Adipose tissue transcriptome reflects variations between subjects with continued weight loss and subjects regaining weight 6 mo after caloric restriction independent of energy intake\textsuperscript{1–3}

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ABSTRACT
Background: The mechanisms underlying body weight evolution after diet-induced weight loss are poorly understood.
Objective: We aimed to identify and characterize differences in the subcutaneous adipose tissue (SAT) transcriptome of subjects with different weight changes after energy restriction–induced weight loss during 6 mo on 4 different diets.
Design: After an 8-wk low-calorie diet (800 kcal/d), we randomly assigned weight-reduced obese subjects from 8 European countries to receive 4 diets that differed in protein and glycemic index content. In addition to anthropometric and plasma markers, SAT biopsies were taken at the beginning (clinical investigation day (CID) 2) and end (CID3) of the weight follow-up period. Microarray analysis was used to define SAT gene expression profiles at CID2 and CID3 in 22 women with continued weight loss (successful group) and in 22 women with weight regain (unsuccessful group) across the 4 dietary arms.
Results: Differences in SAT gene expression patterns between successful and unsuccessful groups were mainly due to weight variations rather than to differences in dietary macronutrient content. An analysis of covariance with total energy intake as a covariate identified 1338 differentially expressed genes. Cellular growth and proliferation, cell death, cellular function, and maintenance were the main biological processes represented in SAT from subjects who regained weight. Mitochondrial oxidative phosphorylation was the major pattern associated with continued weight loss.
Conclusions: The ability to control body weight loss independent of energy intake or diet composition is reflected in the SAT transcriptome. Although cell proliferation may be detrimental, a greater mitochondrial energy gene expression is suggested as being beneficial for weight control. This trial was registered at clinicaltrials.gov as NCT00390637. Am J Clin Nutr 2010;92:975–84.

INTRODUCTION
Body weight maintenance after weight reduction is a major challenge for obesity treatment. Only \approx 20\% of individuals could be considered as successful weight-loss maintainers when weight-maintenance success is defined as intentionally losing \geq 10\% of initial body weight and maintaining that loss for a minimum of 1 y (1, 2). Dietary interventions are the most popular method for weight maintenance compared with exercise or drug-based therapies; however, there is intense debate about what type of macronutrient is the most effective for weight control (3, 4).

Several studies have been published regarding the effect of diet macronutrient composition on weight reduction. Recently, Sacks et al (5) showed no difference between dietary fat, protein, or carbohydrate content on weight loss with the main effect caused

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by energy restriction (ER). Because of their satiating and thermogenic properties, high-protein (HP) diets have been prescribed for both body weight loss and maintenance (6–8). In parallel, research has focused on the type of carbohydrates in diets. The glycemic index (GI) refers to the postprandial blood glucose response expressed as a percentage of the response to an equivalent carbohydrate portion of a reference food, such as white bread or glucose (9). Reducing the insulin response by lowering the GI in diets may lead to an improved blood glucose profile over the late postprandial period (10), which may be translated into a better appetite control. Previous randomized controlled trials showed that overweight or obese people on a low-GI (LGI) diet lost more weight than overweight or obese people on control diet (11). However, despite the great range of programs aimed at weight control, a poor maintenance of weight loss in the long-term has been achieved, and the effect of macronutrients remains to be further investigated (12, 13).

Subcutaneous adipose tissue (SAT) gene expression profiling was shown to be a useful tool to reflect the molecular adaptations to different dietary interventions (14). The SAT transcriptome responds differently to caloric restriction between obese women who were able to lose >3% of fat mass and obese women who were not able to lose >3% of fat mass (15). Also, it seems possible to predict the weight response of subjects to a low-fat hypoenergetic diet by using SAT gene expression profiling before dietary intervention (16). Most of these studies focused on caloric restriction and the effect of diet composition on SAT gene expression during low-calorie programs. Little is known about SAT gene expression during the clinically relevant weight maintenance phase of dietary programs. The current work aimed at identifying SAT gene expression patterns that discriminate successful weight control from weight regain over a 6-mo period in weight-reduced obese women. The dietary intervention consisted of an initial 8-wk ER phase that led to a loss of >8% of body mass followed by a 6-mo randomized controlled follow-up period [after caloric restriction (ACR)] in which participants consumed, ad libitum, one of 4 diets that differed in the GI and protein amounts (17). The identification of genes associated with the success or failure of weight stabilization may affect our understanding of the mechanisms of weight control and provide an essential step in the development of new strategies for obesity management.

SUBJECTS AND METHODS

Dietary intervention study

The study was part of the European integrated Diet, Obesity and Genes (DiOGenes) project (17). A total of 932 overweight and obese adults in 8 European centers (Maastricht, Netherlands; Copenhagen, Denmark; Cambridge, United Kingdom; Heraklion, Greece; Potsdam, Germany; Pamplona, Spain; Sofia, Bulgaria; and Prague, Czech Republic) were enrolled to participate in a dietary intervention study aimed at defining the effect of macronutrient diet composition on weight regain and cardiovascular risk factors after a weight-loss period. The dietary intervention (Figure 1) consisted of an 8-wk ER phase with a low-calorie diet (LCD) (Modifast; Nutrition et Santé, Revel, France) that provided subjects with 3.3 MJ/d with the additional consumption of ≤400 g raw vegetables (total energy intake of 3.3–4.2 MJ/d). A total of 773 subjects who achieved the targeted weight loss (>8% of initial body weight) during ER were selected and randomly assigned to a 6-mo follow-up period referred to as the ACR phase, according to the protocol described by Larsen et al (17). During the ACR phase, all subjects consumed, ad libitum, one of 4 low-fat (25–30% of energy intake) diets that differed in the GI and protein content. More specifically, during the 6-mo follow-up period, participants were randomly assigned into one of the following dietary groups: LGI (LGI)/low-protein (LP), high-GI (HGI)/LP, LGI/HP, and HGI/HP diets. Target intakes in the LP diets were 10–15% of energy intake from proteins and 57–62% of energy intake from carbohydrates, and in HP diets, target intakes were 23–28% of energy intake from proteins and 45–50% of energy intake from carbohydrates. Compared with HGI diets, LGI diets were targeted with a reduction of 15 GI percentage points (18). During the ACR phase, subjects met with a dietitian every 2 wk up to week 6 and once a month thereafter. Weight, adverse events, dietary compliance (by using a compliance questionnaire specifically developed for the study), and other clinical data were collected at each visit.

Ethics

The study was approved by the local ethics committees in the respective countries. The protocol was in accordance with the Declaration of Helsinki (19); all study participants signed an informed consent document after they received verbal and written instructions and according to local legislation. Detailed information about the DiOGenes project is described by Larsen et al (17) and Moore et al (18).

Subject selection and classification

Among the 548 subjects who completed the entire dietary program, a subset of 227 white women was selected. Inclusion criteria were as follows: subjects were between 20 and 50 y old, had plasma markers that indicated that they were non-diabetic and non-dyslipidemic (total cholesterol ≤7 mmol/L, triglycerides ≤3.6 mmol/L, and fasting glucose ≤7 mmol/L), had a high-quality fat biopsy and blood samples, and a full availability of clinical information (Figure 2). Subjects were classified according to body weight variations during the ACR phase. Changes in weight during the ACR period were evaluated by taking into account weight loss during ER, which was defined as...
A total of 548 obese adults completed the program. Among them, 230 women were selected by the DiOGenes program. A total of 548 obese adults completed the Diet, Obesity, and Genes Research, Technology, and Development line 2 (RTD2) according to the criteria (DiOGenes) program. Among them, 230 women were selected by the DiOGenes program. A total of 548 obese adults completed the Diet, Obesity, and Genes Research, Technology, and Development line 2 (RTD2) according to selection criteria (see section entitled “Subject selection and classification”). Subjects were classified according to the $S$ factor, which measures body weight variations during the 6 mo after caloric restriction (ACR) phase in relation to weight loss during the energy restriction (ER) phase. Subjects with the lowest $S$ value represented those subjects who stabilized or who continued to lose weight (i.e., the successful group). Subjects with the highest $S$ value represented subjects who regained weight (i.e., the unsuccessful group) during the ACR phase. A total of 39 successful and 38 unsuccessful subjects were selected. DNA microarrays on whole subcutaneous adipose tissue (AT) biopsies were performed in 22 successful and 22 unsuccessful subjects (5–6 subjects at each diet branch). Validation of differential genes identified during transcriptomic analyses were performed in the entire successful and unsuccessful population. RT-qPCR, reverse transcriptase–quantitative polymerase chain reaction.

\[ S = \frac{\Delta \text{body weight}_{\text{CID2} - \text{CID3}}}{\Delta \text{body weight}_{\text{CID1} - \text{CID1}}} \quad (I) \]

where CID corresponds to the clinical investigation day, CID1 was before the ER, CID2 was after the ER, and CID3 was after the ACR phase (Figure 2). After exclusion of the <10th and >90th deciles, 22 subjects with the lowest $S$ values were classified as successful subjects ($S = -0.292 \pm 0.019; n = 5–6$ at each of the 4 diet branches), whereas 22 subjects with the highest $S$ values were classified as unsuccessful subjects ($S = 0.442 \pm 0.017; n = 5–6$ at each of the 4 diet branches) (see supplemental Figure 1 under “Supplemental data” in the online issue).

**SAT biopsy and total RNA preparation**

Abdominal SAT biopsies were obtained by needle aspiration under local anesthesia after an overnight fast at each of the CIDs (Figure 1). All procedures were carefully harmonized among the different centers involved. The fat samples were stored at −80°C until analysis. Total RNA was extracted with the RNeasy total RNA Mini kit (Qiagen, Courtaboeuf, France). Total RNA concentrations and RNA qualities were estimated by capillary electrophoresis with the Experion analyzer (BioRad, Marnes-la-Coquette, France).

**Microarray analysis**

A total of 250 ng total RNA from each sample was amplified and transcribed into fluorescent cRNA with Agilent’s Low RNA Input Linear Amplification kit (Agilent Technologies, Massy, France). A total of 88 arrays were performed by using a common reference design: Cy5 dye was incorporated into all SAT RNA samples, whereas a reference RNA pool made of the mix of commercial human liver, adipose tissue, and skeletal muscle RNA was labeled with Cy3 dye (Applied Biosystems/Ambion, Foster City, CA). Samples were hybridized to Agilent 44K whole human genome microarrays (Agilent Technologies), which contain over 41,000 unique 60-mer oligonucleotide human sequences and transcripts. Sample preparations and microarray washing were performed according to the manufacturer’s recommendations (Agilent Technologies). Arrays were scanned with an Innoscan 700 scanner (Innopsys, Carbone, France). Images were quantified with MAPIX V3.02 software (Innopsys). Data analysis consisted of loess intraslide normalization followed by quantile interslide intensity normalization with the Limma package for R-bioconductor (http://www.bioconductor.org). Intensity ratios between Cy5 and Cy3 were filtered by using an average intensity of 2 times above the background signal. After normalization and background correction, 8423 unique genes were present across 100% of the microarrays. The generated data set was submitted to the National Center for Biotechnology Information (NCBI) Gene Expression Omnibus (http://www.ncbi.nlm.nih.gov/projects/geo) database (GSE19494).

**Reverse transcriptase–quantitative polymerase chain reaction**

One microgram of adipose tissue total RNA was used for first strand cDNA synthesis by using random hexamers and poly(dT) according to the SuperScript II reverse transcriptase kit (Invtrogen, Cergy Pontoise, France). All measurements were performed in duplicate as previously described (20). A confirmation set was composed of 77 subjects that included subjects selected for the microarray study. mRNA expression levels were normalized with 18S ribosomal RNA expression.

**Statistical analyses**

Changes in biochemical and anthropometric variables between groups (successful compared with unsuccessful groups; and between diet branches) during the dietary intervention were tested by using a general linear model for repeated measures with variables adjusted for basal levels (measurements at CID1). The effect of the diet macronutrient composition within groups was tested by using one-factor analysis of covariance (ANCOVA) and Bonferroni post hoc test. All statistical analyses were performed with SPSS 17.0 software (SPSS Inc, Chicago, IL). The threshold for statistical significance was $P < 0.05$.

The change in gene expression patterns during the ACR phase was evaluated in terms of the fold change after and before the ACR phase (CID3:CID2 ratio) in each group. Log-transformed data were first analyzed by multivariate statistical methods by
using principal components analysis (21, 22). A partial least-square (PLS) projection to latent structures model was used to
find the relation between gene expression profiles and either diets and response groups (PLS discriminant analysis model) or S
variable (PLS1 model). Multivariate modeling was done with
SIMCA-P+ 12 software (Umetrics AB, Umeå, Sweden). Dif-
fferences in gene expression patterns were tested with one-factor
ANCOVA by using total energy intake as a covariate when
testing for differences between successful and unsuccessful
groups; and by 2-factor ANCOVA with diet protein amounts and
the GI as factors and with the S factor as a covariate when testing
for differences in gene expression associated to different diets.
Data were controlled for multiple testing by using Benjamini-
Hochberg P value correction. The threshold for significance was
q < 0.15.

Functional analysis was done with Ingenuity Pathway Analysis
7.5 software (http://www.ingenuity.com; Ingenuity Systems,
Redwood City, CA) and gene set enrichment analysis (GSEA)
v 2.0 software (Broad Institute, Cambridge, MA) (23). Ingenuity
Pathway Analysis 7.5 software (Ingenuity Systems) uses
Fischer’s exact test to calculate a P value that determines the
probability that each biological function and/or disease assigned
to that data set is due to chance alone. P values were controlled
for multiple testing by Benjamini-Hochberg correction. The
GSEA v 2.0 software evaluates a query microarray data set by
using a collection of gene sets annotated in the molecular sig-
nature database and in-house gene sets (24).

RESULTS

Anthropometric and clinical data

Subjects were classified into successful or unsuccessful groups
according to weight changes during the ACR period. Biological
and anthropometrical data for both successful and unsuccessful
groups are shown in Table 1. Importantly, there were no sig-
nificant differences in any of the anthropometrical and clinical
variables before the ER between the a posteriori determined
successful and unsuccessful groups. ER led to a decrease in
body weight, fat mass, waist circumferences, systolic blood
pressure, plasma cholesterol, LDL, C-reactive protein, and in-
sulin concentrations, and homeostatic model assessment insulin
resistance. There were no significant differences in anthropo-
metrical and clinical data after ER between successful and
unsuccessful groups. After the ACR period, differences in an-
thropometrical and clinical measurements between successful
and unsuccessful groups were mainly related to weight varia-
tions between CID2 and CID3. Successful subjects had lower
body weight [Δ body weight (CID2–CID3) = −2.6 ± 1.2 kg], body
mass index, fat mass, and waist circumference, whereas these
variables were higher in unsuccessful subjects [Δ body weight
(CID2–CID3) = +3.9 ± 1.3 kg]. At CID3 (ie, the end of the
ACR phase), unsuccessful subjects had higher C-reactive protein
and fasting plasma glucose concentrations compared with at
CID2 (Table 1). Plasma concentrations of total cholesterol,
HDL, and LDL were increased after the ACR phase in both
groups. Triglycerides, fructosamine, and adiponectin plasma

<table>
<thead>
<tr>
<th>Variable</th>
<th>CID1</th>
<th>CID2</th>
<th>CID3</th>
<th>CID1</th>
<th>CID2</th>
<th>CID3</th>
<th>P_{GLM}</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>39.5 ± 0.9</td>
<td>85.3 ± 2.1</td>
<td>82.3 ± 2.0</td>
<td>41.5 ± 0.7</td>
<td>91.4 ± 2.1</td>
<td>82.2 ± 1.9</td>
<td>0.005</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>96.1 ± 2.3</td>
<td>30.5 ± 0.6</td>
<td>37.8 ± 0.9</td>
<td>32.3 ± 0.9</td>
<td>39.5 ± 1.0</td>
<td>38.5 ± 1.2</td>
<td>0.001</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>34.3 ± 6.9</td>
<td>101.7 ± 1.9</td>
<td>94.0 ± 1.9</td>
<td>34.3 ± 0.9</td>
<td>11.4 ± 1.3</td>
<td>11.9 ± 1.3</td>
<td>0.001</td>
</tr>
<tr>
<td>Waist circumference (cm)</td>
<td>103.1 ± 1.0</td>
<td>94.6 ± 1.5</td>
<td>91.4 ± 1.5</td>
<td>101.7 ± 1.9</td>
<td>94.0 ± 1.9</td>
<td>97.4 ± 1.9</td>
<td>0.001</td>
</tr>
<tr>
<td>SBP (mm Hg)</td>
<td>120.2 ± 2.0</td>
<td>115.2 ± 1.9</td>
<td>117.0 ± 2.0</td>
<td>121.1 ± 2.2</td>
<td>113.0 ± 2.2</td>
<td>120.0 ± 2.1</td>
<td>0.921</td>
</tr>
<tr>
<td>DBP (mm Hg)</td>
<td>74.5 ± 1.5</td>
<td>71.1 ± 1.3</td>
<td>70.7 ± 1.4</td>
<td>73.0 ± 1.6</td>
<td>70.5 ± 1.8</td>
<td>72.6 ± 1.8</td>
<td>0.314</td>
</tr>
<tr>
<td>Cholesterol (mmol/L)</td>
<td>4.7 ± 0.1</td>
<td>4.1 ± 0.1</td>
<td>4.6 ± 0.1</td>
<td>4.8 ± 0.1</td>
<td>4.3 ± 0.2</td>
<td>4.8 ± 0.1</td>
<td>0.761</td>
</tr>
<tr>
<td>HDL (mmol/L)</td>
<td>1.26 ± 0.0</td>
<td>1.16 ± 0.4</td>
<td>1.44 ± 0.05</td>
<td>1.32 ± 0.0</td>
<td>1.21 ± 0.0</td>
<td>1.45 ± 0.06</td>
<td>0.517</td>
</tr>
<tr>
<td>LDL (mmol/L)</td>
<td>2.89 ± 0.1</td>
<td>2.50 ± 1.0</td>
<td>2.69 ± 0.10</td>
<td>2.96 ± 0.11</td>
<td>2.53 ± 0.12</td>
<td>2.82 ± 0.10</td>
<td>0.705</td>
</tr>
<tr>
<td>Triglycerides (mmol/L)</td>
<td>1.28 ± 0.0</td>
<td>1.05 ± 0.0</td>
<td>1.05 ± 0.06</td>
<td>1.29 ± 0.10</td>
<td>1.21 ± 0.15</td>
<td>1.26 ± 0.73</td>
<td>0.719</td>
</tr>
<tr>
<td>Fructosamine (μmol/L)</td>
<td>206.6 ± 3.8</td>
<td>210.9 ± 3.2</td>
<td>213.1 ± 3.0</td>
<td>207.3 ± 3.8</td>
<td>206.2 ± 4.5</td>
<td>216.0 ± 3.4</td>
<td>0.185</td>
</tr>
<tr>
<td>Adiponectin (μg/mL)</td>
<td>9.4 ± 0.7</td>
<td>9.8 ± 0.5</td>
<td>10.6 ± 0.5</td>
<td>9.6 ± 0.7</td>
<td>10.4 ± 0.8</td>
<td>12.2 ± 0.8</td>
<td>0.278</td>
</tr>
<tr>
<td>C-reactive protein (mg/mL)</td>
<td>5.6 ± 0.9</td>
<td>4.7 ± 0.7</td>
<td>3.5 ± 0.6</td>
<td>4.8 ± 0.8</td>
<td>3.0 ± 0.7</td>
<td>4.1 ± 0.9</td>
<td>0.001</td>
</tr>
<tr>
<td>Fasting glucose (mmol/L)</td>
<td>5.0 ± 0.1</td>
<td>4.8 ± 0.1</td>
<td>4.7 ± 0.1</td>
<td>5.1 ± 0.1</td>
<td>4.8 ± 0.1</td>
<td>5.0 ± 0.1</td>
<td>0.018</td>
</tr>
<tr>
<td>Fasting insulin (μIU/mL)</td>
<td>9.9 ± 0.8</td>
<td>7.5 ± 0.7</td>
<td>6.6 ± 0.6</td>
<td>11.4 ± 1.3</td>
<td>8.0 ± 0.8</td>
<td>8.6 ± 0.8</td>
<td>0.115</td>
</tr>
<tr>
<td>HOMA-IR</td>
<td>1.32 ± 0.1</td>
<td>0.96 ± 0.09</td>
<td>0.90 ± 0.07</td>
<td>1.52 ± 0.17</td>
<td>1.12 ± 0.10</td>
<td>1.17 ± 0.11</td>
<td>0.170</td>
</tr>
</tbody>
</table>

All values are means ± SEs. CID1, clinical investigation day before energy restriction; CID2, clinical investigation day after 8-wk caloric restriction; CID3, clinical investigation day 6 mo after the end of caloric restriction; GLM, general linear model; SBP, systolic blood pressure; DBP, diastolic blood pressure; HOMA-IR, homeostasis model assessment of insulin resistance. The table includes anthropometric and plasma variables at CID1, CID2, and CID3 in 39 women with sustained weight loss (ie, the successful group) and 38 women regaining weight (ie, the unsuccessful group) among the 4 dietary arms. There was no significant difference between successful and unsuccessful groups at CID1 and CID2.

GLM for repeated measures was used to test differences in clinical data during the ACR (after caloric restriction) phase between successful and unsuccessful groups. Levels at CID1 were taken as covariates.

P < 0.05 between CID1 and CID2 as determined by one-factor ANOVA and Bonferroni post hoc test between CIDs for each group separately.

P < 0.05 between CID1 and CID3 as determined by one-factor ANOVA and Bonferroni post hoc test between CIDs for each group separately.

P < 0.05 between CID2 and CID3 as determined by one-factor ANOVA and Bonferroni post hoc test between CIDs for each group separately.
concentrations remained constant in successful and unsuccessful subjects after the ACR phase. Diet compositions had no effect on anthropometrical and biochemical variables for either successful or unsuccessful subjects (see supplemental Tables 1 and 2 under “Supplemental data” in the online issue).

Differences in eating behaviors between the 2 groups were evaluated by 3-d dietary records through which the total energy intake at the end of the ACR period was estimated (18). There was no significant difference in energy intake among diet groups, but unsuccessful subjects ingested more energy than successful subjects (6.23 ± 2.60 and 6.78 ± 2.04 MJ/d, respectively; P < 0.02) (data not shown).

Change in adipose tissue gene expression patterns in successful and unsuccessful subjects

A subset of 22 successful and 22 unsuccessful subjects (5 or 6 subjects at each diet branch) with high RNA quality and clinically representative of both groups was selected for microarray analysis. The change in gene expression patterns after ER was tested in terms of fold change after and before the ACR period for each group (CID3:CID2 ratio).

The data set was monitored by both multivariate principal component analysis and univariate 3-factor analysis of variance with or without control for differences in energy intake. All analyses showed that the main source of variation in the gene expression data set was the difference between successful and unsuccessful groups (see supplemental Figure 2 under “Supplemental data” in the online issue).

To determine whether global gene expression changes during ACR could differentiate between subject groups, PLS discriminant analysis was performed. There were no gene expression patterns associated with macronutrient diet composition; however, there was a slight but not significant separation between successful and unsuccessful groups \(R^2 = 0.115\) and \(Q^2 = 0.067\), where \(R^2\) explained the cumulative variation of the 1st 2 factors and \(Q^2\) indicated the variation explained by the model according to cross-validation (21) (Figure 3A). With the use of a model for explaining differences in S values and, hence, differences between successful and unsuccessful subjects, independent of diet compositions, there was a significant separation between successful and unsuccessful groups \(R^2 = 0.813\); \(Q^2 = 0.88\) (Figure 3C). Similarly, differences in clinical data were more associated with the differences in S values than with diet compositions \(R^2 = 0.113\) and \(Q^2 = 0.044\) and \(R^2 = 0.851\).  

![FIGURE 3. Exploratory analyses of gene expression and clinical data. Partial least-squares (PLS) regression was used to determine differences in gene expression patterns (A and C) or to correlate clinical data (B and D) with different groups. A: Score plot of PLS discriminant analysis (PLS-DA) used for explaining differences between groups and diets \((R^2 = 0.115, Q^2 = 0.067)\). B: Loadings bi-plot of PLS-DA correlating clinical data with groups and diets \((R^2 = 0.113, Q^2 = 0.044)\). C: Score plot of PLS1 model for explaining the S factor by gene expression patterns \((R^2 = 0.813, Q^2 = 0.88)\) (successful subjects are shown inside the dark ellipse; unsuccessful subjects are shown inside the gray ellipse). D: Loading bi-plot of PLS1 model showing the correlation of clinical variables with the S factor \((R^2 = 0.851, Q^2 = 0.766)\). \(t[1]\) and \(t[2]\) correspond to the visualized principal components 1 and 2, respectively. Axes in B and D correspond to the correlation coefficient between the first component of the clinical data \((pc(corr)[1])\) and the first component of the gene expression data \((t(corr)[1])\). Ellipses in A and C correspond to the confidence ellipse on the basis of Hotelling’s \(T^2\) at a 0.05 significance level. Ellipses in B and D correspond to the correlation ratios of 0.5, 0.75, and 1 in the dotted, slashed, and solid ellipses, respectively. \(R^2\) explains the variation of the first component, and \(Q^2\) indicates the variation explained by the model according to cross validation. \(Q^2 > 0.5\) indicates a good model. Triangle, low–glycemic index (LGI)/low-protein (LP) diet; box, high-glycemic index (HGI)/LP diet; circle, LGI/high-protein (HP) diet; diamond, HGI/HP diet; black symbols, successful group; gray symbols, unsuccessful group; CRP, C-reactive protein; FM, fat mass; DBP, diastolic blood pressure; SBP, systolic blood pressure; TotEnergy, total energy.]
and $Q^2 = 0.766$, respectively) (Figure 3, B and D). Variables accounting for group separation were selected by using the variable importance in the projection (VIP) score. Variables with a VIP $>1$ were those highly associated to differences in phenotypes. Eighty-eight transcripts had a VIP $>1$ and were considered as differential between successful and unsuccessful groups (see supplemental Table 3 under “Supplemental data” in the online issue).

To avoid confounding effects for differences in total energy intakes between groups during the ACR period, a one-factor ANCOVA with total energy intake as a covariant was performed. There were 1338 significant genes ($P < 0.03; \eta < 0.15$) (see supplemental Table 3 under “Supplemental data” in the online issue). Importantly, 60 of the 88 transcripts with a VIP $>1$ in the multivariate model were also significant by ANCOVA with the unsuccessful populations ($n = 39$ and 38, respectively) (see supplemental Table 4 under “Supplemental data” in the online issue). Among these, 34 genes were up-regulated in unsuccessful subjects and down-regulated in successful subjects and 26 genes were up-regulated in successful subjects and down-regulated in unsuccessful subjects (see supplemental Figure 3 under “Supplemental data” in the online issue).

As suggested by exploratory analyses, differences in SAT gene expression patterns between successful and unsuccessful groups were mainly due to weight variations rather than to differences in diet macronutrient composition. To confirm this, a 2-factor ANCOVA with the 5 factor as a covariant was performed. No significant differences in gene expression were shown ($\eta > 0.90$). Microarray data were confirmed by reverse transcriptase–quantitative polymerase chain reaction (RT-qPCR) analysis for 31 genes. RT-qPCR was performed in the whole successful and unsuccessful populations ($n = 39$ and 38, respectively) (see supplemental Table 4 under “Supplemental data” in the online issue). There was a good concordance between microarray and RT-qPCR data. Changes in expression of all but 4 genes were confirmed.

**Biological and functional analysis**

The main biological processes involving the 1338 differential genes were evaluated by different approaches. For better data interpretation, we calculated the unsuccessful:successful ratio, and all functional analyses were performed by using this ratio. Biological functions analyzed by Ingenuity Pathway Analysis 7.5 software (Ingenuity Systems) showed that cellular growth and proliferation, gene expression, RNA posttranscriptional modification, and cellular function and maintenance functions were enriched in differential genes compared with the biological functions represented on the entire microarray (Figure 4A). The associated networks corresponded to cancer, cell death, gene expression, RNA posttranscriptional modification, and cellular functional modification gene functions (see supplemental Figure 4 under “Supplemental data” in the online issue). Genes in cellular growth and proliferation, gene expression, and cellular function and maintenance biological functions were more expressed in unsuccessful subjects, whereas genes associated with the RNA posttranscriptional modification biological function were more expressed in successful subjects (Figure 4B).

Pathways associated with differentially expressed genes were also assessed with GSEA v 2.0 software. The GSEA v 2.0 software showed 247 gene sets collected on the molecular signature database (MSigDB) of the GSEA software associated with the list of 1338 genes ranked in decreasing expression ratio between unsuccessful and successful subjects. Among these, 14 gene sets were enriched in the unsuccessful group (FDR $\leq 5\%$), and one gene set that was related to mitochondrial function (25) (MOOTHA_VOXPHOS gene set) was enriched in the successful group (FDR $= 2.0\%$) (see supplemental Table 5 under “Supplemental data” in the online issue). Among those gene sets that were enriched in unsuccessful subjects, AT_LONGITUDINAL_WEIGHT_LOSS_WS_DOWN and AT_LONGITUDINAL_WEIGHT_LOSS_DI_DOWN gene sets were in house–generated gene sets that corresponded to abdominal SAT genes down-regulated in subjects maintaining weight after LCD-induced weight loss (26). Four of the gene sets were enriched in successful subjects as follows: INOS_ALL_DN, TGF_BETA_SIGNALING_PATHWAY, NING_COPD_UP, JISON_SCICKLECELL_DIFF, and encompass inflammation-related genes. Two other gene sets, PROLIFERATION_GENES and CELL_PROLIFERATION, were related to cell proliferation.

**DISCUSSION**

An important challenge in obesity management is the long-term maintenance of weight reduction. New strategies for successful body weight maintenance after weight loss are required. To prevent weight regain, the knowledge of the mechanisms involved in weight control should first be elucidated. In the current study, we examined the change in SAT gene expression patterns 6 mo after LCD-induced weight loss. Our results showed differences between subjects who continue to lose weight and subjects who regained weight independent of the diet-macronutrient composition and total energy intake.

The DiOGenes program is a European, multicenter, dietary intervention trial in overweight and obese subjects. Low-fat diets with different protein amounts and GI were assigned to weight-reduced obese subjects (17, 18). Our main purpose was to investigate SAT mRNA to identify gene biomarkers of weight control during the ACR follow-up period. Subject selection was made to better identify genes that accounted for successful weight control without confounding effects that were due to extreme diet-induced weight variations and, in parallel, to search for gene-diet interactions without confounding effects that were due to weight variations. SAT transcriptome changes after the 6-mo ACR period were mainly due to weight variations independent of the diet macronutrient composition; however, it is possible that the subtle effects of varying macronutrient content were masked by weight variations. When subjects from the same group (ie, either the successful or unsuccessful group) were clinically matched across the 4 diet branches, a similar distribution in weight evolution was observed. More specifically, subjects were under conditions of weight regain ($\pm 2.6 \pm 1.2$ kg, which represented 27% of the weight lost while consuming the LCD) or loss (+3.9 $\pm 1.3$ kg, which represented 42% of the weight lost while consuming the LCD) for unsuccessful and successful subjects, respectively. SAT might respond to weight variations first and foremost, as seen in previous works in which ER was the main factor that accounted for changes in SAT gene expression (20, 27).

One of the features of the DiOGenes program was that post-ER diets were given ad libitum (18). As expected, energy intake in unsuccessful subjects was higher than in successful subjects during the 6-mo ACR period. Because SAT responds to energy...
<table>
<thead>
<tr>
<th>Gene symbol</th>
<th>Accession code</th>
<th>Description</th>
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<th>Unsuccessful:successful ratio</th>
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<td>NOMO1</td>
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(Continued)
intake on the basis of an organism’s energy requirements (20, 28), we performed an ANCOVA to avoid confounding effects related to differences in energy intake. The differentially expressed genes shown in the current study represent the expression patterns that differentiated subjects who continued to lose weight from subjects who regained weight, independent of total energy intake. Sixty significant genes were common to both ANCOVA (FDR < 5%) and multivariate analyses (VIP > 1). Although these genes do not belong to a common and unique biological function or pathway, and some of them are currently unknown genes, they do not belong to a common and unique biological function or pathway. Among these genes, E2F transcription factor 4 (E2F4) was up-regulated in unsuccessful subjects. E2F4 is a member of the E2F family of transcription factors. The E2F family plays a crucial role in the control of cell cycle. In SAT, the E2F family plays an important role during adipogenesis by regulating the expression of peroxisome proliferator-activated receptor γ. Specifically, E2F4 represses the expression of peroxisome proliferator-activated receptor γ during terminal adipocyte differentiation (29). The differential expression of E2F4 between successful and unsuccessful subjects suggested a possible deregulation of adipocyte differentiation between subjects who succeeded and subjects who failed weight maintenance. Moreover, 7 genes (i.e., RPL6, RPN1, EIF4A1, RPL0, RPLP0P2, LOC642875, and RL9) that coded for ribosomal proteins were also up-regulated in unsuccessful subjects. This may also suggest a deregulation of adipogenesis in unsuccessful subjects. This coincides with recent findings by Todorcevic et al (30), in which the authors showed that genes coding for ribosomal machinery were down-regulated in adipose tissue–derived stroma-vascular fraction cells that differentiated into adipocytes in Atlantic salmon.

For better interpretation of gene expression data, the 1338 differential genes were ranked according to decreasing fold change between unsuccessful and successful groups. The functional analysis of ranked gene lists showed opposite expression patterns between genes related to inflammation and cell proliferation and genes related to mitochondrial function.

Although genes associated with inflammatory pathways and cell proliferation were up-regulated in unsuccessful subjects and...
Because weight variations could mask possible diet-gene interactions, we are now characterizing the SAT transcriptome of subjects with minimal weight variations during the DiOGenes dietary intervention program.

We are grateful to Sébastien Déjean, Institut de Mathématiques de Toulouse Unité Mixte de Recherche 5219, Paul Sabatier University Toulouse, for advice in statistical analyses.

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