Effects of a Vegetarian Diet on Cardiometabolic Risk Factors, Gut Microbiota, and Plasma Metabolome in Subjects With Ischemic Heart

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ORIGINAL RESEARCH

Effects of a Vegetarian Diet on Cardiometabolic Risk Factors, Gut Microbiota, and Plasma Metabolome in Subjects With Ischemic Heart Disease: A Randomized, Crossover Study

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BACKGROUND: A vegetarian diet (VD) may reduce future cardiovascular risk in patients with ischemic heart disease.

METHODS AND RESULTS: A randomized crossover study was conducted in subjects with ischemic heart disease, assigned to 4-week intervention periods of isocaloric VD and meat diet (MD) with individually designed diet plans, separated by a 4-week washout period. The primary outcome was difference in oxidized low-density lipoprotein cholesterol (LDL-C) between diets. Secondary outcomes were differences in cardiometabolic risk factors, quality of life, gut microbiota, fecal short-chain and branched-chain fatty acids, and plasma metabolome. Of 150 eligible patients, 31 (21%) agreed to participate, and 27 (87%) participants completed the study. Mean oxidized LDL-C (–2.73 U/L), total cholesterol (–5.03 mg/dL), LDL-C (–3.87 mg/dL), and body weight (–0.67 kg) were significantly lower with the VD than with the MD. Differences between VD and MD were observed in the relative abundance of several microbe genera within the families Ruminococcaceae, Lachnospiraceae, and Akkermansiaceae. Plasma metabolites, including L-carnitine, acylcarnitine metabolites, and phospholipids, differed in subjects consuming VD and MD. The effect on oxidized LDL-C in response to the VD was associated with a baseline gut microbiota composition dominated by several genera of Ruminococcaceae.

CONCLUSIONS: The VD in conjunction with optimal medical therapy reduced levels of oxidized LDL-C, improved cardiometabolic risk factors, and altered the relative abundance of gut microbes and plasma metabolites in patients with ischemic heart disease. Our results suggest that composition of the gut microbiota at baseline may be related to the reduction of oxidized LDL-C observed with the VD.

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Key Words: coronary artery disease ■ gut microbiota ■ plasma metabolome ■ randomized controlled trial ■ trimethylamine N-oxide ■ vegetarian diet

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CLINICAL PERSPECTIVE

What Is New?

- Compared with a ready-made meat diet, an isocaloric ready-made vegetarian diet (VD) within an individually adapted diet plan showed secondary prevention potential in patients with ischemic heart disease receiving optimal medical treatment.
- After a 4-week intervention, subjects consuming a VD showed significantly lower oxidized low-density lipoprotein cholesterol, low-density lipoprotein cholesterol, total cholesterol, and body mass index than those on a meat diet.
- Subjects on the VD exhibited reduced relative abundance of fecal microbial taxa and plasma metabolites associated with metabolic disease, including cardiovascular disease, and with increased taxa and metabolites associated with lower cardiometabolic risk than those on a meat diet.

What Are the Clinical Implications?

- A VD in conjunction with optimal medical therapy improves levels of oxidized low-density lipoprotein cholesterol, cardiometabolic risk factors, and phospholipids associated with an elevated risk of coronary events.
- A ready-made VD could be easily implemented in individuals with a history of ischemic heart disease to improve secondary prevention.
- Assessment of gut microbiota in follow-up of patients with ischemic heart disease could help to identify individuals potentially showing a favorable response to a VD.

Nonstandard Abbreviations and Acronyms

APOB	apolipoprotein B
BCFA	branched-chain fatty acid
BMI	body mass index
CVD	cardiovascular disease
HbA1c	hemoglobin A1c
hs-CRP	high-sensitivity C-reactive protein
IHD	ischemic heart disease
LDL-C	low-density lipoprotein cholesterol
MD	meat diet
PCI	percutaneous coronary intervention
SCFA	short-chain fatty acid
TC	total cholesterol
TMAO	trimethylamine N-oxide
VD	vegetarian diet

A western diet, characterized by high consumption of red and processed meat, refined carbohydrates, and high calorie intake, has been associated with increased risk of cardiovascular disease (CVD), including ischemic heart disease (IHD).[1] A global change to an environmentally sustainable healthy diet, with considerable reduction of red meat consumption and increased consumption of plant-based foods, may save ≈11 million premature deaths each year.[1]

Epidemiological studies have shown that a vegetarian diet (VD), primarily based on vegetables, legumes, fruit, grains, nuts, and occasionally eggs or dairy products, is associated with reduced incidence of, and mortality in, IHD as well as all-cause mortality.[2,3] Evidence from some randomized controlled trials supports the effectiveness of a plant-based diet in the prevention of CVD[4] and reduction in CVD risk factors.[5-7] A VD as part of an intensive lifestyle change has been shown to reverse coronary atherosclerosis in patients with IHD.[8] Although mechanisms remain unclear, the effect of a VD in counteracting development of CVD might be attributed to reduced oxidative stress[9,10] and to beneficial effects on factors such as blood lipids, glucose tolerance, and body weight.[4,10,11] Most studies investigating the role of a VD in CVD prevention have comprised healthy participants and not consisted of a homogeneous group of patients on optimal medical therapy (eg, lipid- or blood pressure–lowering medication). The main barriers to adopting a VD have been reported to be enjoyment of eating meat and an unwillingness to alter eating habits.[12]

Analysis of gut microbiota and the plasma metabolome before and after adoption of a VD offers the potential to gain mechanistic insight into nutritional influences on disease-related metabolic processes.[13,14] Research has shown impact of a VD on microbial taxa linked to CVD risk,[14] and plant-based diets have been demonstrated to alter circulating metabolites, such as short-chain fatty acids (SCFAs) produced by gut fermentation of dietary fiber and phosphatidylcholines in multiple biological pathways[15-17] linked to CVD risk.[18,19] Carnitine, produced by ingestion of animal products, and its gut microbiota-derived metabolite, trimethylamine N-oxide (TMAO), have been associated with CVD.[20,21] A recent study reported increased risk of coronary heart disease with higher TMAO concentrations. Regular consumption of plant-based foods could hypothetically lower such risk.[22] Individuals may respond differently to a given diet, and prediction models are being developed to determine the importance of anthropometrics, metabolomics, and microbiota to the outcomes of dietary intervention and to the design and implementation of personalized nutrition regimens.[23,24] Individual variation may contribute to inconsistency in results of dietary intervention studies.[25,26] Recent reports have suggested that responses to dietary

intervention might depend on the gut microbiota composition at baseline,[23,24,27] as well as on metabotype.[28] However, little is known of whether individual baseline microbiota and/or metabolome are associated with the effect of a VD on metabolic CVD risk factors.

We conducted a 4-week randomized crossover study, using subject-specific dietary plans, to investigate effects of a VD on CVD risk factors in subjects with a history of IHD treated by percutaneous coronary intervention (PCI), compared with an isocaloric meat diet (MD). We aimed to determine the effect on oxidized low-density lipoprotein cholesterol (LDL-C) as the primary outcome and the secondary outcomes selected cardiometabolic risk factors, gut microbiota, and plasma metabolome, including TMAO, choline, L-carnitine, and acetyl-carnitine. We also explored whether gut microbiota or plasma metabolome at baseline could predict the level of response to a VD.

METHODS

The data that support the findings of this study are available from the corresponding author on reasonable request.

Study Participants

Patients with IHD who were treated with PCI and receiving optimal medical therapy were recruited from the outpatient clinic at the Department of Cardiology, Örebro University Hospital, Örebro, Sweden. Participant eligibility criteria were age >18 years, stable IHD, PCI conducted >1 month before study initiation, and optimal medical therapy, including aspirin and cholesterol-lowering drugs. Exclusion criteria included age <18 years, unstable coronary disease, PCI treatment during the preceding 30 days, inability to provide informed consent, already following a VD or vegan diet, vitamin B deficiency, known food allergy, previous bariatric surgery, or life expectancy <1 year.

All participants provided written informed consent. The study was performed in compliance with the Declaration of Helsinki, and the regional ethical review board in Uppsala, Sweden, approved the study (Dnr 2016/456). The study is registered at ClinicalTrials.gov (NCT02942628).

Study Design

This was a prospective, open-label, randomized, controlled crossover clinical trial. Subjects consumed isocaloric interventional diets, VD and MD, during 4-week intervention periods separated by a 4-week washout period (Figure 1). The study was performed from September 2017 through June 2018. Subjects were randomly allocated to a preselected intervention sequence, VD-washout-MD or MD-washout-VD, at a 1:1 ratio to ensure balance of sequences. Clinical follow-up was performed on 4 occasions during the study, before and after each intervention period. Follow-up visits were scheduled between 7 AM and 10 AM, and blood sampling was performed after overnight fasting. Patients were asked to collect stool samples in special sealed plastic containers on the day preceding each follow-up visit.

Diets

Dietary interventions were designed on the basis of eating habits in Sweden. They included food items available in standard grocery stores and were in agreement with the Nordic Nutrition Recommendations.[29] The VD was a lacto-ovo-vegetarian diet allowing intake of eggs and dairy products. The MD refers to a conventional diet that was based on the average meat consumption in Sweden and corresponded to a daily intake of 145 g of meat, including red, white, and processed meats.[30] All subjects received a meal plan to follow throughout the study. Lunches and dinners were provided as ready-made frozen meals (Tables S1 and S2). These meals were based on traditional Swedish recipes and produced and supplied by Dafgård, Källby, Sweden. Subjects visited the clinic on a weekly basis to collect meals. At the first study visit, subjects met with a research dietitian who provided information on how to follow the individually energy-adjusted meal plans (Data S1). In addition to the 2 meals provided, subjects were asked to have breakfast, 2 snacks, and a side dish for the main course, every day. The meal plans included 5 to 6 options for breakfast, light meals, and side dishes. The nutrient composition of the diets was calculated using nutrition calculation software (Dietist Net Pro; Kost och Näringsdata AB, Bromma, Sweden) (Table 1).

Adherence to Dietary Intervention

The subjects completed a 3-day weighed food record before intervention, in the final week of each of the interventions, and at the end of the washout period (Table S3). During the intervention, patients were asked to complete a daily diary, recording whether they had consumed the provided lunch and dinner, which options they had chosen for breakfast and light meals, and if there were any deviations from the meal plan.

Primary and Secondary Outcomes

Difference in change in plasma oxidized LDL-C between diets was the primary outcome measure. Secondary outcomes included differences in change of cardiometabolic risk factors (lipids, hemoglobin A1c [HbA1c], hs-CRP [high-sensitivity C-reactive protein],

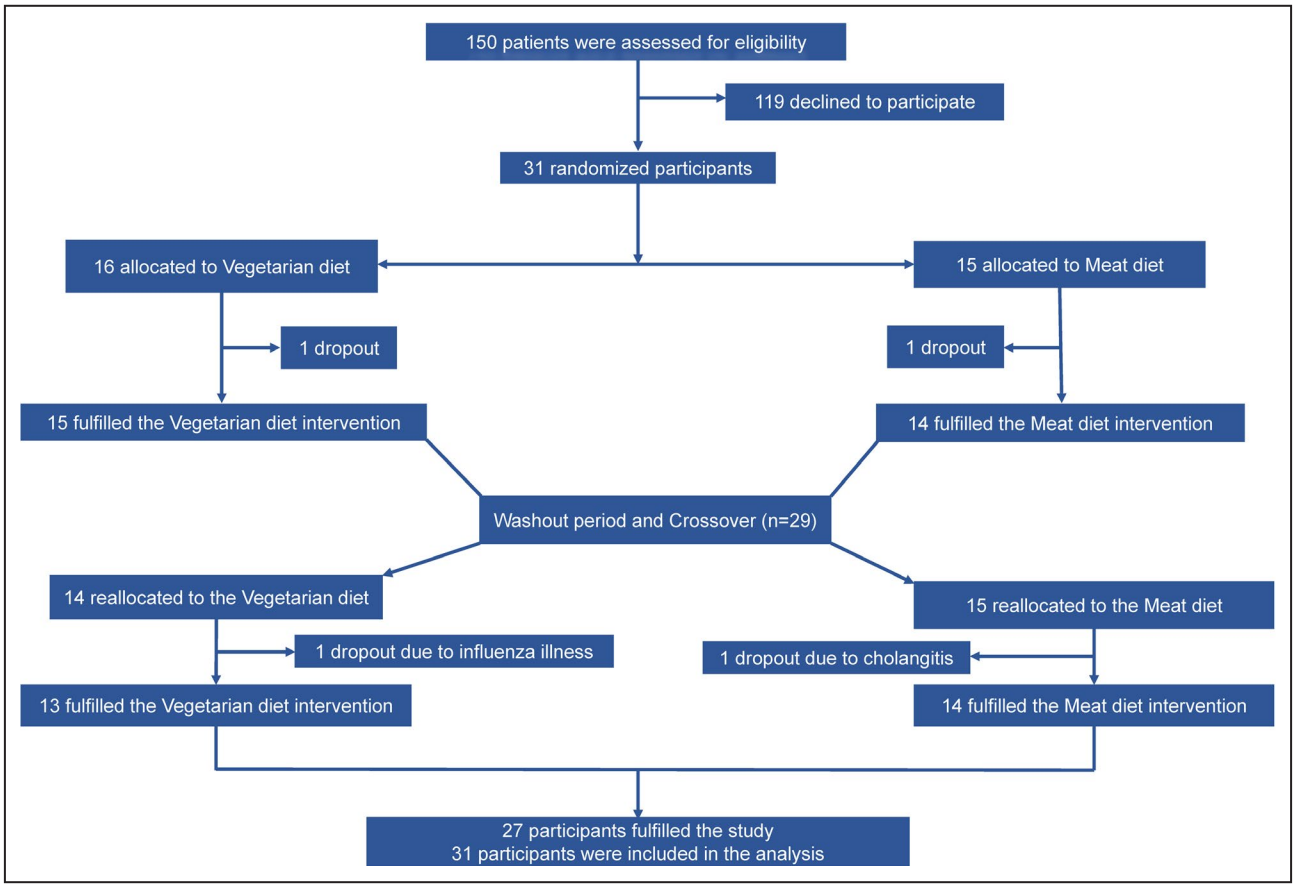

Figure 1. Schedule of study visits and participant flow.

weight, body mass index [BMI], blood pressure, heart rate, quality of life, gut microbiota in fecal samples, fecal SCFAs and branched-chain fatty acids [BCFAs], plasma metabolome, and plasma levels of TMAO, choline, L-carnitine, and acetyl-carnitine).

Oxidized LDL-C and Cardiometabolic Risk Factors

Venous blood samples were collected at the 4 study visits in evacuated plastic tubes (VACUETTE TUBE; Greiner Bio-One GmbH, Kremsmunster, Austria) and centrifuged in a cooling system at 1560*g* for 10 minutes at −40°C and stored at −80°C in aliquots until analyses. An ELISA kit (Mercodia, Uppsala, Sweden) was used for quantitative measure of plasma oxidized LDL-C levels, as described by Holvoet et al,[31] with an intra-assay coefficient of variation <10% (mean, 3.74%) for most samples. Five samples showed a coefficient of variation >10%. Total cholesterol (TC), LDL-C, high-density lipoprotein cholesterol, triglycerides, apolipoprotein A1, apolipoprotein B (APOB), hs-CRP, and

Table 1. Macronutrient Profile of Prescribed Diet

Variable	Energy, kcal	Protein, g	Carbohydrates, g	Fat, g	Saturated Fat, g	Dietary Fiber, g
Vegetarian diet						
According to meal plan*	1394	51.2	169.8	51	20.5	19.5
Intervention food†	999	38.4	104.8	45.7	17	15
Total‡	2393	89.6	274.6	96.7	37.5	34.5
Meat diet						
According to meal plan*	1318	48.9	168.7	43.8	15.2	22.4
Intervention food†	1076	41.8	102.4	55.9	22.2	10.7
Total‡	2394	90.3	275.2	97.5	37.4	33.1

*Bread with topping, side dish, breakfast, and 0 to 3 snacks/light meals.
†Provided frozen dishes, including lunch and dinner.
‡Complete diet.

HbA1c at each study visit were measured at the Clinical Chemistry Laboratory, Örebro University Hospital, according to a standardized protocol (Data S1). Cutoff values of clinical markers routinely monitored after a cardiac event were based on European guidelines on CVD prevention in clinical practice[32]: LDL-C <70 mg/dL (<1.8 mmol/L), systolic blood pressure <130 mm Hg, diastolic blood pressure <80 mm Hg, and BMI <25 kg/m^2. For LDL-C, we used the cutoff according to European guidelines during the study period, <70 mg/dL. A digital automatic sphygmomanometer (Omron m6 ac; Omron Healthcare Co, Ltd, Kyoto, Japan) was used for blood pressure and heart rate measurements. Body height was measured at baseline, and body weight was measured at the 4 study visits. Quality of life was assessed by using the EuroQoL 5-dimension questionnaire at all study visits, including a visual analogue scale and measures of mobility, self-care, usual activities, pain/discomfort, and anxiety/depression.[33] The Lund-Malmö equation was used to determine the estimated glomerular filtration rate.

Gut Microbiota, Fecal Fatty Acids, and Plasma Metabolome

Details of instrumental analysis and preprocessing of raw reads for 16S rRNA gene sequencing analysis, SCFA and BCFA, plasma metabolome, and concentrations of plasma TMAO, choline, L-carnitine, and acetyl-carnitine are described in Data S1.

Fecal samples collected in a sterile stool tube by the participant on the day before each follow-up visit and stored in the home freezer (≈–20°C) were brought to the clinic and stored at –80°C until extraction. DNA was extracted from samples by repeated bead beating and subjected to 16S rRNA gene sequencing in an Illumina Miseq instrument (2×250 bp paired-end reads, V2 kit; Illumina, San Diego, CA) after PCR amplification of the V4 region with the 515F and 806R primers. A total of 1264 zero-radius operational taxonomic units (abundance ≥0.002%) in 102 samples was obtained (Figure S1A), primarily represented by the phyla Firmicutes, Bacteroidetes, Actinobacteria, and Proteobacteria (Figure S1B).

Concentrations of the SCFA acetate, propionate, and butyrate; BCFA isobutyrate and isovalerate; succinate; and lactate in fecal samples were determined using a gas chromatograph mass spectrometer (Agilent Technologies), as previously described.[34]

For untargeted metabolomics, plasma samples were deproteinized using ultracentrifugation and analyzed by high-performance liquid chromatography–quadrupole time-of-flight mass spectrometry (Agilent Technologies).[23] In total, 1882 metabolite features (a molecular entity with a unique mass/charge ratio and retention time, as measured by an instrument) with the coefficient of variation in quality control samples ≤30% were subjected to further analysis. Metabolite identification was based on accurate mass (mass tolerance ≤5 ppm) and tandem mass spectrometry fragmentation (mass tolerance ≤10 ppm) matched against online databases or the literature.

The concentrations of plasma TMAO, choline, L-carnitine, and acetyl-carnitine were analyzed by high-performance liquid chromatography–mass spectrometry on an Exion UHPLC coupled to a QTRAP 6500+ tandem mass spectrometry system, both from AB Sciex LLC (Framingham, MA).

Statistical Analysis

The sample-size calculation was based on previous studies in which a VD or food supplements (nuts, soy-based cereal, or cranberry juice) were shown to reduce oxidized LDL-C by 10% compared with no intervention.[35,36] Considering similar effects in our study and a mean reduction of oxidized LDL-C of 9%, we needed to include 27 patients in a crossover design to be able to reject the null hypothesis that the experimental and control treatments were identical with a probability (power) of 0.80 and a type I error probability of 0.05. On the basis of an estimated 10% dropout rate, we therefore enrolled 31 subjects.

The effects of diets on oxidized LDL-C and cardiometabolic outcomes were evaluated using a generalized linear mixed model that included a fixed effect of the diet, sequence of diet allocation, and their interaction. Missing values were imputed in an intention-to-treat analysis using the last observation carried forward for the subjects (n=2) who were randomized but did not receive intervention and for the subjects who dropped out after the first intervention period (n=2). In addition, we performed on-treatment analysis. A 2-sided $P<0.05$ was considered significant.

A Kruskal-Wallis test was applied to the observed number of microbial species, and the Faith phylogenetic diversity index was used to examine potential differences in α diversity between results of the 2 diets. Principal coordinate analysis of the weighted and unweighted UniFrac distances or the Bray-Curtis dissimilarity was used to analyze the overall composition of gut microbiota. A permutational multivariate ANOVA (Adonis) (n=9999) and analysis of similarities were used to assess the effect of the dietary intervention on principal coordinate analysis scores of β diversity metrics. To identify microbial taxa or plasma metabolites discriminating VD from MD, a random forest modeling approach based on multilevel data analysis was applied[37,38] for pair-wise comparison of zero-radius operational taxonomic unit or metabolite levels of VD and MD (Figure S2, Data S1). The multilevel analysis

deals with dependent data structures and has been successfully used to exploit differences specific to diet in crossover intervention studies. Significance of multivariate models was assessed by permutation tests (n=100). A common baseline effect was assumed for both interventions, because no differences in bacterial genera or plasma metabolome were observed between baseline and the end of the washout period (Figures S3 and S4).

We further assessed the effect of VD versus MD on each selected optimally discriminating zero-radius operational taxonomic unit or metabolite using generalized linear mixed models (R package "lme4"). Fixed factors included diet, sequence of diet allocation, and their interaction with *baseline value* as covariate and *subject* as random factor. The same analysis was applied to the concentrations of fecal SCFAs and BCFAs. Spearman correlation coefficients were calculated for all correlation analyses. The *P* values were adjusted for multiple comparisons using the Benjamini-Hochberg false discovery rate, and a value of $P<0.05$ was considered significant.

In an exploratory analysis, we investigated whether gut microbiota configuration or plasma metabolome at baseline was associated with the influence of VD on metabolic risk factors, including levels of oxidized LDL-C, LDL-C, TC, and BMI. Random forest modeling[37] was used to identify a panel of microbial taxa or plasma metabolites that could enable discrimination of potential responders (subjects who benefitted from VD compared with MD and showed within-individual difference in metabolic risk factors between VD and MD <0) from nonresponders (subjects in whom VD did not improve metabolic risk factors compared with MD and had within-individual difference in metabolic risk factors between VD and MD >0).

RESULTS

Study Population and Diet Adherence

Of 150 patients with a history of IHD treated with PCI and receiving optimal medical therapy who were invited, 31 (21%) agreed to participate and were randomized. Twenty-nine were men (94%), with a median age of 67 years (range, 63–70 years) and a median BMI of 27.5 kg/m^2 (Table 2). Two subjects dropped out because of difficulties adhering to the diet, one because of influenza and one because of cholangitis. Twenty-seven subjects completed the study (Figure 1). Before enrollment, 12 (39%) subjects had experienced an ST-segment–elevation myocardial infarction; 12 (39%) had experienced a non–ST-segment–elevation myocardial infarction; 3 (10%) had unstable; and 5 (16%) had stable angina pectoris. All subjects were receiving statin therapy, 29 (94%) were treated with aspirin, and 20 (65%) received P_2Y_{12} inhibitors (clopidogrel or ticagrelor). During the study, the only change in medical therapy was addition of calcium channel blockers in 2 subjects. Both dietary interventions were well tolerated, and overall adherence based on the self-reported diaries was 88% for both interventions; however, there was a difference in adherence with respect to snacks (Table S4). On the basis of the 3-day food records, there was no significant difference in the intake of macronutrients; however, there was a difference in intake of fiber (Table S3).

Effects on Oxidized LDL-C and Cardiometabolic Risk Factors

Subjects consuming the VD showed significantly lower mean oxidized LDL-C compared with MD (–2.73 U/L) (*P*=0.02) (Figure 2, Table 3). A significant decrease from baseline of oxidized LDL-C after VD intervention was observed, whereas no difference was found after MD (Figure 2, Figure S4).

Subjects on the VD showed lower mean TC (–5.03 mg/dL/–0.13 mmol/L) (*P*=0.01), LDL-C (–3.87 mg/dL/–0.10 mmol/L) (*P*=0.02), body weight (–0.67 kg) (*P*=0.008), and BMI (–0.21 kg/m^2) (*P*=0.009) compared with subjects on the MD (Figure 2, Table 3). No difference between diets was observed for high-density lipoprotein cholesterol, triglycerides, APOB, apolipoprotein, APOB/apolipoprotein A1 ratio, HbA1c, hs-CRP, blood pressure, heart rate, quality of life, or the number of subjects reaching guideline values of clinical markers LDL-C, blood pressure, and BMI (Table 3, Tables S5 and S6). Similar results were obtained by the on-treatment analysis (Table S7).

Compared with baseline, both the VD and MD led to significantly lower mean values of TC (–7.8% and –5.7%, respectively), LDL-C (–11.9% and –7.9%, respectively), high-density lipoprotein cholesterol (–6.5% and –6.3%, respectively), APOB (–9.0% and –3.8%, respectively), and APOB/apolipoprotein A1 ratio (–8.0% and –7.9%, respectively) (Table 3, Figure S5). There were no differences from baseline in triglycerides, apolipoprotein A1, HbA1c, body weight, BMI, hs-CRP, blood pressure, heart rate, quality of life, or number of subjects reaching clinical marker guideline values after the 2 diet interventions (Table 3 and Tables S5 and S6).

Effects on Gut Microbiota, Fecal SCFAs and BCFAs, and Plasma Metabolome

The diets did not alter either richness or overall composition of gut microbiota at the phylum level (Figures S6 and S7) but differed with respect to the relative abundance of several microbial genera (Figure S8, Table S8). Multilevel predictive modeling revealed 46 microbial genera with the potential

Table 2. Baseline Characteristics of the Study Population at First Randomization Intervention

Characteristics	All (n=31)	VD (n=16)	MD (n=15)
Age, median (range), y	67 (63–70)	67 (65–70)	68 (61–70)
Sex, men, n (%)	29 (94)	15 (94)	14 (93)
History before enrollment			
STEMI, n (%)	12 (39)	6 (35)	6 (40)
NSTEMI, n (%)	12 (39)	4 (25)	8 (53)
Instable angina, n (%)	3 (10)	3 (19)	0 (0)
Angina, n (%)	5 (16)	4 (25)	1 (7)
Type 2 diabetes mellitus, n (%)	2 (7)	2 (13)	0 (0)
Hypertension, n (%)	17 (55)	10 (63)	7 (47)
Drug treatment			
Statins, n (%)	31 (100)	16 (100)	15 (100)
Ezetimibe, n (%)	7 (23)	4 (25)	3 (20)
ASA, n (%)	29 (94)	15 (94)	14 (93)
P_2Y_{12} inhibitors, n (%)	20 (65)	8 (50)	12 (80)
β Blockers, n (%)	28 (90)	14 (88)	14 (93)
ACE inhibitors/ARBs, n (%)	27 (87)	13 (81)	14 (93)
CCBs, n (%)	11 (36)	6 (38)	5 (33)
Cardiometabolic risk factors and life quality			
Weight, mean±SD, kg	84±11.0	86±13.6	83±8.6
BMI, mean±SD, kg/m^2	28±2.9	28±3.3	27±2.5
Systolic BP, mean±SD, mm Hg	139±17.4	140±17.4	138±18.0
Diastolic BP, mean±SD, mm Hg	87±9.6	88±10.6	87±8.7
Heart rate, mean±SD, bpm	65.8±9.2	65.1±9.2	66.5±9.5
EQ-5D VAS, mean±SD	80±10.7	78±11.2	82±10.2
Oxidized LDL-C, mean±SD, U/L	40.9±11.7	39.4±11.7	42.1±11.8
Total cholesterol, mean±SD, mg/dL	133.4±23.2	135.7±28.2	130.7±17.0
LDL-C, mean±SD, mg/dL	62.3±16.8	62.3±19.1	62.3±14.7
HDL-C, mean±SD, mg/dL	48.7±13.0	50.6±15.9	46.5±9.0
Triglycerides, mean±SD, mg/dL	94.0±29.8	93.7±32.3	94.2±28.0
APOB, mean±SD, g/L	0.7±0.1	0.7±0.1	0.7±0.1
APOA1, mean±SD, g/L	1.4±0.2	1.4±0.2	1.4±0.1
APOB/APOA1 ratio, mean±SD	0.5±0.1	0.5±0.1	0.5±0.1
HbA1c, median (range), mmol/mol	39 (36–40)	39 (36–42)	39 (36–40)
hs-CRP, median (range), mg/L	0.7 (0.5–1.7)	0.8 (0.4–1.7)	0.7 (0.4–1.7)
eGFR, mean±SD, mL/min per 1.73 m^2	76.4±9.7	75.1±7.6	77.7±11.7

Data are presented as median (interquartile range), number (percentage), or mean±SD. To convert cholesterol markers to millimoles per liter, multiply by 0.02586. To convert triglycerides to millimoles per liter, multiply by 0.01129. ACE indicates angiotensin-converting enzyme; APOA1, apolipoprotein A1; APOB, apolipoprotein B; ARB, angiotensin II receptor blocker; ASA, acetylsalicylic acid; BMI, body mass index; BP, blood pressure; bpm, beats per minute; CCB, calcium channel blocker; eGFR, estimated glomerular filtration rate; EQ-5D, EuroQoL 5-dimension questionnaire (self-reported quality of life); HbA1c, hemoglobin A1c; HDL-C, high-density lipoprotein cholesterol; hs-CRP, high-sensitivity C-reactive protein; LDL-C, low-density lipoprotein cholesterol; MD, meat diet; NSTEMI, non–ST-segment–elevation myocardial infarction; P_2Y_{12} inhibitor, clopidogrel or ticagrelor; STEMI, ST-segment–elevation myocardial infarction; VAS, visual analogue scale; and VD, vegetarian diet.

to distinguish VD from MD (Figure 3A), most belonging to the families Ruminococcaceae (n=13), Lachnospiraceae (n=11), and Eggerthellaceae (n=4). Among them, 12 genera differed in VD and MD when individually assessed by univariate analysis (Figure 3A, Table S8).

The fecal concentrations of acetate, propionate, butyrate, isobutyrate, and isovalerate were 4% 10%, 5%, 3%, and 6% higher, respectively, after 4 weeks of a VD than after MD. These results did not reach significance (Table S9).

The plasma metabolome differed significantly with diet (Figure S9). Thirty-three plasma metabolites distinguished VD from MD with a predictive accuracy of 95%, among them acylcarnitine metabolites and several phosphatidylcholines and

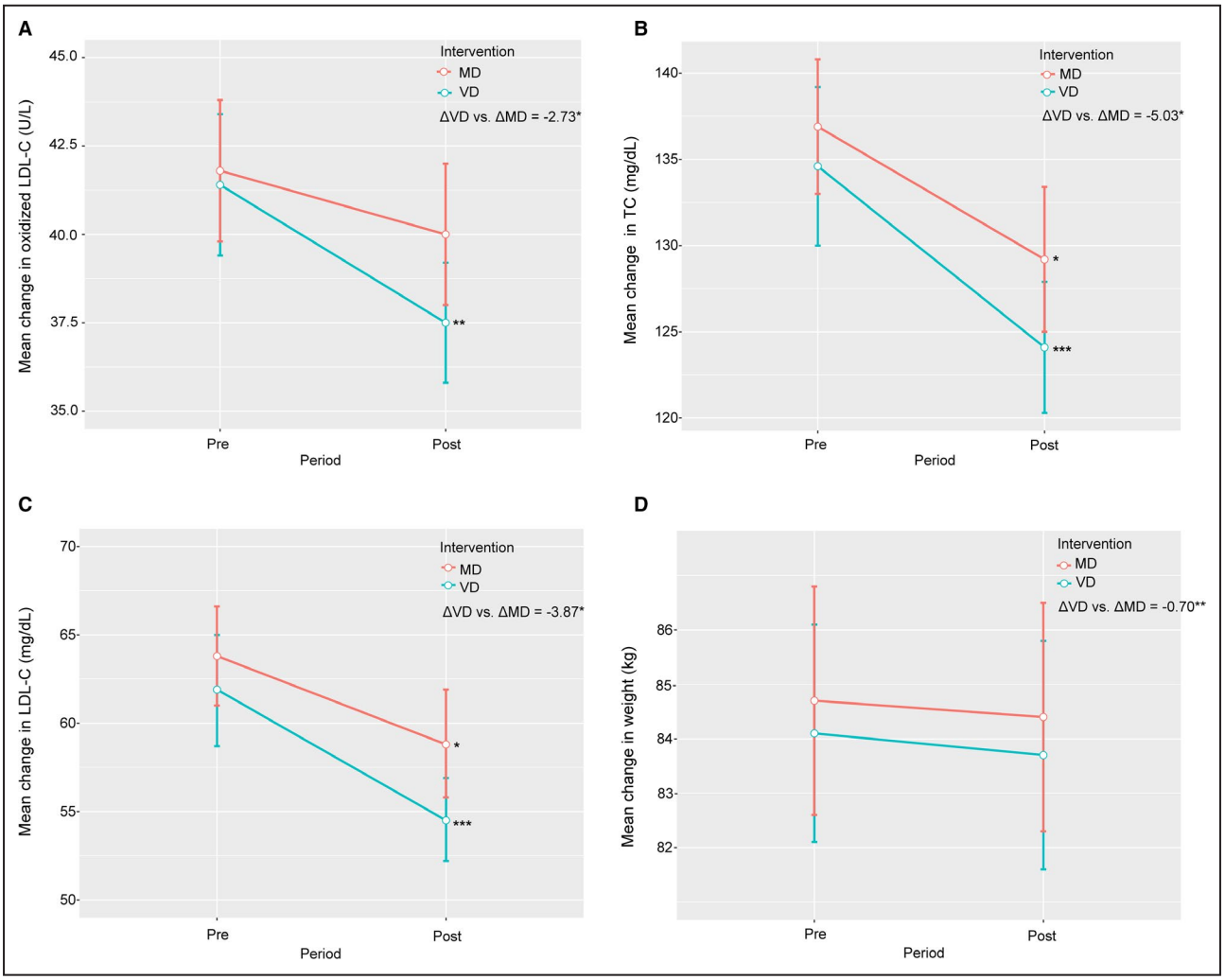

Figure 2. Changes in oxidized low-density lipoprotein cholesterol (LDL-C) and cardiometabolic risk factors according to dietary intervention.

Mean change in oxidized LDL-C (**A**), total cholesterol (TC) (**B**), LDL-C (**C**), and weight (**D**) before and after each intervention. Error bars indicate SEM. ΔVD vs ΔMD indicates differences in risk factors between vegetarian diet (VD) and meat diet (MD) obtained using linear mixed-effects models adjusted for sequence of diet randomization and intervention period. **P*<0.05, ***P*<0.01, ****P*<0.001. Post, 4 weeks after the dietary intervention; Pre, baseline.

phosphatidylethanolamines (Figure 3B, Table S10). When assessed individually using univariate statistics, 28 of 33 metabolites were significantly different from MD in VD (Figure 3B).

We found a significant difference in plasma L-carnitine (–14.77 μmol/L) (95% CI, –21.13 to –8.71 μmol/L; *P*<0.001), but not in TMAO, acyl-carnitine, or choline, between the MD and VD (Figure 4).

The plasma concentration of TMAO and L-carnitine was lower after VD compared with baseline (–1.90 μmol/L [95% CI, –2.87 to –0.93 μmol/L; *P*<0.001] and –14.46 μmol/L [95% CI, –24.75 to –4.17 μmol/L; *P*<0.01]). The concentration of choline increased with the VD (3.09 μmol/L; 95% CI, 1.06–5.12 μmol/L; *P*=0.001) (Figure 4, Figure S10).

We observed multiple correlations of changes in microbiota, metabolites, and cardiometabolic risk factors with diet (Table 4[39-48], Table S11, Figure S11). However, no correlation remained significant after correction for multiple testing. No correlations were observed between fecal SCFAs or BCFAs and assessed clinical risk factors.

Baseline Gut Microbiota and Plasma Metabolites Associated With Clinical Outcome Response to the VD

Although we found significantly lower mean oxidized LDL-C and BMI after VD compared with MD, we observed substantial interindividual difference in response to dietary intervention (Figure 5, Figure S12). Oxidized LDL-C and BMI were lower in 14 and 13 responders (subjects who benefitted from VD compared with MD and showed within-individual difference in

Table 3. Effect of Dietary Intervention on Clinical Parameters

Clinical Parameters	Pre-VD	Post-VD	Pre-MD	Post-MD	Post-VD vs Post-MD*	*P* Value*
Oxidized LDL-C, U/L	41.4 (37.2–45.5)	37.5 (33.8–40.7)†	41.8 (37.7–46.0)	40.0 (35.9–44.2)	−2.73 (−4.9 to −0.6)	0.02
TC, mg/dL	134.6 (124.9–144.2)	124.1 (116.00–131.9)‡	136.9 (129.9–145.0)	129.2 (120.6–137.6)§	−5.03 (−8.89 to −1.16)	0.01
LDL-C, mg/dL	61.9 (55.7–68.4)	54.5 (49.5–59.6)‡	63.8 (58.0–69.6)	58.8 (52.6–65.0)§	−3.87 (−7.35 to −0.77)	0.02
HDL-C, mg/dL	47.6 [42.9–53.0]	44.5 [39.8–49.9]†	49.1 [44.5–54.1]	46.1 [41.4–51.43]§	−1.16 [−2.71 to 0.39]	0.2
Triglycerides, mg/dL	86.8 [76.2–98.3]	92.1 [83.3–102.7]	87.7 [77.1–99.2]	86.8 [77.1–98.3]	5.31 [−1.77 to 13.3]	0.1
APOB, g/L	0.65 (0.60–0.70)	0.59 (0.55–0.63)‡	0.66 (0.62–0.71)	0.61 (0.56–0.65)‡	−0.021 (−0.044 to 0.001)	0.06
APOA1, g/L	1.40 (1.35–1.49)	1.41 (1.34–1.48)	1.44 (1.37–1.51)	1.42 (1.35–1.50)	−0.019 (−0.049 to 0.011)	0.2
APOB/APOA1 ratio	0.45 [0.42–0.48]	0.41 [0.38–0.45]‡	0.46 [0.42–0.5]	0.42 [0.39–0.46]‡	−0.021 [−0.07 to 0.03]	0.4
HbA1c, mmol/mol	38.5 [37.1–40.0]	38.7 [37.2–40.3]	38.6 [37.0–40.4]	38.8 [37.2–40.6]	−0.003 [−0.023 to 0.017]	0.8
Weight, kg	84.1 (80.1–88.2)	83.7 (79.5–87.9)	84.7 (80.5–88.9)	84.4 (80.1–88.6)	−0.7 (−1.1 to −0.2)	0.008
BMI, kg/m^2	27.4 (26.4–28.5)	27.3 (26.2–28.4)	27.6 (26.5–28.7)	27.5 (26.4–28.6)	−0.2 (−0.36 to −0.06)	0.009
hs-CRP, mg/L	0.73 [0.51–1.03]	0.74 [0.50–1.09]	0.81 [0.60–1.09]	0.81 [0.55–1.18]	−0.09 [−0.42 to 0.23]	0.6
Systolic BP, mm Hg	136 (129–143)	133 (127–140)	140 (133–146)	136 (129–142)	−2.3 (−5.4 to 0.8)	0.1
Diastolic BP, mm Hg	86 (82–89)	86 (83–89)	87 (84–91)	87 (83–91)	−1.1 (−3.8 to −1.6)	0.4
HR, bpm	62.7 [59.9–65.7]	63.4 [60.6–66.3]	64.3 [60.9–67.9]	63.5 [60.1–67.1]	−0.001 [−0.04 to 0.04]	0.9

Data are presented as mean (95% CI) or as geometric mean [95% CI]. Within-group change *P* value was calculated with paired *t* test. APOA1 indicates apolipoprotein A1; APOB, apolipoprotein B; BMI, body mass index; BP, blood pressure; bpm, beats per minute; HbA1c, hemoglobin A1c; HDL-C, high-density lipoprotein cholesterol; HR, heart rate; hs-CRP, high-sensitivity C-reactive protein; LDL-C, low-density lipoprotein cholesterol; MD, meat diet; TC, total cholesterol; and VD, vegetarian diet.

*Differences in clinical parameters between VD and MD were examined using linear mixed-effects models adjusted for sequence of diet randomization and period of interventions.

†$P<0.01$.

‡$P<0.001$.

§$P<0.05$.

metabolic risk factors between VD and MD <0), respectively, after VD than after MD, whereas 6 and 7 nonresponders exhibited higher oxidized LDL-C and BMI, respectively, with MD than with VD. In an exploratory analysis, we found that baseline relative abundance of 14 genera could discriminate responders from nonresponders: oxidized LDL-C decreased with the VD in individuals with higher fecal relative abundance of genera of the Ruminococcaceae family, *Ruminococcaceae UCG.010, Ruminococcaceae UCG.002, Ruminococcus 1, Ruminococcaceae UCG.007, Hydrogenoanaerobacterium*, and *Barnesiella* and with low abundance of *GCA.900066575* and *Flavonifractor*. The response of BMI to the VD was not associated with a specific baseline gut microbiota configuration (Figure S8). Plasma metabolites at baseline were not associated with any response to intervention (Figure S13).

DISCUSSION

In this randomized, controlled, crossover study in subjects with IHD, a 4-week VD showed lower oxidized LDL-C and improved cardiometabolic risk factors compared with an isocaloric MD. The VD also influenced the relative abundance of microbial genera and plasma metabolites that have shown links to metabolic disease.[49-52] The change in oxidized LDL-C with the VD occurred in people with a specific baseline gut microbiota showing higher abundance of several genera in the families Ruminococcaceae and *Barnesiella*, a gut microbe that might play an

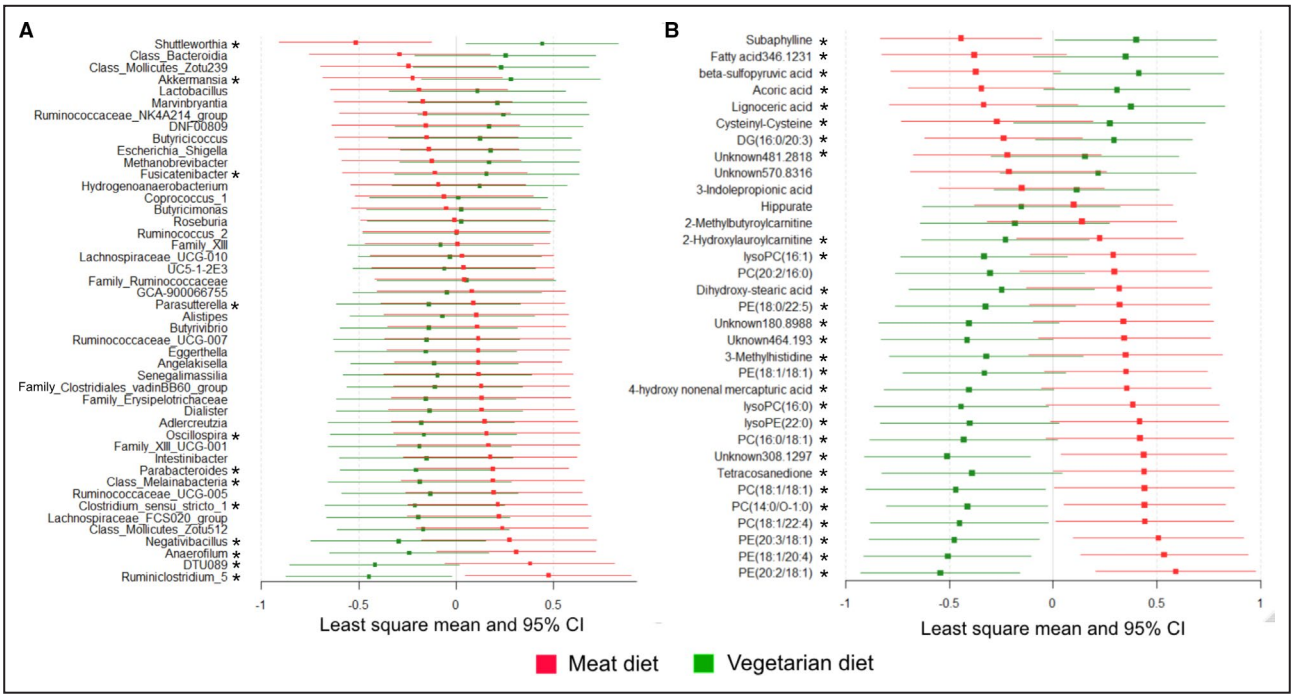

Figure 3. Gut microbiota and plasma metabolites discriminating the vegetarian and meat diets, and selected by multilevel random forest modeling.

Least-squares means and 95% CIs of abundance of zero-radius operational taxonomic units (**A**) and levels of metabolites (**B**) after 4-week intervention of the vegetarian and isocaloric meat diet obtained from random forest multivariate modeling. Standardized values are presented for comparison. *Denotes microbial genera or metabolites significantly differing between meat and vegetarian diet when assessed using generalized linear mixed models. DG indicates diacylglycerol; PC, phosphatidylcholine; and PE, phosphatidylethanolamine.

important role in clearance of intestinal infections and immunomodulation.[53,54]

Diet Effects on Oxidized LDL-C and Cardiometabolic Risk Factors

Conversion of LDL-C to oxidized LDL-C plays a central role in the development and progression of fatty streaks and atherosclerotic plaques.[55] Untreated individuals with IHD have significantly higher levels of oxidized LDL-C compared with people free of IHD.[31] Independent of traditional cardiovascular risk factors, elevated oxidized LDL-C has been shown to be a strong predictor of future IHD events.[6] It has recently been suggested that oxidized LDL-C leads to unstable coronary plaques via complex mechanisms of lipid mediators.[56] Our study indicates that, in subjects with IHD on optimal medical therapy, change in diet was accompanied by a decrease in oxidized LDL-C; hence, adoption of a VD in such patients could be of clinical importance. Studies of the link between diet and oxidized LDL-C are scarce; however, a clinical trial of healthy subjects with no diagnosed CVD showed oxidized LDL-C 5.4 U/L lower after 3 months of a gluten-free vegan diet than seen in a nonvegan diet.[35] We found that 4 weeks on a VD resulted in significantly lower oxidized LDL-C (–2.7 U/L) than with the MD in subjects with IHD treated with PCI, suggesting benefits of implementing VD intervention in addition to optimal medical therapy.

A recent meta-analysis of 11 randomized controlled trials reported a lipid-lowering effect of VD in healthy subjects free of CVD.[7] Most of the included trials comprised subjects not receiving lipid-lowering drugs. The pooled estimated changes in TC and LDL-C were –13.9 and –13.1 mg/dL, respectively, but no significant effects were observed for triglycerides. These effects were greater than those found in the current study. Interventions in the trials included in the meta-analysis were of longer duration, and our subjects had low TC and LDL-C levels at baseline. More important, our results suggest an additive effect of VD on TC and LDL-C in subjects receiving lipid-lowering medication. A 4% decrease in LDL-C may result in a meaningful reduction of coronary events. In agreement with previous studies, we observed a reduction in body weight with the tested VD, supporting a role for a VD on weight control in patients with IHD. The observed effects of VD on oxidized LDL-C and lipid profile may be partly attributed to weight loss.[57] On the other hand, we observed the greatest change in oxidized LDL-C and lipid profile

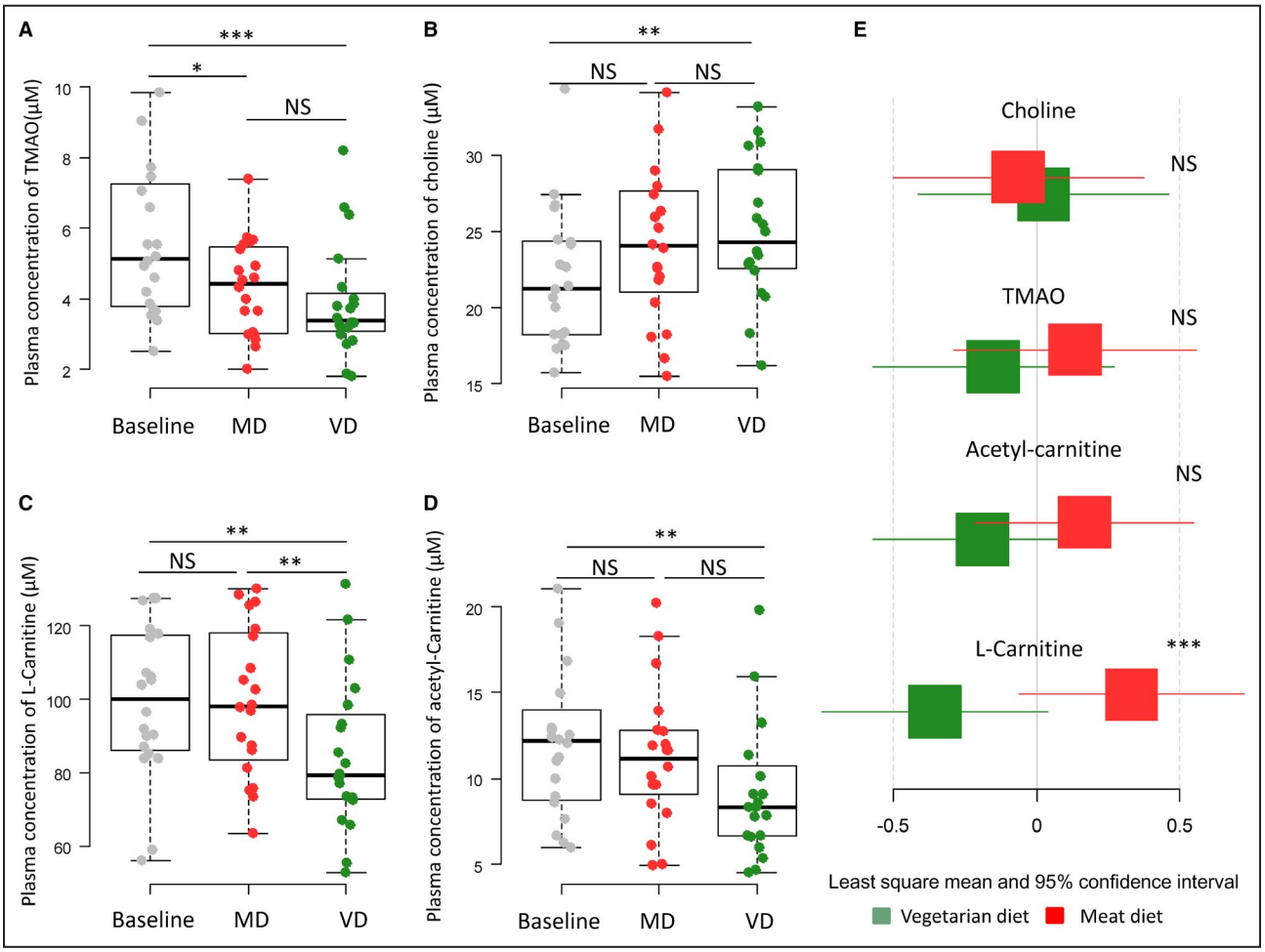

Figure 4. Changes in plasma concentration of trimethylamine N-oxide (TMAO), choline, L-carnitine, and acetyl-carnitine according to dietary intervention.
Boxplots (**A** through **D**) show the concentrations of the metabolites measured at baseline, after the vegetarian diet (VD) and the isocaloric meat diet (MD). Differences were assessed by paired *t* test. Least-squares means and 95% CIs of levels of metabolites (**E**) after 4-week intervention of VD and MD assessed by generalized linear modeling. Standardized values are presented for comparison. **P*<0.05, ***P*<0.01, ****P*<0.001. NS indicates not significant.

compared with baseline after the VD, despite no significant change in weight in this group.

Previous studies have shown benefit of a VD with respect to blood pressure, HbA1c, and hs-CRP compared with an omnivore diet,[6,58,59] which was not supported by our study. The source of the lack of reduction in hs-CRP with the VD may be the fact that all study participants were treated with statins, which show anti-inflammatory properties, or the lack of power to detect changes in hs-CRP.

The baseline treatment did not influence the results, because of the crossover design of the study. Moreover, because no alterations in cholesterol-lowering drugs (statins or ezetimibe) were made during the study period, it is unlikely that medication had an impact on oxidized LDL-C or cholesterol measures. On the other hand, a change in antihypertensive therapy (calcium channel blockers) of 2 subjects may partly explain the lack of effect of VD on blood pressure compared with MD.

Diet Effects on Gut Microbiota and Plasma Metabolome

The 4-week dietary intervention did not alter either the richness or the overall composition of the gut microbiota, in line with previous findings.[60] However, we observed altered relative abundance of bacterial genera that have been associated with human metabolic health status.[13,15,39,40,46,61,62] For example, compared with MD, subjects consuming the VD exhibited higher relative abundance of the genus *Akkermansia*, shown to be enriched after intervention with prebiotic inulin and in polyphenol-rich diets.[39] *Akkermansia* was also linked to beneficial effects on body fat distribution as well as fasting plasma glucose and triglyceride levels

Table 4. Bacterial Genera Discriminating the VD From the MD and Their Correlation With Cardiometabolic Risk Factors and Metabolites as Well as Previously Reported Effects

Genus	Description	VD*	MD	SEM	r†	r‡	Previous Findings
Fusicatenibacter	Class Clostridia, family Lachnospiraceae	898.3	744.0	90.6		3-Indolepropionic acid (0.32), 4-hydroxy nonenal mercapturic acid (0.33), tetracosanedione (0.37)	
Akkermansia	Class Verrucomicrobiae, family Akkermansiaceae	811.5	426.3	203.6		3-Indolepropionic acid (0.37), 2-methylbutyroylcarnitine (–0.32)	Anaerobic genus with health-promoting effect,[14] reported to increase after intake of high fermentable oligosaccharides, disaccharides,[39] and monosaccharides and polyols diets or the dietary-resistant starch.[40]
Clostridium sensu stricto 1	Class Clostridia, family Clostridiaceae_1	353.0	567.8	106.9	BMI (0.29), weight (0.30)	3-Indolepropionic acid (–0.32), cysteinyl-cysteine (0.35), lysophosphatidylethanolamine (22:0) (0.31), phosphatidylethanolamine (18:1/20:4) (0.34)	Pathogenic genus,[41] reported to decrease after a 3-mo VD, and was positively associated with inflammatory markers and LDL-C.[42]
Parabacteroides	Class Bacteroidia, family Tannerellaceae	216.7	309.6	33.5	TC (0.35), LDL-C (0.27)	Lysophosphatidylcholine (16:0) (0.31)	Reported to be a microbial marker for hypertension,[43] and was directly associated with weight gain.[44]
Ruminiclostridium 5	Class Clostridia, family Ruminococcaceae	200.7	390.1	38.4		Lignoceric acid (–0.37), phosphatidylethanolamine (18:1/20:4) (0.33), phosphatidylcholine (20:2/16:0) (0.50), phosphatidylethanolamine (18:0/22:5) (0.32), lysophosphatidylcholine (16:0) (0.31)	Reported to be inversely associated with plant-based diets and several beneficial nutrients (eg, vitamins and magnesium).[15]
Parasutterella	Class Gammaproteobacteria, family Burkholderiaceae	33.7	46.5	4.4		phosphatidylethanolamine (18:1/18:1) (0.32), phosphatidylcholine (18:1/22:4) (0.34)	Reported to be associated with sodium and processed foods.[15]
Negativibacillus	Class Clostridia, family Ruminococcaceae	13.1	24.9	3.9		Phosphatidylcholine (20:2/16:0) (0.32), 4-hydroxy nonenal mercapturic acid (0.40), N-acetylanonaine (0.32)	Reported to be correlated with body weight and obesity-related parameters.[45]
Oscillospira	Class Clostridia, family Ruminococcaceae	11.5	16.4	2.4	LDL-C (–0.28)	3-Indolepropionic acid (–0.48), 2-methylbutyroylcarnitine (0.41), tetracosanedione (–0.36)	Reported to be increased after a 1-y Mediterranean diet in obese population.[46] Ruminococcaceae was positively correlated with plasma indolepropionic acid, whereas was negatively correlated with atherosclerotic disease burden in an apolipoprotein E knockout mice model.[47]
Melainabacteria	Phylum Cyanobacteria	8.3	19.4	4.4		Diacylglycerol (16:0/20:3) (0.40)	
Shuttleworthia	Class Clostridia, family Lachnospiraceae	7.0	0.6	1.2	Oxidized LDL-C (–0.41), TC (–0.32), LDL (–0.28)	Phosphatidylcholine (14:0/O-1:0) (–0.35), lysophosphatidylcholine (16:1) (–0.49), lysophosphatidylethanolamine (22:0) (–0.39), diacylglycerol (16:0/20:3) (0.34), phosphatidylcholine (18:1/18:1) (–0.32)	
DTU089	Class Clostridia, family Ruminococcaceae	7.2	15.4	2.0	TC (0.29)	Lignoceric acid (–0.32), phosphatidylethanolamine (18:1/20:4) (0.32), lysophosphatidylcholine (16:0) (0.32)	
Anaerofilum	Clostridium cluster IV and family Ruminococcaceae	2.0	4.3	0.7	Oxidized LDL-C (0.26), TC (0.27), LDL (0.27)	Phosphatidylethanolamine (18:0/22:5) (0.33)	Reported to decrease after supplements with prebiotic potential based on anaerobic human fecal cultivation study.[48]

BMI indicates body mass index; LDL, low-density lipoprotein; LDL-C, LDL cholesterol; MD, meat diet; TC, total cholesterol; and VD, vegetarian diet.

*The least square mean and SE of genera abundance or metabolite level were obtained from mixed modeling (n=20). Only genera that significantly differed between diets are presented ($P<0.05$). The effect of diet was evaluated using a generalized linear mixed model that included a fixed effect of diet, sequence of allocation, and their interaction.

†Significant Spearman correlations of differences in genera with clinical parameters improved by VD ($P<0.1$).

‡Significant Spearman correlations of differences in genera with plasma metabolites discriminated between the diets ($P<0.05$).

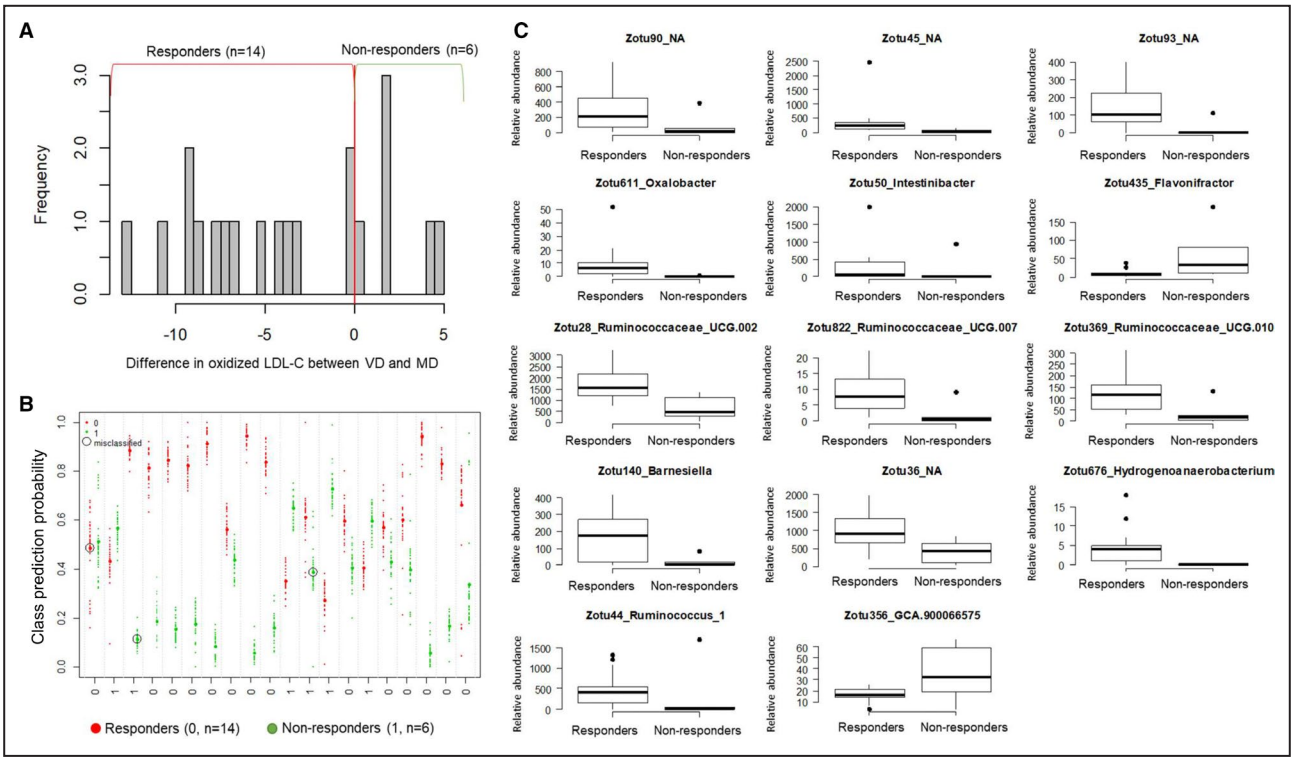

Figure 5. Baseline gut microbiota associated with response to diets in reduction of oxidized low-density lipoprotein cholesterol (LDL-C).

A, Intraindividual difference in oxidized LDL-C between vegetarian diet (VD) and meat diet (MD) is presented. Responders were defined as participants who showed lower oxidized LDL-C after VD than after MD. Patients who had higher oxidized LDL-C after VD than after MD were considered as nonresponders. **B**, Discrimination of responders from nonresponders based on microbial genera at baseline. We applied random forest modeling on relative abundance of zero-radius operational taxonomic units (ZOTUs) at baseline. Of 20 individuals, 17 could be successfully classified as responders or nonresponders. **C**, The optimal set of microbial genera for the successful classification (n=14). Relative abundance of ZOTUs for responders and nonresponders are presented. Boxes represent the interquartile range, and the line within represents the median. Whiskers denote the lowest and highest values within 1.5× interquartile range.

in a 6-week interventional trial of caloric restriction in obese subjects.[49]

The levels of fecal SCFAs were measured to quantify microbiota fiber fermentation capacity. Previous studies have shown effects of a VD or vegan diet on enrichment of SCFA-producing bacteria (eg, *Roseburia*, *Ruminococcus*, and *Blautia*) and subsequent increase in fecal SCFA levels, which may contribute to improved metabolic health.[63,64] We found a trend of increased fecal SCFAs with the VD, consistent with the slightly higher increase of fiber intake compared with the MD. Fecal SCFA level is influenced by the quantity of ingested fiber as well as individual characteristics, including composition of gut microbiota, intestinal gut transit, and rate of intestinal absorption.[65] Therefore, a larger sample size and a greater difference in the ingested fiber content of the diets might have been required to show significant changes in SCFA levels. In the present study, we adjusted the MD meal plans to include higher fiber content of the side dishes, breakfast, and snacks to obtain daily dietary fiber intake similar to that of the VD compared with MD.

We also observed differences in plasma metabolites after VD in subjects with IHD. Subjects consuming the VD exhibited lower levels of the acylcarnitine metabolites 2-hydroxylauroylcarnitine and 2-methylbutyroylcarnitine, as well as of several phospholipids containing fatty acids C14:0, C16:0, C16:1, and C18:1. In addition to traditional risk factors, these metabolites may improve risk prediction for recurrent coronary events.[66]

The VD compared with MD resulted in a reduction of plasma L-carnitine, a metabolite found predominately in red meat, findings that support that most of the subjects were adherent to both interventions and verify the accuracy of the analysis. The conversion of L-carnitine to trimethylamine is gut microbiota dependent,[67] and trimethylamine is absorbed by the portal system and transformed by the liver to TMAO, a potential proatherogenic compound.[21,68] Although no significant difference was observed between diets in TMAO, both VD and MD were shown to reduce its plasma level compared with baseline. These changes may have been caused by the reduced energy intake

designed from individually adapted meal plans rather than dietary composition. However, the results should be interpreted with caution because others have showed a difference in TMAO levels between vegans and omnivores,[18] and there might have been a lack of power in our study to detect significant changes. Moreover, the metabolic control and the renal function may interfere with TMAO levels,[69] although our study subjects had normal HbA1c and estimated glomerular filtration rate at baseline and the crossover design minimizes the likelihood of bias from confounding.

Our results support previously reported correlation of TMAO with genus *Bifidobacterium* (r=−0.31; P=0.05), genera belonging to the family Ruminococcaceae (eg, *Butyricicoccus*: r=−0.42, P=0.01; and *Intestinimonas*: r=−0.40, P=0.02), and several unannotated species of Lachnospiraceae and Ruminococcaceae.[18,51,68] These findings indicate that a short-term VD intervention might have influenced the activity of the gut microbiota in people who are omnivorous.

We observed an effect of VD on potential links among plasma metabolites, bacterial genera, and CVD risk factors. The correlations did not reach significance after false discovery rate correction for multiple testing, possibly because of the small number of participants and the similarity of microbial species in the gut microbiota. Our findings are consistent with previous studies[52,66,70] and indicate that mechanisms underlying the benefits of a short-term VD intervention on CVD risk factors may be explained by modulation of the abundance and metabolism of gut microbes.[39]

Baseline Gut Microbiota Associated With Oxidized LDL-C Response to Diets

Our results underscore the role of individual gut microbiota in specific cardiometabolic risk factor response to a diet,[25-27] such as that of oxidized LDL-C. We observed no significant association of relative abundance of gut bacteria at baseline with change in BMI during the study, in agreement with a recent meta-analysis indicating a weak relationship between gut microbiota and BMI.[71] However, we observed that several genera of the Ruminococcaceae, as well as the genus *Barnesiella*, were more abundant in individuals in whom oxidized LDL-C was reduced to a greater extent (responders) after a 4-week VD; whereas *GCA900066575* in the Lachnospiraceae family was less abundant relative to levels in nonresponders. Accumulating evidence supports a role of inflammation and the immune response in development of atherosclereosis.[72,73] Our results may suggest an interaction between specific gut bacteria and a VD in reduction of oxidized LDL-C, a lipoprotein that has been found to contribute to atherosclerosis-associated inflammation, activating both innate and adaptive immunity.[54,74]

Strengths and Limitations

The major strengths of the reported study include its crossover design, well-characterized subjects receiving optimal medical therapy, and a high rate of study completion. For future implementation, it is also a strength that the dietary interventions included ready-made main meals, because people often state that a VD is inconvenient and that they are unfamiliar with preparing vegetarian food. The availability of acceptable ready-made plant-based foods could facilitate secondary prevention.[75] In our crossover study, effects were only attributed to differences in diet, we found no significant impact in the order of the 2 dietary interventions, and there were no carryover effects.

The study has several limitations. First, the small sample size might have affected results with respect to clinical parameters, such as blood pressure, lipid and apolipoprotein biomarkers, and low-grade inflammation. Second, most of our study participants were men, decreasing generalizability. Third, a short-term intervention period allows only limited conclusions on adherence and clinical impact of diet. Measures of oxidized LDL-C levels in plasma ex vivo may not precisely reflect levels in vivo, as highly oxidized particles are rapidly cleared by scavenger receptors in the liver and antioxidants in blood.[44] We used a sandwich ELISA with a murine monoclonal antibody (mAb-4E6) directed against the oxidized antigenic determinants on the oxidized APOB molecule. This antibody may react with oxidized particles other than LDL-C, such as oxidized phospholipids and lipoproteins.[76] The untargeted metabolomics approach did not include a comprehensive analysis of bile acids, which precluded further investigation into the potential mechanistic role of gut microbiota regulation of bile acid metabolism in the cardiometabolic effects of the VD. We found that bacterial genera in the families Ruminococcaceae and Lachnospiraceae, known to modulate bile acid profile,[77,78] correlated with TC. The association did not remain significant after correction for multiple testing. Finally, information on the micronutrient content of the ready-made dishes was lacking, and a potential difference in the diets might have influenced the study results.

CONCLUSIONS

Our study suggests cardiometabolic benefits of a 4-week VD compared with an isocaloric MD in

subjects with ischemic heart diease on optimal medical treatment. The VD reduced levels of oxidized LDL-C, LDL-C, TC, and body weight compared with MD. The VD intervention also influenced levels of several microbial genera and plasma metabolites known to be linked to metabolic health status, suggesting the role of host-microbiota cometabolism for benefits of VD in people with ischemic heart diease. The composition of gut microbiota at baseline may have been associated with the lower oxidized LDL-C seen with the VD, reinforcing the importance of implementing personalized approaches to nutrition in addition to medical treatment, for effective management of cardiovascular disease.

ARTICLE INFORMATION

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Author Contributions: Djekic, Särnqvist, Bäckhed, Landberg, and Frøbert conceived and planned the clinical trial; Djekic was the principal investigator and performed clinical evaluations, sample collection and analysis, and statistical analyses of the clinical data, interpreted the data, and drafted and revised the manuscript; Shi conducted plasma metabolome analysis, performed statistical analyses on omics data, interpreted the data, and drafted and revised the manuscript; Savolainen performed analysis of plasma trimethylamine N-oxide, choline, L-carnitine, and acetyl-carnitine. Cao supervised data management and performed statistical analyses of the clinical data; Brolin and Tremaroli performed 16s RNA sequencing and participated in data analyses and interpretation; Carlsson revised the meal plan, provided instructions on following the diet plans, and performed dietary data processing; Cao, Bäckhed, Tremaroli, Landberg, and Frøbert supervised data interpretation and revised the manuscript. Frøbert assumed overall responsibility for the project. All authors read and approved the final article.

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Disclosures

None.

Supplementary Materials

Data S1
Tables S1–S11
Figures S1–S13

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SUPPLEMENTAL MATERIAL

Data S1.

Supplemental Methods

Meal plan used in the study for meat diet

- The energy level of the average diet was set at 2400 kcal per day. Adjustments will be made to accommodate individual requirements according to energy intake strata.

Background information

- In the VERDI study we want to investigate the effects on cardiometabolic risk factors of a lacto-ovo-vegetarian diet vs. a diet with meat in quantities corresponding to average meat intake in the Swedish population. You will, during two four-week-periods, follow a meal plan adapted to your calorie requirements so that you do not lose weight during the trial. It is important that you follow the meal plan.

Lunch and dinner

- During two four-week-periods, you will be provided with frozen, ready-made dishes for lunch and dinner. You are advised to consume the entire meal. In addition, you are given an individual meal plan which you should strictly follow.

Meal plan

- You will eat breakfast, snacks, and side dishes according to the meal plan adjusted to meet your energy requirements.
- The meal plan includes a number of alternatives for breakfast, snacks, and side dishes.
- The side dishes consist mainly of bread with toppings and can be consumed along with the main dish for lunch and dinner or between meals.

Please note

- It is important that you complete the daily food diary. In the food diary you record which alternative you have chosen for breakfast, snack, and side dish. Please note any deviations from the meal plan.
- It is important that you follow the meal plan, but you are allowed to deviate from it one day each week. You must still eat the ready-made frozen dishes during this day.

Each day choose **one** of the five breakfast alternatives below. Try to vary your choice from day to day.

- Fill in the food diary and check the alternative you have chosen.
- You do not have to eat the breakfast at any particular time, and you do not have to eat the entire breakfast at one time.

1. Yoghurt with oat cereal, sunflower seeds and apple sauce. Sandwich with butter, ham, and cheese.

Food item	*Quantity*	*Alternative*
Yoghurt (0.5% fat)	2.5 dl	Sour milk (0.5% fat)
Oat cereal	2 dl	Bran flakes, rye cereal
Sunflower seeds	1.5 tbsp	Pumpkin seeds, nuts
Applesauce	2 tbsp	Banana, raisins, jam
Whole grain rye bread	1-1.5 slices (50 g)	Rye crisp bread (3 slices)
Butter (Bregott mellan)	3 tsp	
Ham	2.5 slices (or 5-6 thin slices)	
Bell pepper	2 slices	Tomato, cucumber

2. Oat porridge with raisins and milk. Sandwich with butter, ham and cheese

Food item	*Quantity*	*Alternative*
Rolled oats + water	1 dl + 2 dl	Rye flakes, Buckwheat flakes
Milk (1.5% fat)	2.5 dl	
Raisins	2.5 tbsp	Banana, jam
White bread	1-2 slices (50 g)	Wheat crisp bread (3 slices)
Butter (Bregott mellan)	4 tsp	
Ham	2.5 slices (or 5-6 thin slices)	
Cucumber	4 slices	Bell pepper, tomato

3. Sandwich with butter, ham, eggs and bell pepper. Banana.

Food item	*Quantity*	*Alternative*
White bread	2 large slices (or 3 small)	Wheat crisp bread (3 slices)
Butter (Bregott mellan)	2 tsp	
Ham	2.5 slices (or 5-6 thin slices)	
Eggs	1	Boiled, fried, scrambled
Cheese (17% fat)	2 slices	
Bell pepper	2 slices	Cucumber, tomato
Banana	1 piece	

4. Sandwichs with butter, ham, cheese and tomato. Fruit yoghurt.

Food item	*Quantity*	*Alternative*
Whole grain rye bread	2 slices (80 g)	Rye crisp bread (4 slices)
Butter (Bregott mellan)	4 tsp	
Ham	2.5 slices (or 5-6 thin slices)	
Cheese (17% fat)	1.5 slices	
Tomato	2-3 slices	Cucumber, bell pepper
Fruit yoghurt (0.5%)	2.5 dl	Yoghurt + jam or raisins

5. Yoghurt with muesli and banana. Wheat crisp bread with butter, ham and bell pepper.

Food item	*Quantity*	*Alternative*
Yoghurt (0.5% fat)	2.5 dl	Filmjölk (0.5% fat)
Muesli with fruit and nuts	0,75 dl	
Banana	1	
Wheat crisp bread	2 slices	1 slice white bread
Butter (Bregott mellan)	4 tsp	
Ham	2.5 slices (or 5-6 thin slices)	
Bell pepper	2 slices	Cucumber, tomato

Food items

- *Whole grain rye bread* refers to breads such as Lingongrova, Gott och gräddat, Frökusar, Skördelycka.
- *White bread* refers to breads such as formfranska, bergis, rost/toast.
- *Muesli with fruit and nuts* refers to cereal such as Familjemuesli, F-muesli.
- *Oat cereal (Havrefras)* refers to cereal such as havrefras, Havrekuddar, Havreringar, rågfras, rågkuddar, rågringar

Every day choose **two** of the six light meal/snack alternatives below. Preferably vary your choice from day to day.

- Complete the food diary and check the alternative you have chosen.
- You do not have to eat the light meals/snacks at any particular time, and you can eat them at the same time or separately.

1. Yoghurt with raisins and sunflower seeds. Fruit		
Food item	*Quantity*	*Alternative*
Yoghurt (0.5% fat)	2 dl	Sour milk (0.5%)
Raisins	3 tbsp	Banana, jam
Sunflower seeds	1.5 tbsp	Pumpkin seeds, nuts
Fruit (apple)	1 piece	Pear, orange, nectarine

2. Sandwich with cottage cheese and avocado. Fruit		
Food item	*Quantity*	*Alternative*
White bread	2 slices	Wheat crisp bread (4 slices)
Cottage cheese (4% fat)	2 tbsp	Quark, cream cheese (4%)
Avocado	0.25	
Fruit (apple)	1 piece	Pear, orange, nectarine

3. Cheese sandwich. Fruit		
Food item	*Quantity*	*Alternative*
Whole grain rye bread	2 slices	Rye crisp bread (4 slices)
Butter (Bregott mellan)	2 tsp	
Cheese (17% fat)	1.5 slices	
Fruit (apple)	1 piece	Pear, orange, nectarine

4. Wasa-sandwich and yoghurt drink. Fruit		
Food item	*Quantity*	*Alternative*
Wasa-sandwich	1 piece	Crispbread (2 slices) + Cream cheese 2 tbsp

Yoghurt drink	2.5 dl	Fruit yoghurt (2 dl)
Fruit (apple)	1 piece	Pear, orange, nectarine

5. Rusks with cheese and marmalade and fruit		
Food item	*Quantity*	*Alternative*
Whole grain rusks	1.5 – 2 pieces	
Butter (Bregott mellan)	2 tsp	
Cheese (17%)	2 slices	
Marmalade	1 tbsp	
Fruit (apple)	1 piece	Pear, orange, nectarine

6. Rusks with peanut butter and fruit		
Food item	*Quantity*	*Alternative*
Wheat rusks	2 pieces	
Peanut butter	4 tsp	
Fruit (apple)	1 piece	Pear, orange, nectarine

- The side dishes consist of bread with topping and can be served along with the ready-made lunch or dinner dish or at any time during the day.
- Each day, choose **one** of the five alternatives. Preferably vary your choice from day to day.
- Fill in the food diary and check the alternative you have chosen.

1.	
Crisp bread (Wasa husman)	2 slices
Margarine (Bregott mellan)	1 tsp

2.	
Crisp bread (Finn crisp)	3 slices
Hummus	2 tbsp

3.	
Whole grain rye bread (rågkusar)	0.5 rågkuse
Light mayonnaise (35% fat)	2 tsp

4.	
Crisp bread (Wasa sport)	2 slices
Avocado	0.25 piece

5.	
Whole grain rusks	2 pieces
Peanut butter	1.5 tsp

Analysis of cardiometabolic risk factors, gut microbiota, and plasma metabolome

Anthropometric measurements and assessment of quality of life

A digital automatic sphygmomanometer (OMRON M6 AC, OMRON HEALTHCARE Co., Ltd. Kyoto, Japan) was used to measure blood pressure and heart rate. Blood pressure was measured in the right arm after five minutes of seated rest. Body height in centimeters was measured at baseline. Body weight in kilograms was measured at the four monitoring visits with the participants dressed in light clothing without shoes. BMI was calculated as body weight in kilograms divided by height in meters squared. At all monitoring visits, quality of life was assessed with the EuroQoL five-dimension questionnaire (EQ5D), which assesses mobility, self-care, usual activities, pain/discomfort, and anxiety/depression, with results presented on a visual analogue scale.

Blood sampling and biochemical analyses

Venous blood samples were collected at the four monitoring visits in evacuated plastic tubes (VACUETTE® TUBE, Greiner Bio-One GmbH, Kremsmunster, Austria). Upon collection, the tubes were gently inverted 10 times and placed on ice. Samples were centrifuged in a cooling system at 1560 x g for 10 min at -40°C and stored at -80°C in aliquots for analysis.

Analyses of samples were conducted at the Clinical Chemistry Laboratory, Örebro University Hospital. Total cholesterol (TC), low density lipoprotein cholesterol (LDL-C), high density lipoprotein cholesterol (HDL-C), and triglycerides (TG) were measured by a dry chemistry method using a membrane and colorimetric detection in a Vitros 5.1 FS chemistry system (Ortho Clinical Diagnostics and Johnson & Johnson, Stockholm, Sweden). High-sensitivity CRP (hs-CRP), apolipoprotein A1 (ApoA1), and apolipoprotein B (ApoB) were measured with two-site sandwich assays on a Siemens ADVIA 1800 Chemistry System (Siemens Healthcare,

Upplands Väsby, Sweden). A six-point calibration curve and pooled samples for quality control were also assayed to confirm accurate measurement according to SS-EN ISO/IEC 15 189, STAFS 2011:33 and STAFS 2010:10 (SWEDAC). The HbA_{1c} was measured by the gold standard method on a Tosho G8 High Performance Liquid Chromatography instrument (Tosho Bioscience, Tessenderlo, Belgium).

We used a sandwich ELISA assay kit (Mercodia, Uppsala, Sweden) for quantitative measure of plasma oxidized LDL-C levels. The assay uses the specific murine monoclonal antibody mAb-4E6 directed against the oxidized antigenic determinants on the oxidized ApoB molecule, as described by Holvoet et al. Fresh-frozen plasma (25 µL) was diluted in two steps to a final dilution of 1/6561 and combined with 100 µL of assay buffer and 25 µL of each calibrator. Control and diluted samples were added to appropriate wells on the plate. Plates were incubated in a plate shaker (700–900 rpm) for 2 hours at 20^{o}C and washed six times with 700 µL wash buffer per well using an automatic plate washer. The plates were inverted and tapped against absorbent tissue, and 100 µL of enzyme conjugate was added to each well. Following a second incubation for 1 h on a plate shaker, the plates were washed, and 200 µL of substrate tetramethylbenzidine was added. Finally, the plates were incubated for 15 min on the bench, 50 µL of a solution to stop the reaction was added, and the optical density was measured spectrophotometrically at 450 nm. Two commercially available controls were included on each plate (n=3) for internal quality control. The intra-assay coefficient of variation for oxidized LDL-C concentration was <10% for 95.7% of samples.

16S rRNA gene sequencing for gut microbiome

Fecal samples were collected in sterile stool tubes on the day prior to each baseline/follow-up visit and stored at Örebro biobank at -80°C until extraction. DNA was extracted from each

sample by repeated bead-beating and was subjected to 16S rRNA gene sequencing in an Illumina Miseq instrument (Illumina, San Diego, California, USA) using the 515F and 806R primers and the V2 kit (2 × 250 bp paired-end reads) (Illumina).

Illumina reads were merged using Usearch v. 11 64-bit allowing for up to 30 mismatches in the alignment of the paired-end reads, while discarding reads with a merged length greater than 270 bp and fewer than 230 bp. The merged reads were quality-filtered based on expected errors, removing reads above the threshold of 1.0. The merged reads were converted to zero-radius operational taxonomic units (ZOTU) by compiling the sequences into sets of unique reads and performing error-correction using the UNOISE3 algorithm, discarding sequences with fewer than four reads. The ZOTUs were assigned taxonomy using DADA2's assign Taxonomy (minBoot = 80) and were assigned species, using the properly formatted version of the Silva v. 132 database. A phylogenetic tree of the sequence attributed to each ZOTU was created by aligning the reads using MAFFT v. 7.407 and FastTree v. 2.1.10. The process produced 3,126 ZOTUs after removing dropouts, comprising 8,344,360 reads from 102 samples. The OTU-table was subjected to filtering based on abundance, and ZOTUs below 0.002% of total reads in the table were discarded,[13] resulting in 1,264 amplicon sequence variants in 102 samples (8,253,321 reads). Data were rarefied to the minimum sample sequence depth (56,103 reads) to reduce the effect of sequencing depth.

Measurement of fecal short chain fatty acids and branched chain fatty acids

Fecal concentrations of the short chain fatty acids (SCFA) acetate, propionate, and butyrate and branched chain fatty acids (BCFA) isobutyrate and isovalerate, as well as succinate and lactate, were determined using gas chromatograph-mass spectrometry (Agilent Technologies) as previously described. In brief, 100 mg of frozen fecal material was

transferred to a 16 x 125 mm glass tube fitted with a screw cap, and a volume of 100 µL of internal standard stock solution [(1-13C)acetate, (2H6)propionate 1 M, (13C4)butyrate 0.5 M, (1-13C1)isobutyrate, and (1-13C)isovalerate 0.1 M] was added. Prior to extraction, samples were freeze-dried at -50^{o}C for 3 h. After acidification with 50 µL of 37% HCl, the organic acids were extracted twice in 2 mL of diethyl ether. A 500 µL aliquot of the extracted sample was mixed with 50 µL of N-tert-butyldimethylsilyl-N-methyltrifluoracetamide (Sigma) at 20^{o}C. One µl of the derived material was injected into a gas chromatograph (Agilent Technologies 7890 A) coupled to a mass spectrometer detector (Agilent Technologies 5975 C). Temperature was increased in a linear gradient consisting of initial temperature of 65^{o}C for 6 min, increase to 260 ^{o}C at 15^{o}C min^{-1}, and increase to and held at 280^{o}C for 5 min. The injector and transfer line temperatures were 250^{o}C. Quantitation was completed in ion-monitoring acquisition mode by comparison to labelled internal standards, with the m/z ratios 117 (acetic acid), 131 (propionic acid), 145 (butyric acid), 146 (isobutyric acid), 159 (isovaleric acid), 121 [(2H2)- and (1-13C)acetate], 136 [(2H5)propionate], 146 [(1-13C1)isobutyrate], 149 [(13C4) butyrate], 160 [(1-13C)isovalerate].

Plasma metabolome analysis

Plasma samples were de-proteinized using ultracentrifugation and analyzed by high performance liquid chromatography-quadrupole time-of-flight mass spectrometry (HPLC-qTOF-MS/MS, Agilent qTOF 6520) (Agilent Technologies). Reverse-phase chromatography was applied using an ACQUITY UPLC HSS T3 Column (130Å, 1.8 µm, 2.1 mm×100 mm (Waters) in positive (ESI+) and negative electrospray ionization (ESI-) modes.

The mobile phase delivered at 400 µL/min consisted of eluent A (MilliQ purified water) (Millipore) and eluent B (methanol, methanol CHROMASOLV™ LC-MS Ultra) (Honeywell

Riedel-de Haen), both containing 0.04% (vol:vol) formic acid. The ESI source was operated under the following conditions: gas (nitrogen) temperature 175°C and dry gas flow 10 L/min, nebulizer pressure 45 PSI, capillary voltage 3500 V, fragmenter 125 V, and a skimmer of 65V. For data acquisition, a 2-GHz extended dynamic range mode was used, with the instrument set to acquire data over the m/z range 50–1700. Data were collected in centroid mode at an acquisition rate of 1.67 spectra/s with an abundance threshold of 200 counts. Continuous mass axis calibration was performed in an infusion solution throughout the runs by monitoring reference ions m/z 121.050873 and m/z 922.009798 for positive mode and m/z 112.988900 and 966.000725 for negative mode.

Plasma samples were analyzed in two batches. Within-individual samples were analyzed in the same batch, with full within-batch randomization. The stability and functionality of the system were monitored throughout the instrumental analyses using pooled plasma samples as quality control. Data acquisition used MassHunter Acquisition software (Agilent Technologies).

Raw data acquired in each analytical batch were converted to mzXL format, and deconvolution was performed with the open source R package "XCMS." Key parameters of XCMS including peak detection, alignment, and correspondence were optimized using the R package "IPO" to increase the reliability and stability of processed metabolomics data. The number of obtained metabolite features (mass spectral peak, a molecular entity with a unique mass-to-charge ratio and retention time as measured by LC-MS instrument) with ESI+ and ESI- was 1645 and 1363, respectively. The within- and between- batch measurement errors due to shifts in retention time, mass-to-charge ratio (m/z), and intensity of metabolite features between analytical runs were corrected using R package "batchCorr." After correction,

metabolite features passing the quality control tests (CV<0.3) in both batches were considered qualified features and were subjected to statistical analysis. In total, 840 and 982 features from the ESI+ and ESI- were retained after stringent normalization procedures.

Plasma trimethylamine N-oxide (TMAO), choline, carnitine, and acetyl-carnitine were measured by liquid chromatography tandem mass spectrometry (LC-MS/MS) analyzed on an Exion UHPLC coupled to a QTRAP 6500+ MS/MS system, both from AB Sciex LLC (Framingham, USA). Plasma (50 μL) was thoroughly mixed with methanol (150 μL) and internal standard solution (50 μL) containing d9-TMAO, d11-choline, and d9-carnitine was centrifuged at 15 000 x g at 5°C for 10 min and supernatants collected on vials for analysis. Calibration curves were obtained from a stock solution containing all compounds. The separation used a Waters BEH Amide column (100 x2.1 mm, 1.7 μm) at 35°C with flow of 0.75 mL/min. The gradient was 0% B 0–1.3 min to 80% B at 4.5 min (B was 10mM ammonium formate in acetonitrile and A 10 mM ammonium formate in water, pH 3, injection volume 0.3 μL). The analytes were detected using positive electrospray ionization. Transitions were TMAO 76.1–>58.2, choline 105.1–>61, L-carnitine 163.1–>116.9, acetyl-carnitine 204.1–>85.1.

Statistical analysis

All analyses were performed in R v. 3.5.1. (R Foundation for Statistical Computing, Vienna, Austria).

Packages	Purpose	References and open source tutorials
XCMS	Metabolomics data processing	https://bioconductor.org/packages/release/bioc/vignettes/xcms/inst/doc/xcms.html
IPO	XCMS parameters optimization	https://bioconductor.org/packages/release/bioc/vignettes/IPO/inst/doc/IPO.html

mixOmics	Supervised modelling	http://mixomics.org/
batchCorr	Metabolomics data normalization	https://www.ncbi.nlm.nih.gov/pubmed/27746707
MUVR	Supervised modelling	https://academic.oup.com/bioinformatics/article/35/6/972/5085367
vegan	Microbiota data analyses	http://cc.oulu.fi/~jarioksa/opetus/metodi/vegantutor.pdf
ggplot2	Results visualization and interpretation	https://cran.r-project.org/web/packages/ggplot2/ggplot2.pdf
phyloseq	Microbiome census data analysis	https://joey711.github.io/phyloseq/
Picante	Microbiota diversity calculation	https://cran.r-project.org/web/packages/picante/vignettes/picante-intro.pdf
Hmisc	Descriptive statistics	http://math.furman.edu/~dcs/courses/math47/R/library/Hmisc/html/Overview.html
lme4	Generalized linear mixed model	https://github.com/lme4/lme4/

Descriptive statistics of anthropometric measurements and clinical markers

Normality of distribution of the variables and residuals was visualized using a histogram and tested using the Shapiro-Wilks test. Missing values were imputed in an intention-to-treat analysis using the last observation carried forward method for the participants (n=2) who were randomized but did not receive intervention and for the participants that dropped out after the first intervention (n=2). A two-sided *P* value <0.05 was considered significant.

To investigate whether a VD could alter cardiovascular risk factor in IHD patients, the number of participants exhibiting guideline target values of clinical markers before and after intervention was calculated. Cut-off values of clinical markers routinely measured after a cardiac event were defined based on European Guidelines on cardiovascular disease

prevention in clinical practice: LDL-C <1.8 mmol/L, systolic blood pressure <130 mmHg or diastolic blood pressure <80 mmHg, and BMI <25 kg/m^2.

Descriptive statistics of gut microbiota

The graphic representations and statistical analyses of microbiota were performed using phyloseq v. 1.26 and ggplot2 v. 3. To investigate bacterial richness and phylogenetic diversity within samples, Faith's Phylogentic Diversity and richness were calculated using Picante v. 1.7, and pairwise comparisons of the diets were implemented using a paired Wilcoxon test. To compare gut bacterial patterns of subjects consuming the same and different diets, Bray-Curtis dissimilarity, Weighted UniFrac, and Unweighted UniFrac were calculated using Vegan v. 2.5-4. Principal Coordinates Analysis (PcoA) and a permutation ANOVA (Adonis) was performed to assess differences in the microbiome associated with the diets. A differential abundance analysis of the OTUs was conducted on a subset of the OTUs existing in more than 20 samples using a pairwise paired Wilcoxon test.

Multilevel-predictive modelling for gut microbiota and plasma metabolome

To identify microbial taxa and plasma metabolites discriminating the VD from the MD, random-forest-modeling-based multilevel data analyses (ML-RF) were applied using the R package 'MUVR' for the pair-wise comparison of the ZOTUs or metabolites in subjects consuming the VD and MD. A multilevel analysis deals with dependent data structures and has been successfully used to exploit the differences specific to diet in cross-over human nutrition intervention studies. In this ML-RF algorithm, random forest is applied on the within-subject variation matrix, i.e. the relative abundance of microbial genera observed in the two interventions as independent variables. The model is further incorporated into repeated double cross-validation with unbiased variable selection to reduce statistical overfitting,

improve prediction accuracy, and to identify the most informative features of treatments. Permutation analysis was performed to test overall model validity and degree of overfitting by calculating the cumulative probability of actual model misclassification within a t-distributed H0 population (n=100).

We applied generalized linear mixed modelling to compare VD vs. MD with respect to each of the selected discriminative ZOTUs and metabolites using the R package 'lme4.' Fixed factors included diet, sequence of intervention allocation, and baseline values as covariates and subject as random factor. This analysis was also applied on fecal concentrations of SCFAs. Within-treatment effects were similarly investigated with respect to relative abundance of microbial taxa at baseline and following intervention.

Baseline gut microbiota and plasma metabolites associated with response to diet with respect to clinical outcomes

We investigated whether gut microbiota configuration or plasma metabolome at baseline was associated with the effects a VD on metabolic risk factors including oxidized LDL-C, LDL-C, TC, and BMI. Random forest modelling (R package 'MUVR') was used to identify a panel of baseline microbial genera and plasma metabolites that discriminated subjects who showed lower metabolic risk factors after VD than seen with MD (responders) from those in whom VD did not reduce improve metabolic risk factors compared to MD (non-responders).

Energy-adjusted meal plans

The meal plan was personally adapted according to individual energy requirements and was energy- and macronutrient balanced. A research dietitian calculated the energy requirement for each subject by multiplying the Basal Metabolic Rate (BMR) with the Physical Activity Level (PAL). Henry´s energy equation was used to calculate the BMR and the PAL values according

to Nordic Nutrition Recommendations based on data of physical activity stated by participants at the first study visit.

Table S1. Details of ready-made meat meals.

Week	Day	Meal	Dish	Weight (g)	Energy (kcal)	Protein (g)	Fat (g)	Carbohydrates (g)	Fiber (g)	Saturated fatty acids (g)
1.	1. Mon	Lunch	Dafgårds – Chicken Quiche	240	592.8	22.32	38.4	38.4	2.88	23.28
		Dinner	Dafgårds - Meatballs & Red Peppers	400	480	24	24	36	12	10.8
		Total			1072.8	46.32	62.4	74.4	14.88	34.08
	2. Tue	Lunch	Dafgårds - Spaghetti Bolognese	400	480	24.4	16.4	64	5.6	7.6
		Dinner	Dafgårds – Cheese and Ham Quiche	240	602.4	21.36	40.8	36	1.68	24
		Total			1082.4	45.76	57.2	100	7.28	31.6
	3. Wed	Lunch	Dafgårds – Beef Stew	400	364	20	13.2	35.6	4.8	5.2
		Dinner	Dafgårds – Kebab	400	672	20.4	44	56	7.2	16
		Total			1036	40.4	57.2	91.6	12	21.2
	4. Thur	Lunch	Dafgårds – Greek Beef	380	456	18.24	23.94	41.8	6.84	9.5
		Dinner	Dafgårds – Hash	380	646	14.44	37.62	60.8	4.94	11.78
		Total			1102	32.68	61.56	102.6	11.78	21.28
	5. Fri	Lunch	Dafgårds – Oven roasted chicken	420	504	30.66	13.02	63	4.2	5.88
		Dinner	Dafgårds – Taco plate	390	565.5	19.11	26.52	58.5	3.9	12.09
		Total			1069.5	49.77	39.54	121.5	8.1	17.97
	6. Sat	Lunch	Dafgårds – Fried Falun sausage	400	504	10.4	38.4	48	3.2	13.2
		Dinner	Dafgårds – Fried Pork Loin	420	558.6	22.68	26.04	54.6	7.14	8.82
		Total			1062.6	33.08	64.44	102.6	10.34	22.02
	7. Sun	Lunch	Dafgårds – Chicken Lasagna	420	504	29.4	13.86	67.2	2.52	8.4
		Dinner	Dafgårds – Angus burger	380	558.6	17.1	36.86	38	4.56	14.82
		Total			1062.6	46.5	50.72	105.2	7.08	23.22
2.	8. Mon	Lunch	Dafgårds – Farmer burger	400	640	20.4	38	52	6.8	11.2
		Dinner	Dafgårds - Spaghetti Bolognese	400	480	24.4	16.4	64	5.6	7.6

		Total			1120	44.8	54.4	116	12.4	18.8
	9. Tue	Lunch	Dafgårds - Greek Beef	380	456	18.24	23.94	41.8	6.84	9.5
		Dinner	Dafgårds – Chicken schnitzel	390	624	23.4	29.25	66.3	7.02	7.02
		Total			1080	41.64	53.19	108.1	13.86	16.52
	10. Wed	Lunch	Dafgårds - Cheese and Ham Quiche	240	602.4	21.36	40.8	36	1.68	24
		Dinner	Dafgårds - Meatballs & Red Peppers	400	480	24	24	36	12	10.8
		Total			1082.4	45.36	64.8	72	13.68	34.8
	11. Thur	Lunch	Dafgårds - Fried Falun sausage	400	504	10.4	38.4	48	3.2	13.2
		Dinner	Dafgårds - Chicken Quiche	240	592.8	22.32	38.4	38.4	2.88	23.28
		Total			1096.8	32.72	76.8	86.4	6.08	36.48
	12. Fri	Lunch	Dafgårds - Beef Stew	400	364	20	13.2	35.6	4.8	5.2
		Dinner	Dafgårds – Italian style veal burgers	400	660	24.4	32.4	64	6.4	9.2
		Total			1024	44.4	45.6	99.6	11.2	14.4
	13. Sat	Lunch	Dafgårds - Oven roasted chicken	420	504	30.66	13.02	63	4.2	5.88
		Dinner	Dafgårds – Taco plate	390	565.5	19.11	26.52	58.5	3.9	12.09
		Total			1069.5	49.77	39.54	121.5	8.1	17.97
	14. Sun	Lunch	Dafgårds - Cabbage pudding	400	352	13.6	22.8	37.6	6	5.6
		Dinner	Dafgårds – Kebab	400	672	20.4	44	56	7.2	16
		Total			1024	34	66.8	93.6	13.2	21.6
3.	15. Mon	Lunch	Dafgårds – Angus burger	380	558.6	17.1	36.86	38	4.56	14.82
		Dinner	Dafgårds - Chicken Lasagna	420	504	29.4	13.86	67.2	2.52	8.4
		Total			1062.6	46.5	50.72	105.2	7.08	23.22
	16. Tue	Lunch	Dafgårds - Farmer burger	400	640	20.4	38	52	6.8	11.2
		Dinner	Dafgårds – Pork stew	380	418	20.14	9.88	57	3.04	4.56
		Total			1058	40.54	47.88	109	9.84	15.76
	17. Wed	Lunch	Dafgårds - Fried Pork Loin	420	558.6	22.68	26.04	54.6	7.14	8.82
		Dinner	Dafgårds - Oven roasted chicken	420	504	30.66	13.02	63	4.2	5.88
		Total			1062.6	53.34	39.06	117.6	11.34	14.7
	18. Thur	Lunch	Dafgårds - Cabbage pudding	400	352	13.6	22.8	37.6	6	5.6

		Dinner	Dafgårds - Italian style veal burgers	400	660	24.4	32.4	64	6.4	9.2
		Total			1012	38	55.2	101.6	12.4	14.8
	19. Fri	Lunch	Dafgårds - Hash	380	646	14.44	37.62	60.8	4.94	11.78
		Dinner	Dafgårds - Greek Beef	380	456	18.24	23.94	41.8	6.84	9.5
		Total			1102	32.68	61.56	102.6	11.78	21.28
	20. Sat	Lunch	Dafgårds – Kebab	400	672	20.4	44	56	7.2	16
		Dinner	Dafgårds - Meatballs & Red Peppers	400	480	24	24	36	12	10.8
		Total			1152	44.4	68	92	19.2	26.8
	21. Sun	Lunch	Dafgårds - Spaghetti Bolognese	400	480	24.4	16.4	64	5.6	7.6
		Dinner	Dafgårds - Chicken schnitzel	390	624	23.4	29.25	66.3	7.02	7.02
		Total			1104	47.8	45.65	130.3	12.62	14.62
4.	22. Mon	Lunch	Dafgårds - Chicken Quiche	240	592.8	22.32	38.4	38.4	2.88	23.28
		Dinner	Dafgårds - Angus burger	380	558.6	17.1	36.86	38	4.56	14.82
		Total			1151.4	39.4	75.26	76.4	7.44	38.1
	23. Tue	Lunch	Dafgårds - Cabbage pudding	400	352	13.6	22.8	37.6	6	5.6
		Dinner	Dafgårds - Italian style veal burgers	400	660	24.4	32.4	64	6.4	9.2
		Total			1012	38	55.2	101.6	12.4	14.8
	24. Wed	Lunch	Dafgårds - Cheese and Ham Quiche	240	602.4	21.36	40.8	36	1.68	24
		Dinner	Dafgårds – Pork Stew	380	418	20.14	9.88	57	3.04	4.56
		Total			1020.4	41.5	50.68	93	4.72	28.56
	25. Thur	Lunch	Dafgårds - Beef Stew	400	364	20	13.2	35.6	4.8	5.2
		Dinner	Dafgårds - Hash	380	646	14.44	37.62	60.8	4.94	11.78
		Total			1010	34.44	50.82	96.4	9.74	16.98
	26. Fri	Lunch	Dafgårds - Fried Falun sausage	400	504	10.4	38.4	48	3.2	13.2
		Dinner	Dafgårds - Chicken schnitzel	390	624	23.4	29.25	66.3	7.02	7.02
		Total			1128	33.8	67.65	114.3	10.22	20.22
	27. Sat	Lunch	Dafgårds - Farmer burger	400	640	20.4	38	52	6.8	11.2
		Dinner	Dafgårds - Chicken Lasagna	420	504	29.4	13.86	67.2	2.52	8.4
		Total			1144	49.8	51.86	119.2	9.32	19.6

	28. Sun	Lunch	Dafgårds - Taco plate	390	565.5	19.11	26.52	58.5	3.9	12.09
		Dinner	Dafgårds - Fried Pork Loin	420	558.6	22.68	26.04	54.6	7.14	8.82
		Total			1124.1	41.79	52.56	113.1	11.04	20.91
			Mean/meal		1076	41.43	53.68	106.52	10.7	22.2

Kcal=kilocalorie

Table S2. Details of ready-made vegetarian meals.

Week	Day	Meal	Dish	Weight (g)	Energy (kcal)	Protein (g)	Fat (g)	Carbohydrates (g)	Fiber (g)	Saturated fatty acids (g)
1.	1. Mon	Lunch	Dafgårds - Pea/Sun-dried tomato steak with pasta	400	576	20.4	24	66	7.6	4
		Dinner	Dafgårds - Cheese & Broccoli pie	220	484	19.8	28.6	37.4	2.64	14.52
		Total			1060	40.2	52.6	103.4	10.24	18.52
	2. Tue	Lunch	Dafgårds - Asparagus pie	240	561.6	19.2	38.4	36	2.64	23.04
		Dinner	Dafgårds – Broccoli balls with quinoa and pepper sauce	400	448	27.2	15.2	45.6	10.4	5.6
		Total			1009.6	46.4	53.6	81.6	13.04	28.64
	3. Wed	Lunch	Dafgårds – Vegetable pie with wholegrain crust	220	440	14.96	21.56	41.8	7.04	9.9
		Dinner	Dafgårds - Falafel	400	560	20.4	25.2	64	11.2	3.6
		Total			1000	35.36	46.76	105.8	18.24	13.5
	4. Thur	Lunch	Dafgårds - Pea/Sun-dried tomato steak with pasta	420	508.2	19.32	16.8	65.52	7.14	4.62
		Dinner	Dafgårds - Indian lentil Stew	400	516	23.2	20.4	56	11.6	11.2
		Total			1024.2	42.52	37.2	121.52	18.74	15.82
	5. Fri	Lunch	Dafgårds - Cheese & Broccoli pie	220	484	19.8	28.6	37.4	2.64	14.52
		Dinner	Dafgårds – Veggie burger	400	516	19.2	26.4	48	9.6	4.4
		Total			1000	39	55	85.4	12.24	18.92
	6. Sat	Lunch	Dafgårds - Kale steak	380	338.2	13.68	6.84	53.2	9.12	0.76
		Dinner	Dafgårds - Asparagus pie	240	561.6	19.2	38.4	36	2.64	23.04
		Total			899.8	32.88	45.24	89.2	11.76	23.8
	7. Sun	Lunch	Dafgårds – Broccoli balls with quinoa and pepper sauce	400	448	27.2	15.2	45.6	10.4	5.6
		Dinner	Dafgårds - Mexican bean steak	390	534.3	14.04	24.96	66.3	4.68	8.97
		Total			982.3	41.24	40.16	111.9	15.08	14.57
2.	8. Mon	Lunch	Dafgårds - Cheese & Broccoli pie	220	484	19.8	28.6	37.4	2.64	14.52
		Dinner	Dafgårds - Falafel	400	560	20.4	25.2	64	11.2	3.6
		Total			1044	40.2	53.8	101.4	13.84	18.12

	9. Tue	Lunch	Dafgårds – Vegetable pie with wholegrain crust	220	440	14.96	21.56	41.8	7.04	9.9
		Dinner	Dafgårds - Pea/Sun-dried tomato steak with pasta	400	576	20.4	24	66	7.6	4
		Total			1016	35.36	45.56	107.8	14.64	13.9
	10. Wed	Lunch	Dafgårds – Broccoli balls with quinoa and pepper sauce	400	448	27.2	15.2	45.6	10.4	5.6
		Dinner	Dafgårds - Mexican bean steak	390	534.3	14.04	24.96	66.3	4.68	8.97
		Total			982.3	41.24	40.16	111.9	15.08	14.57
	11. Thur	Lunch	Dafgårds - Indian lentil Stew	400	516	23.2	20.4	56	11.6	11.2
		Dinner	Dafgårds – Veggie burger	400	516	19.2	26.4	48	9.6	4.4
		Total			1032	42.4	46.8	104	21.2	15.6
	12. Fri	Lunch	Dafgårds - Kale steak	380	338.2	13.68	6.84	53.2	9.12	0.76
		Dinner	Dafgårds - Asparagus pie	240	561.6	19.2	38.4	36	2.64	23.04
		Total			899.8	32.88	45.24	89.2	11.76	23.8
	13. Sat	Lunch	Dafgårds – Sun-dried tomato/pea steak with vegetables	420	508.2	19.32	16.8	65.52	7.14	4.62
		Dinner	Dafgårds - Mexican bean steak	390	534.3	14.04	24.96	66.3	4.68	8.97
		Total			1042.5	33.36	41.76	131.82	11.82	13.59
	14. Sun	Lunch	Dafgårds - Falafel	400	560	20.4	25.2	64	11.2	3.6
		Dinner	Dafgårds - Indian lentil Stew	400	516	23.2	20.4	56	11.6	11.2
		Total			1076	43.6	45.6	120	22.8	14.8
3.	15. Mon	Lunch	Dafgårds - Pea/Sun-dried tomato steak with pasta	400	576	20.4	24	66	7.6	4
		Dinner	Dafgårds - Kale steak	380	338.2	13.68	6.84	53.2	9.12	0.76
		Total			914.2	34.08	30.84	119.2	16.72	4.76
	16. Tue	Lunch	Dafgårds - Asparagus pie	240	561.6	19.2	38.4	36	2.64	23.04
		Dinner	Dafgårds – Broccoli balls with quinoa and pepper sauce	400	448	27.2	15.2	45.6	10.4	5.6
		Total			1009.6	46.4	53.6	81.6	13.04	28.64
	17. Wed	Lunch	Dafgårds - Sun-dried tomato/pea steak with vegetables	420	508.2	19.32	16.8	65.52	7.14	4.62
		Dinner	Dafgårds - Falafel	400	560	20.4	25.2	64	11.2	3.6

		Total			1068.2	39.72	42	129.52	18.34	8.22
	18. Thur	Lunch	Dafgårds – Veggie burger	400	516	19.2	26.4	48	9.6	4.4
		Dinner	Dafgårds - Cheese & Broccoli pie	220	484	19.8	28.6	37.4	2.64	14.52
		Total			1000	39	55	85.4	12.24	18.92
	19. Fri	Lunch	Dafgårds - Asparagus pie	240	561.6	19.2	38.4	36	2.64	23.04
		Dinner	Dafgårds - Indian lentil Stew	400	516	23.2	20.4	56	11.6	11.2
		Total			1077.6	42.4	58.8	92	14.24	34.24
	20. Sat	Lunch	Dafgårds - Kale steak	380	338.2	13.68	6.84	53.2	9.12	0.76
		Dinner	Dafgårds - Pea/Sun-dried tomato steak with pasta	400	576	20.4	24	66	7.6	4
		Total			914.2	34.08	30.84	119.2	16.72	4.76
	21. Sun	Lunch	Dafgårds – Vegetable pie with wholegrain crust	220	440	14.96	21.56	41.8	7.04	9.9
		Dinner	Dafgårds - Mexican bean steak	390	534.3	14.04	24.96	66.3	4.68	8.97
		Total			974.3	29	46.52	108.1	11.72	18.87
4.	22. Mon	Lunch	Dafgårds - Veggieburger	400	516	19.2	26.4	48	9.6	4.4
		Dinner	Dafgårds - Sun-dried tomato/pea steak with vegetables	420	508.2	19.32	16.8	65.52	7.14	4.62
		Total			1024.2	38.52	43.2	113.52	16.74	9.02
	23. Tus	Lunch	Dafgårds - Falafel	400	560	20.4	25.2	64	11.2	3.6
		Dinner	Dafgårds – Vegetable pie with wholegrain crust	220	440	14.96	21.56	41.8	7.04	9.9
		Total			1000	35.36	46.76	105.8	18.24	13.5
	24. Wed	Lunch	Dafgårds - Pea/Sun-dried tomato steak with pasta	400	576	20.4	24	66	7.6	4
		Dinner	Dafgårds - Kale steak	380	338.2	13.68	6.84	53.2	9.12	0.76
		Total			914.2	34.08	30.84	119.2	16.72	4.76
	25. Thur	Lunch	Dafgårds – Broccoli balls with quinoa and pepper sauce	400	448	27.2	15.2	45.6	10.4	5.6
		Dinner	Dafgårds - Asparagus pie	240	561.6	19.2	38.4	36	2.64	23.04
		Total			1009.6	46.4	53.6	81.6	13.04	28.64
	26. Fri	Lunch	Dafgårds - Cheese & Broccoli pie	220	484	19.8	28.6	37.4	2.64	14.52

		Dinner	Dafgårds – Veggie burger	400	516	19.2	26.4	48	9.6	4.4
		Total			1000	39	55	85.4	12.24	18.92
	27. Sat	Lunch	Dafgårds - Sun-dried tomato/pea steak with vegetables	420	508.2	19.32	16.8	65.52	7.14	4.62
		Dinner	Dafgårds - Indian lentil Stew	400	516	23.2	20.4	56	11.6	11.2
		Total			1024.2	42.52	37.2	121.52	18.74	15.82
	28. Sun	Lunch	Dafgårds – Vegetable pie with wholegrain crust	220	440	14.96	21.56	41.8	7.04	9.9
		Dinner	Dafgårds - Mexican bean steak	390	534.3	14.04	24.96	66.3	4.68	8.97
		Total			974.3	29	46.52	108.1	11.72	18.87
			Mean/meal		999	38.5	45.7	104.8	15	17

Kcal=kilocalorie

Table S3. Dietary intake before and during the two intervention periods, VD (vegetarian diet) and MD (meat diet), based on 3-day weighed food records+.

	Pre VD		*VD*		*Pre MD*		*MD*	
	Pooled data (n=31)	Original data (n=20)	Pooled data (n=31)	Original data (n=18)	Pooled data (n=31)	Original data (n=18)	Pooled data (n=31)	Original data (n=20)
	mean	median [IQR]	mean	median [IQR]	mean	median [IQR]	mean	median [IQR]
Energy (kcal)	2147	2097 [1929-2360]	2168	2079 [1797-2515]	2373	2266 [2104-2630]	2267	2286 [2042-2567]
Protein (g)	85*	86 [72-101]	78*	79 [65-86]	92**	92 *** [79-99]	80**	81*** [73-88]
Carbohydrates (g)	229	216 [192-294]	247	249 [197-294]	259	253 [201-285]	266	251 [209-293]
Fat (g)	89	89 [73-101]	89	83 [68-102]	96	92 [86-107]	90	85 [71-102]
Saturated fat (g)	36	35 [28-44]	36	35 [27-43]	39	38 [30-49]	34	33 [29-41]
Fiber (g)	24	24 [19-28]	31****	30 [26-37]	26	27 [22-31]	28****	29 [25-33]

♦ Pre VD/MD: Reported dietary intake before VD or MD intervention period (at baseline or end of washout) ♦ VD/MD: Reported dietary intake of VD/MD ♦ ♦ IQR: Interquartile range ♦ Pooled data: Multiple imputation was used for missing values ♦ Wilcoxon Rank Sum test was used for all comparisons ♦ p <0.05 was considered significant ♦

** Borderline significant difference in pooled data (Imputation number/p-values: 1/0.006, 2/0.04, 3/0.01, 4/0.03, 5/0.07) of protein intake of Pre VD and VD*

*** Significant difference in pooled data (Imputation number/p-values: 1/P=0.000, 2/P=0.000, 3/P=0.000, 4/P=0.001, 5/P=0.000) of protein intake between Pre MD and MD. *** Significant difference in original data (p=0.046) of protein intake between Pre MD and MD.*

***** Significant difference in pooled data (Imputation number/p-values: 1/P=0.008, 2/P=0.02, 3/P=0.02, 4/P=0.002, 5/P=0.01) between fiber intake of VD and MD.*

+three-day weighed food information was collected four times during the study: during the week before baseline, the last week of VD, the last week of the washout period, and the last week of MD. 58% (18/31) completed all four registrations. Food records with daily total energy intake:basal metabolic rate <1 were considered as underreported and excluded from the analysis. n of original data refers to the number subjects completing the food record and that remain following exclusion.

Table S4. Adherence to the intervention diets.

	VD		*MD*	
	Pooled data (n=31)	Original data (n=28)	Pooled data (n=31)	Original data (n=27)
	mean %	median % [IQR]	mean %	median % [IQR]
Breakfast	94	100 [96-100]	92	100 [96-100]
Lunch	97	100 [96-100]	98	100 [96-100]
Dinner	94	98 [93-100]	96	96 [95-100]
Side dish	79	92 [73-99]	68	89 [30-100]
Snack (light meals)	67*	76 [43-94]	57*	50 [40-94]
Overall adherence	86**	88 [83-98]	83**	88 [77-96]

♦ *Pooled data: Multiple imputation was used for missing values* ♦ *Wilcoxon signed rank sum test was used for analysis* ♦

**Significant difference in overall adherence of the pooled data (Imputation number/P-values: 1/P=0.01, 2/P=0.02, 3/P=0.01, 4/P=0.02, 5/P=0.01) in snacks of VD and MD.*

***Borderline significant difference in overall adherence (Imputation number/p-values: 1/P=0.05, 2/P=0.03, 3/P=0.03, 4/P=0.04, 5/P=0.1) between VD and MD.*

VD (vegetarian diet) and MD (meat diet), in mean percentage (%) adherence the prescribed diet, calculated from the self-reported study diaries. To assess the adherence to the intervention diets, the participants were asked to conduct a compliance diary every day during the two intervention periods.

Table S5. Participants exhibiting guideline target values of clinical markers/dietary intervention.

	Pre VD (n=31)	Post VD (n=31)	Pre MD (n=31)	Post MD (n=31)	Post VD vs. Post MD
LDL-C <1.8 mmol/L, n (%)	22 (71.0)	27 (87.1)	22 (71.0)	24 (77.4)	-3 (9.7)
BMI <25 kg/m2, n (%)	4 (12.9)	6 (19.4)	5 (16.1)	4 (12.9)	-2 (6.5)
Diastolic Bp <80 mmHg, n (%)	9 (29.0)	8 (25.8)	7 (22.6)	6 (19.4)	-2 (6.5)
Systolic Bp <130 mmHg, n (%)	11 (35.5)	14 (45.2)	9 (29.0)	12 (38.7)	-2 (6.5)

BMI, Body-mass index; Bp, Blood pressure; LDL-C, Low-density lipoprotein cholesterol; MD, meat diet; VD, vegetarian diet.

Table S6. Assessed quality of life relative to dietary intervention.

	Pre VD n=31	Post VD n=31	Pre MD n=31	Post MD n=31	Post VD vs. Post MD
Eq5d, VAS	81.38 (77.25–85.53)	80.84 (76.21–85.47)	80.58 (76.63–84.53)	80.45 (75.55–85.35)	-0.37 (-3.74–2.99)
Mobility					
-No problems, n (%)	27 (87)	27 (87)	28 (90)	27 (87)	0 (0)
-Some problems, n (%)	4 (13)	4 (13)	3 (10)	4 (13)	0 (0)
-Extreme problems, n (%)	0 (0)	0(0)	0 (0)	0 (0)	0 (0)
Self-care					
-No problems, n (%)	31 (100)	31(100)	31 (100)	31(100)	0 (0)
-Some problems, n (%)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
-Extreme problems, n (%)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Usual-activities					
-No problems, n (%)	30 (97)	31 (100)	31(100)	31(100)	0 (0)
-Some problems, n (%)	1 (3)	0 (0)	0 (0)	0 (0)	0 (0)
-Extreme problems, n (%)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Pain/discomfort					
-No problems, n (%)	17 (55)	17 (55)	16 (52)	17 (55)	0 (0)
-Some problems, n (%)	14 (45)	14 (45)	14 (45)	14 (45)	0 (0)
-Extreme problems, n (%)	0 (0)	0 (0)	1 (3)	0 (0)	0 (0)
Anxiety/depression					
-No problems, n (%)	24 (77)	26 (84)	24 (77)	25 (81)	1 (3.2)
-Some problems, n (%)	7 (23)	5 (16)	7 (23)	6 (19)	-1 (-3.2)
-Extreme problems, n (%)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)

Eq5d, the EuroQoL five-dimension questionnaire; VAS, visual analog scale.

Table S7. Effect of dietary intervention on clinical parameters according to on-treatment analysis.

	Pre VD	Post VD	Pre MD	Post MD	Post VD vs Post MD#	p#
Oxidized LDL-C, U/L	41.7 (37.1-46.0)	37.0 (33.6-40.3)**	42.1 (37.7-46.6)	40.2 (35.8-44.6)	-3.16 (-5.53--0.78)	0.02
TC, mg/dL	133.4 (123.0-143.6)	121.4 (113.7-129.2)***	135.1 (127.6-145.0)	127.6 (117.9-136.9)*	-6.2 (-10.1--1.9)	0.005
LDL-C, mg/dL	61.9 (54.5-68.1)	53.4 (48.3-58.8)***	63.8 (57.6-70.4)	58.4 (51.8-65.0)*	-5.0 (-8.5--1.2)	0.008
HDL-C, mg/dL	46.4 [41.8-51.8]	43.3 [38.7-48.3]**	48.3 [43.7-53.4]	44.9 [39.8-50.3]	-1.2 [-3.1-0.4]	0.2
TG, mg/dL	85.0 [73.5-96.5]	90.3 [80.6-100.9]	85.0 [73.5-97.4]	85.0 [74.4-96.5]	5.3 [-2.7-13.3]	0.2
Apo B, g/L	0.64 (0.59-0.70)	0.58 (0.53-0.62)***	0.66 (0.61-0.71)	0.60 (0.55-0.65)**	-0.026 (-0.05--0.001)	0.04
Apo A1, g/L	1.40 (1.33-1.47)	1.39 (1.31-1.46)	1.42 (1.35-1.49)	1.41 (1.34-1.49)	-0.023 (-0.058-0.012)	0.2
Apo B/ApoA1 ratio	0.45 [0.42-0.49]	0.41 [0.38-0.45]**	0.46 [0.42-0.5]	0.42 [0.38-0.46]***	-0.025 [-0.07-0.03]	0.3
HbA_{1c}, mmol/mol	38.0 [36.9-39.2]	38.2 [36.9-39.6]	38.1 [36.9-39.3]	38.2 [37.0-39.5]	0.0001 [-0.022-0.022]	0.9
Weight, kg	82.6 (78.2-86.9)	82.1 (77.7-86.6)	83.2 (78.7-87.7)	82.8 (78.2-87.3)	-0.7 (-1.24--0.15)	0.01
Hs-crp, mg/L	0.69 [0.48-0.99]	0.69 [0.45-1.05]	0.77 [0.57-1.04]	0.76 [0.51-1.14]	-0.10 [-0.48-0.27]	0.4
Systolic Bp, mmHg	134 (127-141)	131 (125-137)	138 (130-145)	134 (127-141)	-3.1 (-6.5-0.3)	0.07
Diastolic Bp, mmHg	85 (81-89)	85 (82-88)	87 (83-90)	87 (83-91)	-1.5 (-4.6-1.6)	0.3
Hr, bpm	62.5 [59.4-65.7]	62.6 [60.1-65.2]	63.8 [60.4-67.5]	63.6 [60.0-67.4]	-0.01 [-0.05-0.03]	0.5

Data are presented as mean (95% C.I) or as geometric mean [95 % C.I]. Within-group change p-value was calculated with paired t-test. *P<0.05,**P<0.01,***P<0.001.

[#]Differences in clinical parameters between vegetarian diet (VD) and meat diet (MD) were examined using linear mixed-effects models adjusted for sequence of randomisation and period of interventions. Apo: Apolipoprotein, Bp; Blood pressure, Bpm; beats per minute, HbA_{1c}; Glycated haemoglobin; HDL-C; High-density lipoprotein cholesterol, Hr; Heart rate, Hs-CRP; High-sensitive c-reactive protein, Kg; Kilograms, LDL-C; Low-density lipoprotein cholesterol, mmHg; millimetres of mercury, MD: meat diet; TC; Total cholesterol, TG; Triglycerides; VD: vegetarian diet. To convert cholesterol markers to millimoles per liter, multiply by 0.02586. To convert triglycerides to millimoles per liter, multiply by 0.01.

Table S8. Gut bacteria genera post-dietary intervention.

Phylum	Class	Family	Genus	Post-MD*	Post-VD*	SEM	p
Firmicutes	**Clostridia**	**Lachnospiraceae**	**Shuttleworthia #**	**0.56**	**7.02**	**1.24**	**0.00**
Firmicutes	**Clostridia**	**Ruminococcaceae**	**DTU089#**	**15.41**	**7.17**	**1.99**	**0.00**
Firmicutes	**Clostridia**	**Ruminococcaceae**	**Ruminiclostridium_5#**	**390.12**	**200.71**	**38.41**	**0.00**
Firmicutes	**Clostridia**	**Clostridiaceae_1**	**Clostridium_sensu_stricto_1#**	**567.77**	**353.04**	**106.86**	**0.00**
Firmicutes	**Clostridia**	**Ruminococcaceae**	**Negativibacillus#**	**24.94**	**13.05**	**3.85**	**0.00**
Firmicutes	**Clostridia**	**Ruminococcaceae**	**Anaerofilum#**	**4.33**	**2.01**	**0.68**	**0.01**
Proteobacteria	**Gammaproteobacteria**	**Burkholderiaceae**	**Parasutterella#**	**46.45**	**33.73**	**4.43**	**0.03**
Firmicutes	**Clostridia**	**Ruminococcaceae**	**Oscillospira#**	**16.38**	**11.52**	**2.38**	**0.04**
Firmicutes	**Clostridia**	**Lachnospiraceae**	**Fusicatenibacter#**	**743.99**	**898.35**	**90.56**	**0.04**
Cyanobacteria	**Melainabacteria**	**NA**	**NA#**	**19.44**	**8.29**	**4.43**	**0.04**
Bacteroidetes	**Bacteroidia**	**Tannerellaceae**	**Parabacteroides#**	**309.61**	**216.68**	**33.51**	**0.05**
Verrucomicrobia	**Verrucomicrobiae**	**Akkermansiaceae**	**Akkermansia#**	**426.26**	**811.53**	**203.63**	**0.04**
Firmicutes	Clostridia	Lachnospiraceae	Lachnospiraceae_FCS020_group#	98.89	85.22	6.00	0.06
Actinobacteria	Coriobacteriia	Eggerthellaceae	Adlercreutzia#	25.77	17.66	2.90	0.06
Firmicutes	Erysipelotrichia	Erysipelotrichaceae	NA#	13.85	7.14	3.82	0.06
Firmicutes	Clostridia	Clostridiales_vadinBB60_group	NA#	25.56	16.79	4.71	0.07
Bacteroidetes	Bacteroidia	NA	NA#	0.98	2.15	0.43	0.07
Actinobacteria	Coriobacteriia	Eggerthellaceae	DNF00809#	4.53	7.36	1.11	0.08
Tenericutes	Mollicutes	NA	NA#	15.37	9.09	3.36	0.08
Firmicutes	Clostridia	Ruminococcaceae	Butyricicoccus#	179.12	220.22	27.20	0.08
Actinobacteria	Coriobacteriia	Eggerthellaceae	Senegalimassilia#	39.67	29.54	5.61	0.08
Tenericutes	Mollicutes	NA	NA#	23.83	60.39	16.18	0.08
Euryarchaeota	Methanobacteria	Methanobacteriaceae	Methanobrevibacter#	110.14	162.22	34.79	0.09
Firmicutes	Clostridia	Ruminococcaceae	Ruminococcaceae_UCG-005#	834.72	677.37	98.87	0.09
Firmicutes	Clostridia	Peptostreptococcaceae	Intestinibacter#	338.76	239.26	49.91	0.09
Firmicutes	Clostridia	Lachnospiraceae	Lachnospiraceae_UCG-010#	24.47	20.36	2.63	0.10

Firmicutes	Negativicutes	Veillonellaceae	Dialister#	618.60	468.50	82.27	0.10
Firmicutes	Bacilli	Lactobacillaceae	Lactobacillus#	33.00	93.27	29.36	0.10
Firmicutes	Clostridia	Ruminococcaceae	Ruminococcaceae_UCG-007#	6.70	5.09	1.11	0.14
Firmicutes	Clostridia	Lachnospiraceae	Marvinbryantia#	121.84	185.73	33.03	0.15
Firmicutes	Clostridia	Ruminococcaceae	Angelakisella#	12.38	9.88	1.69	0.17
Firmicutes	Clostridia	Family_XIII	Family_XIII_UCG-001#	20.74	15.66	2.97	0.17
Bacteroidetes	Bacteroidia	Rikenellaceae	Alistipes#	566.34	484.11	58.10	0.20
Firmicutes	Clostridia	Ruminococcaceae	Ruminococcaceae_NK4A214_group#	301.27	382.87	66.64	0.27
Proteobacteria	Gammaproteobacteria	Enterobacteriaceae	Escherichia/Shigella#	125.64	292.68	106.65	0.28
Actinobacteria	Coriobacteriia	Eggerthellaceae	Eggerthella#	7.20	1.60	4.47	0.29
Firmicutes	Clostridia	Lachnospiraceae	Butyrivibrio#	321.91	107.98	140.78	0.29
Firmicutes	Clostridia	Ruminococcaceae	Hydrogenoanaerobacterium#	3.32	3.89	1.14	0.39
Firmicutes	Clostridia	Lachnospiraceae	UC5-1-2E3#	8.12	5.97	2.13	0.40
Firmicutes	Clostridia	Lachnospiraceae	GCA-900066755#	1.89	1.56	0.51	0.57
Bacteroidetes	Bacteroidia	Marinifilaceae	Butyricimonas#	24.58	26.22	5.23	0.76
Firmicutes	Clostridia	Ruminococcaceae	NA#	776.35	746.43	79.88	0.78
Firmicutes	Clostridia	Lachnospiraceae	Coprococcus_1#	181.22	188.32	23.13	0.79
Firmicutes	Clostridia	Family_XIII	NA#	2.23	2.04	0.62	0.83
Firmicutes	Clostridia	Lachnospiraceae	Roseburia#	480.41	499.21	78.37	0.87
Firmicutes	Clostridia	Ruminococcaceae	Ruminococcus_2#	1233.61	1217.96	142.38	0.89
Firmicutes	Clostridia	Lachnospiraceae	Lachnospira	546.75	800.77	108.00	0.11
Firmicutes	Clostridia	Ruminococcaceae	Ruminococcaceae_UCG-013	121.20	167.57	25.67	0.11
Firmicutes	Clostridia	Lachnospiraceae	Tyzzerella	2.83	1.71	0.65	0.13
Actinobacteria	Coriobacteriia	NA	NA	16.33	12.24	3.04	0.13
Firmicutes	Clostridia	Peptostreptococcaceae	Romboutsia	318.28	200.60	53.86	0.14
Firmicutes	Clostridia	Ruminococcaceae	Ruminococcaceae_UCG-009	7.12	4.67	1.50	0.14
Bacteroidetes	Bacteroidia	Prevotellaceae	Prevotella_9	1344.78	2184.64	675.17	0.15

Firmicutes	Clostridia	Peptococcaceae	NA	2.29	3.58	0.61	0.15
Proteobacteria	Gammaproteobacteria	Pasteurellaceae	Haemophilus	93.83	65.94	27.52	0.15
Actinobacteria	Coriobacteriia	Eggerthellaceae	NA	95.20	80.95	11.11	0.15
Firmicutes	Clostridia	Lachnospiraceae	Lachnospiraceae_UCG-008	31.22	25.32	5.06	0.16
Firmicutes	Clostridia	Family_XIII	Family_XIII_AD3011_group	54.59	44.70	10.09	0.16
Firmicutes	Clostridia	Ruminococcaceae	Candidatus_Soleaferrea	6.82	4.08	1.39	0.18
Proteobacteria	Gammaproteobacteria	Burkholderiaceae	Sutterella	119.28	100.68	13.34	0.18
Firmicutes	Clostridia	Lachnospiraceae	CAG-56	129.53	108.30	16.04	0.19
Actinobacteria	Coriobacteriia	Coriobacteriales_Incertae_Sedis	NA	9.06	5.99	1.60	0.19
Proteobacteria	Deltaproteobacteria	Desulfovibrionaceae	Bilophila	27.30	23.77	4.71	0.21
Firmicutes	Clostridia	Lachnospiraceae	Coprococcus_3	224.07	187.07	27.56	0.22
Firmicutes	Clostridia	Ruminococcaceae	Ruminococcaceae_UCG-003	89.41	108.96	13.98	0.22
Firmicutes	Clostridia	Lachnospiraceae	Howardella	12.54	10.49	2.67	0.24
Firmicutes	Clostridia	Ruminococcaceae	UBA1819	23.47	9.70	8.97	0.24
Firmicutes	Clostridia	Lachnospiraceae	Lachnospiraceae_UCG-001	116.52	149.00	27.09	0.25
Firmicutes	Erysipelotrichia	Erysipelotrichaceae	Merdibacter	11.55	7.83	2.77	0.25
Bacteroidetes	Bacteroidia	Prevotellaceae	Prevotella_7	88.13	43.88	26.66	0.25
Proteobacteria	Deltaproteobacteria	Desulfovibrionaceae	NA	5.57	4.36	1.09	0.26
Firmicutes	Clostridia	Ruminococcaceae	Ruminococcaceae_UCG-010	84.22	71.79	10.01	0.26
Firmicutes	Clostridia	Ruminococcaceae	Fournierella	6.28	4.60	1.16	0.27
Firmicutes	Clostridia	Family_XIII	Mogibacterium	1.57	0.60	0.60	0.27
Firmicutes	Clostridia	Lachnospiraceae	Hungatella	4.93	2.33	1.83	0.27
Firmicutes	Clostridia	Ruminococcaceae	Subdoligranulum	3311.80	2935.40	466.37	0.32
Firmicutes	Erysipelotrichia	Erysipelotrichaceae	Holdemanella	265.34	296.06	50.79	0.32
Bacteroidetes	Bacteroidia	Prevotellaceae	Paraprevotella	30.78	29.03	5.20	0.34
Proteobacteria	Gammaproteobacteria	Enterobacteriaceae	Klebsiella	22.57	76.22	38.88	0.34
Firmicutes	Clostridia	Ruminococcaceae	Ruminiclostridium_6	235.78	183.68	48.31	0.35
Firmicutes	Negativicutes	Acidaminococcaceae	Acidaminococcus	20.93	11.89	6.68	0.35
Firmicutes	Clostridia	NA	NA	1.23	0.97	0.24	0.35

Firmicutes	Clostridia	Lachnospiraceae	Lachnospiraceae_ND3007_group	377.15	409.98	36.50	0.35
Bacteroidetes	Bacteroidia	Marinifilaceae	Odoribacter	47.55	53.98	6.33	0.36
Actinobacteria	Actinobacteria	Bifidobacteriaceae	Bifidobacterium	942.23	1163.59	178.29	0.37
Firmicutes	Clostridia	Ruminococcaceae	Fecalibacterium	5154.16	4869.92	399.52	0.37
Firmicutes	Bacilli	Streptococcaceae	Lactococcus	11.00	15.63	4.44	0.38
Firmicutes	Clostridia	Lachnospiraceae	Coprococcus_2	689.90	804.51	140.26	0.38
Actinobacteria	Coriobacteriia	Eggerthellaceae	Enterorhabdus	66.77	58.18	10.73	0.41
Firmicutes	Clostridia	Lachnospiraceae	GCA-900066575	17.58	15.46	2.08	0.44
Firmicutes	Clostridia	Ruminococcaceae	Ruminococcaceae_UCG-002	1131.35	1052.08	146.65	0.44
Firmicutes	Erysipelotrichia	Erysipelotrichaceae	Erysipelotrichaceae_UCG-003	226.99	192.24	41.24	0.44
Firmicutes	Clostridia	Peptostreptococcaceae	Terrisporobacter	62.71	81.48	21.69	0.45
Firmicutes	Clostridia	Lachnospiraceae	Lachnospiraceae_NK4A136_group	457.00	383.24	68.29	0.45
Firmicutes	Clostridia	Ruminococcaceae	Ruminococcaceae_UCG-014	841.07	754.52	108.58	0.47
Firmicutes	Clostridia	Lachnospiraceae	Lachnospiraceae_AC2044_group	10.98	18.65	7.50	0.48
Firmicutes	Erysipelotrichia	Erysipelotrichaceae	Turicibacter	91.35	111.28	23.94	0.48
Firmicutes	Clostridia	Ruminococcaceae	Ruminococcaceae_UCG-011	1.14	1.56	0.42	0.48
Firmicutes	Negativicutes	Acidaminococcaceae	Phascolarctobacterium	181.57	198.77	41.31	0.50
Firmicutes	Bacilli	Carnobacteriaceae	Granulicatella	0.83	0.60	0.25	0.50
Firmicutes	Clostridia	Ruminococcaceae	Flavonifractor	12.46	11.17	1.75	0.51
Firmicutes	NA	NA	NA	10.54	9.57	2.29	0.51
Actinobacteria	Coriobacteriia	Eggerthellaceae	Gordonibacter	1.35	1.70	0.37	0.52
Firmicutes	Clostridia	Ruminococcaceae	Caproiciproducens	7.87	6.98	1.59	0.53
Firmicutes	Clostridia	Ruminococcaceae	Ruminococcaceae_UCG-004	50.34	44.88	7.47	0.54
Firmicutes	Clostridia	Ruminococcaceae	Phocea	0.47	0.68	0.25	0.55
Firmicutes	Clostridia	Ruminococcaceae	Anaerotruncus	6.00	5.36	2.19	0.59
Bacteroidetes	Bacteroidia	Barnesiellaceae	Barnesiella	145.83	132.43	25.70	0.62

Firmicutes	Clostridia	Lachnospiraceae	NA	1847.16	1918.00	114.99	0.62
Firmicutes	Clostridia	Ruminococcaceae	GCA-900066225	5.69	4.94	1.27	0.63
Firmicutes	Clostridia	Christensenellaceae	Christensenellaceae_R-7_group	1502.70	1417.41	160.78	0.63
Firmicutes	Clostridia	Ruminococcaceae	Intestinimonas	76.14	71.03	10.33	0.63
Firmicutes	Clostridia	Lachnospiraceae	Eisenbergiella	2.93	2.23	1.06	0.63
Firmicutes	Clostridia	Lachnospiraceae	Tyzzerella_3	22.09	27.75	11.21	0.64
Actinobacteria	Coriobacteriia	Atopobiaceae	Olsenella	12.79	13.86	3.33	0.65
Firmicutes	Clostridia	Ruminococcaceae	Ruminiclostridium_9	104.62	109.01	11.21	0.65
Firmicutes	Clostridia	Christensenellaceae	NA	214.41	243.77	60.02	0.65
Firmicutes	Erysipelotrichia	Erysipelotrichaceae	Catenibacterium	284.89	243.09	70.12	0.66
Firmicutes	Clostridia	Lachnospiraceae	Anaerostipes	507.04	553.95	84.98	0.66
Firmicutes	Clostridia	Ruminococcaceae	Oscillibacter	60.39	53.86	11.86	0.68
Firmicutes	Clostridia	Lachnospiraceae	Sellimonas	3.94	4.37	0.77	0.68
Actinobacteria	Actinobacteria	Actinomycetaceae	Actinomyces	20.75	19.50	3.32	0.70
Firmicutes	Clostridia	Ruminococcaceae	Ruminococcus_1	456.75	501.79	83.25	0.71
Firmicutes	Clostridia	Ruminococcaceae	Ruminiclostridium	1.36	1.54	0.37	0.73
Firmicutes	Negativicutes	Veillonellaceae	Veillonella	93.28	106.86	33.72	0.73
Firmicutes	Clostridia	Lachnospiraceae	Blautia	2789.05	2726.91	167.00	0.74
Bacteroidetes	Bacteroidia	Barnesiellaceae	Coprobacter	4.93	5.36	1.77	0.75
Proteobacteria	Gammaproteobacteria	Burkholderiaceae	Oxalobacter	4.41	4.02	1.04	0.75
Actinobacteria	Coriobacteriia	Coriobacteriaceae	Collinsella	722.44	688.30	80.96	0.76
Firmicutes	Clostridia	Ruminococcaceae	Pseudoflavonifractor	2.55	2.34	0.66	0.76
Firmicutes	Clostridia	Lachnospiraceae	Lachnoclostridium	263.34	252.68	40.26	0.79
Firmicutes	Erysipelotrichia	Erysipelotrichaceae	Coprobacillus	3.91	5.07	3.25	0.80
Firmicutes	Clostridia	Ruminococcaceae	CAG-352	242.31	290.23	155.19	0.81
Firmicutes	Clostridia	Lachnospiraceae	Agathobacter	2661.19	2575.52	373.58	0.81
Firmicutes	Clostridia	Lachnospiraceae	Dorea	510.65	518.70	35.98	0.85

Lentisphaerae	Lentisphaeria	Victivallaceae	Victivallis	4.60	4.46	2.12	0.86
Proteobacteria	Alphaproteobacteria	NA	NA	45.86	48.97	20.09	0.89
Firmicutes	Bacilli	Streptococcaceae	Streptococcus	450.58	469.33	124.06	0.92
Actinobacteria	Coriobacteriia	Eggerthellaceae	Slackia	20.54	20.13	3.02	0.92
Firmicutes	Erysipelotrichia	Erysipelotrichaceae	Holdemania	3.49	3.40	0.83	0.93
Firmicutes	Clostridia	Lachnospiraceae	Lachnospiraceae_UCG-004	79.11	80.21	10.32	0.94
Firmicutes	Clostridia	NA	NA	307.27	303.76	104.14	0.95
Firmicutes	Clostridia	Ruminococcaceae	Papillibacter	1.95	1.91	0.53	0.95
Firmicutes	Clostridia	NA	NA	57.60	56.64	28.21	0.97
Firmicutes	Clostridia	Peptostreptococcaceae	NA	1.84	1.87	0.57	0.97
Lentisphaerae	Lentisphaeria	vadinBE97	NA	1.76	1.75	0.79	0.99
Bacteroidetes	Bacteroidia	Bacteroidaceae	Bacteroides	3890.86	3885.54	358.32	0.99
Firmicutes	Erysipelotrichia	Erysipelotrichaceae	Erysipelatoclostridium	7.32	7.34	1.99	0.99

*The least square mean and standard error of genera abundance was obtained by mixed modelling (n=20 subjects). The effects of diet were evaluated using a generalized linear mixed model that included a fixed effect of diet, sequence of allocation, and their interaction. Bold letters denote bacterial genera that significantly differed between meat and vegetarian diets.

Genera selected using multilevel random forest modelling as optimal for discriminating vegetarian diet from meat diet.

Table S9. Fecal levels of short chain fatty acids and branched chain fatty acids relative to dietary intervention.

	Post-MD*	Post-VD*	SEM	Percent difference
Acetate	90.3	94.0	8.3	4.1
Butyrate	34.0	35.7	4.9	5
Propionate	29.1	32	3.6	9.9
Lactate	0.64	0.3	0.3	-53.1
Succinate	0.1	0.1	0.03	12
Isobutyrate	3.23	3.3	0.2	2.2
Isovalerate	2.7	2.8	0.2	5.6
Total BCFAs	5.9	6.2	0.4	5.8
Total SCFAs	154.0	162.0	15.0	5.2

*The least square mean and standard error of fecal fatty acid level was obtained from mixed model (n=20 subjects). The effects of diet were evaluated using a generalized linear mixed model that included a fixed effect of diet, sequence of allocation, and their interaction. Percent difference indicates the median difference in concentration in vegetarian vs. meat diet. Total branched chain fatty acids (BCFA) = the sum of isobutyrate and isovalerate. Total short chain fatty acids (SCFA) = the sum of acetate, butyrate, and propionate.

Table S10. Plasma levels of selected metabolites with respect to dietary intervention.

Metabolite	Ionization	m/z	Retention time	Post MD*	Post VD	SEM	*P Value*
Unknown 180.8988	**RP-**	**182.8988**	**11.38**	**16843.29**	**14557.47**	**633.66**	**0.002**
Cysteinyl-Cysteine	RP-	260.9793	0.70	6631.55	7853.38	492.19	0.066
Lignoceric acid	**RP-**	**367.3586**	**9.02**	**36810.40**	**43195.67**	**1933.87**	**0.025**
dihydroxy-stearic acid	**RP-**	**375.2750**	**7.00**	**196729.93**	**169679.79**	**10085.60**	**0.018**
Unknown 481.2818	**RP-**	**481.2818**	**6.24**	**16467.75**	**26236.58**	**5620.74**	**0.023**
PC (14:0/O-1:0)	**RP-**	**526.3512**	**7.12**	**15228.39**	**11015.75**	**906.87**	**0.004**
lysoPC (16:1)	**RP-**	**552.3308**	**6.82**	**13045.53**	**10808.43**	**680.58**	**0.009**
Unknown 570.8316	RP-	570.8316	0.63	3844.64	4421.80	299.94	0.106
lysoPE (22:0)	**RP-**	**592.3600**	**7.05**	**12193.78**	**8386.79**	**947.67**	**0.011**
DG (16:0/20:3)	**RP-**	**653.4922**	**9.86**	**4283.02**	**5275.64**	**334.29**	**0.027**
PE (18:1/20:4)	**RP-**	**748.5318**	**8.85**	**18748.37**	**12565.47**	**1133.24**	**0.000**
2-Hydroxylauroylcarnitine	**RP-**	**777.5494**	**8.46**	**15513.48**	**13221.52**	**961.62**	**0.035**
PE (18:1/18:1)	**RP-**	**786.5671**	**8.89**	**11655.84**	**8956.61**	**733.66**	**0.004**
PE (20:3/18:1)	**RP-**	**810.5666**	**8.80**	**23648.56**	**17826.32**	**1149.66**	**<0.001**
PE (20:2/18:1)	**RP-**	**812.5822**	**8.96**	**30155.11**	**22896.73**	**1162.19**	**<0.001**
PC (20:2/16:0)	RP-	820.5644	9.23	51624.88	44215.94	2679.98	0.066
PE (18:0/22:5)	**RP-**	**822.5641**	**9.22**	**21394.62**	**16708.67**	**1493.55**	**0.040**
PC (18:1/22:4)	**RP-**	**836.5812**	**8.88**	**13050.88**	**10243.25**	**640.24**	**0.004**
3-[3,5-dihydroxy-4-(sulfo-oxy)phenyl]-2-oxopropanoic acid	**RP+**	**168.9820**	**11.48**	**155146.44**	**182929.37**	**6860.20**	**0.010**
3-Methylhistidine	**RP+**	**170.0908**	**0.63**	**1943.89**	**865.23**	**355.01**	**0.025**
Hippurate	RP+	180.0651	2.90	515914.00	450011.66	58837.15	0.349
3-Indolepropionic acid	RP-	190.0867	4.39	98007.09	122411.46	17291.84	0.259
2-Methylbutyroylcarnitine	RP+	246.1697	2.52	38209.45	32777.99	3639.34	0.181
Subaphylline	**RP+**	**247.1446**	**2.44**	**52076.92**	**118952.27**	**14585.80**	**0.002**
Acoric acid	**RP+**	**286.2014**	**3.91**	**147642.67**	**215322.85**	**17346.72**	**0.002**
4-hydroxy nonenal mercapturic acid	**RP+**	**287.1510**	**5.80**	**84412.33**	**40256.93**	**11244.85**	**0.012**
N-Acetylanonaine	**RP+**	**308.1297**	**5.80**	**27288.67**	**9587.51**	**3534.59**	**0.001**
Fatty acid 346.1231	**RP+**	**346.1231**	**4.55**	**80258.08**	**218453.19**	**39899.08**	**0.024**
Tetracosanedione	**RP+**	**366.3749**	**7.90**	**84840.86**	**45100.68**	**9876.02**	**0.008**
Uknown 464.193	**RP+**	**464.1931**	**4.60**	**19418.79**	**9724.71**	**2506.99**	**0.011**
lysoPC (16:0)	**RP+**	**482.3620**	**7.12**	**95512.54**	**66500.01**	**6965.84**	**0.004**

PC (16:0/18:1)	**RP+**	**742.5793**	**8.87**	**39397.84**	**27681.81**	**2943.77**	**0.004**
PC (18:1/18:1)	**RP+**	**768.5931**	**8.92**	**126228.97**	**82032.18**	**9985.77**	**<0.001**

*The least square mean and standard error of metabolite level was obtained via mixed modelling (n=21 subjects). The effects of diet were evaluated using a generalized linear mixed model that included a fixed effect of diet, sequence of allocation, and their interaction. Bold letters denote the bacterial genera that significantly differed between meat and vegetarian diets.

DG, diacylglycerol, PE, phosphatidylethanolamine; PC, phosphatidylcholine. RP+: reverse phase chromatography positive ionization mode; RP-: reverse phase chromatography negative ionization mode.

Table S11. Spearman correlation of bacterial genera and plasma metabolites with cardiometabolic risk factors, vegetarian diet.

Variables		Oxidized LDL		TC		LDL		BMI		Weight	
		r	*P*	r	*P*	r	*P*	r	*P*	r	*P*
Genus	Zotu1114_GCA.900066755	-0.36	0.02	-0.30	0.05	-0.38	0.01	-0.20	0.21	-0.13	0.42
Genus	Zotu74_Ruminococcaceae_NK4A214_group	-0.51	0.00	-0.30	0.06	-0.37	0.02	-0.19	0.22	-0.25	0.11
Genus	Zotu822_Ruminococcaceae_UCG.007	0.24	0.13	0.26	0.10	0.36	0.02	-0.03	0.83	0.00	1.00
Genus	Zotu647_Eggerthella	0.31	0.04	0.38	0.01	0.35	0.02	0.09	0.58	0.17	0.28
Genus	Zotu62_Coprococcus_1	-0.37	0.02	-0.29	0.06	-0.33	0.03	-0.06	0.69	-0.12	0.46
Genus	Zotu87_Roseburia	0.20	0.21	0.45	0.00	0.32	0.04	-0.12	0.44	-0.06	0.72
Genus	Zotu412_Butyricimonas	0.05	0.76	0.28	0.07	0.31	0.04	0.00	0.99	0.02	0.90
Genus	Zotu10_Ruminococcus_2	-0.34	0.03	-0.23	0.14	-0.31	0.05	-0.14	0.39	-0.12	0.43
Genus	Zotu25_Escherichia.Shigella	0.29	0.06	0.38	0.01	0.31	0.05	-0.21	0.19	-0.16	0.31
Genus	Zotu152_Marvinbryantia	-0.29	0.06	-0.40	0.01	-0.28	0.07	-0.35	0.03	-0.27	0.08
Genus	Zotu397_Oscillospira	-0.11	0.49	-0.11	0.49	-0.28	0.07	0.00	1.00	-0.02	0.89
Genus	Zotu728_Shuttleworthia	-0.43	0.00	-0.32	0.04	-0.27	0.08	-0.05	0.76	-0.10	0.53
Genus	Zotu832_Anaerofilum	0.27	0.09	0.27	0.08	0.27	0.08	-0.09	0.56	0.02	0.90
Genus	Zotu57_Parabacteroides	0.19	0.24	0.35	0.02	0.27	0.09	0.09	0.58	0.09	0.59
Genus	Zotu576_Angelakisella	0.08	0.61	0.28	0.07	0.26	0.10	0.18	0.27	0.23	0.15
Genus	Zotu239_NA	-0.21	0.18	-0.28	0.08	-0.24	0.13	-0.05	0.75	0.01	0.97
Genus	Zotu398_DTU089	0.17	0.30	0.28	0.07	0.22	0.15	0.08	0.63	0.11	0.49
Genus	Zotu788_NA	-0.42	0.01	-0.11	0.48	-0.20	0.20	-0.07	0.66	-0.07	0.66
Genus	Zotu63_Clostridium_sensu_stricto_1	0.02	0.89	0.11	0.50	0.20	0.21	0.28	0.07	0.30	0.05
Genus	Zotu191_Senegalimassilia	0.15	0.36	0.03	0.86	0.19	0.22	0.50	0.00	0.42	0.01
Genus	Zotu676_Hydrogenoanaerobacterium	-0.32	0.04	-0.23	0.14	-0.16	0.32	0.04	0.80	-0.08	0.62
Genus	Zotu58_Butyrivibrio	0.02	0.92	-0.09	0.56	-0.15	0.34	0.31	0.05	0.30	0.06
Genus	Zotu13_Fusicatenibacter	-0.17	0.29	-0.14	0.36	-0.15	0.34	-0.20	0.21	-0.14	0.36
Genus	Zotu50_Intestinibacter	0.12	0.45	0.11	0.48	0.14	0.37	0.05	0.74	0.17	0.29
Genus	Zotu307_NA	0.04	0.81	-0.21	0.18	-0.13	0.42	0.08	0.62	0.01	0.96
Genus	Zotu262_Lachnospiraceae_UCG.010	0.29	0.06	-0.11	0.49	0.13	0.42	-0.03	0.85	0.02	0.90
Genus	Zotu60_Ruminiclostridium_5	0.04	0.81	0.14	0.38	0.12	0.45	0.25	0.11	0.27	0.09

Genus	Zotu145_Butyricicoccus	-0.11	0.50	-0.09	0.59	-0.12	0.46	-0.04	0.82	-0.13	0.40
Genus	Zotu34_Ruminococcaceae_UCG.005	-0.29	0.07	-0.23	0.13	-0.11	0.47	0.01	0.93	-0.10	0.55
Genus	Zotu38_Alistipes	-0.08	0.61	0.17	0.28	0.11	0.49	0.01	0.95	0.00	0.98
Genus	Zotu334_NA	-0.21	0.18	-0.16	0.31	-0.11	0.49	0.23	0.14	0.19	0.24
Genus	Zotu481_UC5.1.2E3	0.36	0.02	0.15	0.33	0.10	0.52	-0.23	0.14	-0.20	0.21
Genus	Zotu512_NA	-0.05	0.73	0.07	0.65	0.10	0.53	0.07	0.67	0.07	0.66
Genus	Zotu169_Lachnospiraceae_FCS020_group	0.25	0.11	0.07	0.66	0.10	0.53	-0.03	0.83	0.02	0.90
Genus	Zotu357_Family_XIII_UCG.001	0.35	0.02	0.12	0.46	0.10	0.53	0.03	0.84	-0.05	0.75
Genus	Zotu629_DNF00809	-0.05	0.76	-0.20	0.21	-0.09	0.56	0.16	0.31	0.09	0.57
Genus	Zotu180_Lactobacillus	0.04	0.81	0.21	0.18	0.09	0.56	0.19	0.22	0.11	0.51
Genus	Zotu36_NA	-0.39	0.01	-0.17	0.27	-0.07	0.65	0.15	0.33	0.12	0.47
Genus	Zotu35_Dialister	-0.15	0.34	-0.18	0.24	-0.07	0.66	0.13	0.41	-0.01	0.95
Genus	Zotu364_Negativibacillus	0.05	0.74	0.08	0.63	0.07	0.66	0.04	0.82	0.14	0.39
Genus	Zotu114_Methanobrevibacter	-0.16	0.32	-0.25	0.11	-0.07	0.66	0.05	0.77	-0.03	0.83
Genus	Zotu279_Adlercreutzia	0.02	0.88	0.13	0.40	0.07	0.66	0.20	0.20	0.18	0.24
Genus	Zotu2541_NA	0.12	0.44	0.17	0.28	0.04	0.78	-0.03	0.84	-0.02	0.88
Genus	Zotu399_NA	-0.29	0.06	0.05	0.75	-0.04	0.79	-0.07	0.67	-0.12	0.45
Genus	Zotu12_Akkermansia	-0.14	0.37	-0.09	0.57	-0.03	0.85	0.13	0.42	0.05	0.76
Genus	Zotu215_Parasutterella	-0.15	0.35	0.12	0.46	-0.03	0.86	-0.10	0.53	-0.23	0.14
Metabolite	PC (18:1/18:1)	0.55	0.00	0.21	0.19	0.33	0.04	-0.14	0.38	-0.17	0.29
Metabolite	lysoPC (16:0)	0.39	0.01	0.39	0.01	0.29	0.07	-0.01	0.95	0.01	0.97
Metabolite	DG (16:0/20:3)	-0.35	0.03	-0.47	0.00	-0.44	0.01	-0.31	0.05	-0.33	0.04
Metabolite	lysoPC (16:1)	0.35	0.03	0.38	0.01	0.25	0.12	0.19	0.23	0.14	0.40
Metabolite	PE (18:0/22:5)	0.34	0.03	0.28	0.08	0.37	0.02	-0.06	0.69	-0.17	0.29
Metabolite	PC (14:0/O-1:0)	0.25	0.12	0.27	0.09	0.23	0.15	-0.03	0.87	-0.13	0.43
Metabolite	Subaphylline	-0.25	0.13	0.01	0.94	-0.08	0.61	-0.06	0.71	-0.08	0.60
Metabolite	PC (20:2/16:0)	0.23	0.15	0.28	0.08	0.28	0.08	0.04	0.80	0.00	0.99
Metabolite	PC (16:0/18:1)	0.23	0.16	0.19	0.25	0.12	0.47	-0.30	0.06	-0.26	0.11
Metabolite	lysoPE (22:0)	0.21	0.18	0.20	0.22	0.21	0.18	-0.08	0.62	-0.09	0.59
Metabolite	Lignoceric acid	-0.20	0.21	-0.37	0.02	-0.31	0.05	-0.04	0.79	-0.07	0.69

Metabolite	Acoric acid	-0.19	0.23	-0.30	0.06	-0.27	0.09	-0.08	0.64	-0.07	0.66
Metabolite	Tetracosanedione	0.18	0.26	0.09	0.60	0.19	0.23	-0.01	0.97	0.00	0.99
Metabolite	PE (20:2/18:1)	0.16	0.32	-0.16	0.34	-0.07	0.65	-0.21	0.19	-0.31	0.05
Metabolite	2-Hydroxylauroylcarnitine	0.15	0.35	0.39	0.01	0.25	0.12	0.26	0.11	0.14	0.39
Metabolite	Hippurate	0.15	0.36	0.28	0.09	0.13	0.42	0.33	0.04	0.24	0.14
Metabolite	3-Indolepropionic acid	0.14	0.38	0.15	0.34	0.25	0.12	0.24	0.14	0.15	0.34
Metabolite	Unknown 464.193	-0.11	0.50	0.13	0.43	0.17	0.28	0.06	0.71	0.02	0.88
Metabolite	Unknown 481.2818	-0.10	0.55	-0.08	0.61	-0.22	0.17	0.31	0.05	0.27	0.09
Metabolite	3-[3.5-dihydroxy-4-(sulfoxy)phenyl]-2-oxopropanoic acid	0.10	0.55	0.15	0.34	0.02	0.89	0.15	0.34	0.21	0.19
Metabolite	N-Acetylanonaine	0.10	0.56	0.35	0.03	0.22	0.17	0.16	0.32	0.18	0.27
Metabolite	Unknown180.8988	0.09	0.57	0.02	0.91	-0.08	0.61	0.36	0.02	0.24	0.14
Metabolite	3-Methylhistidine	-0.09	0.58	-0.10	0.55	-0.16	0.34	-0.13	0.43	-0.20	0.22
Metabolite	PE (18:1/18:1)	-0.09	0.59	-0.10	0.56	-0.11	0.49	-0.20	0.22	-0.27	0.09
Metabolite	2-Methylbutyroylcarnitine	0.08	0.63	0.04	0.81	-0.05	0.78	-0.24	0.14	-0.30	0.06
Metabolite	Cysteinyl-Cysteine	-0.07	0.65	-0.09	0.58	-0.13	0.42	0.36	0.02	0.20	0.21
Metabolite	Unknown570.8316	-0.07	0.67	0.05	0.74	-0.12	0.47	0.06	0.70	0.09	0.57
Metabolite	PE (18:1/20:4)	-0.04	0.81	0.13	0.43	0.07	0.66	0.07	0.65	0.01	0.94
Metabolite	4-hydroxy nonenal mercapturic acid	0.03	0.84	0.22	0.17	0.17	0.29	0.21	0.19	0.17	0.30
Metabolite	PC (18:1/22:4)	0.03	0.87	0.06	0.72	0.06	0.71	-0.12	0.46	-0.26	0.11
Metabolite	dihydroxy-stearic acid	0.02	0.91	0.20	0.22	0.12	0.44	0.14	0.38	0.20	0.23
Metabolite	PE (20:3/18:1)	0.01	0.94	-0.12	0.48	-0.08	0.60	-0.22	0.17	-0.28	0.08
Metabolite	Fatty acid 346.1231	0.00	1.00	0.07	0.67	0.16	0.32	0.16	0.32	0.19	0.23

DG, diglycerides, PE, phatidylethanolamine; PC, phosphatidylcholines.

Figure S1. Descriptive analysis of abundance (A) and prevalence (B) of gut microbiota across samples.

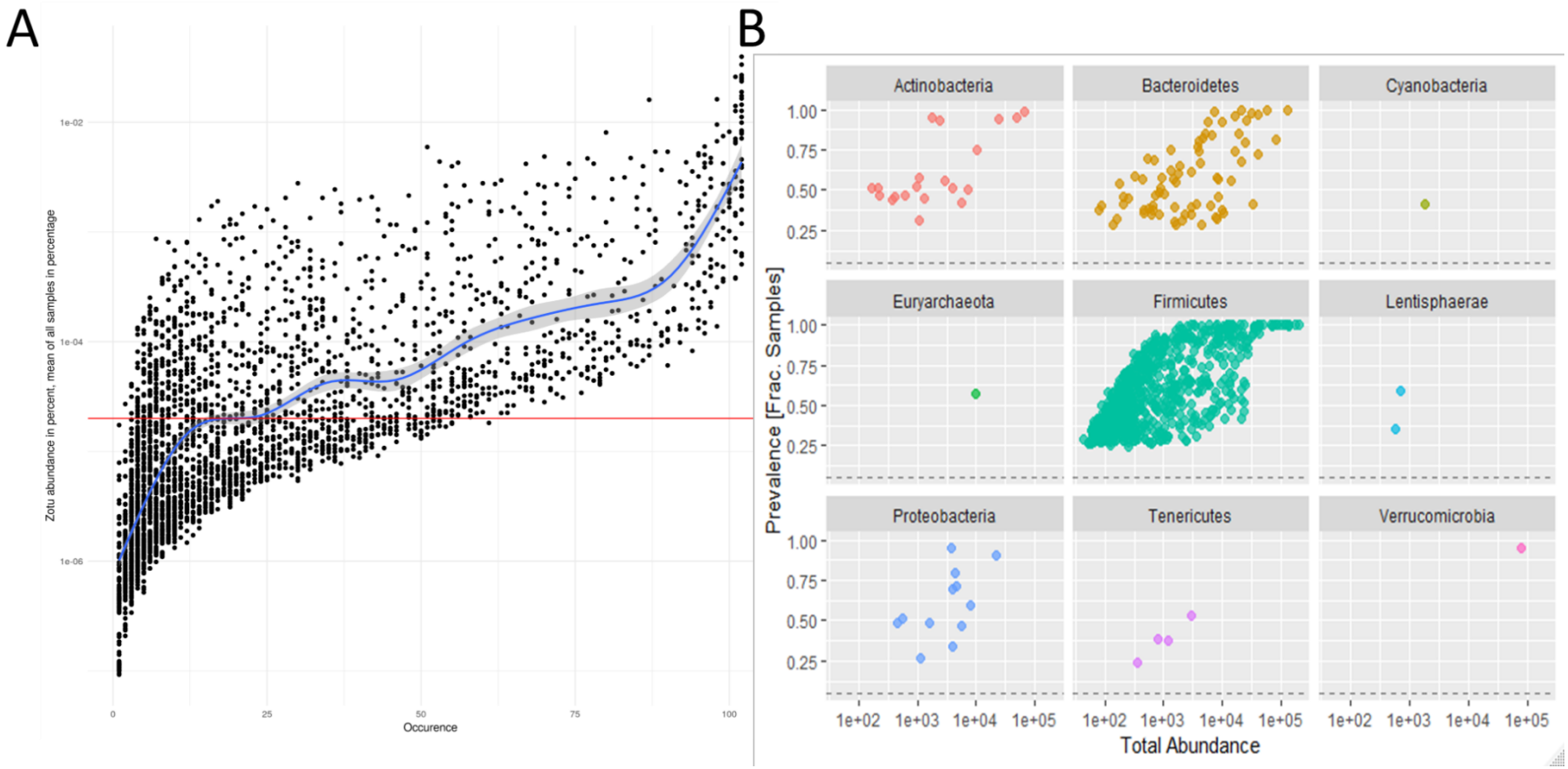


Figure S2. Structure of the effective dataset representing the within-individual variations between treatments. The effective dataset was subjected to multilevel random forest modelling.

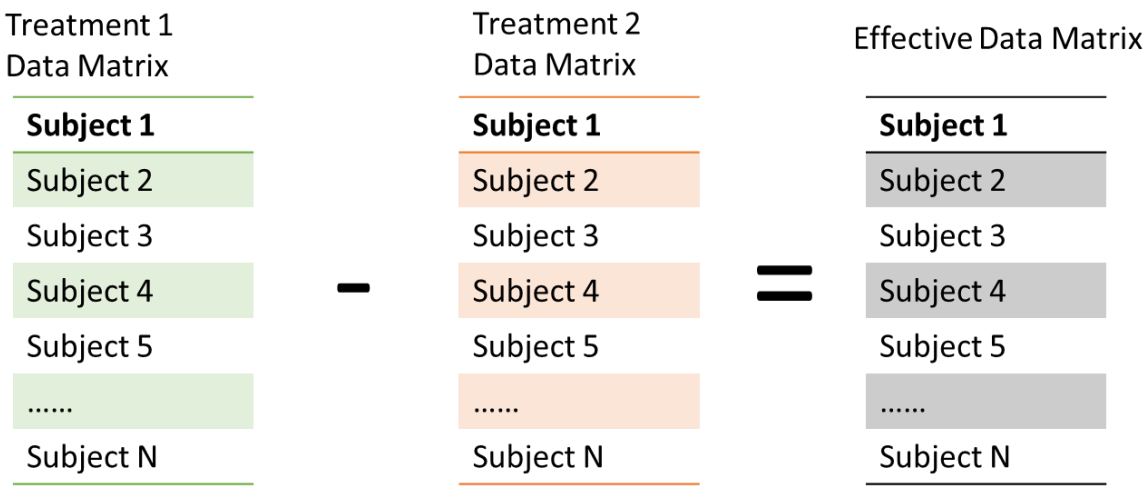
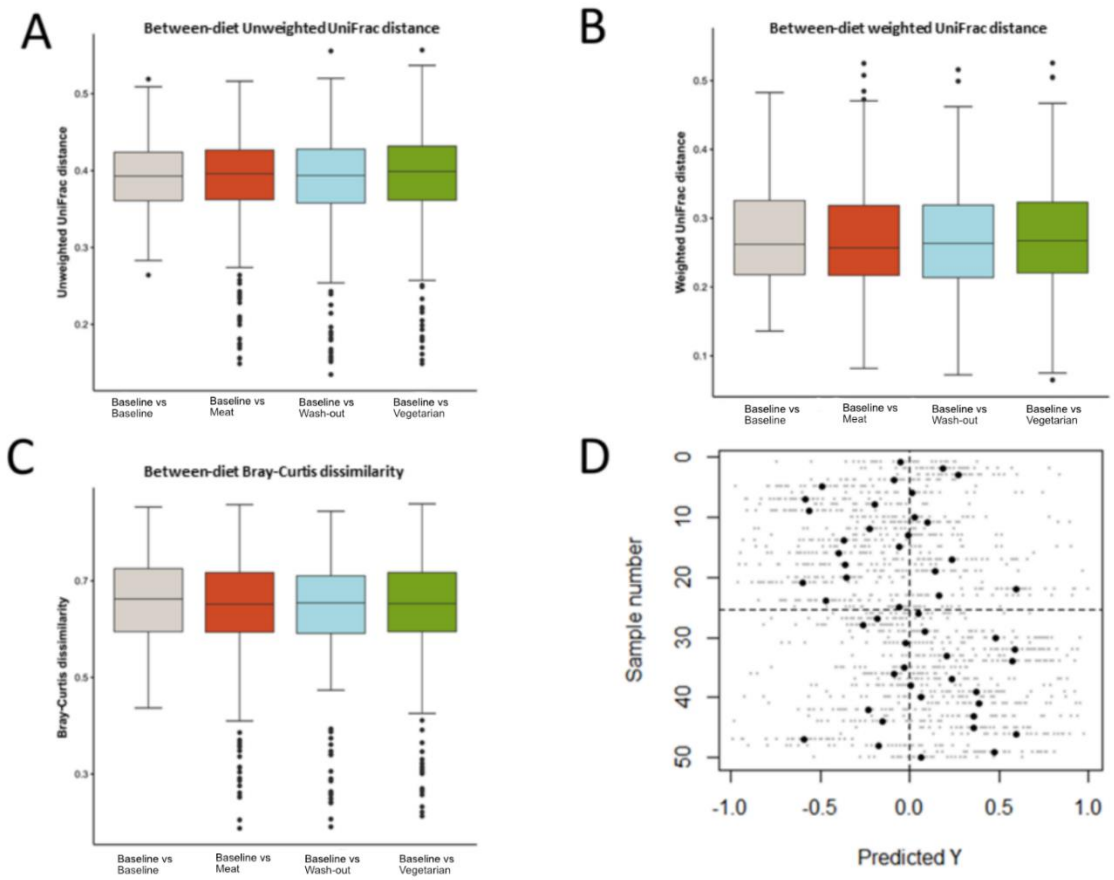
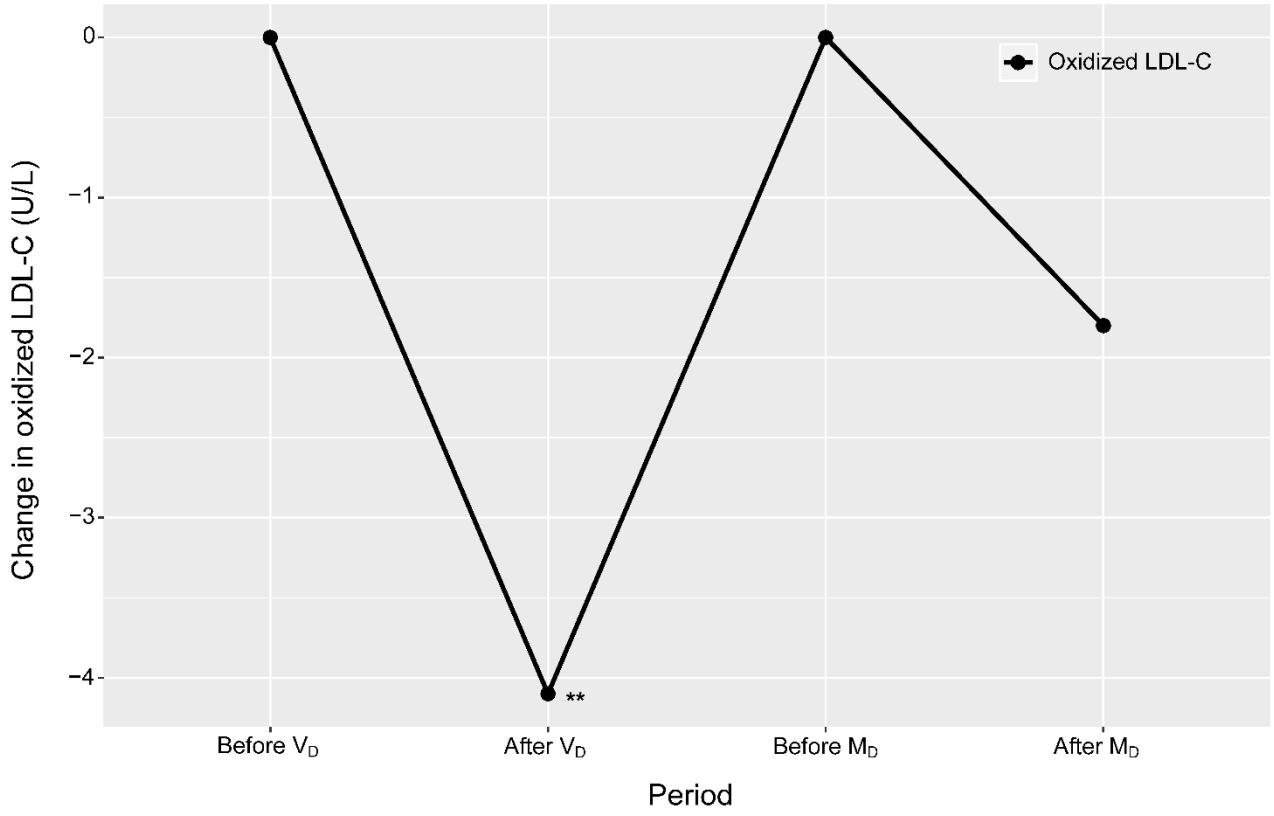


Figure S3. Difference in microbiota diversity and plasma metabolome of baseline and wash-out period.



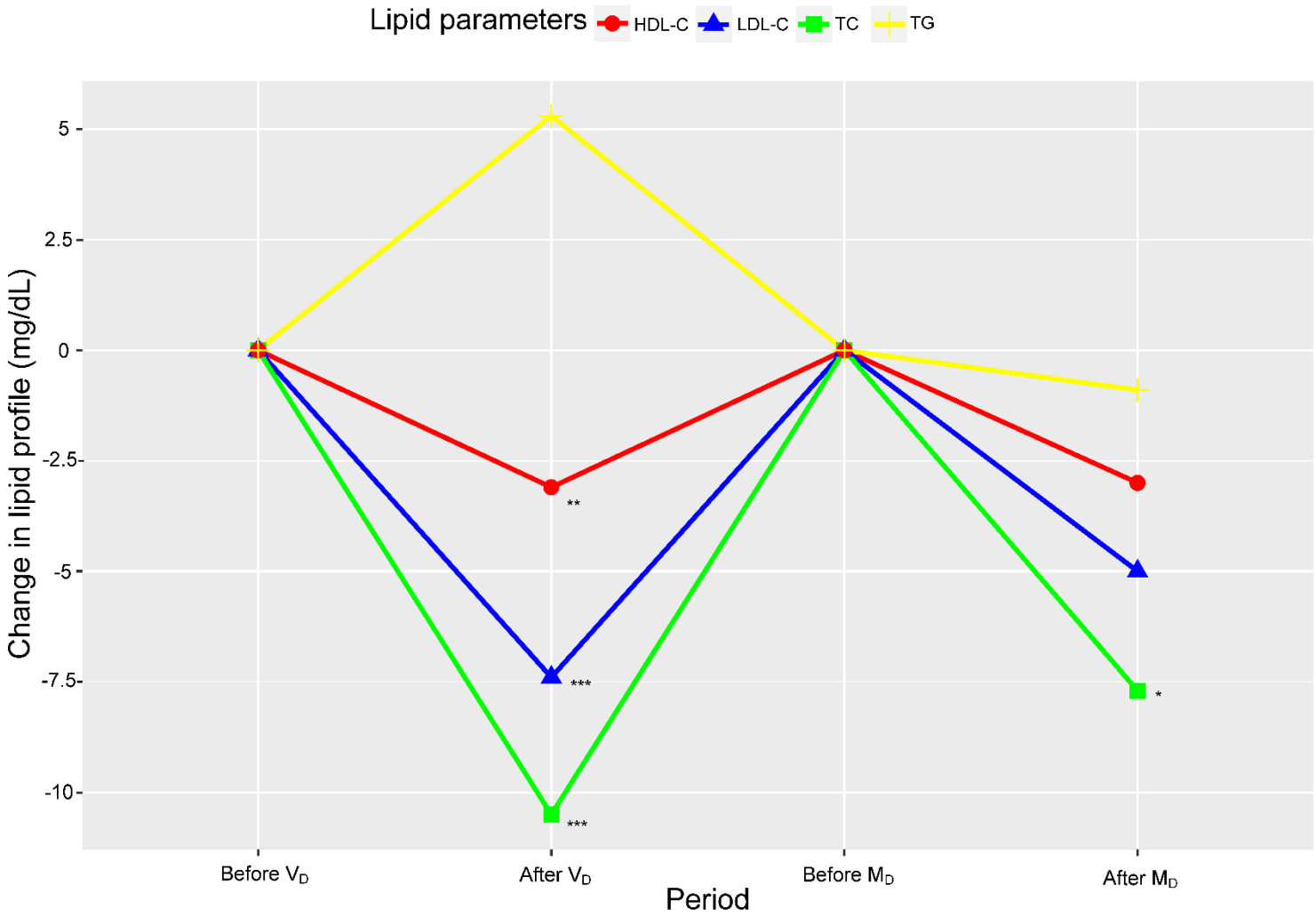
The between-diet distance in gut microbiota for all samples was assessed using (A) unweighted UniFrac, (B) weighted UniFrac and (C) Bray-Curtis. Random forest modeling of plasma metabolome between baseline and wash-out was conducted; model performance is shown in D. No significant difference in metabolome between sampling times was observed.

Figure S4. Time series analysis and mean changes in oxidized LDL according to dietary intervention.



Within-group change P-value was calculated with paired t-test. **P<0.01. LDL-C, low-density lipoprotein cholesterol; MD, meat diet; VD, vegetarian diet.

Figure S5. Time series analysis and mean changes in lipid profile according to dietary intervention.



Within-group change P-value was calculated with paired t-test. *P<0.05; **P<0.01; ***P<0.001. HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; MD, meat diet; TC, total cholesterol; TG, triglycerides; VD, vegetarian diet.

Figure S6. Relative abundance of gut microbiota at phylum (A) and genus level (B) representing the microbiome pattern across individuals with respect to diet period.

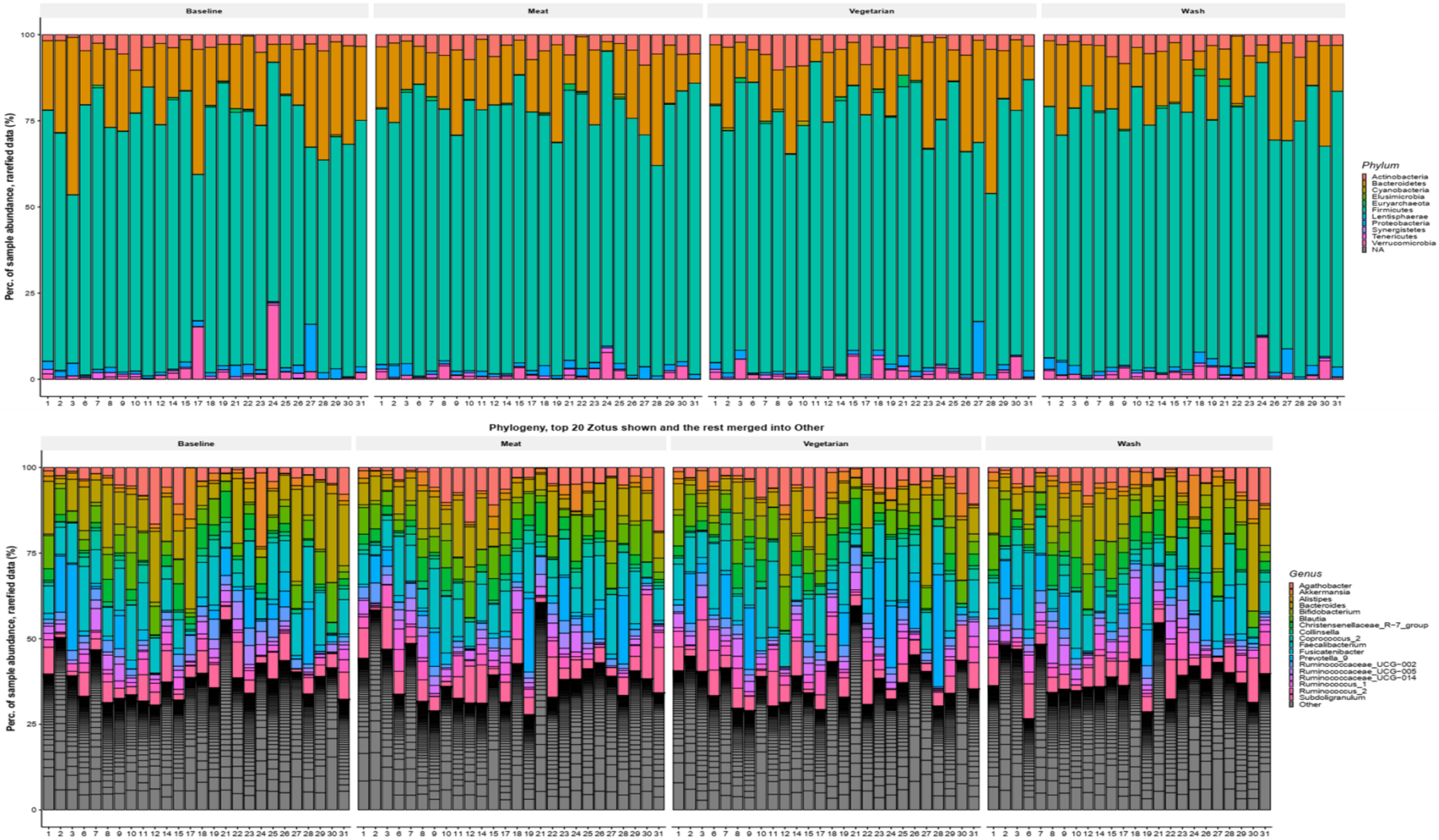
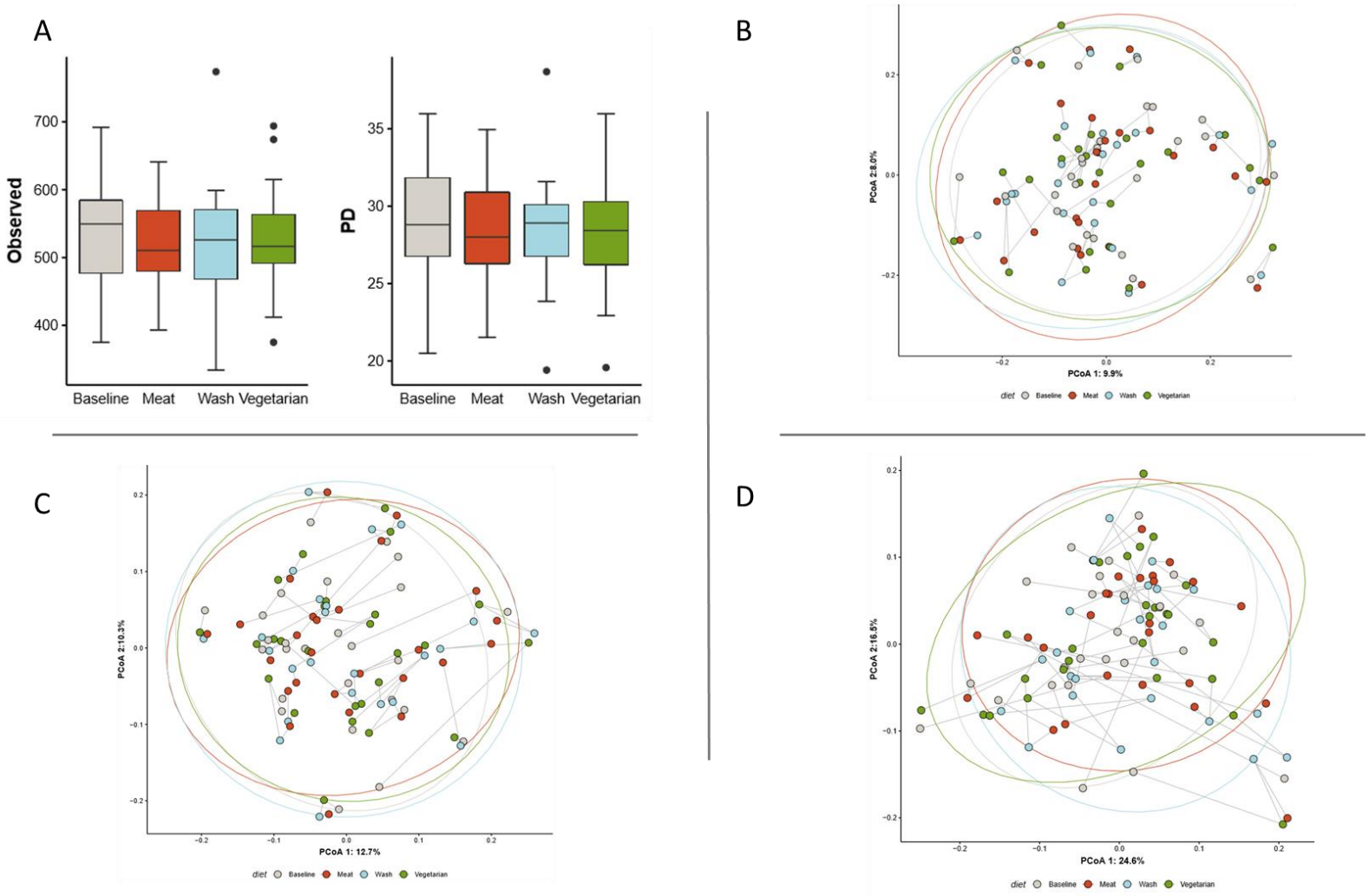
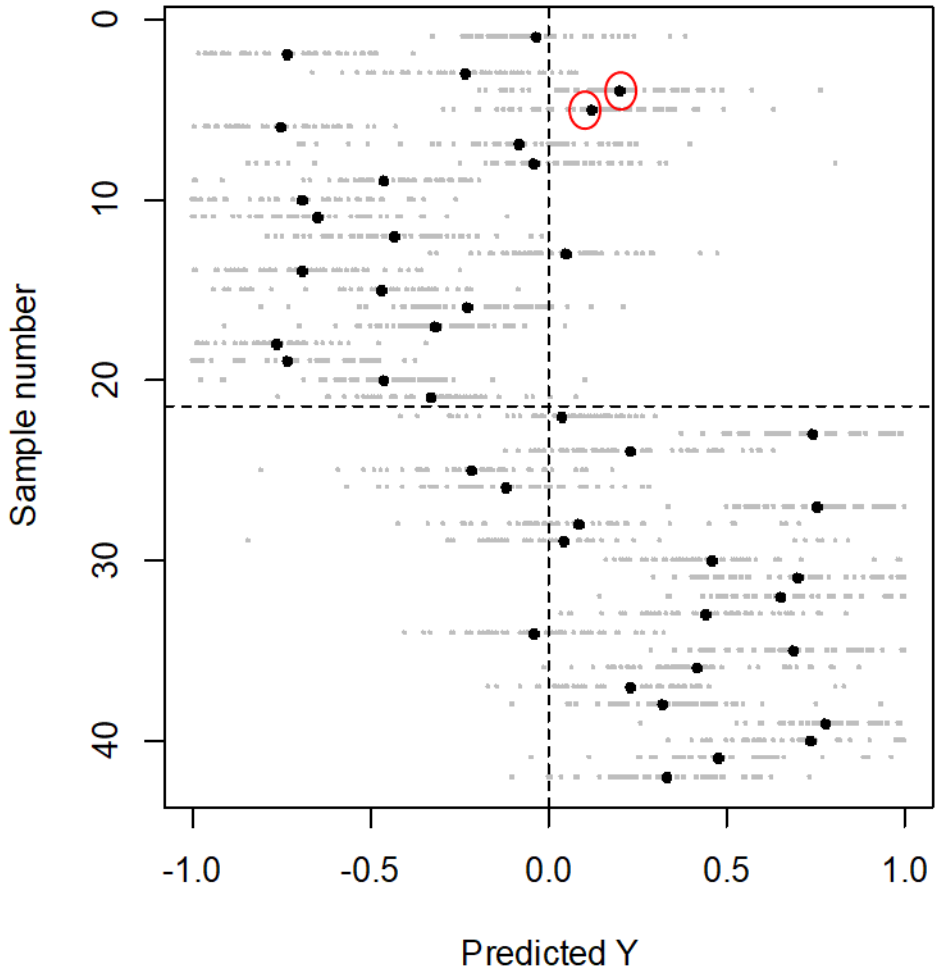


Figure S7. Gut microbiome diversity at four sampling times.



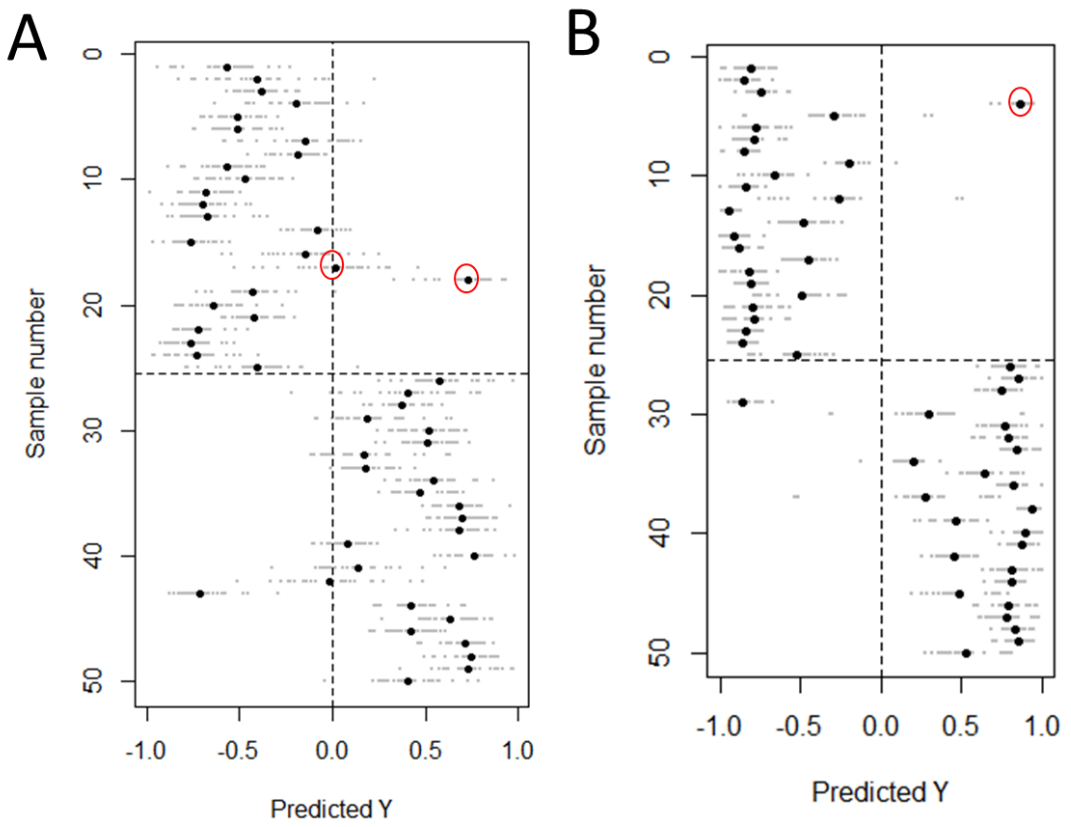
Observed species and Faith's Phylogentic Diversity (A), PCoA plots for Bray-Curtis dissimilarity (B), Unweighted UniFrac (C), and weighted UniFrac (D). Grey lines in panels B, C, and D link samples obtained from an individual at different sampling times.

Figure S8. Prediction of optimal selected bacteria genera using the random forest modeling based multilevel data analysis.



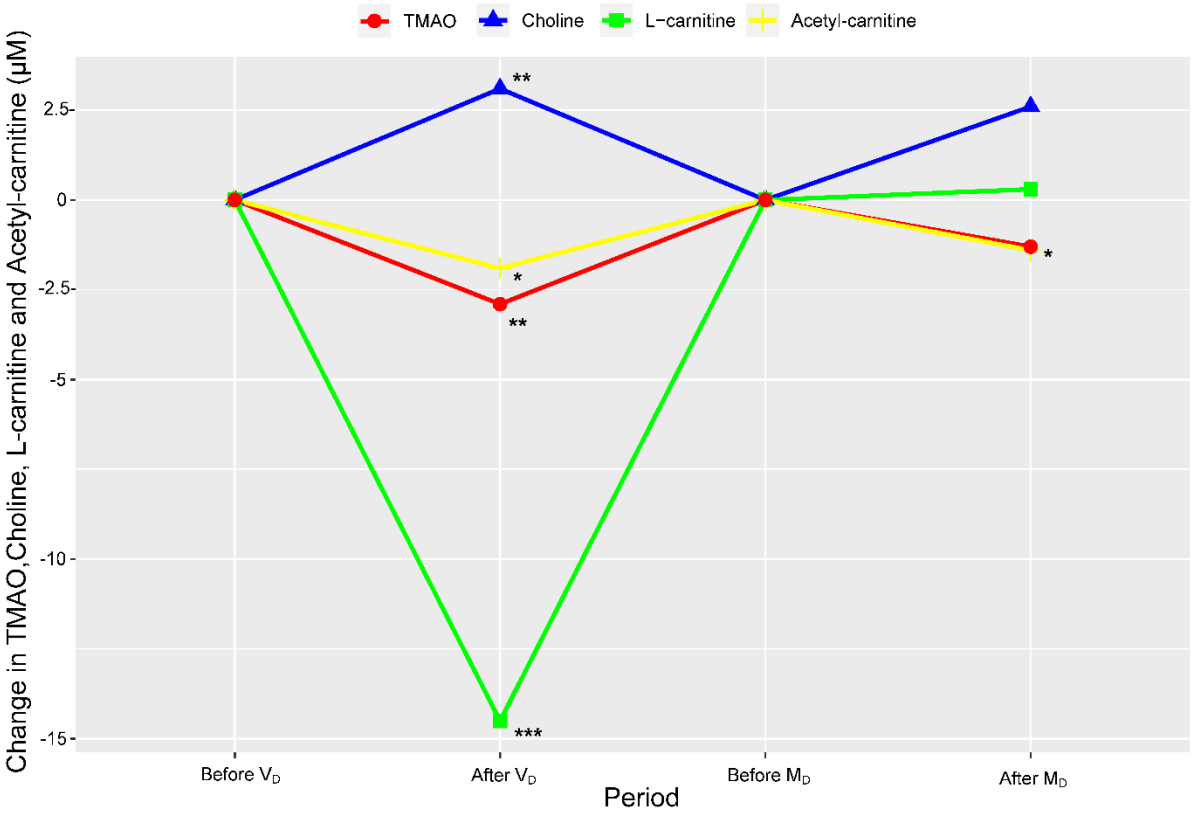
Samples are matched row-wise between upper and lower half for the treatment-effect matrix. Prediction estimates are shown in grey for each repetition of repeat double cross-validation and in black for the prediction estimates averaged over all repetitions. Misclassified samples are circled. The models showed high accuracy in discriminating the vegetarian diet from the meat diet.

Figure S9. Prediction of optimally selected metabolites using the random forest modeling based multilevel data analysis of plasma metabolome by ESI+ (A) and ESI- (B).



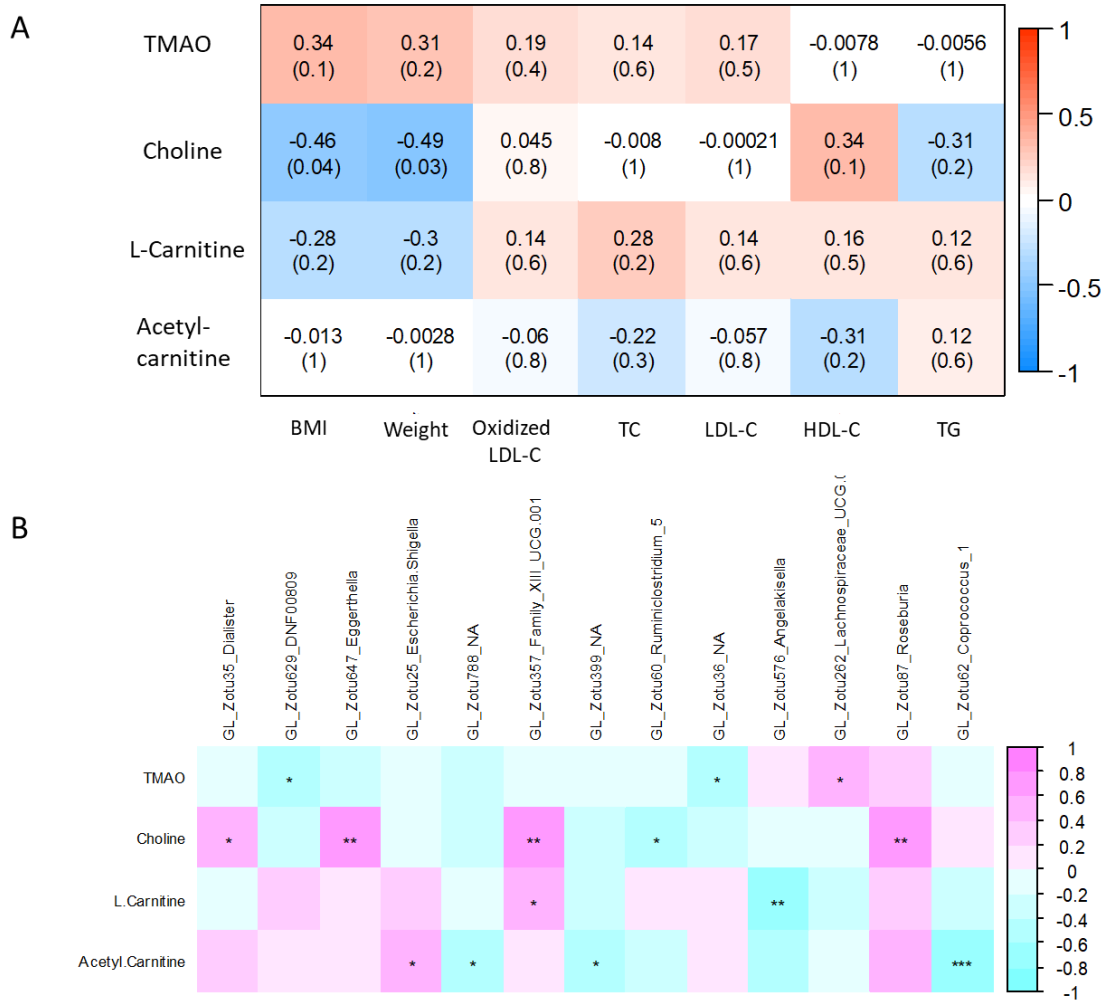
Samples are matched row-wise between upper and lower half for the treatment effect matrix. Prediction estimates are shown in grey for each repetition of repeat double cross-validation and in black for the estimates averaged over all repetitions. Misclassified samples are circled. The models showed high accuracy in discriminating the vegetarian diet from the meat diet.

Figure S10. Time series analysis and mean changes in TMAO, Choline, L-carnitine and Acetyl-carnitine according to dietary intervention.



Within-group change P-value was calculated with paired t-test. *P<0.05; **P<0.01; ***P<0.001. µM, micromolar concentration. MD, meat diet; TMAO, trimethylamine N-oxide; VD, vegetarian diet.

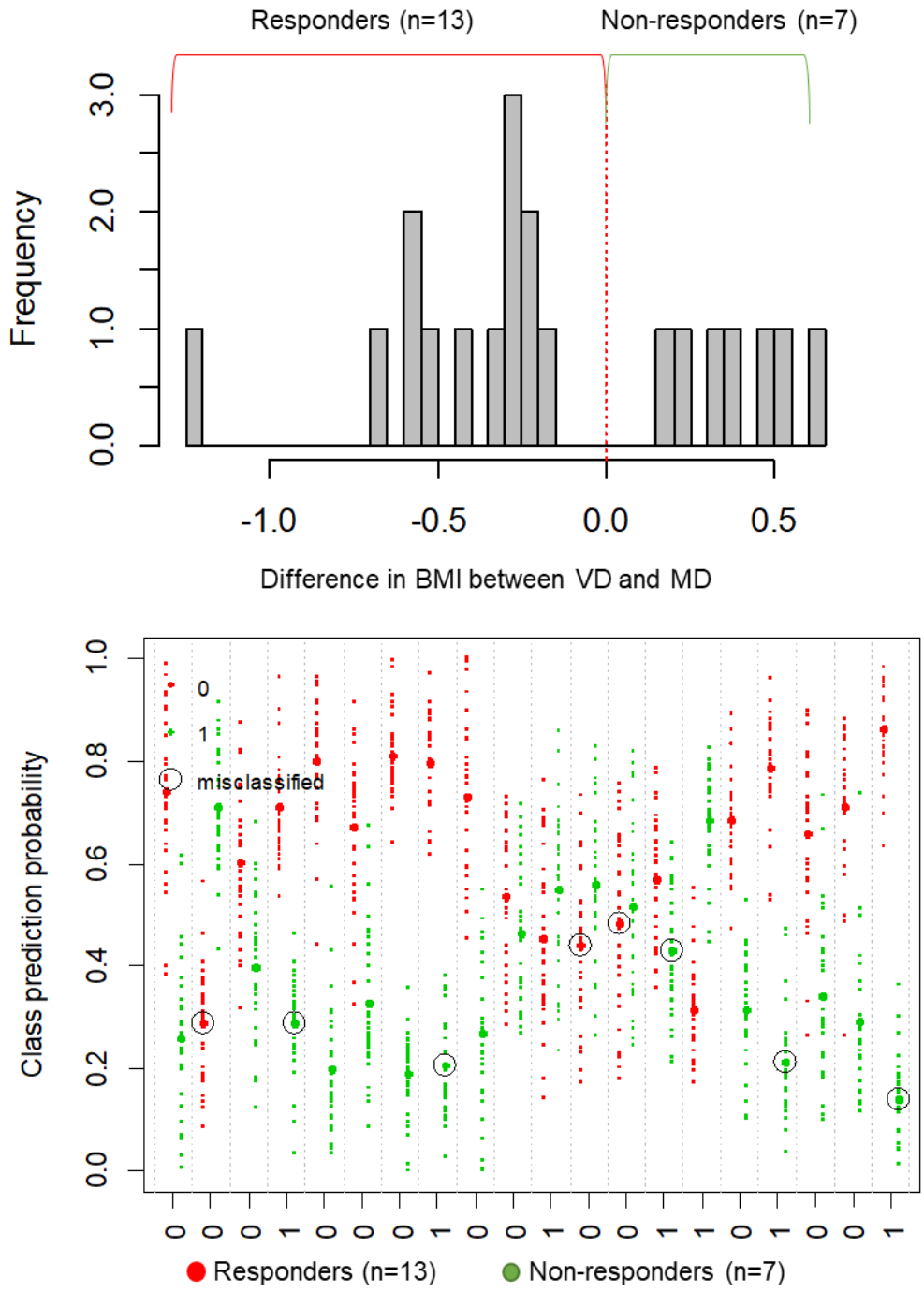
Figure S11. Correlations between plasma concentrations of trimethylamine N-oxide (TMAO), choline, L-carnitine and acetyl-carnitine and cardiometabolic risk factors (A) or the microbial genera (B) that were optimally selected to distinguish the vegetarian diet and the isocaloric meat diet using multilevel random forest algorithm.



Only microbial genera that were significantly correlated with at least one of plasma metabolites are present (B).

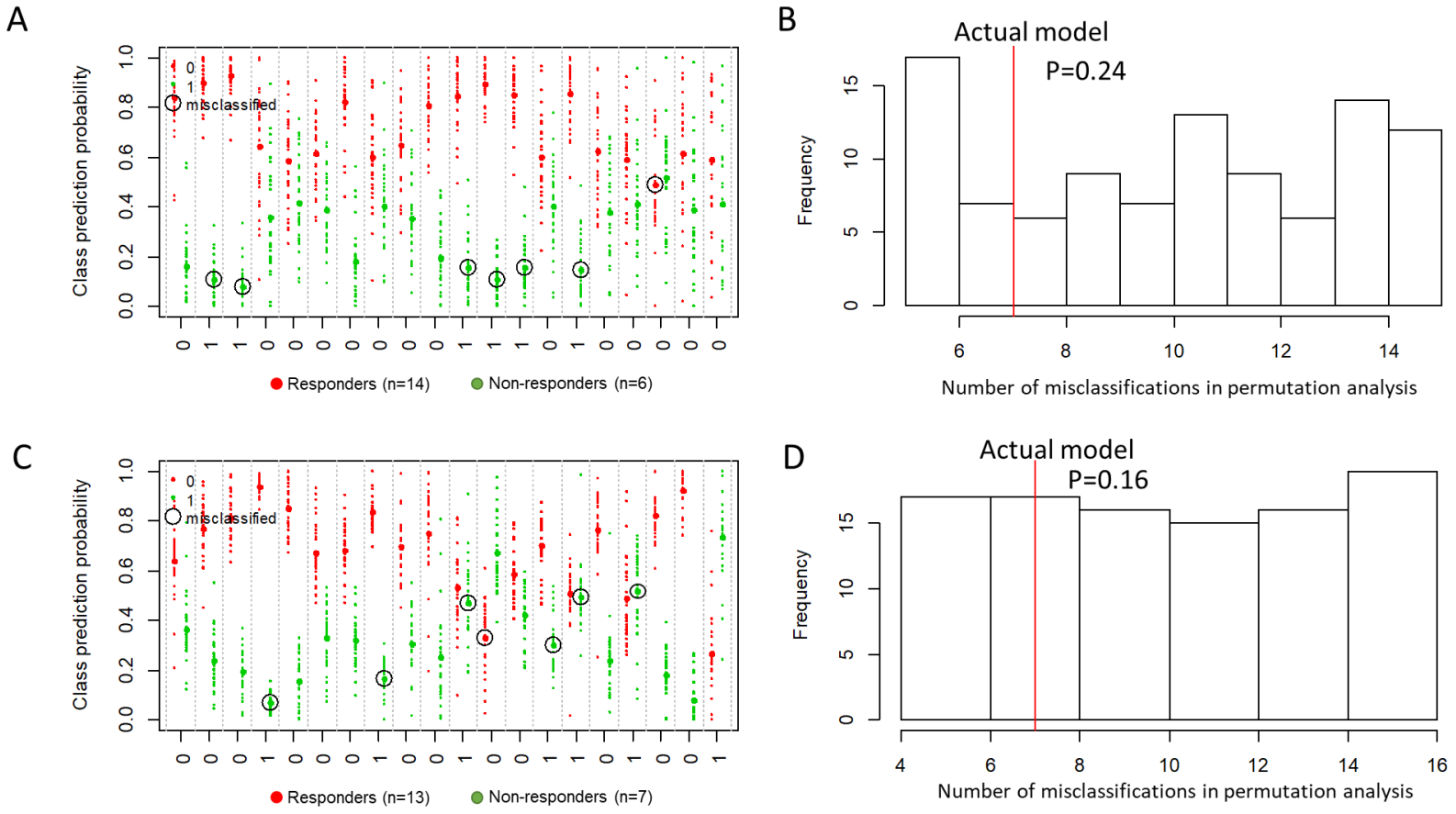
*P<0.05; **P<0.01; ***P<0.001. None of the correlations remained significant after false-discovery-rate correction for multiple testing.

Figure S12. Baseline gut microbiota associated with response to diet with respect to BMI.



Subjects with lower BMI post-VD compared with post-MD were defined as responders. Patients who had higher BMI after VD than MD were categorized as non-responders.

Figure S13. Baseline plasma metabolome showed no association with diet-related change in oxidized LDL-C (A and B) or BMI (C and D).



Accuracy of baseline plasma metabolome in discriminating responders from non-responders using random forest algorithm (A and C) and results from permutation analysis (B and D) is presented.