Adipocyte Gene Expression Is Altered in Formerly Obese Mice and As a Function of Diet Composition[1,2,3]

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Abstract

In the development of obesity, the source of excess energy may influence appetite and metabolism. To determine the effects of differences in diet composition in obesity, mice were fed either a high-carbohydrate diet (HC; 10% fat energy) or a high-fat energy–restricted diet (HFR; 60% fat energy) over 18 wk in weight-matched groups of mice. To identify obesity-associated genes with persistently altered expression following weight reduction, mice were fed either a standard low-fat diet (LF; 10% fat energy), an unrestricted high-fat diet (HF; 60% fat energy), or a HF diet followed by weight reduction (WR). Mice fed a HF diet had significantly greater gonadal fat mass and higher whole blood glucose concentrations than mice fed an HC diet. Of the mice fed a high-fat diet, total body weight and serum insulin concentrations were greater in HF than in HFR. Microarray analysis revealed that HF vs. HC feeding resulted in global differences in adipocyte gene expression patterns. Although we identified genes whose expression was altered in both moderately and severely obese mice, there were also a large number of genes with altered expression only in severe obesity. Formerly obese, WR mice did not differ significantly from lean controls in total body weight or physiological measures. However, microarray analysis revealed distinctly different patterns of adipocyte gene expression. Furthermore, there were 398 genes with altered expression in HF mice that persisted in WR mice. Genes with persistently altered expression following obesity may play a role in rebound weight gain following weight reduction.

Introduction

The incidence of obesity is increasing at an alarming rate in developed countries (1,2). Aside from total energy intake, differences in diet composition may play an important role in the development of obesity and its complications, such as diabetes and cardiovascular disease. Dietary variety and consumption of restaurant foods have both been associated with greater body fat and studies of rats have revealed different metabolic patterns depending on the source of excess energy (3,4). However, the relationship between dietary composition and physiology remains poorly defined (5) and studies have not systematically evaluated the effects of high-carbohydrate feeding vs. high-fat feeding on metabolism.

Given the high prevalence of obesity, there has been interest in identifying factors associated with successful weight reduction. Most human studies have demonstrated that obese individuals typically return to pretreatment weight within 5 y of successful weight reduction, with a substantial proportion exceeding pretreatment weight (6–8). Expansion of the adipocyte population and secretion of small molecules from adipocytes may be involved in the failure to maintain weight loss; however, many factors responsible for rebound weight gain after weight reduction have yet to be identified.

Adipocyte-secreted factors influence diverse processes such as appetite and energy balance, lipid metabolism, insulin sensitivity, and inflammation (9,10) and visceral adiposity has been shown to be more directly related to cardiovascular risk than subcutaneous fat (11,12). We hypothesized that the visceral fat depot may be differentially responsive to diet composition and may produce factors not yet identified that may contribute to difficulty in maintaining weight loss. Using an oligonucleotide microarray, we performed a comprehensive evaluation of adipocyte gene expression in obesity as a function of diet composition (high fat vs. high carbohydrate). We then characterized gene expression in adipocytes derived from weight-reduced mice, identifying genes that may be involved in rebound weight gain following weight reduction.

Materials and Methods

Animals

Male C57BL/6J mice (Jackson Laboratory) were selected because these mice develop obesity and insulin resistance in response to high-fat feeding (13,14). Mice were obtained at 5–6 wk of age and housed 5 animals per cage. Animals were maintained on a 12-h-light/-dark cycle in a temperature-controlled room. Animal care and experimental manipulations were approved by the Institutional Animal Care and Use Committee at the Johns Hopkins University School of Medicine.

We recorded body weight on arrival and at weekly intervals in the morning. Food intake was recorded twice weekly. All mice were given commercially prepared diets (Research Diets; Supplemental Table 1). After 1 wk of acclimatization, mice were divided into 4 groups: mice fed a low-fat diet (LF;[7] 10% energy as fat; catalog no. D12450B); mice fed a high-fat diet (HF; 60% energy as fat; no. D12492); mice fed a high-carbohydrate diet (HC) consisting of LF supplemented with a liquid formula, prepared by dissolving the powdered LF in water to a concentration of 103.5 g/L, thus providing 1674 kJ/L; and mice fed a HF/restricted diet (HFR) provided in sufficient quantity to match total body weight to the HC group. For wk 1, mice in the HFR group were given a quantity of food calculated, based in part on pilot studies, to provide the same energy we estimated would be consumed by the mice fed the HC diet. We weighed mice weekly and adjusted the amount of food provided to match total body weight in the HFR and HC groups. Fresh formula was changed daily and provided to mice in the HC group in place of drinking water to increase total energy intake. After 13 wk, HF-fed mice were subdivided into 2 groups. One-half continued to consume the HF ad libitum and the other half was switched to a LF diet and restricted to 2 g per mouse per day to induce weight reduction (WR).

We used a total of 50 mice for this experiment (10 mice per group). Microarray and RT-PCR data are reported on 42 mice. Data on the remaining 8 mice are not available due to inadequately prepared labeled cRNA or degraded RNA.

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[7]Abbreviations used: HC, high-carbohydrate/-energy; HF, high-fat; HFR, high-fat–restricted; i.p., intraperitoneally; IPA, ingenuity pathways analysis; KRBH, krebs ringer bicarbonate HEPES/albumin/adenosine; LF, low-fat; WR, weight-reduced; NPY, neuropeptide Y; PCA, principal components analysis; TNC, tenascin c; TNF, tumor necrosis factor.

Insulin tolerance testing and fasting insulin concentrations

Insulin tolerance testing was performed at wk 17. After food deprivation overnight, mice were administered 0.75 units/kg insulin (recombinant human regular insulin; Eli Lilly) intraperitoneally (i.p.). Venous tail blood was obtained for glucose determination by glucometer (Accu-Chek Advantage; Roche Diagnostics) at baseline and at 20, 40, and 60 min after insulin injection. The following week, following overnight food deprivation, mice were anesthetized with Avertin (2, 2, 2 tribromoethanol in tert-amyl alcohol; Sigma-Aldrich) 0.5 mg/g i.p. ~100 μL blood was obtained by mandibular bleed, centrifuged at $4000 \times g$; 5 min at 4°C and serum was stored at −80°C for subsequent insulin concentration determined by RIA (Linco Research).

Adipocyte isolation and RNA purification

After 18 wk, mice were killed by CO_2 asphyxiation and gonadal fat pads were harvested and weighed. Adipocytes were purified from adipose tissue according to established methods (15). Gonadal fat pads were weighed and transferred to vials containing 6 mL Krebs ringer bicarbonate HEPES buffer with 5% bovine fraction V albumin (Millipore), 200 nmol/L adenosine (Sigma), and 10 mg collagenase type 1 powder (Worthington Biochemical). Tissue was minced with sharp scissors and samples were incubated in a shaking 37°C water bath for 1 h. Samples were passed through a 500-μm Nitex mesh (Sefar Filtration) into 50-mL conical tubes, centrifuged at $200 \times g$ for 10 min at room temperature and washed in Krebs ringer bicarbonate HEPES/albumin/adenosine (KRBH) buffer followed by 2 cycles of centrifugation at $200 \times g$ for 1 min at room temperature and washing with KRBH buffer.

Immediately after the final wash, total RNA was extracted from adipocytes using Trizol reagent (Invitrogen) according to the manufacturer's protocol. For each sample, 200 μL of adipocytes was homogenized in 1 mL of Trizol and remaining cells were frozen at −80°C. The quality of total RNA samples was assessed using an Agilent BioAnalyzer (Agilent Technologies) and was equivalent across all samples analyzed.

Oligonucleotide microarray

A 0.5-μg aliquot of total RNA from each sample was labeled using the Illumina TotalPrep RNA Amplification kit (Ambion) as previously described (16). A total of 0.85 μg of biotin-labeled cRNA was hybridized for 16 h to Illumina's Sentrix MouseRef-8 Expression BeadChips (Illumina). The arrays were washed, blocked, and then hybridized. Biotinylated cRNA was detected with streptavidin-Cy3 and quantitated using Illumina's BeadStation 500GX Genetic Analysis Systems scanner. Image processing and data extraction were performed using BeadStudio ver. 15 (Illumina) according to previously described methods (17).

RT-PCR confirmation

RT-PCR was performed with the iScript OneStep RT-PCR kit with SYBR Green (Bio-Rad) using the MyiQ Single Color Real-Time PCR Detection system with an iCycler thermocycler (Bio-Rad). RNA for RT-PCR was obtained from the same samples used for the microarray. Reactions were optimized for each primer set (Supplemental Table 2; 18) and PCR products were separated on a 1% agarose gel to verify product size. For every reaction, a standard curve was generated using serial dilutions of mouse adipocyte RNA. Primer concentration was 200 nmol/L for all reactions. All reactions included a dissociation curve analysis at the end of the amplification to confirm a single product at the expected melting temperature. Relative quantitation of gene expression was performed using the threshold cycle. Differences in mRNA expression were calculated after normalizing to 18S expression.

Data analysis

Values for weights, glucose, and insulin concentrations are expressed as means ± SD. Results of RT-PCR are expressed as relative differences in gene expression. Statistical analyses were performed using a *t* test for RT-PCR data. Repeated-measures ANOVA or 1-way ANOVA with Student-Newman-Keuls post test was performed for all other comparisons. Statistical analyses were performed using GraphPad InStat version 3.0 for Windows NT (GraphPad Software). Differences were considered significant at $P < 0.05$.

Microarray data were analyzed using DIANE 6.0, a spreadsheet-based microarray analysis program. Raw microarray data were subjected to Z normalization and tested for significant changes as previously described (19). Genes were determined to be differentially expressed after calculating the Z-ratio and false discovery rate (20). Individual genes with P-value < 0.01, Z-ratio > 2, and false discovery rate < 0.3 were considered significantly changed. Ingenuity pathways analysis (IPA) (Ingenuity Systems) was used to identify biological networks with the greatest number of differentially expressed genes. For each network or pathway, probability scores were calculated using the right-tailed Fisher's exact test. A full discussion of the statistical methodologies employed can be found in Calvano et al. (21)

Results

Weights and food consumption

At the conclusion of the study (18 wk), total body weight did not differ between the LF and WR groups despite a 28% difference prior to weight reduction (Fig. 1*A*; Table 1). Total body weight remained similar between HC and HFR through the duration of the study and did not differ at the time of tissue harvest. Whereas solid food consumption and solid food energy intake were lower in the HC group than in the LF group (Supplemental Fig. 1), consumption of formula in the HC group instead of water resulted in greater total body weight in HC than in LF. Body weight was significantly greater in HF than all other groups ($P < 0.001$) and body weight was greater in HFR than in LF ($P < 0.05$). Despite equivalent total body weights, gonadal fat mass was greater in HFR than in HC (Table 1; $P < 0.05$). Interestingly, although total body weight was greater in HF than in HFR ($P < 0.001$), both groups had equivalent gonadal fat mass. As expected, gonadal fat mass was greater in HF than in both LF and WR ($P < 0.01$).

Glucose metabolism

Insulin and glucose concentrations were measured in all groups of mice after overnight food deprivation. Serum insulin concentrations were higher in HF than in all other groups ($P < 0.01$; Table 1). Although serum insulin concentrations did not differ between LF, HC, and HFR, whole blood glucose concentrations were higher in HFR compared with LF ($P < 0.05$) and HC (Table 1; $P < 0.01$). Whole blood glucose concentrations were also higher in HF than LF, WR, and HC ($P < 0.001$) but not HFR. Thus, differences in glucose and insulin concentrations appeared to be more closely related to adiposity rather than total body weight.

Following insulin administration, whole blood glucose concentrations were lower at 20 min in LF compared with HFR, HF, HC, and WR (Fig. 1*B; P < 0.01$), suggesting greater insulin sensitivity in LF than in all other groups. This physiologic difference between LF and WR indicates there was incomplete recovery from prior obesity. Whole blood glucose concentrations 60 min after insulin administration were lower in LF, WR, and HFR ($P < 0.05$) compared with HF. The latter comparison demonstrates there was an effect of degree of obesity on insulin sensitivity in these mice.

Gene expression

We identified genes differentially expressed in HF vs. LF, HFR vs. HF, HFR vs. LF, HFR vs. HC, WR vs. LF, and WR vs. HF (Table 2; Supplemental Table 3; Supplemental Summary of Analysis). IPA demonstrated altered gene expression in a broad range of cellular functions (Supplemental Table 4). Interestingly, the most highly involved pathway involved PPAR signaling, which was downregulated in WR compared with LF.

Principal components analysis (PCA) and hierarchical clustering demonstrated global differences in adipocyte gene expression between groups (Fig. 2; Supplemental Fig. 2). Despite equivalent body weight at the time of study, HC and HFR did not overlap in PCA or hierarchical clustering and both groups displayed distinct patterns of gene expression from LF controls. Analysis of HF, LF, and WR revealed separation between the 3 groups, indicating that diet-induced obesity and weight reduction resulted in distinct patterns of adipocyte gene expression.

To evaluate changes in gene expression due to the degree of obesity, we compared adipocyte gene expression in HFR and HF. We identified 587 genes that were not altered in HFR vs. LF but were altered in HFR vs. HF, indicating that expression of these genes did not change with moderate obesity (HFR) but did change with severe obesity (HF). In both PCA and hierarchical clustering, there was clear separation between HF and HFR.

We generated a list of genes that were differentially expressed in both WR vs. LF and HF vs. LF. We found that there were 268 genes upregulated and 130 genes downregulated in HF that were similarly differentially expressed in WR. Thus, for a large number of genes, altered expression in diet-induced obesity persisted even after weight reduction had been achieved and maintained in steady state. We used IPA to identify altered pathways (Supplemental Table 4) and to sort genes by location to compile a list of gene products that localize to the extracellular compartment. This resulted in identifying 59 genes differentially expressed in HF and WR (compared with LF) that code for extracellular proteins (Table 3).

Quantitative RT-PCR

Four genes with differential expression in HF vs. LF were analyzed by RT-PCR to validate results from the microarray experiment (Fig. 3). Targets were selected based on robust fold-change in expression and relevance to adipocyte metabolism as identified in previously published studies. Of the 4 targets studied, 2 increased in HF compared with LF and 2 decreased in HF compared with LF. RT-PCR results were consistent with microarray data for all targets evaluated. Gene expression in WR was intermediate for all 4 genes (data not shown), consistent with the PCA and hierarchical clustering, which indicated that the pattern of adipocyte gene expression in WR was unique compared with that of HF and LF.

Discussion

In this study, the source of excess energy played a role in the phenotype of obese mice. When matched for total body weight, HF feeding was associated with higher fasting blood glucose concentrations and greater gonadal fat mass than HC feeding. Similarities in insulin concentrations between HFR and HC may be a result of correlation with weight or, alternatively, may be due to mild β cell toxicity induced by HF, as reported in other studies (22,23).

Several studies have identified differences in physiology and metabolism in high-fat vs. high-carbohydrate–induced obesity in rodents (3,24,25). Using a comprehensive microarray, we discovered that differences in diet composition affect the expression of a large number of genes. The clustering of gene expression data demonstrates that diet and pattern of adipocyte gene expression are associated. There are many signals originating from adipocytes that play a role

in total body insulin sensitivity and hypothalamic control of appetite and the patterns of adipocyte gene expression we have identified may play a role in these complex biological processes.

Our data confirm that changes in degree of obesity are associated with changes in physiologic measures such as fasting insulin concentrations and insulin sensitivity. Not surprisingly, we found that as obesity increases, the altered expression of a number of genes that occurs in moderate obesity (HFR) persists into severe obesity (HF). However, we also determined that there are a large number of genes whose expression levels in moderately obese mice are not different from those in lean mice but are altered only in severe obesity. PCA and hierarchical clustering also demonstrate distinct segregation of HF and HFR mice by adipocyte gene expression. Thus, increasing obesity is associated with qualitative changes in gene expression, with unique patterns of gene expression in HFR and HF.

By merging WR and HF gene expression lists, we were able to identify factors that could play a role in failure to maintain weight loss after energy restriction. By the end of the study, WR mice had maintained weight in a steady state equivalent to the weight of lean controls for 2 wk. Despite near normalization of measured physiologic variables, WR mice displayed a pattern of adipocyte gene expression markedly different from that of either the obese, HF group or the lean, LF mice. PCA and hierarchical clustering revealed broad differences in adipocyte gene expression among the WR, HF, and LF mice. It remains unclear why a number of obesity-related genes continue to have altered expression following weight reduction. A number of different epigenetic phenomena may be involved, including DNA methylation or DNA/protein acetylation (26).

A number of genes we identified as persistently altered following weight reduction have previously been described as playing a role in appetite and adipose tissue development, thus confirming the physiological importance of our findings. Interestingly, a number of factors in this list are cytokines that have been implicated in cachexia related to illness and inflammation. These findings are consistent with previous studies by Clement et al. (27), which showed differential expression of 100 inflammation-related transcripts in adipose tissue of obese individuals that had been on a very low-energy diet for 1 mo. A number of factors that remain upregulated following weight loss are known to act locally in obesity. For example, colony-stimulating factor 1 has been shown to be upregulated in adipose tissue of human subjects who gain weight with overfeeding (28). Matrix metalloproteinases 2, 12, and 14 are strongly induced in adipose tissue in obesity (29) and matrix metalloproteinase 2 is elevated in obese mice (30).

As many signals related to appetite and satiety originate from adipose tissue, we hypothesized that genes differentially expressed in HF and remaining differentially expressed after diet could play a role in the rebound weight gain that often follows dieting and weight loss. Two proteins in Table 3 have been shown to affect appetite in rodents. Tenascin c (TNC) is an extracellular matrix protein expressed in the central nervous system. TNC-knockout mice were found to have lower neuropeptide Y (NPY) mRNA concentrations in limbic structures of the brain (31). As NPY is known to be orexigenic, one could speculate that increased circulating concentrations of TNC could result in increased NPY concentrations and increased appetite. Additional studies are required to determine whether these factors are consistently elevated postobesity and if they act on the hypothalamus to alter appetite.

To our knowledge, this study is unique in demonstrating that in mice of equivalent body weight, differences in diet composition lead to broad differences in adipocyte gene expression. Altered adipocyte gene expression as a consequence of diet composition may be adaptive to a diet high in saturated fat but may also be responsible for increasing the risk of diabetes and other obesity-

related disorders. Although other investigators have shown changes in gene expression in adipocytes and adipose tissue in obesity (32–34), we have demonstrated differences in metabolism and adipocyte gene expression between formerly obese mice and weight-matched controls. Furthermore, this study has demonstrated that there are genes with altered expression in obesity that do not correct following weight reduction. Although a small number of adipocyte-secreted factors are known to be involved in appetite and energy balance, our findings reveal a number of adipocyte-secreted factors with persistently altered expression following weight reduction that may play a role in rebound weight gain following weight loss.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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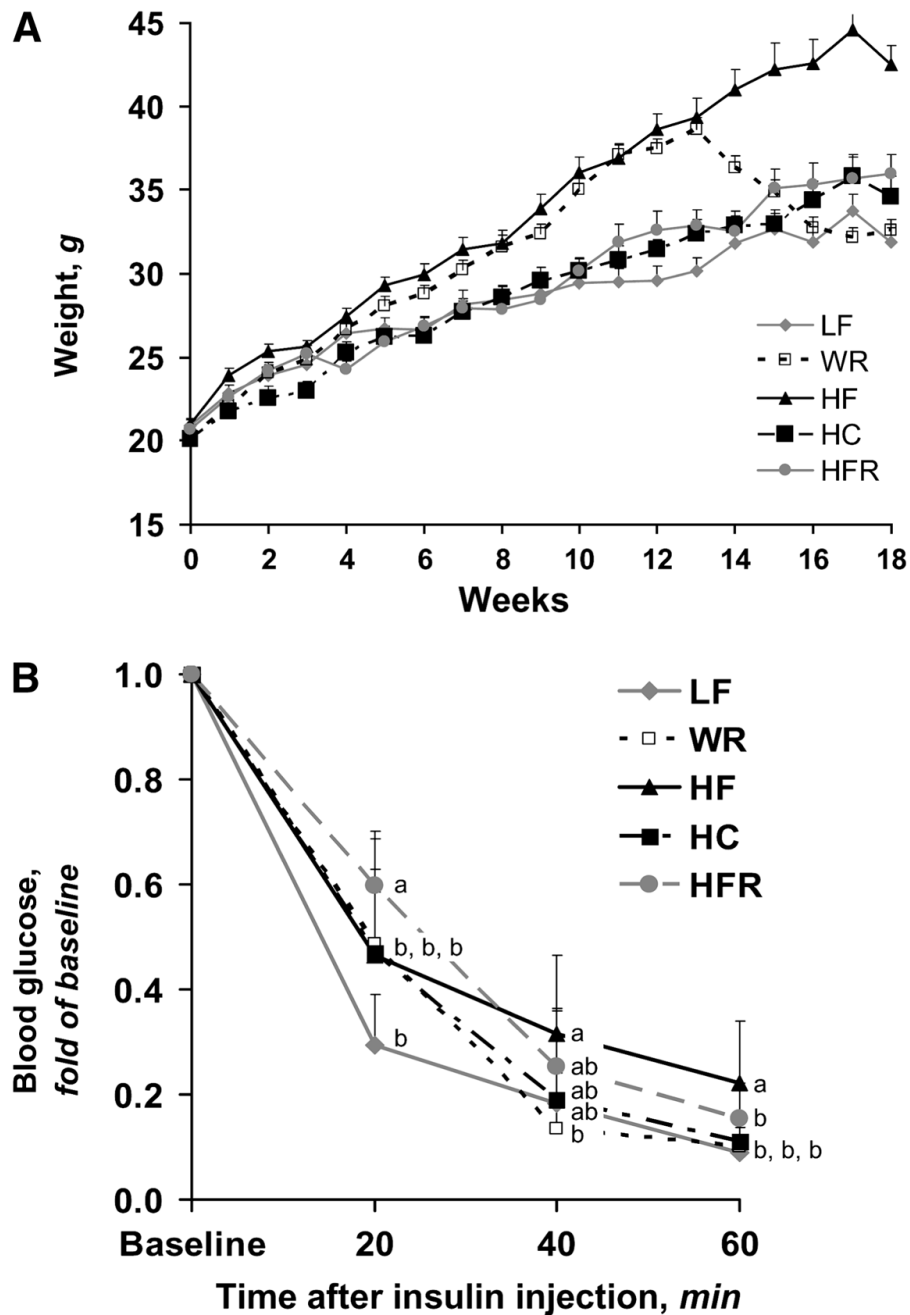
FIGURE 1.
Weekly total body weights (*A*) and proportional decrease in blood glucose concentrations following administration of 0.75 units/kg insulin i.p. to mice fed LF, HF, HFR, HC, or WR diet after overnight food deprivation (*B*). Weight at wk 18 and baseline blood glucose concentrations are reported in Table 1. Values are means + SD, *n* = 10. Means at a time without a common letter differ, *P* < 0.05.
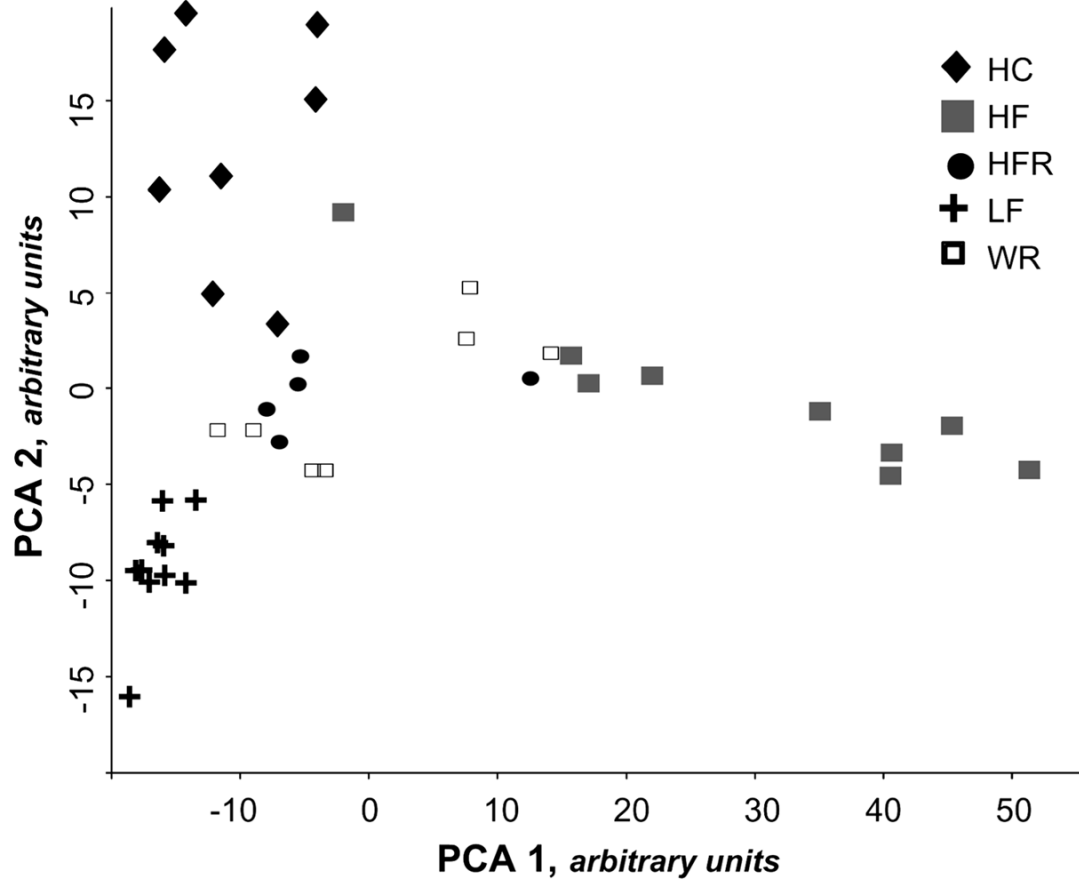
FIGURE 2.
PCA of adipocyte gene expression was performed in DIANE 6.0 to identify clustering within groups. Each data point represents overall adipocyte gene expression data for a single mouse, *n* = 5–10.
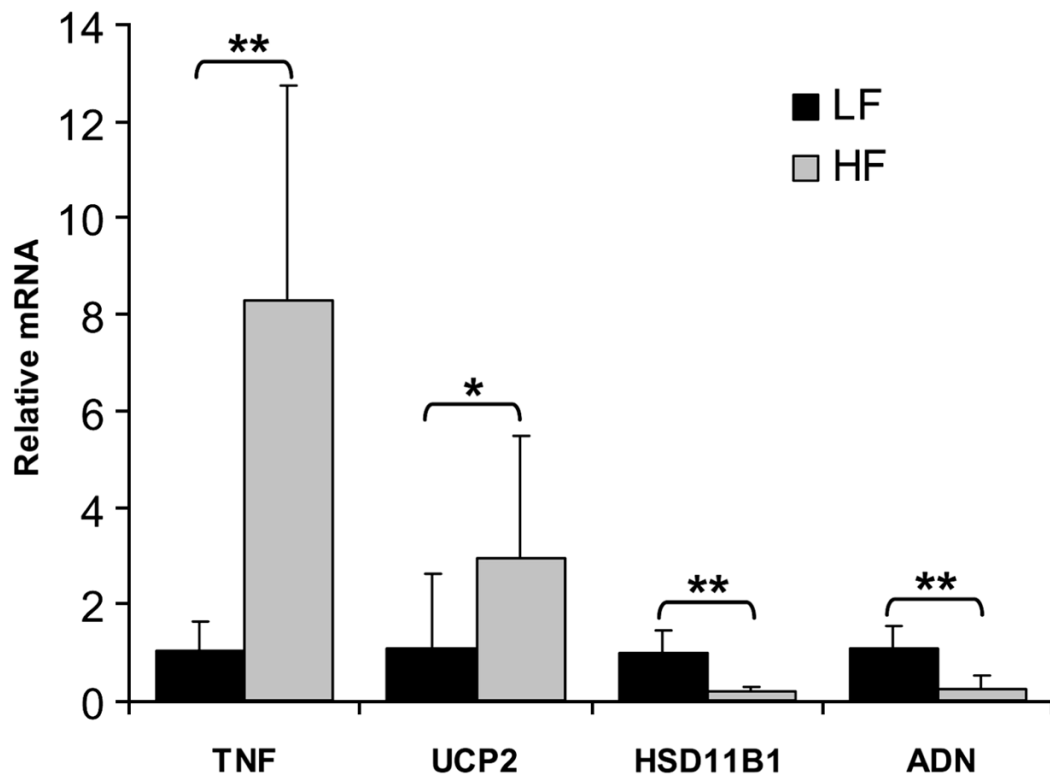
FIGURE 3.
Relative mRNA concentrations for tumor necrosis factor (TNF), uncoupling protein 2 (UCP2), hydroxysteroid 11-β dehydrogenase type 1 (HSD11B1), and adipsin (ADN) in mice fed a LF diet compared with mice fed a HF diet. Values were normalized to 18S ribosomal subunit expression. A 40-ng sample of total RNA per reaction was used to quantitate all templates except 18S RNA, where 160 pg per reaction was used. Values are means + SD, n = 5–10. Asterisks indicate that means differ: *$P < 0.05$, **$P < 0.01$.

TABLE 1

Total body mass, gonadal fat mass, serum insulin, and whole blood glucose concentrations in mice fed a LF, HF, HFR, HC, or WR diet[1]

	Weight	Gonadal fat	Insulin[2]	Glucose[2]
	g		*pmol/L*	*mmol/L*
LF	31.9 ± 2.7^c	1.21 ± 0.37^b	63.7 ± 5.2^b	5.57 ± 1.10^b
HF	42.5 ± 3.8^a	2.23 ± 0.51^a	145 ± 87.0^a	7.85 ± 1.72^a
HFR	36.0 ± 3.6^b	2.02 ± 0.34^a	62.6 ± 8.5^b	7.07 ± 1.45^a
HC	34.6 ± 3.7^{bc}	1.60 ± 0.44^b	57.9 ± 7.7^b	5.24 ± 0.48^b
WR	32.6 ± 1.9^{bc}	1.54 ± 0.30^b	73.3 ± 17.3^b	5.68 ± 1.03^b

[1]Values are means $\pm$ SD, $n = 6$–10. Means in a column without a common letter differ, $P < 0.05$.

[2]Concentrations were measured after overnight food deprivation.

TABLE 2

Numbers of adipocyte genes that were differentially expressed in mice fed a LF, HF, HFR, HC, or WR diet[1]

	HF vs. LF	HFR vs. HF	HFR vs. LF	HFR vs. HC	WR vs. LF	WR vs. HF
				n		
Upregulated	526	298	396	372	390	368
Downregulated	280	531	352	394	227	538

[1] $P < 0.01$ for all genes with significantly different expression between 2 groups.

TABLE 3

Genes that localize to the extracellular compartment that were differentially expressed in adipocytes derived from mice fed a LF or WR diet[1]

Gene symbol	Accession no.	Gene symbol	Accession no.
Upregulated			
ADAMTS4	NM_172845.1	IGFBP5	NM_010518.1
AREG	NM_009704.2	IL10	NM_010548.1
C1QA	NM_007572	IL1B	NM_008361
C1QB	NM_009777.1	LGALS3	NM_010705.1
C1QC	NM_007574.1	LOXL1	NM_010729.2
CCL3	NM_011337.1	LYZ	NM_013590.2
CCL4	NM_013652	MFGE8	NM_008594
CCL5	NM_013653.1	MGP	NM_008597.2
CCL6	NM_009139.1	MMP2	NM_008610.1
CCL9	NM_011338	MMP12	NM_008605.1
CFP	XM_135820.3	MMP14	NM_008608.2
CMTM3	NM_024217.2	PCOLCE2	NM_029620.1
COL16A1	NM_028266.3	PF4	NM_019932.1
COL1A1	NM_007742.2	PLA2G7	NM_013737.2
CSF1	NM_007778.1	PLAU	NM_008873.2
CXCL16	NM_023158.3	PLTP	NM_011125.1
DNASE1L1	NM_027109.1	RETNLA	NM_020509.2
ELA1	NM_033612.1	SRGN	NM_011157.1
FBLN2	NM_007992.1	THBS1	NM_011580.1
FGF13	NM_010200.2	TIMP1	NM_011593
FSTL1	NM_008047.2	TIMP2	NM_011594.2
GDF15	NM_011819.1	TNC	NM_011607.1
GLIPR1	NM_028608.1	TNF	NM_013693
HBEGF	NM_010415.1	TNFSF13	NM_023517.1
Downregulated			
AGT	NM_007428.2	CXCL9	NM_008599.1
AMY2	NM_009669	GPX3	NM_008161.1
AMY2A	NM_007446.1	LGALS4	NM_010706.1
C2	NM_013484.1	PON3	NM_173006.1
C6	NM_016704.1	VEGFA	NM_009505.2
CDC42EP1	NM_027219.1		

[1] $P < 0.01$ for all genes listed.