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Randomized Control Trials

Metabolic profiles reflect weight loss maintenance and the composition of diet after very-low-energy diet

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SUMMARY

Background & aims: Diet and weight loss affect circulating metabolome. However, metabolite profiles induced by different weight loss maintenance diets and underlying longer term weight loss maintenance remain unknown. Herein, we investigated after-weight-loss metabolic signatures of two isocaloric 24-wk weight maintenance diets differing in satiety value due to dietary fibre, protein and fat contents and identified metabolite features that associated with successful weight loss maintenance.

Methods: Non-targeted LC-MS metabolomics approach was used to analyse plasma metabolites of 79 women and men (mean age ± SD 49.7 ± 9.0 years; BMI 34.2 ± 2.5 kg/m^2) participating in a weight management study. Participants underwent a 7-week very-low-energy diet (VLED) and were thereafter randomised into two groups for a 24-week weight maintenance phase. Higher satiety food (HSF) group consumed high-fibre, high-protein, and low-fat products, while lower satiety food (LSF) group consumed isocaloric low-fibre products with average protein and fat content as a part of their weight maintenance diets. Plasma metabolites were analysed before the VLED and before and after the weight maintenance phase. Metabolite features discriminating HSF and LSF groups were annotated. We also analysed metabolite features that discriminated participants who maintained ≥10% weight loss (HWM) and participants who maintained <10% weight loss (LWM) at the end of the study, irrespective of the diet. Finally, we assessed robust linear regression between metabolite features and anthropometric and food group variables.

Results: We annotated 126 metabolites that discriminated the HSF and LSF groups and HWM and LWM groups ($p < 0.05$). Compared to LSF, the HSF group had lower levels of several amino acids, e.g. glutamine, arginine, and glycine, short-, medium- and long-chain acylcarnitines (CARs), odd- and even-chain lysoglycerophospholipids, and higher levels of fatty amides. Compared to LWM, the HWM group in general showed higher levels of glycerophospholipids with a saturated long-chain and a C20:4 fatty acid tail, and unsaturated free fatty acids (FFAs). Changes in several saturated odd- and even-chain LPCs and LPEs and fatty amides were associated with the intake of many food groups, particularly grain and dairy products. Increase in several (lyso)glycerophospholipids was associated with decrease in body weight and adiposity. Increased short- and medium-chain CARs were related to decreased body fat-free mass.

Conclusions: Our results show that isocaloric weight maintenance diets differing in dietary fibre, protein, and fat content affected amino acid and lipid metabolism. Increased abundances of several phospholipid species and FFAs were related with greater weight loss maintenance. Our findings indicate common and

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distinct metabolites for weight and dietary related variables in the context of weight reduction and weight management.
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Abbreviations

BMI	body mass index
CAR	acylcarnitine
ESI	electrospray ionization
(F)FA	(free) fatty acid
FFM	body fat-free mass
FM	body fat mass
HF	high-fat
HSF	higher satiety food
HWM	higher weight loss maintenance
LC-(HR)MS	liquid chromatography combined with (high-resolution) mass spectrometry
LF	low-fat
(L)PC(P)	(lyso)phosphatidylcholine (plasmalogen)
(L)PE(P)	(lyso)phosphatidylethanolamine (plasmalogen)
LSF	lower satiety food
LWM	lower weight loss maintenance
MW	molecular weight
OCFA	odd-chain fatty acid
PCA	principal component analysis
QC	quality control
qTOF	quadrupole-time-of-flight
RT	retention time
Trp	tryptophan
WC	waist circumference
VLED	very-low-energy diet

1. Introduction

Diet is one of the key modifiable lifestyle-related exposures affecting health [1]. Dietary patterns including consumption of whole grain cereal, plant-based products, and low amounts of saturated fats have beneficial effects on human health [2,3] and may support weight management [4,5].

Human plasma metabolome reflects endogenous biological reactions, gut microbial activity, and environmental exposures, such as diet-derived compounds [6]. Circulating metabolites act as signalling molecules and intermediates of glucose, lipid, and amino acid metabolisms participating in multiple molecular pathways related to health and disease [7]. For example, alterations in certain plasma metabolite levels have been considered biomarkers of cardiometabolic disease risk [8,9] and impairments or improvements in glucose and lipid metabolism [10,11]. Dietary patterns [12,13] and foods [14] affect the metabolome. Western diet high in refined grains and saturated fats was discriminated by short-chain acylcarnitines (CARs), long-chain fatty acids (FAs), and several glycerophospholipids from prudent diet high in vegetables and fruits [13]. Moreover, metabolomics approach has proven to be sensitive in identifying circulating biomarkers of individual foods [14].

Investigating metabolite features induced by dietary factors has elicited growing interest among contemporary nutrition research. However, the effect of diet on the metabolome has not been studied extensively in the context of weight management. Caloric restriction for 12 weeks with Mediterranean diet compared to low-fat diet had beneficial effects on the circulating lipid profile [15]. Moderate weight loss with New Nordic Diet higher in plant-based product, whole grain, and fish content compared to an average Danish diet for 26 weeks resulted in differences in e.g. amino acid metabolism and plasma levels of sugar alcohols and ketone bodies, which reflected the healthy dietary pattern and greater weight loss achieved with it [16].

The modulating effect of weight loss on plasma metabolite levels is well established [17–20] typically affecting pathways related to amino acid and lipid metabolism [17,18,21] – the pathways that are also affected by diet [22,23]. Nevertheless, studies with long-term follow up of metabolic changes after weight loss are lacking. Understanding the consequences of weight loss-induced metabolic changes after weight loss is important in order to decipher the influence on weight loss maintenance. Previously, weight loss-induced decrease in several lipid species was found to be associated with adiposity regain during a 12-week weight maintenance phase [21]. Whether these metabolic signatures reflect successful weight management after weight reduction for longer time is yet to be elucidated.

Therefore, the aim of this study was to investigate in participants who had reduced their weight with very-low-energy diet (VLED), the metabolic signatures of two isocaloric diets differing in satiety value due to different dietary fibre, protein, and fat contents. In addition, we wanted to examine which metabolic shifts were associated with improved weight loss maintenance after the weight loss phase. We also analysed in more detail whether the changes in plasma metabolites during weight loss and weight loss maintenance were associated with changes in anthropometric variables and consumption of certain food groups in order to better understand the underlying links between metabolic changes and successful weight management and to dissect those from diet–metabolite interactions.

2. Materials & methods

2.1. Participants

As reported before [24], 99 women and men from Kuopio region in Eastern Finland participated in the study (Fig. 1). Inclusion criteria was age of 30–65 years and body mass index (BMI) of 30–40 kg/m^2. Eligibility was assessed through screening, and subjects were excluded based on the following criteria: BMI >40 or <30 kg/m^2, type 1 or 2 diabetes, pregnancy, polycystic ovary syndrome, diagnosed eating disorder, kidney or thyroid dysfunction, heart or liver disease, alcohol consumption >16 (women) or >24 (men) portions/week, neuroleptic or cortisone medication or any other conditions that may prevent the participants from completing the study. The study was conducted according to the guidelines laid down in Declaration of Helsinki and all procedures involving human subjects were approved by the Ethics Committee

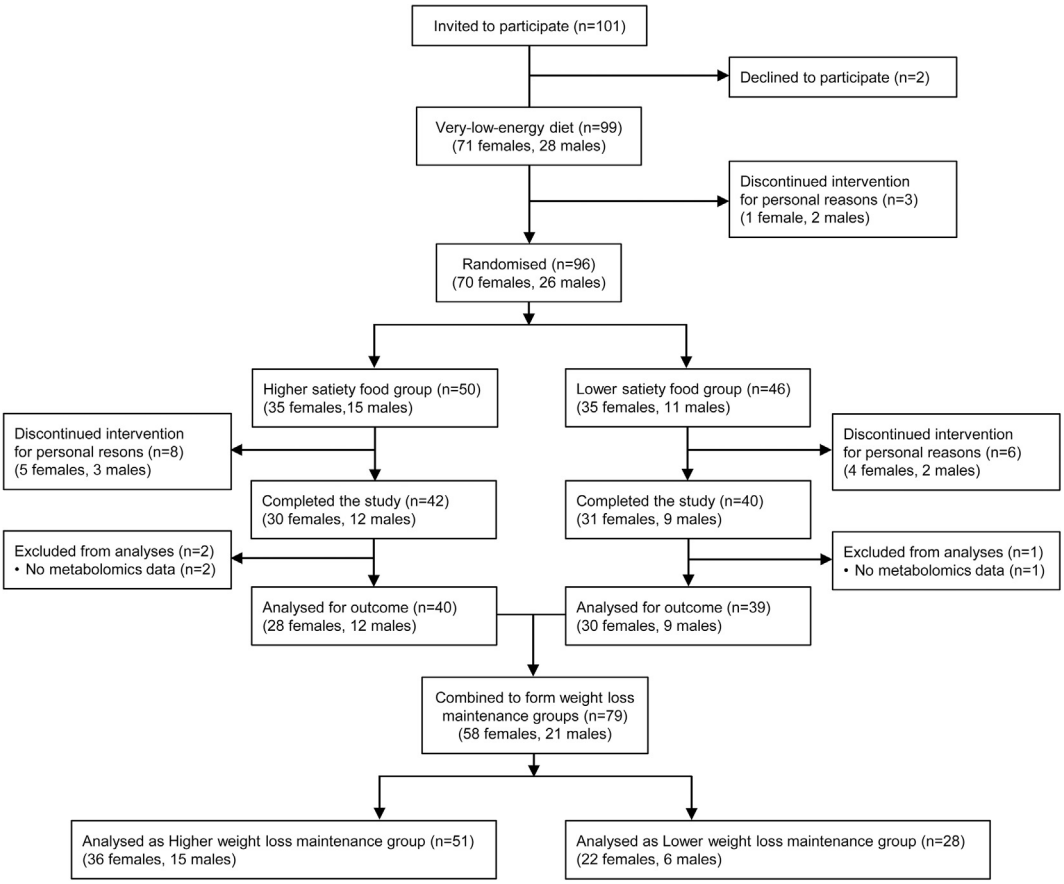

Fig. 1. Flow diagram of the study design.

of the District Hospital Region of Northern Savo (ethics no. 46/2008). All participants signed written informed consent before commencing the intervention.

2.2. Study design

This is a secondary analysis of a previously published parallel-arm study [24]. The study design, randomisation procedure, sample size and energy level calculations, and detailed descriptions of the weight maintenance protocol have been published earlier [24,25]. Briefly, for seven weeks, all the participants adhered to a VLED consuming commercial products providing 600 kcal/day (Nutrifast, Leiras Finland) as well as low-energy vegetables and energy-free beverages. Thereafter, they were randomised (stratified by age and sex) to two intervention groups for a 24-week weight maintenance phase. As a part of their weight maintenance diet, higher satiety food (HSF) group consumed food products that were pre-evaluated as having a higher satiety value, and lower satiety food (LSF) group consumed isocaloric food products with lower predetermined satiety value. Satiety values of the intervention food products were based on evaluations conducted in separate laboratory tests before the intervention (for detailed description see [24]). The products were selected among commercially available food items and among products that were under product development by Finnish food manufacturers. They represented typical Finnish staple food groups: bread, cheese, yoghurt, cold cuts, and vegetable patty (Supplemental Table 1). The foods the HSF group consumed had higher dietary fibre and protein and lower fat content and included whole grain cereal products and low-fat (LF) dairy and meat products, whereas the foods the LSF group consumed had lower dietary fibre and protein and higher fat content and included refined grain cereal products and average/high-fat (HF) dairy and meat products. The intervention food products comprised about 30% of the individually calculated energy content of the weight maintenance diet. The rest of the diet was compiled of freely selected foods by the participants.

Both study groups received the same information about the weight maintenance phase. These comprised written instructions about daily amounts of intervention food products to be consumed and a suggestion of daily amounts of freely selected foods according to the energy level of the diet. However, the participants were advised to consume the freely selected foods *ad libitum* according to their hunger sensations. In addition, they were instructed to aim at maintaining the weight reduction achieved during the VLED without any further weight loss and to keep their physical activity level unaltered throughout the study. The participants participated in group dietary counselling sessions led by a registered dietitian five times during the VLED, one time after the VlLED, and one time at the beginning of the weight maintenance phase.

2.3. Weight loss maintenance groups

We have reported earlier that the amount of maintained weight loss did not differ between the HSF and LSF groups at the end of the study [24,25]. However, there was great inter-individual variability in the weight loss maintenance during the weight maintenance phase (range in relative body weight change during the weight maintenance phase: −8.8% - +9.8%) [24]. Thus, in addition to investigating the metabolic signatures of different diet groups (i.e. HSF and LSF groups), we examined whether metabolic signatures

differ between those participants who maintained ≥10% weight loss (higher weight loss maintenance group, HWM) and those participants who maintained <10% weight loss (lower weight loss maintenance group, LWM) at the end of the study. The 10% weight loss maintenance threshold was chosen as it has been proposed of defining the long-term successful weight loss maintenance [26]. Consequently, metabolite features that discriminated these groups during the weight maintenance phase were analysed.

2.4. Assessment of diet

Food intake was measured by the use of dietary records covering four consecutive days including one weekend day. Dietary records were collected before the VLED (baseline) and at weeks 6, 12, 18, and 24 after the beginning of the weight maintenance phase. Food intake was calculated by food groups using the Micro Nutrica® dietary analysis software version 2.5 (The Social Insurance Institution of Finland). The software is built upon nutrient composition data of foods available in Finland. Food intake was analysed as consumption of food group expressed as grams per day. The software provided 13 main food groups and 71 subgroups which were combined to form 15 food groups of the interest of this study: (1) grain products with ≥6% of fibre (whole grain), (2) grain products with <6% of fibre (refined grain), (3) vegetables (excluding potatoes), (4) fruits and berries, (5) LF (semi-)liquid dairy products (products with <1% of fat; LF dairy), (6) HF (semi-)liquid dairy products (products with ≥1% of fat; HF dairy), (7) fermented dairy products (excluding cheese), (8) cheese, (9) red and processed meat, (10) sausages, (11) fish and shellfish (fish), (12) butter and vegetable oil-butter mixtures (butter), (13) margarines and vegetable oils (excluding coconut oil), (14) sugars, sweets, chocolates, ice cream, and sugar-sweetened beverages (sugar sources), and (15) alcoholic beverages.

2.5. Collection of blood samples and anthropometric measurements

The participants came to laboratory visits at the University of Eastern Finland for blood sampling and measurements of body height, weight, body composition, and waist circumference (WC) after an overnight fast before the VLED at baseline, before the beginning of the weight maintenance phase (week 0), and at 12 and 24 weeks after the beginning of the weight maintenance phase. They were instructed to abstain from alcohol consumption and vigorous exercise for 48 h and 24 h prior the measurements, respectively. Body weight was measured using a digital scale (Vogel & Halke, Hamburg, Germany) and body height using a wall-mounted stadiometer. Body composition was measured using bioelectrical impedance (STA/BIA Body Composition Analyzer, Akern Bioresearch Srl, Firenze, Italy). WC was measured halfway between the lowest crib and iliac crest. Blood samples were frozen and stored in −70 °C. Metabolite profiles were analysed from plasma samples collected before the VLED at baseline, week 0, and week 24. Hence, in this study, we excluded anthropometric data collected at week 12 from the analyses that follow.

2.6. Metabolomics analysis

Liquid chromatography combined with high-resolution mass spectrometry (LC-HRMS) was utilised for non-targeted metabolomics [27,28]. Briefly, sample (100 μl) were thawed on ice and mixed with ice-cold acetonitrile (400 μl) to precipitate plasma proteins. Samples were then centrifuged (700×*g* for 5 min at 4 °C) and the supernatants were collected and transferred to LC-HRMS. A pooled sample was injected after every 12 samples throughout the analysis for quality control (QC). In addition, a solvent blank was prepared and injected at the beginning of the sequence. Samples were randomised before the analysis. In addition, at the beginning and end of the sample analysis, data-dependent product ion scans (MS2) were acquired for each mode.

Amphiphilic metabolites were studied using an ultra-high performance liquid chromatography (Vanquish Flex UHPLC system, Thermo Scientific, Bremen, Germany) coupled online to high-resolution mass spectrometry (HRMS, Q Exactive Focus, Thermo Scientific, Bremen, Germany) [28]. The sample (2 μl) was injected onto a reversed phase column (Zorbax Eclipse XDBC18, 2.1 × 100 mm, 1.8 μm, Agilent Technologies, Palo Alto, CA, USA) and gradient elution technique was used. Mass spectrometry was equipped with heated electrospray ionization (ESI). The positive and negative ionization modes were used to acquire the data from 120 to 1100 (*m/z*).

For the analysis of hydrophilic compounds, we used an ultra-high performance liquid chromatography (1290 LC system, Agilent Technologies, Waldbronn, Karlsruhe, Germany) coupled online to high-resolution mass spectrometry (HRMS, 6540 UHD accurate-mass quadrupole-time-of-flight (qTOF) mass spectrometry, Agilent Technologies, Waldbronn, Karlsruhe, Germany) [27]. The sample (2 μl) was injected onto a column (HILIC, Acquity UPLC® BEH Amide 1.7 μm, 2.1 × 100 mm, Waters Corporation, Milford, MA, USA) and gradient elution technique was used. Mass spectrometry was equipped with a heated ESI source. For data acquisition, an extended dynamic range mode was used in both positive and negative ion modes from 50 to 1600 (*m/z*).

2.7. Statistical analyses for metabolomics data/feature selection

Peak detection and alignment were performed in MSDIAL ver. 4.18 [29]. Peaks were collected on *m/z* value range 50–1500 and all retention times (RTs) were considered. For the peak alignment across samples, the RT tolerance was set to 0.05 min, and the *m/z* tolerance was set to 0.015 Da. The final data matrix contained 12,179 spectral features. Data analysis was conducted separately on each four analytical modes in R version 3.6.3 [30]. The pre-processing procedure is described in more detail in Klåvus et al. (2020) [31]. In summary, the features were corrected for LC-MS-derived drift pattern. The features were log-transformed, and regularised cubic spline regression was fit separately for each feature on the QC samples; the smoothing parameter was chosen from an interval between 0.5 and 1.5 using leave-one-out cross validation to prevent overfitting. Missing values were imputed using random forest imputation [32].

The focus of the metabolic shifts was on the 24-week weight maintenance phase (from week 0 to week 24) after the weight loss period. Relevant features to be annotated were found using MUVR [33] with PLS-DA predicting each target variable and choosing the maximal set of predictive features. For comparison of the HSF and LSF groups, a multi-level MUVR model was used; here, the predictors were changes in metabolite feature levels across the 24-week weight maintenance phase, and the predicted variable was the dietary group. For comparison of HWM and LWM participants, a binary target variable was used, 1 indicating at least 10% weight loss at the end of the study (final weight <0.9 × baseline weight) and 2 indicating lower than 10% weight loss. Next, the relationship between the target variables and relevant features were analysed with feature-wise linear mixed models. Feature levels were used as the dependent variables. The time points (week 0/week 24), group (HSF/LSF; HWM/LWM) and their interaction factors were used as fixed effects and the subjects were used as a random effect. For both the PLS-DA models and the feature-wise linear mixed models, age and sex were used as covariates. The models were run multiple times with and without different covariates. For identification,

features that showed differences (unadjusted models p-value <0.05) between the study groups (HSF, LSF) and/or between the higher and lower weight loss maintainers (HWM, LWM) were selected. The data were further filtered according to RT (≥0.5 min). The filtered data set contained 1920 spectral features that were qualified for metabolite identification. Of these, 817 features were significant in the HSF-LSF comparison, and 1185 features were significant in the HWM-LWM comparison.

2.8. Metabolite identification

Metabolites were annotated based on RT, exact mass, and MS2 spectra (Supplemental Table 3). MS-DIAL [29], Agilent MassHunter Qualitative Analysis B.07.00, and FreeStyle 1.8 SP1 softwares were used to explore the MS2 data. MS2 spectra were compared with spectra that were available in in-house standard library or in electronic databases (HMDB, LIPID MAPS, METLIN). Arachidamide was identified based on Divito et al. (2012) [34]. Metabolite identification confidence levels were determined based on the classification of Chemical Analysis Working Group Metabolomics Standards Initiative: level I refers to validated identification based on a reference standard, level II refers to putative identification using fragmentation data that is available in literature and databases, level III refers to a putative compound characterisation based on the observed physicochemical characteristics of the metabolite feature, and level IV refers to an unknown metabolite [35].

2.9. Statistical analysis

The annotated metabolites, anthropometric and food intake data were further investigated in the study population. Descriptive data are expressed as mean ± standard deviation (continuous variables) and counts (categorical variables). P-value <0.05 indicates statistical significance. Differences in baseline anthropometric and food intake data between the groups were tested using Pearson Chi-Square test for categorical data and independent samples *t* test and Mann–Whitney U test for normally and non-normally distributed continuous data, respectively. Linear mixed models were used to investigate differences in the changes in dietary and anthropometric variables between the groups (Supplemental Methods). Group × time interaction was used as a fixed effect, participant as a subject with random intercept, and age, sex, and energy intake (food groups analyses) as fixed covariates. Variable transformations were performed when needed to fulfil the linearity assumption between the outcome and predictor variables (Supplemental Methods). In case of a significant fixed effect, *post hoc* comparisons of within-group changes and between-group differences in the outcome variables were calculated.

Robust linear regression [36] was used to investigate whether changes in metabolite abundances (outcome) were related to changes in anthropometric variables or the mean consumption of food groups (predictor) (Supplemental Methods). The associations were investigated in the study groups pooled into one sample and separately during the VLED and weight maintenance phase. Metabolites with a p-value <0.005 that discriminated the HSF and LSF groups and the HWM and LWM groups were chosen for these analyses. Age, sex, smoking, baseline metabolite abundance (when modelling VLED phase) or change in metabolite abundance during the VLED (when modelling weight maintenance phase) were entered as covariates in the models. In addition, the models with food groups as predictors included change in BMI and the mean energy intake during the weight maintenance phase as covariates. p-values obtained from the regression analyses were adjusted using Benjamini-Hochberg method to account for multiple testing. However, the results shown are based on the unadjusted p-values from robust linear regression models due to loss of several significant associations while using the adjusted p-values (also shown in Supplemental Table 4). Standardised regression coefficients were visualised in a heatmap with complete linkage hierarchical clustering using Euclidean distance.

Finally, principal component analyses (PCAs) using orthogonal Varimax rotation were conducted using the variables with at least one significant association with another variable in the regression analyses. PCAs were performed separately for VLED and weight maintenance phase (Supplemental Methods). The number of components was determined by eigenvalue >1, examining scree plots, and the interpretability of the components. Variables with standardised factor loadings ≥0.3 were considered when interpreting the components.

Analyses were conducted using R version 4.0.3 [30]. "lmerTest"-package [37] was used for linear mixed modelling, rlm function in "MASS"-package [38] for robust regression modelling, and "sfsmisc"-package [39] for calculating p-values. PCAs were conducted with principal function in the "psych"-package [40].

3. Results

3.1. Participant characteristics and changes in anthropometric measurements during the study

Eighty-two participants completed the whole study. Metabolomics data were missing from three participants. The remaining 79 participants (58 women, 21 men) form the study population of this study (Fig. 1). They were 49.7 ± 9.0 (mean ± SD) years old and had a BMI of 34.2 ± 2.5 kg/m^2 at baseline.

3.1.1. HSF vs. LSF

The baseline characteristics and changes in anthropometric variables in the HSF and LSF groups have been reported earlier [24,25]. In short, the groups did not differ in anthropometric or demographic variables at baseline. During the VLED, the whole study population reduced weight by 12.1 ± 3.5 kg ($p < 0.0001$, paired samples *t* test). During the weight maintenance phase, the HSF and LSF groups were equally successful in maintaining the reduced weight without any statistically significant differences in any of the anthropometric variables between the groups (p-values for group and group × time interaction from linear mixed models, range: 0.351–0.997 [25]). At the end of the study, the whole study population had lost 11.0 ± 4.8 kg of body weight, 7.6 ± 3.6 kg of body fat mass (FM), 3.4 ± 2.4 kg of body fat-free mass (FFM), 4.0 ± 2.7 percentage points of fat percent, and 10.7 ± 3.9 cm of WC from the baseline measurements ($p < 0.0001$ for all).

3.1.2. HWM vs. LWM

The division of participants according to the 10% weight loss maintenance threshold resulted in 51 participants (36 women, 15 men) in the HWM and 28 participants (22 women, 6 men) in the LWM group. At baseline, there were no differences between these groups in mean age, anthropometric variables, or participants randomised to HSF and LSF groups ($p > 0.05$ for all) (Table 1). As expected, linear mixed models adjusted for age and sex showed significant effect of group × time interaction on all anthropometric variables (Table 1). Based on *post hoc* analyses, VLED induced similar changes in body weight and FFM in the HWM and LWM groups. Instead, BMI, FM, fat percent, and WC decreased more in the HWM group compared to the LWM group. During the weight maintenance phase, all anthropometric variables increased in the LWM group, whereas they did not change in the HWM group (Table 1).

Table 1
Baseline characteristics and anthropometric measurements of the study participants divided into groups according to a 10% weight loss maintenance threshold.

	Higher weight loss maintenance group[a] (n = 51)			Lower weight loss maintenance group[a] (n = 28)			Group × time	
	Baseline	0 wk	24 wk	Baseline	0 wk	24 wk	F(df)	p
Intervention groups, HSF/LSF	27/24			13/15			$\chi^2 = 0.3$	0.580
Sex, female/male	36/15			22/6			$\chi^2 = 0.6$	0.442
Age, years	50.5 ± 9.2			48.3 ± 8.6			t = 1.0	0.299
Weight, kg	96.0 ± 12.5	82.7 ± 9.7[c]	82.4 ± 10.6[b]	94.5 ± 11.0	84.5 ± 9.4[c]	88.2 ± 10.5[c,b]	47.2 (2, 154.0)	<0.0001
BMI, kg/m^2	34.0 ± 2.4	29.3 ± 2.0[c,b]	29.2 ± 2.1[b]	34.6 ± 2.8	30.8 ± 2.3[c,b]	32.2 ± 2.6[c,b]	50.0 (2, 154.0)	<0.0001
FM, kg	37.4 ± 6.7	28.1 ± 6.3[c,b]	27.9 ± 6.1[b]	39.3 ± 6.1	31.9 ± 5.1[c,b]	34.7 ± 5.8[c,b]	32.1 (2, 152.9)	<0.0001
FFM, kg	58.6 ± 11.2	54.6 ± 9.2[c]	54.5 ± 10.0	55.4 ± 9.3	52.6 ± 8.3[c]	53.5 ± 8.6[c]	9.1 (2, 153.0)	0.0002
FM, %	39.2 ± 6.0	34.1 ± 6.6[c,b]	34.0 ± 6.4[b]	41.6 ± 5.0	37.8 ± 5.0[c,b]	39.4 ± 5.0[c,b]	14.1 (2, 153.0)	<0.0001
Waist circumference, cm	104.6 ± 10.2	92.2 ± 8.4[c,b]	92.0 ± 9.2[b]	106.0 ± 9.4	95.5 ± 8.6[c,b]	98.2 ± 10.2[c,b]	19.7 (2, 153.0)	<0.0001

Values for continuous variables are mean ± standard deviation and for categorical variables counts.
Baseline differences between the groups were tested using Pearson Chi-Square test for categorical data and Independent samples *t* test (normally distributed variables) and Mann–Whitney U test (non-normally distributed variables) for continuous data.
For repeated outcome measures, effect of group × time -interaction was tested using linear mixed models adjusted for age and sex. F statistics and p values for the interaction term are presented.
Abbreviations: FFM: body fat-free mass; FM: body fat mass; HSF, Higher satiety food group; LSF, lower satiety food group.
[a] Higher weight loss maintenance group: maintained weight loss ≥10% of initial body weight; lower weight loss maintenance group: maintained weight loss <10% of initial body weight.
[b] Between-group difference in the variable in the given study week is statistically significant ($p < 0.05$).
[c] Within-group difference in the variable between the given study week and the previous study week is statistically significant ($p < 0.05$).

3.2. Differences in the consumption of food groups between the study groups

3.2.1. HSF vs. LSF

As expected, the consumption of food groups related to the intervention food products differed between the study groups during the whole weight maintenance phase (Table 2). The HSF group consumed more whole grains and LF dairy and less refined grains, HF dairy, and sausages than the LSF group. The consumption of red and processed meat was higher in the HSF group compared to the LSF group at baseline (difference in the median (IQR) consumption: 49.6 (12.4) g/d, $p = 0.007$) but not at any week of the weight maintenance phase.

3.2.2. HWM vs. LWM

Only the consumption of HF dairy changed differently in the HWM and LWM groups during the study (Supplemental Table 2). *Post hoc* comparisons of study weeks showed that the consumption increased from baseline to week 6 in the LWM group. However, there were no statistically significant between-group differences in HF dairy consumption between the groups at any study week.

3.3. Metabolite profiles of higher and lower satiety food groups

During the 24-week weight maintenance phase, the weight maintenance diet affected especially the metabolism of amino acids and fatty acyls (Supplemental Table 3; Fig. 2; Supplemental Fig. 1). The HSF diet induced changes in arginine biosynthesis and metabolism and in glycine metabolism. Arginine was increased in both groups, and glycine was decreased in both groups. However, the level of both arginine and glycine remained lower in the HSF group in comparison to the LSF group at the end of the weight maintenance phase. Glutamine and ornithine were reduced in the HSF group during the weight maintenance phase, while the same metabolites were increased in the LSF group. At the end of the weight maintenance phase, glutamine and ornithine were on lower level in the HSF group in comparison to LSF group. Phenylalanine was increased in both groups, but the increase was steeper in the HSF group.

For fatty acyls, several short-, medium, and long-chained CARs were found to be reduced in both groups during the weight maintenance phase, but the reduction was more pronounced in the HSF group (Supplemental Fig. 1). As an exception, CAR 11:0, CAR 12:0 and CAR 12:1 were increased in the LSF group, while being reduced in the HSF group. In addition, aminobutyric acid betaine, a precursor of L-carnitine was found to be increased in the LSF group and reduced in the HSF group after the weight maintenance phase. During the VLED, CARs were increased in both groups.

During the weight maintenance phase, certain fatty amides (Supplemental Table 3; Supplemental Fig. 1) were increased in the HSF group, while their level was reduced in the LSF group. Some glycerophospholipids, namely odd- and even-chained lysophosphatidylcholines (LPC) and C18 lysophosphatidylethanolamines (LPE), indicated that this compound group was affected by the dietary intervention (Supplemental Fig. 1). In general, phospholipids were on a slightly lower level in the HSF group in comparison to the LSF group at the end of the weight maintenance phase.

3.4. Metabolite profiles of weight loss maintenance groups

When the HWM group and the LWM groups were compared for the metabolic shifts during the 24-week weight maintenance period, phospholipids were the largest category of metabolites that showed statistically significant differences (Supplemental Table 3; Fig. 3; Supplemental Fig. 2). These included phosphatidylcholines (PC), phosphatidylethanolamines (PE) LPCs, and LPEs. Seventeen out of 25 phospholipids that were on significantly different levels in the HWM and LWM groups and were identified on FA tail level contained a 16:0, an 18:1, and/or a 20:4 FA tail. PEs and PCs with odd-chain FA (OCFA) tails (PE(20:4/17:2), PC(17:0/18:2), PC(15:0/20:5), PC(17:2/20:4)) were on higher levels in the HWM group in comparison to the LWM group both after the VLED and after the weight maintenance phase (Supplemental Fig. 2).

Identified free fatty acids (FFAs) were unsaturated FFAs except for C16:0 FFA (Supplemental Table 3). FFAs also showed an overall higher abundance in the HWM group in comparison to the LWM group and tended to increase after the VLED and decrease after the weight maintenance phase (Supplemental Fig. 2). The C16:0 FFA was increased in the HWM group after the VLED but in the LWM group the level was decreased. After the weight maintenance phase, this FFA was decreased in both groups.

In the weight maintenance phase, amino acid metabolism showed differences between the groups. For example, creatine was on a lower level in the HWM group in comparison to the LWM

Table 2
Food intake (g/day) in the study groups at baseline and at 6, 12, 18, and 24 weeks after the beginning of the weight maintenance phase.

	Higher satiety food group (n = 40)					Lower satiety food group (n = 39)					Group x time	
	Baseline	6 wk	12 wk	18 wk	24 wk	Baseline	6 wk	12 wk	18 wk	24 wk	F(df)	p
Grain products with ≥6% fibre	99.0 ± 50.2	139.2 ± 35.4[e,d]	142.1 ± 39.8[d]	131.4 ± 47.7[d]	135.0 ± 43.5[d]	97.2 ± 61.0	91.8 ± 46.8[d]	97.1 ± 46.9[d]	92.0 ± 55.4[d]	89.7 ± 57.6[d]	6.6 (4, 295.9)	<0.0001
Grain products with <6% fibre	111.8 ± 64.9	56.5 ± 37.0[e,d]	54.5 ± 40.1[d]	66.7 ± 46.2[d]	52.8 ± 36.6[d]	125.8 ± 73.7	104.9 ± 46.8[d]	115.9 ± 57.0[d]	119.2 ± 43.7[d]	113.8 ± 51.6[d]	4.2 (4, 298.3)	0.003
Vegetables	166.8 ± 100.3	250.6 ± 152.0	249.0 ± 149.2	223.7 ± 132.3	207.6 ± 133.8	165.6 ± 73.7	255.9 ± 149.8	208.4 ± 101.4	192.5 ± 98.8	214.4 ± 124.0	1.7 (4, 294.1)	0.154
Fruits and berries	142.2 ± 123.1	135.5 ± 185.2	222.1 ± 161.0	207.4 ± 183.3	134.5 ± 106.3	142.7 ± 108.4	191.1 ± 112.9	247.0 ± 162.9	214.6 ± 153.7	149.1 ± 85.5	0.6 (4, 294.1)	0.627
Low-fat (semi-)liquid dairy	275.8 ± 194.7	322.8 ± 141.2[e,d]	353.5 ± 153.9[d]	383.6 ± 175.1[d]	363.3 ± 176.6[d]	228.0 ± 213.7	184.0 ± 175.8[d]	230.5 ± 188.1[d]	190.5 ± 197.8[d]	160.7 ± 128.7[d]	6.4 (4, 294.8)	<0.0001
High-fat (semi-)liquid dairy	107.7 ± 94.8[c]	68.8 ± 72.1[d]	67.5 ± 54.9[d]	68.7 ± 83.5[d]	60.8 ± 64.6[d]	184.9 ± 135.9[c]	220.8 ± 91.6[e,d]	211.8 ± 69.0[d]	241.3 ± 123.6[d]	250.5 ± 108.5[d]	6.2 (4, 297.0)	<0.0001
Fermented dairy	123.4 ± 152.5	228.5 ± 92.4	231.5 ± 93.6	243.4 ± 119.5	235.7 ± 101.1	127.3 ± 115.0	219.3 ± 116.1	240.2 ± 115.0	211.7 ± 95.3	219.8 ± 84.2	1.2 (4, 296.3)	0.291
Cheese	37.6 ± 29.7	59.4 ± 27.3[e]	66.9 ± 28.3[d]	60.9 ± 23.2[d]	58.0 ± 20.1	36.7 ± 29.9	54.7 ± 33.5[e]	50.0 ± 27.4[d]	48.6 ± 21.1[d]	51.6 ± 24.1	2.5 (4, 296.3)	0.045
Red and processed meat[a]	121.9 ± 62.9[c]	85.5 ± 49.9	85.7 ± 37.2	102.8 ± 75.0	110.3 ± 64.7	86.1 ± 70.3[c]	85.5 ± 48.1	83.2 ± 41.6	94.3 ± 53.5	83.2 ± 48.2	1.6 (4, 296.4)	0.175
Sausage[a]	35.4 ± 47.7	9.6 ± 16.0[e,d]	8.3 ± 13.5[d]	16.4 ± 24.2[d]	24.7 ± 33.4[d]	36.8 ± 48.0	38.9 ± 24.2[d]	45.3 ± 28.1[d]	52.3 ± 37.8[d]	48.1 ± 31.7[d]	7.4 (4, 297.8)	<0.0001
Fish and shellfish[a]	49.1 ± 59.9	23.5 ± 27.5	34.2 ± 39.2	37.4 ± 51.4	28.5 ± 34.9	44.5 ± 49.6	28.5 ± 30.7	32.8 ± 28.0	29.8 ± 42.0	36.1 ± 37.7	1.0 (4, 297.3)	0.424
Butter and vegetable oil-butter spreads	11.4 ± 8.2	5.9 ± 5.4	8.2 ± 5.8	6.9 ± 6.8	6.1 ± 5.5	14.9 ± 13.3	8.2 ± 8.2	8.1 ± 8.0	8.4 ± 8.8	7.1 ± 6.4	1.2 (4, 295.3)	0.298
Margarine and vegetables oils	20.5 ± 15.3	17.3 ± 12.2	24.1 ± 23.1	22.6 ± 17.1	22.8 ± 16.2	15.2 ± 14.9	13.0 ± 10.8	18.1 ± 12.1	15.7 ± 10.4	15.7 ± 14.4	0.3 (4, 296.2)	0.862
Sugar, sweets, chocolates, ice cream, and sugar-sweetened beverages[b]	154.7 ± 188.0	60.6 ± 113.8	61.8 ± 106.2	83.0 ± 172.0	120.6 ± 248.6	129.3 ± 122.0	43.6 ± 59.8	46.0 ± 55.3	41.8 ± 51.0	36.0 ± 39.7	1.7 (4, 292.2)	0.150
Alcoholic beverages[b]	325.7 ± 344.0	114.9 ± 238.2	99.3 ± 149.8	102.3 ± 163.7	198.9 ± 360.4	189.4 ± 280.2	55.8 ± 123.6	88.0 ± 184.7	97.7 ± 259.1	56.9 ± 130.8	0.3 (4, 295.2)	0.866

Values are mean ± standard deviation.
Baseline differences between the groups were tested using independent samples *t* test (normally distributed variables) and Mann–Whitney U test (non-normally distributed variables).
Effect of group x time -interaction was tested using linear mixed models adjusted for age, sex, and mean energy intake during the weight maintenance phase. F statistics and p values for the interaction term are presented.
[a] Square root transformed values were used in the analysis.
[b] Log(natural) transformed values were used in the analysis.
[c] Statistically significant difference between the groups at baseline (high-fat liquid dairy products, p = 0.009, red and processed meat, p = 0.007).
[d] Between-group difference in the dietary variable in the given study week is statistically significant (p < 0.05).
[e] Within-group difference in the dietary variable between the given study week and previous study week is statistically significant (p < 0.05).

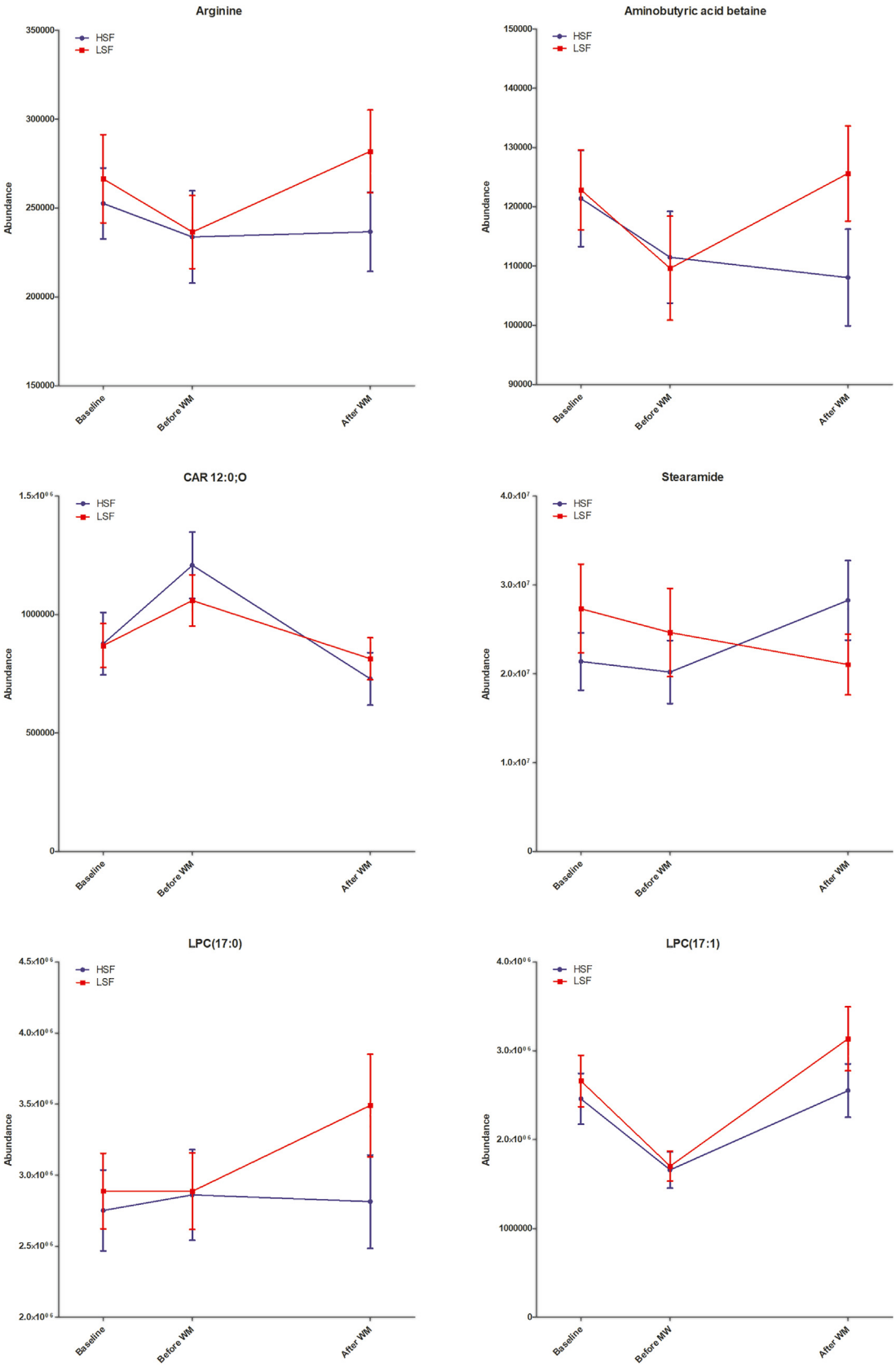

Fig. 2. Examples of metabolites with significantly different levels ($p < 0.05$) between the higher satiety food group (HSF, n = 40, blue line) and the lower satiety food group (LSF, n = 39, red line). The abundances (arbitrary units) are given at baseline, before the weight maintenance phase (WM), and after the weight maintenance phase. Vertical bars indicate 95% confidence intervals. Abbreviations: CAR, acylcarnitine; LPC, lysophosphatidylcholine.

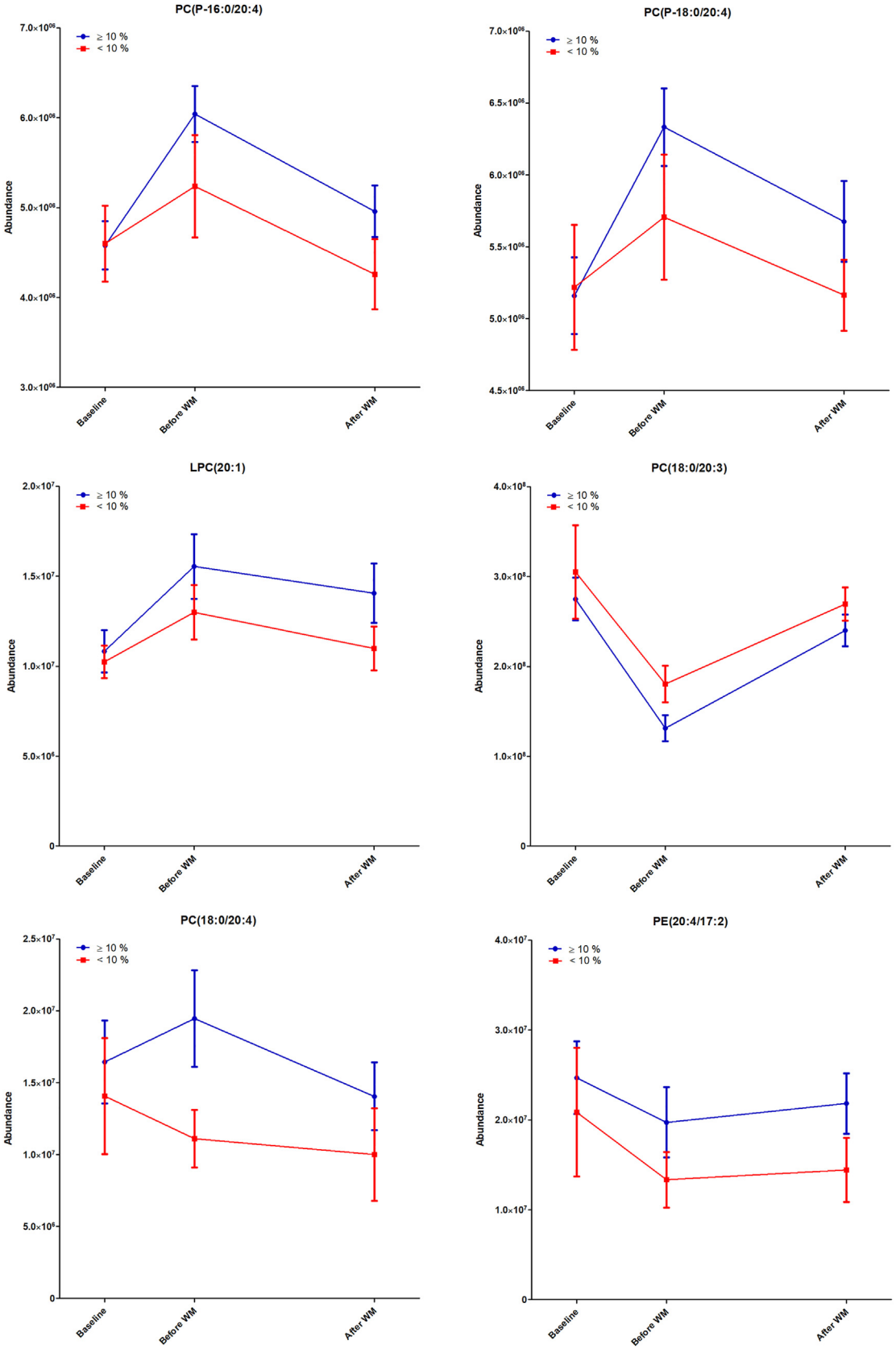

Fig. 3. Examples of metabolites whose abundances were on significantly different levels ($p < 0.05$) between the higher weight loss maintenance group (n = 51, blue line) and the lower weight loss maintenance group (n = 28, red line). The abundance levels are given at baseline, before the weight maintenance phase (WM), and after the weight maintenance phase. Vertical bars indicate 95% confidence intervals. Abbreviations: (L)PC(P), (lyso)phosphatidylcholine (plasmalogen); PE, phosphatidylethanolamine.

group. Tryptophan (Trp) was also on a significantly lower level in the HWM group; further, Trp carbonyl metabolite kynurenine was less abundant while Trp indolyl metabolite hydroxyindoleacetic acid was more abundant in the HWM group in comparison to the LWM group. Both Trp and kynurenine were first decreased after the VLED and then increased again during the weight maintenance phase in both groups. Instead, hydroxyindoleacetic acid was first increased in both weight maintainer groups after the VLED and then decreased after the weight maintenance phase.

3.5. Changes in metabolite abundances associated with anthropometric and food group variables in regression analyses

Next, we wanted to investigate in more detail food–metabolite associations in the context of VLED and weight maintenance, because there may be an underlying effect of diet on the metabolome in our study population. For the purpose, 54 metabolites that discriminated the HSF and LSF groups and/or the HWM and LWM groups having a p-value <0.005 were selected (Supplemental Table 4). The metabolites were subjected for robust linear regression analyses to investigate whether the changes in the abundances of these metabolites were associated with the changes in anthropometric variables (i.e. body weight, BMI, FM, FFM, fat percent, and WC) or food group consumption (Supplemental Table 4). Twenty-six metabolites (13 of which were glycerophospholipids) showed association with both anthropometry and food groups (Fig. 4). Ten metabolites were associated with food group variables only, and 13 with anthropometric variables only of which none were specific to the weight maintenance phase. Of the food groups, most pronounced were whole and refined grains and LF and HF dairy (Fig. 5). An inverse association was more frequent than a positive one between the changes in metabolite abundances and anthropometric variables, indicating an increase in the metabolite abundance with a decrease in body weight, BMI, FM, FFM, body fat percent or WC and vice versa.

3.5.1. Glycerophospholipids

Changes in odd-chain LPCs (LPC(17:0), LPC(17:1)) during the weight maintenance phase were inversely associated with the mean consumption of whole grains, LF dairy, and margarin and vegetable oils, while they had a positive association with refined grains, HF dairy, and butter (latter with LPC(17:1) only) (Fig. 5). The change in LPC(17:0) abundance was inversely related with the changes in body weight, BMI, FM, fat percent, and WC during the weight maintenance phase. On the contrary, the change in LPC(17:1) had a positive association with the changes in body weight, BMI, and FFM during the VLED. The changes in the abundances of lysoglycerophospholipids with a C16:0 or a C18:0 FA tail (LPC(P-18:0), LPE(18:0), and LPE(P-16:0)) were inversely associated with LF dairy consumption; LPC(P-18:0) additionally with red and processed meat consumption, and LPE(18:0) with fruit and berries consumption. All these three compounds had an inverse relationship with the changes in some or all anthropometric variables, especially adiposity indices FM, fat percent and WC (Fig. 5).

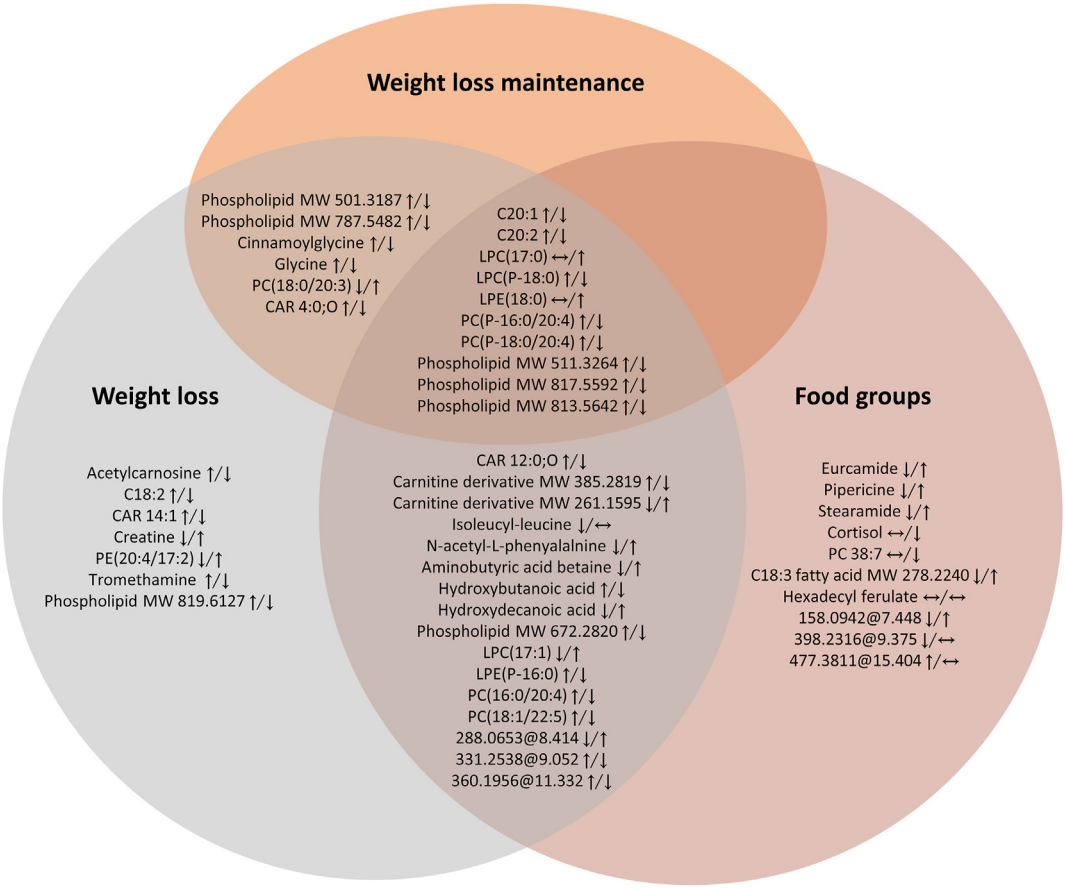

Fig. 4. Venn diagram of changes in metabolite abundances common and distinct for weight loss, weight loss maintenance, and consumption of food groups. The arrows indicate the average direction of change (↑ = increase, ↓ = decrease, ↔ = no change) in the metabolite abundance in the whole study population (n = 79) during the very-low-energy diet/during the weight maintenance phase. Abbreviations: CAR, acylcarnitine; (L)PC(P), (lyso)phosphatidylcholine (plasmalogen); (L)PE(P), (lyso)phosphatidylethanolamine (plasmalogen); MW, molecular weight.

Changes in three unknown phospholipids and identified glycerophospholipids with a saturated and a C20:4 FA tails as well as unsaturated FFAs showed negative associations with the changes in body weight, BMI, and adiposity indices (FM, fat percent, WC) during the VLED and/or weight maintenance phase (Fig. 5). On the contrary, the change in PC(18:0/20:3) abundance was positively related to the changes in body weight, BMI, FM, WC, and FFM during the VLED, and with FFM during the weight maintenance phase.

3.5.2. Acylcarnitines and carnitine-related compounds

CAR(12:0;O) and a carnitine derivative with molecular weight (MW) 385.2819 showed a positive relationship with the mean consumption of sausages (Fig. 5). The L-carnitine precursor aminobutyric acid betaine had a direct association with refined grains and HF dairy and an inverse association with LF dairy. During the VLED phase, CARs with short- or medium-chain FA tail were inversely associated with the reduction of body weight, BMI, FFM, and WC (Fig. 5).

3.5.3. Other compounds

Changes in the abundance of fatty amides erucamide, pipericine, and stearamide had a direct association with the consumption of whole grains and cheese and an inverse association with refined grain and sausage consumption (Fig. 5). Of amino acids and peptides, the change in creatine abundance had a direct relationship with the changes in body weight, BMI, WC, and FFM during the VLED, while the change in glycine abundance showed an inverse association with changes in fat percent and WC (Fig. 5).

3.6. Principal component analysis

PCA was conducted in order to examine whether the univariate relations between metabolites and anthropometry or food groups found in the regression analyses form combinations when considering all the variables simultaneously.

From the VLED data, PCA identified five components explaining 57% of the variance in the data (Supplemental Table 5). Component 1 was characterised by a negative correlation with the changes in body weight and FFM and a positive correlation with the changes in the abundances of FFAs, short- or medium-chain CARs, and glycerophospholipids with a saturated and a C20:4 FA tail (Fig. 6). Component 2 presented a negative correlation with the changes in adiposity and a positive correlation with the abundance changes in LPCs, LPEs, unknown phospholipids, and cinnamoylglycine. Component 3 presented a positive correlation with the changes in body weight, FFM, PC(18:0/20:3), creatine and other amino acids and derivatives. Component 4 was characterised by a positive correlation with the changes in the abundances of PCs, PEs and plasmalogens, and component 5 by a positive correlation with the changes in the abundances of fatty amides.

From the weight maintenance phase data, five components explained 54% of the variance in the data (Supplemental Table 6). Component 1 presented a negative correlation with the changes in the abundances of odd-chain LPCs and a positive correlation with the changes in the abundances of FFAs, CARs, PCs with a saturated and a C20:4 FA tail (Fig. 7). Component 2 presented a positive correlation with the changes in the abundances of LPCs, LPEs, a variety of unknown and known phospholipids. Component 3 presented a positive correlation with the consumption of whole grains and cheese, as well as the changes in the abundances of fatty amides, creatine, and PC(18:0/20:3). Component 4 was characterised by a positive correlation with the consumption of red and processed meat and the changes in body adiposity and a negative correlation with LPC(17:0) and cinnamoylglycine. Component 5 showed positive correlation with the consumption of refined grains, HF dairy, and sausages as well as the changes in the abundances of odd-chain LPCs and a negative correlation with the consumption of whole grains and LF dairy.

4. Discussion

In participants who had undergone weight loss on VLED, HSF diet widely affected amino acid and lipid metabolism compared to LSF diet. Regardless of diet, HWM compared to LWM participants showed alterations especially in lipid metabolism, specifically in FA and glycerophospholipid metabolism. Regression analyses conducted in the whole study population showed that changes in various metabolite abundances were associated with dietary and anthropometric variables, showing both common and distinct metabolites for diet and anthropometry, with both positive and negative associations. In the regression analyses, metabolites specific for weight maintenance were not found, since all associations found during the weight maintenance phase were also found during the VLED. PCA showed that the reduction in anthropometric measures clustered highly with several metabolites, whereas the same was not observed for dietary variables during the weight maintenance phase. The results indicated that severe perturbation of energy balance and thereby great reduction of body weight achieved in 7 weeks may be more prominent modifier of the metabolome than dietary factors during maintenance of the reduced weight.

4.1. Amino acid metabolism and fatty amides associated with dietary factors

In our study, arginine levels were lower and urea levels higher in the HSF compared to the LSF group after the weight maintenance phase. In previous studies, pathways related to arginine metabolism, a urea cycle intermediate, have changed due to weight loss [41] and changes in the amount and quality of dietary protein [23]. High protein intake can lead to increased urea production from arginine [23]. Higher uridine level in the HSF group may indicate increased amino acid flux due to consumption of higher protein foods [23].

Some fatty amides increased in the HSF compared to the LSF group during the weight maintenance phase. Moreover, increased abundances of erucamide, pipericine, and stearamide were associated with higher whole grain and cheese consumption, partly explaining the differences in these compounds between the HSF and LSF groups. Erucamide resembles oleoylethanolamide [42], an endocannabinoid analogue which induces satiety and reduces weight gain [43]. Our observation is interesting since whole grains and cheese were among the higher satiety foods. Whole grains and cheese are rich in dietary fibre and protein, respectively, which induce greater satiety than refined grains and lower protein foods [44,45]. The mechanisms behind the association between dietary factors and fatty amides remains to be elucidated.

4.2. Glycerophospholipids were widely affected by the intervention

We observed that glycerophospholipids, especially LPCs and LPEs, were on different levels in the HSF and LSF groups. Glycerophospholipids also constituted the largest metabolite group whose levels differed between the HWM and LWM groups. LPCs and LPEs with a C16:0, C18:0 or an OCFA tail were mostly affected by diet, specifically grain and dairy products. In line with previous findings [8,46–48], increased abundances of LPC(17:0) and LPC(17:1) were associated with higher HF dairy and LPC(17:0) with lower red and processed meat consumption. Interestingly, they

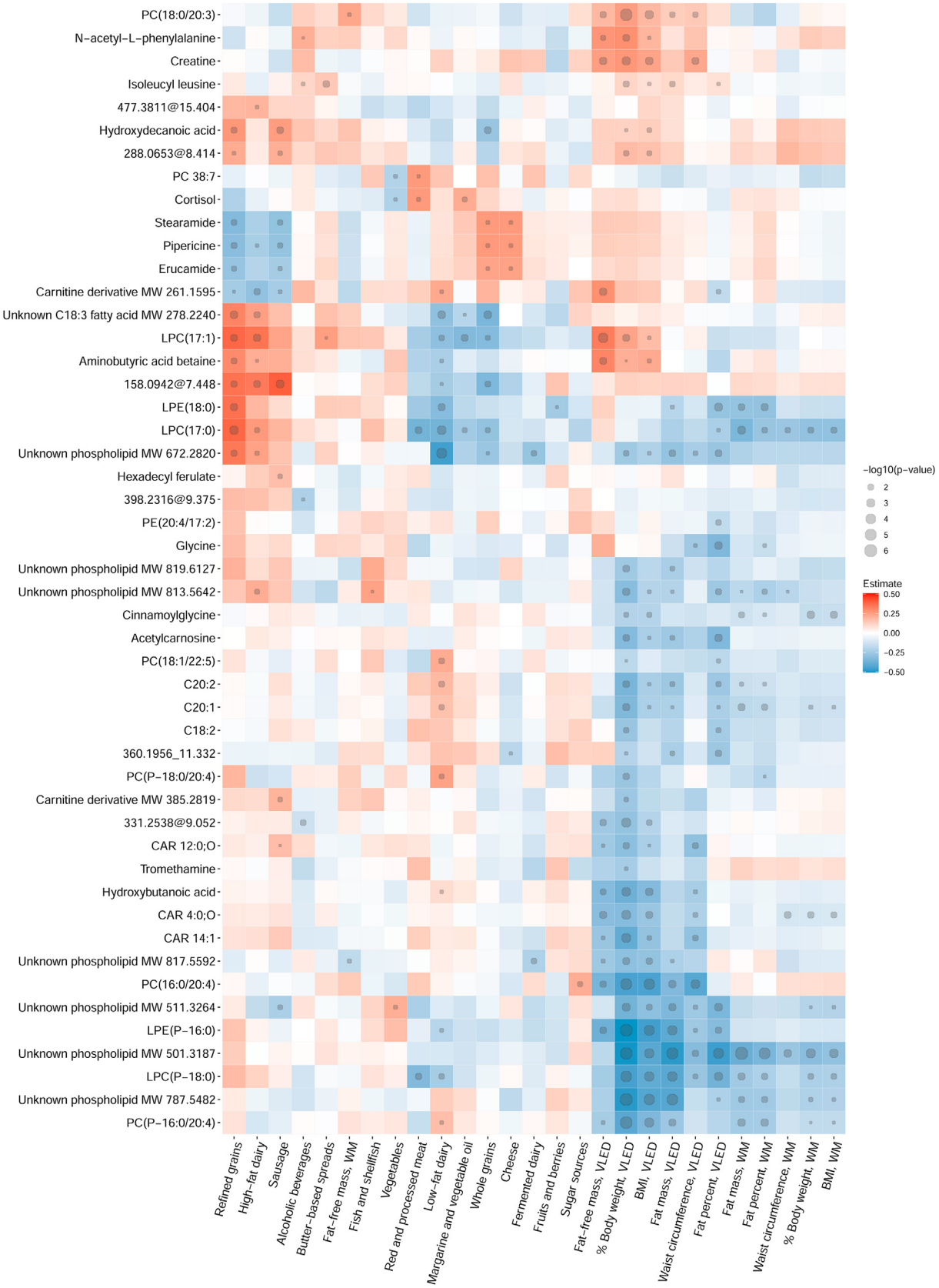

Fig. 5. Heatmap of associations between changes in metabolite abundances and (1) changes in anthropometric variables during the VLED, (2) changes in anthropometric variables during the weight maintenance phase, and (3) mean consumption of food groups during the weight maintenance phase in the whole study population (n = 78–79). Metabolites that were significantly associated with at least one predictor variable are presented in the heatmap. The blocks indicate standardised regression coefficients (estimate) obtained from robust linear regression analyses. Red blocks indicate positive estimates and blue blocks indicate negative estimates. The analyses were adjusted for age, sex, smoking, baseline metabolite abundance (when modelling VLED phase)/change in the metabolite abundance during the VLED (when modelling weight maintenance phase). Models with food group

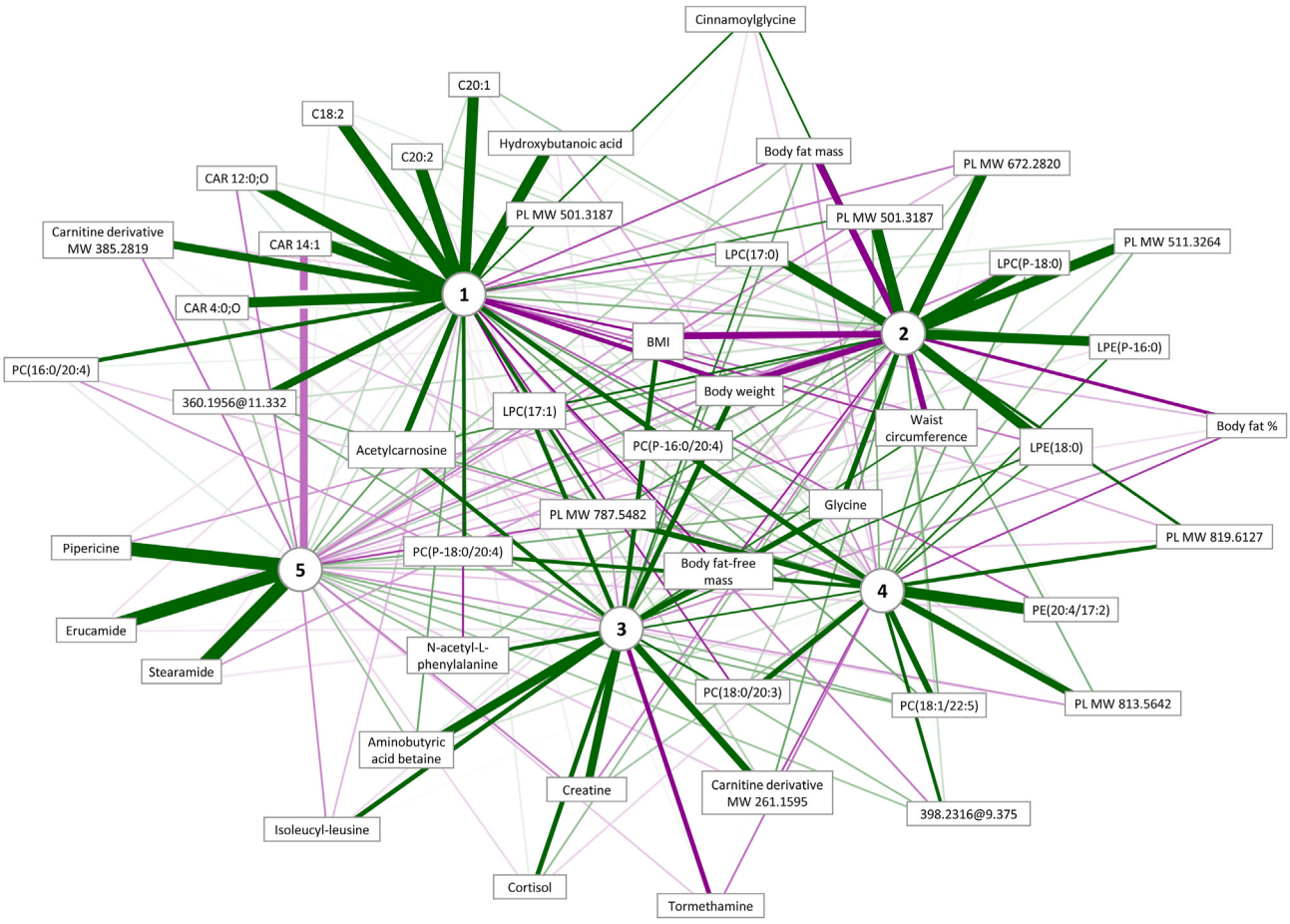

Fig. 6. Network plot of principal component analysis of the very-low-energy diet data (relative change for body weight and delta for all other variables) using Varimax rotation (n = 79). Component numbers are shown in circles. The direction and magnitude of variable loadings into each component are deciphered by the colour and width and length of the network edges, respectively. Green indicates a positive loading and red indicates a negative loading. The width and length of the edges increase and decrease, respectively, with increasing loading. Abbreviations: CAR, acylcarnitine; (L)PC(P), (lyso)phosphatidylcholine (plasmalogen); (L)PE(P), (lyso)phosphatidylethanolamine (plasmalogen); PL, unknown phospholipid; MW, molecular weight.

were associated with lower whole grain intake. Circulating OCFAs are proposed of originating from dairy fat [49], endogenous metabolism [50], or gut microbial-derived propionate [51]. Our finding of inverse association between LPC(17:0) and whole grain intake contradicts the evidence of C17:0 FA production from propionate derived from gut microbial fermentation of dietary fibre [51]. Previously, a direct correlation between whole grain or fibre intake and C17:0-containing lipid levels was found [9,47,52]. However, we and other studies [46,47] found OCFA-containing lipids to be associated with several food groups suggesting possible role of dietary patterns instead of individual foods in affecting their levels. Moreover, our study differs from the previous studies because of the effect of weight loss contributing to the shown results. Our regression analyses showed that body weight and adiposity were negatively related with LPC(17:0) during the weight loss and maintenance phases. The same has also been observed before [53]. Interestingly, our PCA clustered red and processed meat intake with increased adiposity and decreased LPC(17:0), suggesting that LPC(17:0) is related to dietary pattern supporting weight maintenance and cardiovascular health. Saturated OCFAs may have different biological activity than saturated even-chain FAs, indicated by their association with decreased cardiometabolic disease risk [9,47,54,55].

Increased (lyso)glycerophospholipids with a saturated even-chain and a C20:4 FA tail were related to decreased body weight and adiposity in the regression analyses, especially during the VLED. Previous studies have confirmed association between glycerophospholipids and weight loss or BMI [53,56–58], although the direction of the associations has varied. Our PCA results validate the inverse association between most glycerophospholipids and adiposity. Glycerophospholipids constitute most cell membrane lipids and provide FAs for eicosanoid synthesis, producing cell-signalling molecules lysophospholipids [59]. The inverse relationship with decreased adiposity may indicate the release of tissue lipids due to negative energy balance. On the contrary, we found that PC(18:0/20:3) levels paralleled with changes in body weight

variables were further adjusted for change in BMI and the mean energy intake during the weight maintenance phase. Statistically significant ($p < 0.05$) associations are presented with grey circles indicating -log p-value. The changes in metabolite abundances and anthropometric variables were calculated by subtracting the value at the beginning of the given study phase from the value at the end of the corresponding study phase. For body weight, percentage change in relation to the body weight at the beginning of the given study phase was used. Abbreviations: CAR, acylcarnitine; (L)PC(P), (lyso)phosphatidylcholine (plasmalogen); (L)PE(P), (lyso)phosphatidylethanolamine (plasmalogen); MW, molecular weight; VLED, very-low-energy diet; WM, weight maintenance phase.

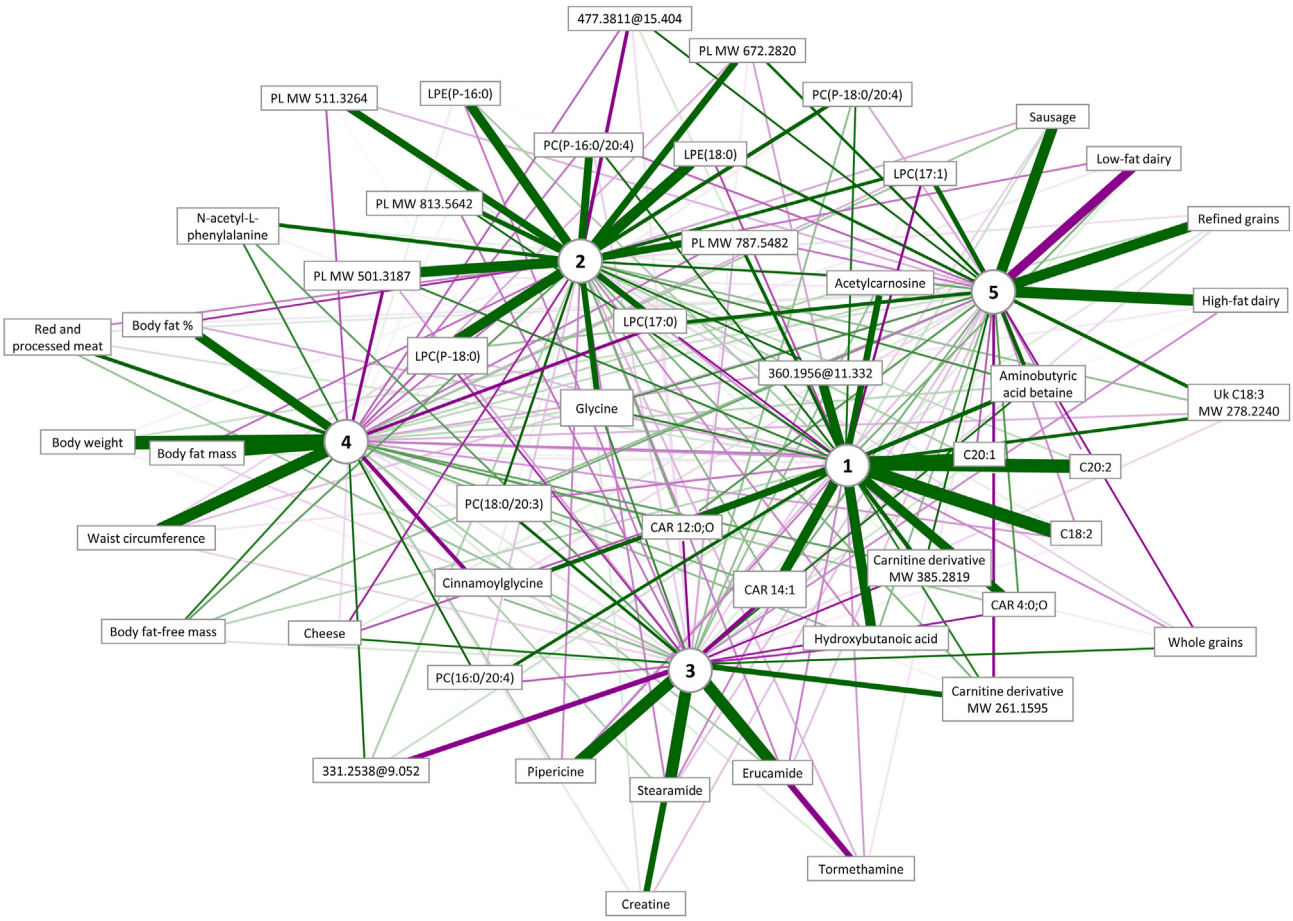

Fig. 7. Network plot of principal component analysis of the weight maintenance phase data (relative change for body weight, mean consumption for food group variables and delta for all other variables) using Varimax rotation (n = 77). Component numbers are shown in circles. The direction and magnitude of variable loadings into each component are deciphered by the colour and width and length of the network edges, respectively. Green indicates a positive loading and red indicates a negative loading. The width and length of the edges increase and decrease, respectively, with increasing loading. Abbreviations: CAR, acylcarnitine; (L)PC(P), (lyso)phosphatidylcholine (plasmalogen); (L)PE(P), (lyso) phosphatidylethanolamine (plasmalogen); PL, unknown phospholipid; MW, molecular weight; Uk, unknown lipid compound.

and FFM during the weight loss and maintenance phases, suggesting that FFM relates to circulating PC(18:0/20:3) levels more strongly than FM. Higher PC(18:0/20:3) levels have previously been positively correlated with BMI [53], increased type 2 diabetes risk [9] and cholesterol levels [60], indicating an interplay between PC(18:0/20:3), body weight, and cardiometabolic health.

4.3. Acylcarnitines indicated interaction between weight reduction and diet

Previously, short- and medium-chain CARs have been associated with red and processed meat intake [12,61,62] reflecting dietary FAs and protein sources [60,63]. Here we showed that most CARs showed slightly steeper reduction in the HSF compared to the LSF group in the weight maintenance phase. Increased abundances of CAR(12:0; O) and carnitine derivative MW 385.2819 were related to higher sausage consumption, indicating the association with processed meat, as shown previously, which may partly explain the milder reduction in CARs in the LSF group. CARs with variable FA chain lengths increased during the VLED, in general, while during the weight maintenance phase, the levels decreased. In the HWM group, the identified CARs increased more compared to the LWM group during the VLED. These observations suggest that weight loss-induced changes in CAR levels are modified by dietary patterns during weight loss maintenance.

We also found that CAR levels were inversely related with body weight and FFM in the regression analyses during the VLED. CAR levels typically increase during weight loss [17,63,64]. The secretion of FFAs from visceral fat has been proposed to influence increased CAR levels [17], and previously, increased hydroxylated short-chain and some long-chain CAR levels correlated with decreased FFM after a 12-week weight loss intervention [64]. CARs are components of β-oxidation of FAs in mitochondria [65]. During negative energy balance, lipid oxidation increases [66], and the low insulin level leads to inefficient suppression of CAR formation [11]. Thus, increased CAR levels may represent increased lipolysis and enhanced FA oxidation in FFM due to negative energy balance.

4.4. Strengths and limitations

The strength of this study was the use of non-targeted metabolomics that enabled discovering novel compounds related to diet and body weight loss maintenance, instead of pre-defined set of metabolites. The inclusion of both weight loss and maintenance phases together with the variation in weight outcomes in the study population enabled investigating metabolites that may underlie successful weight loss maintenance. Measuring food intake four

times during the weight maintenance phase allowed detecting possible transient changes in food consumption. The study has also certain limitations. The intervention foods comprised 30% of the participants' daily energy requirements. Fully controlled diets and thereby differences in the consumption of several other food groups might have been detected as differences across wider range of compound classes. However, the possibility to select most of the food items freely made the diets better representatives of the diets of free-living people. Further, the inclusion of more sample collection points would allow for an improved assessment of metabolic changes when changing from one diet to another. The study population consisted of middle-aged white people, limiting the generalisation of the results to other populations.

5. Conclusion

We demonstrated that diets with differing satiety values and consisting of foods with differing grain product quality and dairy and meat fat contents induced differences in lipid and amino acid metabolism in people who in average maintained an extensive weight reduction for 24 weeks. Furthermore, irrespective of diet, participants who maintained at least 10% weight loss at the end of the study showed differences especially in glycerophospholipid metabolism compared to participants who maintained less than 10% weight loss. In general, lysoglycerophospholipids with a saturated even-chain or an OCFA moiety and fatty amides showed the most consistent associations with whole grain and dairy products. A wide range of glycerophospholipids were inversely associated with body adiposity measures, while CARs were inversely associated with measures of FFM and WC. Overall, our findings indicate common and distinct metabolites for weight- and diet-related variables in the context of weight reduction and weight management thereafter. Moreover, changes in body composition may have different metabolic effects than changes in body weight. Our results provide evidence of metabolic signature in individuals with greater weight loss maintenance as well as of food items associated with favourable or adverse health outcomes. Thus, our findings lay ground for future investigation on the interaction of weight, diet, and metabolic pathways on health.

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Author contribution

MK and LK designed and planned the study, acquired the data and critically revised the manuscript for important intellectual content. KH, OS, and ML managed the LC-MS metabolomics. A Klåvus conducted preprocessing and statistical analyses of metabolomics data. A Kårlund identified the metabolites. MN analysed the final data with consultancy from SM. MN and A Kårlund drafted the manuscript and had primary responsibility for final content. All authors read, commented, and approved the final manuscript.

Conflicts of interest

The authors declare no conflict of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.clnu.2023.05.011.

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