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DISSERTATION

Comparison of fatty acid profiles in vegans and omnivores

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a.m.	ante meridiem
AA	arachidonic acid
AHS-2	Adventist Health Study 2
ALA	α-linolenic acid
ASA	American diabetes association
BfR	Bundesinstitut für Risikobewertung
BMI	Body Mass Index
BTH	Benzothiazole
C	Celsius
CHD	coronary heart disease
CI	confidence interval
cm	centimeter
COX	cyclooxygenase
CVD	cardiovascular disease
d	day
DASH	Dietary approaches to stop hypertension
DHA	docosahexaenoic acid
DPA	docosapentaenoic acid

EDTA	ethylenediaminetetraacetic acid
EPA	eicosapentaenoic acid
EPIC	European Prospective Investigation into Cancer and Nutrition
FAME	fatty acid methyl esters
FAO	Food and Agriculture Organization
FID	flame ionization detector
g	gram
GC	gas chromatography
GLA	gamma-linoleic acid
h	hour
$HbA1_c$	hemoglobin $A1_c$
HDL	high-density lipoprotein
HR	hazard ratio
i.e.	id est
IDH	ischemic heart disease
IL-6	Interleukin 6
IQR	inter-quartile range
kg	kilogram
kPa	kilopascal
L	liter
LA	linoleic acid
LC	long chain
LDL	low density lipoprotein

LOX	lipoxygenase
Lp-PLA-A_2	lipoprotein-associated phospholipase A_2
m	meter
m^2	square meter
MCHC	mean cellular hemoglobin concentration
mg	milligram
mg/dL	milligram per deciliter
min	minute
min^{-1}	revolutions per minute
mm	millimeter
MMA	methylmalonic acid
mmHg	millimeter of mercury
MS	mass spectrometry
MTBE	Methyl tert-butyl ether
MUFA	monounsaturated fatty acids
n	number of individuals
n-3	omega-3 fatty acids
n-6	omega-6 fatty acids
N_2	nitrogen
OR	odds ratio
pmol	picomol
PUFA	polyunsaturated fatty acids
RR	relative risk

SCD	steaoryl-coA-desaturase
SFA	saturated fatty acids
SPE	solid phase extraction
TFA	trans fatty acids
TG	triglycerides
TMSH	trimethyl sulfonium hydroxide
TNF-α	Tumor necrosis factor α
UK	United Kingdom
UV	ultra violet
v/v	volume per volume
WHO	World Health Organization
µm	micrometer
µmol	micromole

1 Abstract

Introduction: A vegan diet is characterized by the abstention from animal products including meat, fish, dairy and eggs. The diet is based on the consumption of grains, legumes, vegetables, fruits, nuts and seeds. Over the last few years a vegan diet has become increasingly popular in Germany. To date, it has been suggested that a vegan diet is generally lower in fat. However, less is known about the impact on fatty acid profiles. Therefore, the present study aimed to investigate plasma phospholipid fatty acid profiles. Furthermore, dietary fatty acid intake, as well as biomarkers of lipid metabolism including total cholesterol, low-density (LDL) and high-density (HDL) cholesterol and triglycerides, was compared between vegans and omnivores.

Methods: For the present cross-sectional study, 72 healthy men and women (36 vegans and 36 omnivores) between 30 and 60 years of age were recruited from January to July 2017 at the Federal Institute of Risk Assessment. Lifestyle factors, anthropometric data and dietary intake (based on three-day weighed food records) were assessed. A fasting blood sample was taken and plasma phospholipid fatty acids were determined using gas chromatography and flame ionization detection.

Results: Compared to omnivores, vegans had lower dietary intakes of total fat, saturated fatty acids (SFA) and monounsaturated fatty acids (MUFA) ($p=0.004$, $p<0.0001$ and $p=0.001$, respectively), and higher intakes in total polyunsaturated fatty acids (PUFA), omega-3 and omega-6 PUFA ($p=0.002$ and $p=0.03$ and $p=0.005$ respectively). With regard to plasma phospholipid profiles, relatively lower proportions of SFA ($p<0.0001$), total trans fatty acids (TFA) ($p<0.0001$) and omega-3-fatty acids ($p<0.001$), but higher proportions of total PUFA ($p=0.009$) and omega-6-fatty acids ($p<0.0001$) were observed. No difference was seen regarding MUFA ($p=0.25$). Vegans had significantly lower concentrations of total cholesterol ($p<0.0001$) and LDL-cholesterol ($p=0.001$). No relevant differences were observed for HDL-cholesterol and triglycerides.

Conclusion: With the exception of omega-3 PUFA, a vegan diet is associated with a more favorable dietary fat intake and more favorable plasma fatty acid profiles, thus it may reduce risk factors for cardiovascular diseases. Therefore, a vegan diet could provide means for prevention and treatment of hyperlipidemia and cardiometabolic disease.

2 Zusammenfassung

Einleitung: Eine vegane Ernährung ist gekennzeichnet durch den Verzicht auf alle tierischen Nahrungsmittel wie Fleisch, Fisch, Eier und Milchprodukte. Sie basiert auf dem Konsum von Getreide, Hülsenfrüchten, Obst, Gemüse, Nüssen und Saaten. Diese pflanzliche Ernährungsweise erfreut sich in Deutschland immer größerer Beliebtheit. Sie zeichnet sich häufig durch einen geringeren Fettanteil aus. Ziel der Studie war es die Bedeutung der veganen Ernährung in Hinblick auf Fettsäureprofile zu untersuchen. Dazu wurden Plasma-Phospholipid-Fettsäure Profile gemessen. Zudem wurden die Aufnahme an Fett und Fettsäuren sowie Parameter des Lipidmetabolismus wie Gesamt, LDL- und HDL-Cholesterin und Triglyzeride zwischen Veganern und Mischköstlern verglichen.

Methoden: In diese Studie wurden 72 gesunde Probanden, 36 Veganer und 36 Mischköstler, zwischen 30 und 60 Jahren eingeschlossen. Die Nahrungsaufnahme wurde mittels 3-Tage-Wiegeprotokollen erfasst. Zusätzlich wurden anthropometrische Daten sowie Lebensstilfaktoren ermittelt. Aus einer Blutprobe wurden Plasma-Phospholipid-Fettsäuren mittels Gaschromatografie und Flammenionisationsdetektor analysiert.

Ergebnisse: Im Vergleich zu Mischköstlern berichteten Veganer in den Wiegeprotokollen von einer geringeren Aufnahme von Gesamtfett, gesättigten Fettsäuren und einfach gesättigten Fettsäuren ($p=0.004$; $p<0.0001$; $p=0.001$) jedoch von einer höheren Aufnahme von mehrfach ungesättigten Fettsäuren sowie Omega-3 und Omega-6 Fettsäuren ($p=0.002$; $p=0.03$; $p=0.005$). Bei den Phospholipiden wurden bei Veganern relativ geringere Anteile von gesättigten Fettsäuren ($p<0.0001$), trans-Fettsäuren ($p<0.0001$) und Omega-3 Fettsäuren ($p<0.0001$) sowie höhere Anteile an mehrfach ungesättigten Fettsäuren ($p=0.009$) insbesondere Omega-6 Fettsäuren ($p<0.0001$) gemessen. Kein Unterschied zeigte sich in Hinblick auf einfach ungesättigte Fettsäuren ($p=0.25$). Veganer zeigten zudem niedrigere Werte in Gesamt ($p<0.0001$) und LDL-Cholesterin ($p=0.001$). Hingegen wurden keine relevanten Unterschiede für HDL-Cholesterin und Triglyzeride festgestellt.

Schlussfolgerung: Die Menge und Zusammensetzung der Aufnahme von Fettsäuren, mit Ausnahme der geringeren Aufnahmemenge an Omega-3 Fettsäuren, sowie die Fettsäureprofile im Blut scheinen bei einer veganen Ernährungsweise im Vergleich zu einer Mischkost vorteilhaft. Entsprechend wurden auch günstigere Lipidwerte

insbesondere LDL-Cholesterin bei Veganern im Vergleich zu den Mischköstlern beobachtet. Diese Zusammenhänge weisen auf mögliche Beiträge der Ernährung zur Prävention und Therapie von Hyperlipidämie und kardio-metabolischen Erkrankungen hin.

3 Introduction

3.1 Definition of veganism and veganism in Germany

A vegan diet is characterized by an elimination of any animal-based foods such as meat, fish, dairy, eggs or honey, and is based on the consumption of grains, legumes, vegetables, fruits, nuts and seeds(1) (2). In contrast, an omnivorous diet includes all food groups from both plant and animal origin (3). The vegan diet has gained popularity among adults in Germany over the last few years (3, 4). According to the “National Consumption Study II” conducted by the Federal Ministry of Food, Agriculture and Consumer Protection in 2008, 0.1% of the German population, which equals approximately 80,000 people, followed a vegan diet (4). In 2016 the percentage of vegans in Germany rose from 0.1% to 1% of the population, i.e. from 81,000 to 810,000 people (3). The latest numbers on Germans following a vegan diet are based on a survey performed by SKOPOS a market research institute, in 2017. In their survey, they claimed that 1.3 million Germans, or 1.5% of the German population follow a vegan diet (5).

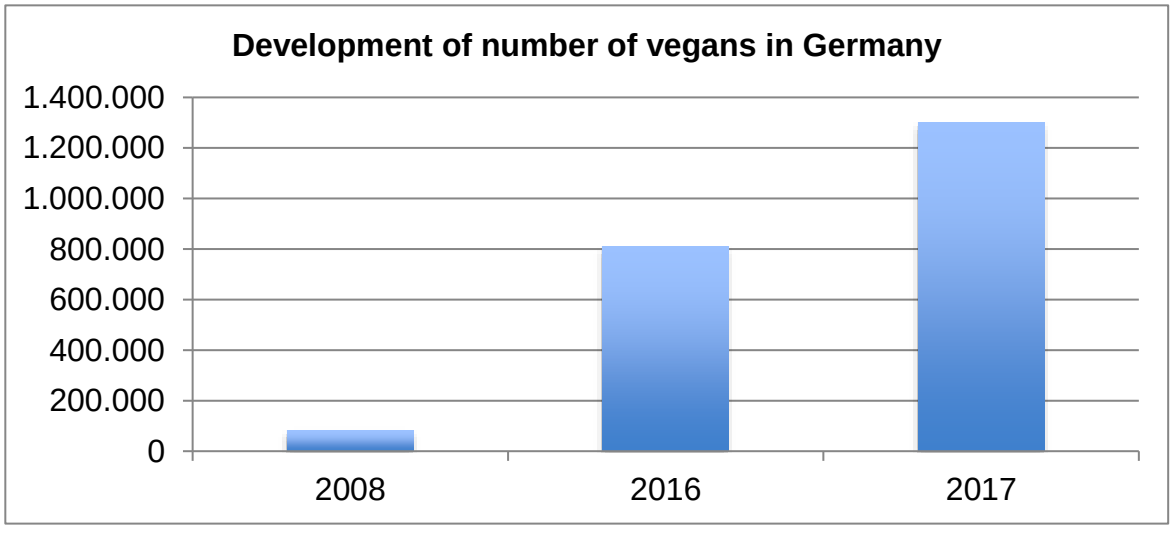

Figure 1: Development of number of vegans in Germany (3-5)

According to Mintel, a market research agency which specializes on the worldwide food market, in 2016 18% of vegan products introduced worldwide were launched in Germany (6). This shows an enormous interest in vegan nutrition (6). Of the three main

reasons for Germans to follow a vegan diet, animal-related motives such as animal welfare or animal rights seems to be the most important reason (7). Additionally, self-related motives including personal well-being, health reasons or weight-loss as well as environmentally-related motives such as concern about climate change or environmental protection, are reasons to change eating habits (7). Similar findings were shown by a study performed by the Federal Institute of Risk Assessment on 42 German vegans in 2015. The study demonstrated that ethical motives were the leading reason for adopting a vegan diet (8, 9).

3.2 Potential health benefits of vegan diets

Several studies state that a vegan diet promotes certain health benefits including a reduced risk of developing type 2 diabetes mellitus and cancer (10). Moreover, a reduction in risk factors for cardiometabolic diseases resulting in a reduced risk for developing cardiovascular diseases (CVD) might be achieved by following a vegan diet (11-14).

3.2.1 Risk factors for cardiometabolic diseases

A vegan diet seems to be the most beneficial diet to improve several risk factors for CVD, such as abdominal obesity, blood pressure, serum lipid profile or blood glucose (12). A large number of studies support the use of a vegan diet to reduce CVD, mainly by limiting these risk factors (11, 13, 14).

In adults overweight is defined as a Body Mass Index (BMI) between 25 kg/m^2 and 29.9 kg/m^2, and obesity as BMI > 30 kg/m^2 (15). Obesity is associated with a higher risk of chronic diseases e.g. hypertension, type 2 diabetes mellitus or CVD (15, 16). Several studies on vegan nutrition have demonstrated that vegans have a lower BMI compared to omnivores (17-19). Moreover, it was observed that vegans gain less weight compared to meat-eaters (20). In addition to this, it has been shown that meat consumption is associated with weight gain, even after adjusting for daily energy intake

(21). It was demonstrated that the risk of obesity was reduced by 7% for each year on a vegan diet (16). Moreover, it was revealed that weight loss is greater when following a vegan diet compared to other diets (16).
In line with this, results from the European Prospective Investigation into Cancer and Nutrition-Oxford (EPIC-Oxford) also showed that vegans have a significantly lower BMI compared to omnivores (22). Here, the age-adjusted differences in mean BMI between vegans and meat eaters was 1.92 kg/m^2 in men and 1.54 kg/m^2 in women (22). Moreover, the authors reported that lifestyle factors contribute to only 3% of the BMI differences among dietary groups, while dietary factors contribute to 50% (22). Furthermore, their analysis demonstrated that after adjusting for lifestyle factors such as smoking, educational level or physical activity, differences in mean BMI between dietary groups were reduced to 0.95 kg/m^2 in men and 0.68 kg/m^2 in women, but still remained significant (22). Moreover, the authors revealed fiber and protein intake as the most important determinants for BMI. Interestingly, increased intake of fiber and decreased protein intake were associated with a lower BMI, and adjusting for these factors led to a smaller difference in the BMI differences between a vegan and non-vegan diet (22).

Several studies revealed that the incidence and prevalence of hypertension are lower in vegans compared to omnivores (17, 23), as animal protein was positively related to blood pressure, while plant protein was correlated inversely (14). Results from the EPIC-Oxford study also showed the lowest prevalence of self-reported hypertension in vegans; however, differences attenuated after adjustment for BMI and differences in measured systolic blood pressure were not significant after adjustments (23).

Additionally, it has already been demonstrated that vegans have lower total cholesterol and LDL-cholesterol levels (13, 14, 24-26). It was shown that a vegan diet can improve fasting and postprandial blood lipids in a similar magnitude as statin therapy (10). When combined with lifestyle changes, such as moderate physical exercise or stress management, a vegan diet may lead to an even greater reduction (1). This was mainly explained due to the differences in fat intake, since meat and dairy are major sources of saturated fatty acids (SFA) (27), whereas plant sources such as vegetable oils, nuts and seeds are rich in polyunsaturated fatty acids (28).

Since vegan diets are associated with a lower BMI, lower waist circumference and more favorable fatty acid profiles, they appear to reduce the risk of developing metabolic syndrome by about one half (1). In addition to limiting these risk factors, vegans consume higher amounts of foods with cardio-protective effects (11). Compared to omnivores, vegans consume higher quantities of fruits and vegetables, which are high in fiber, antioxidants and folic acid. They also consume lower quantities of saturated fats and total fat, which results in lower blood cholesterol concentrations, lower incidents of stroke and lower risk of mortality of ischemic heart disease (11, 12). A higher intake of legumes, nuts and seeds in vegans provides further cardio-protective effects (11).

3.2.2 *Type 2 diabetes mellitus*

Recent studies suggest a prevention of type 2 diabetes when following a vegan diet (14, 17). Accordingly, in the Adventist Health Study-2 (AHS-2), prevalence of diabetes was lowest in vegans. Moreover, it was also shown that compared to meat eaters, a vegan diet is related to a reduced risk for the development of type 2 diabetes (OR 0.23, 95% CI 0.14 – 0.37) (17). Even after adjusting for BMI and other confounders, vegans were 62% less likely to develop diabetes (17).

Moreover, Barnard and colleagues performed a randomized clinical trial to investigate the impact of a low-fat vegan diet compared to the dietary guidelines of the American Diabetes Association (ASA) on glycemic control and cardiovascular risk factors in individuals with type 2 diabetes during a 22 weeks study period (24). The vegan diet consisted of fruit, vegetables, legumes and grains, with approximately 10% of the energy from fat, 15% from protein, and 75% from carbohydrates, whereas the ASA-diet was individualized, based on body weight and contained 15-20% of the energy from protein, < 7% from saturated fats, 60-70% from carbohydrates and < 200 mg from daily cholesterol (24). The authors revealed that 43% of the vegan diet group were able to reduce their diabetes medication, compared to only 26% in the control group (24). Additionally, changes in $HbA1_c$, weight, BMI, waist circumference, total cholesterol and LDL-cholesterol were significantly higher in the vegan group (24). After excluding participants who changed medication, a vegan diet lead to $HbA1_c$ reduction of -1.23% (8.07% at baseline and 6.84% after 22 weeks), whereas $HbA1_c$ decreased by -0.38%

(7.88% at baseline and 7.50% after 22 weeks) in the control group (24). The authors revealed that weight reduction in particular seems to be responsible for increased $HbA1_c$ (24). Indeed, body weight decreased by 6.5 kg among vegans and 3.1 kg in the control group, and LDL-cholesterol fell by 21.2% in the vegan group and 10.7% in the control group (24). However, Barnard and colleagues followed the study participants for an additional year and presented these results after 74 weeks of study in 2009 (29). After the extended period it was demonstrated that both diet groups were associated with weight reduction, this being -4.4 kg in the vegan group and -3.0 kg in the conventional diet group, although no significant differences among these groups were shown (29). $HbA1_c$ changes, without regard to medication adjustments, from baseline to 74 weeks were -0.34% among vegans and -0.14% among the ASA group, and showed no significant difference (p= 0.43) (29). After adjusting analyses for medication changes as a possible confounder, $HbA1_c$ changes were -0.04% among vegans and +0.01% in the conventional group (p= 0.03) (29). Nevertheless, among participants whose medications remained unchanged during the 74 weeks, $HbA1_c$ changes were -0.82% in vegans and -0.21% in the conventional group (p = 0.14) (29).

In the EPIC-Oxford study the association between diet groups and risk of developing diabetes was assessed. Here, compared to meat eaters, vegans and vegetarians showed a lower risk of diabetes (vegetarians: HR = 0.65, 95% CI 0.55–0.76; vegans: HR = 0.53, 95% CI 0.36–0.79). However, this study demonstrated a strong association between BMI and diabetes risk among all dietary groups (30).

3.2.3 Cancer

People following a vegan diet usually consume higher amounts of several vitamins, fibers, carotenoids, flavonoids or phytochemicals than omnivores (11). All these nutrients are suggested to have cancer-protective effects. Moreover, vegans do not consume any red meat, which is strongly related to the risk of developing colorectal cancer (11, 12). Results from the AHS-2 revealed that vegans had the lowest overall cancer incidence (31). Regarding colorectal cancer, vegans were shown to have lower risks compared to meat eaters; however, the results were not shown to be statistically significant (HR, 0.68 (95% CI, 0.43-1.08; p = 0.10) (32). Furthermore, the authors

demonstrated that vegan women experienced fewer female-related types of cancer (31). Results from a recent meta-analysis on vegan nutrition and health outcomes revealed a significant risk reduction of 15% of total cancer incidence when following a vegan diet (10).

3.2.4 Cardiovascular diseases

In 1978 Sanders already showed the positive health effects of vegan nutrition and concluded that vegan diets could be a used as treatment of ischemic heart disease (IHD) (33), angina pectoris and certain types of hyperlipidemias (34).
However, although a vegan diet may reduce several risk factors for CVD, data about CVD-prevention in vegans is limited (11). Nevertheless, the AHS-2 results showed that vegans had a greater risk reduction from CVD when compared to non-vegetarians, and vegan men had a lower mortality risk from CVD. Vegan women, however, did not seem to have lower mortality risk from CVD compared to omnivores (33). Vegans were shown to have a higher intake of fruit and vegetables (35). An inverse association between consumption of fruit and vegetables and mortality from CVD and stroke has been demonstrated (36). A meta-analysis revealed that stroke risk was 26% lower in individuals who consumed five portions of fruit and vegetables a day compared to individuals who only consumed three portions daily (37). However, the influence of vegan diets on the incidence of stroke has not been well investigated so far. To date, only a few studies have investigated this association. They did not find any reduction in stroke incidence by adhering to a vegan diet (28).

Furthermore, potential health benefits in vegans might occur due to the lack of meat consumption (36-39).
Studies have demonstrated that red meat is significantly associated with increased risks for heart disease and stroke (38). Moreover, a meta-analysis showed that each daily serving of red meat was significantly and positively associated with cardiovascular mortality (RR = 1.19, 95% CI 1.13, 1.26; p = 0.001) (37). Additionally it could be shown, that a diet with high amount of saturated fats, like the omnivorous diet, increased CVD risk, knowing that saturated fat increases serum lipids, thus promoting atherosclerosis

and CVD. While conversely, substitution of saturated fatty acids with omega-6 fatty acids from vegetable origins is recommended for decreasing CVD events (36).

However, eating a plant-based diet does not always equal a healthy diet, as there is also plant-based food that may contain high amounts of fat or high amounts of sugar (40). Therefore, a large cohort study analyzed the association of the quality of a plant-based diet and the event of coronary heart disease of 200,000 health workers in the U.S. (40). Based on food frequency questionnaires, the authors distinguished three versions of plant-based diets: an overall plant-based diet, a healthful plant-based diet and an unhealthful plant-based diet (40). A trend for reduced risk of developing coronary heart disease was demonstrated in people with a adherence to an overall plant-based diet, compared to people following an animal-based diet (HR 0.92 95% CI 0.83, 1.01, $p = 0.003$) (40). After analyzing healthful and unhealthful plant-based diets separately, it was revealed that a reduced risk of 25% for developing coronary heart disease could be achieved by a high adherence to a healthful plant-based diet, compared to a low adherence to this diet (HR 0.75 95% CI 0.68, 0.83; $p < 0.001$) (40). Conversely, the highest adherence to a unhealthful plant-based diet was associated with an increased risk to develop coronary heart disease of 32%, compared to a low adherence (HR 1.32, 95% CI 1.20, 1.46; $p < 0.001$) (40).

Recent results from the EPIC-Oxford Study on vegetarians and vegans showed that the risk of hospitalization or death from ischemic heart disease is 32% lower in vegetarians compared to omnivores (41). However, currently sufficient data on vegans currently does not exist and therefore more research is needed to determine the relationship between a vegan diet and cardiovascular events.

3.3 Potential lack of nutrients in vegan diets and related health risks

According to the German Nutrition Society adequate nutrient supply might be difficult or impossible to attain when following a vegan diet (3). Critical nutrients include vitamin B12, calcium and vitamin D, omega-3 fatty acids, protein, zinc, iodine, iron or selenium (3). However, even though a vegan diet might lack critical nutrients, several studies demonstrated that a well-planned vegan diet can supply all the nutrients required (12).

Vegans need to be aware of this, so that by carefully planning their diet, deficiencies can be avoided (11, 12).

3.3.1 Vitamin B12

In comparison to a traditional Western diet, a vegan diet does not contain vitamin B12, since this vitamin can only be found in animal products (11). Indeed, lower plasma vitamin B12 levels and a higher prevalence of vitamin B12 deficiencies were observed in vegans (19, 25, 42). Vitamin B12 deficiency can affect the nervous system in terms of ataxia and paresthesia, and have psychiatric effects such as dementia, mood changes or difficulties with concentration. Moreover, vitamin B12 deficiencies can lead to macrocytic anemia (11). It should be noted that the onset of these deficiency symptoms might begin after several years of following a vegan diet (e.g. serum concentrations below 150 pmol/l for 5 to 10 years are suggested) (25).

However, a Finnish study revealed that 91% of the vegan participants in their study took vitamin B12 supplements (19). Prevalence of vitamin B12 deficiency was low in all groups; however, it was the highest among vegans with four out of 53 participants (19).

Measurement of vitamin B12 deficiency is dominantly assessed by serum B12 status due to its low costs and simple method (43). However, the sensitivity of this method, especially at early stages of deficiency, and the specificity to diagnose decreased vitamin B12 levels, is poor (43). Compared to assessment of serum vitamin B12, it has been suggested that the assessment of holotranscobalamin, a transport protein of vitamin B12, reflects a potential deficiency more accurately that serum vitamin B12 (44). Moreover, a measurement of cobalamin dependent factors like methylmalonic acid (MMA) or total homocysteine could also be helpful in assessing the vitamin B12 status, knowing that both factors correlate inversely with vitamin B12 status (45). Since results are often contradictory when measured independently, Fedosov and colleagues developed a combined indicator to determine vitamin B12 status which includes all four parameters (46). Values were interpreted as high normal +0.4, normal 0, low-normal -0.5, deficient -1.5 and severely deficient -2.5 (46).

3.3.2 *Calcium and vitamin D and bone health*

Compared with omnivores vegans often have a lower intake of calcium and vitamin D (11, 18, 19, 47). Intakes of these nutrients vary widely among vegans and sometimes vegans did not reach the recommendations (12, 19, 25). Since serum calcium is tightly regulated, it cannot be used as parameter for nutritional calcium status (19). Therefore, dietary calcium assessment or excretion of calcium have to be used for the evaluation of calcium deficiency. Low calcium and vitamin D intake is linked to reduced bone density, osteoporosis and risk of bone fracture (48). For vegans, vitamin D status mostly depends on sun exposure and intake of fortified foods or supplements (11). Plant-based sources for vitamin D are enriched foods like plant-milk or margarine (11).

3.3.3 *Omega-3 fatty acids*

Since vegans do not consume any fish or fish oils, they tend to lack the long chain omega-3 (n-3) fatty acids eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), which might have important effects on cardiovascular health, brain development and normal vision (11). Sources of n-3 fatty acids are mainly fish and fish oils; however, EPA and DHA can also be converted endogenously from α-linolenic acid (ALA) (49, 50).

However, there are conflicting findings on n-3 PUFA levels (25, 49, 51-53). While some authors demonstrated significantly lower n-3 PUFA levels in vegans (25, 49, 52) another study showed that vegan women actually had higher n-3 PUFA levels compared to female omnivores (51). Nevertheless, with only 5 female vegan participants included in this analysis, its general implications are limited (51). Other studies revealed that vegans do have low n-3 levels, but not lower than omnivores who also consume low amounts of EPA and DHA (53). In addition to this, vegans also had significantly higher levels of EPA and in comparison to omnivores, DHA levels were not significantly lower (53).

Although vegans seem to have lower plasma n-3 levels than omnivores, the differences in plasma levels were smaller than expected from dietary intake (25, 51).

3.3.4 *Other critical nutrients*

Protein

Even though some studies demonstrate a lack of protein intake (18), protein intakes among vegans normally reach daily recommendations when calorie intake is adequate (12, 25, 47).

Iodine

Vegans who do not consume key iodine sources such as iodized table salt might risk iodine deficiency, since the iodine content of sea vegetables varies widely (12). However, several studies showed that vegans did not reach recommended iodine intakes (18, 25, 47). Therefore, women of child bearing age especially should consider iodine supplements (12).

Selenium

Data on selenium sustenance among vegans vary (18, 19, 25). A Swiss study found no differences in selenium levels between vegans and omnivores (19), whereas a Finnish study showed levels among vegans were lower compared to omnivores, even though these levels were above the recommendations (25). Findings from a Danish study showed low selenium levels in vegans, which did not reach recommended levels (18).

Zinc

Some studies could not demonstrate differences in Zinc intakes among the dietary groups (19, 25), whereas the mean of vegan participants in a German study reached the zinc recommendations; however, 20% of the participants had levels below recommendations (47). Results from a Danish study showed lower zinc levels although vegans reached the recommendations (18).

Iron

Iron deficiency and iron deficiency anemia are the most prevalent nutritional disorders worldwide (54). High risk groups for iron deficiency anemia are children, premenopausal women and pregnant women especially (55). Dietary habits play an important role in the development of iron deficiency, while additionally ethnicity, religion or socioeconomic status have significance in the risk of anemia development (55). The main iron sources for vegans are vegetables, cereals, nuts, seeds and soy (54). Regarding the iron status, it has been shown that despite vegans consuming high amounts in their daily diet, serum ferritin levels were still low (19, 54). This can be explained by the lower bioavailability of plant-based iron compared to iron from animal origins (56). Generally, heme iron that is found in meat is absorbed much easily by the human body when compared to plant-based non-heme iron (56). Additionally, most plants contain polyphenols or phytic acid, natural ingredients that inhibit iron absorption and lead to an even lower bioavailability (56). However, bioavailability of non-heme iron can be increased by dietary ascorbic acid (54). Furthermore, it has been shown that diet has a greater effect on iron absorption when serum ferritin levels are low (12). Depending on iron status and the amount of dietary enhancer and inhibitors, non-heme absorption can vary from 1% to 23% (12). However, it has also been reported that individuals can adapt and increase non-heme iron absorption. Also the effect of enhancers and inhibitors of iron absorption decreased with time (12).

In a Danish study, vegans met daily iron intake recommendations; however, the study did not examine ferritin levels (18). Since vegans generally eat more plants and therefore also more iron absorption inhibitors, a Swiss study was able to demonstrate that iron intake was positively correlated with plasma ferritin in omnivores, but not in vegans (19).

A German vegan study revealed that 40% of young vegan females and 12% of vegan women aged over 50 years were considered as iron deficient based on serum ferritin levels, and 3 women had iron deficiency anemia (54). In this study, no correlation between serum ferritin level and the amount of dietary absorption inhibitors and enhancers was found (54).

3.4 Fatty acid profiles

Fatty acids may have health-promoting as well as deleterious effects. These effects largely depend on the fatty acid composition. Therefore, detailed knowledge about the composition of fatty acid profiles is of high importance for maintaining health (57).

3.4.1 Definition and chemical principles of fatty acids

Fatty acids are structures with a carbon backbone, a methyl group on one end and a carboxyl group on the other end (58). Their nomenclature depends on the amount of carbon atoms, the amount of double bounds and the position of the first double bound (58). Many fatty acids can be synthetized endogenously (58).

Saturated fatty acids (SFA)

Saturated fatty acids have no double bounds and can be synthetized from acetyl-CoA by humans (27, 58). Furthermore, existing fatty acids be can elongated by adding two carbon atoms to create a new fatty acid (58). Dietary sources for saturated fatty acids are animal fats, butter, peanuts and palm oil (58). Dietary intake of saturated fatty acids has been described as a risk factor for cardiovascular disease, inflammation and obesity, and has been shown to result in an a more unfavorable blood lipid profile (59).

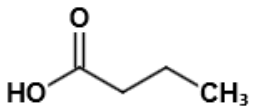

Figure 2: Butanic acid as example of saturated fatty acids

Monounsaturated fatty acids (MUFA)

Monounsaturated fatty acids contain one double bond and can be converted from saturated fatty acids using stearoyl-coA-desaturase (60). Stearoyl-coA-desaturase

(SCD) introduces a cis-double bond in the backbone of C12:0-19:0 saturated fatty acids, resulting in the production of monounsaturated fatty acids (61). A high SCD-activity has been shown to be associated with weight gain and the development of obesity (62). The enzyme activity can be measured by calculating the ratio of the fatty acid product to the substrate. To measure SCD-activity, the ratio of palmitoleic acid (C16:1n7c) to palmitic acid (C16:0) or the ratio of Oleic acid (C18:1n9) to stearic acid (C18:0) can be calculated. Since the C16:0 ratio is less influenced by dietary intake than the C18:0, it has been reported to be the preferred maker of SCD activity (63, 64).

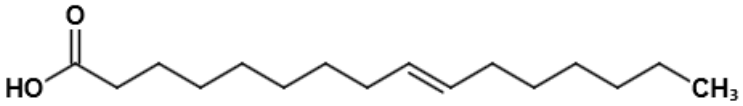

Figure 3: Palmitoleic acid as example of monounsaturated fatty acids

Polyunsaturated fatty acids (PUFA)

Polyunsaturated fatty acids contain more than one double bond and cannot be synthesized endogenously, but can be converted from essential polyunsaturated fatty acids (60). They are important as precursors for eicosanoids, which are signaling molecules that are involved in several physiological and pathophysiological processes such as regulating immune responses or inflammation, and therefore play an important role in human health (58). Different categories of PUFA exist depending on the position of the first double bond relative to the methyl terminus of the chain (60). Omega-3 and omega-6 fatty acids belong to the most important subgroups of PUFA and have their first double bond on the third or sixth carbon from the chain terminus, which is also known as the omega carbon (60).

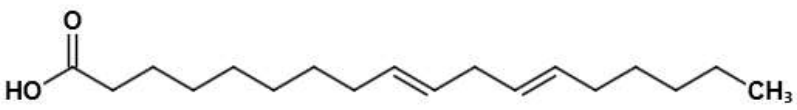

Figure 4: Linoleic acid as example of polyunsaturated fatty acids and omega-6 fatty acids

Essential polyunsaturated fatty acids

There are two essential polyunsaturated fatty acids: linoleic acid (LA, C18:2n-6) as essential omega-6 fatty acid, mostly found in canola oil, flaxseeds or avocado; and α-linolenic acid (ALA, C18:3n-3), the parent essential omega-3 fatty acid mostly found in chia seeds, rapeseeds (canola oil), flaxseeds (linseed oil), walnuts or hempseeds (49, 50).

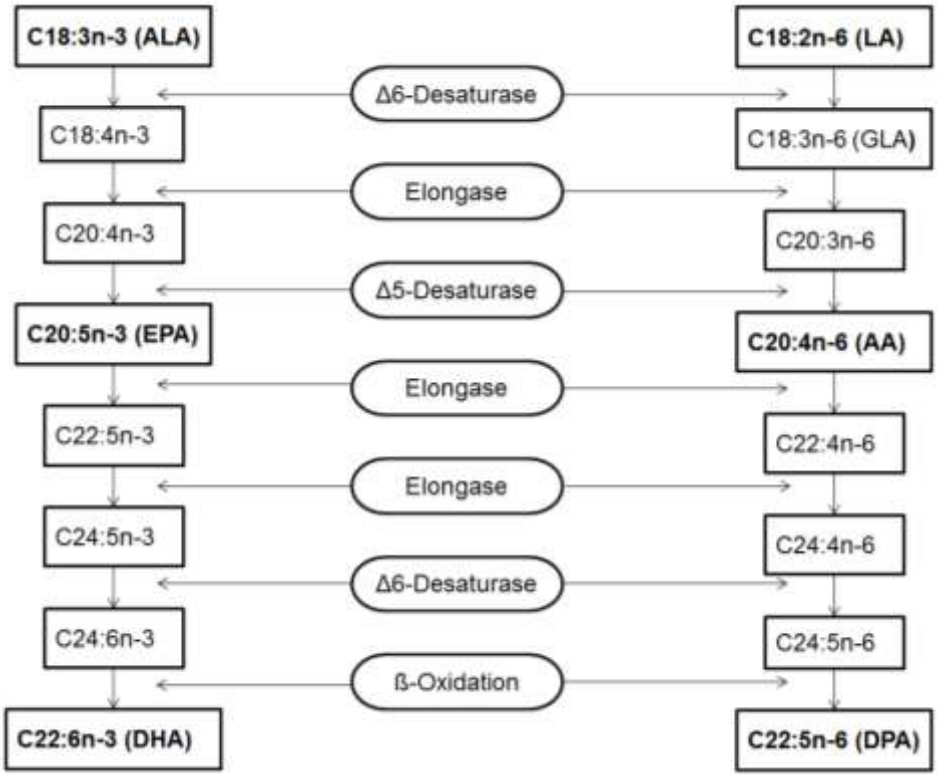

Figure 5: Pathway of metabolic conversion from ALA to EPA and DHA and LA to AA and DPA modified by (65, 66)

Through elongation (adding two carbon atoms) and desaturation (adding a double bond) these fatty acids can be converted into more physiologically active fatty acids. LA can be converted into arachidonic acid (AA) and ALA into EPA and DHA (Figure 5). EPA and DHA can also be found in oily fish, such as in salmon, herring, fish oil or microalgae (49). EPA and DHA play important roles in the normal functioning of the brain, nervous system and vision or cell membrane functions. Moreover, EPA and AA are necessary sources for the production of eicosanoids such as thromboxanes or prostaglandins (67).

While conversion from LA to AA is very effective, the conversion rates from ALA into EPA and DHA depend on Δ5- and Δ6-desaturases and appear to be very low. Only 5-10% is converted into EPA and 2-5% into DHA (49, 68).
However, conversion rates can be influenced by several factors (69). Since they seem to be higher in women of child-bearing age, estrogen might play an important role. Also higher conversion rates are demonstrated in smokers. Moreover, dietary factors have a high impact on conversion rates – for example, high intakes of trans fatty acids depress conversion rates (49). Furthermore it has been shown that alcohol reduces the activity of Δ5- and Δ6-desaturases (49). High amounts of LA negatively influence the ALA to EPA conversion because of competitive inhibition of Δ5- and Δ6-desaturases (51). Since Western diets contain higher amounts of LA than ALA, and both compete for the same desaturases, the conversion is shifted to higher conversion rates from LA into AA (70).

Trans fatty acids (TFA)

Trans fatty acids are fatty acids with one or more double bonds in trans- rather than the usual cis-configuration, resulting in a straighter shape (71). Trans fatty acids occur either naturally in meat and dairy or are formed during the hydrogenation of vegetable fat, as in the production of margarine, and therefore found in snacks, packed baked foods or fried foods (71). Trans fatty acids are linked with an increased risk to develop CVD and therefore the daily intake should be as low as possible (57).

Figure 7: Trans-configured fatty acid

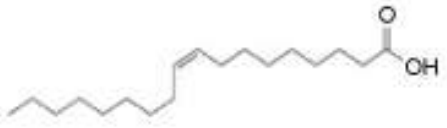

Figure 6: Cis-configured fatty acid

3.4.2 *Measurement of fatty acid profiles*

To measure fatty acid intake objectively, biomarkers offer an alternative to dietary intake (72). For individuals, recognizing and quantifying dietary fat might be difficult as fat especially in processed food is not always distinguishable (58). Moreover, overweight individuals are shown to tend to underreport fat intake due to social implications (58).

Fatty acid profiles are influenced by dietary intake as well as endogenous metabolism (73) and can be measured using free fatty acids, triglycerides, plasma phospholipid fatty acids, erythrocyte membrane fatty acids or adipose tissue (58).

Fatty acids composition measured from adipose tissue seems to be the best biomarker for long-term fatty acid intake, since fatty acid turnover in individuals with stable body weight is relatively low (69). Collection of adipose tissue from abdominal or gluteal tissue is seen as a safe and simple method; however, it is still unusual in larger epidemiological studies due to its invasive method for the collection of the material (58, 72).

However, fatty acids can also be determined in erythrocytes as phospholipid or cholesterol ester fatty acids, or free fatty acids in serum or plasma.

Fatty acids in serum or plasma are often measured due to their bio-accessibility and reflect dietary intake as well as *in vivo* metabolism (74). Free fatty acids reflect the dietary fat intake of the last hours or days, whereas phospholipid or cholesterol ester fatty acids reflect the dietary supply of the last weeks to months (58, 69).

Additionally, fatty acids can be measured in erythrocyte membrane phospholipids. Since erythrocytes have a lifespan of 120 days, it was assumed that they reflect the fatty acid intake of a longer period of time (74). However, it has been revealed that changing dietary fat intake leads to changes in erythrocyte membrane composition within a few days, and therefore erythrocyte membrane fatty acids only reflect fatty acid intake of the past weeks (72).

Since the human body cannot synthesize trans fatty acids or essential fatty acids as LA and ALA, these fatty acids may reflect the dietary fat intake. Furthermore, it was assumed that the synthesis of SFA pentadecanoic acid (C15:0) and heptadecanoic acid (C17:0) is not possible in the human body (58). These fatty acids occur in milk and therefore can be used to reflect diary intake (58). However, recently it has been demonstrated that these odd-chain fatty acids can also be synthetized endogenously, as they were found in the plasma of people following a vegan diet (75). A study by Weitkunat reveals that these odd-chain fatty acids can be synthesized in the liver using gut-derived propionate (76).

3.5 Aim of the study and research questions

To date, very few studies have compared the fatty acid profiles of vegans and omnivores. Furthermore, these studies showed various limitations. The aim of this study was to determine and compare the fatty acid profiles of vegans and omnivores in an apparently healthy study population. Thereby, the present thesis aimed to investigate the correlation between dietary habits and fatty acid profiles.

Additionally, the thesis focused on plasma long chain n-3 fatty acids proportions since these fatty acids are consumed in low quantities in vegan diets. Due to the fact that vegans do not consume any animal products, they depend on an effective synthesis from LA and ALA to EPA and DHA, and therefore vegans must rely on a sufficient Δ5- and Δ6-desaturase activity (77). Thus, the thesis aimed to investigate desaturase activity comparing between vegans and omnivores. Since Δ5- and Δ6-desaturases are affected by several factors such as alcohol or smoking, the study also aimed to reveal how these factors relate to the status of fatty acids.

Furthermore, we wanted to investigate whether the profile of fatty acids correlates to the duration of following a vegan diet, as conversion rates from LA and ALA to EPA and DHA are shown to be low and might not be sufficient to maintain the need for LC n-3 PUFA.

Finally, we wanted to investigate clinical markers of lipid metabolism in vegans compared to omnivores.

4 Methods

4.1 Study design

4.1.1 *Study population*

Participants for the present study were individuals who responded to advertisement posters in vegan and omnivore supermarkets, cafés and restaurants from January 2017 to July 2017. Participants contacted the study center at the German Federal Institute of Risk Assessment (BfR) via phone or e-mail (n = 161), followed by a phone screening consisting of a brief explanation of the study and checking inclusion criteria (age 30-60 years, following the diet for at least one year) and exclusion criteria (BMI > 30 kg/m2, cardiovascular disease, type 2 diabetes mellitus, cancer, pregnancy, breastfeeding, current infection). Vegans were defined as participants who did not consume any meat, fish, eggs or dairy. Vegans were asked whether they make exceptions from the diet, and those who consumed animal products maximally once every 3 months by accident or on purpose were still included in our study. Omnivores were included if they reported at least 3 servings of red or white meat per week, or a combination of ≥ 2 servings of meat and ≥ 2 servings of sausage per week. The final study population was comprised of a sex- and age-matched total of 36 vegans and 36 omnivores. Participants gave their written informed consent and the study was approved by the Ethics Committee of Charité University Medical Center Berlin (EA No. EA4/121/16). The cross-sectional study was conducted at the BfR in Berlin, Germany.

4.1.2 *First visit*

Participants who met our study criteria were invited to the German Federal Institute of Risk Assessment (BfR) located in Berlin. During the first visit, they were extensively informed about the study and all participants gave written informed consent. After this, the participants were instructed in using a three-day weighed food report by trained interviewers. The participants were asked to weigh and note their meals and drinks for two weekdays and one weekend day. These days did not have to be in sequence, they should rather represent their usual eating habits. If the participants were eating out and

did not have any possibility to weigh their food, they were asked to estimate portion size or to take a picture of the dish. To measure nutritional intake the same model of digital scale (Soehnle 65480 Siena) was given to all of our participants.

4.1.3 Second visit

In order to give the participants enough time to collect dietary data, the second visit took place one to four weeks after the first meeting. It began with the assessment of current medications or intake of supplements in the past 4 weeks. Additionally, blood pressure and anthropometric measurements were assessed.

Anthropometrics

Trained and quality-monitored personnel took anthropometric measurements (weight, height, and waist circumference) with participants wearing only light underwear and no shoes. Waist circumference was measured midway between the lower ribs and the iliac crest. Height was taken using a flexible stadiometer (SECA 231). Hip circumference was measured at the biggest scope of the hips. From two measurements, we calculated the mean for further analysis. Weight, muscular mass, visceral fat, total body fat and body mass index were measured using a bioelectrical impedance analysis scale (OMRON BF 511). Age, sex and height were entered in the scale and then the participant had to step on the scale and hold the handles by stretching the arms. For analysis, we calculated the mean out of the two measurements. The Body-Mass-Index was calculated as bodyweight divided by body height squared (BMI = kg/m2). The waist-hip ratio was calculated by dividing waist by hip circumference in centimeter.

Assessment of dietary habits and lifestyle characteristics

Dietary habits were assessed using three-day weighed reports. The evaluation of these protocols was carried out using EAT software version 3.5.5 (University Paderborn), as well as the German Nutrient Database (German: Bundeslebensmittelschlüssel Version 3.02, BLS). This is a nutrition database developed by the Federal Republic of Germany for the assessment of nutritional surveys and covers the nutritional values of 10,000 foods available in Germany. For some vegan products, for example dairy substitutes, food codes were missing in the database. In these cases, new food codes were created based on the ingredients on the packaging or requested from the producers. The nutrients of cooked foods were converted from recipes provided by the participants.

Information on physical activity, educational status and vocational status, smoking habits and medical history was obtained by computer-assisted face-to-face interviews. Physical activity comprises the sum of average hours spent in cycling, sports and gardening during summer and winter per week. Walking contains the sum of average hours per week during summer and winter. The questionnaire to gather physical activity data was established in the European Prospective Investigation into Cancer and Nutrition (EPIC)-Potsdam study and provided by the German Institute of Human Nutrition (http://www.dife.de/forschung/abteilungen/kurzprofil.php?abt=EPI).

Educational status was divided into ‘low education’ (no degree), ‘intermediate education’ (vocational school, technical college) or ‘high education’ (university, university of applied sciences).

Regarding smoking status, we classified participants into non-smokers, ex-smokers and smokers. Non-smokers were characterized as people who never smoked or smoked for a total period of less than three months in their entire life.

Blood pressure

After resting for at least five minutes, blood pressure was measured using a blood pressure monitor, model OMRON 705 IT (HEM-759-E). Blood pressure was measured 3 times with a 2 minute break in between each measurement. For our analysis, we calculated the mean of the second and third blood pressure reading. In order to not influence blood pressure, participants had to remain silent and were not allowed to move during the measurements. The result was communicated after the last measurement.

4.2 Blood collection and laboratory analysis

About 60 mL of venous blood was collected from fasting participants at the BfR study center. Blood was fractionated into serum, plasma and erythrocytes. Measurement of total cholesterol, HDL-cholesterol, LDL-cholesterol, triglycerides and homocysteine, and other biomarkers including complete blood count, lipids, biomarkers of liver and kidney function applying standardized methods was measured in plasma/serum, determined at the accredited medical analytics laboratory (Labor28 GmbH, Berlin, Germany) immediately on each study day using half of the material. The remaining material was stored in freezers (-80ºC) for conservation until time of analysis.

In October 2017, fatty acids in plasma phospholipids were analyzed using gas chromatography (GC) and flame ionization detection (FID) by the German Institute of Human Nutrition in Potsdam-Rehbrücke. The analysis was performed with a strongly modified method based on previous publications (78-80).

To extract fatty acids from plasma, 25 µL of plasma was transferred into vials and 1 mL of distilled water and 3 mL of tert-butyl methyl ether (MTBE)/methanol solution (2/1, v/v) (MTBE contained 0.01% BHT) were added. The antioxidant BHT was added to prevent oxidation of unsaturated fatty acids. The mixture was then vortexed for 15 minutes at 720 min^{-1} using a rotatory shaker and centrifuged for 10 minutes at 2000 x g at 15°C. The upper layer, now containing the lipid fractions, was transferred into another vial and evaporated under a steam of N_2 at 40°C to dryness using a sample collector (SBHCONC/1, Dunn Labortechnik, Asbach, Germany) equipped with a block heater (SBH130D/3, Dunn Labortechnik).

In the next step, phospholipids were separated from other lipids using bonded phase column separation (SPE). To this end, the dried lipids were redissolved into 500 µL of chloroform and the mixture was applied to conditioned 1 mL SPE columns containing 100 mg of aminopropyl-modified silica (Chromabond, Macherey-Nagel GmbH & Co. KG, Düren, Germany). The columns were placed on a vacuum elution apparatus equipped with vents and manometer (Carl Roth GmbH & Co. KG, Karlsruhe, Germany). The columns were washed with 2 x 1 mL of n-hexane and 1 x 1 mL of chloroform/i-propanol (2/1, v/v). The vacuum (~ 10 kPa) was released to prevent columns from becoming dry. In the next step, the samples were added and 4 x 1 mL of chloroform/methanol/acetic acid (100/2/2, v/v) were used to elute neutral lipids and free fatty acids. Phospholipids were eluted with 2 x 1 mL of methanol after changing vials.

Under a steam of N_2 the remaining solvents were evaporated and the dried phospholipids remained.

In order to keep the chemical stability of fatty acids, the phospholipids were methylated and hydrolyzed in the following steps. First, dried phospholipids were redissolved in 200 µL of toluene, vortexed and transferred into vials. Then 10 µL of trimethyl sulfonium hydroxide solution (TMSH, 0.2 mol/l in methanol, Macherey-Nagel) was added to form fatty acid methyl esters (FAME) and the mixture was vortexed for 30 minutes at 40°C using a thermomixer.

Analysis of FAMEs was performed using an Agilent gas chromatography system 7890A equipped with an Agilent 7000 GC/MS Triple Quad (Agilent technologies, Waldbronn, Germany) and a flame ionization detector. 1 µL of sample was injected and FAMEs were separated using a constant helium carrier gas flow of 1 mL/min on a capillary column (HP-88, 100 m x 0.25 mm I.D., 0,2 µm film thickness, Agilent). The initial temperature was 80°C, held for 1 minute, ramp 1 was 10°C/minute to 170°C and held for 5 minutes, ramp 2 was 5°C/minute to 175°C, ramp 3 was 2°C/minute to 190°C, ramp 4 was 5°C/minute to 205°C and held for 17 minutes, and ramp 5 was 5°C/minute to 235°C and held for 8 minutes. The total run time was 57.5 minutes. The fatty acids were identified by reference to standard fatty acid mixtures run on the same column under identical conditions (Figure 8). Therefore, a 37-component FAME mixture (SupelcoTM) was used. All peaks of fatty acids were calculated and fatty acid composition was expressed as area percentage of each fatty acid relative to the total area of all detected fatty acids.

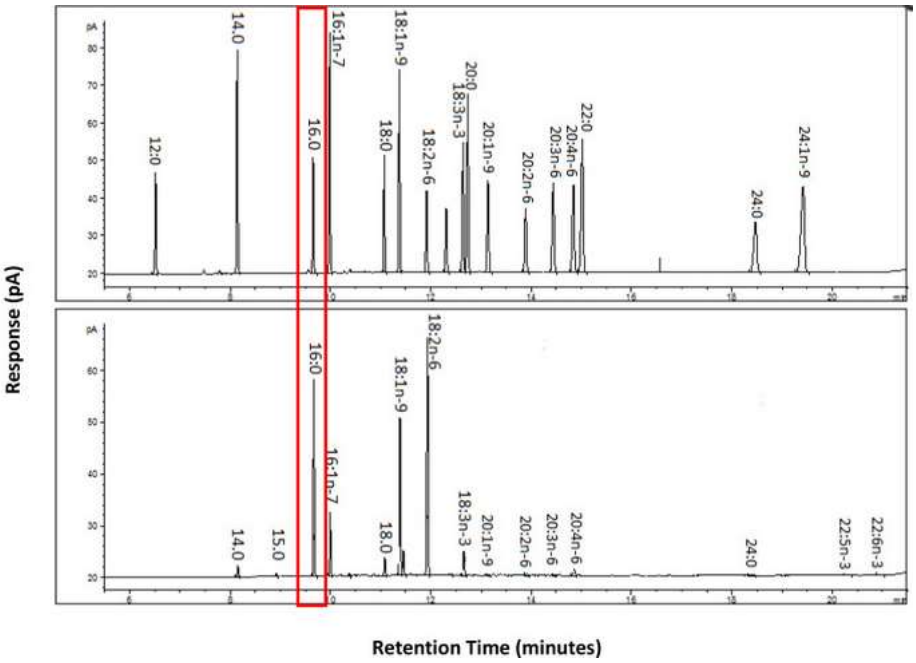

Figure 8: Example of identifying FAMEs using known FAME standard mix (81)

The department for clinical chemistry and laboratory medicine of the University Saarland, Germany, conducted Vitamin B12 and holotranscobalamin measurements. Measurement of the methylmalonic acid (MMA) was performed at BEVITAL AS in Norway. MMA was measured with GC-MS/MS (Within-day CV: 1-4 %; between-day CV: 3-8 %).

4.3 Desaturase activity

Based on fatty acid proportions, the estimated desaturase activity was calculated by calculating the ratio of a fatty acid product to the substrate. The following ratios were measured (82):

$$\text{Estimated } \Delta 5\text{-desaturase activity} = \frac{\text{arachidonic acid (C20:4 n-6)}}{\text{dihomo-γ-linolenic acid (C20:3 n-6)}}$$

$$\text{Estimated } \Delta 6\text{-desaturase activity} = \frac{\text{γ-linolenic acid (C18:3 n-6)}}{\text{linoleic acid (C18:2 n-6)}}$$

$$\text{Estimated stearoyl-CoA-desaturase activity} = \frac{\text{palmitoleic acid (C16:1n7c)}}{\text{palmitic acid (C16:0)}}$$

$$\text{Estimated stearoyl-CoA desaturase activity} = \frac{\text{oleic acid (C18:1n9c)}}{\text{stearic acid (C18:0)}}$$

4.4 Statistical analyses

Normal distribution was tested using the Kolmogorov-Smirnov test. Q-Q-plots and histograms were assessed for visual inspection. Regarding study characteristics, normally distributed variables were reported as mean and standard derivation (SD). Skewed variables were reported as median and interquartile range (IQR) and log-transformed for further analyses (alcohol consumption, physical activity). For comparison between vegans compared to omnivores, a Chi-square test for categorical variables and for continuous variables a Student's t-test (normally distributed variables) or Mann–Whitney U test (not normally distributed variables) was used.

In order to demonstrate a correlation between two metric variables, bivariate correlation analyses were performed using Pearson's correlation coefficient for normally distributed data and Spearman's correlation for skewed distributed data.

Analysis of variance (ANOVA) was used to assess the relationship between plasma phospholipid fatty acid and diet status without adjustment (Model 1). Moreover, multivariable-adjusted analysis of covariance (ANCOVA) was used to further investigate the relationship between plasma phospholipid fatty acid and diet status, adjusted for alcohol intake, educational status, physical activity and smoking status (Model 2). Since most of the measured fatty acids tended to have skewed distributions, fatty acid data were log-transformed for the analyses and data were reported as geometric means and 95%-CI.

For all analyses p-values of < 0.05 were considered statistically significant. However, for multiple comparisons Bonferroni correction was applied assuming $p \leq 0.00128$ for dietary fatty acids based on 39 tests, and $p \leq 0.0017$ for plasma phospholipid fatty acids based on 29 tests.

In order to minimize confounders, we carried out sensitivity analyses. We therefore analyzed fatty acid profiles after excluding participants who consume omega-3 fatty acid supplements regularly, and performed sex specific analyses. Multiplicative interactions

between sex (women/men) and diet (vegans/omnivores) were tested with cross-product terms investigating the association between diet and plasma SFA, MUFA, PUFA, n-3 and n-6 PUFA and TFA.

Statistical analyses were performed using IBM SPSS Statistics for Windows, Version 21.0. (Armonk, NY, USA) or SAS software, version 9.4 (SAS institute, Cary, N.C., USA).

5 Results

5.1 Characteristics of the study population

The distribution of general characteristics of the 72 participants is shown in Table 1, according to vegan (n = 36) or omnivorous diet (n = 36). The median duration of veganism was 4.8 years (IQR: 3.1-8.7). Due to the sex- and age-matched inclusion of the participants, we observed no differences in sex (vegans: 50% male; omnivores: 50% male) and age (vegans: median 37.5 years (IQR: 32.5-44.0); omnivores: median 38.5 years (IQR: 32.0-46.0); p = 0.8). Moreover, we observed no differences in waist-hip-ratio, physical activity, smoking, education or alcohol intake between the groups (all p > 0.05.

Regarding supplement intake, 97.2 % of the vegan participants stated they took supplements on a regular basis, mainly vitamin B12 (91.7%) and vitamin D (50%). No differences were found in serum vitamin B12 levels or combined indicator for vitamin B12 status between diet groups.

Table 1: Basic characteristics of our study population

	Vegans (n=36)	**Omnivores (n=36)**	**p-value**
Duration of vegan diet [years]	4.8 (3.1 - 8.7)		
Age [years]	37.5 (32.5 - 44.0)	38.5 (32.0 - 46.0)	0.8
Sex [male]	50.0% (18)	50.0% (18)	
BMI [kg/m^2]	22.9 (± 3.2)	24.0 (± 2.1)	0.08
Waist-hip ratio			
women	0.8 (± 0.1)	0.8 (± 0.1)	0.7
men	0.9 (± 0.1)	0.9 (± 0.0)	0.3
Systolic blood pressure [mmHg]	111.2 (± 11.0)	114.7 (± 11.9)	0.2

	Vegans (n=36)	Omnivores (n=36)	p-value
Diastolic blood pressure [mmHg]	69.8 (± 7.7)	73.0 (± 7.1)	0.1
Physical activity [h/week]	2.8 (0.9-3.8)	2.3 (1.2-4.1)	0.7
Walking [h/week]	7.0 (5.0-12.0)	5.5 (3.5-12.1)	0.2
Smoking status [%]			0.3
Non-Smoker	66.7 % (24)	58.3 % (21)	
Ex-Smoker	22.2 % (8)	16.7 % (6)	
Smoker	11.1 % (4)	25.0 % (9)	
Alcohol intake [g/d]			
women	0.1 (0.0-4.7)	0.2 (0.0-4.9)	0.2
men	0.0 (0.0-2.0)	3.9 (0.4-16.2)	0.1
Education [%]			0.6
low	0.0 % (0)	2.8 % (1)	
Intermediate	30.6 % (11)	30.6 % (11)	
high	69.5 % (25)	66.7 % (24)	
Supplement intake	97.2 % (35)	33.3 % (12)	*< 0.0001*
Vitamin B12 [pmol/L]	337.9 (218.0-559.1)	267.6 (227.2-364.5)	0.1
Holotranscobalamin [pmol/L]	89.4 (58.9-205.0)	84.3 (67.6-100.4)	0.4
Total homocysteine [µmol/L]	8.6 (6.7-11.3)	8.8 (7.3-10.5)	0.9
Methylmalonic acid [µmol/l]	0.2 (0.2-0.2)	0.2 (0.2-0.2)	0.6
Vitamin B12 indicator*	0.5 (0.1-1.2)	0.4 (0.2-0.7)	0.5

Variables expressed as percentage, mean ± SD or median (IQR). * Combined indicator for vitamin B12.

5.2 Dietary fatty acid intake based on weighed food records

We analyzed and compared the daily intake of total fat and individual fatty acids of our study participants, based on the information from weighed food records (figure 9).
Total energy intake in calories did not differ significantly between both groups (vegans median 2270.1 (IQR 1800.0-2762.3), omnivores median 2385.9 (IQR 2080.9-2737.3), p = 0.3). Regarding total fat intake, the median in vegans was 85.7 g/day (IQR 63.6 – 111.1) and the median in omnivores was 104.1 g/day (IQR 87.8 – 143.3) (p = 0.004).

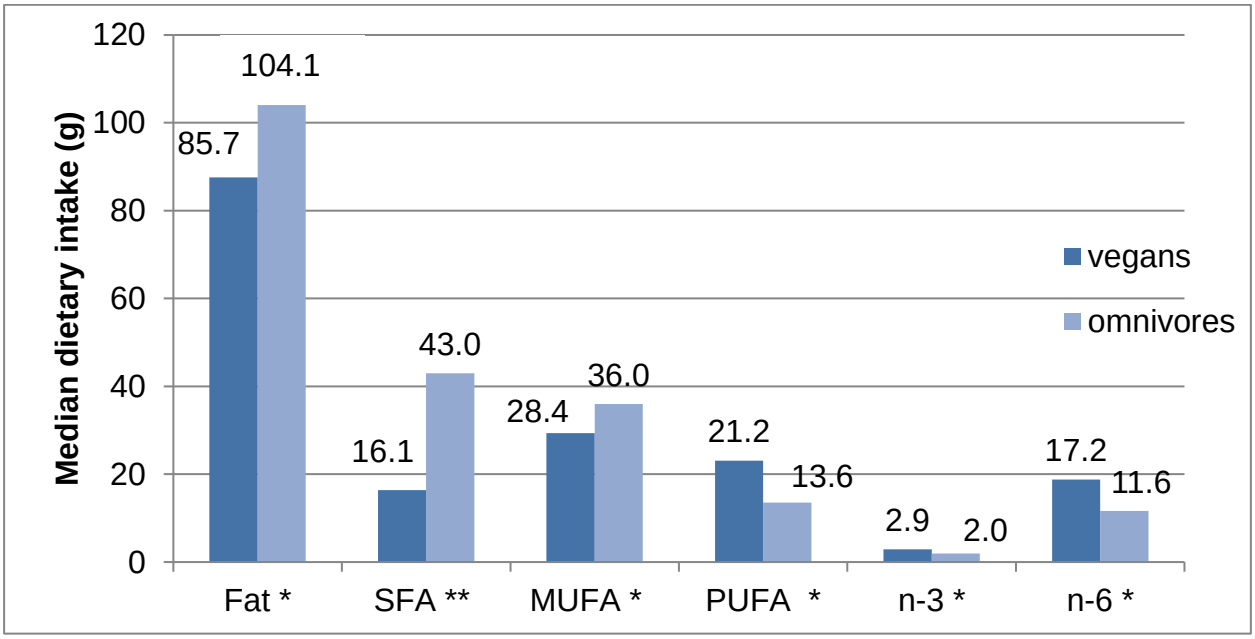

Figure 9: Dietary intake of fatty acids in vegans and omnivores presented as median in grams, ** p < 0.0001, *p < 0.05

The results of different fatty acid intakes are shown in table 2. Vegans had significantly lower dietary intakes of total saturated fatty acids compared to omnivores (p < 0.0001) and lower intake of individual SFA C4:0-C18:0 (all p < 0.0001) except arachidic acid (C20:0) (p = 0.05). Median intake of behenic acid (C22:0) was higher in vegans, however it did not reach significance after Bonferroni correction.
Regarding total monounsaturated fatty acids, it was shown that vegans consume significantly lower amounts (p = 0.001). We observed higher median intakes of C20:1 and C24:1 but no significant differences were found (all p > 0.05).
We revealed that vegans consumed total polyunsaturated fatty acids and omega-3 fatty acids in a significantly higher amount compared to omnivores (p = 0.002 and p = 0.03).

However, differences in omega-3 fatty acid intake did not reach significance after Bonferroni correction. Interestingly the only omega-3 fatty acid that is more highly consumed in vegans is ALA ($p = 0.0002$). Dietary median intake of long-chain omega-3 fatty acids (EPA, DPA and DHA) was 10.9 mg (IQR: 3.7 - 33.5) in vegans and 169.2 mg (IQR: 61.5 - 409.6) in omnivores ($p < 0.0001$).

We could detect similar results regarding omega-6 fatty acids. Vegans consumed higher amounts of total omega-6 fatty acids ($p = 0.002$). However, the only omega-6 fatty acid that was consumed in a significantly higher amount in vegans was LA ($p = 0.003$). Both differences were no longer significant after correction for Bonferroni.

Figure 10 demonstrates the median daily dietary intake of fatty acids based on total energy intake in percent.

The dietary omega-6 to omega-3 ratio did not differ significantly: vegans 5.9:1 (IQR: 4.5:1 – 7.5:1), omnivores 5.3:1 (IQR: 4.5:1 – 7.3:1), $p = 0.8$ (figure 11).

Dietary cholesterol intake was significantly lower in vegans with a median of 8.3 mg (IQR: 1.8 - 17.6), while the omnivore median was 374.9 mg (IQR: 270.1 - 499.0), ($p < 0.0001$).

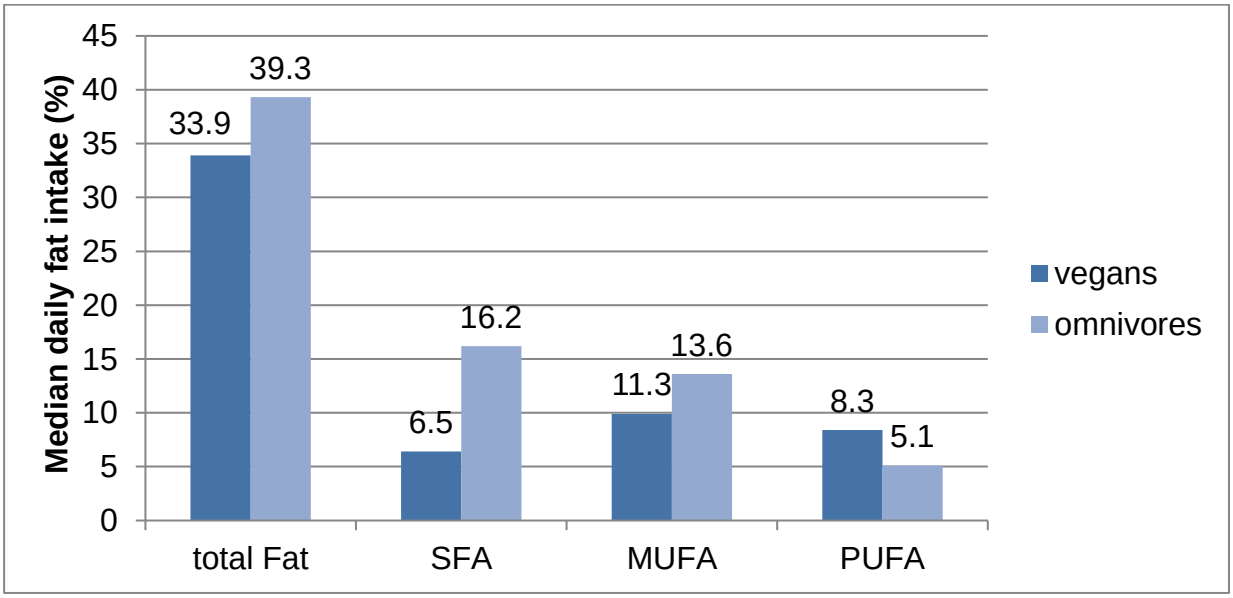

Figure 10: Median daily fat intake as percentage of total daily energy intake

Table 2: Dietary intake of fatty acids in milligrams/day based on weighed food records [a]

	Vegans (n=36)	**Omnivores (n=36)**	**p-value [b]**
SFA			
C4:0 Butyric acid	10.0 (2.3 - 66.5)	1449.0 (969.5 - 2009.0)	*< 0.0001[c]*
C6:0 Capronic acid	14.8 (3.3 -60.4)	822.5 (552.9 - 1251.6)	*< 0.0001[c]*
C8:0 Caprylic acid	73.1 (24.5 - 185.3)	673.8 (489.9 - 823.5)	*< 0.0001[c]*
C10:0 Capric acid	76.3 (44.2 - 174.1)	1223.0 (894.3 - 1654.3)	*< 0.0001[c]*
C12:0 Lauric acid	351.3 (190.4- 1099.5)	2010.3 (1455.0 - 2893.8)	*< 0.0001[c]*
C14:0 Myristic acid	388.2 (233.2 - 808.2)	4935.9 (3572.8 - 6598.7)	*< 0.0001[c]*
C15:0 Pentadecylic acid	19.6 (12.7 - 32.4)	480.9 (347.7 - 636.3)	*< 0.0001[c]*
C16:0 Palmitic acid	8742.7 (6125.9 - 10558.2)	20326.2 (17335.9 - 26618.0)	*< 0.0001[c]*
C17:0 Heptadecanoic acid	56.0 (41.7 - 73.3)	385.7 (278.0 - 476.6)	*< 0.0001[c]*
C18:0 Stearic acid	3168.8 (2399.8 - 4221.3)	9331.1 (7111.7 - 11437.1)	*< 0.0001[c]*
C20:0 Arachidic acid	240.2 (167.2 - 331.6)	304.5 (234.2 - 410.8)	*0.05*
C22:0 Behenic acid	126.3 (81.9 - 188.9)	74.0 (42.7 - 129.0)	*0.004*
C24:0 Lignoceric acid	45.0 (27.8 - 66.3)	34.9 (23.7 - 54.7)	0.2
Sum SFA	16140.3 (10646.4 - 22269.1)	42948.8 (34439.1 - 54640.6)	*< 0.0001[c]*

	Vegans (n=36)	Omnivores (n=36)	p-value [b]
MUFA			
C14:1 Myristoleic acid	5.6 (1.6 - 23.1)	534.2 (416.1 - 715.7)	*< 0.0001*[c]
C15:1 Pentadecanoic acid	1.1 (0.0 - 6.2)	185.4 (136.3 - 312.1)	*< 0.0001*[c]
C16:1n7c Palmitoleic acid	517.7 (345.8 - 676.9)	1887.9 (1489.0 - 2622.1)	*< 0.0001*[c]
C17:1 Heptadecenoic acid	11.2 (6.9 - 17.5)	277.7 (225.5 - 432.8)	*< 0.0001*[c]
C18:1n9c Oleic acid	26703.2 (20020.1 - 34095.6)	32540.3 (27286.4 - 43532.4)	*0.005*
C20:1 Eicosenoic acid	454.0 (268.7 - 656.2)	443.2 (254.6 - 914.2)	0.6
C22:1 Erurcic acid	72.2 (33.1 - 219.3)	81.0 (30.0 - 364.6)	0.4
C24:1 Nervonic acid	12.7 (3.2 - 18.6)	4.1 (1.2 - 11.9)	*0.08*
Sum MUFA	28394.1 (21877.1 - 36384.9)	35950.3 (30771.9 - 48656.4)	*0.001*[c]
n-3 PUFA			
C18:3n3 α-linolenic acid	2855.4 (1937.9 - 4969.8)	1480.6 (1221.7 - 2482.8)	*0.0002*[c]
C18:4 n3 Stearidonic acid (SDA)	0.0 (0.0 - 0.0)	1.2 (0.0 - 6.0)	*< 0.0001*[c]
C20:3 Eicosatrienoic acid	1.1 (0.0 - 5.9)	26.2 (11.4 - 46.6)	*< 0.0001*[c]
C20:5n3 Eicosapentaenoic acid	0.8 (0.1 - 2.9)	41.0 (25.8 - 186.0)	*< 0.0001*[c]
C22:6n3 Docosahexaenoic acid	7.3 (2.9 - 22.8)	126.1 (45.4 - 275.0)	*< 0.0001*[c]

	Vegans (n=36)	**Omnivores (n=36)**	**p-value [b]**
Sum n-3 PUFA	2868.2 (1942.2 - 5019.5)	1962.0 (1494.0 - 3410.2)	*0.03*
n-6 PUFA			
C18:2n6c Linoleic acid	17169.9 (13108.2 - 25354.4)	11381.7 (8632.2 - 16804.4)	*0.003*
C20:2 n6 Eicosadienoic acid	7.2 (4.6 - 11.1)	20.9 (11.8 - 57.1)	*< 0.0001[c]*
C20:4n6 Arachidonic acid	5.5 (3.6 - 11.2)	165.6 (115.3 - 240.1)	*< 0.0001[c]*
C22:2 Docosadienoic acid	0.0 (0.0 - 0.0)	0.0 (0.0 - 0.3)	*0.006*
C22:5n6 Docosapentaenoic acid	0.7 (0.0 -6.1)	49.9 (19.35 - 118.6)	*< 0.0001[c]*
Sum n-6 PUFA	17186.6 (13120.3 - 25364.0)	11608.2 (8737.72 - 17320.4)	*0.005*
C19:3 Nonadecatrienoic acid	0.0 (0.0 - 1.5)	0.0 (0.0 - 15.0)	0.08
C22:3 Docosatrienoic acid	0.0 (0.0 - 0.0)	0.0 (0.0 - 0.0)	*0.04*
C22:4 Docosatetraenoic acid	0.0 (0.0 - 0.0)	0.0 (0.0 - 1.0)	*0.003*
Sum PUFA	21169.1 (17938.6 - 28753.2)	13556.6 (10499.5 - 21401.8)	*0.002*

[a] Data expressed in median and IQR in mg/d

[b] Kruskal-Wallis Test

[c] Statistically significant after Bonferroni correction for multiple comparisons (the threshold is p = 0.00128, when the presented 39 parameters are taken into account)

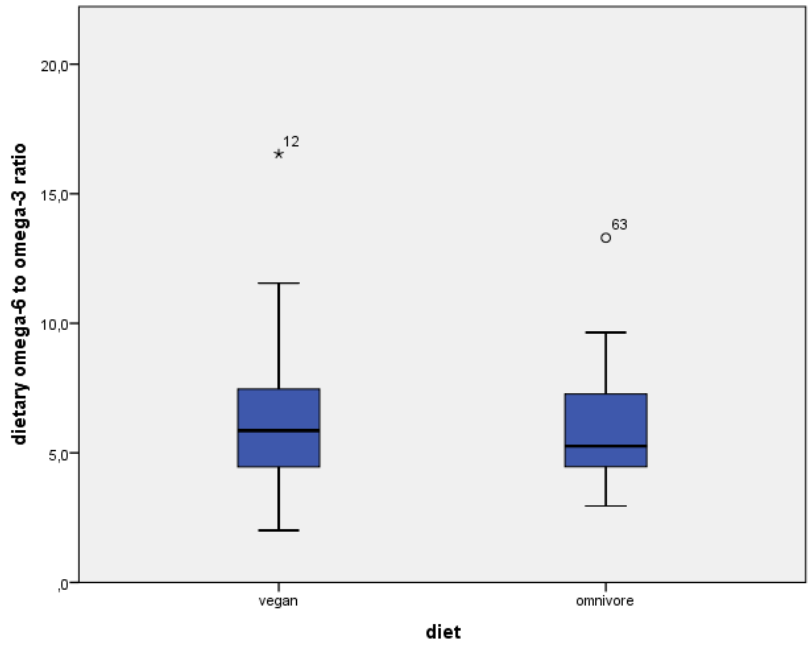

Figure 11: Dietary omega-6 to omega-3 ratio in vegans and omnivores

5.3 Analyses of plasma phospholipid fatty acids in vegans and omnivores

The proportions of fatty acid groups in vegans and omnivores are demonstrated in figure 12. The highest proportion of plasma phospholipid fatty acids in both groups were SFA followed by PUFA, MUFA and TFA. With the exception of total MUFA, we found significant differences between vegans and omnivores within every fatty acid group. Furthermore, we observed a significantly higher plasma omega-6 to omega-3 ratio in vegans compared to omnivores (figure 13).

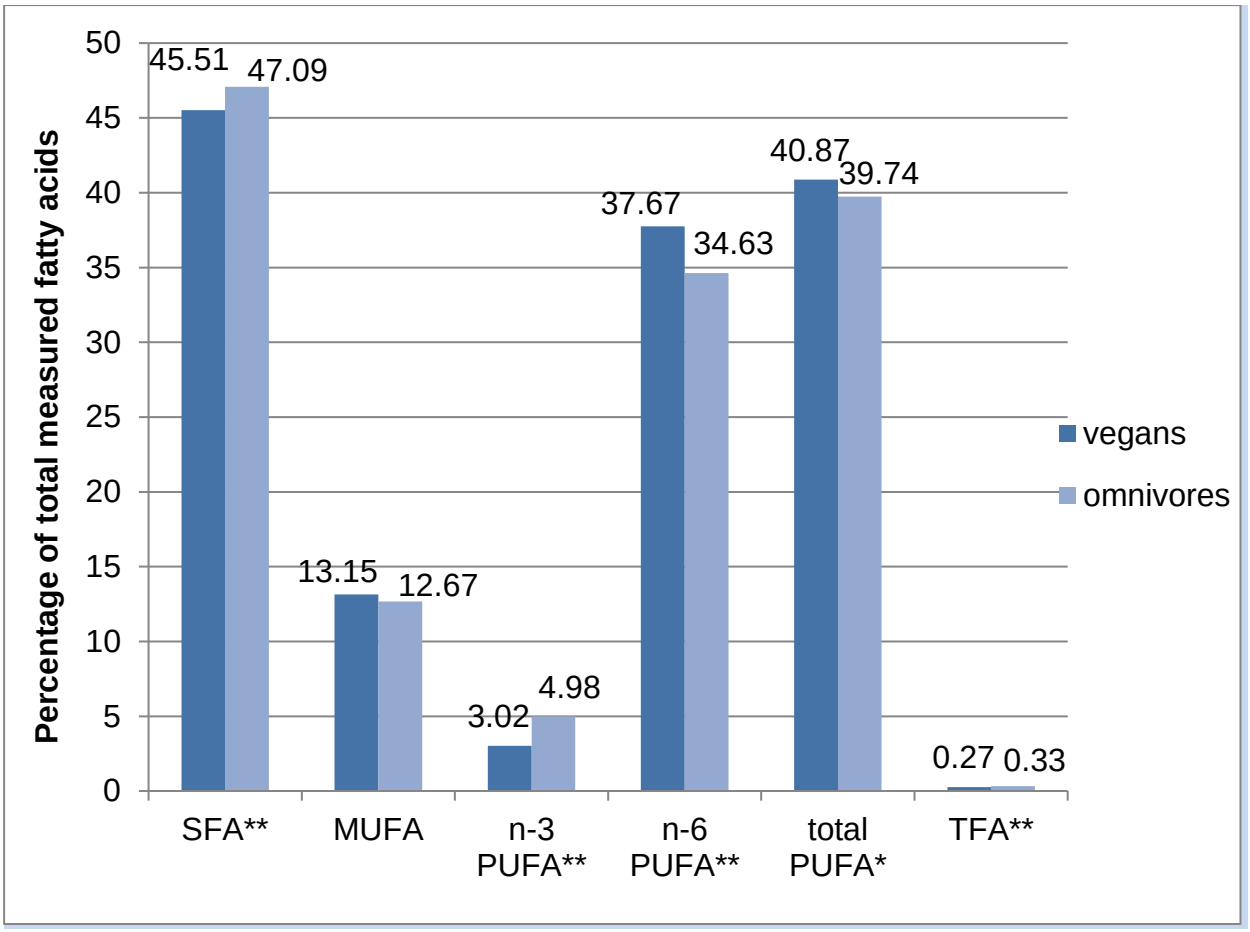

Figure 12: Geometric means (percentage of total fatty acids) of plasma phospholipid fatty acid groups according to diet, *p = 0.009, **p < 0.0001

In table 3 the proportions of individual fatty acids are compared between vegans and omnivores.

Table 3: Plasma phospholipid fatty acid proportions in vegans and omnivores [a]

	Vegans (n=36)	Omnivores (n=36)	p-value
SFA			
C14:0 Myristic acid			
Model1 [b]	0.26 (0.24-0.28)	0.29 (0.27-0.31)	*0.04*
Model 2 [c]	0.26 (0.22-0.31)	0.29 (0.25-0.34)	0.1
C15:0 Pentadecanoic acid			
Model1 [b]	0.15 (0.14-0.16)	0.25 (0.24-0.27)	*< 0.0001* [d]
Model 2 [c]	0.13 (0.12-0.16)	0.24 (0.21-0.27)	*< 0.0001* [d]
C16:0 Palmitic acid			
Model1 [b]	28.27 (27.83-28.71)	30.45 (29.98-30.92)	*< 0.0001* [d]
Model 2 [c]	28.68 (27.68-29.71)	30.71 (29.71-31.75)	*< 0.0001* [d]
C17:0 Heptadecanoic acid			
Model1 [b]	0.38 (0.36-0.40)	0.44 (0.42-0.47)	*< 0.0001* [d]
Model 2 [c]	0.36 (0.31-0.40)	0.42 (0.38-0.48)	*<0.0001* [d]
C18:0 Stearic acid			

	Vegans (n=36)	Omnivores (n=36)	p-value
Model1 [b]	16.21 (15.76-16.68)	15.48 (15.05-15.93)	*0.03*
Model 2 [c]	16.34 (15.29-17.47)	15.66 (14.71-16.66)	*0.05*
C20:0 Arachidic acid			
Model1 [b]	0.15 (0.14-0.16)	0.10 (0.09-0.10)	*< 0.0001* [d]
Model 2 [c]	0.15 (0.13-0.17)	0.10 (0.09-0.11)	*< 0.0001* [d]
Sum SFA			
Model1 [b]	45.51 (45.08-45.94)	47.09 (46.65-47.53)	*< 0.0001* [d]
Model 2 [c]	45.98 (44.96-47.02)	47.47 (46.49-48.47)	*< 0.0001* [d]
MUFA			
C16:1n7c Palmitoleic acid			
Model1 [b]	0.33 (0.29-0.37)	0.45 (0.40-0.51)	*0.0009* [d]
Model 2 [c]	0.37 (0.28-0.49)	0.47 (0.36-0.60)	*0.01*
C18:1n7c *cis*-Vaccenic acid			
Model1 [b]	1.77 (1.69-1.85)	1.40 (1.37-1.47)	*< 0.0001* [d]
Model 2 [c]	1.79 (1.60-1.99)	1.41 (1.28-1.56)	*< 0.0001* [d]
C18:1n9c Oleic acid			

	Vegans (n=36)	Omnivores (n=36)	p-value
Model1 [b]	10.71 (10.20-11.25)	10.61 (10.10-11.14)	0.8
Model 2 [c]	11.55 (10.33-12.90)	11.12 (10.03-12.34)	0.3
C20:1n9 Gondoic acid			
Model1 [b]	0.28 (0.26-0.30)	0.17 (0.16-0.18)	*< 0.0001* [d]
Model 2 [c]	0.26 (0.22-0.31)	0.16 (0.14-0.19)	*< 0.0001* [d]
Sum MUFA			
Model1 [b]	13.15 (12.58-13.75)	12.67 (12.12-13.24)	0.3
Model 2 [c]	13.64 (12.33-15.08)	13.20 (12.01-14.50)	0.06
n-3 PUFA			
C18:3n3 α-Linolenic acid			
Model1 [b]	0.27 (0.24-0.29)	0.25 (0.22-0.27)	0.4
Model 2 [c]	0.31 (0.24-0.39)	0.28 (0.23-0.35)	0.3
C20:5n3 Eicosapentaenoic acid			
Model1 [b]	0.49 (0.42-0.56)	0.96 (0.83-1.10)	*< 0.0001* [d]
Model 2 [c]	0.50 (0.43-0.57)	0.94 (0.81-1.08)	*< 0.0001* [d]

	Vegans (n=36)	Omnivores (n=36)	p-value
C22:5n3 Docosapentaenoic acid n-3			
Model1 [b]	0.70 (0.64-0.76)	0.79 (0.73-0.86)	*0.03*
Model 2 [c]	0.66 (0.54-0.79)	0.74 (0.62-0.88)	0.06
C22:6n3 Docosahexaenoic acid			
Model1 [b]	1.50 (1.36-1.65)	2.90 (2.64-3.19)	*< 0.0001* [d]
Model 2 [c]	1.35 (1.08-1.68)	2.64 (2.15-3.24)	*< 0.0001* [d]
Sum n-3 PUFA			
Model1 [b]	3.02 (2.80-3.26)	4.98 (4.62-5.38)	*< 0.0001* [d]
Model 2 [c]	2.82 (2.36-3.37)	4.66 (3.95-5.51)	*< 0.0001* [d]
n-6 PUFA			
C18:2n6c Linoleic acid			
Model1 [b]	25.70 (24.94-26.48)	21.92 (21.27-22.58)	*< 0.0001* [d]
Model 2 [c]	25.35 (23.64-27.19)	21.79 (20.41-23.27)	*< 0.0001* [d]
C18:3n6 γ-linoleic acid			
Model1 [b]	0.08 (0.07-0.09)	0.08 (0.07-0.09)	0.8
Model 2 [c]	0.09 (0.07-0.13)	0.08 (0.06-0.11)	0.4

	Vegans (n=36)	Omnivores (n=36)	p-value
C20:2n6 Docosadienoic acid			
Model1 [b]	0.37 (0.34-0.39)	0.30 (0.28-0.33)	*0.0005* [d]
Model 2 [c]	0.34 (0.29-0.40)	0.28 (0.24-0.33)	0.0009 [d]
C20:3n6 Dihomo-γ-linolenic acid			
Model1 [b]	2.55 (2.35-2.77)	2.77 (2.55-3.01)	0.2
Model 2 [c]	2.48 (2.04-3.03)	2.71 (2.25-3.26)	0.2
C20:4n6 Arachidonic acid			
Model1 [b]	8.23 (7.69-8.80)	8.78 (8.21-9.39)	0.2
Model 2 [c]	7.67 (6.58-8.95)	8.45 (7.32-9.76)	0.06
C22:4n6 Adrenic acid			
Model1 [b]	0.28 (0.26-0.30)	0.28 (0.26-0.30)	1.0
Model 2 [c]	0.28 (0.24-0.32)	0.28 (0.24-0.32)	0.9
C22:5n6 Docosapentaenoic acid n-6			
Model1 [b]	0.16 (0.14-0.18)	0.23 (0.20-0.25)	*< 0.0001* [d]
Model 2 [c]	0.17 (0.13-0.21)	0.24 (0.19-0.30)	*< 0.0001* [d]
Sum n-6 PUFA			

	Vegans (n=36)	Omnivores (n=36)	p-value
Model1 [b]	37.76 (37.12-38.41)	34.63 (34.04-35.22)	*< 0.0001* [d]
Model 2 [c]	36.60 (35.29-37.97)	34.01 (32.87-35.20)	*< 0.0001* [d]
Sum PUFA			
Model1 [b]	40.87 (40.29-41.46)	39.74 (39.17-40.32)	*0.009*
Model 2 [c]	39.40 (38.23-40.61)	38.78 (37.71-39.90)	0.1
TFA			
C18:1n7t Vaccenic acid			
Model1 [b]	0.05 (0.04-0.06)	0.12 (0.10-0.13)	*< 0.0001* [d]
Model 2 [c]	0.05 (0.03-0.06)	0.11 (0.08-0.14)	*< 0.0001* [d]
C18:1n9t Elaidic acid			
Model1 [b]	0.13 (0.12-0.15)	0.14 (0.13-0.16)	0.4
Model 2 [c]	0.15 (0.12-0.20)	0.16 (0.13-0.20)	0.8
C18:2n6t Linolelaidic acid			
Model1 [b]	0.08 (0.07-0.09)	0.07 (0.06-0.07)	*0.003*
Model 2 [c]	0.08 (0.07-0.10)	0.07 (0.06-0.08)	*0.009*
Sum TFA			

	vegans (n=36)	omnivores (n=36)	p-value
Model1 [b]	0.27 (0.25-0.29)	0.33 (0.31-0.36)	*< 0.0001* [d]
Model 2 [c]	0.28 (0.24-0.33)	0.34 (0.29-0.40)	*0.0004* [d]

[a] All values are presented as percent and geometric means (95 % CI)

[b] unadjusted

[c] adjusted for alcohol intake (g/d), educational level, physical activity (h/week) and smoking status

[d] Statistically significant after Bonferroni correction for multiple comparisons (the threshold is p = 0.0017, when the presented 29 parameters are taken into account)

As demonstrated in Table 3, vegans had significantly lower proportions of saturated fatty acids and of individual fatty acids C15:0, C16:0, C17:0 ($p < 0.0001$). However, SFA that mainly occur in plants as stearic acids (C18:0) and arachidic acid (C20:0) were significantly higher in vegans ($p = 0.03$ and $p < 0.0001$). After adjusting for the main lifestyle factors in Model 2, results for C15:0, C16:0, C17:0, C20:0 and total SFA remained significant, except for myristic acid C14:0 and stearic acid C18:0 ($p = 0.1$ and $p = 0.05$, respectively).

Regarding MUFA we found significant differences within individual fatty acids, but when all MUFA were considered together, no statistical difference could be found ($p = 0.25$ model 1, $p = 0.06$ model 2). Regarding individual MUFA results, these did not change after adjustments.

The proportions of total omega-3 fatty acids were significantly lower in vegans ($p < 0.0001$). Although we could not demonstrate any difference in the proportion of the essential omega-3 fatty acid ALA between vegans and omnivores ($p = 0.37$), the amount of EPA and DHA was significantly higher in omnivores (both $p < 0.0001$). Regarding docosapentaenoic acid (C22:5n3), differences did not reach significance after Bonferroni correction for the unadjusted Model, and no longer reached significance in Model 2. In terms of the remaining omega-3-fatty acids, the results remained significant after adjustments in Model 2.

Total omega-6 fatty acids and the essential omega-6 fatty acid LA reached significantly higher proportions in vegans in both models (both $p < 0.0001$). Additionally docosadienoic acid (C20:2n6) showed higher proportions in vegans ($p = 0.0005$ Model 1 and $p = 0.0009$ Model 2). We could not demonstrate any difference in the proportion of dihomo-γ-linolenic acid (C20:3n6), arachidonic acid (C20:4 n-6) and adrenic acid (C22:4n6) within the dietary groups in both models.

Regarding total PUFA, vegans had significantly higher proportions in our unadjusted model ($p = 0.009$), while it lost statistical significance in Model 2 ($p = 0.1$).

Regarding trans fatty acids, vegans had significantly lower proportions ($p < 0.0001$), but no difference could be found for the principle trans fatty acid, elaidic acid (C18:1 n-9t) ($p = 0.4$).

The plasma omega-6 to omega-3 ratio was significantly different ($p < 0.0001$) vegans median 12.1:1 (IQR: 10.5:1 – 14.6:1), omnivores 6.9:1 (IQR: 6.0:1 – 8.3:1). This is also presented in figure 13.

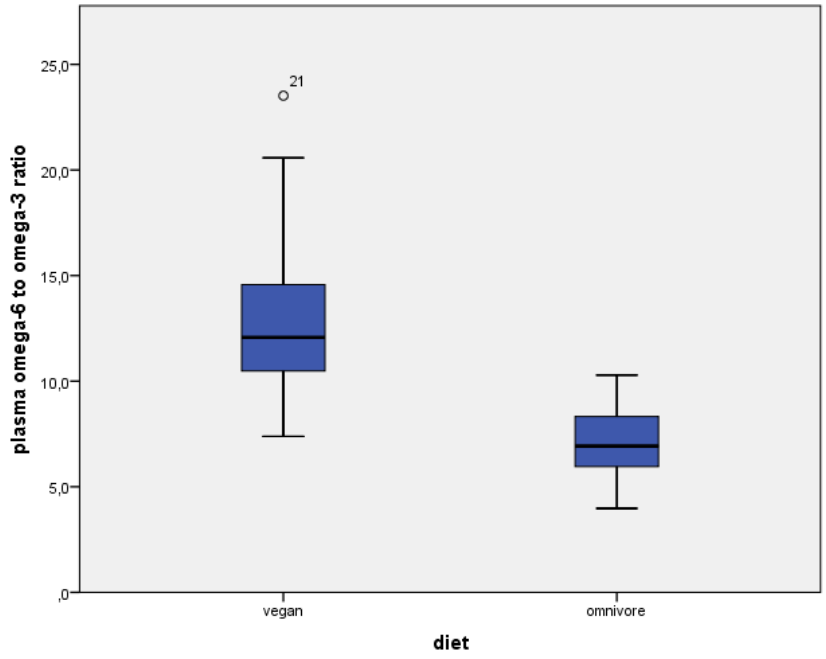

Figure 13: Plasma omega-6 to omega-3 ratio in vegans and omnivores

5.3.1 *Correlation of dietary fatty acids with plasma phospholipid fatty acids*

To reveal the influence of dietary fatty acid intake of fatty acid groups on plasma-phospholipids, we had a closer look at the correlations of these parameters (table 4).
Regarding the whole study population, we saw a positive correlation of SFA intake with SFA in plasma phospholipids ($p < 0.0001$).
When analyzing the different dietary groups separately, we could not find any correlation between fatty acids intake and plasma proportions.

Table 4: Correlation of intake of fatty acid group with plasma phospholipids of the respective fatty acid group [a]

	All participants n = 72		**Vegans n = 36**		**Omnivores n = 36**	
	Correlation coefficient [a]	p-value	Correlation coefficient [a]	p-value	Correlation coefficient [a]	p-value
SFA	0.5	*< 0.0001*	0.1	0.5	0.1	0.6
MUFA	0.2	0.1	-0.1	0.5	0.0	0.9
PUFA	-0.1	0.6	0.5	0.06	0.2	0.2
n-3	-0.1	0.4	0.1	0.7	0.0	1.0
n-6	0.0	0.8	0.1	0.6	0.3	0.1

[a] Spearman's rank correlation coefficient

5.3.2 *Correlation between plasma fatty acids and the duration of a vegan diet*

To investigate the hypothesis that there is a basal conversion rate from ALA to DHA to keep plasma proportions of long chain omega-3 fatty acids stable, we explored the correlation of n-3 proportions and the duration of a participant following a vegan diet (Table 5). We could not demonstrate any significant correlation between the duration of a vegan diet and individual LC-n-3 PUFA, total PUFA and total LC-n-3 PUFA, or LA or n-6 PUFA.

Table 5: Correlation between polyunsaturated fatty acids and duration of a vegan diet [a]

	Correlation coefficient [a]	**p-value**
PUFA	0.2	0.2
n-3 PUFA	0.0	0.9
ALA (C18:3n-3)	0.0	1.0
EPA (C20:5n-3)	-0.1	0.7
DHA (C22:6n-3)	0.0	0.9
Total LC-n3-PUFA	0.0	0.9

	Correlation coefficient [a]	p-value
n-6-PUFA	0.2	0.2
LA (C18:2n-6)	0.1	0.7

[a] Spearman's rank correlation coefficient, n= 36

5.4 Analyses of desaturase activity

The results of the estimated desaturase computations can be seen in table 6. Here, the geometric mean and 95% CI of the estimated enzyme activities are presented. Significant difference in enzyme activity can only be seen for SCD-1 C16:0, which was higher in omnivores, but lost significance after adjusting for the main lifestyle confounders. No differences were observed for Δ5-desaturase, Δ6-desaturase or SCD-1 C18:0.

Table 6: Estimated desaturase activity in vegans and omnivores [a]

	Vegans (n=36)	Omnivores (n=36)	p-value
Δ5-desaturase			
Model 1 [b]	3.23 (2.91-3.58)	3.17 (2.85-3.51)	0.8
Model 2 [c]	3.09 (2.42-3.94)	3.12 (2.48-3.92)	0.9
Δ6-desaturase			
Model 1 [b]	0.003 (0.003-0.004)	0.004 (0.003-0.004)	0.3
Model 2 [c]	0.004 (0.003-0.005)	0.004 (0.003-0.005)	0.8
SCD-1 C16:0			
Model 1 [b]	0.01 (0.01-0.01)	0.02 (0.01-0.02)	*0.006*
Model 2 [c]	0.01 (0.01-0.02)	0.02 (0.01-0.02)	0.06
SCD-1 C18:0			
Model 1 [b]	0.66 (0.62-0.70)	0.69 (0.64-0.73)	0.4
Model 2 [c]	0.71 (0.61-0.82)	0.71 (0.62-0.82)	0.9

[a] Variables are presented as geometric mean (95 % CI)

[b] no adjustments

[c] adjusted for alcohol intake (g/d), educational level, physical activity (h/week) and smoking status

5.5 Analysis of cholesterol and triglyceride within dietary groups

In figure 14 the median concentrations of total cholesterol, HDL- and LDL-cholesterol, as well as triglyceride concentrations (mg/dl) in vegans and omnivores are presented. We found significant differences between vegans and omnivores for total cholesterol and LDL-cholesterol ($p < 0.0001$ and $p = 0.001$), but not for triglycerides and HDL-cholesterol ($p = 0.3$ and $p = 0.2$).

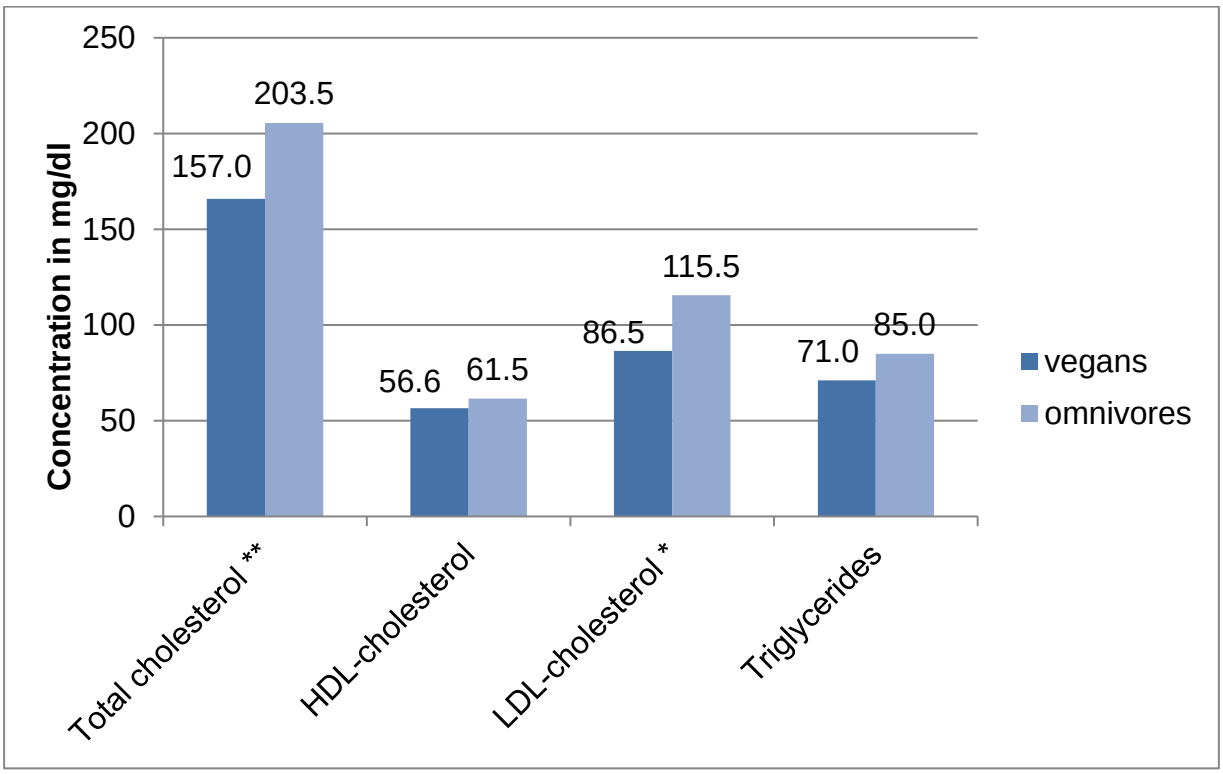

Figure 14: Comparison of cholesterol and triglyceride concentrations in vegans and omnivores
Variables expressed as median in mg/dl; Comparing medians using Mann-Whitney U test, *p = 0.001, **p < 0.0001

After stratification for sex, we revealed HDL-cholesterol levels to be significantly lower in vegan women (64.5 mg/dl (56.0-74.0) vs. 78.5 mg/dl (64.0-83.0), $p = 0.02$) whereas no difference was found in men (vegan 52.0 mg/dl (45.0-57.0), omnivore 51.5 mg/dl (46.0-60.0) $p = 0.9$). No differences were found in triglycerides for sex strata.

5.6 Analysis of plasma phospholipid fatty acids and blood lipids

Correlations of plasma phospholipid fatty acids and blood lipids regarding the whole study population are presented in table 7. When analyzing correlations according to diet we found different results.

In vegans HDL-cholesterol was positively correlated with total plasma phospholipid MUFA (Spearman's correlation coefficient 0.4, p = 0.007) and negatively with plasma phospholipid SFA (Spearman's correlation coefficient -0.3, p = 0.04). We found a negative correlation between LDL-cholesterol and total MUFA (Spearman's correlation coefficient -0.3, p = 0.05) and TFA and triglycerides (Spearman's correlation coefficient -0.5, p = 0.002). In vegans no correlations were found between fatty acid groups and total cholesterol levels, nor did we detect any correlation of total PUFA, omega-3 or omega-6 fatty acids with blood lipids.

In omnivores HDL-cholesterol was correlated with total PUFA and omega-6 PUFA (Spearman's correlation coefficient 0.4, p = 0.02, Spearman's correlation coefficient 0.4, p = 0.02). We found no correlation between LDL-cholesterol and plasma phospholipid fatty acids. We also observed a negative correlation between TFA and triglycerides (Spearman's correlation coefficient -0.4, p = 0.02). Total cholesterol was negatively correlated with total SFA levels (Spearman's correlation coefficient -0.3, p= 0.04).

Table 7: Correlation of plasma phospholipid fatty acids and blood lipids [a]

		SFA	**MUFA**	**PUFA**	**n-3**	**n-6**	**TFA**
HDL [mg/dl]	Correlation Coefficient	-0.1	-0.1	0.0	0.1	-0.1	0.1
	p-value	0.2	0.7	0.9	0.2	0.6	0.2
LDL [mg/dl]	Correlation Coefficient	0.2	0.0	0.0	0.3	-0.2	0.1
	p-value	0.2	0.3	0.9	*0.01*	0.1	0.5
Total Cholesterol [mg/dl]	Correlation Coefficient	0.1	-0.1	-0.1	0.4	-0.3	0.1
	p-value	0.3	0.5	0.6	*0.0003*	*0.01*	0.3

		SFA	MUFA	PUFA	n-3	n-6	TFA
Triglycerides [mg/dl]	Correlation Coefficient	0.1	-0.1	0.0	0.2	-0.2	-0.3
	p-value	0.5	0.4	0.9	0.05	0.2	*0.004*

[a] Spearman's correlation coefficient, n= 72

5.7 Analysis of dietary fatty acids and blood lipids

Regarding the correlation of dietary fat intake with blood lipids in the whole study population, we discovered a significant positive correlation with SFA and LDL-cholesterol as well as total cholesterol. Dietary PUFA intake showed a significant inverse correlation with HDL- and total cholesterol. Regarding omega-3 PUFA, we found an inverse correlation with total cholesterol. Intake of omega-6 PUFA showed a significant inverse correlation with HDL- and total cholesterol. No correlation was found between MUFA intake and blood lipids. Additionally, no significant correlation was found for dietary intake of fatty acids and triglycerides.

Table 8: Correlation of dietary fatty acid intake (based on weighed food records) with blood lipids [a]

		SFA [mg/d]	MUFA [mg/d]	PUFA [mg/d]	n-3 [mg/d]	n-6 [mg/d]
HDL [mg/dl]	Correlation Coefficient	0.1	-0.1	-0.3	-0.2	-0.3
	p-value	0.3	0.5	*0.01*	0.06	*0.009*
LDL [mg/dl]	Correlation Coefficient	0.3	0.1	-0.2	-0.1	-0.2
	p-value	*0.007*	0.4	0.06	0.2	0.08

		SFA [mg/d]	MUFA [mg/d]	PUFA [mg/d]	n-3 [mg/d]	n-6 [mg/d]
Total Cholesterol [mg/dl]	Correlation Coefficient	0.4	0.1	-0.3	-0.3	-0.3
	p-value	*0.0002*	0.6	*0.004*	*0.02*	*0.007*
Triglycerides [mg/dl]	Correlation Coefficient	0.1	0.1	0.0	-0.1	0.0
	p-value	0.3	0.5	1.0	0.4	0.8

[a] Spearman's correlation coefficient, n= 72

After a subdivision into dietary groups, we detected an inverse association between HDL and omega-6 fatty acids and total PUFA in vegans (Spearman's correlation coefficient -0.4, p= 0.007, Spearman's correlation coefficient -0.4 p = 0.02). Furthermore, a positive correlation between SFA and triglycerides was found in vegans (Spearman's correlation coefficient 0.3, p = 0.04). No correlation was detected in omnivores.

5.8 Sensitivity analyses

Regarding sensitivity analyses, we repeated the comparison of plasma phospholipid fatty acid proportions in vegans and omnivores, after excluding participants who consume omega-3 fatty acid supplements (n=3) regularly. We detected no relevant differences in fatty acids groups (data not shown).

Interaction analyses between diet and sex revealed no differences in the association between dietary intake of fatty acids and plasma phospholipid fatty acids: SFA (diet p for interaction = 0.6, plasma p for interaction = 0.1), MUFA (diet p for interaction = 0.4, plasma p for interaction = 0.2), PUFA (diet p for interaction = 0.7, plasma p for interaction = 0.8), n-3 PUFA (diet p for interaction = 0.6, plasma p for interaction = 0.5) n-6 PUFA (diet p for interaction = 0.8, plasma p for interaction = 1.0), and TFA (plasma p for interaction = 0.5).

6 Discussion

6.1 Fatty acid profiles in vegans and omnivores

The aim of this study was to analyze and compare fatty acid profiles in vegans and omnivores regarding dietary intake and plasma phospholipid proportions. Fatty acids play several important roles in the human body: they serve as energy sources, precursors of signaling molecules, components of cell membranes and functional lipids, and play an important role in metabolic homeostasis (83). Furthermore, the elevation of certain circulating fatty acids is associated with chronic diseases such as cardiovascular disease, heart disease, cancer or autoimmune diseases (84). Therefore, it is necessary to differentiate between fatty acid groups as well as individual fatty acids.

6.1.1. Saturated fatty acids

Saturated fatty acids mainly occur in animal sources such as meat, dairy, fish and egg yolks, but they can also be found in plant products like palm oil, or other plant-based oils, chocolate or coconut (85).

Our findings demonstrate that vegans consume lower amounts of total fat and saturated fat compared to omnivores, while their daily energy intake did not differ significantly. The German Nutrition Society recommends a daily intake of 7-10% of energy from SFA and 30% from total fat (86, 87). The WHO recommends a daily intake of SFA below 10% of total energy (88). Our results show that even though vegans and omnivores exceed the dietary recommendations of daily total fat intake, omnivores consume significantly higher amounts of SFA compared to vegans. This is not surprising since the Western diet is rich in meat and dairy, which are major sources for SFA (28). According to the German Nutrition Society dietary fat should mainly come from MUFA and PUFA when exceeding the recommended daily fat intake (87).

In our study, the only SFAs that were consumed in higher amounts in vegans were behenic acid (C22:0) and lignoceric acid (C24:0). These fatty acids are mostly found in peanuts and peanut oil (85).

Regarding plasma fatty acids, vegans had significantly lower plasma phospholipid proportions of SFA, which corresponds with previous findings (25, 52) and can be explained by the fact that vegans do not consume meat and dairy, which are the main dietary sources for SFA in the Western diet (28). Regarding individual plasma SFA, vegans in our study had lower plasma proportions of fatty acids that usually occur in cow's milk such as myristic acid (C14:0), pentadecanoic acid (C15:0), palmitic acid (C16:0) and heptadecanoic acid (C17:0), while fatty acids that mainly come from plants such as stearic acid (C18:0) and arachidic acid (C20:0) were significantly higher among vegans.

Our results on SFA are in line with findings from other studies (18, 19, 25, 52, 89, 90).
Rosell selected 196 meat eaters, 231 vegetarians and 232 vegans from the EPIC-Oxford study to assess dietary intake using food questionnaires and plasma fatty acid composition using gas chromatography (52). In this study, daily intake of saturated fatty acids in vegans was only 40% of that in omnivores ($p < 0.001$) (52). Furthermore, plasma proportions of C14:0 and C16:0 were significantly lower in vegans, whereas no differences were found in plasma C18:0 and C20:0 proportions (52).
Kristensen and colleagues conducted a study among Danish vegans to determine dietary and supplementary intake using four-day weighed food records (18). The authors demonstrated that vegans had lower intakes of saturated fatty acids compared to meat eaters (18).
A Swiss study aimed to assess dietary intake among vegans, omnivores and vegetarians in Switzerland using a three-day weighed food record. The authors demonstrated a significant difference in dietary intake of saturated fatty acids in vegans compared to omnivores (vegans, mean = 20g/d; omnivores, mean = 37g/d) (19).
Furthermore, a Finnish study by Elorinne from 2016 compared dietary intake and status of long-term vegans with non-vegetarians using three-day dietary records (25). Serum fatty acids were detected using gas chromatography (25). The study recruited 22 vegans who followed a vegan diet for at least one year and 19 non-vegans (25). Similarly to our study, it was shown that vegans consumed lower amounts of total fat and saturated fat ($p < 0.001$), while total energy intake did not differ significantly. Furthermore, vegans had lower serum proportions of saturated fatty acids ($p < 0.0001$) (25).

A study from 2014 compared the nutrient intake in vegans, vegetarians, semi-vegetarians, pesco-vegetarians and omnivores (89). Dietary information was estimated using a food-frequency record (89). Here, the lowest intakes for total energy and saturated fatty acids were found in the vegan group compared to omnivores (all $p < 0.01$) (89). The daily intake of saturated fat was 21g in vegans and 54g in omnivores ($p < 0.01$), which is higher when compared to the intake in our study with a mean intake of 16.38g in vegans and 42.95g in omnivores (89).

Interestingly, we were able to additionally demonstrate a significant positive correlation between dietary SFA intake and plasma phospholipid SFA proportion when the whole study population was addressed. However, after stratification for diet no significant correlation could be registered. The significant correlation of dietary SFA and plasma phospholipid proportions in the whole study population might be explained with the wide range of intakes and proportions when considering both vegans and omnivores together.

However, the missing correlation between SFA and plasma proportions was not unexpected. In a study by Forsythe and colleagues, eight weight stable men were fed a carbohydrate restrictive diet over 12 weeks varying in the amount of saturated fat (91). During the first 6 weeks, the participants received a diet which contained 86 g SFA/day, followed by a diet containing a higher amount of unsaturated fat and only 47 g SFA daily for another 6 weeks (91). No association between dietary SFA intake and plasma SFA concentrations could be made even though the intake of SFA was almost halved (91). The authors explain these findings as due to the adaption to a carbohydrate restrictive diet which results in lower insulin levels. Low insulin levels lead to increased lipolysis and decreased de novo lipogenesis (91). Moreover, results from the Swedish National Dietary Survey show that no significant correlation was found for dietary SFA intake and total SFA plasma phospholipid proportions (92). This was explained with the fast metabolization of SFA to MUFA (92).

Regarding the odd-chain fatty acids C15:0 and C17:0 it was assumed that these fatty acids cannot be synthetized endogenously and therefore reflect dairy intake (58), yet it was observed that plasma levels of vegans, omnivores and vegetarians reached comparable concentrations for these fatty acids in several studies (93, 94). Thus, it is suggested that these odd-chain fatty acids must have other dietary sources, or that they

can even be synthesized endogenously either by elongation of shorter odd-chain saturated fatty acids or by chain shortening of very long odd-chain fatty acids (75, 76). They might also be converted from the phytosphingosine of certain glycosphingolipids (75). Furthermore, it was shown recently that these odd-chain fatty acids can be synthetized in the liver using dietary fibers (76).

Regarding the effect of saturated fat on blood lipids, it was shown previously that cholesterol levels can be influenced by the intake of saturated fat (87). This is in line with our findings showing a significant positive correlation between daily SFA intake and LDL-cholesterol and total cholesterol. However, after further subdivision into diet correlation was no longer significant. This may be explained by the wide range of SFA intake as well as cholesterol levels when the whole study population is addressed. Saturated fatty acids, especially the long-chain fatty acids lauric acid (C12:0) myristic acid (C14:0) and palmiticacid acid (C16:0), have been demonstrated to increase total cholesterol and LDL-cholesterol by decreasing LDL-receptor activity (95) (57). This could be one explanation why vegans have lower levels of total and LDL-cholesterol compared to omnivores. Stearic acid has been shown to decrease LDL with neutral or lowering influence on HDL levels (96).

Increased levels of total saturated fatty acids were shown to be associated with insulin resistance, elevated serum glucose and tissue inflammation (59). However, regarding the risk of type 2 diabetes mellitus, the type of saturated fats should also be considered. Several studies have demonstrated an inverse association between C15:0 and C17:0 and type 2 diabetes mellitus (75, 97), while myristic, palmitic and stearic acid are shown to be associated with an increased risk of type 2 diabetes mellitus (98). Furthermore, saturated fat can increase lipid levels and promote atherosclerosis, and therefore affect CVD risk (36). Regarding the association of SFA and CVD, it was shown that the food sources of SFA rather than the amount of SFA influenced CVD risk (99). Additionally, the risk of CVD can be reduced by replacing saturated fat with polyunsaturated fatty acids (99). According to a report by the American Heart Association, replacing SFA with PUFA can reduce CVD risk by approximately 30% (100). Additionally, a meta-analysis points to evidence that the replacement of saturated fats with omega-6 fatty acids reduces CVD events and mortality (101). This was mainly explained by a reduction in LDL-cholesterol and total cholesterol when consuming polyunsaturated instead of

saturated fats. Replacing SFA with MUFA may also lower cholesterol levels but in a smaller extent compared to PUFA (102). Replacing 5% of the energy from SFA with LA reduced the risk of cardiovascular events by 9% and the risk of coronary death was reduced by 13% (101).

To date dietary recommendations on individual fatty acids have been difficult to make since SFA come in combinations in different foods (103). Foods might be high in SFA but may contain high concentrations of other important nutrients. Interestingly, some foods high in SFA were shown to have positive effects on CVD, such as dark chocolate, avocado or nuts (104). Results from previous studies suggest that the matrix of different foods may modify the influence of SFA on CVD events and not the amount of consumed SFA itself (104). Therefore, scientists assume that shifting dietary recommendations on food-based dietary guidelines might be helpful for consumers, so that they choose foods with healthful dietary fats instead of focusing on the amount of dietary fats (104). However, replacing several SFA with other SFA may be still useful for food manufacturers (103).

6.1.2. Monounsaturated fatty acids

Monounsaturated fatty acids mostly occur in oils such as olive or canola oil, but they can also be found in nuts, seeds or animal products such as meat and dairy (85).
As shown in the results, dietary intake of MUFA was significantly higher in omnivores. The German Nutrition Society recommends a daily intake of 10-15% of energy from MUFA (86, 87). We have demonstrated that omnivores and vegans meet the recommendations (13.6% and 11.3% from total energy). Previous studies have shown that foods rich in MUFA in our Western diet often come from animal sources (39).
In particular, MUFA which are mainly found in dairy products (C14:1 C15:1, C16:1n7c, C17:1, C18:1n9) were consumed in a significantly higher amount by omnivores (105).
Previous studies on dietary MUFA intake show mixed results, such as a study by Clarys which demonstrated that vegans have lower dietary intakes of MUFA compared to omnivores ($p < 0.01$) (89), while a Finnish study could not detect any significant difference in dietary MUFA intake in both dietary groups (25). A Swiss study also

showed no significant differences in dietary MUFA intake in vegans and omnivores (vegans, mean = 41g/d; omnivores, mean = 35g/d) (19). Rosell showed a significantly lower intake of MUFA in vegans compared to omnivores ($p < 0.001$) (52).
We did not discover any difference regarding total plasma phospholipid proportions of MUFA, but for individual plasma monounsaturated fatty acid proportions we found significant differences.
Regarding plasma levels, Rosell demonstrated that vegans had significantly lower levels of palmitoleic acid (C16:1n7) ($p < 0.001$), whereas levels of oleic acid (C18:1n9) and erucic acid (C22:1) did not differ significantly (52). Additionally, in our study population the proportions of oleic acid did not differ between dietary groups. This fatty acid represents the topmost dietary MUFA and is mostly found in olive oil (106). In our study vegans had significantly higher levels of C18:1n7c, which is mainly found in safflower or canola oil, and seeds and gondoic acid (C20:1n9), which derives from nuts and also fish oils (85).

In a meta-analysis conducted by Schwingshackl, a risk reduction in all-cause mortality (-11%), cardiovascular mortality (-12%), cardiovascular events (-9%), and stroke (-17%) was demonstrated when comparing the top versus the bottom third of a combination of MUFA subgroups (106). While MUFA from olive oil seemed to be associated with reduced risk, other plant and animal sources did not have any effect on these outcomes, indicating that the source of MUFA should be considered in order to evaluate the benefits of monounsaturated fatty acids (106). Studies on the Mediterranean diet, which is rich in olive oil, showed that replacing MUFA for carbohydrates decreases LDL-levels and increases HDL-cholesterol (39). Moreover, it was revealed that MUFA intake has additional effects on CVD including insulin sensitivity and inflammatory markers (39). Although positive effects of MUFA consumption were shown (57, 106, 107), in prospective studies MUFA intake was not generally associated with CVD risk (39). A possible explanation is that Western food sources rich in MUFA, such as meat and dairy, strongly correlate with SFA (39).

The importance of MUFA from plant-based sources was demonstrated in a study by Wang and colleagues. They examined the association between dietary fat intake and total and cause-specific mortality during a follow-up of up to 32 years including 83,349 female participants from the Nurses' Health Study and 42,884 men from the Health

Professionals Follow-Up Study (107). In this study, the authors found an inverse association between the consumption of MUFA and total mortality (HR 0.89, 95% CI 0.84-0.94, $p < 0.001$), which contradicts the findings of previous studies (106). In these two cohorts, the correlation between MUFA and SFA decreased during follow-up as the majority of food sources were switched from animal products to plant-based foods (107).

6.1.3. Polyunsaturated fatty acids

Polyunsaturated fatty acids can be found in different oils such as safflower, sunflower and flaxseed oils, as well as in soybeans, nuts, seeds and fish (85). In our study vegans received 8.4% of their daily energy from PUFA, omnivores 5.1%. The German Nutrition Society recommends 7-10% of total energy from PUFA (86). We observed that vegans in our population had higher plasma proportions of total PUFA, although results were no longer significant after adjustments for the main lifestyle factors. Higher plasma proportions might be explained with higher dietary intake of PUFAs. In our study, vegans had a significantly higher intake of total PUFA, omega-3 and omega-6 compared to omnivores. These findings correspond with previous results that demonstrate higher blood levels of polyunsaturated fatty acids in vegans, particularly n-6 and n-9 (25, 51-53, 108). This was mainly achieved by significantly greater dietary intake of omega-6 fatty acids compared to omnivores. In a Finnish vegan study, vegans had a higher PUFA intake compared to omnivores (10.9% ± 4.6 % from energy vs. 7.8% ± 2.4 % from energy) 25). The vegan population in the EPIC-Oxford study also consumed significantly higher amounts of PUFA (150%) than omnivores ($p < 0.001$) (52). Interestingly, Welch and colleagues demonstrated that vegan women consume higher amounts of LA compared to omnivores, whereas vegan men consume smaller amounts compared to omnivores (51).

Even though higher plasma PUFA proportions might be explained by higher dietary PUFA intake, we observed that dietary intake of total PUFA did not correlate with total PUFA proportions. These results are in line with findings from the Swedish National Dietary Survey (92).

Regarding the correlation of total PUFA intake and blood lipids, we found a significant negative correlation with HDL- and total cholesterol but no associations with LDL-cholesterol or triglycerides. These results are not in line with findings from a comprehensive systematic review of 49 RCTs on the effects of increasing total PUFA intake on the prevention of cardiovascular disease risk conducted by Abdelhamid et al. (109). Here, total PUFA intake had little or no effect on HDL-, LDL- and total cholesterol, while it possibly reduces triglycerides (109). Our results might be explained by the wide range of PUFA intake when the whole study population including both vegan and omnivorous diets were addressed, since the correlation was no longer significant when analyzing dietary groups separately.

6.1.3.1. Omega-3 polyunsaturated fatty acids

Dietary sources of omega-3 polyunsaturated fatty acids

Omega-3 PUFA occur in plant and animal foods (85). The essential omega-3 fatty acid ALA is mainly found in plant-based foods such as flaxseeds, canola oil and chia seeds (85). Long-chain PUFA EPA and DHA are mainly found in fish such as salmon, herring or sardines (85).

We discovered significantly higher intakes of omega-3 fatty acids in vegans compared to omnivores (2.9 g/d and 2.0 g/d). However, the only omega-3 fatty acid that was consumed in a higher amount in vegans was ALA (2.9 g/d in vegans and 1.5 g/d in omnivores), while long-chain omega-3 fatty acids as EPA and DHA were only consumed in small amounts by vegans (0.0008 g/d and 0.007 mg/d).

These results are inconsistent with findings from the EPIC-Norfolk study, in which dietary data were derived from 7-day food diaries. It was demonstrated that the total n-3 PUFA intake was significantly lower in vegans, with vegans only consuming 0.87 g/d, whereas the total n-3 intake of omnivores was 1.52 g/d (51). Dietary ALA intake in vegan men was 0.84 g/d (omnivores 1.21 g/d), whereas in vegan women ALA intake was 0.71 g/d compared to 0.99 g/d in female omnivores (51). The authors revealed cereals and vegetables to be the main dietary ALA sources in vegans, contributing to 63% of total dietary ALA in vegan men and to 73% in vegan women. Interestingly, cereals and vegetables were also the major ALA source in omnivores, contributing

42%. Fish contributed 13% and meat 12% to the total ALA supply in omnivores (51). The mean dietary intake of EPA in vegan men was 0.009 g/d (± 0.008) which mainly came from spreading fats, compared to 0.13 g/d (± 0.22) in male fish-eaters. The mean dietary intake of EPA in vegan women was 0.002 g/d (± 0.004), compared to 0.02 g/d (± 0.011) in female meat-eaters. The major dietary sources for EPA in vegan women were soups and sauces. In fish eaters, fish supplied 82% of total EPA. The authors also showed that a vegan diet was absent of DHA (51). In our study we were able to detect DHA in a vegan diet, although it was significantly lower compared to omnivores.
Total dietary LC-n-3 intake, which contains the amount of EPA and DHA, of vegan women participating in the EPIC-Norfolk study was 58% compared to fish-eaters, 80% compared to meat-eaters and 82% compared to vegetarians. Vegan men reached 57% of total dietary n-3-PUFA (ALA + DHA + EPA) intake compared to fish-eaters, 76% compared to meat eaters and 75% compared to vegetarians (51).

Regarding the effect of dietary omega-3 fatty acids on blood lipids we detected a significant inverse correlation between omega-3 fatty acids and total cholesterol levels. However, when analyzing dietary groups separately no correlation could be found. This result is in line with findings by Goh (110). However, in this study omega-3 fatty acids did have an effect on lowering triglyceride levels. A possible explanation why triglyceride levels were not affected in our study is that both dietary groups already have very low levels of triglycerides and omega-3 fatty acids might only influence triglycerides at higher levels.

Dietary intake of omega-3 fatty acids did not correlate with plasma phospholipid omega-3 fatty acids. This is contrary to findings from the Swedish National Dietary Survey. Here, plasma phospholipid omega-3 fatty acids correlated strongly with dietary intake (92). Another study also demonstrated increased omega-3 plasma phospholipid levels after supplementation of 1 g omega-3 fatty acids for 3 months in patients with heart failure (111). However, even when analyzing dietary groups separately, no significant correlation of dietary omega-3 fatty acids and plasma proportions was found.

Plasma proportions of omega-3 polyunsaturated fatty acids

Even though vegans reported higher omega-3 intakes, we observed significantly lower plasma proportions of total omega-3 and long chain omega-3 polyunsaturated fatty acids in vegans compared to omnivores. This is not surprising since vegans do not consume any fish, the main dietary source of omega-3 PUFA. Proportions of ALA, however, did not differ significantly between both groups ($p = 0.37$). Different findings on plasma LC-n-3 PUFA proportions have been demonstrated in previous studies (25, 49, 52, 53, 112). Even though dietary intake of n-3 PUFA in the EPIC-Norfolk study was significantly lower in vegans, total plasma LC-n-3 PUFA levels, which contained the proportion of plasma ALA, EPA and DHA were, according to the authors, higher than expected from dietary intake. LC-n-3 PUFA levels in vegan men were 89% compared to fish eaters, 98% compared to meat eaters and 92% compared to vegetarians. Vegan women had plasma n-3 PUFA levels of 104% compared to fish eaters, 114% compared to meat eaters and 120% compared to vegetarians. These differences were significant with $p < 0.001$ in women and $p = 0.002$ in men (51).

Sarter and colleagues compared LC-n-3 PUFA levels of long-term vegans to omnivores (53). In this study, 165 vegans who followed a vegan diet for at least one year and did not consume any fatty acid supplements were recruited. The omega-3 index, which is calculated as the sum of EPA and DHA levels in red blood cell membranes, was analyzed using the dried blood spot methodology. The results were compared to the preexisting data of 76 omnivores. The authors revealed that vegans do have low n-3 levels, but not lower than omnivores who also consume low amounts of EPA and DHA. In addition to this, vegans had significantly higher proportions of EPA ($p < 0.003$), while DHA levels were lower compared to omnivores, although the difference was not statistically significant (53).

In the EPIC-Oxford study the proportion of plasma LA was 37.12 in vegans and 30.42 in meat-eaters ($p < 0.001$) (52). However, the authors could not demonstrate elevated EPA and DHA levels in vegans. In their study each of the LC-n-3 PUFAs was lower in vegans compared to meat eaters. The proportion of EPA was 0.34 in vegans and 0.72 in omnivores and the proportion of DHA was 0.70 in vegans and 1.69 in meat-eaters (both $p < 0.001$) (52).

Furthermore, results from a Finnish study could not demonstrate such high n-3 PUFA levels. The total plasma LC-n-3 PUFA of vegans was only 38% of omnivores. However, it must be taken into account that in this study 8 of 17 omnivores took omega-3 supplements, whereas only one vegan participant took n-3 supplements (25).

Other studies state that although total n-3 intake in vegans and omnivores does not differ, vegans were shown to have significantly lower plasma concentrations of EPA and DHA compared to people who eat fish (49, 53).

In our study, vegans had significantly lower proportions of total omega-3 fatty acids as well as EPA and DHA compared to omnivores, while ALA proportions did not differ significantly. The plasma proportions of omega-3 fatty acids result from dietary intake as well as endogenous conversion from ALA. As previously described, the dietary intake of EPA and DHA was very low compared to omnivores. However, on the basis of a very low dietary intake of long-chain omega-3 fatty acids, vegans presented relatively high plasma proportions. This could be explained by the conversion from ALA into EPA and DHA, although conversion rates are expected to be relatively low (68). However, we did not find a significant difference in desaturase activity in vegans. Furthermore, metabolism of n-3 fatty acids must be taken into account. As mentioned before omega-3 fatty acids can be used as storage, in cell membranes, as precursors for eicosanoids or for EPA and DHA and in ß-oxidation (113), and they might therefore no longer be traced in plasma phospholipids. This might also explain why we could not demonstrate a significant correlation of dietary omega-3 fatty acid intake with omega-3 plasma phospholipid fatty acid proportions.

Health effects of omega-3 polyunsaturated fatty acids

Data on the association between dietary intakes of n-3 PUFA and CVD risk is inconsistent. While some studies did not find any association (114, 115), others revealed a significant risk reduction from CVD, though only for specific outcomes including ischemic stroke, sudden cardiac death, fatal CHD and CHD (116, 117). In a study conducted by Wang and colleagues, a modest inverse association between marine n-3 PUFA intake and total mortality was shown (HR = 0.96, 95% CI, 0.93-1.00, p =0.002) (107). Furthermore, a recent Scandinavian study found an increase in CVD risk at very low LC-n-3 PUFA intakes of ≤ 0.06 g/d, compared to > 0.73 g/d in women aged 16-47 with a follow-up of up to 12 years (118). In our study the mean daily intake of total omega-3 fatty acids was 2.87g, which was higher than the average daily intake of n-3 PUFA in vegans in the EPIC-Norfolk study (0.89g) (51). On the other hand, a study including over 44,000 healthy men found a significant risk reduction with increasing ALA

intakes and low LC-n-3 PUFA intakes (< 100 mg/d) (119). At higher omega-3 PUFA intakes an association could no longer be shown (119). This might be of relevance for our vegan population since they consume only low amounts of LC-n-3 PUFA in their diet (median 10.9 mg/d).

Interestingly, we revealed a significant negative correlation between dietary omega-3 fatty acids and total cholesterol in the whole study population, whereas no association could be observed with LDL-cholesterol. When analyzing dietary groups separately no correlation could be detected. This can be explained by the wide range of cholesterol levels as well as dietary omega-3 intake when the whole population was addressed.

Relevance of omega-3 fatty acid supplementation

The health effects of EPA and DHA supplementation are currently being discussed in literature. In the early 2000s, study results supported the recommendation to increase n-3 PUFA intake, since EPA and DHA promote certain health effects such as preventing CVD, and lowering plasma triglycerides, blood pressure, heart rate and also the risk of thrombosis (120, 121). Moreover, EPA and DHA seemed to have important protective influences on CVD risk such as antithrombotic and anti-inflammatory effects (50). However, results from recent studies failed to show similar benefits for n-3 PUFA and therefore the dietary recommendations are being questioned (120, 122, 123). Assumed possible reasons for these null findings include the low dosage of supplementation, on average 1 g/d of EPA and DHA supplements, along with short treatment periods, small sample sizes or a higher background of omega-3 intake. The current use of other established pharmacological therapy for CVD prevention is also a possible explanation (120, 122).

A protective role of omega-3 PUFA from sudden cardiac death was seen in the Italian Gruppo Italiano per lo Studio della Sopravvivenza nell'Infarto miocardico (GISSI) study (124). Here, participants with a myocardial infarction in the last three months were given a low dose omega-3 fatty acid supplement (1 g/d) over a follow-up of 3.5 years. The authors demonstrated a reduction from sudden cardiac death within 4 months after myocardial infarction when supplementing omega-3 fatty acids ($p = 0.048$). The mechanism for this benefit was explained with the reduction of ventricular arrhythmia, the most common cause of sudden cardiac death after myocardial infarction (124).

Furthermore, a randomized clinical trial found protective CVD benefits from EPA but not from DHA (125). In a blinded randomized 6-week trial, 121 participants received either olive oil placebo (6 g/d), EPA (600 mg/d), EPA (1800 mg/d) or DHA (600 mg/d) (125). Compared to the placebo group, low dose EPA supplementation did not show any significant effect on CVD factors like total cholesterol, LDL-cholesterol, HDL-cholesterol or triglycerides (125). High dosages of EPA (1800 mg/d) were shown to lower Lipoprotein-associated phospholipase A_2 (Lp-PLA_2) by 14.1% ($p = 0.003$). Lp-PLA_2 is considered as an inflammatory marker (125, 126). This enzyme is released by macrophages in atherosclerotic plaques and produces precursors of arachidonic acid, and therefore propagates pro-inflammatory response (125, 126). Elevated Lp-PLA_2-levels over 200 ng/ml are a risk factor for stroke, coronary disease and overall mortality (126).

Regarding DHA, supplementation was shown to increase fasting and postprandial LDL-levels by 20% ($p = 0.0005$) and total cholesterol by 6.3 % ($p = 0.015$), while it lowered postprandial triglyceride levels by 20% ($p = 0.04$) compared to placebo (125). No significant differences were seen regarding fasting triglycerides (125).

Sanders and colleagues conducted a randomized double-blind placebo-controlled trial to investigate the effects on DHA supplementation on CVD risks in healthy participants over a period of 4 weeks (127). Subjects received 4 g/d DHA oil derived from schizochytrium (DHA-S). This is a marine algae that is a primary DHA source in the marine food chain. The placebo consisted of 4 g/d refined olive oil (127). The treatment did not lower serum triglycerides compared to placebo (127). The authors found a significant increase in arachidonic acid and total cholesterol as well as LDL- and HDL-cholesterol ($p = < 0.001$) (127). Since the LDL to HDL ratio did not change, the supplementation of DHA-S seems to have a neutral effect on CVD risk (127).

Nonetheless, DHA supplementation was also shown to decrease production of thromboxane A_2, Leukotriene B_4, IL-6 and TNF-α and therefore LC-n-3 PUFA may have anti-inflammatory effects (50).

Contrarily, recent randomized controlled trials of EPA and DHA supplementation show no benefit of omega-3 PUFA supplementation on CVD risk or mortality (50, 120, 123). Moreover, a recent meta-analysis involving 77,000 individuals from 10 randomized controlled trials supplementing omega-3 fatty acids, in dosages of between 226-1800 mg/d over at least one year, did not find any significant association between supplementation and coronary heart disease death (rate ratio [RR], 0.93; 99% CI, 0.83-

1.03; p = 0.05), non-fatal myocardial infarction (RR, 0.97; 99% CI, 0.87-1.08; p = 0.43) or any coronary heart disease (RR, 0.96; 95% CI, 0.90-1.01; p = 0.12) (123). Nor did they find any association between omega-3 fatty acid supplementation and major vascular events (RR, 0.97; 95% CI, 0.93-1.01; p = 0.10) (123). However, results from ongoing trials are needed to affirm the authors assumption, that cardio-protective effects of EPA and DHA are only seen at high daily intakes above 3 g/d, which are above daily intakes, since most diets contain less than 0.5 g/d (50, 123).

As mentioned previously, vegans consume only low amounts of EPA and DHA and therefore have lower long chain n-3 PUFA proportions. However, the impact of these low plasma omega-3 proportions on CVD risk is not yet established. Nonetheless, vegans have lower arterial stiffness, arterial aging and risk factors for CVD (50). A systematic review with a meta-analysis of 108 studies conducted by Dinu analyzed the association of vegan diets and multiple health outcomes (10). Here, vegans showed a significantly reduced risk of incidence and/or mortality from ischemic heart disease, though no significant reduced risk for total cardiovascular disease could be demonstrated (10). It might be the case, however, that the lack of EPA and DHA mitigates the favorable risk factor for CVD in vegans (50, 108). The importance of low n-3 levels in vegans is not known to date, and so far there is no proof that a vegan diet which is low in long chain n-3 fatty acids has negative effects on health or cognitive functions (13, 128). However, a randomized controlled trial showed that a daily supplementation of 2.2 g fish oil over 26 weeks significantly improved executive functions and significantly reduced the development of brain atrophy in older healthy adults (129).

Even though some authors advise vegans to take omega-3 PUFA supplements to maintain health (94, 108), general recommendations for vegans to take these supplements remain unclear (50, 130).

Moreover, it has been shown that elevation of plasma DHA can only be achieved by supplementation of DHA itself, as supplementations of precursor substances such as ALA or EPA do not elevate plasma DHA (27). Also DHA supplements should be taken with caution. Although they can lower plasma triglycerides, elevated plasma LDL, prolonged bleeding time and impaired immune response can occur (11, 127). Furthermore, it was shown that the average LDL increase caused by DHA supplements equals an increased intake of saturated fatty acids by 6% of energy, so this should therefore be considered when recommending DHA supplementation (50).

6.1.3.2 Omega-6 polyunsaturated fatty acids

Omega-6 fatty acids mostly occur in oils such as canola, corn, soybean or nuts and seeds (walnuts, sunflower) (85). We demonstrated a significantly higher dietary intake and a significantly higher proportion of plasma phospholipid omega-6 fatty acids in vegans. However, looking at individual dietary omega-6 fatty acids, only LA was consumed in higher amounts in vegans. Regarding plasma phospholipid fatty acids, vegans had significantly higher proportions of LA and docosadienoic acid (C20:2n6) compared to omnivores and significantly lower proportions for DPA n-6 (C22:5n6). These results are in line with findings from EPIC-Oxford. Regarding plasma phospholipid fatty acids, vegans had higher proportions of LA ($p < 0.05$), whereas no difference was found between dihomo-γ-linolenic acid (C20:3n6) and arachidonic acid (C20:4n6) (52).

Results from a Finnish study demonstrated higher serum proportions of total omega-6 fatty acids in vegans ($p < 0.001$). However, regarding individual omega-6 fatty acids, LA (C18:2n6), GLA (C18:3n6), DGLA (C20:3n6) and adrenic acid (C22:4n6) showed significantly higher serum proportions in vegans (25).

A significantly higher intake of total omega-6 fatty acids was also described by Schüpbach ($p < 0.05$) (19), Rosell ($p < 0.001$) (52) and Kristensen (18). A Dutch study revealed a higher intake of omega-6 fatty acids, but only in vegan women (77). However, the study population in this study only consisted of 12 vegan and 15 omnivore participants (77).

An inverse association between total plasma PUFA and LA and CVD-mortality and total mortality has been demostrated (99, 101, 107, 131, 132). Higher proportions of LA were also demonstrated to reduce risks of stroke or transient ischemic attack (99). Wu et al. followed 2,700 participants from 1999-2010 who were free of CVD events and found that higher plasma LA proportions were associated with lower risk for CVD-mortality (HR 0.51, 95% CI 0.32, 0.82, $p = 0.001$) (131). Moreover, they demonstrated the importance of both plasma LA and n-3 fatty acids for cardiovascular health, as participants with the highest circulating levels of both fatty acid groups had a 54% lower mortality risk (HR 0.46, 95% CI 0.30, 0.69) compared to participants with the lowest plasma concentrations (131). Moreover, an increased intake in PUFA, especially n-6 PUFA from 3% to 6% of total energy, may reduce the risk of developing type 2 diabetes mellitus (99).

Regarding blood lipids we found a negative correlation between dietary intake of omega-6 fatty acids and HDL- as well as total cholesterol. After subdivision into diet groups, negative correlation between HDL-cholesterol and omega-6 intake remained significant in vegans but could no longer be detected in omnivores. Previous studies presented mixed results. A systematic review including 19 RCTs on the effect of omega-6 fatty acids on blood lipids showed little to no effect of omega-6 fatty acids on HDL-levels (133). In a Spanish study on 20 obese (BMI > 40kg/m^2) men an inverse correlation on omega-6 fatty acids and triglycerides and a positive correlation with HDL-cholesterol was found (134). However, studies have also demonstrated that diets high in omega-6 fatty acids might lower HDL-cholesterol, as was presented in a review by DiNicolantonio in 2018 (135). It has been shown that high intakes of LA tend to lower HDL-cholesterol, while diets high in omega-3 fatty acids tend to increase HDL-cholesterol (135).

6.1.3.2. The role of the n-6 to n-3 ratio

As already described, LA intake is generally higher in vegans than in omnivores, and long chain n-3 intake in vegans is low, which may result in elevated dietary n-6 to n-3 ratios in vegans compared to omnivores. In order to maintain health a ratio of 5:1 - 10:1 was suggested by the WHO (136). However, recommendations are ambiguous as the FAD does not recommend any specific ratio as long as dietary intakes of omega-3 and omega-6 fatty acids lie within recommendations (88). On the other hand, an elevated n-6 to n-3 ratio was shown to be associated with weight gain and development of obesity, due to increased leptin and insulin resistance (66) as well as chronic inflammatory diseases such as coronary heart disease (51). For maintenance of health and reducing the risk of developing these diseases, adequate n-3 PUFA supply is needed (51). According to the FAO, the minimum dietary requirement for ALA is 0.5% of energy, and 0.25 % of energy of EPA and DHA for adult men and non-pregnant women. For pregnant and lactating women 0.3 % of energy from EPA and DHA, from which 0.2% of energy should be DHA, are recommended (88). There is insufficient evidence to recommend a minimum daily intake (88). Vegans are considered to have a ratio of <14:1 compared to <10:1 in omnivores (49). In a German vegan study the authors revealed a ratio of 10:1 in strict vegans and 7.9:1 in moderate vegans (42). In our study

we detected a dietary omega-6 to omega-3 ratio of 5.9:1 in vegans and 5:3:1 in omnivores, while the plasma ratio was 12.1:1 in vegans and 6.9:1 in omnivores. These findings are in line with previous studies (77, 94). Kornsteiner and colleagues explain this by means of omega-3 fatty acid substitution in cell membranes due to low n-3 intakes in vegans (94).

To ensure a sufficient conversion from ALA to EPA and DHA in vegans, a ratio of 2:1-4:1 is recommended (49). A decreased ratio can be achieved by limiting the intake of LA and increasing dietary ALA, or by taking DHA supplements (49).

As mentioned previously, it has been demonstrated, that high amounts of LA negatively influence conversion from ALA to EPA (51). Since Western diets, especially vegan diets, contain higher amounts of LA than ALA, with both competing for the same desaturases, the conversion is shifted to higher conversion rates from LA into AA because of competitive inhibition of Δ5- and Δ6-desaturases (51, 70). Through desaturases and elongases LA is converted into arachidonic acid (AA), and through cyclooxygenases (COX) and lipoxygenases (LOX) AA can be converted into eicosanoids such as prostaglandins, leukotrienes or thromboxanes. Eicosanoids derived from LA mainly have pro-inflammatory properties, whereas eicosanoids derived from ALA are less inflammatory and even have anti-inflammatory effects (67). An elevated n-6 to n-3 ratio might lead to increased conversion from LA to AA and therefore tends to shift metabolism into a pro-inflammatory and pro-thrombotic state. According to findings from previous studies, this might result in vasospasm, vasoconstriction and increased blood viscosity, and may enhance the development of diseases associated with these conditions (67).

Even though vegans in our population had significantly higher plasma proportions of LA, we did not find any difference regarding the proportion of AA. Similar results were presented by Toohey and colleagues (137). Fokkema and colleagues explain this as due to higher C20:4n-6 conversion in the liver, while omnivores receive this fatty acid from meat (77). Recent studies have even demonstrated that high levels of the fatty acids LA, arachidonic acid, the mainly endogenously produced n-6 PUFA, gamma-linoleic acid (GLA) or dihomo-GLA were not associated with increased inflammation in men (138). In contrast LA, the main dietary n-6 PUFA, was inversely associated with CRP levels (139). Similar results were presented in a Finnish study on 2,169 subjects (140). In this study, the authors demonstrated high n-6 PUFA levels, and furthermore that the ratio of n-6 to n-3 was negatively associated with CRP-levels and LDL-oxidation

(140). A study conducted by Mozaffarian and colleagues on the interplay between different PUFA and coronary heart diseases in men was not able to demonstrate that omega-6 PUFA in any way counteracts the effects of LC-n-3 PUFA on heart disease (119). We found that high proportions of LA are inversely correlated with EPA and DHA in vegans and EPA only in omnivores. These results are in accordance with findings by Goyens (141). However, a prospective cohort study on 3,277 healthy participants conducted by Vedofte and colleagues observed no evidence that high intakes of LA modify the effect of ALA on ischemic heart diseases (116). Interestingly, Wang and colleagues demonstrated that an elevated n-6 to n-3 ratio was not associated with increased mortality, but with a slightly lower total mortality, as well as mortality from CVD and cancer (107).

6.1.4. Trans fatty acids

Trans fatty acids occur either naturally in meat and dairy or are formed during hydrogenation of vegetable fat as in the production of margarine (85). They are found in processed industrial food sources like chips, pastries, and fried or convenience foods (57, 71). Regarding total plasma trans fatty acid proportions, we found significantly lower proportions in vegans. No difference occurred regarding the principal trans fatty acid, elaidic acid, which is mainly found in vegetable fat such as margarine. Our results are in line with findings by Kristensen, showing that vegans consume lower amounts of TFA (18). Trans fatty acids have been shown to increase LDL-cholesterol, total triglycerides and insulin levels, as well as lowering protective HDL-cholesterol levels (71, 142). Therefore they may have a negative effect on the relation of LDL- to HDL-cholesterol with respect to CVD-risk (142).

Moreover, trans fatty acids were shown to increase further risk factors for CVD as they increase systemic inflammation and thrombogenesis, and reduce endothelial functions (59). It is suggested that TFA influence metabolic and signaling pathways in monocytes, adipocytes, hepatocytes and endothelial cells; however, to date the precise mechanisms are not established (59). A meta-analysis demonstrated that although no association was found between trans fatty acids and type 2 diabetes mellitus, trans fatty acids were shown to be associated with all-cause mortality, total CHD and CHD-

mortality (143). Hence, trans fatty acids are linked with an increased risk to develop CVD and therefore the daily intake should be as low as possible (57).

6.1.5 *Discrepancy of dietary and plasma phospholipid fatty acids*

It should be noted that plasma phospholipid fatty acids may reflect dietary fat intake over the last few weeks or months (58). However, they can reflect only a part of fatty acid profiles. Fatty acid status is a result of several factors as a consequence of bioavailability, post ingestion metabolism by gut microbiota, enterohepatic circulation, nutrient interactions, tissue storage, turnover, metabolism as desaturation or elongation, de novo lipogenesis, and excretion, as well as external factors such as alcohol intake or lifestyle (144-146). Furthermore, not every fatty acid correlates with dietary intake. To determine whether plasma fatty acids can be used as markers to reflect dietary PUFA intake, Astorg et al. correlated plasma fatty acids with 24-h food recalls in 533 volunteers. They revealed that ALA cannot be used as marker for habitual level of intake, whereas levels of LA, AA, EPA and DHA were significantly correlated with measured intake based on food recalls (147). Another reason for low correlation might be the assessment of dietary intake. Participants might tend to underreport dietary intake and also the calculation of nutrient intake from recipes might be a source of error. In addition, the method of determination of plasma phospholipid fatty acid might be disadvantageous, as studies have reported that dietary fatty acid intake correlates stronger with fatty acids measured in erythrocytes (148).

6.2. Estimated desaturase activity

For PUFA desaturation Δ5- and Δ6-desaturases are rate-limiting enzymes (97). In our study, activity for Δ5- and Δ6-desaturase and stearoyl-coA-desaturase was estimated by calculating the ratio of the fatty acid product to the fatty acid precursor. Direct measurement of desaturase activity is only possible via tissue biopsy, which is difficult to justify in healthy individuals (149). Therefore estimated enzyme activities are calculated. The product-to-precursor ratio indirectly estimates the desaturase activities

(149). This method has been used in previous studies (51, 69) and is based on the assumption that the ratio of a product to its precursor reflects the balance of enzymatic conversion.

The importance of desaturase activities in the maintenance of health has been described previously. It was demonstrated that elevated Δ5-desaturase activity was associated with a lower diabetes risk and high Δ6-desaturase activity with a higher risk to develop type 2 diabetes (97). Additionally, Δ6-desaturase activities were increased and Δ5-desaturase activities decreased in individuals who are obese and have metabolic syndrome (62). Moreover, decreased Δ5-activity has been reported as an independent risk factor for myocardial infarction (62). Furthermore, a diet high in MUFA and PUFA was shown to be associated with lower estimated Δ6- and Δ9-desaturase activities and higher estimated Δ5-desaturase activity. Elevated SCD-desaturase activities have been revealed to be high in conditions like diabetes, atherosclerosis, obesity and metabolic syndrome (150).

Since vegans do not consume any animal products, they depend on an effective synthesis from ALA to EPA and DHA (77). As described earlier, data on n-3 levels in vegans is mixed as some studies found lower levels in vegans (25, 52, 77, 94), while others demonstrated elevated levels compared to omnivores (51, 53). According to the authors, elevated levels in vegans might occur due to a higher conversion rate from plant derived ALA to circulating LC-n-3 PUFA (51). Our results could not confirm these assumptions of elevated desaturase activities in vegans. Furthermore, our adjusted model did not show that enzyme activity was affected by smoking status or alcohol intake as described before (49, 51, 70). Findings from previous studies demonstrate that high LA intake affects the plasma LC-n-3 PUFA proportion (52). Even though diet might influence endogenous conversion, interestingly, results from EPIC-Oxford interestingly demonstrate that LC-n-3 levels were not influenced by dietary fish intake, although 30% of the omnivore study population claimed to eat oily, fried or other white fish at least once a week and 43% consumed 2-3 servings a month (52).

We could not find any association between the duration of following a vegan diet and DHA proportions. Similar findings were presented by Rosell and colleagues who assume that there is a basal endogenous conversion rate from ALA to DHA to keep DHA levels low but stable (52).

Regarding activities of stearoyl-coA-desaturase, we revealed significantly higher activities of SCD-C16 in omnivores in the unadjusted model. Elevated activities of SCD-

16 were recently shown to be associated with the development of obesity (62). Additionally, it was shown that n-6 were inversely associated with SCD-C16 activity indicating that n-6 could be relevant in regulating SCD-C16 and therefore influencing body weight (151).

6.3. Cholesterol and triglycerides in vegans and omnivores

We detected significant differences between total cholesterol and LDL-cholesterol in vegans and omnivores in both men and women. HDL-cholesterol was significantly lower in vegan women compared to omnivore women and no significant difference was found for triglycerides in either gender. The differences in total and LDL-cholesterol levels can be explained with a higher consumption of PUFA and lower intakes of SFA in vegan diets (152). Even though results from a Taiwanese study on pre- and postmenopausal women demonstrated that a vegan diet was shown to lower HDL-cholesterol, while it did not affect LDL-cholesterol in premenopausal women (153), most studies reveal the LDL-lowering effects of vegan diets (25, 154-156).

In a Finnish vegan study, LDL-cholesterol was 25% lower in vegans and total cholesterol was 20% lower compared to omnivores (25). These results correspond with findings from a previous meta-analysis that assessed the effect of vegetarian (including vegan) diets on blood lipids (154). In this study, vegetarian and particularly vegan diets were shown to significantly lower total and LDL-cholesterol and HDL-cholesterol without influencing triglyceride levels (154). These results were replicated in a meta-analysis by Yokoyama et al. that included 30 observational studies and 19 controlled trials (155). The authors could not demonstrate any triglyceride-lowering effects of vegan or vegetarian diets (155). Furthermore, observational studies revealed that consumption of plant-based diets was associated with lower LDL-cholesterol concentrations (mean -22.9 and -12.2 mg/dl) and total cholesterol concentrations (-29.2 and -12.5 mg/dl) compared to consumption of omnivorous diets (155). Statin use alone reduced LDL-cholesterol by 70 mg/dl depending on statin type (155). Although diet might not alter LDL-cholesterol in the same magnitude as pharmacological therapy, combining both might be an effective treatment and in some cases might make statin therapy

redundant, since the intake of statins can be associated with side effects or interfere with medical compliance (155).

The importance of addressing dietary change in hyperlipidemia was also shown in a randomized trial conducted by Jenkins and colleagues (156). The authors wanted to determine whether a diet rich in fiber, soy proteins, plant sterols and almonds leads to cholesterol reduction compared to statins. LDL reduction due to diet was 28.4% in 4 weeks and did not show any significant difference in efficacy compared to statin therapy (156).

Current data on the effect of vegan diets on triglyceride levels is mixed (137, 154, 155, 157-159). One study demonstrated significantly lower triglyceride levels in vegans compared to omnivores (0.94 ± 0.07 mmol/l vs. 1.17 ± 0.04 mmol/l, p = 0.05) (137). In another study, vegans were reported to have significantly lower levels of total cholesterol, LDL-cholesterol and triglycerides even after adjustments for age, gender or smoking habits (157). However, several studies did not show significantly reduced triglyceride levels in vegans (154, 155). Barnard and colleagues revealed that a low-fat vegan diet high in carbohydrates reduced HDL and LDL-cholesterol in premenopausal women, but mean triglyceride levels increased by 18.7% (p = 0.01) (159). However, studies also suggest that when changing eating habits to a high carbohydrate diet, like a vegan diet, changes in triglyceride levels are transient and resolve without further dietary adjustment (158).

6.4. Methods

In the present study, fatty acids were measured from plasma phospholipids. The FA profile in blood and tissues partly reflects dietary FA intake, but it is also strongly determined by the endogenous FA metabolism (97). Plasma phospholipid levels represent the fatty acid intake over several weeks; however, these levels do not reflect tissue concentrations (58, 69). These could only be measured by tissue biopsy, which is ethically difficult to justify in healthy individuals. We used GC and FID to measure plasma phospholipid fatty acids. This method is suited to measuring fatty acids that occur in higher proportions. Regarding trans fatty acids, these amounts were extremely low, and when using GC and FID inaccuracies in proportions of trans fatty acids might

occur. The most reliable method for an exact quantitation of trans fatty acids is the combination of silver-ion thin layer chromatography (Ag-TLC) with GC (73, 160).

6.5. Strengths and limitations

Several strengths of this study should be mentioned. For a better comparison of dietary groups, and in order to reduce confounding effects, participants were matched with regard to sex and age. Using detailed questionnaires, lifestyle parameters and general characteristics of our participants were analyzed in depth. Qualified personnel took anthropometric measurements. Blood samples were collected after a fasting period of at least 8 hours between 07:30 and 09:00 a.m. and processed under standardized conditions, which included standardized centrifugation and storage at -80°C until further analysis. The measurement of fatty acids was blinded.

Furthermore, dietary intake was assessed using three-day weighed food records, which might reflect vegan dietary intake in particular the most accurately compared to other methods for dietary assessment. Existing dietary questionnaires, such as 24-h food recalls, mostly do not include vegan products or food alternatives like soy yoghurt or different sources of plant milk. Furthermore, most vegan dairy alternatives contain added vitamins or minerals such as vitamin B12 or calcium. Using weighed food records, the dietary intake of our study participants might be assessed most accurately, since added nutrients can be considered as well. All participants received the same model of a kitchen scale for standardization and a very detailed explanation of how to fill in the food records. However, this is demanding for the participants and can therefore lead to misreporting or to changing dietary behavior (161, 162).

Furthermore, we analyzed a wide spectrum of plasma phospholipid fatty acids including 24 individual fatty acids and 34 dietary fatty acids.

A limitation to our study is the relatively small study population. The study was primarily designed to be able to detect significant differences between vegans and omnivores in bone health, but not for the detection of differences in the fatty acid proportions (163). Therefore, the ability to make several comparisons in our study might be limited. Participants were only recruited in Berlin. The transferability of our results into other regions in Germany, especially rural areas, might be limited. In Berlin, there is a huge

range of vegan supermarkets and restaurants, and also discounters that offer a high variety of vegan food for the vegan community. Furthermore, the study included middle-aged healthy participants. Therefore, the results cannot be applied to other populations. Finally, we have to mention that the study was cross-sectional, and therefore we can only represent the data of our population at a certain point of time.

7 Summary

In this study we assessed dietary fatty acid intake and plasma phospholipid fatty acid profiles in vegans and omnivores. As compared to omnivores, we detected lower total fat, SFA and MUFA intake in vegans but higher intakes of PUFA, n-3 and n-6. In plasma phospholipids, more favorable fatty acid proportions were seen in vegans in terms of saturated fatty acids, polyunsaturated fatty acids, n-6 fatty acids in particular, and trans fatty acids. On the other hand, vegans had significantly lower proportions for total-n-3 fatty acids and LC-n-3 PUFA.

Lower SFA intake and SFA plasma phospholipid proportions were shown to have positive health effects. The health impact of low LC-n-3 levels is inconsistent. However, to date there is no proof that such low n-3 levels in vegans have any deleterious effect, and therefore no general recommendation exists to supplement n-3. Studies on CVD-prevention by consuming n-3 supplements show mixed results. Therefore, further prospective studies on vegans and CVD outcome are needed. Also RCTs on n-3 supplementation in vegans and CVD outcome are relevant so as to investigate whether low n-3 levels in vegans might influence CVD risk in vegans. To date, the pathophysiological importance of decreased omega-3 levels has not been established.

We could not demonstrate any correlation between the duration of following a vegan diet and LC-n-3 PUFA levels, and therefore share the assumption made previously by other authors that conversions from ALA to LC-n-3 PUFA in vegans remain low but stable.

Furthermore, no differences were detected in the activities of Δ5-, Δ6-desaturase or SCD-C18. Regarding SCD-C16, we found significantly higher activities among omnivores in the unadjusted Model. This enzyme has previously been associated with weight gain and obesity.

We also revealed that vegans had significantly lower levels of total cholesterol and LDL-cholesterol. However, in line with previous studies, we also observed lower concentrations of HDL-cholesterol in vegan women compared to omnivore women. No differences were found regarding triglycerides.

Overall it can be said that a vegan diet is associated with a more favorable dietary fat intake, with the exception of omega-3 PUFA plasma fatty acids, resulting in more favorable profiles which might reduce risk factors for cardiovascular diseases. Therefore, a vegan diet could provide the means for the prevention and treatment of hyperlipidemia and cardio-metabolic diseases.

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9 Statutory Declaration

“I, Alessa Longrée by personally signing this document in lieu of an oath, hereby affirm that I prepared the submitted dissertation on the topic: Comparison of fatty acid profiles in vegans and omnivores, 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 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 shall comply with the regulations of Charité – Universitätsmedizin Berlin on ensuring good scientific practice.

I declare that I have not yet submitted this dissertation in identical or similar form to another Faculty.

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

10 Curriculum Vitae

My curriculum vitae does not appear in the electronic version of my paper for reasons of data protection.

11 List of publications

Alessa Longrée contributed the following to the below listed publications:

Publication 1: Juliane Menzel, Ronald Biemann, Alessa Longrée, Berend Isermann, Knut Mai, Matthias B. Schulze, Klaus Abraham, Cornelia Weikert, Association of a vegan diet with inflammatory biomarkers, Scientific report, 2020

Contribution: Recruitment of participants and distribution of advertisement posters. Instructions in using three-day weighed food reports.
Assessment of current medications and intake of supplements, blood pressure and anthropometric measurement. Assessment of lifestyle characteristics and dietary intakes. Plausibility checks of three-day weighed food reports.

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Alessa Longrée

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