RESEARCH ARTICLE

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Changes in Circulating Metabolites During Weight Loss are Associated with Adiposity Improvement, and Body Weight and Adiposity Regain During Weight Loss Maintenance: The SATIN Study

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Scope: To examine the relationship between changes in circulating metabolites during diet-induced weight loss and changes of adiposity. This study also investigates changes in these metabolites in relation to body weight and adiposity regain during a weight loss maintenance period.
Methods and Results: This cohort study is nested within the Satiety Innovation (SATIN) study. Participants (n = 162) achieving ≥8% weight loss during an initial 8-week low-calorie formula diet (LCD) are included in a 12-week weight loss maintenance period. A targeted metabolite profiling (123 metabolites) approach is applied using three different platforms (proton nuclear magnetic resonance, liquid chromatography mass spectrometry, gas chromatography mass spectrometry). Changes in several lipid species and citric acid are significantly associated with greater reduction of body weight, total fat, and abdominal adiposity distribution during the LCD. Decreases in the concentrations of lysophosphatidylcholines (LPCs) 14:0, LPC 20:3, phosphatidylcholine (PC) 32:2, PC 38:3, sphingomyelin (SM) 32:2, and increases in citric acid concentrations during the LCD are associated with adiposity regain and loss, respectively, during the weight loss maintenance period.
Conclusions: The results show that weight loss is associated with changes in lipid species and citric acid. These changes are related to subsequent weight and adiposity regain identifying the adipose lipid metabolism as an important factor for the maintenance of lost weight and adiposity.

1. Introduction

Overweight and obesity are amongst the biggest health concerns worldwide as they are major risk factors for other highly prevalent chronic health conditions, including cardiometabolic diseases,[1] and several types of cancer.[2] Prevention and treatment of overweight and obesity are therefore critical issues from a public health perspective. Both weight loss and weight regain prevention have great health impact. Of these, it is widely accepted that the main challenge for obesity treatment is weight loss maintenance. Several studies indicate that a healthy weight loss of 5–10% can be achieved regardless of which behavioral or pharmacological treatments are employed,[3] but weight is gradually regained in the vast majority of individuals.[4,5] Yet, strategies and physiological mechanisms supporting the maintenance of an achieved weight loss are less well explored.

Weight loss maintenance is hindered by a complex interaction of environmental, biological, behavioral, and

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cognitive factors, which are only partly known.[6] Difficulties in maintaining weight loss may stem from the metabolic and physiologic responses resisting the maintenance of decreased body weight.[7–10] Metabolomics, through a systematic evaluation of small-molecule metabolites in biological fluids, may help to identify these metabolic alterations. Although various low-calorie weight loss diets are effective for the treatment of obesity and the improvement of body composition and fat distribution,[11] little is known about how metabolic responses during weight loss are associated with changes of adiposity,[12,13] body composition,[13] and fat distribution.[13] Furthermore, whether changes in metabolomic profiles elicited by weight loss and associated with adiposity changes could be associated with successful maintenance weight loss and adiposity reductions remains unknown.

Therefore, we examined the relationship between changes in circulating metabolites during a dietary intervention inducing weight loss (≥8% of initial body weight) and adiposity reduction. We also investigated whether changes in these metabolites were associated with weight and adiposity regain during a weight-loss maintenance period.

2. Experimental Section

2.1. Study Design and Participants

The current study was nested within the FP-7 European Commission project Satiety Innovation (SATIN) work package 5, including participants from Denmark and Spain.

SATIN was a two-phase, double blinded parallel, randomized multicenter study, including women and men aged between 20 and 65 years with an initial body mass index (BMI) of 27.0–35.0 kg m^{-2}, fat mass of no less than 23%, and without comorbidities. Participants with significant weight changes (±3kg in the last 3 months), severe chronic medical conditions (type 1 or 2 diabetes, cardiovascular diseases, hypertension, chronic kidney diseases, liver diseases, active inflammatory bowel diseases, cancer, bariatric surgery and other interventions, psychological or behavioral problems, psychiatric disorders), drug addictions, regular alcohol consumption above recommendations and smoking (including smoking cessation within the last three months prior to study) were excluded from the study.

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The study comprises an initial 8-week low-calorie diet (LCD) period where participants were instructed to follow the Modifast (Nutrition et Santé SAS) formula diet to achieve at least an 8% of weight reduction needed for the inclusion in the randomized part of the study. Participants reaching the pre-defined weight loss entered in a 7–10 days run-in period for diet stabilization and then were randomly allocated in a 1:1 ratio to one of the two intervention groups; (1) including an active satiety enhancing product (active intervention group) or (2) including a similar control product without satiety enhancing properties (control group) for 12-weeks (weight-loss maintenance period). Detailed information of the study protocol and procedures has been published elsewhere.[14,15]

All procedures were conducted in accordance with the ethical principles set forth in the current version of the Declaration of Helsinki (Fortaleza, Brazil, October 2013). The protocol was approved by the local institutional review boards and Ethics Committees of all the recruiting centers and all participants provided written informed consent. This trial was registered in clinicaltrials.gov (identifier: NCT02485743). Also, all study procedures were aligned between sites before initiation of the study and on-site monitoring visits were carried out by an independent monitor.

Out of 236 participants with blood samples available at baseline, we have included in this analyses a total of 162 participants randomized to the weight-loss maintenance period with available blood samples (Figure S1, Supporting Information) (75 subjects from Reus and 87 from Copenhagen). Seven participants were excluded because lack of adiposity measures after the weight loss maintenance period (Figure S1, Supporting Information).

2.2. Assessment of Energy Intake and Physical Activity

Nutritional data were collected at baseline, after the 8 weeks of LCD and at the end of a 12-week weight loss maintenance period using 3-day dietary records, and energy intakes were calculated using Danish and Spanish food composition tables.[16]

Participants were asked to wear an ActiGraph tri-axis accelerometer monitor (GT3X+) on the right hip using an elastic belt for 7 consecutive days at baseline, after the 8 weeks of LCD and at the end of a 12-week weight loss maintenance period. They were only allowed to remove the accelerometer during water activities (i.e., showering or swimming). At the end of the observation period, data were reintegrated to 60-s epochs and analyzed using ActiLife6 (the ActiGraph 2012, ActiLife version 6). Before analysis of physical activity, self-reported sleep duration and non-wear time defined as 60 min of consecutive zeros using vector magnitude were removed, allowing for 2 min of non-zero interruptions with a maximum of 100 counts min^{-1} (CPM). Total physical activity (CPM) was expressed as vector magnitude of the total tri-axial counts from monitor wear time divided by monitor wear time.

2.3. Body Composition and Biochemical Measurements

Anthropometric measures (body weight, height, and sagittal abdominal diameter) were determined by trained personnel at

baseline, after the 8 weeks of LCD and at the end of a 12-week weight loss maintenance period. BMI was calculated. Body composition was assessed by dual-energy X-ray absorptiometry (DXA) (Lunar Prodigy Primo, GEHealthcare, Little Chalfont, UK, in participants from Reus) and GE Lunar iDXA, Encore software version 16.2 (in participants from Copenhagen). Fasting blood samples were obtained at baseline and after the 8-weeks weight-loss period. Glucose and insulin concentrations were measured using standard enzymatic automated methods and the insulin resistance index (HOMA-IR) was estimated.[17] Total cholesterol, high-density lipoprotein (HDL-C) cholesterol and low-density lipoprotein (LDL-C) cholesterol levels were measured using standard enzymatic automated methods (COBAS; Roche Diagnostics Ltd.).

2.4. Multiplatform Targeted Metabolomics

Metabolites were analyzed at baseline and after the 8 weeks of LCD using a multiplatform approach including gas chromatography coupled to high resolution mass spectrometry (GC-HRMS), liquid chromatography coupled to high resolution mass spectrometry (LC-HRMS), and proton nuclear magnetic resonance (^{1}H-NMR). The analytical procedures are specified in the Supplemental Methods. Information about the mass to charge ratio, retention time, repeatability and reproducibility (expressed as RSD) for each metabolite is shown in Table S1, Supporting Information.

2.5. Statistical Analyses

Baseline results were expressed as mean ± standard deviation (SD) for continuous variables and percentages for categorical variables. Eight-weeks and between the end of the 8-week intervention and the conclusion of the 12-week weight-loss maintenance period changes are expressed as mean (95% confidence interval [CI]). Statistical significance of changes after 8-weeks of LCD and 12-weeks weight loss maintenance, respectively, was evaluated using paired *t*-test. Individual metabolites with equal or more than 20% missing values were excluded, leaving 123 metabolites for further analyses. Data on metabolites were log-transformed to improve normality. We first analyzed changes in metabolites concentrations between baseline and 8-weeks LCD period using paired *t*-test. Two-sided *p* values were reported according to an alpha level = 0.0004 (alpha = 0.05 with Bonferroni correction for 123 independent tests [including 123 metabolites]). For those metabolites found to change significantly, we assessed their associations with improvements in body weight and adiposity measures (fat mass, sagittal abdominal diameter) during the 8-week LCD period. With respect to metabolites, we first calculated the difference between 8-week log-transformed value and baseline log-transformed value and then scaled these differences to multiples of 1 SD. Linear regression models were fitted to examine these associations adjusting for age, sex, BMI at baseline (except for the outcome body weight change), value for the respective outcome traits at the baseline examination, and the respective metabolite at baseline. The Bonferroni correction for 60 independent tests (including 60 metabolites) for each respective outcome trait was applied and significance was reported according to an alpha level = 0.0008 (alpha corrected according to the significant changes in **Table 1**). Metabolites found significantly associated with body weight and adiposity were assigned into specific metabolic pathways based on the pathway analysis using MetaboAnalyst 5.0. To test whether changes in the metabolites found to associate significantly with improvements in adiposity measures were significantly associated with changes in adiposity measures during the 12-week weight loss maintenance period, linear regression models were fitted for each outcome trait. Multivariate-adjusted models were performed, including age, sex, BMI at baseline (except for the outcome body weight change), value for the respective outcome traits at the baseline examination, intervention group (enhancing satiety foods, control foods), and the respective metabolite at baseline. Bonferroni correction for the number of tests based on the number of metabolites found to associate significantly with adiposity measures was applied (alpha corrected for weight and total fat in **Table 2**). To test the robustness of these associations, we conducted two sensitivity analyses: (1) adding into the multivariable model 2 covariates such as changes in energy intake and physical activity during 8 weeks and (2) further adjusting the multivariable model for change in the respective outcome traits during 8 weeks instead of their value at the baseline examination. A logistic regression model was also performed to calculate odds ratios (ORs) and 95% CIs for adiposity regain according to 1 SD log-transformed changes in the metabolites. For this analysis, participants were categorized into either the body weight or total fat regain group or sustained weight loss or total fat reduction group (reference group). Statistical analyses were performed using Stata 14.1 (Stata Corp.).

3. Results

3.1. Characteristics of the Study Participants

Descriptive characteristics are shown in **Table 3**. The mean age of the study population was 47.3 ± 9.9 years with body weight of 88.1 ± 10.7 kg and BMI of 30.9 ± 2.0 kg m^{-2} when initiating the 8-week LCD period. No significant differences in the general characteristics of the 162 participants included in the present analyses and those participants initially recruited in the SATIN study were observed (Table S2, Supporting Information). The average weight loss for the 162 participants achieving ≥8% during the LCD was 9.7 kg ($p < 0.001$). During this period, participants also reduced BMI (–3.1 kg m^{-2}) ($p < 0.001$), total fat (–7.2 kg) ($p < 0.001$) and sagittal abdominal diameter (−3.1 cm) ($p < 0.001$). Furthermore, total energy intake and physical activity significantly decreased and increased, respectively. After the 12-week weight loss maintenance period, seven participants did not have adiposity measures and the 155 remaining regained on average 1.0 kg ($p < 0.001$). Of the study participants, 69% showed weight regain (2.3kg), whereas 31% showed further weight loss (−1.9 kg). Correspondingly, 61% of the participants regained (1.6 kg) and 39% lost (–1.9 kg) additional total fat. During this period, both energy intake and physical activity did not significantly change.

Table 1. Changes in adiposity measures at 8 weeks of a low-calorie diet (LCD) per 1SD log-transformed changes in the concentrations of metabolites.

Change in metabolite between baseline and 8 weeks LCD	Change in body weight	Change in fat mass	Change in sagittal abdominal diameter
Free cholesterol	0.19 (−0.25, 0.64)	0.27 (−0.07, 0.62)	0.005 (−0.21, 0.22)
Esterified cholesterol	0.34 (−0.07, 0.75)	0.32 (−0.010, 0.64)	0.03 (−0.17, 0.24)
Total cholesterol	−0.14 (−0.55, 0.27)	−0.05 (−0.37, 0.26)	0.04 (−0.15, 0.24)
Triglycerides	0.45 (0.02, 0.89)	0.46 (0.12, 0.80)	0.09 (−0.13, 0.31)
Phosphatidylcholine	0.42 (−0.003, 0.85)	0.29 (−0.04, 0.63)	0.02 (−0.19, 0.24)
Lysophosphatidylcholine	0.59 (0.17, 1.00)	0.36 (0.03, 0.69)	0.08 (−0.13, 0.29)
Sphingomyelin	−0.16 (−0.62, 0.30)	0.04 (−0.31, 0.39)	−0.21 (−0.43, 0.009)
Fatty acyl chains	0.40 (−0.07, 0.87)	0.49 (0.13, 0.85)	0.06 (−0.17, 0.30)
Monounsaturated fatty acid	0.08 (−0.29, 0.47)	0.09 (−0.21, 0.39)	−0.02 (−0.20, 0.16)
LPC 14:0	1.53 (1.09, 1.98)*	0.83 (0.45, 1.20)*	0.38 (0.14, 0.62)
LPC 20:3	1.02 (0.59, 1.45)*	0.52 (0.15, 0.88)	0.26 (0.05, 0.48)
PC 30:0	1.36 (0.89, 1.84)*	0.79 (0.40, 1.17)*	0.19 (−0.07, 0.45)
PC 32:1	0.94 (0.50, 1.37)*	0.66 (0.32, 0.99)*	0.11 (−0.11, 0.33)
PC 32:2	1.39 (0.96, 1.83)*	0.78 (0.41, 1.15)*	0.21 (−0.02, 0.45)
PC 33:1	1.04 (0.58, 1.51)*	0.65 (0.29, 1.01)	0.09 (−0.16, 0.33)
PC 34:4	1.42 (0.96, 1.88)*	0.91 (0.52, 1.29)*	0.25 (0.009, 0.50)
PC 35:1	0.58 (0.09, 1.07)	0.36 (−0.02, 0.74)	0.12 (−0.13, 0.37)
PC 36:1	1.10 (0.65, 1.55)*	0.60 (0.24, 0.96)	0.15 (−0.07, 0.39)
PC 36:4e	0.09 (−0.46, 0.64)	0.03 (−0.40, 0.47)	−0.15 (−0.42, 0.11)
PC 36:5	0.36 (−0.04, 0.77)	0.33 (0.01, 0.64)	0.03 (−0.16, 0.23)
PC 38:3	1.26 (0.89, 1.63)*	0.80 (0.49, 1.10)*	0.27 (0.08, 0.46)
PC 38:4	0.42 (−0.09, 0.93)	0.38 (−0.02, 0.78)	0.05 (−0.20, 0.29)
PC 38:4e	0.09 (−0.39, 0.57)	0.04 (−0.34, 0.42)	−0.05 (−0.29, 0.18)
PC 40:4	0.76 (0.27, 1.25)	0.36 (−0.02, 0.75)	0.10 (−0.14, 0.34)
PC 40:6	−0.07 (−0.51, 0.35)	0.17 (−0.16, 0.50)	0.03 (−0.17, 0.23)
PE 36:5e	0.42 (−0.02, 0.86)	0.07 (−0.28, 0.43)	0.07 (−0.16, 0.29)
PE 38:5e	0.16 (−0.30, 0.63)	−0.19 (−0.56, 0.18)	0.009 (−0.22, 0.24)
PE 38:6e	0.54 (0.03, 1.05)	0.11 (−0.31, 0.52)	0.09 (−0.16, 0.34)
SM 32:1	0.91 (0.47, 1.35)*	0.64 (0.29, 0.99)*	0.07 (−0.15, 0.30)
SM 32:2	1.17 (0.69, 1.66)*	0.77 (0.39, 1.16)*	0.28 (0.04, 0.53)
SM 33:1	−0.24 (−0.72, 0.23)	−0.07 (−0.44, 0.29)	−0.13 (−0.35, 0.10)
SM 35:1	−0.53 (−0.95, −0.10)	−0.13 (−0.46, 0.21)	−0.20 (−0.41, 0.01)
SM 36:0	−0.81 (−0.31, −0.32)	−0.31 (−0.71, 0.09)	−0.19 (−0.47, 0.07)
SM 36:1	−0.59 (−1.02, −0.16)	−0.11 (−0.45, 0.23)	−0.10 (−0.32, 0.11)
SM 38:1	0.76 (0.35, 1.17)*	0.59 (0.26, 0.91)	0.11 (−0.09, 0.32)
SM 40:1	0.74 (0.28, 1.20)	0.49 (0.12, 0.86)	0.21 (−0.02, 0.43)
SM 40:2	0.42 (−0.05, 0.89)	0.30 (−0.07, 0.67)	−0.01 (−0.24, 0.22)
SM 41:1	0.76 (0.27, 1.24)	0.42 (0.03, 0.81)	0.15 (−0.09, 0.39)
SM 41:2	0.20 (−0.42, 0.82)	−0.09 (−0.57, 0.39)	0.32 (0.03, 0.61)
SM 42:1	0.67 (0.17, 1.18)	0.38 (−0.02, 0.79)	0.18 (−0.06, 0.43)
TG 50:2	0.41 (−0.03, 0.86)	0.43 (0.08, 0.78)	0.17 (−0.05, 0.39)
Lactic acid	0.42 (−0.01, 0.87)	0.33 (−0.03, 0.68)	0.10 (−0.12, 0.32)
Glycolic acid	0.05 (−0.45, 0.56)	−0.41 (−0.78, −0.03)	−0.03 (−0.27, 0.21)
Valine	0.46 (0.02, 0.91)	0.10 (−0.25, 0.45)	0.004 (−0.21, 0.22)
Glutamate	0.69 (0.28, 1.10)	0.39 (0.06, 0.72)	0.30 (0.10, 0.50)
Glucose	0.32 (−0.13, 0.78)	0.28 (−0.08, 0.63)	0.03 (−0.19, 0.25)
Tyrosine	0.65 (0.23, 1.08)	0.42 (0.08, 0.76)	−0.05 (−0.26, 0.16)
LPC 16:0	0.14 (−0.38, 0.67)	0.02 (−0.38, 0.43)	0.03 (−0.22, 0.29)
LPC 16:1e	−0.50 (−0.92, −0.08)	−0.34 (−0.67, −0.01)	−0.17 (−0.38, 0.04)

(*Continued*)

Table 1. Continued.

Change in metabolite between baseline and 8 weeks LCD	Change in body weight	Change in fat mass	Change in sagittal abdominal diameter
LPC 18:1	0.13 (−0.33, 0.59)	−0.10 (−0.46, 0.25)	−0.04 (−0.27, 0.19)
LPC 18:2	0.26 (−0.16, 0.68)	−0.05 (−0.38, 0.28)	−0.03 (−0.23, 0.17)
LPC 20:0	0.27 (−0.21, 0.76)	0.001 (−0.39, 0.39)	0.08 (−0.16, 0.32)
LPC 20:1	−0.23 (−0.66, 0.20)	−0.17 (−0.50, 0.16)	0.04 (−0.17, 0.26)
LPC 20:4	−0.12 (−0.54, 0.30)	−0.04 (−0.37, 0.28)	0.01 (−0.19, 0.22)
LPC 22:6	−0.15 (−0.53, 0.23)	−0.05 (−0.35, 0.25)	−0.02 (−0.20, 0.17)
PC 34:2e	−0.88 (−1.32, −0.44)*	−0.89 (−1.22, −0.56)*	−0.34 (−0.55, −0.13)
PC 42:5e	−0.75 (−1.15, −0.36)*	−0.43 (−0.74, −0.11)	−0.15 (−0.35, 0.04)
SM 42:3	−1.13 (−1.52, −0.75)*	−0.35 (−0.68, −0.02)	−0.27 (−0.47, −0.06)
Glycine	−0.41 (−0.82, −0.001)	−0.29 (−0.61, 0.03)	−0.06 (−0.27, 0.14)
Citric acid	−1.43 (−0.80, −1.06)*	−0.91 (−1.21, −0.61)*	−0.45 (−0.65, −0.26)*

Values presented as beta estimates (95% confidence interval) and each regression was adjusted for age, sex, BMI at baseline (except for the outcome body weight change), value for the respective outcome traits at the baseline examination, and the respective metabolite at baseline. BMI, body mass index; LPC, lysophosphatidylcholine; PC, phosphatidylcholine; PE, phosphatidylethanolamine; SD, standard deviation; SM, sphingomyelin; TG, triglyceride. *Significant after Bonferroni correction for 60 tests.

Table 2. Changes in adiposity measures after the 12-week weight loss maintenance period per 1SD log-transformed changes in the concentrations of metabolites during the initial 8 weeks.

Change in metabolite between baseline and 8 weeks	Change in body weight	Change in fat mass
LPC 14:0	0.90 (0.34, 1.46)*	1.11 (0.61, 1.62)*
LPC 20:3	0.84 (0.36, 1.32)*	NA
PC 30:0	0.35 (-0.24, 0.94)	0.64 (0.10, 1.18)
PC 32:1	0.25 (-0.25, 0.75)	0.37 (-0.09, 0.83)
PC 32:2	0.76 (0.24, 1.28)	1.03 (0.54, 1.51)*
PC 33:1	0.17 (-0.37, 0.71)	NA
PC 34:4	0.78 (0.23, 1.33)	1.08 (0.57, 1.59)*
PC 36:1	0.53 (0.01, 1.05)	NA
PC 38:3	0.82 (0.40, 1.24)*	1.09 (0.70, 1.47)*
SM 32:1	0.19 (-0.31, 0.70)	0.31 (-0.17, 0.79)
SM 32:2	0.76 (0.21, 1.32)	0.94 (0.42, 1.46)*
SM 38:1	0.63 (0.19, 1.07)	NA
PC 34:2e	-0.59 (-1.09, -0.11)	-0.65 (-1.11, -0.19)
PC 42:5e	-0.18 (-0.63, 0.26)	NA
SM 42:3	-0.54 (-1.00, -0.08)	NA
Citric acid	-0.48 (-0.95, -0.02)	-0.81 (-1.21, -0.40)*

Values presented as beta estimates (95% confidence interval) and each regression was adjusted for age, sex, BMI at baseline (except for the outcome body weight change), value for the respective outcome traits at the baseline examination, intervention group (satiety controlling foods, control foods), and the respective metabolite at baseline. NA indicates no analysis performed for the metabolite and adiposity measure because in Table 2 these parameters were not significantly associated. BMI, body mass index; LPC, lysophosphatidylcholine; PC, phosphatidylcholine; SD, standard deviation; SM, sphingomyelin. *Significant after Bonferroni correction for 15 and 10 tests for the outcomes weight and total fat, respectively

3.2. Association Between Changes in Metabolite Levels and Changes in Adiposity Measures During the 8-week LCD

After correction for multiple testing, 60 metabolites showed a significant change from baseline to the end of the 8 weeks LCD period (Table S3, Supporting Information). Changes in metabolite concentrations associated with changes in adiposity measures are shown in Table 1. Weight loss-induced decrease in lysophosphatidylcholines (LPCs) 14:0, LPC 20:3, phosphatidylcholines (PCs) 30:0, PC 32:1, PC 32:2, PC 33:1, PC 34:4, PC 36:1, PC 38:3, sphingomyelins (SMs) 32:1, SM 32:2, SM 38:1, and increase in PC 34:2e, PC 42:5e, SM 42:3 and citric acid (Table S3, Supporting Information) were associated with a decreased body weight (Table 1). A decrease in the concentrations of LPC 14:0, PC 30:0, PC 32:1, PC 32:2, PC 34:4, PC 38:3, SM 32:1, SM 32:2, and an increase in the concentrations of PC 34:2e and citric acid (Table S3, Supporting Information) were also associated with a decrease in total fat (Table 1). Finally, an increase in citric acid concentrations was found to be associated with a decrease in sagittal abdominal diameter. Similar results were found after adjusting analyses for changes in energy intake and physical activity except for the association of SM 38:1 and PC 42:5e with body weight, of PC 32:1, PC 32:2 and SM 32:2 with total fat, and of citric acid with abdominal measure (Table S4, Supporting Information). Eight distinct metabolic pathways were identified, mapping to the metabolite set significantly associated with body weight and total fat changes (**Figure 1**). The metabolic pathway with the highest impact (based on *p* values derived from pathway analyses) was related to glycerophospholipid metabolism, while a moderate impact was found for linoleic acid metabolism, alpha-linolenic acid metabolism, citric acid cycle, and sphingolipid metabolism.

3.3. Association Between Changes in Metabolite Levels During 8-Weeks LCD and Changes in Adiposity Measures During the 12-Week Weight-Loss Maintenance Period

We then investigated whether the initial (8 weeks) changes in these metabolites were significantly associated with changes in adiposity measures during the 12-week weight-loss maintenance period. We observed that decreases in LPC 14:0, LPC 20:3, and PC 38:3 were associated with body weight regain (Table 2). Also,

Table 3. General characteristics of the study subjects at baseline, after the 8-week LCD and after the 12-week weight loss maintenance period.

	Baseline (n = 162)	8 weeks change (n = 162)	Weight loss maintenance change (n = 155)	*p* value for 8 weeks change	*p* value for weight loss maintenance change
Sex (%Women)	75.0	NA	NA	NA	NA
Age [yrs]	47.5 ± 9.9	NA	NA	NA	NA
Height [m]	1.68 ± 0.09	NA	NA	NA	NA
Weight [kg]	88.1 ± 10.7	−9.7 (−10.2, −9.2)	1.0 (0.6, 1.4)	<0.001	<0.001
BMI [kg m^{-2}]	30.9 ± 2.0	−3.4 (−3.5, −3.2)	0.3 (0.2, 0.5)	<0.001	<0.001
Total fat [kg]	36.1 ± 5.6	−7.2 (−7.5, −6.9)	0.003 (−0.3, 0.4)	<0.001	0.984
Bone mass [kg]	2.8 ± 0.5	−0.01 (−0.02, −0.0005)	−0.009 (−0.02, 0.0006)	0.040	0.065
Sagittal diameter [cm]	23.2 ± 2.4	−3.1 (−3.3, −2.8)	0.6 (0.4, 0.8)	<0.001	<0.001
Glucose [mg dL^{-1}]	94.9 ± 11.2	−2.9 (−4.1, −1.8)	−0.5 (−1.5, 0.6)	<0.001	0.367
Insulin [mcUI mL^{-1}]	9.1 ± 6.4	−3.2 (−3.9, −2.5)	0.2 (−0.2, 0.7)	<0.001	0.361
HOMA−IR	2.2 ± 1.7	−0.8 (−1.0, −0.6)	0.04 (−0.07, 0.1)	<0.001	0.482
TChol [mg dL^{-1}]	197.6 ± 34.9	−19 (−23.4, 16.2)	15.1 (11.6, 18.6)	<0.001	<0.001
HDL−C [mg dL^{-1}]	56.3 ± 15.9	−5.2 (−6.5, −3.8)	10.3 (8.9, 11.7)	<0.001	<0.001
LDL-C [mg dL^{-1}]	120.6 ± 30.9	−12.3 (−15.5, −9.2)	5.9 (2.9, 8.9)	<0.001	<0.001
EI [kcal d^{-1}]	1953.5 ± 633.5	−400.8 (−502.3, −299.2)	57.5 (−26.7, 141.8)	<0.001	0.179
TPA (CPM)	608.9 ± 187.9	47.9 (23.5, 72.4)	−14.9 (−46.4, 16.5)	<0.001	0.348

Baseline data are presented as mean ± standard deviation unless otherwise indicated. Data are presented as mean (95% confidence interval) for changes after 8 weeks of LCD and changes between the end of the 8-week intervention and the conclusion of the 12 week weight maintenance period. The paired *t* test was used to assess changes in variables between baseline and 8 weeks of LCD and after the 12 weeks of weight loss maintenance period. BMI, body mass index; CPM, counts min^{-1}; EI, energy intake; HDL-C, high-density lipoprotein-cholesterol; LCD, low-calorie diet; LDL-C, low-density lipoprotein-cholesterol; TChol, total cholesterol; TPA, total physical activity.

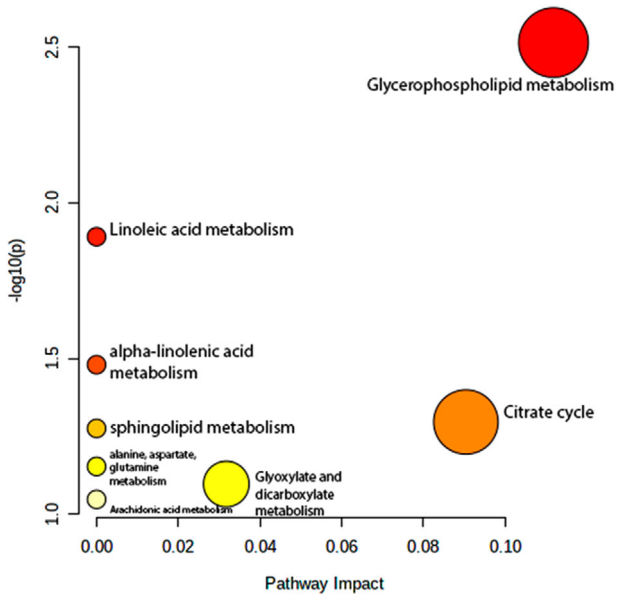

Figure 1. Metabolic pathway analysis.

initial decreases in LPC 14:0, PC 32:2, PC 34:4, PC 38:3, SM 32:2 were associated with total fat increase, whereas increases in citric acid was associated with total fat decreases (Table 2). In sensitivity analyses, the associations between decreases in these metabolites and total fat regain persisted after adjustment for changes in total fat (Table S5, Supporting Information). On the other hand, only decreases in PC 38:3 remained significantly associated with body weight regain after adjustment for weight changes (Table S5, Supporting Information). Finally, we investigated whether the initial changes in metabolites were associated with failure to maintain weight loss and total fat loss during the weight loss maintenance period. We found that the initial decreases in PC 32:2 and PC 38:3 concentrations were associated with an increased risk of body weight regain (OR 2.66; 95% CI 1.41, 5.02 and OR 2.18; 95% CI 1.39, 3.41, respectively) (**Figure 2**A). We also observed that initial decreases in LPC 14:0, PC 32:2, PC 34:4, and PC 38:3 were associated with increased risk of total fat regain (OR 4.60; 95% CI 2.27, 9.33, OR 4.51; 95% CI 2.22, 9.16, OR 5.48; 95% CI 2.42, 12.39, and OR 2.81; 95% CI 1.74,4.54, respectively) (Figure 2B). Conversely, initial increases in the concentrations of PC 34:2e and citric acid were associated with decreased risk of total fat regain (OR 0.47; 95% CI 0.29, 0.76 and OR 0.32; 95% CI 0.19, 0.53, respectively) (Figure 2B). On the other hand, no significant associations between 8 weeks changes in citric acid and changes in sagittal abdominal diameter during the 12-week weight loss maintenance period were observed.

4. Discussion

In the present study, we found that changes in several metabolites involved in different metabolic pathways, including glycerophospholipid, linoleic acid/alpha-linolenic acid and sphingolipid metabolism, and citric acid cycle, during an 8-week LCD were associated with a reduction in body weight and total fat. Further, our results indicated that changes in certain lipid species and citric acid during weight loss were related to weight and adiposity regain after 12 weeks of weight loss maintenance.

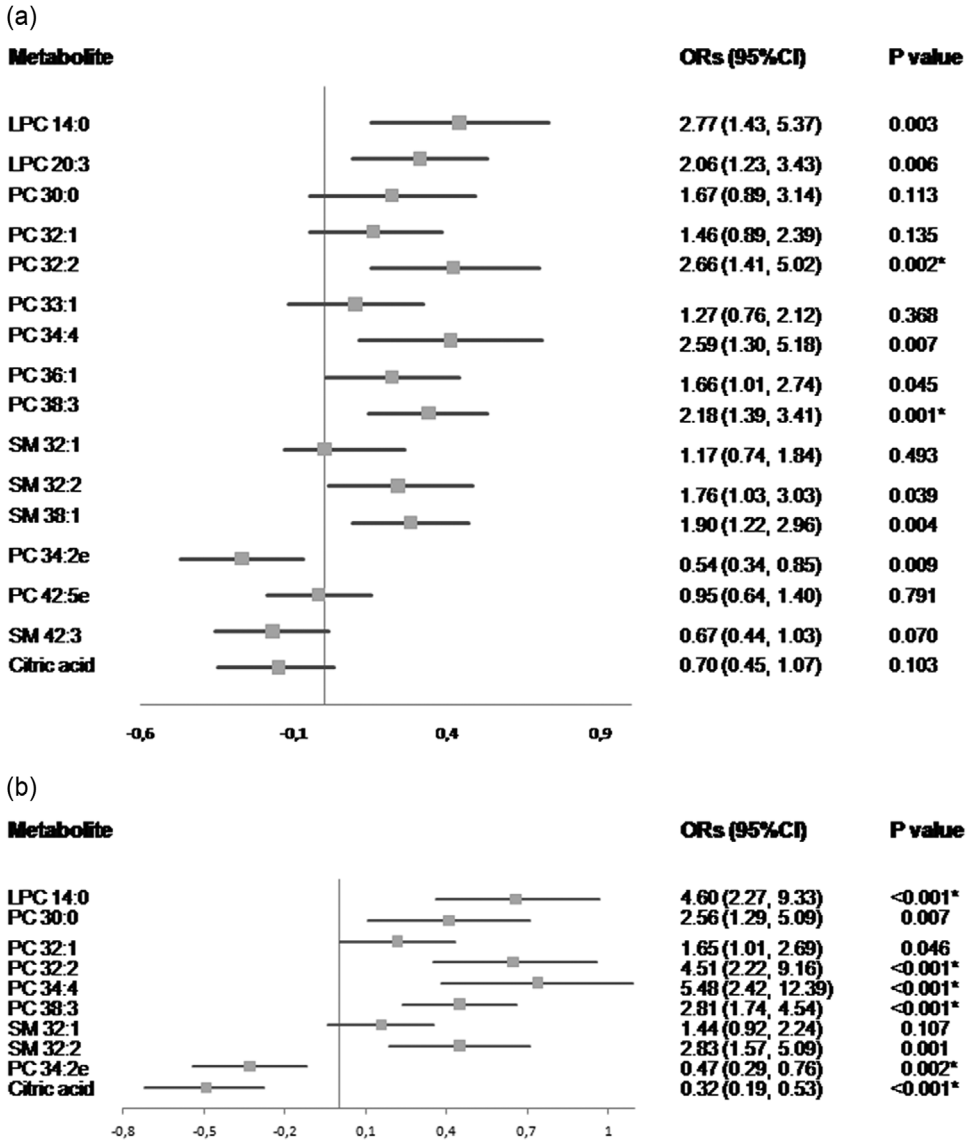

Figure 2. Probability of weight gain at 12-weeks according to 1SD log-transformed changes in the concentrations of metabolites during the initial 8 weeks low calorie diet. Figure 2B. Probability of total fat increase at 12-weeks according to 1SD log-transformed changes in the concentrations of metabolites during the initial 8 weeks low calorie diet. Odds ratios with 95% confidence interval (log scale) after adjustment for age, sex, BMI at baseline (except for the outcome body weight change), value for the respective outcome traits at the baseline examination, intervention group (satiety controlling foods, control foods), and the respective metabolite at baseline. *Significant after Bonferroni correction for 15, 10 tests for the outcomes body weight and total fat, respectively. Abbreviations: BMI; body mass index, LPC; lysophosphatidylcholine, PC; phosphatidylcholine, SD, standard deviation; SM; sphingomyelin.

According to previous studies, obesity results in generally higher lipid load in circulation characterized by increased content of cholesterol fatty acid, PC, PE, and LPC species.[18,19] After 8 weeks of an LCD, we observed reductions in the concentrations of cholesterol and lipid species (mainly PCs and SMs, two LPCs), in line with the findings of a previous study,[10] whereas eight LPC species increased. Contradictive findings related to alterations of LPC in obesity have been reported. Some studies showed decreased levels of these compounds,[20–22] while other studies have demonstrated increases with obesity.[17] Schwab et al.[23] could not observe significant alterations in circulating LPC levels after a 33-week period of weight loss in nine obese subjects. On the contrary, we found a significant relationship of decreases in the concentrations of LPC 14:0 and LPC 20:3 with reduction in adiposity. We also observed that decreases in five PCs (PC 30:0, PC 32:1, PC 32:2, PC 34:4, PC 38:3) and three SMs (SM 32:1, SM 32:2, SM 38:1) were consistently associated with reductions in body weight and total fat at 8 weeks, linking their modulation with adiposity improvement.[22,23] On the other hand, increases in two PCs (PC 34:2e, PC 42:5e) and SM 42:3 were associated with adiposity reduction. These findings imply that the observed changes were entirely dependent

upon changes in adiposity, unlike the other metabolites (NMR lipids, phosphatidylethanolamine species, organic acids, amino acids) that may be influenced by different mechanisms. Previous studies revealed decreased adipose lipid turnover and lipolytic activity in subjects with overweight and obesity,[24] while substantial weight loss and adiposity reduction is explained by increased lipid removal and decreased lipid storage. Changes in the concentrations of circulating phospholipids and SMs during weight loss may reflect altered synthesis and efflux from adipose tissue.[25] Besides this, our findings in relation to decreases in lipid species including glycerophospholipids and sphingolipids may also be explained via the lipotoxicity hypothesis. This hypothesis states that an oversupply of dietary fats, which exceed the capacity of adipocytes to store them, leads to storage in other tissues, such as hepatocytes.[26] A reduction in adiposity during the 8-week LCD may have resulted to their reduced storage in the liver and excretion into the bloodstream.[22] Since elevated PC (<5 double bonds) and SM levels have been found to be associated with increased risk of coronary artery disease and mortality,[27,28] it could be speculated that the decreases in PC and SM could be involved in the beneficial health effects of weight loss.[29]

Our study also showed that decreases in the concentrations of PC 38:3 during the LCD were related to weight regain and increases in total fat during the 12-week weight loss maintenance period. Similar results were obtained for decreases in PC 32:2 and weight/total fat increases as well as for decreases in PC 34:4 and total fat increases. Conversely, early increases in PC 34:2e were associated with further fat loss. Early decreases in LPC 14:0 and LPC 20:3 were also associated with adiposity gain after the weight loss maintenance period. Similarly, initial decreases in plasma SM 32:2 concentrations were also associated with total fat increases after 12 weeks. In the DiOGenes intervention study, participants with morbid obesity and high circulating PC and SM levels were most successful in losing weight upon an 8-week LCD period,[10] while these compounds decreased. The authors suggested that these observations might be due to a higher lipid turnover during the weight loss period which corresponds to a higher energy metabolism at baseline.[10] Recently, a prospective study revealed that individuals with a low baseline lipid removal rate were more likely to remain weight-stable after weight loss.[30] On the contrary, our results suggest that an initial increase in lipid metabolism induced by weight loss was associated with adiposity regain identifying the adipose-related alterations in lipid metabolism with decreased adiposity as an important factor for the maintenance of lost weight and adiposity.

Our findings are not in agreement with those from previous studies showing associations of changes in amino acids with weight loss.[12,31] These studies,[12,31] used long-term weight-loss diet interventions, whereas our participants followed a shorter LCD. In a previous study, weight loss by short-term LCD intervention did not lower circulating amino acids.[32] However, differences in study design can only partly explain these discrepancies.

Citric acid, an intermediate of the citric acid cycle, was found to increase after the first 8 weeks and was associated with weight loss and decreases in all adiposity measures during this period. Increases in citric acid have been noted in plasma of adults with overweight and obesity after a 12-week LCD and subsequent weight loss ≥7.2 kg.[8] The plasma citric acid homeostasis is mainly regulated and maintained by the bone and about 90% of the total plasma citric acid in the body resides in bone.[33] During weight loss, changes in bone metabolism may occur. We found a significant reduction in total bone mass after 8 weeks. Previous diet-induced weight loss interventions of longer duration (ranging from 6 months to 1 year) have indicated that a weight loss of ≈10% (similar to our study) results in a 1–2% bone loss at the hip and total body.[34,35] According to a meta-analysis, interventions of 2 or 3 months in duration induced significant increases in serum concentrations of osteocalcin, indicating an early effect of such weight loss programs to promote a bone catabolic state.[36] Therefore, we speculate that weight loss might have induced bone breakdown releasing citric acid from bone to plasma contributing to the increases in plasma citric acid concentrations observed in our study after the LCD.[33] Interestingly, our results indicated that initial (8-weeks) increases in citric acid concentrations were associated with total fat reduction and total fat reduction during the weight loss maintenance period. Whether such a metabolite could be used as marker of total fat reduction and whether bone metabolism changes might have persisted during the weight loss maintenance period are hypotheses that require further investigation.

Our study has several strengths. A comprehensive metabolite profiling was performed using combinations of different metabolomic platforms to quantitatively analyze a wide range of metabolites. Our study participants were free of type 2 diabetes or cardiovascular diseases and were non-smokers, all factors that may affect the concentrations of these metabolites. The consistent findings of the metabolites and adiposity measurements during the LCD period strengthen our conclusion.

Concerning limitations, participants were overweight/obesity without comorbidities and this may limit the generalizability of the findings to overweight/obese individuals with obesity-related comorbidities. Furthermore, although we adjusted for several potential confounders, residual confounding cannot be ruled out. An additional limitation could be that the SATIN sample lacked a control group to rule out that the observed changes in plasma metabolite concentrations may be related to the time course rather than to the change in body weight or adiposity. Finally, the use of a targeted metabolomic approach only, may have limited the discovery of new metabolites associated with body weight or fat mass changes. Untargeted metabolomics could complement our approach providing a more comprehensive view of the metabolic changes that accompany weight loss and could be associated with weight and adiposity regain.

In conclusion, changes in circulating concentrations of several lipid species including PCs, LPCs, and SMs were associated with greater improvement of body weight and adiposity in adults with overweight/obesity following a LCD. These data are intriguing in light of a growing body of literature suggesting that weight loss and adiposity reductions are associated with changes in lipid metabolism. Decreases in the concentrations of LPC 14:0, LPC 20:3, PC 32:2, PC 38:3, SM 32:2, and increases in citric acid concentrations during weight loss, which were related to adiposity regain and loss, respectively, could help us to understand the potential role of metabolic responses for the ability to resist weight-loss regain. Further studies are warranted to replicate these results in other populations and try to establish metabolites as potential biomarkers for successful weight loss maintenance.

Supporting Information

Supporting Information is available from the Wiley Online Library or from the author.

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Conflict of Interest

The authors declare no conflict of interest.

Author Contributions

M.B., A.S., J.C.G.H., C.P. designed research; C.P., J.G.-G., L.C.-B., T.T.H., J.C.G.H., A.S., J.H., M.B. conducted research; A.S., J.C.G.H., M.B. were the coordinators of subject recruitment at the outpatient clinics; the metabolomics analyses were performed with the equipment of the Centre for Omic Sciences (COS), Joint Unit of the Universitat Rovira i Virgili and Eurecat, and considered a unique scientific and technical infrastructure (ICTS) supervised by M.B.; C.P. analyzed the data; M.B., C.P., J.C.G.H., A.S. interpreted statistical analysis and data; C.P. drafted the paper; M.B. supervised the study and M.B., J.C.G.H., A.S. had full access to all of the data in the study and took responsibility for the integrity of the data and the accuracy of the data analysis. All authors revised the manuscript for important intellectual content, read and approved the final manuscript.

Data Availability Statement

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

Keywords

metabolomics, SATIN, weight loss, weight maintenance

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