30 (108)	0.940	>0.999	M2	29.89	-63.91, 122.39	0.575	>0.999	M2	-	-
SBP, mmHg	CG^{6w}	137.22 ± 2.80	139.84 ± 3.66	137.38 ± 4.63	131.03 ± 3.30	-6.19 ± 2.64	**0.033***	0.231	M1	3.88	-3.77, 12.01	0.316	>0.999	M1	0.503	>0.999
	OG^{6w}	134.89 ± 3.10	136.73 ± 3.29	135.83 ± 2.61	133.37 ± 3.16	-1.52 ± 2.88	0.606	>0.999	M2	4.54	-3.35, 12.69	0.235	>0.999	M2	0.516	>0.999

		Before (V1)	After 2 weeks (V2)	After 4 weeks (V3)	After 6 weeks (V4)	Δ	*P*	$P^{adj.4}$		β	95 % CI	*P*	$P^{adj.4}$		*P*	$P^{adj.4}$
DBP, mmHg	CG^{6w}	86.52 ± 2.44	89.27 ± 2.64	87.05 ± 2.82	84.62 ± 2.48	-1.90 ± 1.48	0.217	>0.999	M1	1.81	-2.81, 6.59	0.477	>0.999	M1	0.743	>0.999
	OG^{6w}	88.03 ± 2.28	90.16 ± 2.59	88.05 ± 2.18	87.53 ± 2.18	-0.50 ± 1.89	0.795	>0.999	M2	1.71	-3.27, 6.51	0.476	>0.999	M2	0.737	>0.999
Lipid metabolism																
TC, mg/dL	CG^{6w}	212.41 ± 10.34	210.69 ± 10.70	210.31 ± 9.87	216.29 ± 11.69	3.88 ± 4.70	0.421	>0.999	M1	-10.22	-28.22, 7.37	0.260	>0.999	M1	0.583	>0.999
	OG^{6w}	211.18 ± 11.34	208.50 ± 10.60	206.00 ± 9.20	204.94 ± 11.63	-6.24 ± 7.41	0.412	>0.999	M2	-11.40	-32.27, 12.86	0.254	>0.999	M2	0.59	>0.999
LDL-C, mg/dL	CG^{6w}	138.53 ± 9.19	133.94 ± 9.78	134.38 ± 9.41	139.53 ± 9.78	1.00 ± 3.76	0.794	>0.999	M1	-4.05	17.46, 9.62	0.566	>0.999	M1	0.902	>0.999
	OG^{6w}	139.71 ± 9.26	136.50 ± 8.27	133.81 ± 7.10	136.53 ± 9.26	-3.18 ± 5.85	0.595	>0.999	M2	-5.16	-18.42, 11.24	0.484	>0.999	M2	0.905	>0.999
HDL-C, mg/dL	CG^{6w}	54.94 ± 4.82	55.75 ± 5.30	57.50 ± 5.18	54.94 ± 4.82	0.41 ± 1.01	0.690	>0.999	M1	-3.58	-5.68, -1.57	**0.008 ****	0.088	M1	0.095	0.950
	OG^{6w}	50.18 ± 3.01	49.63 ± 2.50	48.75 ± 2.66	47.59 ± 2.40	-2.59 ± 1.09	**0.030***	0.330	M2	-4.01	-6.10, -1.75	**0.004 ****	**0.044***	M2	0.097	0.970
TG, mg/dL	$CG^{6w(a)}$	147.00 (88.50)	164.50 (121.25)	134.50 (117.50)	122.00 (59.00)	-3.00 (50.50)	0.586	>0.999	M1	-0.55	-36.96, 38.84	0.972	>0.999	M1	0.834	>0.999
	$OG^{6w(a)}$	135.00 (56.50)	131.50 (73.25)	136.00 (45.75)	119.00 (70.00)	3.00 (52.50)	0.675	>0.999	M2	-0.17	-39.87, 43.88	0.994	>0.999	M2	0.828	>0.999
NEFAs, mmol/L	$CG^{6w(a)}$	0.570 (0.320)	-	-	0.606 (0.370)	0.03 (0.310)	0.848	>0.999	M1	-0.04	-0.19, 0.16	0.659	>0.999	M1	-	-
	$OG^{6w(a)}$	0.594 (0.370)	-	-	0.594 (0.290)	-0.06 (0.300)	0.546	>0.999	M2	-0.05	-0.18, 0.15	0.567	>0.999	M2	-	-
Lipase, U/L	CG^{6w}	30.00 (15.00)	35.00 (17.50)	38.00 (20.50)	37.00 (15.50)	1.00 (4.50)	0.243	>0.999	M1	-4.72	-22.88, 7.33	0.486	>0.999	M1	0.283	>0.999
	$OG^{6w(a)}$	31.00 (16.00)	29.00 (13.50)	33.00 (15.75)	31.00 (21.50)	1.00 (9.00)	0.887	>0.999	M2	-4.29	-20.97, 6.63	0.509	>0.999	M2	0.286	>0.999

		Before (V1)	After 2 weeks (V2)	After 4 weeks (V3)	After 6 weeks (V4)	Δ	*P*	$P^{adj.4}$		β	95 % CI	*P*	$P^{adj.4}$		*P*	$P^{adj.4}$
Bili-rubin, mg/dL	CG^{6w}	0.54 (0.40)	0.57 (0.37)	0.54 (0.35)	0.47 (0.62)	-0.05 (0.20)	0.365	>0.999	M1	-0.06	-0.19, 0.06	0.514	>0.999	M1	0.183	>0.999
	$OG^{6w(a)}$	0.49 (0.33)	0.51 (0.31)	0.52 (0.26)	0.44 (0.30)	-0.02 (0.25)	0.390	>0.999	M2	-0.07	-0.18, 0.04	0.499	>0.999	M2	0.17	>0.999
γ-GT, U/L	CG^{6w}	21.00 (13.00)	19.50 (16.00)	20.00 (15.00)	20.00 (15.00)	0.00 (3.50)	0.125	>0.999	M1	-1.29	-5.05, 2.83	0.440	>0.999	M1	0.465	>0.999
	OG^{6w}	22.00 (23.00)	23.00 (21.25)	23.50 (19.75)	27.00 (23.00)	0.00 (4.00)	0.878	>0.999	M2	-1.43	-5.68, 3.09	0.463	>0.999	M2	0.481	>0.999
ALT, U/L	CG^{6w}	17.00 (13.00)	19.00 (5.50)	20.00 (11.75)	19.00 (11.00)	2.00 (7.50)	0.197	>0.999	M1	-3.54	-10.46, 3.87	0.797	>0.999	M1	0.250	>0.999
	$OG^{6w(a)}$	25.00 (25.50)	25.50 (24.25)	26.50 (19.25)	24.00 (19.00)	0.00 (10.50)	0.314	>0.999	M2	-3.56	-10.31, 4.14	0.87	>0.999	M2	0.243	>0.999
AST, U/L	$CG^{6w(a)}$	23.00 (10.50)	23.50 (9.00)	21.50 (7.75)	23.00 (9.50)	0.00 (4.00)	0.643	>0.999	M1	-0.62	-5.39, 4.51	0.798	>0.999	M1	0.669	>0.999
	$OG^{6w(a)}$	25.00 (10.0)	27.00 (8.00)	27.50 (7.50)	25.00 (10.50)	-1.00 (9.50)	0.739	>0.999	M2	-1.63	-6.92, 4.26	0.443	>0.999	M2	0.671	>0.999
FLI	CG^{6w}	68.90 (23.35)	72.97 (18.68)	74.56 (26.60)	72.93 (22.46)	-0.55 (8.17)	0.580	>0.999	M1	1.18	-2.47, 5.15	0.862	>0.999	M1	0.747	>0.999
	OG^{6w}	79.52 (28.95)	81.43 (23.69)	82.78 (19.49)	80.34 (16.58)	0.01 (9.92)	0.619	>0.999	M2	1.17	-2.64, 5.32	0.795	>0.999	M2	0.748	>0.999
Glucose metabolism																
Glu-cose, mg/dL	CG^{6w}	94.29 ± 1.40	99.63 ± 1.84	98.44 ± 2.10	97.06 ± 1.57	2.76 ± 2.19	0.225	>0.999	M1	-0.72	-6.05, 4.69	0.771	>0.999	M1	0.444	>0.999
	OG^{6w}	105.12 ± 3.96	105.19 ± 3.81	105.31 ± 3.74	106.18 ± 4.31	1.06 ± 1.35	0.443	>0.999	M2	-0.88	-7.17, 6.08	0.754	>0.999	M2	0.452	>0.999
Insulin, mU/L	$CG^{6w(a)}$	14.00 (9.25)	10.95 (7.35)	12.10 (5.82)	12.10 (5.60)	-1.20 (6.60)	0.186	>0.999	M1	1.94	-1.08, 4.58	0.185	>0.999	M1	0.495	>0.999
	$OG^{6w(a)}$	14.90 (7.50)	14.60 (4.68)	13.10 (5.70)	14.40 (6.65)	-0.40 (8.70)	0.796	>0.999	M2	2.05	-1.21, 5.09	0.195	>0.999	M2	0.498	>0.999
HOMA-IR	$CG^{6w(a)}$	3.11 (2.12)	2.62 (2.18)	3.09 (1.73)	2.96 (1.51)	-0.35 (2.14)	0.345	>0.999	M1	0.72	-0.12, 1,53	0.125	0.875	M1	0.726	>0.999
	$OG^{6w(a)}$	3.89 (2.43)	3.82 (1.55)	3.24 (1.83)	4.03 (3.25)	-0.15 (2.54)	0.858	>0.999	M2	0.75	-0.16, 1.76	0.151	>0.999	M2	0.727	>0.999

		Before (V1)	After 2 weeks (V2)	After 4 weeks (V3)	After 6 weeks (V4)	Δ	*P*	$P^{adj.4}$		β	95 % CI	*P*	$P^{adj.4}$		*P*	$P^{adj.4}$
HbA1c[a], %	CG^{6w}	5.60 (0.50)	5.60 (0.35)	5.45 (0.48)	5.50 (0.40)	0.00 (0.30)	0.430	>0.999	M1	0.09	-0.04, 0.22	0.211	>0.999	M1	0.532	>0.999
	OG^{6w}	5.60 (0.55)	5.60 (0.38)	5.65 (0.30)	5.80 (0.80)	0.00 (0.25)	0.772	>0.999	M2	0.09	-0.04, 0.21	0.262	>0.999	M2	0.535	>0.999
hsCRP, mg/dL	$CG^{6w(a)}$	1.89 (2.45)	1.80 (2.13)	1.74 (3.25)	1.88 (2.65)	0.00 (1.31)	0.567	>0.999	M1	2.34	0.52, 5.09	0.075	0.600	M1	0.072	0.576
	$OG^{6w(a)}$	2.55 (4.20)	3.01 (4.58)	3.08 (3.22)	5.06 (4.73)	0.83 (3.51)	**0.039***	0.312	M2	2.34	0.16, 5.38	0.097	0.776	M2	0.075	0.600
TyG	CG^{6w}	8.73 ± 0.11	8.88 ± 0.13	8.76 ± 0.12	8.73 ± 0.12	-0.01 (0.07)	0.723	>0.999	M1	0.01	-0.19, 0.23	0.957	>0.999	M1	0.696	>0.999
	OG^{6w}	8.88 ± 0.11	8.93 ± 0.11	8.91 ± 0.10	8.85 ± 0.12	-0.03 (0.08)	0.763	>0.999	M2	0.01	-0.23, 0.29	0.948	>0.999	M2	0.69	>0.999
HOMA-β	$CG^{6w(a)}$	148.91 (108.81)	125.51 (79.41)	118.30 (73.16)	129.33 (61.48)	-24.89 (48.16)	**0.021***	0.168	M1	5.67	-22.36, 32.68	0.696	>0.999	M1	0.078	0.576
	$OG^{6w(a)}$	121.76 (111.91)	136.92 (98.29)	114.52 (61.98)	133.71 (63.87)	-0.82 (73.90)	0.704	>0.999	M2	5.30	-25.27, 36.35	0.730	>0.999	M2	0.076	0.600
QUICKI	CG^{6w}	0.323 (0.040)	0.331 (0.030)	0.323 (0.030)	0.325 (0.030)	0.004 (0.040)	0.350	>0.999	M1	-0.01	-0.02, 0.01	0.283	>0.999	M1	0.817	>0.999
	OG^{6w}	0.313 (0.030)	0.314 (0.020)	0.321 (0.020)	0.311 (0.040)	0.002 (0.040)	0.860	>0.999	M2	-0.01	-0.02, 0.01	0.360	>0.999	M2	0.824	>0.999
Kidney function																
Crea-tinine, mg/dL	CG^{6w}	0.89 ± 0.04	0.90 ± 0.04	0.88 ± 0.04	0.90 ± 0.03	0.01 ± 0.02	0.545	>0.999	M1	-0.06	-0.11, -0.01	**0.037***	0.132	M1	0.435	>0.999
	OG^{6w}	0.82 ± 0.03	0.82 ± 0.03	0.81 ± 0.03	0.79 ± 0.03	-0.03 ± 0.02	0.157	0.588	M2	-0.06	-0.13, -0.01	**0.048***	0.180	M2	0.440	>0.999
GFR, mL/min	CG^{6w}	81.24 ± 2.42	79.51 ± 2.39	81.81 ± 2.76	80.50 ± 2.28	-0.73 ± 1.48	0.626	>0.999	M1	5.45	0.62, 10.06	**0.033***	0.132	M1	0.524	>0.999
	OG^{6w}	87.76 ± 2.33	88.38 ± 2.17	90.00 ± 2.47	90.58 ± 2.26	2.82 ± 1.85	0.147	0.588	M2	5.43	0.71, 9.64	**0.045***	0.180	M2	0.524	>0.999
Urea, mg/dL	$CG^{6w(a)}$	29.40 (13.00)	28.25 (8.70)	28.60 (10.10)	27.30 (11.95)	3.10 (13.75)	0.500	>0.999	M1	-0.07	-4.02, 3.78	0.971	0.971	M1	0.980	>0.999
	$OG^{6w(a)}$	29.80 (8.15)	29.35 (11.25)	29.50 (11.35)	28.90 (7.85)	-0.20 (4.50)	0.949	0.949	M2	0.79	-3.24, 4.75	0.709	0.709	M2	0.980	>0.999

		Before (V1)	After 2 weeks (V2)	After 4 weeks (V3)	After 6 weeks (V4)	Δ	*P*	$P^{adj.4}$		β	95 % CI	*P*	$P^{adj.4}$		*P*	$P^{adj.4}$
Uric acid, mg/dL	CG^{6w}	5.84 ± 0.38	5.83 ± 0.35	5.79 ± 0.42	6.01 ± 0.37	0.16 ± 0.14	0.265	>0.999	M1	-0.31	-0.64, 0.00	0.095	0.190	M1	0.398	>0.999
	OG^{6w}	5.69 ± 0.28	5.51 ± 0.27	5.74 ± 0.23	5.56 ± 0.26	-0.13 ± 0.13	0.342	0.684	M2	-0.36	-0.69, -0.04	0.079	0.180	M2	0.374	>0.999

Data are shown as mean ± SEM or median (IQR) depending on data distribution. V1 and V4: *n* = 34, V2 and V3: *n* = 32. [a]CG^{6w}: *n* = 16. [b]OG^{6w}: *n* = 15. Absolute change (Δ) calculated as pre-intervention value (V1) subtracted from post-intervention value (V4). [1]*P*-values refer to paired Student's *t*-test or Wilcoxon test depending on data distribution. In case log-transformed data were used for the Student's *t*-test, the intervention group is labelled with a superscript a.[2]*P*-value refers to linear regression model with the control group (CG^{6w}) as the reference adjusted for pre-intervention value (M1) and additionally adjusted for BMI, age, and sex (M2: $LnR^{adj.}$). [3]*P*-value refers to linear mixed model with the interaction term (time point x diet group) to test for differences in parameter progression over time between the two diet groups. [4]$P^{adj.}$ is corrected for multiple testing via the Bonferroni-Holm method.

Abbreviations: ALT, alanine transaminase; AST, aspartate transaminase; BMI, body mass index; CG^{6w}, six-week control group; DBP, diastolic blood pressure; FLI, fatty liver index; GFR, glomerular filtration rate; HbA1c, glycated hemoglobin; HDL-C, high-density lipoprotein cholesterol; HOMA-IR, homeostasis model assessment-estimated insulin resistance; HOMA-β, homeostasis model assessment of beta-cell function; hsCRP, high-sensitivity C-reactive protein; LDL-C, low-density lipoprotein cholesterol; NEFAs, non-esterified fatty acids; OG^{6w}, six-week oat group; QUICKI, quantitative insulin sensitivity check index; REE, resting energy expenditure; SBP, systolic blood pressure; TC, total cholesterol; TG, triglycerides; TyG, triglyceride-glucose index; V, visit; WC, waist circumference; WHtR, waist-to-height ratio; γ-GT; gamma-glutamyl transferase.

Table 4-7: Postprandial clinical markers before and after the six-week intervention

		Within-group comparison[1]					Between-group comparison[2]				
		Before (V1)	After six weeks (V4)	Δ	*P*	$P^{adj.3}$		β	95 % CI	*P*	$P^{adj.3}$
AUC											
TG, mg * dL^{-1} * min	$CG^{6w(a)}$	14,835.00 (11,619.00)	14,993.00 (5,124.75)	-588.50 (4,453.50)	0.989	>0.999	M1	532.75	-2,698.31, 4,509.69	0.468	>0.999
	$OG^{6w(a)}$	15,390.00 (6,619.00)	17,340.00 (7,788.50)	-232.00 (8,801.00)	0.986	>0.999	M2	517.56	-3,307.64, 4,939.56	0.441	>0.999
NEFAs, mmol* L^{-1} * min	$CG^{6w(a)}$	30.18 (10.69)	29.76 (24.07)	-1.10 (14.49)	0.646	>0.999	M1	-2.43	-12.17, 9.74	0.915	>0.999
	$OG^{6w(a)}$	34.36 (23.62)	36.27 (16.70)	-6.39 (19.81)	0.491	>0.999	M2	-2.53	-12.25, 9.64	0.974	>0.999
Glucose, mg * dL^{-1} * min	CG^{6w}	19,961.50 (5,638.50)	20,655.50 (5,266.75)	209.50 (3,881.00)	0.962	>0.999	M1	1524.08	-351.44, 3,3557.66	0.160	>0.999
	OG^{6w}	24,863.00 (9,469.00)	22,508.00 (12,724.00)	878.00 (3,660.00)	0.246	>0.999	M2	1259.05	-723.78, 3,700.13	0.236	>0.999
Insulin, mU * L^{-1} * min	$CG^{6w(a)}$	12,405.00 (9,882.50)	11,467.00 (7,426.25)	-839.50 (6,302.75)	0.160	>0.999	M1	1975.69	-1,536.69, 4,894.58	0.289	>0.999
	OG^{6w}	19,073.00 (13,530.00)	18,711.00 (9,443.00)	-2,362.00 (8,910.00)	**0.048***	0.384	M2	1767.63	-1,738.70, 5,118.76	0.329	>0.999
Indices											
Disposition index[a]	$CG^{6w(a)}$	2.54 (3.61)	3.73 (2.04)	0.73 (5.46)	0.133	>0.999	M1	-1.43	-3.64, 0.44	0.098	0.784
	$OG^{6w(a)}$	1.76 (2.99)	2.01 (4.14)	0.23 (1.05)	0.600	>0.999	M2	-1.28	-3.20, 0.37	0.126	>0.999
Insulinogenic index[a]	$CG^{6w(a)}$	0.87 (1.27)	1.02 (0.80)	-0.03 (0.70)	0.390	>0.999	M1	-0.14	-0.56, 0.24	0.471	>0.999
	$OG^{6w(a)}$	0.90 (0.74)	0.95 (0.70)	-0.10 (0.53)	0.512	>0.999	M2	-0.06	-0.51, 0.34	0.500	>0.999
OGIS	CG^{6w}	394.06 ± 16.55	391.42 ± 15.95	-2.64 ± 10.31	0.801	>0.999	M1	-6.59	-34.84, 24.62	0.633	>0.999
	OG^{6w}	332.92 ± 19.53	341.26 ± 15.72	8.33 ± 10.62	0.444	>0.999	M2	-4.87	-36.81, 19.38	0.700	>0.999
Insulin sensitivity index	$CG^{6w(a)}$	2.99 (2.34)	3.12 (1.26)	0.30 (1.71)	0.291	>0.999	M1	-0.18	-0.96, 0.50	0.726	>0.999
	$OG^{6w(a)}$	1.88 (1.11)	2.05 (1.16)	0.45 (1.47)	0.235	>0.999	M2	-0.01	-0.91, 0.71	0.988	>0.999

Data are shown as mean ± SEM or median (IQR) depending on data distribution. n= 33 (CG^{6w}: n = 16, OG^{6w}: n = 17). [a]CG: *n* = 13. Absolute change (∆) calculated as pre-intervention value (V1) subtracted from post-intervention value (V4). [1]*P*-values refer to paired Student's *t*-test or Wilcoxon test depending on data distribution. In case log-transformed data were used for the Student's *t*-test, the intervention group is labelled with a superscript a. [2]*P*-value refers to linear regression model with the control group (CG^{6w}) as the reference adjusted for pre-intervention value (M1) and additionally adjusted for BMI, age, and sex (M2: $LnR^{adj.}$). [3]$P^{adj.}$ is corrected for multiple testing via the Bonferroni-Holm method.

Abbreviations: AUC, area under the cure; CG^{6w}, six-week control group; NEFAs, non-esterified fatty acids; OG^{6w}, six-week oat group; OGIS, oral glucose insulin sensitivity index; TG, triglycerides; V, visit.

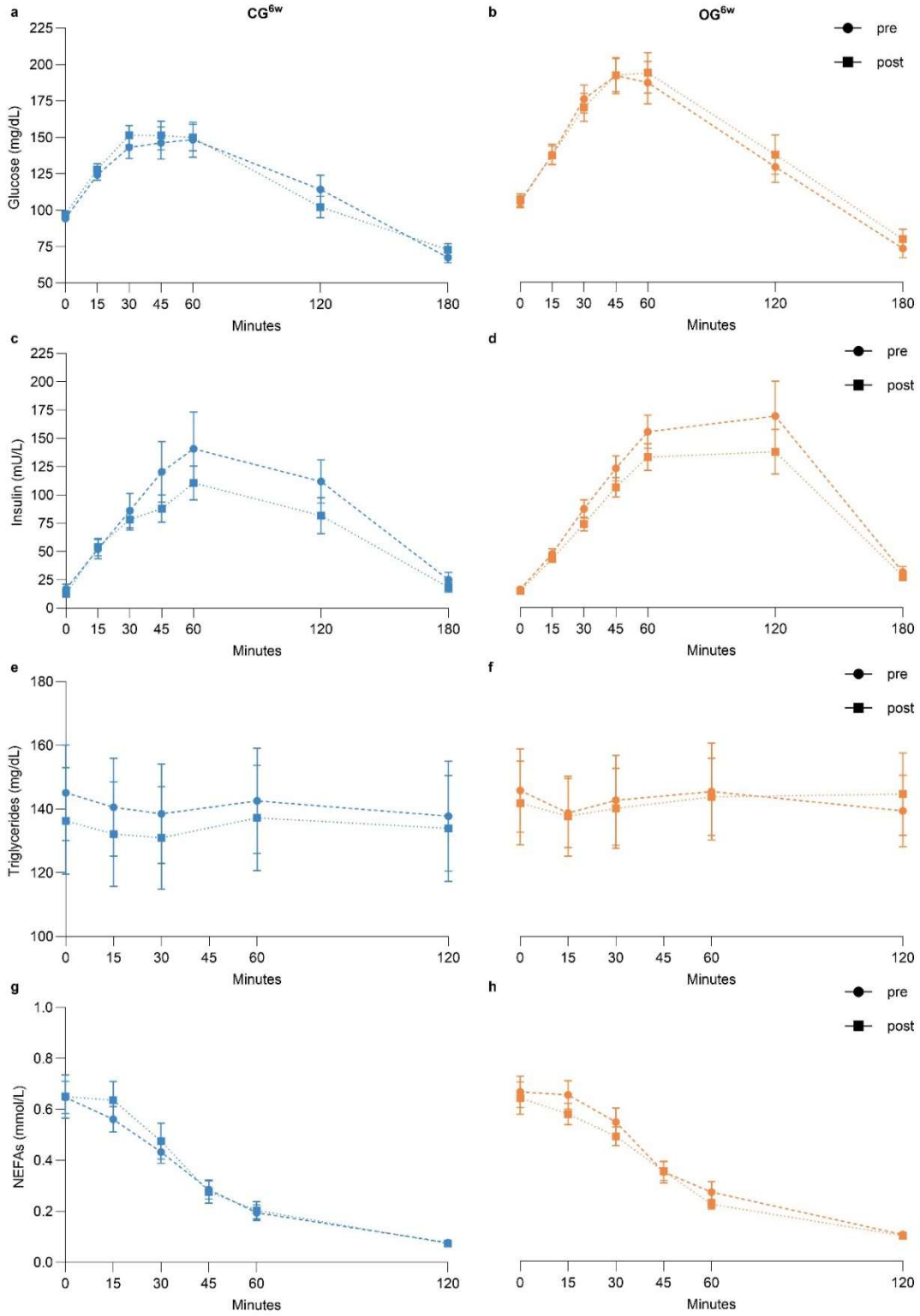

Figure 4-5: Diet-induced changes in postprandial metabolism during the six-week intervention

Figure 4-5: Diet-induced changes in postprandial metabolism during the six-week intervention

Changes within the CG^{6w} and OG^{6w} in (**a** + **b**) plasma glucose, (**c** + **d**) serum insulin, (**e** + **f**) serum triglycerides, and (**g** + **h**) serum NEFA concentration during the oral glucose tolerance test (OGTT). Data are shown as mean ± SEM. The circle markers show the measurements before the six-week intervention period (pre), square markers show the measurements after the intervention period (post); lines are linearly interpolated between subsequent data. Blue lines refer to the CG^{6w} (pre: n = 17, post: n = 16), orange lines refer to the OG^{6w} (n = 17).

Abbreviations: CG^{6w}, six-week control group; OG^{6w}, six-week oat group; NEFAs, non-esterified fatty acids.

Table 4-8: Structure of the final sPLS-DA models

Data set	Intervention	No. of variables	No. of components	No. of variables per component					
				Comp 1	Comp 2	Comp 3	Comp 4	Comp 5	Comp 6
Clinical markers	Short-term	26	2	3	19				
	Six-week	26	1	26					
Bacterial genera	Short-term	143	2	1	41				
	Six-week	146	1	1					
KEGG pathways	Short-term	145	2	10	5				
	Six-week	146	6	35	13	2	2	5	5
Global plasma metabolomic profile	Short-term	1,128	2	30	95				
	Six-week	1,128	1	15					
Global fecal metabolomic profile	Short-term	1,054	2	91	971				
	Six-week	1,054	1	31					
Subgroup analysis:									
Bacterial genera (diet-induced change)	Short-term	141	2	10	10				
	Six-week	146	2	12	19				
Bacterial genera (baseline differences)	Short-term	148	2	10	1				

Abbreviation: Comp, component; KEGG, Kyoto encyclopedia of genes and genomes.

4.4 Effects on Global Plasma Metabolomic Profile

After the two-day intervention period, a strong separation between OG and CG in the non-targeted global plasma metabolomic profile, which was generated by UPLC-MS/MS[260,261], was observed using sPLS-DA (AUC = 0.99 (P = 1.47 x 10^{-6}, BER = 0.04; **Figure 4-6a**; **Table 4-8**). Phenolic compounds, including 2-acetamidophenol sulfate, 2-aminophenol sulfate, 4-hydroxyhippurate, 4-vinylphenol sulfate, and vanilloylglycine, were among the most distinguishing metabolites (**Figure 4-6b**) and showed a significant increase in OG compared with CG (FDR corrected q < 0.05, LnR^{adj}; **Figure 4-6b**,**c**). Thus, the results suggest that a short-term, high-dose oat diet leads to a distinct shift in the plasma metabolomic profile, particularly characterized by an increase in microbially produced phenolic compounds.

A clear difference in the plasma metabolomic profile was also observed between OG^{6w} and CG^{6w} after the six-week intervention period (AUC = 0.927 (P = 2.1 x 10^{-5}), BER = 0.176; **Figure 4-6d**; **Table 4-8**). The phenolic compounds 2-acetamidophenol sulfate and 2-aminophenol sulfate were among the most important distinguishing metabolites and showed a significant increase in OG^{6w} compared with CG^{6w} (q < 0.05; **Figure 4-6e**,**f**). Notably, an increase in these phenols was also detected in the OG, supporting the assumption that this increase is related to oats. These results thus indicate that a six-week, moderate oat diet leads to shifts in the global plasma metabolomic profile that are consistent with those of a short-term, high-dose oat diet, although the effects are less pronounced.

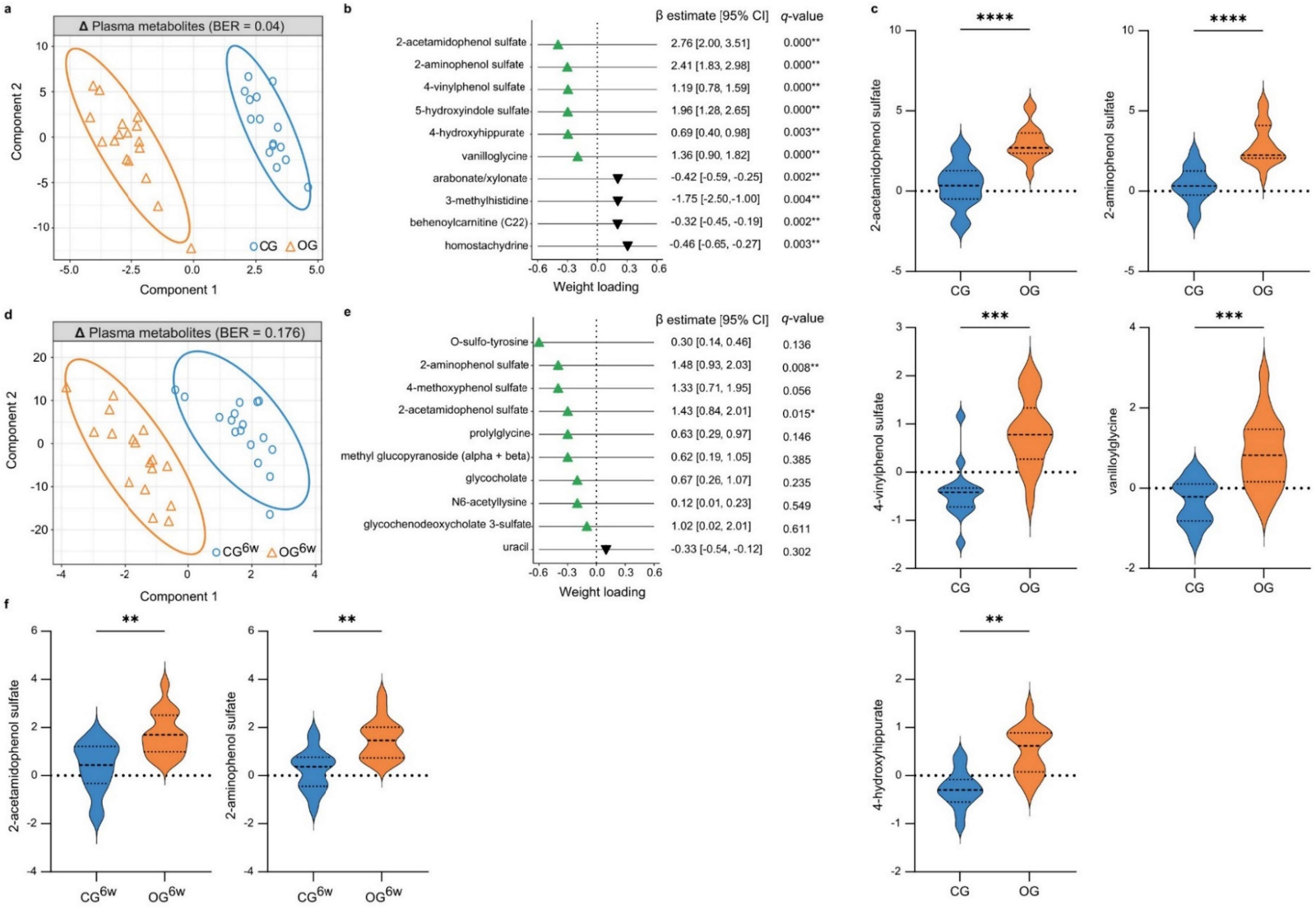

Figure 4-6: Oat diet enhanced microbially produced phenols in plasma

Figure 4-6: Oat diet enhanced microbially produced phenols in plasma

(**a**) Two-component sample plot of the sPLS-DA on the changes in the global plasma metabolomic profiles in OG and CG. (**b**) Weight loadings of the top 10 selected plasma metabolites in component 1 (green or black triangle: increase or decrease in OG vs. CG). Coefficient and 95% CI were calculated using linear regression models adjusted for baseline concentration, age, sex, BMI ($LnR^{adj.}$). (**c**) Violin plots showing log fold change of selected phenolic metabolites (dashed center line: median; dotted line: upper and lower quartiles). Between-group differences were analyzed using linear regression models adjusted for baseline concentration, age, sex, BMI ($LnR^{adj.}$). ****q*-value < 0.001, ***q*-value < 0.01, **q*-value < 0.05. (**d**) Two-component sample plot of the sPLS-DA on the changes in the global plasma metabolomic profiles in OG^{6w} and CG^{6w}. **e** Weight loadings of the top 10 selected plasma metabolites in component 1 (green or black triangle: increase or decrease in OG^{6w} vs. CG^{6w}). Coefficient and 95% CI were calculated using linear regression models adjusted for baseline concentration, age, sex, BMI ($LnR^{adj.}$). **f** Violin plots showing log fold change of selected phenolic metabolites (dashed center line: median; dotted line: upper and lower quartiles). Between-group differences were analyzed using linear regression models adjusted for baseline concentration, age, sex, BMI ($LnR^{adj.}$). ***q*-value < 0.01. (**a**–**c**) OG: *n* = 17, CG: *n* = 15. (**d**–**f**) OG^{6w}: *n* = 17, CG^{6w}: *n* = 17.

Abbreviations: BER, balanced error rate; CG, control group; CG^{6w}, six-week control group; OG, oat group; OG^{6w}, six-week oat group.

4.5 Effects on Global Fecal Metabolomic Profile

Investigating the diet-induced modulation of the non-targeted global metabolomic profiles in feces[260,261], sPLS-DA revealed a strong separation between OG and CG after the two-day intervention period (AUC = 0.93 ($P = 1.16 \times 10^{-4}$), BER = 0.177; **Figure 4-7a**; **Table 4-8**). Amino acids and their (microbial) degradation products including 8-methoxykynurenate, indolepropionate, 3-hydroxyphenylacetate, and N-acetyltryptophan, as well as pyridoxate, a metabolite of the vitamin B6 metabolism, were among the most influential compounds for differentiating the diet groups (**Figure 4-7b**). 8-methoxykynurenate and pyridoxate increased in OG compared to CG, while indolepropionate and 3-hydroxyphenylacetate decreased ($q < 0.05$, $LnR^{adj.}$; **Figure 4-7b**,**c**). Shifts in the fecal metabolomic profile induced by the short-term, high-dose oat diet thus appear to be mainly linked to amino acid-related pathways, which have a significant relevance for cholesterol metabolism[290].

After the six-week intervention period, different shifts in the fecal metabolomic profile were also revealed between OG^{6w} and CG^{6w} (AUC = 0.75 ($P = 0.014$), BER = 0.353; **Figure 4-7d**; **Table 4-8**). The most influential metabolites contributing to group differentiation were lipid metabolites; however, these metabolites did not differ significantly between the diet groups according to $LnR^{adj.}$ ($q < 0.05$; **Figure 4-7e**). Thus, the results suggest that a six-week, moderate oat diet has less pronounced effects on the global fecal metabolomic profile compared with a short-term, high-dose oat diet and might lead to a shift in the lipid metabolite pattern rather than to changes in individual lipids.

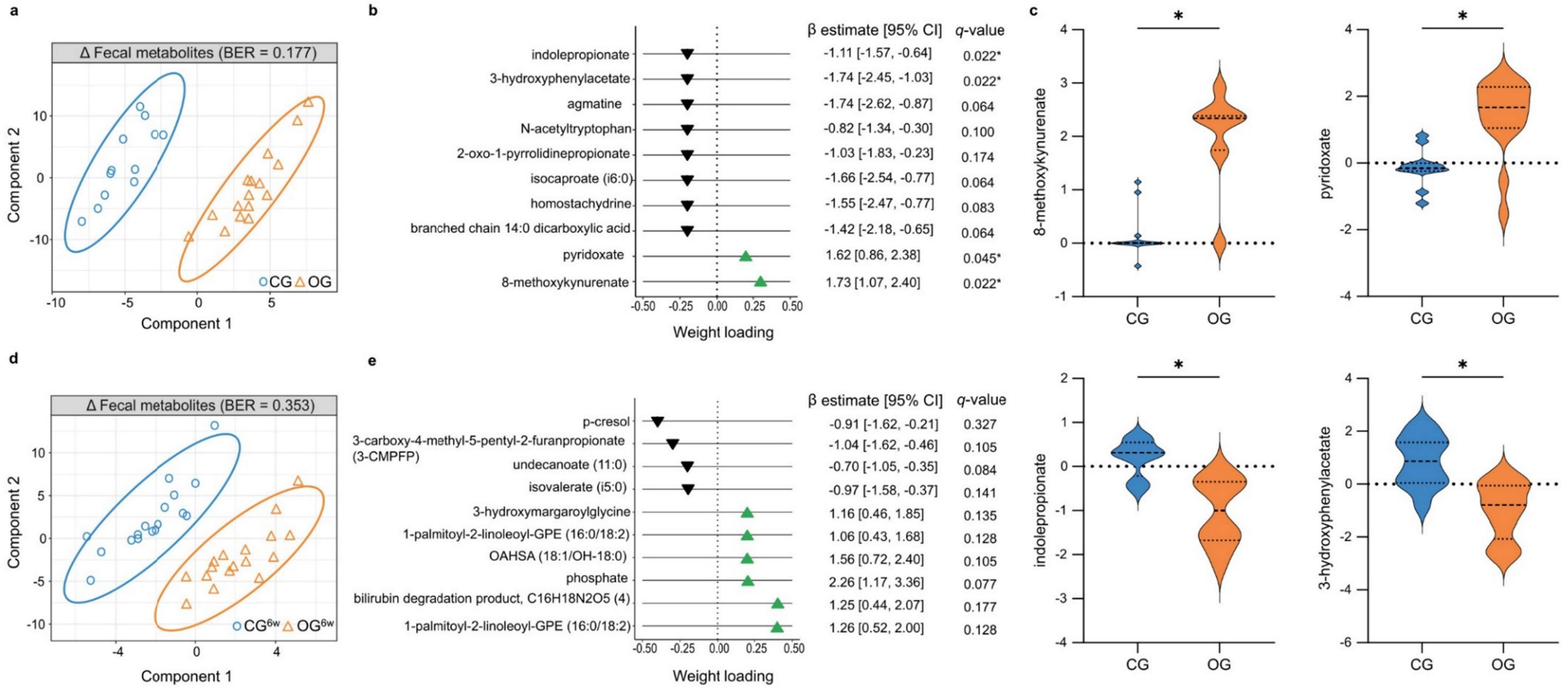

Figure 4-7: Oat diets modulated fecal amino acid and lipid metabolites

Figure 4-7: Oat diets modulated fecal amino acid and lipid metabolites

(**a**) Two-component sample plot of the sPLS-DA on the changes in the global fecal metabolomic profiles in OG and CG. (**b**) Weight loadings of the top 10 selected fecal metabolites in component 1 (green or black triangle: increase or decrease in OG vs. CG). Coefficient and 95% CI were calculated using linear regression models adjusted for baseline concentration, age, sex, BMI ($LnR^{adj.}$). (**c**) Violin plots showing log fold change of selected metabolites (dashed center line: median; dotted line: upper and lower quartiles). Between-group differences were analyzed using linear regression models adjusted for baseline concentration, age, sex, BMI ($LnR^{adj.}$). ****q*-value < 0.001, ***q*-value < 0.01, **q*-value < 0.05. (**d**) Two-component sample plot of the sPLS-DA on the changes in the global fecal metabolomic profiles in OG^{6w} and CG^{6w}. (**e**) Weight loadings of the top 10 selected fecal metabolites in component 1 (green or black triangle: increase or decrease in OG^{6w} vs. CG^{6w}). Coefficient and 95% CI were calculated using linear regression models adjusted for baseline concentration, age, sex, BMI ($LnR^{adj.}$). (**a**–**c**) OG: *n* = 16, CG: *n* = 12. (**d**–**f**) OG^{6w}: *n* = 17, CG^{6w}: *n* = 17.

Abbreviations: BER, balanced error rate; CG, control group; CG^{6w}, six-week control group; OG, oat group; OG^{6w}, six-week oat group

4.6 Shifts in Gut Microbiota Composition

Investigating dietary modulation of the gut microbiota composition based on 16S rRNA gene sequencing using sPLS-DA, a significant difference was observed between OG and CG after the two-day intervention period (AUC = 0.83 (P = 2.96 x 10^{-3}), BER = 0.219; **Figure 4-8a**; **Table 4-8**). *Erysipelotrichaceae* UCG-003 was the most important genus for differentiating the diet groups (weight loading component 1: -1.0) and showed a significant increase in OG compared with CG according to LinDA[269] (log_2 fold change = 1.01, q = 0.025; **Figure 4-8b**). This indicates that the increase is related to oats, which is further supported by the visible reduction during the oat-free follow-up (**Figure 4-8c**).

After the six-week intervention period, differences in the modulation of the microbial composition were also observed between OG^{6w} and CG^{6w} (AUC = 0.79 (P = 0.004), BER = 0.265; **Figure 4-8d**; **Table 4-8**). *Ruminococcus torques* group, a mucolytic bacterium that has been linked to negative health outcomes[291], was most relevant for differentiating the diet groups (weight loading component 1: -1.0) and tended to decrease in OG^{6w} compared with CG^{6w} (log_2 fold change = -1.58, q = 0.07, LinDA; **Figure 4-8e**,**f**).

These results suggest that both a short-term, high-dose and a six-week, moderate oat diet led to specific microbial shifts while maintaining the core microbiome composition with a stable overall ecological structure and diversity (**Figure 4-9**).

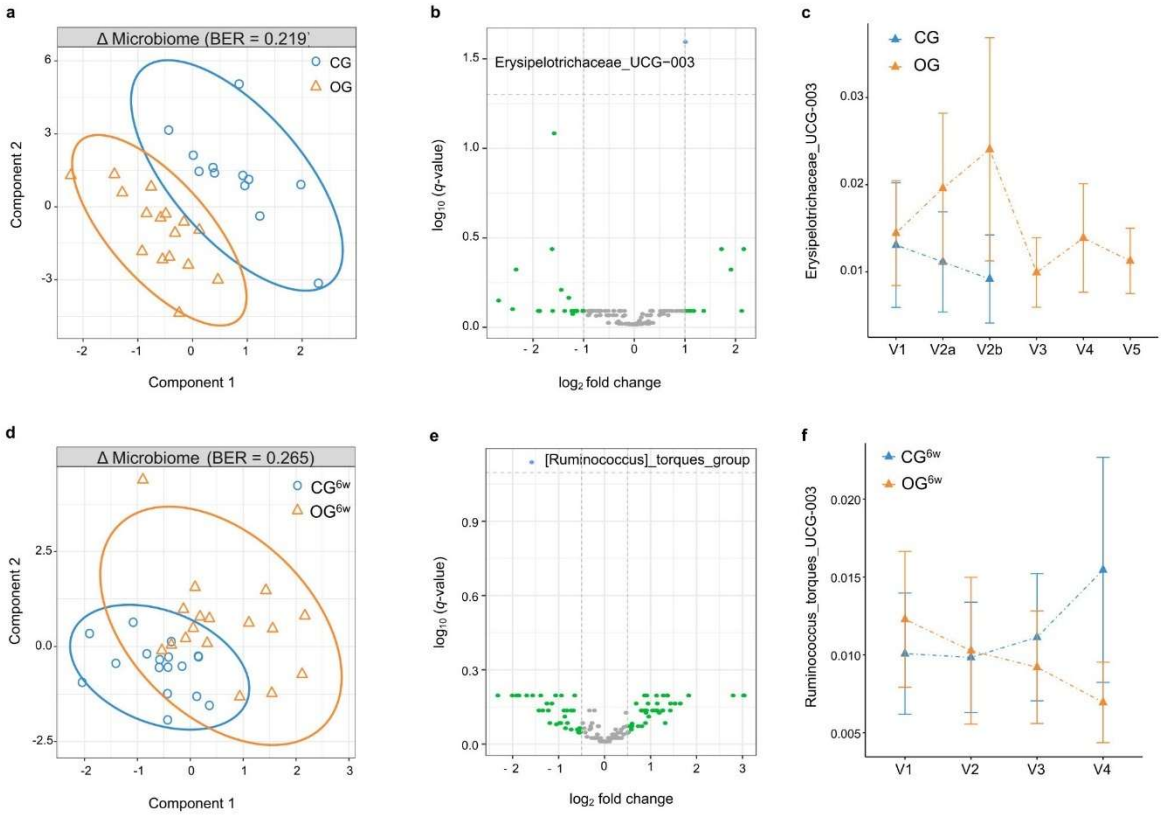

Figure 4-8: Oat-induced modulation of gut microbiota composition

(**a**) Two-component sample plot of the sPLS-DA on the shifts in microbial composition in OG and CG. **(b**) Volcano plot showing the changes in microbial composition on genus level identified using LinDA, presented as log2 fold change of each genus against its statistical significance, reported as negative log10-transformed *q*-value, for the interaction term visit*group. (**c**) Relative abundance of Erysipelotrichaceae UCG-003 (mean ± 95% CI) before (V1), during (V2a) and after the two-day intervention (V2b) as well as during the six-week follow-up period (V3-V5) in OG and CG. (**d**) Two-component sample plot of the sPLS-DA on the shifts in microbial composition in OG^{6w} and CG^{6w}. (**e**) Volcano plot showing the changes in microbial composition on genus level identified using LinDA, presented as log2 fold change of each genus against its statistical significance, reported as negative log10-transformed *q*-value, for the interaction term visit*group. (**f**) Relative abundance of *Ruminococcus torques* group (mean ± 95% CI) before (V1), during (V2, V3) and after the six-week intervention period (V4) in OG^{6w} and CG^{6w}. (**a**–**c**) OG: *n* = 16, CG: *n* = 12. (**d**–**f**) OG^{6w}: *n* = 17, CG^{6w}: *n* = 17.

Abbreviations: BER, balanced error rate; CG, control group; CG^{6w}, six-week control group; OG, oat group; OG^{6w}, six-week oat group.

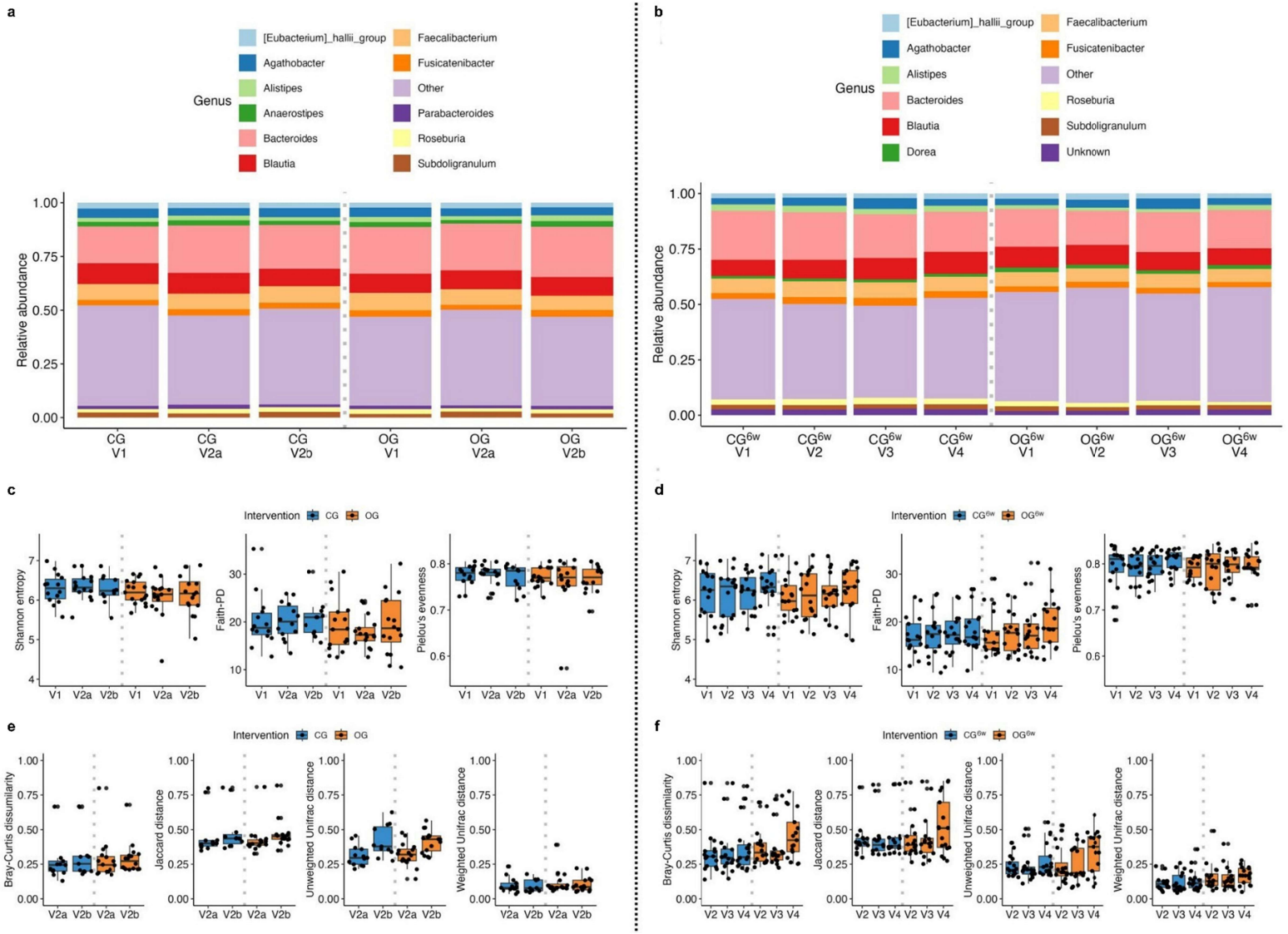

Figure 4-9: Diet-induced changes in microbial composition and diversity

Figure 4-9: Diet-induced changes in microbial composition and diversity

Relative abundance of the 11 most abundant genera in the (**a)** short-term and (**b**) six-week intervention study. These genera comprised approximately 50% of the fecal microbiome, whereas the remaining 133 genera made up the remaining 50%. Alpha diversity indices (Shannon entropy, Faith-PD, and Pielou's evenness) of the (**c**) short-term intervention study (OG: *n* = 17 [V1], *n* = 16 [V2a, V2b]; CG: *n* = 14 [V1, V2a], *n* = 11 [V2b]) and (**d**) the six-week intervention study (OG^{6w}: *n* = 17 [V1+V4], *n* = 16 [V2, V3]; CG^{6w}: *n* = 17 [V1+V4], *n* = 16 [V2, V3]). Beta diversity metrics (Bray Curtis dissimilarity, Jaccard distance, unweighted and weighted Unifrac distance) for the (**e**) short-term intervention study (OG: *n* = 16 [V2a, V2b]; CG: *n* = 13 [V2a], *n* = 11 [V2b]) and (**f**) six-week intervention study (OG^{6w}: *n* = 16 [V2, V3], *n* = 17 [V4]; CG^{6w}: *n* = 16 [V2, V3], *n* = 17 [V4]). (**c-f**) Diversity scores are shown per individual as black dots, as median (center line) and quartiles (box limits: Q1, Q3) for CG and CG^{6w} in blue and OG and OG^{6w} in orange (whiskers extend to 1.5x interquartile range).

Abbreviations: CG, control group; CG6w, six-week control group; OG, oat group; OG6w, six-week oat group; V, visit.

4.7 Shifts in Gut Microbiota Functional Capacity

Whether microbial functional capacity changed in response to the dietary interventions was investigated based on the KEGG database[271] by quantifying pathway abundance using PICRUSt2[270]. According to sPLS-DA, OG and CG modulated the microbial functions differently (AUC = 0.69 (P = 0.08), BER = 0.395; **Figure 4-10a**; **Table 4-8**); even though no clear separation between the diet groups was observed after the two-day intervention period, several pathways seemed to be of relevance for differentiation, including carbohydrate digestion and absorption, aminobenzoate degradation, selenocompound metabolism, naphthalene degradation, and phosphotransferase system (PTS) (**Figure 4-10b**). An increase in aminobenzoate degradation and PTS as well as a decrease in naphthalene degradation and carbohydrate digestion and absorption was identified using $LMM^{adj.}$ (P < 0.05; **Figure 4-10b**), suggesting that benzoate and related pathways might be of relevance and potentially associated with the observed increase in the phenolic metabolites in plasma[292].

Furthermore, a different modulation of the microbial functional capacity between the diet groups of the six-week intervention study was revealed (AUC = 0.68 (P = 0.07), BER = 0.353; **Figure 4-10c**; **Table 4-8**) with phenylalanine metabolism, proximal tubule bicarbonate reclamation, beta-lactam resistance, fructose and mannose metabolism, and PTS seemed to be the most relevant pathways for differentiation (**Figure 4-10d**) even though no clear separation between OG^{6w} and CG^{6w} was observed. An increase in OG compared to CG was shown in phenylalanine metabolism and proximal tubule bicarbonate reclamation, while a decrease was observed in the other pathways using $LMM^{adj.}$ (P < 0.05; **Figure 4-10d**). Thus, the results suggest that a the six-week, moderate oat diet might modulate a variety of different microbial functions, which is in line with the heterogenous metabolic response.

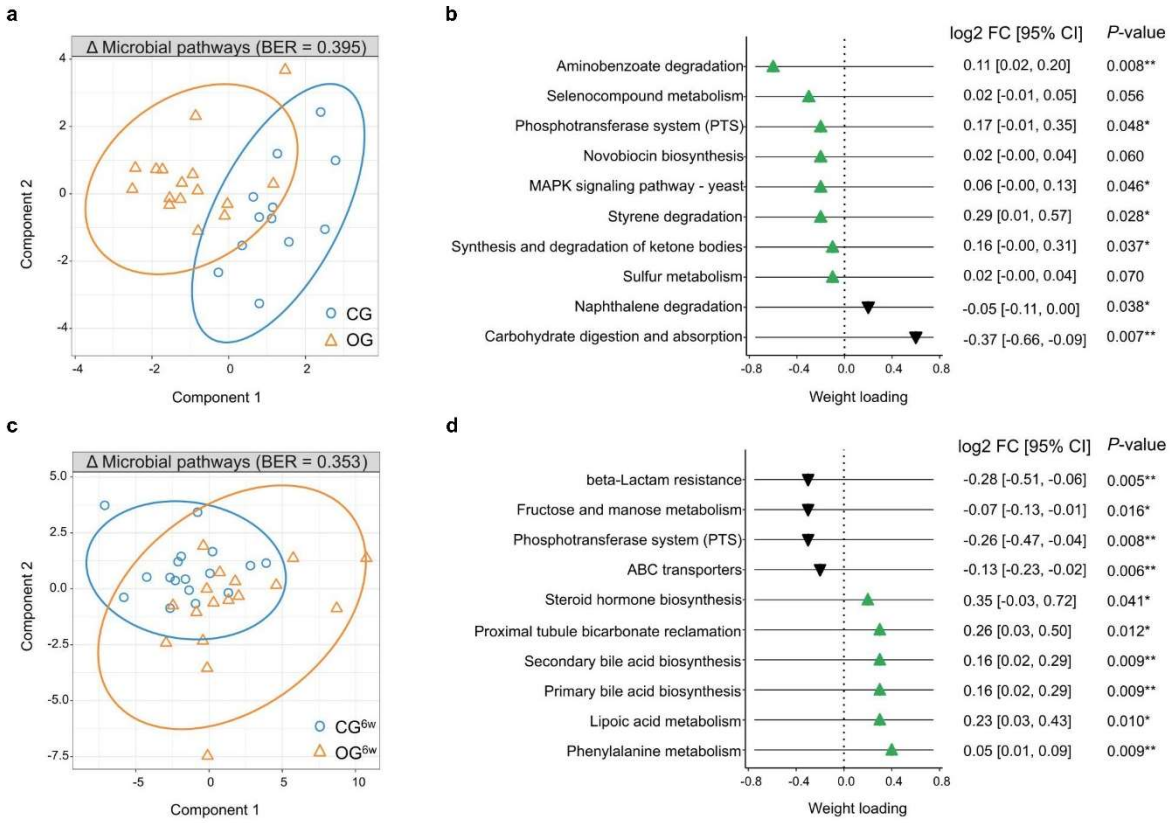

Figure 4-10: Oat-induced modulation of the gut microbiota functional capacity

(**a**) Two-component sample plot of the sPLS-DA on the changes in microbial functional capacity in OG and CG. (**b**) Weight loadings of the top 10 selected microbial pathways in component 1 (green or black triangle: increase or decrease in OG vs. CG). Log2 fold change and 95% CI were calculated using linear mixed models adjusted for baseline concentration, age, sex, BMI ($LMM^{adj.}$). (**c**) Two-component sample plot of the sPLS-DA on the changes in microbial functional capacity in OG^{6w} and CG^{6w}. (**d**) Weight loadings of the top 10 selected microbial pathways in component 1 (green or black triangle: increase or decrease in OG^{6w} vs. CG^{6w}). Log2 fold change and 95% CI were calculated using linear mixed models adjusted for baseline concentration, age, sex, BMI ($LMM^{adj.}$). (**a**+**b**) OG: *n* = 16, CG: *n* = 12. (**c**+**d**) OG^{6w}: *n* = 17, CG^{6w}: *n* = 17.

Abbreviations: BER, balanced error rate; CG, control group; CG^{6w}, six-week control group; OG, oat group; OG^{6w}, six-week oat group.

4.8 Interactions between metabolism, gut microbiome, and metabolomic profiles

DIABLO revealed strong correlations between the changes in metabolism, plasma and fecal metabolites, as well as microbial composition (model 1.1: AUC = 0.94 (P = 5.03 x 10^{-3}), BER = 0.07; **Figure 4-11a**) and functional capacity (model 2.1: AUC = 0.92 (P = 5.11 x 10-3), BER = 0.04; **Figure 4-11b**) induced by the short-term, high-dose oat diet. Notably, the reduction in serum cholesterol levels was associated with an increase in several plasma phenolic compounds including FA, DHFA, 2-acetamidophenol sulfate, 2-aminophenol sulfate, 4-hydroxyhippurate, vanilloylglycine, 4-vinylphenol sulfate, 2-methoxyhydroquinone sulfate (1), and 3-methoxycatechol sulfate (2). These inverse associations were additionally largely confirmed by pairwise correlation analyses (P < 0.05; **Figure 4-11c**). In addition, PLS regression models revealed that the change in plasma phenolic compounds solely predicted 13.6% of the variation in cholesterol levels (Q^2 = 0.136) and explained 13.5% of the variance in TC (R^2 = 0.135) and 19.3% of the variance in LDL-C (R^2 = 0.193). Thus, the results suggest that phenolic compounds play an important role in the cholesterol-lowering effect of oats.

Moreover, associations of microbial phenol degradation products including DHFA and 4-hydroxyhippurate with genera such as *Erysipelotrichaceae* UCG-003 and microbial pathways such as aminobenzoate and naphthalene degradation were found (see below), indicating that the increase in plasma phenols is driven by oat-induced alterations in the gut microbiota. Shifts in *Erysipelotrichaceae* UCG-003, *Marvinbryantia* and naphthalene degradation were associated with changes in circulating cholesterol levels (see below), with the inverse association between *Erysipelotrichaceae* UCG-003 and cholesterol levels also identified by pairwise correlation analysis (P < 0.05; **Figure 4-11d**). In addition, these changes in the gut microbiome solely predicted 13.5% of the variation in cholesterol levels (Q^2 = 0.135) and explained 11.1% of the variance in TC (R^2 = 0.111) and 19.6% of the variance in LDL-C (R^2 = 0.196). This supports the assumption that alterations in microbial composition and functional capacity may contribute to lowering cholesterol levels.

Besides the phenolic compounds, various plasma amino acid metabolites such as 3-methylhistidine, N-acetyl-3-methylhistidine, and 1-methyl-5-imidazolelactate were positively associated with the oat-induced change in circulating cholesterol levels,

which was also shown by pairwise correlations ($P < 0.05$; see below). These metabolites, especially 3-methylhistidine, were additionally linked to shifts in *Erysipelotrichaceae* UCG-003 and microbial naphthalene degradation, indicating that a decrease in histidine metabolites due to oat-induced shifts in microbial composition and functional capacity may contribute to lowering cholesterol levels; however, the observed associations were not as strong as for the phenolic compounds.

Moreover, the changes in cholesterol levels were positively associated with fatty acids, including plasma 2S,3R-dihydroxybutyrate, fecal branched-chain 14:0 dicarboxylic acid, and fecal isocaproate (i6:0), and negatively associated particularly with fecal endocannabinoids, which have been linked to anti-inflammatory effects[293] (see below). These results were largely confirmed by additional pairwise correlation analyses ($P < 0.05$; **Figure 4-11c**,**e**). Notable, shifts in *Erysipelotrichaceae* UCG-003 and microbial naphthalene degradation were associated with many of the above-mentioned metabolites. This suggests that changes in the lipidome contribute to the reduction in circulating cholesterol levels potentially via an oat-induced modulation of the gut microbiota; however, the correlations were also less pronounced compared to those with the phenolic compounds.

In the six-week intervention study, DIABLO revealed several correlations between changes in metabolism, plasma and fecal metabolites, as well as the microbial composition (model 1.2: AUC = 0.85 ($P = 4.42 \times 10^{-3}$), BER = 0.32; **Figure 4-12a**) and functional capacity (model 2.2: AUC = 0.85 ($P = 0.004$), BER = 0.32; **Figure 4-12b**). However, most of the selected features did not differ between OG^{6w} and CG^{6w}, indicating that the identified correlations might not be related to the moderate oat diet and should be interpreted with caution.

Details on the structure of all DIABLO models performed, including the selected number of features per component, are shown in **Table 4-9**.

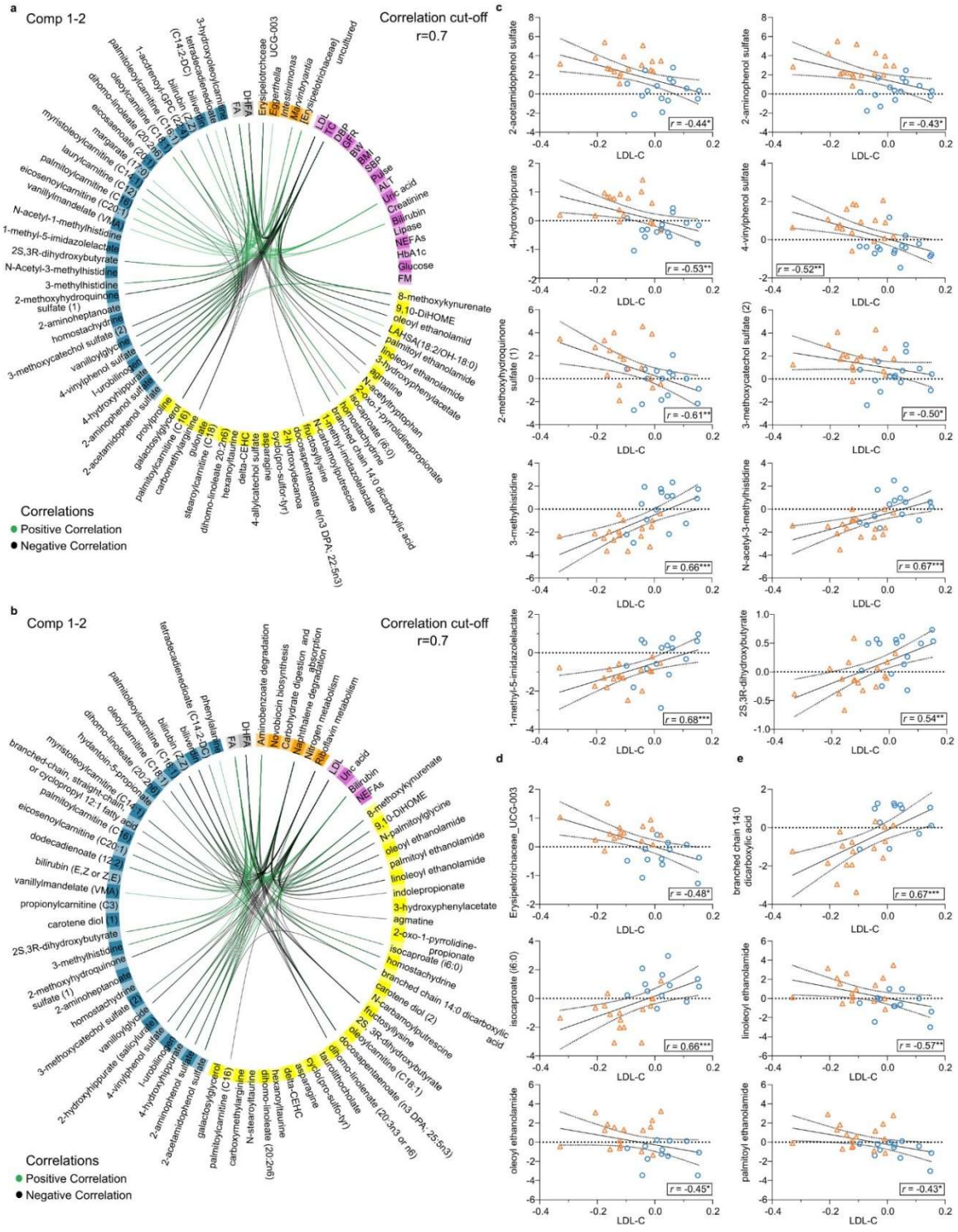

Figure 4-11: Oat-derived bioactive phenolic compounds reduce cholesterol through the gut microbiome

Figure 4-11: Oat-derived bioactive phenolic compounds reduce cholesterol through the gut microbiome

Circos plot shows positive (green) and negative (black) correlations (cutoff r = ±0.7) between the selected variables in the five data sets along component 1 and 2 derived from the DIABLO evaluations ("models") (n = 28). (**a**) Model 1.1 includes the data sets gut microbiome composition (orange), clinical marker (violet), fecal metabolites (yellow), plasma metabolites (blue), and targeted plasma metabolomic profile (DHFA, FA) (grey). (**b**) Model 2.1 includes the data sets microbial pathways (orange), clinical marker (violet), fecal metabolites (yellow), plasma metabolites (blue), and targeted plasma metabolomic profile (DHFA, FA) (grey). Intra-block correlations are not presented. Scatter plots show the associations between the log fold changes in LDL-cholesterol and (**c**) selected plasma metabolites, (**d**) Erysipelotrichaceae UCG-003, and (**e**) selected fecal metabolites in OG (presented as orange triangles) and CG (presented as blue circles). Pairwise correlations were assessed using Spearman's rank correlation coefficient. (**a**,**b**,**d**,**e**) n = 28 (OG: n = 16, CG: n = 12). (**c**) n = 32 (OG: n = 17, CG: n = 15).

Abbreviations: ALT, alanine aminotransferase; BMI, body mass index; BW, body weight; DHFA, dihydroferulic acid; DBP, diastolic blood pressure; GFR, glomerular filtration rate; FA, ferulic acid; FM, fat mass; LDL-C, low-density lipoprotein cholesterol; NEFAs, non-esterified fatty acids; SBP, systolic blood pressure; TC, total cholesterol.

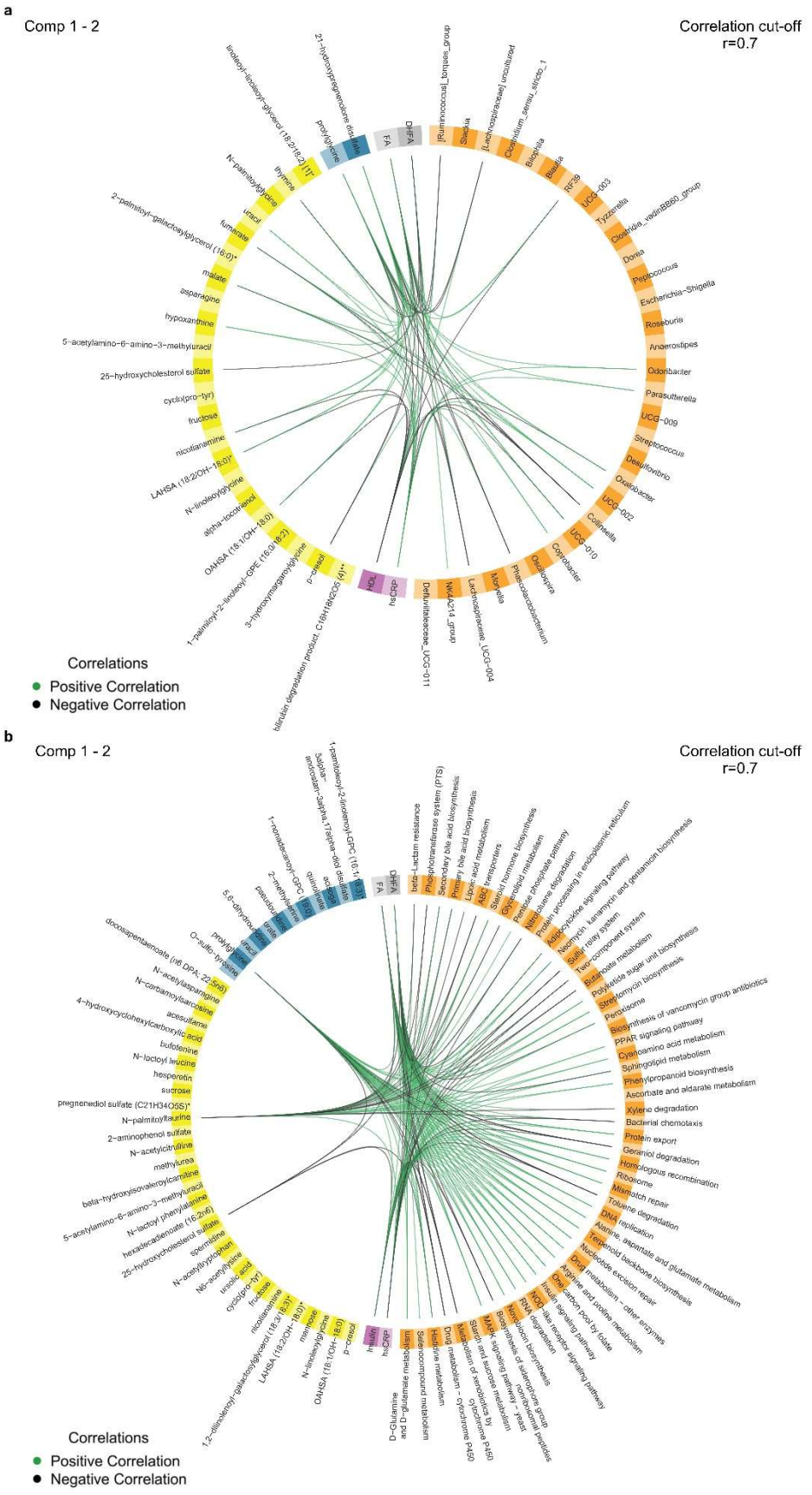

Figure 4-12: Associations between the change in clinical markers, metabolites and gut microbiota following the six-week dietary intervention

Figure 4-12: Associations between the change in clinical markers, metabolites and gut microbiota following the six-week dietary intervention

Circos plot shows positive (green) and negative (black) correlations (cutoff $r = \pm 0.7$) between the selected variables in the five different data sets along component 1 and 2, derived from the DIABLO evaluations ("models"). (**a**) Model 1.2 includes the following data sets: gut microbiome composition (orange), clinical marker (violet), fecal metabolites (yellow), plasma metabolites (blue), and targeted plasma metabolomic profile (DHFA, FA) (grey). (**b**) Model 2.2 includes the following data sets: microbial pathways (orange), clinical marker (violet), fecal metabolites (yellow), plasma metabolites (blue), and targeted plasma metabolomic profile (DHFA, FA) (grey). (**a**, **b**) Intra-block correlations are not presented. Log-fold change was used as the input to the models (n = 34 (OG^{6w}: n = 17), CG^{6w}: n = 17)).

Abbreviations: DHFA, dihydroferulic acid; FA, ferulic acid; HDL-C, high-density lipoprotein cholesterol; hsCRP, high-sensitivity C-reactive protein.

Table 4-9: Structure of the final DIABLO models

Model	Intervention	No. of Comp	No. of variables per component and data set					
			Comp	Targeted plasma metabolomic profile	Clinical markers	Bacterial genera or KEGG pathways	Global plasma metabolomic profile	Global fecal metabolomic profile
1.1	Short-term	2	1	1	3	4	15	15
			2	1	15	1	15	15
1.2	Six-week	2	1	1	1	21	1	11
			2	1	1	11	1	11
2.1	Short-term	2	1	1	1	5	15	15
			2	1	3	2	15	15
2.2	Six-week	2	1	1	1	31	1	31
			2	1	1	21	11	1
Subgroup analysis								
				Plasma SCFAs	**Clinical markers**	**Bacterial genera**		
3.1	Short-term	2	1	1	4	16		
			2	1	2	66		
3.2	Six-week	2	1	1	3	117		
			2	7	1	137		

Abbreviations: Comp, component; KEGG; Kyoto encyclopedia of genes and genomes; SCFAs, short-chain fatty acids.

4.9 *In vitro* Experiment

4.9.1 *In vitro* Cell Culture Experiment

In PBMCs fed with alkyne cholesterol, DHFA led to a significant decrease in the molar fraction of alkyne cholesterol (-15.71 ± 6.33 mol% (mean ± SEM), $P = 0.042$), while total lipidome remained stable (**Figure 4-13a**). In addition, in MHCs, the molar fraction of alkyne cholesterol esters tended to be lower in the PBMCs treated with DHFA (-5.54 ± 1.94 mol%, $P = 0.065$; **Figure 4-13a**). These results support that microbially produced phenolic compounds such as DHFA mediate the oats' cholesterol-lowering effects by reducing alkyne cholesterol and cholesterol esters incorporation into PBMCs relative to the total lipidome.

In HuH7 cells fed with ^{13}C labeled acetate and alkyne fatty acid 182, DHFA seemed to decrease the molar fraction of ^{13}C labeled-alkyne cholesterol esters and alkyne cholesterol esters of the total lipidome, while the total lipidome itself increased by trend (**Figure 4-13b**). Consistent with this, a decrease in the molar fraction of the unlabeled cholesterol esters tended to take place (**Figure 4-14a**). No differences in total lipidome, the molar fraction of alkyne cholesterol esters and alkyne cholesterol were observed between HuH7 cells either treated with or without DHFA and additionally fed with alkyne cholesterol (**Figure 4-14b**). These results indicate that DHFA might impact different effects i) the totality of lipids, ii) the *de novo* synthesis of cholesterol esters and iii) the esterification of cholesterol in HuH7 cells, supporting that phenolic compounds such as DHFA might contribute to the cholesterol-lowering properties of oats.

4.9.2 Fecal Batch Culture Fermentation of *in vitro* Digested Oats

To prove whether the identified phenolic compounds stem from the microbial metabolization of oats, anaerobic fecal batch culture fermentations of *in vitro* digested oat flakes were conducted. This demonstrated that the gut microbes are capable of producing a variety of metabolites including phenolic compounds by directly interacting with the provided oats. In particular, a significant increase in 2-aminophenol was shown in both oat groups (POG vs. PCG: $P < 0.001$ (6 h, 36 h), $P < 0.01$ (24 h), SOG vs. SCG: $P < 0.01$ (24 h, 36 h); **Figure 4-13c**), which is in line with the increase in the plasma level of 2-aminophenol sulfate observed in the RCTs.

In addition, a rapid production of microbial products such as DHFA and vanillic acid were observed in both oat groups by a simultaneous reduction of oat phenolic compounds such as FA and vanillin mainly within 6 h (POG vs. PCG: $P < 0.01$ (DHFA, FA, vanillin), $P < 0.05$ (vanillic acid); SOG vs. SCG: $P < 0.05$ (DHFA, FA, vanillin); **Figure 4-14c**), emphasizing that the microbial activity results in the production of phenolic oat-derived metabolites.

Moreover, as expected, shifts in the concentrations of various SCFAs, in particular an increase in acetic acid (POG vs. PCG: all $P < 0.05$) and succinate (SOG vs. SCG: $P = 0.033$ (36 h)) and a decrease in isobutyric acid (POG vs. PCG: $P = 0.002$ (24 h), SOG vs. SCG: $P = 0.001$ (6 h), $P = 0.047$ (36 h); **Figure 4-13c**), were found. Furthermore, significantly higher abundances of known SCFA-producing bacteria such as *Faecalibacterium*, *Fusicatenibacter*, *Bifidobacterium*, *Blautia*, and *Roseburia* were observed in POG compared with PCG, especially after 36 h (all $P < 0.05$; **Figure 4-14d**). In addition, *Erysipelotrichaceae* UCG-003 seemed to increase in POG compared to PCG after 36h of incubation ($P = 0.172$; **Figure 4-13d**), supporting that the identified increase in the abundance of *Erysipelotrichaceae* UCG-003 in the RCT is induced by the short-term, high-dose oat intake.

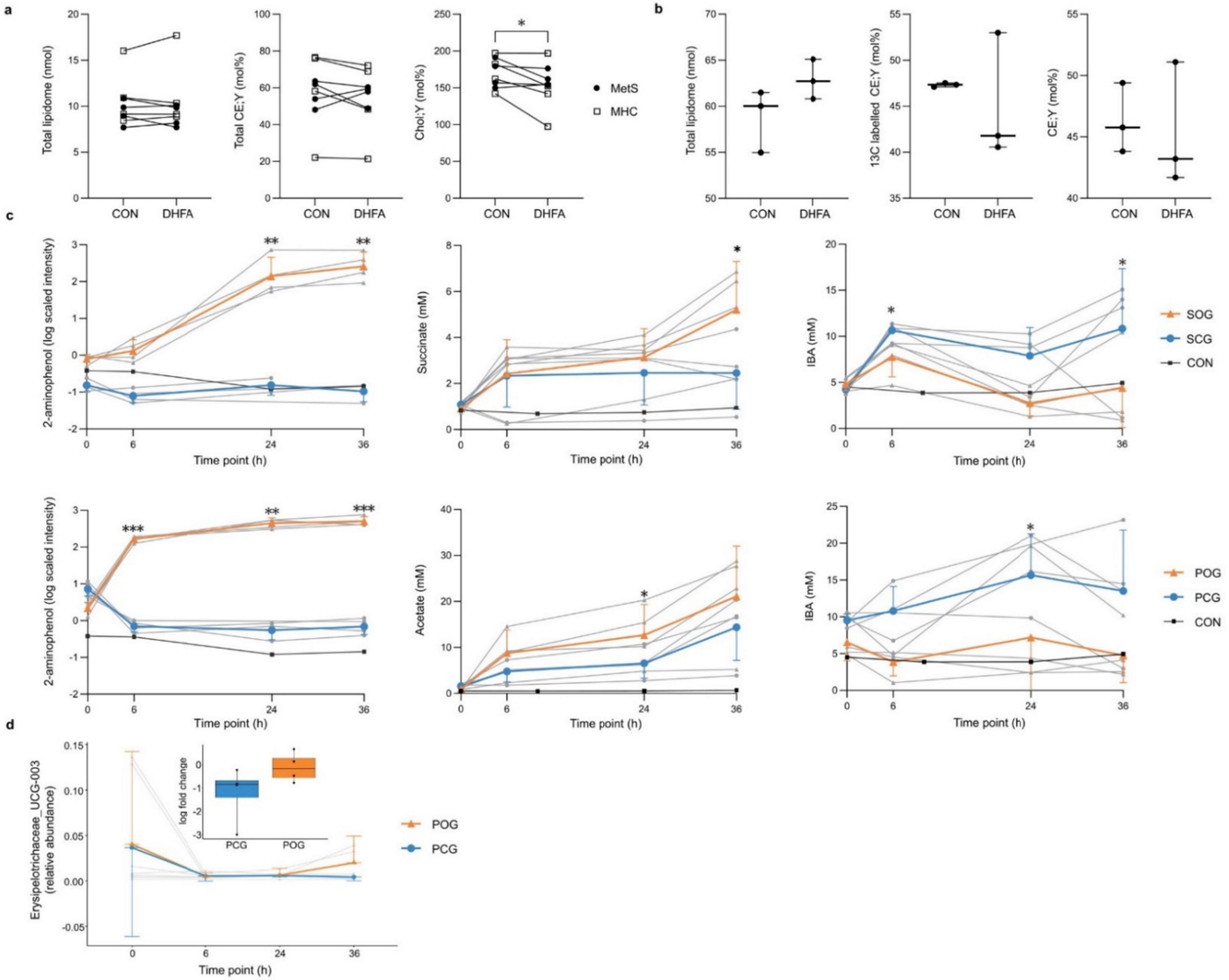

Figure 4-13: Effects of microbially produced phenolic compounds on cholesterol metabolism *in vitro*

Figure 4-13: Effects of microbially produced phenolic compounds on cholesterol metabolism *in vitro*

(**a**) Impact of DHFA on the total lipidome and the molar fraction (mol%) of alkyne cholesterol esters and alkyne cholesterol of the total lipidome of PBMCs fed with alkyne cholesterol. Individual data points are shown for n = 8 participants (black circles: individuals with MetS, white squares: MHCs). Differences between the treatment groups (DHFA vs. CON) were analyzed using paired Student's t-test. *P < 0.05. (**b**) Impact of DHFA on the total labeled lipidome and the molar fraction (mol%) of ^{13}C labeled-alkyne cholesterol esters and alkyne cholesterol esters of HuH7 cells fed with ^{13}C labeled acetate and alkyne fatty acid 182. Samples are triplicate for each condition (n = 3) and presented as median and range. Differences between the treatment groups (DHFA vs. CON) were analyzed using unpaired Student's t-test. (**c**) Microbially induced changes in 2-aminophenol (log fold change) and selected SCFAs (mM) *in vitro* over 36 h. Data are shown as individuals data points (grey triangles: SOG or POG, grey dots: SCG or PCG, n = 4 each; black squares: control) and as mean ± SD (orange: SOG or POG, blue: SCG or PCG). Differences between the treatment groups were analyzed using paired Student's t-test with the log fold change as input. *P < 0.05, **P < 0.01, ***P < 0.001. (**d**) Shifts in the relative abundance of Erysipelotrichaceae UCG-003 *in vitro* over 36 h. Data are shown as individuals data points (grey triangles: POG, grey dots: PCG, n = 4 each) and as mean ± SD (orange: POG, blue: PCG). Boxplots show the corresponding log fold change at 36 h (dashed center line: median; whiskers: min and max; dots: individual data points, n = 4 each). Differences between the groups were analyzed using paired Student's t-test with the fold change of the CLR-transformed count data as input (relative abundance threshold: 0.01%).

Abbreviations: CON, control treatment (without DHFA); DHFA, dihydroferulic acid; MHCs, metabolically healthy controls; MetS, metabolic syndrome; PCG, physiological control group; POG, physiological oat group; SCG, starving control group; SOG, starving oat group.

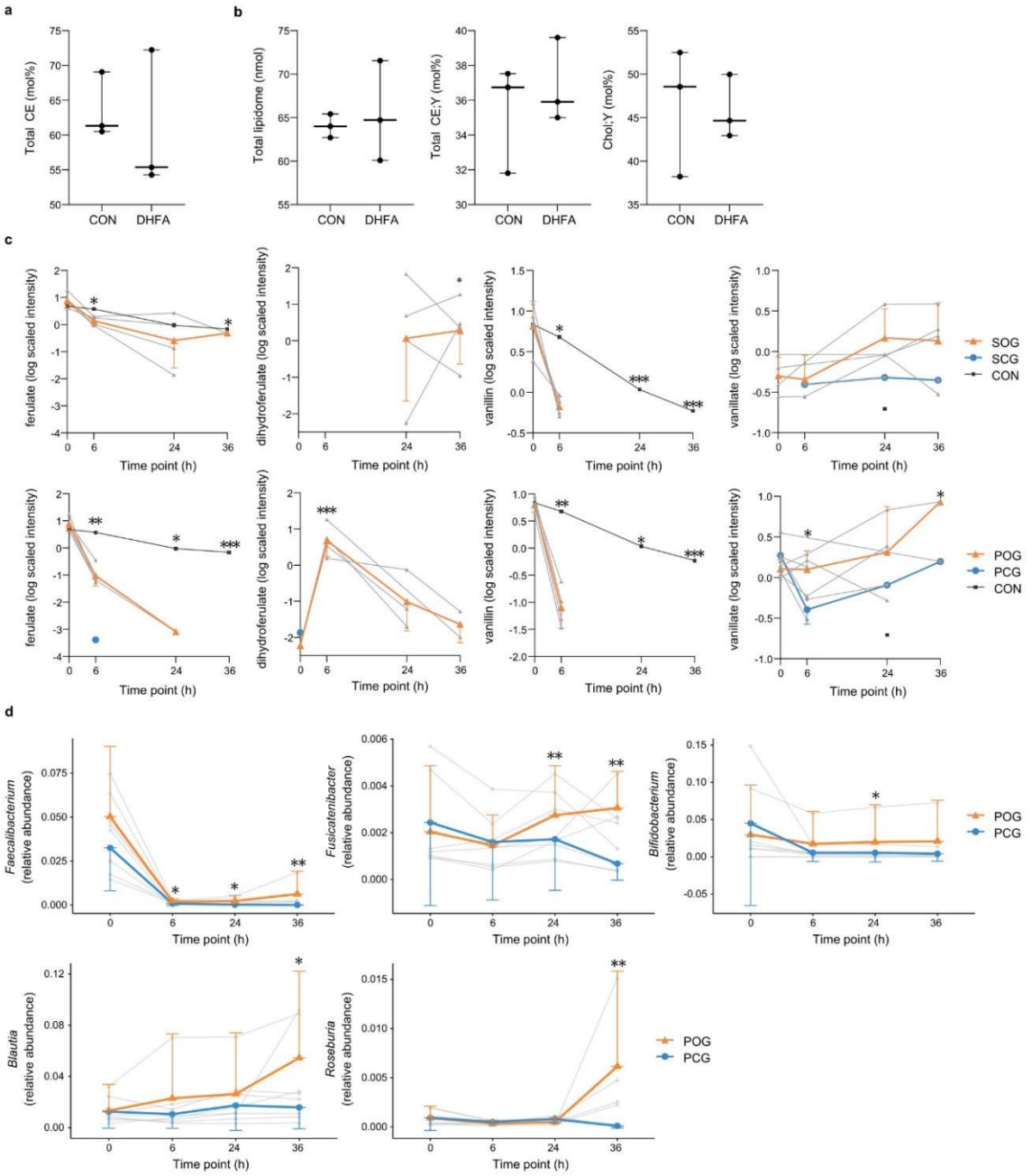

Figure 4-14: Impact of microbially produced phenolic compounds from oats on cholesterol metabolism *in vitro* and changes in selected genera

Figure 4-14: Impact of microbially produced phenolic compounds from oats on cholesterol metabolism *in vitro* and changes in selected genera

Impact of DHFA on (**a**) the molar fraction (mol%) of total unlabeled cholesterol esters of the total lipidome (labeled + unlabeled) of HuH7 cells fed with13C labeled acetate and alkyne fatty acid 182 as well as on (**b**) the total lipidome (labeled + unlabeled) and the molar fraction (mol%) of alkyne cholesterol esters and alkyne cholesterol of HuH7 cells fed with an alkyne cholesterol. Samples are triplicate for each condition (n = 3) and presented as median and range. Differences between the treatment groups (DHFA vs. CON) were analyzed using unpaired Student's t-test. (**c**) Microbially induced changes in phenolic metabolites *in vitro* over 36 h. Data are shown as individuals data points (grey triangles: SOG and POG, grey dots: SCG and PCG; n = 4 each; black squares: control) and as mean ± SD (orange: SOG and POG, blue: SCG and PCG). Differences between the treatment groups were analyzed using paired Student's t-test with the log fold change as the input. *P < 0.05, **P < 0.01, ***P < 0.001. (**d**) Shifts in the relative abundances of selected significant genera between POG and PCG *in vitro* over 36 h. Data are shown as individuals data points (grey triangles: POG, grey dots: PCG; n = 4 each) and as mean ± SD (orange: POG, blue: PCG). Differences between the groups were analyzed using paired Student's t-test with the fold change of the CLR-transformed count data as input (relative abundance threshold: 0.01%). *P < 0.05, **P < 0.01.

Abbreviation: CON, control treatment (without DHFA); DHFA, dihydroferulic acid; PCG, physiological control group; POG, physiological oat group; SCG, starving control group; SOG, starving oat group.

4.10 Subgroup Analysis

4.10.1 Participants Characteristics

Participants of the short-term intervention included in this subgroup analysis (*n* = 27) had a mean age of 58.8 ± 7.8 years, were 56% females, and had a BMI of 32.1 ± 3.4 kg/m². Included participants of the six-week intervention (*n* = 22) were 59.1 ± 7.6 years, 55% females, and had a BMI of 31.9 ± 3.1 kg/m². A consort diagram of participants flow is provided in **Figure 4-15**. In both interventions, no significant differences in gut permeability, inflammatory status and plasma SCFAs concentrations were observed between the study groups at baseline (**Table 4-10**).

4.10.2 Gastrointestinal Tolerability and Well-Being

Overall, the study diets were well tolerated, with generally low symptom scores during both intervention periods (**Table 4-11**). Participants in the OG reported slightly more adverse effects than those in the CG, particularly on the second intervention day ($P = 0.011$). The most frequently reported diet-related adverse events in both studies were flatulence (GI tolerability) and exhaustion (well-being).

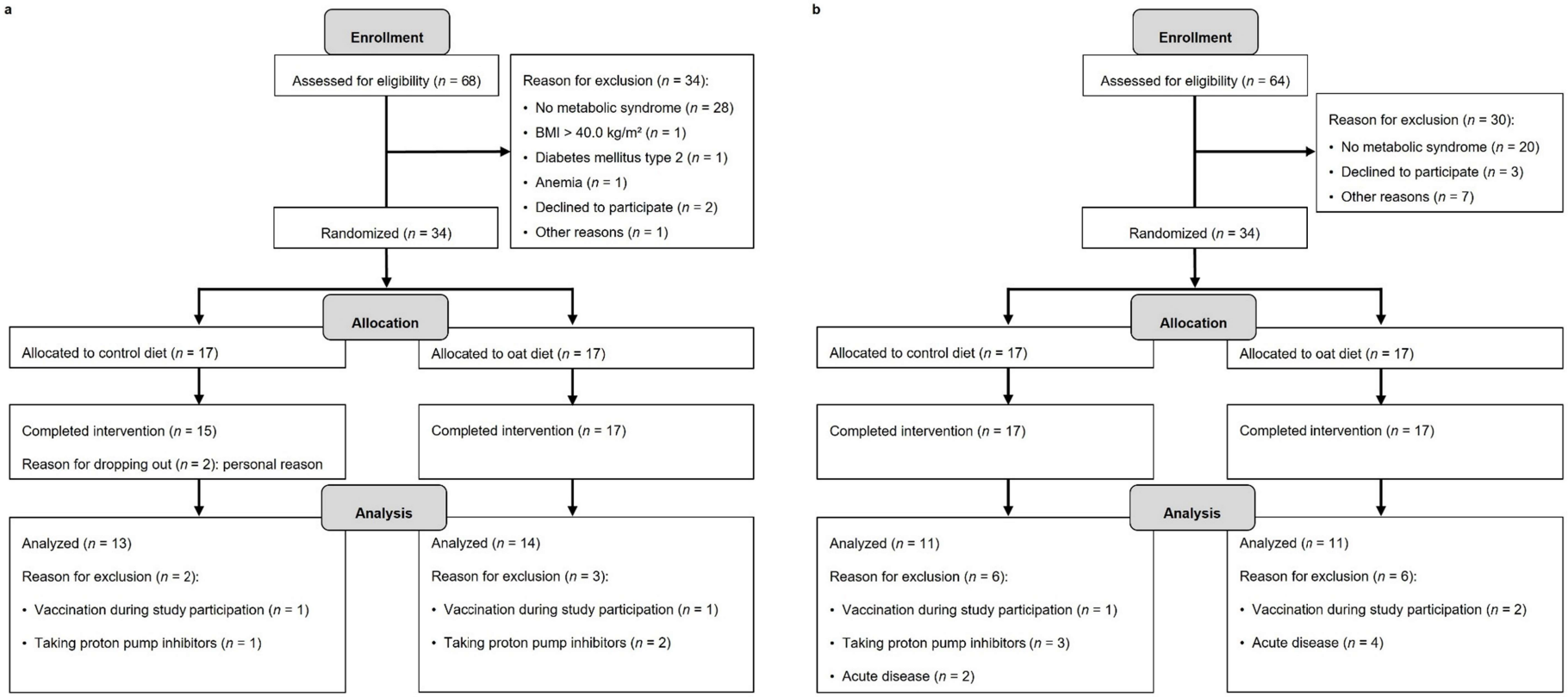

Figure 4-15: Participants flow diagram for the subgroup analysis in (a) the short-term and (b) the six-week dietary interventions based on the CONSORT flow diagram

Table 4-10: Baseline characteristics of participant subgroups in the short-term and the six-week interventions[1]

Parameter	Short-term intervention				Six-week intervention			
	All participants (*n* = 27)	**CG (*n* = 13)**	**OG (*n* = 14)**	***P***	**All participants (*n* = 22)**	**CG^{6w} (*n* = 11)**	**OG^{6w} (*n* = 11)**	***P***
Zonulin, ng/mL	290.2 (467.3)	251.0 (348.3)	456.0 (419.0)	0.282	477.3 (813.4)	781.1 (820.7)	310.2 (796.7)	0.243
LPS, pg/mL	45.7 (61.3)	70.7 (59.5)	35.3 (60.5)	0.302	27.1 (75.4)	25.0 (88.5)	29.2 (74.9)	1.000
hsCRP, mg/L	2.3 (3.0)	1.8 (3.4)	2.9 (3.0)	0.289	2.0 (3.5)	1.9 (3.6)	2.6 (4.1)	0.631
IL-6, pg/mL	1.5 (1.9)	1.5 (2.4)	1.5 (1.5)	0.570				
Acetic acid, ng/mL	2,774.2 (1,584.2)	2,794.9 (2,290.1)	2,678.9 (1716.0)	0.283	3,059.2 ± 1,391.1	3,320.2 ± 1,787.2	2,798.3 ± 848.6	0.396
Propionic acid, ng/mL	81.3 (31.0)	83.8 (39.3)	81.1 (27.6)	0.516	86.2 (24.0)	83.3 (32.0)	86.7 (20.5)	0.435
Butyric acid, ng/mL	43.4 (27.2)	42.7 (32.8)	51.4 (28.9)	0.622	35.5 (26.1)	37.4 (28.5)	33.3 (17.7)	0.562
Isobutyric acid, ng/mL	18.9 (8.3)	18.1 (9.0)	19.1 (6.8)	0.914	23.3 ± 6.7	20.9 ± 6.6	25.7 ± 6.2	0.095
2-MBA, ng/mL	43.7 ± 7.7	43.3 ± 9.2	44.1 ± 6.5	0.796	42.0 (7.8)	38.9 (9.3)	45.4 (7.7)	0.090
Isovaleric acid, ng/mL	44.0 (27.3)	55.7 (25.0)	38.8 (28.5)	0.303	56.0 ± 27.8	50.2 ± 31.0	61.9 ± 24.1	0.336
Valeric acid, ng/mL	19.3 ± 4.0	19.1 ± 4.2	19.4 ± 3.9	0.863	17.8 (5.2)	18.8 ± 2.7	19.5 ± 6.2	0.921
Hexanoic acid, ng/mL	75.7 ± 27.2	68.8 ± 20.6	82.1 ± 14.1	0.060	69.3 ± 14.2	68.0 ± 11.2	70.5 ± 17.1	0.700

[1]Data are presented as mean ± SD or median (IQR) for skewed data. Differences between the groups were assessed using unpaired Student's *t*-test or Mann-Whitney U-test for normal or skewed data, respectively.

Abbreviations: CG, control group; CG6w, six-week control group; hsCRP, high-sensitivity C-reactive protein; IL-6, interleukin 6; LPS, Lipopolysaccharides; OG, oat group; OG6w, six-week oat group; 2-MBA, 2-Methylbutyric acid.

Table 4-11: Gastrointestinal (GI) tolerability of the study diets and well-being[1]

	Short-term intervention			Six-week intervention		
	CG (*n* = 13)[2]	**OG (*n* = 14)**	***P***	**CG^{6w} (*n* = 11)**	**OG^{6w} (*n* = 11)**	***P***
GI tolerability (score: 0 – 12)						
First intervention day	0.0 (2.0)	0.5 (2.0)	0.721	0.0 (2.0)	0.0 (2.0)	0.883
Last intervention day	0.0 (1.0)	1.5 (1.0)	**0.011***	0.0 (2.0)	0.0 (2.0)	1.000
Average	0.0 (0.9)	1.0 (1.1)	0.107	0.0 (1.0)	0.0 (2.0)	0.472
GI tolerability over the six-week intervention period (score: 0 – 168)	x	x	x	1.5 (21.0)	6.5 (42.0)	1.000
Well-being (score: 0 – 18)						
First intervention day	0.0 (3.0)	1.0 (4.0)	0.888	1.0 (4.0)	0.0 (1.0)	0.421
Last intervention day	0.0 (1.0)	3.5 (7.0)	0.317	0.0 (3.0)	0.0 (1.0)	0.186
Average	0.3 (1.5)	3.8 (5.6)	0.161	0.5 (3.5)	0.0 (1.0)	0.307
Well-being over the six-week intervention period (score: 0 – 252)	x	x	x	3.0 (9.0)	3.0 (10.0)	1.000

[1]Data are shown as median (IQR). P-value refers to Fisher's exact test (*$p < 0.05$). The lower the score, the better the GI tolerability and well-being. [2]Last intervention day: $n = 12$.

Abbreviations: CG, control group; CG6w, six-week control group; OG, oat group; OG6w, six-week oat group.

4.10.3 Effects on Gut Permeability and Inflammatory Status

Following the 2-day energy-restricted high-dose oat diet, zonulin level decreased compared to baseline (Δ -131.91 ± 27.91 ng/mL (mean ± SEM), $P^{adj.}$ = 0.002), whereas it remained stable in the CG (Δ -48.75 ± 24.84 ng/mL, $P^{adj.}$ = 0.156) (**Figure 4-16a**). However, no significant difference was found between the groups (-75.9 [-162.1, -1.8] ng/mL (β estimate OG vs. CG adjusted for baseline [95% CI], $P^{adj.}$ = 0.216). LPS (**Figure 4-16b**), hsCRP, and IL-6 concentrations remain unchanged and did not differ between the two diet groups (**Table 4-12**). During the six-week intervention period, the markers for gut permeability and inflammation remained stable with no differences between OG^{6w} and CG^{6w}. Comparable results were also observed after additional adjustment for sex. (**Table 4-12**)

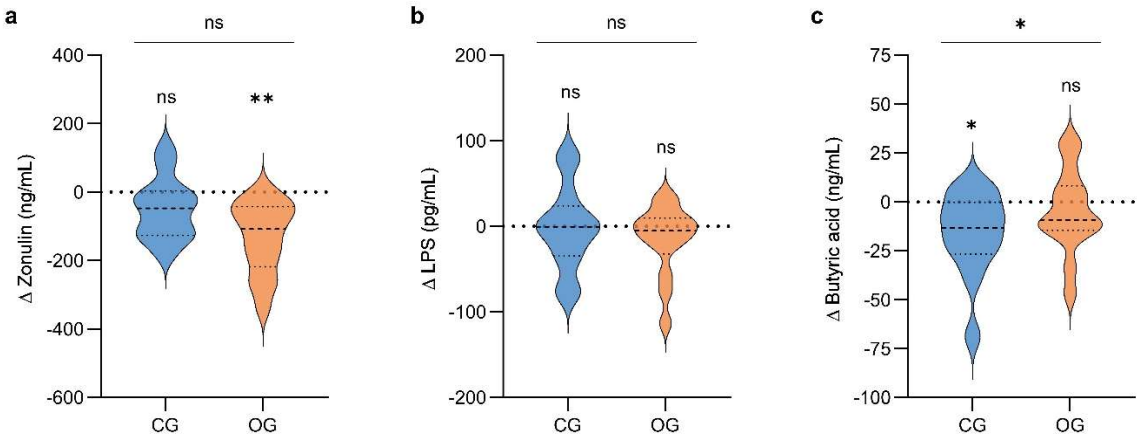

Figure 4-16: Changes in intestinal permeability markers and butyric acid level following the short-term intervention

Absolute change (shown as Δ) in (**a**) serum zonulin, (**b**) serum LPS, and (**c**) plasma butyric acid concentrations in the CG (blue, *n* = 13) and OG (orange, *n* = 14), presented as violin plots (dashed center line: median, dotted line: upper and lower quartiles). a+b: ** $P^{adj.}$ < 0.01, ns: $P^{adj.}$ > 0.05 (Bonferroni Holm), c: *P < 0.05, ns: P > 0.05.

Abbreviations: CG, control group; CG^{6w}, six-week control group; LPS, lipopolysaccharides; OG, oat group; OG^{6w}, six-week oat group.

Table 4-12: Gut permeability and inflammatory markers before and after each dietary intervention

		Within-group comparison[1]					Between-group comparison[2]					
		Before	**After**	**Δ**		***P*adj.**		**Model**	**β estimate**	**95% CI**	***P***	***P*adj.**
Zonulin, ng/mL	CG	251.00 (348.25)	224.30 (328.45)	-26.70 (129.65)	0.052	0.156	2DI	(i)	-75.871	-162.051; -1.841	0.054	0.216
	OG	457.95 (418.95)	223.05 (289.28)	-108.25 (175.63)	**<0.001*** **	**0.002****		(ii)	-79.832	-178.972; 4.560	0.075	0.300
	CG^{6w}	781.13 (820.71)	815.29 (802.55)	12.75 (159.11)	0.110	0.330	6WI	(i)	-75.302	-328.871; 136.276	0.573	0.573
	OG^{6w}	310.23 (796.66)	293.43 (761.70)	61.58 (250.30)	0.294	0.588		(ii)	-75.22	-352.886; 168.058	0.580	0.580
LPS, pg/mL	CG	70.73 (59.46)	45.68 (52.05)	0.00 (58.37)	0.905	0.905	2DI	(i)	-18.614	-45.746; 4.560	0.200	0.600
	OG	35.30 (60.53)	31.57 (27.99)	-5.09 (42.05)	0.311	0.933		(ii)	-15.755	-45.416; 9.700	0.319	0.957
	CG^{6w}	24.97 (88.51)	39.11 (97.87)	15.46 (56.08)	0.110	0.330	6WI	(i)	-32.946	-71.108; 3.912	0.118	0.354
	OG^{6w}	29.20 (74.89)	12.44 (55.95)	0.00 (47.64)	0.515	0.588		(ii)	-32.437	-68.444; 3.696	0.117	0.351
hsCRP, mg/L	CG	1.81 (3.36)	2.47 (5.77)	0.31 (1.82)	**0.036***	0.144	2DI	(i)[a]	-1.523	-3.167; 0.122	0.894	1.000
	OG	2.85 (2.97)	2.52 (4.42)	-0.29 (1.19)	0.423	0.933		(ii)	-1.349	-3.634; 0.538	0.897	1.000
	CG^{6w}	1.86 (3.60)	1.88 (2.45)	-0.14 (1.10)	0.130	0.330	6WI	(i)	1.355	-0.356; 4.123	0.142	0.354
	OG^{6w}	2.55 (4.10)	3.16 (3.97)	0.61 (4.27)	0.179	0.537		(ii)	1.308	-0.365; 4.008	0.151	0.351
IL-6, pg/mL	CG	1.48 (2.44)	2.10 (1.41)	0.15 (0.64)	0.389	0.788	2DI	(i)	-0.007	-0.672; 0.659	0.984	1.000
	OG	1.50 (1.49)	1.84 (1.69)	0.06 (0.61)	0.582	0.933		(ii)	-0.020	-0.752; 0.713	0.956	1.000

[1]Data are presented as median (IQR). Absolute change (Δ) was calculated as follows: pre-intervention (baseline) value subtracted from the respective value post-intervention. *P*-value refers to paired Student's *t*-test or Wilcoxon rank test depending on the distribution of the data. [2]Data are presented as β estimate and 95% CI using BCa-bootstrapping with 1,000 BCa-samples. *P*-value refers to a linear regression model adjusted for (i) the baseline value of the endpoint and (ii) additionally for sex, with the control group (CG or CG6w) as the reference. [a]Model was performed with log-transformed data. Estimates and 95% CI are shown from the models with untransformed data, while *P*-value refers to the model with log-transformed data. $^{**}P^{adj.} < 0.01$ (Bonferroni-Holm correction), $^{***}P < 0.001$, $^{*}P < 0.05$. Short-term intervention: $n = 27$, six-week intervention: $n = 22$.

Abbreviations: CG, control group; CG^{6w}, six-week control group; hsCRP, high-sensitivity C-reactive protein; IL-6, interleukin 6; LPS, Lipopolysaccharides; OG, oat group; OG^{6w}, six-week oat group; 2DI, 2-day intervention; 6WI, 6-week intervention.

4.10.4 Effects on Plasma SCFA Concentrations

After the 2-day energy-restricted high-dose oat diet, higher plasma butyric acid levels were observed in the OG compared to CG (14.8 [5.4, 22.3] ng/mL, $P = 0.015$; **Figure 4-16c**). While the butyric acid concentration remained stable in the OG (Δ -4.8 ± 5.7 ng/mL, $P = 0.514$), a reduction was found in the CG (Δ -16.1 ± 5.9 ng/mL, $P = 0.011$). After the six-week intervention period, a different change in hexanoic acid levels was observed between the two groups (11.6 [3.8, 19.3] ng/mL, $P = 0.040$). Hexanoic acid level remained stable in the OG^{6w} (Δ 0.43 ± 5.03, $P = 0.934$), while it decreased in the CG^{6w} (Δ -9.36 ± 4.03 ng/mL, $P = 0.042$). These effects persisted when additionally adjusted for sex. (**Table 4-13**).

Table 4-13: Plasma short-chain fatty acid (SCFA) concentration before and after each dietary intervention

		Within-group comparison[1]				Between-group comparison[2]				
		Before	After	Δ	*P*		Model	β estimate	95% CI	*P*
Acetic acid, ng/mL	CG	2,794.9 (2,290.1)	3,312.2 (2,066.7)	983.3 (1,487.7)	0.116	2DI	(i)	-233.267	-2,054.366; 1,426.531	0.928
	OG	2,678.9 (1716.0)	2,818.4 (1537.7)	-56.0 (1506.1)	0.526		(ii)	440.851	1,192.827; 1,890,206	0.889
	CG^{6w}	3,320.18 ± 538.86	2,971.44 ± 337.97	-348.74 ± 579.17	0.560	6WI	(i)	-756.976	-1,517,526; -84.116	0.086
	OG^{6w}	2,798.32 ± 255.88	2,169.52 ± 195.12	-628.80 ± 333.77	0.089		(ii)	-758.407	-1,509.396; 34.094	0.080
Propionic acid, ng/mL	CG	83.8 (39.3)	76.6 (36.2)	-5.9 (69.4)	0.526	2DI	(i)	7.673	-12.492; 28.477	0.484
	OG	81.1 (27.6)	87.6 (47.1)	-15.3 (50.3)	0.550		(ii)	13.623	-7.527; 35.859	0.26
	CG^{6w}	83.27 (32.03)	89.72 (20.47)	-0.64 (45.48)	0.849	6WI	(i)	11.252	-9.084; 29.998	0.309
	OG^{6w}	86.73 (20.47)	89.72 (28.35)	20.62 (43.20)	0.223		(ii)	9.013	-11.469; 26.870	0.423
Butyric acid, ng/mL	CG	42.7 (32.8)	36.8 (14.2)	-13.3 (26.5)	**0.011***	2DI	(i)	14.798	5.385; 22.298	**0.015***
	OG	51.4 (28.9)	50.2 (32.1)	-9.2 (22.8)	0.514		(ii)	14.190	4.507; 21.289	**0.027***
	CG^{6w}	37.44 (28.54)	42.98 (42.75)	3.77 (25.37)	0.500	6WI	(i)[a]	5.242	-11.005; 22.109	0.827
	OG^{6w}	33.28 (17.72)	45.73 (46.78)	7.61 (43.21)	0.248		(ii)[a]	2.702	-14.522; 19.053	0.882
Isobutyric acid, ng/mL	CG	18.1 (9.0)	21.3 (7.2)	0.7 (7.3)	0.701	2DI	(i)	1.254	-2.098; 5.069	0.514
	OG	19.1 (6.8)	20.4 (7.7)	1.3 (3.6)	0.622		(ii)	1.303	-2.334; 5.499	0.501
	CG^{6w}	18.17 (10.79)	20.61 (6.81)	-0.20 (5.46)	0.869	6WI	(i)	0.940	-2.701; 3.952	0.646
	OG^{6w}	24.42 (11.07)	24.08 (6.60)	-0.17 (8.13)	0.605		(ii)	0.686	-3.154; 3.981	0.752
2-MBA, ng/mL	CG	43.3 ± 2.5	40.8 ± 3.3	-2.5 ± 3.6	0.496	2DI	(i)	-0.331	-8.735; 9.723	0.932
	OG	44.1 ± 1.7	40.8 ± 2.5	-3.3 ± 2.3	0.186		(ii)	-1.612	-10.451; 7.055	0.656
	CG^{6w}	38.91 (9.26)	36.98 (5.56)	-1.82 (11.32)	0.186	6WI	(i)	5.211	-2.619; 11.843	0.216
	OG^{6w}	45.36 (7.68)	44.58 (17.21)	-4.71 (14.44)	0.440		(ii)	3.884	-3.649; 10.232	0.301

		Before	After	Δ	*P*		Model	β estimate	95% CI	*P*
Isovaleric acid, ng/mL	CG	55.7 (25.0)	43.5 (28.6)	-8.9 (36.4)	0.577	2DI	(i)[a]	-3.480	-29.797; 15.370	0.948
	OG	38.8 (28.5)	37.1 (29.8)	0.9 (13.5)	0.899		(ii)[a]	2.805	-22.231; 19.035	0.437
	CG^{6w}	44.72 (30.60)	47.00 (17.83)	10.05 (27.42)	0.871	6WI	(i)[a]	8.921	-8.667; 26.153	0.431
	OG^{6w}	66.32 (45.21)	46.30 (34.17)	-3.91 (24.97)	0.748		(ii)[a]	6.474	--9.101; 21.172	0.524
Valeric acid, ng/mL	CG	19.1 ± 1.2	16.8 ± 1.5	-2.3 ± 1.2	0.082	2DI	(i)	2.175	-1.701; 6.037	0.286
	OG	19.4 ± 1.0	19.1 ± 1.6	-0.2 ± 1.4	0.867		(ii)	1.211	-2.598; 5.121	0.529
	CG^{6w}	18.07 (4.13)	18.67 (3.56)	-0.17 (5.92)	0.722	6WI	(i)	2.233	-0.764; 5.260	0.295
	OG^{6w}	17.62 (6.11)	19.14 (6.86)	1.11 (4.46)	0.707		(ii)	1.679	-1.537; 4.983	0.44
Hexanoic acid, ng/mL	CG	65.1 (30.9)	73.8 (38.2)	5.4 (38.3)	0.437	2DI	(i)	-6.570	-24.576; 8.711	0.395
	OG	78.6 (23.3)	72.1 (28.0)	-3.7 (33.4)	0.237		(ii)	-13.651	-34.382; 8.206	0.165
	CG^{6w}	68.05 ± 3.37	58.69 ± 2.53	-9.36 ± 4.03	**0.042***	6WI	(i)	11.554	3.777; 19.299	**0.040***
	OG^{6w}	70.46 ± 5.16	70.88 ± 4.17	0.43 ± 5.03	0.934		(ii)	10.052	2.582; 16.017	**0.038***

[1]Data are presented as mean ± SEM. or as median (IQR) depending on the distribution of the data (n = 27). Absolute change (Δ) was calculated as follows: pre-intervention (baseline) value subtracted from the respective value post-intervention. *P*-value refers to paired Student's *t*-test or Wilcoxon rank test (*P <0.05); [2]Data are presented as β estimate and 95% CI using BCa-bootstrapping with 1,000 BCa-samples. *P*-value refers to a linear regression model adjusted for (i) the baseline value of the end point and (ii) additionally for sex, with control group (CG or CG6w) as the reference. [a]Model was performed with log-transformed data. Estimates and 95% CI are shown from the models with untransformed data, while *P*-value refers to the model with log-transformed data. Short-term intervention: n = 27, six-week intervention: n = 22.

Abbreviations: CG, control group; CG^{6w}, six-week control group; OG, oat group; OG^{6w}, six-week oat group; 2DS, 2-day intervention; 2-MBA, 2-Methylbutyric acid; 6WS, 6-week intervention.

4.10.5 Shifts in Gut Microbiota Composition

sPLS-DA revealed diet-induced changes in microbial composition at the genus level that significantly distinguished the two diet groups of the short-term intervention (BER = 0.23, AUC = 0.78 (*P* = 0.03); **Figure 4-17a**). The most important bacteria contributing to the differentiation were *Erysipelotrichaceae* UCG-003, *Parasutterella*, *Ruminiclostridium*, *Eggerthella* and *Marvinbryanthia*, with the first four showing an increase and the last a decrease in the OG compared to CG (**Figure 4-17b**). In the six-week intervention, a tendency towards a different modulation of the microbial composition at the genus level between the two diet groups was observed (BER = 0.32, AUC = 0.71 (*P* = 0.09); **Figure 4-17c**). The bacteria that seemed to be most important for differentiating between OG^{6w} and CG^{6w} were *Ruminococcus torques* group, *Slackia*, *Bilophila*, *Enterorhabdus* and *Eggerthellaceae* uncultured, all of which decreased in OG^{6w} compared to CG^{6w} (**Figure 4-17d**). Details on the structure of the final sPLS-DA model and all selected bacteria as well as their weight loadings are provided in **Table 4-8** and **Table 4-14**.

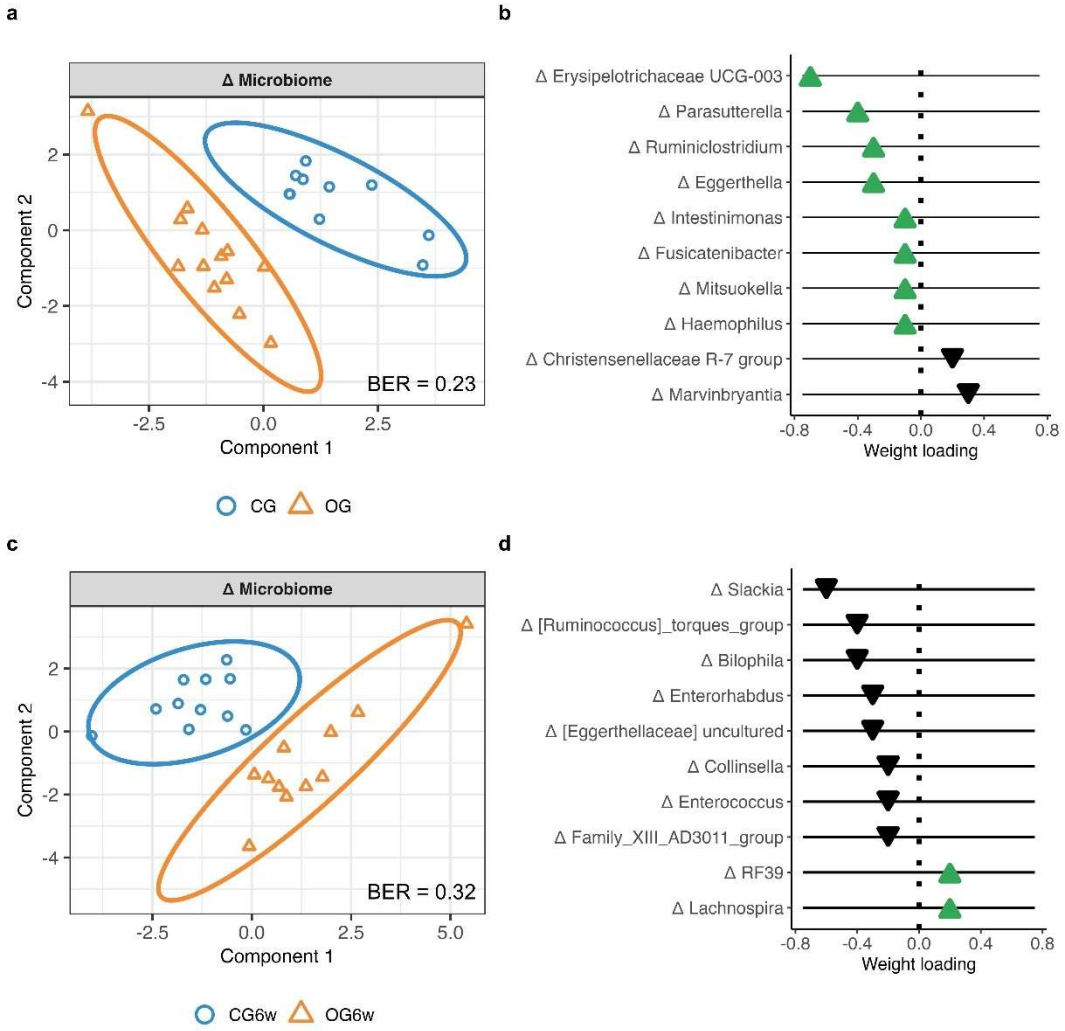

Figure 4-17: Shifts in microbial composition following the short-term and six-weeks intervention

(**a**) Two-component sample plots of the sPLS-DA investigating the diet-induced change in microbial composition at the genus level following the short-term intervention. Data points are shown as blue circles for the CG (*n* = 10) and orange triangles for the OG (*n* = 13). (**b**) Weight loadings of selected bacterial genera in component 1 (green or black triangle: increase or decrease in OG vs. CG). (**c**) Two-component sample plots of the sPLS-DA investigating the diet-induced change in microbial composition at the genus level following the six-week intervention. Data points are shown as blue circles for the CG^{6w} (*n* = 11) and orange triangles for the OG^{6w} (*n* = 11). (**d**) Weight loadings of the top 10 selected bacterial genera in component 1 (green or black triangle: increase or decrease in OG^{6w} vs. CG^{6w}).

Abbreviations: BER, balanced error rate; CG, control group; CG^{6w}, six-week control group; OG, oat group; OG^{6w}, six-week oat group.

Table 4-14: Weight loadings of the sPLS-DA models for identifying diet-induced changes in microbial composition

Intervention	Component	Group contribution	Importance	Median change (oat vs. control)	Oat group	Control group
					OG	CG
Short-term	**Component 1**					
	Erysipelotrichaceae UCG-003	OG	-0.726	increase	0.593	-0.770
	Parasutterella	OG	-0.388	increase	0.475	-0.617
	Ruminiclostridium	OG	-0.315	increase	0.449	-0.584
	Marvinbryantia	CG	0.278	decrease	-0.436	0.567
	Eggerthella	OG	-0.275	increase	0.435	-0.566
	Christensenellaceae R-7 group	CG	0.170	decrease	-0.399	0.518
	Intestinimonas	OG	-0.134	increase	0.386	-0.502
	Fusicatenibacter	OG	-0.114	increase	0.379	-0.493
	Mitsuokella	OG	-0.079	increase	0.367	-0.477
	Haemophilus	OG	-0.060	increase	0.360	-0.468
	Component 2					
	Anaerostipes	OG	0.613	increase	0.124	-0.161
	UCG-005	OG	-0.416	increase	0.050	-0.065
	NK4A214 group	OG	-0.412	increase	0.006	-0.008
	UCG-002	OG	-0.408	increase	0.156	-0.203
	Intestinibacter	CG	-0.231	decrease	-0.053	0.069
	Clostridium innocuum group	CG	0.127	decrease	-0.163	0.212
	Romboutsia	CG	-0.123	decrease	-0.059	0.077
	Muribaculaceae	CG	-0.121	decrease	-0.215	0.279
	Sarcina	OG	0.112	increase	0.121	-0.158
	CAG-56	CG	-0.063	decrease	-0.128	0.167

Intervention	Component	Group contribution	Importance	Median change (oat vs. control)	Oat group	Control group
					OG^{6w}	CG^{6w}
Six-week	**Component 1**					
	Slackia	CG^{6w}	-0.576	decrease	-0.568	0.568
	[Ruminococcus]_torques_group	CG^{6w}	-0.396	decrease	-0.504	0.504
	Bilophila	CG^{6w}	-0.351	decrease	-0.488	0.488
	Enterorhabdus	CG^{6w}	-0.328	decrease	-0.480	0.480
	[Eggerthellaceae]_uncultured	CG^{6w}	-0.291	decrease	-0.467	0.467
	Collinsella	CG^{6w}	-0.243	decrease	-0.450	0.450
	Enterococcus	CG^{6w}	-0.166	decrease	-0.423	0.423
	Family_XIII_AD3011_group	CG^{6w}	-0.161	decrease	-0.421	0.421
	[Barnesiellaceae]_uncultured	CG^{6w}	-0.010	decrease	-0.368	0.368
	UCG-003	OG^{6w}	0.135	increase	0.412	-0.412
	RF39	OG^{6w}	0.178	increase	0.427	-0.427
	Lachnospira	OG^{6w}	0.181	increase	0.428	-0.428
	Component 2					
	Acidaminococcus	OG^{6w}	-0.430	increase	0.248	-0.248
	[Eubacterium]_eligens_group	OG^{6w}	-0.370	increase	0.254	-0.254
	Bacteroides	OG^{6w}	-0.252	increase	0.019	-0.019
	Phascolarctobacterium	CG^{6w}	-0.234	decrease	-0.236	0.236
	Lactobacillus	CG^{6w}	-0.203	decrease	-0.024	0.024
	Oscillibacter	OG^{6w}	-0.172	increase	0.147	-0.147
	[Ruminococcus]_gnavus_group	OG^{6w}	-0.147	increase	0.045	-0.045
	Veillonella	OG^{6w}	-0.134	increase	0.232	-0.232
	Lachnospiraceae_NK4A136_group	CG^{6w}	-0.078	decrease	-0.056	0.056

	Component	Group contribution	Importance	Median change (oat vs. control)	Oat group	Control group
					OG^{6w}	CG^{6w}
	Component 2					
Six-week	Fusobacterium	OG^{6w}	-0.015	increase	0.243	-0.243
	Izemoplasmatales	OG^{6w}	0.009	increase	0.202	-0.202
	GCA-900066575	CG^{6w}	0.034	decrease	-0.062	0.062
	[Erysipelotrichaceae]_uncultured	CG^{6w}	0.042	decrease	-0.220	0.220
	Turicibacter	OG^{6w}	0.088	increase	0.218	-0.218
	Dorea	CG^{6w}	0.121	decrease	-0.232	0.232
	Lactococcus	CG^{6w}	0.233	decrease	-0.302	0.302
	Subdoligranulum	OG^{6w}	0.280	increase	0.002	-0.002
	Marvinbryantia	OG^{6w}	0.349	increase	0.018	-0.018
	[Eubacterium]_ventriosum_group	CG^{6w}	0.404	decrease	-0.075	0.075

Abbreviations: CG, control group; CG^{6w}, 6-week control group; OG, oat group; OG^{6w}, 6-week oat group.

4.10.6 Associations between Changes in Gut Permeability, Microbial Composition and SCFAs

In the short-term intervention, several correlations between shifts in zonulin, SCFAs and specific microbes were revealed using DIABLO (r = ±0.6); however, the separation between the two diet groups did not reach significance (model 3.1: BER = 0.39, AUC = 0.79 (P = 0.11); **Figure 4-18a**). Details on the structure of the final DIABLO models are provided above (see **Table 4-9**). Looking more closely at the associations with the change in butyric acid which showed an increase in OG compared with CG, inverse associations tended to be observed with shifts in zonulin and hsCRP levels as well as with the genera *Marvinbryantia*, Christensenellaceae R-7 group, *Muribaculaceae* and *Enterorhabdus*, while positive associations tended to be found with shifts in *Lachnospiraceae* UCG-008, *Clostridium methylpentosum* group, *Ruminiclostridium*, *Eggerthella*, *Intestinimonas* and *Erysipelotrichaceae* UCG-003. Interestingly, the first three genera mentioned showed a decrease and the last four an increase in OG compared with CG (**Table 4-14**).

In the six-week intervention, DIABLO identified numerous correlations between changes in LPS and hsCRP levels and various SCFAs and microbes; however, the separation between the two diet groups also did not reach significance (model 3.2: BER = 0.45, AUC = 0.80 (P = 0.08); **Figure 4-18b**). Looking more closely at the associations with the change in hexanoic acid which showed an increase in OG^{6w} compared with CG^{6w} (see above), a trend towards an inverse association was found with shifts in LPS levels and various microbes including *Ruminococcus torques* group and *Eggerthellaceae* uncultured. Interestingly, these genera showed a decrease in the OG^{6w} compared to CG^{6w} (**Table 4-14**). In contrast, a trend towards positive associations were observed with shifts in genera such as RF39 and UCG-003 (**Figure 4-18b**), which showed an increase in the OG^{6w} compared to CG^{6w} (**Table 4-14**).

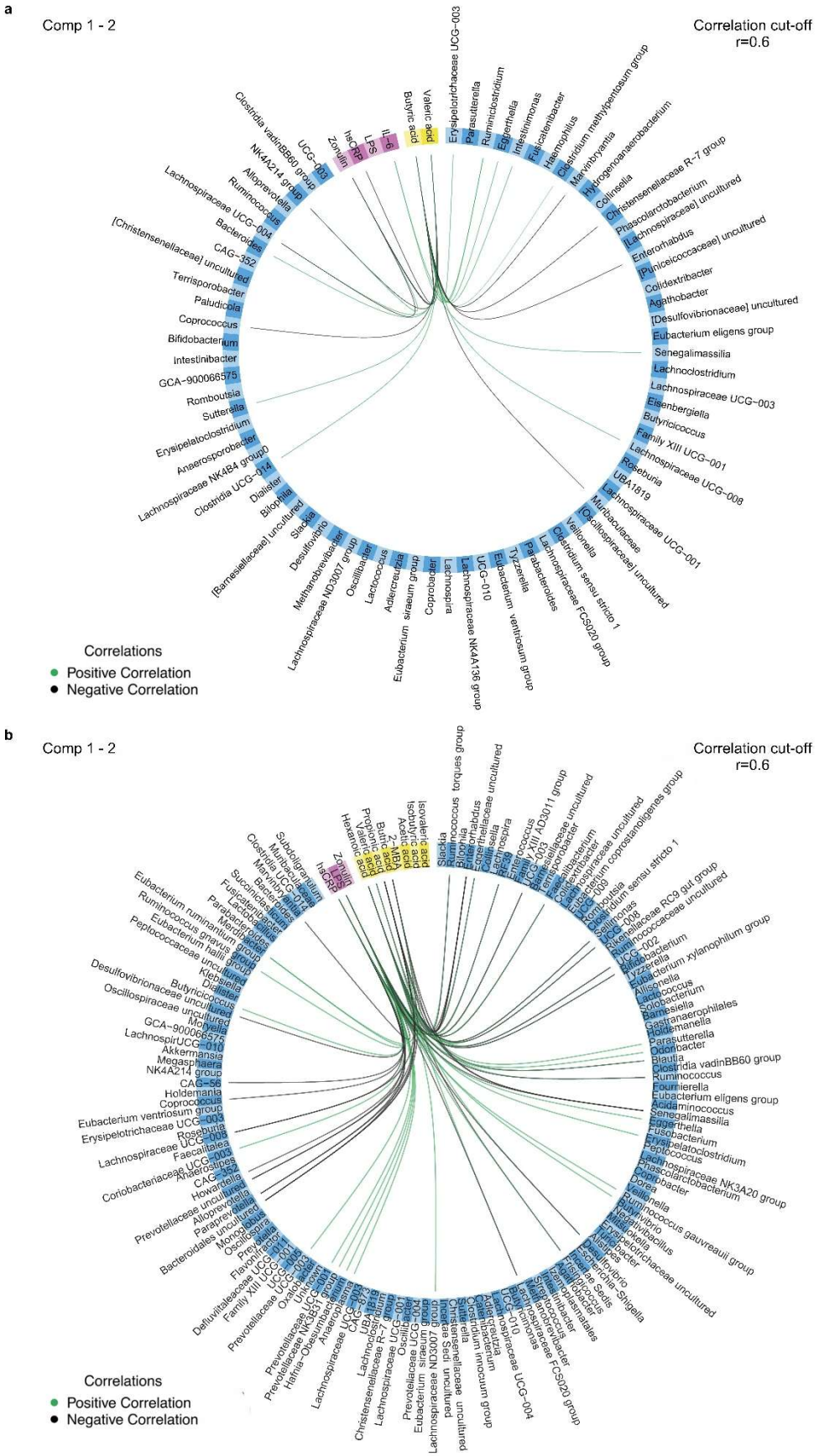

Figure 4-18: Associations between shifts in microbial composition and changes in gut permeability and SCFAs

Figure 4-18: Associations between shifts in microbial composition and changes in gut permeability and SCFAs

Circos plot shows positive (green) and negative (black) correlations (cut-off value: $r = \pm 0.6$) between the selected variables in the three data sets (clinical outcomes (violet), plasma SCFAs (yellow), microbial genera (blue)) along component 1 and 2 derived from the DIABLO in the (**a**) short-term intervention (model 3.1, $n = 23$) and (**b**) the six-week intervention (model 3.2, $n = 22$). Intra-block correlations are not presented.

Abbreviations: DHFA, dihydroferulic acid; FA, ferulic acid; hsCRP, high-sensitivity C-reactive protein; IL-6, interleukin 6; LPS, lipopolysaccharides; 2-MBA, 2-Methylbutyric acid.

4.10.7 Association Between Zonulin Reduction and SCFA Response in Each Study Group

Looking more closely at the relationships between changes in clinical markers and SCFA levels within each diet group, several inverse correlations were observed between the change in zonulin and shifts in valeric acid (r = -0.81, $P = 4.32 \times 10^{-4}$; Spearman's rank), butyric acid (r = -0.60, $P = 0.022$), acetic acid (r = -0.53, $P = 0.049$), and hexanoic acid (r = -0.53, $P = 0.049$) in the OG (**Figure 4-19**). Positive correlations between the change in zonulin and shifts in butyric acid (r = 0.73, $P = 0.011$) and 2-methylbutyric acid (r = 0.65, $P = 0.032$) were observed in the OG^{6w}. In the CG, a negative correlation was found between the shifts in zonulin and isobutyric acid (r = -0.56, $P = 0.046$), while no associations were observed within the CG^{6w}.

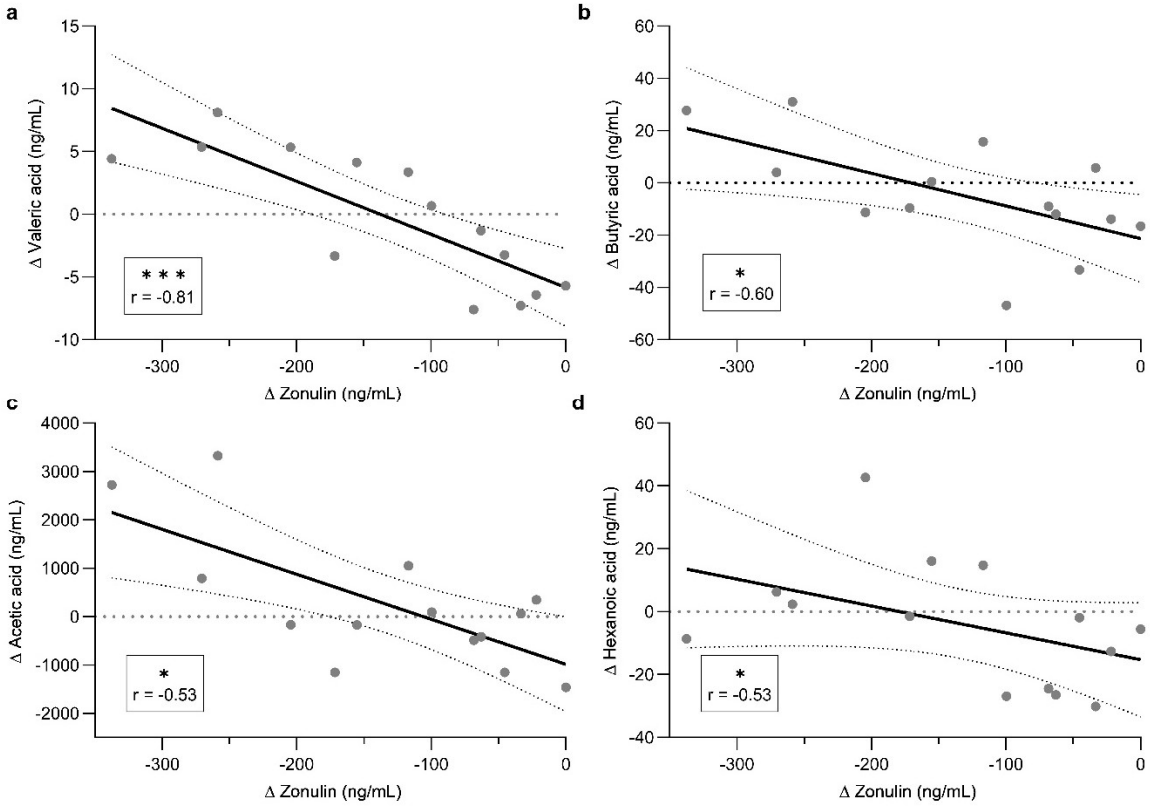

Figure 4-19: Associations between changes in zonulin and SCFA levels in the OG

Correlation between the absolute change (shown as Δ) in zonulin and (**a**) valeric acid, (**b**) butyric acid, (**c**) acetic acid, and (**d**) hexanoic acid levels ($n = 14$). ***$P < 0.001$, *$P < 0.05$.

4.10.8 Explaining Changes in Gut Permeability and SCFAs by Shifts in Gut Microbiota Composition

Following the 2-day energy-restricted high-dose oat diet, sPLS regression analysis revealed that shifts in 25 of 135 bacterial genera were associated with changes in zonulin and four SCFAs, namely acetic, butyric, propionic and valeric acids (**Figure 4-20a**). Shifts in bacterial genera associated with a reduction in zonulin, such as *Intestinibacter* and *Ruminococcus*, were simultaneously associated with an increase in SCFA levels, especially acetic acid and butyric acid. Comparable associations were observed with *Lachnospiraceae* NK4A136 group, *Eubacterium eligens* group, *Terrisporobacter,* and Clostridia UCG-014. In contrast, shifts in bacterial genera associated with an increase in zonulin, such as *Coprococcus*, *Tyzzerella, Ruminococcus gnavus* group and *Parasutterella*, were associated with a decrease in SCFA levels.

Following the six-week isocaloric moderate oat diet, sPLS regression analysis revealed that shifts in 17 of 146 bacterial genera were associated with changes in zonulin, LPS, hsCRP and all SCFAs except propionic acid (**Figure 4-20b**). Shifts in bacterial genera negatively associated with LPS and hsCRP, such as RF39, were associated with an increase in SCFA levels, except for acetic acid. In contrast, shifts in bacterial genera positively associated with LPS and hsCRP, such as *Bilophila,* were associated with a decrease in SCFA levels, except for acetic acid. No clear pattern was observed for zonulin.

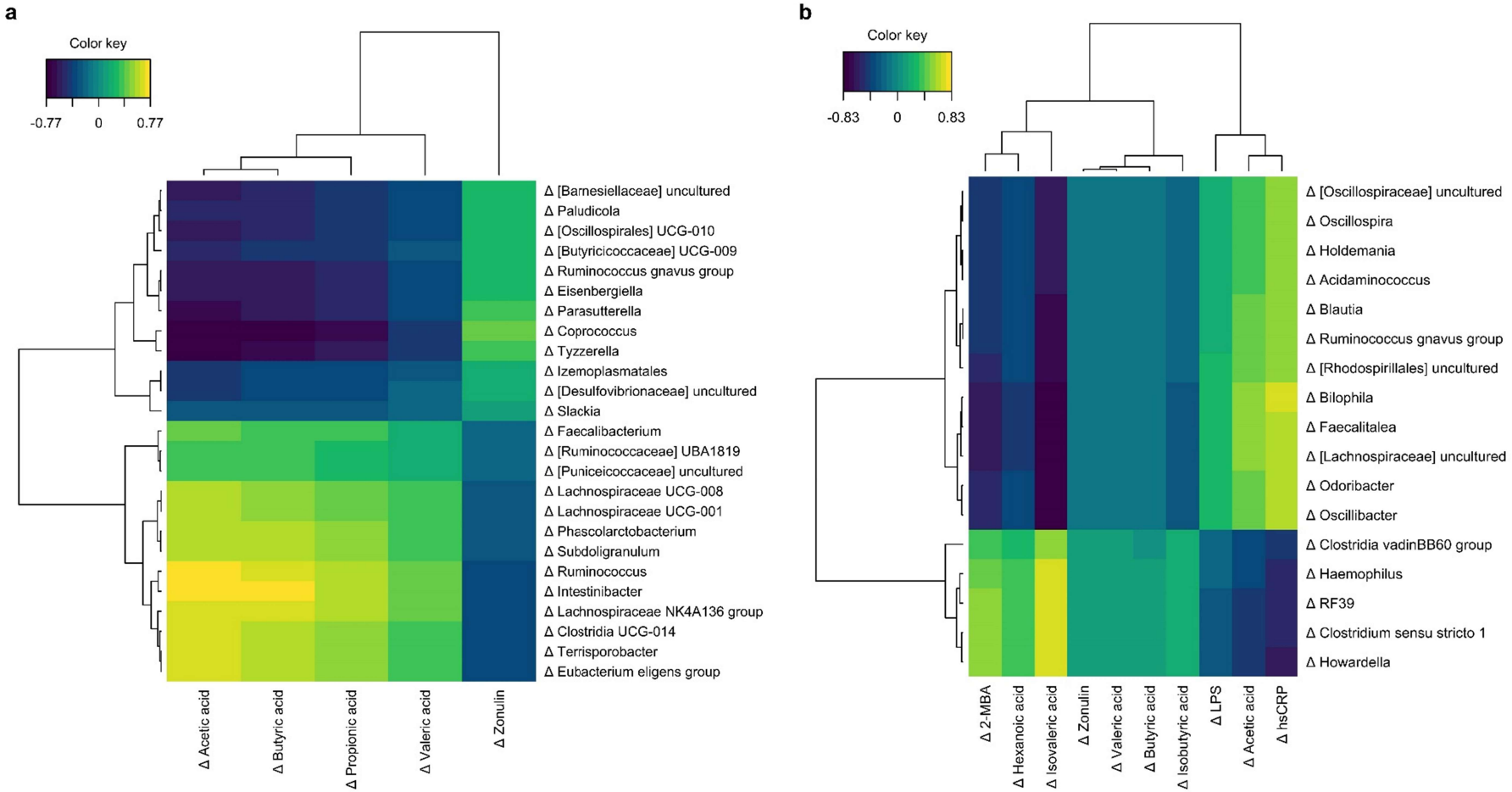

Figure 4-20: Associations between shifts in microbial composition and changes in zonulin and SCFA levels

Visualization of the sPLS model in regression mode, explaining changes in clinical markers and SCFAs through shifts in the gut microbiome composition at genus level following the (**a**) short-term, high-dose oat diet (OG: *n* = 13) and (**b**) six-week, moderate oat diet (OG^{6w}: *n* = 11). Abbreviations: hsCRP, high-sensitivity C-reactive protein; LPS, lipopolysaccharides; 2-MBA, 2-methylbutyric acid.

4.10.9 Relevance of Valeric Acid for Zonulin and SCFA Response

Because of the negative correlation between the changes in zonulin and valeric acid in the OG (see above), responders and non-responders were classified according to the relative change (%Δ) in valeric acid, with the cut-off value defined by the upper tertile: responders (VA-R) %Δ > 6.5% (*n* = 6) and non-responders (VA-NR) %Δ ≤ 6.5% (*n* = 8).

As expected, VA-R showed a considerably greater reduction in zonulin (-170.0 [-279.8, -47.7] ng/mL, *P* = 0.024; **Figure 4-21a**) and an increase in valeric acid levels (9.2 [6.5, 11.7] ng/mL, *P* = 0.002; **Figure 4-21b**) compared to VA-NR. Moreover, an increase in butyric acid (20.7 [6.4, 33.7] ng/mL, *P* = 0.031; **Figure 4-21c**) and hexanoic acid levels (28.56 [15.1, 37.7] ng/mL, *P* = 0.020; **Figure 4-21d**) was observed in the VA-R compared to VA-NR, primarily driven by SCFA reductions in the VA-NR. These effects persisted when additionally adjusted for sex, apart from hexanoic acid (**Table 4-15**). In addition, an increase in acetic acid (1,526.7 [357.1, 2445.8] ng/ml, *P* = 0.045) and propionic acid levels (44.0 [24.5, 60.5], *P* = 0.009) was found in the VA-R compared to VA-NR when sex was included as a covariate in the analysis (**Table 4-15**).

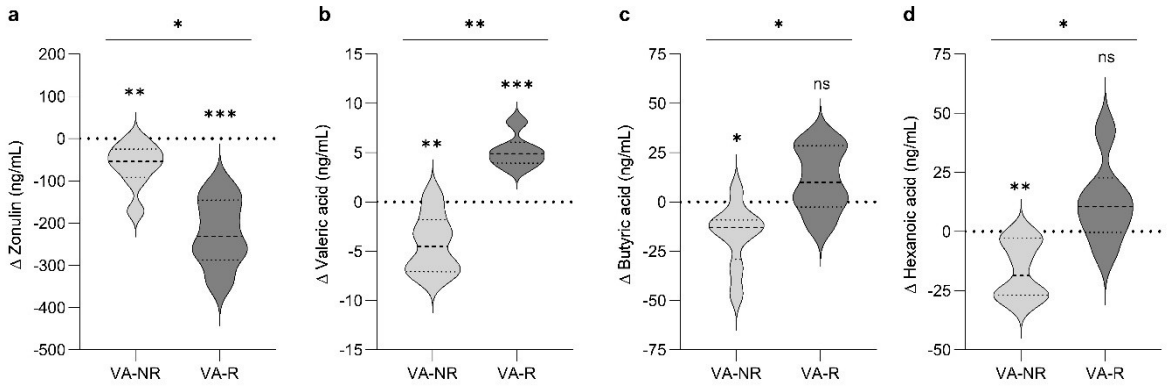

Figure 4-21: Differences in zonulin and SCFA responses between valeric acid responders (VA-R) and non-responders (VA-NR)

Absolute change (shown as Δ) in (**a**) zonulin, (**b**) valeric acid, (**c**) butyric acid, and (**d**) hexanoic acid in VA-NR (light gray, *n* = 8) and VA-R (dark gray, *n* = 6), shown as violin plots (dashed center line: median, dotted line: upper and lower quartiles). ***$P < 0.001$, **$P < 0.01$, *$P < 0.05$, ns $P > 0.05$.

Abbreviations: VA-R, valeric acid responders; VA-NR, valeric acid non-responders.

Table 4-15: Clinical markers and SCFA concentrations before and after the intervention in valeric acid responders (VA-R) and non-responders (VA-NR) within the OG

		Within-group comparison[1]				Between-group comparison[2]			
		Before	After	Δ	*P*	Model	β estimate	95% CI	*P*
Zonulin, ng/mL	VA-R	591.95 (255.30)	401.05 (324.38)	-231.65 (141.60)	**0.001*** **	(i)	-170.04	-279.759; -47.691	**0.024***
	VA-NR	275.70 (381.18)	194.5 (271.85)	-54.25 (67.03)	**0.002****	(ii)	-158.473	-284.506; -37.305	**0.042***
LPS, pg/mL	VA-R	43.56 (66.85)	34.87 (20.39)	-13.17 (76.15)	0.294	(i)	-10.748	-34.537; 15.651	0.376
	VA-NR	34.14 (58.18)	31.57 (56.99)	1.34 (31.73)	0.889	(ii)	-10.083	--42.617; 21.739	0.518
hsCRP, mg/L	VA-R	4.12 (2.89)	2.96 (3.26)	-0.58 (2.09)	0.31	(i)[a]	-0.578	-3.424; 0.894	0.772
	VA-NR	1.57 (2.36)	1.36 (4.69)	-0.17 (1.05)	0.789	(ii)[a]	0.202	-2.286; 1.648	0.113
IL-6, pg/mL	VA-R	1.88 ± 0.26	2.13 ± 0.39	0.25 ± 0.46	0.609	(i)[a]	0.238	-0.834; 1.254	0.374
	VA-NR	1.90 ± 0.41	1.90 ± 0.41	0.01 ± 0.12	0.936	(ii)[a]	0.440	-0.732; 1.636	0.269
Acetic acid, ng/mL	VA-R	1,663.65 (1,026.79)	3,336.45 (2163.37)	918.61 (3043.68)	0.116	(i)	995.923	-815.267; 2,133.253	0.086
	VA-NR	3,352.90 (807.29)	2,589.40 (1,389,91)	-451.12 (1238.75)	0.093	(ii)	1,526.67	357.098; 2,445.761	**0.045***
Propionic acid, ng/mL	VA-R	82.74 (31.26)	96.12 (21.70)	18.00 (48.0)	0.250	(i)	25.081	-2.609; 49.847	0.097
	VA-NR	76.00 (42.05)	59.54 (55.60)	-20.50 (26.0)	0.104	(ii)	44.016	24.457; 60.507	**0.009****
Butyric acid, ng/mL	VA-R	46.85 ± 5.62	58.15 ± 5.90	11.30 ± 6.74	0.154	(i)	20.728	6.420; 33.706	**0.031***
	VA-NR	67.22 ± 13.69	50.32 ± 9.63	-16.90 ± 5.71	**0.021***	(ii)	23.125	3.881; 40.066	**0.037***
Isobutyric acid, ng/mL	VA-R	20.09 ± 0.73	22.89 ± 2.17	1.51 (6.59)	0.298	(i)	3.509	-1.139; 8.767	0.290
	VA-NR	20.40 ± 2.35	19.59 ± 2.45	0.49 (3.82)	0.662	(ii)	5.814	-0.678; 11.530	0.204
2-MBA, ng/mL	VA-R	43.72 ± 1.40	45.77 ± 3.63	2.05 ± 3.44	0.577	(i)	9.099	-2.663; 19.489	0.072
	VA-NR	44.36 ± 2.94	37.11 ± 3.01	-7.25 ± 2.48	**0.022***	(ii)	6.951	-3.308; 16.079	0.194
Isovaleric acid, ng/mL	VA-R	37.02 (24.22)	40.51 (29.07)	3.99 (18.35)	0.483	(i)	3.903	-6.003, 17.207	0.466
	VA-NR	39.31 (29.61)	34.72 (32.14)	0.77 (14.57)	0.458	(ii)	8.276	-3.144; 19.926	0.214

		Before	After	Δ	*P*	Model	β estimate	95% CI	*P*
Valeric acid, ng/mL	VA-R	18.60 ± 1.55	23.73 ± 1.43	5.13 ± 0.67	**0.001*** **	(i)	9.234	6.520; 11.656	**0.002** **
	VA-NR	19.97 ± 1.45	15.70 ± 1.68	-4.28 ± 1.05	**0.005** **	(ii)	9.825	7.807; 12.873	**0.004** **
Hexanoic acid, ng/mL	VA-R	78.64 (27.38)	85.62 (36.62)	10.54 (23.15)	0.116	(i)	28.551	15.128; 37.691	**0.020***
	VA-NR	78.20 (21.53)	66.42 (20.15)	-18.56 (24.01)	**0.007** **	(ii)	20.78	0.289; 42.687	0.116

[1]Data are presented as mean ± SEM. or as median (IQR) depending on the distribution of the data (n = 14). Absolute change (Δ) was calculated as follows: pre-intervention concentration subtracted from the respective value post-intervention. *P*-value refers to paired Student's *t*-test or Wilcoxon rank test; [2]Data are presented as β estimate and 95% CI using BCa-bootstrapping with 1,000 BCa-samples. *P*-value refers to a linear regression model with valeric acid non-responders (VA-NR) as the reference adjusted for (i) the baseline concentration of the end point and (ii) additionally for sex. [a]Model was performed with log-transformed data. Estimates and 95% CI are shown from the models with untransformed data, while *P*-value refers to the model with log-transformed data. ***$P < 0.001$, **$P < 0.01$, *$P < 0.05$.

Abbreviations: hsCRP, high-sensitivity C-reactive protein; IL-6, interleukin 6; LPS, lipopolysaccharide; VA-NR, valeric acid non-responders; VA-R, valeric acid responder; 2-MBA, 2-Methylbutyric acid.

4.10.10 Comparison of Baseline Characteristics between Valeric Acid Responder (VA-R) and Non-Responder (VA-NR)

Comparison of the baseline characteristics between VA-R and VA-NR showed a higher diastolic BP in the VA-R than VA-NR (-8.54 [-15.81, -1.27] mmHg (mean differences [95% CI], $P = 0.025$) and a median difference of -1,689 ng/mL for plasma acetic acid ($P = 0.043$) (**Table 4-16**). Moreover, differences in the sex distribution were observed ($P = 0.085$), as all the VA-R were female, whereas the VA-NR group comprised an equal number of female and male persons.

According to sPLS-DA taking into account the unequal sex distribution, a microbial signature was identified that clearly distinguishes VA-R from VA-NR at baseline (BER = 0.23, AUC = 0.83 ($P = 0.04$); **Figure 4-22a**). The most important bacteria for differentiation were *Tyzerella*, *Lachnospiraceae* NK4B4 group, *Negativibacillus*, *Bacteroides pectinophilus* group, and *Eubacterium eligens* group (top five of ten genera from component 1) as well as Clostridia UCG-014 (single genus from component 2) (**Table 4-17**). While *Tyzerella*, *Negativibacillus*, *B. pectinophilus* group and Clostridia UCG-014 were more abundant in the VA-R than VA-NR, Lachnospiraceae NK4B4 group and *Eubacterium eligens* group were less abundant (**Table 4-17**). Spearman correlation analysis revealed positive associations between component 1 obtained from the sPLS-DA and changes in hexanoic acid ($r = 0.78$, $P = 0.001$), valeric acid ($r = 0.74$, $P = 0.002$), and butyric acid ($r = 0.66$, $P = 0.01$) and a negative association with the change in zonulin ($r = -0.69$, $P = 0.008$) (**Figure 4-22b**). In addition, a negative association was observed between component 2 obtained from the sPLS-DA and the change in isovaleric acid ($r = -0.64$, $P = 0.01$).

Table 4-16: Baseline characteristics of valeric acid responders (VA-R) and non-responders (VA-NR) within the OG[1]

Parameter	VA-R (*n* = 6)	VA-NR (*n* = 8)	*P*
Sex (female)	6 (100 %)	4 (50 %)	0.085
Age, years	57.3 ± 8.8	59.8 ± 8.2	0.607
BW, kg	87.9 ± 10.8	92.9 ± 12.7	0.456
BMI, kg/m²	30.8 ± 3.2	31.8 ± 3.4	0.601
Fat mass, %	42.1 ± 1.8	38.6 ± 8.9	0.314
WC, cm	99.9 ± 5.4	105.3 ± 11.0	0.286
BP systolic, mmHg	141.8 ± 10.9	135.4 ± 10.2	0.281
BP diastolic, mmHg	97.9 ± 6.6	89.4 ± 5.8	**0.025***
Triglycerides, mg/dL	144.8 ± 15.3	148.8 ± 56.2	0.855
HDL cholesterol, mg/dL	52.0 (11.3)	56.5 (17.5)	0.950
Glucose, mg/dL	102.8 ± 12.6	98.0 ± 6.0	0.414
Insulin, mU/L	21.5 ± 12.4	16.3 ± 9.3	0.385
HOMA-IR	5.4 ± 2.8	4.0 ± 2.4	0.332
Zonulin, ng/mL	592.0 (255.3)	275.7 (381.2)	0.058
LPS, pg/mL	43.6 (66.9)	34.1 (58.2)	0.698
hsCRP, mg/L	4.1 (2.9)	1.6 (2.4)	0.082
IL-6, pg/mL	1.9 ± 0.6	1.9 ± 1.2	0.975
Acetic acid, ng/mL	1,663.7 (1,026.8)	3,352.9 (807.3)	**0.043***
Propionic acid, ng/mL	82.7 (31.3)	76.0 (42.1)	0.699
Butyric acid, ng/mL	46.9 ± 13.8	67.2 ± 38.7	0.246
Isobutyric acid, ng/mL	20.1 ± 1.8	20.4 ± 6.6	0.904
2-MBA, ng/mL	43.7 ± 3.4	44.4 ± 8.3	0.862
Isovaleric acid, ng/mL	37.0 (24.2)	39.3 (29.6)	0.636
Valeric acid, ng/mL	18.6 ± 3.8	20.0 ± 4.1	0.536
Hexanoic acid, ng/mL	81.9 ± 17.2	82.3 ± 12.6	0.962

[1]Data are presented as mean ± SD or as median (IQR) for non-normally distributed data, respectively, or numbers (%) (*n* = 14). Differences between the groups were assessed using Fisher's exact test for categorical variables and unpaired Student's *t*-test or Mann-Whitney U-test for normal or skewed continuous variables. **P* < 0.05.

Abbreviations: BMI, body mass index; BP, blood pressure; BW, body weight; HDL, high density lipoprotein; hsCRP, high-sensitivity C-reactive protein; HOMA-IR, homeostasis model assessment of insulin resistance; IL-6, interleukin 6; LPS, Lipopolysaccharides; VA-NR, valeric acid non-responder; VA-R, valeric acid responder; WC, waist circumference; 2-MBA, 2-Methylbutyric acid.

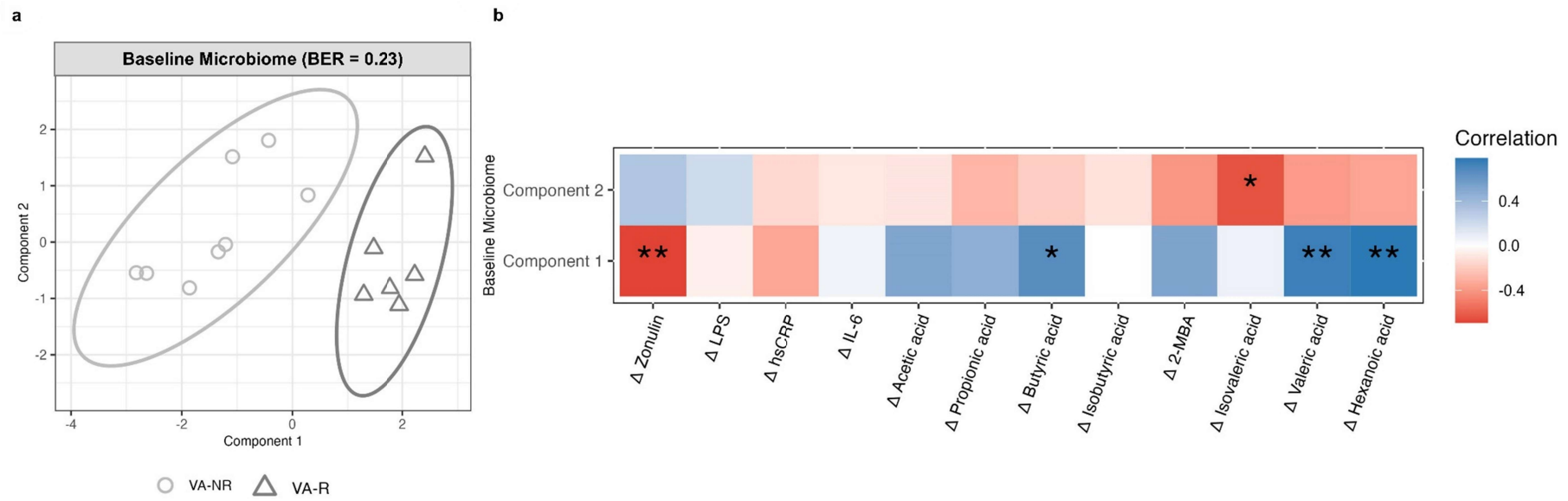

Figure 4-22: Differences in microbial composition between valeric acid responder s(VA-R) and non-responders (VA-NR) at baseline and associations with zonulin and SCFA responses

(**a**) Two-component sample plot of the sPLS-DA identifying a microbial signature at genus level that distinguished VA-R (light gray circles, *n* = 8) and VA-NR (dark gray triangles, *n* = 6) at baseline. (**b**) Correlations between baseline microbiome composition based on the components obtained from the sPLS-DA and changes in clinical markers and SCFAs. ****P* < 0.001, ***P* < 0.01, **P* < 0.05, ns *P* > 0.05.

Abbreviations: VA-R, valeric acid responders; VA-NR, valeric acid non-responders; 2-MBA, 2-methylbutyric acid

Table 4-17: Weight loadings of the sPLS-DA to identify a microbial signature that distinguishes VA-R from VA-NR at baseline

	Group contribution	Importance	Median change (VA-R vs. VA-NR)	VA-R	VA-NR
Component 1					
Tyzzerella	VA-R	0.655	increase	0.833	-0.625
Lachnospiraceae NK4B4 group	VA-NR	-0.488	decrease	-0.760	0.570
Negativibacillus	VA-R	0.367	increase	0.708	-0.531
Bacteroides pectinophilus group	VA-R	0.294	increase	0.675	-0.507
Eubacterium eligens group	VA-NR	-0.195	decrease	-0.632	0.474
Eubacterium ruminantium group	VA-NR	-0.154	decrease	-0.615	0.461
GCA-900066575	VA-R	0.141	increase	0.609	-0.456
Clostridia vadinBB60 group	VA-NR	-0.130	decrease	-0.604	0.453
[Peptococcaceae] uncultured	VA-NR	-0.088	decrease	-0.585	0.439
[Oscillospiraceae] NK4A214 group	VA-NR	-0.071	decrease	-0.578	0.434
Component 2					
Clostridia UCG-014	VA-R	-1	increase	0.326	-0.244

Abbreviations: VA-NR, valeric acid non-responders; VA-R, valeric acid responder.

5 DISCUSSION

In this thesis, the interactions between metabolism, the gut microbiome, and microbiome-derived metabolites were investigated in the context of two different dietary interventions to optimize human health. It was shown that a short-term, high-dose oat diet and a six-week, moderate oat diet led to a significant increase in the plasma concentration of various (microbially produced) phenolic metabolites including (DH)FA in individuals with MetS. In addition, it was convincingly demonstrated that the short-term, high-dose oat diet improved obesity-related dyslipidemia and has the potential to reduce gut permeability. These improvements were linked to shifts in the gut microbiome and global metabolomic profiles. In particular, microbially produced phenolic metabolites and SCFAs have been shown to be driving factors for the beneficial effects of oats on lipid metabolism and gut permeability. Moreover, the gut microbiome before the intervention was identified as a potential modulator of the microbial and metabolic response to the short-term, high-dose oat diet. Thus, new knowledge on the interplay between host metabolism, the gut microbiome and dietary interventions, especially oats, was generated and important insights into potential underlying mechanisms were gained.

5.1 Microbiome-Derived Metabolites as Mediators of Metabolic Outcomes in Dietary Interventions

As shown in this thesis, microbiome-derived metabolites play a decisive mediating role in the effects of dietary strategies on host metabolism. Improvements in lipid metabolism and gut permeability following a short-term, high-dose oat diet were linked to an oat-induced increase in microbially produced phenolic metabolites and SCFAs in plasma. Given that different bacteria without taxonomic similarity often share the capacity for similar functions (functional redundancy), the functional capacity of the gut microbiota is considered highly conserved and less diverse compared to the microbial composition.[33,294,295] It is therefore postulated that a functional core microbiome is preferable to taxonomic profiling.[296] Thus, functional analysis of the microbiome combined with global metabolomic profiling is a promising approach to elucidate the interplay between the gut microbiome, diet and host metabolism and to develop effective microbiome-targeted dietary strategies.

5.1.1 Cholesterol-Lowering Effects of Oats Induced by Microbially Produced Phenolic Metabolites

The results of this thesis suggest that phenolic compounds in oats, and particularly their microbial degradation products, are driving factors for the oat-induced improvements in lipid metabolism, characterized by a decrease in serum cholesterol levels. Moreover, changes in a number of metabolites of lipid and amino acid metabolism might contribute to the cholesterol-lowering effect via oat-induced shifts in the gut microbiome composition and function. As observed in the short-term intervention, microbially produced metabolites, especially phenolic compounds such as DHFA, may play a more relevant role in oat-induced metabolic improvements than bioactive compounds originating from oats. In the moderate six-week intervention, however, individual differences in lipid metabolism and microbial composition and functional capacity might be more important. (**Figure 5-1**)

The differences in the health effects of the two oat interventions may be explained by different underlying mechanisms such as calorie restriction, dose-dependent exposure to the bioactive compounds and potential synergistic effects.

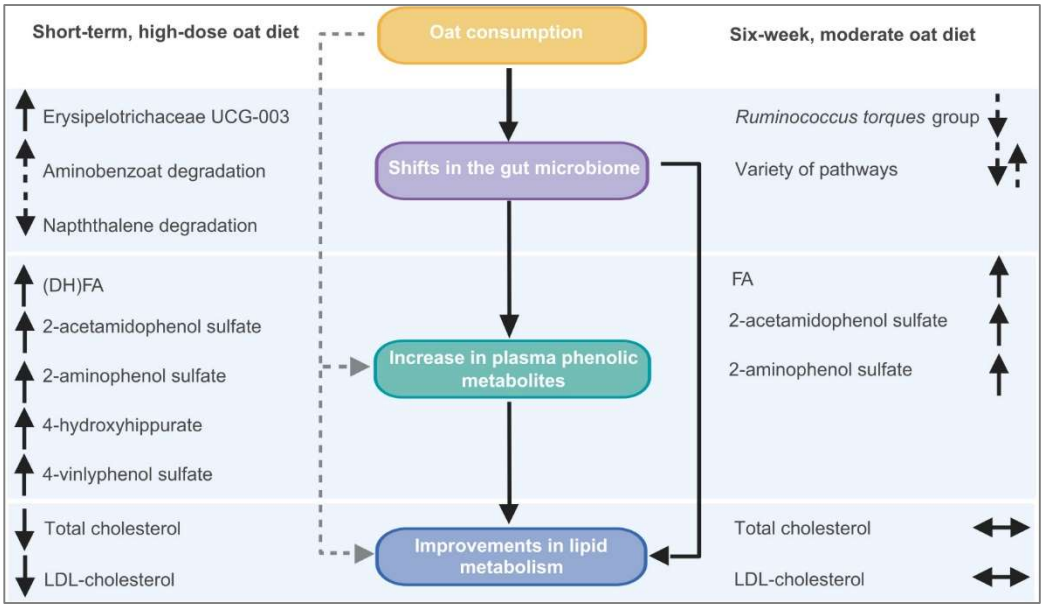

Figure 5-1: Summary of cholesterol-lowering effects of oats induced by microbially produced phenolic metabolites

Solid arrow: significant change; dashed arrow: trend; ↑ increase; ↓ decrease; ↔ no change.

Abbreviations: DHFA, dihydroferulic acid; FA, ferulic acid; LDL, low-density lipoprotein.

As the gut microbiota is able to metabolize dietary phenolic compounds into more biologically active metabolites, it is thought to play crucial roles in the digestion and absorption of these compounds.[76] Indeed, in the *in vitro* batch culture experiment, a rapid microbial production of phenolic compounds from oat fermentation was demonstrated. Accordingly, a significant increase in plasma FA levels after both oat diets and an increase in plasma DHFA concentrations, one of the main microbial derivatives of FA, after the short-term, high-dose oat diet were observed compared with the respective controls. These increases were associated with decreased TC and LDL-C levels, demonstrating that phenolic metabolites derived from oats, such as FA, can decrease cholesterol levels.[187] The proposed mechanism underlying the cholesterol-lowering properties of FA is the inhibition of 3-hydroxy-3-methyl-glutaryl-coenzyme A reductase (HMGCR), which controls cholesterol synthesis and modulates lipogenic gene expression in the liver.[297] However, the potential of FA to improve lipid metabolism has to date mainly been investigated in animal studies[297,298], while the potential of DHFA and other (microbially produced) phenolic metabolites to improve lipid metabolism has not yet been investigated. Therefore, the finding that DHFA also mediates the cholesterol-lowering effect by modulating cholesterol metabolism in PBMCs and tends to affect total lipids, *de novo* synthesis of cholesterol esters, and esterification of cholesterol in HuH7 cells is quite remarkable and offers new therapeutic approaches.

Moreover, elevated levels of several other phenolic compounds related to oats and metabolites linked to the microbial degradation of phenolic compounds, including 4-hydroxyhippurate[299], 2-hydroxyhippurate[300], 2-acetamidophenol sulfate[301], 2-aminophenol sulfate[301], 3-methoxycatechol sulfate[302], vanilloylglycine[303], 4-vinylphenol sulfate[304], and 2-methoxyhydroquinone sulfate[305] were observed. To the best of my knowledge, such a connection between these metabolites and cholesterol reduction, as observed in the short-term intervention study, has not yet been demonstrated. Thus, the results of the present work indicate that a short-term, high-dose oat diet and, to a lesser extent, the six-week, moderate oat diet significantly increase the plasma concentration of phenolic compounds[306] and their microbial degradation products[300], thereby confirming the bioavailability of these metabolites and contributing to cholesterol reduction. Cell and animal studies suggest several mechanisms by which phenols may affect lipid metabolism,

including suppression of hepatic cholesterol synthesis[307,308], inhibition of lipogenic genes in the liver[309,310], and regulation of hepatic lipolysis and fatty acid *β*-oxidation[308,311]. These findings emphasize the lipid-lowering potential of phenolic metabolites, as demonstrated in the present study.

The attenuated effect of the six-week oat intervention on plasma phenolic metabolite levels may be attributed to the lower oat consumption (80 g/day) when integrating a single oat meal into the habitual diet, compared to the short-term, high-dose oat diet (300 g/day) where three oat meals replaced the habitual diet entirely. The single meal leads to a relatively lower intake of oat bioactive compounds, including phenols, which may impact their biological effects such as their hypolipidemic activity[297]. It has been shown that the concentration of (microbially produced) metabolites is a decisive factor for their effect, as they exert various biological activities at different concentrations.[312,313] The compound's concentration threshold for one biological function may therefore differ from that required for another activity.[77] Higher plasma concentrations of potentially health-promoting microbial metabolites, such as phenolic compounds, after the short-term, high-dose oat diet compared to the six-week, moderate oat diet might therefore contribute to the differences in metabolic effects. In addition, maintaining the habitual Western dietary pattern alongside a single oat meal introduced greater variability in foods and nutrient exposure, potentially covering intervention efficacy. It can therefore be assumed that the impact of a single oat meal may not be strong enough to compensate for the inter-individual differences in habitual food intake during the study period. Heterogeneity of oat meal preparation[314] as recommended in the OG^{6w} in combination with inter-individual differences in the response to oats based on the gut microbiome and host genetic phenotype may also contribute to the moderate effect in the six-week intervention.[315] These observations suggest that personalized nutrition strategies may optimize oat interventions at moderate doses, where individual parameters seem to exert greater influence compared to a high-dose protocol. Despite the high individual variability, almost 20% of the variance in LDL-C in the short-term study could be explained solely by changes in the plasma phenolic compounds. Furthermore, it is worth noting that the participants in the short-term intervention additionally underwent calorie restriction, while the dietary intervention in the six-week study was isocaloric. The observed differences in the health effects

of the two oat interventions may therefore be explained by different underlying mechanisms such as calorie restriction[316], dose-dependent exposure to the bioactive compounds and potential synergistic effects.

Notably, in the short-term, high-dose oat diet, the increase in phenolic compounds was associated with a specific oat-induced modulation of the gut microbiota, characterized by an increase in *Erysipelotrichaceae* UCG-003, which is associated with a healthy aging process[317]. Because of the strong positive correlation between this genus and various phenolic compounds and its inverse correlation with cholesterol levels, it can be proposed that *Erysipelotrichaceae* UCG-003 may metabolize the phenolic compounds in oats and thus contribute to lowering cholesterol levels. This oat-induced increase in *Erysipelotrichaceae* UCG-003 and its inverse correlation with cholesterol levels in humans, however, should be confirmed in further clinical studies. Presently this bacterium is an uncultivated genus whose physiological properties have not been described. However, the KEGG metabolic pathways for the family *Erysipelotrichaceae* include pathways for the degradation of aromatic compounds, specifically benzoate degradation.[318] In addition, an oat-induced increase in aminobenzoate degradation was observed, which is in line with a previous study[217] showing the upregulation of pathways associated with the bacterial degradation of aromatic compounds[319]. This supports the finding of a positive correlation between aminobenzoate degradation and DHFA levels. Aminobenzoate degradation is linked to cardio-metabolic health owing to its inverse correlation with body weight.[320] Therefore, an oat-induced increase in this specific pathway may be considered beneficial, particularly in individuals with MetS, as investigated.

Previous studies have suggested that dietary L-histidine can induce hypercholesterolemia.[321] Therefore, the changes in histidine metabolism observed in the short-term, high-dose oat diet are notable, as a reduction in dietary histidine intake resulted in lower levels of 3-methylhistidine in plasma.[322] These alterations may have contributed to the oat-induced cholesterol reduction. In addition, metabolites produced by microbes through the degradation of histidine, specifically imidazole propionate, have negative effects on human health by impairing glucose tolerance and insulin signaling.[68,69] A reduction in potentially harmful microbially produced metabolites of histidine, such as 1-methyl-5-imidazolelactate and

1-methyl-5-imidazoleacetate, may contribute to the cholesterol-lowering effect, as observed in the short-term, high-dose oat diet.

To date, the properties of oats to improve lipid metabolism have mainly been attributed to β-glucan, which can reduce the intestinal absorption of dietary cholesterol by forming a viscous gel in aqueous solutions and inhibit the enterohepatic circulation of bile acids by increasing their fecal excretion.[180,323] Furthermore, the effects of SCFAs produced by the bacterial fermentation of dietary fiber on lipid metabolism have already been studied in detail[56,324]; however, little is known about the role of other bioactive substances contained in oats, such as phenolic compounds. In the present work, even a two-day, high-dose oat diet improved lipid metabolism by lowering serum TC (-8%) and LDL-C concentrations (-10%), confirming the long-postulated cholesterol-lowering effect of oats[286,325] and highlighting the role of oat-derived phenolic compounds and their respective microbial metabolites as possible contributing factors. So far, little is known about the effects of short-term, high-dose oat diets.[326] Since nutritional intervention, particularly a two-day treatment, is expected to have only subtle treatment effects, the significant reduction in cholesterol, as shown in this study, is quite notable. Compared to the mean effect size of cholesterol-lowering drugs, e.g., statins, which cause an average relative reduction in LDL-C of 15% to 58%–depending on the specific product, dose (5-80 mg) and the continuous intake[327]–the clinical implications of the oat diet can be considered as relevant. Especially since cholesterol levels tended to remain below the baseline value during the six-week follow-up phase of the short-term intervention study, it would be advisable to consume a high-dose oat diet intermittently. Given the increasing prevalence of MetS, it is particularly important to identify cost-effective, easy-to-implement (e.g., concepts of intermittent fasting[328]), and sustainable strategies such as a short-term, high-dose oat diet to improve metabolism.

In summary, the present study showed that oat consumption, particularly a short-term, high-dose oat diet, confers metabolic health benefits in individuals with MetS by increasing circulating microbially produced phenolic metabolites, which in turn lower serum cholesterol levels. This mechanism suggests that phenolic metabolites mediate the cholesterol-lowering properties of oats, besides SCFAs, highlighting the significant role of oat-gut microbiome interactions in this health-promoting effect.

5.1.2 Reduction of Gut Permeability Induced by Calorie-Restricted Oat Diet is Associated with Short-Chain Fatty Acid Response

In addition to its beneficial effects on lipid metabolism mediated by microbially produced phenolic compounds, the results of the subgroup analysis suggests that the short-term, calorie-restricted, high-dose oat diet has the potential to reduce gut permeability in individuals with MetS, who are at risk for intestinal barrier dysfunction.[329,330] While the six-week moderate oat intake stabilized gut permeability and inflammatory markers and had mild effects on the SCFA levels and gut microbiota composition, the high-dose oat diet induced a notable reduction in serum zonulin concentration compared to baseline (-31%) and led to a significant shift in the gut microbiome compared to the control diet within two days. Interestingly, the reduction in circulating zonulin correlated inversely with changes in SCFA levels, particularly butyric and valeric acids, and with shifts in specific gut microbes. Oat-induced modulation of the microbial composition and function may therefore be a potential driving factor for this improvement.

Given the increasing prevalence of non-communicable diseases associated with an impaired gut barrier[331,332], identifying cost-effective and easy-to-implement strategies to restore intestinal barrier function is crucial. In particular, dietary approaches are promising as they can have a wide range of applications–regardless of the metabolic state–without severe side effects, as shown in the present work. In recent decades, a growing body of evidence from *in vitro* and animal studies has shown that dietary fibers such as oat fiber are able to improve intestinal permeability.[333–336] In this context, SCFAs produced by the microbial fermentation of dietary fibers are ascribed a key role in maintaining and supporting intestinal barrier function by serving as an energy source for enterocytes and acting as signaling molecules.[337] Especially butyric acid has been shown to exert positive effects on the gut barrier function[54,338], which is in line with the observed inverse association between the change in zonulin and butyric acid levels in the short-term, high-dose oat diet. In addition, valeric acid has been linked to protective effects on the GI tract[339,340] and is considered a potential therapeutic agent as shown *in vitro*[341] and potentially in individuals with MetS. A strong correlation between the change in valeric acid and zonulin levels was observed in the short-term oat intervention, indicating an important barrier-stabilizing effect of valeric acid in individuals with

MetS. Molecular mechanisms by which SCFAs affect gut permeability include histone deacetylation[54,341] and the modification of the MAPK pathway[342]. While the relationship between diet, SCFAs, and gut permeability has been investigated extensively in cell and animal studies, human trials are largely lacking. One RCT investigating the connection between the Mediterranean diet, SCFAs, and intestinal permeability identified butyric and propionic acids as key mediators[343], which is in line with the findings of this thesis.

Consistent with the butyric acid responses upon the two short-term dietary interventions, a diet-induced modulation of the microbial composition was observed, mainly characterized by a higher abundance of genera of the phylum Bacillota (synonym Firmicutes) in the OG compared to the CG. Bacillota represents the primary butyrate-producing bacterial phylum in the human colon[344], and previous studies reported an oat-induced increase in Bacillota abundance[345] as well as a positive association between Bacillota and β-glucan fermentation[346]. This indicates that the difference in the butyric acid responses observed between the diet groups may be related to a different dietary modulation of the microbiome. Looking more closely at the genus level, an increase in *Erysipelotrichaceae* UCG-003 and *Intestinimonas*, both of which associated with an increase in butyric acid, was observed in the OG compared to the CG. Moreover, *Erysipelotrichaceae* UCG-003 has been associated with healthy aging, potentially by strengthening the intestinal barrier through SCFA production.[317] *Intestinimonas* is recognized as a butyrate-producing species due to its ability to metabolize sugars and lysine into butyric acid.[347] This is particularly interesting, as oats contain relatively high levels of lysine[348], and the lysine-to-butyrate pathway is estimated to be the second dominant butyrogenic pathway in the intestine.[349] This suggests a key role of *Intestinimonas* in intestinal butyrate production[350], which is in line with the present results.

Apart from the diet-induced changes in the gut microbiome, the baseline microbiome also appears to play a decisive role in the SCFA response and the related zonulin reduction, as a distinct microbial signature was identified between VA-R and VA-NR in the OG. Especially the class Clostridia, which is attributed a specific role in colonocyte metabolism through the production of SCFAs[351], seems to be of relevance here.

Since caloric restriction also affects gut permeability[352], it is worth mentioning that both diets of the short-term intervention were hypocaloric. In line with this, a reduction of zonulin was also found in the CG, although this did not reach statistical significance. Possible underlying mechanisms are a reduction in body weight[353], as observed in both diet groups[354], and an induction of autophagy[355], which is involved in numerous aspects of intestinal physiology[356], including the maintenance of the gut barrier integrity by regulating tight junction (TJ) proteins[357,358]. In addition, calorie restriction and related weight loss have been shown to influence the composition and function of the gut microbiome, which in turn affect SCFA concentrations.[359–361] It can therefore be assumed that the reduction in butyric acid levels observed in the CG might be caused by calorie restriction. This effect may have been compensated for by the high-dose oat diet through compositional and functional gut microbiota remodeling, suggesting that the specific prebiotic properties of oats[362] might contribute to lowering intestinal permeability by maintaining the SCFA concentration stable despite calorie restriction. Interestingly, a mechanistic link between butyric acid and autophagy has also been discussed.[363] It can therefore be hypothesized that the reduction in zonulin levels observed in the OG compared to baseline was caused by the combined effect of an oat-induced shift in the gut microbiome leading to stable SCFA levels and calorie restriction, with the latter being an important additional factor. The stable zonulin and SCFA concentrations and the almost unchanged microbial composition as observed in the isocaloric six-week oat intervention might also be explained following this hypothesis. Additionally, a single oat meal integrated in the habitual Western diet under isocaloric conditions may not be sufficient[364] to improve gut permeability in individuals at risk of gut barrier dysfunctions. Furthermore, high individual variation in clinical markers at baseline may mask potential treatment-associated changes. In particular, the expected modest effects of the six-week moderate oat intervention may be masked by inter-individual differences in the response to oats based on the gut microbiome.[315]

In summary, the results of the present subgroup analysis suggest that, within a calorie-restricted diet, a 2-day high-dose oat intake potentially decreases gut permeability in individuals with MetS, possibly mediated by the combined effects of oat-induced microbiome alterations leading to subsequent shifts in SCFA levels and calorie restriction.

5.1.3 Link Between Gut Permeability and Metabolic Improvements

A growing body of evidence from both human and animal studies suggests that intestinal barrier impairments play an important role in the pathogeneses of MetS.[157,329,365] Specifically, gut barrier disorders—often caused by microbial dysbiosis—can lead to increased intestinal permeability and the translocation of microbial endotoxins such as LPS into the systemic circulation, thereby inducing metabolic endotoxemia and subsequent systemic low-grade inflammation.[159,160] Interestingly, a link between systemic inflammation and dyslipidemia has been demonstrated.[366,367] Animal and observational studies have shown that LPS-induced endotoxemia is linked to high concentrations of triglycerides, very-low-density lipoprotein, intermediate-density lipoprotein, LDL and total fatty acids, and low levels of HDL-C, with associations being particularly strong in individuals with MetS.[368–370] Conversely, lowering metabolic endotoxemia by strengthening gut barrier integrity has been shown to improve dyslipidemia.[371,372] It can therefore be assumed that the reduction in gut permeability observed following the short-term, high-dose oat diet—relative to baseline—may be an additional mechanism contributing to the oat-induced improvements in lipid metabolism.

Moreover, low-grade systemic inflammation has been shown to play a crucial role in the development of insulin resistance by interfering with insulin signaling pathways in different metabolic tissues, thereby impairing glucose uptake and metabolism.[373,374] In addition, gut barrier integrity is essential for maintaining liver and kidney homeostasis, and increased intestinal permeability has been linked to hepatic and renal disorders such as fatty liver disease and chronic kidney disease by triggering chronic inflammation.[329,375–377] Thus, a decrease in gut permeability might also play a role in the observed improvements in fasting and postprandial glucose metabolism, as well as in markers of liver and kidney function, following the short-term, high-dose oat diet compared to baseline. This is of particular relevance, as disturbance in glucose metabolism are a hallmark of MetS[221], and individuals with MetS face a substantially increased risk of developing chronic liver and kidney diseases.[378,379]

Furthermore, there is increasing evidence that life-style associated metabolic disorders such as MetS are associated with an increased risk of neurodegenerative diseases[380,381]. It has been shown that obesity and related metabolic disorders are

linked to pathologic brain aging, involving adverse changes in brain morphology[382], impaired synaptic plasticity[383,384], and cognitive impairments[385,386]. In particular, neuroinflammation, also potentially caused by obesity-related systemic low-grade inflammation, is considered an important factor in the pathophysiology of neurodegenerative diseases.[387,388] Thus, it is highly interesting that microbiome-derived metabolites such as phenolic compounds[81] and SCFAs[389,390] have been shown to exert neuroprotective properties, for example by enhancing blood-brain barrier integrity[391] and reducing neuroinflammation[392,393]. It can therefore be speculated that consuming oats may also have neuroprotective effects due to its interaction with the gut microbiome and the resulting health-promoting metabolites. However, to date, the evidence is limited and detrimental effects of SCFAs on brain health have also been reported, especially in germ-free mouse models.[394] Further studies are therefore needed to elucidate the neuroprotective effects of oats, focusing on the gut-brain axis, which is a vital component in the intricate relationship between host metabolism, gut microbiome and diet[395].

5.1.4 Potential Synergistic Effects of Microbiome-Derived Metabolites

Beyond individual effects of microbiome-derived metabolites, previous studies have shown that dietary fibers, SCFAs and phenolic compounds may have synergistic effects on the gut microbiome and host health. For example, an *in vitro* experiment demonstrated that oats as a whole plant food exerted a greater bifidogenic effect than the isolated, single bioactive compounds.[198] In addition, a synergistic anti-inflammatory effect of phenolic metabolites and butyrate has been shown in a tumor necrosis factor alpha (TNF-α)-induced Caco-2 cell model, suggesting that a combination of both metabolites has more pronounced effects on gut health than the individual compounds.[396] In line with this, stronger effects of FA and β-glucan combined than of FA alone were observed on intestinal barrier functions, glucose metabolism, and the gut microbiota in mice.[397] Interestingly, butyric acid has been shown to increase the absorption and consequently the concentration of circulating phenolic acids including FA.[398] Consistent with this, fiber-bound FA have been shown to be more bioavailable (i.e., extension of the time in blood and decrease in urinary excretion) than free FA.[399] These findings suggest that interactions between different (microbiome-derived) metabolites play an important role in the health

effects of dietary interventions. Investigating the effects of whole-plant foods such as oats instead of isolated bioactive compounds is therefore an exciting approach as it enables the examination of synergistic effects among various nutrients and combined signals mediated by different microbial degradation products. This is essential considering that metabolic activities of the gut microbes are closely interconnected and metabolites do not act independently.[42,400,401] In line with this, the results of this thesis suggest that a pattern of different microbial metabolites rather than a single metabolite contributes the cholesterol-lowering effect of oats. Moreover, holistic dietary approaches are more easily transferred to 'real-life scenarios', as people do not consume individual nutrients but food such as whole-grain oats.

5.2 Oats as a Holistic Microbiome-Targeted Dietary Approach for Optimizing Host Health

Considering the increasing prevalence of lifestyle-associated diseases[31,32] linked to microbial dysbiosis such as T2DM[27,402] and CVD[403,404], the development of effective microbiome-targeted strategies that can be easily integrated into everyday life is crucial. This is especially relevant for people already at high risk such as individuals with MetS. Among various strategies, dietary approaches such as increasing oat consumption are promising. Oats are considered to be versatile, easily integrated into the daily diet and well tolerated, which is also reflected by the high adherence to the applied oat diets in the present RCTs. In addition, oats are affordable and readily available[405], making them a cost-effective and sustainable option, especially compared to other dietary strategies such as the supplementation of prebiotics and synbiotics.

Synbiotics are defined as "a mixture comprising live microorganisms and substrate(s) selectively utilized by host microorganisms that confers a health benefit on the host" (Swanson *et al*., 2020, p. 688)[406] and have been shown to beneficially modulate the gut microbiome by increasing the abundance of health-promoting bacteria and SCFA concentration[407–409]. Compared to food-based approaches, synbiotics, like prebiotics, offer a more precise and targeted strategy to modulating the gut microbiome and host health, as they can be developed specifically for particular health issues by providing specific strains and substrates.[406] However,

current evidence of the health effects of synbiotics is still limited, especially regarding long-term effects and possible side effects[410]. Moreover, synbiotics are not inherently superior to a probiotic and may even attenuate probiotic-induced improvements[411], which suggests that synbiotics must be well designed to achieve the desired health effect. Even though synbiotics as well as prebiotics are easily incorporated into the diet and everyday life like oats, they are linked to a significantly higher financial outlay compared to food-based strategies. As they are often expensive and must be taken continuously over a long period of time to be effective (e.g., improvements in metabolic health usually require at least 3 months of administration)[406], long-term use can be costly. For example, the cost of the freely available synbiotics 'Biotic Junior', applied in one of the above-mentioned studies[407], amounts to approximately €40 per month.[412] This aligns with the costs estimated by the German consumer advice center[413] and accounts for more than 15% of the monthly expenditure on a healthy diet.[414,415] Since these supplements augment rather than replace a meal, their costs represent an additional expense beyond basic food expenditures. Therefore, cost-effective strategies based on changes to a single meal (e.g., replacing a Western meal with an oat meal) and/or changes to the entire diet (e.g., following a short-term, high-dose oat diet instead of a Western diet) may be a more appropriate approach to optimizing health.

Oat consumption, particularly a short-term, high-dose oat diet, is a suitable and rapid approach to alleviate obesity-related lipid-disorders in individuals with MetS, as convincingly demonstrated in this thesis. Specifically, it was shown that a high-dose oat intake reduced circulating TC and LDL-C by 8% and 10%, respectively, even in two days, emphasizing the clinical relevance of dietary strategies in lowering blood lipids compared to lipid-lowering drugs[327], especially considering the risk of side effects of medical treatments[416,417]. In addition, the short-term, high-dose oat diet was identified as a potential valuable tool of alleviating obesity-related intestinal barrier permeability. The broad effect spectrum of oats is particularly beneficial when multiple metabolic disorders are to be tackled together, as is the case of MetS.

Interestingly, a recent study demonstrated that increasing fiber intake from whole plant-based foods in combination with a synbiotic supplement was more effective in combating obesity than either approach alone.[418] It can therefore be assumed that a combination of two dietary strategies, such as the regular implementation of a

short-term, high-dose oat diet and occasional synbiotics intake, is a promising strategy for optimizing the gut microbiome and metabolic health in the long-term. However, further research is needed to enable evidence-based recommendations regarding the combined use of various nutritional strategies, including food-based approaches and supplementation.

5.3 Factors Influencing the Individual Response to Dietary Interventions

There is growing evidence that there are huge inter-individual differences in the response to dietary interventions (responders vs. non-responders), indicating that a 'one-size-fits-all' approach to achieving health is not feasible.[419] In particular, the unique composition of a person's gut microbiome has been shown to significantly influence the impact of specific dietary changes on host health.[87,420] In line with this, a microbial signature was identified, that influenced the SCFA response to the short-term, high-dose oat diet applied in the present work, as well as the associated improvements in gut permeability in individuals with MetS. This finding suggests that the metabolic changes might be modulated by the baseline gut microbiome. Animal and human studies showed that inter-individual variations in the gut microbiome are causally linked to different effects of dietary fibers on microbial composition and function (e.g., SCFA production), as well as on host metabolism.[421–425] Thus, in accordance with the current literature, the results of this thesis provide further evidence that the host response to a dietary intervention is highly individualized and that unique microbiome signatures contribute considerably to this heterogeneity. Varying capacities of different microbial species to degrade food compounds and their preferences for certain food components are potential underlying explanations, as they affect the bioavailability and metabolization of dietary compounds into bioactive molecules.[426,427] This, in turn, results in high inter-individual variations in the metabolome and the subsequent metabolic responses.[18,45] Recent studies also suggest that inter-individual variability in the gut mycobiome[428,429], gut virome[430,431], and gut phageome[432,433] may influence the microbial and metabolic response to diet by modulating host-microbiome interactions. However, further research is needed to fully elucidate the roles of viruses, phages and fungal species in the host-diet-microbiome interactions. Moreover, it has been shown that humans exhibit a person-specific mucosal colonization resistance which may also contribute to the

individualized impact of dietary interventions on the microbial community.[434] The intestinal colonization resistance is influenced by several factors, including microbial composition, host genetics, and environmental factors such as diet.[435]

Habitual diet is considered to be an important modulator of the metabolic effects of dietary interventions targeting the microbiome. Dietary fibers play a particular crucial role in gut homeostasis by serving as an energy source for enterocytes and as substrates for microbial fermentation to SCFAs[93], which have various effects on host metabolism (details see **Chapter 1.2**) and can also bolster colonic resistance to pathogens[435]. Conversely, insufficient fiber intake has been associated with detrimental effects on gut health, including a loss in microbial diversity and reduced SCFA production.[127,128] This may impact the stability and resilience of the gut microbiome[39,436,437] and thus the extent of dietary interventions to modulate the gut microbiota. It is assumed that the more stable and resistant the microbiome is, the weaker its response to an intervention. This assumption is based on the fact that transitioning from one stable state to another requires a significant perturbation, namely the crossing of a tipping point.[437,438] While a 'healthy' gut microbiome is considered to exhibit remarkable resistance allowing it to maintain its stability and functional integrity in the face of disturbances[437,439], a 'dysbiotic' microbiome might be less stable and resilient to interference. In line with this, it was shown that individuals with low habitual fiber intake benefited more from a 7-week synbiotic supplementation, characterized by a greater increase in known SCFA-producing bacteria, than those with initially high fiber intake.[407] The gut microbiome of individuals with low habitual fiber intake may therefore not only have a greater potential for improvements, but might be more amenable to diet-induced changes than that of individuals with a high fiber intake. Indeed, different microbial signatures were observed between the individuals with low and high habitual fiber intake, particularly characterized by lower abundances of genera associated with SCFA production such as *Butyrivibrio*[440] and *Lachnospiraceae* UCG-008[441] in individuals with low habitual fiber intake.[407] It can therefore be assumed that differences in the habitual dietary fiber intake may result in distinct microbial compositions, which in turn affects the response of the gut microbiome to dietary interventions.[407] However, in cases where an aberrant microbiome demonstrates high resilience and stability, it can impede treatment efforts.[439]

In addition to the individual gut microbiome and dietary behavior, the person's genetic background and metabolic state prior to the intervention are other important factors influencing the response to dietary modifications (e.g., improvements are easier to achieve starting form a pathophysiological state).[442,443] It is widely recognized that host genetics contribute considerably to inter-individual variations in the gut microbiome[444,445] and influence the microbial and metabolic responses to diet[149,446]. Interestingly, recent studies revealed that the apolipoprotein E (*ApoE*) genotype, a key modulator of circulating cholesterol levels, is associated with specific microbial signatures[447,448] and with different metabolic responses to dietary fibers and whole grains[449,450]. While carrying the ε2 allele (*ApoE2)* is associated with lower LDL-cholesterol levels, the ε4 allele (*ApoE4*) is linked to hypercholesterinemia and an increased risk of CVD.[451] It can therefore be assumed that the individual genetic phenotype may have influenced the cholesterol-lowering effect of the oat diets applied in the present RCTs. In addition, it is speculated that variations in the *ApoE* genotype modulated the impact of the oat interventions on circulating triglycerides levels and glucose metabolism, as *ApoE* polymorphisms have also been identified as genetic risk factors for hypertriglyceridemia[452,453] and T2DM[454,455]. Furthermore, *ApoE* polymorphism has been demonstrated to be a genetic determinant of neurodegenerative disorders, particularly Alzheimer's disease, with the ε4 allele conferring increased risk and earlier onset.[456,457] Future studies investigating the effects of oat consumption on metabolism and gut microbiome should therefore also consider the host genetic background to further uncover the complex host-diet-microbiome interactions.

Moreover, age has been identified as an important factor influencing the interactions between host, diet and the gut microbiome.[458] Thus, the relatively broad age range of the participants in the RCTs presented, representing a large proportion of the general adult population, enables good generalizability of the results and a high degree of transferability into clinical practice.

The highly complex and person-specific interactions between diet, metabolism and the gut microbiome, as shown in this thesis, pose a challenge in developing effective microbiome-targeted approaches to optimize human health. Due to the large heterogeneity in the individual response, the effects of dietary interventions may be masked or underestimated. This may also explain the moderate effects of a single

oat meal integrated into the participants' habitual Western diet on host metabolism and the gut microbiome during the 6-week intervention study. Personalized nutritional strategies taking into account different modifiable factors (e.g., habitual diet, gut microbiome, lifestyle) as well as non-controllable factors (e.g., genetic disposition, biological sex, age) therefore hold great potential for improving host health.[459,460] In this context, continuous monitoring of various biomarkers enables the recording of day-to-day variations in metabolic markers and metabolomic profiles, and facilitates to evaluate the effectiveness of nutritional interventions.[461] Considering that changes in the microbial metabolome may occur with a time lag to the diet-induced shift in the gut microbiome[42] and that diurnal oscillation in the microbial metabolome has been observed[462], one-time measurements might lead to misinterpretations. Despite the great potential, however, the current scientific evidence for data-driven personalized nutritional recommendations is limited and implementation in clinical practice is still restricted.[463,464] To date, most evidence in humans is derived from observational studies which do not provide proof of causality and cannot determine whether microbiome shifts are the cause or the consequence of a disease.[465,466] Further well-designed RCTs considering various types of data or 'omics' (e.g., habitual diet, metabolic markers, gut microbiome, metabolomic profiles), allowing in-depth characterization, such as the RCTs presented in this thesis, are therefore needed to strengthen the potential and (clinical) application of personalized nutrition recommendations.

5.4 Challenges in Studying Host-Diet-Microbiome Interactions

When investigating host-diet-microbiome interactions, methodological challenges must be kept in mind. The fecal microbiome, which is most frequently analyzed in clinical studies, is a surrogate marker for the gut microbiome. Although research demonstrates that it reflects the microbial composition of the large intestine[467,468], differences between the composition of fecal samples and colonic biopsies have also been reported[469–471]. In addition, it has been shown that the metabolome profiles in feces and in the intestine differ considerably.[472,473] Data from fecal samples may thus not fully represent the microbiome and metabolites present in the gut, especially those of the small intestine. Using smart capsules to collect microbiome samples along the gut, in addition to stool samples, is therefore

desirable in future human intervention studies, gaining a more detailed insight in microbial composition, functional potential and the interactions with the host.[473–475] Differences in the experimental designs and procedures, including fecal sample collection, preservation and storage, DNA extraction and sequencing protocols, as well as differences in the bioinformatic pipelines also pose challenges, which lead to technology-related bias and limit the comparability of results across studies[11,476-478]. Some researchers therefore suggest that an international standard protocol for studying host-diet-microbiome interactions is essential to generate reliable and reproducible results.[479] However, there is an ongoing debate regarding the need for a single standardized method, as the most appropriate method for one microbial community might not be suitable for another.[480]

Besides, current microbiome analysis usually generates compositional data with relative abundances but without quantitative information, potentially causing misinterpretation of results and limiting the ability to truly characterize host-microbiome interactions.[111,481] The implementation of bioinformatic pipelines that enable quantitative microbiome profiling may therefore be a suitable approach to further elucidate the complex diet-host-microbiome interactions.[482,483] Addressing these challenges will enhance our understanding of the complex interplay between the gut microbiome, nutrition and host, and enable the development of effective and reliable dietary strategies to optimize health by modulating the gut microbiome.

5.5 Strengths and Limitations

A notable strength of the research presented in this thesis is the investigation of host-diet-microbiome interactions in two different dietary interventions, comparing a short-term, hypocaloric, high-dose oat diet and a six-week, isocaloric, moderate oat diet with corresponding control diets. In addition, the randomized, controlled design of the study is a significant strength, as it allows for the demonstration of causality between the dietary strategies and the observed changes in host metabolism, metabolomic profiles and the gut microbiome. By generating and analyzing various (‘multi-omic’) data sets, including metabolic blood parameters in fasting and in postprandial state, gut microbiome composition and functional capacity, as well as plasma and fecal metabolomic profiles, an in-depth characterization of the female and male participants was conducted. This holistic approach enhanced the

understanding of the complex interplay between host metabolism, diet and the gut microbiome and enabled the identification of the underlying mechanisms for the oat-induced improvements in circulating cholesterol levels and gut permeability in humans. Furthermore, both trials were conducted under ‘real-life conditions’, confirming the feasibility of the intervention and the relevance of the results. The isocaloric design of the six-week oat study additionally enabled the evaluation of the isolated effect of this dietary strategy without introducing bias from weight loss. Thus, well-tolerated, easy-to-implement and successful nutritional strategies for improving metabolic and gut health by modulating the gut microbiome were demonstrated.

However, it should be acknowledged that baseline differences–including sex distribution, blood lipid and liver enzymes levels– were present, both between the diet groups within each intervention and between the two interventions, which may have implications when comparing and interpreting the results. However, these differences also reflect the complexity and heterogeneity of the MetS, enabling the investigation of intervention effects across a broader metabolic spectrum. Importantly, all analyses were adjusted for relevant confounding factors including sex, increasing the robustness and generalizability of the results presented.

Since host genetic background was not taken into account, the impact of the genetic phenotype on host-diet-microbiome interactions could not be assessed. However, more than 25% of the variation in cholesterol levels in the short-term, high-dose oat study could be predicted by changes in the metabolomic profiles and the gut microbiome according to additional PLS regression models, which include the most important features identified with the DIABLO models as input (correlation cut-off value $r = \pm 0.5$). In addition, considering these features, >20% of the variance in TC and >30% of the variance in LDL-cholesterol could be explained. These findings emphasize that interactions between diet and the gut microbiome play a key role in the health effects of dietary interventions, especially by the production of microbial metabolites. However, no conclusions can be drawn about the effects of a regularly repeated short-term, high-dose oat diet or long-term effects beyond the intervention period of six weeks.

Regarding the subgroup analysis, it is also important to acknowledge that commercially available ELISA kits do not specifically detect zonulin (prehaptoglobin-2) but rather the zonulin family of peptides.[484] Nevertheless, zonulin serves as a surrogate marker for gut barrier assessment, as circulating zonulin levels positively correlate with the lactulose/mannitol ratio, a well-established indicator of intestinal permeability.[485,486] Therefore, it seems plausible that the reduction in serum zonulin observed upon the short-term intervention reflects a decrease in gut permeability.

To confirm the findings on lipid metabolism, gut permeability, the gut microbiome and microbially produced metabolites presented in this thesis and to elucidate the role of host genetics, further well-designed randomized controlled intervention studies are needed.

6 CONCLUSION AND OUTLOOK

In conclusion, the research presented in this thesis contributes significantly to the understanding of the complex interaction between metabolism, diet and the gut microbiome, emphasizing its key role for human health and offering new possibilities for innovative therapeutic and preventive strategies.

In particular, it has been convincingly demonstrated that microbial metabolites such as phenolic compounds and SCFAs produced during the breakdown of dietary phenols and fiber, respectively, are driving factors for the health-promoting effects of whole-grain oats. Oat consumption, particularly a short-term, high-dose oat diet, provides metabolic health benefits for individuals with MetS by increasing circulating microbially produced phenolic metabolites, thereby lowering serum cholesterol levels. This identified mechanism shows that phenolic metabolites, besides SCFAs, are driving factors for the cholesterol-lowering properties of oats. In addition, within a calorie-restricted diet, a 2-day high-dose oat intake potentially decreases gut permeability in individuals with MetS. This effect might be mediated by the combined effects of oat-induced microbiome alterations leading to subsequent shifts in SCFA levels, and calorie restriction. This research thus provides new insights into the health effects of microbiota-targeted dietary interventions and their underlying mechanisms.

This open new avenues for microbiota-targeted nutritional therapies, considering microbially produced metabolites, particularly phenolic degradation products, as potential agents to improve metabolic disorders linked to obesity. Given the global increase in lifestyle-associated diseases, there is an urgent need to develop tailored microbiota-targeted dietary strategies for the primary and secondary prevention of T2DM and CVD caused by Western dietary pattern and concomitant microbial dysbiosis.

The observations made in this thesis implicate that there is a great potential of a short-term, high-dose oat diet as a successful, well-tolerated and easy-to-implement dietary strategy to alleviate obesity-related lipid and gut barrier disorders and thus to optimize host health by modulating the gut microbiome. The research presented is therefore a basis for future studies investigating the effects of a regularly implemented high-dose oat diet on metabolism and the gut microbiome. Based on

these findings, modulation of the gut microbiota functions by dietary interventions such as oats can be considered as a promising approach to optimize human health and prevent and treat obesity-related metabolic disorders.

Due to the demonstrated importance of (microbially produced) phenolic metabolites for the metabolic improvements by oats in addition to the known mechanisms of oat β-glucan, it can also be concluded that a combination of dietary fibers and phenolic compounds is a valuable nutritional strategy and might, for example, improve the efficacy of synbiotics as a common prebiotic component. Future RCTs applying whole-grain oats, isolated oat β-glucans and oat phenols as interventions could provide further insights into potential synergistic effects of the different bioactive compounds and their microbial degradation products, and deliver important knowledge for the development of new nutritional strategies. In addition, studies assessing the gut microbiome together with neurological biomarkers and an individual's cognitive performance could reveal the impact of plant-based foods such as oats and their bioactive components on brain health, which is crucial due to the increased risk of neurodegenerative disorders in individuals with lifestyle-associated metabolic diseases.

Furthermore, the research presented in this thesis indicates that personalized nutrition approaches are necessary to enhance the health benefits of moderate dietary interventions, such as the six-week oat diet, by accounting for inter-individual variability in responses to oats based on person-specific characteristics. This strategy is aligned with current literature and critical for tackling the global rise in the prevalence of metabolic disorders. However, further well-designed intervention studies are needed to address this challenge and continue unravelling the complex host-diet-microbiome interactions. It remains to be determined whether the identified oat-derived phenolic metabolites contribute to lowering cholesterol levels across different populations and in a specific pattern. Moreover, there is still a plethora of unknown microbiome-derived metabolites that have yet to be discovered.

So far, dietary interventions such as oat consumption are effective, safe and feasible strategies to optimize human health by modulating gut microbiota composition and enhancing the production of microbial bioactive metabolites, including phenolic compounds and SCFAs.

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8 APPENDIX

8.1 *In vitro* cell culture experiments

Blood was collected in EDTA-containing cuvettes. Subsequently, the PBMCs were isolated by density gradient centrifugation with Pancoll (density 1.077 g/mL, PAN Biotech, # P04-601000). After washing of the cells with PBS and removal of platelets, PBMCs were plated in RPMI Medium (PAN Biotech, # P04-22100) supplemented with Penicillin/Streptomycin and 10% Fetal Bovine Serum with a density of $2*10^6$/mL into a 96 well round bottom plate (Sarstedt, # 83.3925). After 2 h incubation, PBMCs were exposed to medium with or without 10 µM DHFA (SigmaAldrich, #17803-5G). Additionally, the medium was supplemented with 20 µg/mL Alkyne cholesterol. After 24 h, PBMCs were washed first with 200 µL ice-cold 0.5% DL-BSA (Carl Roth, #0052.3) in PBS and then with 200 µL ice-cold 155 mM Ammonium acetate in H2O. Between every washing step, plates were centrifuged at 610 x g for 5 min and supernatant was decanted. Subsequently, 250 µL of PBMC extraction mix (230 µl MeOH/$CHCl_3$ 5/1, 20 µl internal standard mix containing alkynelabeled lipids: 103 pmol TG 48:3-d8, 14.6 pmol DG 32:3-d8, 26 pmol CE 17:2-d7, 15.8 pmol dh-alkyne-Cer15:1-d8, 13 pmol PA 32:3-d8, 113 pmol PC 32:3-d8, 36 pmol PE 32:3-d8, 30 pmol PI 32:3-d8, 34 pmol PS 32:3-d8 and 20 pmol of double-labeled TG 49:5-d8 and nonalkyne labeled lipids: 250 pmol PE 33:1-d7, 472 pmol PC 33:1-d7, 98 pmol PS 31:1, 84 pmol PI 34:0, 56 pmol PA 31:1, 51 pmol PG 28:0, 39 pmol LPA 17:0, 35 pmol LPC 17:1, 38 pmol LPE 17:0, 32 pmol Cer 17:0, 240 pmol, SM 18:1-d9, 55 pmol GlcCer 12:0, 339 pmol TG 50:1-d4, 111 pmol, CE 18:1-d6 and 64 pmol DG 31:1) was added to each well and plates were placed into an ultrasound bath for 20s. Samples were collected and then subjected to lipid extraction.

HuH-7 cells (JCRB0403) were cultivated in growth medium (DMEM 4.5 g l^{-1} glucose, 10% FCS plus penicillin/streptomycin), seeded into 12-well plates and allowed to grow for 48 h. HuH-7 cells were exposed to medium with or without 10 µM DHFA (SigmaAldrich, #17803-5G). Additionally, the medium contained either 20 µg/mL Alkyne cholesterol or 5 mM 1,2-13C2 sodium acetate (CIL, #CLM-440-1) and 100 µM alkyne fatty acid 182, and incubated for 24 h. Cells on 12-well plate were washed once with ice-cold 0.5% DL-BSA in PBS and quickly once with 155 mM ammonium acetate, taking care to remove the liquid after the last wash as complete

as possible. To each well, 500 µl of extraction mix (480 µl MeOH/$CHCl_3$ 5/1, 20 µl internal standard mix containing alkyne-labeled lipids: 206 pmol TG 48:3-d8, 29.2 pmol DG 32:3-d8, 52 pmol CE 17:2-d7, 31.6 pmol dh-alkyne-Cer15:1-d8, 26 pmol PA 32:3-d8, 226 pmol PC 32:3- d8, 72 pmol PE 32:3-d8, 60 pmol PI 32:3-d8, 68 pmol PS 32:3-d8 and 41 pmol of double-labeled TG 49:5-d8 and nonalkyne labeled lipids: 500 pmol PE 33:1-d7, 944 pmol PC 33:1-d7, 196 pmol PS 31:1, 168 pmol PI 34:0, 112 pmol PA 31:1, 102 pmol PG 28:0, 78 pmol LPA 17:0, 70 pmol LPC 17:1, 76 pmol LPE 17:0, 64 pmol Cer 17:0, 480 pmol, SM 18:1-d9, 110 pmol GlcCer 12:0, 678 pmol TG 50:1-d4, 222 pmol, CE 18:1-d6 and 128 pmol DG 31:1) was added and the entire plate was sonicated for 1 min in a bath sonicator. The extracts including the cell remnants were collected into 1.5-ml original Eppendorf tubes and centrifuged at 20,000g for 5 min to pellet protein. The supernatants were transferred into fresh tubes. After addition of 400 µl of CHCl3 and 600 µl of water, samples were shaken for 30 s and centrifuged for 5 min at 20,000g. The upper phase was removed, and the lower phase transferred into a fresh tube and dried for 20 min at 45 °C in a speed-vak. CHCl3 (8 µl) was added and the tubes briefly vortexed. To each tube, 40 µl of Click mix was added (prepared by mixing 20 µl of 50 mM C171 in 50% MeOH (stored as aliquots at −80 °C) with 200 µl of 5 mM Cu(I)AcCN4BF4 in AcCN and 800 µl of ethanol), followed by sonication for 5 min and incubation at 40 °C for 16 h. 250 µl of $CHCl_3$ and 600 µl of water were added per sample and briefly shaken and centrifuged for 2 min at 20,000g. The upper phase was removed and the lower phase dried in a speed-vac as above. 1,000 µl of Spray buffer (2-propanol/methanol/water 8:5:1 + 10 mM ammonium acetate) was added, the tubes sonicated for 5 min and the dissolved lipids analyzed by mass spectrometry (MS).

Mass spectra were recorded on a Thermo Q-Exactive Plus spectrometer equipped with a standard HESI ion source using direct injection from a Thermo Dionex AS-AP autosampler driven by an AXP-MS pump under the control of Xcalibur software. MS1 spectra (resolution 280,000) were recorded in 100 m/z windows from 250-1,200 m/z (positive mode) followed by recording MS/MS spectra (resolution 280,000) by data independent acquisition in 1 m/z windows from 250-1,200 m/z (positive mode). The raw data were converted to .mzML files using MSConvert and analyzed with LipidXplorer software. For further analysis, absolute amounts were

calculated using internal standard intensities, followed by the calculation of the molar fraction for the identified lipids.

8.2 Fecal batch fermentation of *in vitro* digested oats

In preparation, oat flakes (Demeterhof Schwab GmbH & Co. KG, Windsbach, Germany) were digested *in vitro* according to a modified method previously described by Kristek *et al.*[1]. Briefly, 60 g of oat flakes was homogenized in 150 ml of sterile distilled water and microwaved for one minute at 950 W. Subsequently, 20 mg α-Amylase (from *Aspergillus oryzae*, Sigma-Aldrich; dissolved in 6,25 ml of 1 mM $CaCl_2$) was added and the mixture incubated for 30 min at 37 °C and 200 rpm on an incubation shaker. After incubation, the pH of the solution was adjusted to 2 using 6 M HCl, and 2.7 g Pepsin (Carl Roth) in 25 ml of 0.1 M HCl was added, followed by 2 h of incubation at 37°C and 200 rpm. From there, 560 mg of pancreatin (from porcine pancreas, Sigma-Aldrich), 3.5 g bile salts (Sigma-Aldrich), and 125 ml of 0.5 M $NaHCO_3$ were added, and the pH was adjusted to 7.0 with 5 M NaOH. After 3 h of further incubation, the solution was transferred into a regenerated cellulose dialysis tubing (Spectra/Por® 7, Carl Roth) with 1 kDa MWCO and dialyzed against 0.01 M NaCl at 5°C for 15 h. Subsequently, the dialysis fluid was exchanged, and dialysis continued for 2 h. Finally, the dialyzed solution was lyophilized for 5 days to remove any fluid content.

For the fecal batch culture experiments, 500 mg of stool samples that were each taken from four representative participants prior to the short-term intervention study were homogenized in 5 ml of sterile, anoxic basal buffer (0.5 g/l bile salts (Sigma-Aldrich), 0.01 g/l $CaCl_2$ x 2 H_2O, 0.04 g/l K_2HPO_4, 0.01 g/l $MgSO_4$ x 7 H_2O, 0.04 g/l KH_2PO_4, 0.1 g/l NaCl, 2 g/l $NaHCO_3$, flushed with 90% N_2/10% CO_2). Subsequently, the slurry was divided into 2.5 ml aliquots until further use. To mimic a fasting period for the fecal microbiota in comparison to physiological conditions, one aliquot per participant was used to inoculate either 50 ml of sterile, anoxic basal buffer (starvation group) or 50 ml of sterile anoxic Brain Heart Infusion medium (BHI, flushed with 90% N_2/10% CO_2; physiological group) in serum flasks sealed with butyl rubber stoppers. After incubation overnight at 37°C, the starvation and the physiological groups were each used to inoculate (3%v/v) 50 ml of sterile, anoxic basal medium (see Kristek *et al.*[1]; flushed with 90% N_2/10% CO_2) either with or

without 3.3 g of anoxic, sterile *in vitro* digested oat flakes (from 66 g/l stock solution in 5 mM potassium phosphate buffer, pH 7.5, flushed with N_2; corresponding to an oat meal based on a colon volume of 1.5 l) in serum flasks sealed with butyl rubber stoppers. Finally, the cultures were incubated at 37°C over 36 h. Samples for global metabolomic profiling, including phenolic compounds, short-chain fatty acids (SCFAs), and microbiome analysis, were taken directly after inoculation, after 6 h, 24 h, and 36 h of incubation time.

SCFA profiling

The concentrations of SCFAs were determined using isocratic HPLC (Agilent 1260 Infinity II System equipped with a VWD dual wavelength detector, Agilent Technologies). Briefly, samples were centrifuged for 2 min at 12,000 x g and the supernatants were sterile filtered (Filtropur S, pore size: 0.2 µm, Sarstedt). The filtrate was diluted 1:5 with 5 mM TFA and finally analyzed on an Aminex HPX-87H 300 x 7,8 mm column (Bio-Rad Laboratories) at 60 °C with 5 mM TFA as the mobile phase (flow rate of 0.5 ml/min). To determine the absolute concentrations of every SCFA species in each sample, UV absorption at 210 nm was quantified against respective standard curves using OpenLab CDS software version 2.6.

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