Lipid Metabolism Predicts Changes in Body Composition during Energy Restriction in Overweight Humans\textsuperscript{1–3}

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Abstract

Dietary weight loss regimens could be more effective by selectively targeting adipose while sparing lean mass (LM) if predictive information about individuals’ lipid metabolic responses to an intervention were available. The objective of this study was to examine the relationships among changes in 4 anthropometric outcomes, weight, waist circumference (WC), percent body fat (BF), and percent LM, and comprehensive circulating lipid metabolites in response to energy reduction in overweight participants. This was a cohort study (n = 46) from a larger multi-center (n = 105) weight loss trial. We used stepwise regression to examine relationships among baseline plasma fatty acids of 7 lipid classes, biochemical metabolites, and diet to explain the variance of 4 anthropometric outcomes after intervention. No predictor variables explained the variance in the percent change in body weight. The circulating concentration of FFA 18:1(n-9) at baseline explained 31% of the variance in percent change of WC, with adjustment for energy intake at 12 wk. Circulating concentrations of phosphatidylcholine 18:0 and FFA 18:1(n-9) at baseline together explained 33% of the variance in percent LM change. The circulating concentration of phosphatidylcholine 18:0 at baseline explained 23% of the variance in the change in percent BF. This study determined relationships among comprehensive and quantitative measurements of complex lipid metabolites and metabolic outcomes as changes in body composition. Measurements of plasma circulating metabolites explained 20–30% of the variance in changes in body composition after a weight loss intervention. Thus, circulating lipids reflect lipid metabolism in relation to changes in body composition. J. Nutr. 139: 222–229, 2009.

Introduction

Variations in the development and consequences of obesity are proposed to depend on genetic predisposition combined with various environmental factors that lead to unbalanced energy intake and expenditure (1). Current treatments for weight loss involve lifestyle modification through energy restriction and exercise to balance energy intake with expenditure (2). Yet, weight loss regimens that blindly prescribe energy restriction to achieve long-term weight loss are only partially successful and can produce undesirable effects (3,4). An emphasis on weight loss as a function of the change in body weight overlooks the physiological consequences of changes in body composition. Ideally, a dietary intervention spares lean mass (LM)\textsuperscript{10} and primarily results in body fat (BF) loss. Energy intake alone cannot account for other influences on weight and body composition such as genetics, physical activity, nonexercise activity thermogenesis, diet composition, and metabolic phenotype.

Metabolic phenotype has been described as the sum of an individual's genetic blueprint, the environment, and their interaction to manifest a physical and biochemical characteristic (5). Metabolic phenotype is reflected in the measurement of biological molecules whose concentrations represent substrates and products of biochemical pathways. In particular, quantitative

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\textsuperscript{3} Supplemental Tables 1–6 and Supplemental Figures 1 and 2 are available with the online posting of this paper at jn.nutrition.org.

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\textsuperscript{10} Abbreviations used: ADJ, adjusted; BF, body fat; \% BF, change in percent body fat; CE, cholesterol ester; D, Cook’s Distance; DAG, diacylglycerol; HOMA-IR, homeostasis model assessment for insulin resistance; LM, lean mass; \% LM, change in percent lean mass; LPC, lysophosphatidylcholine; MUFA, monounsaturated fatty acids; PC, phosphatidylcholine; PE, phosphatidylethanolamine; TAG, triacylglycerol; WC, waist circumference; \% WC, percent change in waist circumference; \% weight, percent change in weight.

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and comprehensive measurements of the lipid metabolome in tissues and biofluids can be mapped to their functions in biochemical pathways (6,7). Circulating lipids are highly dynamic, interactive biological molecules that make up most cellular components and signaling molecules. As key regulators in both anabolic and catabolic pathways, circulating lipids and their varying fatty acid compositions are influenced by dietary (8–11), pharmacologic (12), hormonal (13,14), and environmental triggers (15). Identifying metabolic phenotypes through comprehensive and quantitative measurement of lipids as surrogates of integrative metabolism in response to a dietary treatment, drug, or environmental exposures can provide valuable information.

Analytical approaches to quantifying complex lipid structures can identify several serum lipid metabolites as diagnostic indices for both early and late stages of metabolic syndrome. Examination of tissue and biofluid fatty acid composition of complex lipid structures has great potential to reveal mechanistic interplay on the metabolic phenotype. For example, elevated concentrations of serum total FFA, and of palmitic acid (16:0) in particular (16), are associated with metabolic syndrome through their induction of lipotoxicity in peripheral tissues, including muscle, liver, and pancreas (17). Epidemiologically, impaired glucose tolerance and type 2 diabetes are associated with higher concentrations of serum cholesterol palmitate and palmitoleate compared with normal controls (18). Independent prospective case-cohort studies have demonstrated a positive association between plasma phospholipid stearic acid (18:0) and incidence of type 2 diabetes (19,20). These data suggest that lipid metabolism is reflective in circulating lipids and describes the metabolic phenotype.

The primary aim of this cohort study to the parent multicenter weight loss trial (21) was to examine predictive relationships between comprehensive and quantitative measurements of the circulating concentration of fatty acids within 7 lipid classes at baseline and changes in body composition during moderate energy restriction over a 12-wk period in overweight and obese individuals. It is our hope that these data will stimulate future studies that examine mechanistic relationships between circulating lipid species and metabolic phenotype.

**Methods**

**Participants.** In the parent project, 105 overweight and mildly obese individuals were recruited from the faculty, staff, and student populations of each of the 4 participating institutions (University of Tennessee, Purdue University, The Ohio State University, and the USDA, Agricultural Research Service, Western Human Nutrition Research Center at the University of California-Davis) (21). A subset of data from the parent project is presented herein. Data from only the University of Tennessee, Purdue University, and the USDA, ARS, Western Human Nutrition Research Center at the University of California, Davis were used and The Ohio State University was dropped from the analysis due to the high attrition rate of participants at this site. With the exclusion of The Ohio State University, 79 participants completed the trial and 63 met the parent study’s a priori weekly compliance criteria. To generate statistical outcomes: 1) percent change in weight (%Δ weight); 2) percent change in WC (%Δ WC); 3) change in percent LM (%Δ%LM); and 4) change in percent BF (%Δ BF). These outcomes were determined using the following equations:

\[
\%\Delta \text{Weight} = \left\{ \frac{\text{[weight (kg)}_{12\text{wk}} - \text{weight (kg)}_{\text{baseline}}]}{\text{weight (kg)}_{\text{baseline}}} \right\} \times 100
\]

\[
\%\Delta \text{WC} = \left\{ \frac{\text{[WC (cm)}_{12\text{wk}} - \text{WC (cm)}_{\text{baseline}}]}{\text{WC (cm)}_{\text{baseline}}} \right\} \times 100
\]

\[
\%\Delta \text{LM} = \%\text{LM}_{12\text{wk}} - \%\text{LM}_{\text{baseline}}
\]

\[
\%\Delta \text{BF} = \%\text{BF}_{12\text{wk}} - \%\text{BF}_{\text{baseline}}
\]

Participants maintained daily food records throughout the 12-wk intervention period and we assessed compliance by weekly subject interview and review of the diet diary and pill-counts. A priori weekly compliance criteria are reported elsewhere (22).

Nutrient assessment was computed by averaging 7-d diet records for the 2-wk lead-in period and averaging weekly 3-d diet records for intervention wk 0–11 using Nutritionist Pro Food Processor Plus software. Baseline micro- and macronutrient intakes were determined by averaging the pooled nutrient data from the 2-wk lead-in period. Micro- and macronutrient intake during the 12-wk intervention period was determined by averaging the pooled nutrient data from wk 2–11. Data were not included for baseline or wk 1 to allow participants a 2-wk adaptation period to accurately log dietary intake data.

**Anthropometric measurements.** Measurement of body weight was done during the 2-wk lead-in period and weekly thereafter; height was measured at baseline and waist circumference (WC) at baseline and 12 wk (22). BMI was calculated as kg/m². BF and LM were assessed via dual energy X-ray absorptiometry at baseline and 12 wk of the study (21,22).

We used statistical models to predict the following anthropometric outcomes: 1) percent change in weight (%Δ weight); 2) percent change in WC (%Δ WC); 3) change in percent LM (%Δ%LM); and 4) change in percent BF (%Δ BF). These outcomes were determined using the following equations:
% LM and BF were determined as:

\[
% \text{LM} = \left[\frac{\text{LM (kg)}_{\text{baseline}}}{\text{weight (kg)}_{\text{baseline}}} \times 100
\]

\[
% \text{BF} = \left[\frac{\text{BF (kg)}_{\text{baseline}}}{\text{weight (kg)}_{\text{baseline}}} \times 100
\]

\[
% \text{LM}_{12\text{wk}} = \left[\frac{\text{LM (kg)}_{12\text{wk}}}{\text{weight (kg)}_{12\text{wk}}} \times 100
\]

\[
% \text{BF}_{12\text{wk}} = \left[\frac{\text{BF (kg)}_{12\text{wk}}}{\text{weight (kg)}_{12\text{wk}}} \times 100
\]

Physical activity assessment. Participants were instructed not to change their usual physical activity habits during the study. To ensure that physical activity remained unchanged, 3-d physical activity records were collected from all participants at baseline and 12 wk (21,22). Physical activity level was scored as metabolic equivalent levels (23) and metabolic equivalent levels per hour per week at baseline was used to adjust regression models for changes in body composition.

Metabolite measurements. Fasting levels of plasma lipids (triglycerides (TAG) and total, LDL, and HDL cholesterol), glucose, and insulin were measured at baseline and 12 wk.

Plasma glucose, TAG, LDL cholesterol, and HDL cholesterol were measured at each site’s clinical medical laboratory with a Beckman Lxi-725 auto-analyzer. Plasma insulin was measured using a commercially available RIA kit (Linco Research) at each site’s clinical medical laboratory. Insulin resistance at baseline and 12 wk was measured using the homeostasis model assessment for insulin resistance (HOMA-IR) (24). Baseline HOMA-IR values were used to adjust regression models for changes in body composition.

Analysis of the fatty acid composition of plasma lipids. Blood was collected from all participants into EDTA evacuated tubes, centrifuged immediately at 1,500 g, 10 min at 20°C, portioned into aliquots, and stored at <−80°C until analyzed. Fatty acid analyses of circulating lipid classes were determined by high-throughput methods developed by Lipomics Technologies. The lipids from plasma (200 µL) were extracted using a modified Folch extraction in chloroform:methanol (2:1, v:v) (25) in the presence of a panel of quantitative authentic internal standards. Extracted concentrated lipids were analyzed by HPLC for phospholipid separation TLC for nonpolar lipid classes. Lipid classes were trans-esterified in 3 mol/L methanolic HCl in sealed vials under a nitrogen atmosphere at 100°C for 45 min. The resulting FAME were extracted from the mixture with hexane separated and quantified by capillary GC using an Agilent 6890 gas chromatograph equipped with a 30-m DB-88 capillary column (Agilent Technologies) and a flame-ionization detector (26). Fatty acids of each lipid class were determined quantitatively (µmol/L) and expressed as a percent of total fatty acids within that class (mol %). Fatty acids in which 20% of the data were missing or below the limit of quantification were dropped from the analyses and considered not determined in the “Results” section.

Statistical analyses. All statistical procedures were conducted using SPSS version 12 for Windows. Means ± SD are reported for baseline and 12-wk anthropometric and clinical characteristics, and baseline fatty acid composition of cholesterol ester (CE), FFA, lysophosphatidylcholine (LPC), phosphatidylcholine (PC), phosphatidylethanolamine (PE), and TAG of study participants. All variables were analyzed for normality, and extreme values through the SPSS Explore procedure were removed from further analysis. Cases with values >3 box-lengths from the 75th percentile or 25th percentile were deemed outliers and removed from all analyses.

Paired t tests were performed to determine differences of anthropometric and clinical characteristics between baseline and 12 wk with an α set at 0.05. Stepwise regression was used to generate predictive models between 10 sets of predictor variables and 4 anthropometric outcomes: % Δ weight, % Δ WC, % Δ LM, and % Δ BF. Eleven sets of predictor variables were used to predict these 4 anthropometric outcomes and included the following independent variables: site and treatment both converted into dummy variables (−1, 0, 1), sex, age, respective baseline anthropometric measurement, and values of plasma fatty acids for: CE, diacylglycerol (DAG), FFA, LPC, PC, PE, TAG, total plasma lipid classes, fasting clinical metabolites, and reported micro- and macronutrient intake at baseline and at 12 wk.

Due to high variation in abundance among circulating lipid classes, only fatty acids with a mean abundance of ≥1.0% were used in each stepwise regression. Estimation of metabolic flux through desaturases and elongases determined as product-precursor ratios were also included in stepwise regressions. The ratios of circulating 16:1(n-7):16:0 and 18:1(n-9):18:0 were used as surrogates for Δ9 desaturase activity; the ratios of 18:3(n-6):18:2(n-6) and 20:4(n-6):20:3(n-6) were used as surrogates for Δ6 and Δ5 desaturase activity, respectively; and the ratios of 18:1(n-7):16:1(n-7), 18:0:16:0, and 20:2(n-6):18:2(n-6) were used as surrogates for elongase activities (27). Stepwise regression analyses were conducted using baseline fatty acids of each circulating lipid class, clinical metabolites, and reported dietary intake to generate 11 different models to predict 4 anthropometric outcomes independent of treatment. The specific variables used for each of the 11 stepwise regression models to predict each of the 4 anthropometric outcomes are reported (Table 1).

For each stepwise regression, the F statistic probability was set at an α between 0.05 and 0.06. Normality for each stepwise regression model was determined by generating normal probability plots of the regression standardized residual. Equal variance for each regression model across each dependent variable was determined by plotting the standardized predicted dependent variable by the standardized residuals. Outlying cases that strongly influenced each stepwise regression model were tested by Cook’s distance (D). Data points with larger D values than the rest of the data were considered highly influential and were deleted. The models with deleted observations with large D values were re-regressed and compared with ensure the model was statistically relevant and not a product of 1 highly influential data point. Multicollinearity between baseline lipids selected by stepwise regression was checked by a Variance Inflation Factor of ≤4.0. If variables demonstrated a Variance Inflation Factor >4.0, they were dropped from the final regression model.

Final linear regressions were performed to predict anthropometric outcomes by including all the predictor variables selected by the initial stepwise regression. Site, sex, and age were not used to adjust for any of the final models, because they did not influence any of the anthropometric outcomes. Treatment influenced 3 of the 4 anthropometric outcomes and when included in the equation, strengthened the model by 10%. However, treatment was not used to adjust the final models to explore the practical predictability of lipid metabolism on changes in body composition independent of any treatment. The effect of treatment on changes in anthropometry is reported elsewhere (Smilowitz JT, Wiest MM, Watkins SM, Tegarden D, Zemel MB, Van Loan MD, German JB, unpublished data). Final models are shown with and without adjustment for physical activity and HOMA-IR at baseline.

To determine whether reported micro- and macronutrient intakes influenced predictor variables, stepwise regressions were performed using micro- and macronutrient intake, sex, and age as independent variables and circulating lipids as dependent variables at baseline and 12 wk. To determine whether circulating lipids influenced baseline anthropometry, stepwise regressions were performed using circulating lipid predictors at baseline, with sex and age as independent variables and baseline anthropometric measurements (weight, WC, % LM, and % BF) as dependent variables.

ANCOVA was performed on each stepwise regression to confirm the results of the final adjusted model in which the dependent variable was the anthropometric outcome. Covariates included any of the selected baseline circulating lipids, clinical metabolites, and/or reported dietary intake selected by the original stepwise regression models. Interactions between all possible covariates and independents were determined by ANCOVA.

Results

Baseline and 12-wk anthropometric and clinical characteristics. There were significant reductions in weight, BMI, LM, total BF, % BF, WC, and plasma cholesterol, insulin, and
HOMA-IR and a significant increase in % LM between baseline and 12 wk (Table 2). Physical activity levels did not change during the study (data not shown).

**Changes in anthropometric measurements.** The % Δ weight, % Δ WC, Δ % BF, and Δ % LM between baseline and 12 wk did not significantly differ between sites or sex, nor were they influenced by age (Table 3).

**Baseline fatty acid composition of plasma lipids.** Concentrations of fatty acids of plasma CE, FFA, TAG, LPC, PC, and PE at baseline are reported in Supplemental Tables 1 and 2, respectively.

**Relationships between predictor variables and changes in weight and WC.** In this cohort, no baseline predictor variables were selected by stepwise regression to explain the variance in the % Δ weight (data not shown). Of all possible sets of predictors, circulating concentrations of FFA 18:1(n-9) and FFA 20:4(n-6):20:3(n-6) (data not shown) at baseline were associated with the % Δ weight. After deleting 1 highly influential data point, the significance of FFA 20:4(n-6):20:3(n-6) disappeared. Yet circulating FFA 18:1(n-9) remained highly significant and explained 25% \((P = 0.0005)\) of the variance for the % Δ weight (Fig. 1A). WC, gender, age, site, physical activity, insulin, HOMA-IR, glucose, reported intake of micro- and macronutrients, including monounsaturated fatty acids (MUFA) (% total fat) and oleic acid, and clinical metabolites at baseline were not associated with the % Δ WC. Reported intake of energy at 12 wk was associated with the % Δ WC \(R^2 = 0.149; r = -0.411; P = 0.006\) and positively associated with baseline weight \(ADJ R^2 = 0.283; P = 0.0005\) (data not shown). Final models for the % Δ WC include reported intake of energy at 12 wk and adjusted for HOMA-IR and physical activity at baseline (Table 4).

**Relationships between predictor variables and changes in BF and LM.** The Δ % BF was positively associated with plasma concentrations of PC 18:0, PC 18:3(n-6):18:2(n-6), and TAG 18:3(n-6):18:2(n-6) at baseline and negatively associated with concentrations of plasma CE 18:1(n-7), FFA 18:1(n-7), and total FFA at baseline (Supplemental Table 3). The Δ % LM was

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### TABLE 1 Specific sets of predictor variables used in stepwise regression to generate predictive models for anthropometric outcomes

<table>
<thead>
<tr>
<th>Model</th>
<th>Specific variables</th>
</tr>
</thead>
<tbody>
<tr>
<td>CE2</td>
<td>14:0, 16:0, 18:0, 16:1(n-7), 18:1(n-9), 18:2(n-6), 20:4(n-6), 16:1(n-7):16:0, 18:1(n-9):18:0, 20:4(n-6):20:3(n-6), 18:3(n-6):18:2(n-6), 18:1(n-7):16:1(n-7), 18:0:16:0</td>
</tr>
<tr>
<td>DAG2</td>
<td>14:0, 15.0 16:0, 18:0, 16:1(n-7), 18:1(n-9), 18:2(n-6), 20:4(n-6), total dimethyl acetal, 16:1(n-7):16:0, 18:1(n-9):18:0, 18:3(n-6):18:2(n-6), 18:1(n-7):16:1(n-7), 18:0:16:0</td>
</tr>
<tr>
<td>FFA2</td>
<td>Age, sex, site, treatment, 14:0, 16:0, 18:0, 16:1(n-7), 18:1(n-9), 18:2(n-6), 18:3(n-3), 20:4(n-6), 16:1(n-7):16:0, 18:1(n-9):18:0, 18:1(n-7):16:1(n-7), 18:0:16:0</td>
</tr>
<tr>
<td>LPC2</td>
<td>14:0, 15.0 16:0, 18:0, 18:1(n-9), 18:1(n-7), 18:2(n-6), 20:3(n-6), 20:4(n-6), 16:1(n-7):16:0, 18:1(n-9):18:0, 20:4(n-6):20:3(n-6), 18:1(n-7):16:1(n-7), 18:0:16:0</td>
</tr>
<tr>
<td>PC2</td>
<td>16:0, 18:0, 18:1(n-9), 18:1(n-7), 18:2(n-6), 20:3(n-6), 20:4(n-6), 22:5(n-3), 16:1(n-7):16:0, 18:1(n-9):18:0, 20:4(n-6):20:3(n-6), 20:2(n-6):18:2(n-6), 18:1(n-7):16:1(n-7), 18:0:16:0</td>
</tr>
<tr>
<td>PE2</td>
<td>14:0, 16:0, 18:0, 18:1(n-9), 18:1(n-7), 18:2(n-6), 20:3(n-6), 20:4(n-6), 22:5(n-3), 22:6(n-3), dimethyl acetal 18:0, dimethyl acetal 18:1(n-9), total dimethyl acetal, 16:1(n-7):16:0, 18:1(n-7):18:0, 18:0:16:0, 18:1(n-9):18:0, 18:2(n-6):20:4(n-6), 20:2(n-6):18:2(n-6), 18:1(n-7):16:1(n-7), 18:0:16:0</td>
</tr>
<tr>
<td>TAG2</td>
<td>14:0, 16.0, 16:1(n-7), 18:0, 18:1(n-9), 18:1(n-7), 18:2(n-6), 18:3(n-3), 20:4(n-6), 16:1(n-7):16:0, 18:1(n-9):18:0, 20:4(n-6):20:3(n-6), 18:1(n-7):16:1(n-7), 18:0:16:0</td>
</tr>
<tr>
<td>Total lipid classes3</td>
<td>CE, DAG, FFA, LPC, PC, PE, TAG</td>
</tr>
<tr>
<td>Clinical metabolites</td>
<td>Fasting circulating glucose,4 TAG,4 total cholesterol,4 HDL cholesterol,4 LDL cholesterol,4 insulin,5 total cholesterol:HDL,6 Total lipid classes</td>
</tr>
<tr>
<td>Daily mean dietary intake at baseline and 12 wk</td>
<td>Age, sex, site, treatment, energy,7 carbohydrate, protein, fat,8 oleic acid, linoleic acid, α-linolenic acid, eicosapentaenoic acid, docosahexaenoic acid,9 cholesterol:energy,10 tryptophan, threonine, isoleucine, leucine, lysine, methionine, cysteine, phenylalanine, tyrosine, valine, histidine11, biotin, folate, vitamin B-12, vitamin D,12 calcium, niacin, pantothentic acid, riboflavin, thiamin, vitamin B-613</td>
</tr>
</tbody>
</table>

1 Each of the 11 sets of possible predictor variables was used in stepwise regression to predict changes in anthropometric outcomes: % Δ weight, % Δ WC, Δ % BF, and Δ % LM. Age, sex, site, and treatment were included as possible predictor variables in each stepwise regression. 2 Circulating fatty acids of each lipid class as mol % and their ratios were included as possible predictor variables in corresponding stepwise regression models. 3 Circulating metabolites as mmol/L were included as possible predictor variables in corresponding stepwise regression models.
Anthropometric and clinical characteristics of study participants at baseline after 12 wk of energy restriction

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Baseline</th>
<th>12 wk</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>25.4 ± 4.9</td>
<td>25.4 ± 4.9</td>
</tr>
<tr>
<td>Sex, n, (%)</td>
<td>69 (76.1)</td>
<td>69 (76.1)</td>
</tr>
<tr>
<td>Female</td>
<td>11 (23.9)</td>
<td>11 (23.9)</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>81.4 ± 12.7</td>
<td>76.6 ± 12.0*</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>29.0 ± 3.1</td>
<td>27.2 ± 2.9*</td>
</tr>
<tr>
<td>LM, kg</td>
<td>46.0 ± 10.7</td>
<td>45.3 ± 9.9**</td>
</tr>
<tr>
<td>% LM</td>
<td>56.1 ± 6.5</td>
<td>58.9 ± 6.4*</td>
</tr>
<tr>
<td>Total BF, kg</td>
<td>32.1 ± 6.0</td>
<td>28.0 ± 5.9*</td>
</tr>
<tr>
<td>% BF</td>
<td>39.7 ± 6.1</td>
<td>36.8 ± 6.1*</td>
</tr>
<tr>
<td>WC, cm</td>
<td>89.4 ± 11.2</td>
<td>82.5 ± 10.6*</td>
</tr>
<tr>
<td>TAG², mmol/L</td>
<td>1.33 ± 0.72</td>
<td>1.21 ± 0.65</td>
</tr>
<tr>
<td>Total cholesterol,²,³ mmol/L</td>
<td>4.50 ± 0.92</td>
<td>4.37 ± 0.91³</td>
</tr>
<tr>
<td>LDL cholesterol,² mmol/L</td>
<td>2.71 ± 0.91</td>
<td>2.63 ± 0.84</td>
</tr>
<tr>
<td>HDL cholesterol,² mmol/L</td>
<td>1.27 ± 0.37</td>
<td>1.25 ± 0.35</td>
</tr>
<tr>
<td>Total-HDL cholesterol²</td>
<td>3.87 ± 1.3</td>
<td>3.76 ± 1.1</td>
</tr>
<tr>
<td>Insulin,² pmol/L</td>
<td>59.4 ± 35.8</td>
<td>47.2 ± 33.5*</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>2.1 ± 1.3</td>
<td>1.7 ± 1.2 *</td>
</tr>
</tbody>
</table>

¹ Values are expressed as means ± SD, n = 46 unless otherwise specified. Different from baseline, *P < 0.0005; **P < 0.01; †P < 0.05.
² Metabolites were measured in plasma obtained from fasting participants; n = 45.
³ n = 44.

Relationships between dietary intake and plasma fatty acids (mol %). Reported intake of SFA (% of total fat) at baseline was positively, but weakly, associated with the circulating concentration of PC 18:0 at baseline (data not shown). There was no relationship between reported SFA intake and circulating PC 18:0 at 12 wk, respectively. Additionally, there were no correlations between reported intake of MUFA (% total fat) or oleic acid at baseline and 12 wk and circulating FFA 18:1(n-9) at baseline and 12 wk, respectively.

Relationships between baseline plasma fatty acid predictors as quantitative data (μmol/L) and changes in anthropometry. To confirm the predictive validity of baseline circulating fatty acids as mol %, we generated stepwise and linear regressions using the same set of baseline circulating lipid species as quantitative data (μmol/L) against the same anthropometric outcomes. Stepwise regression selected the ratio of baseline circulating PC 18:0:16:0 (Supplemental Fig. 1) to explain 20% of the variance in Δ % BF (Supplemental Table 5). Stepwise regression selected the ratio of PC 18:0:16:0 (Supplemental Fig. 2) and FFA 18:1(n-9) (μmol/L) to explain 26% of the variance in Δ % LM (Supplemental Table 5).

Relationships among plasma fatty acids (mol %). To discover possible metabolic relationships between circulating fatty acid predictors for changes in body composition, we generated stepwise regressions between baseline predictor variables and fatty acids of the same lipid classes (Supplemental Table 6). The circulating concentration of FFA 18:1(n-9) at baseline was negatively associated with the ratio of FFA 16:1(n-7):16:0 (Fig. 2A), a surrogate for Δ9 desaturase activity (r = 0.60; ADJ $R^2 = 0.34; P < 0.0005$). Baseline circulating PC 18:0 was negatively associated with PC 16:0 (Fig. 2B) (r = -0.80; ADJ $R^2 = 0.64; P < 0.0005$).

Discussion

The objective of this study was to develop statistical models that target circulating lipid metabolites associated with changes in body composition. Using 11 sets of predictor variables that encompassed the comprehensive analyses of circulating fatty acids, clinical metabolites, and reported micro- and macronutrient intake, stepwise regression selected PC 18:0 and FFA 18:1(n-9) as prominent predictor variables for changes in body composition. Despite the large variation in baseline insulin sensitivity among participants, the variance and significance of the adjusted models did not greatly change from unadjusted models.

Our results showed that individuals with higher circulating baseline FFA 18:1(n-9) experienced greater reductions in WC. Additionally, individuals with higher baseline levels of FFA 18:1(n-9) also exhibited higher % LM at the end of the 12-wk dietary intervention. These results were independent of diet based on reported intake of MUFA (% total fat) or oleic acid.

### TABLE 2

Changes in anthropometric and clinical characteristics of study participants in response to energy restriction for 12 wk

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Baseline</th>
<th>12 wk</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight change, %</td>
<td>-5.91 ± 2.95 (-12.4 to -1.8)</td>
<td></td>
</tr>
<tr>
<td>WC change, %</td>
<td>-7.74 ± 3.51 (-14.5 to -0.32)</td>
<td></td>
</tr>
<tr>
<td>BF, % body weight</td>
<td>-2.98 ± 2.23 (-9.0 to -2.1)</td>
<td></td>
</tr>
<tr>
<td>LM, % body weight</td>
<td>2.77 ± 2.29 (-1.9 to -9.2)</td>
<td></td>
</tr>
</tbody>
</table>

¹ Values are means ± SD (range), n = 46 unless otherwise specified.
² n = 44.
Baseline reported intake of MUFA (% total fat) and oleic acid (g/d) were not associated with baseline circulating FFA 18:1(n-9), the % WC, or % LM. Thus, if diet records were accurate, circulating FFA 18:1(n-9) was not associated with dietary MUFA (% total fat) or with oleic acid intake.

Circulating levels of FFA 18:1(n-9) could derive from several metabolic consequences: desaturation by adipose tissue, lipolysis by adipose tissue and TAG-rich lipoproteins in plasma, reduced uptake by peripheral tissues and liver, decreased cellular transport, and/or any combination of these factors. We propose that individuals with higher baseline circulating FFA 18:1(n-9) have greater rates of adipose tissue lipolysis than individuals with lower baseline circulating concentrations.

Elevated circulating fatty acids are associated with the fasted condition, exercise (28), higher lipolytic activity in adipose tissue due to genetic polymorphisms (29,30), obesity (30), and insulin resistance (31,32). Yet it is unknown whether the fatty acid composition compared with total circulating fatty acids better reveals an individual’s metabolic state (i.e. insulin resistant, postprandial, postabsorptive, etc.). Increased circulating FFA 18:1(n-9) could result from increased output, how the individual fatty acid lipolysis products are preferentially taken up by

| TABLE 4 | Linear regression models for predicting changes in body composition using baseline circulating lipid metabolites of study participants undergoing a 12-wk energy-restriction intervention |
|------------------|------------------|------------------|------------------|------------------|------------------|------------------|------------------|
| Final models | Predictors | Partial r | Model ADJ $R^2$ | SEE | Intercept ± SEM | $\beta$ Coefficients ± SE | $P$ |
| % WC $^{2,3}$ | FFA 18:1(n-9)$^4$ | -0.421 | 0.31 | 2.9 | 17.3 ± 5.7 | -0.547 ± 0.168 | 0.0005 |
| Energy $^2$ | -0.413 | -0.001 ± 0.000 |
| % WC $^{2,5}$ | FFA 18:1(n-9)$^4$ | -0.439 | 0.28 | 3.0 | 15.5 ± 7.6 | -0.530 ± 0.176 | 0.0020 |
| Energy $^2$ | -0.458 | -0.001 ± 0.000 |
| % BF $^3$ | PC 18:0$^4$ | 0.498 | 0.23 | 2.0 | -10.0 ± 1.9 | 0.582 ± 0.153 | 0.0005 |
| % BF $^6$ | PC 18:0$^4$ | 0.422 | 0.23 | 2.0 | -14.1 ± 3.4 | 0.506 ± 0.160 | 0.0050 |
| % LM $^3$ | PC 18:0$^4$ | -0.474 | 0.33 | 1.9 | -0.395 ± 3.8 | -0.522 ± 0.149 | 0.0005 |
| % LM $^6$ | FFA 18:1(n-9)$^4$ | 0.417 | 0.31 | 1.9 | 3.3 ± 5.9 | 0.465 ± 0.158 | 0.0010 |
| % LM $^6$ | PC 18:0$^4$ | -0.427 | 0.31 | 1.9 | 3.3 ± 5.9 | 0.465 ± 0.158 | 0.0010 |
| FFA 18:1(n-9)$^4$ | 0.360 | 0.277 ± 0.115 |

$^1$ Relationships between predictor variables and anthropometric outcomes were determined by linear regression, n = 46 unless otherwise indicated.

$^2$ n = 43.

$^3$ Model is unadjusted.

$^4$ Circulating lipids are expressed as mol %.

$^5$ Model is adjusted with HOMA-IR and physical activity at baseline.

$^6$ Model is adjusted with HOMA-IR, physical activity, and WC at baseline.
extrahepatic tissues, or represent greater substrate availability for fatty acid oxidation by extrahepatic tissues, thereby contributing quantitatively to their concentrations in circulation. Unfortunately, fatty acid oxidation was not measured in this cohort and could not be regressed against circulating FFA 18:1(n-9). Future mechanistic studies should be designed in which labeled fatty acids released by lipolysis are measured as oxidized fatty acid products over a 24-h period.

In addition to circulating FFA 18:1(n-9), PC 18:0 at baseline also predicted changes in body composition. Compared with individuals with higher circulating PC 18:0, individuals with lower baseline circulating PC 18:0 experienced greater reductions in total BF and higher % LM. These relationships were independent of sex, age, research site, and WC, % BF, LM, and saturated fat intake at baseline and 12 wk.

The goal of our study was to establish the extent to which composition of circulating phospholipids reflect metabolic status. Epidemiologically, circulating phospholipid 18:0 was positively associated with the incidence of type-2 diabetes (19,20), independent of dietary intake (19). Circulating lipids represent indices of metabolism independent of dietary intake. In our study, reported intake of SFA at baseline was weakly associated with circulating baseline PC 18:0. However, this relationship was not observed between reported SFA intake and circulating concentrations of PC 18:0 at 12 wk. Additionally, there were no relationships between reported baseline macronutrient intake and the 4 anthropometric outcomes.

More mechanistically, plasma PC 18:0 was reduced in PE-N-methyltransferase-deficient mice, suggesting that this metabolite is generated through the PE-N-methyltransferase pathway and not via the cytidine diphosphate-choline pathway (33). Induction of diabetes in streptozotocin-treated rats was increased hepatic PE-N-methyltransferase abundance and activity by 50% and was prevented by insulin treatment (34). Currently, to our knowledge, the mechanistic relationships between PC synthetic pathways and changes in body composition is unknown.

Baseline circulating PC 18:0, a predictor of the Δ % BF and Δ % LM, was strongly and negatively associated with baseline circulating PC 16:0. It is not known if this inverse relationship reflects metabolic flux through elongase or PC biosynthesis pathways. Future research should be designed to identify circulating metabolites and annotate their origins to specific tissues and pathways.

In addition to the small number of participants used to analyze large numbers of variables used to generate linear regression models, there were several other limitations to this study. Because circulating lipids are reflective of diet and endogenous metabolism, differentiating between the 2 is challenging unless diet composition is controlled. There were large variations in dietary intake of saturated fat between the 3 testing, sites because participants were not provided with a standardized dietary protocol. We used weekly self-reported 3-d diet records to determine the relationships between circulating lipids and dietary intake. We cannot confirm the validity of these data.

Overall, metabolic pathways are in place to respond to the diet. A nutritional approach to understanding diet and lipid metabolism can now take advantage of the comprehensive measurements of products and substrates of entire metabolic pathways. This study measured plasma lipid metabolites prior to a dietary weight loss intervention to identify those that reflect a preexisting metabolic phenotype associated with subsequent changes in body composition. Despite the large number of variables used to generate predictive models for changes in anthropometry, our dataset represents an unusual opportunity to develop various hypotheses relating metabolism and changes in body composition during energy restriction. Epidemiological data and biochemical inference corroborate our findings; however, future studies are warranted to identify the metabolic pathways involved and to further understand the relationship between metabolic phenotype and subsequent health status.

**Literature Cited**


