Metabolic adaptation characterizes short-term resistance to weight loss induced by a low-calorie diet in overweight/obese individuals

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ABSTRACT

Background: Low-calorie diet (LCD)–induced weight loss demonstrates response heterogeneity. Physiologically, a decrease in energy expenditure lower than what is predicted based on body composition (metabolic adaptation) and/or an impaired capacity to increase fat oxidation may hinder weight loss. Understanding the metabolic components that characterize weight loss success is important for optimizing weight loss strategies.

Objectives: We tested the hypothesis that overweight/obese individuals who had lower than expected weight loss in response to a 28-d LCD would be characterized by *1*) impaired fat oxidation and *2*) whole-body metabolic adaptation. We also characterized the molecular mechanisms associated with weight loss success/failure.

Methods: This was a retrospective comparison of participants who met their predicted weight loss targets [overweight/obese diet sensitive (ODS), $n = 23$, females $= 21$, males $= 2$] and those that did not [overweight/obese diet resistant (ODR), $n = 14$, females $= 12$, males $= 2$] after a 28-d LCD (900–1000 kcal/d). We used whole-body (energy expenditure and fat oxidation) and tissue-specific measurements (metabolic proteins in skeletal muscle, gene expression in adipose tissue, and metabolites in serum) to detect metabolic properties and biomarkers associated with weight loss success.

Results: The ODR group had greater mean ± SD metabolic adaptation (−175 ± 149 kcal/d; +119%) than the ODS group (−80 ± 108 kcal/d) after the LCD ($P = 0.030$). Mean ± SD fat oxidation increased similarly for both groups from baseline (0.0701 ± 0.0206 g/min) to day 28 (0.0869 ± 0.0269 g/min; $P < 0.001$). A principal component analysis factor comprised of serum 3-hydroxybutyric acid, citrate, leucine/isoleucine, acetylcarnitine, and 3-hydroxylbutyrlcarnitine was associated with weight loss success at day 28 (std. $\beta = 0.674$, $R^2 = 0.479$, $P < 0.001$).

Conclusions: Individuals who achieved predicted weight loss targets after a 28-d LCD were characterized by reduced metabolic adaptation. Accumulation of metabolites associated with acetyl-CoA excess and enhanced ketogenesis was identified in the ODS group. This trial was registered at clinicaltrials.gov as NCT01616082. *Am J Clin Nutr* 2021;114:267–280.

Keywords: overweight, obese, low-calorie diet, weight loss, metabolic adaptation, metabolomics

Introduction

Over 650 million people globally are obese (1). Obesity is linked to increased risk of type 2 diabetes, cardiovascular complications, and certain cancers (2). Moderate weight loss between 5% and 10% body weight is a proposed target for achieving improvements in metabolic function and health outcomes (3). Although low-calorie diets (LCDs) (800–1400 kcal/d) can reduce weight by 8% over a 6-mo period (4, 5) there is notable response heterogeneity among cohorts. This weight loss response heterogeneity exists despite adherence and compliance to a weight loss diet (6). Characterizing the metabolic components of controlled weight loss success will be crucial in predicting the efficacy of weight loss interventions in obese populations and developing future personalized treatment plans.

Weight loss failure has been linked to metabolic adaptation (7), which is defined as a reduction in resting metabolic rate (RMR) beyond what is anticipated from changes in body composition (8, 9). Historically, metabolic adaptation is seen as a protective survival mechanism against prolonged periods of caloric restriction, purported to be mediated by decreased leptin concentrations due to decreases in fat mass (FM) (7). In the modern-day obesogenic environment this metabolic adaptation can blunt the weight loss effects of an LCD (7).

Reducing FM through caloric restriction requires the engagement of multiple tissues. Triglycerides stored in adipocytes are initially hydrolyzed into fatty acids (FAs) which are released into the circulation (10). FAs are transported to tissues capable of FA oxidation such as skeletal muscle and liver. Upon cellular uptake, long-chain FAs enter the mitochondria via the carnitine-shuttle enzyme complex (11). Acetyl-CoA produced from β-oxidation enters the tricarboxylic acid (TCA) cycle which mediates complete oxidation. Dysregulation at any step of this

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coordinated response could theoretically diminish FM loss during an LCD.

Profiling of serum metabolites provides an overview of whole-body metabolic processes and offers an investigative approach for multisystemic disorders such as obesity and type 2 diabetes (12, 13). Metabolomics tools have been useful for identifying defects in lipid metabolism and oxidation (14, 15), whereas specific profiling of serum acylcarnitines (ACs) has aided in the identification of long-chain FA oxidation disorders (16). Previous research has identified metabolomic markers in response to various weight loss strategies (17, 18). Combining these metabolomic analyses with muscle and adipose tissue analyses may provide greater insight into whole-body and tissue-specific markers of weight loss success in overweight/obese individuals during an acute weight-loss intervention.

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Supplemental Figures 1–4 and Supplemental Tables 1–4 are available from the "Supplementary data" link in the online posting of the article and from the same link in the online table of contents at https://academic.oup.com/ajcn/.

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Abbreviations used: AA, amino acid; *ABDH5,* 1-acylglycerol-3-phosphate O-acyltransferase; AC, acylcarnitine; ACC, acetyl-CoA carboxylase; *AQP7*, aquaporin-7; BL, baseline; C2, acetyl-carnitine; C3, propionyl carnitine; C4/Ci4, citrate and butyryl/isobutyryl carnitine; C5:1, tiglyl carnitine; C5/C4=O, isovalerylcarnitine/acetoacetylcarnitine; C4-OH, 3-hydroxylbutyrlcarnitine; C5-OH/C3-DC, malonyl carnitine; C4-DC/Ci4-DC, methylmalonyl/succinyl carnitine; C6-OH-DC, hydroxyhexanoyl-dicarboxylic-carnitine; C8:1, octenoyl carnitine; C8/C5:1-DC, octanoyl/mesaconyl carnitine; C5-DC, glutaryl carnitine; C8:1-OH/C6:1-DC, octenoyl carnitine3-hydroxy-cis-5-octenoyl/hexenedioyl carnitine; C6-DC, adipoyl carnitine; C10:3, decatrienoyl carnitine; C10:2, decadienoyl carnitine; C10:1, decenoyl carnitine; C10, decanoyl carnitine; C8:1-DC, octenoyl carnitine; C10-OH/C8-DC, suberoyl carnitine; C12:1, dodecenoyl carnitine; C12, lauroyl carnitine; C12:OH/C10:2-DC, sebacoyl carnitine; C14:2, tetradecadienoyl carnitine; C14:1, tetradecenoyl carnitine; C14, myristoyl carnitine; C16:1, exadecenoyl carnitine C16, palmitoyl carnitine; C18:2, linoleyl carnitine; C18:1, oleyl carnitine; C18, stearoyl carnitine; *CD36,* cluster of differentiation 36; *CPT1*, carnitine palmitoyltransferase I; *DGAT1,* diacylglycerol O-acyltransferase 1; EE, energy expenditure; ESI, electrospray ionization; FA, fatty acid; *FAS,* fatty-acid synthase; FFM, fat-free mass; FM, fat mass; *GIPR,* Gastric Inhibitory Polypeptide Receptor; *G0S2,* G0/G1 Switch 2; *GPAT1,* glycerol-3-phosphate acyltransferase 1; *HSL,* hormone sensitive lipase; LCD, low-calorie diet; *LPL,* lipoprotein lipase; *MCAD,* medium-chain acyl-CoA dehydrogenase; *MLYCD,* malonyl-CoA decarboxylase; MRM, multiple reaction monitoring; NIDDK, National Institute of Diabetes and Digestive and Kidney Diseases; *NRPA,* natriuretic peptide receptor-A; *NPRB,* natriuretic peptide receptor-B; OA, organic acid; ODR, overweight/obese diet resistant; ODS, overweight/obese diet sensitive; PA, physical activity; PCA, principal component analysis; *PGC1-α,* peroxisome proliferator-activated receptor gamma coactivator 1-alpha; *PLIN1,* perilipin-1; *PPARγ1,* peroxisome proliferator-activated receptor gamma-1; *PPARγ2,* peroxisome proliferator-activated receptor gamma-2; RMR, resting metabolic rate; ROS, reactive oxygen species; RQ, respiratory quotient; *SCD1,* stearoyl-CoA desaturase; *SIRT1*, sirtuin 1; *SREBP1*, sterol regulatory element-binding protein 1; TCA, tricarboxylic acid; TRI, Translational Research Institute; VCO_2, carbon dioxide production; VLCAD, very long-chain acyl-CoA dehydrogenase; VO_2, oxygen consumption; 3-HBA, 3-hydroxybutyric acid.

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We hypothesized that overweight/obese individuals with a diminished weight loss response to a 28-d LCD would exhibit impaired fat oxidation and whole-body metabolic adaptation. Our primary outcome variable was fat oxidation. We used whole-body and tissue-specific measurements to detect metabolic mechanisms associated with weight loss success. By predicting weight loss targets that considered baseline (BL) body composition, age, sex, RMR, and physical activity (PA) using the Hall model (19), we stratified participants into those who did and did not meet their expected weight loss targets.

Methods

Ethical approval and screening

The study protocol was approved by the AdventHealth Institutional Review Board and carried out in accordance with the Declaration of Helsinki. All participants provided their written consent to take part in the study. Before enrollment, all participants were evaluated for eligibility. Inclusion/exclusion criteria can be found at clinicaltrials.gov (NCT01616082). To detect changes in fat oxidation, we recruited 50 overweight/obese (BMI $\geq$27 and $\leq$45 kg/m^2) men and women between the ages of 18 and 55 y (**Supplemental Figure 1**). Thirty-nine participants completed the 28-d dietary intervention: 2 participants demonstrated increases in body weight throughout the LCD and were excluded from further analyses, leaving 37 included in this analysis. All participants were in good health and without evidence or history of clinically significant metabolic and cardiovascular disorders including diabetes.

Experimental protocol

Figure 1 depicts a summary of the time points for sample collection and procedures. RMR and respiratory quotient (RQ) were measured by indirect calorimetry on days −14, 0, 7, 14, and 28. Body composition was determined by DXA on days 0 and 28. Muscle and adipose tissue samples were obtained at days 0 and 14. The LCD started on day 0 and finished on day 28. Blood samples and weight measurements were obtained at every time point. All measurements were obtained in the morning after an overnight fast.

LCD

Participants received an LCD of ∼900–1000 kcal/d for 28 d. To achieve this, 2 meals (breakfast and lunch) were replaced with a total of 600 kcal of meal-replacement shakes (HMR®70 PLUS, Health Management Resources Corporation). A portion-controlled dinner of ∼300–400 kcal made up the balance of the calories. Shakes were provided to participants and dinners were self-selected by participants from an approved list of Lean Cuisine® and Healthy Choice® brand meals. Verbal compliance and barriers for weight loss were monitored throughout the study during weekly dietary counseling visits.

Model predictions

Weight loss predictions over the 28-d LCD were based on the National Institute of Diabetes and Digestive and Kidney

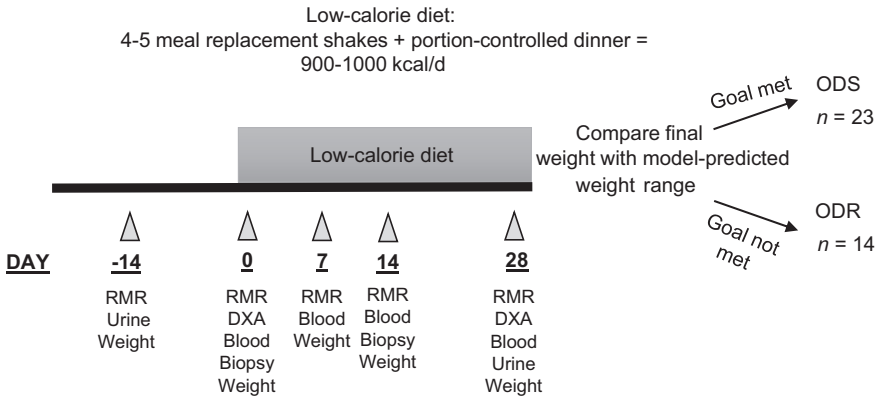

FIGURE 1 Study schematic. ODR, overweight/obese diet resistant; ODS, overweight/obese diet sensitive; RMR, resting metabolic rate.

Diseases (NIDDK) mathematical model (https://www.niddk.nih.gov/bwp) using expert mode and advanced features (19). The model predicted a target weight range throughout the 900- to 1000-kcal/d LCD based on sex, age, height, weight, body fat percentage, PA level, and RMR for each participant. Importantly, stratification based on this model accommodates for differences in BL body composition. We prespecified day 28 as the time point to stratify participants on weight loss success because it was a long enough duration for known electrolyte and water balance alterations to dissipate (20) while still acute enough to monitor early metabolic changes that represent the trajectory to weight loss success. At day 28, participants who did not achieve the upper target weight of the model's predicted weight range were classified as overweight/obese diet resistant (ODR). Conversely, participants who met the model-predicted target weight range were classified as overweight/obese diet sensitive (ODS).

DXA

Body composition was measured using a GE Lunar iDXA, running enCORE software version 13.3 (GE Medical Systems). All DXA scans were analyzed in the Imaging Core using enCORE software to determine fat-free mass (FFM; kg) and FM (kg). Test–retest CVs averaged 0.7% across a range of body composition.

Indirect calorimetry.

Indirect calorimetry was performed with a MAX II Metabolic cart (AEI Technologies) to measure RMR and RQ at BL and days 7, 14, and 28. A transparent plastic hood connected to the cart was placed over the head of the participant who was lying in a semi-Fowler position for 30 min. The first 10 min of data were discarded to ensure the accuracy of the resting measurement (21). Calculations of oxygen consumption (VO_2) and carbon dioxide production (VCO_2) were made from continuous measurements of carbon dioxide and oxygen concentrations in inspired and expired air diluted in a constant air flow (~40 L/min) generated by the analyzer.

RMR was calculated using the following equation (22):

$$\text{RMR (kcal/d)} = \text{VO}_2\,\text{(L/24 h)} \times [4.686 + (\text{RQ} - 0.707) \times 0.361/0.293] \quad (1)$$

where $\text{RQ} = \text{VCO}_2/\text{VO}_2$.

On the day −14 and day 28 visits, urine was collected to measure the rate of urinary nitrogen production (g/24 h) in order to calculate fat oxidation according to the Frayn equation (23):

$$\text{Fat oxidation (g/min)} = [1.67 \times \text{VO}_2\ \text{(L/d)}] - [1.67 \times \text{VCO}_2\,\text{(L/min)}] - [1.92 \times n\,\text{(g/min)}] \quad (2)$$

Metabolic adaptation

A stepwise multiple linear regression was performed to determine predictors of RMR at BL for this cohort. The following independent variables were entered into the model: age, sex, race/ethnicity, FM, and FFM, which produced the following equation:

$$\text{RMR (kcal/d)} = 563.9 + [24.33 \times \text{FFM (kg)}] - [7.62 \times \text{age (y)}] - (74.66 \text{ if race} = \text{black}) \quad (3)$$

This equation was used to predict RMR at BL and after the LCD at day 28. Predicted RMR was subtracted from RMR measured by indirect calorimetry to determine the RMR residual at day 0 and day 28. The day 28 RMR residual was subtracted from the day 0 RMR residual to determine metabolic adaptation as described by Galgani and Santos (8).

PA monitor

Indexes of PA [daily energy expenditure (EE), active EE, and number of steps] were measured with a triaxial activity

monitor (SenseWear Pro3 Armband, BodyMedia Inc.). The monitor integrated motion sensor data with a variety of heat-related sensors to estimate the energy cost of free-living activity. Participants wore the monitor for 7 d before the LCD, except while showering or bathing.

Muscle tissue biopsy

Percutaneous muscle biopsies on the medialis vastus lateralis were performed in the morning after an overnight fast. The muscle biopsy sample was obtained from 10–15 cm above the knee under local anesthetic (bupivacaine 0.25%, 1% lidocaine) with a 6-mm Bergstrom needle with suction (24). Excess blood, visible fat, and connective tissue were removed from the muscle biopsy sample (~150 mg) before being snap frozen in liquid nitrogen and registered in the Translational Research Institute (TRI) biorepository.

Adipose tissue biopsy

An abdominal adipose tissue biopsy was performed after the muscle biopsy. The adipose biopsy sample was obtained from the mid-abdomen ~5–8 cm lateral to the umbilicus under local anesthetic (bupivacaine 0.25%, 1% lidocaine) with a 3- to 4-mm Mercedes Liposuction needle. The adipose tissue sample (~1000 mg) was washed in sterile PBS before being snap frozen in liquid nitrogen and registered in the TRI biorepository.

Tissue and blood analyses

Targeted serum metabolomics.

Serum 3-hydroxybutyric acid (3-HBA) was measured by GC/MS (ThermoFisher Trace GC Ultra/DSQ II single quadrupole mass spectrometer). Calibration standards (1–1000 μM) were prepared in water and spiked with ^{13}C 3-HBA (500 μM) as an internal standard. 3-HBA was extracted from samples using a series of extraction and derivatization steps to form the corresponding trimethylsilyl derivative. Detection of the derivatized 3-HBA was achieved by single ion monitoring after GC separation.

Detailed protocols for the LC-MS/MS assays of ACs, amino acids (AAs), and organic acids (OAs) were described previously (25). Working calibration standard mixes were prepared for ACs, AAs, and OAs. The calibrators and sample were spiked with a mixture of heavy isotope–labeled internal standards for ACs, AAs, or OAs. Measurement of derivatized ACs and AAs was achieved using multiple reaction monitoring (MRM) of calibration solutions and study samples on an Agilent 1290 HPLC/6490 triple quadrupole mass spectrometer. Measurement of OAs was performed by single reaction monitoring using a Thermo Scientific Quantiva triple quadrupole mass spectrometer (Thermo Scientific). The raw data were processed using Mass hunter quantitative analysis software (Agilent) and Xcalibur 3.0 (Thermo Scientific).

Extraction and LC/MS/MS measurement of malonyl CoA.

The working calibration standard was prepared for malonyl CoA. The calibrator and samples were spiked with heavy isotope–labeled internal standard for malonyl CoA. Malonyl CoA was then extracted using Oasis HLB 1cc (30 mg) Extraction Cartridges (Waters Corporation). The extracted samples were dried under nitrogen, reconstituted in 10 mM ammonium carbonate, pH 9.5, and separated on an Acquity UPLC BEH C18 2.1 × 50 mm, 1.7 μm column (Waters Corporation) using a 2.35-min linear gradient with 10 mM ammonium carbonate, pH 9.5 and acetonitrile. Measurement of malonyl CoA was achieved using MRM for the product ion for malonyl CoA. Calibration solutions and study samples were analyzed using an Agilent 1290 HPLC/6490 triple quadrupole mass spectrometer. The mass spectrometer was operated in positive ion mode using electrospray ionization (ESI) with an ESI capillary voltage of 3500 V. The electron multiplier voltage was set to 400 V. The ion transfer tube temperature was 325°C and vaporizer temperature was 325°C. The ESI source sheath gas flow was set at 10 L/min. The mass spectrometer was operated with a mass resolution of 0.7 Da and an N_2 collision gas pressure of 30 psi.

Muscle western blotting.

Homogenates from snap-frozen skeletal muscle tissue were prepared as previously described (26). For acetyl-CoA carboxylase (ACC) quantification, muscle homogenates were first purified for biotinylated proteins with immobilized recombinant streptavidin (Pierce™ Streptavidin Plus UltraLink™ Resin, ThermoFisher) with an overnight incubation at 4°C. Proteins were separated on Criterion 4%–15% Tris-HCl precast SDS polyacrylamide gels (BioRad) and then transferred to polyvinylidene difluoride membranes (Millipore). Membranes were blocked in 50% Odyssey Blocking Buffer (LI-COR)/50% TBS for 1 h and then incubated overnight at 4°C with primary antibodies, followed by incubation with species-specific secondary antibodies for 1 h (IRDye 800CW anti-rabbit IgG No. 926-32211 and IRDye 680RD anti-mouse IgG No. 926-68070; Li-Cor Biosciences). For ACC, membranes were incubated with α-Streptavidin 680 nm (Li-Cor, cat#926-68031) allowing ACC1 and ACC2 quantification at the 265-kDa and 280-kDa molecular weights, respectively. ACC membranes were further incubated with primary antibody α-phospo ACC (ser79; 1:1000; Cell Signaling) overnight at 4°C before 1 h secondary antibody incubation with IRDye 800CW anti-rabbit IgG (Li-Cor, No. 926-32211) allowing quantification of ACC1 and ACC2 phosphorylation. Protein bands were visualized with the Odyssey 9120 Infrared Imaging System (LI-COR, Bioscience) and quantified using ImageJ software (NIH). Protein loading was controlled by normalizing bands of interest to either α-tubulin or α-actin. Gel-to-gel variation was controlled by using standardized samples on each gel.

Adipose RT-PCR.

Total RNA was extracted from adipose tissue using the lipid RNeasy kit (Qiagen). *Total lipin, lipin 1A, lipin 1B,* peroxisome proliferator-activated receptors gamma 1 (*PPARγ1*), *peroxisome proliferator-activated receptors gamma 1* (*PPARγ2*), cluster of differentiation 36 (*CD36*), carnitine palmitoyltransferase I (*CPT-1*), medium-chain acyl-CoA dehydrogenase (*MCAD*), malonyl-CoA decarboxylase (*MLYCD*), peroxisome proliferator-activated

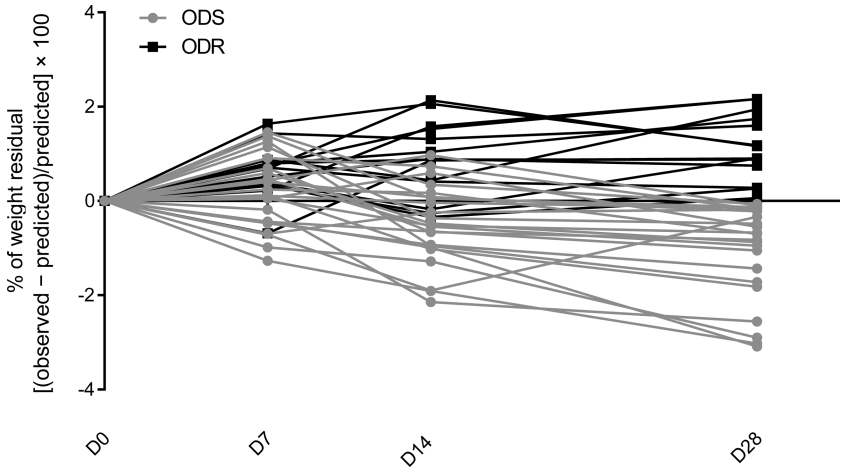

FIGURE 2 Percentage of weight residuals over the 28-d low-calorie diet period. Observed weight loss as a percentage of predicted weight loss for time points D0, D7, D14, and D28. Weight loss predictions were based on the mathematical model established by Hall et al. (19) with inputs described in the Methods. The upper weight of the predicted range was used as the target for weight loss. A positive value at D28 indicates weight loss that did not meet the high end of the predicted range and is classified as ODR ($n = 14$). A negative value at D28 indicates weight loss that did meet the high end of the predicted range and is classified as ODS ($n = 23$). D, day; ODR, overweight/obese diet resistant; ODS, overweight/obese diet sensitive.

receptor gamma coactivator 1-alpha (*PGC1-α*), peroxisome proliferator activated receptor alpha (*PPAR-α*), lipoprotein lipase (*LPL*), and ribosomal protein lateral stalk subunit (*PRPLP0*) primer-probe sets were designed and sequences are shown in **Supplemental Table 1** (LGC Biosearch Technologies). The remaining genes were predesigned primer probe sets (Life Technologies). qRT-PCR reactions were performed as previously described (27, 28) using the TaqMan Fast Virus 1-step reaction mix Standard protocol (Life Technologies) on a ViiA7 Real-Time PCR system (Applied Biosystems). Samples were normalized to either *RPLP0* or the geometric mean of hypoxanthine phosphoribosyltransferase 1 (*HPRT1*), peptidylprolyl isomerase B (*PPIB*), and glyceraldehyde-3-phosphate dehydrogenase (*GADPH*).

Statistical analysis

For metabolomic data, at BL all serum metabolites were log transformed to reduce heteroscedasticity and approximate a normal distribution. Principal component analysis (PCA) with varimax rotation was used to reduce the dimensionality of the data. Components with eigenvalues >1 were retained and individual metabolites with a component load >0.4 for a given PCA-derived factor were included. To assess whether changes in serum metabolite concentrations were associated with weight loss success, PCA following the same criteria as aforementioned was also conducted on the change in serum metabolites compared with BL at day 7, day 14, and day 28 (**Supplemental Tables 2–4**). The independent *t* test was used to compare differences in PCA component scores at BL and at each time point between ODS and ODR. Multiple linear regression was used to assess the association between PCA component scores and the weight loss residual at day 28 after adjusting for age, sex, and ethnicity. PCA analyses were performed on SPSS (IMB) statistics version 26.

A repeated-measures ANCOVA with body mass as a covariate was used to determine changes in energy expenditure and fat oxidation rates over the LCD and between groups. A 2-factor repeated-measures ANOVA was used to detect changes in body composition, muscle protein expression, adipose gene expression, and serum ACs over the LCD and between groups. Post hoc analysis was achieved with Stepdown Bonferroni adjustment. Significance was set to $P < 0.05$. Participants with missing time points were excluded from the analysis. Statistical analyses were performed on SAS version 9.4 (SAS). Data are reported as means ± SDs including the figures.

Results

Weight loss success

Of the 39 participants that completed 28 d of LCD, 16 participants did not achieve the upper target weight of the model's predicted weight range and were classified as ODR (**Figure 2**). Of these 16, 2 participants demonstrated increases in body weight throughout the LCD and were excluded from further analyses (Supplemental Figure 1). Twenty-three participants achieved the model-predicted target weight range and were classified as ODS (Figure 2). A comparison of weight, BMI, waist circumference, vital signs, and laboratory parameters for ODS and ODR participants before the initiation of the LCD was performed (**Table 1**). There were no significant differences in BL laboratory parameters measuring overall metabolic health. The ODS group did have a significantly higher BMI than the ODR group ($P < 0.05$) but there was no significant difference in body mass at BL (Table 1).

Body composition

After the 28-d LCD both groups displayed had significant reductions in body mass, BMI, FM, and FFM ($P < 0.05$) (**Table 2**) as expected. There was an interaction effect for group and time for body mass, BMI, FM, and FFM indicating that the ODS group had significantly greater reductions in each of

TABLE 1 Baseline participant characteristics[1]

	ODS	ODR
n	23	14
Age, y	37.1 ± 8.2	41.6 ± 9.6
Sex, F/M	21/2	12/2
Race/ethnicity[2]	3B, 9W-H, 11W-NH	3B, 4W-H, 7W-NH
Body mass, kg	98.3 ± 10.6	92.6 ± 10.3
BMI, kg/m^2	36.2 ± 3.1	33.8 ± 2.4*
$\dot{V}O_2$ max, mL · min^{-1} · kg^{-1}	20.3 ± 3.1	18.4 ± 4.2
Daily EE,[3] kcal	2589 ± 441	2176 ± 772
Active EE,[3] kcal	247 ± 136	204 ± 109
Daily steps	6088 ± 2249	5668 ± 1863
DBP, mm Hg	74 ± 6	73 ± 7
SBP, mm Hg	121 ± 6	123 ± 9
ALT, units/L	22 ± 15	15 ± 8
AST, units/L	19 ± 7	18 ± 4
CK total, units/L	90 ± 45	104 ± 43
CRP, mg/L	7.51 ± 6.78	4.25 ± 3.51
Glucose, mg/dL	91 ± 9	91 ± 8
Hb, g/dL	13.3 ± 1.1	13.0 ± 0.9
Cholesterol, mg/dL	182 ± 36	174 ± 33
HDL, mg/dL	48 ± 11	52 ± 11
LDL, mg/dL	112 ± 31	104 ± 29
VLDL, mg/dL	22 ± 8	18 ± 7
Triglycerides, mg/dL	112 ± 39	92 ± 33
RBCs, 10^6/mm^3	4.69 ± 0.30	4.69 ± 0.55
WBCs, 10^3/mm^3	7.01 ± 1.96	6.24 ± 1.52

[1] Values are mean ± SD. *Significant difference between groups ($P < 0.05$). ALT, alanine transaminase; AST, aspartate transaminase; B, black; CK, creatine kinase; CRP, C-reactive protein; DBP, diastolic blood pressure; EE, energy expenditure; Hb, hemoglobin; ODR, obese/overweight diet resistant; ODS, obese/overweight diet sensitive; SBP, systolic blood pressure; $\dot{V}O_2$ max, maximal oxygen uptake; WBC, white blood cell; W-H, white-Hispanic; W-NH, white-non Hispanic.

[2] Race and ethnicity were self-reported.

[3] EE values obtained from accelerometry measures.

these parameters after the LCD than the ODR group ($P < 0.05$) (Table 2).

Substrate oxidation and energy expenditure

There were no significant differences in fasting RQ between the groups throughout the LCD. Fasting RQ decreased at day 7 compared with BL ($P < 0.001$) and was suppressed throughout the rest of the treatment period (**Figure 3**C). Fasting fat oxidation rates were similar between groups at BL and day 28 (Figure 3D). Fasting fat oxidation rates increased similarly from BL (0.0701 ± 0.0206 g/min) to day 28 (0.0869 ± 0.0269 g/min) in both groups ($P < 0.001$) (Figure 3D).

The anticipated weight loss effect of a fixed energy intake was confirmed by a significant relation between BL FFM and absolute weight loss ($R^2 = 0.232$, $P = 0.03$). There was no significant difference for RMR between the groups at any time point when adjusted for body mass (day 0, 7, 14, or 28) (Figure 3A). RMR decreased at day 14 compared with BL ($P = 0.01$) and remained lower at day 28 (Figure 3A). Metabolic adaptation was calculated by the differences in the RMR residuals at day 0 and day 28, where the RMR residual is the difference between indirect calorimetry–measured RMR and predicted RMR by our

TABLE 2 Effect of low-calorie diet on body composition[1]

	ODS BL	Day 7	Day 14	Day 28	ODR BL	Day 7	Day 14	Day 28
Body mass, kg[†]	98.3 ± 10.6	95.8 ± 10.2*	94.3 ± 10.0*	92.2 ± 9.5*	92.6 ± 10.3	90.8 ± 10.5*	90.0 ± 10.2*	88.7 ± 9.9*
BMI,[†] kg/m^2	36.2 ± 3.1#	35.3 ± 3.0#*	34.7 ± 3.0*	33.9 ± 2.8*	33.8 ± 2.4	33.2 ± 2.4*	32.8 ± 2.5*	32.3 ± 2.5*
Fat mass,[†] kg	46.4 ± 6.3			42.5 ± 5.8*	43.1 ± 5.8			40.4 ± 5.6*
Fat-free mass,[†] kg	52.4 ± 6.4			50.3 ± 6.1*	49.8 ± 6.1			49.1 ± 6.1
Body fat, %	46.9 ± 3.5			45.7 ± 3.6*	46.4 ± 3.9			45.1 ± 3.6*

[1] Values are mean ± SD. ODR, $n = 14$; ODS, $n = 23$. Data were analyzed with a 2-factor repeated-measures ANOVA. BL, baseline; ODR, overweight/obese diet resistant; ODS, overweight/obese diet sensitive.

*Significant difference: time point compared with BL, within group ($P < 0.05$).

#Significant difference: ODS compared with ODR, within day ($P < 0.05$).

[†]Significant time × group interaction ($P < 0.05$).

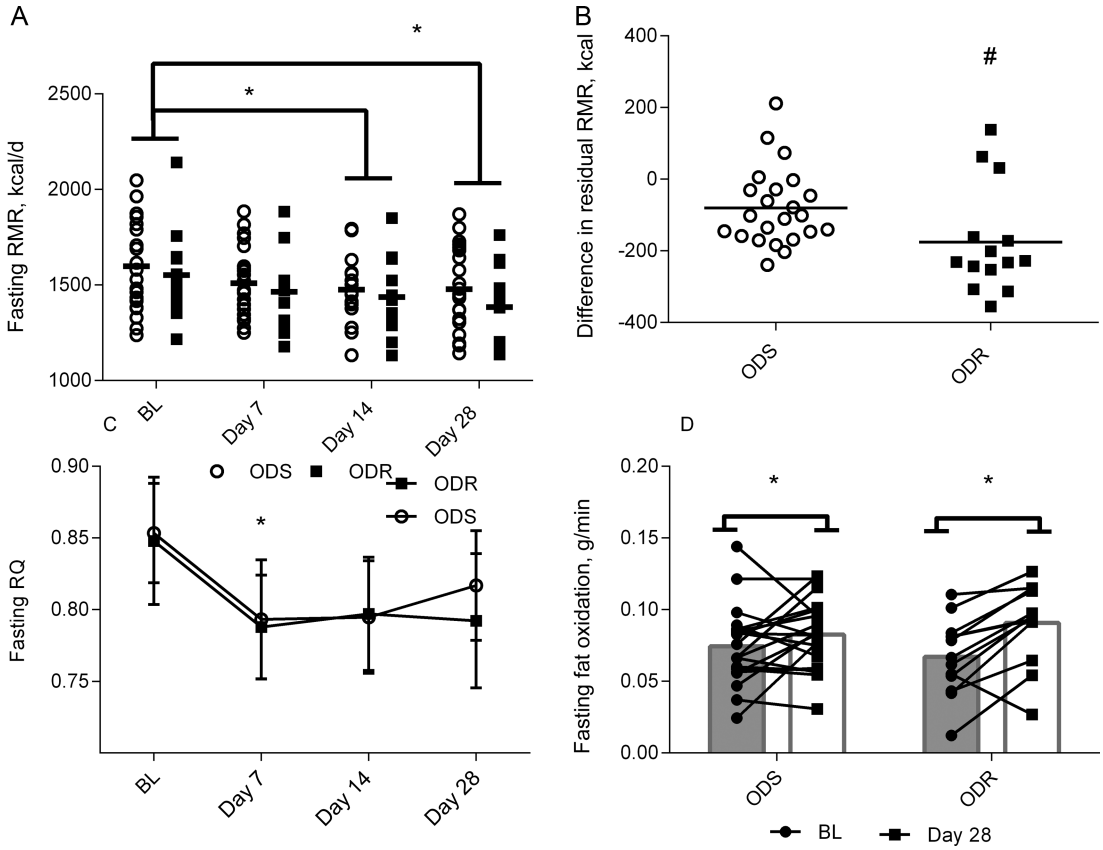

FIGURE 3 Changes in energy expenditure and overnight fasting substrate oxidation during the 28-d LCD. (A) RMR was significantly lower at days 14 and 28 than at BL for both the ODS and ODR groups. Metabolic adaptation was calculated as the difference in RMR residuals from day 28 to day 0, where the RMR residual is the predicted RMR subtracted from the measured RMR by indirect calorimetry. (B) Metabolic adaptation was greater in the ODR group than in the ODS group. (C) Fasting RQ decreased at day 7 compared with BL and remained suppressed for the remainder of the LCD in both groups (P = nonsignificant for ODS compared with ODR). (D) Fasting rates of fat oxidation increased from BL to day 28 in both groups. There were no significant group × time interactions for any of the variables. Data are mean ± SD. Data were analyzed with repeated-measures ANCOVA with body mass as a covariate. ODR, $n = 14$; ODS, $n = 23$. #Significant difference between groups ($P < 0.05$). *Significant difference compared with BL ($P < 0.05$). BL, baseline; LCD, low-calorie diet; ODR, overweight/obese diet resistant; ODS, overweight/obese diet sensitive; RMR, resting metabolic rate; RQ, respiratory quotient.

cohort-specific equation. The ODR group had greater metabolic adaptation (−175 ± 149 kcal/d; +119%) (negative difference in residual RMR) than the ODS group (−80 ± 108 kcal/d; $P = 0.030$).

PA

Daily average EE measured by accelerometry at BL was not significantly different between the ODS and ODR groups (Table 1) ($P > 0.05$). BL active EE was also similar between the ODS and ODR groups (Table 1) ($P > 0.05$). There was also no difference in the average number of steps per day between the ODS and ODR groups (Table 1) ($P > 0.05$) at BL.

Biomarkers of weight loss success

A comprehensive assessment of biomarkers reported to influence weight loss success was conducted to reveal potential mechanisms that contribute to observed differences at the whole-body level.

PCA.

We began our investigation with an analysis of the data from the target metabolite assays. PCAs were used to group highly correlated serum metabolites into fewer factors at BL and during the LCD (**Table 3**, Supplemental Tables 2–4). At BL, serum PCA produced 13 factors (Table 3). BL PCA factors 8 (α-ketoglutarate, citrate and butyryl/isobutyryl carnitine) and 11 [arginine, methylmalonyl/succinyl carnitine, glutaryl carnitine (C5-DC), and 3-hydroxy-*cis*-5-octenoyl/hexenedioyl carnitine] were significantly different between the ODS and ODR groups ($P < 0.05$) (Table 3). However, neither factor had a significant relation with the weight loss residual at day 28.

PCA for the change in serum metabolites at days 7, 14, and 28 compared with BL yielded 12 factors each (Supplemental Tables 2–4). D7-BL PCA factors 1 (various chain-length ACs) and 9 (lactate and pyruvate) were significantly different between the ODS and ODR groups (Supplemental Table 2). Only D7-BL PCA factor 1 had a significant relation with the weight loss residual at day 28 (std. $\beta = 0.379$, $P = 0.026$). PCA for the change in serum metabolites at day 14 and day 28 compared

TABLE 3 PCA for baseline serum metabolites[1]

Factor	Metabolites (component loading)	Eigenvalue	Variance, %	ODS vs. ODR (*P* value)[2]
1	C2 (0.547), C8/C5:1-DC (0.858), C8:1-OH/C6:1-DC (0.499), C6-DC (0.736), C10:2 (0.592), C10:1 (0.852), C10 (0.924), C8:1-DC (0.629), C10-OH/C8-DC (0.837), C12:1 (0.922), C12 (0.929), C12:2-OH/C10:2-DC (0.753), C14:2 (0.866), C14:1 (0.904), C14 (0.624), C16 (0.612), C18:1 (0.711), valine (−0.485)	11.923	22.929	0.965
2	Lactate (0.684), pyruvate (0.917), succinate (0.917), alanine (0.495), glycine (−0.513), serine (−0.600)	4.242	8.158	0.264
3	C3 (0.853), C5/C4=O (0.846), C5-OH/C3-DC (0.769), C4-DC/Ci4-DC (0.456)	3.820	7.347	0.825
4	C8:1 (0.848), C10:3 (0.856), C10:2 (0.613)	3.431	6.599	0.758
5	Leucine/isoleucine (0.651), methionine (0.446), phenylalanine (0.592), tyrosine (0.846), valine (0.532), C4/Ci4 (0.530)	3.186	6.128	0.916
6	Alanine (0.592), arginine (0.631), methionine (0.684), proline (0.729), serine (0.448)	2.562	4.926	0.969
7	3-HBA (0.695), C2 (0.414), C4-OH (0.830), C18:2 (0.570)	2.485	4.779	0.170
8	α-KG (0.860), citrate (0.682), C4/Ci4 (0.467)	2.268	4.361	0.010[#]
9	Citrulline (0.773), ornithine (0.842)	2.219	4.267	0.666
10	C5:1 (0.860), C18 (0.528)	2.127	4.091	0.306
11	Arginine (0.470), C4-DC/Ci4-DC (0.405), C5-DC (0.812), C8:1-OH/C6:1-DC (0.439)	2.075	3.991	0.040[#]
12	Asparagine/aspartic acid (0.775), glutamine/glutamic acid (0.600)	1.990	3.827	0.673
13	Histidine (0.800), C16 (0.406)	1.756	3.377	0.073

[1]C2, acetyl-carnitine; C3, propionyl carnitine; C4/Ci4, citrate and butyryl/isobutyryl carnitine; C4-DC/Ci4-DC, methylmalonyl/succinyl carnitine; C4-OH, 3-hydroxylbutyrlcarnitine; C5/C4=O, isovalerylcarnitine/acetoacetylcarnitine; C5-DC, glutaryl carnitine; C5:1, tiglyl carnitine; C5-OH/C3-DC, malonyl carnitine; C6-DC, adipoyl carnitine; C8/C5:1-DC, octanoyl/mesaconyl carnitine; C8:1, octenoyl carnitine; C8:1-OH/C6:1-DC, 3-hydroxy-*cis*-5-octenoyl/hexenedioyl carnitine; C8:1-DC, octenoyl carnitine; C10-OH/C8-DC, suberoyl carnitine; C10, decanoyl carnitine; C10:1, decanoyl carnitine; C10:2, decadienoyl carnitine; C10:3, decatrienoyl carnitine; C12, lauroyl carnitine; C12:1, dodecenoyl carnitine; C12:OH/C10:2-DC, sebacoyl carnitine C14, myristoyl carnitine; C14:1, tetradecenoyl carnitine; C14:2, tetradecadienoyl carnitine; C16, palmitoyl carnitine; C18:1, oleyl carnitine; C18:2, linoleyl carnitine; ODR, overweight/obese diet resistant; ODS, overweight/obese diet sensitive; PCA, principal component analysis; 3-HBA, 3-hydroxybutyric acid; α-KG, α-ketoglutarate.

[2]Independent t test was used to compare differences in PCA component scores between the ODS group ($n = 23$) and the ODR group ($n = 14$).

[#]Significant difference: ODS compared with ODR ($P < 0.05$).

with BL produced similar factors to serum D7-BL factor 1 consisting of ACs of various lengths (D14-BL PCA factor 1, D28-BL PCA factor 1). However, this was not significantly different between the groups (Supplemental Tables 3, 4) and did not have a significant relation with the weight loss residual at day 28.

D14-BL PCA factor 3 had a significant relation to the weight loss residual at day 28 (std. $\beta = -0.560$, $P = 0.008$) (**Figure 4**B). This factor was similar in composition to D7-BL PCA factor 3 comprising of 3-HBA, citrate, leucine/isoleucine, acetyl-carnitine (C2), and 3-hydroxylbutyrylcarnitine (C4-OH). This D7-BL PCA factor 3 had a trend toward a relation with the weight loss residual at day 28 but was not statistically significant (std. $\beta = -0.418$, $P = 0.059$) (Figure 4A). A similar PCA factor was found at day 28 (D28-BL PCA factor 2) which had a stronger relation to the weight loss residual at day 28 (std. $\beta = -0.674$, $P < 0.001$) (Figure 4C) and was significantly different between groups ($P = 0.008$). The metabolites that comprise D28-BL PCA factor 2 which exhibited the strongest relation to the weight loss residual at day 28 are displayed over the 28-d LCD in **Supplemental Figure 2**.

Serum ACs.

Owing to D7-BL PCA factor 1 being related to the weight loss residual at day 28 and consisting of a plethora of ACs, differences in ACs between groups were interrogated further. At BL, only C5-DC was higher in the ODR group than in the ODS group ($P = 0.037$) (**Figure 5**A). At day 7 of the LCD, the ODR group compared with the ODS group had significantly higher concentrations of the following ACs: octanoyl/

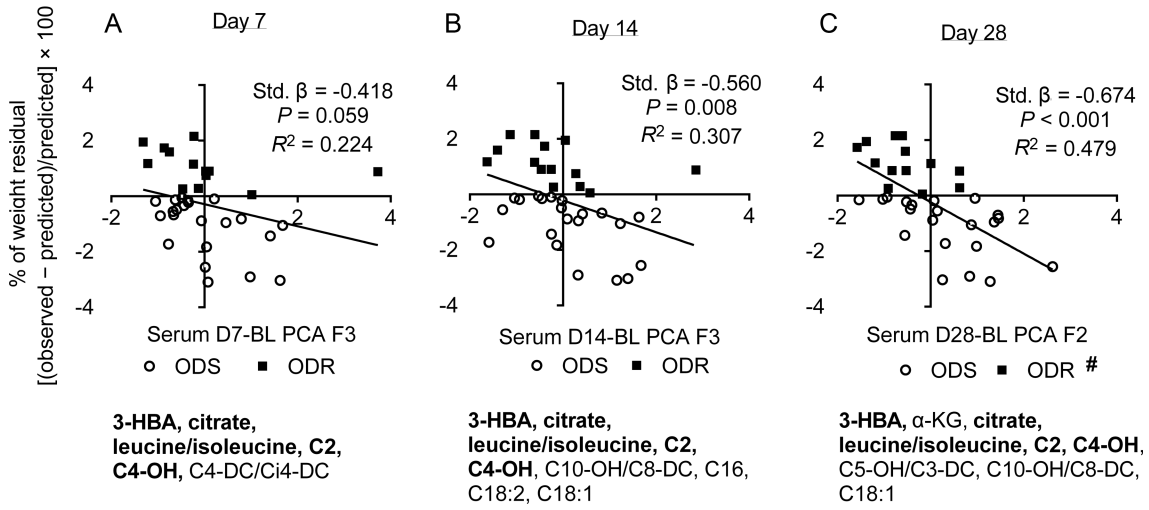

FIGURE 4 Relation between weight loss residuals at day 28 and PCA score factors from change in metabolites at day 7 (A), day 14 (B), and day 28 (C). Multiple linear regression was used to assess the association between PCA component scores and weight loss residuals at day 28 after adjusting for age, sex, and ethnicity. (A) At day 7 PCA factor 3 had a trend ($P = 0.059$) toward a relation with the weight loss residual. (B) At day 14 PCA factor 3 had a significant ($P = 0.008$) and stronger relation with the weight loss residual. (C) At day 28 PCA factor 2 had a significant ($P < 0.001$) and stronger relation with the weight loss residual. PCA factors at each time point were comprised of reoccurring metabolites (highlighted in bold). ODR, $n = 14$; ODS, $n = 23$. #Significant difference for ODS compared with ODR ($P < 0.05$). BL, baseline; C2, acetyl-carnitine; C4-DC/Ci4-DC, methylmalonyl/succinyl carnitine; C4-OH, 3-hydroxylbutyrlcarnitine; C5-OH/C3-DC, malonyl carnitine; C10-OH/C8-DC, suberoyl carnitine; C16, palmitoyl carnitine; C18, stearoyl carnitine; C18:2, linoleyl carnitine; D, day; F, factor; ODR, overweight/obese diet resistant; ODS, overweight/obese diet sensitive; PCA, principal component analysis; 3-HBA, 3-hydroxybutyric acid; α-KG, α-ketoglutarate.

mesaconyl carnitine, glutaryl (C5-DC), decanoyl (C10:1), decanoyl carnitine, dodecenoyl carnitine, lauroyl carnitine, tetradecadienoyl carnitine, tetradecenoyl carnitine, and myristoyl carnitine ($P < 0.05$) (Figure 5B). At day 14 of the LCD, only C5-DC and decadienoyl carnitine were higher in the ODR group than in the ODS group ($P < 0.05$) (**Supplemental Figure 3**A). By day 28 of the LCD, propionyl carnitine, tiglyl carnitine, C5-DC AC, and C10:1 AC were higher in the ODR group than in the ODS group ($P < 0.05$) (Supplemental Figure 3B), whereas C4-OH AC was significantly elevated in the ODS group compared with the ODR group ($P < 0.05$) (Supplemental Figure 3B).

Adipose gene expression.

Adipose tissue is a key regulator of whole-body energy balance that affects weight loss success. We hypothesized that being ODR would upregulate the lipogenic gene expression profile and downregulate lipolytic genes. To test this hypothesis, we measured a panel of genes in these pathways in adipose tissue biopsy samples in a subset of the most diverse participants based on weight loss success (ODS, $n = 6$; ODR, $n = 7$). Inconsistently with our hypothesis, at BL the ODS group compared with the ODR group had higher expression of genes relating to lipogenic transcription factors (*total lipin, lipin 1A*, and *PPARγ1*), FA uptake and β-oxidation (*MLYCD* and *ACC2*), and lipogenesis [*ACC1* and diacylglycerol O-acyltransferase 1 (*DGAT1*)] ($P < 0.05$) (**Figure 6**A). At day 14 of the LCD the ODS group had significantly higher expression of genes related to FA uptake and β-oxidation (*CD36, CPT1, MLYCD, ACC2*, and *PPARα*) and lipogenesis [fatty acid synthase (*FAS*) and *DGAT1*] ($P < 0.05$) (Figure 6B).

Muscle malonyl-CoA and expression of muscle metabolic proteins.

ACC is a key enzyme with several important roles in fat metabolism and overall body energy metabolism. ACC catalyzes the carboxylation of acetyl-CoA to malonyl-CoA and exists in 2 isoforms: ACC1 (located primarily in liver and adipose tissue) and ACC2 (located primarily in muscle and heart but also reported to be present in adipose tissue). ACC2 regulates FA oxidation via its product, malonyl-CoA, which inhibits the mitochondrial FA transporter carnitine palmitolytransferase. We hypothesized that ODR participants would display an inability to accelerate fat oxidation, as evidenced by elevated malonyl-CoA concentrations. To test this hypothesis, we measured malonyl-CoA in skeletal muscle. Muscle malonyl-CoA content was not significantly different at BL between the ODS and ODR groups ($P = 0.546$) and did not change with the LCD ($P = 0.424$) (**Supplemental Figure 4**A). We also quantified MCAD and very long-chain acyl-CoA dehydrogenase (VLCAD) responsible for the first step of medium- and long-chain β-oxidation, respectively. At BL the ODS group had significantly higher protein expression of MCAD and VLCAD ($P < 0.05$) (Supplemental Figure 4B). At day 14 of the LCD there was no significant difference in protein expression of the metabolic proteins measured ($P > 0.05$) (Supplemental Figure 4C).

Discussion

The primary aim of the current study was to determine whether weight loss failure after a 28-d LCD in overweight/obese individuals was associated with impaired fat oxidation. Secondly, we aimed to characterize the metabolic adaptation and molecular

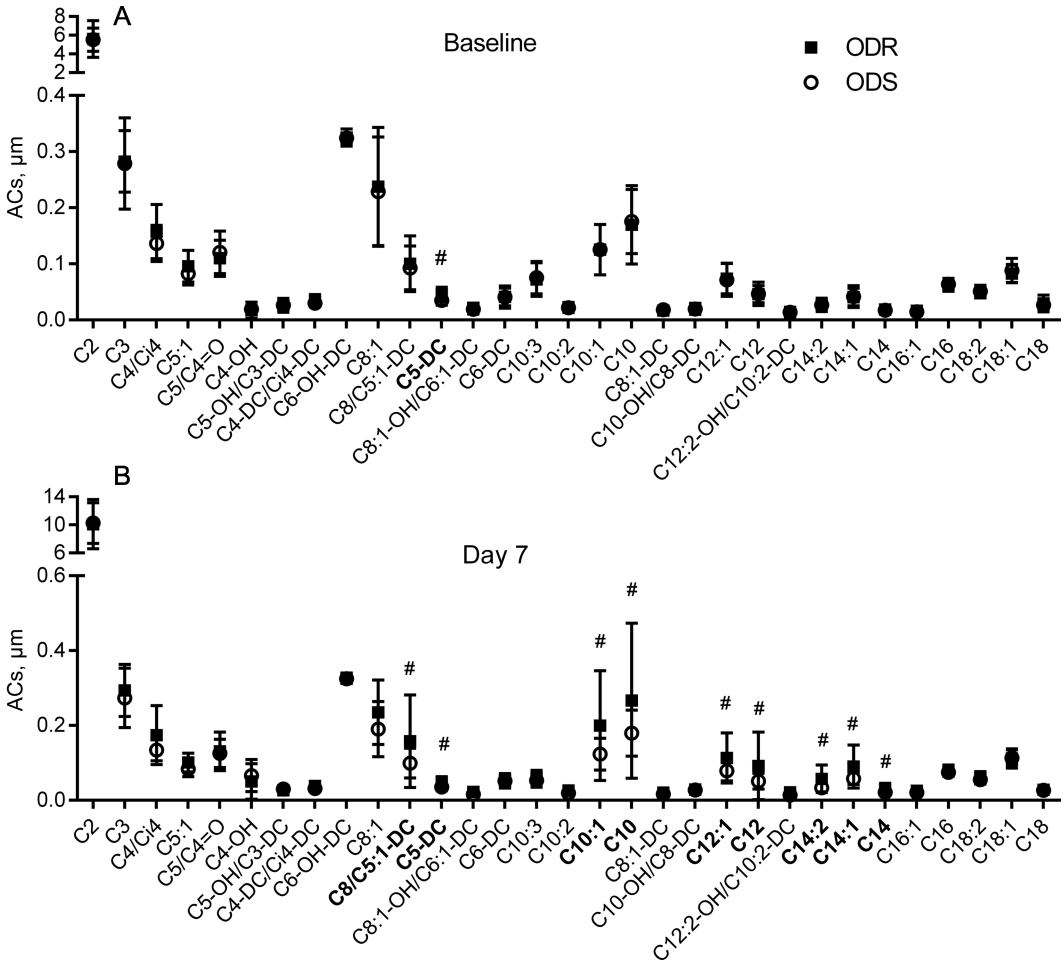

FIGURE 5 Medium- and long-chain ACs were increased at day 7 of the LCD in the ODR group only. Serum ACs quantified with LC/MS/MS (A) at baseline and (B) at day 7 of the LCD. At baseline the only difference was that C5-DC was higher in the ODR group than in the ODS group. After 7 d of the LCD, ODR groups had higher concentrations of C8/C5:1-DC, C5-DC, C10:1, C12:1, C14:2, and C14:1. Data are mean ± SD. Data were analyzed with a 2-factor repeated-measures ANOVA. ODR, $n = 14$; ODS, $n = 23$. #Significant difference between groups, $P < 0.05$. AC, acylcarnitine; C2, acetyl-carnitine; C3, propionyl carnitine; C4/Ci4, citrate and butyryl/isobutyryl carnitine; C5:1, tiglyl carnitine; C5/C4=O, isovalerylcarnitine/acetoacetylcarnitine; C4-OH, 3-hydroxylbutyrlcarnitine; C5-OH/C3-DC, malonyl carnitine; C4-DC/Ci4-DC, methylmalonyl/succinyl carnitine; C6-OH-DC, hydroxyhexanoyl-dicarboxylic-carnitine; C8:1, octenoyl carnitine; C8/C5:1-DC, octanoyl/mesaconyl carnitine; C5-DC, glutaryl carnitine; C8:1-OH/C6:1-DC, octenoyl carnitine3-hydroxy-cis-5-octenoyl/hexenedioyl carnitine; C6-DC, adipoyl carnitine; C10:3, decatrienoyl carnitine; C10:2, decadienoyl carnitine; C10:1, decenoyl carnitine; C10, decanoyl carnitine; C8:1-DC, octenoyl carnitine; C10-OH/C8-DC, suberoyl carnitine; C12:1, dodecenoyl carnitine; C12, lauroyl carnitine; C12:OH/C10:2-DC, sebacoyl carnitine; C14:2, tetradecadienoyl carnitine; C14:1, tetradecenoyl carnitine; C14, myristoyl carnitine; C16:1, exadecenoyl carnitine C16, palmitoyl carnitine; C18:2, linoleyl carnitine; C18:1, oleyl carnitine; C18, stearoyl carnitine; LCD, low-calorie diet; ODR, overweight/obese diet sensitive; ODS, overweight/obese diet sensitive.

mechanisms associated with weight loss success/failure. Participants were stratified based on whether they met their weight loss predicted targets (ODS) or did not (ODR). ODS participants lost more weight yet were characterized by a lower metabolic adaptation with comparable fat oxidation rates as compared with ODR participants.

Metabolic adaptation refers to reduced RMR compared with what is anticipated based on body composition (8, 9). ODR participants had a greater metabolic adaptation than ODS participants. In other words, the ODR group had greater reductions in actual RMR compared with what was expected based on FFM, age, and race. This difference existed despite both groups having comparable RMR throughout the LCD. Metabolic adaptation after long-term weight loss has been established (29–34). In the present study we have shown that metabolic adaptation exists over a short duration (28 d) in agreement with previous research (35) and more notably characterizes individuals who have a blunted response to weight loss. Although metabolic adaptation is believed to hinder weight loss, others have postulated that metabolic adaptation is beneficial in reducing the rate of aging (36). Maintaining a higher mass-adjusted metabolic rate is associated with disease risk (37), likely through increased reactive oxygen species (ROS) production (33). Caloric restriction can induce mitochondrial biogenesis (29, 38) which is proposed to reduce oxidative stress owing to diminished ROS production (39). Although the absence of metabolic adaptation during an LCD characterizes weight loss success, to be physiologically beneficial it may require a concomitant increase in mitochondrial mass to alleviate ROS production.

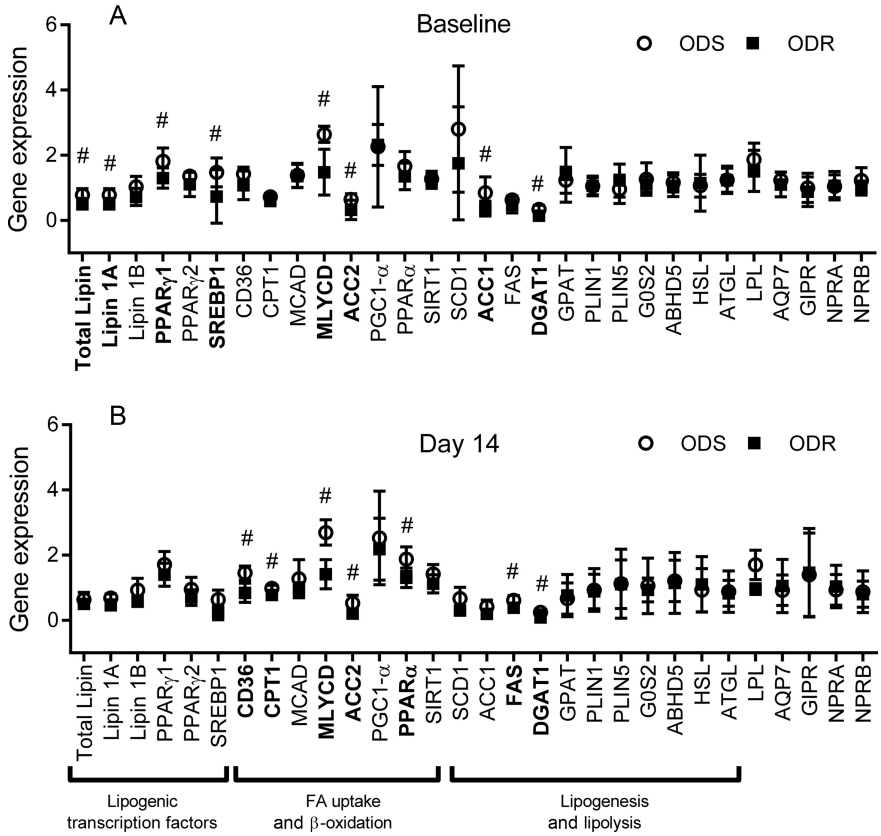

FIGURE 6 Comparison of the expression of genes in adipose tissue (qRT-PCR) at baseline and at day 14 of the low-calorie diet for the ODS and ODR groups. At baseline the ODS group had higher expression of genes relating to lipogenic transcription factors (*total lipin, lipin 1A, PPARγ1*, and *SREBP1*), FA uptake and *β*-oxidation (*MLYD* and *ACC1*), and lipogenesis (*ACC2* and *DGAT*). At day 14 ODS participants had significantly higher expression of *CD36, CPT1, MLYCD, PPARα, FAS*, and *DGAT1*. Data are mean ± SD. Data were analyzed with a 2-factor repeated-measures ANOVA. ODR, $n = 7$; ODS, $n = 6$. #Significant difference between groups ($P < 0.05$). *ABDH5,* 1-acylglycerol-3-phosphate O-acyltransferase; *ACC1,* acetyl-CoA carboxylase 1; *ACC2*, acetyl-CoA carboxylase 2; *AQP7;* aquaporin-7, *CD36,* cluster of differentiation 36*; CPT1,* carnitine palmitoyltransferase I; *DGAT1,* diacylglycerol O-acyltransferase *1;* FA, fatty acid; *FAS,* fatty-acid synthase; *GIPR,* gastric inhibitory polypeptide receptor; *G0S2,* G0/G1 switch 2; *GPAT1,* glycerol-3-phosphate acyltransferase 1; *HSL,* hormone sensitive lipase; *LPL*, lipoprotein lipase; *MCAD*, medium-chain acyl-CoA dehydrogenase; *MLYCD,* malonyl-CoA decarboxylase; *NRPA*, natriuretic peptide receptor-A; *NPRB,* natriuretic peptide receptor-B*;* ODR, overweight/obese diet resistant; ODS, overweight/obese diet sensitive; *PGC1-α,* peroxisome proliferator-activated receptor gamma coactivator 1-alpha; *PLIN1,* perilipin-1; *PPARγ1,* peroxisome proliferator-activated receptor gamma-1*; PPARγ2,* peroxisome proliferator-activated receptor gamma-2; *SCD1,* stearoyl-CoA desaturase; *SIRT1,* sirtuin 1; *SREBP1*, sterol regulatory element-binding protein 1.

We performed PCA analysis of serum metabolites at different time points throughout the LCD. PCA factors comprised of overlapping metabolites (3-HBA, citrate, leucine/isoleucine, C2, and C4-OH) reoccurred at each time point. Importantly, this PCA component score showed correlation to weight loss success defined as percentage of weight loss residual at day 28. The majority of these metabolites (3-HBA, citrate, C2, and C4-OH) are associated with pathways to remove excess acetyl-CoA from the cell and/or mitochondria (40–42). Excess acetyl-CoA in the mitochondria can impair insulin action in addition to acetylating and deactivating proteins involved in the FA oxidation pathway (43, 44). Examination of individual metabolites comprising these PCA factors revealed that the main differences between the ODR and ODS groups existed for 3-HBA and C4-OH, which were also consistently the highest factor loads in each of the PCA factors with C2. Ketogenesis in the liver forms an additional outlet of excess acetyl-CoA by transformation into ketone bodies such as 3-HBA (45). C4-OH is the carnitine-modified form of 3-HBA (46). Elevated circulating 3-HBA has been identified as an acute marker of long-term favorable glucose and lipid responses after metabolic surgery (17) and is elevated after caloric restriction (45, 46). The catabolism of leucine produces both acetoacetate (a ketone body) and acetyl-CoA, indicating the inclusion of this metabolite in this PCA factor may be due to its role in ketogenesis during the LCD. Together, this group of metabolites may indicate that the ODS group was fueling the TCA cycle for oxidation to the extent that overflow pathways, such as ketogenesis, were upregulated to prevent toxic acetyl-CoA accumulation in cells.

Excess acetyl-CoA inhibits *β*-oxidation resulting in accumulation of long-chain acyl-CoAs within the mitochondria (14). It is assumed the increased serum AC concentrations reflect accumulation of their respective acyl-CoA precursors in the cell/mitochondria due to dysregulation of transport into the mitochondria or reduced *β*-oxidation (15, 47). At day 7 the ODR group had significantly higher medium- and long-chain ACs than the ODS group, indicating impaired complete fat oxidation. A major site of FA oxidation is muscle. The only

difference observed in our measures of muscle FA oxidative capacity was BL MCAD and VLCAD protein content was higher in the ODS than in the ODR group. There was, however, upregulation of genes regulating FA uptake and β-oxidation (PPARα, ACC2, MLYCD, CPT1, and CD36) in adipose tissue at day 14 in the ODS compared with the ODR group. Upregulation of FA oxidation genes in adipose tissue has been reported in individuals who lost weight in comparison with individuals who regained weight after dietary intervention (48). Mitochondrial biogenesis in adipose tissue is also compromised in individuals with obesity and type 2 diabetes (49, 50), suggesting that adipose FA oxidation may be important in regulating adipose tissue loss/gain. Despite elevated serum ACs and downregulated adipose FA oxidation genes existing in the ODR group, whole-body complete fat oxidation rates measured with indirect calorimetry were similar between groups throughout the LCD.

Habitual PA levels were similar between groups at BL. A limitation of the current study is we did not measure PA during the intervention and cannot exclude that PA influenced weight loss success. The benefits of PA-induced EE for weight loss alone are negligible (51) unless substantial amounts of PA (200–300 min/wk) are performed (52, 53), which was not observed during BL measurements in this cohort. Reduced body weight influences EE during PA owing to altered skeletal muscle work efficiency (54). Whether the ODR group had reduced EE during PA, when the time spent performing PA was kept constant, warrants further investigation. If the ODR group participated in less PA or had reduced EE during PA it may be pertinent that these individuals require more volitional exercise during LCD to maintain an elevated total daily EE.

Although we did not observe differences in our primary outcome of fat oxidation between the 2 groups, a retrospective power analysis revealed that we were underpowered to be able to detect these changes. A further limitation of the current study is that whole-body fat oxidation rates were only measured for 30 min during fasting conditions. A decreased ability to switch from low to high fat oxidation may predispose weight gain or impair weight loss (55). Future studies performing a 24-h measure of fat oxidation with whole-room calorimetry and 24-h urinary nitrogen collection with a larger sample size are warranted to reveal differences in fat oxidation throughout the day including postprandial measures. The NIDDK model does not consider whether weight loss is achieved from reductions in FM or FFM. Whereas it is well established that FM loss is beneficial for metabolic health, reductions in FFM can be detrimental and even result in body weight regain (56). It should be noted that although the ODS group lost significantly more FM they also had reductions in FFM. Future studies should characterize whole-body and tissue-specific measurements of interventions that specifically target FM loss while preserving FFM. We also acknowledge participants may have been nonadherent to the calorie restriction. This possibility contrasts against the greater metabolic adaptation of ODR than of ODS participants. In other words, if they were nonadherent their metabolic adaptation should have been less. These novel findings may be limited to females only because they represent the majority of the cohort. Furthermore, we cannot speculate about the influence of the menstrual cycle on these results because this was not tracked during the study.

In conclusion, individuals who achieved weight loss predicted targets after an acute 28-d LCD were characterized by reduced metabolic adaptation and accumulation of metabolites associated with acetyl-CoA excess and enhanced ketogenesis. Future research should be conducted to monitor the potency of these biomarkers over a weight loss period extending into weight loss maintenance combined with metabolic flux measurements to determine lipolysis and lipogenesis across 24 h. Strict environmental oversight in a metabolic ward should be implemented to eliminate nonadherence to the caloric restriction.

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The authors' responsibilities were as follows—SAP, FY, HX, and SRS: were responsible for the conception and design of the study; SAP, AP, CPB, KPJ, JSS, and SRS: were responsible for conducting the clinical trial and collection of data; KLW, KDC, SAP, AP, JSS, FY, HX, CJP, SJG, and SRS: contributed to the analysis and interpretation of the data; KLW, KDC, and SRS: wrote the manuscript; and all authors: contributed to the manuscript and read and approved the final manuscript. The authors report no conflicts of interest.

Data Availability

Data described in the manuscript will be made available upon request pending application and approval.

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