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Habitual Physical Activity and Plasma Metabolomic Patterns Distinguish Individuals with Low vs. High Weight Loss during Controlled Energy Restriction^{1–4}

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Abstract

Background: Total weight loss induced by energy restriction is highly variable even under tightly controlled conditions. Identifying weight-loss discriminants would provide a valuable weight management tool and insights into body weight regulation.

Objective: This study characterized responsiveness to energy restriction in adults from variables including the plasma metabolome, endocrine and inflammatory markers, clinical indices, body composition, diet, and physical activity.

Methods: Data were derived from a controlled feeding trial investigating the effect of 3–4 dairy product servings in an energy-restricted diet (2092 kJ/d reduction) over 12 wk. Partial least squares regression was used to identify weight-loss discriminants in 67 overweight and obese adults. Linear mixed models were developed to identify discriminant variable differences in high- vs. low-weight-loss responders.

Results: Both pre- and postintervention variables ($n = 127$) were identified as weight-loss discriminants (root mean squared error of prediction = 1.85 kg; $Q^2 = 0.43$). Compared with low-responders (LR), high-responders (HR) had greater decreases in body weight (LR: 2.7 ± 1.6 kg; HR: 9.4 ± 1.8 kg, $P < 0.01$), BMI (in kg/m^2 ; LR: 1.0 ± 0.6 ; HR: 3.3 ± 0.5 , $P < 0.01$), and total fat (LR: 2.2 ± 1.1 kg; HR: 8.0 ± 2.1 kg, $P < 0.01$). Significant group effects unaffected by the intervention were determined for the respiratory exchange ratio (LR: 0.86 ± 0.05 ; HR: 0.82 ± 0.03 , $P < 0.01$), moderate physical activity (LR: 127 ± 52 min; HR: 167 ± 68 min, $P = 0.02$), sedentary activity (LR: 1090 ± 99 min; HR: 1017 ± 110 min, $P = 0.02$), and plasma stearate [LR: $102,000 \pm 21,000$ quantifier ion peak height (QIPH); HR: $116,000 \pm 24,000$ QIPH, $P = 0.01$].

Conclusions: Overweight and obese individuals highly responsive to energy restriction had accelerated reductions in adiposity, likely supported in part by higher lipid mobilization and combustion. A novel observation was that person-to-person differences in habitual physical activity and magnitude of weight loss were accompanied by unique blood metabolite signatures. This trial was registered at clinicaltrials.gov as NCT00858312. *J Nutr* 2015;145:681–90.

Keywords: weight loss, metabolomics, obesity, physical activity, statistical modeling, body composition, branched-chain amino acids, respiratory exchange ratio, calorie restriction

Introduction

Weight loss is the physical manifestation of a multifaceted adaptation to negative energy balance, requiring a concert of

biological and behavioral factors. Indeed, there are substantial differences in individual responses to energy restriction even in tightly controlled interventions. Although this variability is generally associated with genetic variation (1–4) or dietary

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³ Reference to a company or product name does not imply approval or recommendation of the product to the exclusion of equivalent materials. The USDA is an equal opportunity provider and employer.

⁴ Supplemental Figure 1 and Supplemental Tables 1–4 are available from the “Online Supporting Material” link in the online posting of the article and from the same link in the online table of contents at <http://jn.nutrition.org>.

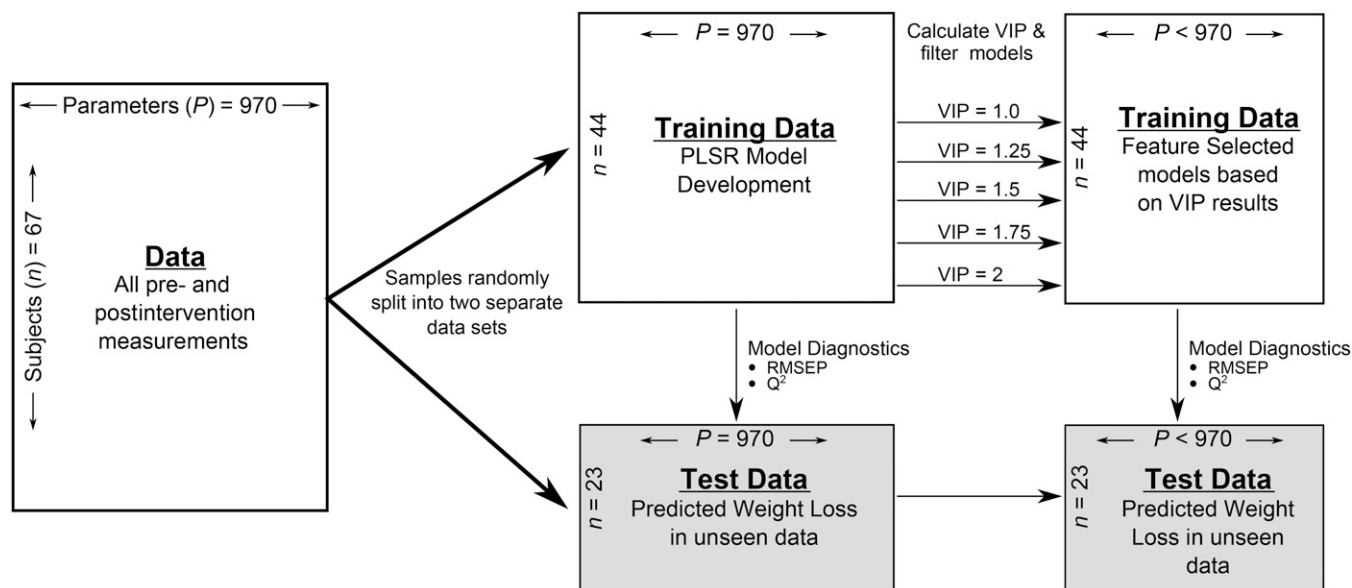


FIGURE 1 Schematic of multivariate statistical modeling approach of weight loss in overweight and obese subjects consuming an energy deficit of 2092 kJ/d (500 kcal/d) for 12 wk. PLSR, partial least squares regression; RMSEP, root mean square error of prediction; VIP, variable importance in projection.

compliance (5–7), results have been inconsistent, likely reflecting diversity among studied populations, dietary treatments, and outcome measurements (8). Clearly, differences in metabolic and behavioral factors driving person-to-person variability in weight loss remain to be fully elaborated.

Many behavioral and biological factors have been assessed individually to examine their effect on energy balance and cellular metabolism. For example, physical activity increases energy expenditure and weight loss when combined with energy restriction (9, 10). Resting metabolic rate and resting respiratory exchange ratio (RER)⁸ are directly related to energy balance and are associated with weight loss and gain (11–13). Other studies have identified cellular (2, 14) and endocrine (15–17) markers that affect energy metabolism. However, few studies have integrated these factors in a unifying model to describe weight-loss variability.

Based on these and other findings, weight loss is likely the result of behavior (volitional or habitual physical activity), as well as person-to-person differences in thermogenesis and mitochondrial fuel partitioning. To better understand factors underlying this complexity, and to unmask connections between these factors, this study combined metabolomic, clinical, body composition, endocrine, inflammatory, dietary, and physical activity data to define a weight-loss “responder” phenotype in overweight and obese adults consuming an energy-restricted, controlled diet. Using multivariate statistical modeling, we identified determinants of weight loss that provide insight into potential mechanisms that could drive individual responsiveness to energy restriction.

Methods

Subjects

Overweight and obese [BMI (in kg/m²): 28–37] men and women were recruited ($n = 71$) from the Davis and Sacramento, California, communities. Main inclusion criteria included age (women: 20–45 y;

men: 20–50 y), habitually low dairy consumption (≤ 1 serving of dairy/d), and typical calcium intake ≤ 600 mg/d. Detailed selection criteria and consort diagrams are published elsewhere (18). The availability of paired pre- and postintervention plasma aliquots limited the sample size to 67 subjects for this analysis.

Study design

This study constitutes a secondary analysis of a randomized control trial originally designed to investigate the effect of 3–4 servings of dairy products on weight loss during energy restriction. In-depth details regarding the study design and results pertaining to the primary intervention have been previously reported (18). Participants were studied for a total of 15 wk with all foods provided. Weeks 1–3 of the study were considered a run-in period and were designed to establish each participant’s total energy requirements. Energy intake to maintain body weight was initially estimated from gender-specific prediction equations for overweight individuals adjusted for self-reported physical activity (19, 20). Participants were then provided weighed foods based on these estimates. Food consumption was observed during this period by study personnel. Body weight was monitored daily during the run-in period, and energy intake was adjusted based on increases or decreases in total weight of $\pm 3\%$ for 3 consecutive days. An individual was considered in energy balance when body weight was stable for ≥ 5 consecutive days. The following 12 wk (study weeks 4–15) were designed as the intervention period. During the intervention period, final energy intake requirements established from the 3-wk run-in period were reduced by 2092 kJ/d (500 kcal/d) and subjects were randomly assigned to either an adequate dairy (3–4 servings of dairy/d) or a low dairy (≤ 1 serving of dairy/d) group. One dairy serving was equal to a 240 mL serving of milk or yogurt, a 56 g serving of processed cheese, or a 42 g serving of natural cheese. Preintervention data were collected near the end of the run-in period (study week 3) and postintervention data were collected near the end of the intervention period (study week 15), except for physical activity. Physical activity measurements were conducted during study weeks 4 and 14 because of study-related commitments during weeks 3 and 15. All procedures were approved by the Institutional Review Board for the Protection of Human Subjects at the University of California, Davis. All subjects were informed of the study requirements and provided written informed consent before participation.

Dietary intake and compliance

All run-in and intervention diets contained comparable amounts of macronutrients and fiber (fat, carbohydrates, and protein at $\sim 35\%$, 49% , and 16% of total energy, respectively, and fiber at 8–10 g/4186 kJ).

⁸ Abbreviations used: AC, activity count; FDR, false discovery rate; PLSR, partial least squares regression; RER, respiratory exchange ratio; RMSEP, root mean square error of prediction; VCO₂, carbon dioxide production; VIP, variable importance in projection; VO₂, oxygen consumption.

Participants consumed 2 of the 3 meals/d at the USDA Agricultural Research Service Western Human Nutrition Research Center throughout the run-in period and during weeks 1 and 2 of the intervention period. Between intervention weeks 3 and 9, meals were “packaged to go” and consumed off-site. During these weeks, all food was weighed and measured before distribution and compliance was monitored by returned food containers and food diary records twice weekly. Subjects agreed not to use any dietary supplements during the course of the study. Each individual’s caffeine intake was assessed at baseline and kept constant throughout intervention. A priori compliance determinations included adherence to diet treatment (consumption of low dairy or adequate dairy servings), energy intake within 837 kJ of prescription, and consumption by the adequate dairy group of 95% of the 295 total dairy servings during the intervention. Only individuals who met all compliance criteria in a given week were counted as compliant for the week. Total study compliance was defined as meeting compliance for ≥ 10 intervention weeks and all participants achieved this criterion.

Body composition measurements

Body weight was measured to the nearest 0.1 kg in light clothing (shoes and jewelry removed). Height was measured with the use of a wall-mounted stadiometer and recorded to the nearest 0.1 cm. BMI was calculated as kilograms per meter squared. Waist circumference was measured with a metal nonstretchable tape measure to the nearest 0.1 cm in the standing position against bare skin with the abdomen relaxed and arms at sides. Total and regional fat measurements were assessed with the use of DXA (GE Lunar, Prodigy model). Daily calibration procedures were performed according to manufacturer instructions. To reduce

variance in the data, DXA scans were analyzed by a single operator. Intra-abdominal adipose tissue was measured with the use of computed tomography transabdominal slices (Siemens, Somatom 16 CT Scanner) as previously described (18).

Assessment of physical activity

Participants were instructed to maintain their typical physical activity throughout the study. Physical activity was monitored with the use of an omnidirectional accelerometer (Actical; Philips Electronics). This sensor integrates motion amplitude and frequency, producing an electrical current that increases with intensity of motion that is stored as activity counts (ACs). Sedentary, light, moderate, and vigorous activity are defined as <100 ACs, 100–1535 ACs, 1535–3962 ACs, and >3962 ACs, respectively (21). Participants were instructed to wear the Actical attached to a belt and worn over the left hip, except during bathing or swimming, for 7 d. At the end of the week, participants returned the Actical to the Western Human Nutrition Research Center, where the data were downloaded and analyzed. A day was considered complete if the Actical contained at least 12 h of data. Data are reported as physical activity over a 24 h period averaged over all days worn. Physical activity data were analyzed for sedentary, light, moderate, and vigorous activity and expressed as energy expenditure for each level of activity and time spent at each activity level.

Resting metabolic rate

Subject resting metabolic rate was measured in the morning after a 12 h fast. Before the measurement, subjects were made comfortable in a hospital bed and instructed to rest quietly in a reclined position for 15 min. Oxygen consumption (VO_2) and carbon dioxide production (VCO_2) were measured in reclining subjects with the use of an automated metabolic measuring cart (TrueMax 2400 Metabolic Measurement System, ParvoMedics). The RER ($VCO_2:VO_2$) was determined from the resting steady-state VCO_2 and VO_2 values, whereas energy expenditure was

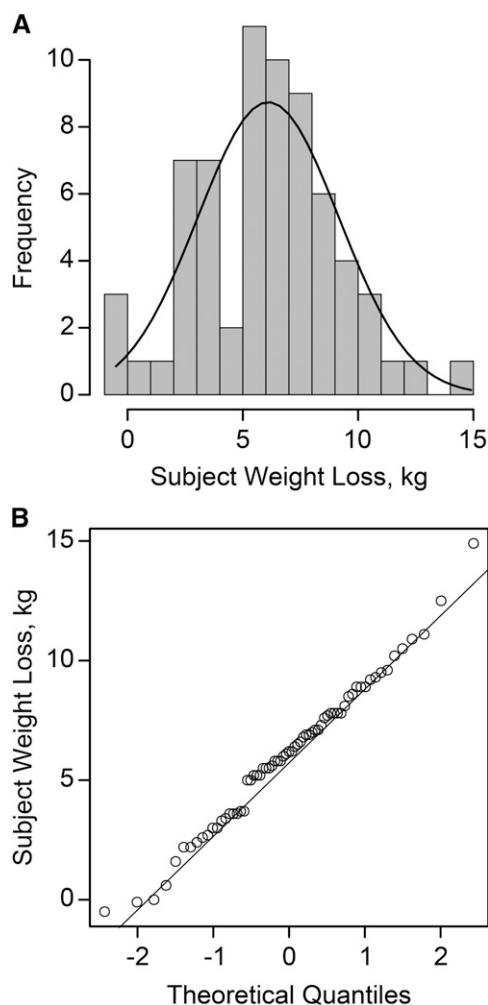


FIGURE 2 Distribution histogram (A) and Q-Q plot (B) of total weight loss in overweight and obese subjects ($n = 67$) after consuming an energy deficit of 2092 kJ/d (500 kcal/d) for 12 wk. Q, quantile.

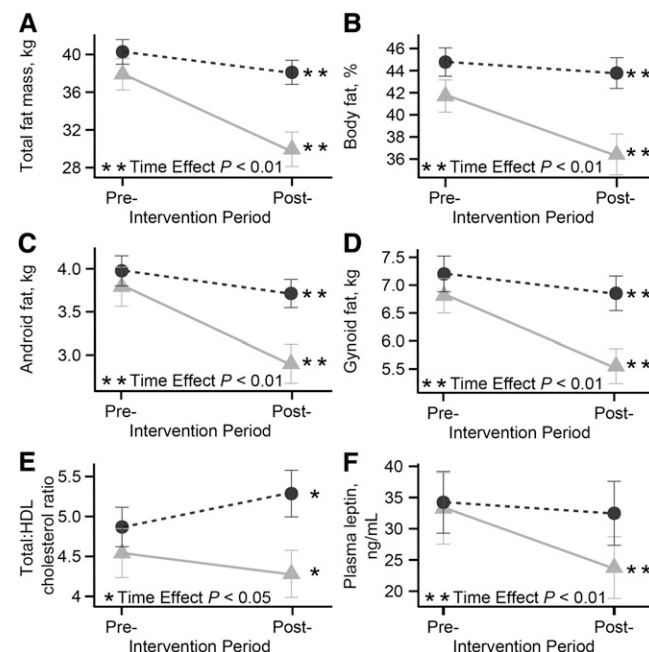


FIGURE 3 Pre- and postintervention changes in selected clinical, adiposity, and endocrine indices including total fat mass (A), body fat (B), android fat (C), gynoid fat (D), total:HDL cholesterol ratio (E), and plasma leptin (F) in high- and low-weight-loss responders. Variables presented in the figure had a significant interaction term in linear mixed model analyses. High-responder (circles) and low-responder (triangles) represent high- and low-weight-loss tertiles, respectively. Serum cholesterol, HDL cholesterol, and leptin were measured after an overnight fast. Values are means \pm SEMs, $n = 22$ for both groups. Post-, postintervention; Pre-, preintervention.

calculated with the use of the Weir equation (22) and converted to kilojoules per day as previously described (18).

Blood collection and analytical analyses

Blood was collected by venipuncture after an overnight fast, and serum and plasma were stored at -80°C until analyzed. Clinical assessment of serum total cholesterol, HDL cholesterol, LDL cholesterol, glucose, hematocrit, and hemoglobin were performed at the University of California, Davis, Medical Center. Serum insulin, leptin, IL-1 β , IL-6, IL-8, and TNF- α were measured with multiplex technology (Millipore; Bio-Plex, Bio-Rad). Serum C-reactive protein was measured with a chemiluminescence analyzer and high-sensitivity C-reactive protein kit (Immulite, Siemens Diagnostics). Serum 25-hydroxycholecalciferol and 1,25-dihydroxycholecalciferol were measured by RIA (DiaSorin and Immunodiagnosics Systems, respectively) as previously described (23). As reported previously (24), the following variables were measured by commercial ELISAs: bone-specific alkaline phosphatase (Metra BAP, Quidel); pyridinoline (Metra PYD, Quidel); osteocalcin (Metra Osteocalcin, Quidel); C-terminal telopeptide of type I collagen (CrossLaps, Nordic Bioscience Diagnostics); plasma plasminogen activator inhibitor-1 (Technozym Actibind PAI-1, Technoclone). Heparin plasma zinc was assessed on a Varian Vista inductively coupled plasma atomic emission spectrophotometer.

Plasma metabolite analysis

Plasma samples were extracted and derivatized by silylation/methyloximation before analysis by GC/time-of-flight MS for untargeted metabolomics (25). The resulting data were processed with the use of the BinBase database (26) to match metabolic feature retention indices and mass spectral data against the Fiehn mass spectral library of 1200 authentic metabolite spectra and the 2005 National Institute of Standards and Technology/Environmental Protection Agency/National Institutes of Health Mass Spectral Library. Metabolites were reported if present within at least 50% of each study design group (27). Metabolite quantifier ion peak heights were normalized to the sum intensities of all known metabolites and used for statistical analyses.

Statistics

All statistical analyses were conducted in R version 3.0.1 (28). Data are presented as means \pm SDs in text. α was set at 0.05 for all analyses.

Pre- and postintervention comparisons. Mann-Whitney *U* tests were used to compare pre- and postintervention differences in all measurements. This nonparametric test was conducted on untransformed data because of the prevalence of data that were unable to achieve a normal distribution after log transformations. Based on Anderson-Darling tests (29) at $\alpha = 0.05$, 80% of the data were not normally distributed. False

TABLE 1 Clinical, serum endocrine, and energy balance characteristics of overweight and obese subjects responsive or nonresponsive to energy restriction before and after a 12 wk weight-loss intervention¹

Variable	High-responder		Low-responder		Linear mixed model analysis ²		
	Preintervention	Postintervention	Preintervention	Postintervention	Responder Group	Week	Interaction
Clinical variables							
Total body weight, kg	91.8 \pm 12	82.5 \pm 12	91.4 \pm 11	88.7 \pm 11	0.90	<0.01*	<0.01*
BMI, kg/m ²	32.5 \pm 2.9	29.2 \pm 3.0	32.4 \pm 2.5	31.4 \pm 2.3	0.89	<0.01*	<0.01*
Total cholesterol, mg/dL	157 \pm 27	155 \pm 29	162 \pm 33	164 \pm 40	0.67	0.55	0.40
HDL cholesterol, mg/dL	37.7 \pm 13	38.6 \pm 10	34.8 \pm 9.9	32.6 \pm 10	0.37	0.64	0.25 ³
LDL cholesterol, mg/dL	101 \pm 24	97.4 \pm 22	108 \pm 23	112 \pm 32	0.42	0.27	0.12 ³
Total:HDL cholesterol ratio	4.50 \pm 1.4	4.30 \pm 1.4	4.90 \pm 1.2	5.30 \pm 1.4	0.42	0.08	<0.01*
TGs, mg/dL	91.4 \pm 39	93.0 \pm 60	96.1 \pm 44	100 \pm 40	0.74	0.82	0.81
Glucose, mg/dL	83.5 \pm 7.0	84.7 \pm 7.1	82.2 \pm 8.4	85.7 \pm 9.2	0.60	0.40	0.28
Total fat mass, kg	37.9 \pm 7.8	29.9 \pm 8.6	40.3 \pm 6.1	38.1 \pm 6.0	0.28	<0.01*	<0.01 ^{3*}
Body fat, %	41.7 \pm 6.8	36.4 \pm 8.7	44.8 \pm 6.0	43.8 \pm 6.5	0.16	<0.01*	<0.01 ^{3*}
Android fat, kg	3.80 \pm 1.1	2.90 \pm 1.1	4.00 \pm 0.80	3.70 \pm 0.80	0.52	<0.01*	<0.01 ^{3*}
Gynoid fat, kg	6.80 \pm 1.4	5.50 \pm 1.5	7.20 \pm 1.5	6.80 \pm 1.5	0.38	<0.01*	<0.01 ^{3*}
Total lean mass, kg	49.9 \pm 9.3	49.0 \pm 9.7	47.1 \pm 9.4	46.5 \pm 9.7	0.33	0.01	0.54
Endocrine variables							
Insulin, pmol/L	38.7 \pm 1.0	32.1 \pm 18	46.4 \pm 26	46.1 \pm 22	0.26	0.06	0.20
HOMA-IR	2.60 \pm 1.4	2.20 \pm 1.3	3.00 \pm 1.6	3.20 \pm 1.6	0.32	0.10	0.11
Leptin, $\mu\text{g/L}$	33.2 \pm 26	23.8 \pm 0.20	34.2 \pm 23	32.5 \pm 24	0.89	<0.01*	0.01 ^{3*}
25-hydroxycholecalciferol, nmol/L	36.6 \pm 19	42.5 \pm 18	32.3 \pm 13	36.5 \pm 10	0.36	0.01	0.56 ³
1,25-dihydroxycholecalciferol, pmol/L	126 \pm 22	118 \pm 32	120 \pm 27	111 \pm 26	0.51	0.19	0.88 ³
Energy balance variables							
Energy intake, kJ/d	10,700 \pm 1900	8620 \pm 1900	11,100 \pm 1700	9070 \pm 1700	0.36	<0.01*	0.04
Energy restriction, %	20.1 \pm 3.5	24.3 \pm 5.8	19.1 \pm 2.7	23.0 \pm 4.6	0.45	<0.01*	0.76
Total PA-EE, kJ/d	3650 \pm 1400 ^a	3260 \pm 1400 ^{a,b}	2910 \pm 100 ^{a,b}	2610 \pm 910 ^b	0.05	0.02	0.60
Average PA-EE, kJ/min	2.60 \pm 1.2 ^a	2.30 \pm 1.0 ^{a,b}	2.10 \pm 0.70 ^{a,b}	1.80 \pm 0.7 ^b	0.04	0.02	0.59
Total activity, ACs/d	22,000 \pm 15,000	20,100 \pm 14,000	17,500 \pm 8300	13,700 \pm 6000	0.19	0.29	0.51
Average activity, ACs/min	160 \pm 110	144 \pm 99	124.5 \pm 58	97.0 \pm 43	0.16	0.25	0.56
RER	0.81 \pm 0.03 ^b	0.82 \pm 0.03 ^b	0.86 \pm 0.06 ^a	0.86 \pm 0.05 ^a	<0.01*	0.79	0.94 ³
RMR-EE, kJ/d	6780 \pm 1100	7450 \pm 1300	7280 \pm 1500	7470 \pm 1100	0.37	0.01*	0.24

¹ Values are means \pm SDs, $n = 22$ per group. High-responder and low-responder represent high- and low-weight-loss tertiles, respectively. Pairwise comparisons of means were conducted on variables with a significant responder group fixed effect. Serum glucose and all serum endocrine measurements were conducted after an overnight fast. Means without a common letter differ with the use of Tukey's honestly significant difference tests ($P < 0.05$). AC, activity count; PA-EE, physical activity energy expenditure; RER, respiratory exchange ratio; RMR-EE, resting metabolic rate energy expenditure; VIP, variable importance in projection.

² Subject identification was input as a random effect. Statistical significance was set at $P \leq 0.05$. *Statistically significant ($P_{\text{adjusted}} \leq 0.05$) after false discovery rate correction (38).

³ Featured in partial least squares regression modeling weight loss at a VIP cutoff of 1.5.

discovery rates (FDR) for Mann-Whitney *U* tests were assessed with the use of Storey's *q*-value (30). A *q*-value of 0.2 (i.e., 20% false discovery at $\alpha = 0.05$) was selected as the FDR control level.

Partial least squares regression. Before analysis, pre- and post-intervention data were separated, missing data points were imputed with a singular value decomposition-based method (31, 32), and the resulting data were condensed into a single data frame. Missing data accounted for 0.22% of all data and were never >3.7% for a single variable. Variables that did not have a normal distribution were log transformed.

Principal component analyses were used to identify potential confounders, including measurement time (pre- and postintervention), treatment (low and adequate dairy), gender (male and female), and season of intervention (winter, spring, summer, and fall). Gender-specific clusters were identified in the nonmetabolomics data (e.g., body composition, serum endocrine and inflammation markers, and physical activity) (Supplemental Figure 1A). Therefore, nonmetabolomics variables were gender-adjusted with the use of multiple linear regressions, and subsequent principal component analyses of residuals verified adjustment success (Supplemental Figure 1B). Gender-adjusted residuals were used in further multivariate analyses. The metabolomics data did not require adjustment.

Because of the high dimensionality and collinear nature of the data, partial least squares regression (PLSR) was used to identify variables associated with participant change in weight. We used a development/validation scheme to evaluate PLSR models and selected discriminant variables using a filter approach with the development dataset. A diagram of the model development and validation is presented in Figure 1. In short, data were stratified by week, and one-third of row-wise samples were randomly removed as an independent external validation dataset to assess model performance. The orthogonal scores algorithm was implemented with the use of the R "pls" package (33). Model performance and quality assessment was determined with the use of root mean square error of prediction (RMSEP) and the Q^2 statistic. Development data were scaled and centered to unit variance before model development. Validation data were scaled and centered with development scales and centered values.

Because our objective was to evaluate the simultaneous interactions and influences of multiple physiologic systems on weight loss, all variables were included in an initial PLSR model with weight change as the dependent variable. Variable importance in projection (VIP) scores, a weighted measure of the contribution of each variable according to the variance explained in the dependent variable, were assessed for all variables (34, 35). Subsequent PLSR models were fitted based on selected VIP cutoffs and assessed for model performance. Final model selection minimized complexity (i.e., low number of explanatory variable), while maintaining model performance.

Analysis of high- and low-weight-loss responders. Total weight loss was assessed as pre-intervention minus postintervention weight loss and was assessed for normal distribution. ANOVA was used to determine if weight loss differed by treatment, season, or gender. Total weight loss was visually assessed for normality with a frequency distribution plot and a Q-Q plot and also tested via the Anderson-Darling test. Study data were then stratified based on weight-loss tertiles with "high-responders" (i.e., individuals with the greatest weight loss) defined as those with weight loss ≥ 7.2 kg and "low-responders" (i.e., individuals with the least weight loss) defined as those with weight loss <5.2 kg. Individuals who lost between 5.2 and 7.2 kg were considered "responders." Weight-loss cutoffs were determined to allow equal populations among tertiles ($n = 22, 23,$ and 22 for high-responder, responder, and low-responder tertiles, respectively). Responders were removed from further analyses to highlight the differences between high- and low-responders.

Linear mixed model analysis was used to assess differences in pre- and postintervention time points and high- and low-responder classes with subject identification as a random effect (36). Post hoc analysis of fixed effects used pairwise contrast analysis (37) adjusted by FDR correction (38). Significant interactions were further assessed for pre- and postintervention differences within high- and low-responder classes by linear mixed model analysis.

Variables selected in the final PLSR model and determined to have either a significant group fixed effect or interaction were evaluated with the use of Spearman correlations and hierarchical cluster analysis.

Miscellaneous. Other R packages essential for manuscript preparation, graphics, and additional analyses in no particular order of importance include reshape2 (39), plyr (40), ggplot2 (41), ggthemes (42), ReporteRs (43), multcompView (44), and Hmisc (45).

Results

Participant and variable characteristics. The majority of participants were women (71.6%) aged 19–49 y (32.9 ± 9.2 y). A total of 940 pre- and postintervention variables were assessed and all changes from pre- to postintervention are provided in Supplemental Table 1.

Differences in clinical, endocrine, and energy balance variables. Total weight loss ranged from -0.5 to 14.9 kg and was normally distributed (Figure 2). No differences in weight

TABLE 2 Physical activity characteristics of overweight and obese subjects responsive or nonresponsive to energy restriction before and after a 12 wk weight-loss intervention¹

Variable	High-responder		Low-responder		Linear mixed model analysis ²		
	Preintervention	Postintervention	Preintervention	Postintervention	Responder group	Week	Interaction
Time sedentary, min/d	1000 \pm 120 ^b	1030 \pm 110 ^{a,b}	1080 \pm 99 ^a	1100 \pm 100 ^a	0.02	0.10	0.46
Time sedentary, %	71.8 \pm 8.7	73.2 \pm 7.6	77.0 \pm 6.4	77.1 \pm 6.4	0.02	0.21	0.39
Sedentary activity, ACs/d	3660 \pm 820	3680 \pm 930	3840 \pm 810	400 \pm 960	0.47	0.85	0.65
Time light, %	15.8 \pm 4.5	15.4 \pm 3.6	13.6 \pm 4.0	14.1 \pm 3.8	0.08	0.65	0.38
Time light, min/d	219 \pm 63	215 \pm 49	189 \pm 56	199 \pm 52	0.09	0.78	0.38
Light activity, ACs/d	19,700 \pm 7000	19,200 \pm 5400	16,600 \pm 6200	17,800 \pm 6000	0.12	0.76	0.32
Light energy expenditure activity, kJ/d	933 \pm 28	858 \pm 205	816 \pm 290	828 \pm 250	0.13	0.06	0.14
Time moderate, %	12.2 \pm 5.1 ^a	11.9 \pm 4.8 ^{a,b}	9.3 \pm 3.8 ^{a,b}	8.8 \pm 3.8 ^b	0.03	0.49	0.98
Time moderate, min/d	169 \pm 71 ^a	166 \pm 67 ^{a,b}	130 \pm 52 ^{a,b}	123 \pm 53 ^b	0.04	0.59	0.95
Average moderate activity, ACs/d	954 \pm 320	895 \pm 270	1050 \pm 320	900 \pm 180	0.26	0.28	0.25
Active days, n/wk	6.9 \pm 0.9	6.7 \pm 0.8	6.8 \pm 0.5	6.2 \pm 1	0.72	0.58	0.19

¹ Values are means \pm SDs, $n = 22$ per group. Physical activity indices featured in partial least squares regression modeling weight loss at a VIP cutoff of 1.5. Pairwise comparisons of means were conducted on variables with a significant responder group fixed effect. Means without a common letter differ with the use of Tukey's honestly significant difference tests. AC, activity count; VIP, variable importance in projection.

² Subject identification was input as a random effect. Statistical significance was set at $P \leq 0.05$. No physical activity indices were found to be significant at $\alpha = 0.05$ after false discovery rate correction (38).

loss were detected by treatment, season, gender, or initial BMI classification.

High- and low-responders did not differ by plasma insulin, glucose, TGs, or total cholesterol (Table 1). Compared with low-responders, high-responders had greater decreases over time in total body weight, BMI, total and percentage body fat, gynoid and android fat, and serum leptin concentrations (Table 1 and Figure 3). The total-to-HDL cholesterol ratio decreased in high-responders but increased in low-responders (Table 1 and Figure 3).

High-responders had a significantly lower RER at both pre- and postintervention than did low-responders, but had no differences in energy intake, percentage restriction of energy intake, or resting energy expenditure (Table 1). Total physical activity energy expenditure was greater in high-responders over the entire study than in low-responders. Both groups had an ~10% decline in total physical activity after the intervention.

Identification of weight-loss discriminants. Important discriminants of weight loss were ranked based on VIP scores calculated from the initial PLSR model (Supplemental Table 2). Subsequent PLSR models were fitted based on VIP cutoffs and

assessed for model performance (Supplemental Table 3). PLSR models fitted with VIP cutoffs of 1 and 1.25 resulted in models with the lowest RMSEP of weight loss in participants in the validation set (1.62 kg and 1.63 kg for VIP score = 1 and VIP score = 1.25, respectively). However, these models still retained 33% and 23% of all variables measured, respectively. Restricting the VIP cutoff to 1.5 resulted in a PLSR model with a slightly higher RMSEP of 1.81 kg, but retained only 13% of measured variables; this was thus the model with the lowest complexity with performance statistics comparable to the inclusive PLSR model (Supplemental Table 3). This model was chosen as the optimal model.

The optimal PLSR model discriminated total weight loss with 127 variables. Many of the clinical, endocrine, and energy balance variables described above were featured in this model (e.g., total body weight, BMI, adiposity measurements, serum leptin, total-to-HDL cholesterol ratio, and RER). HDL and LDL cholesterol, 25-hydroxycholecalciferol and 1,25-dihydroxycholecalciferol were also included. Conversely, whereas total physical activity energy expenditure differed between high- and low-responders, they were not featured in the PLSR model. Physical activity

TABLE 3 Plasma metabolite characteristics of overweight and obese subjects responsive or nonresponsive to energy restriction before and after a 12 wk weight-loss intervention¹

Variable	High-responder		Low-responder		Linear mixed model analysis ²		
	Preintervention	Postintervention	Preintervention	Postintervention	Responder loss group	Week	Interaction
Stearic acid, QIPH	121,000 ± 22,000 ^a	111,000 ± 25,000 ^{a,b}	102,000 ± 23,000 ^b	101,000 ± 20,000 ^b	0.01	0.09	0.28
Oleic acid, QIPH	10,700 ± 5200 ^a	9080 ± 3700 ^{a,b}	7970 ± 3000 ^b	7390 ± 3300 ^b	0.02	0.14	0.50
2-ketoisocaproic acid, QIPH	1960 ± 1100 ^{b,c}	1880 ± 990 ^e	3450 ± 2600 ^{a,b}	3940 ± 2700 ^a	0.02	0.87	0.41
Myo-inositol, QIPH	13,400 ± 2700 ^a	13,300 ± 3200 ^a	11,700 ± 2400 ^{a,b}	10,600 ± 1900 ^b	0.03	0.86	0.30
Palmitic acid, QIPH	26,900 ± 5500 ^a	24,700 ± 5500 ^{a,b}	23,100 ± 6000 ^b	22,300 ± 6400 ^b	0.04	0.16	0.51
Citric acid, QIPH	39,800 ± 8900	46,700 ± 13,000	35,400 ± 9800	33,200 ± 8400	0.17	0.01	0.01
Dihydro-3-coumaric acid, QIPH	259 ± 110	267 ± 100	304 ± 98	229 ± 88	0.15	0.78	0.02
Dodecanol, QIPH	234 ± 50	266 ± 59	272 ± 73	251 ± 71	0.05	0.06	0.03
Arachidic acid, QIPH	3440 ± 790	3160 ± 1500	3500 ± 760	4380 ± 1600	0.87	0.46	0.03
Succinic acid, QIPH	767 ± 170	844 ± 200	805 ± 170	725 ± 190	0.50	0.16	0.04
Threonine acid, QIPH	8710 ± 2700	9990 ± 3300	9350 ± 3600	8320 ± 2700	0.50	0.12	0.05
Glycerol- α -phosphate, QIPH	402 ± 120	419 ± 100	350 ± 65	374 ± 77	0.07	0.53	0.86
Conduritol- β -epoxide, QIPH	1960 ± 1100	1780 ± 1100	1470 ± 700	1260 ± 590	0.07	0.44	0.94
4-deoxythreonic acid/ 4-deoxyerythronic acid, QIPH	1650 ± 600	1830 ± 1000	1260 ± 510	1420 ± 700	0.09	0.26	0.92
Isoheptadecanoic acid, QIPH	2310 ± 500	2100 ± 440	2060 ± 540	1910 ± 480	0.10	0.10	0.73
1-monopalmitin, QIPH	169 ± 33	185 ± 44	188 ± 47	210 ± 52	0.14	0.22	0.78
Cholesterol, QIPH	402,000 ± 220,000	351,000 ± 180,000	308,000 ± 230,000	303,000 ± 240,000	0.16	0.07	0.25
3-hydroxybutanoic acid, QIPH	12,400 ± 7200	18,700 ± 17,000	10,800 ± 9500	11,500 ± 8200	0.64	0.03	0.17
Uric acid, QIPH	37,100 ± 15,000	33,300 ± 14,000	31,400 ± 12,000	31,300 ± 15,000	0.19	0.11	0.27
Proline, QIPH	91,700 ± 37,000	85,900 ± 39,000	111,000 ± 77,000	114,000 ± 41,000	0.22	0.70	0.66
N-acetylmannosamine, QIPH	320 ± 96	336 ± 86	351 ± 68	349 ± 70	0.22	0.52	0.62
Methionine sulfoxide, QIPH	5870 ± 2600	6440 ± 2600	4900 ± 3100	4180 ± 2400	0.24	0.46	0.24
Urea, QIPH	158,000 ± 77,000	200,000 ± 100,000	198,000 ± 120,000	206,000 ± 150,000	0.27	0.16	0.42
Phosphoric acid, QIPH	91,500 ± 17,000	90,700 ± 23,000	86,200 ± 25,000	94,700 ± 31,000	0.48	0.90	0.33
Behenic acid, QIPH	468 ± 190	471 ± 170	588 ± 240	673 ± 770	0.35	0.98	0.64
3-methoxytyrosine, QIPH	226 ± 49	247 ± 46	217 ± 53	215 ± 80	0.62	0.22	0.35
5- β -cholestanol, QIPH	180 ± 92	188 ± 71	213 ± 200	176 ± 130	0.41	0.81	0.36
5-methoxytryptamine, QIPH	466 ± 200	553 ± 500	580 ± 680	565 ± 340	0.42	0.54	0.61
β -alanine, QIPH	944 ± 360	833 ± 310	927 ± 410	893 ± 240	0.87	0.24	0.57
Taurine, QIPH	3980 ± 1800	4110 ± 1800	4020 ± 3400	3810 ± 2000	0.96	0.84	0.72

¹ Values are means ± SDs, $n = 22$ per group. Annotated metabolites featured in partial least squares regression modeling weight loss at a VIP cutoff of 1.5. Pairwise comparisons of means were conducted on variables with a significant responder group fixed effect. All plasma metabolite measurements were conducted after an overnight fast. Means without a common letter differ with the use of Tukey's honestly significant difference tests. QIPH, quantifier ion peak height; VIP, variable importance in projection.

² Subject identification was input as a random effect. Statistical significance was set at $P \leq 0.05$. No metabolites were found to be significant at $\alpha = 0.05$ after false discovery rate correction (38).

variables selected in the PLSR model are provided in Table 2. Based on linear mixed model analyses, only time and percentage time in sedentary and moderate activity were significantly different between high- and low-responders, with high-responders engaging in less sedentary and greater moderate activity (Table 2).

Several metabolites were found to be differentially affected in high- and low-responder groups and retained in the discriminant model. Annotated metabolites featured in the PLSR model are provided in Table 3, whereas nonannotated metabolites are provided in Supplemental Table 4. Plasma stearic (18:0), oleic (18:1n-9), and palmitic (16:0) acids were significantly greater in high- than in low-responders, likely indicative of the enhanced adiposity loss and lower RER. Additionally, the product of leucine catabolism, 2-ketoisocaproic acid, was lower, whereas myo-inositol was greater in high- than in low-responders. Threonic acid, succinic acid, citric acid, dodecanol, and dihydro-3-coumaric acid either increased or were unchanged in high-responders, whereas they generally decreased in low-responders (Figure 4). In contrast, arachidic acid (20:0) was unchanged in high-responders and increased in low-responders.

Variables featured in the final model that were significantly different between high- and low-responders were combined into a Spearman correlation matrix to assess factor inter-relations (Figure 5). Body fat indices, physical activity variables, tricarboxylic acids, and many nonannotated metabolites were highly correlated. In high- but not low-responders, oleic and palmitic acids were positively correlated to percentage body fat, whereas palmitic acid was also correlated to total fat and leptin in high-responders. Conversely, oleic and palmitic acids were positively

correlated with succinic and citric acids in low- but not high-responders. The total-to-HDL cholesterol ratio was negatively correlated to percentage body fat and gynoid fat in low-responders, but not in high-responders and, although not apparent in low-responders, the ratio was positively correlated to sedentary activity and negatively correlated to moderate activity in high-responders.

Discussion

An integrative approach allowed us to identify multiple physiologic and behavioral factors associated with higher weight loss in overweight and obese subjects consuming a 2092 kJ/d (500 kcal/d) energy deficit over 12 wk. We observed distinct differences in factors associated with lipid distribution and oxidation in subjects losing the greatest amount of weight as compared with those who lost the least. As expected, subjects with the greatest weight loss had accelerated losses in regional and total adiposity. The observation that high-responders have a lower RER and elevated serum FAs is consistent with other reports of increased FA oxidation in individuals more responsive to dietary energy restriction (2, 14, 46). The difference in RER is particularly interesting because the preintervention RER was measured during the weight maintenance phase of the study, and thus before energy restriction-associated changes in RER.

Individual differences in habitual activity may be a key factor in determining an individual's response to weight-loss intervention. We suspect that engagement in moderate activity as opposed to sedentary activity was the driving force behind the high-responders' apparent ability to better mobilize and utilize their lipid reserves compared with low-responders. It is well known that physical activity leads to weight loss with energy restriction (47–49) and is a key treatment option for metabolic syndrome and insulin resistance (50, 51). Our results suggest that physical activity results in a beneficial metabolic phenotype, possibly manifested through improved skeletal muscle lipid oxidation (52).

By leveraging metabolomics, we observed several plasma metabolites that were significantly different between high- and low-responders, indicating that this phenotype has an impact on metabolism beyond lipids. For example, high-responders had lower 2-ketoisocaproic acid abundance than did low-responders. This metabolite is a direct product of leucine catabolism and may suggest higher mitochondrial flux of this amino acid through the rate-limiting branched chain ketoacid dehydrogenase complex in branched-chain amino acid-consuming tissues. Intriguingly, higher plasma concentrations of leucine, other branched-chain amino acids, and branched-chain ketoacids have been correlated with insulin resistance in humans (25, 53, 54) and reduced activity of the branched chain ketoacid dehydrogenase complex associated with the insulin-resistant state may be the mechanism behind these observations (55).

Tricarboxylic acids succinate and citrate were disparately regulated in high- and low-responders and negatively correlated to oleic and palmitic acid in low-responders. Increases in citrate and succinate oxidation have been noted in skeletal muscle biopsies from sedentary obese adults after a 16 wk moderate-exercise intervention (56); thus, these observations may suggest alterations in tricarboxylic acid cycle dynamics related to FA oxidation. Still, it is difficult to conclusively relate plasma changes of carboxylic acids to changes in tissue mitochondria, and a targeted analysis of muscle biopsies would be required to confirm this interpretation.

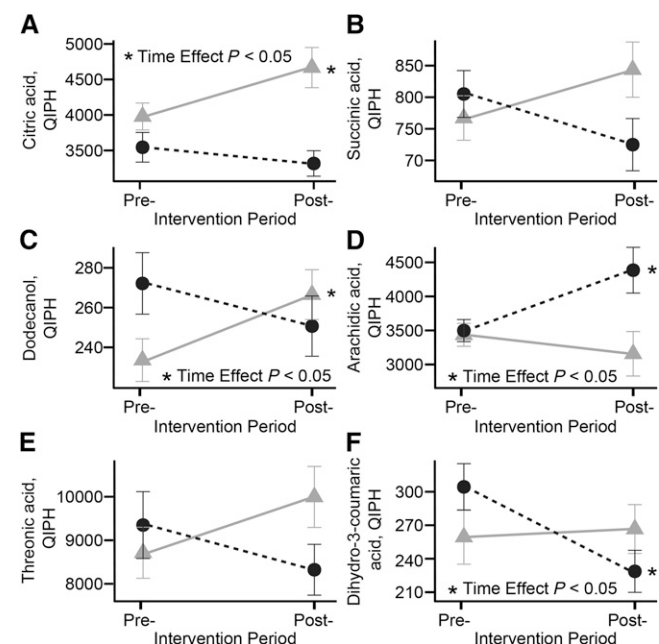


FIGURE 4 Pre- and postintervention changes in selected plasma metabolites, including citric acid (A), succinic acid (B), dodecanol (C), arachidic acid (D), threonic acid (E), and dihydro-3-coumaric acid (F) in high- and low-weight-loss responders. Metabolites presented in the figure had a significant interaction term in linear mixed model analyses. High-responder (circles) and low-responder (triangles) represent high- and low-weight-loss tertiles, respectively. Plasma metabolites were measured after an overnight fast. Values are means \pm SEMs, $n = 22$ for both groups. Post-, postintervention; Pre-, preintervention; QIPH, quantifier ion peak height.

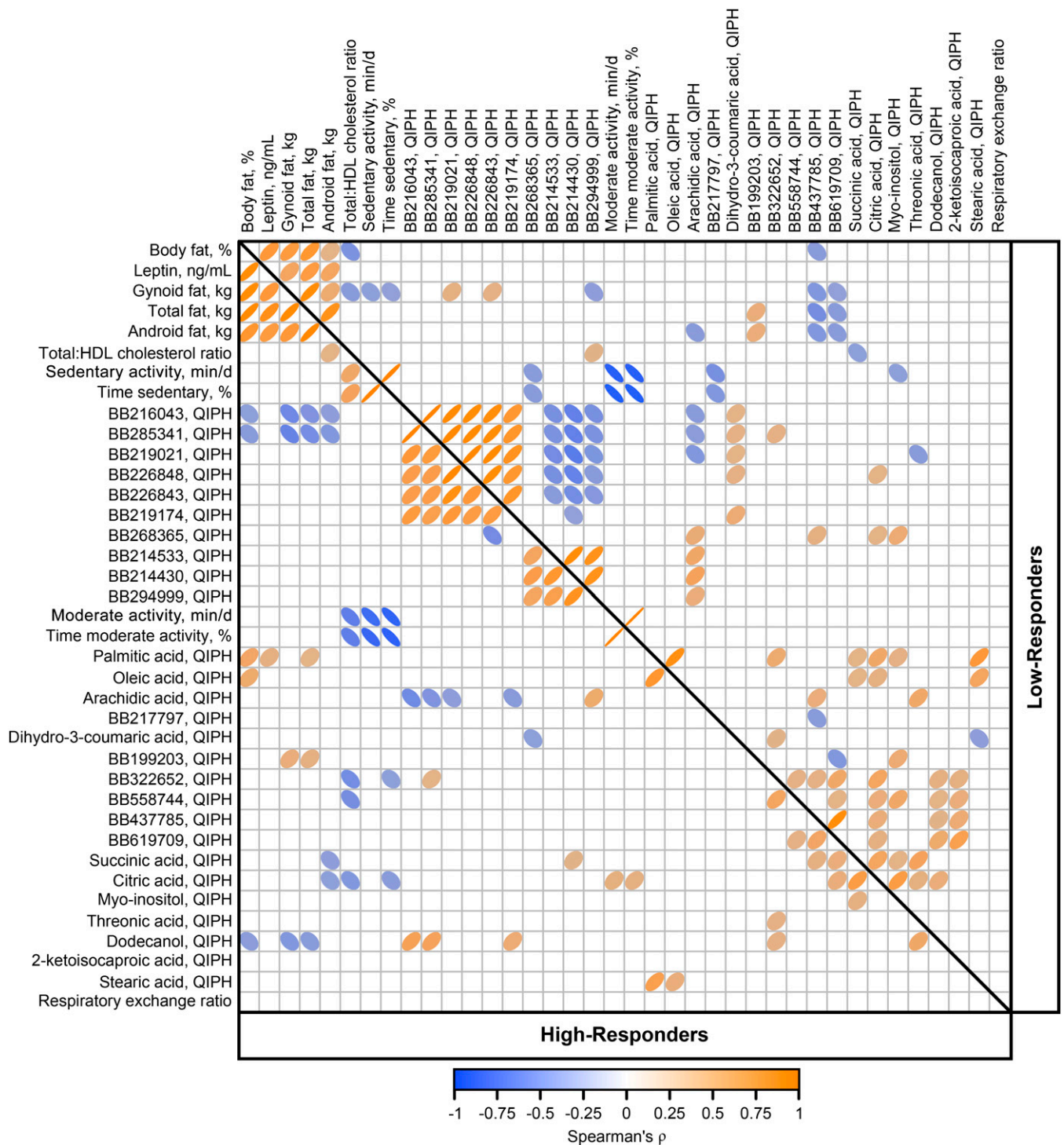


FIGURE 5 Spearman correlation matrix of variables associated with weight-loss responsiveness in overweight and obese men and women ($n = 67$) consuming an energy deficit of 2092 kJ/d (500 kcal/d) for 12 wk. Variables were selected by partial least squares regression as discriminant of weight loss and found to be significantly different between high- and low-responders in linear mixed model analyses. High-responder and low-responder represent high- and low-weight-loss tertiles ($n = 22$ for both groups), respectively. Direction or color of ellipses represent positive or negative correlation, respectively. Darker color and thinner ellipses represent strength (Spearman's ρ) of correlations. Only significant correlations at $\alpha = 0.05$ are displayed. BB, BinBase identification numbers; QIPH, quantifier ion peak height.

Although metabolomics allowed us to link biological determinants of weight loss to behavioral and clinical outcomes, it is also accompanied by additional study limitations. The statistical analysis of such large datasets is subject to over-fitting and over-interpretation because of the “small n /large P ” paradigm. We have attempted to minimize model over-fitting through data

training/test splits. Furthermore, global metabolomic results should be considered to be hypothesis-generating until confirmed with more targeted approaches. Additionally, this study was a secondary study from a tightly controlled feeding study and, although biological variation is likely reduced, we may not have enough power to assess true high-responders from

low-responders. Moreover, these subjects were overweight and obese, but still considered metabolically healthy. Therefore, our results may not extend to overweight and obese individuals with metabolic disorders. Lastly, we did not have access to daily physical activity data and report measurements over a 24 h period (including activity associated with sleeping). Therefore, when considering physical activity associated with waking hours, sedentary activity measurements reported herein are overestimated whereas time-adjusted light, moderate, and vigorous physical activity indices are underestimated.

One exciting, yet challenging outcome of the current research was the observation that the majority of determinants of weight loss were metabolites that do not have full structural identification and annotation. Uniform correlations among these metabolites suggest they are either associated with similar metabolic processes or partially derivatized products of a single or highly-related compounds. Additionally, many of these unknown metabolites had strong correlations with body composition markers and other known metabolites, making their identification a priority for future studies.

In summary, we assessed blood metabolomics and clinical chemistry, along with hormonal and inflammatory biomarkers, body composition, and dietary and physical activity measurements to identify a broad range of variables that characterize weight loss in overweight and obese adults consuming a 12 wk energy-restricted diet in a controlled feeding intervention. With the use of multivariate analyses, ~13% of the measured pre- and postintervention variables could be used to accurately model weight loss. Our findings suggest that overweight and obese individuals highly responsive to energy restriction are characterized by greater losses in total and regional adiposity that are supported by a lower RER and characterized by greater lipid mobilization. Person-to-person differences in habitual moderate physical activity and sedentary behavior are associated with this phenotype. The current research supports the idea that persons who incorporate regular light-to-moderate physical activity into daily activity will benefit most in terms of total weight and adipose reduction achieved by dietary interventions.

Acknowledgments

NLK, SHA, and MDVL designed the research; BDP, NLK, OF, SHA, and MDVL conducted the research; NLK, OF, SHA, and MDVL provided essential reagents and materials; BDP and JWN analyzed the data; BDP, NLK, SHA, MDVL, and JWN interpreted the data; BDP wrote the manuscript; and BDP and JWN had primary responsibility for the final content. All authors read and approved the final manuscript.

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