Original Research Communications

A distinct adipose tissue gene expression response to caloric restriction predicts 6-mo weight maintenance in obese subjects[1–3]

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ABSTRACT

Background: Weight loss has been shown to reduce risk factors associated with cardiovascular disease and diabetes; however, successful maintenance of weight loss continues to pose a challenge.

Objective: The present study was designed to assess whether changes in subcutaneous adipose tissue (scAT) gene expression during a low-calorie diet (LCD) could be used to differentiate and predict subjects who experience successful short-term weight maintenance from subjects who experience weight regain.

Design: Forty white women followed a dietary protocol consisting of an 8-wk LCD phase followed by a 6-mo weight-maintenance phase. Participants were classified as weight maintainers (WMs; 0–10% weight regain) and weight regainers (WRs; 50–100% weight regain) by considering changes in body weight during the 2 phases. Anthropometric measurements, bioclinical variables, and scAT gene expression were studied in all individuals before and after the LCD. Energy intake was estimated by using 3-d dietary records.

Results: No differences in body weight and fasting insulin were observed between WMs and WRs at baseline or after the LCD period. The LCD resulted in significant decreases in body weight and in several plasma variables in both groups. WMs experienced a significant reduction in insulin secretion in response to an oral-glucose-tolerance test after the LCD; in contrast, no changes in insulin secretion were observed in WRs after the LCD. An ANOVA of scAT gene expression showed that genes regulating fatty acid metabolism, citric acid cycle, oxidative phosphorylation, and apoptosis were regulated differently by the LCD in WM and WR subjects.

Conclusion: This study suggests that LCD-induced changes in insulin secretion and scAT gene expression may have the potential to predict successful short-term weight maintenance. This trial was registered at clinicaltrials.gov as NCT00390637. *Am J Clin Nutr* 2011;94:1399–409.

INTRODUCTION

Obesity is associated with an increased risk of cardiovascular disease, diabetes, metabolic syndrome, and a number of cancers; however, weight loss of 5–10% has repeatedly been shown to convey modest to significant reductions in the risk of these downstream complications (1). The most common strategy to promote weight loss in obesity involves modifying lifestyle via changes in dietary and exercise habits. Although reduced caloric intake and increased physical activity favor a reduction in body weight body fat mass and improvements in metabolic parameters, one of the greatest difficulties for obesity management is weight maintenance after successful weight loss.

Several meta-analyses have shown that energy restriction, increased physical activity, or both can lead to successful short-term weight loss; however, the long-term effectiveness of these interventions appears challenging (2, 3). Numerous factors have been shown to influence successful weight maintenance, including behavior (4), physical activity (2), eating habits (5), the length of time an individual has maintained weight loss (6), the

degree of energy deficit and consequent weight loss (3, 7), and the influence of altering dietary macronutrient content (ie, carbohydrate, protein, and fat) (8–11). It is now widely accepted that body weight and body composition are also influenced by a genetic component (which encompasses genetic polymorphisms, epigenetics, and gene transcription); however, our understanding of how these genetic determinants contribute to successful weight maintenance remains limited (12).

Diet-induced weight loss in overweight and obese individuals decreases the expression of genes associated with PUFA metabolism, inflammation, and cell death and modifies the expression of genes encoding components of the extracellular matrix (13–17). Previous attempts to predict an individual's response (ie, high compared with low weight loss) using only scAT[4] gene expression profiles appear to be limited, suggesting that alternate approaches may be required to improve prediction accuracy (18, 19).

Studying changes in gene expression has provided novel insight to help clarify the molecular basis for the metabolic improvements associated with diet-induced weight loss. For example, Capel et al (20) highlighted the interplay between immune cells and adipocytes during the various phases of a weight-loss program (caloric restriction and weight stabilization) by monitoring scAT gene expression profiles. Recently, Márquez-Quiñones et al (11) focused on the weight-maintenance phase of the DiOGenes study and found that unsuccessful participants (ie, subjects who regained weight after an LCD phase) had an increased expression of genes related to cellular growth and differentiation (11).

The present study was designed to further contribute to our understanding of the interindividual variability regarding successful weight maintenance by determining whether scAT gene expression profiles during an LCD can be used to differentiate and predict subjects who experience successful short-term weight maintenance from participants who experience weight regain. This study provides important knowledge that may prove beneficial in the long term for the development of personalized strategies to improve successful weight maintenance.

SUBJECTS AND METHODS

Dietary intervention study

This study is part of the European Framework project entitled Diet, Obesity, and Genes (DiOGenes). For a thorough description of the overall objective and goals of this dietary intervention, *see* Larsen et al (21) and Moore et al (22). Briefly, the project consisted of 2 phases: an initial weight-loss phase and a 6-mo weight-maintenance phase. A total of 932 overweight and obese white adults were recruited from across 8 European countries to study the effects of dietary macronutrients on weight regain and cardiovascular risk factors. Inclusion and exclusion criteria for study participation were outlined previously (21). Of relevance

to the current study, all subjects were weight stable (<3 kg change in body weight) during the 2-mo period before initiating the study. The initial weight-loss phase consisted of an 8-wk LCD (3300 kJ/d, ~800 kcal; Modifast; Nutrition et Santé). Only those participants who achieved the targeted weight loss (≥8% of initial body weight) were invited to continue the protocol. Subjects were randomly assigned to the weight-maintenance phase as described (21). During this weight-maintenance phase, participants consumed ad libitum 1 of 4 low-fat (20–25% of energy intake) diets that differed in GI and protein content (23). More specifically, subjects adhered to one of the following diets: LGI/LP, HGI/LP, LGI/HP, or HGI/HP. Target energy intakes in the LP diets were 10–15% protein and 57–62% carbohydrates and in the HP diets were 23–28% protein and 45–50% carbohydrates. The goal was to achieve a difference of ~15 GI points between the LGI and HGI diets. During this weight-maintenance period, subjects met with a dietitian at regular intervals.

The study was approved by local ethics committees in the various countries. The protocol was in accordance with the Declaration of Helsinki. All study participants provided written consent.

Blood sampling

Fasting blood samples were obtained at each of the 3 CIDs for the analysis of blood metabolites, as outlined in Larsen et al (21). An OGTT lasting 120 min was also performed at each CID after the consumption of 75 g glucose.

Subject selection

Of the 548 subjects that completed the entire dietary protocol, a subset of 227 women were selected according to the following criteria: age between 20–50 y, nondiabetic (fasting glucose ≤7 mmol/L), nondyslipidemic (fasting cholesterol ≤7 mmol/L and fasting triglycerides ≤3.6 mmol/L), availability of a fat biopsy at the required time points, and a complete clinical evaluation during the protocol (see Supplementary Figure 1 under "Supplemental data" in the online issue). Body weight and energy intake measurements at the 3 time points were necessary: CID 1 (before commencing the LCD), CID 2 (at the end of the LCD phase), and CID 3 (at the end of the 6-mo weight-maintenance phase) (21).

Previously, Márquez-Quiñones et al (11) reported negligible differences in scAT expression profiles between participants in the various weight-maintenance diets; therefore, subjects in the 4 different dietary branches were considered all together. Subjects were classified according to changes in body weight during the weight-maintenance period, which was expressed as a percentage of weight lost during the LCD period. Subjects who experienced 0–10% and 50–100% weight regain during the weight-maintenance period were classified as weight maintainers (WMs) and weight regainers (WRs), respectively. Of the 227 women available, a subset of 20 subjects were randomly selected for each group and matched for the following bioclinical variables at both CID 1 and CID 2 time points: body weight (kg), BMI (in kg/m²), total energy intake (kJ/d), and fasting cholesterol (mmol/L), triglycerides (mmol/L), HDL cholesterol (mmol/L), fructosamine μmol/L), adiponectin (μg/mL), C-reactive protein (mg/L), glucose (mmol/L), insulin (μIU/mL), and insulin resistance (HOMA-IR).

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[4]Abbreviations used: CID, clinical investigation day; DiOGenes, Diet, Obesity, and Genes; EHMN, Edinburgh human metabolic network; GI, glycemic index; HGI, high glycemic index; HP, high protein; KEGG, Kyoto Encyclopedia of Genes and Genomes; LCD, low-calorie diet; LGI, low glycemic index; LP, low protein; OGTT, oral-glucose-tolerance test; RT-PCR, reverse transcriptase polymerase chain reaction; scAT, subcutaneous adipose tissue; WM, weight maintainer; WR, weight regainer.

It is noteworthy to mention that WM and WR subjects were not individually matched, but rather the WM and WR groups as a whole were matched (ie, by using average values for each variable).

Sample preparation and microarray analysis

scAT samples from the periumbilical area were obtained by needle aspiration under local anesthesia after an overnight fast at each of the time points. For the present prediction study only biopsies at CID 1 and CID 2 were required. All procedures were standardized between study centers across Europe, and biopsy samples were stored at −80°C until analysis. Total RNA was extracted by using the RNeasy total RNA Mini kit (Qiagen). Total RNA concentration and quality were confirmed by using the Agilent 2100 Bioanalyzer (Agilent Technologies). Two hundred nanograms of total RNA from each sample were amplified and transcribed into fluorescent complementary RNA by using Agilent's Low RNA Input Linear Amplification kit (Agilent Technologies). Cyanine-5 dye was incorporated into all scAT samples, whereas the reference pool was labeled with cyanine-3 dye. The reference pool consisted of a commercial mix of human liver, adipose tissue, heart, intestine, and skeletal muscle RNA (AMBION/Applied Biosystems). A total of 80 samples (40 paired samples from CID 1 and CID 2) were randomly hybridized to Agilent 4 × 44K whole human genome microarrays, which are composed of >41,000 unique 60-mer oligonucleotide human sequences and transcripts. Sample preparation, hybridization, and microarray washing were performed according to the manufacturer's recommendations (Agilent Technologies). Arrays were scanned by using a GenePix 4000A Scanner (Axon Instruments-Molecular Devices). The complete data set is available in the NCBI Omnibus (http://www.ncbi.nlm.nih.gov/geo/) through the series accession number GSE24432.

Real-time RT-PCR analysis

A subset of genes was validated by real-time RT–PCR in 17 WMs and 17 WRs (sufficient RNA was not available for all 40 subjects). Reverse transcription was performed with 0.5 mg total RNA and random hexamer primers, according to the manufacturer's instructions (Promega). RT-PCR amplification was performed by using an ABI 7300 (Applied Biosystems) with the following thermal cycling conditions: 2 min at 50°C, 10 min at 95°C, followed by 40 cycles at 95°C for 15 s and at 60°C for 1 min for denaturation, annealing, and elongation. All samples were normalized to 18S gene expression (18S rRNA control kit; Eurogentec). Differences in gene expression were assessed by using a 2-tailed, homoscedastic Student's t test. Specific primers and probes were designed by using Universal ProbeLibrary Assay Design Center by Roche Applied Science (https://www.roche-applied-science.com).

Statistical analyses

Changes in bioclinical and anthropometric variables between groups (WMs compared with WRs) and between times (CID 1 compared with CID 2) were analyzed by using JMP Genomics version 4.1 software (SAS Institute). A mixed-model ANOVA was generated by using groups, times, and the interaction between group and time (group × time) as fixed effects and the subject ID as a random effect (to account for repeated measurements). Least-square means were estimated for group × time conditions. A post hoc Student's t test was used to determine significance in specific pairwise analyses. The AUC was calculated by using the trapezoid rule for both glucose and insulin response to the 120-min OGTT. All data are presented as means ± SEMs.

Microarray normalization was carried out by subtracting the median intensity background signal before intraslide Loess normalization of log-transformed data [Goulphar version 1.1.3 package (24)]. All data were then uploaded into the JMP Genomics version 4.1 platform (SAS Institute) and further normalized by using a quantile interslide intensity method. Multiple probes corresponding to the same gene were averaged to provide a single value for each GeneID per microarray. A mixed-model ANOVA was generated by using groups (WMs, WRs), times (CID 1, CID 2), and the interaction between group and time (group × time) as fixed effects. Because each of the 40 individuals provided a biopsy at both time points, the model included subjects as a random effect. Least-square means were estimated for group-by-time conditions. A false discovery rate of 0.01 was used to account for multiple testing.

FunNet analysis

The functional analysis of gene expression data was performed by using FunNet (25). FunNet identifies KEGG biological pathways that are overrepresented in gene expression data lists while accounting for tests of multiplicity (false discovery rate = 0.05). Four lists of significant GeneIDs were obtained from the ANOVA and used for the functional analyses: *1)* weight maintainers, which corresponded to genes up- and downregulated by the LCD that were identified in WM subjects only; *2)* weight regainers, which corresponded to genes up- and downregulated by the LCD that were identified in WR subjects only; *3)* directional concordance, which corresponded to genes up- and downregulated by the LCD that were identified in both WM and WR subjects; and *4)* oppositely regulated, which corresponded to genes that were regulated in both WM and WR subjects during the LCD but in an opposite manner. More specifically, upregulated pathways correspond to those genes that are downregulated in WMs and upregulated in WRs, whereas downregulated pathways correspond to those genes that are upregulated in WMs and downregulated in WRs.

Reporter metabolite analysis

The global predictive analysis of enzyme-induced transcriptional changes on metabolite concentrations was performed by reporter metabolite analysis (26, 27) based on the EHMN reconstruction, which represents a high-confidence reconstructed network of metabolism (28). The metabolic reconstruction forms a bipartite network containing 2 kinds of nodes, enzymes and metabolites. A metabolite is connected to an enzyme if it is catalyzed (ie, produced or consumed) by that particular enzyme. Therefore, metabolites will be linked only to enzymes and never to each other, whereas enzymes will be linked only to metabolites and never to each other. The reporter metabolite algorithm relies on a rigorous statistical framework to identify metabolite nodes

that are enriched in differentially expressed enzymatic genes among their connected enzyme nodes. We compared our results with those previously reported by Capel et al (20). Note that, although the EHMN was used in both studies, the number of enzymes detected in each network differed because the quality control measures applied to the gene expression data were not identical. The present analysis covered 1860 enzymes (80% of the EHMN), 2166 metabolic reactions (76%), and 2225 metabolites in the EHMN. [*See* Supplementary Table 1 under "Supplemental data" in the online issue for the EHMN coverage for the Capel et al (20) analysis.] The reporter metabolite values were generated by using Cytoscape 2.7.0 software (29).

RESULTS

Anthropometric and bioclinical data during the protocol

Subjects were classified into WM or WR groups according to the percentage weight regain during the 6-mo weight-maintenance phase, where WMs and WRs corresponded to participants who regained 0–10% and 50–100% of the weight lost after the LCD, respectively (**Figure 1**). Importantly, WM and WR groups were established by ensuring that there was no difference at CID 1 (baseline) or CID 2 (after the LCD) for the anthropometric and bioclinical variables listed in **Table 1**. All subjects lost a minimum of 8% of their initial body weight during the LCD phase. Energy restriction led to significant decreases in body weight, BMI, cholesterol, triglycerides, HDL cholesterol, glucose, and insulin in both WM and WR subjects (Table 1). Fructosamine, adiponectin, C-reactive protein, and HOMA-IR were not significantly changed by the LCD in a group-specific manner.

A group × time interaction analysis revealed that the reduction in circulating triglycerides observed after the LCD in WM and WR subjects was borderline different ($P = 0.0627$). A
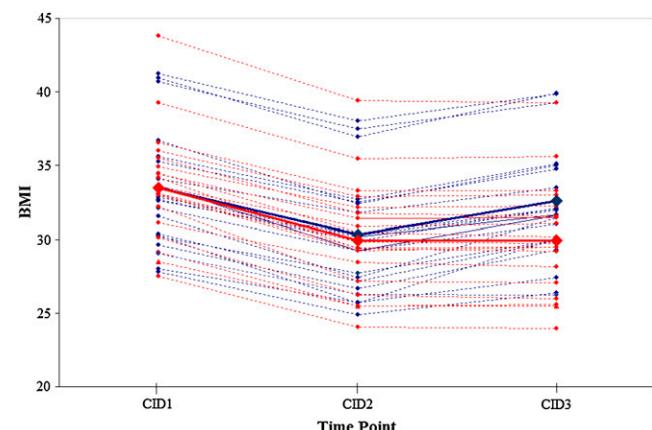
FIGURE 1. BMI evolution over the course of the intervention period. Each dotted line depicts the weight curve of an individual; red and blue lines represent WMs and WRs, respectively. The solid red and blue lines depict the group average for WMs and WRs, respectively. Both WMs and WRs experienced significant reductions in BMI during the 8-wk caloric restriction. A group × time interaction analysis showed that the decreases in BMI during the LCD were similar between the 2 groups. CID1 refers to the time point before caloric restriction; CID2 refers to the time point after 8 wk of caloric restriction; CID3 refers to the time point after the 6-mo weight-maintenance phase. CID, clinical investigation day; LCD, low-calorie diet; WMs, weight maintainers; WRs, weight regainers.

group-specific analysis found that triglyceride concentrations were significantly reduced after the LCD in WMs (CID 1: 1.3 ± 0.1 mmol/L; CID 2: 1.0 ± 0.1 mmol/L; $P = 0.0007$) and not in WRs (CID 1: 1.1 ± 0.1 mmol/L; CID 2: 1.0 ± 0.1 mmol/L; $P = 0.3522$). Although the differences in fasting insulin between WM and WR groups were not statistically different at CID 1 and CID 2, the group × time interaction analysis showed that there was a difference in the reductions in fasting insulin after the LCD between the 2 groups ($P = 0.0479$). Indeed, the reductions in fasting insulin after the LCD appeared to be somewhat greater in WM (CID 1: 10.9 ± 1.1 μIU/mL; CID 2: 6.2 ± 0.9 μIU/mL; $P < 0.0001$) than in WR (CID 1: 8.9 ± 0.8 μIU/mL; CID 2: 6.2 ± 0.6 μIU/mL, $P = 0.0004$) subjects.

Energy intake during the protocol

Energy intake in all subjects was assessed at CID 1, 2 wk after CID 2, and at CID 3 by using 3-d dietary records. Energy intake during the LCD was fixed at 3300 kJ/d to ensure that energy intake at CID 2 was equivalent for all study participants. Although energy intake decreased significantly from CID 1 to CID 2 in both groups, there was no difference in energy intake between the groups at either CID 1 or CID 2 (data not shown). When measuring mean energy intake at the end of the 6-mo weight-maintenance phase (CID 3), no significant differences ($P = 0.5770$) were found between the WM (6226 ± 533 kJ/d) and WR (6788 ± 875 kJ/d) groups. Nevertheless, and although not significant, WM subjects consumed, on average, ~500 kJ/d less than did WR subjects.

Changes in insulin secretion associated with 6-mo weight maintenance

The AUC for glucose and insulin response after an OGTT was calculated in all subjects before and after the LCD. No significant differences in glycemic response were detected between WMs and WRs at either CID 1 or CID 2 (data not shown). In contrast, the OGTT-induced insulin secretion was markedly higher in the WM group than in the WR group at CID 1 (WM insulin AUC: 8245 ± 881 μIU · min · mL^{-1}; WR insulin AUC: 5674 ± 509 μIU · min · mL^{-1}; $P < 0.0001$), despite similar baseline fasting insulin concentrations. A group × time interaction analysis indicated that changes in insulin secretion after the LCD differed between the 2 groups ($P = 0.0123$). More specifically, the LCD resulted in a significant decrease in insulin secretion in the WM group only. At CID 2 there was no significant difference in insulin secretion between the 2 groups (WM insulin AUC: 5189 ± 414; WR insulin AUC: 5235 ± 482; $P < 0.9888$). This was further reflected by changes in the individual data (*see* Supplementary Figure 2 under "Supplemental data" in the online issue). When plotting changes (ie, CID 2 – CID 1) in insulin secretion at each time point during the OGTT for each of the 40 subjects, it was apparent that considerable variability exists between individuals with regards to LCD-induced changes in insulin response. Despite this interindividual variability, the WM group experienced a significant decrease in insulin secretion ($P < 0.05$) at each time point during the OGTT (30, 60, 90, and 120 min) at CID 2 compared with CID 1. In contrast, the WR group showed no change in insulin secretion at CID 2 compared with CID 1.

TABLE 1
LCD response in each group, as shown in commonly measured bioclinical variables[1]

Bioclinical variable	WMs ($n = 20$)		WRs ($n = 20$)		P value	
	CID 1	CID 2	CID 1	CID 2	Main effect, time	Interaction, group × time[2]
Weight (kg)	91.8 ± 2.7	82.1 ± 2.6	91.9 ± 2.8	83.2 ± 2.6	<0.0001	0.1568
BMI (kg/m^2)	33.5 ± 0.9	29.9 ± 0.8	33.5 ± 0.9	30.3 ± 0.9	<0.0001	0.1053
Fasting cholesterol (mmol/L)	5.1 ± 0.2	4.2 ± 0.2	4.9 ± 0.2	4.1 ± 0.2	<0.0001	0.5989
Fasting triglycerides (mmol/L)	1.3 ± 0.1	1.0 ± 0.1	1.1 ± 0.1	1.0 ± 0.1	0.0024	0.0627
Fasting HDL (mmol/L)	1.2 ± 0.1	1.1 ± 0.1	1.3 ± 0.1	1.1 ± 0.1	0.0055	0.1552
Fasting fructosamine (μmol/L)	206 ± 5	207 ± 4	202 ± 4	200 ± 5	0.8120	0.6489
Fasting adiponectin (μg/mL)	9.2 ± 1.0	10.4 ± 1.0	9.8 ± 1.0	10.4 ± 1.0	0.1072	0.5832
Fasting CRP (mg/L)	4.6 ± 0.8	3.4 ± 0.9	4.1 ± 0.6	4.8 ± 1.3	0.7991	0.1238
Fasting glucose (mmol/L)	5.0 ± 0.1	4.7 ± 0.1	5.0 ± 0.1	4.7 ± 0.1	<0.0001	0.7126
Fasting insulin (μIU/mL)	10.9 ± 1.1	6.2 ± 0.9	8.9 ± 0.8	6.2 ± 0.8	<0.0001	0.0479
HOMA-IR	2.8 ± 0.4	2.2 ± 0.5	2.3 ± 0.4	1.6 ± 0.4	0.1326	0.8705

[1] All values are means ± SEMs. The table includes bioclinical variables at CID 1 and CID 2 in 20 women classified as WMs and in 20 women classified as WRs. A mixed-model ANOVA was generated by using groups, times, and the interaction between group and time (group × time) as fixed effects and the subject ID as a random effect (to account for repeated measurements). There was no group effect at either CID 1 or CID 2, reinforcing that groups were well matched. CID 1 refers to the time point before caloric restriction; CID 2 refers to the time point after 8 wk of caloric restriction. CID, clinical investigation day; CRP, C-reactive protein; LCD, low-calorie diet; WMs, weight maintainers; WRs, weight regainers.

[2] A significant interaction was observed for fasting insulin, and a borderline interaction was observed for fasting triglycerides.

Gene expression analysis

Gene expression differences between WMs and WRs before and after the LCD

Adipose tissue gene expression in WMs and WRs was first examined at CID 1 and CID 2 independently. Although there were no significant differences in bioclinical variables at CID 1 between WM and WR subjects, a gene expression analysis showed that 1292 genes were differentially expressed between the 2 groups before commencing the LCD. Despite this large number of differentially expressed genes, a functional pathway analysis failed to detect any differences in KEGG biological pathways between the 2 groups. At CID 2, the 2 groups of subjects appeared to be more similar with regard to their scAT gene expression profiles, with only 77 genes identified as differentially expressed between WMs and WRs. Again, no KEGG pathways were found to differ significantly between the groups.

Effects of LCD-induced weight loss on gene expression in WMs and WRs

The primary goal of the current study was to assess the differences in LCD-induced changes in gene expression in subjects classified as WMs and WRs. When comparing changes in gene expression between CID 1 and CID 2, 1291 and 1298 genes were differentially expressed in WMs and WRs, respectively. More specifically, 583 genes were upregulated and 708 were downregulated in the WMs, whereas 628 genes were upregulated and 670 were downregulated in the WRs. The most significant downregulated gene in both WM and WR was stearoyl-CoA desaturase (*SCD1*; WMs: −3.4-fold; WRs: −2.5-fold), the rate-limiting enzyme responsible for the conversion of SFAs into MUFAs (30). Expression changes for *SCD1* were confirmed by real-time RT-PCR (WMs: −1.8-fold; WRs: −2.0-fold; *P* < 0.05). In WMs, the most upregulated gene was cell death–inducing DFFA-like effector a (*CIDEA*; +2.2-fold), which was not differentially regulated in WRs. This gene plays an important role in adipose tissue energy expenditure and lipid accumula-

tion, in particular in increasing fat oxidation (31). We confirmed the increase in *CIDEA* expression during the LCD in WMs by real-time RT-PCR (+1.9-fold; *P* < 0.05). In WRs, the most upregulated gene was vimentin (*VIM*; +2.0-fold). This gene, expressed in fibroblasts and preadipocytes, was unique to WRs and is thought to play an important role in the cellular remodeling that occurs during adipocyte differentiation (32). We were unable to confirm a significant increase in *VIM* expression in WRs with real-time RT-PCR (+1.2-fold, *P* = 0.17).

The 2 gene lists for WMs and WRs were further dissected to better explore the shared and unique gene expression responses to the LCD between the 2 groups. A total of 1027 and 1034 genes were uniquely regulated in WMs and WRs, respectively (**Figure 2**). Although there were a large number of differentially expressed genes unique to the 2 groups, the functional analysis showed that these genes tended to belong to similar functional pathways (Figure 2). The LCD caused an increase in ribosomal genes and decreases in oxidative phosphorylation and metabolism pathways in both groups. The genes associated with oxidative phosphorylation are also found in other pathways, which is why pathways related to Alzheimer, Huntington, and Parkinson disease appear in Figure 2; however, it is the oxidative phosphorylation pathway that is most relevant when considering adipose tissue gene expression. The LCD caused a decrease in the valine, isoleucine, and leucine degradation pathway (related to 10 genes: *ABAT*, *ACAA2*, *ALDH6A1*, *AOX1*, *BCKDHB*, *DLD*, *HIBADH*, *HMGCS1*, *HSD17B10*, and *MCCC1*) in WM subjects, whereas the LCD caused a decrease in the fructose and mannose metabolism pathway (related to 6 genes: *ALDOA*, *ALDOB*, *KHK*, *MPI*, *PFKM*, and *PFKP*) in WR subjects.

After removing genes uniquely regulated in the 2 groups, 264 genes were differentially expressed in both WMs and WRs; however, directional concordance was not always maintained. As depicted in Figure 2, 170 genes were in directional concordance, meaning that the LCD had a similar effect on gene expression in both WM and WR groups. In contrast, 94 genes were regulated oppositely, meaning that the LCD had a different effect on gene expression in each group.
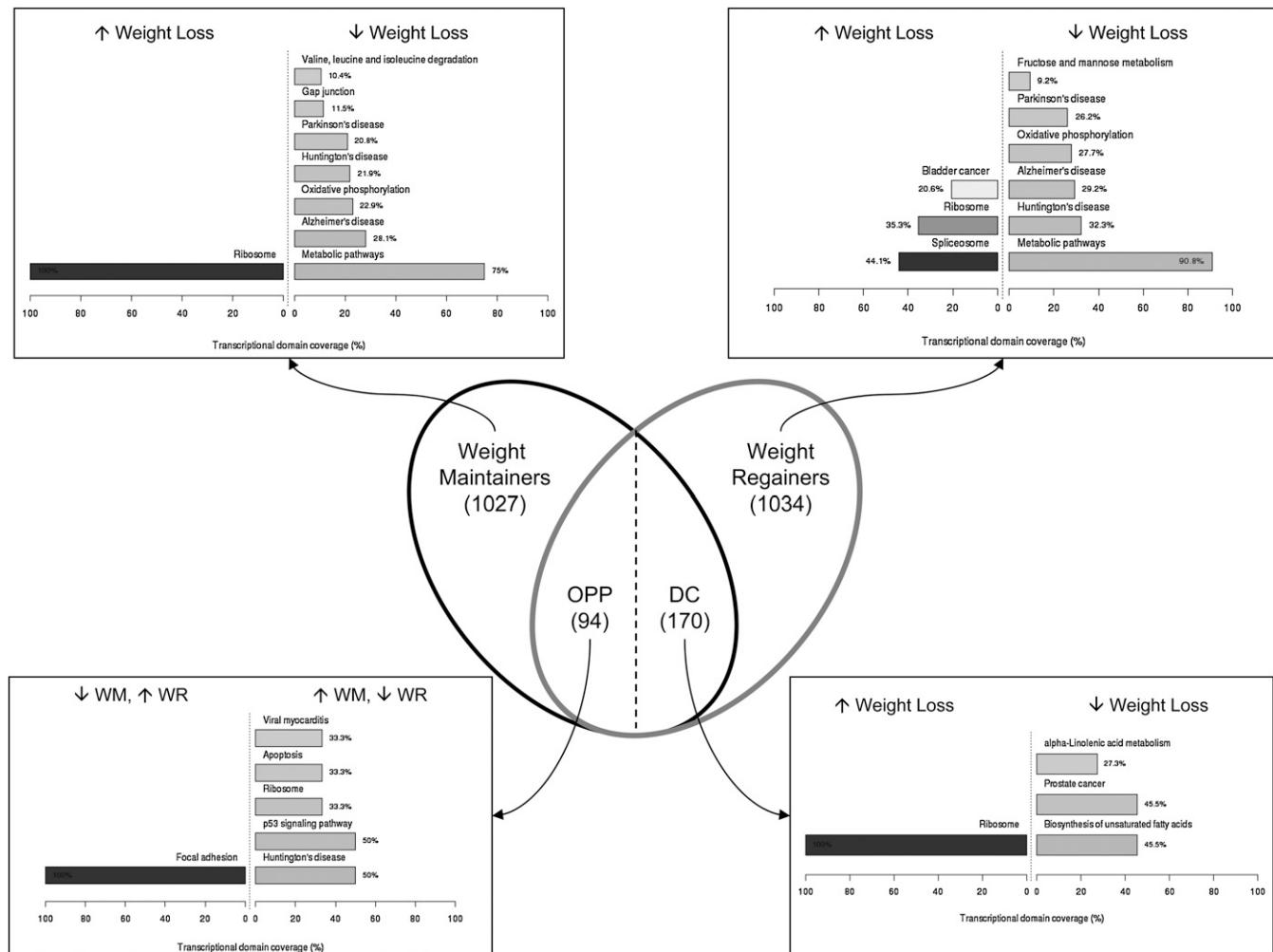
FIGURE 2. Venn diagram showing the overlap in differentially expressed genes after the LCD in WMs and WRs. Functional analyses showed that although the genes regulated by the LCD in WMs and WRs differ, they are related to similar functional processes. The LCD caused a significant decrease in the valine, leucine, and isoleucine degradation pathway in WM subjects. The LCD caused a significant decrease in the fructose and mannose metabolism pathway in WR subjects. Genes that are differentially expressed by the LCD in both WMs and WRs were not always directionally concordant. Oxidative phosphorylation and biosynthesis of unsaturated fatty acid pathways decreased with the LCD in both WMs and WRs. The apoptosis pathway was upregulated by the LCD in WMs compared with WRs. ↑ and ↓ indicate an increase and decrease, respectively, during the LCD. DC, genes in directional concordance (ie, up or down in WM and WR subjects); LCD, low-calorie diet; OPP, genes that are not directionally concordant (ie, up in WM and down in WR subjects or vice versa); WM, weight maintainer; WR, weight regainer.

Those genes in directional concordance suggest that the LCD caused a decrease in the biosynthesis of unsaturated fatty acids and α-linoleic metabolism pathways in both WMs and WRs. These pathways include such genes as fatty acid desaturase 1 (*FADS1*), fatty acid desaturase 2 (*FADS2*), acyl-CoA oxidase 1 (*ACOX*), and stearoyl-CoA desaturase (*SCD1*). Changes in *FADS1* expression were confirmed by real-time RT-PCR (WMs: −1.9-fold; WRs: −1.8-fold; $P < 0.05$). In addition, a subset of genes related to ribosomal pathways was upregulated by the LCD in WMs and WRs.

We also examined pathways that were oppositely regulated by the LCD ("OPP" in Figure 2) in the WM and WR groups. Several genes related to focal adhesion functions were upregulated in WRs and downregulated in WMs after the LCD: catenin β1 (*CTNNB1*), fibronectin 1 (*FN1*), mitogen-activated protein kinase 1 (*MAPK1*), PTK2 protein tyrosine kinase 2 (*PTK2*), β-actin (*ACTB*), and caveolin 1 (*CAV1*). These genes play important roles in the coordination of the extracellular matrix and

mediate processes such as cell growth and differentiation and intracellular signaling, suggesting that an LCD had different effects on extracellular matrix remodeling in the 2 groups. Interestingly, the LCD resulted in the increased expression of genes related to apoptosis and the p53 signaling pathway in WM subjects and not in WR subjects. More specifically, caspase 3 (*CASP3*) and caspase 8 (*CASP8*) were upregulated in WMs, whereas these genes were downregulated in WRs. The upregulation of *CASP8* gene expression in WMs was validated by real-time RT-PCR (+1.7 fold; $P < 0.05$), as was the downregulation in WR subjects (−1.2 fold; $P < 0.05$). Both *CASP3* and *CASP8* play crucial roles in initiating programmed cell death, suggesting that greater cell death in scAT during an LCD may underlie successful short-term weight maintenance.

Reporter metabolite analysis

To assess how the transcriptional differences in WMs and WRs most likely affected downstream metabolism, we overlaid our
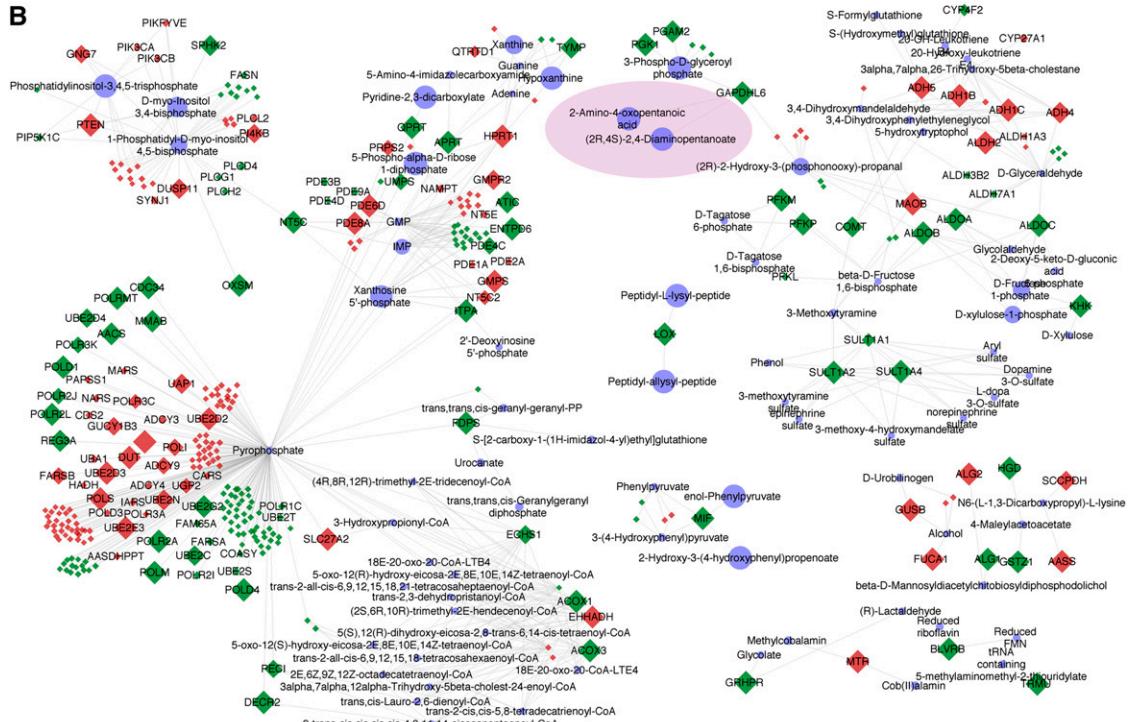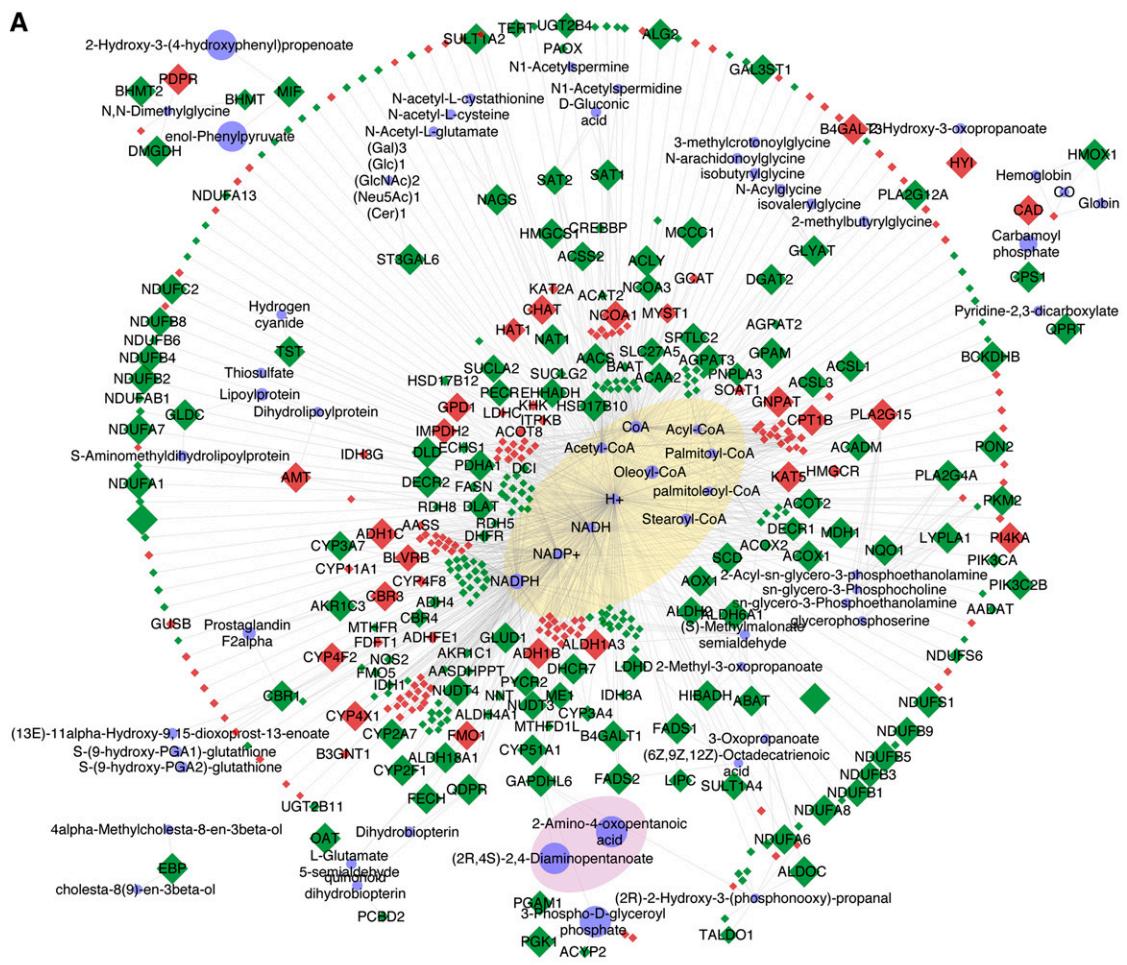
FIGURE 3. Metabolic reconstruction network analysis corresponding to LCD-induced changes in gene expression in WMs (A) and WRs (B). We used a high-confidence metabolic network reconstruction to search for metabolites that are catalyzed by enzymes that exhibit coordinated changes in gene expression levels during the LCD. The metabolic networks consist of metabolites (circles) that are connected to enzymes (diamonds) that catalyze the metabolites. Only metabolites linked to enzymes for which the underlying genes are differentially expressed during the LCD are shown. Metabolite circles are scaled according to their significance (ie, larger circles reflect smaller P values), in which the P value denotes the significance of being a metabolic "hot spot." A metabolite that is connected to a large number of differentially regulated enzymes (between CID1 and CID2) will be depicted by a larger circle. The ranges of P values, which correspond to circle size, are (A) 2.0×10^{-5} to 0.05 and (B) 8.9×10^{-10} to 0.05. For enzymes, red indicates upregulation and green indicates downregulation in gene expression. The impact of the LCD on scAT gene expression is more highly coordinated in WMs than in WRs, as represented by the dense and highly interconnected network. In WMs (A), the LCD elicits a marked coordinated downregulation of genes coding for enzymes associated with fatty acid metabolism, citric acid cycle, and oxidative phosphorylation (shaded in yellow). That signature is absent in the WR analysis (B) in which alternate sites in metabolism are active. Highly significant metabolites common to both WMs and WRs are shaded in purple. CID, clinical investigation day; LCD, low-calorie diet; scAT, subcutaneous adipose tissue; WMs, weight maintainers; WRs, weight regainers.

gene expression data with the EHMN reconstruction (28) and used the reporter metabolite analysis (26) to identify metabolites that may represent biomarkers for successful weight maintenance. It is apparent by the network structure that the LCD induces a more highly coordinated response in WM subjects than in WR subjects, as represented by the dense and highly interconnected network (**Figure 3,** A and B); however, there are some shared and distinct features within these 2 networks that are noteworthy.

Metabolites identified as significantly downregulated by the LCD in both WMs and WRs were (2R,4S)-2,4-diaminopentanoate and 2-amino-4-oxopentanoic acid from the D-arginine and D-ornithine pathway ($P = 4.5 \times 10^{-4}$ and $P = 2.0 \times 10^{-6}$, respectively). Their significance was driven by the *GAPDH* gene, which was downregulated in both groups during caloric restriction (WMs: −1.16-fold; WRs: −1.17-fold).

Interestingly, we observed a global pattern in the metabolite network that was unique to WMs and related to a large number of differentially expressed enzymes. The LCD resulted in a marked coordinated downregulation in enzymes associated with fatty acid metabolism, citric acid cycle, and oxidative phosphorylation in WM subjects, a signature that was absent in WRs. More specifically, several metabolites displayed differential expression in their associated enzymes: NADPH, NADP+, NADH, H+, CoA, acetyl-CoA, acyl-CoA, stearoyl-CoA, oleoyl-CoA, palmitoyl-CoA, and palmitoleoyl-CoA (**Table 2**). The majority of these metabolites (9 out of 11) were previously identified in the Capel et al (20) study, in which the authors also reported a marked downregulation in the same enzymes during energy restriction. Most of these metabolites were related to a large number of enzymes (shown by the numerous connections to several enzymes in the metabolic network), which reinforced the differences in network connectivity observed between WMs and WRs, as these metabolites were not significantly regulated in the WR group.

DISCUSSION

Considerable interindividual variability in weight maintenance after caloric restriction has been observed. The present study was designed to assess whether changes in scAT gene expression profiles during the weight-loss phase of a dietary intervention protocol could be used to predict changes in body weight during a subsequent 6-mo weight-maintenance phase. A unique aspect of this work lies with the fact that the subjects used for the current study were selected out of the larger DiOGenes cohort to ensure that there were no differences in the bioclinical values used as selection criteria (Table 1). This analysis showed that an 8-wk LCD triggered distinct changes in scAT gene expression in

subjects classified as WMs (0–10% weight regain) compared with WRs (50–100% weight regain). Furthermore, only the WM group experienced changes in plasma triglyceride concentrations and insulin secretion during the LCD.

Fasting triglyceride concentrations decreased significantly with the LCD in the WM group only. Schwab et al (33) previously reported decreases in triglycerides enriched in saturated and short-chain fatty acids after energy restriction, which were associated with improved insulin sensitivity. A larger follow-up study failed to find significant decreases in plasma triglycerides (15), suggesting variability in response to an energy-restricted diet. Weight maintenance was not assessed in either study; therefore, it is unclear whether a relation exists between the changes in triglycerides and successful 6-mo weight maintenance.

Both groups of subjects experienced significant decreases in fasting insulin concentrations at CID 2 compared with CID 1. Although the decrease in fasting insulin appeared to be greater in the WM group than in the WR group, it is important to note that both groups experienced the expected decreases in fasting insulin concentrations associated with LCD-induced weight loss. Therefore, our data do not suggest that fasting insulin is a reliable predictor of weight maintenance. This agrees with a previous study by Crujeiras et al (34), in which baseline fasting plasma insulin concentrations were reported to not predict weight regain.

A significant reduction in insulin secretion after the LCD was observed in the WM but not in the WR group (*see* Supplementary Figure 2 under "Supplemental data" in the online issue). This is an intriguing finding that suggests that studying insulin secretion in response to a glucose challenge at various stages during a weight-loss and weight-maintenance intervention study may prove interesting. In the current study, we used fasting insulin concentrations at CID 1 to match our WM and WR groups; however, it is important to note that data regarding insulin secretion in response to an OGTT are not routinely used for bioclinical matching of different subject groups. When we subsequently examined insulin secretion data at CID 1, we found that the WM group had significantly higher insulin secretion than did the WR group. Moreover, weight loss induced by the LCD led to significant reductions in insulin secretion in the WM group only. It is therefore tempting to suggest that an individual's capacity to improve insulin sensitivity after weight loss is a possible predictor of successful weight maintenance. However, as shown in Supplementary Figure 2, significant variability in the change in insulin secretion after the LCD was observed between individuals, thereby reinforcing the inherent challenge associated with identifying predictors and the absolute necessity for their independent validation. A number of studies have attempted to determine whether insulin secretion plays a role in long-term

TABLE 2
Reporter metabolites identified in WMs and WRs and their agreement with an independent study[1]

Reporter metabolite	WMs			WRs			Capel et al (20)		
	P value[2]	↑ Reactions	↓ Reactions	P value[2]	↑ Reactions	↓ Reactions	P value[2]	↑ Reactions	↓ Reactions
NADPH	0.008	57	88	0.217	75	70	0.026	6	16
Oleoyl-CoA	0.009	4	4	0.111	2	6	0.020	0	2
CoA	0.009	42	67	0.427	45	64	0.002	6	15
Acetyl-CoA	0.012	24	46	0.768	26	44	0.035	2	10
Stearoyl-CoA	0.013	5	8	0.164	3	10	0.020	0	2
NADP+	0.019	58	90	0.237	76	72	0.020	6	16
H+	0.031	128	204	0.117	149	183	0.037	9	38
NADH	0.033	50	74	0.217	75	70	0.085	3	21
Palmitoyl-CoA	0.039	10	17	0.105	7	20	0.054	1	2
Acyl-CoA	0.044	18	18	0.202	13	23	<0.001	0	6
Palmitoleoyl-CoA	0.050	7	8	0.154	6	9	0.005	0	2

[1] To assess how the transcriptional differences in WMs and WRs most likely affected downstream metabolism, we overlaid our gene expression data with the EHMN reconstruction and used the reporter metabolite algorithm to identify metabolites that vary between the 2 groups. We compared our results with those of Capel et al (20). Note that even though EHMN reconstruction was used in both studies, the number of enzymes detected in each network differed because of the different algorithms used to identify differentially regulated genes. ↑ and ↓, the number of metabolic reactions in which the metabolite is catalyzed by an enzyme that is either up- or downregulated during the low-calorie diet; EHMN, Edinburgh human metabolic network; WMs, weight maintainers; WRs, weight regainers.

[2] The unadjusted P values of reporter metabolites denote their significance of being metabolic "hot spots." In other words, these metabolites are connected to more differentially regulated enzymes (between clinical investigation days 1 and 2) than is expected by chance. $P < 0.05$ for significant changes in reporter metabolites.

body-weight regulation (35, 36); however, previous research has tended to examine whether insulin secretion affects weight gain. Schwartz et al (35) showed that reduced insulin secretion was a significant predictor for weight gain, and Chaput et al (37) showed that 30-min insulin concentrations during an OGTT were positively associated with 6-y weight gain. It is difficult to directly compare the outcomes of our study and these other studies because of different experimental designs; however, our data suggest that changes in insulin secretion after weight loss may be associated with changes in body weight.

The present study provides a novel contribution to the existing literature by studying whether LCD-induced changes in scAT gene expression can be used to predict successful short-term weight maintenance. Functional analysis of gene expression data showed that focal adhesion, apoptosis and p53 signaling pathways were differentially regulated during an LCD in WMs and WRs. In the WM group, subjects experienced a decrease in the focal adhesion pathway, which consists of extracellular matrix genes associated with diverse functions such as inflammation and cell growth and differentiation. Because the present study used a discovery-based approach and the genes related to focal adhesion have wide-ranging roles in various signaling pathways, it is difficult to predict whether extracellular remodeling is higher or lower in each group. Rather, we report here that an LCD has different effects on the extracellular matrix in WM and WR subjects.

The LCD caused an increase in caspase gene expression in WM subjects (ie, apoptosis pathway), suggesting that these subjects may be experiencing an increase in scAT apoptosis. In addition, *CIDEA*, the most highly upregulated gene in WMs has also been shown to regulate apoptosis in different cell types, including in adipocytes (38). Little previous work has examined the impact of diet-induced weight loss on adipose tissue apoptosis. Aubin et al (39) studied obese subjects and found that an

inhibitor of cellular adipose apoptosis was higher in the stroma-vascular fraction of scAT after weight loss. This work aligns with that of Alkhouri et al (40) who recently showed that caspase-3 was upregulated (ie, increased apoptosis) in diet-induced obese mice. Our results suggest the opposite: an LCD increased *CASP3* and *CASP8* expression only in individuals experiencing successful short-term weight maintenance. Although our study and that of Alkhouri et al appear to conflict, there are several noteworthy differences. Firsy, different fat depots were used in these studies, suggesting that omental and subcutaneous fat depots may regulate apoptosis pathways differently after changes in body weight. Second, Alkhouri et al compared morbidly obese and lean individuals, whereas we recruited only moderately obese participants. Despite these differences, both studies show that changes in body weight may influence adipose tissue apoptosis. Because of the variable response observed between individuals after caloric restriction, it appears likely that stratifying our population into WM and WR groups better highlighted subtle differences in scAT apoptosis. The notion that greater scAT apoptosis during an LCD may predispose individuals to successful weight maintenance is intriguing; however, future studies are required to confirm this finding, to identify the specific adipose tissue cell type in which the apoptosis pathway is increased, and to determine the physiologic outcome for this increase.

Interestingly, the metabolic network analysis was able to pick up several metabolites related to fatty acid metabolism, the citric acid cycle, and oxidative phosphorylation that were specifically regulated by the LCD in WMs and associated with a large number of downregulated enzymes. It is most likely that this analysis was able to detect these differences because it incorporates metabolic network topology, a feature that is often lacking in classical bioinformatic functional analyses such as FunNet. The marked differential expression and the major downregulation in enzymes

catalyzing fatty acid metabolism, the citric acid cycle, and oxidative phosphorylation observed during the LCD in WMs suggest that individuals predisposed to successful weight maintenance may be able to decrease fat accumulation by co-ordinating a better overall metabolic response.

Some limitations of the current study are worth noting. We classified subjects as WMs or WRs based solely on changes in body weight during the weight-loss and weight-maintenance phases, as previously reported (11, 41); however, we acknowledge that the regulation of body weight is multifactorial and can be influenced by environmental (eg, diet), lifestyle/behavioral (eg, physical activity, psychological), and molecular (eg, gene polymorphisms, gene expression) factors. The goal of the current study was to assess the molecular contribution to 6-mo weight maintenance; however, differences in energy intake and physical activity may have also contributed to weight regain. We used 3-d dietary records and pedometers to estimate energy intake and physical activity; however, there are caveats to consider. The first is that food records are widely recognized to have limitations, such as underreporting food intake and portion size (42, 43), in particular in the obese. Although we did not identify a significant difference in energy intake between WMs and WRs at CID 3, the WM group consumed ~500KJ/d less than did the WR group. We cannot exclude that an additional 500 KJ/d over the course of 6 mo could contribute to differences in weight gain. Our data regarding physical activity were incomplete; therefore we were unable to account for the effect of physical activity on weight maintenance/regain. Despite these limitations, our data suggest that individuals who experience a highly coordinated transcriptomic response to an LCD may provide new insight in understanding the genetic/genomic contribution to successful weight maintenance.

In conclusion, the current study shows that LCD-induced changes in bioclinical variables and scAT gene expression may foreshadow successful weight maintenance. The relation between changes in insulin secretion and weight maintenance is intriguing, yet requires validation in other populations that vary in sex and ethnicity before it can be considered a valid predictor for successful weight maintenance. Furthermore, our study reinforces the continued need to explore the relevance of genetic and metabolic factors in predicting changes in body weight and paves the way to further explore to what extent LCD-induced changes in gene expression can be used to confidently predict short-term weight maintenance.

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