Aus dem Institut für Sozialmedizin, Epidemiologie und Gesundheitsökonomie
der Medizinischen Fakultät Charité – Universitätsmedizin Berlin
und
Bundesinstitut für Risikobewertung, Berlin

DISSERTATION

A vegan diet and gut health - associations with microbiota and fecal bile acid concentrations
Vegane Ernährung und Darmgesundheit – Zusammenhänge mit Mikrobiota und Gallensäurekonzentrationen im Stuhl

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von

Iris Trefflich

aus Bergisch Gladbach

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1. Abstract

A vegan diet is often supposed to be rich in dietary fiber and low in fat intake compared to an omnivorous diet. Following a vegan diet is discussed with health benefits, such as a low risk of the onset of obesity or for total cancer. A diet also plays a major role in microbiota composition, which may impact the health status. Bile acids are strongly related to diet, in particular to fat and meat intake as they mediate fat digestion. Some bacteria of the microbiota convert primary bile acids into secondary bile acids, which are discussed to increase the risk of colorectal carcinoma. So far, only few studies have investigated the differences in microbiota and fecal bile acids in a meatless diet compared to an omnivorous diet.
First, this cumulative thesis compared the microbiota composition of vegans and vegetarians with omnivores in a systematic review. In the second publication, fecal and serum bile acids of vegans were compared with omnivores. A dietary pattern should identify food groups contributing most to fecal bile acid concentrations.

Both publications were conducted with data from the cross-sectional study “Risks and benefits of a vegan diet” (RBVD) with 36 vegans and 36 omnivores. In RBVD, the diet was assessed with 3-day weighing protocols. Each participant provided a complete stool sample for bile acids and microbiota analyses. The dietary pattern was derived by reduced rank regression with food groups as predictor and fecal bile acid concentrations as response variables. In the systematic review, a literature research was conducted in PubMed and Embase. Studies that compare the microbiota composition of healthy vegans or vegetarians with omnivores were selected. Furthermore, the RBVD results were considered in this review.

In the systematic review, 16 studies were included. The number of significant differences in bacterial abundances in vegans or vegetarians compared to omnivores was small.
In RBVD, fecal secondary and conjugated bile acids were significantly lower in vegans than in omnivores ($p<0.01$). The derived dietary pattern can be described as a fatty om-

nivorous pattern, and it was associated with increasing bile acids across the score. Processed meat, fried potatoes, fish, margarine and coffee correlated positively with higher bile acid concentration, whereas muesli was inversely associated.

The systematic review could not reveal a “vegan” or “vegetarian” microbiota composition, which may be attributed to the high individuality of microbiota and different analyzing methods. Lower concentrations of fecal secondary bile acids in vegans compared to omnivores could refer to a more favorable risk profile towards the development of a colorectal carcinoma. Yet, the cross-link of diet, microbiota and bile acid metabolism is not fully understood, so further research is needed.

2. Zusammenfassung

Eine vegane Diät ist häufig reicher an Ballaststoffen und ärmer an Fett als eine Mischkost und wird mit gesundheitlichen Vorteilen, wie z.B. einem geringeren Risiko für Adipositas oder Krebs diskutiert. Ernährung beeinflusst auch die Zusammensetzung der Mikrobiota, welche auf den Gesundheitsstatus wirken kann. Der Gallensäuren Stoffwechsel steht eng im Zusammenhang mit der Ernährung, insbesondere mit Fett- und Fleischverzehr. Einige Bakterien der Mikrobiota wandeln primäre Gallensäuren in sekundäre Gallensäuren um, die ein Risiko für die Entstehung eines Kolonkarzinoms darstellen können. Unterschiede in der Mikrobiota und fäkalen Gallensäuren bei einer fleischfreien Ernährung im Vergleich zu Mischkost sind bisher nur wenig untersucht.
Für diese kumulative Promotion wurde zunächst in einem systematischen Review die Mikrobiota von Veganern und Vegetariern mit der von Mischköstlern verglichen. In der zweiten Publikation wurden Assoziationen zwischen veganer Ernährung und Gallensäuren in Stuhl und Serum im Vergleich zu Mischkost untersucht. Über die Herleitung von Ernährungsmustern wurden Lebensmittel identifiziert, die den größten Einfluss auf fäkale Gallensäuren haben könnten.

Für beide Publikationen wurden Daten der Querschnittstudie “Risiken und Benefits einer veganen Diät” (RBVD) mit je 36 Veganern und Mischköstlern genutzt. Die Ernährung wurde mit 3-Tage-Wiegeprotokollen erhoben. Jeder Teilnehmer gab eine Stuhlprobe für die Analyse der Mikrobiota und der Gallensäuren ab. Ernährungsmuster wurden mittels Reduced Rank Regression hergeleitet mit Lebensmitteln als Prädiktoren und Gallensäuren als Responder. Für den systematischen Review wurde eine Literaturrecherche in PubMed und Embase durchgeführt. Es wurden Studien ausgewählt, welche die Zusammensetzung der Mikrobiota von Veganern bzw. Vegetariern mit der von Mischköstlern verglichen. Im Review wurden Ergebnisse aus der RBVD Studie berücksichtigt.

In dem systematischen Review wurden 16 Studien aufgenommen. Die Zahl der signifikanten Unterschiede an Bakterien zwischen Veganern bzw. Vegetariern im Vergleich zu Mischköstlern war gering.
Fäkale sekundäre Gallensäuren waren bei Veganern niedriger als bei Mischköstlern ($p<0.01$). Das hergeleitete Ernährungsmuster ging mit steigenden Konzentrationen aller

fäkalen Gallensäuren einher und kann als fettreiches Mischkost-Muster beschrieben werden. Verarbeitetes Fleisch, frittierte Kartoffeln, Fisch, Margarine und Kaffee waren positiv, Müsli war dagegen invers assoziiert.

In dem systematischen Review konnte keine „vegane“ oder „vegetarische“ Mikrobiota identifiziert werden. Eine hohe Interindividualität der Mikrobiota und verschiedene Auswertungsmethoden könnten dazu beigetragen haben. Niedrige sekundäre Gallensäuren bei Veganern im Stuhl könnten im Vergleich mit Mischköstlern auf ein günstigeres Risikoprofil hinsichtlich der Entstehung eines Kolonkarzinoms hinweisen. Das Zusammenspiel von Diät, Mikrobiota und Gallensäure-Stoffwechsel bedarf weiterer Forschung.

3. Introduction and objectives

3.1 Plant-based diets and impact on health

Given the increasing amount of vegan cooking books, newspapers and magazines, as well as vegan options in restaurants or supermarkets, it could be assumed that the majority of the German population follows a vegan diet. A vegan diet, also called a strict vegetarian diet is defined as a diet without meat, poultry, fish as well as dairies and eggs [1]. Differentiated from this type of diet, are pescetarian and lacto-ovo-vegetarian diets, which include either fish or milk products or eggs [1] (Table 1). There has been an increasing interest in plant-based diets, which included vegetarian and vegan diets, especially in Western countries in recent years. Though reliable evaluations about the frequency of veganism are rare, estimations suggest that 0.1 - 1 % of the German population follows a vegan diet [2].

In a qualitative data survey, 42 vegans from Berlin and Brandenburg were interviewed about their motivations for following a vegan diet [3]. The answers of the interviewed persons revealed that veganism is more than a diet and is linked with lifestyle. The main motivations for veganism were ethical reasons and animal protection. Health reasons or ecological aspects played a minor role. In this survey, vegans were predominantly female and characterized by a higher level of education. An already existing vegetarian diet and a vegan social surrounding, favored the decision for following a vegan lifestyle [3].

Table 1: Definition of different types of plant-based diets

Type of diet	Meat	Poultry	Fish	Dairy	Eggs
Omnivorous	✓	✓	✓	✓	✓
Pescetarian	×	×	✓	✓	✓
Ovo-vegetarian	×	×	×	×	✓
Lacto-vegetarian	×	×	×	✓	×
Ovo-lacto-vegetarian	×	×	×	✓	✓
Vegan	×	×	×	×	×

Categorization of diets based on [1].

A vegan diet is linked to several health benefits. The increased consumed quantities of vegetables and fruits in a plant-based diet are associated with lower blood cholesterol levels and a decreased risk of heart diseases [4]. Additionally, the consumption of grains and cereals is supposed to be higher in a vegan diet compared to an omnivorous diet.

Due to the high amount of vegetables and fruits in a vegan diet, the intakes of vitamin C, E, magnesium and secondary plant compounds are higher compared to an omnivorous diet [2, 4]. Moreover, vegans have higher intakes of fiber, magnesium and potassium than omnivores [2]. Furthermore, due to the omission of dairy and food with animal origin the fat intake is lower in a vegan diet than in omnivorous diets [4]. Results of the Adventist Health study, a religious community in the United States of America which emphasize healthy lifestyle with restricted alcohol, cigarettes, and meat consumption, described positive effects of a vegetarian and vegan diet, towards the onset of adiposity or type-2 diabetes [1]. A comprehensive meta-analysis including 28 studies in vegan and 96 studies in vegetarian populations demonstrated, that a vegetarian diet is associated with a decreased risk of ischemic heart disease and a vegan diet is associated with a decreased risk of cancer of any site [5].

Nevertheless, the waiver of any animal-based food items could cause an inadequate supply of nutrients, especially for vulnerable populations like pregnant and breastfeeding women, children, and adolescents [2]. Therefore, the German Society of Nutrition (DGE) took position to the risks of a vegan diet [2]. The most critical nutrient in a vegan diet is vitamin B12, which occurs only in food with animal origin. Low vitamin B12 levels in serum are associated with neurological disorders and anemia and subordinated with elevated plasma levels of homocysteine [2, 4]. A long-term supplementation with vitamin B12 is therefore recommended when following a vegan diet [2]. Due to the lack of dairies as sources for calcium, a vegan diet is discussed as unfavorable regarding bone health. Other critical nutrients in a vegan diet are the trace elements zinc, selenium and iodine [2, 4]. Consequently, a targeted selection of food items which are rich in these nutrients is recommended for vegans.

Despite the increasing interest in vegan nutrition and the discussions about the risks and benefits of this type of diet, the proportion of vegans in large cohort studies is small and data about nutritional status of vegans are rare [6]. For Germany, studies that examine dietary intake, health status and lifestyle of healthy and adult vegans are out of date. The cross-sectional “Gießener Vegetarier-Studie” was conducted in 1983 and of the 588 participants, approximately 80 % were lacto-ovo vegetarian and 9.3 % were vegan [7]. Dietary intakes of vegan and vegetarian populations were pooled together and published as vegetarian, so that based on the available data, unambiguous statements about a vegan population are difficult to make. Almost twenty years later, the cross-sectional “German

Vegan Study" (GVS) was conducted in 98 vegan and 56 moderate vegans [8]. Moderate vegans were defined by a consumption of eggs and diary less than 5 % of total energy intake [8] and were more adherent to a vegetarian diet from today´s perspective. Until now, no nationwide and representative study focusing on diet in an adult vegan population has been conducted in Germany.

3.2 Human gut microbiota

The composition of all living microorganisms across the human body is summarized as "microbiota", with the most microorganisms living in the gut system [9]. The microbiota, an ecosystem itself, consists mostly of bacteria alongside viruses, fungi or protozoans with a commensal relationship for the host [9, 10]. The amount of bacteria varies from their location in the intestine, from $10^1 – 10^3$ cfu/ml in the duodenum and ileum to $10^{11} – 10^{12}$ cfu/ml in the colon [11]. Analyses of stool samples of healthy subjects revealed 11,831 different bacterial RNAs and 395 different bacterial phylotypes, whereas the most frequent phyla are Firmicutes and Bacteroidetes, Actinobacteria, Verrucomicrobia, and Proteobacteria [12]. The hierarchical classification of bacteria within the taxonomic ranks is presented in figure 1.

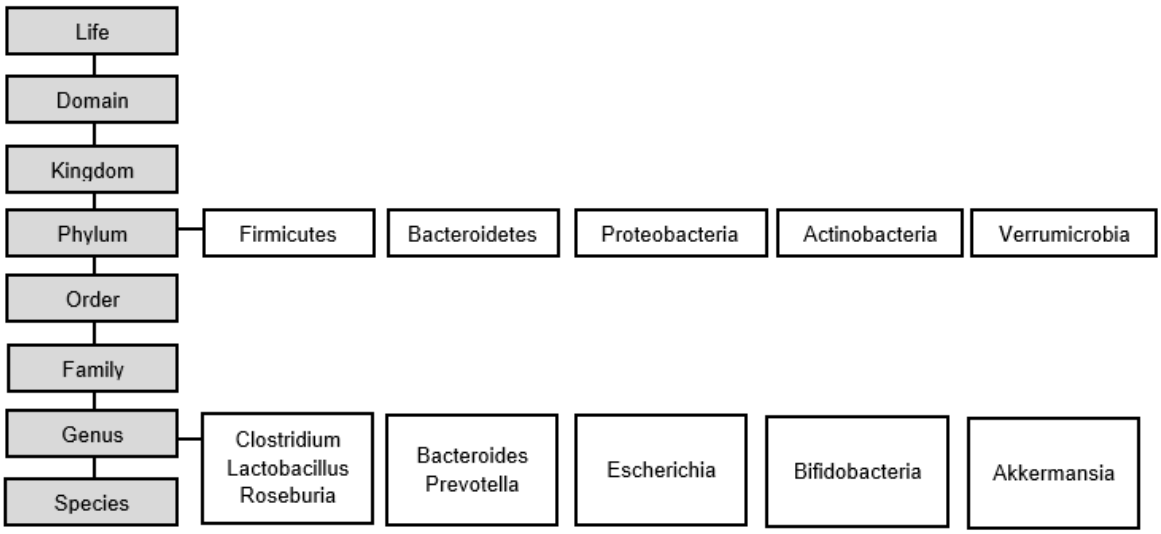

Figure 1: Classification of bacteria in the taxonomic ranks
Examples of most abundant phyla and genera in human gut microbiota are presented in white boxes [9, 12].

The variability and diversity of the microbiota composition is high across geographic areas and racial origins, nevertheless a core of abundant species exists worldwide in humans

[10, 13]. The normal microbiota is attributed to be beneficial to human health and is involved in nutrient metabolism; it is assumed to have protective functions, such as displacement of pathogens, and has structural functions as an intestinal barrier [9].

The composition of the microbiota is influenced by many factors (Figure 2); such as the mode of delivery which is seen as an early colonization factor, or the intake of antibiotics [9]. Over a lifetime, diet is supposed to be a strong contributor to microbiota composition [14, 15].

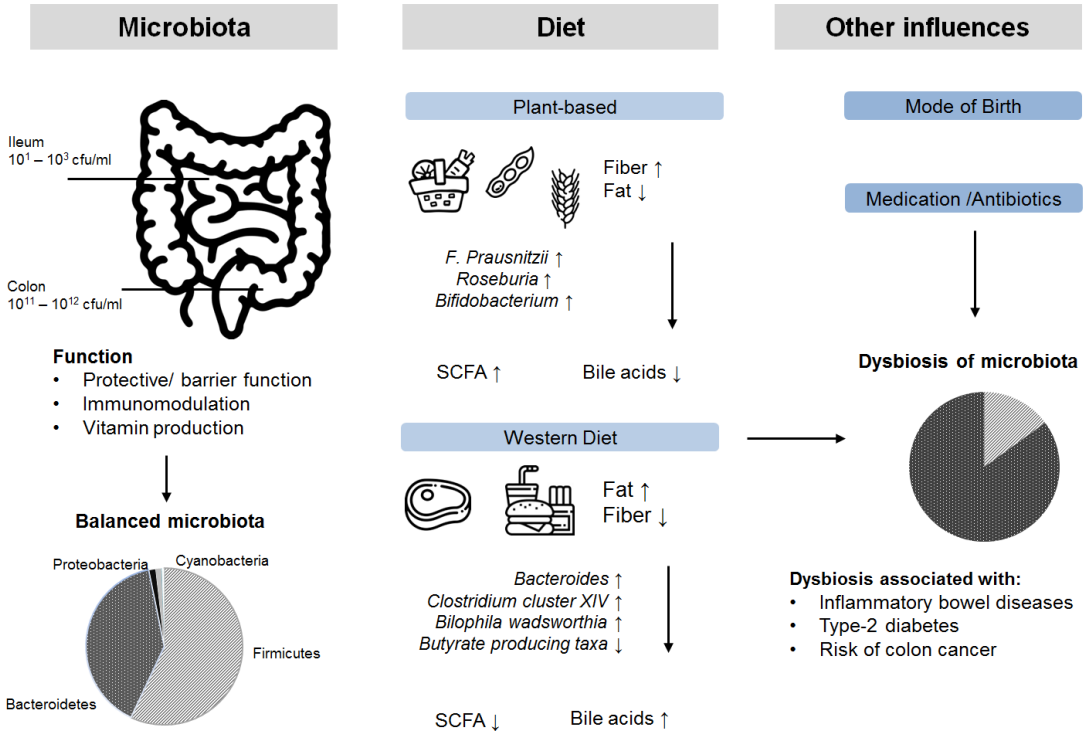

Figure 2: Role of microbiota and impact of diet on microbiota composition
Constitution and function of the microbiota. Diet, mode of delivery, and medications are supposed to influence microbiota colonization most [9, 11, 16-18].

Diets with high proportion of fiber and low in fat, as it occur in diets with high amounts of plant-based food or in populations with original and traditional way of life, are associated with a higher bacterial richness and diversity than Western diets [10, 15]. For example, dietary fiber can be fermented by bacteria of the microbiota into short chain fatty acids (SCFA), which are energy substrate for colonocytes [14, 16]. Bacteria such as *Roseburia spp.*, *Faecalibacterium prausnitzii*, *Ruminococcus spp.* or *Prevotella spp.* metabolize fiber and produce SCFA [16]. An often-cited cross-sectional study compared the microbiota composition in children from Burkina Faso with children from Italy. Children from Burkina

Faso, following a fiber-rich diet, showed significant higher abundances of Bacteroidetes and lower Firmicutes than Italian children [19]. After a food-exchange intervention, the impact on microbiota activity was larger in the animal-based diet group than in the plant-based diet group. Moreover, in the animal-based diet fiber degrading bacteria decreased compared to the plan-based diet [17].

3.3 Bile acids

Bile acid metabolism is also suggested to be strongly related to diet, in particular to dietary fat and meat intake [20, 21]. In traditional diets and plant-based diets with low or any meat intake, fecal bile acid concentrations were lower than in Western diets [22, 23].
The primary bile acids cholic acid (CA) and chenodeoxycholic acid (CDCA) are synthesized from cholesterol in the liver. To increase their solubility, CA and CDCA are conjugated with taurine or glycine and released out of the bile post-prandial [20, 21]. Bile acids are emulsifier and are involved in lipid digestion, as they support absorption of dietary cholesterol, triglycerides, and fat-soluble vitamins [20].

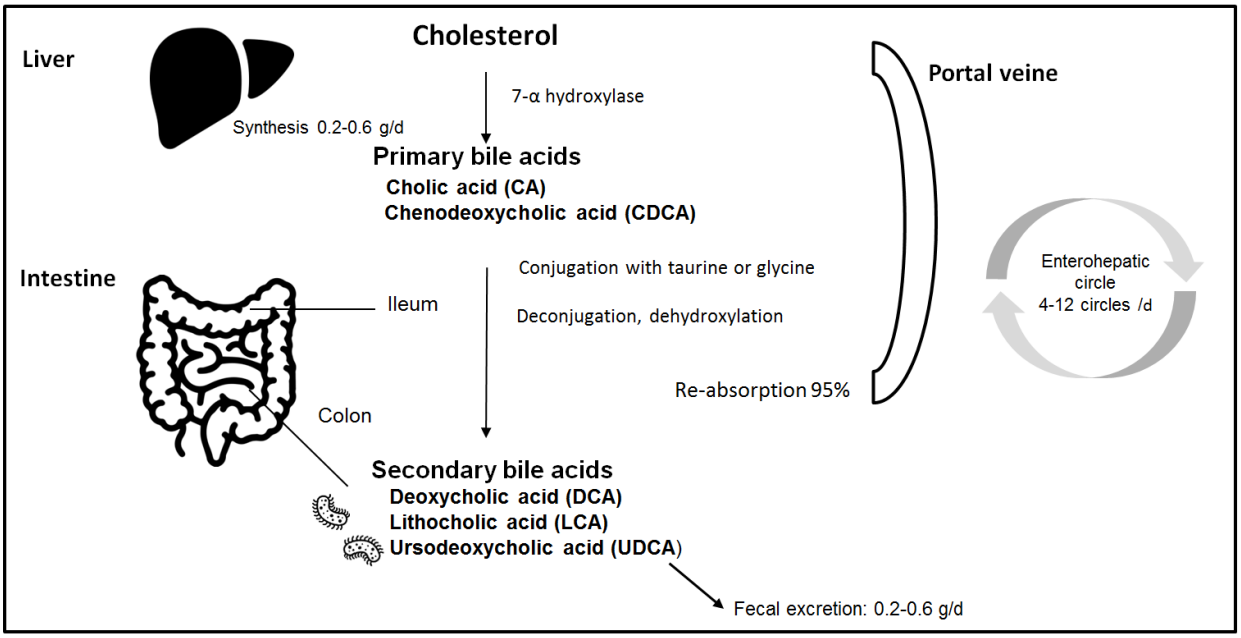

Figure 3: Synthesis and metabolism of primary and secondary bile acids
Adapted from DiCiaula et al. [21]

In the ileum, 95 % of the bile acids are reabsorbed and transported back to the liver to lead back into the enterohepatic circulation [20, 21]. Gut bacteria such as *Clostridium cluster XIVa* and *Bilophila wadsworthia* are involved in converting the remaining primary bile acids into the secondary bile acids deoxycholic acid (DCA), lithocholic acid (LCA),

and ursodeoxycholic acid (UDCA) [18]. Approximately 5 % of the bile acids are excreted via feces (Figure 3). Bile acids act as signaling molecules and can modify gene expressions, which are involved in cell proliferation or apoptosis [18, 20]. In this context, elevated bile acid concentrations may be associated with an increased risk of colorectal cancer [20, 24].

3.4 Dietary pattern

General diets consist of combinations of different food groups and nutrients which interact with each other [20]. In contrast to a single nutrient approach, a dietary pattern analysis may offer a better perception of nutrient interaction and a health or disease outcome [20, 25]. Dietary pattern can be either defined with a priori approaches, where previous to the study available data and evidences are used [25]. Examples for this “a priori” approach are dietary guidelines or health indices, based on scientific evidence of diet-disease relationships, but not on obtained study data [25, 26]. The second approach “a posteriori” is explorative, where study data on food and nutrient intakes are modelled statistically to derive a dietary pattern [26, 27]. Examples for this approach are principal component analysis and cluster analyses, where prior knowledge about disease relationship is not taken into account [26]. The established statistical method of reduced rank regression (RRR) combines both approaches and has been applied in nutritional epidemiology [10, 27]. Dietary patterns are derived explorative and based on current knowledge, which link diet to health outcomes [27]. In nutritional epidemiology, RRR explains linear combinations of food groups or food items (used as predictor variables) with a maximum of possible variation in disease-related nutrients or biomarkers (used as response variables) [20, 26, 27].

3.5 Objectives

Despite the increasing interest in a vegan diet, hardly any study has been conducted in an adult vegan population in Germany in recent times. The available data came from the 1980s [7] and the 1990s [8] and may not reflect the dietary behavior of today’s vegans. To receive up-to-date information about dietary behavior, macro- and micro nutritional intakes of vegans, a cross-sectional study of 36 vegans and 36 omnivores as controls was conducted at the research unit *Risks of Subpopulations and Human studies* at the Federal Institute for Risk Assessment (BfR), Berlin. Aim of this study was to identify health

risks and benefits of a vegan diet ("*Risks and Benefits of a Vegan Diet study*", RBVD). Based on the data of RBVD, several research topics like nutrient status [28], bone health, inflammation markers [29] and exposition to contaminants associated with a vegan diet are currently investigated by our research unit.
The cross-link between diet, gut microbiota, and health status, is of increasing interest and importance in science. Vegan and vegetarian diets, supposed to be rich in fiber and low in fat, are discussed with an altered microbiota composition compared to a "Western" omnivorous diet, rather rich in fat. An altered microbiota and changes in the bacterial metabolism might also result in changes in bacterial metabolites, such as secondary bile acids.

This thesis aimed to investigate microbiota composition and concentrations of secondary bile acids as markers of gut health in vegans and omnivores. Some of the results of this thesis are included and in the first [10] and second publication [20]. The aim of the first publication was to summarize current evidence on associations of a vegan or vegetarian diet with microbiota composition compared with an omnivorous diet in a systematic review [10]. The second publication [20] aimed to compare bile acid concentrations of vegans and omnivores in stool and also serum. Furthermore, a dietary pattern related to stool bile acid concentrations was derived [20].

4. Methods

This thesis is based on several underlying methods, which enabled the preparation of the two publications [10, 20].

4.1 RBVD study

4.1.1 Study population and study design

Study participants were recruited by adverts in supermarkets, cafés and restaurants in Berlin from January to July 2017 [10, 20]. Participants should be between 30 and 60 years old and should be vegan or omnivorous respectively for at least one year (Figure 4). Omnivorous participants should consume at least 3 portions meat per week or at least 2

portions of meat and 2 portions of processed meat [20]. Persons with BMI ≥ 30, prevalent serious diseases such as cardiovascular disease or cancer, a current acute infection, taking of glucocorticoids or proton pump inhibitors were not included in the study [10]. Pregnant and breastfeeding women were excluded, too. In total, 36 vegans and 36 omnivores (sex- and age-matched, 5-year strata) were included into the final study population [10]. All participants were invited twice to the study center [10, 20]. The cross-sectional study was approved by the ethics committee of the Charité – Universitätsmedizin Berlin (No. EA4/121/16) and was conducted at the BfR in Berlin, Germany [10, 20].

4.1.2 Assessment of anthropometry and lifestyle characteristics

Body weight, fat and muscle mass and the level of visceral fat were measured with the bio-impedance scale BF511 by study personnel at the second study visit [10]. Body height was measured in a standing position with the portable stadiometer Seca 213 and hip and waist circumferences were taken with a measurement tape with participants wearing no outerwear and shoes [10]. Physical activity, educational level, smoking habits, alcohol consumption and medical history were assessed by using computer-based questionnaires [10, 20].

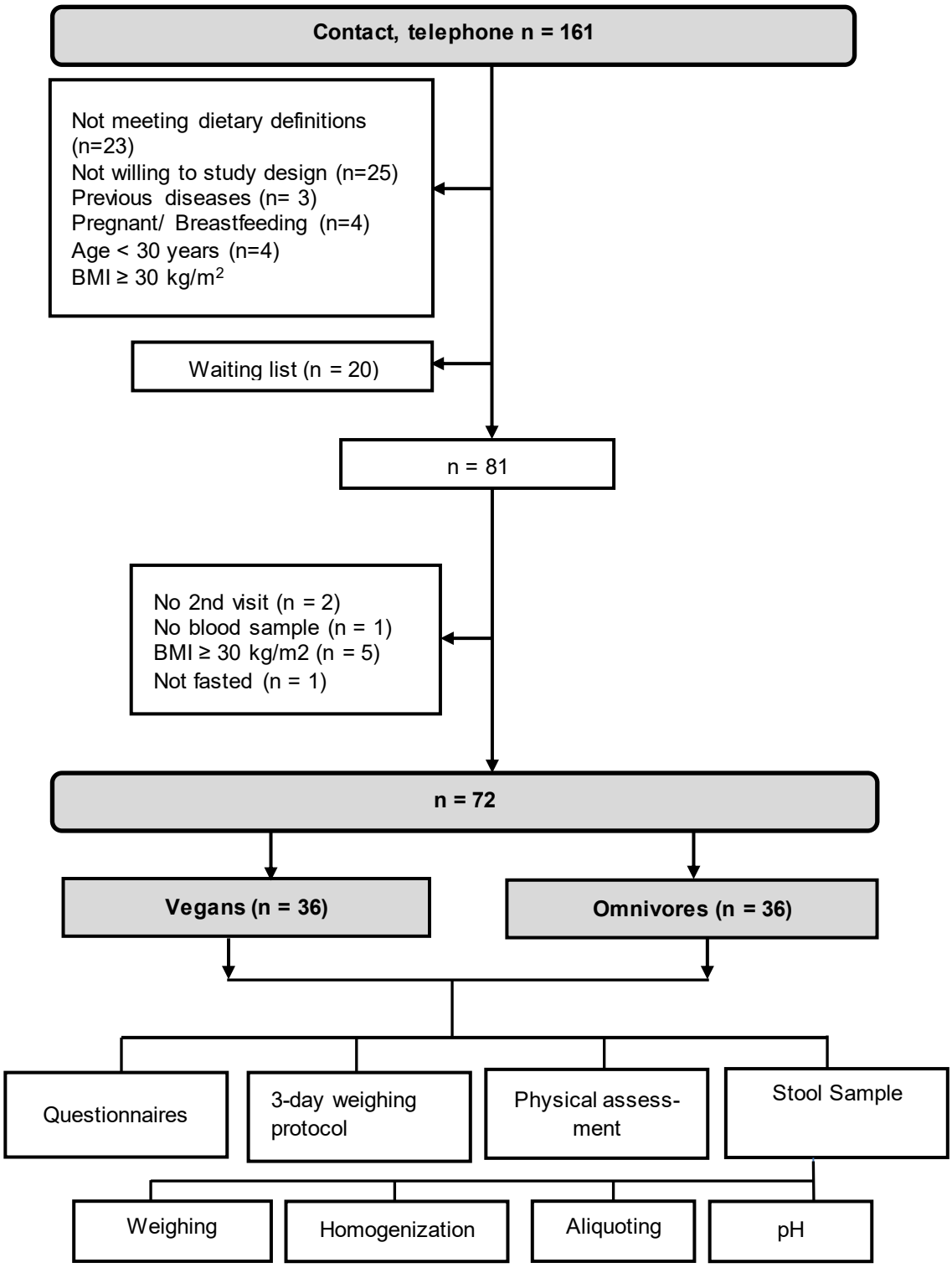

Figure 4: Study flowchart and design of the "Risk and Benefits of a Vegan Diet" study
Figure amended from Trefflich et al. [20] and Weikert et al. [28].

4.1.3 Processing of stool samples

All participants were asked to collect a complete stool at home in the morning of the second examination day and brought it in a collection device (Fecotainer®, AT Medical BV, Netherlands), to the study center [10, 20]. If the participants were not able to deliver a stool sample for the second examination day, the samples were picked up at participants´ homes or work places by study staff at later times [10].
According to the SOP of our study, the complete stool sample was weighed and homogenized with a stomacher for 15 minutes [10, 20]. Native stool was allocated to six different Sarstedt aliquots for the analysis of microbiota composition (5 g), fatty acids, bile acids (each 1-2 g) and the inflammation marker calprotectin (10 g). Two additional samples (10-15 g) were used as retention samples. All aliquots were labelled and stored at -80 ºC for further analyses [10, 20]. The pH value of fresh stool sample was measurement with a pH electrode (Knick Portamess 752), which was held into the remaining sample.

4.1.4 Assessment of microbiota

Due to the development of modern technologies in the recent years, it has become easier and quicker to analyze the huge quantity of bacteria in the microbiota. There are several methods for identifying bacterial communities and gene sequencing is a common method nowadays. The method of 16S ribosomal ribonucleic acid (rRNA) gene sequencing is currently widely used and described comprehensively by Morgan et al. [30]. 16S rRNA sequencing seeks to identify which bacteria are present in the sample and their quantity. 16S rRNA sequencing is conducted in the following steps: The DNA of the sample is extracted and then amplified in order to receive copies of DNA sequences. The extraction of the whole bacterial genome is too complex; therefore DNA of selected regions is chosen and these were then used as markers. A marker uniquely tags distinct genomes [30] and is defined by the presence of each member of the investigated population and discriminates individuals with different genomes [30]. The 16S region of the ribosomal RNA gene fulfills these criteria and is therefore broadly used as a marker in gene sequencing. The 16S region has a small size of 1.5 Kb and is mostly fixed, which leads to homogenous sections [9, 30]. The variable sites between these fixed regions are sequenced to receive information about the unique and distinctive bacterial communities. Broadly used variable regions are V3, V4 and V9 [9, 30]. The next step in DNA analyzing is the classification of the genes to single bacteria. This has to be done by grouping similar sequences into

clusters, the so-called operational taxonomy units (OTU). Mostly a cut-off between 95 % and 99 % identity is used to classify the units into genera and species [30]. The designation of OTUs to phyla, genera and species is performed by comparing the sequences with databases such as the NCBI Bacterial 16S rRNA database.

For the RBVD study, 16S rRNA gene sequencing was performed by CeMeT GmbH (Tübingen, Germany) [10]. In the systematic review, the first ten abundant bacteria in descending order were presented for each taxonomic level, [10].

4.1.5 Assessment of bile acids

Bile acids in feces and serum in the RBVD study were analyzed with ultra-performance liquid chromatography-tandem mass spectrometry (UPLCMS/MS) at the Department of Molecular and Clinical Medicine/Wallenberg Laboratory, University of Gothenburg [20]. For the chromatographic separation, 50µl of the serum samples and 30-80 mg of stool samples were extracted in 500µl methanol respectively containing deuterated standards of bile acids [20].

4.1.6 Dietary assessment

The dietary assessment in the RBVD study and for this thesis was also described in parts in the second publication [20].
For the present study a 3-day weighing protocol was used for the assessment of dietary intake. In a weighing protocol, all consumed food and beverages are recorded with a kitchen scale for several days [31]. The participants in the present study were provided with an electronic kitchen scale and kept a weighing protocol for three days on two week days and on one weekend day [20]. The recorded days did not have to be consecutive. At the BfR study site, the participants were instructed on how to fill out the protocols and how to weigh all consumed meals and snacks. Type of consumed food and drinks, brand name, consumed amount, type of packaging, way of further processing as well as time and place of consumption, were entered by the participants into the protocols. The weighing protocols were implemented in the Children's Nutrition Survey to establish food consumption study (KiESEL) conducted at the BfR, and adopted for an adult and vegan population. The data of the food protocols were evaluated with the software EAT version

3.5.5, and each food was allocated to the national food code (Bundeslebensmittelschlüssel Version 3.02, BLS) [20]. If a food item was not included in the BLS, a new food code was developed based on nutritional information stated on the packaging on request of producers [20]. For cooked and prepared dishes, recipes were attached by the participants to the weighing protocols. Ingredients of cooked dishes were converted from recipes to effective quantities of consumed portions with the following formula:

$$\frac{\text{Recipe ingredient (g)}}{\text{Weight of total dish (g)}} * \text{consumed portion (g)}$$

Yield and retention factors of different food items were considered [32] for the estimation of the weight of cooked food [20]. Food items such as pasta, rice, beans or grains absorb water during cooking and increase in volume. Processing with high temperatures lead to a loss of water e.g. in meat. Factors of single food products [32] were grouped into su food categories to reduce the amount of yield and retention factors (also published as supplemental S1 in [20]). To estimate the portion size of dishes eaten out of home, the participants could send pictures taken with mobile phones to the study personnel. All 72 participants filled out the protocols completely. When the protocols were returned by the participants on the second examination day, the protocols were checked by study personnel for completeness and ambiguities were requested directly. Entered and proofed for quality data were merged with BLS to obtain macro- and micronutrients data [20]. All food data from the three single days were summarized and divided by three to achieve daily food intakes [20].

For a better overview of the results of the weighing protocols, the food items were categorized into 49 groups according to the food groups used in EPIC Potsdam [20, 33]. Plant-based milk and milk products, meat alternatives, savory vegetable spreads were added as new food groups. Additionally, carbonated and non-alcoholic drinks were summarized into the food group soft drinks, so that in total 53 food groups were received (also published as supplemental S2 in [20]). We assessed food groups separately, which could be vegan or non-vegan like pasta with eggs or cookies and sweets.

4.2 Systematic review

A systematic review is a helpful way to summarize results of several studies and to give an overview of one research question at a glance. Results of the included studies are

systematically extracted and compared to each other, to reveal homogeneity or heterogeneity. In cases of small study sizes and small statistical power in single studies, evaluations regarding one research topic can be improved with a systematic review [34].
To identify eligible studies, a systematic review follows these steps: 1) Expression of the research question; 2) Identification of the relevant literature; 3) Rating of the literature; 4) Summarization of outcomes; 5) Interpretation of results. A guidance tool such as PICOS [35] should be used to conduct these steps. PICOS stands for population (P), intervention or exposure (I), comparison (C), outcome (O) and study design (S). In contrast to a narrative summary of several studies, the systematic review is therefore characterized by a clearly defined research question, as well as a priori defined inclusion and exclusion criteria to identify eligible studies [34, 36]. At least two different scientific databases, such as PubMed, Embase or other, are searched systematically for eligible studies on the basis of predetermined search terms by at least two team members. The processes of study selection and reasons for excluding studies have to be documented. In this systematic review [10], the selection of eligible studies has been done on basis of the PRISMA statement (Preferred Reporting System for Systematic reviews and Meta-Analyses) [36]. This statement has been developed to increase the quality of systematic reviews and serves as guidance in conducting systematic reviews [36]. Figure 7 shows the process of the PRISMA flowchart [36] used in the present systematic review [10].

Table 2: PICOS tool for eligibility criteria

PICOS format	Description
Population	Healthy subjects, aged ≥18 years
Intervention or Exposure	*Vegetarian diet* (dairy, egg, no meat and fish) *Vegan diet* (no animal products)
Comparisons	Control diet (omnivore, Western-type, non-vegetarians/vegans)
Outcome	Gut microbiota composition
Study Design	Cross-sectional, prospective cohort studies and randomized controlled trails of either parallel or crossover design.

Steps for choosing eligible studies in systematic reviews according to the PICOS scheme [35]. This table has been amanded from Trefflich et al. [10].

For the systematic review, a literature research was conducted in PubMed and Embase (up to May 2018) with pre-defined search terms, and studies with microbiota composition

as an outcome in vegan and/or vegetarian population were included. Studies, conducted in children, without omnivorous participants as control group, or any modified vegan or vegetarian diets, were excluded [10] (
Table 2).

The reported microbiota in the reviewed studies was presented at phylum, family, genus and species level, so far as specified in the included studies. Results of the microbiota composition in vegans/ vegetarians compared to omnivores were presented by contrasting significant with non-significant results (significance level $p \leq 0.05$) [10]. Additionally, the results of the RBVD study were included in this review. The study protocol of the systematic review was prospectively registered on the International Register of Systematic Reviews (PROSPERO), registration number CRD42018091139 [10].

4.3 Statistical analysis

Characteristics, status of vitamins and nutrients, microbiota data and other biochemical variables of vegan and omnivorous fecal samples were presented with means and standard deviation for normal distributed continuous variables or with median and interquartile range for non-normal distributed variables. Categorical variables are presented as percentages. For categorical variables, Chi-square or Fisher's Exact test were used, and Mann-Whitney-U for continuous variables, or T-Test (for normally distributed variables) were applied. All statistical analyses were conducted with SAS Enterprise software package version 9.3 (SAS Institute Inc., Cary, NC, USA) [10, 20].

4.3.1 Reduced Rank Regression

Of particular interest is whether the consumption of certain food groups influence bile acid concentrations. Therefore, a modern statistical method used in nutritional epidemiology, the reduced rank regression (RRR), was applied to identify these food groups and to derive dietary pattern in the second publication [20]. RRR uses the variables x1, …,xn as predictor variables and y1, ….yn as response variables [26]. In the second publication [20], fecal secondary bile acids LCA, DCA and UDCA, and the sum of primary and secondary conjugated bile acids with were chosen as response variables. All five bile acids

were log-transformed because of non-normal distribution before calculation with RRR. The average intakes (g/d) of the 53 food groups were used as predictor variables [20].
RRR extracts linear combinations of the predictor variables which explain the maximum of variance in the response variables [20, 26, 27]. These combinations are called factors. For deriving factors, RRR uses eigenvalues of the covariance matrix of the response variables [26]. The response score, which is a linear function of responses variables (here bile acid concentrations), is then calculated with weights from the eigenvalues in decreasing order. The number of derived response scores is equal to the number of response variables [26], so that in our study five response scores were derived [20]. Then, the response scores are projected onto the space of predictor variables and create factor scores out of a linear combination of predictors [26]. The derived factors are called dietary pattern.
Only food groups with a factor loading > 0.2 were defined as crucial contributors to the pattern and were used for the calculation of the dietary pattern [20]. A factor loading is the product of the standardized score parameter and the Pearson correlation coefficient. Behavior of bile acid concentrations across the first score was calculated in a logistic model by categorizing the score into tertiles [20].

5. Results

5.1 Results of the cross-sectional study

5.1.1 Study population of the RBVD study

Median age of vegans was 37.5 years (IQR 32.5-44.0) and of omnivores 38.5 years (IQR 32.0-46.0) (Table 3). Vegan participants followed their diet for 4.8 years (IQR 3.1 -8.7). BMI was lower in vegans (22.9 ± 3.2) than in omnivores (24.0 ± 2.1), but remained not significant (p = 0.08). The majority of all participants were higher educated and non-smoker. Among 13 smokers, four were vegans and nine were omnivores [10, 20]. Median physical activity was 2.8 h/ week (IQR 0.88-3.75) for vegans and 2.3 h/ week (IQR 1.2-4.1) for omnivores (p = 0.69).

Table 3: Characteristics of RBVD study population

	Vegan (n = 36)	**Omnivore (n = 36)**	***p-value***
Male (%)	50 %	50 %	
Age (years)	37.5 (32.5 – 44.0)	38.5 (32.0 – 46.0)	*0.75*
Body weight (kg)	70.1 (± 13.9)	73.6 (± 10.3)	*0.24*
BMI	22.9 (± 3.2)	24.0 (± 2.1)	*0.08*
Duration of vegan diet (years)	4.8 (3.1 – 8.7)	n.a.	
Education [n (%)]			*0.60*
Low	0 (0.0)	1 (2.8)	
Intermediate	11 (30.6)	11 (30.6)	
High	25 (69.5)	24 (66.7)	
Physical activity (h/week)	2.8 (0.88 – 3.75)	2.3 (1.2 – 4.1)	*0.69*
Smoking behavior [n (%)]			*0.30*
Never smoker	24 (66.7)	21 (58.3)	
Ex-Smoker	8 (22.2)	6 (16.7)	
Smoker	4 (11.1)	9 (25)	

Results presented as mean (±) or median (Q1-Q3) or as absolute numbers and in percentage
Low education: no degree; intermediate education: vocational school, technical college; High education: university, university of applied sciences [10, 20].

5.1.2 Results of the dietary assessment

The evaluation of the protocols revealed no significant difference in energy and carbohydrate intake between vegan and omnivorous participants (Table 4). Vegans had a higher fiber intake (45.6 g/d, IQR 33.7 - 56.4) than omnivores (23.7 g/d, IQR 18.6 - 29.9) ($p < 0.0001$), whereas the intake of fat was lower in vegans (85.7 g/d, IQR 63.6 - 111.1) compared to omnivores (104.1 g/d, IQR 87.8 - 143.3, $p = 0.044$) [20]. The intake of saturated fatty acids was higher in omnivores (43.0 g/d, IQR 34.4 - 54.6) than in vegans (16.1 g/d, IQR 10.7 - 22.3) ($p = 0.001$).

The intakes of several micronutrients differed significantly between vegans and omnivores [28]. The intakes of vitamin B12 and vitamin D were significantly lower in vegans than in omnivores ($p < 0.0001$). The dietary intakes of folic acid, vitamin B6, vitamin E and vitamin K were significant higher in vegans compared to omnivores. Vegans also showed a higher intake of potassium (2237.9 mg/d, IQR 1523.0 - 33408.5), magnesium (634.3 mg/d, IQR 496.4 - 713.7) and iron (22.0 mg/d, IQR 15.5 - 26.4) than omnivores; whereas iodine (0.12 mg/d, IQR 0.08 - 0.2) intake was higher in omnivores than in vegans (0.08 mg/d, IQR 0.05 - 0.1) (Table 4).

Table 4: Daily macro and micro-nutrient intake of the study population

	Vegan (n = 36)	Omnivore (n = 36)	*p*
Total energy (kcal)	2270.1 (1800.0 – 2762.3)	2385.9 (2080.9 – 2737.3)	*0.32*
Macronutrients (g/d)			
Fiber	45.6 (33.7 – 56.4)	23.7 (18.6 – 29.9)	*<0.0001*
Protein	72.2 (54.9 – 91.8)	86.3 (71.4 – 107.0)	*0.02*
Fat	85.7 (63.6 – 111.1)	104.1 (87.8 – 143.3)	*0.004*
Saturated fatty acids	16.1 (10.7 – 22.3)	43.0 (34.44 – 54.6)	*<.0001*
Unsaturated fatty acids	28.39 (21.88 – 36.38)	35.95 (30.8 – 48.7)	*0.001*
Carbohydrate	258.7 (211.5 – 371.2)	230.3 (199.3 – 291.0)	*0.12*
Vitamins (mg/d)			
Vitamin B12 (µg/d)	0.3 (0.1 – 0.9)	5.2 (4.2 – 8.0)	*<0.0001*
Vitamin D (µg/d)	0.9 (0.3 – 1.9)	2.5 (1.9 – 4.3)	*<0.0001*
Folic acid (µg/d)	446 (311 – 607)	296 (249 – 343)	*0.0005*
Vitamin B1	1.7 (1.3 – 2.1)	1.3 (1.1 – 1.7)	*0.049*
Vitamin B2	1.5 (1.0 – 2.1)	2.0 (1.6 – 2.3)	*0.01*
Vitamin B6	2.2 (1.7 – 2.7)	1.8 (1.4 – 2.1)	*0.02*
Vitamin C	173.4 (108.2 – 275.7)	131.0 (98.3 – 173.7)	*0.11*
Vitamin E	26.9 (16.1 – 37.6)	13.4 (11.1 – 18.6)	*<0.0001*
Vitamin K	0.3 (0.3 – 0.4)	0.1 (0.1 – 0.2)	*<0.0001*
Minerals (mg/d)			
Potassium	2237.9 (1523.0 – 33408.5)	2866.4 (2121.6 – 3986.2)	*0.02*
Calcium	898.9 (649.9 – 1256.6)	1049.1 (822.3 – 1456.6)	*0.04*
Magnesium	634.3 (496.4 – 713.7)	417.4 (332.6 – 496.5)	*<0.0001*
Iodine	0.08 (0.05 – 0.1)	0.1 (0.1 – 0.2)	*0.002*
Iron	22.0 (15.5 – 26.4)	14.0 (11.5 – 17.0)	*0.0001*

Energy and nutrient intake based on 3-day weighing protocols (g/d) presented as median (Q1-Q3). Mann-Whitney-U-test was used for statistical testing. The data of this table has been published in parts in Weikert et al. [28].

Furthermore, the intakes of selected food groups were compared (Figure 5). Vegans consume significantly more raw ($p = 0.1$) and cooked vegetables ($p = 0.008$), nuts and seeds ($p = 0.01$), than omnivores [20]. The intake of plant-based oils was higher in vegans (19.3 g/d, IQR 11.83-32.2) than in omnivores (9.2 g/d, IQR 5.2 -14.7) (p = 0.0006). Vegans consumed 271.2 g/d (IQR 133.8-488.7) plant-based milk products compromising soy, cereal and nut-based products. Omnivores consumed a comparable amount of 240.2 g/d milk (IQR 136.5- 431.8). There was no difference in the consumption of sweets between vegans and omnivores (p = 0.17). The intake of selected food groups is shown in figure 5. Detailed information about the intake of all food groups in the present study is shown in supplemental S3 of the second publication [20].

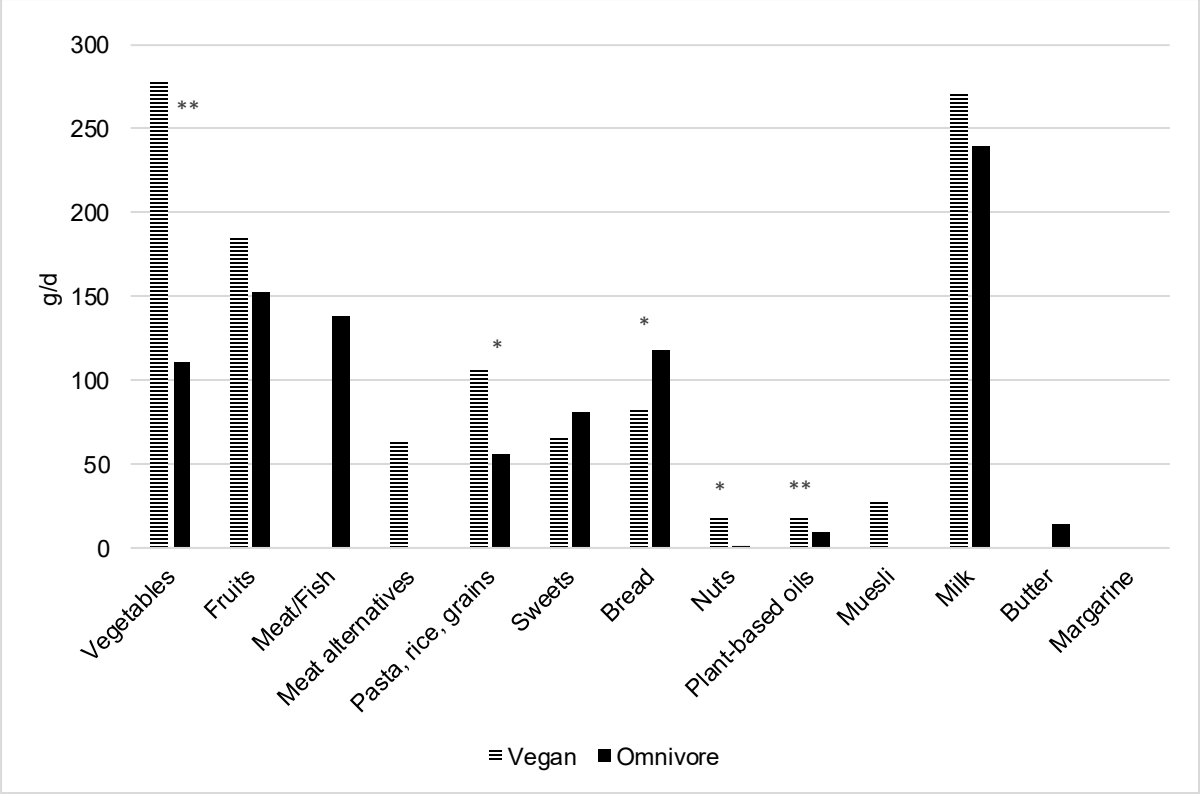

Figure 5: Intakes of selected food groups in RBVD (g/d)

Data presented as median. Mann-Whitney-U-test was used for statistical testing * p< 0.05, **p< 0.001. Vegetables summarized intakes of cooked vegetable, raw, legumes and cabbage. Meat/Fish summarized intake of red meat, poultry, fish, and processed meat. Sweets summarized dessert, cake, confectionary, jam. Milk compared plant-based milk with dairy.

5.2 Microbiota

The composition of the microbiota in the RBVD study is presented in figure 6. Parts of these results were considered in the systematic review [10].

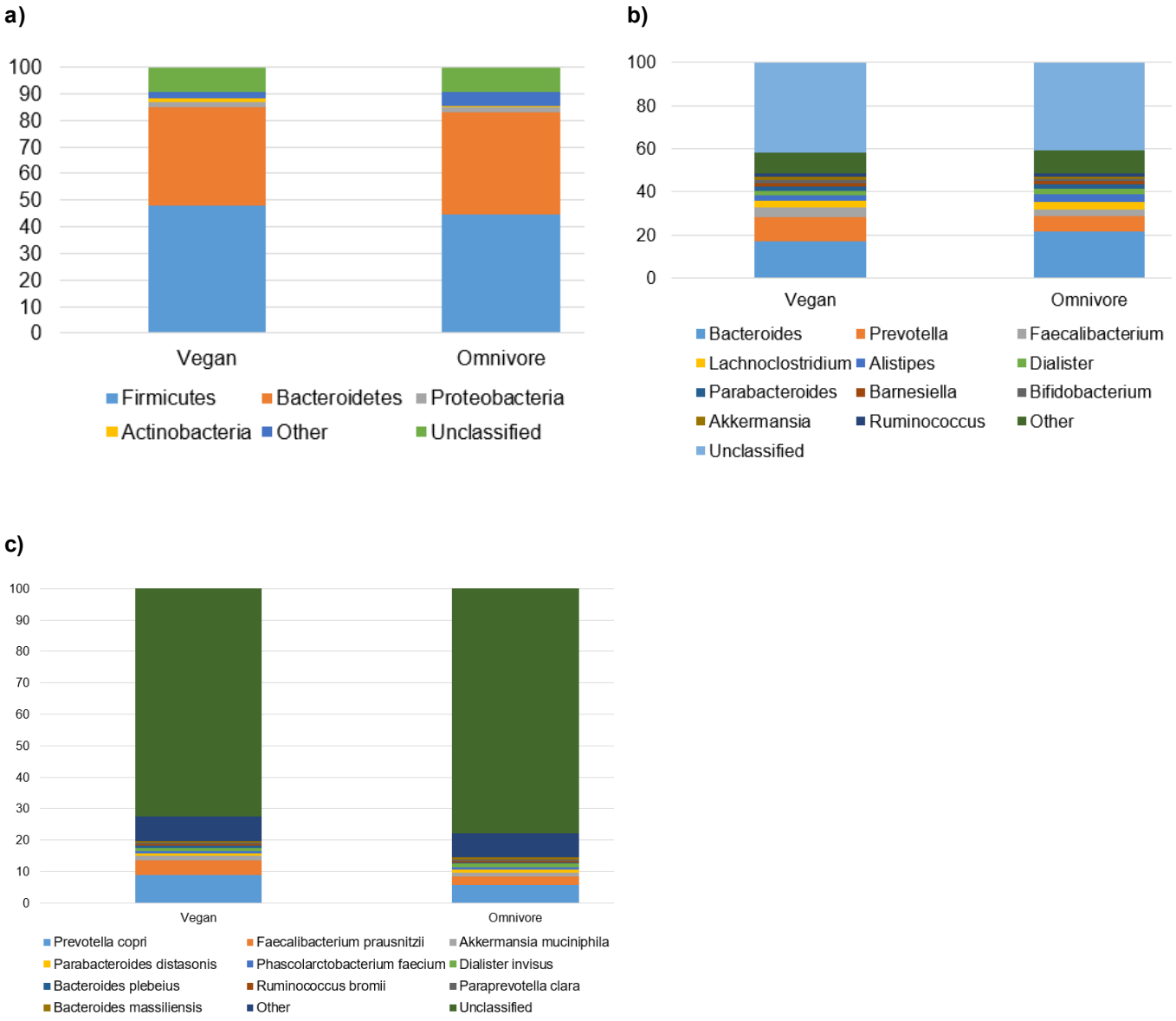

Figure 6: Microbiota composition of vegans and omnivores in the RBVD study

Out of total microbiota results, the most 10 abundant taxa at a) phylum, b) genus and c) species level are presented on percentage skale.

Firmicutes, Bacteroidetes, Proteobacteria and Actinobacteria were the most abundant phyla in our study, but there was no significant difference between vegans and omnivores (Figure 6). At family level, *Bacteroidaceae*, *Lachnospiraceae*, *Ruminococcaceae* and *Prevotellaceae* were the most abundant taxa in the study population, but without any difference between vegan and omnivores. At genus level, vegans had significant lower abundances of *Lachnoclostridium* than omnivores. *Prevotella copri, Faecalibacterium prausnitzii* and *Akkermansia muciniphila* were the three most abundant species in the RBVD study, but without significant differences between vegans and omnivores. Omnivorous participants had significantly higher levels of *Dialister invisus* compared to vegans. But all other taxa did not differ significantly between vegans and omnivores. The high proportion of unclassified taxa is noteworthy

The database research conducted for the systematic review identified 3363 citations and after exclusion of duplicates and non-eligible studies, 16 studies were finally included [10] (Figure 7). Characteristics of the included studies are described in table 5. The detailed references of the studies are listed in the published review [10]. All studies were cross-sectional studies and the majority of them were conducted during the years 2009 – 2019. In total, all included studies comprised 1229 healthy participants, with 389 vegetarians, 342 vegans and 498 omnivores. Out of 96 different genera, *Bacteroides, Prevotella* and *Bifidobacterium, Lactobacillus*, *Enterococcus,* and *Clostridium* were the most reported genera in all dietary groups. *Escherichia coli*, the *Bacteroides fragilis group*, *Prevotella copri* and *Faecalibacterium prausnitzii* were the most reported species out of 177 species. Surprisingly, the number of significant differences between vegans or vegetarians compared to omnivores was low across all taxonomic levels in the reviewed studies. At all taxonomic levels the results were heterogeneous [10].

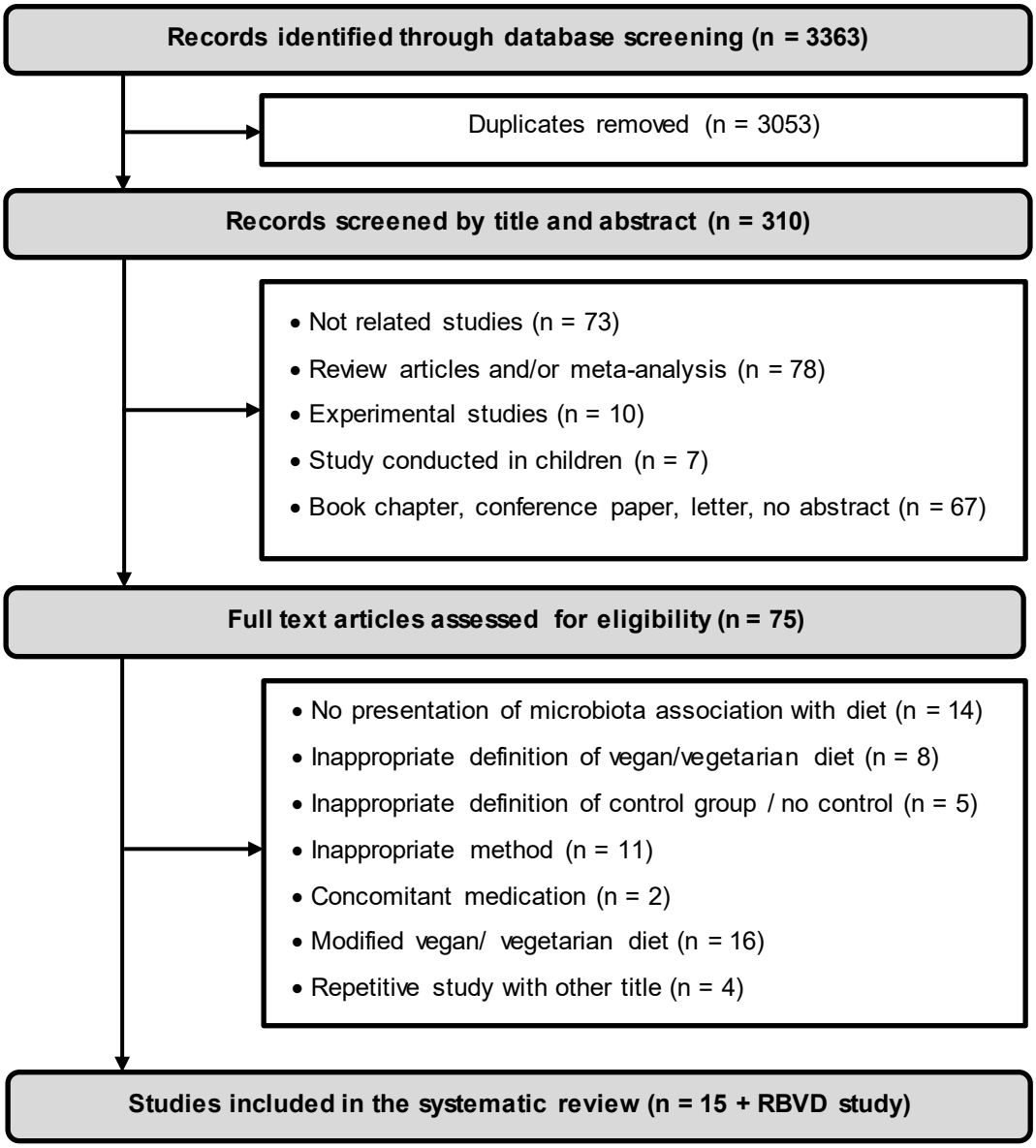

Figure 7: PRISMA flow chart of study selection

This figure has been amended from Trefflich et al. [10], PRISMA flow chart adapted from [36].

Table 5: Characteristics of included studies in the systematic review

Author, country (Year)	Study design	Study population (n)	Size male % (n)	Vegetarian (n) Age [years] Duration	Type of diet	Vegan (n) Age [years] Duration	Omnivore (n) Age [years]	Methods
Aries et al., Great Britain (1971)	Cross-sectional	(*n* = 58)	NR	_____	_____	(*n* = 18) NR	(*n* = 40) NR	Bacterial counting
Goldberg et al., United States of America (1977)	Cross-sectional	(*n* = 28)	39.2 % (*n* = 11)	_____	_____	(*n* = 14) 47 (22 – 66) >10 y	(*n* = 14) 45 (15 – 61)	Bacterial counting
Finegold et al., United States of America (1977)	Cross-sectional	(*n* = 27)	NR	(*n* = 13) 57.6 ± 9.5 all of their life	OLV	_____	(*n* = 14) 51.8 ± 12.6	Bacterial counting
Kabeerdoss et al. India (2012)	Cross-sectional	(*n* = 56)	0 % (*n* = 0)	(*n* = 32) 19 (18-19) NR	LV	_____	(*n* = 24) 19 (18 – 20)	16S r-RNA sequencing
Zimmer et al. Germany (2012)	Cross-sectional	(*n* = 138)	60.8 % (*n* = 84)	(*n* = 46) 47.80 ± 12.09 > 1 m	OLV	(*n* = 46) 46.50 ± 12.62 > 1 m	(*n* = 46) 46.50 ± 12.26	Bacterial counting
Ruengsomwong et al. Thailand (2014)	Cross-sectional	(*n* = 13)	NR	(*n* = 7) 54.2 ± 6.6 > 3 y	OLV, LV	_____	(*n* = 6) 61.8 ± 8.4	Real time quantitative PCR, rRNA-DGGE
Ferrocino et al.*, Italy (2015)	Cross-sectional	(*n* = 153)	49.6 % (*n* = 76)	(*n* = 51) 38 ± 9.8 > 1 y	OLV	(*n* = 51) 38 ± 9.8 > 1 y	(*n* = 51) 38 ± 9.8	Real time quantitative PCR, rRNA-DGGE
De Filippis et al. *, Italy (2016)	Cross-sectional	(*n* = 153)	41,8 % (*n* = 64)	(*n* = 51) 39 ± 9 > 1 y	NR	(*n* = 51) 37 ± 10 > 1 y	(*n* = 51) 39 ± 9	16S r-RNA sequencing
Ruengsomwong et al. Thailand (2016)	Cross-sectional	(*n* = 72)	_____	(*n* = 36) 50.9 ± 5.9 > 3 y	OLV, LV, V	_____	(*n* = 36) 51.8 ± 8.1	16S r-RNA sequencing
Schwiertz et al., Germany(2016)	Cross-sectional	(*n* = 19)	36.8 % (*n* = 7)	_____	_____	(*n* = 9) 31 (29 – 44) > 0.5 y	(*n* = 10) 27 (25 – 44)	Real time quantitative PCR

Author, country (Year)	Study design	Study population (n)	Size male % (n)	Vegetarian (n) Age [years] Duration	Type of diet	Vegan (n) Age [years] Duration	Omnivore (n) Age [years]	Methods
Wu et al., United States of America (2016)	Cross-sectional	(*n* = 31)	NR	_____	_____	(*n* = 15) NR > 0.5 y	(*n* = 16) NR	16S r-RNA sequencing
Federici et al., Italy (2017)	Cross-sectional	(*n* = 29)	48.2 % (*n* = 14)	(*n* = 12) 39 ± 10 > 1 y	OLV	(*n* = 10) 33 ± 7 > 1 y	(*n* = 7) 41 ± 9	Bacterial counting
Franco-De-Moraes et al., Brazil (2017)	Cross-sectional	(*n* = 268)	45.8 % (*n* = 123)	(*n* = 102) 49.6 ± 8.6 ≥ 1 y	OLV	(*n* = 66) 49.6 ± 8.5 ≥1 y	(*n* = 100) 49.1 ± 8.2	16S r-RNA sequencing
Losasso et al., Italy (2018)	Cross-sectional	(*n* = 101)	31.7 % (*n* = 33)	(*n* = 32) 42.3 ± 13.2 ≥ 1 y	NR	(*n* = 26) 39.4 ± 11.1 ≥1 y	(*n* = 43) 45.0 ± 13.9	16S r-RNA sequencing
Zhang et al., Sweden (2018)	Cross-sectional	(*n* = 11)	50 % (*n* = 2)	(*n* = 4) 34.5 ± 7.4 NR	OLV	_____	(*n* = 7) 35.7 ± 10.4	16S r-RNA sequencing
RBVD, Germany (2018)	Cross-sectional	(*n* = 72)	*n* = 36 (50 %)	_____	_____	(*n* = 36) 37.5 (32.5–44.0) 4.8 y	(*n* = 36) 38.5 (32.0–46.0)	16S r-RNA sequencing

*Authors used data of the same study population. Age of study population in years (y) presented as mean and standard deviation. NR = not reported. OLV = ovo-lacto vegetarian diet. LV = lacto-vegetarian diet. rRNA-DGGE = ribosomal RNA Denaturing Gradient Gel Electrophoresis. The results of this table, including full references of the included studies have been published in Trefflich et al. as table 3 [10].

5.3 Bile acids in stool and serum

The following results were presented partly in the second publication [20]. The fecal bile acid profile showed significant differences between a vegan and an omnivorous diet. Secondary and conjugated bile acids, except tauro-lithocholicacid and tauro-ursodeoxycholicacid, were significantly higher in omnivores than in vegans (Table 6). Primary fecal bile acids and ursodeoxycholicacid did not differ between the two types of diet.

Table 6: Fecal bile acid concentrations in vegans and omnivores

Bile acids (µmol/g)	Vegan	Omnivore	*p*
Cholicacid	0.74 (0.18 - 9.43)	1.87 (0.57 - 6.82)	*0.17*
Chenodeoxycholic	0.00 (0.00 - 2.93)	0.00 (0.00 - 0.40)	*0.34*
Deoxycholicacid	215.5 (49.8-644.5)	798.7 (283.6 - 2345.2)	*0.002*
Lithocholicacid	105.11 (53.9 - 294.1)	308.8 (170.8 - 725.4)	*0.002*
Ursodeoxycholicacid	0.05 (0.00 - 0.64)	0.21 (0.00 - 4.16)	*0.32*
gamma Cholicacid	0.65 (0.22 - 1.88)	1.85 (0.67 - 4.76)	*0.008*
gamma Chenodeoxycholic	0.86 (0.31 - 1.95)	1.89 (1.01 - 3.20)	*0.007*
gamma Deoxycholicacid	0.62 (0.12 - 1.02)	1.35 (0.45 - 2.80)	*0.003*
gamma Lithocholicacid	0.00 (0.00 - 0.00)	0.00 (0.00 - 0.00	*1.00*
gamma Ursodeoxycholicacid	0.04 (0.00 - 0.13)	0.15 (0.01 - 0.34)	*0.01*
tauro Cholicacid	0.16 (0.05 - 0.55)	0.65 (0.30 - 1.43)	*0.0006*
tauro Chenodeoxycholic	0.16 (0.02 - 0.53)	0.57 (0.21 - 1.43)	*0.0009*
tauro Deoxycholicacid	0.08 (0.00 - 0.21)	0.41 (0.13 - 1.27)	*0.0002*
tauro Lithocholicacid	0.00 (0.00 - 0.05)	0.00 (0.00 - 0.13)	*0.39*
tauro Ursodeoxycholicacid	0.00 (0.00 - 0.00	0.00 (0.00 - 0.07)	*0.17*

Data presented as median and IQR for fecal bile acids. Mann Whitney U-test was used for bile acids. Gamma = glycine conjugated bile acids, tauro = taurine conjugated bile acids.

Table 7: Serum bile acid concentrations in vegans and omnivores

Serum bile acids (µmol/L)	vegan	Omnivore	*p*
Cholicacid	0.07 (0.04 - 0.38)	0.04 (0.02 - 0.07)	*0.002*
Chenodeoxycholic	0.14 (0.04 - 0.46)	0.05 (0.01 - 0.28)	*0.01*
Deoxycholicacid	0.43 (0.17 - 0.54)	0.23 (0.13 - 0.45)	*0.15*
Lithocholicacid	0.02 (0.02 - 0.02)	0.02 (0.01 - 0.02)	*0.12*
Ursodeoxycholicacid	0.02 (0.01 - 0.06)	0.02 (0.01 - 0.06)	*0.48*
gamma Cholicacid	0.21 (0.10 - 0.45)	0.09 (0.05 - 0.22)	*0.005*
gamma Chenodeoxycholic	0.82 (0.42 - 1.33)	0.39 (0.19 - 0.56)	*0.0007*
gamma Deoxycholicacid	0.26 (0.12 - 0.56)	0.15 (0.08 - 0.26)	*0.03*
gamma Lithocholicacid	0.00 (0.00 - 0.00)	0.00 (0.00 - 0.00)	*1.00*
gamma Ursodeoxycholicacid	0.06 (0.02 - 0.09)	0.04 (0.02 - 0.08)	*0.37*
tauro Cholicacid	0.02 (0.01 - 0.02)	0.02 (0.01 - 0.02)	*0.72*
tauro Chenodeoxycholic	0.06 (0.03 - 0.11)	0.05 (0.03 - 0.08)	*0.36*
tauro Deoxycholicacid	0.02 (0.00 - 0.05)	0.02 (0.01 - 0.04)	*0.42*
tauro Lithocholicacid	0.00 (0.00 - 0.00)	0.00 (0.00 - 0.00)	*0.58*
tauro Ursodeoxycholicacid	0.00 (0.00 - 0.00)	0.00 (0.00 - 0.00)	*0.79*

Data presented as median and IQR for serum bile acids. Mann Whitney U-test was used for bile acids. Gamma = glycine conjugated bile acids, tauro = taurine conjugated bile acids.

In serum, primary bile acids and glycine-conjugated bile acids were significant higher in vegans than in omnivores (Table 7). Serum secondary bile acids tended to be higher in vegans, though this difference was not significant [20].

5.4. Reduced rank regression and dietary pattern

The first dietary pattern score for fecal bile acids explained 47.4 % of the bile acids variance, with a high proportion of the explained variance in glycine conjugated bile acids (69.1 %) and taurine conjugated bile acids (54.8 %) [20]. In the first pattern coffee, fish, margarine, fried potatoes, bread and processed meat were identified with positive loadings; and muesli intake was negatively loaded (Figure 8) [20].

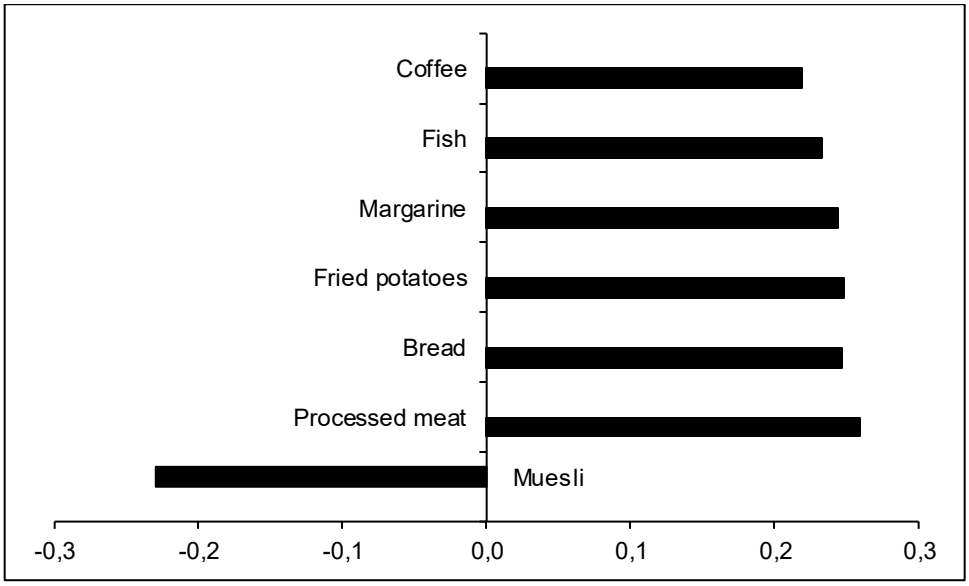

Figure 8: Factor loadings of food groups contributing to the first dietary pattern score
This figure has been published in Trefflich et al. as figure 3 [20].

The concentrations of the response bile acids increased across the tertiles of the dietary pattern score (DCA, LCA, TBA, GBA: $p < 0.0001$; UDCA: $p < 0.02$) [20]. The proportion of vegans decreased across the score instead (Table 8). [20].

Table 8: Concentrations of fecal bile acids across first pattern score

	Tertiles of dietary pattern score			
Bile acids (nmol/g)	**1 (75 % vegans)**	**2 (50 % vegans)**	**3 (25 % vegans)**	***p-value for trend***
DCA	74.23 (3.22 – 195.81)	586.21 (283.57 – 1041.85)	1327.30 (644.51 – 3037.96)	< *0.0001*
LCA	64.20 (13.99 – 93.47)	198.93 (120.73 – 327.30)	417.49 (305.10 – 1070.67)	< *0.0001*
UDCA	0.01 (0.00 – 0.16)	0.16 (0.00 – 0.64)	1.07 (0.00 – 16.91)	*0.02*
Glycine con.	0.73 (0.32 – 1.52)	3.45 (2.43 – 5.95)	9.06 (5.76 – 18.81)	< *0.0001*
Taurine con.	0.26 (0.05 – 0.50)	1.05 (0.49 – 2.00)	3.25 (1.71 – 8.65)	< *0.0001*

Concentrations of fecal bile acids (nmol/g) presented as median (Q1-Q3). DCA = deoxycolicacid, LCA = lithocholicacid, UDCA = ursodeoxycholicacid, con = conjugated. This table has been published in Trefflich et al. as table 2 [20].

6. Discussion

The interest in a vegan diet has been increasing in recent years. Once a vegan diet remained a niche for people with a very ecological lifestyle, but nowadays it is getting more popular for a broader population. Nevertheless, studies examining dietary intake, health status and lifestyle of healthy and adult vegans in Germany are rare and out of date.
Therefore, a cross-sectional study in vegans was conducted at the research unit *Risks of Subpopulations and Human studies* at the Federal Institute for Risk Assessment (BfR). The research unit aimed to investigate risks but also benefits of a vegan diet.
For this thesis, data of the RBVD study was used to compare microbiota composition in vegans and omnivores. Concentrations of bile acids [20], SCFA as well as fecal pH were investigated as markers of gut health. Furthermore, the association of a vegan and vegetarian diet on microbiota composition was summarized in a systematic review [10].

Diet

For the assessment of the nutrient intake, 3-day weighing protocols were chosen in the cross-sectional study RBVD [20]. A weighing protocol is a very precise method for assessing dietary intake. Consumed food and beverages are weighed and recorded in blank protocol templates by the participants [31]. It is recommended, to weigh and record consumed food directly after consumption, to avoid memory gaps as this could occur in a 24-h recall or a food frequency questionnaire (FFQ) [31]. It is known, that normal nutritional behavior can be influenced through food recording, so participants tend to eat less than usual. Protein and energy intake can therefore be underestimated in a range of 4 - 37 % [31]. Furthermore, the post processing of the weighing protocols, such as calculation of portion size from recipes, can be very time-consuming.
In line with a cross-sectional study of 22 Finnish vegans [37] and a Swiss study of 53 vegans [38] we chose weighing protocols in RBVD as a dietary assessment method. This method is more comprehensive than FFQ or a 24-h recalls. Furthermore, FFQ or 24-h recalls may not contain specific vegan food items, so that vegan participants might have difficulties selecting suitable food items. “The Gießener Vegetarier Studie” and the German Vegan Study assessed the diet by dietary protocols for 14 days and 9 days respectively [7, 8], which queried the frequency of consumed food, but not quantities.

We could not find any significant difference regarding energy and carbohydrate intakes between vegans and omnivores, whereas protein intake was higher in omnivores [20]. These findings are in line with the Finnish study [37] and the Swiss study [38]. Also in line with these studies, the intake of fiber was higher in vegans than in omnivores [37, 38]; and the intake of fat and saturated fatty acids was lower in vegan participants [37]. As expected and in line with findings of other studies [37, 38], intakes of vitamin B12 and D were considerably lower in vegans than in omnivores [28]. In line with other studies, vegans in our study had lower intakes of iodine [37] and calcium [38]. As in other studies, the intakes of iron [37, 38], magnesium [38], and folic acid [37, 38] were higher in vegans than in omnivores of our study [28] These findings were in line with the evaluation of a vegan diet of DGE [2] and a meta-analysis [4].

Nevertheless, the consumption of certain food groups differed in our study compared to other cross-sectional studies. Vegans in our study consumed significant more vegetables than omnivores, which did not correspond to Finnish vegans [37] or findings of the GVS [8]; in both studies fruit consumption was higher in vegans than in omnivores. Also in contrast to these studies, vegans of our study consumed more rice, cereals and grains [8, 37]. Interestingly and despite lower fat intakes, vegans of our study consumed more fat and oils than omnivores, which might be attributed to the use of oils for cooking and preparation of salads.

Microbiota

Despite these differences in diet, we could observe only minor differences in microbiota composition at all taxonomic levels between vegans and omnivores in our study. In line with our results are the findings of Wu et al. (cited in [10]). In this cross-sectional study, a similar composition of microbiota in Westernized 15 vegans and 16 omnivores was observed. Bacteroidetes and Firmicutes were the most abundant phyla in our study population, with a higher proportion of Firmicutes than Bacteroidetes in vegans and omnivores. This is not entirely in line with literature, assigning Firmicutes to a rather Western style diet than to a plant-based diet [19, 22]. Fiber degrading bacteria, such as *Prevotella* and *Faecalibacterium* tended to be higher vegans than in omnivores, but the differences were not significant in our study [10]. However, Bacteroides tended to be higher in omnivores than in vegans in our study. This has also been observed in other omnivorous populations [39] and animal-based diets [17]. We observed only a difference for the genus *Lachnoclostridium* and the species *Dialister invisus*, which were both higher in omnivores than

in vegans. Comparing with the results of the systematic review, lower abundances of *Dialister invisus* were reported by one vegetarian study (Zhang et al., 2016. cited in [10]), but no findings for Lachnoclostrium were reported.

As in our own study, we could not reveal a "vegan" or "vegetarian" microbiota composition in the systematic review [10]. As discussed comprehensively in the first publication [10], we observed a heterogeneity in the presented taxa, which could occur due to many factors: The time period of the conduction of the reviewed studies ranged from the 1970s to 2019 and therefore classical methods like bacterial counting were used alongside modern 16S rRNA sequencing methods to analyze the microbiota composition. However, even with modern technologies the detected microbiota is dependent on the choice of DNA-extracting methods and the selected sequencing libraries for the taxonomic classification [9], which could explain in part the heterogeneous results of the reviewed studies [10]. Though the reviewed studies compared populations from the same origin respectively, the geographical regions of each single study varied strongly. This could also contribute to different results in microbiota composition. Although a worldwide core microbiota exists [13], it remains doubtful if the microbiota compositions of populations with different regional origins can be compared in a systematic review. For example, in this review studies conducted in South American and in Asia were included, thus conducted in populations with different consumed food items, environmental exposure or lifestyle which could affect the microbiota in their own manner [10].
The choice of methods is crucial for the analysis of microbiota composition and it is recommended to use validated dietary assessment methods, standardized protocols for stool sample management and validated statistical methods to ensure reliable and reproducible results.

Bile acids and dietary pattern

Bile acids are strongly associated with diet and with microbiota composition. Members of the *Clostridium cluster IX* and *Bilophila wadsworthia* are involved in the conversion of primary bile acids into secondary bile acids [10, 18]. However, the abundances of these bacteria differed not significantly between vegans and omnivores in our study and in the other reviewed studies [10].
Elevated secondary bile acid concentrations are discussed with stimulated oxidative stress and an increased risk of colorectal cancer [20, 23, 24] and may act as negative

markers of gut health. In the second publication, bile acids in feces were compared with in vegans and omnivores [20]. Vegans had significantly lower fecal bile acids concentrations than omnivores, which may be explained by higher fat intake and meat consumption of omnivores, which is in line with other studies [22, 23]. The elevated concentrations of fecal secondary bile acids in omnivores suggest a higher bacterial activity and bile acid conversion attributed to diet. Moreover, in the second publication we applied a reduced rank regression and identified a dietary pattern characterized by a high intake of processed meat, margarine and fried potatoes, and a low intake of muesli [20]. Bile acid concentrations and the intake of fatty or animal derived food groups were positively associated and increased across the tertiles of this pattern. However, the proportion of vegans decreased across the dietary pattern [20].

In contrast, serum bile acid concentrations differed from our findings of fecal bile acid concentrations. Serum primary bile acids and glycine-conjugated bile acids were higher in vegans than in omnivores. As discussed comprehensively in the second publication [20], higher primary serum bile acids in vegans may be explained by higher degrees of reabsorption of bile acid, thus resulting in lower total fecal bile acid concentrations. Nevertheless, 7-alpha-hydroxy-4-cholesten-3-one (C4), as a precursor in bile acid synthesis did not differ between vegans and omnivores. Thus, diet seems to impact bile acid metabolism in later metabolic steps.

Limitations and strengths

Our study had some limitations. The study size of 72 participants in our study could result in a small statistical power to compute significant differences in microbiota composition between vegans and omnivores. Moreover, this study was initially powered to detect differences in bone health and not in microbiota composition. Only one stool sample was collected, which could make it difficult to represent dietary changes and therefore changes in bacterial abundances or bacterial metabolites over time. Nevertheless, the fast processing of the stool samples shortly after defecation can be seen as strength, for changes in bacteria and pH value were reduced. The composition of diet on the day before collecting the stool sample was not known of all participants, because the 3-day weighing protocols were recorded anytime during the first and second study visit. All participants were highly motivated to take part in this study and more than 67 % of the participants had a high-school degree. This high educational level may explain the low numbers of smokers and moderate levels of physical activity. On the other hand, this can be

seen as a limitation, because participants of epidemiological or nutritional studies are in general interested in health and diet, and their nutritional intake and lifestyle may not represent the general population.

Outlook

The role of microbiota and the pathways of its metabolites and thus the impact on human health status have not been fully understood. This also applies for bile acids, which are metabolites of the microbiota, and as well signaling molecules, affecting metabolic regulation. The microbiota, its metabolites and diet as an influencing factor, are a complex inter-play, and correlations should be seen in a network. Modern technologies and bioinformatics methods, such as genomics or metabolomics [30] follow this approach.

At our research unit it is planned to characterize the microbiota data of the RBVD study by their number (richness) and diversity in a more in-depth statistical analysis. In this planned study, microbiota data, metabolome and dietary datasets could be correlated. Clustering methods could be used to compare vegans and omnivores. Other fecal markers, such as short chain fatty acids out of bacterial fiber fermentation, ammonium and fecal pH may refer to microbiota activity and intestinal health status. This broadened perspective may contribute to understanding metabolic processes in more detail and to the role of a vegan diet in disease prevention in further research.

Western diets, rich in fat and sugar and low in fiber, are made responsible for an increasing prevalence of diet-related diseases in many industrialized but also in emerging countries. Simultaneously, solutions have to be found to achieve sustainable food security for the growing population throughout the world. Therefore, an international commission on nutrition advised in a recently published report, the doubling of the consumption of fruits, vegetables and legumes, and on the other hand the reduction by half of the consumption of meat [40]. Nutritional societies in westernized countries may consider advising a change of our nutritional behavior towards a plant-based diet.

Against this background, information about both the risks and benefits of a plant-based diet is becoming a public health topic and should be obligated by health professionals and educational institutions to reach the general public. Up-to-date, larger-scale studies observing the health status of vegans and vegetarians and dietary intakes are rare, particularly in Germany. Thus, a further study with a larger size is planned at the BfR. This study will investigate short and long term risks of as well as benefits of different types of

plant-based diets (vegan, vegetarian, pescetarian) compared to an omnivorous diet. The dietary assessment will be one of the main topics of this study. During the evaluation of our study results, the importance of a suitable nutrient database emerged. Due to the variety of new food items coming into market, especially high processed vegan food products, available databases may be not complete or out of date. The dietary assessment of vegans with detailed information about macro- and micronutrients may be limited when using these databases. Thus, the development of a suitable database is important to assess a vegan diet in future studies successfully.

Among other research questions like body composition or bone health, the microbiota and its metabolites will be a matter of interest in the planned study.

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8. Statutory Declaration

I, Iris Trefflich, by personally signing this document in lieu of an oath, hereby affirm that I prepared the submitted dissertation on the topic “A vegan diet and gut health - associations with microbiota and fecal bile acid concentrations” (“Vegane Ernährung und Darmgesundheit – Zusammenhänge mit Mikrobiota und Gallensäurekonzentrationen im Stuhl”), independently and without the support of third parties, and that I used no other sources and aids than those stated.
All parts which are based on the publications or presentations of other authors, either in letter or in spirit, are specified as such in accordance with the citing guidelines. The sections on methodology (in particular regarding practical work, laboratory regulations, statistical processing) and results (in particular regarding figures, charts and tables) are exclusively my responsibility.

My contributions to any publications to this dissertation correspond to those stated in the below joint declaration made together with the first supervisor. All publications created within the scope of the dissertation comply with the guidelines of the ICMJE (International Committee of Medical Journal Editors; www.icmje.org) on authorship. In addition, I declare that I am aware of the regulations of Charité – Universitätsmedizin Berlin on ensuring good scientific practice and that I commit to comply with these regulations.

The significance of this statutory declaration and the consequences of a false statutory declaration under criminal law (Sections 156, 161 of the German Criminal Code) are known to me.

Date Signature

9. Declaration of publications

Iris Trefflich had the following work share in the creation of the following manuscripts:

Publication 1

Authors: Iris Trefflich, Afraa Jabakhanji, Juliane Menzel, Michael Blaut, Andreas Michalsen, Alfonso Lampen, Klaus Abraham, Cornelia Weikert
Title: Is a vegan or a vegetarian diet associated with the microbiota composition in the gut? Results of a new cross-sectional study and systematic review
Journal: Critical review in food science and nutrition
Year: 2019

Contribution
The study design was planned in close collaboration with Prof. Dr. Cornelia Weikert. The literature research in the databases was conducted independently by two colleagues, Iris Trefflich was one of them. Iris Trefflich wrote the manuscript and discussed the progress and results with Prof. Dr. Weikert frequently. All statistical analyses were performed by Iris Trefflich. All tables and figures in the manuscript were created by Iris Trefflich (in this thesis, figure 6, figure 7, table 2, table 3, table 5). During the publication process, Iris Trefflich was responsibility for the communication with the co-authors and implemented their suggestions into the manuscript. As a corresponding author, she managed the communication and contact to the journal during the peer view process.

Publication 2:

Authors: Iris Trefflich, Hanns-Ulrich Marschall, Romina di Giuseppe, Marcus Ståhlman, Andreas Michalsen, Alfonso Lampen, Klaus Abraham, Cornelia Weikert
Title: Associations between dietary patterns and bile acids – results from a cross-sectional study in vegans and omnivores
Journal: Nutrients
Year: 2019

Contribution

The study design was planned in close collaboration with Prof. Dr. Cornelia Weikert. The statistical method was chosen in collaboration with All statistical analyses were performed by Iris Trefflich.. All statistical analyses were performed by Iris Trefflich. Iris Trefflich wrote the manuscript and discussed the progress and results with Prof. Dr. Weikert frequently. All tables and figures in the manuscript were created by Iris Trefflich based on her calculation (in this thesis, figures 4, figure 5, figure 8, table 3, table 4, table 6 - 8). Due to the use of a special graphical program, a colleague supported the creation of figures 1 and 2 in the second manuscript. During the publication process, Iris Trefflich was responsibility for the communication with the co-authors and implemented their suggestions into the manuscript. As a corresponding author, she managed the communication and contact to the journal during the peer view process.

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Date Signature

10. Printed copies of publications

Publication 1:

Trefflich I, Jabakhanji A, Menzel J, Blaut M, Michalsen A, Lampen A, Abraham K, Weikert C. Is a vegan or a vegetarian diet associated with the microbiota composition in the gut? Results of a new cross-sectional study and systematic review. Critical reviews in food science and nutrition. 2019 (published online October 21, 2019). DOI: 10.1080/10408398.2019.1676697

Extract of Journal Summary List

Journal Data Filtered By: **Selected JCR Year: 2017** Selected Editions: SCIE,SSCI Selected Categories: **"NUTRITION and DIETETICS"** Selected Category Scheme: WoS

Gesamtanzahl: 81 Journale

Rank	Full Journal Title	Total Cites	Journal Impact Factor	Eigenfactor Score
1	Annual Review of Nutrition	5,528	8.886	0.005230
2	PROGRESS IN LIPID RESEARCH	5,302	8.435	0.006750
3	Advances in Nutrition	3,937	6.853	0.012870
4	AMERICAN JOURNAL OF CLINICAL NUTRITION	58,213	6.509	0.055760
5	CRITICAL REVIEWS IN FOOD SCIENCE AND NUTRITION	10,197	6.015	0.011670
6	NUTRITION REVIEWS	7,526	5.788	0.010600
7	International Journal of Behavioral Nutrition and Physical Activity	8,371	5.548	0.019780
8	CLINICAL NUTRITION	10,558	5.496	0.016870
9	PROCEEDINGS OF THE NUTRITION SOCIETY	5,238	5.347	0.006230
10	INTERNATIONAL JOURNAL OF OBESITY	22,185	5.151	0.032040
11	FOOD CHEMISTRY	90,665	4.946	0.101120
12	NUTRITION RESEARCH REVIEWS	2,164	4.586	0.001840
13	CURRENT OPINION IN CLINICAL NUTRITION AND METABOLIC CARE	4,842	4.534	0.007130
14	EUROPEAN JOURNAL OF NUTRITION	5,669	4.423	0.011650
15	JOURNAL OF NUTRITIONAL BIOCHEMISTRY	9,815	4.414	0.014150
16	JOURNAL OF NUTRITION	38,804	4.398	0.029930
17	JOURNAL OF PARENTERAL AND ENTERAL NUTRITION	5,287	4.249	0.007990
18	Nutrients	12,031	4.196	0.032520
19	Obesity	17,578	4.042	0.037840
20	Journal of the Academy of Nutrition and Dietetics	3,687	4.021	0.014370
21	INTERNATIONAL JOURNAL OF EATING DISORDERS	8,732	3.897	0.010160
22	NUTRITION	10,167	3.734	0.013010
23	BRITISH JOURNAL OF NUTRITION	26,011	3.657	0.035400
24	Nutrition Journal	4,484	3.568	0.009540

Bei der nachfolgenden Publikation handelt es nicht um eine Open Access Publikation.

https://doi.org/10.1080/10408398.2019.1676697

Publication 2:

Trefflich I, Marschall HU, di Giuseppe R, Ståhlman M, Michalsen A, Lampen A, Abraham K, Weikert C: Associations between dietary patterns and bile acids – results from a cross-sectional study in vegans and omnivores. Nutrients. 2019;12(1).

Extract of Journal Summary List

Journal Data Filtered By: **Selected JCR Year: 2018** Selected Editions: SCIE,SSCI
Selected Categories: **"NUTRITION and DIETETICS"** Selected Category Scheme: WoS
Gesamtanzahl: 86 Journale

Rank	Full Journal Title	Total Cites	Journal Impact Factor	Eigenfactor Score
1	PROGRESS IN LIPID RESEARCH	5,839	12.540	0.007160
2	Annual Review of Nutrition	5,565	8.422	0.004320
3	Advances in Nutrition	4,896	7.240	0.012150
4	CRITICAL REVIEWS IN FOOD SCIENCE AND NUTRITION	12,591	6.704	0.015020
5	AMERICAN JOURNAL OF CLINICAL NUTRITION	59,513	6.568	0.053470
6	CLINICAL NUTRITION	12,594	6.402	0.018240
7	International Journal of Behavioral Nutrition and Physical Activity	9,914	6.037	0.020780
8	NUTRITION REVIEWS	8,400	5.779	0.009290
9	NUTRITION RESEARCH REVIEWS	2,313	5.595	0.001920
10	FOOD CHEMISTRY	104,574	5.399	0.103870
11	PROCEEDINGS OF THE NUTRITION SOCIETY	5,722	5.017	0.005460
12	INTERNATIONAL JOURNAL OF OBESITY	22,929	4.514	0.030070
13	JOURNAL OF NUTRITIONAL BIOCHEMISTRY	10,544	4.490	0.012700
14	EUROPEAN JOURNAL OF NUTRITION	6,730	4.449	0.012210
15	JOURNAL OF NUTRITION	39,454	4.416	0.026850
16	Nutrients	19,332	4.171	0.047140
17	Journal of the Academy of Nutrition and Dietetics	4,815	4.141	0.013970
18	JOURNAL OF PARENTERAL AND ENTERAL NUTRITION	5,848	4.109	0.008210
19	Obesity	18,844	3.969	0.036270
20	NUTRITIONAL NEUROSCIENCE	1,778	3.950	0.002260

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Associations between Dietary Patterns and Bile Acids—Results from a Cross-Sectional Study in Vegans and Omnivores

Iris Trefflich [1,2,*], Hanns-Ulrich Marschall [3], Romina di Giuseppe [4], Marcus Ståhlman [3], Andreas Michalsen [2], Alfonso Lampen [1], Klaus Abraham [1] and Cornelia Weikert [1,2]

1 Department of Food Safety, German Federal Institute for Risk Assessment, 10589 Berlin, Germany; Alfonso.Lampen@bfr.bund.de (A.L.); klaus.abraham@bfr.bund.de (K.A.); cornelia.weikert@bfr.bund.de (C.W.)
2 Institute for Social Medicine, Epidemiology and Health Economics, Charité-University Medicine, 10589 Berlin, Germany; andreas.michalsen@charite.de
3 Department of Molecular and Clinical Medicine/Wallenberg Laboratory, University of Gothenburg, 405 30 Gothenburg, Sweden; hanns-ulrich.marschall@gu.se (H.-U.M.); Marcus.Stahlman@wlab.gu.se (M.S.)
4 Institute for Epidemiology, University Kiel, 24105 Kiel, Germany; romina.digiuseppe@epi.uni-kiel.de
* Correspondence: iris.trefflich@bfr.bund.de

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Abstract: Bile acids play an active role in fat metabolism and, in high-fat diets, elevated concentrations of fecal bile acids may be related to an increased risk of colorectal cancer. This study investigated concentrations of fecal and serum bile acids in 36 vegans and 36 omnivores. The reduced rank regression was used to identify dietary patterns associated with fecal bile acids. Dietary patterns were derived with secondary and conjugated fecal bile acids as response variables and 53 food groups as predictors. Vegans had higher fiber ($p < 0.01$) and lower fat ($p = 0.0024$) intake than omnivores. In serum, primary and glycine-conjugated bile acids were higher in vegans than in omnivores ($p \leq 0.01$). All fecal bile acids were significantly lower in vegans compared to omnivores ($p < 0.01$). Processed meat, fried potatoes, fish, margarine, and coffee contributed most positively, whereas muesli most negatively to a dietary pattern that was directly associated with all fecal bile acids. According to the pattern, fat intake was positively and fiber intake was inversely correlated with bile acids. The findings contribute to the evidence that, in particular, animal products and fat may play a part in higher levels of fecal bile acids.

Keywords: vegan diet; fecal and serum bile acids; dietary pattern; reduced rank regression

1. Introduction

An increasing trend toward plant-based diets and, in particular, vegan diets was observed in developed countries over the last few years [1,2]. A vegan diet is defined as a diet without consumption of any animal products and is supposed to be rich in fiber and low in fat [3]. Due to this dietary composition, vegans and vegetarians tend to have a lower body mass index (BMI), which is considered beneficial to positive health effects against the onset of obesity or type 2-diabetes [4,5]. Two comprehensive meta-analyses demonstrated that a vegan diet is associated with lower prevalence of cardio-metabolic risk factors [6] and a lower risk of total cancer [7]. Colorectal cancer (CRC) is one of the most common cancer types, and incidence rates are increasing in Western countries [8]. CRC is strongly associated with meat intake, whereas fiber intake shows an inverse relationship [9]. Due to its absorptive and viscous properties, fiber interacts with cholesterol and, thus, with bile acid

metabolism [10]. This association between diet and fecal bile acid concentrations was observed in several studies [11–14].

One step in the complex synthesis of bile acids from cholesterol is the formation of 7-alpha-hydroxy-4-cholesten-3-one (C4) in the liver. C4 is a valid serum biomarker for the synthesis of primary bile acids cholic acid (CA) and chenodeoxycholic acid (CDCA) [15,16]. Primary bile acids are conjugated in the liver with glycine or taurine to increase solubility before excretion into bile; the conjugation ratio of glycine–taurine is about 3:1 in humans [17]. Primary bile acids are released postprandially [18], and bile acid concentrations in plasma increased and remained elevated for a longer period after a high-fat/high-energy intake than after carbohydrate intake [19]. Bile acids build micelles, which contribute to the absorption of intestinal cholesterol, triglycerides, and fatty acids [20]. Bacteria of the intestinal microbiota are involved in dehydroxylation at the C-7 atom of CA and CDCA into the secondary bile acids deoxycholic acid (DCA) and lithocholic acid (LCA). Gut bacteria are also involved in epimerization of ursodeoxycholic acid (UDCA) from primary or secondary bile acids [18].

Approximately 95% of bile acids are reabsorbed from the intestine, transported back to the liver via the portal vein, and excreted into bile, to start the enterohepatic circulation once again. The amount of bile acids excreted with feces is equal to the synthesized amount of 0.2–0.6 g/day in adults [21].

Bile acids can stimulate oxidative stress and DNA damage due to their hydrophobicity [22], and they induce the apoptosis resistance of epithelial colon cells [23], which could be related to both inflammatory bowel diseases and colorectal cancer etiology [17,21,23–26]. Furthermore, bile acids are also signaling molecules. They are principally involved in metabolic regulation and energy expenditure [27], and they are linked to lipid and glucose metabolism via the farnesoid X receptor (FXR), a regulator of the enterohepatic circulation of bile acids [28].

Nutrients are not consumed separately, and diets consist of combinations of different food groups and nutrients. Therefore, dietary pattern analyses provide a better understanding of nutrient interaction [29]. One method identifying dietary patterns is reduced rank regression (RRR), an established method to derive dietary patterns, which was applied in nutritional epidemiology [30,31]. RRR establishes linear combinations of foods or food items (used as predictor variables) with a maximum of a possible variation in disease-related nutrients or biomarkers (used as response variables) [32,33]. RRR is yet to be used within the context of any fecal biomarkers.

However, to date, the effect of a vegan diet on fecal bile acid levels was only investigated in small short-term (a few days or weeks) intervention studies [34,35], but not in a population adhering to a vegan diet for a longer period—at least one year. In addition, there are no data available on serum bile acids in vegans.

The aim of this study was twofold. Firstly, we aimed to compare serum and fecal bile acid concentrations between vegans and omnivores. Secondly, we aimed to identify dietary patterns explaining the variation in fecal bile acid concentrations to identify combinations of food groups contributing to bile acid levels in stools.

2. Materials and Methods

Between January and July 2017, 36 vegan and 36 omnivorous persons were recruited for the Risk and Benefits of a Vegan Diet study (RBVD) at the Federal Institute for Risk Assessment, Berlin (BfR) (see study flow chart, Figure S1, Supplementary Materials). Inclusion criteria were ages between 30 and 60 years and a body mass index < 30 kg/m^2. Each type of diet was to have been followed for at least one year. Exclusion criteria were acute infection, serious diseases, pregnancy or breastfeeding, and taking of proton pump inhibitors or glucocorticoids. Furthermore, the consumption of at least three portions of meat per week or two portions of meat and two portions of processed meat per week was defined as an omnivorous diet, whereas a vegan diet was defined by no consumption of any animal food products. All participants visited the study center twice. On the first visit, the participants were comprehensively informed about the study details and signed the informed consent. On the second visit, lifestyle and basic characteristics were recorded using questionnaires, and a fasting blood

sample of 60 mL was taken. Blood lipids, and parameters of the hemogram were analyzed in 30 mL of the sample in a certificated laboratory on the same day. The remaining half was stored at −80 °C for further analysis for serum bile acids, among other elements. The study was approved by the Ethics Committee of the Charité University Medical Center Berlin (No. EA4/121/16).

2.1. Collection of Fecal Samples

The participants received a collection device (Fecotainer®, AT Medical BV, Enschede, The Netherlands) to collect an entire stool sample at home on the morning of the second visit to the study center. The time of bowel movement, beginning of storage, and time of delivery to the study center were documented by the participants on a form, as well as the frequency of bowel movements and intake of antibiotics within the last two months. To avoid microbial changes, samples were no older than four hours when processed at the study center. Stool samples were weighed and homogenized for 15 min using a stomacher, partitioned into different aliquots, and stored at −80 °C for further analysis.

2.2. Analysis of Bile Acids in Stool and Serum

Determination of bile acids in serum and stool was performed in a randomized and blinded manner at the University of Gothenburg, Department of Molecular and Clinical Medicine/Wallenberg Laboratory, Sweden.

For serum, 50 µL of sample was extracted with 500 µL of methanol containing deuterated internal standards (d4-TCA, d4-GCA, d4-GCDCA, d4-GUDCA, d4-GLCA, d4-UDCA, d4-CDCA, d4-LCA d4-C4; 50 nM of each). After 10 min of vortex and 10 min of centrifugation at 20,000× *g*, the supernatant was evaporated under a stream of nitrogen and reconstituted in 200 µL of methanol–water (1:1).

For feces, 30–80 mg of the sample (wet weight) was extracted in 500 µL of methanol containing 2.5 µM of the internal standards stated above. The extraction was made in 2-mL polypropylene tubes filled with six zirconium oxide beads (3 mm). Extensive homogenization and extraction took place for 10 min at 25 Hz using a TissueLyzer II instrument (Retsch GmbH, Haan, Germany). After centrifugation at 20,000× *g*, the samples were diluted 1:100 in methanol–water [1:1].

The samples were injected (5 µL) and bile acids were separated on a C18 column (1.7 µm, 2.1 × 100 mm; Kinetex, Phenomenex, Torrance, CA, USA) using water with 7.5 mM ammonium acetate and 0.019% formic acid (mobile phase A) and acetonitrile with 0.1% formic acid (mobile phase B). The chromatographic separation started with 1-min isocratic separation at 20% B. The B-phase was then increased to 35% over 4 min. During the next 10 min, the B-phase was increased to 100%. The B-phase was held at 100% for 3.5 min before returning to 20%. The total runtime was 20 min. Bile acids were detected using multiple reaction monitoring (MRM) in negative mode (C4 in positive mode) using a QTRAP 5500 mass spectrometer (Sciex, Concord, Canada), and quantification was carried out using external standard curves.

Non-labeled bile acids were attained from Sigma-Aldrich (Stockholm, Sweden), and the deuterated acids were from CDN Isotopes (Quebec, Canada) or Toronto Research Chemicals (North York, Canada) (d4-TCA).

2.3. Dietary Assessment

On the first visit, the participants received a detailed explanation of how to record foods and beverages from the study center, and they were provided with three-day weighed food records and digital kitchen scales to record their diet for two weekdays and one weekend day. All 72 participants fully completed their food records when they returned for the second visit to the study center. The data of the food records were captured in EAT software, version 3.5.5 (University of Paderborn, Paderborn, Germany), where each food item was assigned to the German national food code (Bundeslebensmittelschlüssel Version 3.02, BLS). For food items without available food codes, new codes were generated on the basis of ingredients lists on the packaging or were requested from the producers. Ingredients of cooked dishes were converted from recipes into effective quantities of

consumed portions. To estimate the weight of cooked food items, yield and retention factors were taken into account [36]. Yield and retention factors of single food items were summarized into superordinate food groups for simplification (Table S1, Supplementary Materials). After data entry, as well as quality and plausibility checks, the food data were merged with the BLS to assign macro- and micronutrients. All food data were averaged to achieve daily food intakes.

After assigning the food items to the BLS code, they were categorized into 49 food groups according to food groups used in European Prospective Investigation into Cancer and Nutrition (EPIC) Potsdam study [37]. New food groups with items typical for a vegan diet, namely, plant-based milk alternatives, meat alternatives, savory vegetable spreads, and soft drinks, were defined and added to the original food groups; thus, consequently, 53 food groups were available (Table S2, Supplementary Materials). Food groups, with vegan and non-vegan food items such as egg found in pasta, cookies, and sweets, were captured separately for each diet.

2.4. Assessment of Lifestyle Characteristics

The body weight, height, and waist circumference of the study participants were measured by trained study personnel. Levels of education, lifestyle characteristics such as smoking behavior, alcohol consumption, and physical activity, and medical history were acquired using computer-assisted questionnaires. Physical activity was defined as the sum of average number of hours spent cycling, doing sports, and gardening during the summer and winter per week.

2.5. Statistics

Characteristics of the study population, status of nutrients, food groups, and biochemical continuous variables are presented as means and standard deviation for normally distributed variables or as medians and interquartile range (IQR) for variables which are not normally distributed. Categorical variables are presented as percentages. For categorical variables, chi-square or Fisher's exact test were used, and, for continuous variables, the Mann–Whitney U test or *t*-test (for normally distributed variables) were applied.

The method of reduced rank regression (RRR) is applied in nutritional epidemiology [32] to derive dietary patterns and to identify the linear combination of predictor variables which explain the largest proportion of variation in response variables [38]. In this analysis, the standardized intake of 53 food groups out of the three-day weighed food records was used as predictors, and log-transformed secondary bile acids DCA, LCA, and UDCA, as well as the sum of primary and secondary conjugated bile acids with glycine or taurine, were as responses. The secondary bile acids were selected due to their association with an increased risk of inflammatory bowel diseases and CRC [17,21,23–26], and conjugated bile acids were chosen as response variables due to their close relationship to diet [17]. The number of derived patterns is equal to the number of response variables; thus, five patterns were identified. To guarantee that the observed variation of fecal markers mirrored the different profiles of vegans and omnivores, the RRR patterns were derived within the pooled data of vegans and omnivores and not by splitting the data by diet [39]. Trends of biomarker concentrations and macronutrient intakes across pattern scores were calculated in logistic models by categorizing the score into tertiles. To calculate the dietary pattern, each food group with a factor loading >0.2 was identified and taken into account.

All statistical analyses were conducted using SAS (version 9.3, SAS Institute Inc., Cary, NC, USA).

3. Results

3.1. Study Population

In total, 36 vegans and 36 omnivores were included in the RBVD study. The main characteristics of the study population are described in Table 1. No significant differences in body weight, BMI,

education level, physical activity, and smoking status were observed between the vegan and omnivorous study group.

Table 1. Characteristics of the Risk and Benefits of a Vegan Diet study (RBVD) population.

	Vegan (*n* = 36)	**Omnivore (*n* = 36)**	***p***
Male (%)	50%	50%	
Age (years)	37.5 (32.5–44.0)	38.5 (32.0–46.0)	0.75
Body weight (kg)	70.1 ± 13.9	73.6 ± 10.3	0.24
BMI	22.9 ± 3.2	24.0 ± 2.1	0.08
Cholesterol (mg/dL)	165.9 ± 34.9	205.4 ± 41.6	<0.0001
Duration of vegan diet (years)	4.8 (3.1–8.7)	n.a.	
Education (*n* (%))			0.60
Low	0 (0.0)	1 (2.8)	
Intermediate	11 (30.6)	11 (30.6)	
High	25 (69.5)	24 (30.6)	
Physical activity (h/week)	2.8 (0.88–3.75)	2.3 (1.2–4.1)	0.69
Smoking behavior (*n* (%))			0.30
Never smoker	24 (66.7)	21 (58.3)	
Ex-smoker	8 (22.2)	6 (16.7)	
Smoker	4 (11.1)	9 (25)	
Stool			
Weight (mg)	102.6 (39.9–185.9)	97.3 (47.4–157.6)	0.79
Processing time (h:min)	2:48 (1:49–3:45)	2:32 (1:34–3:38)	0.47

Data are reported as means (± SD) for normally distributed variables, as medians (quartile (Q)1–Q3) for variables not normally distributed, or as percentage (%). Low education: no degree; intermediate education: vocational school, technical college; high education: university, university of applied sciences. Statistical tests were carried out using the *t*-test for normally distributed variables, Mann–Whitney U test for variables not normally distributed, and chi^2 for categorical variables. BMI = body mass index; n.a. = not applicable.

3.2. Intake of Macro-Nutrients and Food Groups Derived from Three-Day-Weighing Records

Total energy intake did not differ significantly between vegans ($p = 0.49$). Vegans had a significantly lower intake of fat (87.6 mg/day, IQR 64.1–116.2), compared to omnivores (fat 104.1 mg/day, IQR 87.8–143.3) ($p = 0.024$). Fiber intake was significantly higher in vegans (45.6 g/day, IQR 33.7–58.2) than in omnivores (23.7 g/day, IQR 18.6–29.9) ($p < 0.0001$). Median intakes of all food groups are shown in Table S3 (Supplementary Materials).

3.3. Concentrations of Bile Acids in Feces and Serum

The fecal bile acid profiles showed significant differences between a vegan and omnivorous diet (Figure 1). Vegans had significantly lower levels of total bile acids (564 nmol/g, IQR 195–1261) than omnivores (1667 nmol/g, IQR 804–5092) ($p < 0.01$). In particular, all secondary and both glycine- and taurine-conjugated bile acids were lower in vegans than in omnivores ($p < 0.01$). Levels of primary CA tended to be higher in the omnivorous group (1.87 nmol/g, IQR 0.57–6.82) than in the vegan group (0.74 nmol/g, IQR 0.18–9.43) ($p = 0.18$). Moreover, there was no difference in CDCA between vegans (0.00 IQR 0.00–2.93) and omnivores (0.00, IQR = 0.00–0.40) ($p = 0.77$).

Vegan participants had significantly higher total bile acid serum concentrations than omnivores ($p = 0.001$) (Figure 2). Primary bile acids CA ($p = 0.002$) and CDCA ($p = 0.01$) were higher in vegans compared to the omnivorous group. Vegans had higher levels of total glycine-conjugated bile acids (1.32 µmol/L, IQR 0.81–2.53) than omnivores (0.79 µmol/L, 0.47–1.04) ($p = 0.001$). The secondary bile acids DCA, LCA, and UDCA did not differ significantly between vegans and omnivores. C4 levels in serum did not differ between vegans (35.62, IQR 23.19–50.91) and omnivores (30.13 nmol/L, IQR 16.21–47.41) ($p = 0.36$).

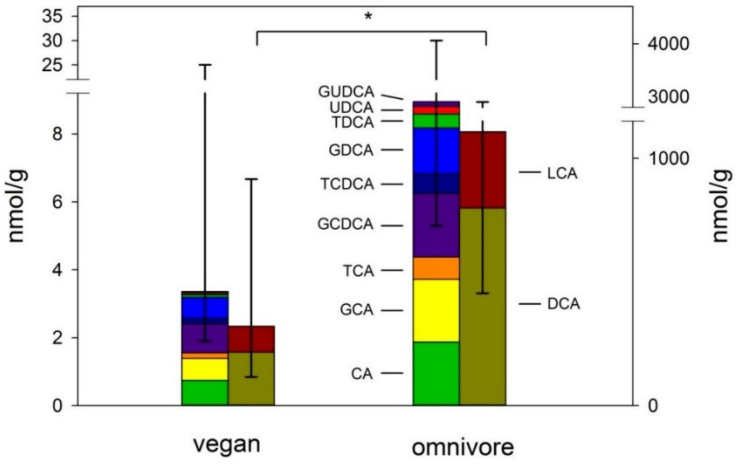

Figure 1. Fecal bile acid concentrations in vegans and omnivores of the Risk and Benefits of a Vegan Diet study (RBVD) study (nmol/g). Data are presented as medians and interquartile range (IQR) for total fecal bile acids. Mann–Whitney U test was used for bile acids (* $p \leq 0.001$). Values for secondary bile acids are presented on right *y*-axis, while primary and conjugated bile acids are presented on left y-axis. CA= Cholic acid, GCA = glycine-conjugated CA, TCA = taurine-conjugated CA, GCDCA = glycine-conjugated Chenodeoxycholic acid, TCDCA = taurine-conjugated CDCA, DCA = Deoxycholic acid, GDCA = glycine-conjugated DCA, TDCA = taurine-conjugated DCA, UDCA = Ursodeoxycholic acid, GUDCA = glycine-conjugated UDCA, LCA = Lithocholic acid, DCA = Deoxycholic acid.

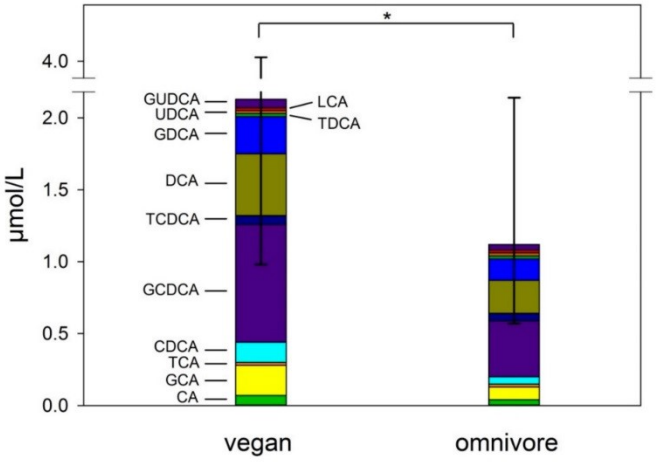

Figure 2. Serum bile acids concentrations in vegans and omnivores of the RBVD study (µmol/L). Data are presented as medians and IQR for total serum bile acids. Mann–Whitney U test was used for bile acids (* $p \leq 0.001$). CA = Cholic acid, GCA = glycine-conjugated CA, TCA = taurine-conjugated CA, CDCA = Chenodeoxycholic acid, GCDCA= glycine-conjugated CDCA, TCDCA = taurine-conjugated CDCA, DCA = Deoxycholic acid, GDCA = glycine-conjugated DCA, TDCA = taurine-conjugated DCA, UDCA = Ursodeoxycholic acid, GUDCA = glycine-conjugated UDCA, LCA = Lithocholic acid.

3.4. Dietary Pattern Explaining Variance in Fecal Bile Acids

The first reduced rank regression-derived dietary pattern explained 47.4% of the variance in bile acids, mostly driven by the explained variance in glycine-conjugated bile acids (69.1%) and taurine-conjugated bile acids (54.8%). This pattern consisted of positive loadings for coffee, fish, margarine, fried potatoes, bread, and processed meat, and a negative loading for muesli (Figure 3). Factor loadings of all food groups are presented in Table S4 (Supplementary Materials).

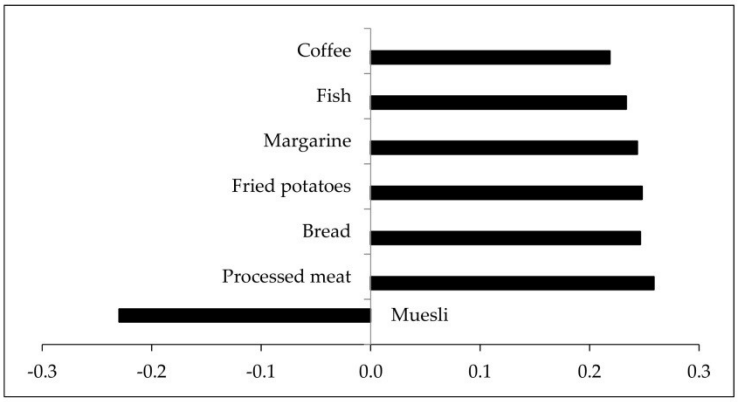

Figure 3. Factor loadings of all 53 food groups in first dietary pattern score. Factor loadings are correlations between food groups and the dietary pattern score. Food groups with factor loadings >0.2 were chosen for the dietary pattern.

The concentrations of DCA, LCA, and conjugated bile acids used as response variables were significantly higher across the tertiles of the dietary pattern score (Table 2), whereas the proportion of vegans was lower across the score. The first tertile included 75% vegans, the second tertile included 50% vegans, and the third tertile included 25% vegans.

Table 2. Concentrations of fecal bile acids across first pattern score.

	Tertiles of Dietary Pattern Score			
Bile Acids (nmol/g)	**1 (75% Vegans)**	**2 (50% Vegans)**	**3 (25% Vegans)**	***p*-Value for Trend**
DCA	74.23 (3.22–195.81)	586.21 (283.57–1041.85)	1327.30 (644.51–3037.96)	<0.0001
LCA	64.20 (13.99–93.47)	198.93 (120.73–327.30)	417.49 (305.10–1070.67)	<0.0001
UDCA	0.01 (0.00–0.16)	0.16 (0.00–0.64)	1.07 (0.00–16.91)	0.02
Total glycine-con.	0.73 (0.32–1.52)	3.45 (2.43–5.95)	9.06 (5.76–18.81)	<0.0001
Total taurine-con.	0.26 (0.05–0.50)	1.05 (0.49–2.00)	3.25 (1.71–8.65)	<0.0001

Concentrations of fecal bile acids are presented as medians (Q1–Q3). DCA = deoxycholic acid, LCA = lithocholic acid, UDCA = ursodeoxycholic acid, con. = conjugated.

Fiber intake decreased across the tertiles of the first dietary pattern ($p = 0.01$). Intakes of fat, as well as saturated and unsaturated fatty acids, increased across the tertiles of the pattern, although this was only significant for saturated fatty acids ($p = 0.0005$) (Table 3).

Table 3. Intake of macronutrients according to tertiles of the first dietary pattern.

	Tertiles of Dietary Pattern Score			
Macronutrients (g/day)	**1 (75% Vegans)**	**2 (50% Vegans)**	**3 (25% Vegans)**	***p*-Value for Trend**
Fiber	37 (25–55)	32 (27–50)	24 (17–34)	0.01
Protein	75 (53–122)	90 (68–107)	78 (69–103)	0.43
Fat	92 (64–109)	100 (83–124)	98 (85–152)	0.05
Carbohydrates	238 (203–310)	236 (205–297)	270 (211–356)	0.37
Sucrose	46 (34–61)	53 (37–68)	60 (41–81)	0.19
Unsaturated fatty acids	34 (23–40)	34 (28–43)	33 (27–49)	0.25
Saturated fatty acids	19 (10–33)	36 (16–44)	34 (26–52)	0.0005

Data are presented as medians (Q1–Q3).

The second dietary pattern explained 27.5% of the bile acid variance. This pattern was characterized by positive loadings for fried potatoes (0.4), margarine (0.35), high-fat plant-based milk products (0.27), potato chips (0.26), sauce (0.24), bread (0.21), and confectionery (0.20). DCA levels were lower across the tertiles of this dietary pattern, whereas levels of UDCA and taurine-conjugated bile acids were higher.

The remaining three dietary patterns explained only 5.1%, 2.7%, and 0.5% of bile acid variation and are not further discussed in this study.

4. Discussion

To the best of our knowledge, this is the first study investigating associations between serum bile acids in vegans and omnivores, and data on fecal bile acids in vegans are rare.

As expected, we observed higher dietary fiber intake and lower fecal bile acids in vegans compared to omnivores. Therefore, our results may confirm the association of lower fecal bile acids concentrations with high-fiber and low-fat diets [11,12,34], such as a vegan diet. In contrast, our results in serum in vegans compared to omnivores were rather unexpected. We found increased serum but decreased fecal bile acid concentrations in vegans as compared to omnivores. The increased amounts of serum bile acids in vegans were mostly due to increased levels of unconjugated primary bile acids, CA and CDCA, and glycine-conjugated bile acids. This might be explained by higher degrees of reabsorption of bile acid from terminal ileum (glycine-conjugated bile acid) and unconjugated primary bile acids (CA and CDCA) from colon, thus resulting in lower total fecal bile acid concentrations. These changes are nonetheless within physiological ranges, as hepatic bile acid synthesis, estimated by C4, was similar in both vegans and omnivores.

Although we observed significant differences in concentrations of bile acids in serum and feces between vegans and omnivores, the composition of bile acids differed only modestly between the two groups. Overall, the compositions of serum and fecal bile acids profiles in our study were in line with other observations of healthy populations with a high proportion of secondary bile acids in feces and conjugated bile acids in serum [40].

In healthy populations, glycine-conjugated bile acids have the highest proportion of total bile acids in serum [19,41–43], followed by the secondary bile acid DCA [41,42], in agreement with the observed serum bile acid profiles in both vegans and omnivores of our study. The conjugation of bile acids with either amino acid taurine or glycine may depend on diet. A diet high in animal products contains more taurine, meaning that taurine conjugation is more strongly linked to meat consumption [25], for example. Despite higher excreted taurine-conjugated bile acids in stools of omnivores, serum levels of taurine-conjugated bile acids did not differ between vegans and omnivores in our study. The conjugation of bile acids with glycine is associated with the intake of vegetables and carbohydrates due to the abundance of this amino acid in these food groups [20,25]. Thus, the higher intake of these food groups in vegans compared to omnivores in our study was in line with higher glycine-conjugated bile acids in serum in vegans and may confirm this hypothesis. Interestingly, fecal glycine-conjugated

bile acid concentrations were lower in vegans than in omnivores, and this observation needs to be confirmed in further studies before drawing any conclusion.

In line with other cross-sectional studies in vegan populations [44–46], we observed a significant higher fiber intake in vegan participants compared to omnivorous participants. Due to their absorptive and viscous properties, fibers are suggested to "bind" bile acids by forming micelles with bile acids [47]. This interaction depends on the hydrophobic structure of bile acids, and, in an in vitro study, the more hydrophobic bile acids DCA and CDCA were absorbed more than CA [48]. The absorption rate may also depend on the type of fiber; thus, cellulose showed a higher binding capacity than lignin [10], and, among vegetables, kale showed a high binding of bile acids [49] in vitro. The micelles are suggested to inhibit the re-absorption of bile acids [50], and to enhance their excretion in stool [47]. However, our findings of higher fecal bile acid concentrations in omnivores and higher serum bile acid levels in vegans do not support this hypothesis and further human studies are needed to clarify the relevance of this mechanism in vivo.

We observed a significantly lower fat intake in vegans than in omnivores, which is line with other European cross-sectional studies investigating nutrient intake in vegan populations [44–46]. Fat intake alters bile acid concentrations [18]. In a previous study of healthy participants after three months of a high-fat/high-beef diet, fecal total and secondary bile acids increased compared to the levels observed in those participants following an ongoing mixed diet [14]. The effects of changes from animal-based to plant-based diets or vice versa on fecal bile acid concentrations were investigated in very small intervention studies. In a Dutch study, 12 participants were randomized to a vegetarian, vegan, or mixed diet group for 20 days, and total fecal bile acids and DCA decreased in the vegan and vegetarian groups compared to the mixed group [35]. In another recent cross-over study with 10 participants, total fecal bile acids and DCA increased after five days of consuming a diet with animal products compared to the plant-based diet [34]. After a two-week diet change from a high-fiber rural diet to a high-fat Westernized diet, fecal secondary bile acids increased in 20 native Africans [11]. In our cross-sectional study, concentrations of total fecal bile acids and secondary bile acids were lower in vegans than in omnivores and in agreement with the results of these short-term intervention studies.

Additionally, we applied RRR to derive dietary patterns explaining the variation in fecal bile acids. Another method for deriving dietary pattern is principal component analysis (PCA), which explains the maximum variation of food intakes. In contrast to PCA, the RRR approach allows the derivation of dietary patterns associated with biomarkers, thereby relating them to specific pathways or disease risk [32]. This is of interest in our study, since secondary bile acids are discussed with an increased risk of CRC, and, due to their role as signaling molecules, bile acids are part of metabolic diseases such as dyslipidemia [51] or type 2 diabetes [52].

A high score for the first pattern was characterized by a positive correlation with processed meat, fish, margarine, fried potatoes, and coffee, and a negative correlation with muesli. Although the consumption of fish, fried potatoes, and margarine was only observed in the 75th percentile of the participants, the derived dietary pattern explained 47.4% variance of bile acids and confirmed the applicability of the RRR as a statistical method.

As expected due to known [12,14,18] and discussed associations between diet and bile acid metabolism, dietary fat showed a positive, but fiber intake a negative correlation with this dietary pattern. The proportion of vegans decreased across the pattern score.

Secondary fecal bile acid concentrations increased across the tertiles of the first pattern. Accordingly, the concentrations of DCA and LCA reached higher levels in the pattern compared to observed concentrations in stools of omnivores. This might be explained by the increasing fat intake across the pattern and, thus, the increasing proportion of omnivores across the pattern. The variance of the derived pattern was mostly driven by glycine-conjugated bile acids, which is in line with the high proportion of glycine-conjugated bile acids in enterohepatic circulation.

This positive correlation of bile acids with the derived pattern confirms the impact of food products rich in fat and particularly of animal origin on elevated bile acid concentrations [11,12,17], as also

described in previous studies. In particular, processed meat, which is high in cholesterol and saturated fatty acids [53], contributed to this dietary pattern. Increasing intake of saturated fatty acids in the derived pattern was in line with this observation. Moreover, our derived dietary pattern included fried potatoes, which were enriched with fat after processing, and their intake was correlated with higher bile acid concentration across the pattern. Yet, due to their fat content, the identification of processed meat, margarine, and fried potatoes as major food groups in this pattern was in line with a recent population based study, which observed a positive correlation between intakes of meat, processed meat, potatoes, and vegetables oils and fecal bile acid concentrations [54].

Nevertheless, the first derived pattern was inversely correlated with muesli intake, a food containing cereals and rich in fiber. This can be interpreted as in line with a study in Finnish women, which showed that, after adding rye bread (high in fiber) to the normal diet for two weeks, total fecal bile acids were lower compared to the baseline diet or after the consumption of low-fiber bread [55]. Moreover, the inverse association between fiber intake and the pattern score may support the hypothesis of the bile acid-binding capacities of fiber.

A few limitations merit consideration. A weakness of our study is that we did not estimate fecal bile acid excretion rates. Rather, bile acid concentrations were analyzed in aliquots of homogenized fresh stool samples instead of dry matter. Measurements of fecal bile acid excretion rates would have demanded complete stool collections over several days, which is notoriously infeasible in an ambulatory study setting outside dedicated clinical trial facilities. Rather, we presented significant differences in fecal bile acid concentrations between vegans and omnivores in aliquots of carefully homogenized morning bowel movements. We believe that this approach is justified as we did not observe significant differences in the total weights of these stool samples. Of note, this approach is not only in agreement with similar studies [35,56], but also supported by comparisons of bile acid concentrations between wet and dried fecal samples that did not find differences [40]. Moreover, our observations of increased fecal bile acids in omnivores compared to vegans are totally in line with literature [11,12,34,35] and confirm the associations of a plant-based diet and decreased bile acids in wet fecal mass.

Our aim was to identify a dietary pattern explaining the variation in fecal bile acid levels by implementing the RRR technique. Due to this narrow focus, our study did not address cross-linked information about microbiota composition or fecal short-chained fatty acids as bacterial metabolites out of fiber degradation. The investigation into these associations needs further research.

Three-day weighed food records were used to assess dietary intake. Although weighed food records are called the gold standard for assessing dietary intake, it is recognized in nutritional epidemiology that weighing dietary records are time-consuming for the participants and can be biased by influencing the eating habits during the protocol days or as a result of under-reporting [57]. Nevertheless, this instrument was chosen because the specific food items of a vegan diet were captured more reliably in weighing protocols than in standardized food frequency questionnaires. The small sample size of both groups and the high educational level of the participants may suggest that our study population lacked a good representation of the general population. However, the high degree of education could explain the low numbers of smokers and moderate levels of physical activity. Furthermore, the similar distribution of education and lifestyle characteristics in both groups may minimize the risk of confounding.

5. Conclusions

In our study, we observed a higher dietary fiber and lower fat intake, in line with lower fecal bile acid concentrations, in vegans compared to omnivores. We provide the first data on serum bile acids in vegans compared to omnivores.

Our findings could suggest that a vegan diet, which is low in fat and high in fiber intake, is related to lower fecal bile acid concentrations, and it may play a protective role in the development of CRC.

Due to their role in metabolic regulation, bile acids may not only be relevant to the development of CRC, but also to metabolic diseases such as dyslipidemia [51] or type 2 diabetes [52].

In conclusion, despite an increasing trend toward veganism in Western countries, studies investigating associations between a vegan diet and metabolic changes are still scarce. If replicated in larger studies, the association between a vegan diet and fecal bile acids may be established as an important underlying mode of action to explain protective effects in the development of CRC and other metabolic diseases.

Supplementary Materials: The following are available online at http://www.mdpi.com/2072-6643/12/1/47/s1: Table S1: Yielding factors for prepared food items; Table S2: Definition of food groups; Table S3: Intake of food groups of RBVD population; Table S4: Factor loadings of all 53 food groups in first dietary pattern score; Figure S1: Study flow chart.

Author Contributions: I.T. performed the statistical analyses and data interpretation, as well as wrote the manuscript. C.W. and K.A. designed the study and were responsible for recruiting and dealing with participants. C.W. supervised the writing of the manuscript and was primarily responsible for the final content. H.-U.M. and M.S. analyzed the bile acids and provided expertise on the interpretation of bile acid data. R.d.G. provided statistical and methodological expertise. All authors contributed to the interpretation of the data and revised the manuscript. All authors have read and agreed to the published version of the manuscript.

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11. Curriculum vitae

Mein Lebenslauf wird aus datenschutzrechtlichen Gründen in der elektronischen Version meiner Arbeit nicht veröffentlicht

12. Complete list of publications

11.1 Research papers

2017

Andres S, Ziegenhagen R, **Trefflich I,** Pevny S, Schultrich K, Braun H, Schänzer W, Hirsch-Ernst KI, Schäfer B, Lampen A. Creatine and creatine forms intended for sports nutrition. Mol Nutr Food Res. 2017 Jun;61(6)

2018

Weißenborn A, Bakhiya N, Demuth I, Ehlers A, Ewald M, Niemann B, Richter K, **Trefflich I**, Ziegenhagen R, Hirsch-Ernst, K I, Lampen A. Höchstmengen für Vitamine und Mineralstoffe in Nahrungsergänzungsmitteln. J Consum Prot Food Saf (2018) 13: 25

Trefflich I, Jahn C, Jannasch F, Jäger S, Schulze MB, Mühlenbruch K (2018). Application of diabetes risk scores in health checkups. A comparison of the German Diabetes Risk Score (GDRS) and FINDRISK test. Ernahrungs Umschau 65(11): 180–18

2019

Trefflich I, Jabakhanji A, Menzel J, Blaut M, Michalsen A, Lampen A, Abraham K, Weikert C. Is a vegan or a vegetarian diet associated with the microbiota composition in the gut? Results of a new cross-sectional study and systematic review. Critical reviews in food science and nutrition. 2019. 10.1080/10408398.2019.1676697

Trefflich I, Marschall HU, di Giuseppe R, Ståhlman M, Michalsen A, Lampen A, Abraham K, Weikert C. Associations between dietary patterns and bile acids – results from a cross-sectional study in vegans and omnivores. Nutrients. 2019;12(1).

2020

Weikert C, **Trefflich I,** Menzel J, Obeid R, Longree A, Dierkes J, Meyer K, Herter-Aeberli I, Mai K, Stangl GI, Müller S, Schwerdtle T, Lampen A, Abraham A. Versorgungsstatus mit Vitaminen und Mineralstoffen bei veganer Ernährungsweise. Dtsch Arztebl Int 2020; 117: 575–82

11.2 Scientific conference presentation

2018

Trefflich, I.; Menzel, J.; Lampen, A.; Abraham, K.; Weikert, C. Association between a vegan diet and stool pH. Complement Med Res 2018;25:1–20. (poster presentation)

2019

Trefflich I, Marschall HU, Blaut M, Lampen A, Abraham K, Weikert C. Zusammenhänge zwischen veganer Ernährungsweise und Gallensäuren sowie kurzkettigen Fettsäuren im Stuhl. Deutsche Gesellschaft für Ernährung e.V.: Proc. Germ. Nutr. Soc., Vol. 25 (2019) (oral presentation)

Trefflich I, Marschall HU, Lampen A, Abraham K, Weikert C. Lebensmittelverzehr und Ernährungsmuster bei Veganern sind mit niedrigeren Gallensäuren im Stuhl assoziiert. DGEpi Ulm (oral presentation)

Trefflich I, Marschall HU, Blaut M, Lampen A, Abraham K, Weikert C. Associations between a vegan diet, bile acids and short chained fatty acids in stool. VESNA conference Prague (oral presentation)

2020

Trefflich I, Jabakhanji A, Menzel A, Abraham A, Weikert C. Bestehen Zusammenhänge zwischen einer veganen oder vegetarischen Ernährung und der Komposition der Darmflora? Ergebnisse einer neuen Querschnittstudie und eines systematischen Reviews. Deutsche Gesellschaft für Ernährung e.V.: Proc. Germ. Nutr. Soc., Vol. 26 (2020) (oral presentation, congress cancelled due to COVID-19)

Weikert C, **Trefflich I,** Menzel J, Lampen A, Abraham K. Makro- und Mikronutrientenstatus bei veganer Ernährungsweise in Deutschland. Deutsche Gesellschaft für Ernährung e.V.: Proc. Germ. Nutr. Soc., Vol. 26 (2020)

13. Acknowledgments

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