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*Research Article*

# Differential Effects of Calorie Restriction and Exercise on the Adipose Transcriptome in Diet-Induced Obese Mice

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We tested the hypothesis that obesity reversal by calorie restriction (CR) versus treadmill exercise (EX) differentially modulates adipose gene expression using 48 female C57BL/6 mice administered a diet-induced obesity (DIO) regimen for 8 weeks, then randomized to receive for 8 weeks either: (1) a control (AIN-76A) diet, fed ad libitum (DIO control); (2) a 30% CR regimen; (3) a treadmill EX regimen (with AIN-76A diet fed ad libitum); or (4) continuation of the DIO diet. Relative to the DIO controls, both CR and EX reduced adiposity by 35–40% and serum leptin levels by 80%, but only CR increased adiponectin and insulin sensitivity. Gene expression microarray analysis of visceral white adipose tissue revealed 209 genes responsive to both CR and EX, relative to the DIO group. However, CR uniquely altered expression of an additional 496 genes, whereas only 20 were uniquely affected by EX. Of the genes distinctly responsive to CR, 17 related to carbohydrate metabolism and glucose transport, including glucose transporter (GLUT) 4. Chromatin immunoprecipitation assays of the Glut4 promoter revealed that, relative to the DIO controls, CR significantly increased histone 4 acetylation, suggesting epigenetic regulation may underlie some of the differential effects of CR versus EX on the adipose transcriptome.

## 1. Introduction

More than two thirds of all adults in the USA are either overweight or obese [1]. Obesity is associated with an increased risk of developing several chronic diseases, including atherosclerosis, type 2 diabetes and many types of cancer [2–4]. At the crux of obesity-related diseases is metabolic dysregulation characterized by insulin resistance and elevated levels of circulating insulin, glucose, and several other metabolic factors directly linked to excess adiposity. In the context of low adiposity, insulin activates signaling through the insulin receptor, resulting in translocation of the glucose transporter 4 (Glut4) to the cell membrane to increase glucose uptake into the adipocyte [5]. In contrast, high levels of adiposity are marked by enlarged adipocytes which are unresponsive to insulin levels even under conditions of hyperinsulinemia [6]. In the insulin-resistant state, adipose tissue secretes adipokines and proinflammatory factors that reduce insulin sensitivity in peripheral tissues, thereby affecting whole-body glucose homeostasis [7, 8]. Unfortunately, the mechanisms underlying these changes in insulin responsiveness in adipocytes are poorly understood. Furthermore, mechanism-based lifestyle strategies for effectively offsetting obesity-induced insulin resistance are lacking.

Increased energy expenditure and decreased energy intake are the two most commonly recommended lifestyle changes to reduce adiposity and restore insulin sensitivity [9]. Calorie restriction (CR) and exercise (EX) are both effective at improving insulin sensitivity and decreasing both body weight and percent body fat [10, 11], although the differential effects of these two antiobesity interventions on weight reduction, body composition, and chronic disease

risk are well established [9, 11]. There are also conflicting reports within the human literature concerning the effectiveness of EX in improving body fat distribution and adipokine secretion, two key predictors of insulin resistance [10, 12–14]. Furthermore, studies on the beneficial effects of EX have focused mainly on molecular changes in the skeletal muscle and liver, while considerably less is known about changes in adipose tissue [11, 15, 16]. Therefore, the aim of this study was to compare the effect of CR and EX on visceral white adipose tissue (VWAT) gene expression, along with changes in body composition and insulin resistance, in diet-induced obese mice. We utilized an animal model of postmenopausal obesity because postmenopausal women are especially at risk for developing diseases associated with obesity such as type 2 diabetes [17] and breast cancer, the second leading cause of cancer death in women [4].

In the present study, we show that, despite comparable reductions in adiposity in obese mice, CR was more effective than EX at increasing adiponectin, improving insulin sensitivity, and altering the adipose transcriptome. Although both CR and EX qualitatively affected a shared set of genes related to metabolism, CR had a stronger quantitative effect on these genes. Furthermore, CR induced a dramatic change in expression of an additional set of genes related to carbohydrate metabolism and transport in VWAT that was not observed in the EX mice.

## 2. Materials and Methods

*2.1. Animal Study Design.* All animal protocols were approved by the University of Texas at Austin Institutional Animal Care and Use Committee. To model the postmenopausal state, 6-week-old ovariectomized C57BL/6 mice were used (Charles River Labs, Inc. Frederick, Md, USA). Ovariectomized mice exhibit characteristics of the postmenopausal state in humans: decreased levels of circulating estrogen, loss of bone mineral density, and cessation of estrous cycles [18]. Upon arrival, mice had ad libitum access to water and chow diet and were on a 12 : 12 h light/dark cycle.

To compare the effects of CR and EX on reversal of obesity and insulin resistance, and other metabolic perturbations, 48 mice were singly housed upon receipt and put on a diet-induced obesity (DIO) regimen for 8 wks consisting of ad libitum access to a 60 kcal% fat diet (D12492; Research Diets, Inc, New Brunswick, NJ, USA), beginning one week after arrival. At week 9, the mice were randomized into the following treatment groups ($n$ = 12/group): (1) DIO control (AIN-76A diet fed ad libitum); (2) 30% CR; (3) treadmill exercise regimen, fed AIN-76A diet ad libitum (EX); or (4) continuation on the DIO regimen. In animal models, CR diet regimens, typically involving a 20–40 reduction in carbohydrate calorie intake and designed to limit total energy intake while insuring adequate nutrition, represent the most potent dietary approach to prevent and/or reverse obesity and inhibit tumor growth [7]. The DIO control and EX groups were switched from the DIO regimen to a modified AIN-76A diet (D12450B, that is 10 kcal% fat and is the base diet of our CR regimen; Research Diets, New Brunswick, NJ, USA) consumed ad libitum. The DIO control group was used as a feeding control for determining CR feed intake and to ensure that EX mice were not overeating to compensate for increased energy expenditure. We have previously shown that switching DIO mice to the control (AIN-76A) diet maintains adiposity near the peak level achieved during the 8 weeks of DIO [19], and this was confirmed in the current study. Since body weight and body composition data on this DIO control diet were comparable to continuous DIO, the DIO control group was used as the comparator for all analyses. This also provided control for changes in expression due to differences in diet composition/fat consumption, since the diets for the DIO control, CR, and EX groups all based on the AIN-76A diet. The CR group consumed a modified diet (D0302702, administered in daily aliquots) providing 30% fewer calories from carbohydrates compared to the control diet, with all other components being isonutrient when intake was limited to 70% of mean kcal consumption of the diet control group. The EX group were run on a variable speed treadmill 5 days/wk on a 5% grade, beginning with 10 min/day at 12 m/min. Time and intensity were increased gradually over the next two weeks until the EX group reached 40 min/day at a maximum rate of 20 m/min. The DIO control, continuous DIO and CR mice were all placed on the treadmill but not run. Body weights and feed intake were measured weekly.

At the beginning of week 17, when the CR and EX mice achieved comparable reductions in adiposity relative to the DIO controls, mice were euthanized. In the morning the mice were killed, all mice received their respective dietary or exercise treatment, followed by a 6-hr fast. Mice were anesthetized with isofluorane for terminal blood collection via the retro-orbital venous plexus, and then killed by cervical dislocation. Whole blood was allowed to clot at room temperature for 30 min prior to centrifugation at 1000 ×g for 10 min. The serum was removed and stored at −80°C for analyses. A 1-gram sample of VWAT was collected from each mouse and stored at −80°C until further analyses. Carcasses were stored at −20°C. Percent body fat and lean mass were determined using dual energy X-ray absorptiometry (DXA) (GE Lunar Piximus II, Madison, WI, USA) as described previously [20].

To further characterize the effect of CR on the histone code (which required different tissue processing procedures than the gene expression microarray analysis), an additional group of 15 mice received the AIN-76A control diet (labeled overweight mice), CR diet (labeled lean mice), or DIO (labeled obese mice) for 8 weeks ($n$ = 5/diet group). Body composition on these mice was determined using quantitative magnetic resonance (Echo Medical Systems, Houston, TX, USA). Animals were then killed after an 8-hr fast, serum collected as described above, and tissues (including VWAT, liver and mammary glands 4 and 9) were excised, formadehyde treated to crosslink proteins, and immediately flash frozen for analysis by the chromatin immunoprecipitation assay described below.

*2.2. Glucose Tolerance Test.* To determine the effects of CR and EX on glucose regulation following weight loss, we conducted a glucose tolerance test (GTT) on the 48 mice

on study at week 15. GTT was performed after a 6-hour fast by administration of 20% glucose (2 g/kg body weight IP). Blood samples were taken from the tail and analyzed for glucose concentration using an Ascencia Elite XL 3901G glucose analyzer (Bayer Corporation, Mishawaka, Ind). Glucose levels were determined at baseline, 15, 30, 60, and 120 min after injection of glucose.

*2.3. Serum Hormones.* Leptin, insulin, and adiponectin were measured in serum collected at the terminal bleed, using mouse adipokine LINCOplex Multiplex Assays (Millipore, Inc., Billerica, MA, USA) analyzed on a BioRad Bioplex 200 analysis system (Biorad, Inc. Hercules, CA, USA).

*2.4. Gene Expression Microarray Analysis.* Total RNA was isolated from VWAT tissues using an organic extraction and precipitation protocol with a DNAseI treatment step (Asuragen Inc., Austin, TX, USA). Biotin-labeled targets were prepared using modified MessageAmp-based protocols (Ambion Inc., Austin, TX, USA) and hybridized to MOE 430A 2.0 arrays (Affymetrix, Santa Clara, CA, USA). The arrays were scanned on an Affymetrix GeneChip Scanner 3000 7G. A summary of the image signal data, detection calls, and gene annotations for every gene interrogated on the array was generated using Affymetrix Statistical Algorithm MAS 5.0 (GCOS v1.3), with all arrays scaled to 500. Sample normalization was carried out using the Robust Multichip Average (RMA) followed by multiple group analysis comparison using ANOVA. Pairwise comparisons were performed to identify expression fold differences with false discovery rate (FDR) set at 0.05. Genes with expression differences equal or greater than 2-fold compared to DIO controls, were selected to be analyzed using the Database for Annotation, Visualization and Integrated Discovery (DAVID; [21]). The resulting Gene Ontology (GO) analysis was used to identify genes relevant to the different effects of CR and EX in reversing obesity, some of which were selected for further analysis. In the DAVID analysis, genes that were represented more than once in the microarray output were filtered. Some of the genes in the Gene Ontology analysis belonged to more than one functional category and are tabulated accordingly. Expression changes were verified in VWAT from a separate cohort of mice that underwent CR or EX following DIO, as described above, using Taqman Gene Expression Assay (Applied Bioystems Inc., Carlsbad, CA, USA). Gene expression data were normalized to the housekeeping gene $\beta$-actin.

*2.5. Chromatin Immunoprecipitation (ChIP) Assay.* ChIP assays were performed per manufacturer's instructions (Millipore). Briefly, proteins from VWAT were formaldehyde crosslinked to DNA. After homogenization, lysis, and sonication, proteins were incubated overnight with antibodies to acetyl-histone H4 or trimethyl histone H4 (Millipore). The DNA-protein complexes were washed, DNA was eluted, and crosslinking was reversed by heating to 65°C overnight. DNA was purified using QIAGEN PCR purification kit (QIAGEN, Valencia, CA, USA). Quantitative, real-time PCR was performed using SYBR Green (ABI) with the following *Slc2a4* primers: forward primer 5′-CCCTTTAAGGCTCCA-TCTCC-3′ and reverse primer 5′-TGTGTGTATGCCCCG-AAGTA-3′ (ABI). *GAPDH* was used as the internal control for analysis of acetylation with the following primers: forward primer 5′-CATGGCCTTCCGTGTTCCTA-3′ and reverse primer 5′-CCTGCTTCACCACCTTCTTGAT-3′. For analysis of methylation, *p16* was used as the internal control with the following primers: forward primer 5′-ACACTCCTTGCCTACCTGAA-3′ and reverse primer 5′-CGAACTCGAGGAGAGCCATC-3′.

*2.6. Statistics.* Values are presented as mean ± standard error (SE). One-way analysis of variance (ANOVA) followed by Tukey's Honestly Significant Differences test was used to assess the effects of diet on mean weekly body weight at weeks 8 and 16, body composition data at week 16, serum adipokine levels, and fasting glucose levels. Repeated measures analysis was used to evaluate glucose tolerance tests. For serum insulin, mRNA levels (as measured by qRT-PCR), and relative quantification of Glut 4 in ChIP experiments means, were compared using Student's $t$-test. For all tests SPSS software was used (SPSS Inc., Chicago, IL, USA), and $P \leq .05$ was considered statistically significant.

## 3. Results

*3.1. Both CR and EX Decrease Adiposity, Insulin and Leptin Levels, but Only CR Increases Adiponectin and Restores Insulin Sensitivity in DIO Mice.* During the first 8 weeks, the DIO regimen increased mean body weight of the 48 mice on study from 20.3 ± 0.5 g to 30.7 ± 0.5 g, and % body fat to 52.3%. As shown in Table 1, one week after randomization (week 9 of the study), the DIO control, EX, CR, and continuous DIO groups did not differ in body weight. However, by week 16 of the study, the DIO control group (30.8 ± 1.6 g) was significantly heavier than the EX mice (26.0 ± 0.9 g) and the CR mice (19.9 ± 0.5 g), but not the continuous DIO group (33.2 ± 1.5 g). The body weight data closely correlated with calorie intake (for weeks 9–16: 709 ± 10.0 kcal for DIO controls; 556 ± 4.6 kcal for the EX mice; 413.0 ± 4.3 kcal for the CR; and 722 ± 11.6 kcal for the continuous DIO group) and % body fat (Table 1). Although the CR mice weighed significantly less than the EX mice (primarily due to the increase in lean mass in the EX group relative to the CR mice), there was no difference in percent body fat, with both groups exhibiting >25% reductions in % body fat compared to DIO control mice. Achieving meaningful reductions in adiposity in obese mice via CR and EX was a goal of the study design, given that percent body fat is associated with insulin resistance and other key metabolic changes associated with DIO [22]. Since body weight, kcal consumption and body composition data for mice on the DIO control diet were comparable to mice on the continuous DIO diet, the DIO control group was used as the comparator for all analyses. This allowed us to eliminate the possibility that any changes observed in hormones and gene expression could have been due to differences in diet composition/fat consumption, since the diets for the DIO control, CR, and EX groups were all based on the same AIN-76A diet composition.

Table 1: Body composition after 8 weeks of DIO followed by 8 weeks of control diet, exercise, or calorie restriction.

| Group | Body weight week 9 (g) | Body weight week 16 (g) | Percent body fat (%) | Lean mass (g) |
|---|---|---|---|---|
| DIO Control | $30.1 \pm 0.9^{a}$ | $30.8. \pm 0.6^{a}$ | $51.1 \pm 3.8^{a}$ | $12.6 \pm 0.2^{a}$ |
| Exercise | $29.3 \pm 0.6^{a}$ | $26.0 \pm 0.9^{b}$ | $38.9 \pm 2.7^{b}$ | $13.6 \pm 0.2^{b}$ |
| Calorie Restriction | $31.3 \pm 0.6^{a}$ | $19.9 \pm 0.5^{c}$ | $33.7 \pm 1.4^{b}$ | $10.5 \pm 0.1^{c}$ |
| ContinuousDIO* | $30.3 \pm 1.2^{a}$ | $33.2 \pm 1.5^{a}$ | $57.3 \pm 2.9^{a}$ | $11.8 \pm 0.2^{d}$ |

Data are presented mean ± SEM. Significant differences ($P < .05$) beween data within a column are indicated by different superscripts; $n = 12$/group.

As shown in Figure 1(a), this also allowed us to limit our hormone and microarray analyses to 3 groups (DIO control, CR, and EX), without the continuous DIO group, thus increasing the number of mice per group analyzed within our budget constraints.

At the end of the study we also measured circulating leptin and adiponectin levels, two adipokines that are positively and negatively correlated with adiposity, respectively [23]. Consistent with decreased adiposity, leptin levels were roughly 80% lower in the CR and EX mice (Figure 1(b)). However, only CR increased adiponectin levels compared to DIO control mice (Figure 1(c)), even though percent body fat in CR and EX mice did not statistically differ. The higher levels of adiponectin observed in the CR mice were associated with decreased fasting insulin levels (Figure 1(d)), decreased fasting glucose levels (Figure 1(e)), and increased insulin sensitivity as indicated by significantly lower blood glucose levels at every time point following glucose challenge (Figure 1(e)). In contrast, the EX mice did not display increased insulin sensitivity or decreased fasting insulin levels compared to sedentary DIO. Taken together, these data demonstrate that CR and EX differentially affected adipose tissue metabolism.

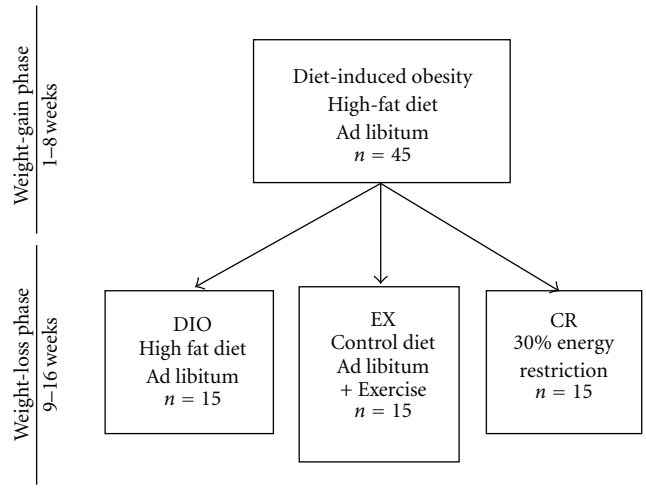
*3.2. Transcriptional Changes Common to CR and EX in VWAT.* Gene expression microarray analysis was performed on VWAT collected following the 8-wk weight-loss phase after DIO. Pairwise comparisons of DIO versus CR and DIO versus EX revealed that 725 transcripts were significantly altered (±2.0 fold, $P < .05$, Figure 2(a)). Of those 725 transcripts, 209 were common to CR and EX (Figure 2(a)), possibly representing a suite of genes most sensitive to energy balance. GO analysis was used to categorize these genes according to function and revealed that the majority of genes altered both by CR and EX were related to metabolic process, immune response, and stress response (Figure 2(b)). Within the metabolic process category, 24 of the genes were related to lipid metabolism, and overall the response of the genes to CR and EX was qualitatively similar. More specifically, a number of genes involved in fatty acid synthesis and transport were upregulated (Figure 2(c)). These included stearoyl-CoA desaturase (Scd1), fatty acid synthase (Fasn), carnitine palmitoyltransferase 1 (Cpt1), and elongation of long chain fatty acids 3 and 6 (ELOVL3 and ELOVL6). In addition, 9 genes related to glucose metabolism were affected by CR and EX, including pyruvate dehydrogenase E1 alpha 1(Pdha1), leptin (Lep), and glycerol phosphate dehydrogenase 2 (Gpd2). As in lipid metabolism, genes related to carbohydrate metabolism were qualitatively responsive to both CR and EX, although CR had a stronger quantitative effect.

Reductions in adiposity are accompanied by lower levels of immune cell infiltrates into adipose tissue, which mediate the proinflammatory state associated with obesity [24]. As expected, the reduced adiposity in CR and EX mice was associated with decreased expression of genes related to immune response (Figure 2(b)). These immune-related genes also comprised the majority of the genes in the stress response category, including downregulation of transcripts that code for chemokines that attract and are produced by monocytes and macrophages, specifically Chemokine (C-C motif) ligand (Ccl) 2, 6, 7, and 9.

*3.3. Unique Transcriptional Changes in Response to CR or EX in VWAT.* CR uniquely affected expression of 496 genes, whereas a mere 20 genes were responsive only to EX (Figure 2(a)). GO analysis of the genes uniquely responsive to EX revealed that only the grouping of genes related to mitochondrial transport was significant. Specifically, uncoupling proteins Ucp1 and Ucp2 were both upregulated by EX. Given the robust transcriptional response to CR, we focused our analysis on those genes whose expression was affected by CR but not EX (Table 2). GO analysis showed that in every category of genes altered by both CR and EX, CR impacted an additional set of genes unaffected by EX. For example, in genes relating to cellular lipid metabolic processes, which was the largest subset of transcripts uniquely altered by CR, soluble carrier family 27 (Slc27a1) and Acetyl-Coenzyme A carboxylase alpha (Acaca) were upregulated. CR also uniquely increased expression of sterol regulatory element binding transcription factor 1 (Srebp1), a master regulator for lipid metabolism in adipocytes. With respect to immune response and stress response, CR resulted in a downregulation of gene expression, whereas expression of genes related to biosynthesis of steroids was upregulated.

In addition to affecting more genes in each functional category than EX, CR affected the transcription of genes in another category not modulated by EX, specifically 4 genes related to glucose transport. Complementing this increase in transcription of glucose transport genes, CR resulted in upregulation of another 14 genes related to carbohydrate metabolism processes. Taken together, these data are suggestive of increased glucose flux into the adipose tissue, which may underlie the enhanced insulin sensitivity observed in response to CR.

*3.4. Real-Time RT-PCR Confirmation of Microarray Results.* Given that the DIO mice were on a high-fat diet, and the CR




(a) Animal Study Design for Microarray

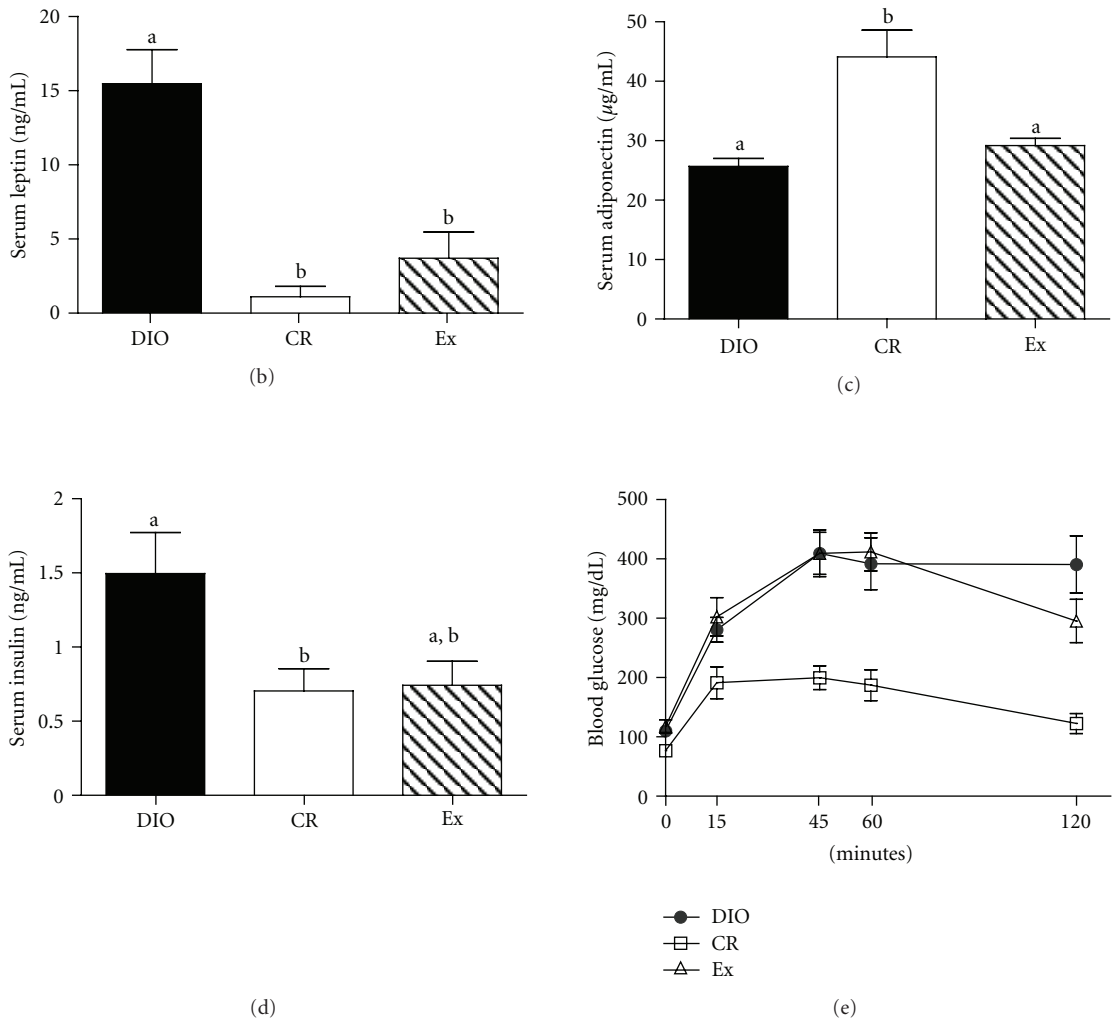
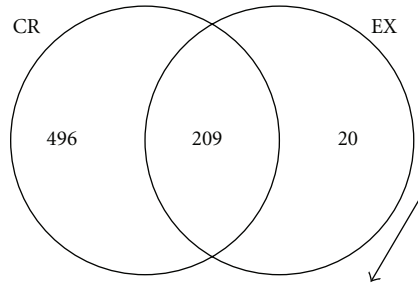


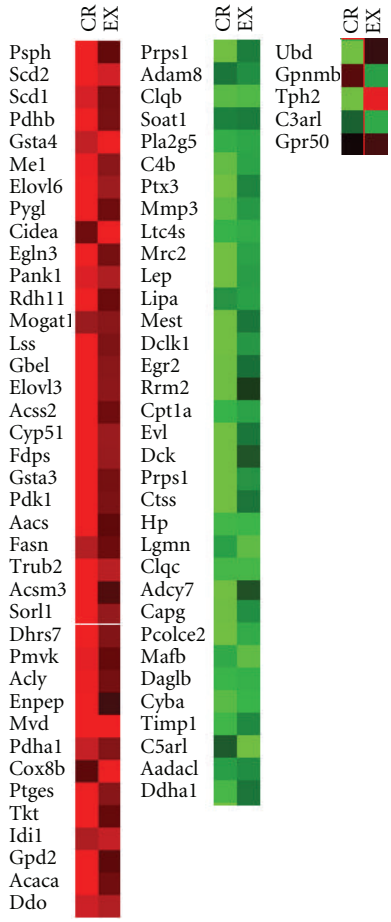

Figure 1: Effect of calorie restriction or exercise in diet-induced obese mice on serum hormones and glucose tolerance. (a) Animal study design for gene expression microarray experiments. (b) Serum leptin levels, (c) serum adiponectin levels, and (d) serum insulin levels after 8 weeks of intervention, ($n = 11$ for DIO group; $n = 10$ for CR group; $n = 10$ for EX group). (e) Blood glucose concentrations during a glucose tolerance after 7 weeks of intervention. Data shown are mean ± SE. DIO (•), EX (△), CR (□), $n = 12$/group. Significance ($P \leq .05$) between groups is denoted by different letters.




(a) Venn diagram comparing CR and EX

| Gene Ontology | Number of genes | Percentage of input | *P*-value |
|---|---|---|---|
| Metabolic process | 79 | 44.13% | .02 |
| Immune response | 17 | 9.5% | <.001 |
| Response to stress | 15 | 8.38% | .009 |
| Steroid biosynthetic process | 6 | 3.35% | <.001 |

(b) DAVID analysis of genes common to CR and EX




(c) Heat map for metabolic genes common to both CR and EX

| Gene ontology | Number of genes | Percentage of input | *P*-value |
|---|---|---|---|
| Response to stress | 30 | 6.82% | .007 |
| Cellular lipid metabolic process | 30 | 6.82% | <.001 |
| Cellular carbohydrate process | 14 | 3.18% | .007 |
| Immune response | 7 | 1.59% | .04 |
| Steroid biosynthetic process | 7 | 1.59% | .004 |
| Glucose transport | 4 | 0.91% | .02 |

(d) DAVID analysis of genes unique to CR

Figure 2: Effect of weight loss induced by calorie restriction or exercise on mRNA expression in VWAT. (a) Venn Diagram of genes differentially expressed by CR and EX compared to DIO controls. (b) Classification of genes targeted by both CR and EX. (c) Heat map of genes related to metabolic processes affected by both CR and EX. (d) Classification of genes targeted uniquely by CR ($n = 6$/group).

and EX consumed a low-fat diet, we were concerned that the observed differences in expression of metabolic genes might be due to differences in the macronutrient contents of the diets and not energy balance *per se*. To address this concern, confirmatory analysis of mRNA expression was done using the diet control mice as the reference group. A gene that was responsive to both CR and EX (Lep), two genes uniquely responsive to EX (Ucp1 and Ucp2), and three genes uniquely responsive to CR and relating to carbohydrate metabolism and transport (Slc2a4, Acly, and Sh2b) were selected for validation. RT-PCR analysis verified that Ucp1, but not Ucp2, was significantly increased by EX only (Figure 3). Although according to the microarray analyses, Lep was reduced by both CR and EX, RT-PCR analyses revealed that only CR significantly reduced Lep expression (Figure 3). All three genes relating to carbohydrate metabolism and transport, Acly,

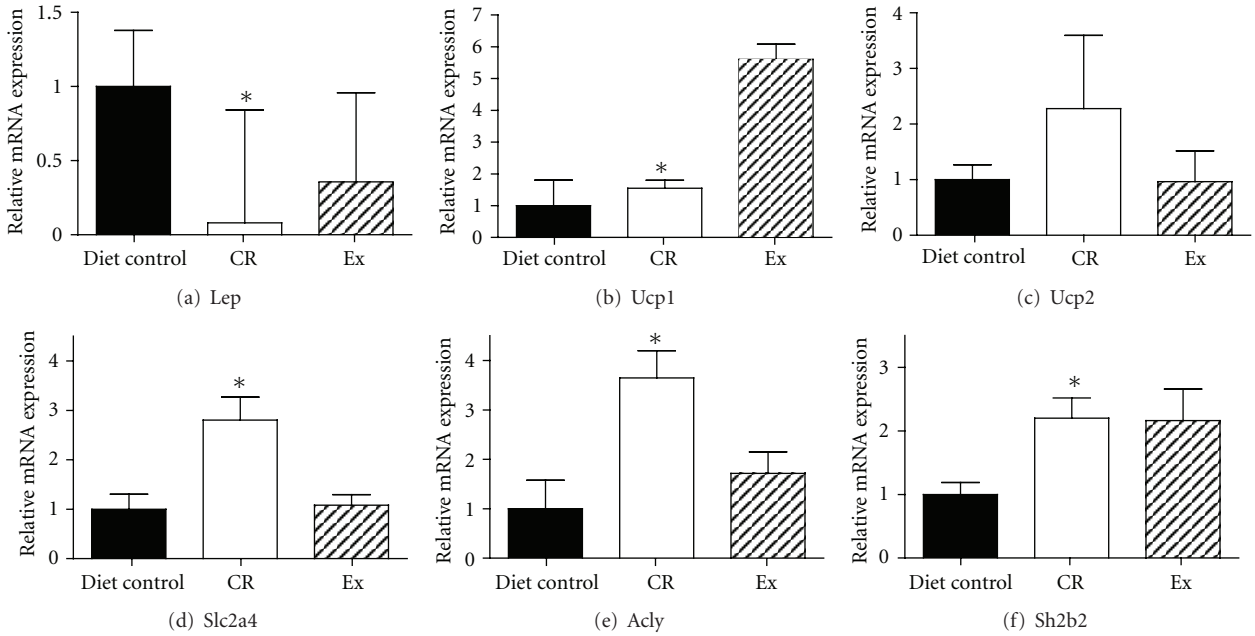



Figure 3: Confirmation of microarray data analysis of mRNA expression in VWAT. Expression of mRNA transcripts in VWAT from DIO control, CR and EX mice ($n = 7$/group). (a) Leptin (Lep), (b) Uncoupling protein 1 (Ucp1), (c) Uncoupling protein 2 (Ucp2), (d) Solute carrier 2, family 4 (Slc2a4) (e) ATP-citrate lyase (Acly), and (f) SH2B adapter protein 2 (Sh2b2) (Data shown are mean ± SE, $^*(P \leq .05)$).

Slc2a4, and Sh2b2, were indeed significantly increased by CR only (Figure 3). Importantly, Slc2a4 codes for the insulin-responsive glucose transporter, Glut4. Translocation of Glut4 from the cytosol to the plasma membrane in response to insulin signaling is the rate-limiting step of glucose transport into the adipocyte. Furthermore, downregulation of Glut4 at the messenger RNA and protein levels has been implicated in obesity and insulin resistance. Although we lacked sufficient VWAT samples for an extensive protein analysis, due to the use of these tissues for genomic and other analyses, Western blot analyses for Glut4 protein expression on 3 VWAT samples/group showed similar trends as observed with the mRNA analyses. Specifically, the lowest Glut 4 protein expression was observed in VWAT from a control mouse, the highest expression was in a CR sample, and the samples from the exercise group were similar to the controls (data not shown). Finally, increases in the enzyme ATP-citrate lyase (Acly), which was also upregulated by CR but not EX, has recently been linked to increases in Glut4 mRNA levels [25]. For these reasons, we focused our analyses on elucidating how CR resulted in increased transcription of Glut4.

*3.5. CR Results in Acetylation of Histone 4 at the GLUT4 Promoter.* Since regulation of acetylation of histones has been shown to be nutrient dependent [25], we hypothesized that increased Glut4 mRNA expression in CR mice may be the result of histone modifications at the Glut4 promoter. To test this hypothesis, we generated obese (DIO) and lean (CR) mice (relative to overweight control mice consuming AIN-76A diet ad libitum) through an 8-week diet intervention. Body weight and percent body fat were positively associated with fasting blood glucose levels and inversely related to Glut4 mRNA levels in the VWAT (Figures 4(b), 4(c), and 4(d)). Modifications to the histone code such as methylation, which can result in decreased transcription [26] or acetylation, which can result in increased transcription [25] may account for the differences in Glut4 transcription in VWAT, so both forms of epigenetic alteration were assessed. There were no differences in trimethylation of histone 4 at the Glut4 promoter (Figure 5(a)). However, CR significantly increased histone 4 acetylation at the Glut4 promoter compared to control mice (Figure 5(b)), which was associated with higher levels of Glut4 mRNA and increased insulin sensitivity.

## 4. Discussion

With over two thirds of American adults classified as overweight or obese [1], increased understanding of how best to reverse the harmful effects of obesity is urgently needed. Given the critical role of adipose tissue in regulating glucose homeostasis and other aspects of metabolism, analysis of the changes that occur in adipose tissue after weight loss could reveal novel targets for prevention or treatment of obesity-related diseases. To our knowledge, this is the first study to compare the effects of CR and EX (the two most commonly recommended lifestyle modifications to prevent or reverse obesity) on gene expression in adipose tissue in a model of DIO. The direct comparison of these two obesity reversal interventions revealed the following novel findings: (1) CR led to altered expression of more than 20 times the number of genes in the adipose tissue than were uniquely affected by EX; (2) alteration of expression of carbohydrate transport genes (particularly GLUT4) was uniquely affected by CR and correlated with the increased insulin sensitivity exhibited by

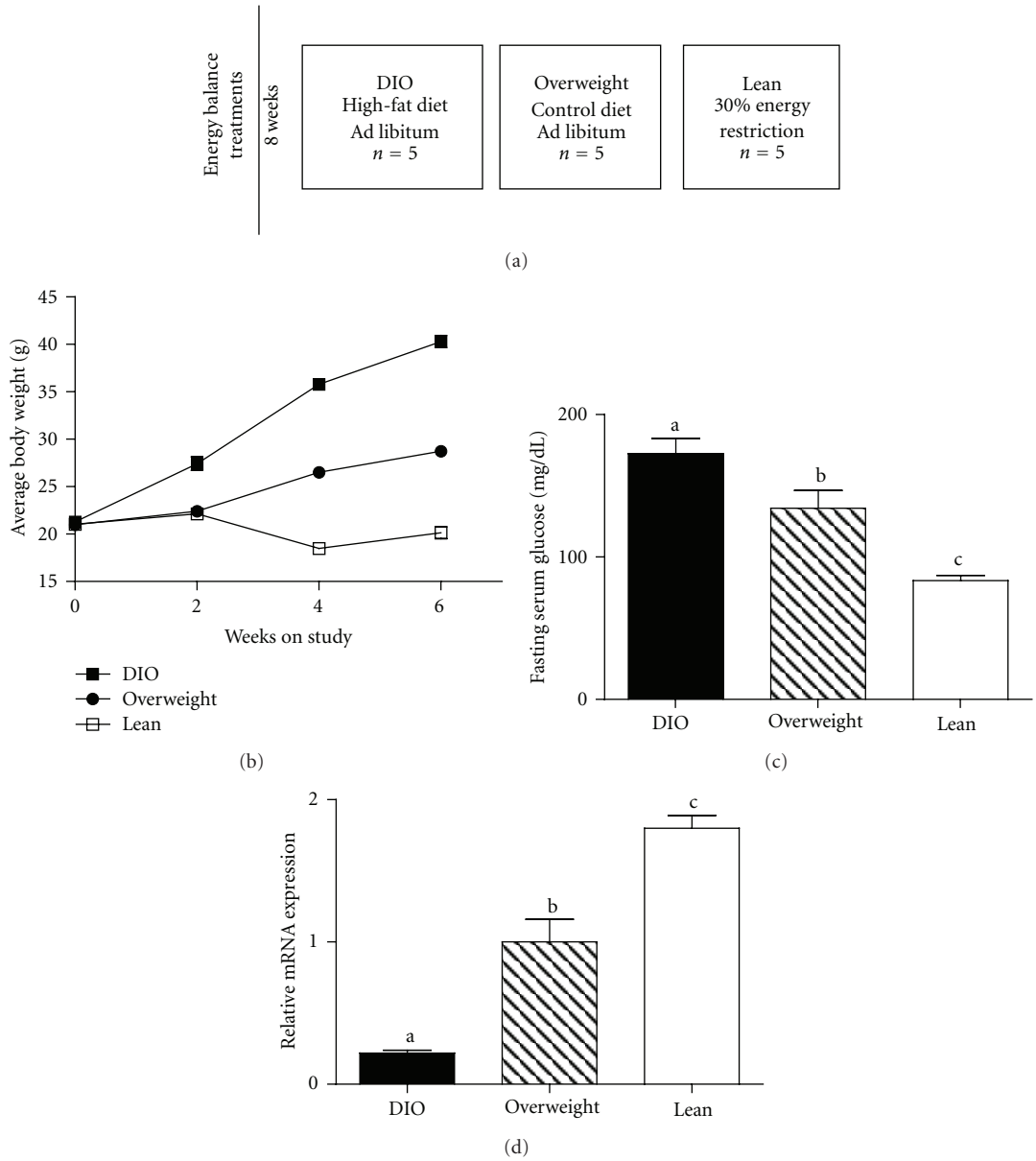



Figure 4: Lean phenotype is associated with lower blood glucose levels and elevated levels of Glut4 mRNA relative to control mice. (a) Study design for chromosomal immunoprecipitation experiments. (b) Average body weight of mice on DIO, overweight (control AIN-76A) diet, or a lean (CR) regimen to generate obese, overweight or lean mice ($n = 5$/group). Data shown are mean ± SE. (c) Fasting blood glucose levels of mice after 8 weeks of respective dietary regimen ($n = 5$/group). Data shown are mean ± SE. Significance ($P < .05$) between groups is denoted by different letters. (d) Relative mRNA expression of Glut4 in VWAT ($n = 5$/group). Significance ($P < .05$) between groups is denoted by different letters.

CR; (3) upregulation of Glut 4 by CR may be explained in part by our finding that CR increased acetylation of histone 4 at the Glut4 promoter.

CR and EX both resulted in significant weight loss compared to sedentary DIO controls, which remained obese with a % body fat >50%. Although CR, and EX groups displayed comparable levels of percent body fat at the end of the intervention, only CR significantly improved insulin sensitivity. Exercise has been shown to increase insulin sensitivity in mice and humans [27, 28], although these effects are less clear in obese individuals such as the DIO mice used in this report. The relatively short intervention in our study may explain why EX was not as affective as CR at altering indices of insulin resistance. In other rodent studies that showed a significant affect of EX, the intervention was either more than 10 weeks long [10, 28, 29] or the intervention period was longer than the period of diet-induced obesity [15]. These differences in study design suggest that, in the short term, EX may not be as effective as CR in restoring insulin sensitivity.

Table 2: Transcriptional changes in response to calorie restriction or exercise in visceral white adipose tissue.

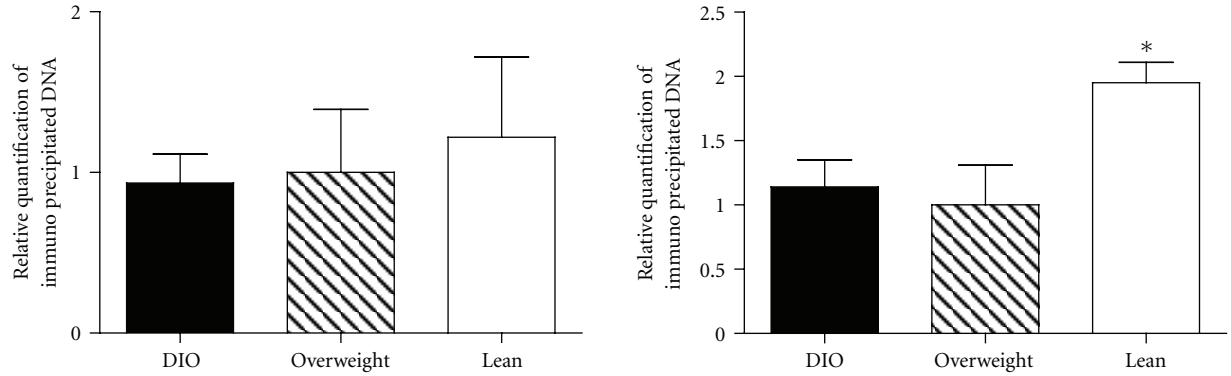
| Gene Symbol | Gene title | Fold change | |
|---|---|---|---|
| Cellular lipid metabolic process | | CR × DIO | |
| Slc27a1 | Solute carrier family 27 (fatty acid transporter) | 2.53 | up |
| Fads2 | Fatty acid desaturase 2 | 2.18 | up |
| Ces3 | Carboxylesterase 3 | 2.51 | up |
| Sult1a1 | Sulfotransferase family 1A | 2.22 | up |
| Ptges | Prostaglandin E synthase | 2.25 | up |
| Sgpp1 | Sphingosine-1-phosphate phosphatase 1 | 2.73 | down |
| Echs1 | Enoyl Coenzyme A hydratase | 2.00 | up |
| Hsd11b1 | Hydroxysteroid 11-beta dehydrogenase 1 | 3.28 | up |
| Apoc3 | Apolipoprotein C-III | 5.71 | up |
| Srebf1* | Sterol regulatory element binding transcription factor 1 | 2.98 | up |
| Aldh1a7 | Aldehyde dehydrogenase family 1 | 2.18 | up |
| Hpgd | Hydroxyprostaglandin dehydrogenase 15 (NAD) | 2.09 | down |
| Rdh11 | Retinol dehydrogenase 11 | 4.59 | up |
| Rarres2 | Retinoic acid receptor responder (tazarotene induced) 2 | 2.61 | down |
| Nsdhl* | NAD(P) dependent steroid dehydrogenase-like | 2.65 | up |
| Gpam | Glycerol-3-phosphate acyltransferase | 2.06 | up |
| Hmgcs1* | 3-hydroxy-3-methylglutaryl-Coenzyme A synthase 1 | 3.01 | down |
| Abat | 4-aminobutyrate aminotransferase | 2.88 | down |
| Sorl1 | Sortilin-related receptor | 2.77 | up |
| Pip4k2a | Phosphatidylinositol-5-phosphate 4-kinase | 2.02 | down |
| Acaca | Acetyl-Coenzyme A carboxylase alpha | 4.60 | up |
| Tm7sf2* | Transmembrane 7 superfamily member 2 | 3.69 | up |
| Sc5d* | Sterol-C5-desaturase | 2.11 | up |
| Fdft1* | Farnesyl diphosphate farnesyl transferase 1 | 2.68 | up |
| Hsd17b12* | Hydroxysteroid (17-beta) dehydrogenase 12 | 2.17 | up |
| Pcx* | Pyruvate carboxylase | 2.88 | up |
| Cellular carbohydrate metabolic process | | | |
| Fn3k | Fructosamine 3 kinase | 4.05 | up |
| Chst1* | Carbohydrate (keratan sulfate Gal-6) sulfotransferase 1 | 2.00 | up |
| Dlat | Dihydrolipoamide S-acetyltransferase | 2.59 | up |
| Pkm2 | Pyruvate kinase | 2.37 | up |
| Pmm1 | Phosphomannomutase 1 | 2.72 | up |
| Ppp1r1a | Protein phosphatase 1 | 2.61 | up |
| Pgd | Phosphogluconate dehydrogenase | 2.40 | up |
| Agl | Amylo-1 | 2.16 | up |
| Oxct1 | 3-oxoacid CoA transferase 1 | 2.17 | down |
| Pdk1 | Pyruvate dehydrogenase kinase | 3.07 | up |
| Gpd1 | Glycerol-3-phosphate dehydrogenase 1 (soluble) | 2.02 | up |
| Taldo1 | Transaldolase 1 | 2.03 | up |
| Glucose transport | | | |
| Sh2b2* | SH2B adaptor protein 2 | 3.26 | up |
| Slc2a4 | Solute carrier family 2 (facilitated glucose transporter) | 3.43 | up |
| Pcx* | Pyruvate carboxylase | 2.88 | up |
| Klf15 | Kruppel-like factor 15 | 2.36 | up |
| Immune response | | CR × DIO | |
| Malt1 | Mucosa associated lymphoid tissue Lymphoma translocation gene 1 | 2.55 | down |
| Bcl6* | B-cell leukemia/lymphoma 6 | 2.90 | down |
| Clec7a* | C-type lectin domain family 7 | 2.89 | down |
| Cfb | Complement factor B | 2.53 | down |
| Cd55* | CD55 antigen | 2.25 | Down |
| Thy1 | Thymus cell antigen 1 | 2.44 | down |

Table 2: Continued.

| Gene Symbol | Gene title | Fold change | |
|---|---|---|---|
| Biosynthesis of Steroids | | | |
| Lss | Lanosterol synthase | 2.13 | up |
| Hmgcs1* | 3-hydroxy-3-methylglutaryl-Coenzyme A synthase 1 | 3.01 | down |
| Tm7sf2* | Transmembrane 7 superfamily member 2 | 3.69 | up |
| Sc5d* | Sterol-C5-desaturase | 2.11 | up |
| Fdft1* | Farnesyl diphosphate farnesyl transferase 1 | 2.68 | up |
| Hsd17b12* | Hydroxysteroid (17-beta) dehydrogenase 12 | 2.17 | up |
| Nsdhl* | NAD(P) dependent steroid dehydrogenase-like | 2.65 | up |
| Sh2b2* | SH2B adaptor protein 2 | 3.26 | up |
| Stress response | | | |
| Thbs1 | Thrombospondin 1 | 2.29 | down |
| Tfpi2 | Tissue factor pathway inhibitor 2 | 4.32 | down |
| Gp1bb | Glycoprotein Ib | 2.06 | up |
| Taok3 | TAO kinase 3 | 2.21 | down |
| Sod3 | Superoxide dismutase 3 | 2.17 | down |
| Dusp10 | Dual specificity phosphatase 10 | 2.62 | down |
| Adrb3 | Adrenergic receptor | 2.09 | up |
| F2r | Coagulation factor II (thrombin) receptor | 2.22 | down |
| Ccnd1 | Cyclin D1 | 2.91 | down |
| Evl | Ena-vasodilator stimulated phosphoprotein | 2.32 | down |
| Ctsb | Cathepsin B | 2.00 | down |
| Ly86 | Lymphocyte antigen 86 | 2.65 | down |
| Fabp4 | Fatty acid binding protein 4 | 2.03 | up |
| Rad50 | RAD50 homolog (S. cerevisiae) | 2.27 | down |
| Tsc22d2 | TSC22 domain family 2 | 2.41 | down |
| Ptger3 | Prostaglandin E receptor 3 (subtype EP3) | 2.21 | up |
| Lcp1 | Lymphocyte cytosolic protein 1 | 3.06 | down |
| Pros1 | Protein S (alpha) | 2.09 | down |
| Hspa12a | Heat shock protein 12A | 2.40 | down |
| Anxa2 | Annexin A2 | 2.01 | down |
| Uhrf1 | Ubiquitin-like | 2.84 | down |
| Cdkn1a | Cyclin-dependent kinase inhibitor 1A (P21) | 2.28 | down |
| Srebf1* | Sterol regulatory element binding transcription factor 1 | 2.98 | up |
| Chst1* | Carbohydrate (keratan sulfate Gal-6) sulfotransferase 1 | 2.00 | up |
| Bcl6* | B-cell leukemia/lymphoma 6 | 2.90 | down |
| Clec7a* | C-type lectin domain family 7 | 2.89 | down |
| Cd55* | CD55 antigen | 2.25 | down |
| Exercise unique | | | |
| Mitochondrial Transport | | EX × DIO | |
| Ucp1 | Uncoupling protein | 8.94 | up |
| Ucp2 | Uncoupling protein 2 | 2.09 | down |

* Genes represented in two different categories.

CR has been shown to decrease expression of genes related to aging and tumorigenesis in multiple tissues [30]. However, there is a paucity of studies examining the effect of CR on adipose tissue following weight loss. More importantly, there are no studies, to our knowledge, directly comparing the effect of CR and EX on gene expression in adipose tissue. To our knowledge there are only two microarray studies comparing CR to EX; one was performed by our group in the mouse mammary gland [31] and the other by Lu et al. in mouse skin [32]. In these reports CR and EX exhibited distinct effects on gene expression, with CR impacting more than 4 times the number of genes than EX. In the present study, we found that this differential impact was more pronounced in adipose tissue, with CR affecting more than 20 times the number of genes altered by EX. Not only did CR induce a stronger quantitative effect than EX on




(a) H3K9 Trimethylation of Glut4 promoter

(b) H4 Acethylation of Glut4 promoter

Figure 5: Calorie restriction increases histone H4 acetylation at the GLUT 4 promoter. (a) Relative quantification of Glut4 DNA immunoprecipitated with anti-trimethyl H4 antibody. (b) Relative quantification of Glut4 DNA immunoprecipitated with anti-acetyl H4 antibody ($n = 3$/group). Data shown are mean ± SE, $^*(P \leq 0.05)$.

genes that were qualitatively similar in their response to both CR and EX, but CR affected an additional 48 genes related to metabolism that were unaffected by EX. Of those genes, there was an overall upregulation of genes related to carbohydrate metabolism and glucose transport, including Glut4.

We also found that DIO downregulates multiple genes that play a role in lipid metabolism and upregulates a profile of genes related to immune/inflammatory response [33, 34]. Furthermore, many of the lipid metabolism genes shown to be decreased by DIO were increased by CR and EX in our study [33]. Likewise, immune response genes that have been shown to be increased by DIO were decreased after weight loss induced by CR or EX [33]. Together these data support previous findings that in the obese state, there is diminished fatty acid synthesis and transport, characteristic of insulin-resistant adipose tissue rich in immune cell infiltrates. Importantly, our data show that these processes are sensitive to both CR and EX interventions.

Many of the transcripts related to lipid and carbohydrate metabolism that were affected by both CR and EX in the present study were also shown by Shankar et al. to be induced by a high-carbohydrate diet [35]. Increased transcription of these genes is consistent with increased uptake of glucose and fatty acids into the adipose tissue. In the study by Shankar et al. these transcriptional changes were measured in rats fed a high-carbohydrate diet for 4 weeks, during which time the rats gained weight and the adipocytes hypertrophied, whereas the mice in our study first underwent DIO but then lost weight for 8 weeks before analysis. The similarities between the two studies are indicative of increased signaling through the insulin receptor/phosphatidylinositol 3-kinase (IR/PI3K) pathway that mediates glucose uptake and the lipogenic effects of insulin in adipose tissue.

Glucose uptake into adipose tissue is mediated by two different Glut isoforms: Glut1 and Glut4. Glut1 mediates basal uptake of glucose into adipocytes. Although others have reported that Glut1 mRNA increases with obesity [36], we did not observe any changes in Glut1 mRNA expression in the microarray. Translocation of the GLUT4 transporter from the cytosol to the membrane is the rate-limiting step in insulin-mediated glucose uptake in adipocytes and skeletal muscle [37]. The importance of Glut4 function in adipose tissue is underscored by the finding that overexpression of Glut4 in adipocytes rescues insulin resistance in mice with muscle-specific knockout of Glut4 [38]. However, expression of Glut4 in the muscle does not compensate for lack of Glut4 activity in adipose tissue [39], further implicating adipose tissue as a key metabolic organ in the etiology of insulin resistance. There is considerable evidence that Glut4 mRNA levels in adipose tissue decrease with obesity [40], and that increases in Glut4 mRNA in adipose tissue can ameliorate insulin resistance [41, 42]. Indeed, our finding that Glut4 mRNA levels were significantly increased by CR, but not by EX, and that this increase was associated with improved insulin sensitivity, supports this idea. Therefore, increased transcription of Glut4 in VWAT during weight loss may be a critical event in reversing insulin resistance.

Studies into the transcriptional regulation of Glut4 in skeletal muscle implicate a histone deacetylase (HDAC5) as a crucial mediator of changes to Glut4 mRNA levels in response to exercise [43]. Raychaudhuri et al. have also described a series of histone modifications mediated by histone deacetylases and histone methyltransferases that culminate in a metabolic knockdown of the Glut4 gene in the skeletal muscle of rats that had experienced intrauterine growth restriction [44]. Collectively, these studies suggest that transcriptional regulation of the Glut4 gene is highly responsive to changes in energy balance. This led to our hypothesis that Glut4 mRNA levels in adipose tissue could be subjected to similar transcriptional regulation. In support of this hypothesis, Wellen et al. recently discovered that during adipocyte differentiation, levels of global histone acetylation are dependent on glucose availability [25]. More specifically, acetylation of histones 3 and 4 at the Glut4 promoter is linked to increased Glut4 mRNA expression in response to higher concentrations of glucose during differentiation. Our *in vivo* ChIP data extend the in vitro findings and show that increased acetylation of histone 4 at the Glut4 promoter,

which was associated with higher levels of Glut4 mRNA, occurred in lean mice that were highly insulin sensitive as indicated by significantly decreased fasting glucose levels. Taken together, these data suggest that insulin-responsive adipose tissue maintains H4 acetylation. This leads to increased transcription of Glut4 to facilitate continued glucose uptake. However, as adiposity increases so does insulin resistance [3]. Deregulation of signal transduction downstream of the insulin receptor results in decreased trafficking of Glut4 to the cell membrane [45, 46] and a decline in glucose flux into the adipocyte [47]. According to the findings of Wellen et al., limited glucose availability results in diminished histone acetylation and decreased Glut4 mRNA expression [25]. Therefore, in the context of obesity and insulin resistance, the lower levels of Glut4 mRNA expressed in adipose tissue may be a consequence of decreased insulin-mediated uptake of glucose that results in diminished histone acetylation at the Glut4 promoter. Further analyses are required to determine if other modifications to the histone code at the Glut4 promoter may be contributing to transcriptional repression of Glut4 mRNA in obesity.

In conclusion, these findings show that obesity reversal by CR versus EX results in many shared, but also many differential, changes in the adipose transcriptome. In particular, CR has specific and significant effects on the expression of key metabolic genes and pathways associated with obesity-related disease. In addition, some of the effects of these antiobesity interventions on VWAT gene expression and metabolism may result from chromatin remodeling, as illustrated by CR's effect on histone acetylation of the GLUT4 promoter. Taken together, these studies provide insights regarding new targets, including potential epigenetic-related regulation of key metabolic genes, such as Glut4 acetylation, for preventing or treating obesity-related diseases.

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## References

[1] K. M. Flegal, M. D. Carroll, C. L. Ogden, and L. R. Curtin, "Prevalence and trends in obesity among US adults, 1999–2008," *Journal of the American Medical Association*, vol. 303, no. 3, pp. 235–241, 2010.

[2] T. Y. Li, J. S. Rana, J. E. Manson et al., "Obesity as compared with physical activity in predicting risk of coronary heart disease in women," *Circulation*, vol. 113, no. 4, pp. 499–506, 2006.

[3] C. Meisinger, A. Döring, B. Thorand, M. Heier, and H. Löwel, "Body fat distribution and risk of type 2 diabetes in the general population: are there differences between men and women? The MONICA/KORA Augsburg Cohort Study," *American Journal of Clinical Nutrition*, vol. 84, no. 3, pp. 483–489, 2006.

[4] E. E. Calle, C. Rodriguez, K. Walker-Thurmond, and M. J. Thun, "Overweight, obesity, and mortality from cancer in a prospectively studied cohort of U.S. Adults," *New England Journal of Medicine*, vol. 348, no. 17, pp. 1625–1638, 2003.

[5] R. T. Watson and J. E. Pessin, "GLUT4 translocation: the last 200 nanometers," *Cellular Signalling*, vol. 19, no. 11, pp. 2209–2217, 2007.

[6] A. S. Greenberg and M. S. Obin, "Obesity and the role of adipose tissue in inflammation and metabolism," *American Journal of Clinical Nutrition*, vol. 83, no. 2, pp. 461S–465S, 2006.

[7] S. Crowe, L. E. Wu, C. Economou et al., "Pigment epithelium-derived factor contributes to insulin resistance in obesity," *Cell Metabolism*, vol. 10, no. 1, pp. 40–47, 2009.

[8] P. Sartipy and D. J. Loskutoff, "Monocyte chemoattractant protein 1 in obesity and insulin resistance," *Proceedings of the National Academy of Sciences of the United States of America*, vol. 100, no. 12, pp. 7265–7270, 2003.

[9] S. General, *The Surgeon General's Call to Action to Prevent and Decrease Overweight and Obesity*, U.S. Department of Health and Human Services, 2001.

[10] E. P. Weiss, S. B. Racette, D. T. Villareal et al., "Improvements in glucose tolerance and insulin action induced by increasing energy expenditure or decreasing energy intake: a randomized controlled trial," *American Journal of Clinical Nutrition*, vol. 84, no. 5, pp. 1033–1042, 2006.

[11] R. H. Coker, R. H. Williams, S. E. Yeo et al., "The impact of exercise training compared to caloric restriction on hepatic and peripheral insulin resistance in obesity," *Journal of Clinical Endocrinology and Metabolism*, vol. 94, no. 11, pp. 4258–4266, 2009.

[12] I. Giannopoulou, L. L. Ploutz-Snyder, R. Carhart et al., "Exercise is required for visceral fat loss in postmenopausal women with type 2 diabetes," *Journal of Clinical Endocrinology and Metabolism*, vol. 90, no. 3, pp. 1511–1518, 2005.

[13] A. S. Ryan, B. J. Nicklas, D. M. Berman, and D. Elahi, "Adiponectin levels do not change with moderate dietary induced weight loss and exercise in obese postmenopausal women," *International Journal of Obesity*, vol. 27, no. 9, pp. 1066–1071, 2003.

[14] B. J. Nicklas, X. Wang, T. You et al., "Effect of exercise intensity on abdominal fat loss during calorie restriction in overweight and obese postmenopausal women: a randomized, controlled trial," *American Journal of Clinical Nutrition*, vol. 89, no. 4, pp. 1043–1052, 2009.

[15] A. D. Krisan, D. E. Collins, A. M. Crain et al., "Resistance training enhances components of the insulin signaling cascade in normal and high-fat-fed rodent skeletal muscle," *Journal of Applied Physiology*, vol. 96, no. 5, pp. 1691–1700, 2004.

[16] K. Y. Lee, S. J. Kim, Y. S. Cha et al., "Effect of exercise on hepatic gene expression in an obese mouse model using cDNA microarrays," *Obesity*, vol. 14, no. 8, pp. 1294–1302, 2006.

[17] C. C. Cowie, K. F. Rust, D. D. Byrd-Holt et al., "Prevalence of diabetes and impaired fasting glucose in adults in the U.S. population: National Health and Nutrition Examination Survey 1999–2002," *Diabetes Care*, vol. 29, no. 6, pp. 1263–1268, 2006.

[18] S. Z. Haslam, J. R. Osuch, A. M. Raafat, and L. J. Hofseth, "Postmenopausal hormone replacement therapy: effects on normal mammary gland in humans and in a mouse postmenopausal model," *Journal of Mammary Gland Biology and Neoplasia*, vol. 7, no. 1, pp. 93–105, 2002.

[19] K. E. Wheatley, E. A. Williams, N. C. P. Smith et al., "Low-carbohydrate diet versus caloric restriction: effects on weight loss, hormones, and colon tumor growth in obese mice," *Nutrition and Cancer*, vol. 60, no. 1, pp. 61–68, 2008.

[20] D. Berrigan, J. A. Lavigne, S. N. Perkins, T. R. Nagy, J. C. Barrett, and S. D. Hursting, "Phenotypic effects of calorie restriction and insulin-like growth factor-1 treatment on body composition and bone mineral density of C57BL/6 mice: implications for cancer prevention," *In Vivo*, vol. 19, no. 4, pp. 667–674, 2005.

[21] D. W. Huang, B. T. Sherman, and R. A. Lempicki, "Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources," *Nature Protocols*, vol. 4, no. 1, pp. 44–57, 2009.

[22] M. È. Piché, S. J. Weisnagel, L. Corneau, A. Nadeau, J. Bergeron, and S. Lemieux, "Contribution of abdominal visceral obesity and insulin resistance to the cardiovascular risk profile of postmenopausal women," *Diabetes*, vol. 54, no. 3, pp. 770–777, 2005.

[23] M. Matsubara, S. Maruoka, and S. Katayose, "Inverse relationship between plasma adiponectin and leptin concentrations in normal-weight and obese women," *European Journal of Endocrinology*, vol. 147, no. 2, pp. 173–180, 2002.

[24] J. M. Bruun, J. W. Helge, B. Richelsen, and B. Stallknecht, "Diet and exercise reduce low-grade inflammation and macrophage infiltration in adipose tissue but not in skeletal muscle in severely obese subjects," *American Journal of Physiology*, vol. 290, no. 5, pp. E961–E967, 2006.

[25] K. E. Wellen, G. Hatzivassiliou, U. M. Sachdeva, T. V. Bui, J. R. Cross, and C. B. Thompson, "ATP-citrate lyase links cellular metabolism to histone acetylation," *Science*, vol. 324, no. 5930, pp. 1076–1080, 2009.

[26] K. Tateishi, Y. Okada, E. M. Kallin, and Y. Zhang, "Role of Jhdm2a in regulating metabolic gene expression and obesity resistance," *Nature*, vol. 458, no. 7239, pp. 757–761, 2009.

[27] L. L. Frank, B. E. Sorensen, Y. Yasui et al., "Effects of exercise on metabolic risk variables in overweight postmenopausal women: a randomized clinical trial," *Obesity Research*, vol. 13, no. 3, pp. 615–625, 2005.

[28] V. J. Vieira, R. J. Valentine, K. R. Wilund, N. Antao, T. Baynard, and J. A. Woods, "Effects of exercise and low-fat diet on adipose tissue inflammation and metabolic complications in obese mice," *American Journal of Physiology*, vol. 296, no. 5, pp. E1164–E1171, 2009.

[29] Z. A. Zarins, M. L. Johnson, N. Faghihnia et al., "Training improves the response in glucose flux to exercise in postmenopausal women," *Journal of Applied Physiology*, vol. 107, no. 1, pp. 90–97, 2009.

[30] W. R. Swindell, "Genes and gene expression modules associated with caloric restriction and aging in the laboratory mouse," *BMC Genomics*, vol. 10, article 585, 2009.

[31] M. Padovani, J. A. Lavigne, G. V. R. Chandramouli et al., "Distinct effects of calorie restriction and exercise on mammary gland gene expression in C57BL/6 mice," *Cancer Prevention Research*, vol. 2, no. 12, pp. 1076–1087, 2009.

[32] J. Lu, L. Xie, J. Sylvester et al., "Different gene expression of skin tissues between mice with weight controlled by either calorie restriction or physical exercise," *Experimental Biology and Medicine*, vol. 232, no. 4, pp. 473–480, 2007.

[33] R. C. Moraes, A. Blondet, K. Birkenkamp-Demtroeder et al., "Study of the alteration of gene expression in adipose tissue of diet-induced obese mice by microarray and reverse transcription-polymerase chain reaction analyses," *Endocrinology*, vol. 144, no. 11, pp. 4773–4782, 2003.

[34] R. S. Miller, K. G. Becker, V. Prabhu, and D. W. Cooke, "Adipocyte gene expression is altered in formerly obese mice and as a function of diet composition," *Journal of Nutrition*, vol. 138, no. 6, pp. 1033–1038, 2008.

[35] K. Shankar, A. Harrell, P. Kang, R. Singhal, M. J. J. Ronis, and T. M. Badger, "Carbohydrate-responsive gene expression in the adipose tissue of rats," *Endocrinology*, vol. 151, no. 1, pp. 153–164, 2010.

[36] I. Talior, M. Yarkoni, N. Bashan, and H. Eldar-Finkelman, "Increased glucose uptake promotes oxidative stress and PKC-$\delta$ activation in adipocytes of obese, insulin-resistant mice," *American Journal of Physiology*, vol. 285, no. 2, pp. E295–E302, 2003.

[37] E. Karnieli and M. Armoni, "Transcriptional regulation of the insulin-responsive glucose transporter GLUT4 gene: from physiology to pathology," *American Journal of Physiology*, vol. 295, no. 1, pp. E38–E45, 2008.

[38] E. Carvalho, K. Kotani, O. D. Peroni, and B. B. Kahn, "Adipose-specific overexpression of GLUT4 reverses insulin resistance and diabetes in mice lacking GLUT4 selectively in muscle," *American Journal of Physiology*, vol. 289, no. 4, pp. E551–E561, 2005.

[39] E. D. Abel, O. Peroni, J. K. Kim et al., "Adipose-selective targeting of the GLUT4 gene impairs insulin action in muscle and liver," *Nature*, vol. 409, no. 6821, pp. 729–733, 2001.

[40] C. N. Lumeng, S. M. Deyoung, and A. R. Saltiel, "Macrophages block insulin action in adipocytes by altering expression of signaling and glucose transport proteins," *American Journal of Physiology*, vol. 292, no. 1, pp. E166–E174, 2007.

[41] M. Furuta, Y. Yano, E. C. Gabazza et al., "Troglitazone improves GLUT4 expression in adipose tissue in an animal model of obese type 2 diabetes mellitus," *Diabetes Research and Clinical Practice*, vol. 56, no. 3, pp. 159–171, 2002.

[42] E. Tozzo, L. Gnudi, and B. B. Kahn, "Amelioration of insulin resistance in streptozotocin diabetic mice by transgenic overexpression of GLUT4 driven by an adipose-specific promoter," *Endocrinology*, vol. 138, no. 4, pp. 1604–1611, 1997.

[43] S. L. McGee, B. J. W. Van Denderen, K. F. Howlett et al., "AMP-activated protein kinase regulates GLUT4 transcription by phosphorylating histone deacetylase 5," *Diabetes*, vol. 57, no. 4, pp. 860–867, 2008.

[44] N. Raychaudhuri, S. Raychaudhuri, M. Thamotharan, and S. U. Devaskar, "Histone code modifications repress glucose transporter 4 expression in the intrauterine growth-restricted offspring," *Journal of Biological Chemistry*, vol. 283, no. 20, pp. 13611–13626, 2008.

[45] B. Emanuelli, P. Peraldi, C. Filloux et al., "SOCS-3 inhibits insulin signaling and is up-regulated in response to tumor necrosis factor-$\alpha$ in the adipose tissue of obese mice," *Journal of Biological Chemistry*, vol. 276, no. 51, pp. 47944–47949, 2001.

[46] J. E. Friedman, T. Ishizuka, S. Liu et al., "Reduced insulin receptor signaling in the obese spontaneously hypertensive Koletsky rat," *American Journal of Physiology*, vol. 273, no. 5, pp. E1014–E1023, 1997.

[47] J. Shao, H. Yamashita, L. Qiao, and J. E. Friedman, "Decreased Akt kinase activity and insulin resistance C57BL/KsJ-Lepr(db/db) mice," *Journal of Endocrinology*, vol. 167, no. 1, pp. 107–115, 2000.