Distinct skeletal muscle fiber characteristics and gene expression in diet–sensitive vs. diet–resistant obesity

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Abbreviations: ODR, obese diet-resistant; ODS, obese diet-sensitive; PLS-DA, partial least squares discriminant analysis; BMI, body mass index; PAE, physical activity energy expenditure; GSEA, gene set enrichment analyses
**Abstract** Inter-individual variability in weight gain and loss under energy surfeit and deficit conditions, respectively, are well recognized but poorly understood phenomena. We documented weight loss variability in an intensively supervised clinical weight loss program and assessed skeletal muscle gene expression and phenotypic characteristics related to variable response to a 900 kcal regimen. Matched pairs of healthy, diet-compliant obese diet-sensitive (ODS) and diet-resistant (ODR) subjects were defined as those in the highest and lowest quintiles for weight loss rate. Physical activity energy expenditure was minimal and comparable. Following program completion and weight stabilization, skeletal muscle biopsies were obtained. Gene expression analysis of *rectus femoris* and *vastus lateralis* indicated upregulation of genes and gene sets involved in oxidative phosphorylation, glucose and fatty acid metabolism in ODS, as compared to ODR. In *vastus lateralis* there was a higher proportion of oxidative (type I) fibers in ODS compared to ODR women and to lean controls; fiber hypertrophy in ODS compared to ODR women and lean controls; and lower succinate dehydrogenase in oxidative and oxidative-glycolytic fibers in all obese compared to lean subjects. Intramuscular lipid content was generally higher in obese vs. lean, and specifically higher in ODS vs. lean women. Altogether, findings demonstrate differences in muscle gene expression and fiber composition related to clinical weight loss success.

**Supplementary key words:** mitochondria, oxidative phosphorylation, fiber type, thermogenesis
Obesity is reaching epidemic proportions in developed countries and represents a significant risk factor for cardiovascular disease, diabetes, and cancer (1). Enrolment in clinical and non-clinical obesity treatment programs is unprecedented. Success in obesity treatment programs is highly variable, related in part to compliance and program characteristics (e.g., type and duration of hypocaloric diets, educational components, and/or exercise-associated energy expenditure). While it is generally well accepted that there is substantial inter-individual variability in the susceptibility to weight gain in response to overfeeding (2, 3), less well understood is the impact of biological factors on weight loss success. However, studies of monozygotic twins have shown greater inter-pair than intra-pair variation in weight loss (4), consistent with the idea that there are important genetic determinants of weight loss success. We have studied the molecular and cellular determinants of variable weight loss in highly compliant subjects in an intensively supervised and interactive hypocaloric clinical obesity treatment program at the Ottawa Hospital. We previously reported differences in muscle mitochondrial energy inefficiencies between program participants exhibiting high versus low weight loss success (5). Here we extend these findings by demonstrating distinct differences in skeletal muscle gene expression profiles and in structural and metabolic characteristics between individuals who exhibit markedly different degrees of weight loss success in a clinical obesity treatment program.

Factors affecting skeletal muscle energy metabolism are relevant since skeletal muscle is a major determinant of resting energy expenditure (~20%), largely due to the fact that it accounts for ~ 40% of adult body weight (6). Yet the idea that differences in skeletal energy expenditure characteristics can explain variability in weight loss success has not been well explored. However, available evidence is consistent with this idea. Briefly, physiological and gene expression studies demonstrate that low oxidative capacity in muscle is characteristic of obesity and type 2 diabetes mellitus, and predisposition to the latter (7-11). In a long-term follow-up study of obese men, lower proportions of oxidative (type I) fibers were associated with a higher prevalence of obesity (12). Notably, Tanner et al. showed that weight loss following gastric bypass surgery was correlated with proportions of type I fibers in rectus abdominus muscle (13). Others have demonstrated that whole body resting energy expenditure is correlated with forearm muscle oxygen uptake – a phenomenon hypothesized to explain obesity susceptibility (6).
Finally, in rodent obesity studies, a lower proportion of type I fibers was associated with increased risk for high fat diet-induced obesity compared to obesity-resistant rats (14).

We have investigated phenotypic and gene expression differences in rectus femoris and vastus lateralis muscle biopsies from obese diet-sensitive (ODS) and obese diet-resistant (ODR) subjects in an intensively supervised, multidisciplinary clinical weight loss program. Our findings reveal marked increases in the proportion of oxidative fibers and fiber size in vastus lateralis muscle of ODS compared to ODR and lean subjects. Obesity (ODS and ODR) was associated with decreased muscle mitochondrial content. Moreover, exploratory gene expression analysis of rectus femoris and vastus lateralis muscles from ODR and ODS subjects indicate upregulation of gene sets involved in oxidative phosphorylation, glucose and fatty acid metabolism in ODS, as compared to ODR. These findings provide new insights into factors contributing to weight loss success in obesity.

METHODS

Clinical Protocol

The Human Research Ethics Committees of the Ottawa Hospital and the University of Ottawa Heart Institute approved the study. Signed consent was obtained from all participants of our study. A total of 2,878 patients with a Body Mass Index (BMI) of 30-50 kg/m2 entered the Ottawa Hospital Weight Management Program from September 1992 to the end of December 2006. This intensively supervised, multidisciplinary program uses the Optifast 900® total meal replacement for the first 6 or 12 weeks to accomplish safe weight loss in a timely fashion [2,3,4]. Percent weight loss was evaluated in the first 6 weeks of the 900 kcal meal replacement. We excluded 316 patients who had insufficient baseline or 6 weeks data or who failed to complete the initial 6 weeks of study. Males were excluded from study since total numbers were relatively small. This left 1,868 evaluable women who were ranked according to percent weight loss.

Selection of Patients

As in our previous report (5), we studied healthy highly compliant women enrolled in the Ottawa Hospital Weight Management Program. Because adherence to protocol is a major reason for poor response to dietary intervention, every effort was made to remove noncompliant patients.
Selection of compliant patients was maximized by studying subjects during the first 6 weeks of an 8 or 12 month weight program with substantial cost to the patient ($1700-$2800). In addition, patients were excluded if they completed less than 75% of the 26 weekly visits, or were absent for more than two visits during the initial 6 weeks on meal replacement, or there were physician notes expressing reservations about self-reported compliance, or there was inadequate completion of the laboratory testing protocol.

Patients were also excluded on the basis of medical conditions possibly affecting rate of weight loss, including thyroid indices (TSH, free T3) out of normal range at week 1 or week 13, diabetes mellitus treated with insulin or oral hypoglycaemic agents, cigarette smoking, congestive heart failure, obstructive sleep apnea, active malignancy, immobility, or previous bariatric surgery. Patients treated with weight-altering medications including tricyclic antidepressants, paroxetine, mirtazepine, lithium, valproate, gabapentin and typical and atypical antipsychotics, fluoxetine in doses greater than 20mg, bupropion, topiramate, systemic glucocorticoids and weight management drugs were also excluded. Women meeting these criteria and who were in the top quintile of percent weight loss in the first 6 weeks of meal replacement were matched according to initial BMI and age with women in the bottom quintile for the rate of weight loss.

**Data Management**

Clinical information, including medical history, medication, weekly compliance assessments, anthropometric measurements, physicians’ notes and laboratory values were collected as reported in detail elsewhere [5]. Exclusions and rate of weight loss percentiles were described using software developed in this clinic [2] and by individual chart reviews on all patients to verify data.

**Physical activity energy expenditure**

Activity at work and planned physical activity of obese subjects were assessed by questionnaire prior to weight loss and were maintained at pre-study levels over the first 6 weeks of meal replacement. Activity at work was assessed using a 5-point Likkert scale with a value of 1 defined as an “inactive” (e.g., seated almost exclusively) and a value of 5 defined as a “very active” (e.g., walking nearly continuously) work environment. Subjects also recorded the frequency, duration, and type of planned weekly exercise activities. Daily physical activity
energy expenditure (PAE in kcal \cdot d^{-1}) was calculated using total body mass and specific energy expenditure coefficients (kcal \cdot kg^{-1} \cdot min^{-1}) of each planned exercise \cite{6}. (see also Results)

**Muscle Biopsies**

A subset of well matched women meeting the above criteria who were in the top (ODS) or bottom (ODR) quintile of percent weight loss in the first 6 weeks of meal replacement underwent *rectus femoris* open muscle biopsy. Biopsies were carried out between 07:00 and 09:00 after a 12 hour fast and several months after completion of the meal replacement program and after a period of at least 4 weeks of weight stabilization. For gene expression analyses, RNA was extracted from biopsied *rectus femoris* muscle of 6 matched pairs of ODS and ODR women. For replication studies needle biopsies of the *vastus lateralis* muscle were collected from 7 pairs of similarly matched obese diet-sensitive and obese diet-resistant women. For analyses of muscle fiber size, type and for mitochondrial analyses, a 100-150 mg needle biopsy of the *vastus lateralis* was collected using a Bergstrom needle from an additional group consisting of 6 matched pairs of ODS and ODR women.

**Gene Expression Analyses**

*mRNA extraction.* Frozen tissue was homogenized in Trizol and mRNA isolated using Qiagen oligo-dT latex beads. RNA samples were treated with amplification grade DNase1 and RNA concentration determined spectrophotometrically using OD260/280.

*Whole genome expression analyses using GeneChip® microarrays.* Hybridization samples were prepared as recommended (www.affymetrix.com) and hybridized to the Affymetrix GeneChip U133A 2.0 Array consisting of 18,400 transcripts and variants including 14,500 well characterized human genes. Sufficient RNA was obtained from 6 obese diet-sensitive and 6 obese diet-resistant subjects for microarray analyses. Gene expression signals were scaled to a target intensity of 150 for all chips. Replicates were compared against each other to spot outliers and none were detected. Genes with a maximum present call in >50% replicates in either group were kept for further analysis. Multiple statistical tests were performed to increase confidence in the set of genes that differed significantly between the ODS and ODR groups. These tests included the classical t-test, a modified t-test incorporating technology specific error models \cite{14} class distinction based on signal to noise \cite{15}, and partial least squares discriminant analysis.
(PLS-DA). Multivariate analysis PLS-DA was performed in SIMCA P+ software (Umetrics, Uppsala, Sweden) to identify candidate genes that could distinguish ODS from ODR subjects. Models were validated for fit (R2) and predictability (Q2) by randomly permuting the samples 200 times and recalculating R2 and Q2.

**Global pathway analyses**

Affymetrix gene expression data from ODS and ODR subjects was subjected to global pathway analysis by use of the Gene set enrichment analysis (GSEA) algorithm (15, 16). Since the number of samples are relatively small (6 of each class), GSEA was conducted by permuting gene-sets instead of phenotypes (samples). GSEA was conducted on data from the full chip with no prior filtering. One thousand permutations were used to obtain the nominal P value and gene sets with a false discovery rate (FDR) <0.05 were considered to be significantly regulated.

**Data sharing**

All microarray data was deposited in the Gene Expression Omnibus repository (http://www.ncbi.nlm.nih.gov/geo/) and is publicly available (accession number GSE 17371)

**Quantitative RealTime RT-PCR**

Determination of transcript quantity in key candidate genes was carried out using TaqMan quantitative reverse-transcription PCR (RT-PCR) on cDNA from individual vastus lateralis biopsy samples. Real-time PCR results were generated using a 5’ nuclease assay (TaqMan) and the ABI 7900 Sequence Detection System (Applied Biosystems, Foster City, CA, USA). Following outlier identification and removal, all data were subjected to normalization using beta actin and cyclophilin as endogenous reference transcripts. Following normalization, all data were subjected to statistical analysis using ANOVA, with significance criteria of p value < 0.05 and fold change > 1.5.

**Histological Analyses**

Muscle was immediately chilled in ice-cold PBS, and transferred to a 1:2 mixture of ice-cold 20% sucrose in PBS: OCT (Phosphate Buffered Saline: Optimal Cutting Temperature) compound over 2 hours. It was then frozen in OCT compound using a liquid-nitrogen cooled
isopentane slurry (-140°C) and stored at -80°C. Muscle was sectioned (10 μm) by cryostat and mounted onto slides at -20°C.

**Intramuscular neutral lipid content** was assessed by Oil Red O staining followed by densitometric analysis of grey-scale images. Briefly, frozen sections were fixed with paraformaldehyde (4% in PBS) for 10 min, then exposed to oil red O (Sigma) for 1 h followed by 2 or 3 washes with distilled water. Oil Red O staining was quantified using ImageJ software (NIH). Images were converted to 8-bit grey-scale, and the integrated pixel density and area were obtained per muscle fiber. Two images from two different sections were analyzed per subject. For each group, the total numbers of fibers/subject analyzed were (mean ± SEM): 106 ± 6 (ODS), 118 ± 8 (ORD), 136 ± 23 (L).

**Mitochondrial content** was assessed using the total succinate dehydrogenase (SDH) activity assay in frozen muscle sections (described above). Sections were thawed and activity was assayed over a period of one hour at 25 °C. It was confirmed that the assay was linear over this period of time. Activity at the individual fiber level was visualized colorimetrically and quantified densitometrically from images captured with a Zeiss Axiovert microscope and a LCD camera. All fibers/section were included in the analysis of a section. See below for details of image analysis protocol.

**Myofibrillar size analyses** were conducted using laminin/meromycin staining. Briefly, the sections analyzed for succinate dehydrogenase activity were fixed for 30 minutes on ice in 3.7% paraformaldehyde. After rinsing, immunostaining was performed for laminin (LAM-89; Sigma; L8271) at 1/125 of stock, and subsequently for sarcomeric myosin (MF-20c; DSHB) at 1/5. Primary antibodies were detected using standard ABC amplification techniques (Vector; PK-7100). See below for details of image analysis protocol.

**Fiber Typing.** Sections immediately adjacent to the SDH/Laminin/MF-20 material were air dried (60 min.; room temperature) and stained for type IIa (N2.26, IgG1; DSHB) and type IIx fibers (212-F, IgG1; P. Merrifield). Although 212F reacts with both type IIx and IIb proteins, fibers were categorized as IIx because human muscle does not translate MyHC-IIb mRNA [7]. Staining was enhanced with a biotin-avidin amplification step (M.O.M Basic Kit; Vector BMK 2202, Avidin/Biotin Blocking kit; Vector SP-2001). IIa fibers were detected with Avidin-488 (Invitrogen; A-21370). IIx fibers were detected using alkaline phosphatase (Vector Blue AP Kit III; SK-5300).
Sections from tissue also adjacent to the SDH/Laminin/MF-20 sections (opposing direction) were double labeled for type I (A4.840 IgM; DSHB) and type IIA (N2.26, IgG1; DSHB). Ila staining was repeated to detect hybrid fibers. Antibodies for type I fibers were diluted to 5% of stock, and illuminated with Avidin-TXR (Invitrogen; A-820) diluted to 4%. Slides were mounted in anti-fade medium and stored at -20 °C.

Image analysis. Images were taken at equal exposure settings between patients (SDH at 0.13S, 212F at 0.16S, N.2.261 at 5.0S, A4.840 at 5.0S, LAM89 at 6.5S, MF-20 at 5.0S). Laminin and meromycin image sets were used to create analyzable region of interest (ROI) maps using Image-J software, as follows. Contrast was enhanced by eye in the laminin and meromycin photographs. Images were then merged and converted to binary images using Image-J software (http://reb.info.nih.gov/ij/). Each binary image was then completely watershed using the white-pen erasing tool, and damaged/incomplete fibers were removed. A 25x25 unit background control ROI was added to each ROI map to assess background intensity. Each fiber was identified as type I, type IIA, type IIX or hybrid. Hybrid fibers comprised less than 1% of total fibers and were excluded from analysis. SDH images were analyzed for size and pixilation using the ROI maps and the “Analyze-Measure” function (Image J protocol). Differences in lighting and assay conditions were normalized using a background subtraction calculation. On average 297 fibers (SD: 29) were examined from each patient.

Statistical analyses of muscle histology. Means were compared using ANOVA with Tukey’s post hoc tests using GraphPad Prism software (Version 4). Data are presented as group means ± SEM unless otherwise indicated.

RESULTS

Whole Genome Expression Analyses

Subject characteristics for the entire cohort of 24 subjects were previously reported (5). For analyses of gene expression we examined RNA from *rectus femoris* biopsies of 6 matched pairs of subjects (Table 1). The per cent weight loss in the first 6 weeks of meal replacement was 49% greater (P< 0.0001) for the ODS subjects versus the ODR subjects.
Upregulation of gene sets involved in oxidative phosphorylation, glucose and fatty acid metabolism in ODS as compared to ODR women

Partial least squares discriminant analysis (PLS-DA) of whole-genome expression data displayed significant separation of the ODS and ODR subjects (Figure 1), suggestive of significant differences in gene expression between the two groups. The top differentially expressed genes (Table 2) included FRZB (frizzled-related protein B), a negative regulator of canonical Wnt signaling, which, based on two probe sets, had 2.5 fold higher expression in diet-sensitive subjects. Expression of NR1D2 (REV-ERBβ), a transcriptional silencer, which regulates expression of lipid uptake genes in skeletal muscle (17), was significantly decreased in skeletal muscle of diet-sensitive subjects, as was expression of ATP1B1 (Na/K-transporting ATPase beta-1 chain) and SLC24A3 (sodium/potassium/calcium exchange member 3). Gene set enrichment analysis revealed upregulation of gene sets in ODS versus ODR subjects relevant to oxidative phosphorylation, cell cycle and protein turnover (Table 3).

We further explored these relationships in a second group of similar subjects, where we obtained vastus lateralis muscle biopsies from matched ODS and ODR subjects (n=7 in each group). By Q-RT-PCR, the top differentially expressed genes with higher expression in ODS versus ODR included PPARD, relevant to mitochondrial biogenesis (p=0.01) and SLC25A3 (p=0.0009), which functions to catalyze the co-transport of phosphate and a proton into the mitochondrial matrix (Table 4). Expression of IRS2 was also higher (p=0.02) in diet-sensitive versus diet-resistant subjects. Although to a lesser extent as compared to above studies of rectus femoris biopsies, vastus lateralis FRZB expression was increased by 11% in ODS subjects as compared to ODR subjects (p=0.02).

Characterization of Subjects Involved in Muscle Fiber Phenotype Analyses.

Fifteen additional pairs of ODS and ODR subjects were recruited for these analyses. Age, height, body weight, and BMI were similar for all ODS and ODR subjects at the onset of calorie restriction (Table 5). Similarly, age and height of the lean control subjects were comparable to those of obese subjects at the time of muscle biopsy. After 6 weeks of calorie restriction, ODS and ODR groups had lost 11.2 ± 0.34 and 7.3 ± 0.33 percent (p < 0.001) of initial body weight, respectively (Figure 2A). Rate of weight loss was 45% greater for ODS than ODR subjects (1.52 ± 0.10 and 1.05 ± 0.08 kg/wk, respectively) at 6 weeks of calorie restriction (p < 0.001; Table 5).
Physical activity level does not account for weight loss differences

To exclude the possibility that differences in weight loss were affected by physical activity energy expenditure, we assessed both activity at work and planned activity. As part of the program, a physical activity questionnaire was administered prior to the onset of calorie restriction. Results were used to ensure that activity was maintained at pre-program levels over the first 6 weeks of the program. Activity at work was assessed using a 5-point Likert scale with a value of 1 defined as an “inactive” work environment (e.g., seated almost exclusively) and a value of 5 defined as a “very active” work environment (e.g., walking nearly continuously). ODS and ODR subjects had similar levels of work-related activity (Figure 2B). Similarly, subjects were asked to record the frequency, duration, and type of planned weekly exercise activities. There were no differences in planned activity between ODS and ODR cohorts (h/wk; Figure 2C). We then calculated the physical activity energy expenditure (in kcal · wk⁻¹) using the specific energy expenditure coefficients (kcal · kg⁻¹ · min⁻¹) of each planned exercise (18). There were no significant differences between ODS and ODR groups (Figure 2D). To explore the theoretical energy cost associated with the difference in weight loss at the six week time point (3.9 kg) between ODS and ODR groups, we then calculated the energy expenditure that would be required for ODS subjects to lose this ‘additional’ weight. We used the estimated energy densities of lean body mass (7.6 MJ/kg) and fat body mass (39.5 KJ/kg) from Hall (19) and the MRI whole body results from Ross et al. (20). The latter reported body weight loss (5.2 kg), fat mass loss (4.1 kg), and skeletal muscle mass loss (0.5 kg) with calorie restriction in a non-exercised obese group. We then assumed that the remainder of the weight, not accounted for by either fat mass loss or skeletal muscle mass, also fell into the lean mass category (i.e., total lean = 1.1 kg), equivalent to an 80/20 split for fat/lean mass percentages. This conservatively underestimates the ratio for our study subjects (5) since they were slightly more obese than the group studied by Ross et al. (20). This theoretical energy content (lost as body weight) is almost four-fold greater than the calculated physical activity energy expenditure (PAE) (Figure 2D), arguing against a role for differences in activity underlying the weight loss variability.

Increased proportion of oxidative fibers in obese diet-sensitive subjects

Six of the latter 15 pairs of ODS and ODR subjects consented to vastus lateralis biopsies. Fiber typing analyses revealed that the proportion of type I oxidative fibers was 52% higher in the
ODS as compared to the ODR group (p < 0.001). It was also significantly greater in the ODS than in the lean subjects (p < 0.05). Of additional importance is the significantly decreased proportion of type I fibers in the ODR compared to the lean subjects (p < 0.05). Type I fibers comprised only 36.7 ± 2.5 % of all fibers in ODR subjects whereas type I fibers in ODS and lean subjects accounted for 55.6 ± 1.1 % and 45.2 ± 3.0 % of fibers, respectively (Figure 3 A and B).

While there were no fiber-specific differences in fiber size, the proportional contributions of the fiber types to muscle cross sectional area differed between subject groups. The proportion of muscle area as type I oxidative fibers were 55% greater in ODS than in ODR muscle (p < 0.001; Figure 3C). Interestingly, the contribution of type I fibers to muscle cross-sectional area was 28% greater in ODS than in muscle from lean subjects. There were no proportional differences by area in the contribution of type I fibers between the ODR and lean groups. The proportion of muscle area as type IIa fibers was 41% and 40% lower in ODS as compared to ODR and lean subjects, respectively (p < 0.001). There were no significant differences in the proportion of area as type IIx fibers between groups.

Myofibrillar hypertrophy in obese diet-sensitive subjects

We then sought to characterize differences in fiber size. The cross sectional area of type I fibers was 27% and 60% greater in ODS than in ODR and lean subjects (p < 0.05; p< 0.001), respectively (Figure 4A). Type IIa cross sectional area was 23% and 46% greater in ODS muscle as compared to ODR and lean muscle (p < 0.01; p< 0.001), respectively. Finally, the IIx area was 35% and 69% greater in ODS than in ODR and lean muscle (p < 0.01; p< 0.001), respectively. Thus, regardless of fiber type, the cross sectional fiber area was greater in muscle of ODS subjects.

Lower muscle fiber-specific and mass-specific mitochondrial content in obesity

To determine possible differences in mitochondrial content between groups, we assessed total activity of succinate dehydrogenase (SDH). When SDH activity was analyzed on a fiber-specific basis (Figure 4B), activity was lower in types I and IIa in ODS and ODR, compared to lean subjects. In type IIx fibers, activity was lower in ODR than in lean subjects (p < 0.05). When analyzed on a mass-specific basis (Figure 4C), activity was lower in muscle of ODR as
compared to lean subjects (p < 0.05). Interestingly, there were no differences in SDH activity between ODS and ODR muscle (Figure 4B and C; see Discussion).

**Higher intramuscular neutral lipid content in obesity**

Greater levels of intramuscular lipid have been reported in obese as compared to lean subjects (21-23), as well as in type I vs. type II fibers from *vastus lateralis* (21, 22, 24, 25). It was therefore of interest to determine intramuscular lipid content in ODS, ODR and L muscle *ex vivo*. Images of oil red O-stained muscle sections were converted to grey-scale, quantified densitometrically, and values expressed per total area of muscle analyzed. Lipid content/muscle area was ~26% higher in muscle from obese subjects (ODR and ODS combined) as compared to L subjects (P = 0.02, unpaired t-test; not shown). When the obese subjects were separated into ODS and ODR, only lipid content of ODS muscle was significantly higher, by ~33%, than that of L muscle (P < 0.05, one-way ANOVA, Bonferroni post test, ODS vs. L; Figure 5).

**DISCUSSION**

**Distinct gene expression signatures in ODS compared to ODR and lean control women**

Skeletal muscle capacity for oxidative metabolism is an important determinant of whole body energy metabolism, relevant to obesity and weight loss success. Previous reports have revealed that gene sets involved in oxidative phosphorylation (OXPHOS) and mitochondrial function are downregulated in subjects with type 2 diabetes as compared to controls (8, 9). In support of the hypothesis that differences in weight loss in response to calorie restriction are in part determined by innate differences in skeletal muscle characteristics, global gene expression profiles allowed separation of the ODS and ODR subjects (Figure 1). Due to the phenotypic similarities between ODS and ODR subjects, (both groups being obese) the differences in gene expression were often subtle. Limiting sample sizes, high variability in human samples, and small differences in gene expression reduced the statistical power of the study and consequently, too much reliance on statistical significance, especially multiple testing adjustments, would have resulted in false negatives. We therefore searched for biological relevance in addition to statistical significance with greater emphasis on the former. In this regard, we have essentially
followed the procedures outlined in Mootha et al. (8) and have used the permutation-based p-value and the magnitude of the normalized enrichment scores (NES) as the criterion for pathway evaluation without further consideration for multiple-testing corrections. By gene set enrichment analysis of microarray data from *rectus femoris* biopsies, we have demonstrated upregulation of gene sets relevant to oxidative phosphorylation, cell cycle and protein turnover in ODS compared with ODR subjects.

Pertinent to the role of Wnt signaling in the determination of muscle fiber type, we have demonstrated increased *rectus femoris* expression of frizzled related protein-3 (*FRZB*) in ODS compared to ODR women. Wnts are expressed in skeletal muscle and modulate myocyte differentiation and the expression of oxidative versus glycolytic fibers (26). *FRZB* is a negative regulator of Wnt/Frizzled-1 signaling via the β catenin pathway but does not impair Wnt-5a signaling via the Ca/PKC pathway and thus may promote oxidative fiber formation (27). Further studies are required to address this hypothesis.

Differential regulation of genes relevant to mitochondrial biogenesis and function were confirmed by Q-RT-PCR in the second study. In particular, expression of *PPARD* was increased by 1.5 fold in ODS versus ODR women. In concert with PGC1α, PPARδ promotes a switch to type I fibers and markedly enhances the transcription of oxidative phosphorylation and uncoupling protein genes. Consistent with enhanced oxidative capacity, the expression of mitochondrial phosphate carrier protein (*SLC25A3*) was increased 1.6 fold and that of mitochondrial glycerol-3-phosphate acyltransferase (*GPAM*) by 1.8 fold in *vastus lateralis* of ODS versus ODR subjects.

**Muscle fiber phenotypic characteristics**

In human muscles, unlike those of small mammals (*e.g.*, rodents), there is substantial variability in fiber type proportions (28, 29). Despite wide acceptance of inter-individual fiber type heterogeneity and its importance in exercise performance variability amongst athletes (30), the role of fiber type heterogeneity in weight loss is very poorly understood. Our results indicate that a greater proportion of type I fibers in ODS is a predominant feature distinguishing the ODS from ODR and lean subjects. Comprehensive investigations of fiber type composition of human *vastus lateralis* muscle have shown that type I fibers typically account for 51 to 55 % of all fibers,
regardless of age (31). Our results indicate that, among obese individuals, fiber composition does vary and may impact propensity for weight loss.

It appears likely that intrinsic energetic efficiency is lower in type I, compared to type II fibers. The latter is supported by in vivo spectroscopy results of higher in vivo uncoupling in muscles that are largely oxidative than in those that are largely glycolytic (32). Indeed this is consistent with our findings of increased mitochondrial proton leak in rectus femoris of ODS compared to ODR subjects (5).

**Potential mechanisms contributing to greater oxidative fiber content**

Fiber type is determined predominantly by genetic and developmental factors. Importantly, weight loss in obese subjects does not change muscle fiber type proportions (33). However, hypoxia, endurance exercise training and motor neuron activity promote conversion of type IIx to type IIa fibers by increasing intracellular Ca++ and activating transcription of myoglobin, MEF2, IGF, PPARGC1A and gene sets important in mitochondrial biogenesis and oxidative metabolism (34-42). In our study there were no differences in physical activity in ODS and ODR subjects. Moreover the amount of activity required for fiber type conversion (i.e., from the highly glycolytic type IIx to the oxidative glycolytic type IIa fibers) is much greater than the low intensity day-to-day activity of our subjects (e.g., (43)). Indeed recent findings (11) demonstrate in overweight/obese adults that 16 weeks of diet, or diet and exercise (3-5 d/wk of 30-40 min/d of moderate intensity treadmill walking), resulted in no change in vastus lateralis fiber types. Thus, the observed differences in fiber composition between ODS and ODR subjects may have genetic or epigenetic origins.

**Hypertrophy of muscle fibers**

The greater cross-sectional area of all fiber types in ODS compared to ODR does not appear to be affected by subject age. We studied subjects ranging in age from 36 to 62 years; all but one was younger than 60 years. This is consistent with observations that age-associated sarcopenia, which predominantly reflects a loss of fiber number and to a lesser degree fiber size, which are not substantially manifested until after age 60 (31). Moreover, while loss of muscle mass with aging has been associated with reduced fiber size and number (31, 44), the relative proportion of type I to type II fibers in vastus lateralis (45) and other skeletal muscles (46) is not
altered with aging. Thus age does not appear to be a factor contributing to the observed differences in fiber size in the present investigation.

**Increased oxidative fiber content, but not mitochondrial content, is associated with greater rate of weight loss in diet-sensitive subjects**

Since the lower mass-specific mitochondrial succinate dehydrogenase activity in type I and II fibers is comparable in ODS and ODR groups compared to the lean group (Figure 4 B and C), the loss of mitochondrial content, *per se*, does not appear to be an immediate determinant of poor diet responsiveness. However, we found increased type I and decreased type IIa fibers in ODS subjects with reciprocal decrease and increases in types I and IIa fibers in ODR subjects. As both types I and IIa fibers have high mitochondrial contents compared to the highly glycolytic type IIx fibers (21, 47), the absence of significant differences in total mitochondrial content between ODS and ODR muscle is not surprising. Type IIa fibers are estimated to have approximately 80% of the mitochondrial content as type I fibers (21, 47). Notably muscle mitochondrial capacity is not altered (*e.g.*, increased) by weight loss (11), despite increased insulin sensitivity and decreased intramyocellular lipid. Thus, while reduced mitochondrial content may reduce overall weight loss responsiveness in obese individuals, it does not contribute to the weight loss-differential observed here. While speculative, but consistent with the lower energetic efficiency of type I fibers (32), our findings support the idea that mitochondria from type I fibers are energetically distinct from those in type IIa fibers, and further research is needed.

**Intramuscular neutral lipid content**

Intramuscular lipid content has been reported to be higher in obese as compared to lean subjects (21-23). Our results also demonstrate greater intramuscular lipid in obese (ODS and ODR, combined) as compared to L muscle. Higher lipid content has been observed in type I vs. type II fibers from vastus lateralis (21, 22, 24, 25). Thus, the greater lipid content of ODS (but not ODR) as compared to L muscle is generally consistent with the higher proportion of type I fibers in ODS muscle. That the increase (~33%) in lipid content of ODS vs. L was slightly greater than the increase (~26%) in the proportion of type I fibers suggests that type I fibers within ODS muscle contain slightly more lipid as compared to L muscle. Exercise training is associated with increased muscle lipid content (48, 49); however, there were no differences in
physical activity between ODS and ODR subjects. Weight loss is also associated with a reduction in intramuscular triglyceride levels (23). The lower degree of weight loss exhibited by ODR subjects may have resulted in a reduced lipid loss in ODR muscle, leading to the greater than predicted lipid content, based on type I fiber content, of ODR compared to ODS muscle.

**Rate of weight loss was not compromised by initial body composition**

Initial body composition can influence rate of weight loss at a given level of calorie restriction and total energy expenditure (TEE) (19, 50). Ideally, stored fat is mobilized during energy deficit to forestall excessive catabolism of lean tissue. However, compared to fat mass (39.5 MJ · kg⁻¹), ~ 5.2 times more lean mass (7.6 MJ · kg⁻¹) must be lost to satisfy an energy deficit (51). Thus, the greater weight loss ODS vs. ODR could potentially reflect a greater proportional mobilization of energy from lean reserves.

In our study, average body fat mass of ODS and ODR subjects prior to calorie restriction was well above 30 kg and comparable between groups. In calorie-restricted obese subjects with an initial body fat mass of greater than 30 kg, fat is the predominant component lost (51). MRI-based estimates of calorie-restricted obese women, revealed that muscle and body fat accounted for ~ 10% and 80% of weight lost, respectively, when initial body fat mass was 39.2 kg (20). Thus, it seems unlikely that the greater weight loss by the ODS group reflects a greater mobilization of lean body mass, implying that ODS subjects had a greater intrinsic TEE than ODR subjects.

**Estimates of energy expenditure**

Daily TEE of ODS and ODR subjects during calorie restriction would equal the energy consumed (900 kcal/day) plus the estimated energy content of body mass lost. The energy content of weight loss during the first six weeks for ODS and ODR subjects (11.4 and 7.5 kg, respectively), was approximately 40,600 ± 1,960 kcal (170 ± 8.2 MJ) and 25,900 ± 1190 kcal (108 ± 5.0 MJ), respectively, according to the estimates of Hall (51). The estimated daily TEE for ODS and ODR subjects is 1,867 ± 47 kcal (7812 ± 197 KJ) and 1,517 ± 28 kcal (6347 ± 117 KJ), respectively. The TEE of the ODS group is thus 350 kcal/day (or 23%) greater than that of the ODR group (P < 0.001). There were no differences in total body mass, fat mass, or fat-free mass (Table 5), consistent with intrinsic differences in TEE between the groups.
Estimates of PAE revealed no differences in either expenditure related to physical activity at work, or planned physical activity. It is noteworthy that the PAE in ODS and ODR (1397 ± 420 kcal · d⁻¹ and 1254 ± 129 kcal · d⁻¹) account for only ~25% of the difference theoretically required to explain the weight loss difference.

In summary, these studies demonstrate altered expression of genes relevant to fuel uptake and oxidation in a comparison of well-matched obese individuals who differ solely in their propensity for weight loss. Our findings of vastus lateralis fiber hypertrophy and a higher proportion of oxidative (type I) and lower oxidative-glycolytic (type IIa) fibers in ODS compared to ODR subjects and to lean controls, in conjunction with no differences in mass specific mitochondrial content are consistent with the idea that the differences in the bioenergetic demands of types I and IIa fibers (32) are quantitatively important. Overall, these findings support the conclusion that skeletal muscle capacity for oxidative metabolism influences the propensity to gain or lose weight.

Acknowledgments

We are thankful to our research volunteers for their generous participation in this study. We also are grateful for the exceptional support from Heather Doelle, Brenda Bradley, and Sybil Hebert for their work in recruitment and clinical data collection; from Linda Jui and Mahmoud Salkhordeh for their technical expertise in histology; and for Dr. Mark Tarnopolsky (McMaster University) for technical advice on muscle biopsy procedures. The antibody for type IIx fibers was generously provided by Dr. Peter Merrifield (University of Western Ontario).
REFERENCES


Figure Legends

Figure 1. Partial least squares discriminant analysis (PLSDA) scoreplot. PLSDA demonstrates a clear separation between the ODS (red) and ODR (black) subjects based on global gene expression profiles.

Figure 2. Weight loss and physical activity characteristics of obese subjects involved in *vastus lateralis* muscle phenotype analyses. A. Rate of weight loss, expressed as a percentage of initial body weight in ODS (filled symbols) and ODR (open symbols) subjects. (Mean ± SEM; ODS: N = 15; ODR: N = 15). B. Work-related physical activity in arbitrary units (1 = inactive; 5 = very active). C. Planned physical activity (hours per week). D. Planned physical activity energy expenditure (kcal/wk): actual and the calculated amount required for the additional weight loss of ODS compared to ODR subjects. The energy required for the additional weight loss of ODS compared to ODR subjects was calculated for subjects involved in this study, and in our previous study (5).

Figure 3. Increased proportion of oxidative (type I) fibers in ODS than in ODR and lean subjects. A. Representative images of *vastus lateralis* sections from ODS, ODR and lean subjects Scale bar represents 250 um. B. Fiber type proportions, expressed as a proportion of total fibers in ODS, ODR and lean groups (mean +/- SEM; N = 6 matched pairs of subjects). C. Fiber type proportions, expressed as a percentage of muscle surface area in ODS, ODR and lean groups (mean +/- SEM; N = 6 matched pairs of subjects).

Figure 4. Fiber surface area and SDH activity analyses. A. Hypertrophy of types I, IIa and IIx in *vastus lateralis* of ODS compared to ODR and lean subjects. Average fiber cross-sectional area in ODS, ODR and lean groups (mean +/- SEM; N = 6 matched pairs of subjects). Mitochondrial Content: B. Succinate dehydrogenase (SDH) activity per unit area of the three major fiber types in *vastus lateralis* of ODS, ODR and lean subjects (mean +/- SEM; N = 6 matched pairs of subjects). C. Overall SDH activity per unit area of *vastus lateralis* of ODS, ODR and lean subjects (Mean +/- SEM; N = 6 matched pairs of subjects).

Figure 5. Intramuscular lipid content. Intramuscular lipid content was determined by oil red O staining followed by densitometric analyses of muscle fibers. Optical densities (OD) were expressed per total area of muscle analyzed. Lipid content of muscle from L subjects was significantly lower than that from ODS subjects (*: P < 0.05; one-way ANOVA, Bonferroni post hoc test, L vs. ODS). Values are means ± SEM; n = 6/group. ODS: obese diet-sensitive; ODR: obese diet-resistant; L: lean.
### TABLE 1. Characteristics of study subjects for muscle whole genome expression analysis

<table>
<thead>
<tr>
<th></th>
<th>Obese Diet-Sensitive</th>
<th>Obese Diet-Resistant</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age (yrs)</strong></td>
<td>46.7 (6.1)</td>
<td>45.0 (11.6)</td>
</tr>
<tr>
<td><strong>N</strong></td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td><strong>Baseline at program entry</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>98.1 (10.4)</td>
<td>104.7 (16.9)</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>35.8 (3.8)</td>
<td>40.7 (6.4)</td>
</tr>
<tr>
<td><strong>Six weeks of 900 kcal diet</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>87.9 (9.5)</td>
<td>97.5 (16.1)</td>
</tr>
<tr>
<td>Weight lost (kg)</td>
<td>10.1 (1.1)</td>
<td>7.2 (1.1)*</td>
</tr>
<tr>
<td>% weight loss</td>
<td>10.4 (0.6)</td>
<td>7.0 (0.8)**</td>
</tr>
<tr>
<td><strong>At biopsy</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>89.0 (13.2)</td>
<td>98.8 (21.5)</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>32.5 (5.2)</td>
<td>35.6 (4.1)</td>
</tr>
</tbody>
</table>

Mean (SD). *, P<0.001; **, P<0.0001. Study subjects with microarray data. The entire study cohort of 12 ODS and 12 ODR subjects were described previously (5).
TABLE 2. Whole genome expression analyses of *rectus femoris* muscle from obese diet-sensitive vs obese diet-resistant subjects

<table>
<thead>
<tr>
<th>Probe ID Symbol</th>
<th>Gene Symbol</th>
<th>Gene Name</th>
<th>Mean ODR</th>
<th>Mean ODS</th>
<th>ODS vs ODR</th>
<th>log ratio</th>
<th>p value</th>
<th>p value</th>
<th>p value</th>
<th>Resolver</th>
</tr>
</thead>
<tbody>
<tr>
<td>203697_at</td>
<td>FRZB</td>
<td>frizzled related protein</td>
<td>69.3</td>
<td>174.7</td>
<td>up</td>
<td>1.33</td>
<td>0.001</td>
<td>0.000</td>
<td></td>
<td></td>
</tr>
<tr>
<td>202814_s_at</td>
<td>HIS1</td>
<td>HEXIM1 (HMBA inducible)</td>
<td>323.0</td>
<td>197.7</td>
<td>down</td>
<td>-0.71</td>
<td>0.002</td>
<td>0.020</td>
<td></td>
<td></td>
</tr>
<tr>
<td>202814_s_at</td>
<td>NRID2</td>
<td>REV_ERBβ</td>
<td>867.67</td>
<td>493.23</td>
<td>down</td>
<td>-0.81</td>
<td>0.002</td>
<td>0.010</td>
<td></td>
<td></td>
</tr>
<tr>
<td>208029_s_at</td>
<td>LC27</td>
<td>putative integral membrane transporter</td>
<td>1819.6</td>
<td>1316.1</td>
<td>down</td>
<td>-0.47</td>
<td>0.014</td>
<td>0.005</td>
<td></td>
<td></td>
</tr>
<tr>
<td>201242_S_AT</td>
<td>ATP1B1</td>
<td>ATPase Na+/K+ transporting β1 polypeptide</td>
<td>4802.6</td>
<td>3076.0</td>
<td>down</td>
<td>-0.64</td>
<td>0.021</td>
<td>0.002</td>
<td></td>
<td></td>
</tr>
<tr>
<td>57588_at</td>
<td>SLC24A3</td>
<td>solute carrier family Na+/K+/Ca++ exchanger 3</td>
<td>24486.0</td>
<td>295.7</td>
<td>down</td>
<td>-0.72</td>
<td>0.034</td>
<td>0.050</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The top differentially expressed genes identified through microarray analysis are shown. Columns 1-3 contain the Affymetrix reported probeset ID, the gene symbol and the gene name respectively. Columns 4 and 5 contain the average gene expression signals recorded for the obese, diet-resistant (ODR) and obese, diet-sensitive (ODS) groups respectively. Columns 6 and 7 show the direction of change and the magnitude of change (log2) in gene expression in the ODS group compared to the ODR group. Columns 8 and 9 show the statistical significance of differential expression for the genes as ascertained by a classical t-test or by a modified t-test based on the Resolver error model for gene expression analysis (Rosetta Inpharmatics, Seattle, WA).
### TABLE 3. Gene set enrichment analysis

<table>
<thead>
<tr>
<th>Name</th>
<th>Size</th>
<th>ES</th>
<th>NES</th>
<th>Nominal P-value</th>
<th>FDR Q-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>RIBOSOMAL_PROTEINS</td>
<td>92</td>
<td>0.72</td>
<td>3.16</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>PROTEASOME</td>
<td>17</td>
<td>0.65</td>
<td>1.91</td>
<td>0.002</td>
<td>0.04</td>
</tr>
<tr>
<td>CELL_CYCLE_REGULATOR</td>
<td>24</td>
<td>0.57</td>
<td>1.86</td>
<td>0</td>
<td>0.06</td>
</tr>
<tr>
<td>MOOTHA_VOXPHOS</td>
<td>77</td>
<td>0.41</td>
<td>1.74</td>
<td>0.008</td>
<td>0.18</td>
</tr>
<tr>
<td>ELECTRON_TRANSPORT_CHAIN</td>
<td>90</td>
<td>0.38</td>
<td>1.64</td>
<td>0</td>
<td>0.379</td>
</tr>
</tbody>
</table>

All genes on the chip were ranked by differences in expression between ODR and ODS subjects using the t-test. An enrichment score (ES) was calculated for each gene set. NES: enrichment score normalized for differences in gene set size; FDR q-value: false discovery rate.
TABLE 4. Top differentially expressed genes in *vastus lateralis* muscle biopsies from obese diet -sensitive *versus* diet -resistant subjects

<table>
<thead>
<tr>
<th>Gene ID</th>
<th>Name</th>
<th>ODS</th>
<th>ODR</th>
<th>Fold Difference ODS vs ODR</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>SLC25A3</td>
<td>mitochondrial phosphate carrier protein</td>
<td>244215±11324</td>
<td>155457±14401</td>
<td>1.6</td>
<td>0.0009</td>
</tr>
<tr>
<td>IRS2</td>
<td>insulin receptor substrate-2</td>
<td>9900±1502</td>
<td>5653±744</td>
<td>1.8</td>
<td>0.0390</td>
</tr>
<tr>
<td>GPAM</td>
<td>mitochondrial glycerol-3-PO₄ acyltransferase</td>
<td>66168±13975</td>
<td>35912±4452</td>
<td>1.8</td>
<td>0.0367</td>
</tr>
<tr>
<td>PPARD</td>
<td>PPAR delta</td>
<td>11269±870</td>
<td>7527±850</td>
<td>1.5</td>
<td>0.0132</td>
</tr>
</tbody>
</table>

n=7 in each group; p values <0.05 and fold change > 1.5
**TABLE 5:** Characteristics of subjects for *vastus lateralis* muscle phenotype analyses.

<table>
<thead>
<tr>
<th></th>
<th>Obese Diet-Sensitive</th>
<th>Obese Diet - Resistant</th>
<th>Lean</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>15</td>
<td>15</td>
<td>15</td>
</tr>
</tbody>
</table>

**Baseline at program entry**

<table>
<thead>
<tr>
<th></th>
<th>Obese Diet-Sensitive</th>
<th>Obese Diet - Resistant</th>
<th>Lean</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yrs)</td>
<td>45.4 ± 2.3</td>
<td>46.7 ± 2.7</td>
<td>---</td>
</tr>
<tr>
<td>Body mass (kg)</td>
<td>102.8 ± 2.9</td>
<td>101.9 ± 3.1</td>
<td>---</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>38.5 ± 0.9</td>
<td>38.5 ±1.3</td>
<td>---</td>
</tr>
</tbody>
</table>

**Six weeks of CR**

<table>
<thead>
<tr>
<th></th>
<th>Obese Diet-Sensitive</th>
<th>Obese Diet - Resistant</th>
<th>Lean</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body mass (kg)</td>
<td>91.4 ± 2.5</td>
<td>94.4 ± 2.9</td>
<td>---</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>34.2 ± 0.7</td>
<td>35.7 ± 1.3**</td>
<td>---</td>
</tr>
<tr>
<td>Mass lost (kg)</td>
<td>11.4 ± 0.6</td>
<td>7.5 ± 0.4**</td>
<td>---</td>
</tr>
<tr>
<td>Rate of mass loss (g/day)</td>
<td>217.2 ± 13.0</td>
<td>149.4 ± 11.6**</td>
<td>---</td>
</tr>
</tbody>
</table>

**Six months weight management**

<table>
<thead>
<tr>
<th></th>
<th>Obese Diet-Sensitive</th>
<th>Obese Diet - Resistant</th>
<th>Lean</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body mass (kg)</td>
<td>78.0 ± 1.9</td>
<td>85.0 ± 2.6</td>
<td>---</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>29.2 ± 0.4</td>
<td>32.2 ± 1.2*</td>
<td>---</td>
</tr>
<tr>
<td>Mass lost (kg)</td>
<td>13.3 ± 1.3</td>
<td>9.4 ± 1.2*</td>
<td>---</td>
</tr>
<tr>
<td>Rate of mass loss (g/day)</td>
<td>45.6 ± 7.3</td>
<td>37.3 ± 8.0</td>
<td>---</td>
</tr>
</tbody>
</table>

**At biopsy**

<table>
<thead>
<tr>
<th></th>
<th>Obese Diet-Sensitive</th>
<th>Obese Diet - Resistant</th>
<th>Lean</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yrs)</td>
<td>48.5 ± 2.1</td>
<td>50.7 ± 2.6</td>
<td>51.1 ± 2.4</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>163.4 ± 1.6</td>
<td>162.9 ± 1.6</td>
<td>163.0 ± 2.0</td>
</tr>
<tr>
<td>Body mass (kg)</td>
<td>91.1 ± 3.2</td>
<td>93.5 ± 3.1</td>
<td>51.9 ± 1.5**</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>34.1 ± 1.1</td>
<td>35.3 ±1.3</td>
<td>19.5 ± 0.4**</td>
</tr>
<tr>
<td>Adipose mass (kg)</td>
<td>40.4 ± 1.9</td>
<td>43.9 ± 2.2</td>
<td>12.3 ± 1.0**</td>
</tr>
<tr>
<td>Fat-free mass (kg)</td>
<td>50.6 ± 1.4</td>
<td>49.7 ± 1.1</td>
<td>39.3 ± 0.6**</td>
</tr>
</tbody>
</table>

CR, calorie restriction. *, P<0.05 ; **, P<0.01. Data are mean ± SEM
Figure 1

PLS-DA analysis of ODS and ODR subjects

R²X[1] = 0.130378
R²X[2] = 0.112126

Ellipse: Hotelling T² (0.95)
Figure 2

A  
Body Weight Loss (%) Initial Wt  
Time (wk)  

B  
Planned Physical Activity (h/wk)  
ODS  ODR

C  
Activity at Work (AU)  
ODS  ODR

D  
Planned Activity (Kcal/wk)  
ODS - Actual  ODS - This Study  ODS - Jasper et al, 2002

Gerrits et al
Figure 3

A

ODS

ODR

L

B

% of total fibers

Type I  Type Ila  Type IIx

C

% of total surface area

Type I  Type Ila  Type IIx
Figure 4

A

Cross sectional area (μm²)

B

SDH Density (OD/μm²)

C

SDH (OD/μm²)

Type I  Type IIa  Type IIx

Type 1  Type 2A  Type 2X

ODS  ODR  L