International Journal of Hygiene and Environmental Health 237 (2021) 113808

Contents lists available at ScienceDirect

International Journal of Hygiene and Environmental Health

journal homepage: www.elsevier.com/locate/ijheh

Internal exposure to perfluoroalkyl substances (PFAS) in vegans and omnivores

Juliane Menzel [a,b,*], Klaus Abraham [a], Stefan Dietrich [a], Hermann Fromme [c], Wolfgang Völkel [d], Tanja Schwerdtle [a], Cornelia Weikert [a,b]

[a] German Federal Institute for Risk Assessment, Department of Food Safety, 10589, Berlin, Germany
[b] Institute of Social Medicine, Epidemiology and Health Economics, Charité – Universitätsmedizin Berlin, corporate member of Freie Universität Berlin and Humboldt-Universität zu Berlin, 10117, Berlin, Germany
[c] Institute and Clinic for Occupational, Social and Environmental Medicine, University Hospital, LMU Munich, 80336, Munich, Germany
[d] Department of Chemical Safety and Toxicology, Bavarian Health and Food Safety Authority, 80538, Munich, Germany

ARTICLE INFO

Keywords:
Internal exposure
Perfluoroalkyl substances (PFAS)
Vegans
Omnivores
Cholesterol

ABSTRACT

Perfluoroalkyl substances (PFAS) are a complex group of anthropogenic compounds with exceptional properties. Due to their high persistence and mobility, they have caused ubiquitous environmental contamination and in part accumulate in the food chain. In the general population, diet is the main source of PFAS exposure, with the important sources fish and meat. As a vegan diet implies the complete exclusion of any animal products, it might be expected that vegans have lower blood levels of PFAS compared to omnivores. Furthermore, lower levels of cholesterol is one of the well-documented nutritional effects in vegans, but cholesterol levels were also found to be associated with higher PFAS levels in epidemiological studies.

To examine the relations of internal PFAS levels and the levels of cholesterol in vegans and omnivores, the cross-sectional "Risks and Benefits of a Vegan Diet" (RBVD) study was used involving 36 vegans and 36 omnivores from Berlin/Germany. Nine perfluoroalkyl substances were quantified in plasma using a triple-stage quadrupole mass spectrometer.

Lower median plasma concentrations were found in vegans compared to omnivores for perfluorooctane sulfonic acid (PFOS) (2.31 vs. 3.57 ng/ml, respectively; $p = 0.02$) and for perfluorononanoic acid (PFNA) (<0.25 vs. 0.41 ng/ml, respectively; $p < 0.0001$). No significant differences of the median concentrations were observed for perfluorooctanoic acid (PFOA) (1.69 vs. 1.44 ng/ml, respectively, $p = 0.26$) and perfluorohexane sulfonic acid (PFHxS) (1.96 vs. 1.79 ng/ml, respectively; $p = 0.70$). The strongest correlations with food groups, derived from a food frequency questionnaire, were observed between levels of PFOA and water consumption (in case of the total study population, $n = 72$), and between levels of PFOS as well as PFNA and the consumption of 'meat and meat products' (in case of the omnivores, $n = 36$). Levels of Low Density Lipoprotein (LDL) cholesterol were confirmed to be considerably lower in vegans compared to omnivores (86.5 vs. 115.5 mg/dl, respectively; $p = 0.001$), but no associations between the four main PFAS and LDL cholesterol were observed (all $p > 0.05$) at the low exposure level of this study.

According to the results of our study, a vegan diet may be related to lower PFAS levels in plasma. We highlight the importance of the adjustment of dietary factors like a vegan diet in case of epidemiological studies dealing with the impact of PFAS on the levels of blood lipids.

1. Introduction

Perfluoroalkyl substances (PFAS) are a complex group of man-made chemicals composed of a fluorinated carbon backbone of varying length, primarily terminated by a carboxylate (perfluoroalkyl carboxylic acids, PFCAs) or a sulfonate (perfluorooctane sulfonic acids, PFSAs) as

* Corresponding author. German Federal Institute for Risk Assessment (Department of Food Safety), Max-Dohrn-Str. 8-10, 10589, Berlin, Germany.
E-mail addresses: Juliane.Menzel@bfr.bund.de (J. Menzel), klaus.abraham@bfr.bund.de (K. Abraham), stefan.dietrich@bfr.bund.de (S. Dietrich), Hermann.Fromme@med.uni-muenchen.de (H. Fromme), Wolfgang.Voelkel@lgl.bayern.de (W. Völkel), tanja.schwerdtle@bfr.bund.de (T. Schwerdtle), cornelia.weikert@bfr.bund.de (C. Weikert).

https://doi.org/10.1016/j.ijheh.2021.113808
Received 29 April 2021; Received in revised form 13 July 2021; Accepted 13 July 2021
Available online 20 July 2021

functional group. The combination of the polar and non-polar structure makes PFAS 'amphiphilic' providing water and oil repellency, and the strength of their carbon-fluorine bonds results in extremely high chemical and thermal stability. Since decades, the compounds have been used for the production of many consumer products like nonstick cookware, breathable textiles or protective coatings for paper, food packing materials, and carpets. From these everyday objects, PFAS are released and have been found – due to their high persistence and mobility – to cause ubiquitous environmental contamination and in part to accumulate in the food chain (Sunderland et al., 2019).

Consumption of food and drinking water is the main route of background exposure in humans. Internal exposure to PFAS in individuals can easily be determined by an analysis of serum or plasma. Four compounds, namely the PFCAs perfluorooctanoic acid (PFOA) and perfluorononanoic acid (PFNA), and the PFSAs perfluorooctane sulfonic acid (PFOS) and perfluorohexane sulfonic acid (PFHxS), typically represent more than 90% of detectable PFAS in serum/plasma of adults in industrialized countries. In the European adult population, median concentrations of PFOS, PFOA, PFHxS, and PFNA were found to be 7.7, 1.9, 0.67 and 0.61 ng/ml, respectively, based on studies from 2007/2008 and onwards (EFSA Panel on Contaminants in the Food Chain, 2020). This pattern results from the occurrence in food and drinking water on the one hand, and from accumulation due to half-lives up to several years in humans on the other hand. The latter is due to missing metabolic degradation and low urinary excretion (EFSA Panel on Contaminants in the Food Chain, 2020).

According to the recent evaluation of the European Food Safety Authority (EFSA), 'Fish and other seafood' was the most important contributor to the mean Lower Bound (LB) exposure in case of PFOS and PFOA, followed by 'Eggs and egg products', 'Meat and meat products', and 'Fruit and fruit products' (EFSA Panel on Contaminants in the Food Chain, 2020). Therefore, internal background exposure to these two substances in humans can be expected to be influenced by their dietary habits. Over the last years, plant-based diets have become increasingly popular in Germany and many other western countries, not merely due to increasing awareness of suffering animals or environmental problems, but also because of expected health benefits (Janssen et al., 2016). As a vegan diet implies the complete exclusion of any animal products, it might be expected that vegans have lower blood levels of PFAS compared to omnivores. Studies on this issue are yet missing. Therefore, the first aim of this investigation was to compare the internal PFAS exposure of German vegans and omnivores. For this purpose, PFAS was analyzed in samples of the 'Risks and Benefits of a Vegan Diet' (RBVD) study in 36 vegans and 36 omnivores aged 30–60 years (Menzel et al., 2020, 2021; Weikert et al., 2020).

While a broad spectrum of toxic effects of different PFAS was observed in experimental animals primarily at higher doses, epidemiological studies conducted in recent years revealed associations of certain biological parameters and levels of PFAS in serum/plasma even in the higher background range (EFSA Panel on Contaminants in the Food Chain, 2020). Using data of reduced formation of vaccine antibodies in one-year old children (Abraham et al., 2020), EFSA derived a tolerable weekly intake (TWI) of 4.4 ng/kg body weight for the sum of PFOS, PFOA, PFHxS and PFNA. According to the modelling of EFSA, such an intake corresponds to an internal level of 6.9 ng/ml for the sum of these four PFAS in women at the age of 35 years (EFSA Panel on Contaminants in the Food Chain, 2020).

Regarding possible changes of lipid metabolism, positive associations have been observed especially between high background levels of PFOS/PFOA and levels of Low Density Lipoprotein (LDL) cholesterol (Frisbee et al., 2010; Steenland et al., 2009). In this context, a vegan diet may be an undervalued confounding factor: An on average lower level of LDL cholesterol is one of the well-documented nutritional effects in vegans compared to omnivores (Yokoyama et al., 2017), resulting from the missing intake of animals fats. As outlined above, vegans may concurrently have lower external and internal PFAS exposure, resulting from missing intake of foods of animal origin with relatively high PFAS content. Therefore, the second aim of this investigation was to compare the impact of internal PFAS exposure and of a vegan diet on blood lipid levels, especially with regard on levels of LDL cholesterol in the RBVD study.

2. Methods

2.1. Study population

Participants of the present RBVD study were recruited by announcement (flyer) in (organic/vegan) supermarkets and investigated between January 2017 and July 2017 at the German Federal Institute for Risk Assessment (BfR) in Berlin (Weikert et al., 2020). A phone screening was performed including a brief explanation of the study and checking inclusion criteria (age 30–60 years, following the diet at least one year) and exclusion criteria (BMI $\geq$30, cardiovascular disease, type 2 diabetes, cancer, pregnancy, breastfeeding, current infection). Hypercholesterolemia and taking lipid-lowering medications were no study exclusion criteria. The final study population comprises 36 vegans and 36 omnivores, who were matched by sex and age. In the present study, an omnivorous diet was defined as the consumption of at least three portions of meat per week or two portions of meat and two portions of processed meat (e.g. cold cuts, sausages) per week, whereas a vegan diet was defined as no consumption of any animal food products. Each participant visited the study center twice - on their first visit, participants gave their written informed consent, received instructions to document their diet, and got material to collect urine. At the second visit, a fasting blood sample was collected, anthropometric measurements were performed and lifestyle characteristics as well as a food frequency questionnaire were assessed. The time span was on average 2 weeks between the two visits in the study center (minimum 1 week to maximum 4 weeks). The study was approved by the Ethics Committee of Charité – Universitätsmedizin Berlin (No. EA4/121/16) and was conducted in accordance with the Declaration of Helsinki.

2.2. Assessment of lifestyle characteristics

Anthropometric measurements, i.e. weight, height, and waist circumference, were taken by trained and quality-monitored personnel on participants wearing only light underwear. Body weight was assessed by an electronic digital scale (Omron BF511, Omron Healthcare Ltd., Kyoto, Japan) and the height was measured using a flexible anthropometer (SECA 213, Hamburg, Germany). Waist circumference was defined as in the horizontal plane midway between the lowest ribs and the iliac crest. Information on physical activity, educational level and smoking status was assessed by computer-based questionnaires. In the RBVD study the physical activity has been determined by a physical activity questionnaire used in the European Prospective Investigation into Cancer and Nutrition (EPIC)-study, provided by the Human Study Center of the German Institute of Human Nutrition Potsdam-Rehbruecke (InterAct et al., 2012). Physical activity contains the sum of average hours in summer and winter per week spent on cycling, sports and gardening. Walking comprises the sum of average hours per week during summer and winter. Further, occupational activity was assessed in the RBVD study. The validated EPIC-Potsdam Study food frequency questionnaire (FFQ) collects semi-quantitatively for each food item information on the usual portion size and the average frequency of intake of 102 food items during the past 12 months (Nothlings et al., 2007). Portion size for each item was estimated via image of different portion sizes or with standard portion sizes e.g. a cup (150 ml). Food groups were derived from the FFQs and available as g/d. Individual food groups were summed up to derive food groups, as a basis serves the classification of EFSA: 'Fruits', 'Vegetables (including fungi)', 'Starchy roots and tubers', 'Waters', 'Grains and grain based products', 'Meat and meat products', 'Fish and other seafood' and 'Eggs' (supplemental Table 1).

2.3. Blood collection and laboratory analysis

About 60 ml of venous blood was collected from fasting participants at the BfR study center.

The accredited medical laboratory (Labor 28 GmbH, Berlin, Germany) measured routine biomarkers including plasma concentrations of total cholesterol, HDL cholesterol, LDL cholesterol and triglycerides on the same day. Plasma samples used for PFAS assessment were stored at −80 °C in freezers until time of analysis. PFAS were measured at the Bavarian Health and Food Safety Authority. The following compounds were analyzed using 100 µl plasma: perfluorodecanoate (PFDA), PFNA, PFOA, perfluorohexanoic acid (PFHxA), PFOS, PFHxS, perfluorobutane sulfonate (PFBS), perfluorododecanoate (PFDoDA), and 3H-perfluoro-3-[(3-methoxy-propoxy) propanoate] (ADONA). Sample preparation, analysis and quality criteria have been previously described in detail by Mosch et al. (2010). In brief, an online extraction LC-MS/MS system was used, and the compounds were quantified with a triple-stage quadrupole mass spectrometer (API 5500 QTRAP™ Applied Biosystems, Darmstadt, Germany) equipped with a TurboIonSpray® interface. The perfluorinated substances and the corresponding isotope-labeled internal standards were purchased from Wellington Laboratories (Ontario, Canada). Limit of quantification (LOQ) in plasma was 0.25 ng/ml, based on a tenfold peak-to-noise ratio. Values below the LOQ were assigned the half value. The sum of PFAS was defined including PFOS, PFOA, PFHxS and PFNA ('PFAS sum'). Accuracy of the analysis was ensured by External Quality Assurance Schemes (EQUAS) for PFOS and PFOA (http://www.g-equas.de/).

2.4. Statistics

Normally distributed variables were reported as mean and standard derivation (SD). Skewed variables were reported as median and interquartile range (IQR). Categorical variables were reported as percentage. A Student's *t*-test or Mann-Whitney *U* test was used to compare continuous variables between vegans and omnivores, and a chi square test was used for categorical variables.

To investigate the association of veganism with biomarkers of the lipid metabolism, compared to omnivores, an analysis of variance (ANOVA) was performed for model 1 (unadjusted). Additionally, a multivariable adjusted analysis of covariance (ANCOVA) was conducted to detected differences between vegans and omnivores in model 2 (adjusted for several PFAS and the PFAS sum) and model 3 (additionally adjusted for age, sex, smoking status, education, waist circumference, and physical activity). The model was not adjusted for recent weight changes as the study did not assess data on weight changes. Blood lipid concentrations were skewed, thus variables were log-transformed for ANOVA or ANOVA, afterwards back-transformed and expressed as geometric means and 95%-confidence intervals (95%-CI).

To investigate a potential relationship between PFAS plasma levels and blood lipids, we used linear regression models and also a restricted cubic spline (RCS) regression analyses to investigate nonlinear associations. Three knots were used, located at the 5th, 50th and 95th percentiles. The RCS regression models fitted with generalized estimating equations were constructed using the SAS macro %RCS_Reg (v1.50) developed by Desquilbet and Mariotti (2010). Not only levels of blood lipids, but also those of PFAS were skewed distributed. Therefore, all variables were log-transformed for the analyses. The analyses were performed in unadjusted models (model 1), adjusted for type of diet (model 2) as well as additionally adjusted for age, sex, smoking status, education, waist circumference, and physical activity (model 3).

To investigate potential correlations between PFAS plasma levels and food groups, we calculated Spearman (partial) correlations for the total and the omnivorous sample. Correlation analyses between individual PFAS and individual food groups were performed in an unadjusted model (model 1) for omnivores, and for the total sample, model 1 was adjusted for type of diet. Model 2 was additionally adjusted for age, sex, smoking status, education, waist circumference, and physical activity.

The statistical analyses were performed using SAS software, version 9.4 (SAS institute, Cary, N.C., USA), IBM SPSS Statistics for Windows, Version 26.0 (Armonk, NY: IBM Corp) and R software (version 3.6.3). Test findings with p values of <0.05 were considered statistically significant.

3. Results

The general characteristics of the 72 sex- and age matched participants are shown in Table 1, according to vegan or omnivorous diet (n = 18 men and 18 women each). The median duration of veganism was 4.8 years (IQR: 3.1–8.7). Median age was 37.5 years (range 30–57) in vegans and 38.5 years (range 30–57) in omnivores, respectively. No relevant differences in anthropometric measurements, physical activity, smoking, education, were observed between the groups. Regarding occupational activity, 16.7% (n = 6) of vegans and 8.4% (n = 3) of omnivores reported a high level of intensive occupational activity. 61.1% (n = 22) of vegans and 77.8% (n = 28) of omnivores stated a high level of sedentary occupational activity. None of the participants took any lipid-lowering medications.

The following PFAS were analyzed: PFOS, PFOA, PFHxS, PFNA, PFDA, PFBS, PFHxA, PFDoDA and ADONA. Levels of the main four contaminants, PFOS, PFOA, PFHxS and PFNA are given in Table 2. PFOS and PFOA were quantifiable in all the 72 participants, whereas PFHxS and PFNA were below the LOQ in two samples and 22 samples, respectively. In case of PFDA, 58 samples were below the LOQ. Of the 14 samples quantifiable (range of 0.26–0.49 ng/ml), 13 were from omnivores. These values were not considered in the following evaluation. Levels of the other four compounds (PFBS, PFHxA, PFDoDA and ADONA) were not found above the LOQ.

Table 1
Characteristics of the study population according to vegan or omnivorous diet.

	Vegans (n = 36)	Omnivores (n = 36)	*p-value*
Duration vegan diet [years]	4.8 (3.1–8.7)		
Men	50.0% (18)	50.0% (18)	
Age [years]	37.5 (32.5–44.0)	38.5 (32.0–46.0)	*0.75*
Anthropometry			
BMI [kg/m^2]	22.9 ± 3.2	24.0 ± 2.1	*0.08*
Waist circumference [cm]			
Women	73.1 ± 6.9	77.2 ± 6.2	*0.07*
>80	8.3% (3)	13.9% (5)	
Men	84.5 ± 8.9	86.0 ± 6.1	*0.56*
>94	5.6% (2)	5.6% (2)	
Education [%]			*0.60*
Low	0.0% (0)	2.8% (1)	
Intermediate	30.6% (11)	30.6% (11)	
High	69.4% (25)	66.7% (24)	
Lifestyle			
Physical Activity [h/week]	2.8 (0.88–3.75)	2.3 (1.2–4.1)	*0.69*
Walking [h/week]	7.0 (5.0–12.0)	5.5 (3.5–11.8)	*0.15*
Smoking status			*0.30*
Non-smoker	66.7% (24)	58.3% (21)	
Ex-Smoker	22.2% (8)	16.7% (6)	
Smoker	11.1% (4)	25.0% (9)	
Blood lipids			
Total cholesterol [mg/dl]	157.0 (137.0–180.5)	203.5 (178.5–222.5)	<0.0001
HDL cholesterol [mg/dl]	56.5 (50.5–71.5)	61.5 (51.5–80.5)	0.21
LDL cholesterol [mg/dl]	86.5 (68.5–97.0)	115.5 (93.5–136.0)	0.001
Triglyceride [mg/dl]	71.0 (53.0–90.5)	85.0 (52.0–120.5)	0.26

Variables expressed as percentage (n), mean ± SD or median (IQR).

Table 2
PFAS according to a vegan or omnivorous diet (n = 72).

	Vegans (n = 36)		Omnivores (n = 36)		
	Median (IQR)	Min - Max	Median (IQR)	Min - Max	*p-value*
PFOS [ng/ml]	*n > LOQ: 36*		*n > LOQ: 36*		
All (n = 36)	2.31 (1.37–3.59)	0.34–6.70	3.57 (1.94–5.14)	0.84–11.1	*0.02*
Men (n = 18)	2.31 (1.37–4.47)	0.34–6.70	4.65 (3.28–5.86)	0.84–10.8	*0.04*
Women (n = 18)	2.31 (1.38–2.75)	0.59–6.36	2.62 (1.87–3.96)	1.38–11.1	*0.21*
PFOA [ng/ml]	*n > LOQ: 36*		*n > LOQ: 36*		
All (n = 36)	1.69 (1.35–2.75)	0.26–4.24	1.44 (0.98–2.61)	0.62–4.65	*0.26*
Men (n = 18)	1.66 (1.46–2.79)	0.26–4.24	1.68 (1.18–2.92)	0.62–4.65	*0.73*
Women (n = 18)	1.75 (1.28.2.10)	0.72–3.80	1.18 (0.92–2.04)	0.64–3.35	*0.23*
PFHxS [ng/ml]	*n > LOQ: 35*		*n > LOQ: 35*		
All (n = 36)	1.96 (0.88–3.75)	<LOQ –11.2	1.79 (0.92–2.74)	<LOQ – 6.09	*0.70*
Men (n = 18)	2.14 (1.06–3.76)	<LOQ – 8.97	1.93 (1.33–2.70)	0.38–5.08	*0.76*
Women (n = 18)	1.74 (0.69–3.74)	0.26–11.2	1.79 (0.85–3.11)	<LOQ– 6.09	*0.83*
PFNA [ng/ml]	*n > LOQ: 16*		*n > LOQ: 34*		
All (n = 36)	<LOQ (<LOQ -0.30)	<LOQ – 0.49	0.41 (0.33–0.58)	<LOQ - 1.05	*<0.0001*
Men (n = 18)	<LOQ (<LOQ -0.30)	<LOQ – 0.42	0.49 (0.39–0.65)	<LOQ– 1.05	*<0.0001*
Women (n = 18)	<LOQ (<LOQ -0.30)	<LOQ – 0.49	0.35 (0.29–0.48)	<LOQ– 0.92	*0.003*
PFAS sum [ng/ml]					
All (n = 36)	6.41 (4.08–9.38)	0.84–21.4	7.65 (5.02–11.1)	2.02–21.6	*0.33*
Men (n = 18)	6.93 (3.98–9.96)	0.84–18.5	8.85 (7.28–12.0)	2.02–21.6	*0.18*
Women (n = 18)	5.96 (4.18–8.25)	1.78–21.4	6.18 (4.70–8.55)	2.44–20.6	*0.80*

LOQ: limit of quantification. PFAS sum: PFOS + PFOA + PFHxS + PFNA.

In the total study population (n = 72), median concentrations (IQR) of PFOS: 2.71 ng/ml (1.64–4.67), PFOA: 1.62 ng/ml (1.14–2.71), PFHxS: 1.84 ng/ml (0.91–3.19) and PFNA: 0.32 ng/ml (<0.25–0.44) were observed. The median concentration (IQR) of the PFAS sum was 7.05 ng/ml (4.72–9.85). The distributions of plasma levels of PFAS were skewed, as depicted in Fig. 1 for the lead compounds PFOS and PFOA in vegans and omnivores. According to the dietary group, data on plasma levels of the all four PFAS evaluated are compiled in Table 2 for the whole study group and separately for men and women. Regarding PFOA, no significant differences were observed between vegans and omnivores (p = 0.26), however, vegans were with tendency more likely to have higher PFOA concentrations compared to omnivores. In case of PFOS, the levels were significantly higher in omnivores (median 3.57 ng/ml, IQR: 1.94–5.14) compared to vegans (median 2.31 ng/ml, IQR: 1.37–3.59) (p = 0.02). The strongest difference was seen for PFNA with median values below the LOQ of 0.25 ng/ml (IQR: <0.25–0.30) for vegans compared to 0.41 ng/ml (IQR: 0.33–0.58) for omnivores (p < 0.0001). Of the 22 PFNA measurements below the LOQ, two were from omnivores, and 20 from vegans. No significant differences between vegans and omnivores were seen for PFHxS (p = 0.70) and the PFAS sum (p = 0.33). Considering the duration of the vegan diet, long-time vegans were with tendency more likely of lower level of the PFAS sum (PFOS + PFOA + PFHxS + PFNA, Fig. 2).

Regarding the level of the PFAS sum in women of childbearing age (≤45 years of age), a median value of 5.82 ng/ml (IQR: 4.44–7.76, range 1.78–21.4) was observed (n = 28, 15 vegans and 13 omnivores, median PFAS sum 5.74 vs. 5.91 ng/ml, respectively; p = 0.53). Ten of these 28 women (36%) exceeded the EFSA derived plasma level of 6.9 ng/ml corresponding to the TWI in women at the age of 35 years.

Correlations between PFAS and eight selected food groups are visually summarized in Fig. 3, and specific correlation coefficients are presented in supplemental Table 2. In the total population (n = 72), the strongest correlations were observed between PFOA and water consumption (model 2 correlation coefficient 0.34, p = 0.01, supplemental Table 2). Regarding the consumption of 'meat and meat products' in omnivores (n = 36), the strongest correlations were observed with the concentrations of PFOS (model 2 correlation coefficient 0.38, p = 0.04) and PFNA (model 2 correlation coefficient 0.50, p = 0.01, supplemental Table 2). Supplemental Table 3 shows the intake of the food groups based on FFQ data in vegans and omnivores.

Levels of LDL and total cholesterol were considerably lower in vegans compared to omnivores (Tables 1 and 3). As shown in Table 3, after adjustment of several PFAS (model 2), none of the investigated PFAS, neither the PFAS sum, alter the differences of LDL cholesterol between vegans and omnivores (Table 3) indicating no relevant impact of PFAS on the association between vegans/omnivores and LDL cholesterol. After further adjustment for lifestyle factors (model 3), the difference between both diet groups got smaller, especially omnivores had lower concentrations of LDL cholesterol. Nevertheless, further adjustment of PFAS sum (model 3, Table 3) as well as for PFOS, PFOA, PFHxS or PFNA did not alter the difference of blood lipid concentrations. We observed no relevant linear or non-linear associations between LDL cholesterol and different PFAS (on the log-scale), as depicted by splines in the supplemental Figure 1. Concerning other blood lipids, concentrations of HDL and total cholesterol as well as of triglycerides in vegans and omnivores also did not change after adjustment for different PFAS (data not shown). In line, linear regression analyses detected no associations between PFAS and blood lipids (supplemental Table 4).

4. Discussion

The present cross-sectional study is the first study investigating differences in PFAS levels in vegans compared to omnivores. Significantly lower concentrations of PFOS and PFNA were observed in vegans compared to omnivores. Accordingly, we observed correlations with food groups expected to contribute most to the internal exposure with PFAS. At the present level of internal PFAS exposure, we did not observe relevant associations between PFAS and blood lipids in particular under consideration of the large differences in LDL and total cholesterol levels between vegans and omnivores.

Only very few data of recent years on internal exposure to PFAS are available for German adults. The internal plasma levels of PFAS measured in our total study group (residing in Berlin) were found to be – despite the high proportion of vegans – relatively high. The best

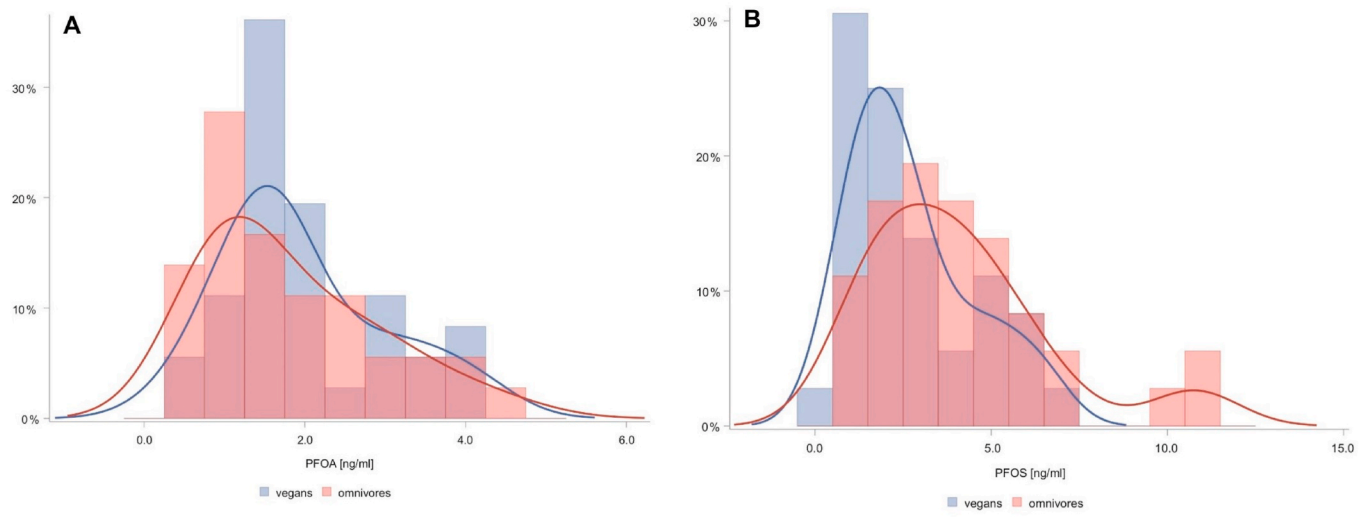

Fig. 1. Distributions of plasma PFOA and PFOS levels according to vegans and omnivores. Distribution of plasma PFOA levels [ng/ml] (A) and PFOS levels [ng/ml] (B) of the study population. Histograms depicted vegans in blue and omnivores in red. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

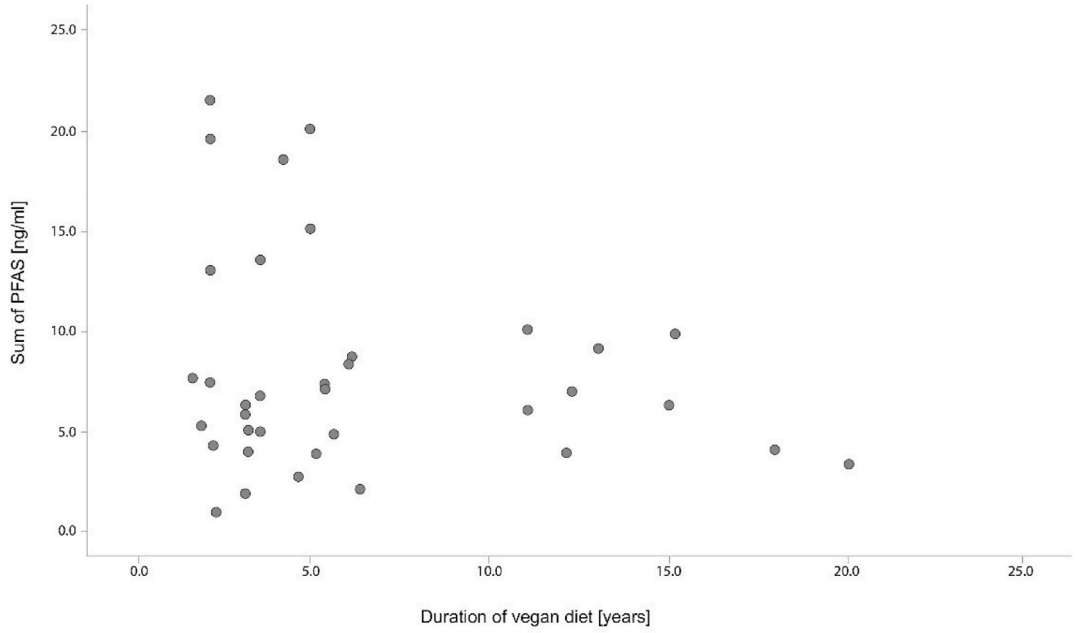

Fig. 2. Scatter plot of sum of PFAS (PFOS + PFOA + PFHxS + PFNA) according to the duration following a vegan diet. Scatter plot of sum of PFAS levels [ng/ml] to the duration of vegan diet [years] in vegans (n = 36).

comparison within Germany is possible with a group from Munich ("Side C", n = 158 blood donors, median age 39.5 years) investigated 2016 (Fromme et al., 2017), serving as a control for a contaminated region in Bavaria/Germany. Results revealed median levels of PFOS, PFOA, PFHxS, PFNA and the PFAS sum to be 2.1, 1.1, 0.5, 0.4 and 4.1 ng/ml, respectively (PFAS sum: personal communication of Prof. Fromme). Furthermore, data are available from the German Environmental Specimen Bank (n = 40 students aged 20–29 years from Münster), with median levels for the 2017/2019 sampling of PFOS, PFOA, PFHxS, PFNA and the PFAS sum of 2.6, 1.7, 0.5, 0.4 and 5.8 ng/ml, respectively (Gockener et al., 2020). The main difference between these investigations and our study are the surprising high levels of PFHxS, with a 3.7-fold higher median in Berlin (PFHxS median: 0.5 vs. 1.8 ng/ml). This may be due to regionally different nutritional habits or to higher concentrations in drinking water as well as due to differences in the socio-economic status. However, sample sizes of all studies were rather small and therefore also a chance finding cannot be excluded.

In the three investigations mentioned from Munich, Münster and Berlin, the sex ratios were exactly or roughly 1:1, and confirmed the higher levels of PFAS in men compared to women observed in many studies. As presented by EFSA, this is due to several factors. Besides a higher external exposure (e.g. due to a higher meat consumption), physiological differences including urinary elimination, menses, and use of oral contraceptives (2 vegans, 6 omnivores) have to be considered, as well as pregnancy and lactation (EFSA Panel on Contaminants in the Food Chain, 2020). In our study, the median levels of the PFAS sum were 8.2 ng/ml in men and 6.0 ng/ml in women. According to the recent risk assessment of EFSA, an internal level of the PFAS sum of up to 6.9 ng/ml corresponds to an external intake below the TWI in women of the childbearing age (EFSA Panel on Contaminants in the Food Chain,

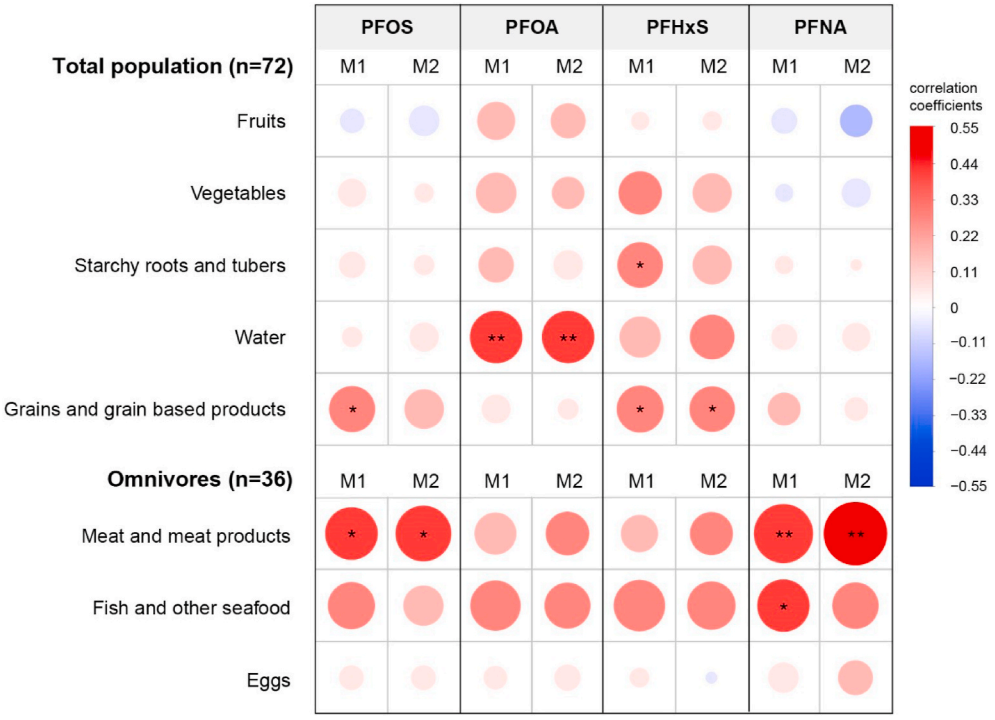

Fig. 3. Heatmap on correlations of 8 relevant food groups with PFAS plasma concentrations. Total population (n = 72) M1: adjusted for type of diet (vegan or omnivores), M2: additional adjustment for age, sex, smoking status, education, waist circumference, and physical activity; Omnivores (n = 36) M1: unadjusted model, M2: additional adjustment for age, sex, smoking status, education, waist circumference, and physical activity; **p < 0.01, *p < 0.05; Water includes drinking water, coffee, tea, and herbal tea.

Table 3
LDL cholesterol concentrations according to a vegan or omnivorous diet (n = 72).

	Vegans (n = 36)	Omnivores (n = 36)	*p-value*
LDL cholesterol [mg/dl]			
Model 1			
Unadjusted	86.3 (77.7–95.9)	110.3 (99.3–122.5)	*0.002*
Model 2			
PFOS	85.9 (77.1–95.8)	110.8 (99.4–123.5)	*0.002*
PFOA	86.2 (77.6–95.9)	110.4 (99.3–122.7)	*0.002*
PFHxS	86.3 (77.7–96.0)	110.3 (99.2–122.6)	*0.002*
PFNA	84.2 (74.6–95.1)	113.1 (100.1–127.7)	*0.003*
PFAS Sum	86.2 (77.6–95.9)	110.4 (99.3–122.8)	*0.002*
Model 3			
Lifestyle factors	86.1 (68.4–108.5)	103.6 (83.0–129.2)	*0.02*
Lifestyle factors + PFOS	85.7 (67.8–108.3)	104.0 (83.2–130.1)	*0.02*
Lifestyle factors + PFOA	86.3 (68.4–109.0)	103.5 (82.8–128.3)	*0.02*
Lifestyle factors + PFHxS	85.6 (67.8–108.0)	102.1 (81.5–128.0)	*0.02*
Lifestyle factors + PFNA	83.3 (65.5–106.0)	106.4 (84.7–133.6)	*0.01*
Lifestyle factors + PFAS Sum	85.7 (68.0–108.2)	103.4 (82.8–129.1)	*0.01*

expressed as geometric mean (95%-CI); Model 1: unadjusted, Model 2: adjusted for several PFAS, Model 3: additional adjusted for lifestyle factors i.e. age, sex, smoking status, education, waist circumference, physical activity; PFAS sum: PFOS + PFOA + PFHxS + PFNA.

2020). For this group (women between 18 and 45 years of age), 10 of 28 women (36%, maximum level 21.4 ng/ml) exceeded the level on our study, while 1 of 52 women exceeded it in Munich 2016 (2%, maximum level 7.2 ng/ml (Fromme et al., 2017)) and 6 of 20 women in Münster 2017/2019 (30%, maximum level 16.3 ng/ml (Gockener et al., 2020). These numbers demonstrate large regional differences in internal exposure to PFAS, and representative studies are necessary to get a more reliable picture of the proportion of women in the German population exceeding EFSA's internal level for the PFAS sum of 6.9 ng/ml corresponding the TWI.

The study detected significant differences in PFOS and PFNA concentrations between both diet groups, showing 54% and 240% higher median concentrations in omnivores compared to vegans, respectively. Obviously, this is due to the relatively high PFAS concentrations in food products of animals. According to the exposure assessment of EFSA (Annex A) for German Adults (EFSA Panel on Contaminants in the Food Chain, 2020), 'Fish and other seafood' was the most important contributor to the mean LB exposure, followed by 'Meat and meat products', 'Fruit and fruit products' and 'Eggs and egg products' in case of PFOS. In case of PFNA, 'Fruit and fruit products' and 'Fish and other seafood' were the most important contributors to the mean LB exposure. Therefore, the differences observed between vegans and omnivores are obviously better to explain by the diet in case of PFOS than in case of PFNA. Regarding the heat map generated from the FFQ data (Fig. 3), the pattern of PFOS and PFNA in omnivores seem comparable, with highest correlations for 'Meat and meat products' and 'Fish and other seafood'. However, due to the small number of participants of the study, the nutritional data from the FFQ with respect to the internal exposure to PFAS should be interpreted with caution. Nevertheless, our results are in line with another study. Lin et al. noticed that participants (n = 941 adults with pre-diabetes) with high consumption of meat, fried fish, and other fish/shellfish (but not omega-3 rich fish) had higher plasma concentrations of PFOS, PFHxS and PFNA (Lin et al., 2020).

Currently, PFAS levels in many food groups are found to be nearly completely below the presently available LOQs (EFSA Panel on Contaminants in the Food Chain, 2020). Therefore, more sensitive analytical methods are needed for the quantification of PFAS in foods. A higher proportion of quantified PFAS levels in food groups may lead to a better estimation of the contributions of different food groups to the exposure of different PFAS via food consumption and possibly to a change of the pattern of these contributions especially in case of PFNA.

Regarding PFOA and PFHxS in the two diet groups, no relevant differences were detected in vegans compared to omnivores. In case of PFOA, this is surprising, as EFSA identified 'Fish and other seafood', 'Eggs and egg products' as well as 'Meat and meat products' as most

important contributors to the mean LB exposure of PFOA (and PFOS) (EFSA Panel on Contaminants in the Food Chain, 2020). Interestingly, besides the above mentioned food categories, 'Alcoholic beverages' and 'Drinking water' were reported to be also important contributors to the mean LB exposure in case of PFOA in Germany (EFSA Panel on Contaminants in the Food Chain, 2020). Indeed, PFOA levels were found to have the highest correlations with the consumption of 'Water' in the total study group (see heat map, Fig. 3). In case of PFHxS, 'Fruit and fruit products', 'Alcoholic beverages' and 'Drinking water' were the only contributors to the mean LB exposure in Germany (EFSA Panel on Contaminants in the Food Chain, 2020). This is reflected in the heat map in case of 'Water' only, but as in case of PFNA, more sensitive analytical methods may change the pattern of the contributors to external exposure.

Epidemiological studies provided consistent findings of associations between serum levels of PFOS/PFOA and levels of cholesterol in populations with relatively high exposure (EFSA Panel on Contaminants in the Food Chain, 2020). In 2018, EFSA even used these associations with serum cholesterol levels to derive TWIs for both PFOS and PFOA (EFSA Panel on Contaminants in the Food Chain, 2018). However, a clear mode of action is missing. Since lower LDL cholesterol levels in vegans are one of the most highlighted health benefits of this diet (Benatar and Stewart, 2018), we thought to also analyze the relation between different PFAS and LDL cholesterol in our small study sample. Although a wide range of concentrations of LDL cholesterol was observed in our study population, we did not find any relevant relation between PFAS and LDL cholesterol. This may be due to the relatively low concentrations of PFAS in comparison to previous studies (EFSA Panel on Contaminants in the Food Chain, 2020). The distinctly lower levels of LDL cholesterol in vegans with concurrently lower levels of PFOS and PFNA strikingly raises the issue of confounding by diet in case of the above-mentioned epidemiological studies, and the extent of necessary adjustment to avoid false interpretations. Interestingly, in most studies investigating associations between PFAS and blood lipids, statistical models were adjusted – beside age, sex, smoking, alcohol intake and education – only for body mass index or waist circumference (EFSA Panel on Contaminants in the Food Chain, 2018). Only very few studies adjusted for further dietary variables such as saturated or animal fat intake (Eriksen et al., 2013; Nelson et al., 2010) or food groups such as meat or fish intake (Canova et al., 2020; Lin et al., 2020; Skuladottir et al., 2015) or healthy diet score (Donat-Vargas et al., 2019). Taking altogether, evidence for a causal relationship between PFAS and blood lipids is still not convincing and residual confounding still cannot be completely excluded to explain the observed associations between PFAS and blood lipids in many but not all studies. Further studies are necessary to clarify at one hand possible mechanisms and to investigate associations with improved statistical models including more potential confounders in particular dietary factors on the other hand.

Different methods exist for dietary assessments, and each has its advantages and disadvantages. Because of the long half-life time of PFAS, FFQ might be a good method assessing the past long-term diet. The EPIC-FFQ (Nothlings et al., 2007) captured the time span of the previous 12 months. We see the limitation of the present FFQ, which did not cover all variety of a vegan diet, as some food groups will not be fully assessed, thus the diet of vegans might be underestimated. However, in the present evaluation we analyzed only predetermined food groups, eaten by both omnivores and vegans, for example fruits, vegetables or water. Therefore, the use of the FFQ for our study purpose is acceptable. For the food groups of meat, fish or eggs, the analyses were only performed in omnivores to avoid bias due to non-consumption of the vegan population. Nevertheless, this study underlines the need for assessment tools in science for a past long-term diet also for participants following a plant-based diet (e.g. vegan, vegetarian) or the modification of already existing tools.

Limitations of our study deserve to be mentioned. The present RBVD study is relatively small (n = 72), including middle aged vegans and omnivores from a relatively small area (Berlin, Germany); therefore, the results may not be generalizable to other populations. Nevertheless, the RBVD study provides comprehensive high-quality data as a result of the standardized procedures in combination with extensive information from computer-based questionnaires and anthropometric measurements. Regarding FFQ, we see also some limitations concerning recall bias and under/over-reporting, attributed to reliance on participant's memory, inability to accurately estimate portion sizes and misinterpretation of the questions, or social desirability bias (Hooson Jzh et al., 2020). Other routes of exposure than diet such as ingestion of house dust, inhalation of indoor air, and dermal absorption may substantially contribute to the exposure to PFAS on an individual basis (Poothong et al., 2020), but these routes could not be considered in our study. Further, some packing materials and take-away food, as well as kitchen utensils might be potential sources of exposure to PFAS.

5. Conclusion

Lower levels of PFOS and PFNA, but not of PFOA and PFHxS were observed in vegans compared to omnivores. FFQ data allowed the identification of relevant food groups contributing to the levels of these four PFAS. The strong impact of a vegan diet on levels of blood lipids, especially on LDL cholesterol, was confirmed in our study. In contrast, the association of PFAS and LDL cholesterol was found to be negligible, possibly due to the relatively low levels of PFAS observed. However, we highlight the importance of the adjustment of dietary factors like a vegan diet in case of epidemiological studies dealing with the impact of PFAS on the levels of blood lipids.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

We thank all participants for their cooperation during the RBVD study. We also thank Elektra Polychronidou, Corinna Genrich, and Christel Rozycki for technical assistance, who contributed to the success of our study with great commitment. Furthermore, we would also like to thank Marius Menzel for illustrational work.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijheh.2021.113808.

References

Abraham, K., Mielke, H., Fromme, H., Volkel, W., Menzel, J., Peiser, M., Zepp, F., Willich, S.N., Weikert, C., 2020. Internal exposure to perfluoroalkyl substances (PFASs) and biological markers in 101 healthy 1-year-old children: associations between levels of perfluorooctanoic acid (PFOA) and vaccine response. Arch. Toxicol. 94, 2131–2147.

Benatar, J.R., Stewart, R.A.H., 2018. Cardiometabolic risk factors in vegans; A meta-analysis of observational studies. PloS One 13, e0209086.

Canova, C., Barbieri, G., Zare Jeddi, M., Gion, M., Fabricio, A., Dapra, F., Russo, F., Fletcher, T., Pitter, G., 2020. Associations between perfluoroalkyl substances and lipid profile in a highly exposed young adult population in the Veneto Region. Environ. Int. 145, 106117.

Desquilbet, L., Mariotti, F., 2010. Dose-response analyses using restricted cubic spline functions in public health research. Stat. Med. 29, 1037–1057.

Donat-Vargas, C., Bergdahl, I.A., Tornevi, A., Wennberg, M., Sommar, J., Koponen, J., Kiviranta, H., Akesson, A., 2019. Associations between repeated measure of plasma perfluoroalkyl substances and cardiometabolic risk factors. Environ. Int. 124, 58–65.

EFSA Panel on Contaminants in the Food Chain, Knutsen, H.K., Alexander, J., Barregard, L., Bignami, M., Bruschweiler, B., Ceccatelli, S., Cottrill, B., Dinovi, M., Edler, L., Grasl-Kraupp, B., Hogstrand, C., Hoogenboom, L.R., Nebbia, C.S., Oswald, I.P., Petersen, A., Rose, M., Roudot, A.C., Vleminckx, C., Vollmer, G., Wallace, H., Bodin, L., Cravedi, J.P., Halldorsson, T.I., Haug, L.S., Johansson, N., van

Loveren, H., Gergelova, P., Mackay, K., Levorato, S., van Manen, M., Schwerdtle, T., 2018. Risk to human health related to the presence of perfluorooctane sulfonic acid and perfluorooctanoic acid in food. EFSA J 16, e05194.

EFSA Panel on Contaminants in the Food Chain, Schrenk, D., Bignami, M., Bodin, L., Chipman, J.K., Del Mazo, J., Grasl-Kraupp, B., Hogstrand, C., Hoogenboom, L.R., Leblanc, J.C., Nebbia, C.S., Nielsen, E., Ntzani, E., Petersen, A., Sand, S., Vleminckx, C., Wallace, H., Barregard, L., Ceccatelli, S., Cravedi, J.P., Halldorsson, T.I., Haug, L.S., Johansson, N., Knutsen, H.K., Rose, M., Roudot, A.C., Van Loveren, H., Vollmer, G., Mackay, K., Riolo, F., Schwerdtle, T., 2020. Risk to human health related to the presence of perfluoroalkyl substances in food. EFSA J 18, e06223.

Eriksen, K.T., Raaschou-Nielsen, O., McLaughlin, J.K., Lipworth, L., Tjonneland, A., Overvad, K., Sorensen, M., 2013. Association between plasma PFOA and PFOS levels and total cholesterol in a middle-aged Danish population. PloS One 8, e56969.

Frisbee, S.J., Shankar, A., Knox, S.S., Steenland, K., Savitz, D.A., Fletcher, T., Ducatman, A.M., 2010. Perfluorooctanoic acid, perfluorooctanesulfonate, and serum lipids in children and adolescents: results from the C8 Health Project. Arch. Pediatr. Adolesc. Med. 164, 860–869.

Fromme, H., Wockner, M., Roscher, E., Volkel, W., 2017. ADONA and perfluoroalkylated substances in plasma samples of German blood donors living in South Germany. Int. J. Hyg Environ. Health 220, 455–460.

Gockener, B., Weber, T., Rudel, H., Bucking, M., Kolossa-Gehring, M., 2020. Human biomonitoring of per- and polyfluoroalkyl substances in German blood plasma samples from 1982 to 2019. Environ. Int. 145, 106123.

Hooson Jzh, J., Hutchinson Jyh, J., Warthon-Medina, M., Hancock, N., Greathead, K., Knowles, B., Vargas-Garcia, E., Gibson, L.E., Bush, L.A., Margetts, B., Robinson, S., Ness, A., Alwan, N.A., Wark, P.A., Roe, M., Finglas, P., Steer, T., Page, P., Johnson, L., Roberts, K., Amoutzopoulos, B., Burley, V.J., Greenwood, D.C., Cade, J. E., 2020. A systematic review of reviews identifying UK validated dietary assessment tools for inclusion on an interactive guided website for researchers. Crit. Rev. Food Sci. Nutr. 60, 1265–1289. www.nutritools.org.

InterAct, C., Peters, T., Brage, S., Westgate, K., Franks, P.W., Gradmark, A., Tormo Diaz, M.J., Huerta, J.M., Bendinelli, B., Vigl, M., Boeing, H., Wendel-Vos, W., Spijkerman, A., Benjaminsen-Borch, K., Valanou, E., de Lauzon Guillain, B., Clavel-Chapelon, F., Sharp, S., Kerrison, N., Langenberg, C., Arriola, L., Barricarte, A., Gonzales, C., Grioni, S., Kaaks, R., Key, T., Khaw, K.T., May, A., Nilsson, P., Norat, T., Overvad, K., Palli, D., Panico, S., Ramon Quiros, J., Ricceri, F., Sanchez, M.J., Slimani, N., Tjonneland, A., Tumino, R., Feskins, E., Riboli, E., Ekelund, U., Wareham, N., 2012. Validity of a short questionnaire to assess physical activity in 10 European countries. Eur. J. Epidemiol. 27, 15–25.

Janssen, M., Busch, C., Rodiger, M., Hamm, U., 2016. Motives of consumers following a vegan diet and their attitudes towards animal agriculture. Appetite 105, 643–651.

Lin, P.D., Cardenas, A., Hauser, R., Gold, D.R., Kleinman, K.P., Hivert, M.F., Fleisch, A.F., Calafat, A.M., Sanchez-Guerra, M., Osorio-Yanez, C., Webster, T.F., Horton, E.S., Oken, E., 2020. Dietary characteristics associated with plasma concentrations of per- and polyfluoroalkyl substances among adults with pre-diabetes: cross-sectional results from the Diabetes Prevention Program Trial. Environ. Int. 137, 105217.

Menzel, J., Abraham, K., Stangl, G.I., Ueland, P.M., Obeid, R., Schulze, M.B., Herter-Aeberli, I., Schwerdtle, T., Weikert, C., 2021. Vegan diet and bone health-results from the cross-sectional RBVD study. Nutrients 13.

Menzel, J., Biemann, R., Longree, A., Isermann, B., Mai, K., Schulze, M.B., Abraham, K., Weikert, C., 2020. Associations of a vegan diet with inflammatory biomarkers. Sci. Rep. 10, 1933.

Mosch, C., Kiranoglu, M., Fromme, H., Volkel, W., 2010. Simultaneous quantitation of perfluoroalkyl acids in human serum and breast milk using on-line sample preparation by HPLC column switching coupled to ESI-MS/MS. J Chromatogr B Analyt Technol Biomed Life Sci 878, 2652–2658.

Nelson, J.W., Hatch, E.E., Webster, T.F., 2010. Exposure to polyfluoroalkyl chemicals and cholesterol, body weight, and insulin resistance in the general U.S. population. Environ. Health Perspect. 118, 197–202.

Nothlings, U., Hoffmann, K., Bergmann, M.M., Boeing, H., 2007. Fitting portion sizes in a self-administered food frequency questionnaire. J. Nutr. 137, 2781–2786.

Poothong, S., Papadopoulou, E., Padilla-Sanchez, J.A., Thomsen, C., Haug, L.S., 2020. Multiple pathways of human exposure to poly- and perfluoroalkyl substances (PFASs): from external exposure to human blood. Environ. Int. 134, 105244.

Skuladottir, M., Ramel, A., Rytter, D., Haug, L.S., Sabaredzovic, A., Bech, B.H., Henriksen, T.B., Olsen, S.F., Halldorsson, T.I., 2015. Examining confounding by diet in the association between perfluoroalkyl acids and serum cholesterol in pregnancy. Environ. Res. 143, 33–38.

Steenland, K., Tinker, S., Frisbee, S., Ducatman, A., Vaccarino, V., 2009. Association of perfluorooctanoic acid and perfluorooctane sulfonate with serum lipids among adults living near a chemical plant. Am. J. Epidemiol. 170, 1268–1278.

Sunderland, E.M., Hu, X.C., Dassuncao, C., Tokranov, A.K., Wagner, C.C., Allen, J.G., 2019. A review of the pathways of human exposure to poly- and perfluoroalkyl substances (PFASs) and present understanding of health effects. J. Expo. Sci. Environ. Epidemiol. 29, 131–147.

Weikert, C., Trefflich, I., Menzel, J., Obeid, R., Longree, A., Dierkes, J., Meyer, K., Herter-Aeberli, I., Mai, K., Stangl, G.I., Muller, S.M., Schwerdtle, T., Lampen, A., Abraham, K., 2020. Vitamin and mineral status in a vegan diet. Dtsch Arztebl Int 117, 575–582.

Yokoyama, Y., Levin, S.M., Barnard, N.D., 2017. Association between plant-based diets and plasma lipids: a systematic review and meta-analysis. Nutr. Rev. 75, 683–698.