Contribution of Energy Restriction and Macronutrient Composition to Changes in Adipose Tissue Gene Expression during Dietary Weight-Loss Programs in Obese Women

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Context: Hypoenergetic diets are used to reduce body fat mass and metabolic risk factors in obese subjects. The molecular changes in adipose tissue associated with weight loss and specifically related to the dietary composition are poorly understood.

Objective: We investigated adipose tissue gene expression from human obese women according to energy deficit and the fat and carbohydrate content of the diet.

Design and Setting: Obese subjects recruited among eight European clinical centers were followed up 10 wk of either a low-fat (high carbohydrate) or a moderate-fat (low carbohydrate) hypoenergetic diet.

Subjects: Two sets of 47 women in each dietary arm were selected among 648 subjects matched for anthropometric and biological parameters.

Main Outcome Measure: We measured adipose tissue gene expression changes in one set using a candidate gene approach. The other set was used to survey 24,469 transcripts using DNA microarrays. Results were analyzed using dedicated statistical methods. Diet-sensitive regulations were confirmed on the other set of subjects.

Results: The two diets induced similar weight loss and similar changes for most of the biological variables except for components of the blood lipid profile. One thousand genes were regulated by energy restriction. We validated an effect of the fat to carbohydrate ratio for five genes (*FABP4*, *NR3C1*, *SIRT3*, *FNTA*, and *GABARAPL2*) with increased expression during the moderate-fat diet.

Conclusions: Energy restriction had a more pronounced impact on variations in human adipose tissue gene expression than macronutrient composition. The macronutrient-sensitive regulation of a subset of genes may influence adipose tissue function and metabolic response. (*J Clin Endocrinol Metab* 93: 4315–4322, 2008)

Obesity is a global health problem of increasing severity because of the associated risk of life-threatening health complications such as cardiovascular disease, type 2 diabetes, and cancer. Moderate weight loss has repeatedly proved to be beneficial with a reduced risk of type 2 diabetes (1). It is widely recommended to decrease energy intake to reach these objectives, but there remains a long-standing debate over the optimal

macronutrient composition of the diet. Various weight loss outcomes have been reported with hypoenergetic diets with different amounts of fat and carbohydrate (2–4).

Proper glucose and lipid homeostasis depends on adipose tissue metabolism, which is altered in response to obesity-induced changes in adipose tissue gene expression. Modification of gene expression toward a nonobese profile could contribute to the

Abbreviations: aRNA, Amplified RNA; BMI, body mass index; Cy, cyanine dyes; HOMA, homeostasis model assessment; Inserm, Institut National de la Santé et de la Recherche Médicale; LF, low-fat, high-carbohydrate diet; MF, moderate-fat, low-carbohydrate diet; NUGENOB, Nutrient-Gene Interaction in Human Obesity; RT-qPCR, RT-quantitative PCR; SAM, significance analysis of microarray.

beneficial effects of weight loss (5). *In vitro* studies identified several classes of transcription factors mediating the impact of fat and glucose on the regulation of gene expression (6, 7). Moreover, much progress has been achieved in the understanding of the metabolic effects of calorie restriction (8). However, the *in vivo* molecular adaptations of adipose tissue during energy deprivation and in response to macronutrients are mostly unknown. The characterization of dietary signatures that influence the risk of diet-related diseases may help in identifying new potential risk factors and indicators of effects of dietary components (9). Such information is lacking for dietary treatment of obesity. The complexity of the response of obese subjects suggests that large cohorts and specific technical and statistical tools should be used.

The Nutrient-Gene Interaction in Human Obesity (NUGENOB) study trial, a large randomized intervention carried out in 771 obese subjects to compare the effects of a low-fat/high-carbohydrate and a moderate-fat/low-carbohydrate hypoenergetic diet, is ideally suited to address the respective influence of calorie restriction and macronutrient composition on adipose tissue gene expression (10). Two gene expression studies have already been performed in the NUGENOB program. On a subset of the cohort and without a whole genome approach, a limited number of genes were shown regulated by calorie restriction, and none could be identified as diet-sensitive genes (11, 12). Using the two dietary arms of the NUGENOB cohort and a pangenomic DNA microarray approach combined with specific statistical and bioinformatic tools, we investigated the regulation of transcript levels according to energy deficit and the fat to carbohydrate ratio. The work was completed by a candidate gene approach and cross-validation on an independent set of subjects using RT-quantitative PCR (RT-qPCR).

Subjects and Methods

Subjects and study design

Subjects were participants in the European multicenter NUGENOB study (www.nugenob.org). Subjects were randomly assigned to 10-wk dietary intervention of two hypoenergetic diets: a low-fat, high-carbohydrate diet (LF) or a moderate-fat, low-carbohydrate diet (MF). Both diets were designed to provide 600 kcal/d less than the individual's estimated energy requirement. Fat provided 20–25 and 40–45%, carbohydrates provided 60–65 and 40–45% of total energy in the LF and the MF diet, respectively. Both diets derived 15% of total energy from protein. Details on volunteer recruitment, dietary intervention, and com-
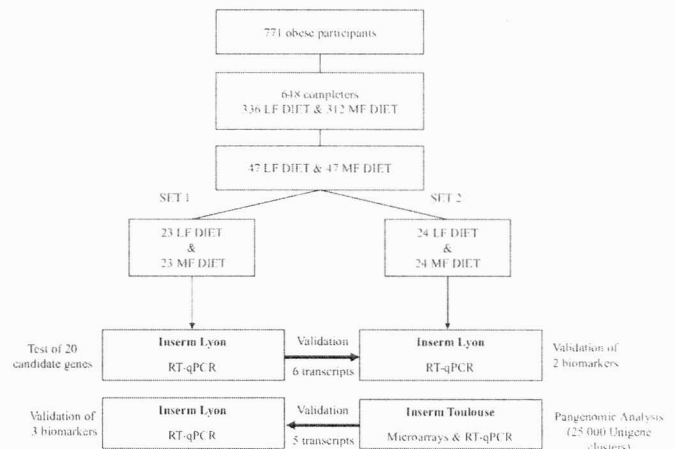

FIG. 1. Schematic representation of experimental design. A total of 771 obese subjects were enrolled in a 10-wk dietary intervention comparing a LF and MF hypoenergetic diets. Among the 648 subjects who completed the program, 47 subjects were selected within each dietary group and further randomly assigned to set 1 or 2. Set 1 was used for the candidate gene study and the validation of biomarkers from set 2. Set 2 was used for pangenomic DNA microarray analysis and validation of biomarkers obtained in set 1.

pliance as well as procedures about the measurements of anthropometric and blood parameters were previously described (10).

From a total of 771 participants, 648 completed the intervention, among whom 47 women per diet were selected, matched for high quality of adipose tissue RNA, weight, height, body mass index (BMI), waist to hip ratio, energy intake, fat, carbohydrate, protein, and alcohol energy intakes before the intervention and similar changes in weight, BMI, percent of body fat mass, and energy intake during the intervention. Each dietary group was randomly divided into two similar sets of subjects (Fig. 1). Set 1 was used for a candidate gene approach using RT-qPCR, which was further confirmed on set 2 [experiments performed by the Institut National de la Santé et de la Recherche Médicale (Inserm) Lyon laboratory].

Set 2 was used for microarray experiments and RT-qPCR measurements (experiments performed by the Inserm Toulouse laboratory). Results from set 2 were confirmed on set 1 by RT-qPCR in the Inserm Lyon laboratory. Experiments in each laboratory were performed with different RT-qPCR technical platforms (see below).

Subcutaneous adipose tissue biopsy and total RNA preparation

An abdominal sc fat specimen (~1 g) was obtained by needle aspiration under local anesthesia after an overnight fast before and after the dietary intervention. Biopsies were washed and stored in RNA Later preservative solution (QIAGEN, Courtaboeuf, France) at −80 C until analysis. Total RNA was extracted using the RNeasy total RNA minikit (QIAGEN) in the Inserm Toulouse laboratory. Total RNA concentration and integrity were estimated using Agilent 2100 bioanalyzer (Agilent Technologies, Massy, France).

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Microarray experiments

One μg of total RNA from each sample from set 2 was amplified with the MessageAmp RNA kit (Ambion, Austin, TX). Quantity and quality of amplified RNA (aRNA) were systematically checked using an Agilent 2100 bioanalyzer. Fluorescent targets were synthesized from 1 μg of aRNA with cyanine dyes (Cy) using the CyScribe first-strand cDNA postlabeling kit (Amersham Biosciences, Orsay, France). Fluorescent probes obtained before and after the dietary intervention for a same subject were cohybridized on cDNA pangenomic microarrays containing 41,760 spots representing 24,469 Unigene clusters (http://genome-www5.stanford.edu/). Dye switch of aRNA labeling was done to remove dye-bias effect. For half of the subjects, aRNA from the fat biopsy obtained before dietary intervention was labeled with Cy3, whereas Cy5 was used for aRNA from the biopsy obtained after the intervention. For the second half of subjects, the labeling was reversed.

Hybridization was performed according to the standard protocol described elsewhere (http://cmgm.stanford.edu/pbrown/protocols/index) with slight modifications. Arrays were scanned using a GenePix 4000A scanner (Axon Instruments-Molecular Devices, Sunnyvale, CA).

Data analysis

Images were analyzed using Genepix pro 5.0 software (Axon Instruments-Molecular Devices). The loess normalization smoothing procedure was applied for intensity normalization using the Limma package with R software. Intensity ratios between Cy5 and Cy3 were filtered using an average intensity 2.5 times above the background signal. The SD of the $\log_2$ ratio was set to 1 after data normalization. Clones with more than five missing values within each dietary group were excluded from the analysis. Thus, the $\log_2$ Cy5 to Cy3 signal ratios were retrieved for 18,442 spots. When labeling was reversed, values were multiplied by -1. Analysis of the function of regulated genes was done using the Protein Analysis Through Evolutionary Relationships (PANTHER) database with focus on the biological process ontology. For genes down-regulated by calorie restriction, biological processes overrepresented among the list of differentially expressed genes were considered significant at $P < 0.05$, determined by the binomial statistic with Bonferroni correction for multiple testing. We performed further analysis using the Ingenuity Pathways Analysis software 6.0 (Ingenuity Systems, Redwood City, CA) to identify networks between these genes.

mRNA level measurements by RT-qPCR

First-strand cDNA was synthesized from 1 μg of adipose tissue total RNA after deoxyribonuclease treatment using random hexamers and poly(dT) according to the SuperScript II reverse transcriptase kit (Invitrogen, Cergy-Pointoise, France). All measurements were performed in duplicate using the Sybergreen method and Applied Biosystems 7500 real-time PCR (Applied Biosystems, Foster City, CA) system (Inserm Toulouse) or a Roche Light Cycler (Roche Diagnostics, Meylan, France) system (Inserm Lyon). mRNA expression level was normalized by 18S rRNA expression.

Statistical analysis

Biological and anthropometric parameters were compared between sets and dietary groups by ANOVA. All changes (anthropometric, bio-logical parameters, mRNA levels) during energy restriction were analyzed by paired-sample t test. Diet effect on the change in variables after dietary intervention was analyzed by a univariate general linear model on the measurement after energy restriction with an adjustment for measurement before energy restriction (13).

To analyze microarray experiments, the significance analysis of microarray (SAM) procedure was used to compare the two dietary groups and analyze gene expression changes induced by energy restriction (14). Next, two distinct statistical methods were used to identify the genes differentially regulated in response to the two diets. Random forests (15, 16) and nearest shrunken centroids (17) are two classifiers, already used in the context of microarray data analysis, including a feature selection mechanism that enable to extract the most discriminative genes. In the random forest classifier, genes can be ordered according to their importance based on the change in classification accuracy when expression ratio value is randomly permuted in the other dietary group. This method was applied on normalized after-to-before expression ratios from microarray features without missing data among the 48 microarray experiments. The nearest shrunken centroids method (included in the Prediction Analysis of Microarray software) improve the more usual nearest centroid method, which computes an average gene expression (centroid) for each gene in each group divided by the within-class SD for that gene. This method was used on normalized expression ratios of the 100 most over up- and down-regulated transcripts by each dietary program as determined with SAM. To select transcripts for RT-qPCR validation analysis, the effect of the diets on after-to-before dietary intervention gene expression ratio was analyzed by Student t test. Pearson correlation coefficient was determined on log-transformed data with normal distribution to study relationship of gene expression and plasma parameters. Spearman rho correlation coefficient was used on data without normal distribution.

Results

Subject characteristics

Comparison of the two dietary groups is presented on Table 1. Averaged fat and carbohydrate intakes before the dietary program (35 and 46% of total energy intake, respectively) were similar between the LF and MF groups. During energy restriction, in the LF group, about 23 and 59% of total energy supply was derived from fat and carbohydrates, respectively. In the MF group, about 42 and 41% of total energy was derived from fat and carbohydrates, respectively. The differences in fat or carbohydrate intakes between the two dietary groups were highly significant. There was no difference in energy intake derived from proteins.

Before the 10-wk dietary intervention, LF and MF groups had similar BMI, fat-free mass, fat mass, waist to hip ratio, blood lipid and cholesterol profile, leptinemia, insulinemia, and glycemia (Table 2). Energy restriction induced a significant and sim-

TABLE 1. Macronutrient intake of subjects before and during the LF and MF energy restricted diets

Intake	LF (n = 47)		MF (n = 47)	
	Before	During	Before	During
Energy intake (kcal)	1951 ± 66	1459 ± 38[a]	1937 ± 67	1535 ± 32[a]
Energy from fat (%)	34.5 ± 1.1	23.3 ± 0.4[a]	35.9 ± 0.9	41.9 ± 0.4[a,b]
Energy from cholesterol (%)	47.6 ± 1.2	59.0 ± 0.6[a]	45.9 ± 1.1	41.3 ± 0.5[a,b]
Energy from protein (%)	16.4 ± 0.5	17.5 ± 0.4	16.8 ± 0.5	16.7 ± 0.3

Values are means ± SEM. Differences between groups were determined by variance analysis.

[a] $P \leq 0.05$ before vs. during the dietary intervention.

[b] $P < 0.001$ between the diets.

TABLE 2. Characteristics of subjects following a LF and MF diets

Parameters	LF (n = 47)		MF (n = 47)	
	Before	After	Before	After
Age (yr)	37.1 ± 1.2		38.4 ± 1.1	
Weight (kg)	94.1 ± 1.8	87.3 ± 1.8[a]	96.8 ± 1.7	89.9 ± 1.6[a]
BMI (kg . m^{-2})	35.1 ± 0.5	32.5 ± 0.5[a]	35.5 ± 0.6	33.0 ± 0.6[a]
FFM (kg)	53.0 ± 0.8	51.5 ± 0.8[a]	54.2 ± 0.7	52.4 ± 0.7[a]
FM (kg)	41.0 ± 1.2	35.8 ± 1.2[a]	42.7 ± 1.3	37.5 ± 1.2[a]
Body fat (%)	43.3 ± 0.6	40.6 ± 0.6[a]	43.7 ± 0.6	41.4 ± 0.6[a]
Waist to hip ratio	0.86 ± 0.01	0.84 ± 0.01[a]	0.86 ± 0.01	0.84 ± 0.01[a]
Triacylglycerols (μmol/liter)	1003 ± 71	1032 ± 73	1031 ± 76	901 ± 53[a,b]
Free fatty acids (μmol/liter)	533 ± 19	526 ± 18	525 ± 19	527 ± 24
Free glycerol (μmol/liter)	113 ± 11	119 ± 13	117 ± 12	110 ± 10
Total cholesterol (mmol/liter)	4.90 ± 0.14	4.48 ± 0.12[a]	5.04 ± 0.11	4.79 ± 0.11[a,b]
HDL-cholesterol (mmol/liter)	1.20 ± 0.05	1.06 ± 0.04[a]	1.19 ± 0.05	1.14 ± 0.05[a,b]
LDL-cholesterol (mmol/liter)	3.25 ± 0.13	2.95 ± 0.12[a]	3.38 ± 0.1	3.25 ± 0.09[a,b]
VLDL-cholesterol (mmol/liter)	0.29 ± 0.02	0.30 ± 0.02	0.29 ± 0.02	0.26 ± 0.01[a,b]
Insulin (μU/ml)	9.60 ± 0.78	8.21 ± 0.70[a]	9.82 ± 0.96	8.40 ± 0.92[a]
Leptin (ng/ml)	33.8 ± 1.9	22.0 ± 1.5[a]	33.1 ± 1.9	20.9 ± 1.5[a]
Cortisol (nmol/liter)	231 ± 17	299 ± 25[a]	205 ± 21	253 ± 20[a]
Glucose (mmol/liter)	5.20 ± 0.05	5.13 ± 0.07	5.27 ± 0.07	5.17 ± 0.07[a]
HOMA insulin secretion	116 ± 10	102 ± 09	110 ± 09	99 ± 09
HOMA insulin resistance	2.24 ± 0.19	1.92 ± 0.18[a]	2.36 ± 0.25	2.00 ± 0.27[a]

Values are means ± SEM. HDL, High-density lipoprotein; LDL, low-density lipoprotein; VLDL, very low-density lipoprotein; FFM, fat-free mass; FM, fat mass.

[a] $P \leq 0.05$ before *vs.* after the dietary intervention.

[b] $P \leq 0.05$ for change following the intervention between the diets.

ilar weight loss (-6.8 ± 0.2 kg), fat mass (-5.2 ± 0.2 kg), fat-free mass (-1.6 ± 0.2 kg), and BMI (-2.5 ± 0.1 kg . m^{-2}) decrease in the two dietary groups. Mean values for plasma cortisol ($+ 58 \pm 20$ nmol/liter), insulin (-1.41 ± 0.46 μU/ml), and leptin (-12.0 ± 0.8 ng/ml) levels and the insulin resistance index homeostasis model assessment (HOMA) (-0.34 ± 0.12) were also significantly and similarly modulated by the LF and MF diet. As observed in the whole population of the NUGENOB program (10), changes in plasma levels of triacylglycerols and cholesterol fractions differed between the two dietary groups.

The LF and MF groups were divided to constitute two sets of subjects (Fig. 1). There was no difference between sets 1 and 2 regarding food intake, anthropometric and biological parameters. Changes induced by the diets were similar in the two sets (data not shown).

Changes in candidate gene expression in adipose tissue during low-calorie diets

Information related to the selected genes is presented in supplementary Table 1, published as supplemental data on The Endocrine Society's Journals Online Web site at http://jcem.endojournals.org. Selection was based on experimental evidence of *in vitro* or *in vivo* regulation of mRNA levels by fatty acid or glucose in adipocytes or potential activation during energy restriction. Nuclear or membrane receptors, transcriptional modulators, and secreted factors that may be activated by a hypoenergetic diet were also considered.

The effects of the two hypoenergetic diets were determined on sc adipose tissue mRNA levels by RT-qPCR in set 1. The mRNA levels of stearoyl-CoA desaturase (*SCD*), acetyl-coenzyme A carboxylase-α (*ACACA*), fatty acid synthase (*FASN*), and adipo-

nutrin (*ADPN*) were significantly reduced after a LF or a MF diet (Fig. 2). Five genes, fatty acid binding protein 4 (*FABP4*), liver X receptor-α (*LXRα*), adipose triglyceride lipase (*ATGL*), and sirtuin 2 and 3 (*SIRT2*, *SIRT3*), showed a differential expression between the diets. When the two sets of subjects were combined (n = 43–47 for each diet), a diet effect was observed for the five genes ($P < 0.05$).

Variations in global gene expression in adipose tissue during calorie restriction

To gain a global insight on changes in gene expression, pangenomic microarray analysis was performed on adipose tissue samples from set 2 (Fig. 1). As a preliminary approach, the SAM two-class unpaired procedure was used to compare changes induced between the LF and MF diets and showed no significant difference in gene expression. We next performed the analysis of the effect of energy restriction on the entire set combining the MF and LF groups. With a false discovery rate of 5 and 10%, one and 81 cDNA clones were up-regulated, and 925 and 1283 clones were down-regulated during energy restriction, respectively (supplementary Table 2).

To validate these data, we quantified adipose tissue mRNA levels by RT-qPCR before and after each diet in set 2 for 10 genes with similar expression changes in both diets. We selected six down-regulated genes: glycerol-3-phosphate acyltransferase mitochondrial (*GPAM*), hydroxysteroid 17β-dehydrogenase 12 (*HSD17 β12*), aldolase C fructose-bisphosphate (*ALDOC*), cyclin D1 (*CCND1*), NAD(P)H dehydrogenase quinone 1 (*NQO1*), and enoyl coenzyme A hydratase domain-containing 1 (*ECHD*). The four remaining genes were up-regulated genes: proline-rich nuclear receptor coactivator 1 (*PNRC1*), glutathi-
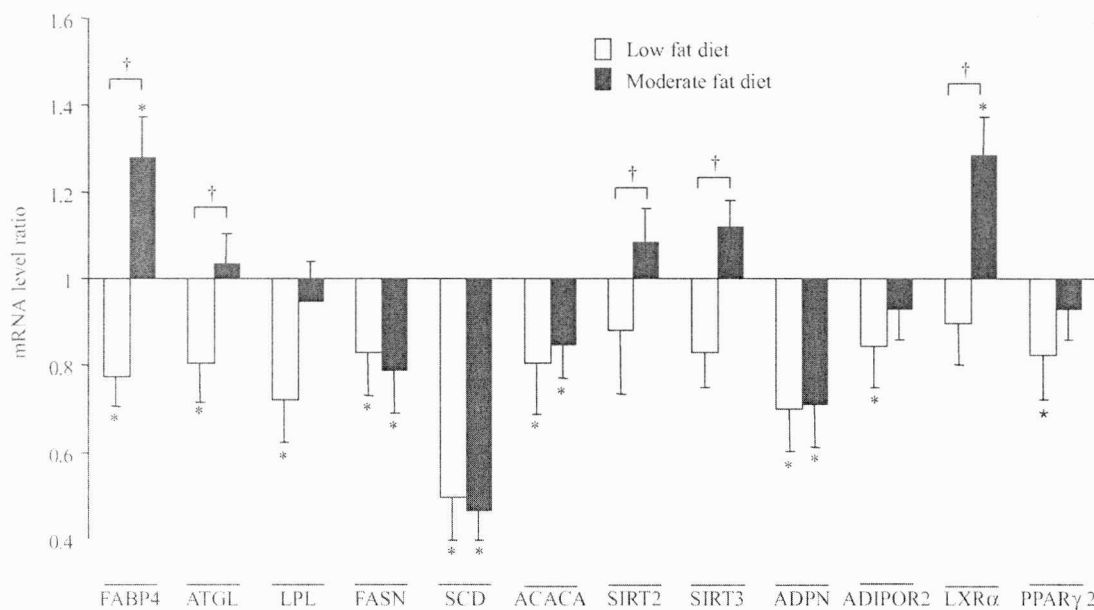
FIG. 2. Adipose tissue mRNA expression of candidate genes after 10 wk of LF or MF hypoenergetic diet. *Bar charts* represent means ± SEM (n = 17–22 values per group) of after-to-before dietary intervention ratio of adipose tissue mRNA expression estimated by RT-qPCR in the two dietary groups on set 1. *, P < 0.05 after vs. before the dietary intervention; †, P < 0.05 between the two diets.

one peroxidase 3 (*GPX3*), alcohol dehydrogenase 1C (*ADH1C*), and *N*-deacetylase/*N*-sulfotransferase 1 (*NDST1*). Except for *NDST1*, results were in complete agreement with microarray data (Table 3). Moreover, the down-regulation of *SCD* and *ACACA* mRNA levels detected with microarray experiments was in accordance with the decrease observed in the two dietary groups of set 1 by RT-qPCR (Fig. 2). Energy, lipid, and carbohydrate metabolisms are the main biological processes involving genes down-regulated by calorie restriction (Fig. 3). The most relevant and dense networks assembled by Ingenuity Pathways Analysis with down-regulated genes (see supplementary Figure 1, published as supplemental data on The Endocrine Society's Journals Online Web site at http://jcem.endojournals.org, and supplementary Table 3) are related to lipid metabolism, cellular

assembly, organization, and small molecule biochemistry. Network 1 illustrates possible coregulation of electron transport chain complex I and acyl CoA synthase. Network 2 shows that TNFα could play a central role in the interaction between enzymes involved in β-oxidation and fat storage. The third network shows linkage between electron transport chain complex IV, heat shock proteins, and the release of cytochrome c.

Identification of transcripts regulated by dietary intake

The candidate gene analysis revealed genes differentially regulated in the two diets. We extended our study using whole-genome DNA microarray analysis of set 2 to analyze whether more transcripts could be detected. We used two statistical meth-

TABLE 3. Changes in adipose tissue gene expression during a 10-wk hypoenergetic diet

| | | Ratio of gene expression levels | |
Gene Symbol	Gene name	Microarray	RT-qPCR
Down-regulated genes			
SCD	Stearoyl-CoA desaturase (δ-9-desaturase)	0.62 ± 0.05	0.48 ± 0.06[a]
ALDOC	Aldolase C, fructose-bisphosphate	0.76 ± 0.05	0.70 ± 0.06[a]
GPAM	Glycerol-3-phosphate acyltransferase, mitochondrial	0.78 ± 0.05	0.70 ± 0.04[a]
ECHDC1	Enoyl coenzyme A hydratase domain-containing 1	0.81 ± 0.03	0.73 ± 0.04[a]
CCND1	cyclin D1	0.82 ± 0.03	0.85 ± 0.04[a]
NQO1	NAD(P)H dehydrogenase, quinone 1	0.84 ± 0.02	0.82 ± 0.05[a]
HSD17β12	Hydroxysteroid (17-β) dehydrogenase 12	0.85 ± 0.02	0.86 ± 0.04[a]
ACACA	Acetyl-coenzyme A carboxylase-α	0.96 ± 0.02	0.83 ± 0.07[a]
Up-regulated genes			
PNRC1	Proline-rich nuclear receptor coactivator 1	1.12 ± 0.03	1.24 ± 0.08[a]
GPX3	Glutathione peroxidase 3 (plasma)	1.14 ± 0.04	1.38 ± 0.12[a]
ADH1C	Alcohol dehydrogenase 1C (class I), γ-polypeptide	1.24 ± 0.06	1.13 ± 0.06[a]
NDST1	N-deacetylase/N-sulfotransferase 1	1.25 ± 0.06	0.87 ± 0.05[a]

Changes in mRNA levels were determined using microarrays and RT-qPCR before and after energy restriction in set 2 (n = 48), except for *SCD* and *ACACA* genes for which RT-qPCR measurements were performed in set 1 (n = 43). Data represent means ± SEM (ratio of mRNA levels after the dietary intervention divided by values before the dietary intervention).

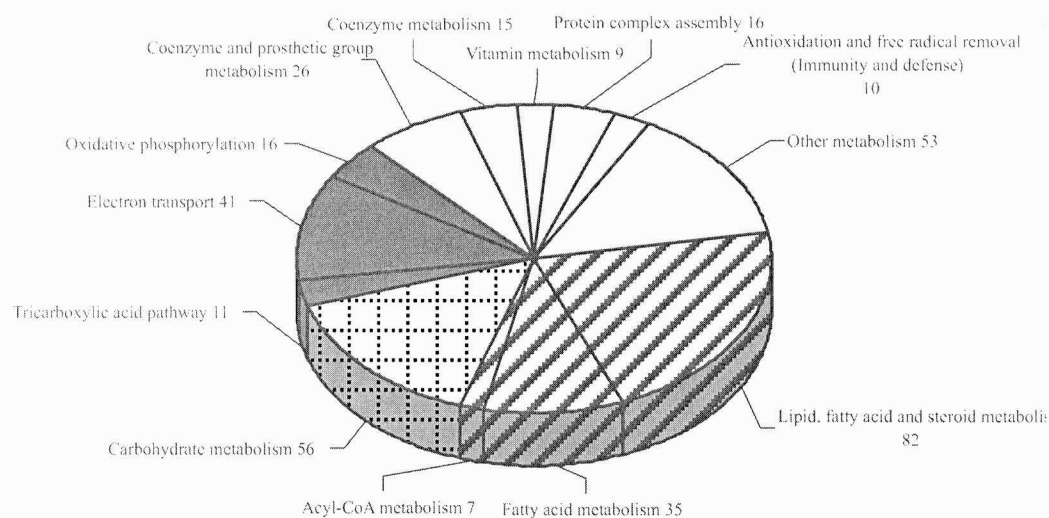[a] P ≤ 0.05, before vs. after dietary intervention.

FIG. 3. Ontology analysis of biological processes of genes down-regulated during calorie restriction. The Protein Analysis Through Evolutionary Relationship (PANTHER) was performed on 843 transcripts. Significantly enriched pathways are shown with the number of genes. Main classes of processes are combined as follows: energy production (*gray area*), carbohydrate metabolism (*gridded area*), lipid metabolism (*line-filled area*), and other metabolism (*white area*).

ods dedicated to classification and selection of genes that are more relevant than SAM.

In the random forest classification analysis, genes with the highest importance in the classification accuracy (significance value under 0.05) were selected. A list of 64 cDNA clones as potential markers of macronutrient composition of the diet was obtained (supplementary Table 4). Independently, Prediction Analysis of Microarray identified the most specific changes in gene expression induced by each diet. Different values of threshold shrinkage were tested and the best accuracy of class prediction by cross validation (77%) was obtained with a list of 57 cDNA clones (supplementary Table 5).

The transcripts identified by the two methods map to 84 genes. Combining the output from microarray and candidate gene approaches 89 genes could be responsive to changes of the fat to carbohydrate ratio of energy-restricted diet. Ontological classification identified eight genes involved in oncogenesis, 12 in apoptosis, 24 in signal transduction, seven in cell cycle control, three in angiogenesis, and nine in lipid and steroid metabolism.

Of the 84 genes identified by the microarray approach as differentially expressed according to the macronutrient content of the diet, we restricted the list excluding clones not linked to known genes in the GenBank database. Moreover, only clones presenting a significant difference in after-to-before energy restriction expression ratio between the MF and LF groups were selected. A list of 12 genes was obtained (supplementary Table 6).

Confirmatory analyses of biomarkers

To validate the accuracy of the selection methods, adipose tissue mRNA levels of the 12 selected genes were quantified before and after dietary intervention by RT-qPCR in samples from set 2 (Fig. 1). According to the general linear model analysis, a differential effect of diet ($P < 0.1$) tended to be confirmed for nuclear receptor subfamily 3, group C, member 1 (*NR3C1*), GABA A receptor-associated protein-like 2 (*GABARAPL2*), ornithine decarboxylase antizyme 2 (*OAZ2*), phosphatase and tensin homolog (*PTEN*), farnesyltransferase CAAX box α (*FNTA*),

and amyloid-βA4 precursor protein (*APP*). A similar trend was observed on set 1 for *FNTA* ($P = 0.06$), *NR3C1* ($P = 0.08$), and *GABARAPL2* ($P < 0.05$). If the two sets of subjects were combined (n = 43–47 for each diet), we detected a significant effect of the macronutrient composition of the diet on *NR3C1*, *GABARAPL2* (both at $P < 0.01$), and *FNTA* ($P < 0.05$) expression change. After an adjustment for BMI and other matching criteria, similar results were obtained (data not shown).

Using candidate gene and pangenomic approaches, cross-validation on two independent sets of subjects and stringent statistical procedures, a differential expression of five genes, *FABP4*, *SIRT3*, *NR3C1*, *GABARAPL2*, and *FNTA*, was observed between LF and MF diets (Fig. 4). Of note, a strong correlation was found between *NR3C1* and *FABP4* mRNA levels (rho = 0.41, $P < 0.0001$, n = 89). We studied the relationship in diet-induced changes of plasma lipid (Table 2) and mRNA levels (Fig. 4) of parameters influenced by the composition of the diet on the entire group of subjects (n = 91). *NR3C1* expression correlated with total and low-density lipoprotein-cholesterol (r = 0.25, $P = 0.02$ and r = 0.24, $P = 0.02$, respectively) whereas *GABARAPL2* expression correlated with total and high-density lipoprotein-cholesterol (r = 0.22, $P = 0.04$ and r = 0.23, $P = 0.03$, respectively).

Discussion

Concomitant with the escalating prevalence of obesity has been the rise in the use of hypocaloric diets to induce weight loss, many of which alter macronutrient composition, especially the proportion of fat and carbohydrate (18). The *in vivo* adaptations to calorie restriction and to changes in diet composition are poorly understood, although the molecular bases of such adaptations are being unraveled (7, 8). We performed on a large group of subjects an extensive analysis of the effect of two hypoenergetic diets with different fat to carbohydrate ratios on adipose tissue gene expression. The subjects were divided into two sets to identify and validate the variations in mRNA levels.
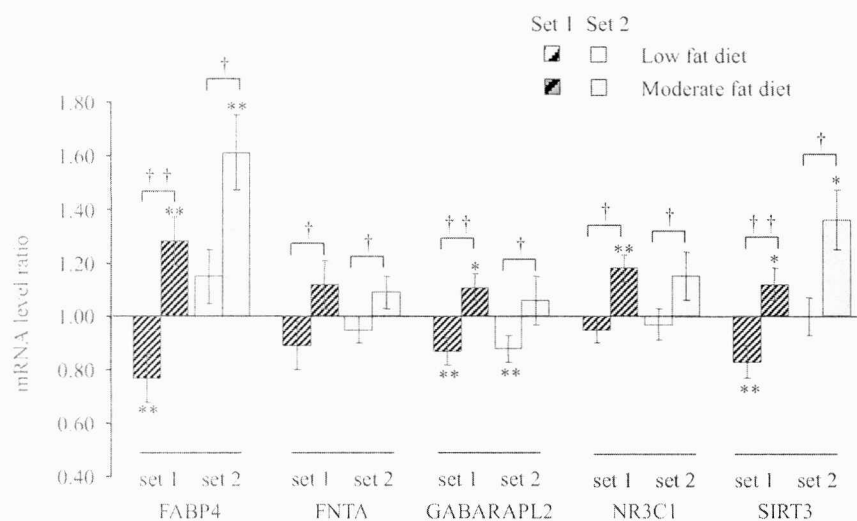
FIG. 4. Variations in adipose tissue mRNA expression of transcripts regulated by macronutrient content of hypoenergetic diet. Bar charts represent means ± SEM (n = 19–24 values/group) of after-to-before dietary intervention ratio of adipose tissue mRNA expression estimated by RT-qPCR in the two sets and dietary groups. *, $P < 0.1$, **, $P < 0.05$ after vs. before the dietary intervention. †, $P < 0.1$, ††, $P < 0.05$ between the diets. Dashed and filled bars represent sets 1 and 2, respectively.

As reported for variations in clinical parameters (10), we found a predominant impact of energy restriction and/or weight loss over macronutrient composition on adipose tissue gene expression. Compared with the previous studies on the NUGENOB cohort restricted to a subset of subjects and limited number of transcripts, the list of genes regulated by energy restriction is considerably expanded by our microarray analysis (11, 12). In agreement with these studies, the vast majority of the genes are down-regulated during the diets. Among the most down-regulated transcripts are genes involved in lipid, carbohydrate, and energy metabolism, which represented the majority of genes regulated during the hypocaloric diets. The genes included rate-limiting enzymes of de novo lipogenesis such as FASN and ACACA involved in saturated fatty acid synthesis; SCD, fatty acid desaturase 1 and 2 (FADS1, FADS2) involved in unsaturated fatty acid synthesis; and ELOVL family member 5, elongation of long chain fatty acids (ELOVL5), a long-chain fatty acid elongase. A decrease in triacylglycerol storage capacity was also suggested by the down-regulation of ADPN, GPAM, and lipoprotein lipase (LPL).

Another important result of this study is the differential expression of transcripts according to the dietary fat to carbohydrate ratio. This was achieved because of our unique experimental design. Five transcripts, SIRT3, FABP4, NR3C1, GABARAPL2, and FNTA, showed a reproducible differential expression between the two diets. Of note, the genes showed a similar pattern of regulation, i.e. up-regulation or no change during the diet with an elevated proportion of lipids and down-regulation or no change during the low-fat diet. Expression of these genes was 15–65% higher in the MF than the LF diet.

The regulation of SIRT3 is of interest in the context of metabolic adaptations to various fat and carbohydrate intakes. SIRT3 is found in the matrix of the mitochondrion, the importance of which in the adaptation to energy restriction is increas-

ingly documented (19, 20). SIRT3 deacetylates the mitochondrial enzyme, acetyl-coenzyme A synthetase 2, thereby allowing the entry of carbon from acetate, generated following a diet enriched with fat, into the tricarboxylic cycle (21, 22). This step might be important during times of food limitation to both harvest dietary acetate and make use of acetate generated by the liver during ketogenesis (23).

We also showed a differential regulation of FABP4 by the two diets. FABP4 is involved in the intracellular traffic of fatty acids in the adipocyte and could be a critical mediator of insulin sensitivity (24). An increased FABP4 expression may allow proper handling of the flux of fatty acids. The gene is induced by fatty acids in adipocytes, an effect likely to be mediated by peroxisomal proliferator-activated receptors (25, 26).

Increased expression of the glucocorticoid receptor NR3C1 could increase the cortisol-mediated transcriptional activity, contributing to the regulation of FABP4 and potentially other glucocorticoid receptor targets (27). The very high correlation between NR3C1 and FABP4 gene expression supports this notion. These transcriptional effects could modify the metabolic profile induced by weight loss as suggested by the correlation between NR3C1 adipose tissue gene expression and plasma cholesterol parameters.

The relationship between macronutrients and GABARAPL2 and FNTA has never been documented. GABARAPL2 modulates intra Golgi transport through an interaction with soluble N-ethylmaleimide-sensitive factor attachment protein receptor complexes (28), which was described to control adipocyte GLUT4 translocation in response to insulin (29, 30). The role of the farnesyltransferase FNTA in adipose tissue remains elusive. Of note, it has been suggested that farnesylation of proteins may influence adipose tissue development (31).

To increase the relevance of this work, highly stringent criteria were applied to transcripts identified as sensitive to dietary intake. It is highly likely that other genes selected by the classification and prediction methods show real differential expression between the MF and LF diets.

To conclude, during hypoenergetic diets, the primary determinant of changes in adipose tissue gene expression is energy restriction rather than the composition in fat and carbohydrate. The regulation in energy metabolism-related processes and coregulatory pathways may explain the variations in anthropometric and biological parameters. However, the macronutrient content of the diets influences expression of a subset of genes, which may contribute to differential response in blood lipid profile.

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