Blood Gene Expression Reveal Pathway Differences Between Diet-Sensitive and Resistant Obese Subjects Prior to Caloric Restriction

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Weight loss in response to caloric restriction displays significant interindividual heterogeneity. To develop early predictors of weight-loss success, we have compared whole-blood gene expression profiles of obese, diet-sensitive vs. obese, diet-resistant subjects prior to the initiation of clinically supervised caloric restriction. Pathway enrichment analysis of gene expression profiles by multiple applications converged on the "oxidative phosphorylation" (OXPHOS) pathway, and to a lesser extent the "proteasome" pathway, as statistically significantly upregulated in obese, diet-sensitive subjects compared to the diet-resistant subjects. The finding of increased OXPHOS is consistent with earlier observations of increased proton leak, increased expression of *OXPHOS* genes, and increased oxidative muscle fibers in skeletal muscle of obese, diet-sensitive subjects. The current study further highlights the utility of blood as a sentinel tissue reflecting systemic states and provides a potential modality to predict future weight-loss success, relevant to the design of individualized bariatric treatment programs.

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INTRODUCTION

Weight loss in response to caloric restriction is highly variable. Even in a clinically controlled environment, a four- to tenfold variation in weight loss is evident, after correction for initial body weight, gender, and age (1). The unpredictability of weight-loss success represents a significant barrier in the medical and surgical management of obesity. If the extent of weight-loss success can be predicted early in treatment, it would allow the physician to tailor the intervention, follow-up, and counseling to the individual patient and may also allow the choice for the most appropriate surgical procedure. For example, the response to caloric restriction might help to determine different subsets of patients more suitable for the duodenal switch or the Roux-en-Y procedures. The alarming rise in global obesity and its associated public health burden underscores a pressing need for early predictors of weight-loss success.

As the first step toward addressing this need, we have investigated the transcriptional profile of prospectively collected blood samples from a cohort of carefully screened, obese diet-sensitive (ODS) and obese diet-resistant (ODR) subjects (2) in the highest and lowest quintiles of a weight-loss distribution spectrum following 6 weeks of a clinically supervised, 900 kcal meal replacement program at the Ottawa Hospital Weight Management Clinic (3). In our earlier studies, we detected differential transcriptional response in biologically relevant pathways in skeletal muscle from ODS and ODR subjects and also demonstrated differential proportions of oxidative (type 1) muscle fibers between the two groups. We were therefore interested in determining to what extent gene expression in blood might recapitulate some of the same pathway differences. Justification for this approach is supported by several recent publications reporting differential blood gene expression signatures for nonhematologic disorders (4–6), paving the way for potential biomarker discoveries. We selected individuals from the extremes of weight-loss success to increase our chances of identifying differentially expressed signals. Finally we interrogated the gene expression signatures via multiple pathway analysis tools as a way of relating our findings to biological processes with greater confidence.

METHODS

The study protocol was approved by the Human Research Ethics Committees of the Ottawa Hospital and the University of Ottawa Heart Institute and informed consent was obtained from all participants prior to their enrolling into the program. Blood was drawn by standard

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venipuncture on the day of initiation of caloric restriction (day 1) and again on week 6 of caloric restriction and processed for microarray analysis on Affymetrix U133 Plus 2.0 genechips (data published in Gene Expression Omnibus (GEO), GSE 18897). Expression signals were generated via GC-RMA (7). Probesets were filtered such that probes with normalized average expression ≤50 units in both groups were removed. Gene signals were log transformed to base 2 and an average log signal was calculated for each gene for each group. Differences in the average log signals between ODS and ODR (log ratios) was calculated as an index of differential expression. The statistical significance of differential gene expression was calculated via a regularized *t*-test utilizing Bayesian statistics (CyberT, http://cybert.microarray.ics.uci.edu/) (8) and the filtered dataset was subjected to pathway analysis via three independent applications—gene-set enrichment analysis (GSEA; http://www.broadinstitute.org/gsea/), Ingenuity pathway analysis (IPA; http://www.ingenuity.com/) and Database for Annotation, Visualization and Integrated Discovery (DAVID; http://david.abcc.ncifcrf.gov). Results were examined for the magnitude of pathway enrichment and its statistical significance (measured as false discovery rate (FDR), P_{FDR}). All other statistical analyses were conducted in the JMP software package (SAS, Cary, NC).

RESULTS AND DISCUSSION

The two cohorts were demographically and phenotypically matched (**Table 1**). They differed significantly by percent weight loss at 6 ($P < 0.0001$) and 26 weeks ($P < 0.01$) after initiation of caloric restriction (**Supplementary Figure S1** online). Percent weight loss was independent of initial body weights (**Figure 1**).

Table 1 Demographic and phenotypic characteristics of the obese, diet-sensitive (ODS) and obese, diet-resistant (ODR) subjects

Variable[a]	Obese diet-sensitive	Obese diet-resistant
n	9	9
Female (%)	5 (56%)	5 (56%)
Age (years)	50.7 (8.4)	52.7 (12.1)
BMI at baseline (kg/m^2)	47 (7.9)	40 (6)
Weight (kg)	130.7 (32.1)	114.8 (20.7)
Fat free mass (kg)	67.9 (11.7)	63.9 (11.4)
Fat mass (kg)	62.3 (22.5)	50.2 (13.8)
Body fat (%)	46.8 (6.8)	43.5 (6.3)
Waist circumference (cm)	128.6 (17.8)	116.1 (13.5)
Glucose (mmol/l)	6.9 (2.9)	5.5 (0.7)
Insulin (pmol/l)	113.4 (72.5)	80.3 (34.3)
HbA_{1c} (%)	6.04 (0.99)	5.71 (0.46)
SBP (mm Hg)	142.3 (7.3)	137.1 (21.2)
DBP (mm Hg)	85.4 (10.2)	79.8 (9.5)
Total cholesterol (mmol/l)	4.82 (0.90)	5.38 (0.63)
Triglycerides (mmol/l)	2.35 (2.58)	1.80 (0.67)
LDL-C (mmol/l)	2.91 (0.58)	3.52 (0.62)
HDL-C (mmol/l)	1.05 (0.23)	1.03 (0.14)
TSH (mU/l)	2.4 (1.3)	2.7 (1.4)

DBP, diastolic blood pressure; HbA_{1c}, hemoglobin A_{1c}; HDL, high-density lipoprotein; SBP, systolic blood pressure; LDL, low-density lipoprotein; TSH, thyroid-stimulating hormone.
[a]Values for continuous measures are reported as average (s.e.). No significant difference (at $P < 0.05$ level) was observed for any variable between the groups.

GSEA detects statistically significant differences in *a priori* defined gene sets (pathways) through a weighted Kolmogorov–Smirnov statistic (Normalized Enrichment Score (NES)) based on over-representation of gene-set members toward the top or bottom of a list of genes ranked by the strength of their correlation to the phenotypes (ODS and ODR). In this context, it is important to remember that the results obtained from GSEA (or other pathway analysis programs) are directly dependent on the pathway databases these tools interrogate. The information content in different databases can vary, both in the number of pathways contained and in the identities of genes constituting closely related pathway descriptors. An example of this discrepancy was noted in the current analysis and is discussed later.

GSEA identified "oxidative phosphorylation (OXPHOS)" as the top scoring Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway upregulated in the ODS cohort (NES 2.09, FDR-adjusted $P = 0.00$; **Figure 2a**). **Figure 2b** is a MA-plot depicting the distribution of gene expression differences (log ratios) between the ODS and ODR cohorts against log average expression of the genes in the two groups. Non-OXPHOS pathway genes, shown in gray, show a symmetric distribution around a log ratio of zero, indicating overall parity in the number of up- and downregulated genes in each group, at all intensity levels. However, examination of individual expression values of the 84 *OXPHOS* genes between ODS and ODR subjects (shown as black crosses) demonstrate that despite modest differences of expression in the individual genes (~20% between ODS and ODR), the upregulation was consistent across the set (72/84 or ~85% of the probesets showing higher expression in ODS). This finding is consistent with an earlier report by Mootha *et al.* (9) demonstrating coordinate downregulation of *OXPHOS* genes in type 2 diabetic subjects (compared to normoglycemic controls). In that report, the authors also identified a subset of

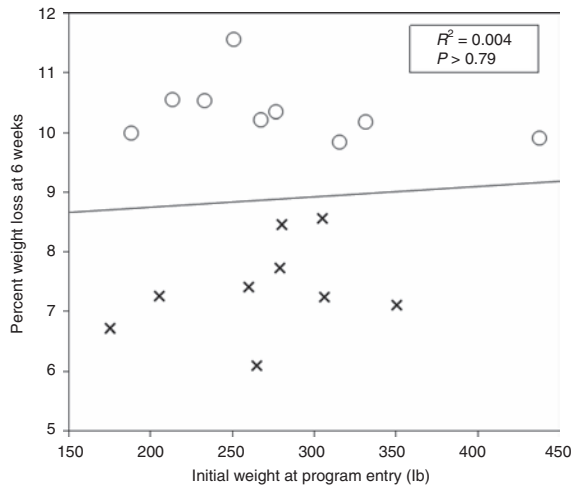

Figure 1 Weight-loss properties of obese diet-sensitive (ODS; circles) and obese diet-resistant (ODR; crosses) subjects. Independence of percent weight loss (week 6) to body weights at entry into the caloric restriction program for ODS or ODR subjects. Initial weights are plotted on the x-axis and percent weight loss after 6 weeks of caloric restriction are plotted on the y-axis.

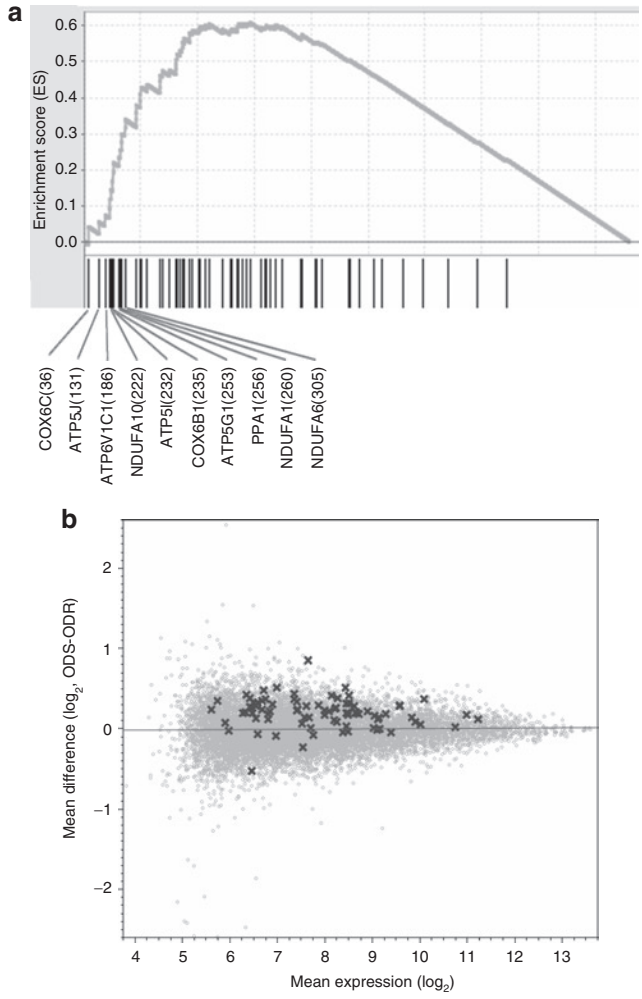

Figure 2 Pathway enrichment by gene-set enrichment analysis. (**a**) Enrichment plot for "oxidative phosphorylation". The gray curve is the running enrichment score, ES (defined under Materials and Methods) for the "oxidative phosphorylation" pathway over the ranked list of genes. The score at the peak of the plot (the score furthest from 0.0) is the ES for the gene set. The relative ranks of oxidative phosphorylation (*OXPHOS*) genes (based on expression differences in obese diet-sensitive (ODS) subjects and obese diet-resistant (ODR) are indicated by the lines at the bottom of the plot. Symbols and ranks of the top 10 *OXPHOS* genes are indicated. (**b**) MA plots of log intensity-ratios (mean differences between ODS vs. ODR log signals, y-axis) vs. log intensity-averages (mean expression, x-axis) for (i) all filtered genes on the microarray (gray) and (ii) only *OXPHOS* pathway-specific genes (black crosses). An excess of positive values on the y-axis indicates upregulation of the majority of genes of the OXPHOS pathway in the ODS cohort.

OXPHOS genes (*OXPHOS-CR*) whose expression was coordinately regulated across multiple tissues (based on a mouse tissue expression atlas). We examined whether the *OXPHOS-CR* subset contributed differentially to pathway enrichment by comparing *OXPHOS-CR* and non-*OXPHOS-CR* memberships among the genes contributing to core enrichment of *OXPHOS*. GSEA assigns genes within a pathway as contributing to its core enrichment (or lack thereof) based on the statistical significance of the gene's differential expression between the two groups. In our analysis, no differential representation was observed for the *OXPHOS-CR* or non-*OXPHOS-CR* genes for core enrichment (data not shown) suggesting that the observed results are not solely due to co-regulation. We also did not detect any structural or functional relationships among the ~12% *OXPHOS* genes that were downregulated in ODS (these include the genes *COX7C*, *NDUFAB1*, *ATP5L*, *ATP5C1*, *NDUFB8*, *COX15*, *NDUFC2*, and *MPHOSPH8*). Detailed results of pathway analysis of the ODS and ODR cohorts on day 1 are shown in **Supplementary Table S1** online. The relative expression signals of the *OXPHOS* genes in the ODS and ODR cohorts on day 1 are shown in **Supplementary Table S2** online. The gene components of the OXPHOS pathway, as represented in KEGG, are illustrated in **Supplementary Table S3** online. In order to determine whether the findings from GSEA analysis was influenced by gender, we carried out independent GSEA analysis on subsets of male and female ODS and ODR subjects. The results, shown in **Supplementary Table S4** online, demonstrate that *OXPHOS* was identified as one of the top two differentially regulated pathways in both males and females, suggesting that the original results are independent of gender. Similarly, we addressed the question of whether the menopausal status of the women subjects included in the analysis had any bearing on the final results. The study had 2/3 or 66% postmenopausal women in the ODS group and 3/6 or 50% postmenopausal women in the ODR group. Since these numbers were too small to draw any meaningful conclusions on the influence of menopausal status on gene expression, we analyzed public domain gene expression data downloaded from GEO (GEO study no. GS12517 investigating peripheral blood mononuclear cell gene expression in 11 premenopausal and 12 postmenopausal women). Specifically, we investigated the expression patterns of genes belonging to the OXPHOS pathway between the pre- and postmenopausal groups. The results (**Supplementary Figure S2** and **Supplementary Table S5** online) demonstrate no systematic trends in the expression patterns of *OXPHOS* genes between premenopausal and postmenopausal women, suggesting that the results obtained in our study are unlikely to be influenced by menopausal status. Finally, we interrogated the gene expression signals to determine whether the expression of the *OXPHOS* genes were significantly influenced by fat mass or insulin levels. Nonparametric rank correlation (Spearman's ρ) of *OXPHOS* gene expression to fat mass and insulin levels showed that although some individual genes showed a statistically significant correlation to fat mass or insulin levels ($P < 0.05$ level), the overall impact on the gene-set was low (e.g., only 7.9% and 18.4% of the *OXPHOS* genes were correlated to fat mass and insulin levels respectively at $P < 0.05$ level). These results are elaborated in **Supplementary Table S6** online.

Based on the GSEA results, we focused on pathway enrichment analysis among genes upregulated in the ODS cohort on day 1 (ODS/ODR $\log_2$ ratio ≥ 0.1) in subsequent analyses. We employed two different pathway analysis tools, DAVID and IPA, which use variants of Fisher's exact test to determine pathway enrichment and, unlike GSEA, do not take gene ranks into consideration. DAVID provides biological

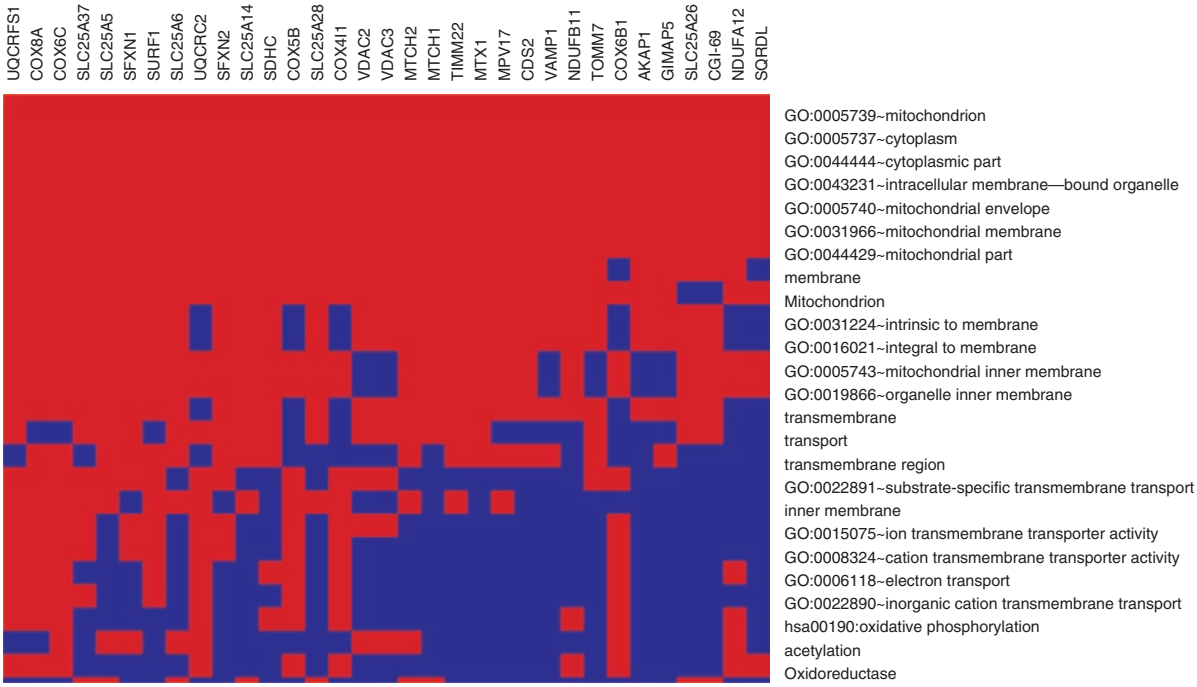

Figure 3 Pathway enrichment analysis in DAVID (Database for Annotation, Visualization and Integrated Discovery). Functional annotation clustering of "mitochondria"-related terms, including "oxidative phosphorylation" among genes upregulated in obese diet-sensitive subjects. Clustered annotation terms are represented in rows and genes represented in columns. Red areas indicate established gene-annotation overlap and blue areas indicate nonoverlap. The top 25 clustered terms are shown.

pathway enrichment statistics from gene expression data through a conservative Fisher's exact test known as the EASE (Expression Analysis Systematic Explorer) score (10,11). In our analysis, DAVID identified *OXPHOS* as the top KEGG pathway enriched in ODS (2.2-fold enrichment, $P < 2.8E\text{-}4$; **Supplementary Table S7** online). In addition to KEGG pathway enrichment analysis, we applied the Gene Functional Classification Tool within DAVID to classify the ODS-upregulated genes into functionally related gene groups for identifying major biological signatures. The functional annotation clustering analysis, based on a gene-to-gene functional similarity matrix, revealed a concentration of "mitochondria" related terms in a highly significant gene cluster (14.4-fold enrichment in the gene cluster compared to the full list of genes) further supporting an enhanced involvement of mitochondrial processes in the ODS (**Figure 3**).

We next examined pathway enrichment via the core analysis module within IPA. IPA leverages the Ingenuity knowledge-base (a curated database of chemical and biological relationships extracted from the literature) to interrogate data for enrichment of biological processes, pathways and molecular networks. We initially focused on the "canonical pathways" category in IPA (a combination of KEGG and other IPA-curated pathways) to help compare results obtained from GSEA and DAVID. A pathway enrichment analysis, using Fisher's exact test, identified OXPHOS as the fourth most significant pathway upregulated in the ODS (FDR-adjusted $P < 1.4 \times 10^{-5}$), following "protein ubiquitination," "mitochondrial dysfunction," and "polyamine regulation" (**Supplementary Table S8** online). The "mitochondrial dysfunction" pathway is a collection of genes that are associated with mitochondrial dysfunction through genetic polymorphisms. Since several of these genes relate to *OXPHOS*, we observed significant overlap between the genes in these two pathways (27/39 genes in *OXPHOS* overlapped with 36 genes in mitochondrial dysfunction). Next, we performed a network overlap analysis to identify the canonical pathways that significantly overlap with the networks (gene and protein regulatory relations) dynamically generated from our data in the IPA knowledge-base. We restricted the network size to a maximum of 35 genes (range 27–34 genes) and examined the top 20 IPA networks for pathway overlap by comparing the percent overlap between the top canonical pathway and the network genes for each network. The largest pathway overlap was noted for the OXPHOS pathway that overlapped 62% with a network of 31 genes (network 13 in **Figure 4**, 21/34 genes in common). Additionally, 3 of the 20 networks contained either OXPHOS or a related canonical pathway named "mitochondrial dysfunction" as the top canonical pathway with 20% or greater overlap (the highest overlap observed for "mitochondrial dysfunction" was 55% (network 13). **Figure 4** compares the percent pathway overlap obtained for all 20 networks. The results indicate that the OXPHOS pathway provides the strongest overlap among all canonical pathways tested. **Supplementary Figure S3** online further depicts the genes within network 13 that are in common with genes of the OXPHOS pathway.

OXPHOS is an index of cellular energetics and involves mitochondrial electron transport during cellular respiration

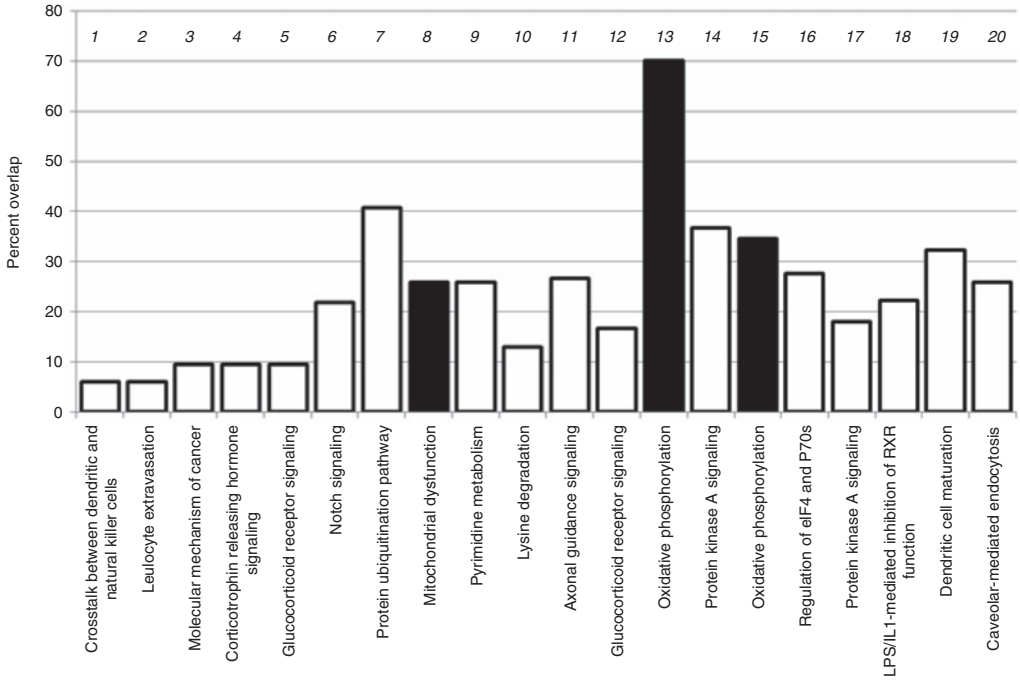

Figure 4 Network overlay analysis in ingenuity pathway analysis (IPA). Analysis of the top 20 networks generated in IPA from genes upregulated in obese diet-sensitive and the highest overlapping canonical pathway for each network. Percent overlap is plotted for each network (numbered in italics) against the pathway identity. Networks containing overlap with oxidative phosphorylation and mitochondrial dysfunction pathways are indicated in black.

and adenosine triphosphate production. The relationship between resting energy expenditure and weight-loss success has been established from previous studies demonstrating reduced mitochondrial proton leak in skeletal muscle biopsies from ODR subjects following program completion and weight stabilization (2). Additionally, genes related to OXPHOS were upregulated in ODS *rectus femoris* biopsies, while *vastus lateralis* biopsies showed a higher proportion of oxidative (type 1) fibers in ODS compared to ODR subjects. The greater capacity for electron transfer will allow for greater utilization of electron-donors such as NADH (nicotinamide adenine dinucleotide) and $FADH_2$ (flavin adenine dinucleotide) which are obtained, in part, from fatty acid β-oxidation. These changes, in skeletal muscle, may explain a portion of the accelerated weight loss observed in the ODS. Notably, the current study points to very similar changes occurring in whole-blood, underscoring the importance of blood as a reflector and an indicator of systemic states. Also, both the earlier (2) and current studies were performed in subjects at a time when they were not undergoing supervised caloric restriction. The persistence of differences in the absence of an environmental stimulus (caloric restriction) suggests that the differences in OXPHOS in ODS and ODR are partly under genetic control. The presence of a genetic component is further consistent with the observation that weight-loss success upon caloric restriction in murine models is strain-dependent and exhibits 40–50% heritability (12).

To identify additional biological pathways that could differentiate ODS from ODR subjects, we compared the top 10 scoring pathways from each analysis (**Table 2**). OXPHOS was identified by all three approaches whereas a second pathway named as "protein-ubiquitination pathway" was identified within the top 10 pathways for IPA and DAVID, but not GSEA (the closest term match in GSEA was the "ubiquitin-mediated proteolysis" pathway, NES 0.62, FDR-adjusted $P = 1.0$). Closer inspection revealed that in GSEA, the gene components of "ubiquitin-mediated proteolysis" were restricted only to the ubiquitination enzymes, whereas in both IPA and DAVID, the "protein ubiquitination pathway" contained ubiquitination enzymes as well as subunit components of the proteasome. Several components of the proteasome pathway were upregulated in the ODS, leading to the pathway's high rank in DAVID and IPA. We next investigated the "proteasome pathway" in GSEA and identified it to be the 21st most upregulated pathway in the ODS (NES 1.47, 15% FDR; **Supplementary Table S1** online). These results lead to the hypothesis of an activated proteasome in the ODS cohort, that could result in basally enhanced protein turnover. Upon the introduction of caloric restriction, this could further augment the catabolism of proteins and contribute to increased weight loss in the ODS. However, the relationship between protein turnover and obesity appears to be unclear at present. Thus, while proteosomal subunit mRNA expressions have previously been reported to be inversely correlated to BMI (13,14), other reports have shown an increase in ubiquitin-mediated proteolysis in obesity and a reduction of proteolytic activity following caloric restriction (15,16). A similar examination in our dataset identified two proteasomal subunit genes, *PSMA4* and *PSMC6* as inversely correlated to BMI ($P < 0.1$ level), independent of diet-sensitivity status

Table 2 Comparison of top 10 biological pathways identified by different pathway analysis tools

	Enrichment score			Nominal *P* value			Adjusted *P* value			Rank		
Pathway	GSEA	IPA	DAVID	GSEA	IPA	DAVID	GSEA	IPA	DAVID	GSEA	IPA	DAVID
HSA00190 oxidative phosphorylation	2.09	0.23	2.22	0.00×10^{0}	5.13×10^{-10}	1.42×10^{-6}	0		0.002	1	2	1
HSA04940 type 1 diabetes mellitus	1.79			1.25×10^{-3}			0.037			2		
HSA01032 glycan structures degradation	1.78			2.87×10^{-3}			0.028			3		
HSA00561 glycerolipid metabolism	1.71			4.15×10^{-3}			0.058			4		
HSA05120 epithelial cell signaling in *Helicobacter pylori* infection	1.67			4.93×10^{-3}			0.075			5		
HSA00260 glycine serine and threonine metabolism	1.62			1.97×10^{-2}			0.109			6		
HSA00480 glutathione metabolism	1.61			1.71×10^{-2}			0.108			7		
HSA00010 glycolysis and gluconeogenesis	1.6			1.15×10^{-2}			0.11			8		
HSA04612 antigen processing and presentation	1.58			8.54×10^{-3}			0.128			9		
HSA00020 citrate cycle	1.54			2.11×10^{-2}			0.173			10		
Mitochondrial dysfunction		0.21			3.98×10^{-11}						3	
Polyamine regulation in colon cancer		0.35			2.34×10^{-9}						4	
Ubiquinone biosynthesis		0.18			8.91×10^{-8}						5	
EIF2 signaling		0.24			1.35×10^{-6}						6	
Glucocorticoid receptor signaling		0.18			4.37×10^{-6}						7	
IL8 Signaling		0.19			8.91×10^{-6}						8	
Role of NFAT in regulation of immune response		0.18			1.10×10^{-5}						9	
CCR5 signaling in macrophages		0.21			1.62×10^{-5}						10	
HSA04640 hematopoietic cell lineage			2.23			1.10×10^{-4}			0.011			2
HSA04120 ubiquitin-mediated proteolysis		0.25	1.94		1.29×10^{-6}	1.13×10^{-4}			0.008		1	3
HSA03050 proteasome			3.64			2.98×10^{-4}			0.015			4
HSA05212 pancreatic cancer			1.89			8.13×10^{-3}			0.28			5
HSA05020 Parkinson's disease			2.77			1.80×10^{-2}			0.456			6
HSA05220 chronic myeloid leukemia			1.77			2.01×10^{-2}			0.441			7
HSA04650 natural killer cell mediated cytotoxicity			1.52			2.54×10^{-2}			0.475			8
HSA00970 aminoacyl t-RNA biosynthesis			2.11			2.83×10^{-2}			0.473			9
HSA05213 endometrial cancer			1.89			3.42×10^{-2}			0.503			10

A measure of pathway enrichment, statistical significance, multiple testing correction and rank of pathways are shown. The KEGG pathway database was used for GSEA and DAVID whereas the "canonical pathway" data was used for IPA analysis. A filtered list of 12,127 probesets, containing both up and downregulated genes was used for GSEA analysis. A filtered list of 2,923 probesets, all upregulated in the ODS cohort (vs. ODR at day 1) at a log ratio of 0.1 or higher, was used as input for IPA and DAVID analysis. The enrichment score is defined as "normalized enrichment score" in GSEA, "fold enrichment" in DAVID and "ratio" in IPA. *P* value is based on permutation testing for GSEA, modified Fisher exact *P* value for DAVID and Fisher exact *P* value for IPA. A Benjamini–Hochberg type FDR was calculated for all three methods.

DAVID, Database for Annotation, Visualization and Integrated Discovery; FDR, false discovery rate; GSEA, gene-set enrichment analysis; IL8, interleukin 8; IPA, ingenuity pathway analysis; NFAT, nuclear factor of activated T-cells; t-RNA, transfer RNA.

(**Supplementary Table S9** and **Supplementary Figure S4** online).

The identification of OXPHOS and proteasome pathways as being differentially regulated in the ODS and ODR subgroups prior to caloric restriction led us to further investigate the status of these pathways once caloric restriction had started. Thus, we examined by GSEA, whole-blood gene expression data from ODS and ODR subjects after 6 weeks of caloric restriction. At week 6, the OXPHOS pathway was no longer differentially regulated between the ODS and ODR cohorts (NES −0.63, FDR-adjusted $P = 1.0$), reflecting a general adjustment of this pathway in both groups due to reduced substrate availability from caloric restriction. A comparison of day 1 and week 6 data in the ODS further revealed a downward trend for this pathway at week 6 (NES −1.52, nominal $P = 0.005$, FDR-adjusted $P = 1.0$; **Supplementary Figure S5** online). In contrast, the proteasome pathway continued to show differential regulation and was the top-ranked ODS-upregulated pathway at week 6 (NES 1.62, nominal $P = 0.008$, FDR-adjusted $P = 0.73$; **Supplementary Figure S6** online). Despite a high FDR, the consistency of the upregulation of the proteasome pathway in the ODS at day 1 and week 6 is suggestive of a continued capacity for higher protein turnover in the ODS cohort.

The current study is exploratory in nature and certain limitations exist. First, the selection of matched individuals from the top and bottom quintiles of weight loss resulted in small sample sizes. Second, although matched for initial weights, there was a small (although statistically insignificant) difference in the initial BMI between the two groups. Third, since whole blood was utilized for genomic profiling, the individual contributions of the different blood cell types to the observed differences remain unknown. Finally, due to small sample sizes, we have purposefully not further split the cohorts into training and replication sets but have depended on the consistency of results from multiple analytic approaches to increase confidence in our findings.

This pilot study provides proof of concept that it is indeed possible to differentiate fast and slow weight losers based on plausible biological mechanisms derived from gene expression profiling in blood. Importantly, as the results were obtained prior to the initiation of caloric restriction, it supports the feasibility of identifying early weight-loss indicators. We consider these findings as the first step toward the identification of mechanism-based predictors of weight-loss success in response to caloric restriction and assistance in subsequent treatment modalities such as the design of individualized bariatric treatment programs.

SUPPLEMENTARY MATERIAL

Supplementary material is linked to the online version of the paper at http://www.nature.com/oby

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DISCLOSURE

S.G. and J.S. have equity in GlaxoSmithKline. R.M., R.D. and M.E.H. have no disclosures.

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