British Journal of Nutrition (2017), **117**, 669–685
doi:10.1017/S0007114517000629

A comparison of heart rate variability, *n*-3 PUFA status and lipid mediator profile in age- and BMI-matched middle-aged vegans and omnivores

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(Submitted 5 September 2016 – Final revision received 8 February 2017 – Accepted 22 February 2017)

Abstract

Low heart rate variability (HRV) predicts sudden cardiac death. Long-chain (LC) *n*-3 PUFA (C20–C22) status is positively associated with HRV. This cross-sectional study investigated whether vegans aged 40–70 years (*n* 23), whose diets are naturally free from EPA (20 : 5*n*-3) and DHA (22 : 6*n*-3), have lower HRV compared with omnivores (*n* 24). Proportions of LC *n*-3 PUFA in erythrocyte membranes, plasma fatty acids and concentrations of plasma LC *n*-3 PUFA-derived lipid mediators were significantly lower in vegans. Day-time interbeat intervals (IBI), adjusted for physical activity, age, BMI and sex, were significantly shorter in vegans compared with omnivores (mean difference −67 ms; 95 % CI −130, −3·4, $P<0·05$), but there were no significant differences over 24 h or during sleep. Vegans had higher overall HRV, measured as 24 h standard deviation of normal-to-normal intervals (SDNN) (mean adjusted difference 27 ms; 95 % CI 1, 52, $P=0·039$). Conversely, vegans presented with decreased 8 h day-time HRV: mean adjusted difference in SDNN −20 ms; 95 % CI −37, −3, $P=0·021$, with no differences during nocturnal sleep. Day-time parameters of beat-to-beat HRV (root of the mean of the sum of the squares of differences between adjacent normal-to-normal intervals, percentage of adjacent normal-to-normal intervals that differ by >50 % and high-frequency power) were similarly lower in vegans, with no differences during sleep. In conclusion, vegans have higher 24 h SDNN, but lower day-time HRV and shorter day-time IBI relative to comparable omnivores. Vegans may have reduced availability of precursor markers for pro-resolving lipid mediators; it remains to be determined whether there is a direct link with impaired cardiac function in populations with low-*n*-3 status.

Key words: Vegans: *n*-3 PUFA: CHD: Heart rate variability: Inflammation: Eicosanoids: Lipid mediators

The longer chain (LC) *n*-3 PUFA (C20–C22), EPA (20 : 5*n*-3) and DHA (22 : 6*n*-3), are mainly derived from seafood, although small amounts are provided by meat, eggs and dairy products. Consequently, vegans consume a diet devoid of 20 : 5*n*-3 and 22 : 6*n*-3[1]. The main *n*-3 PUFA in vegan diets is *α*-linolenic acid (ALA; 18 : 3*n*-3), derived from plant foods, particularly soya and seed oils such as rapeseed oil. (LC) *n*-3 PUFA as percentages of total fatty acids in blood fractions are, in vegans, only a third of the level in meat- and fish-eaters[2]. 20 : 5*n*-3 and 22 : 6*n*-3 can be endogenously synthesised from 18 : 3*n*-3 by desaturation and elongation enzymes, but the rate of this conversion is restricted to a narrow range in adults[3]. Stable isotope studies have suggested that conversion of 18 : 3*n*-3 to 22 : 6*n*-3 can vary from undetectable amounts up to 4 % in men, and 9 % in women[4]. An observational study suggested that conversion from dietary 18 : 3*n*-3 to LC *n*-3 PUFA might be increased in non-fish-eaters[5]. Furthermore, dietary supplementation of vegans with 18 : 3*n*-3 has not been found to increase 22 : 6*n*-3 in blood lipids, including plasma choline phosphoglycerides and platelet phosphoglycerides[6,7] and erythrocytes, platelets and plasma cholesteryl esters, phospholipids and TAG[6,7]. It is unclear whether the lack of 20 : 5*n*-3 and 22 : 6*n*-3 intake in vegans has adverse effects on cardiovascular health[8,9] especially as BMI[10,11], blood cholesterol[12,13] and blood pressure[14,15] are lower than in meat-eaters.

LC *n*-3 PUFA, especially 22 : 6*n*-3, are rapidly incorporated into cellular lipids, primarily membrane phospholipids, in a variety of cells including cardiomyocytes and neural tissue,

Abbreviations: %E, percentage of energy; HDHA, hydroxydocosahexaenoic acid; HF, high-frequency power; HR, heart rate; HRV, heart rate variability; IBI, interbeat intervals; LC, long chain; LF, low-frequency power; pNN50, percentage of adjacent normal-to-normal intervals that differ by >50 %; RMSSD, root of the mean of the sum of the squares of differences between adjacent normal-to-normal intervals; SDANN, standard deviation of the average 5-min normal-to-normal intervals; SDNN, standard deviation of normal-to-normal intervals; SPM; specialised pro-resolving lipid mediators; VLF, very-low-frequency power.

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thereby influencing membrane properties and function of membrane proteins. Fish oil consumption reduces heart rate (HR) in humans[16]. Increasing LC *n*-3 PUFA content in cardiomyocyte membranes by 3 weeks of dietary fish oil in rabbits decreases HR in isolated hearts, and reduces pacemaker activity and pacemaker current in sinoatrial node cells[17]; mechanisms are likely to be related to increased membrane fluidity and direct interaction with a hyperpolarisation-activated I_f channel protein[17], altering ion channel currents and reducing intrinsic pacemaker rate, reviewed by Billman[18].

Raised HR are associated with a high degree of sympathetic activity and suppressed parasympathetic activity (vagal activity slows HR) resulting in low heart rate variability (HRV); a reduced capacity to self-regulate the HR in response to physiological demands. Low HRV is associated with mortality after a myocardial infarction[19–21], risk of sudden death in patients with CHD[22], and risk of cardiac events in the general population[23]. Higher *n*-3 PUFA tissue status or fish consumption has been positively associated with HRV[24,25]. Since HRV is under the control of the autonomic nervous system, regulation of HR may be influenced by *n*-3 PUFA status of neuronal and cardiac tissue. The brain is particularly rich in 22 : 6*n*-3, and incorporation of dietary LC *n*-3 PUFA into neuronal tissue influences gene expression, membrane protein signalling, neurotransmission and signal transduction pathways[26]. This may influence autonomic function by enhancing parasympathetic and/or reducing sympathetic activity, thus reducing HR and increasing HRV. Therefore, impairment of cardiac autonomic function due to depleted LC *n*-3 PUFA content in the central or peripheral nervous tissue would reduce the responsivity of the heart.

A further mechanism whereby cardiac function may be modulated by neuronal LC *n*-3 PUFA status is via the production of eicosanoids and related PUFA-derived lipid mediators that may reduce inflammation and terminate ('resolve') acute inflammatory events, preventing further neuronal tissue damage. PUFA can be oxygenated into numerous bioactive lipid mediators[27], and some of the 20 : 5*n*-3- and 22 : 6*n*-3-derived species act as precursors of the specialised pro-resolving lipid mediators (SPM), resolvins, protectins and maresin, which are autocoid substances actively involved in the resolution of local inflammation[27–29]. Neuroprotectin D1 is a neuroprotective lipid mediator derived from 22 : 6*n*-3 which might be particularly relevant to the preservation of optimal cardiac autonomic function[30].

This study aims to compare HRV between vegans and age/sex/BMI-matched omnivores, representing populations with low and adequate tissue *n*-3 PUFA status, respectively. The primary hypothesis of the study is that vegans have higher HR/shorter interbeat intervals (IBI) and lower HRV compared with omnivores. Exploratory analysis of plasma 20 : 5*n*-3- and 22 : 6*n*-3-derived lipid mediator concentrations was conducted in order to provide mechanistic hypothesis-generating data that may help explain differences in HR/IBI/HRV between low and high LC *n*-3 PUFA status groups.

Methods

The present study was conducted according to the guidelines laid down in the Declaration of Helsinki, and all procedures involving human subjects were approved by the research ethics committee of King's College London (BDM/12/13-84). Written informed consent was obtained from each subject. In all, twenty-three healthy, non-smoking men and women, aged 40–70 years who had been following a vegan diet for at least 2 years were compared with 24 age- and BMI-matched healthy participants who followed a mixed diet including meat, fish, eggs and dairy-containing foods (omnivores). Primary outcome variables were HR/IBI and time-domain parameters of different components of HRV: standard deviation of normal-to-normal intervals (SDNN): the most commonly reported marker of HRV and an indication of overall HRV, mainly determined by day/night differences) and root square root of the mean of the sum of the squares of differences between adjacent normal-to-normal intervals (RMSSD); an indicator of beat-to-beat, respiration-driven variability representing parasympathetic cardiac regulation). Secondary outcome variables included: other time and frequency-domain and non-linear parameters of HRV, erythrocyte and plasma fatty acid composition, plasma oxygenated lipid mediator profile, fasting plasma lipid profile, vitamin B_{12}, serum 25-hydroxyvitamin D, IL-6, fasting plasma glucose, blood pressure, body composition and background diet in order to compare risk factors for CVD in vegans and omnivores. A sample size of twenty-three in each group has a 80 % power to detect a difference between SDNN means of 25 ms and between RMSSD means of 15 ms with a significance level of 0·05 (two-tailed), based on SD of 30 and 18 ms, respectively, obtained from sleep-time HRV recordings in a previous cohort of middle-aged to older healthy men and women[31]. Participants were recruited by distributing adverts to vegan organisations and societies. Omnivore participants were recruited through internal and external email circulars and posters amongst university students and staff. The study was also promoted via social media, flyer distributions to vegan restaurants and vegan food shops throughout London, and at various vegan food events. Volunteers who responded to advertisements were given more information about the study, completed an initial eligibility questionnaire via telephone or email and, if eligible, were provided with a study information sheet. Exclusion criteria included a reported history of CVD, diabetes, cancer (excluding basal cell carcinoma) in the past 5 years, chronic renal, liver or inflammatory bowel disease, history of drug or alcohol abuse (previous weekly alcohol intake >60 units/men or 50 units/women), current self-reported weekly alcohol intake exceeding twenty-eight units, current use of marine *n*-3 supplements, pregnancy, weight change of >3 kg in the previous 2 months, and BMI <18·5 and >35 kg/m^2. Vegan subjects were enrolled on the study along with omnivore controls, aiming to match for sex, age (±5 years) and BMI (±2 kg/m^2). A validated FFQ[32] was used to verify self-classification of dietary status of eligible volunteers and to provide supplementary information on habitual dietary intakes. Analysis was carried out using an Excel spreadsheet that incorporated additional food composition data on LC *n*-3 PUFA contents of foods other than fish (meat, dairy products and eggs).

Participants attended one study visit, which took place in the morning. Volunteers were instructed to fast for 12 h before the visit and consume nothing but water until attending the clinic. Once written informed consent was obtained, seated blood pressure was measured in triplicate using an A&D

Medical UA-767Plus upper arm automatic blood pressure monitor (A&D Instruments Limited), in accordance with guidelines from the British Hypertension Society. Height, body weight and percentage body fat and waist circumference (WC) were measured using a stadiometer, a Tanita weighing scale (model: BC-418 MA; Tanita UK Ltd) and a tape measure, respectively. Participants completed the FFQ, which was checked for completeness and any missing data verified directly with the participant. Fasting plasma glucose and serum lipids, serum liver function markers and whole blood haematology was analysed on the same day in fresh blood samples, and further plasma aliquots were frozen at −70°C until analysis of fatty acid and lipid mediator profiles could take place. Erythrocytes were washed with saline and lysed. The erythrocyte lysate was de-proteinised in the presence of butylated hydroxytoluene, chloroform was added to extract lipids then centrifuged as previously described[33]; supernatant was frozen at −20°C until analysis for fatty acid composition could be conducted[33]. An Actiheart monitor was fitted on the chest (CamNtech Ltd), which they wore for 24 h. A diary was provided during the recording period to keep a register of all the daily activities (activities/exercise, meals or naps).

Heart rate variability measurements

IBI and continuous HR were measured for approximately 24 h using Actiheart monitors, which are small, light-weight (<10 g) waterproof devices that also contain piezoelectric sensors to record acceleration in the vertical plane (counts per minute) as a measure of physical activity[34]. Before the monitor could be fitted, the area of skin was prepared including shaving of chest hair where required, using alcohol wipes to clean and dry the skin and use of an abrasive pad (Unilect™) to remove the top layer of skin cells. Two electrocardiogram (ECG) electrodes (SP-50, 50 mm round; Pulse Medical) were placed on the chest to fit the Actiheart monitor. A short signal test involving a 5 min walk was performed before programming for the 24 h recording to confirm adequate signal:noise ratio. Data processing of the 24 h IBI recordings was carried out using the Actiheart software (version 4.0.91; CamNtech Ltd) and Kubios HRV analysis software (Biosignal Analysis and Medical Imaging Group, Department of Physics, University of Kuopio)[35]. HRV, HR/IBI and accelerometry data[34] were analysed for the full length of recording time (minimum of 18 h, up to 24 h). Further analysis was carried on a standardised day-time period of 8 h and sleep-time period of 2 h to remove the influence of variability in recording duration on HRV parameters. HRV outcomes included time and frequency-domain parameters; time-domain parameters are based on the time intervals between adjacent QRS (Q, R and S being points on the R wave seen on an ECG during ventricular depolarisation, and R being the peak upward deflection) complexes (normal-to-normal (NN) intervals) whereas frequency-domain parameters employ power spectral analysis of NN intervals to determine the power (variance) within frequency bands[36]. Time-domain parameters included SDNN, standard deviation of the average normal-to-normal intervals in 5 min segments of the whole recording (SDANN), RMSSD, the percentage of adjacent normal-to-normal intervals that differed by >50 % (pNN50) and triangular index (TI), the integral of the density distribution (the number of all NN intervals) divided by the maximum of the density distribution. Frequency-domain parameters included high-frequency (HF), low-frequency (LF) and very-low-frequency (VLF) power, and the ratio of the LF and HF band powers (LF:HF). A non-linear parameter using Poincaré plots of short-term variability (SD1) against long-term variability (SD2) was also calculated as a measure of complexity of HRV distribution over the duration of the recording. SDNN, LF and TI represent overall variability. Short-term (beat-to-beat) components of HRV include RMSSD, pNN50 and HF. SDANN and VLF reflect longer-phase components of variability.

Fatty acid analysis

Proportions of fatty acids in whole plasma and erythrocyte membranes were analysed by GC (Agilent 7890A GC; Agilent Technologies) with a BPX70 GC column (length 25 m, internal diameter 0·32 mm, film thickness 0·25 μm) custom designed for separation of fatty acid methyl esters (SGE Analytic Science) following transesterification, as previously described[37], but substituting toluene for benzene and using pentadecanoic acid as an internal standard. Since total plasma concentrations of fatty acids differ in vegans compared with omnivores, individual plasma fatty acids were compared between groups as weight percentages of the sum of fatty acids (% weight)[38] The Omega-3 Index was defined as the sum of % weight EPA + DHA in erythrocytes.

Mediator lipidomics

Prostanoids and hydroxy fatty acids derived from dihomo-γ-linolenic acid (20 : 3*n*-6), linoleic acid (LA; 18 : 2*n*-6), 18 : 3*n*-3, arachidonic acid (AA; 20 : 4*n*-6), 20 : 5*n*-3 and 22 : 6*n*-3 were extracted from plasma and analysed by ultraperformance liquid chromatography with electrospray ionisation and tandem MS as previously described[39]. In brief, samples were extracted in 15 % (v/v) methanol, and internal standards were added (20 ng each of PGB_2-*d*4, 12-hydroxyeicosatetraenoic acid (HETE)-*d*8, 8,9-epoxyeicosatetraenoic acid-*d*11 and 8(9)-dihydroeicosatetraenoic acid-*d*11. Lipid extracts were semi-purified using solid phase extraction (C18-E cartridges; 500 mg, 6 ml; Phenomenex), and dried under N, before reconstitution in ethanol for analysis. Chromatographic separation was performed on a C18 column (Acquity UPLC BEH, 1·7 μm, 2·1 × 50 mm; Waters) using a gradient of acidified acetonitrile and water (Acquity Ultraperformance Liquid Chromatography and Xevo triple quadrupole mass spectrometer; Waters). Analytes were recorded using multiple reaction monitoring assays, using the transitions reported in Astarita *et al.*[27] and quantified using calibration lines constructed with commercially available standards (Cayman).

Blood biochemistry analysis

Blood samples were collected into fluoride oxalate tubes for glucose analysis and SST™ II tubes for TAG, total cholesterol, and HDL-cholesterol, vitamin B_{12}, 25-hydroxy vitamin D and

IL-6 analysis; plasma and serum were stored frozen at −40°C until analysis (Becton Dickinson). Analyses of full blood counts, plasma glucose, serum lipids, vitamins and IL-6 were determined by a clinical pathology accredited clinical biochemistry laboratory (ViaPath, Kings College Hospital). Glucose and lipids were analysed following enzymatic methods using reagents supplied by Bayer Diagnostics Europe Ltd (Bayer House) using an ADVIA 2400 analyser (Siemans Healthcare Diagnostics). IL-6 was analysed using a high-sensitivity cytokine chip array assay (Human cytokine HS X biochip; Randox Laboratories Limited). Serum vitamin D and B_{12} concentrations were analysed using the ADVIA Centaur total vitamin D and vitamin B_{12} immunoassays (Siemens Healthcare Diagnostics Ltd).

Statistical analysis

Statistical analyses were performed using IBM SPSS Statistics 21.0 (Statistical Product and Service Solutions; IBM Corp.). χ^2 tests for categorical variables and independent samples *t* test for continuous variables were used to assess the differences between vegan and omnivore subjects' characteristics, dietary intakes, erythrocyte and plasma fatty acids and lipid mediators. Non-normally distributed data were normalised by natural logarithm (LN) (results shown as geometric means and 95 % CI) before analysis by independent *t* test. If LN transformation failed to yield a normal distribution, a Mann–Whitney *U* test was applied to compare groups (results shown as medians with lower and upper quartiles). In the case of lipid mediators, results from non-normally distributed data analysed by Mann–Whitney *U* test were shown as medians with minimum and maximum values due to the proportion of undetectable concentrations of LC *n*-3 PUFA-derived mediators in omnivores as well as vegans.

For HRV analysis, normally distributed raw data or LN transformed data were analysed by univariate ANCOVA, adjusted for sex, age, BMI and, in the case of day-time and 24 h data, physical activity (accelerometry data). Results are expressed as estimated marginal means (95 % CI), adjusted for sex, age, BMI and 24 h activity for 24 h HRV and sleep-time – day-time HRV, or sex, age, BMI and 8 h activity for 8 h day-time HRV, or adjusted for sex, age and BMI only for 2 h sleep-time. Estimated marginal means and 95 % CI from data that were LN transformed before analysis by ANCOVA were back-transformed and expressed as geometric means and 95 % CI. Data that could not be normalised by LN transformation were analysed using Mann–Whitney *U* test and significance values are presented unadjusted, with results shown as medians (lower and upper quartiles).

Results

Participant characteristics

Fig. 1 shows the flow of participants through the study. Subject characteristics of the forty-seven participants who completed the study are presented in Table 1. The mean ages of vegans (eight men, fifteen women) and omnivores (twelve men, twelve women) were 49 (SD 8) and 54 (SD 9) years, respectively, and there were no significant differences in mean age or BMI, or distributions of sex between groups, although the sex distributions were not fully balanced across groups. Furthermore there were no significant differences in other markers of body composition (% body fat, WC). Seated resting HR was on average 7 beats per minute higher and systolic blood pressure was 7 mmHg lower in vegans compared with omnivores; there was no difference in mean seated diastolic blood pressure (Table 1). Fasting serum total and LN serum LDL-cholesterol concentrations were lower in vegans compared with omnivores, but there were no differences in mean fasting plasma glucose, LN serum TAG, serum HDL-cholesterol, serum vitamin B_{12}, 25-hydroxy vitamin D or IL-6 concentrations, nor blood Hb concentrations, indicating that the vegan group did not differ in vitamin D status and were likely to be taking dietary vitamin B_{12} supplements. Analysis of FFQ (Table 1) showed that 61 % of vegans reported taking vitamin B_{12} supplements, and suggested that vegans and omnivores had comparable total energy and percentage of energy (%E) as fat intakes. Omnivores reported significantly higher protein (%E), SFA (%E) and food-derived vitamin B_{12} (μg) intakes and vegans had significantly higher carbohydrate (%E), total PUFA (%E) and 18 : 2*n*-6 (g) intakes. There were no differences in reported 18 : 3*n*-3 (g) intakes. As expected, vegans reported no dietary intake of 20 : 5*n*-3 and 22 : 6*n*-3, hence omnivores obtained significantly higher intake of these fatty acids, with estimated median intakes of 0·14 (interquartile range (IQR) 0·09, 0·24) and 0·45 (IQR 0·30, 0·81) g/d for 20 : 5*n*-3 and 22 : 6*n*-3, respectively. A subset (twelve omnivore and eight vegan participants) completed 4-d food diaries (data not shown); analyses of these supported the FFQ data.

Fatty acid and lipid mediator profiles

Vegans had a significantly higher proportion of plasma and erythrocyte 18 : 2*n*-6, plasma 18 : 3*n*-3 and erythrocyte 20 : 3 *n*-6 compared with omnivores (Table 2). Both whole plasma and erythrocyte membrane proportions of 20 : 5*n*-3 and 22 : 6*n*-3, plasma palmitic acid (16 : 0), and erythrocyte docosapentaenoic acid *n*-3 (22 : 5*n*-3) and palmitoleic acid (16 : 1*n*-7) were significantly lower in vegans compared with omnivores. Vegans had a significantly lower Omega-3 Index, with a geometric mean of 2·7 % compared with 5·4 % in omnivores, although both groups would be considered below the proposed Omega-3 Index cut-off of >8 % for optimal CVD protection[8]. Erythrocyte 18 : 2*n*-6:18 : 3*n*-3 ratios were inversely correlated with erythrocyte 20 : 5*n*-3 contents in vegans (r −0·541, P = 0·008, n 23), but not 22 : 5*n*-3 or 22 : 6*n*-3 contents; with no significant correlations in the erythrocyte lipids of omnivores. In plasma, the ratio of 18 : 2*n*-6:18 : 3*n*-3 was inversely correlated with plasma 22 : 5*n*-3 (r −0·576, P = 0·004, n 23) and 22 : 6*n*-3 (r −0·498, P = 0·016, n 23) in vegans and plasma 22 : 5*n*-3 only in omnivores (r −0·474, P = 0·019, n 24).

Table 3 shows *n*-3 and *n*-6 PUFA-derived lipid mediators evaluated in the fasting plasma of the two study groups. A complete diagrammatic list of all lipid mediators included in the analysis protocol, including those that were and were not detectable in the plasma of this study population is given in Fig. 2(a) and (b). In general, the lipid mediators derived from

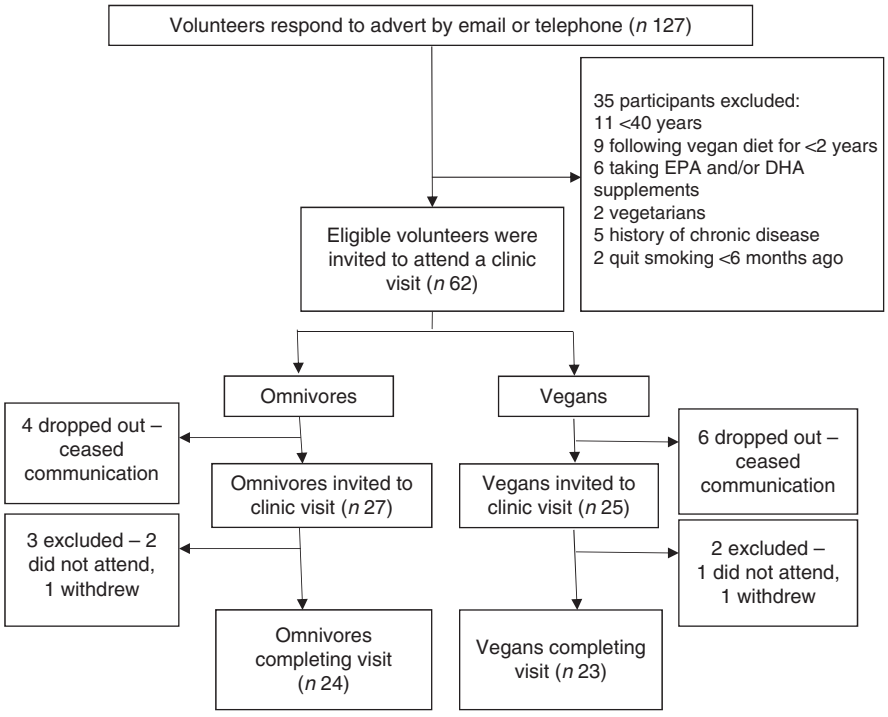

Fig. 1. Consort diagram.

n-6 PUFA (mainly 20 : 3*n*-6 and 18 : 2*n*-6), and plant-derived *n*-3 PUFA (18 : 3*n*-3) were higher in vegans compared with omnivores, and the mediators derived from 20 : 5*n*-3 and 22 : 6*n*-3 were lower in vegans compared with omnivores, showing a clear difference in the lipidomic profile between the groups. SPM (resolvins, protectins and maresins) were not detectable in the fasting plasma samples. Notably, in vegans there were markedly lower fasting plasma concentrations of 18-hydroxyeicosapentaenoic acid (HEPE), an 20 : 5*n*-3-derived precursor marker for resolvin E1 (RvE1), and undetectable concentrations of 17-hydroxydocosahexaenoic acid (HDHA), a 22 : 6*n*-3-derived precursor marker for RvD1, RvD2 and PDX, an isomer of protectin D1. 14-HDHA, another mediator arising from 22 : 6*n*-3 and a precursor marker for the macrophage-derived maresin 1 (MaR1), was also much lower in vegan fasting plasma compared with omnivores (Table 3). In summary, these data show that vegans have increased blood concentrations of oxygenated metabolites of 18 : 2*n*-6 and 18 : 3*n*-3 compared with omnivores, and very low or undetectable concentrations of LC *n*-3 PUFA-derived oxygenated metabolites.

Heart rate and heart rate variability

24 h. The average duration of the 24 h IBI recording was 21.02 (95 % CI 20.11, 21.52) hours; for day-time analysis it was 13.08 (95 % CI 12.38, 13.38) hours and for sleep-time analysis it was 05.56 (95 % CI 05.25, 06.27) hours. Only recordings with a minimum of 18 h were included in the 24 h physical activity, IBI, HR and SDNN analysis.

Vegans had higher 24 h HRV than omnivores as shown in Table 4: SDNN, SDANN and VLF were higher in vegans. Differences in these parameters indicate greater variability in longer-phase cycles in the vegan group during the 24 h period, whereas beat-to-beat (parasympathetically driven) variability (RMSSD, PNN50 and HF) and IBI/HR were not different between groups over the 24 h period. This is substantiated by much greater sleep-time minus day-time differences in mean IBI/HR, indicating that vegans experience a greater drop in HR from day to night compared with omnivores, due to having faster day-time HR.

Day-time. Day-time analysis was carried out on the first 8 h segment of data following fitting of the monitor on the morning of the study visit, excluding noisy sections where signal was poor, in order to standardise the length of recording. There was no difference in physical activity levels as assessed by accelerometry between vegans and omnivores. IBI was significantly shorter in vegans (reciprocal to HR, for which group differences fell just short of statistical significance) during the day compared with omnivores (Table 4). In contrast to the 24 h measurement period, HRV was reduced in vegans during the day compared with omnivores: this was observed in parameters of overall variability (SDNN, LF, although not in TI) and the beat-to-beat parameters of variability (RMSSD, PNN50, HF). To summarise, during day-time waking hours, vegans had shorter IBI/faster HR and in accordance with this, they had reduced beat-to-beat HRV, compared with omnivores, even after adjusting for physical activity and other covariates.

Nocturnal sleep-time. Sleep-time analysis was carried out on the first 2 h of sleep data, excluding periods of awakening as determined by increases in accelerometry counts per minute, in

Table 1. Characteristics and background dietary intakes of vegan and omnivore participants
(Numbers and percentages; mean values and standard deviations; geometric means and 95 % confidence intervals; medians and interquartile ranges (IQR))

	Omnivore (*n* 24)		Vegan (*n* 23)		
	n	%	*n*	%	*P**
Sex					
Male	12	50	8	35	
Female	12	50	15	65	0·292†
Ethnicity					
White	21	88	21	91	0·289†
Black African/Caribbean	0	0	1	5	
South Asian	2	8	0	0	
Other	1	4	1	5	
Vitamin B_{12} supplement use	8	33	14	61	0·059†
	Mean	SD	Mean	SD	
Age (years)	54	9·1	49	7·9	0·081
BMI (kg/m^2)	23·3	2·8	23·5	4·4	0·896
Waist circumference (cm)					
Male	91·7	6·5	97·2	5·9	0·165
Female	88·8	12·5	86·0	14·8	0·617
% body fat					
Male	19·3	7·9	21·1	5·9	0·572
Female	30·6	7·7	29·4	9·9	0·751
Systolic BP (mmHg)	118	9·8	111	9·9	0·032
Diastolic BP (mmHg)	75	9·3	74	8·2	0·703
Heart rate (bpm)	63	10·8	70	9·0	0·017
Plasma glucose (mmol/l)	5·2	0·4	5·1	0·4	0·616
Serum TAG (mmol/l)					0·849
Geometric mean	0·77		0·76		
95 % CI	0·66, 0·92		0·64, 0·90		
Serum total cholesterol (mmol/l)	4·9	0·86	4·1	0·66	0·001
Serum LDL-cholesterol (mmol/l)					0·002
Geometric mean	2·81		2·16		
95 % CI	2·49, 3·17		1·94, 2·41		
Serum HDL-cholesterol (mmol/l)					
Male	1·52	0·35	1·29	0·23	0·118
Female	1·71	0·24	1·66	0·42	0·725
Energy intake (MJ)	8·17	2·00	7·67	2·77	0·477
Protein intake (%E)	16·6	2·2	13·3	2·4	<0·001
Carbohydrate intake (%E)	49·2	7·3	56·5	11·6	0·013
Total fat intake (%E)	33·8	5·9	30·9	9·5	0·216
SFA intake (%E)	11·8	2·6	6·3	1·7	<0·001
MUFA intake (%E)					0·136‡
Median	13·5		11·6		
IQR	12·1, 15·0		9·3, 14·4		
PUFA intake (%E)	6·0	1·2	9·6	3·1	<0·001‡
18 : 2*n*-6 intake (g)					0·025‡
Median	7·6		10·5		
IQR	5·9, 10·2		7·3, 18·5		
18 : 3*n*-3 intake (g)					0·425
Median	0·7		0·8		
IQR	0·5, 1·0		0·5, 1·2		
20 : 5*n*-3 intake (g)					<0·001‡
Median	0·14		0·00		
IQR	0·09, 0·24		0·00, 0·00		
22 : 6*n*-3 intake (g)					<0·001‡
Median	0·45		0·01		
IQR	0·30, 0·81		0·01, 0·01		
Serum vitamin B_{12} (ng/l)	442	216	358	117	0·108
Hb (g/l)					
Male	14·4	0·6	14·4	0·8	0·805
Female	13·2	1·0	13·5	1·0	0·460
Serum 25-hydroxy vitamin D (nmol/l)	54·3§	20·9	55·6	26·5	0·854
IL-6 (ng/l)					0·701‡
Median	0·93§		1·07		
IQR	0·25, 1·54		0·23, 3·05		

BP, blood pressure; bpm, beats per minute; %E, percentage of energy18 : 2*n*-6, linoleic acid; 18 : 3*n*-3, *α*-linolenic acid; 20 : 5*n*-3, EPA; 22 : 6*n*-3, DHA.
* Use of independent samples *t* test.
† χ^2 test.
‡ Mann–Whitney *U* test.
§ *n* 23 due to sample loss.

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Table 2. Plasma and erythrocyte fatty acid composition in vegan and omnivore participants (*n* 47)
(Mean values and 95 % confidence intervals; mean differences (vegan) and 95 % confidence intervals (omnivore); geometric means and 95 % confidence intervals; medians and interquartile ranges (IQR))

	Omnivore (*n* 24)		Vegan (*n* 23)		Difference between groups		
Plasma and erythrocyte fatty acids (weight %)	Mean	95 % CI	Mean	95 % CI	Mean difference	95 % CI	*P**
Plasma							
16 : 0	20·8	20·4, 21·3	19·3	18·6, 20·0	−1·49	−2·31, −0·67	0·001
16 : 1*n*-7	1·79	1·48, 2·10	1·11	0·91, 1·32	−0·67	−1·04, −0·31	0·001
18 : 0	7·62	7·30, 7·94	7·60	7·15, 8·04	−0·02	−0·55, 0·50	0·931
18 : 1*n*-9	18·5	17·6, 19·3	18·9	17·9, 19·8	0·37	−0·89, 1·62	0·559
18 : 2*n*-6	27·1	26·0, 28·2	33·1	31·9, 34·4	6·06	4·43, 7·68	<0·001
18 : 3*n*-3							0·006
Geometric mean	0·53		0·71		1·34†		
95 % CI	0·48, 0·59		0·59, 0·85		1·09, 1·64		
20 : 3*n*-6	1·42	1·28, 1·55	1·42	1·28, 1·57	0·01	−0·18, 0·20	0·952
20 : 4*n*-6	6·68	6·12, 7·25	6·55	5·94, 7·16	−0·13	−0·94, 0·68	0·745
20 : 5*n*-3							<0·001
Geometric mean	1·03		0·47		0·46†		
95 % CI	0·79, 1·34		0·40, 0·55		0·34, 0·62		
22 : 4*n*-6							0·036
Geometric mean	0·20		0·23		1·14†		
95 % CI	0·19, 0·21		0·21, 0·25		1·01, 1·28		
22 : 5*n*-6	0·26	0·21, 0·30	0·20	0·15, 0·26	−0·05	−0·12, 0·02	0·146
22 : 5*n*-3	0·59	0·53, 0·64	0·51	0·44, 0·59	−0·07	−0·16, 0·02	0·113
22 : 6*n*-3							<0·001
Geometric mean	2·23		0·91		0·41†		
95 % CI	1·94, 2·57		0·80, 1·05		0·34, 0·49		
Erythrocyte							
16 : 0	16·8	15·4, 18·2	17·6	16·7, 18·6	0·81	−0·87, 2·48	0·337
16 : 1*n*-7							0·016‡
Median	0·41		0·31		–		
IQR	0·30, 1·57		0·21, 0·50		–		
18 : 0							0·135
Geometric mean	15·6		16·3		1·05†		
95 % CI	14·9, 16·3		15·6, 17·1		0·99, 1·14		
18 : 1*n*-9	15·7	15·1, 16·3	15·4	14·7, 16·2	−0·23	−1·15, 0·68	0·609
18 : 2*n*-6	11·7	11·0, 12·3	13·3	12·5, 14·1	1·64	0·64, 2·64	0·002
18 : 3*n*-3							0·610
Geometric mean	0·34		0·32		0·92†		
95 % CI	0·26, 0·45		0·27, 0·38		0·67, 1·27		
20 : 3*n*-6							0·042
Geometric mean	1·78		2·02		1·13†		
95 % CI	1·64, 1·94		1·84, 2·22		1·01, 1·28		
20 : 4*n*-6	15·9	14·9, 16·9	15·6	14·4, 16·9	−0·27	−1·82, 1·27	0·725
20 : 5*n*-3	1·26	1·07, 1·45	0·67	0·52, 0·81	−0·59	−0·83, −0·36	<0·001
22 : 4*n*-6	2·75	2·47, 3·03	3·83	3·50, 4·16	1·08	0·66, 1·50	<0·001
22 : 5*n*-6	0·38	0·27, 0·49	0·52	0·40, 0·64	0·14	−0·02, 0·30	0·078
22 : 5*n*-3	2·62	2·36, 2·88	2·15	1·94, 2·36	−0·47	−0·80, −0·15	0·005
22 : 6*n*-3							<0·001
Geometric mean	4·19		2·07		0·49†		
95 % CI	3·63, 4·83		1·85, 2·32		0·41, 0·59		
Omega-3 Index							<0·001
Geometric mean	5·42		2·71		0·50†		
95 % CI	4·73, 6·20		2·40, 3·05		0·42, 0·60		

16 : 0, palmitic acid; 16 : 1*n*-7, palmitoleic acid; 18 : 0, stearic acid; 18 : 1*n*-9, oleic acid; 18 : 2*n*-6, linoleic acid; 18 : 3*n*-3, *α*-linolenic acid; 20 : 3*n*-6, dihomo-*γ*-linolenic acid; 20 : 4*n*-6, arachidonic acid; 20 : 5*n*-3, EPA; 22 : 5*n*-3, docosapentaenoic acid *n*-3; 22 : 6*n*-3, DHA.

* *P* value obtained using independent samples *t* test.

† Exponents of mean differences in Ln values (the ratio of the geometric mean in vegans:that in omnivores, with 95 % CI of the geometric mean ratios).

‡ Use of Mann–Whitney *U* test where data remained not normally distributed following LN transformation. Total plasma fatty acid concentrations were (geometric means with 95 % CI): omnivores (1869 mg/l, 1660, 2104; *n* 24), vegans (1998 mg/l, 1755, 2274; *n* 23); there were no significant differences between groups.

order to standardise the length of recording. Longer segments were not available for all participants and therefore were not included in the analysis. There were no significant differences for any of the parameters between groups:

Nocturnal sleep–time–day–time differences in HR/IBI and beat–to–beat HRV.

Circadian changes are a key determinant of variability in HR over 24 h, measured as 24 h SDNN. Differences in mean nocturnal sleep-time and day-time IBI are a significant factor in the size of the SDNN value. As described above, the sleep-time minus day-time differences in HR/IBI were statistically significant, with the mean decrease in HR/increase in IBI from day-time to sleep-time being distinctly larger in vegans

Table 3. Plasma concentrations of *n*-6 and *n*-3 PUFA-derived lipid mediators in vegan and omnivore participants (*n* 47)
(Mean values and 95 % confidence intervals; geometric means and 95 % confidence intervals; medians and minimum and maximum values)

	Omnivore (*n* 24)		Vegan (*n* 23)		
Compounds (ng/l)	Mean	95 % CI	Mean	95 % CI	*P**
n-6 PUFA derived					
20 : 3*n*-6-derived					
15-HETrE	51·82	36·94, 66·70	53·87	39·78, 67·96	0·533
13,14-dihydro-15-keto PGE_1					0·519
Geometric mean	17·45		13·93		
95 % CI	9·52, 31·99		7·82, 24·78		
13,14-dihydro PGE_1					0·001†
Median	0·000		0·000		
Minimum and maximum values	0·000, 4·332		0·000, 76·928		
18 : 2*n*-6 derived					
9-HODE					<0·001
Geometric mean	2433		5045		
95 % CI	1982, 2988		4067, 6260		
9 OxoODE					<0·001
Geometric mean	477		994		
95 % CI	398, 571		771, 1282		
13-HODE					<0·001
Geometric mean	3320		6536		
95 % CI	2717, 4056		5483, 7791		
13 OxoODE	335	291, 379	537	467, 606	<0·001
12,13-EpOME	389	326, 451	769	622, 917	<0·001
12,13-DiHOME	2820	2159, 3480	5544	4527, 6561	<0·001
9,10-EpOME					<0·001
Geometric mean	258		426		
95 % CI	220, 303		351, 518		
9,10-DiHOME					<0·001
Geometric mean	3199		7400		
95 % CI	2482, 4125		5910, 9267		
Trans-EKODE					<0·001
Geometric mean	300		560		
95 % CI	248, 362		425, 738		
20 : 4*n*-6 derived					
6-keto $PGF_{1\alpha}$					<0·001†
Median	8·20		0·000		
Minimum and maximum values	0·000, 59·14		0·000, 4·49		
13,14-dihydro $PGF_{2\alpha}$					<0·001
Geometric mean	48·4		20·0		
95 % CI	34·57, 67·91		14·82, 26·97		
13,14-dihydro-15-keto $PGF_{2\alpha}$					0·016†
Median	0·000		0·000		
Minimum and maximum values	0·000, 25·56		0·000, 12·79		
13,14-dihydro-15-keto PGE_2					0·459
Geometric mean	3·67		3·07		
95 % CI	2·584, 5·217		2·172, 4·347		
TXB2					0·968
Geometric mean	10·3		10·5		
95 % CI	7·57, 14·14		6·08, 18·05		
5-HETE					0·738
Geometric mean	124		132		
95 % CI	96·4, 160·4		103·2, 168		
8-HETE	70·2	56·77, 83·57	74·5	62·33, 86·60	0·626
9-HETE					0·478†
Median	12·82		0·000		
Minimum and maximum values	0·00, 70·99		0·000, 70·99		
11-HETE	62·3	49·55, 75·13	59·7	49·68, 69·64	0·736
12-HETE					0·580†
Median	146·48		143·6		
Minimum and maximum values	96·76, 1214·43		82·20, 1625·76		
15-HETE	174	145, 203	186	158, 214	0·533
20-HETE					0·896
Geometric mean	284		290		
95 % CI	232, 348		229, 367		
5,6-DHET					0·635
Geometric mean	55·4		52·0		
95 % CI	47·09, 65·19		41·29, 65·37		

Table 3. *Continued*

Compounds (ng/l)	Omnivore (*n* 24) Mean	95 % CI	Vegan (*n* 23) Mean	95 % CI	*P**
8,9-DHET					0·238
Geometric mean	71·6		82·1		
95 % CI	62·75, 81·81		67·40, 99·95		
11,12-DHET	204	173, 235	233	207, 260	0·147
14,15-DHET	260	231, 290	312	272, 353	0·036
n-3 PUFA derived					
18 : 3*n*-3 derived					
9-HOTrE					0·007
Geometric mean	139		206		
95 % CI	111, 173		170, 250		
13-HOTrE					0·005
Geometric mean	150		245		
95 % CI	114, 198		201, 299		
20 : 5*n*-3 derived					
5-HEPE					<0·001†
Median	75·7		21·4		
Minimum and maximum values	0·000, 462·2		0·000, 107·5		
8-HEPE					<0·001†
Median	15·2		0·000		
Minimum and maximum values	0·000, 219·0		0·000, 21·0		
18-HEPE					<0·001†
Median	74·8		0·000		
Minimum and maximum values	0·000, 624·8		0·000, 150·2		
22 : 6*n*-3-derived					
4-HDHA					<0·001†
Median	75·800		45·734		
Minimum and maximum values	38·181, 413·405		0·000, 102·331		
7-HDHA					0·581†
Median	0·000		0·000		
Minimum and maximum values	0·000, 202·374		0·000, 125·813		
10-HDHA					<0·001†
Median	20·529		0·000		
Minimum and maximum values	0·000, 197·107		0·000, 14·974		
11-HDHA					0·022†
Median	0·000		0·000		
Minimum and maximum values	0·000, 191·074		0·000, 0·000		
13-HDHA					<0·001†
Median	19·255		0·000		
Minimum and maximum values	0·000, 87·861		0·000, 0·000		
14-HDHA					<0·001†
Median	41·255		0·000		
Minimum and maximum values	0·000, 299·686		0·000, 57·819		
17-HDHA					0·001†
Median	0·000		0·000		
Minimum and maximum values	0·000, 364·505		0·000, 0·000		
20-HDHA					<0·001†
Median	107·538		25·446		
Minimum and maximum values	44·088, 748·889		0·000, 105·464		
19,20-DiHDPA					0·040
Geometric mean	1448		1098		
95 % CI	1240, 1690		878, 1374		

20 : 3*n*-6, dihomo-γ-linolenic acid; HETrE, hydroxyeicosatrienoic acid; 18 : 2*n*-6, linoleic acid; HODE, hydroxyoctadecadienoic acid; OxoODE, oxooctadecadienoic acid; EpOME, epoxyoctadecenoic acid; DiHOME, dihydroxyoctadecenoic acid; EKODE, epoxyketooctadecenoic acid; 20 : 4*n*-6, arachidonic acid; TX, thromboxane; HETE, hydroxyeicosatetraenoic acid; DHET, dihydroeicosatetraenoic acid; 18 : 3*n*-3, α-linolenic acid; HOTrE, hydroxyoctadecatrienoic acid; 20 : 5*n*-3, EPA; HEPE, hydroxyeicosapentaenoic acid; 22 : 6*n*-3, DHA; HDHA, hydroxydocosahexaenoic acid; DiHDPA, dihydroxydocosapentaenoic acid.

* *P* value obtained using independent samples *t* test.

† Use of Mann–Whitney *U* test where data remained not normally distributed following LN transformation.

compared with omnivores (Table 4). The lack of difference in sleep-time HR between groups, together with observations of shorter IBI (and non-statistically significant faster HR) during the day in vegans, suggests that the larger night-day difference in vegans is a result of greater circadian fluctuations in sympathetic–parasympathetic balance. Sleep-time minus day-time HRV also reflects the degree of circadian modulation of autonomic regulation of HR in both vegans and omnivores. The larger increases in beat-to-beat HRV parameters (RMSSD, HF, PNN50) during nocturnal sleep may indicate a greater suppression of parasympathetic regulation during day-time waking hours in vegans when considered alongside

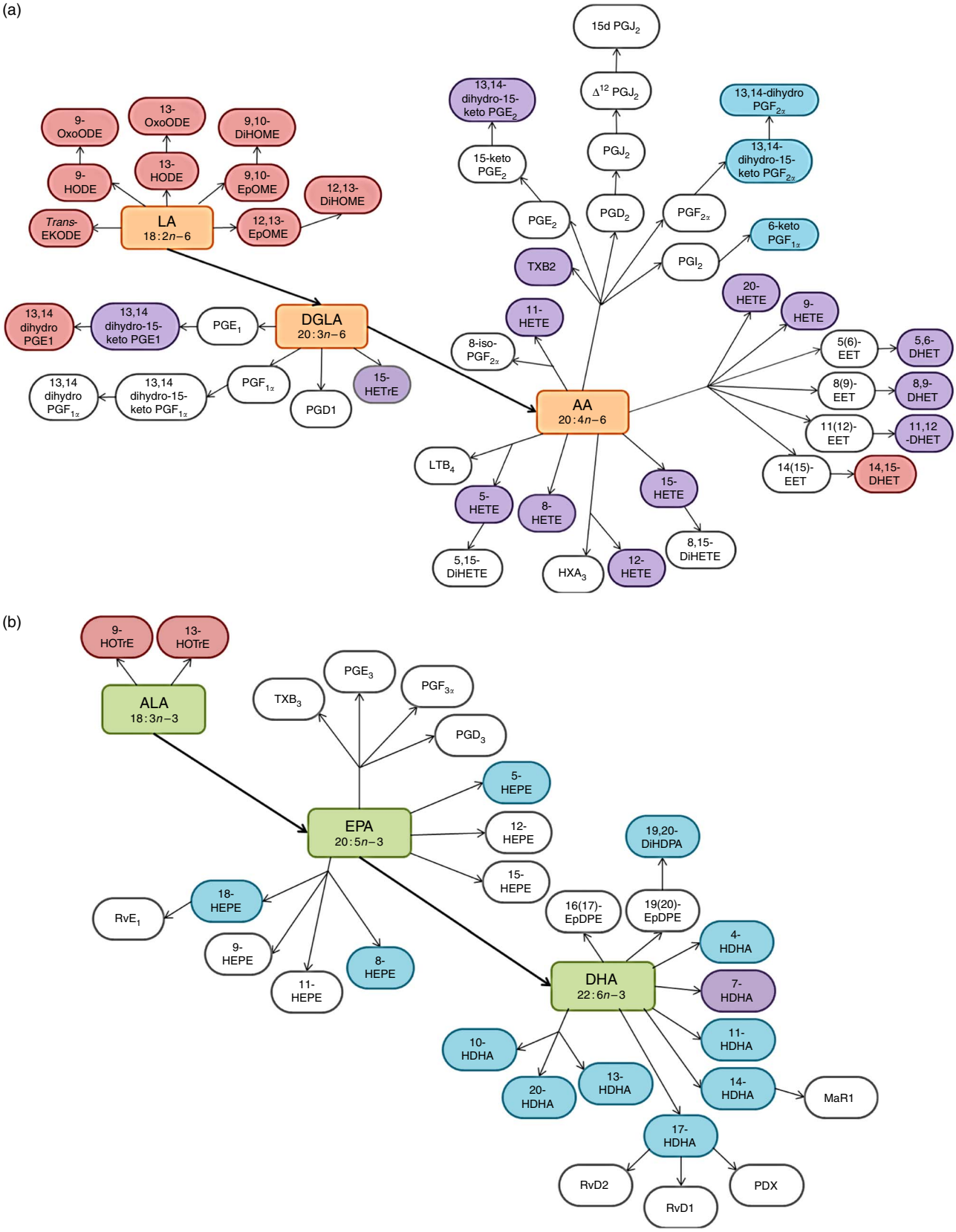

Fig. 2. *(For caption see following page)*

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the shorter mean day-time IBI in this group compared with omnivores.

Discussion

Low HRV is associated with mortality after a myocardial infarction[21,40,41] and risk of cardiac events in the general population[23]. Associations between increased *n*-3 PUFA consumption and higher HRV[42–45], and lower HR[16], suggests that populations with very low *n*-3 PUFA tissue status might be at greater risk of arrhythmic events or sudden cardiac death. Vegetarians/vegans in the Adventist Health Study[46], European Prospective Investigation into Cancer and Nutrition (EPIC)-Oxford cohort[47] and five combined cohorts[48] have been reported to have lower risk of CHD than non-vegetarians. However, a recent study of two combined population cohorts (EPIC-Oxford and the earlier Oxford Vegetarian Study cohort) reported similar rates of all-cause mortality and no clear differences between vegans and comparable regular meat-eaters, fish-eaters and vegetarians in mortality from CHD up to the age of 90 years[49], despite the fact that vegan populations have lower CHD risk factors such as blood pressure[14,15], plasma lipids[12,13] and lower BMI [10,11] compared with populations that eat foods of animal origin. Although the latter findings do not preclude a lower risk of premature CHD in vegans, the notion that cardiovascular health of elderly vegans might be further optimised by increased intakes of dietary LC *n*-3 PUFA remains a possibility.

We hypothesised that a population with low tissue LC *n*-3 PUFA status would have higher HR and lower HRV, and vegans were chosen as a clearly defined group that could be considered as a model to test this hypothesis. As expected, we observed marked differences between vegans and omnivores in their tissue *n*-3 PUFA status, as represented by erythrocyte lipid fatty acid composition (an indicator of longer-term PUFA intake due to the 4-month lifespan of an average erythrocyte[50]). These findings were supported by differences in the plasma fatty acid composition and self-reported dietary LC *n*-3 PUFA intakes. The average erythrocyte Omega-3 Index in the omnivore group was lower than indices reported previously for a meat- and fish-eating UK population[51–53], but differences between the groups studied here were clear-cut. Inverse relationships were observed between erythrocyte 18 : 2*n*-6:18 : 3*n*-3 ratios and erythrocyte 20 : 5*n*-3 in the vegan group. This supports existing evidence that higher dietary intakes of 18 : 2*n*-6, an *n*-6 PUFA which is abundant in omnivore diets but even more so in vegan/vegetarian diets[5], may inhibit conversion of 18 : 3*n*-3 to LC *n*-3 PUFA[54].

The observed group differences in HR and HRV were more complex than hypothesised, mainly due to divergence in night/day differences. Differences in all primary outcome variables – HR/IBI, SDNN (overall HRV), and RMSSD (beat-to-beat HRV) – were observed between groups but the nature of the difference depended on whether analysis was carried out over the full 24 h or only during day-time waking hours. In line with the hypothesis, mean day-time HR was higher/IBI shorter and overall (SDNN) and beat-to-beat HRV (RMSSD, PNN50 %, HF) was lower in vegans, even following adjustment for physical activity during the same 8 h period. These observations might indicate that low *n*-3 status could lead to either a predominance of sympathetic regulation, a greater withdrawal of parasympathetic activity, or possibly, due to depletion of LC *n*-3 PUFA in cardiomyocyte membranes, there is a greater stimulation of pacemaker activity despite a normal level of sympathetic neural transmission during waking hours. However, it is also possible that the differences in HRV observed in vegans and omnivores are unrelated to LC *n*-3 PUFA tissue status; this would require investigation with a dietary intervention trial. A recent review on *n*-3 fatty acids and effects on HR and HRV has argued that, according to evidence from animal models, it is more likely that 22 : 6*n*-3 is acting to reduce HR via modulation of pacemaker activity rather than changes in cardiac autonomic neural regulation[18], although the role of the 22 : 6*n*-3-derived SPM, neuroprotectin D1 (PD1), in protecting the nervous system from inflammation-related injury shows that 22 : 6*n*-3-dependent physiological mechanisms exist in synapses and neural circuits in order to sustain neuronal function[55,56]. The stable precursor to PD1 and RvD1, 17-HDHA , was not detected in the fasting plasma of any vegan subjects, whereas nine out of twenty-four omnivores had detectable concentrations. There were also marked differences in concentrations of LC *n*-3 PUFA-derived precursor markers to RvE1 (from 18-HEPE) and MaR1 (from 14-HDHA). Venous blood plasma concentrations of lipid mediators in whole fasting plasma are likely to be an insensitive marker of capacity for autacoid release and activity in specific sites of inflamed tissue. Nevertheless, higher circulating plasma concentrations of SPM precursor markers may indicate ease of bioavailability for conversion to SPM at times of need, which presents clear functional implications for populations with low tissue 20 : 5*n*-3 and 22 : 6*n*-3 stores.

Although the vegan group were not deficient in other nutrients that are related to HRV, such as vitamin D[57] and vitamin B_{12}[58], the nature of the study design means that we cannot exclude the influence of other dietary or lifestyle factors associated with the vegan lifestyle. The vegans reported almost half the intake of SFA (%E) as the omnivores, in agreement with results reported in larger vegan populations[59], and correspondingly lower amounts of 16 : 0 as a proportion of total plasma fatty acids and lower serum concentrations of

Fig. 2. *(For figure see previous page)* (a) Schematic outline of oxygenated species derivatives of *n*-6 PUFA, linoleic acid (LA; 18 : 2*n*-6), dihomo-γ-linolenic acid (DGLA; 20 : 3*n*-6) and arachidonic acid (AA; 20 : 4*n*-6), analysed in blood plasma of vegans and omnivores. 20: 3*n*-6 (DGLA)-, 18: 2*n*-6 (LA)- and 20: 4*n*-6 (AA)-derived lipid mediators assayed in study participants' plasma. ■, Higher in vegans, ■, higher in omnivores, ■, no difference, □, not detected or below the limit of detection. OxoODE, oxooctadecadienoic acid; HODE, hydroxyoctadecadienoic acid; EpOME, epoxyoctadecenoic acid; DiHOME, dihydroxyoctadecenoic acid; EKODE, epoxyketooctadecenoic acid; TX, thromboxane; HETE, hydroxyeicosatetraenoic acid; EET, epoxyeicosatetraenoic acid; DHET, dihydroeicosatetraenoic acid; HETrE, hydroxyeicosatrienoic acid; DiHETE,dihydroxyeicosatetraenoic acid; LT, leukotriene; HX, hepoxilin. (b) Schematic outline of oxygenated species derivatives of *n*-3 PUFA, α-linolenic acid (ALA; 18 : 3*n*-3), EPA (20 : 5*n*-3) and DHA (22 : 6*n*-3), analysed in blood plasma of vegans and omnivores. 18 : 3*n*-3 (ALA)-, 20 : 5*n*-3 (EPA)- and 22 : 6*n*-3 (DHA)-derived lipid mediators assayed in study participants' plasma. ■, Higher in vegans, ■, higher in omnivores, ■, no difference, □, not detected or below the limit of detection. HOTrE, hydroxyoctadecatrienoic acid; TX, thromboxane; HEPE, hydroxyeicosapentaenoic acid; DiHDPA,dihydroxydocosapentaenoic acid; Rv, resolvin; EpDPE, epoxydocosapentaenoic acid; HDHA, hydroxydocosahexaenoic acid; MaR, maresin; PD, protectin D.

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Table 4. Physical activity, heart rate and heart rate variability parameters of vegan and omnivore participants over 24 h, day-time and sleep-time, with sleep – day differences (*n* 47)*
(Estimated marginal means and 95 % confidence intervals; geometric means and 95 % confidence intervals; medians and interquartile ranges (IQR))

	Omnivore (*n* 24)		Vegan (*n* 22†)		
	Mean	95 % CI	Mean	95 % CI	*P*‡
24-h measurement					
Activity, IBI and HR					
Physical activity (cpm)§					0·181
Geometric mean	236		285		
95 % CI	196, 284		233, 348		
IBI (ms)§	845	798, 892	811	760, 861	0·336
HR (bpm)§	75	71, 79	78	74, 83	0·210
Time-domain HRV parameters					
TI	38	33, 42	41	36, 46	0·294
SDNN (ms)§	145	129, 162	172	154, 189	0·039
SDANN (ms)	128	114, 143	155	139, 170	0·018
RMSSD (ms)					0·905
Geometric mean	35		35		
95 % CI	31, 40		30, 40		
PNN50 (%)					0·299
Geometric mean	8·9		7·0		
95 % CI	6·5, 12·2		5·0, 9·7		
Frequency-domain HRV parameters					
LF (ms^2)					0·130
Geometric mean	971		761		
95 % CI	786, 1198		608, 953		
HF (ms^2)					0·567
Geometric mean	350		309		
95 % CI	262, 467		227, 421		
VLF (ms^2)					0·022
Geometric mean	12 619		17 980		
95 % CI	10 351, 15 379		14 547, 22 204		
Non-linear methods					
SD1:SD2 (Poincaré ratio)	0·13	0·11, 0·14	0·11	0·10, 0·12	0·051
Day-time (8 h) measurement					
Activity, IBI and HR					
Physical activity (cpm)					0·935
Geometric mean	437		443		
95 % CI	347, 552		345, 570		
IBI (ms)	787	745, 830	721	675, 766	0·039
HR (bpm)	80	76, 84	86	81, 91	0·062
Time-domain HRV parameters					
TI	31	28, 34	27	24, 31	0·135
SDNN (ms)	121	109, 132	101	89, 113	0·021
SDANN (ms)	100	89, 111	84	73, 96	0·056
RMSSD (ms)	34	30, 38	25	20, 30	0·009
PNN50 (%)					0·001
Geometric mean	6·6		2·7		
95 % CI	4·6, 9·4		1·8, 3·9		
Frequency-domain HRV parameters					
LF (ms^2)	908	785, 1032	628	495, 761	0·004
HF (ms^2)					0·002
Geometric mean	260		135		
95 % CI	198, 342		100, 181		
VLF (ms^2)					0·078
Geometric mean	8325		6470		
95 % CI	6894, 10 052		5277, 7929		
Non-linear methods					
SD1:SD2 (Poincaré ratio)	0·15	0·13, 0·16	0·13	0·11, 0·15	0·125
Sleep-time (2 h) measurement					
IBI and HR					
IBI (ms)	965	903, 1026	991	925, 1056	0·568
HR (bpm)	64	61, 68	62	59, 66	0·475
Time-domain HRV parameters					
TI	19	16, 22	18	16, 21	0·762
SDNN (ms)	78	68, 88	85	75, 96	0·324
SDANN (ms)					0·717
Geometric mean	42		44		
95 % CI	36, 51		37, 54		

Table 4. *Continued*

	Omnivore (*n* 24)		Vegan (*n* 22†)		
	Mean	95 % CI	Mean	95 % CI	*P*‡
RMSSD (ms)					0·214
Geometric mean	38		44		
95 % CI	32, 45		37, 53		
PNN50 (%)					0·826
Geometric mean	10·9		11·7		
95 % CI	7·0, 16·9		7·3, 18·7		
Frequency-domain HRV parameters					
LF (ms^2)					0·925
Geometric mean	882		902		
95 % CI	646, 1205		647, 1256		
HF (ms^2)					0·601
Geometric mean	403		464		
95 % CI	280, 580		315, 684		
VLF (ms^2)					0·304
Geometric mean	2881		3519		
95 % CI	2215, 3744		2661, 3744		
Non-linear methods					
SD1:SD2 (Poincaré ratio)					0·740
Geometric mean	0·27		0·28		
95 % CI	0·23, 0·32		0·24, 0·33		
Sleep-time – day-time differences					
IBI and HR					
IBI (ms)	183	139, 227	270	223, 317	0·012
HR (bpm)	−16	−19, −13	−24	−28, −20	0·003
Time-domain beat-to-beat HRV parameters					
RMSSD (ms)	8	1, 14	22	15, 29	0·006
PNN50 (%)	6·4	0·9, 11·8	14·2	8·5, 20·1	0·058
Frequency-domain beat-to-beat HRV parameters					
HF (ms^2)					0·095¶
Median	127		234		
IQR	−12, 417		84, 988		

IBI, interbeat interval (also known as RR interval), the time interval between R spikes of the QRS complex of the electrocardiogram; HR, heart rate; Cpm, counts per minute; bpm, beats per minute; HRV, heart rate variability; NN, normal-to-normal; TI, triangular index (total number of all NN intervals divided by the height of the histogram of all NN intervals); SDNN, standard deviation of all NN intervals (NN intervals, similar to R-R, but on normalised IBI data); SDANN, standard deviation of the averaged NN intervals, calculated from 5 min epochs; RMSSD, the square root of the mean of the sum of squares of differences between adjacent NN intervals; PNN50, percentage of adjacent NN intervals that differed by >50 %; LF, low-frequency power; HF, high-frequency power; VLF, very-low-frequency power; SD1:SD2, the ratio of the SD of beat-to-beat IBI variability (SD1) against the SD of long-term IBI variability (SD2).

* Adjusted for sex, age, BMI and 24 h activity for 24 h HRV and sleep-time – day-time HRV, or 8 h activity for 8 h day-time, and adjusted for sex, age and BMI only for sleep-time. Sleep-time – day-time represents HR/IBI and beat-to-beat HRV during a standardised 2 h nocturnal sleep period minus a standardized 8 h day-time period, to indicate the difference between night and day.

† Missing data from one subject due to unusable day-time HRV recording.

‡ *P* value obtained using ANCOVA for normally distributed raw or LN transformed data (adjusted for sex, age, BMI and activity for 24 h, day-time and sleep-time – day-time differences, and adjusted for sex, age and BMI only for sleep-time), except for sleep-time – day-time differences in HF.

§ Only recordings >18 h included for 24 h physical activity, SDNN, IBI and HR data analysis, *n* 21 for omnivores and *n* 19 for vegans.

¶ Use of an unadjusted non-parametric test, the Mann–Whitney *U* test, where data remained not normally distributed following LN transformation.

LDL-cholesterol. However, these differences are less likely to exert a major influence on cardiac electrophysiology. Animal studies have shown that PUFA feeding decreased vulnerability to arrhythmia compared with high SFA feeding without any reduction in the proportion of membrane SFA, and high-MUFA feeding did not reduce arrhythmia compared with high-SFA diets[60]. This suggests that SFA membrane composition is not a major determinant of vulnerability to arrhythmias and addition of LC *n*-3 PUFA (replacing mainly 18 : 1 and *n*-6 PUFA) might be the most important determinant. In fact our small cross-sectional study showed that erythrocyte SFA proportions were not different between groups and that vegans had lower day-time HRV, and therefore potentially a greater risk of arrhythmia if there was also coronary atherosclerosis present, despite lower SFA intake.

There may be other explanations for higher HR and reduced HRV in vegans that are not related to *n*-3 PUFA status and were not measured as part of this study, for example, susceptibility to psychological stress (although reduced self-reported stress and anxiety has been observed in 109 vegans compared with 228 omnivores[61]), and job-related activities, and possibly frequency/duration of using a bicycle (which would not have been detected by accelerometry). The effects of physical activity on HRV depend on the type and intensity of activity involved, but higher parasympathetically regulated HRV parameters are associated with greater levels of habitual physical activity[62]. As parasympathetically regulated HRV parameters were lower in vegans during the day-time, then it suggests that either habitual physical activity levels were lower in vegans or some other factor associated with vegan diet and lifestyle, such as the depletion in tissue 20 : 5*n*-3 + 22 : 6*n*-3 content, counteracted the effect of habitual physical activity levels.

No differences were observed between groups during a standardised 2 h sleep period. Previous research from our

group showed increased longer-phase HRV (SDANN and VLF) in a middle-aged population at moderate risk of CVD during nocturnal sleep following 12 months fish oil supplementation at doses of 0·45–1·8 g/d LC *n*-3 PUFA compared with a refined olive oil placebo[31]. Consistent with this, fish consumption was positively related to VLF in a large cohort of older adults[25]. Low VLF is associated with increased risk of mortality post-myocardial infarction, particularly arrhythmic death[40]. Since SDANN and VLF represent slowly changing periodic variability in HR in response to thermoregulatory and hormonal shifts that may particularly occur during sleep, then it is likely that the 2 h standardised period in the current study was too short to detect longer-phase differences in HRV between vegans and omnivores during sleep.

Contrasting observations were made for longer-phase components of 24 h HRV, which represent changes in HR over sustained periods in response to periodic fluctuations in neurohormonal and circadian physiology rather than beat-to-beat variability. These components of HRV (SDNN, SDANN and VLF) were higher, and Poincaré ratio was lower, over 24 h in vegans compared with omnivores; this may represent more pronounced neurohormonal rhythms in vegans, or they may just reflect the higher HR and reduced HRV experienced by vegans during waking hours relative to sleep-time due to reasons discussed above.

Sub-clinical markers of inflammation have been linked to risk of cardiovascular events, vascular inflammation being the key, self-amplifying component of atherogenesis[63–65]. Resolution of acute inflammatory responses is a critical, programmed factor in tissue repair and prevention of further pathological changes to tissues. SPM derived from LC *n*-3 PUFA take over from the initiating lipid mediators, prostaglandins and leukotrienes, during the neutrophil-monocyte sequence, and play a functional role in ending acute inflammatory events by inhibition of neutrophil influx to the site of trauma, counter-regulating pro-inflammatory cytokines, and stimulating resolving macrophages to clear the products of the inflammatory response, thereby allowing the injured area to heal[66]. In theory, low tissue availability of 20 : 5*n*-3, 22 : 5*n*-3 and 22 : 6*n*-3 could compromise resolution of acute inflammatory events increasing risk of chronic inflammation, although this is purely speculative at present. Increased circulating concentrations of RvE1 and precursor markers of resolvins (18-HEPE, 17-HDHA), and maresins (14-HDHA) have been demonstrated following *n*-3 PUFA supplementation[67], the same precursor markers that were found to be different in our comparison of vegans and omnivores. Our data show that a population with no dietary intake of marine *n*-3 PUFA have much lower or zero fasting plasma concentrations of these SPM precursor markers. It is not known whether individuals with low *n*-3 status have increased rates of 20 : 5*n*-3-/22 : 6*n*-3-derived mediator turnover as an adaptive mechanism to avoid compromising SPM availability. If this were the case, then it would be expected that having low pools of SPM precursors would have no functional consequences in vegans. Future research in this area should address whether populations with low-*n*-3 status are more at risk of having a pro-inflammatory profile.

Vegans had greater concentrations of 18 : 3*n*-3- and 18 : 2*n*-6-derived lipid mediators that have a variety of deleterious and cytoprotective effects[68,69]. In the case of 18 : 2*n*-6, this is likely to be due to higher dietary intakes, as supported by FFQ estimates, proportions of total plasma fatty acids, and incorporation into erythrocyte membrane lipids[68,70]. Although plasma 18 : 3*n*-3 proportions of total fatty acids were higher in vegans, reported dietary intakes were not different; however, FFQ estimates of intakes are likely to underestimate true intakes due to incomplete food composition data. Vegans also had lower concentrations of markers of AA-derived prostanoid production (6-keto $PGF_{1\alpha}$ – a marker of PGI_2 synthesis, and 13,14-dihydro $PGF_{2\alpha}$,/13,14-dihydro-15-keto $PGF_{2\alpha}$ – markers of $PGF_{2\alpha}$ production). There were no differences between groups for a range of AA-derived LOX-catalysed mediators (HETE), suggesting that the lipid mediator profile of vegans may not necessarily be entirely pro-inflammatory relative to omnivores. Few of these lipid mediators have been fully characterised regarding their functional effects, and evidence in animal and cell models to date suggests that 18 : 2*n*-6- and 20 : 4*n*-6-derived lipid mediators comprise a complex array of diverse bioactive molecules that induce a range of physiological effects in various tissues[71–73].

Previous work has also demonstrated that circulating pro-inflammatory cytokines may be reduced by fish oil supplementation, as reviewed by Calder[74,75]. We included a measure of low grade inflammation, IL-6, in our comparison between vegans and omnivores, but found no differences between groups. However, this does not necessarily indicate that there are no differences between groups in their capacity to inhibit or resolve acute inflammatory events since circulating cytokine concentrations have limited utility as biomarkers of inflammation that may be occurring in localised areas of tissue. Previous studies have shown that serum IL-6 concentrations were inversely correlated with HRV in men with renal disease[76], the metabolic syndrome[77] and young healthy subjects[78], although not not all studies agree[79]. Down-regulation of inflammatory cytokine gene expression plus increased production of pro-resolving lipid mediators are two potential mechanisms whereby cardiac function might possibly be preserved by increased 20 : 5*n*-3 and 22 : 6*n*-3 intakes, by reducing inflammatory tissue damage in the brain and autonomic nerves, and also in the heart tissue itself.

Limitations of the present study

The cross-sectional design limits our findings to being exploratory in nature and the associations between low *n*-3 status and reduced HRV require confirmation by a randomised controlled trial of 20 : 5*n*-3 + 22 : 6*n*-3 supplementation in a population with an Omega-3 Index of <3 %. The sample population size is small and although statistical power calculations were conducted for the primary HRV outcomes, the study may be underpowered to detect group differences in other more variable outcomes such as beat-to-beat HR. Multiple statistical testing was carried out to explore group differences in short and long term, and time and frequency-domain HRV, increasing the risk of generating false-positive results. There is no agreed upon method for correcting statistical analyses that involve the full set of HRV measures, but the data set represents

groupings of related outcomes rather than a large collection of disparate variables. The data presented here are consistent when comparing variables that represent similar physiological phenomenon. For example, there are two time-domain (RMSSD, pNN50) and one frequency-domain (HF) parameters of beat-to-beat variability. These are all vagally regulated and all show consistently that day-time beat-to-beat HRV is lower in vegans compared with omnivores. Therefore, although type I errors cannot be ruled out with complete certainty, it is reassuring that statistically significant differences between groups are supported by analogous parameters. The stated aim was to match groups for age, sex and BMI, but matching for sex was not wholly achieved. Any influence of this imbalance in sex distribution on HRV results was minimised by adjusting for age, sex and BMI, in addition to activity levels for 24 h and day-time HRV, in the statistical model. Technical problems in obtaining good quality sleep-time HRV data limited the standardised duration of nocturnal HRV to 2 h which may have led to effects on longer phase HRV parameters being missed. However, the fact that HRV was lower and mean IBI was shorter in vegans only during the day, and not over the whole 24 h period, suggests that there may be a diet-mental stress interaction during waking hours that resulted in a greater degree of sympathetic nervous system activity relative to parasympathetic activity. Future studies could investigate this further by measuring HRV responses under controlled mental stress conditions in populations with very low Omega-3 Indices compared with populations with optimum Omega-3 Indices.

Summary

The differences observed in parameters of cardiac electrophysiology and circulating lipid mediator concentrations between vegans and omnivores may contribute to the sum effect of diet and lifestyle on CVD risk. The lower availability of LC *n*-3 PUFA-derived lipid mediators in vegans may influence anti-inflammatory capacity, although other differences in LA- and ALA-derived mediators feed into an array of disparate inflammatory pathways and the sum effect is difficult to predict. Crucially, this study presents novel information on associations between free-living, unsupplemented dietary PUFA intakes with lipid mediator profiles in humans.

Acknowledgements

The authors thank Anne-Catherine Perz, MaryJo Searle and Catherine Kidd (King's College London) for assistance in blood sample processing.

This research was supported by King's College London and received no specific grant from any funding agency, commercial or not-for-profit sectors.

W. L. H., T. A. B. S. and A. M. P. conceived the research question and devised the study. A. M. P. conducted the study and analysed the data, with the assistance of H. A.-K. A. N., A. C. K. and R. G. provided lipidomic analytical expertise. All authors contributed to writing and editing the manuscript.

The authors declare that there are no conflicts of interest.

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