Weight Loss after Gastric Bypass Surgery in Human Obesity Remodels Promoter Methylation

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SUMMARY
DNA methylation provides a mechanism by which environmental factors can control insulin sensitivity in obesity. Here, we assessed DNA methylation in skeletal muscle from obese people before and after Roux-en-Y gastric bypass (RYGB). Obesity was associated with altered expression of a subset of genes enriched in metabolic process and mitochondrial function. After weight loss, the expression of the majority of the identified genes was normalized to levels observed in normal-weight, healthy controls. Among the 14 metabolic genes analyzed, promoter methylation of 11 genes was normalized to levels observed in the normal-weight, healthy subjects. Using bisulfite sequencing, we show that promoter methylation of PGC-1α and PDK4 is altered with obesity and restored to nonobese levels after RYGB-induced weight loss. A genome-wide DNA methylation analysis of skeletal muscle revealed that obesity is associated with hypermethylation at CpG shores and exonic regions close to transcription start sites. Our results provide evidence that obesity and RYGB-induced weight loss have a dynamic effect on the epigenome.

INTRODUCTION
Obesity is a chronic lifelong condition (Ogden et al., 2002) that results from the interaction between heritable factors with environmental influences (O’Rahilly, 2009). Excess accumulation of white adipose tissue due to an imbalance between energy intake and expenditure causes obesity (Rosen and Spiegelman, 2006). The increasing prevalence of obesity is recognized as a major risk for a wide range of diseases including type 2 diabetes, cardiovascular disease, cancer, and musculoskeletal disorders (Pi-Sunyer, 2009). Conventional strategies for the treatment of obesity, including lifestyle modifications of diet and exercise behavior are often insufficient and pharmacological options are limited (Blevins, 2010). Roux-en-Y gastric bypass (RYGB) surgery, combining gastric volume restriction and an intestinal bypass, leads to the diminution of daily calorie absorption and sustainable weight loss (Kral and Näslund, 2007), which ultimately improves long-term mortality (Adams et al., 2007; Sjöström et al., 2004, 2007). RYGB surgery dramatically improves insulin-sensitivity often before weight loss has occurred, and leads to the clinical resolution or remission of type 2 diabetes (Buchwald et al., 2009; Greenway et al., 2002; Rubino et al., 2010). Early alterations in the release of incretin hormones have been implicated (Falkén et al., 2011), but the molecular mechanism is incompletely resolved.

Skeletal muscle is clinically recognized as a primary organ involved in the development of insulin resistance in type 2 diabetes (Martin et al., 1992). Skeletal muscle insulin resistance is a common feature of many metabolic disorders including cardiovascular disease, hypertension, polycystic ovary syndrome, and obesity (Reaven, 2005). Defects in insulin-stimulated glucose transport in skeletal muscle account for whole body insulin resistance in people with severe obesity and type 2 diabetes (Dohm et al., 1988; Goodyear et al., 1995; Zierath et al., 1994). Impairments in lipid oxidation and mitochondrial dysfunction have also been linked to the development of skeletal muscle insulin resistance in obesity and type 2 diabetes due to an accumulation of intracellular lipid metabolites and feedback inhibition on insulin signal transduction to glucose transport (Kim et al., 2000; Petersen et al., 2004; Ritov et al., 2005). Cultured myotubes derived from type 2 diabetic patients (Bouzakri and Zierath, 2007; Henry et al., 1995) or severely obese humans (Bell et al., 2010; Hulver et al., 2005) retain defects in insulin signaling and metabolism reminiscent of the clinical phenotype, suggesting epigenetic modifications impact the metabolic memory of skeletal muscle and contribute to the development of insulin resistance.

Previously, we have identified that a subset of promoters for genes involved in mitochondrial function are differentially methylated in skeletal muscle from insulin resistant type 2 diabetic patients compared to normal glucose-tolerant healthy men (Barrès et al., 2009). Epigenetic control through DNA methylation affects gene transcription by regulating the accessibility of the transcription machinery to the chromatin. DNA methylation may provide a mechanism linking environmental influences with gene
transcription and physiological responses controlling insulin sensitivity and metabolism. Here, we investigated the role of obesity and weight loss after RYGB surgery on global, as well as promoter-specific DNA methylation. We studied peroxisome proliferator-activated receptor γ coactivator-1 α (PGC-1α) and pyruvate dehydrogenase kinase, isoenzyme 4 (PDK4), two genes involved in mitochondrial function and fuel utilization in skeletal muscle. We also performed a genome-wide DNA methylation analysis of skeletal muscle to determine whether obesity is associated with global changes in promoter methylation.

RESULTS

Clinical Characteristics of the Study Participants
To determine the role of weight loss on glucose and lipid metabolism, a cohort of obese women with a body mass index (BMI) >35 kg/m² was studied before and 6 months after RYGB surgery. Sixteen age-matched nonobese (normal weight) women were studied for comparative purposes. Before surgery, insulin, high density lipoprotein (HDL) cholesterol, triglycerides, and nonesterified fatty acids (NEFA) were normalized after RYGB surgery. Moreover, leptin, MCP-1, and C-reactive protein (CRP), were increased in the obese women, whereas the level of the proinflammatory chemokine monocyte chemotactic protein-1 (MCP-1) was decreased. Although RYGB surgery markedly reduced body weight, BMI remained above normal. Fasting glucose, insulin, total cholesterol, low density lipoprotein (LDL), triglycerides, and NEFA levels were normalized after RYGB surgery. Moreover, leptin, MCP-1, and CRP levels were improved after RYGB surgery. In contrast, inflammatory markers, including IL6, IL8, and tumor necrosis factor-α (TNF-α) were unaltered 6 months after RYGB surgery. To further examine a possible participation of TNF-α, we measured circulating levels of TNF-α receptors I and II and found no difference in the groups studied (Figure S1).

Global Cytosine Methylation in Skeletal Muscle with Obesity after Weight Loss
We determined whether obesity and weight loss alters global DNA methylation in vastus lateralis skeletal muscle using a lumi-nometric assay. Methylation of the inner cytosine within the CCGG sequence (CpG methylation) or the CCA/TGG sequence (non-CpG methylation) showed that global methylation levels at CpG and non-CpG sites in skeletal muscle were similar before and after RYGB surgery-induced weight loss (Figure S2). Global methylation was similar between obese and nonobese women. To control for adipose tissue contamination in the skeletal muscle biopsy material, we determined mRNA expression of the adipose tissue-enriched gene Adipin. mRNA expression of Adipin was similar in vastus lateralis muscle obtained before versus after RYGB surgery (Figure S3), which suggests the reduction in adipose tissue due to weight loss surgery did not mask any putative variation in global DNA methylation in skeletal muscle. Collectively, our results provide evidence that global CpG and non-CpG methylation in human skeletal muscle is unaltered by either obesity or RYGB surgery-induced weight loss.

RYGB Surgery-Induced Weight Loss Partially Normalizes the Transcriptomic Profile of Metabolic Genes
To investigate the potential contribution of epigenetic modifications in the control of gene expression in obese humans, we first identified genes differentially expressed in obese versus normal-weight women (clinical characteristics are presented in Table S2) and then analyzed the respective promoter methylation. Using a genome-wide microarray analysis of skeletal muscle, we found that obesity was associated with altered expression of a subset of 896 genes in skeletal muscle (Tables S3, S4, and S5), enriched in the Gene ontology terms GO:0006629 lipid metabolic process and GO:0005739 mitochondrion (Figure 1A). After weight loss, the expression of the majority of the identified genes was normalized to levels observed in the normal-weight, healthy women (Figures 1A and 1B). Promoter methylation of a subset of genes was investigated using a methyl-CpG binding protein-based system for methylated DNA enrichment. Enriched methylated fractions were detected by quantitative PCR. Of the 14 genes studied, promoter methylation was affected in 11 (78%) genes. We found eight (57%) genes with a negative association between gene expression and promoter methylation, whereas three (21%) genes showed a positive association (Figure 1C). Promoters with high CpG ratio constitute a gene signature that is stably expressed in somatic cells (Weber et al., 2007). However, we found various CpG ratio signatures among the 16 promoters studied, regardless of their methylation behavior in obesity (Figure S4). This finding suggests the CpG ratio does not predict methylation behavior in response to environmental factors.

 Bisulfite Sequencing Analysis of PGC-1α and PDK4 Promoter Methylation
Previously, we used a methylated DNA immunoprecipitation (MeDIP) array to reveal gene-specific changes in DNA methylation in skeletal muscle from type 2 diabetic patients (Barrés et al., 2009). Our earlier study highlighted promoter-specific differential methylation of PGC-1α and PDK4 in insulin-resistant type 2 diabetic patients compared to insulin-sensitive normal glucose-tolerant men (Barrés et al., 2009). Our transcriptomic analysis revealed that RYGB surgery-induced weight loss led to an increase in PGC-1α mRNA expression and a decrease in PDK4 mRNA expression in skeletal muscle (Table S6, p < 0.002 and p < 0.0001, respectively). The inverse relationship between these two metabolic genes is consistent with previous evidence from clinical and experimental studies, whereby PGC-1α and PDK4 have an inverse regulation in insulin-resistant conditions (Jeoung and Harris, 2008). We first validated the microarray results using quantitative PCR (Figures 2A and 3A) and then used bisulfite sequencing to determine the DNA methylation pattern of the PGC-1α and PDK4 promoters on proximal region relative to the transcription start site. RYGB surgery decreased PGC-1α promoter methylation (Figures 2B and 2C) and conversely increased PDK4 promoter methylation (Figures 3B and 3C). The majority of methylated cytosines of the
PGC-1α (Figure 2B) and PDK4 (Figure 3B) promoters were found within non-CpG sites. In a previous study (Barres et al., 2009), we demonstrated that PGC-1α promoter methylation was associated with a decrease in gene activity. Here, we tested the effect of methylation at non-CpG sites on PDK4 promoter activity. Methylation of the CpC site at position /C0112 relative to the transcription start site decreased gene activity 19% (p < 0.005, Figure 3E), providing evidence that non-CpG methylation can alter gene expression.

Environmental Factors Involved in PGC-1α and PDK4 Promoter Methylation

The relationship between PGC-1α and PDK4 methylation levels and clinical variables was determined using a Pearson correlation analysis. Individual PGC-1α methylation levels were positively correlated, whereas PDK4 methylation levels were negatively correlated with BMI, C-reactive protein, and leptin levels (Figure S5). Insulin levels correlated with methylation levels of PDK4, but not PGC-1α. Conversely, triglyceride levels correlated with methylation level of PGC-1α, but not PDK4. These data indicate that PGC-1α and PDK4 promoter methylation may be affected by systemic factors in vivo. Expression of DNA methyltransferases (DNMTs) plays a direct role in DNA methylation levels in normal and pathological states (Robertson et al., 2000). Western blot analysis of DNMT1, DNMT3A, and DNMT3B protein in vastus lateralis showed altered DNMTs could be involved in the remodeling of DNA methylation after RYGB-induced weight loss.

Genome-wide Methylation Analysis

We used a methyl-CpG binding protein-based system for methylated DNA enrichment to determine the effect of RYGB surgery-induced weight loss on genome-wide DNA methylation. In a separate cohort of obese men (clinical characteristics are presented in Table S7), deep sequencing of enriched DNA fractions revealed 409 differentially methylated regions (DMR) between skeletal muscle biopsies obtained pre- and post-RYGB (Tables S8 and S9). DMRs were almost exclusively (397 out of 409) lower reads in post- versus pre-RYGB conditions (Table S9), suggesting that RYGB-induced weight loss-induced DNA hypomethylation. Of the 409 DMRs, 38 were proximal to coding genes (Table S10), but strikingly a large majority of DMRs (371) were found close to noncoding Ensembl annotations (Figure 4A) and specifically enriched within proximity of transcription start sites, CpGs, CpG shores, or exons (Figures 4B and 4C), suggesting a possible contribution in initiation of transcription. To examine a possible association between gene expression and DNA methylation across the cohorts, we assessed whether the coding genes proximal to the DMRs that were found in the obese men were also differentially expressed in the obese women. We found no...
overlap between the gene array and the methyl-seq results when applying the p value < 0.05 and FDR < 0.05 criteria. However, when applying selection by p value < 0.05 only, we found that 23 of the 40 differentially methylated genes were differentially expressed (Table S10), suggesting a certain degree of association between methylation and gene expression across the two cohorts.

**DISCUSSION**

Skeletal muscle insulin resistance in obesity and type 2 diabetes develops from an interaction between genetic and environmental factors that impair signaling and enzymatic cascades controlling glucose and lipid metabolism (Doria et al., 2008). In type 2 diabetes, epigenetic regulation through DNA methylation is involved in the transcriptional control of genes related to mitochondrial function and metabolic regulation in skeletal muscle and pancreatic β-cells (Barrès et al., 2009; Ling et al., 2008). Here, we provide evidence that global cytosine methylation is unaltered with obesity or weight loss. Rather, a dynamic change in mRNA and DNA methylation of promoters was observed in skeletal muscle with human obesity and RYGB surgery-induced weight loss. Of potential clinical relevance is the inverse relationship between DNA methylation level of the PGC-1α and PDK4 promoters with BMI, C-reactive protein, leptin, triglyceride, and insulin levels. Thus, DNA methylation appears to play a role in orchestrating environmentally-induced transcriptional responses in somatic tissues to impact the physiological regulation of metabolism in human obesity.

With human obesity and RYGB surgery-induced weight loss, we observed differences in promoter-specific DNA methylation of a subset of genes involved in the regulation of lipid metabolism in skeletal muscle. Similar to our previous study of PGC-1α in normal glucose-tolerant and type 2 diabetic men (Barrès et al., 2009), an opposing pattern between DNA methylation and mRNA expression of PGC-1α was observed between normal-weight and obese women, suggesting DNA methylation is involved in driving mRNA expression. Previously, we provided evidence that in vitro methylation of a single cytosine residue of the PGC-1α promoter (located −260 residues relative to the +1 transcription start site) caused a marked reduction of gene activity (Barrès et al., 2009). Here, we report that methylation of a single cytosine residue located within a non-CpG site in the PDK4 promoter is sufficient to drive down promoter activity, which further suggests non-CpG methylation plays a causative role in regulating gene expression. DNA methylation at transcriptional repressor binding sites also leads to gene activation (Ando et al., 2000; Pipaon et al., 2005). We found that a subset of genes showed a positive association between gene expression and promoter methylation suggesting an identical phenomenon. Collectively, our results suggest that DNA methylation is involved in the physiological control of gene transcription and clinical parameters associated with insulin-sensitivity after weight loss from RYGB surgery.
Our genome-wide DNA methylation analysis failed to retrieve PGC-1α, PDK4, or any of the 14 genes investigated in Figure 1C. We speculate that differences in assay sensitivity across the various methods used to detect changes in DNA methylation (qPCR, Sanger, or deep sequencing) may account for this difference in retrieval rate. Moreover, given that we studied both male and female cohorts, we cannot exclude the possible influence of sex-dependent differences on DNA methylation between the cohorts, rather than assay variation, that accounted for the difference in retrieval rate.

Elevated levels of nutrients and circulatory cytokines, characteristic of the insulin resistant phenotype, can acutely increase DNA methylation of the PGC-1α promoter in differentiated myotubes cultured in vitro (Barrès et al., 2009). Overfeeding in humans also increases DNA methylation of PGC-1α in skeletal muscle (Brons et al., 2010). Collectively, these observations are consistent with a role for de novo methylation in nondividing, differentiated cells. Thus, changes in clinical chemistry after weight loss surgery may influence DNA methylation and subsequently mRNA expression patterns. Selective silencing of the DNA methyltransferase 3B (DNMT3B), but not DNMT1 or DNMT3A, prevented lipid-induced non-CpG methylation of PGC-1α and decreased mtDNA and PGC-1α mRNA (Barrès et al., 2009). Whether DNA methyltransferase activity is modified after weight loss is unknown. Because the epigenetic changes reported here are reversed by weight loss, approaches to modify promoter-specific DNA methylation events may hold promise for the treatment of insulin resistance in obesity or type 2 diabetes.

Rapid changes in clinical biomarkers reflecting improvements in glucose and lipid metabolism and insulin action often occur after RYGB surgery before any major weight loss is achieved (Buchwald et al., 2009; Rubino et al., 2010; Thaler and Cummings, 2009). Although the underlying mechanisms are unknown, gut hormones and the switch from lipid storage to lipid oxidation may play a role (Falkén et al., 2011; Rubino et al., 2010; Thaler and Cummings, 2009). Diet supplementation with methyl donors or mono carbon metabolites, such as folic acid and homocysteine, modulate DNA methylation (Cooney et al., 2002; Waterland et al., 2005) and may contribute to the promoter-specific changes in DNA methylation reported here. However, plasma levels of homocysteine and folic acid were unaltered by RYGB surgery in our cohort, indicating the RYGB surgery-induced weight-loss-induced changes in promoter-specific DNA methylation do not occur from any deficiency in dietary intake or absorption of methyl donors. Previously, we provided evidence that elevated levels of free fatty acids or TNFα induce acute hypermethylation of the PGC-1α and TFAM promoters (Barrès et al., 2009). However, plasma TNFα level was unchanged by weight loss, suggesting the dynamic remodeling of DNA methylation was independent of changes in this cytokine. Moreover, the transcriptomic analysis of muscle biopsies revealed expression of TNFα and TNFα receptor I and II mRNA.

Figure 3. PDK4 Promoter Methylation Is Increased after RYGB
(A) Real-time PCR quantification of PDK4 mRNA expression in vastus lateralis.
(B) Visualization of the representative bisulfite sequencing results as analyzed by Methtools 2.0 (Grunau et al., 2000) showing a representative pattern of methylation of the region −446 to −160 relative to the transcription start site of the PDK4 promoter in vastus lateralis. Each line represents a sequence read from an individual clone of sequencing plasmid. Each dot represents methylation at CpG (black) or non-CpG (colored).
(C) Bisulfite sequencing quantification showing the percentage of cytosine methylation of the PDK4 promoter in vastus lateralis. Results for normal-weight women (hatched bar; n = 6), obese women pre-RYGB (open bar; n = 5), and obese women post-RYGB (closed bar; n = 5). Graphs show mean ± SEM results. *p < 0.05 pre- versus post-surgery. #p < 0.05 versus normal-weight women.
(D) Suppression of PDK4 promoter activity by non-CpG methylation. Firefly luciferase vector containing an unmethylated (PDK4 promoter) or in vitro methylated (Me-PDK4 promoter) promoter of PDK4 was cotransfected with an empty Renilla luciferase vector in HEK293 cells. Firefly luciferase activity was assayed at 48 hr after transfection and normalized to Renilla luciferase activity. Results are mean ± SEM, n = 3 (*p < 0.005).

See also Figures S2, S3, S5, S6, and Tables S1, S2, S3, and S4.
was unaltered in obesity. Methylation levels of PDK4 and PGC-1α correlated with BMI, leptin, and C-reactive protein levels, implicating a clinical association with adiposity. Interestingly, in this cohort of normal-weight and obese women, triglycerides levels correlated with PGC-1α, but not PDK4 methylation levels whereas insulin levels correlated with PDK4, but not PGC-1α methylation levels. Thus, body mass index appears to be a common factor, whereas insulin or triglycerides may have promoter-specific effects on DNA methylation. Our finding of altered DNMTs expression after weight loss, but not in obese versus normal-weight women, suggest that factors other than DNMT1 and DNMT3B protein levels account for alterations in the DNA methylation profile observed with obesity or weight loss.

In summary, obesity and weight loss are associated with promoter-specific changes in DNA methylation in PGC-1α and PDK4 in skeletal muscle. Genome-wide DNA methylation analysis also reveals that weight loss is associated with changes in methylation at CpG shores and exonic regions close to transcription start sites. Dynamic changes in DNA methylation may be an early event that orchestrates metabolic gene transcription involved in the regulation of insulin sensitivity in human obesity. Our results highlight the potential importance of environmental factors in forming the metabolic memory of somatic cells.

EXPERIMENTAL PROCEDURES

Patients
This study was approved by the Regional Ethics Committee of Stockholm. All participants provided informed written consent. Clinical characteristics are shown (Table S1) for the obese women before and after RYGB surgery (n = 8) and nonobese (normal weight) glucose-tolerant age-matched women (n = 16). Participants were selected for the transcriptomics analysis (Table S2), obese women before and after RYGB surgery (n = 5) and nonobese (normal weight) glucose-tolerant age-matched women (n = 6); for bisulfite analysis (Table S3), obese women before and after RYGB surgery (n = 8) and nonobese (normal weight) glucose-tolerant age-matched women (n = 9); and for genome-wide methylation analysis (Table S4), obese men before and after RYGB surgery (n = 6). Clinical chemistry was assessed as described in the Extended Experimental Procedures.

Surgery and Skeletal Muscle Biopsies
A standard laparoscopic RYGB with a 1 m Roux limb was performed. The patients were not subjected to a preoperative fast. Skeletal muscle biopsies (50–100 mg) were obtained from the vastus lateralis portion of the quadriceps femoris using a Weils-Blakesly contochome from the obese and nonobese (normal weight) subjects. Biopsies were obtained during the RYGB surgery after the induction of general anesthesia. Only nonglucose-containing intravenous solutions were administered before the biopsy was taken during the RYGB surgery. Biopsies taken from the obese subjects 6 months after RYGB surgery and from the nonobese (normal weight) women were obtained under local anesthesia (5 mg/ml of lidocaine hydrochloride) in the morning after a 12 hr fast. Biopsy samples for mRNA and DNA analysis were immediately frozen and stored in liquid nitrogen until analysis. The patients were prescribed a liquid diet for 1 month after surgery and then solid food. All patients were prescribed multivitamin, B12, folic acid, vitamin D, and calcium supplementation once daily.

Nucleic Acid Purification and Real-Time PCR
Details regarding nucleic acid purification and real-time PCR are provided in the Extended Experimental Procedures.

Bisulfite Sequencing
Bisulfite treatment was performed as described (Olek et al., 1996), with the adaptations as described in the Extended Experimental Procedures.
**Luminometric Methylation Assay**

Global CpG and non-CpG methylation was determined by luminometric methylation assay (LUMA) as previously described (Barrérs et al., 2009; Karimi et al., 2006). Details of the LUMA assay are presented in the Extended Experimental Procedures. Visualization of bisulfite sequencing results as analyzed by Methtools 2.0 (Grunau et al., 2000) (http://genome.imb-jena.de/methtools/).

**Skeletal Muscle Transcriptomics**

Affymetrix Human Genome U219 arrays were used to probe for transcripts differentially expressed between normal-weight (n = 6) woman, obese women pre-RYGB (n = 5), and obese women post-RYGB (n = 5). Probe-set data were normalized using a multi-array average (MMA) method. Gene expression levels between obese women pre-RYGB versus post-RYGB were compared using univariate paired testing, with a false discovery rate <5% and p < 0.002. Gene ontology terms were calculated using DAVID (Huang et al., 2009).

**Methylated DNA Enrichment**

Genomic DNA was extracted from cells and tissue using a Qiagen DNeasy blood and tissue kit and methylated DNA enrichment was achieved as described in the Extended Experimental Procedures.

**Luciferase Assay**

PDK4 promoter constructs were generated by PCR amplification of human PDK4 promoter region (−189 to +45 relative to the transcription start site), and subcloned into pCpG-basic vector containing a firefly luciferase gene. The external cytosome residue of the sequence CCGG was methylated by MspI methyltransferase (New England BioLabs) supplemented with fresh S-adenosylmethionine. Details of the luciferase experiments are found in the Extended Experimental Procedures.

**Genome-wide Methylation Analysis**

Sequencing quality was assessed using FastQC. Adapter dimers were removed with the FastX toolkit. The remaining reads were quality filtered with the following procedure: 3' nucleotides were trimmed if the Phred quality score was below 20, 5' nucleotides were trimmed if more than 10% of the reads had a quality below 20 at that position of sequence reads, and sequences possessing more than 70% of nucleotides with a quality below 20 were removed. The remaining reads were aligned to the human genome (hg19) with the short read aligner Bowtie (Langmead et al., 2009). Reads with more than one best alignment were discarded. Because each read originated from a fragment of 500 nucleotides on average, we extended each aligned read by 450 nt at the 3' end. To estimate aligned reads located in introns, exons, or intergenic regions, we used the Ensemble gene and exons database from Ensemble BioMart with the BedTools intersectBed (Quinlan and Hall, 2010). CGIs and CGI shores were estimated from the UCSC Table Browser (Hinrichs et al., 2006). Analysis and annotation of differentially methylated regions (DMRs) was performed using the Bioconductor package MEDIPS (Chavez et al., 2010) ChiPeakAnno, and biomaRt (Durinck et al., 2009). Peak calling was performed with MACS (Feng et al., 2011) and differential peaks using the Bioconductor package DiffBind.

**Statistics**

Results are presented as mean ± SE. The data were tested for normal distribution using the Skewness and Kurtosis test in SPSS Statistics Software 17.0. When a normal distribution was observed, the statistical difference between age-matched normal-weight women and obese women before surgery was determined using a two-tailed unpaired Student’s t test. The statistical differences before versus after surgery were determined using a two-tailed paired Student’s t test. The relationship between PGC-1α and PDK4 methylation levels and clinical variables was determined using a Pearson correlation analysis. p < 0.05 was considered significant.

**ACCESSION NUMBERS**

The Gene Expression Omnibus (GEO) accession number for the DNA-methylation data sets is GSE45747.

**SUPPLEMENTAL INFORMATION**

Supplemental Information includes Extended Experimental Procedures, six figures, and ten tables and can be found with this article online at http://dx.doi.org/10.1016/j.celrep.2013.03.018.

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