Aus dem Helmholtz Zentrum München – Deutsches Forschungszentrum für Gesundheit und Umwelt (GmbH), Selbständige Forschungruppe für Klinische Epidemiologie,
Leiter: Prof. Dr. Jakob Linseisen

und der

Ludwig-Maximilians-Universität München, Lehrstuhl für Epidemiologie, Universitäres Zentrum für Gesundheitswissenschaften am Klinikum Augsburg (UNIKA-T),
Lehrstuhlinhaber: Prof. Dr. Jakob Linseisen

Habitual Diet, the Gut Microbiota, Fecal Metabolites and their Associations with Metabolic Diseases:

Results from a Population-based Study

Dissertation

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Taylor Alyssa Jones Breuninger

aus

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Berichterstatter:	Prof. Dr. Jakob Linseisen
Mitberichterstatter:	Prof. Dr. Julia Seiderer-Nack
	Prof. Dr. Bärbel Otto
Dekan:	Prof. Dr. med. dent. Reinhard Hickel
Tag der mündlichen Prüfung:	14.09.2021

Affidavit

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Breuninger, Taylor Alyssa Jones

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Abbreviations

Abbreviation	Meaning
BMI	Body mass index
CVD	Cardiovascular disease
DASH	Dietary Approaches to Stop Hypertension
FXR	Farnesoid X receptor
HbA1C	Hemoglobin A1c
HDL-c	High-density lipoprotein cholesterol
KORA	Cooperative Health Research in the Region of Augsburg
LDA	Latent Dirichlet Allocation
LDL-c	Low-density lipoprotein cholesterol
NCEP ATP	National Cholesterol Education Program Adult Treatment Panel
rRNA	Ribosomal ribonucleic acid
SCFA	Short-chain fatty acids
TMA	Trimethylamine
TMAO	Trimethylamine N-oxide
T2DM	Type 2 diabetes mellitus

Publication List

1. Breuninger TA, Wawro N, Meisinger C, Artati A, Adamski J, Peters A, Grallert H, Linseisen J. Associations between fecal bile acids, neutral sterols, and serum lipids in the KORA FF4 study. Atherosclerosis 2019, 288:1-8. doi: https://doi.org/10.1016/j.atherosclerosis.2019.06.911

2. Breuninger TA, Wawro N, Breuninger J, Reitmeier S, Clavel T, Six-Merker J, Pestoni G, Rohrmann S, Rathmann W, Peters A, Grallert H, Meisinger C, Haller D, Linseisen J. Associations between habitual diet, metabolic disease, and the gut microbiota using Latent Dirichlet Allocation. Microbiome 2021, 9:61. doi: https://doi.org/10.1186/s40168-020-00969-9

1 Detailed description of author's contribution to each publication

1.1 Contribution to Publication I

The doctoral candidate completed the literature search independently and developed an analysis plan for the publication. She conducted the statistical analysis and interpreted the results of the analysis autonomously. The doctoral candidate also drafted the manuscript independently. Additionally, she was responsible for selecting the target journal and managing the submission process, including communicating with the editors and other representatives of the journals as the corresponding author. Throughout the entire process, she consulted with and received feedback and guidance from her supervisor Prof. Dr. Jakob Linseisen.

1.2 Contribution to Publication II

The analysis plan for the publication was drafted by the doctoral candidate. She also completed the literature search, statistical analysis, and interpretation of the results. The doctoral candidate independently drafted the manuscripts and selected the target journal. As corresponding author, she was responsible for submitting the manuscript and communicating with journal representatives. During the review process, she responded to and implemented suggestions for changes to the manuscripts and conducted additional sub-analyses. She consulted with and received feedback and guidance from her supervisor Prof. Dr. Jakob Linseisen during each step.

2 Introduction

2.1 The burden of metabolic disease in Germany and Europe as a whole

Metabolic diseases, including dyslipidemia, obesity, type 2 diabetes mellitus (T2DM), and hypertension, are a major public health problem. It is estimated that at least 26% of the adult population in Germany have obesity while 28% have high blood pressure [1]. Although the prevalence of raised blood pressure has decreased over the past two decades in Germany, the prevalence of obesity has continued to steadily increase [1], and dyslipidemia has an even higher prevalence of approximately 65% in adults under 80 years old [2]. At the same time, prevalence of T2DM has increased by more than 50% in many European countries over the past decade [3]. In Germany, it is currently estimated that at least 7.2%-9.9% of the adult population is living with diabetes, and these numbers continue to grow [4, 5]. Recent estimates predict an increase in T2DM prevalence of 54-77% in Germany between the years 2015 and 2040, meaning that between 10 and 12 million people could be living with T2DM in Germany in the next 20 years [4].

Metabolic diseases are complex, stemming from a number of both modifiable and unmodifiable factors that are often intertwined. Several major modifiable risk factors are poor diet, smoking, and inadequate physical activity [6-9]. However, metabolic diseases themselves are also risk factors for the development of cardiovascular disease (CVD), which is a leading cause of morbidity and mortality worldwide (**Figure 1**) [1, 7, 10, 11]. In Europe as a whole, CVD causes over 3.9 million deaths each year, or 45% of all deaths, and it has been estimated that CVD costs the European Union €210 billion a year [3]. In Germany specifically, CVD is estimated to have accounted for 37% of deaths in 2016 [1]. Although age-standardized mortality rates from CVD in Europe have declined over the past few decades, absolute numbers have climbed along with the increasing age of the population, and the burden remains immense [3].

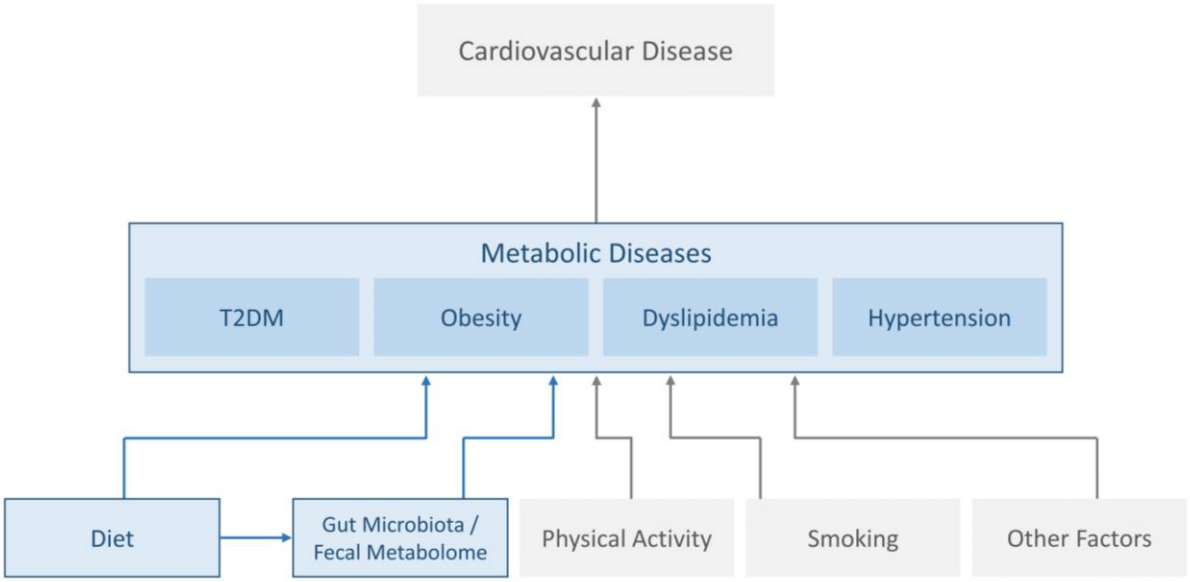

Figure 1: A simplified schema of the relationship between cardiovascular disease, metabolic diseases, and their major risk factors. The metabolic diseases and risk factors that are the focus of this dissertation are highlighted in blue. Arrows indicate the directionality of each relationship. T2DM: Type 2 diabetes mellitus.

It was recently determined that high blood pressure was the top medical risk factor for death due to both CVD worldwide between 1980 and 2010, responsible for at least 40% of these deaths [12]. An additional 15% of these deaths could be attributed to overweight/obesity and elevated blood glucose each, and high serum cholesterol was estimated to be responsible for an additional 10% of CVD and T2DM deaths [12], while another source estimated that altered lipoprotein metabolism represents approximately half of the population-attributable risk of CVD [11]. As a result, interventions that favorably modify major metabolic risk factors at the population level stand to reduce the burden of both metabolic and cardiovascular diseases enormously. However, despite intense research focus and public health efforts in the past decades, the prevalence of metabolic diseases continues to rise, and a deeper understanding of the risk factors implicated in the development of metabolic diseases is essential.

2.2 Nutrition as a major modifiable risk factor

Nutrition is a major modifiable risk factor for both metabolic diseases and CVD. While raised systolic blood pressure is considered the largest medical contributor to CVD mortality across Europe, nutrition is the largest behavioral contributor, responsible for an estimated 49.2% of all CVD deaths [3, 6]. Diet is also one of the most important modifiable risk factors for many metabolic diseases. Along with increased physical activity, nutrition therapy is the cornerstone of prevention and treatment of obesity. Additionally, several dietary factors, most notably sodium and potassium, are involved in the regulation of blood pressure; a low-sodium, high-potassium diet in particular has been shown to be effective in reducing the risk for hypertension [13]. Furthermore, it has been estimated that lifestyle changes, such as weight loss, dietary modifications, and increased physical activity, can provide a 40%-70% reduction in relative risk of the progression from prediabetes to T2DM and may delay or prevent the development of diabetes-related comorbidities [14-18]. Nutrition therapy for T2DM has also been demonstrated to lower hemoglobin A1c (HbA1C) levels by 1-2 HbA1c percentage points on average, as well as resulting in weight loss, improved lipid levels, and lower blood pressure [9, 14]. A number of studies have also confirmed that dietary intervention is an effective non-pharmaceutical strategy for improving serum lipid levels [19, 20].

Suboptimal diet has consistently been associated with the development and progression of metabolic diseases, for example by raising blood glucose levels, leading to the derangement of serum lipid levels, raising blood pressure, or contributing to excess body weight and increased inflammation [8, 20]. In the past, macronutrient distribution was a main cornerstone of dietary recommendations for the prevention and treatment of metabolic diseases. Due to the evidence for a relationship between high saturated fat and cholesterol intake and a higher risk for dyslipidemia and metabolic dysfunction, a low fat, moderate carbohydrate diet was promoted [21, 22]. However, recent studies point to a differential association between dietary fat and metabolic diseases or biomarkers. While the fat content of a diet itself is not consistently related to metabolic diseases/ biomarkers, replacing saturated fat with polyunsaturated fats is often associated with reduced risk, while replacing saturated fats with carbohydrates (especially refined carbohydrates) has no effect or even increases risk [21, 23, 24]. Additionally, although a low-fat, moderate-carbohydrate diet with caloric restriction has also long been recommended for prevention and treatment of metabolic

diseases, a number of recent studies show a low-carbohydrate, high fat diet may be equally effective in managing blood glucose control and weight loss, at least in the short term [25-27].

Further research has also identified a number of foods and nutrients associated with metabolic diseases. In general, diets high in fruits and vegetables, whole grains, unsaturated (especially omega-3) fats, nuts and legumes have consistently been inversely associated with inflammation and metabolic diseases [8, 20, 28, 29]. These foods closely align with dietary patterns such as the Mediterranean diet, DASH diet, or a plant-based diet, which have also shown great promise in preventing and treating metabolic diseases [20, 30-32].

Conversely, a "Western" diet, high in processed foods, red meats, refined grains, excess sodium, saturated fat, alcohol and added sugars, promotes inflammation and has been positively associated with the development of metabolic diseases [8, 20, 33, 34]. As a result, the current focus in nutrition research has shifted away from macronutrient composition alone and has been placed more on individual foods, nutrients, and increasingly, dietary patterns.

It is clear that a full understanding of how individual foods and nutrients, dietary patterns, and the quantity and quality of macronutrients are related to metabolic diseases is crucial to making useful and effective recommendations for public health measures. Because nutrition is strongly associated with metabolic biomarkers and outcomes, it is a major target for behavioral modification and offers huge potential in the prevention and treatment of metabolic diseases. It was recently estimated that nearly half (45.4%) of all cardiometabolic deaths in the United States could be attributed to suboptimal diet, and that suboptimal diet is responsible for over 18% of all T2DM, ischemic heart disease, and stroke costs in the United States, translating to $50.4 billion per year [35, 36]. Consequently, well-informed dietary recommendations (along with public adherence) could translate to a major reduction in metabolic disease-related morbidity, mortality and the associated economic costs.

2.3 The role of the gut microbiota and fecal metabolome in metabolic diseases

The human gut microbiome has become an area of immense research interest in recent decades [37]. It has been associated with various disease states, ranging from obesity to irritable bowel disease to rheumatoid arthritis and T2DM [38-42]. The composition of the gut microbiome is influenced by a number of factors such as age, geography, medications, diet, and even mode of delivery at birth [37, 43, 44]. The gut microbiome plays an integral role in host health and regulating host physiology [45]. The gut microbiota aid in the digestion and absorption of nutrients and produce a wide range of metabolites and even vitamins [46, 47]. Subsequently, the composition and activity of the gut microbiota plays a large role in determining the composition of the fecal metabolome [48].

At the same time, nutrition inevitably plays a major role in shaping gut microbiota composition as the main source of substrate for microbes residing within the gastrointestinal tract [49-52]. A diet rich in certain nutrients selects for microbes that preferentially utilize them as substrates. Additionally, the presence or absence of certain microbes may impact the effect a dietary component exerts on the host. Although nutrition is independently a major risk factor for metabolic diseases, the gut microbiota (and the metabolites it produces) appears to be one way by which diet-disease associations are mediated (**Figure 2**) [53]. Probably the most well studied example of this is the fermentation of dietary fiber in the gut. Dietary fiber is associated with a number of favorable health outcomes, though it is resistant to digestion by the host. While some benefits may be mechanical, such as increased bulk, potentially leading to increased satiety and decreased intestinal transit time, others are a result of short-chain fatty acids (SCFA), mainly butyrate, acetate, and propionate, which are metabolites produced a result of the fermentation of dietary fiber by SCFA-producing bacteria [46]. SCFA aid in maintaining an ideal pH in the colon, maintain barrier function, have anti-cancer properties, and are absorbed into the bloodstream and play a role in regulating host physiology [54].

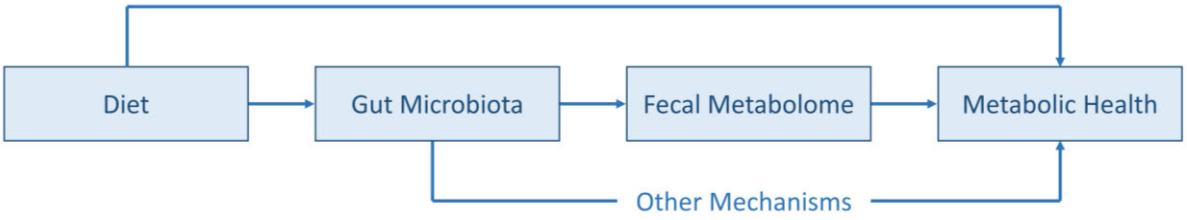

Figure 2: The relationship between diet, the gut microbiome, the fecal metabolome and metabolic health, as examined in this dissertation. Diet affects the composition of the gut microbiota and in turn, the metabolites it produces, many of which affect metabolic health. However, diet has also been independently associated with metabolic health. Arrows indicate the directionality of each relationship.

Another example is the conversion of dietary phosphatidylcholine or L-carnitine to Trimethylamine (TMA) by gut microbiota. TMA is the precursor to Trimethylamine N-oxide (TMAO), which has been associated with the development of atherosclerotic lesions and the risk of CVD, though some studies have been conflicting and research continues in this area [53, 55]. The gut microbiota play an essential role in the conversion of phosphatidylcholine to TMAO, and as a result, the observed associations between TMAO and CVD. Additionally, the gut microbiota has also been associated with metabolic diseases independently of diet. For example, bile acid-modifying gut bacteria play an important role in modulating lipid metabolism by converting primary to secondary bile acids, which act as signaling molecules and which also results in increased excretion and de novo production of bile acids [53, 56, 57]. The de novo production of bile acids depletes the body's cholesterol stores and may therefore lower serum cholesterol levels. Additionally, bile acids modulate lipid metabolism by binding to intestinal and hepatic farnesoid X receptor (FXR) [58].

However, despite intensive research into the gut microbiome and human health in recent years, it is still not clear what a "healthy" microbiota composition is or how to best modify the gut microbiome favorably for disease prevention or treatment [46, 59]. In addition to high inter-individual variability in gut microbiota composition, another complicating factor is functional redundancy, meaning that multiple taxa within an environment may perform similar roles [59]. As a result, the microbiota composition of two individuals could look very different while fulfilling

the same functions. Furthermore, the best strategy to assess microbiota composition in relation to health remains controversial. More and more, researchers are analyzing the fecal metabolome in addition to or even instead of microbiota composition, therefore assessing the functions of an individual's microbiota based on the metabolites it produces, rather than only assessing the composition of the microbes themselves [48]. Still, many unanswered questions remain in this field, and further study into the relationship between diet, the gut microbiome, and metabolic diseases is essential.

2.4 Scientific challenges

While the areas of nutrition, the gut microbiome, and metabolic diseases have been the subject of extensive research, there are many questions that remain unclarified. Associations between diet and metabolic diseases are often conflicting, and dietary recommendations for the prevention and treatment of metabolic diseases have changed distinctly in recent decades [22, 23, 28]. Furthermore, the etiology of metabolic diseases is still not completely understood, despite decades of research in this area. Although it seems clear that dysbiosis of the gut microbiota plays some role in metabolic disease risk, the depth of its role remains poorly understood. Associations between the gut microbiota and metabolic risk factors and disease have been identified in several studies, but associations found between individual taxa and disease states are often conflicting [53, 54]. The degree to which associations between nutrition and metabolic diseases are mediated by the gut microbiome also requires further investigation. Furthermore, the best strategy for assessing disease risk based on gut microbiota composition remains undecided. There are countless methods in use for describing and analyzing gut microbiota composition and its associations with various disease states. These efforts are complicated by the high complexity, sparsity, and of compositionality of microbiome data, as well as by functional redundancy and that taxa can frequently not be reliably identified beyond the genus level due to the methods currently used in many microbiome studies.

Although nutrition and the gut microbiota has been studied extensively, many findings have not yet been confirmed in population-based studies with large sample sizes. The same problem persists for studies on gut microbiota and metabolic diseases. Sufficient studies combining detailed data on habitual diet, gut microbiota composition, and metabolic disease risk in one joint analysis are

also lacking, and even fewer studies analyzing fecal metabolites in this context exist. Although it is clear that these factors are involved in the development of metabolic diseases, the extent to which both the gut microbiome and/or fecal metabolites play a role in modifying the association between diet and metabolic diseases remains unclear.

2.5 Aims and objectives of the Dissertation

The aim of this dissertation is to address the previously specified gaps in the literature by analyzing the role of habitual diet, the gut microbiota, and fecal metabolites in the development of metabolic diseases in a large, well-defined, population-based cohort. The dissertation is based on two first-author publications of the doctoral candidate, both of which have been published in international journals and were peer reviewed. The first publication focused on fecal metabolites and serum lipids, while the second publication investigated both habitual diet and the gut microbiota in the context of metabolic diseases (serum lipids, BMI, waist circumference, hypertension, and T2DM). The first publication aimed to identify a relationship between selected fecal metabolites and biomarkers of dyslipidemia. The aims of second publication were 1) to identify an appropriate method for dimensionality reduction of the available gut microbiota data, 2) to identify differential associations of food items with microbial subgroups and 3) to examine the relationship between microbial subgroups and metabolic diseases.

2.6 Description of the analyses and contribution to the problem at hand

The first publication examined the relationship between fecal metabolites and markers of dyslipidemia in 1,387 participants of the KORA FF4 study, which was conducted in 2013/2014 in the Augsburg region as the second follow-up to the original KORA S4 study. Twenty-five fecal metabolites were selected from over 800 available metabolites based on their previously identified associations with diet and dyslipidemia in the literature. The selected metabolites, which belong to four categories, are given in **Table 1**.

Table 1. Selected fecal metabolites examined in Publication I

Primary Bile Acids	Secondary Bile Acids	Plant Sterols	Animal Sterols
• Cholate • Glycochenodeoxy-cholate • Glycocholate	• 12-dehydrocholate • 3b-hydroxy-5-cholenoic acid • 6-oxolithocholate • 7,12-diketolithocholate • 7-ketodeoxycholate • Dehydrolithocholate • Deoxycholate • Glycodeoxycholate • Glycolithocholate sulfate • Glycoursodeoxycholate • Hyocholate • Lithocholate • Ursocholate • Ursodeoxycholate	• Stigmasterol • Sitostanol • Beta-sitosterol • Ergosterol • Campesterol	• Coprostanol • Cholesterol

Serum LDL-c, HDL-c, total cholesterol and triglycerides were considered as markers of dyslipidemia, and were analyzed both continuously and as binary variables, with cut-offs for the binary variables chosen according to the 2003 National Cholesterol Education Program Adult Treatment Panel (NCEP ATP) III Guidelines as follows [60]:

- LDL-c: ≥ 3.36 mmol/l
- HDL-c: < 1.03 mmol/l in men and < 1.29 mmol/l in women
- Total cholesterol: ≥ 5.17 mmol/l
- Triglycerides: ≥ 1.69 mmol/l

Multivariate linear and logistic regression models were used to assess the relationship between each of the fecal metabolites and serum lipid levels. This publication identified significant associations between fecal bile acids and fecal cholesterol and serum lipids, and contributed to knowledge of how fecal metabolites, particularly bile acids, are related to dyslipidemia.

The second publication addressed the relationship of habitual diet and gut microbiota composition with metabolic diseases. Gut microbiota composition was assessed in 1,992 participants of the KORA FF4 study using 16S rRNA gene sequencing. As one of the aims of the analysis was to identify an appropriate method for dimensionality reduction and as an alternative to classical clustering strategies, the machine learning method Latent Dirichlet Allocation was implemented to identify 20 latent microbial subgroups within the data. A unique combination of the 20 subgroups can be used to describe the composition of each participant's gut microbiome. Habitual diet was evaluated using a combination of repeated 24-hour food lists and one food frequency questionnaire. **Table 2** displays the 22 food groups and subgroups, seven nutrients, and two dietary scores were selected for the analysis. Analyses including dietary data were limited to 1,442 participants. The metabolic diseases or biomarkers that were assessed in this publication were:

- Serum lipids (LDL-c, HDL-c, total cholesterol and triglycerides)
- BMI
- Waist circumference
- Hypertension (normotensive; known, controlled; known, uncontrolled; known, untreated; undetected), and
- T2DM (normal blood glucose tolerance, prediabetes, undetected T2DM, and known T2DM).

Table 2: Dietary factors examined in Publication II

Food Groups and Subgroups			Nutrients	DQS
• Potatoes	• Dairy	• Sweets	• Alcohol	• AHEI
• Vegetables	• Cheese	• Cake	• Total protein	• MDS
• Fruit	• Yogurt	• SSB	• Total fat	
• Legumes	• Whole grains	• Coffee	• Total carbohydrates	
• Nuts/seeds	• Refined grains	• Wine	• Total fiber	
• Plant oil	• Fresh red meat	• Beer	• Soluble fiber	
• Animal fat	• Processed meat		• Insoluble fiber	
• Eggs	• Fish			

AHEI, Alternate Healthy Eating Index; DQS, diet quality scores; MDS, Mediterranean Diet Score; SSB, sugar-sweetened beverages.

Two sets of Dirichlet regression models were used to evaluate associations between the selected variables: one assessing associations between dietary factors and the 20 subgroups, and another assessing the associations between metabolic diseases and the 20 subgroups. This publication was able to confirm existing associations between diet and the gut microbiota, as well as associations between metabolic diseases and the gut microbiota, in a large, population-based study. Additionally, it identified a number of new associations that should be explored further in future studies. Finally, the publication presented a promising method for the assessment of gut microbiota composition in relation to diet and human health, and which holds promise for the identification of subgroups of microbes that are of importance to human health.

3 Summary

Metabolic diseases are a major public health challenge both in Germany and worldwide. With increasing life expectancy and decreasing birth rates, the population of Germany is aging, leading to an increased burden of metabolic diseases. Nutrition is an important modifiable risk factor of metabolic diseases and has been the focus of research and public health interventions for decades. However, official recommendations have been conflicting, and while the basics of a preventative diet seem clear, specific questions remain unanswered and the mechanisms behind diet-disease associations often remain uncertain. The gut microbiome and metabolome have emerged as a major focus of research in recent decades as it becomes clear that dysbiosis of the gut microbita is associated with a wide range of human diseases. However, exactly how important the composition and function of the gut microbiota and its metabolites are to metabolic health remains elusive, and many questions still remain. Furthermore, many previously identified associations have not been validated in large cohort studies.

This dissertation, which encompasses two publications, aims to gain new insight into the complicated relationship between habitual diet, the gut microbiome, and metabolic diseases, while also confirming previously identified associations in a large, population-based study. The analyses from both publications included in the dissertation use data from the Cooperative Health Research in the Region of Augsburg (KORA FF4) study, conducted in 2013/2014, in which 2,279 participants of the original S4 study (1999-2001, n=4,261) returned for a second follow-up survey.

The first publication investigated the relationship between selected fecal metabolites and serum lipid levels in 1,387 participants of the KORA FF4 study. Metabolite concentrations were measured and identified by Metabolon Inc (Durham, NC, USA). Plant and animal sterols, as well as primary and secondary bile acids were selected from the set of 807 metabolites. Serum LDL-cholesterol, HDL-cholesterol, total cholesterol, and triglycerides were selected as outcome variables to represent dyslipidemia. All four variables were analyzed both as continuous variables and as binary variables with cutoffs for the binary variables chosen according to the 2003 National Cholesterol Education Program Adult Treatment Panel III Guidelines. Multivariate linear regression models were utilized to assess associations between 25 metabolites (and the sums of the four categories) and serum lipid variables. Multivariate logistic regression models were applied

to assess associations between fecal metabolites and the four binary outcome variables. In this publication, most primary and secondary bile acids, as well as some plant sterols and fecal coprostanol and cholesterol were found to be significantly positively associated with serum lipid levels (especially serum triglycerides and hypertriglyceridemia). These results indicated that fecal bile acids may play a role in the development or progression of dyslipidemia, and suggest that this is an important area for future research.

The second publication examined the relationship between habitual diet, gut microbiota composition, and metabolic diseases or biomarkers (BMI; waist circumference; serum LDL-c, HDL-c, total cholesterol, and triglycerides; T2DM; and hypertension) in the KORA FF4 study. Fecal microbiota composition was assessed using 16S rRNA gene amplicon sequencing, and data were available for 1,992 participants. Habitual dietary intake (22 food groups or subgroups, seven nutrients and two diet quality scores) was evaluated using repeated 24-hour food lists and one food frequency questionnaire. Dietary data was available for a subset of 1,442 of these individuals. Latent Dirichlet Allocation (LDA) was applied to learn 20 latent microbial subgroups within the data set, and multivariate Dirichlet regression models were used to identify associations between habitual diet or metabolic diseases/biomarkers and the 20 subgroups. Eight of the 20 subgroups were significantly associated with one or more dietary factors, and nine of the 20 subgroups were significantly associated with one or more metabolic disease or biomarker. Participants with a higher intake of foods such as fruits and whole grains were characterized with a higher probability for subgroups 5 and 14 in particular, while participants with T2DM had a lower probability for subgroups 5 and 14. This publication identified new subgroups of bacteria that may be relevant for metabolic diseases, and which present avenues for further research. Additionally, LDA was demonstrated as a powerful and effective tool for the identification of latent subgroups within microbiome data, with high potential for use in exploratory studies.

The publications comprising this dissertation expand upon current knowledge of the relationship between diet, the gut microbiome and metabolome, and metabolic diseases. The cross-sectional nature of the analyses do not allow causal associations to be drawn, but were able to confirm previously-identified associations in a large cohort, demonstrate the application of a promising new machine learning method in the field, and identified important areas of study for future research.

4 Zusammenfassung

Stoffwechselerkrankungen sind sowohl in Deutschland als auch weltweit eine große Herausforderung für die öffentliche Gesundheit. Mit steigender Lebenserwartung und sinkenden Geburtenraten altert die Bevölkerung in Deutschland, was zu einem erhöhten Auftreten von metabolischen Erkrankungen führt. Die Ernährung ist ein wichtiger modifizierbarer Risikofaktor für Stoffwechselerkrankungen und steht seit Jahrzehnten im Fokus von Forschung und öffentlichen Gesundheitsmaßnahmen. Allerdings haben sich die offiziellen Empfehlungen über die Jahre hinweg geändert, und während die positiven Effekte einer präventiven gesundheitsförderlichen Ernährung klar zu sein scheinen, bleiben spezifische Fragen, zum Beispiel die Mechanismen hinter den Assoziationen zwischen Ernährung und Krankheiten, oft unbeantwortet. Das Darmmikrobiom und das Metabolom haben sich in den letzten Jahrzehnten zu einem wichtigen Forschungsschwerpunkt entwickelt, da deutlich wird, dass eine Dysbiose des Darmmikrobioms mit einer Reihe von menschlichen Krankheiten in Verbindung gebracht werden kann. Wie wichtig die Zusammensetzung und Funktion der Darmmikrobiota und ihrer Stoffwechselprodukte für die Stoffwechselgesundheit sind, ist jedoch nach wie vor nicht genau bekannt. Darüber hinaus sind viele zuvor identifizierte Assoziationen noch nicht in großen Kohortenstudien validiert worden.

Diese Dissertation, die zwei begutachtete Publikationen umfasst, zielt darauf ab, neue Erkenntnisse über den komplexen Zusammenhang zwischen der gewöhnlichen Ernährung, dem Darmmikrobiom und Stoffwechselerkrankungen zu gewinnen und gleichzeitig zuvor identifizierte Assoziationen in einer großen, bevölkerungsbasierten Studie zu bestätigen. Die Analysen der beiden in der Dissertation enthaltenen Publikationen nutzen Daten der 2013/2014 durchgeführten Studie Kooperative Gesundheitsforschung in der Region Augsburg (KORA FF4), bei der 2.279 Teilnehmer der ursprünglichen S4-Studie (1999-2001, n=4.261) an einer zweiten Nachbefragung teilnahmen.

In der ersten Publikation wurde der Zusammenhang zwischen ausgewählten Stuhl-Metaboliten und Serumlipidspiegeln bei 1.387 Teilnehmern der KORA FF4-Studie untersucht. Die Metaboliten-Konzentrationen wurden von Metabolon Inc (Durham, NC, USA) gemessen und identifiziert. Aus dem Set von 807 Metaboliten wurden pflanzliche und tierische Sterole sowie

primäre und sekundäre Gallensäuren ausgewählt. Der Outcome Dyslipidämie wurde mit Hilfe von Serum-LDL-Cholesterin, HDL-Cholesterin, Gesamtcholesterin und Triglyceriden dargestellt. Alle vier Variablen wurden sowohl als kontinuierliche Variablen als auch als binäre Variablen analysiert, wobei die Cutoffs für die binären Variablen gemäß den 2003 National Cholesterol Education Program Adult Treatment Panel III Guidelines gewählt wurden. Multivariate lineare Regressionsmodelle wurden verwendet, um Assoziationen zwischen 25 Metaboliten (und den Summen der Kategorien pflanzliche und tierische Sterole sowie primäre und sekundäre Gallensäuren) und Serumlipidvariablen zu identifizeiren. Multivariate logistische Regressionsmodelle wurden angewandt, um Zusammenhänge zwischen Stuhl-Metaboliten und den vier binären Ergebnisvariablen zu modellieren. In dieser Publikation wurde festgestellt, dass sowohl die Mehrheit der primären und sekundären Gallensäuren als auch manche pflanzliche Sterole, Coprostanol und Cholesterin signifikant positiv mit den Serumlipidwerten (insbesondere Serumtriglyceride und Hypertriglyceridämie) assoziiert sind. Diese Ergebnisse deuten darauf hin, dass diese Gallensäuren eine Rolle bei der Entwicklung oder dem Fortschreiten von Dyslipidämie spielen können und legen nahe, dass dies ein wichtiges Gebiet für zukünftige Forschung ist.

Die zweite Publikation untersuchte den Zusammenhang zwischen dem gewöhnlichen Verzehr, der Zusammensetzung der Darmmikrobiota und Stoffwechselerkrankungen oder Biomarkern (BMI, Taillenumfang, Serum-LDL-c, HDL-c, Gesamtcholesterin und Triglyceride, T2DM und Bluthochdruck) in der KORA FF4-Studie. Die Zusammensetzung der Stuhl-Mikrobiota wurde mittels 16S rRNA-Gen-Amplikon-Sequenzierung bestimmt, und Daten waren für 1.992 Teilnehmer verfügbar. Die gewöhnliche Verzehr (29 Lebensmittelgruppen oder Nährstoffe und zwei Ernährungsqualitäts-Scores) wurde anhand von wiederholten 24-Stunden-Nahrungsmittellisten und einem Fragebogen zur Verzehrshäufigkeit von Nahrungsmitteln bewertet. Ernährungsdaten waren für eine Subgruppe von 1.442 dieser Personen verfügbar. Latent Dirichlet Allocation (LDA) wurde angewandt, um 20 latente mikrobielle Subgruppen innerhalb des Datensatzes zu identifizieren, und multivariate Dirichlet-Regressionsmodelle wurden verwendet, um Assoziationen zwischen dem gewöhnlichen Verzehr oder Stoffwechselerkrankungen oder Biomarkern und den 20 Subgruppen zu identifizieren. Acht der 20 Subgruppen waren signifikant mit einem oder mehreren Ernährungsfaktoren assoziiert, und neun der 20 Subgruppen waren signifikant mit einer oder mehreren Stoffwechselerkrankungen oder Biomarkern assoziiert. Teilnehmer mit einem höheren Verzehr von Lebensmitteln wie Obst

und Vollkornprodukten wurden mit einer höheren Wahrscheinlichkeit insbesondere für die Subgruppen 5 und 14 charakterisiert, während Teilnehmer mit T2DM eine geringere Wahrscheinlichkeit für die Subgruppen 5 und 14 hatten. Diese Publikation identifizierte neue Subgruppen von Bakterien, die für Stoffwechselerkrankungen relevant sein könnten und die Möglichkeiten für weitere Forschung eröffnen. Darüber hinaus wurde gezeigt, dass LDA ein leistungsfähiges und effektives Werkzeug für die Identifizierung latenter Subgruppen innerhalb von Mikrobiomdaten ist, mit hohem Potenzial für den Einsatz in explorativen Studien.

Die in dieser Dissertation enthaltenen Publikationen erweitern den aktuellen Wissensstand über die Zusammenhänge zwischen Ernährung, Darmmikrobiom und Metabolom sowie Stoffwechselerkrankungen. Der Querschnittscharakter der Analysen erlaubt es nicht, kausale Schlussfolgerungen zu ziehen, konnte aber zuvor identifizierte Assoziationen in einer großen Kohorte bestätigen. Die Anwendung einer vielversprechenden neuen Methode des maschinellen Lernens auf diesem Gebiet wurde so demonstriert und wichtige Untersuchungsbereiche für zukünftige Forschung identifiziert.

5 Publication I: Associations between fecal bile acids, neutral sterols, and serum lipids in the KORA FF4 study

[Breuninger TA, Wawro N, Meisinger C, Artati A, Adamski J, Peters A, Grallert H, Linseisen J. Associations between fecal bile acids, neutral sterols, and serum lipids in the KORA FF4 study. Atherosclerosis 2019, 288:1-8. doi: https://doi.org/10.1016/j.atherosclerosis.2019.06.911]

6 Publication II: Associations between habitual diet, metabolic diseases, and the gut microbiota using Latent Dirichlet Allocation

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