Gene expression profiling of human skeletal muscle in response to stabilized weight loss[1–3]

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ABSTRACT

Background: Diet-induced weight reduction promotes a decrease in resting energy expenditure that could partly explain the difficulty in maintaining reduced body mass. Whether this reduction remains after stabilized weight loss is still controversial, and the molecular mechanisms are unknown.

Objective: The objective was to investigate the effect of a stabilized 10% weight loss on body composition, metabolic profile, and skeletal muscle gene expression profiling.

Design: Obese women were assigned to a 4-wk very-low-calorie diet, a 3–6-wk low-calorie diet, and a 4-wk weight-maintenance program to achieve a 10% weight loss. Resting energy expenditure, body composition, plasma variables, and skeletal muscle transcriptome were compared before weight loss and during stabilized weight reduction.

Results: Energy restriction caused an 11% weight loss. Stabilization to the new weight was accompanied by an 11% decrease in the resting metabolic rate normalized to the body cellular mass. A large number of genes were regulated with a narrow range of regulation. The main regulated genes were slow/oxidative fiber markers, which were overexpressed, and the gene encoding the glucose metabolism inhibitor PDK4, which tended to be down-regulated. The knowledge-based approach gene set enrichment analysis showed that a set of genes related to long-term calorie restriction was up-regulated, whereas sets of genes related to insulin, interleukin 6, and ubiquitin-mediated proteolysis were down regulated.

Conclusions: Weight loss–induced decreases in resting metabolic rate persist after weight stabilization. Changes in skeletal muscle gene expression indicate a shift toward oxidative metabolism. *Am J Clin Nutr* 2008;88:125–32.

INTRODUCTION

Energy restriction is a useful method for achieving weight loss, but long-term maintenance of the reduced body mass is difficult to achieve. Dieting may have significant effects on body composition and metabolic rate, which lead to weight fluctuation. The rate of weight loss decreases with time when the same hypocaloric diet is maintained. The body mass lost during dieting consists of both fat and lean tissue. This is accompanied by a rapid reduction in energy expenditure that, in turn, exacerbates the tendency to regain lost weight (1). The mechanisms controlling the decrease in resting energy expenditure observed during weight loss are not fully understood. The resting metabolic rate (RMR) is the largest portion of total daily energy expenditure and represents the energy spent for the maintenance of basic body functions and homeostasis. RMR is primarily related to the magnitude of lean body mass (LBM). Although skeletal muscle mass contributes only 20% of the RMR, skeletal muscle metabolism is a major determinant of variability in resting energy expenditure (2). Previous studies that aimed to examine the effects of altered body weight were undertaken during dynamic weight change and most of them showed that weight stability was not achieved. As such, examining the steady state after weight reduction is equally important, because, during the dynamic phase of weight reduction, many metabolic and hormonal perturbations may confound the adaptations occurring during weight loss itself.

Gene expression profiling combined with clinical investigations permit a novel approach to understanding physiologic and pathophysiological processes (3). The investigation of the skeletal muscle transcriptome may be the key for understanding metabolic diseases. Several groups, including ours, have successfully used DNA microarrays to examine human skeletal muscle mRNA expression in vivo after physiologic and hormonal challenges, which have led to the discovery of unexpected pathways (4–8). Little is known regarding the mechanisms that

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TABLE 1
Body composition and indirect calorimetry[1]

	Before weight loss	After weight loss	*P* value
Weight (kg)	88.8 ± 10.0	78.7 ± 9.6	0.005[2]
BMI (kg/m^2)	33.8 ± 2.3	29.7 ± 2.1	0.005[2]
Fat mass (kg)	42.6 ± 6.4	34.2 ± 6.7	0.005[2]
LBM (kg)	43.6 ± 5.2	42.2 ± 5.0	0.037[2]
BMC (kg)	2.9 ± 0.3	2.8 ± 0.3	0.005[2]
ECW (L)	18.1 ± 2.2	18.0 ± 2.2	0.594[2]
BCM (kg)	26.2 ± 3.7	24.7 ± 3.6	0.028[2]
Actual RMR (kJ/d)	7077 ± 769	5956 ± 882	0.005[2]
Predicted RMR (kJ/d)		6795 ± 519	0.01[3]
RMR/BCM (kJ · d^{-1} · kg^{-1})	264 ± 9	244 ± 2	0.022[2]
RRQ	0.84 ± 0.05	0.85 ± 0.04	0.89[2]

[1] All values are $\bar{x}$ ± SD; n = 10. LBM, lean body mass; BMC, bone mineral content; RMR, resting metabolic rate; ECW, extracellular water; BCM, body cellular mass; RMR/BCM, RMR normalized for BCM; RRQ, resting respiratory quotient. Linear regression was used to generate equations for predicting RMR using BCM. For 10 subjects, actual RMR before weight reduction = 34.62 × BCM + 765, R^2 = 0.4058. Predicted RMR after weight reduction was calculated by using BCM after weight reduction.

[2] Comparison with obese before weight loss.

[3] Comparison between actual and predicted RMR after weight reduction.

connect RMR to skeletal muscle gene expression in humans. To the best of our knowledge, no studies have examined global mRNA expression after stabilized weight loss.

The goal of the present study was to investigate the effect of stabilized 10% weight loss on body composition, the metabolic profile, and skeletal muscle gene expression in obese women. To gain insight into the molecular basis of the adaptive mechanisms to stabilized weight loss, we performed skeletal muscle gene expression profiling in obese women before and after stabilized weight loss.

SUBJECTS AND METHODS

Subjects

Ten obese, white, premenopausal women aged 27–48 y with stable weight over the previous 3 mo were recruited at the Toulouse Clinical Investigation Center. The subjects were consuming their usual diet before the study, and none were engaged in physical activity training. The characteristics of the subjects are presented in **Table 1**. The protocol was approved by the Ethics Committee of Toulouse University Hospitals, and all subjects gave written informed consent.

Study design

The program of weight loss included 3 phases. The first phase involved a 4-wk very-low-calorie liquid-formula diet (3.3 MJ/d, GAYELORD HAUSER, Distriborg France, Saint Genis Laval, France). The formula included 62 g protein, 104 g carbohydrate, and 13 g fat. The second phase involved a 3- to 6-wk low-calorie diet (4–5 MJ/d, 15% protein, 50% carbohydrate, and 35% lipid) until the subjects achieved a 10% reduction in initial body weight. The last phase was a 4-wk isocaloric diet (mean: 5.8 MJ/d; 15% protein, 50% carbohydrate, and 35% lipid), planned to stabilize the reduced body weight. The subjects were instructed to continue with their usual level of physical activity during the study.

The same investigations were performed before and after the weight-loss program as follows. After the subjects fasted overnight for 12 h and rested for 45 min, oxygen consumption and carbon dioxide production were monitored over 30 min with an open-circuit ventilated-canopy system (Deltatrac II monitor; Datex Instrumentarium Corp, Helsinki, Finland). RMR was derived from oxygen consumption and carbon dioxide production using indirect calorimetry. Blood samples were drawn for determinations of hormonal and metabolic variables, and a percutaneous biopsy sample of the vastus lateralis muscle was obtained as previously described (5). Body composition was assessed by dual-energy X-ray absorptiometry performed with a total body scanner (DPX, Software 3.6; Lunar Radiation Corp, Madison, WI) (9). The extracellular water compartment was determined by bromide dilution. Bromide concentration in serum ultrafiltrate was determined with HPLC, and the corrected bromide space was calculated according to Miller et al (10).

Plasma biochemical determinations

Blood glucose was assayed by using the glucose oxidase technique (Biotrol, Paris, France). Plasma nonesterified fatty acids were assayed with an enzymatic method (Unipath, Dardilly, France). Serum insulin was measured by using a Bi-insulin immunoradiometric assay kit (Sanofi Diagnostics Pasteur, Marne-La-Coquette, France). Adiponectin, interleukin-6 (IL-6), and leptin were measured with Quantikine enzyme-linked immunosorbent assay tests (R&D Systems, Minneapolis, MN). Other variables were determined by using standard clinical biochemistry methods.

Total RNA preparation and amplification

Skeletal muscle biopsy samples were obtained at the beginning and at the end of the protocol. Total RNA from frozen biopsy samples was prepared and amplified as previously described (5). Total and amplified RNA (aRNA) concentrations and quality checks using the Agilent 2100 bioanalyzer and RNA 6000 labChip Kit (Agilent Technologies, Massy, France) led to the exclusion of 2 samples. A flow chart of the sample processing and decision tree is presented in **Figure 1**.

Probe labeling and microarray hybridization

Fluorescent probes were synthesized from 1 μg of aRNA by using the CyScribe First Strand cDNA Labeling Kit (Amersham Biosciences, Orsay, France). Cy3 (before weight reduction) and Cy5 (after weight reduction) probes from the same subject were purified and hybridized as described by Viguerie et al (5). The arrays were scanned by using a GenePix 4000B scanner (Axon Instruments, Union City, CA).

Microarray analysis

The cDNA microarrays produced at Stanford University (Internet: http://genome-www5.stanford.edu) consisted of polymerase chain reaction (PCR)–amplified cDNAs printed on glass slides with 41 473 cDNA spots representing 23 267 genes listed in the unigene database. The images were analyzed as previously described (5). The quality of the hybridization and of the lowess normalization was checked by using an M/A plot. In the end, we

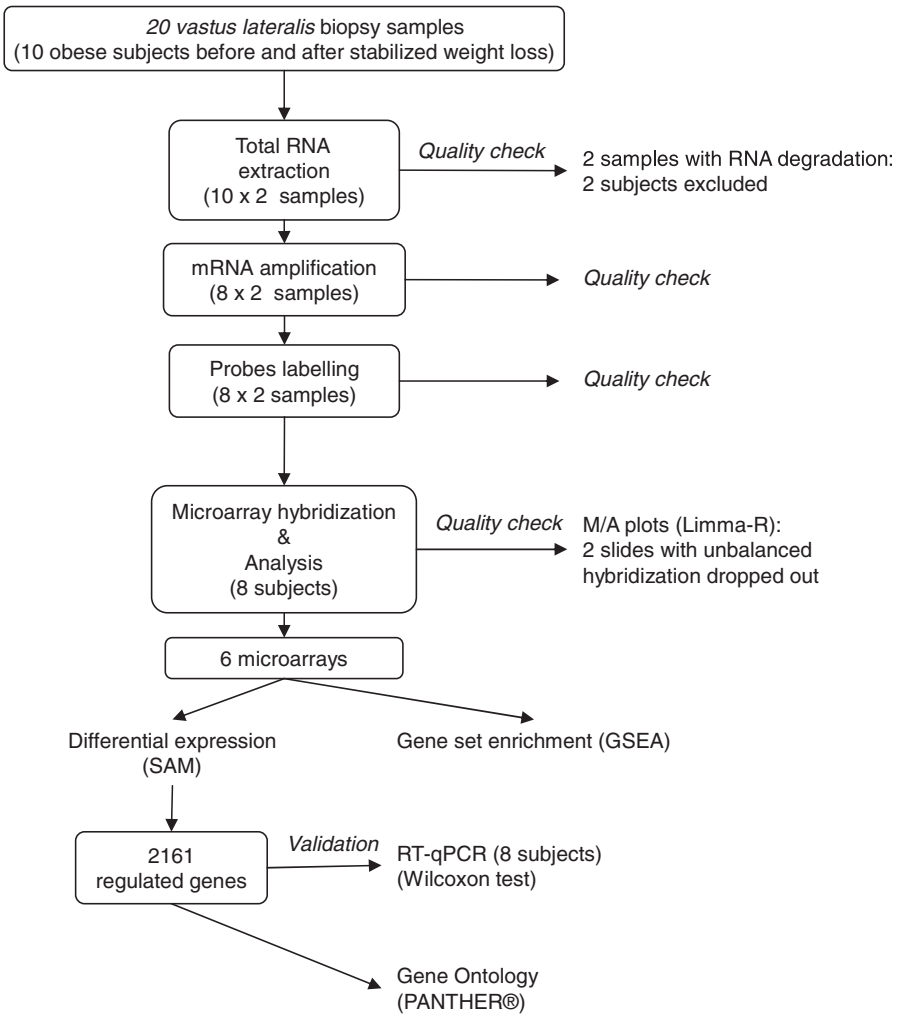

FIGURE 1. Flow chart of sample processing and decision tree. A brief description of the different steps of the analyses is given in Methods.

performed the statistical analysis using microarray data from 6 different subjects. These 6 subjects did not differ from the whole group regarding their decrease in RMR. The mean fold change in expression for a gene is the ratio of mean signal values from 6 experiments; 23 267 spots were exploitable in ≥5 of 6 slides. Data were analyzed by using the one-class significance analysis of microarray (SAM) procedure (11). To analyze the function of the differential genes, we used the PANTHER (Protein ANalysis THrough Evolutionary Relations) database (Internet: http://panther.appliedbiosystems.com). Biological processes over-represented among the list of differentially expressed genes were considered significant if $P < 0.05$, as determined by the binomial statistic (12).

To test for the enrichment of sets of genes in expression data sets, we used version 2.0.1 of the computational method Gene Set Enrichment Analysis (GSEA) (13) and the 1687 curated gene sets from the c2 collection of the Molecular Signatures Database, MSigDB (http://www.broad.mit.edu/gsea/). GSEA comparison of skeletal muscle gene expression before and after stabilized weight loss was performed using the signal-to-noise ratio metric of 19 738 features to produce rank-ordered gene lists in the human data set.

Real-time quantitative PCR

Reverse transcription was performed with the use of 1 μg aRNA for each sample from the 8 patients, as previously described (5). Primers were designed by using the Primer express software (*see* Table S1 under "Supplemental data" in the online issue). We used the geometric mean of TATA-box binding protein and hypoxanthine phosphoribosyl transferase 1 (Taqman Control Assays; Applied Biosystems, Foster City, CA) mRNA concentrations to normalize gene expression.

Conventional statistical analysis

General statistical analysis was performed by using SPSS for WINDOWS 13.0 (SPSS Inc, Chicago, IL). Significant differences were determined by nonparametric paired Wilcoxon's test and were considered significant if $P < 0.05$.

Microarray data set

The data discussed in this publication were deposited in NCBIs Gene Expression Omnibus (GEO; Internet: http://www.ncbi.nlm.nih.gov/geo/) and are accessible through GEO Series accession number GSE9917.

TABLE 2
Plasma variables[1]

	Before weight loss	After weight loss and stabilization	*P* value
Glucose (mmol/L)	5.29 ± 0.69	5.24 ± 0.33	0.81
Insulin (mU/L)	10.00 ± 4.01	8.00 ± 2.00	0.11
QUICKI	0.33 ± 0.03	0.35 ± 0.01	0.17
Total cholesterol (g/L)	1.88 ± 0.38	1.70 ± 0.31	0.01
HDL cholesterol (g/mL)	0.41 ± 0.07	0.42 ± 0.10	0.63
LDL cholesterol (g/L)	1.24 ± 0.30	1.10 ± 0.24	0.25
Triacylglycerol(g/L)	1.11 ± 0.48	0.87 ± 0.49	0.14
NEFA (mmol/L)	0.53 ± 0.19	0.39 ± 0.13	0.14
TSH (mU/L)	1.79 ± 0.86	1.52 ± 0.62	0.09
Free T_3 (pg/mL)	3.72 ± 0.36	4.67 ± 3.7	0.55
Free T_4 (pg/mL)	13.0 ± 1.4	11.6 ± 3.2	0.17
Leptin (ng/mL)	50.1 ± 25.2	24.2 ± 12.6	0.01
Adiponectin (μg/mL)	5.4 ± 2.9	6.0 ± 3.6	0.26
CRP (mg/L)	4.84 ± 4.10	4.04 ± 4.90	0.11
IL-6 (pg/mL)	2.81 ± 1.50	1.63 ± 0.42	0.02

[1] All values are $\bar{x}$ ± SD; $n = 10$. QUICKI, quantitative insulin sensitivity check index; NEFA, nonesterified fatty acids; CRP, C-reactive protein; IL-6, interleukin 6; TSH, thyrotropin; T_3, triiodothyronine; T_4, thyroxine.

RESULTS

Effects of a nutritional challenge on anthropometric, metabolic, and plasma variables

Ten obese women (BMI, in kg/m^2: 34 ± 2) were submitted to an energy-restricted diet until they lost 10% of their initial body weight. They were then fed an isocaloric diet for 4 wk. As targeted, the mean weight loss was 11.4 ± 2.9%, whereas the decrease in fat mass reached 20.1 ± 5.5% (Table 1). Stabilized weight reduction induced a slight but significant decrease in LBM. Because variations in LBM can be due to variations in extracellular water (14), this compartment was determined by using bromide dilution space assessment and was taken into account when calculating body cellular mass (BCM = LBM − extracellular water). The decrease in body weight was accompanied by a significant decrease in RMR, which remained significant even after normalization of RMR using BCM (RMR/BCM). Because the intercept of the regression line between RMR and BCM did not pass through zero, we calculated the equation of the regression line before weight reduction and used this equation to calculate the predicted RMR after weight reduction. After weight loss, the actual RMR was significantly lower than predicted RMR. The resting respiratory quotient was not affected by weight reduction.

As expected, weight reduction lowered plasma total cholesterol, leptin, and IL-6 in obese subjects (**Table 2**). Plasma glucose did not change, and insulin was not significantly decreased after the stabilized weight loss. Thyroid status was not affected by the loss of weight.

Changes in skeletal muscle mRNA expression

Gene expression profiling was carried out to gain insight into the molecular mechanisms of the adaptive process that take place in skeletal muscle after stabilized weight loss. Total RNA was considered correct for before and after samples from each of the 8 subjects out of the 10 enrolled in the program. Gene expression profiling analysis was performed using data from 6 subjects. A flow chart of the microarray-based gene expression analysis is shown in Figure 1.

SAM resulted in the selection of 2839 differentially expressed cDNA with a false discovery rate (FDR) of 5%. These cDNA represented 2161 genes listed in the UniGene database; 1623 cDNAs were down-regulated and 1216 were up-regulated (*see* Table S2 and S3 under "Supplemental data" in the online issue). We found no change in adipose-specific transcripts such as leptin, adiponectin, lipin, or perilipin, which indicated that the changes in transcript levels observed here were not due to changes in the putative adipose tissue content of skeletal muscle biopsy samples. Of these 2161 genes, 1902 were found in the PANTHER database. Fourteen biological processes were specifically overrepresented in this list (**Figure 2**). Between 25% and 50% of the genes belonging to these selected biological processes were differentially expressed after stabilized weight loss. The extent of variation in gene expression was low, as >98% of the significant genes had <60% of variation (equivalent to a 1.6-fold change). Selection of genes with more than a 1.5-fold increase or decrease in expression level resulted in the selection of 102 cDNA (34 down-regulated and 68 up-regulated), including 78 assigned in the UniGene database (**Table 3** and **Table 4**).

Microarray data validation of genes selected among the most relevant biological processes was performed on 8 subjects with good-quality RNA using real-time quantitative PCR (RT-qPCR) (**Table 5**). We confirmed the changes observed for troponin I type 1 (TNNI1), troponin T type 1 (TNNT1), troponin C type 1 (TNNC1; $P = 0.07$), NADH dehydrogenase (ubiquinone) 1 α subcomplex (NDUFA13/GRIM-19), myosin light chain 3 (MYL3, $P = 0.09$), and pyruvate dehydrogenase kinase isozyme 4 (PDK4, $P = 0.07$). The up-regulated genes encoded slow/oxidative skeletal muscle (TNNI1, TNNT1, TNNC1, and MYL3) or mitochondrial markers (NDUFA13). The mRNA level of PDK4, the inhibitor of the pyruvate dehydrogenase complex, tended to decrease during weight loss.

Because most of the genes had a mean fold change of <1.5, we applied the GSEA method. We searched the c2 collection of MSigDB, which includes metabolic and signaling pathways as well as gene expression signatures of genetic and chemical perturbations coming from experimental results in the literature. Because of the large number of down-regulated pathways according to the criteria recommended by Subramanian et al (FDR < 25%) (13), we increased the stringency of the analysis and selected 34 gene sets with an FDR ≤ 10% (*see* Table S4 and S5 under "Supplemental data" in the online issue). Of the top ranked sets, only 2 were specifically designed from skeletal muscle gene expression data, CALRES_MOUSE_UP and ROME_INSULIN_2F_UP. The first one contains genes that were overexpressed in the gastrocnemius muscle of aged mice subjected to long-term energy restriction (15) (*see* Table S6 under "Supplemental data" in the online issue). As expected, in the present study, CALRES_MOUSE_UP gene set was up-regulated after the stabilized weight loss. The genes from the ROME_INSULIN_2F_UP gene set were up-regulated in human vastus lateralis muscle during a 3-h hyperinsulinemic euglycemic clamp (7) (*see* Table S7 under "Supplemental data" in the online issue). Here, this gene set was more expressed in the skeletal muscle before weight loss. Of the top ranked gene sets,

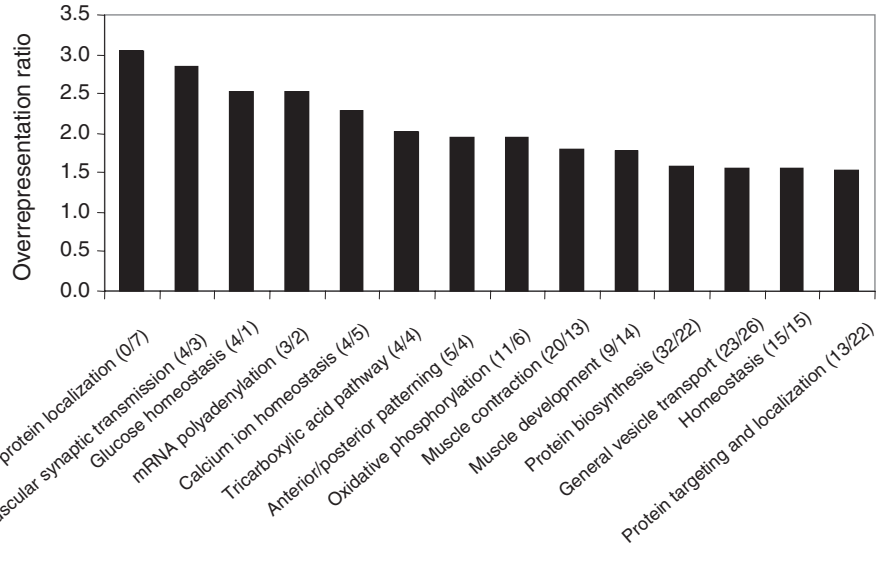

FIGURE 2. Biological processes significantly overrepresented among the list of differentially expressed skeletal muscle mRNA during weight stabilization. The number of up- and down-regulated genes in each process, respectively, is shown in parentheses. The ratios represent the numbers of differentially expressed genes divided by the number of expected genes that is the number of genes randomly expected for this biological process based on the draw of 1902 genes from the 11957 unique transcripts detected on the arrays. The biological processes with a ratio > 1.5 are shown.

2 sets related to IL6, KRETZSCHMAR_IL6_DIFF and BROCKE_IL6, included genes down-regulated after stabilized weight loss. The UBIQUITIN_MEDIATED_PROTEOLYSIS gene set was also down-regulated after weight loss.

DISCUSSION

The adaptive mechanisms to weight reduction remain poorly understood in humans. Here, we combined a comprehensive study of the metabolic adaptations to a stabilized weight loss with a transcriptomic analysis of skeletal muscle to assess the molecular mechanisms occurring along with the reduction in body weight. The adaptations that took place at the skeletal muscle gene expression level suggested an improved aerobic capacity.

Long-term maintenance of weight loss appears to be a crucial obstacle for most people (16). Weight fluctuation has led to speculation that metabolic efficiency increases after weight reduction and then predisposes individuals to regain the weight they have lost (1). Although a proportional decrease in metabolic rate is expected with a loss of LBM, a greater than expected decrease in metabolic rate has been hypothesized to explain weight fluctuations (1, 17). The extent to which the change in RMR persists after weight stabilization remains controversial. Some studies have suggested that there is a persistent decrease in energy expenditure after weight loss (18, 19), whereas other showed no change (20–22). To address this debate, we aimed at a focused assessment of body composition and thus of RMR adjusted for BCM. In agreement with previous investigations (1, 18, 19), the present study showed that an 11% body weight loss was accompanied by an 11% decrease in RMR adjusted for BCM. It has been proposed that changes in the circulating concentrations of metabolic hormones are involved in the adaptation to reduced calorie intake (23). A possible effect of the decrease in the circulating concentration of the thermogenic hormone leptin remains controversial in humans (24). Indeed, leptin administration reverses the decrease in total energy expenditure but does not modify RMR (25). Thyroid status was not modified by weight loss and cannot explain the decrease in RMR observed in the present study.

The precise determination of the change in RMR constituted a valid basis for deciphering the molecular adaptations occurring in skeletal muscle during steady weight loss accompanied with a decrease in RMR. After weight loss, ≈9% of the skeletal muscle transcriptome was affected, but the magnitude of the changes was modest. A low level of regulation is often observed in vivo in human transcriptomic studies as has been reported in skeletal muscle (4, 5, 7, 26). We previously used microarrays to study in vivo changes in the gene expression of human skeletal muscle after intravenous epinephrine infusion (5). Of the ≈40 000 transcripts studied, only 4% were regulated by the catecholamine. Although the hormone was infused for 6 h, the magnitude of the skeletal muscle gene response to epinephrine was small, as $<1\%$ of the catecholamine-responsive genes exhibited more than a 2-fold change in expression.

In the present study, 14 biological processes were significantly altered by weight loss. Of them, glucose homeostasis, the tricarboxylic acid pathway, and oxidative phosphorylation seem to be strongly related to the metabolic rate. Unfortunately, of the 5 regulated genes belonging to the glucose homeostasis biological process (glucagon-like peptide 1 receptor, suppressor of cytokine signaling 7, Fem-1 homolog a, glycoprotein hormones α polypeptide, protein tyrosine phosphatase, and non-receptor type1; data not shown), only protein tyrosine phosphatase seems to be related to glucose utilization. The implication of the 8 genes belonging to the tricarboxylic acid cycle in the metabolic rate is most obvious, but 4 were up-regulated and 4 were down-regulated. Similarly, of the 17 regulated genes belonging to the oxidative phosphorylation pathway, 11 were up-regulated and 6 were down-regulated. This kind of analysis did not allow us to determine which gene was clearly involved in the decrease in RMR.

TABLE 3
Up-regulated genes during weight loss in vastus lateralis muscle[1]

Gene name	Gene symbol	Mean fold change
LAG1 homolog, ceramide synthase 5	*LASS5*	2.36
Troponin T type 1	*TNNT1*	2.12
Ribosomal protein S6 kinase, 90 kDa, polypeptide 4	*RPS6KA4*	1.84
Troponin C type 1	*TNNC1*	1.83
Glycophorin A	*GYPA*	1.69
Zinc finger with KRAB and SCAN domains 1	*ZKSCAN1*	1.68
Vacuolar protein sorting 45A	*VPS45A*	1.67
Desmocollin 1	*DSC1*	1.66
Troponin I type 1	*TNNI1*	1.66
Hypothetical protein FLJ21816	*FLJ21816*	1.65
T, brachyury homolog	*T*	1.63
Adaptor-related protein complex 1, gamma 2 subunit	*AP1G2*	1.62
α -2-macroglobulin	*A2 M*	1.61
SET domain containing 4	*SETD4*	1.61
Eukaryotic translation initiation factor 4A, isoform 1	*EIF4A1*	1.60
Non-metastatic cells 1, protein (NM23A) expressed in	*NME1*	1.60
Ring finger protein 152	*RNF152*	1.60
RNA binding motif protein 19	*RBM19*	1.60
Myosin, light polypeptide 3, alkali; ventricular, skeletal, slow	*MYL3*	1.59
Major histocompatibility complex, class I, E	*HLA-E*	1.58
Ribosome binding protein 1 homolog 180 kDa	*RRBP1*	1.58
UDP-Gal:betaGlcNAc β 1,4- galactosyltransferase, polypeptide 1	*B4GALT1*	1.58
Hypothetical protein LOC645757	*LOC645757*	1.57
RAB6 interacting protein 2	*RAB6IP2*	1.57
Transmembrane and coiled-coil domain family 1	*TMCC1*	1.57
Glutamate receptor, ionotropic, *N*-methyl D-aspartate 2A	*GRIN2A*	1.56
Interferon regulatory factor 5	*IRF5*	1.56
Myosin, heavy polypeptide 11, smooth muscle	*MYH11*	1.56
A kinase (PRKA) anchor protein 8-like	*AKAP8L*	1.55
Coiled-coil domain containing 69	*CCDC69*	1.55
Ring finger protein 13	*RNF13*	1.55
Hypothetical protein BC008326	*LOC89944*	1.54
NADH dehydrogenase (ubiquinone) 1 α subcomplex, 13	*NDUFA13*	1.54
WD repeat and SOCS box-containing 1	*WSB1*	1.54
Adenylate kinase 1	*AK1*	1.53
ATPase type 13A3	*ATP13A3*	1.53
BAI1-associated protein 2-like 1	*BAIAP2L1*	1.53
Pre-mRNA cleavage factor I, 59 kDa subunit	*FLJ12529*	1.53
Spondin 2, extracellular matrix protein	*SPON2*	1.53
Sterile alpha motif domain containing 14	*SAMD14*	1.53
DEAD (Asp-Glu-Ala-Asp) box polypeptide 42	*DDX42*	1.52
PI-3-kinase-related kinase SMG-1	*SMG1*	1.52
SECIS binding protein 2	*SECISBP2*	1.52
ST3 β-galactoside α-2,3-sialyltransferase 2	*ST3GAL2*	1.52
Trafficking protein particle complex 3	*TRAPPC3*	1.52
Klotho β	*KLB*	1.51
Ring finger protein 19	*RNF19*	1.51
RNA binding motif protein 5	*RBM5*	1.51
Testes-specific heterogenous nuclear ribonucleoprotein G-T	*HNRNPG-T*	1.51
Flavin containing monooxygenase 4	*FMO4*	1.50

[1] The genes were selected by using significance analysis of microarray with a false discovery rate <0.05 and a mean fold change >1.5.

GSEA analysis was designed to study discrete variations in gene expression (13). This strategy is a knowledge-based approach that uses gene sets defined on the basis of prior biological knowledge. The present GSEA analysis showed variations in sets of genes involved in response to long-term calorie restriction, IL-6, insulin, and ubiquitin mediated proteolysis. Down-regulation of gene sets involved in IL-6 and insulin response agrees with the observed decrease in plasma insulin and IL-6, stressing that GSEA is an appropriate method to analyze microarray data from clinical studies. Down-regulation of the ubiquitin-mediated proteolysis gene set, as revealed by GSEA and SAM analysis, is related to the presence of 3 ubiquitin-conjugating enzymes in the list of most down regulated genes (Table 4) and could be responsible for a decrease in protein turnover explaining the observed decrease in RMR. Indeed, the protein turnover contributes to $\approx$20% of the basal metabolic rate,

TABLE 4
Down-regulated genes during weight loss in vastus lateralis muscle[1]

Gene name	Gene symbol	Mean fold change
CAP, adenylate cyclase-associated protein, 2	*CAP2*	0.54
Ubiquitin-conjugating enzyme E2D 1	*UBE2D1*	0.54
Stress-associated endoplasmic reticulum protein 1	*SERP1*	0.56
Sarcolipin	*SLN*	0.58
Ubiquitin-conjugating enzyme E2B	*UBE2B*	0.58
Syntaxin 3	*STX3*	0.59
General transcription factor IIIC, polypeptide 2, β	*GTF3C2*	0.60
Chaperonin containing TCP1, subunit 8 (θ)	*CCT8*	0.61
Heterogeneous nuclear ribonucleoprotein D-like	*HNRPDL*	0.61
Ubiquitin-conjugating enzyme E2E 3	*UBE2E3*	0.61
Coagulation factor II (thrombin)	*F2*	0.62
Peroxiredoxin 3	*PRDX3*	0.62
Four and a half LIM domains 1	*FHL1*	0.63
Nexilin	*NEXN*	0.63
Poly(rC) binding protein 1	*PCBP1*	0.63
Armadillo repeat containing, X-linked 6	*ARMCX6*	0.64
Dimethylarginine dimethylaminohydrolase 1	*DDAH1*	0.64
E74-like factor 2	*ELF2*	0.64
Pyruvate dehydrogenase kinase, isozyme 4	*PDK4*	0.64
Trophoblast-derived noncoding RNA	*TncRNA*	0.64
Eukaryotic translation initiation factor 1A pseudogene 1	*EIF1AP1*	0.65
Leucine rich repeat containing 2	*LRRC2*	0.65
ELAV-like 2	*ELAVL2*	0.66
Mortality factor 4 like 2	*MORF4L2*	0.66
RAP2A, member of RAS oncogene family	*RAP2A*	0.66
Shwachman-Bodian-Diamond syndrome pseudogene	*SBDSP*	0.66
Similar to mouse 2310016A09Rik gene	*LOC134147*	0.66
Ubiquitin carboxyl-terminal esterase L1	*UCHL1*	0.66

[1] The genes were selected by using significance analysis of microarray with a false discovery rate <0.05 and a mean fold change <0.66.

and the synthesis and breakdown of muscle protein are principally responsible for the energy expenditure of resting muscle (27, 28).

PDK4 was the only gene implicated in glucose metabolism, which was down-regulated by weight loss. It encodes an enzyme that inactivates the pyruvate dehydrogenase complex, which links glycolysis to the mitochondrial tricarboxylic acid cycle and ATP production (29). Reduced PDK4 expression was previously shown to be associated with an increased insulin sensitivity in previously obese patients (30). The decrease in PDK4 expression indicates a probable enhancement of glucose oxidation by increasing pyruvate dehydrogenase activity, although the resting respiratory quotient was not affected by weight loss.

In the present study, the majority of genes regulated during weight loss are involved in muscular contraction. The vastus lateralis skeletal muscle contains mixed slow and fast fibers. A shift in skeletal muscle fiber type occurs in various physiologic conditions, such as aging or physical training (31, 32). The increase in mRNA of all 3 subunits of the slow fiber specific troponin TNNI1, TNNT1, and TNNC1; the slow fiber specific myosin light chain MYL3; and the NADH dehydrogenase subcomplex NDUFA13 suggest an enhanced muscular aerobic capacity. Troponins and myosin are involved in muscular contraction. The function of NDUFA-13 in the respiratory chain complex I is not yet fully understood. The implication of the products of these genes in lowering RMR remains to be investigated.

Overexpression of FOXO1, a known regulator of metabolism from the FOXO family of transcription factors, has been shown to regulate type I fiber genes, including TNNC, TNNT, and

TABLE 5
Comparison of fold changes in mRNA concentrations in 8 selected genes determined by real-time quantitative polymerase chain reaction (RT-qPCR)

UniGene name	UniGene symbol	Array ratio	RT-qPCR ratio (after/before)	*P* value[1]
Troponin T type 1	*TNNT1*	2.12	1.45	0.04
Troponin C type 1 (slow)	*TNNC1*	1.83	1.25	0.07
Troponin I type 1	*TNNI1*	1.66	1.48	0.01
Myosin, light chain 3	*MYL3*	1.59	1.39	0.09
NADH dehydrogenase 1 α subcomplex, 13	*NDUFA13*	1.54	1.30	0.01
Adenylate kinase 1	*AK1*	1.53	1.22	0.21
Pyruvate dehydrogenase kinase, isozyme 4	*PDK4*	0.64	0.52	0.07
Sarcolipin	*SLN*	0.58	0.85	0.21

[1] RT-qPCR data were compared by using nonparametric Wilcoxon's test ($n = 8$).

myosin light chain among other transcripts (33). Skeletal muscle FOXO1 expression negatively regulates glucose metabolism via activation of PDK4 transcription (34). In the present study, PDK4 mRNA concentrations were down-regulated after weight loss. The lack of change in FOXO1 gene expression after weight loss (data not shown), combined with the absence of correlation between regulation of PDK4 and troponin genes, does not favor the implication of FOXO1.

To summarize, the present study confirmed that steady weight loss in humans is accompanied by a decrease in RMR adjusted to BCM. This decrease is concomitant with a significant modification of skeletal muscle transcriptome. The causal relation between the decrease in RMR and the changes in skeletal muscle gene expression remain to be clarified, but the present study provides new insight about the general transcriptomic adaptation occurring in muscle during and after stabilized weight loss.

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