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Increases in Insulin Sensitivity among Obese Youth are Associated with Gene Expression Changes in Whole Blood

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Abstract

Objective—Lifestyle intervention can improve insulin sensitivity in obese youth yet few studies have examined the molecular signatures associated with these improvements. Therefore, the purpose of this study was to explore gene expression changes in whole-blood that are associated with intervention-induced improvements in insulin sensitivity.

Design and Methods—Fifteen (7M/8F) overweight/obese (BMI percentile=96.3±1.1) Latino adolescents (15.0±0.9 years) completed a 12-week lifestyle intervention that included weekly nutrition education and 180 minutes of moderate-vigorous exercise per week. Insulin sensitivity was estimated by an oral glucose tolerance test and the Matsuda Index. Global microarray analysis profiling from whole blood was performed to examine changes in gene expression and to explore biological pathways that were significantly changed in response to the intervention.

Results—A total of 1,459 probes corresponding to mRNA transcripts (717 up, 742 down) were differentially expressed with a fold change ≥1.2. These genes were mapped within 8 significant pathways identified, including insulin signaling, type 1 diabetes, and glycerophospholipid metabolism. Participants that increased insulin sensitivity exhibited five times the number of significant genes altered compared to non-responders (1,144 vs. 230).

Conclusions—These findings suggest that molecular signatures from whole blood are associated with lifestyle-induced health improvements among high-risk Latino youth.

Keywords

Gene Expression; Exercise; Diabetes; Insulin Sensitivity; Adolescent

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Conflicts of interest

Authors have no conflicts of interest to disclose.

Introduction

Obesity and type 2 diabetes have reached epidemic proportions and disproportionately impact Latino youth. Nearly 30% of overweight Latino youth exhibit an abnormal metabolic profile and as many as 30% are prediabetic (1). Lifestyle modification that includes both nutrition and exercise is the first line intervention for obese youth and while lifestyle interventions have been shown to reduce obesity-related risk factors (2) few studies have examined the molecular signatures associated with these health improvements.

The pathogenesis of insulin resistance and type 2 diabetes is multifactorial and involves genetic and environmental factors. In obese adults, improvements in cardiometabolic risk factors following a lifestyle modification can result in changes in mRNA expression levels in skeletal muscle (3). A historical challenge to conducting molecular research in children is the need for invasive procedures to acquire specimens in key regulatory tissues such as liver or muscle. However, advances in microarray technology applied to blood samples of youth have been used to examine gene expression changes contributing to the pathophysiology of diabetes (4) as well as response to exercise (5). Data in adults further support the utility of microarray gene expression from blood for the identification of metabolic derangements in insulin resistant populations (6) and response to exercise training (5). To date, no studies have applied this approach to examine the molecular signatures in response to lifestyle intervention among obese youth. Therefore, the purpose of this study was to apply a microarray based, transcriptomic profiling approach to examine gene expression changes following a 12-week diabetes prevention program for obese Latino youth. A secondary purpose was to explore whether changes in gene expression may be associated with a differential response to the program.

Methods and Procedures

Study Design and Participants

The present manuscript describes gene expression changes in whole-blood among 15 overweight / obese Latino youth (7 male/8 female, age 15.0±0.9, BMI percentile = 96.3±1.1) who completed a comprehensive lifestyle intervention. Details of the study design along with changes in health outcomes have been described elsewhere (2). Briefly, the 12-week intervention included weekly nutrition education classes along with three, 60-minute moderate to vigorous physical activity sessions per week and led to significant improvements in insulin sensitivity and glucose tolerance. The study was approved by the Institutional Review Board at Arizona State University, and participants and a parent or legal guardian provided written informed assent and consent.

Clinical Procedures

Participants were assessed for anthropometrics (height, weight, waist circumference), a fasting (10–12 hours) blood sample was collected for gene expression studies, and insulin sensitivity and glucose tolerance were assessed via an oral glucose tolerance test (OGTT). A standardized, 75-gram OGTT was administered with blood samples collected at 0, 30, 60, 90, and 120 minutes for measurement of plasma glucose (glucose oxidase, YSI INC.,

Yellow Springs, OH) and insulin (ELISA, ALPCO Diagnostics, Windham, NH). Insulin sensitivity was estimated by the whole-body insulin sensitivity (WBISI) using plasma glucose and insulin concentrations as described by Matsuda and Defronzo (7). Gene expression and OGTT samples were collected at baseline and 24–48 hours after the last exercise session.

RNA extraction, RNA amplification, labeling, microarray hybridization, and data analysis

Baseline and post-intervention blood samples were collected in PAXgene blood RNA tubes containing a proprietary reagent that stabilizes RNA (BD Diagnostics, Franklin Lakes,NJ). Total RNA was isolated using the PAXgene Blood RNA Kit (Qiagen, Valencia, CA) according to the manufacturer's instructions. Total RNA (500 ng) and RNA spike-in kit, was amplified and labeled using the Agilent Quick Amp, two-color labeling kit (Agilent Technologies), as per manufacturer's directions. RNA integrity was assessed using Agilent RNA 6000 Nano Kit (Agilent Technologies, Palo Alto, CA) and quantified using the NanoVue Spectrophotometer (GE Healthcare). The RNA integrity numbers (RIN) and RNA absorbance ratio (A_{260}/A_{280}) was 8.3 ± 0.066, and 2.1 ± 0.004, respectively. Complementary RNA (cRNA) was purified using RNeasy Mini Kit (Qiagen). After labeling, cRNA was quantified using NanoVue Spectrophotometer (GE Healthcare) for cyanine 3 (Cy3) and cyanine 5 (Cy5) dye labeled cRNA concentration, RNA absorbance ratio (260 nm/280 nm), A260 values, ADye values, and dye/probes values. Labeled cRNA samples were fragmented using Agilent Gene Expression Hybridization Kit (Agilent Technologies, Palo Alto, CA), as per the instructions. The fragmented cRNA was hybridized onto whole human genome 4×44K 60-mer oligonucleotide arrays (G4112F, Agilent Technologies, Santa Clara, CA, USA) using a SureHyb DNA Microarray Hybridization chamber for 17 hours at 65°C in a rotating incubator. Pre and post-fragmented cRNA for each individual were hybridized to a microarray chip. After the washing with wash Buffers (Agilent Technologies, Palo Alto, CA), the slides were scanned using an Agilent Microarray Scanner (Agilent Technologies, Palo Alto, CA) and the hybridization signals were extracted using the Agilent Feature Extraction software, version 10.5.1.1 (Agilent Technologies, Palo Alto, CA).

Expression values were analyzed using GeneSpring GX 11.5 (Agilent Technologies, Palo Alto, CA) to identify differentially expressed genes. Data were first normalized by Lowess to account for the 2-color labeling, quantile normalization, and baseline to median of all samples. The probes below 20% expression were filtered and the control probes were excluded from the analysis. The remaining 16,264 probes were subjected to a paired student-t test applying Benjamini-Hochberg multiple testing correction and only those that achieved a fold change ≥1.2 were included, $P<0.05$. Database for Annotation, Visualization, and Integrated Discovery (DAVID) (http://david.niaid.nih.gov) was used to classify the genes into the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways. Only pathways with Expression Analysis Systematic Explorer (EASE) scores $P<0.05$ are presented in this analysis. EASE score is a modified Fisher Exact p-value in DAVID system used for gene-enrichment analysis. EASE score p-value= 0 represents perfect enrichment.

Quantitative Real-Time Polymerase Chain Reaction (Q-RT-PCR)

Microarray findings were confirmed by Q-RT-PCR on select genes using the ABI PRISM 7000 Sequence Detection System (Applied Biosystems, Life Technologies, Carlsbad, CA). One microgram of RNA was reverse transcribed using the High Capacity cDNA Reverse Transcription Reagents kit (Applied Biosystems) according to the manufacturer's instructions. TaqMan Universal Fast PCR master mix reagents and the Assay-On-Demand gene expression primer pair and probes (Applied Biosystems, Life Technologies, Carlsbad, CA) were used to determine the quantity of each gene in each sample. Actin beta was used as the endogenous control. Paired sample t-test were used to compare changes between Q-RT-PCR and microarray and Pearson correlation coefficient was used to examine the association between fold-change between methods.

Results

As previously reported, BMI and waist circumference decreased significantly and improvements in glucose tolerance and insulin sensitivity were observed (2). These improvements included a decrease in 2-hour glucose (10.8%) and 2-hour insulin (23.6%), which, corresponded to a 29.2% increase in insulin sensitivity as measured by the WBISI (2.4 ± 0.3 to 3.1 ± 0.3; P = 0.02). Table 1 presents changes in selected cardiometabolic risk factors in response to the intervention.

A total of 1,459 probes (717 up, 742 down) were differentially expressed in whole blood with a fold change ≥ 1.2 and P <0.05. A complete list of these genes is provided in the supplemental material (Supplemental Table 1). Table 2 presents the top 10 up-regulated genes, which significantly increased by approximately 1.5 – 1.7 fold over baseline. Genes of metabolic interest in this group include hexokinase 3 (*HK3)*, ATPase, H+ transporting V0 subunit e2 (*ATPV0E*), and sterol regulatory element binding transcription factor 1 (*SREBF1*). Table 3 presents the top 10 down-regulated genes, which significantly decreased approximately 1.7–2.0 fold from baseline. Down-regulated genes of metabolic interest included suppressor of cytokine signaling 1 (*SOCS1*), endothelial cell adhesion molecule (*ESAM*), and hexose-6-phosphaste dehydrogenase (*H6PD*). In order to identify whether the intervention was associated with changes in biological pathways, the 1,459 differentially expressed probes were characterized into pathways using DAVID Bioinformatics Resources 6.7 (8). Eight pathways were found significant (Table 4) with the insulin signaling pathway exhibiting the largest number of significantly altered genes. Table 5 presents the individual genes along with the magnitude of change for these significant genes within the insulin signaling pathway; Figure 1 presents a schematic of the genes and where they lie in the insulin signaling pathway. Five genes within the insulin signaling pathway were used to confirm microarray findings by Q-RT-PCR. These genes included ARAF, HK3, MKNK2, PDK1, and MAP2K2. No difference was found for mean fold-change over baseline for these genes by method (1.3±0.3 vs 1.1±0.1, p=0.1) while a strong correlation between fold change by microarray and Q-RT-PCR was noted (r=0.9, p=0.01).

Although insulin sensitivity increased significantly in response to the intervention, considerable heterogeneity was noted in this response where 9 of the 15 participants exhibited improvements (>10%) over baseline, while six did not (Figure 2). Given that up-

regulation in the insulin signaling pathway is associated with increases in whole-body insulin sensitivity (9), we explored whether differences in gene expression profiles existed between those who did improve insulin sensitivity compared to those who did not. Participants that increased insulin sensitivity exhibited almost five times the number of significant genes altered by at least 1.2 fold compared to those who did not improve (1,144 genes in responders versus 230 genes in non-responders). The two groups shared only 111 genes changes in common (Figure 3). The genes unique to responders and non-responders as well as those that were shared between groups are presented in supplemental tables 2–4.

Discussion

Insulin resistance is a key pathophysiologic process associated with type 2 diabetes as well as cardiovascular disease risk in youth (10). Therefore reducing insulin resistance (i.e. increasing insulin sensitivity) is an important intervention target for improving health in this population. Lifestyle interventions that incorporate a combination of diet and exercise have been shown to reduce obesity and its comorbidities (2, 11). However, very little is known about the molecular signatures associated with these conditions in youth (6, 12) and even less is known about the biological pathways by which lifestyle intervention can lead to health improvements.

Using global gene expression profiling from whole blood, we identified 1,459 probes corresponding to mRNA transcripts that were significantly altered in response to lifestyle intervention. The genes represent a robust and diverse response, which could be a function of either the nature of the intervention (nutrition and exercise) or the tissue sampled (whole blood). Dietary changes and exercise are known to have independent as well as interactive effects on health (13) but effects of these interventions on gene expression are largely unknown (14). Nonetheless, many of the observed changes in gene expression (both up-regulation and down-regulation) discovered in this study may provide insight into the changes in health outcomes.

Among the up-regulated genes of interest are v-akt murine thymoma viral oncogene (*AKT2*) (1.20 fold change), *SREBF1* (1.33 fold change), and MAP kinase interacting serine/threonine kinase 2 (*MKNK2*) (1.35 fold change). The phosphatidylinositol 3-kinase/Akt pathway has been shown to play an essential role in GLUT4 translocation (15). Akt substrates are well known for their role in signaling via AS160, which cause the accumulation of GTP-bound forms of a subset of Rab proteins, enhancing the GLUT4 vesicle trafficking (16). Reduction in *AKT2* gene expression has been reported in pancreatic islets from T2DM patients (17). In circulating leukocytes from insulin resistant patients, *AKT2* mRNA levels inversely correlate with both plasma insulin levels and HOMA-IR and are reduced compared to insulin sensitive individuals (18). Gonzalez-Navarro et al. also observed defects in AKT2 signaling which may contribute to accelerated atherosclerosis in patients with metabolic syndrome (18). The *SREBF1* gene produces SREBP-1a and SREBP-1c by specific promoters and alternative splicing (19). SREB-1a and SREBP-1c are transcription factors controlling lipid homeostasis in humans (20). SREBP-1c mediates the transcriptional effects of insulin on genes encoding enzymes involved in glycolysis, lipogenesis, and gluconeogenesis (21). In a French case-control study, the *SREBF1* gene C-

G polymorphism was shown to associate more frequently with obesity and type 2 diabetes than the C-allele carriers (22). In addition, the *SREBF1* G952G single nucleotide polymorphism significantly affects cholesterol synthesis (23). Therefore, *SREBF1* gene expression regulation may be result in the improved metabolic parameters observed in the youth as a result of the intervention.

Down-regulated genes *ESAM* (−1.33 fold change) and *H6PD* (−1.43 fold change) have been linked to obesity-related heath outcomes. ESAM contributes to the integrity of tight junctions and is thought to modulate endothelial function (24). ESAM has been linked to experimental diabetic nephropathy (25) and its soluble fraction is related to atherosclerosis in humans (26). ESAM is higher in patients with diabetes than in controls and correlates with diabetes duration and control (27). *H6PD* mRNA levels were significantly higher in patients with type 2 diabetes than controls in both visceral and subcutaneous adipose tissues (28). In this study adolescents decreased their waist circumference by 3.6% (2) which may have initiated or elicited a change in *H6PD* expression.

Given that changes in individual genes provide only limited information on underlying disease mechanisms and processes in humans, we further explored how these individual changes in gene regulation may function in a coordinated fashion to influence metabolic and physiological mechanisms. The 1,459 Agilent probe IDs were entered into DAVID Bioinformatics Resources 6.7 in order to identify biological pathways that may be changed in response to intervention. Of the eight pathways found significant using DAVID, the insulin signaling pathway exhibited the largest number of genes represented.

Within this pathway, there are a number of genes that may serve as molecular signatures for improvements in whole-body insulin sensitivity. There are a number of up-regulated genes that may contribute to the improvement of insulin sensitivity downstream of the insulin receptor and also at the level of glucose uptake. For example, we observed a 1.69 fold increase in hexokinase 3 (*HK3*) which is involved in phosphorylation of glucose to produce glucose-6-phosphate, the initial step in most glucose metabolism pathways (29). Insulin resistant subjects after exercise have been shown to exhibit increased insulin sensitivity, due in part to an increase in insulin-stimulated glucose transport-phosphorylation, specifically glucose-6-phosphate (30). Furthermore, we observed increases in genes involved in glycogen storage such as glycogen phosphorylase B (*PYGB*). This enzyme is positively regulated by AMP and negatively regulated by ATP, ADP, and glucose-6-phosphate (31). *PYGB* catalysis is the rate-determining step in glycogen degradation. Single nucleotide polymorphisms in PYGB have been associated with acute insulin response to glucose in Hispanic-Americans (32). Among the other genes up-regulated in the insulin signaling pathway were calmodulin 2 (*CALM2*), MAP kinase interacting serine/threonine kinase 2 (*MAP2K2*), *AKT2*, and *SREBF1*. Suppressor of cytokine signaling 1 (*SOCS1*) was down regulated. *SOCS1* expression can be induced by a subset of cytokines and the protein encoded by this gene functions downstream of cytokine receptors and takes part in a negative feedback loop to attenuate cytokine signaling (33). Mooney et al. concluded that SOCS proteins may be inhibitors of insulin receptor signaling and could mediate cytokine-induced insulin resistance and contribute to the pathogenesis of type 2 diabetes (34). This is

the first study to show these markers using whole blood in response to lifestyle intervention in youth.

In addition to insulin signaling, several other pathways identified in this study have been associated with obesity-related health. For example, a recent genome-wide association study mapped several genes within the glycerophospholipid metabolism pathway to type 2 diabetes and coronary artery disease in adults (35). Another pathway identified was related to type 1 diabetes. Although the pathophysiology of type 1 diabetes is thought to be related to auto-immune processes rather than obesity and insulin resistance, Kaizer and colleagues used microarray analysis on peripheral blood monocytes to show that children with type 2 diabetes share 18 of the 22 most highly differentially expressed genes with children diagnosed with type 1 diabetes (4). The authors suggest that these commonalities may be related to β-cell dysfunction and contribute to the shared phenotype of hyperglycemia in these diseases. Whether the type 1 diabetes pathway may be better described as a β-cell dysfunction pathway warrants further study.

Although, it is often thought that lifestyle changes such as dietary modification and exercise will lead to health improvements, biological variability must be taken into account as some individuals may not improve in certain outcomes and may even regress (36). For example, Boule and colleagues (37) found that a standardized exercise training program resulted in a significant increase in insulin sensitivity of 10%. However, approximately 42% of participants either did not change or actually exhibited a decrease in insulin sensitivity. The authors suggested that genetic factors might contribute to individual variability in measures of glucose homeostasis following exercise training. Following a 20-week exercise regimen, Wilmore et al (38) observed varied body weight and composition responses in the HERITAGE Family Study. This finding is further supported by Leon and HERITAGE co-workers (39) who reported significant effects of APOE genotype on training induced responses in 10 of 16 lipoprotein-lipid levels in whites, but in only 2 of 16 blacks. To explore whether molecular signatures were associated with variability in changes in insulin sensitivity in the present study, we compared the gene expression profiles of the youth who improved insulin sensitivity (n=9) to those who did not (n=6). Analyzing these groups separately, we noted considerably more changes in the responders (1,144 probes) compared to the non-responders (230 probes). Due to the small sample size and limited number of genes, it is difficult to draw definitive conclusions about how these genes may influence response to lifestyle intervention or whether these genes can be mapped to significant pathways. Nonetheless, these data present an interesting platform for future studies with larger numbers of participants to examine in whom lifestyle intervention may be most appropriate. In the age of personalized medicine, this information will be critical for physicians who choose to prescribe exercise as medicine.

Although the intervention was sufficient to elicit an improvement in insulin sensitivity, we cannot exclude the possibility that changes in gene expression in whole blood were the result of changing proportions of the cell types in circulation or from shifting cells with either higher or lower expression of a particular gene. Moreover, we did not have sufficient power to detect whether sex-specific differences existed in gene expression changes. While boys and girls both exhibited significant increases in insulin sensitivity, the magnitude was

larger in boys compared to girls (44% vs. 17%, respectively). Whether this effect is mediated by molecular factors or is a reflection of the higher baseline insulin sensitivity in girls (3.0±0.5) compared to boys (1.8±0.2) warrants further examination. Due to the global nature of this study, our goal was to determine the range and magnitude of genomic response to lifestyle intervention among obese youth. We used a stringent method to remove low expressed genes and also used Benjamini-Hochberg multiple testing correction to evaluate the gene expression regulation. Nonetheless, the expression levels observed in this study in response to intervention may be lower than those typically reported in other tissues such as skeletal muscle. This may be a function of examining changes in whole blood, however, microarray analysis in blood has been used frequently to study the molecular signatures associated health and health changes (4–6, 40).

Given the increasing prevalence of obesity and type 2 diabetes in youth, there is a pressing need to better understand these conditions so that appropriate interventions can be developed and implemented. To our knowledge, this is the first study to examine the molecular signatures following lifestyle intervention among obese youth. These data suggest that increases in insulin sensitivity are associated with significant changes in gene expression which may function in a coordinated fashion through pathways such as the insulin signaling pathway to improve health. Moreover, there may be biological differences between children who do and do not respond to lifestyle interventions. Whether difference in response is intervention-specific warrants further examination in larger, more diverse populations.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

GQS, LJM, DKC conceived the experiments. DNM, DKC, GQS carried out the experiments and analyzed the data. DKC and LJM provided comments and advice. GQS and DNM were involved in writing the paper and all authors had final approval of the submitted and published versions. We would like to thank the children and families who participated in the intervention, the promotoras from St. Vincent de Paul who delivered the lifestyle education classes, and Mrs. Erica Hoppin, MFA for her help in designing and delivering the exercise sessions. We also thank Mr. Darwin Tsinajinnie for his technical assistance. This work was supported by a grant from the Southwest Interdisciplinary Research Center an Exploratory Center of Excellence in Health Disparities Research and Training funded through the National Institute of Minority Health and Health Disparities - P20 MD002316.

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What is already known about this subject?

- Insulin resistance is associated with type 2 diabetes in youth.
- Lifestyle interventions can improve insulin sensitivity in obese youth.
- Response to lifestyle intervention may be mediated by molecular factors.

What does this study add?

- Changes in insulin sensitivity following lifestyle intervention are associated with distinct molecular signatures in blood
- Molecular signatures may be useful biomarkers to predict response to lifestyle intervention in obese youth

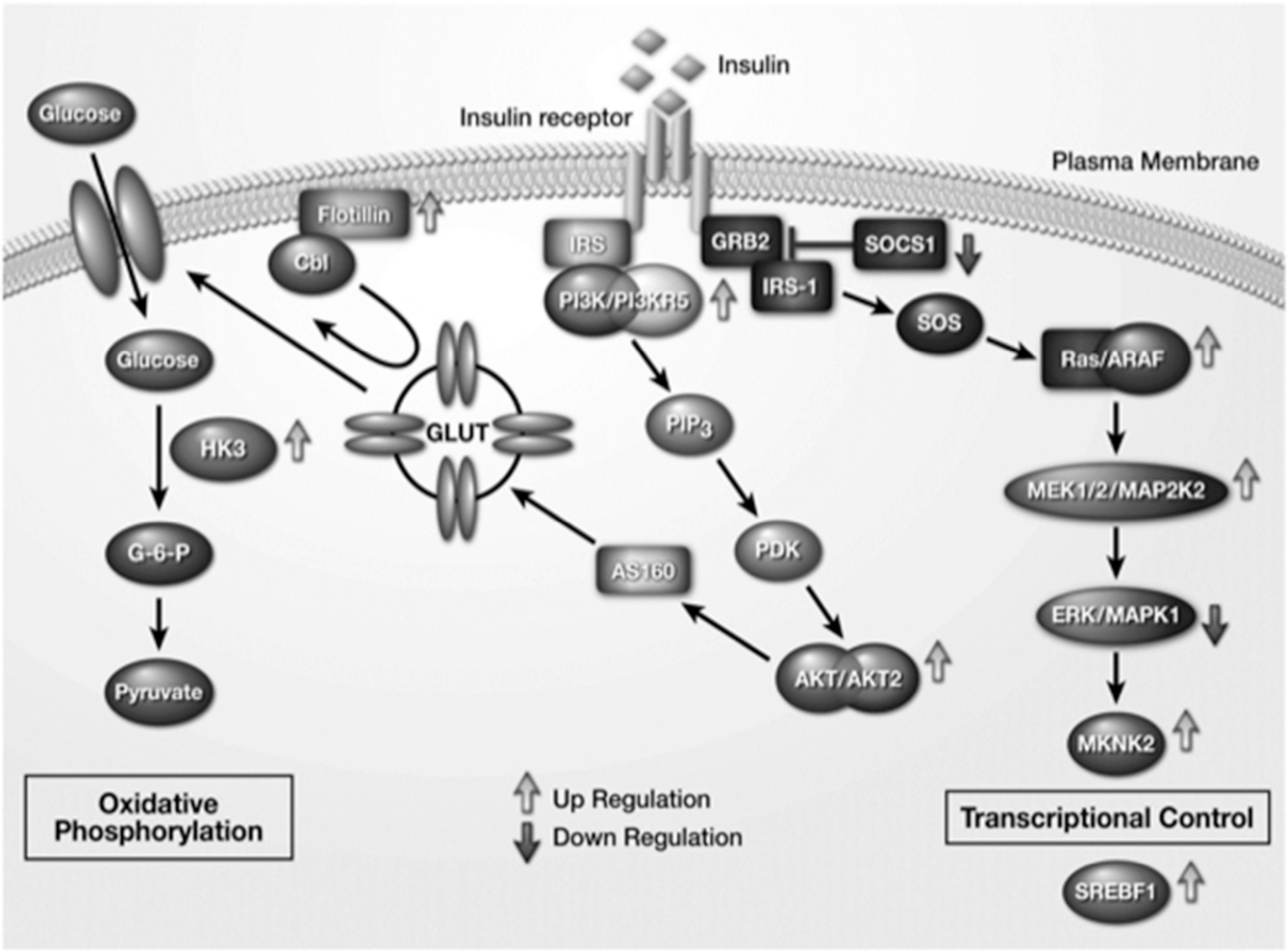

Figure 1. Significant genes within the insulin signaling pathway

This schematic depicts how the observed changes in gene expression within the insulin signaling pathway may contribute to improvements in insulin sensitivity.

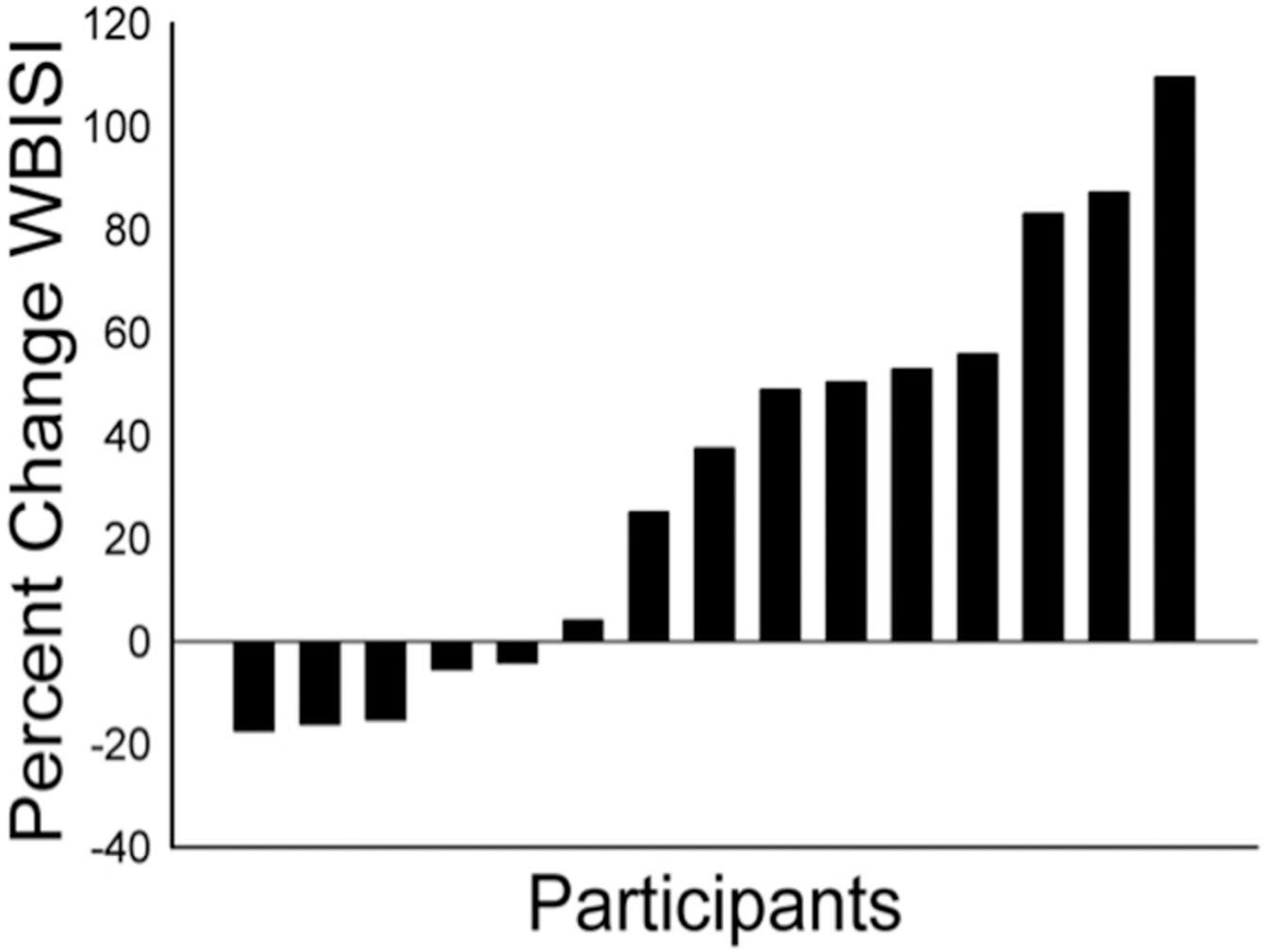

Figure 2. Individual changes in insulin sensitivity

Improvements in insulin sensitivity following intervention in the 15 participants listed in rank order from least improved to most improved.

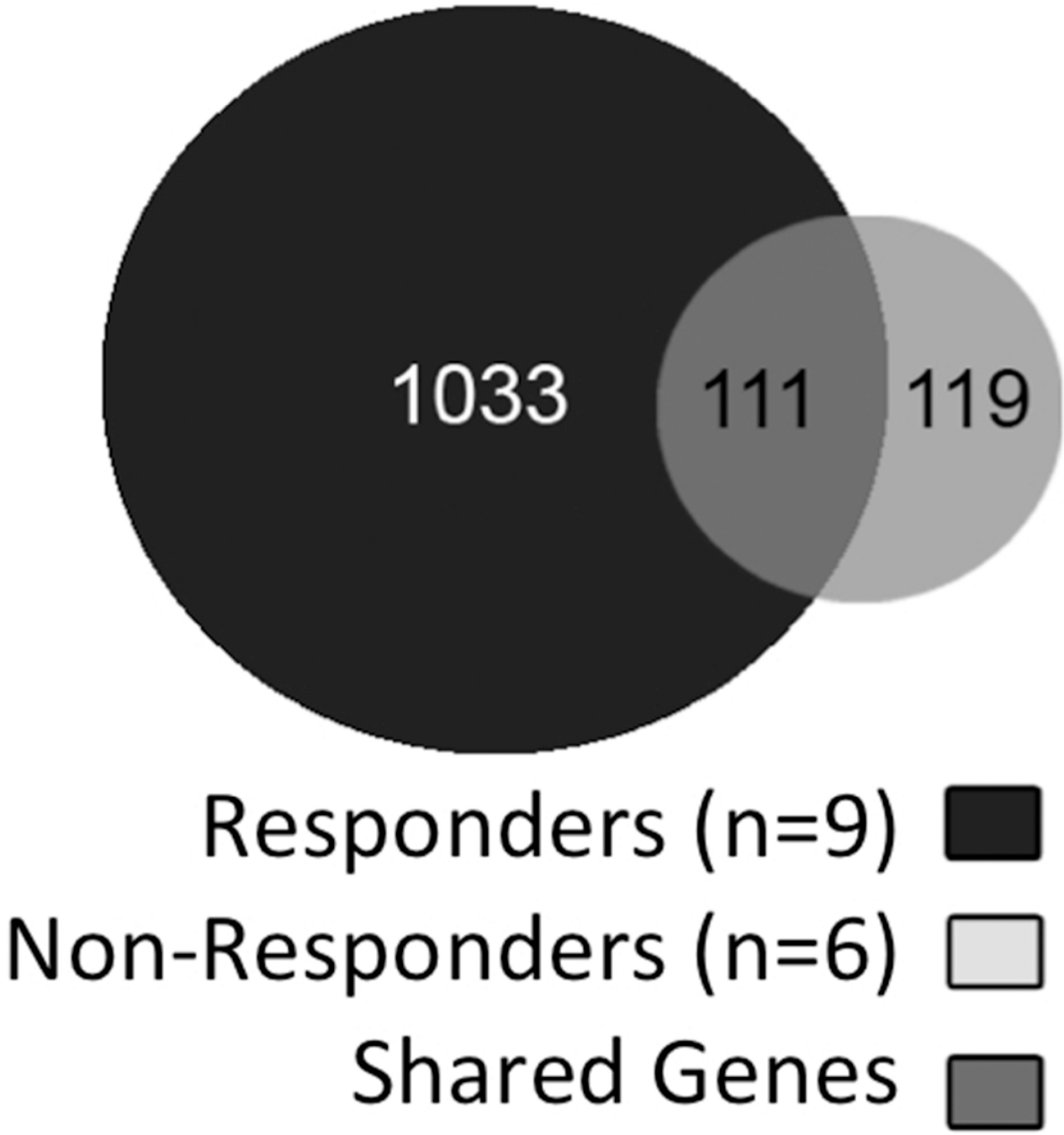

Figure 3. Gene expression by changes in insulin sensitivity

Venn diagram of the total number of significant genes that changed by at least 1.2 fold over baseline in those who did (Responders n=9) and those who did not (Non-responders n=6) increase their insulin sensitivity by at least 10% following the intervention. The overlapping portion is the number of genes shared by both groups.

Table 1

Metabolic changes in response to the intervention.

	Mean ± SE	Mean ± SE	% change	p-value
Triglycerides (mg/dl)	141.2 ± 14.3	98.2 ± 9.9	−30.5	0.002
Total Cholesterol (mg/dl)	153.7 ± 7.0	133.2 ±7.9	−13.3	0.002
HDL-Cholesterol (mg/dl)	40.0 ± 1.8	39.2 ±1.9	−2.0	0.621
LDL-Cholesterol (mg/dl)	90.1 ± 6.1	75.7 ±6.5	−6.0	0.002
Fasting glucose (mg/dl)	89.1 ± 2.0	89.9 ± 2.0	0.9	0.78
2-hr glucose (mg/dl)	117.2 ±4.8	104.5 ± 2.7	−10.8	0.01
Insulin Sensitivity (WBISI)	2.4 ± 0.3	3.1 ± 0.3	29.2	0.02

Values are means ± SE (*n*=15): 7 boys, 8 girls. HDL, high density lipoprotein; LDL, low density lipoprotein; WBISI, whole body insulin sensitivity index. Changes in were evaluated by Wilcoxin Signed Rank.

Table 2

Top 10 up-regulated genes

Gene Symbol	Gene	Fold Change
HK3	hexokinase 3 (white cell), nuclear gene encoding	1.693
FCN1	ficolin (collagen/fibrinogen domain containing) 1	1.619
CAMK1	calcium/calmodulin-dependent protein kinase I	1.589
ABTB1	ankyrin repeat and BTB (POZ) domain containing 1,	1.537
ZMAT5	zinc finger, matrin type 5, transcript variant 1	1.535
NLRC5	NLR family, CARD domain containing 5	1.532
ARFGAP2	ADP-ribosylation factor GTPase activating protein 2	1.528
TSR2	T20S rRNA accumulation, homolog (S. cerevisiae)	1.526
FGR	Gardner-Rasheed feline sarcoma viral (v-fgr) oncogene	1.523
ATP6V0E2	ATPase, H+ transporting V0 subunit e2, transcript	1.521

Table 3

Top 10 down-regulated genes

Gene Symbol	Gene	Fold Change
OXTR	oxytocin receptor	−1.735
S100A5	S100 calcium binding protein A5	−1.760
MYOG	myogenin (myogenic factor 4)	−1.768
UBE2QP1	ubiquitin-conjugating enzyme E2Q family pseudogene	−1.768
PLCXD2	phosphatidylinositol-specific phospholipase C, X	−1.769
TJP3	tight junction protein 3 (zona occludens 3)	−1.797
FAM189B	family with sequence similarity 189, member B,	−1.815
RERG	RAS-like, estrogen-regulated, growth inhibitor	−1.983
MT-1E	Metallothionein-1E (MT-1E)(Metallothionein-IE)	−2.004
ACOT12	acyl-CoA thioesterase 12	−2.022

Table 4

Biological pathways mapped from individual genes that were identified as significant by Database for Annotation, Visualization and Integrated Discovery (DAVID) v6.7.

KEGG Pathway	Total Genes	# Up-Regulated	# Down-Regulated	p-value
Insulin signaling pathway	17	12	5	0.016
Neurotrophin signaling pathway	16	11	5	0.017
Leukocyte transendothelial migration	14	8	6	0.048
Antigen processing and presentation	13	10	3	0.008
Fc gamma R-mediated phagocytosis	12	9	3	0.049
Glycerophospholipid metabolism	10	7	3	0.035
Type I diabetes mellitus	8	7	1	0.019
Autoimmune thyroid disease	8	7	1	0.05

Table 5

Differentially expressed genes by 1.2 fold change involved in the Insulin Signaling Pathway (by DAVID KEGG pathways)

Probe	Gene	Description	Fold Change
A_24_P222599	PDPK1	3-phosphoinositide dependent protein kinase-1	−1.242
A_24_P929754	MKNK2	MAP kinase interacting serine/threonine kinase 2	1.346
A_23_P304237	RAPGEF1	Rap guanine nucleotide exchange factor (GEF) 1	1.212
A_23_P326170	CALM2	calmodulin 2 (phosphorylase kinase, delta) (CALM2)	1.228
A_23_P314526	FLOT1	flotillin 1 (FLOT1)	1.503
A_23_P213584	HK3	hexokinase 3 (white cell) (HK3), nuclear gene	1.693
A_24_P37962	HK3	hexokinase 3 (white cell) (HK3), nuclear gene	1.474
A_24_P237265	MAPK1	mitogen-activated protein kinase 1 (MAPK1),	−1.213
A_23_P208835	MAP2K2	mitogen-activated protein kinase kinase 2	1.213
A_23_P66543	PIK3R5	phosphoinositide-3-kinase, regulatory subunit 5	1.220
A_23_P331670	PYGB	phosphorylase, glycogen; brain	1.254
A_24_P461118	PRKCI	protein kinase C, iota	−1.322
A_23_P338890	PTPN1	protein tyrosine phosphatase, non-receptor type 1	−1.231
A_24_P390784	SREBF1	sterol regulatory element binding transcription	1.325
A_23_P420196	SOCS1	suppressor of cytokine signaling 1	−1.257
A_23_P208870	AKT2	v-akt murine thymoma viral oncogene homolog 2,	1.203
A_24_P270814	CRK	v-crk sarcoma virus CT10 oncogene homolog	1.213
A_23_P73511	ARAF	v-raf murine sarcoma 3611 viral oncogene homolog	1.402