



Published in final edited form as:

*Am J Physiol Endocrinol Metab.* 2008 May ; 294(5): E910–E917. doi:10.1152/ajpendo.00607.2007.

## Effect of acute physiological hyperinsulinemia on gene expression in human skeletal muscle in vivo

Dawn K. Coletta<sup>1</sup>, Bogdan Balas<sup>1</sup>, Alberto O. Chavez<sup>1</sup>, Muhammad Baig<sup>1</sup>, Muhammad Abdul-Ghani<sup>1</sup>, Sangeeta R. Kashyap<sup>2</sup>, Franco Folli<sup>1</sup>, Devjit Tripathy<sup>1</sup>, Lawrence J. Mandarino<sup>3</sup>, John E. Cornell<sup>1</sup>, Ralph A. DeFronzo<sup>1</sup>, and Christopher P. Jenkinson<sup>1</sup>

<sup>1</sup>Division of Diabetes, Department of Medicine, University of Texas Health Science Center at San Antonio, Texas

<sup>2</sup>Cleveland Clinic Foundation, Endocrinology, Diabetes and Metabolism, Cleveland, Ohio

<sup>3</sup>Arizona State University, School of Life Sciences, Tempe, Arizona

### Abstract

This study was undertaken to test the hypothesis that short-term exposure (4 h) to physiological hyperinsulinemia in normal, healthy subjects without a family history of diabetes would induce a low grade inflammatory response independently of glycemic status. Twelve normal glucose tolerant subjects received a 4-h euglycemic hyperinsulinemic clamp with biopsies of the vastus lateralis muscle. Microarray analysis identified 121 probe sets that were significantly altered in response to physiological hyperinsulinemia while maintaining euglycemia. In normal, healthy human subjects insulin increased the mRNAs of a number of inflammatory genes (CCL2, CXCL2 and THBD) and transcription factors (ATF3, BHLHB2, HES1, KLF10, JUNB, FOS, and FOSB). A number of other genes were upregulated in response to insulin, including RRAD, MT, and SGK. CITED2, a known coactivator of PPAR $\alpha$ , was significantly downregulated. SGK and CITED2 are located at chromosome 6q23, where we previously detected strong linkage to fasting plasma insulin concentrations. We independently validated the mRNA expression changes in an additional five subjects and closely paralleled the results observed in the original 12 subjects. A saline infusion in healthy, normal glucose-tolerant subjects without family history of diabetes demonstrated that the genes altered during the euglycemic hyperinsulinemic clamp were due to hyperinsulinemia and were unrelated to the biopsy procedure per se. The results of the present study demonstrate that insulin acutely regulates the levels of mRNAs involved in inflammation and transcription and identifies several candidate genes, including HES1 and BHLHB2, for further investigation.

### Keywords

gene expression; muscle; insulin action; euglycemic hyperinsulinemic clamp; inflammation

INSULIN RESISTANCE is a common pathological state associated with obesity, hypertension, atherosclerotic cardiovascular disease, and type 2 diabetes mellitus (11). This cluster of abnormalities, referred to as the insulin resistance or metabolic syndrome, is thought to be related to the underlying insulin resistance (34). Despite the severe impairment in insulin action in insulin resistant states, glucose tolerance remains normal because the pancreatic  $\beta$ -cells are able to augment their insulin secretory capacity to offset the insulin resistance (14, 19). Hyperinsulinemia (increased insulin levels) occurs as a result of the increase in insulin secretion from the  $\beta$ -cells. Over time, the excessive rates of insulin secretion cannot be maintained, leading to the development of glucose intolerance and type 2 diabetes (11, 14).

Despite intensive investigation, the underlying cause of insulin resistance remains unknown. Many cellular defects have been shown to contribute to the pathophysiology of insulin resistance in type 2 diabetes, including impaired insulin signaling (4, 10), reduced insulin-stimulated glucose uptake, decreased hexokinase II expression and activity, diminished glycogen synthase activity, and decreased pyruvate dehydrogenase activity (8, 15, 26, 30, 39, 42). Impaired mitochondrial function has been associated with an increase in toxic lipid metabolites (fatty acyl-CoA, diacylglycerol, ceramide) in skeletal muscle of subjects with prediabetes and type 2 diabetes, and these toxic lipid metabolites have been implicated as a cause of the insulin resistance (1, 20, 23, 27, 29). We (36) previously reported that a physiological increase in plasma FFA concentration in normal healthy subjects resulted in changes in gene expression in skeletal muscle that are consistent with an inflammatory response. In addition, we also demonstrated that changes in the composition of the extracellular matrix are a general characteristic feature of insulin-resistant muscle (7).

Insulin has been shown to be a risk factor for the development of atherosclerotic cardiovascular disease (11). The effects of insulin on arterial tissues include proliferation of smooth muscle cells, enhanced cholesterol synthesis and low-density lipoprotein-receptor activity, increased formation and decreased regression of lipid plaques, stimulation of connective tissue synthesis, and stimulation of growth factors (11). In the present study, we set out to test the hypothesis that short-term exposure (4 h) to physiological hyperinsulinemia in normal, glucose-tolerant, healthy subjects without a family history of diabetes would induce a low-grade inflammatory response, independently of glycemic status, in skeletal muscle. By combining the euglycemic insulin clamp technique (13) with microarray profiling, we tested this hypothesis and, at the same time, identified novel pathways that are regulated by insulin in skeletal muscle.

## MATERIALS AND METHODS

### Subjects

Healthy, normal glucose-tolerant subjects without a family history of diabetes participated in the study. All subjects were free of any organ system disease as determined by medical history, physical examination, screening chemistries, complete blood cell count, urinalysis, and electrocardiogram. Normal glucose tolerance was established by a 75-g oral glucose tolerance test (OGTT) performed 1 wk prior to the clamp study. Percent body fat was determined by DEXA using a Hologic Delphi A scanner (Hologic, Bedford, MA). Subjects were not taking any medications and demonstrated stable body weight ( $\pm 3$ lbs) over the 6 mo

prior to the study. None of the subjects participated in any heavy exercise, and they were instructed not to exercise for 3 days before the study. All subjects gave informed written consent prior to their participation in the study. The study protocol was approved by the Institutional Review Board of the University of Texas Health Science Center at San Antonio.

### Study design

All subjects consumed a weight-maintaining diet containing 50% carbohydrate, 35% fat, and 15% protein over the 3 days prior to study and were instructed not to participate in any heavy exercise. After a 10-h overnight fast, all subjects reported to the General Clinical Research Center (GCRC) at 0800, and catheters were placed into an antecubital vein (for infusion of all test substances) and retrogradely into a vein on the dorsum of the hand (for blood withdrawal). The hand was placed in a heated box (65°C) to achieve arterialization of the venous blood. Twelve subjects received a 4-h euglycemic hyperinsulinemic (80 mU·m<sup>-2</sup>·min<sup>-1</sup>) clamp, which was performed with infusion of [3-<sup>3</sup>H]glucose to determine rates of glucose appearance and disposal, as previously described (10, 13). Briefly, 120 min prior to the start of the insulin clamp, a primed (25 µCi) continuous (0.25 µCi/min) infusion of [3-<sup>3</sup>H]glucose (DuPont-NEN, Boston, MA) was started and continued throughout the clamp to determine rates of total body glucose disposal and endogenous (primarily hepatic) glucose production (13). During the 30 min prior to the start of the insulin infusion and throughout the insulin clamp, plasma glucose concentration was determined every 5 min, and a variable glucose infusion was adjusted on the basis of the negative feedback principle to maintain the plasma glucose concentration at each subject's basal value. Plasma insulin and plasma tritiated glucose specific activity were measured every 10–15 min. Under euglycemic hyperinsulinemic conditions, the majority (~85%) of glucose disposal occurs in skeletal muscle (12). Biopsies of the vastus lateralis muscle were taken at -60, 30, and 240 min of the insulin infusion, as previously described (10). A control saline (0.2 ml·m<sup>-2</sup>·min<sup>-1</sup>) infusion was performed in three additional subjects, with a vastus lateralis muscle biopsy taken basally and at the end of the saline infusion. After completion of the microarray/RT-PCR determinations (see below), we studied an additional five subjects to validate our original observations. These five subjects received a 3-h euglycemic hyperinsulinemic (80 mU·m<sup>-2</sup>·min<sup>-1</sup>) clamp with tritiated glucose, as described above. Vastus lateralis muscle biopsies were performed at 60 min and after 180 min of insulin infusion. Muscle biopsy specimens (~200 mg) were frozen immediately in liquid nitrogen and stored until processed.

### Muscle biopsy processing

Muscle biopsies from each subject were homogenized in RNAsat solution (Tel-Test, Friendswood, TX), using a Polytron homogenizer (Brinkmann Instruments Westbury, NY). Total RNA was purified with RNeasy and DNase I treatment (Qiagen, Chatsworth, CA). RNA isolated from the insulin infusion biopsies was used in the microarray and quantitative RT-PCR studies described below. The RNA isolated from the control saline infusion biopsies was used for the quantitative RT-PCR studies.

## Microarray analysis: target preparation, hybridization, staining, scanning, and image analysis

RNA was prepared for hybridization to Affymetrix (Santa Clara, CA) HG-U133A arrays according to the manufacturer's instructions. A description of the protocol has been described previously (36). The probe arrays were scanned twice and the stored images aligned and analyzed using the GeneChip Operating System software (GCOS; Affymetrix). GeneChip quality assessments using GCOS demonstrated that the average percent present call across all arrays was  $28 \pm 1\%$ . Average scaling values and noise levels were  $6.8 \pm 0.9$  and  $4.7 \pm 0.2$ , respectively. *bioB*, *bioC*, *bioD* and *cre* were present on all arrays with the exception of *bioB*, which was present only 50% of the time, as expected. 3'/5' GAPDH and actin expression ratios were well within the acceptable range:  $1.2 \pm 0.1$  and  $0.8 \pm 0.1$ , respectively.

## Microarray data expression and analysis

The Affymetrix data acquisition programs in GCOS automatically generate a cell intensity file (CEL) from the stored images that contain a single intensity value for each probe cell on the array. The CEL data files have been deposited in the Gene Expression Omnibus (GEO) database as series GSE9105. The CEL files were imported into the R software package (<http://www.r-project.org>), and the probe level data were converted to expression measures using the Affy package from Bioconductor. Expression values for each mRNA were obtained by GC-Robust Multi-Array Analysis (GCRMA). CEL files were normalized together, and the expression values obtained were submitted to analysis with linear models of microarray data (LIMMA). To correct for multiple testing, *P* values were adjusted using the method of Benjamini and Hochberg by setting the false discovery rate (FDR) to 5% and a fold change of  $>1.5$ .

Functional classification of the significantly increased or decreased genes was performed using database for annotation, visualization and integrated discovery (DAVID) (<http://david.niaid.nih.gov>), Onto-Express (<http://vortex.cs.wayne.edu/ontoexpress/>), and expression analysis systemic explorer (EASE; downloaded from DAVID). DAVID uses clustering algorithms to classify related genes into functionally related groups. EASE discovers categories of genes that are overrepresented in the significant gene list compared with the total set of genes represented on the microarray. Such overrepresented categories represent biological "themes" of a given list of significant genes. The categories are sorted into three major gene ontology (GO) groups: 1) molecular function, 2) biological processes, and 3) cellular components. Onto-Express was used in the present study to translate the list of differentially expressed insulin-regulated genes into chromosome location.

## Analysis of published microarray data

We compared the results of our microarray analysis with other previously published datasets by using the Web-based approach described by Parikh et al. (28). Briefly, we obtained normalized data files that were downloaded from either the Diabetes Genome Anatomy Project (DGAP; <http://www.diabetesgenome.org>) or the GEO database (<http://www.ncbi.nlm.nih.gov/geo/>). Using the Mann-Whitney *U*-test, a nonparametric statistical technique to identify mRNA expression changes, we compared the results in the DGAP and

GEO databases with our list of mRNAs that were significantly altered following insulin infusion.

### Quantitative real-time PCR

Muscle expression of various genes was determined using a one-step quantitative real-time PCR (QRT-PCR) from total RNA (Applied Biosystems 7900HT). The quantity of mRNA for each gene of interest for each sample was normalized to that of 18S ribosomal RNA, using the comparative ( $2^{-CT}$ ) method as described previously (36). Statistical comparisons were done using paired *t*-tests. Sequences of the primers and probes used for mRNA quantification by QRT-PCR can be supplied upon request.

### Substrate and hormone determinations

Plasma glucose concentration was determined by the glucose oxidase method on a Beckman Glucose Analyzer (Beckman Instruments, Fullerton, CA). Plasma insulin was determined by radioimmunoassay (Diagnostic Products, Los Angeles, CA). Plasma tritiated glucose specific activity was determined on Somogyi precipitates of plasma as previously described (5).

### Statistical analysis

Data were expressed as the means  $\pm$  SE. Statistical significance of the difference between means was determined using paired or nonpaired Student's *t*-tests where appropriate. Correlations were analyzed using Pearson's product moment test. For all analyses,  $P < 0.05$  was considered to be statistically significant. Statistical analysis of microarray data and QRT-PCR data were described above.

## RESULTS

### Subjects

Twelve healthy subjects without a family history of diabetes (5 females and 7 males) with a mean age ( $\pm$ SE) of  $38 \pm 4$  yr and body mass index of  $25.1 \pm 0.8$  kg/m<sup>2</sup> participated in the 4-h euglycemic hyperinsulinemic clamp study. These subjects had a lean body mass and percent body fat of  $52.9 \pm 4.9$  kg and  $29.6 \pm 4.1\%$ , respectively. The fasting plasma glucose (FPG) and fasting plasma insulin (FPI) concentrations were  $5.2 \pm 0.2$  mmol/l, and  $27.8 \pm 6.9$  pmol/l, respectively. The 2-h plasma glucose concentration during the OGTT was  $5.7 \pm 0.3$  mmol/l and the insulin-stimulated glucose disposal rate ( $R_d$ ) was  $10.3 \pm 0.5$  mg·kg<sup>-1</sup>·min<sup>-1</sup>. Steady-state plasma glucose (SSPG) and insulin (SSPI) concentrations during the insulin clamp were  $5.3 \pm 0.1$  mmol/l and  $826.5 \pm 48.6$  pmol/l, respectively. Basal endogenous glucose production (basal EGP) was  $2.0 \pm 0.2$  mg·kg<sup>-1</sup>·min<sup>-1</sup> and was suppressed to  $0.10 \pm 0.01$  mg·kg<sup>-1</sup>·min<sup>-1</sup> during the clamp. An additional five subjects matched for age, body mass index, FPG, and  $R_d$  ( $45 \pm 3$  yr,  $27.2 \pm 1.4$  kg/m<sup>2</sup>,  $5.3 \pm 0.1$  mmol/l, and  $7.1 \pm 1.1$  mg·kg<sup>-1</sup>·min<sup>-1</sup>, respectively) participated in a 3-h euglycemic hyperinsulinemic clamp study. Three subjects (age  $28 \pm 4$  yr, body mass index  $23.8 \pm 2.4$  kg/m<sup>2</sup>, FPG  $5.2 \pm 0.3$  mmol/l, and  $R_d$   $8.0 \pm 1.4$  mg·kg<sup>-1</sup>·min<sup>-1</sup>) participated in the control saline infusion.

## Analysis of microarray data

Microarray analysis was performed on the muscle biopsies obtained from the 12 subjects who received a 4-h euglycemic hyperinsulinemic clamp (Fig. 1). Using a false discovery rate of <5% and a fold change of >1.5, there were no individual probe sets with significantly increased or decreased expression after 30 min of insulin infusion compared with the basal state. In contrast, 121 probe sets were significantly altered (117 upregulated and 4 downregulated) after 240 min of insulin infusion compared with the basal state [Supplementary Tables 1 and 2, respectively (see online)]. The 117 upregulated probe sets represent in total 93 genes, since most genes are represented more than once due to the presence of more than one probe set on the HG-U133A GeneChip. The expression of 105 probe sets (96 increased and 9 decreased) was altered after 240 min compared with their expression 30 min after the start of the insulin infusion (Supplementary Tables 3 and 4, respectively). The 96 probe sets with significantly increased expression at 240 vs. 30 min of insulin infusion also were significantly altered at 240 min vs. the basal state. We identified five additional probe sets that were decreased at 240 vs. 30 min of the insulin infusion (Supplementary Table 4). Of note, one of these genes was thioredoxin-interacting protein (TXNIP), which has been implicated in insulin-stimulated peripheral glucose uptake in humans (28). The results of the present study indicate that most of the detectable changes occurred 30 min after the start of insulin.

## Functional classification of genes

Gene functional classification clustering analysis was performed in DAVID on the genes that were upregulated ( $n = 93$ ) by insulin. Four functional groups were identified from the list of upregulated genes: 1) transcription binding, activity, and regulation, 2) protein kinase activity, 3) inflammation and chemokine activity, and 4) metal ion binding (Table 1). Additional information on the clustering of the genes in each group is displayed in Supplementary Figs. 1–4. No functional groups were identified for the four genes downregulated by insulin.

EASE analysis on the genes that were upregulated ( $n = 93$ ) by insulin yielded 42 significant gene categories, divided among three GO systems: biological process ( $n = 28$ ), cellular component ( $n = 1$ ), and molecular function ( $n = 13$ ). The gene categories identified for each significant GO system are depicted in Supplementary Table 5. Briefly, for the biological process system, the majority of the genes coded for proteins involved in cell growth (50%) and regulation of transcription (31%). In addition, genes in the molecular function system were involved in RNA/DNA binding (74%) and transcription (33%). The only cellular component system identified was the nucleus, where 43% of the genes were localized.

The chromosomal location of the differentially expressed genes following insulin infusion was determined using the program Onto-Express. The location of the insulin-regulated genes was distributed among all chromosomes except Y, 13, and 7. Statistical analysis performed in Onto-Express yielded no chromosome that contained more insulin-regulated genes than expected (Supplementary Fig. 5).

### Insulin signaling gene analysis

Because the microarray analysis identified significant increases in some genes involved in the insulin-signaling pathway including RRAD, KRAS, KLF10, and FOS (Supplementary Table 1), we looked at the microarray expression values of other insulin-signaling genes. Following 240 min of insulin infusion, the expression of 24 insulin-signaling genes increased (all  $P < 0.05$ , without correcting for multiple testing; Table 2). Many of these mRNA expression changes did not achieve statistical significance when corrected for multiple testing.

### Analysis of published datasets

Parikh et al. (28) examined mRNA expression in human muscle biopsies from normal glucose-tolerant subjects before and after a 2-h euglycemic hyperinsulinemic clamp. Analysis of the normalized dataset of Parikh et al. using a Mann-Whitney  $U$ -test identified six genes (KCNJ2, SOCS3, BHLHB2, HES1, MT1H, and TXNIP) that were significantly altered and in common with our insulin-regulated genes (Supplementary Table 6). Hansen et al. (21) reported that the expression of three of these genes (HES1, BHLHB2, and TXNIP) was significantly increased by insulin in human myotubes. Rome et al. (37) also reported that HES1 mRNA expression was significantly increased during a 3-h euglycemic hyperinsulinemic clamp. Analysis of the normalized dataset of Patti et al. (29), using a Mann-Whitney  $U$ -test, identified a significant increase in the mRNA expression of HES1 in type 2 diabetics compared with normal, healthy subjects without a family history of diabetes (Supplementary Table 6). Similarly, BHLHB2 and MT1H mRNAs were both significantly increased in diabetic subjects compared with normal glucose-tolerant subjects when we analyzed the dataset of Mootha et al. (27).

### Correlations of microarray data with subject characteristics

To identify associations between insulin-regulated gene expression and insulin-stimulated glucose disposal, we performed correlation analysis using Pearson's product moment test. Insulin-stimulated whole body (primarily represents muscle) glucose disposal was strongly and inversely correlated with ADH1B, CYR61, and BHLHB2 mRNA expression ( $r = -0.74$ ,  $-0.65$ , and  $-0.57$ , respectively). We also observed a negative correlation between basal EGP and serum/glucocorticoid-regulated kinase (SGK) mRNA expression ( $r = -0.74$ ,  $P < 0.01$ ), while DNAJB1, HBEGF, and TRIB1 mRNA gene expression was strongly correlated with fasting plasma insulin concentration ( $r = 0.74$ ,  $0.65$ , and  $0.58$ , respectively). These and other correlations are depicted in Supplementary Table 7.

### QRT-PCR analysis to confirm microarray data

To validate the microarray data, we quantitated the changes in mRNA levels of 20 genes (18 of which increased and 2 of which decreased) using QRT-PCR in the twelve subjects who received a 4-h euglycemic hyperinsulinemic clamp. These genes displayed wide differences in their response to insulin on the microarray (from  $-2.3$  to  $7.7$ -fold change). The correlation coefficient ( $r$ ) between the genes quantitated by QRT-PCR and microarray analysis was  $0.79$  ( $P < 0.01$ ) (Supplementary Fig. 6). Of note, the QRT-PCR fold changes were larger than the changes observed with microarray analysis. This is consistent with previous studies from our

laboratory in which microarray analysis tends to underestimate the changes in gene expression (36). In addition, we independently validated the mRNA expression changes in an additional five subjects who received a 3-h euglycemic hyperinsulinemic clamp. mRNA expression in these 5 subjects, who were studied after the microarray/QRT-PCR analyses were completed in the first 12 subjects, closely paralleled that in the original 12 individuals (Table 3). We also observed significant interindividual variation in the basal mRNA levels in these normal, healthy subjects. This natural variation in mRNA expression most likely represents the complex interplay of intrinsic (i.e., age, sex, ethnicity, and genes), physiological, and environmental factors. This observation is consistent with other studies that have identified significant interindividual variation in basal mRNA expression in healthy subjects (18, 33, 43).

### QRT-PCR analysis performed on control saline infusion samples

To verify that the changes observed during the insulin clamp were due to insulin action on the muscle and not due to the biopsy procedure per se, we performed QRT-PCR analysis on the muscle biopsies from subjects who received saline infusion. For this study, we analyzed the same genes that were used to validate the insulin infusion microarray analysis. The results of the saline infusion study are presented in Table 4. Of the 20 genes examined, three genes were significantly altered compared with the basal state. ETS2 and RRAD mRNA levels were significantly increased during the saline infusion, by  $1.24 \pm 0.03$  and  $1.78 \pm 0.09$ , respectively (both  $P < 0.05$ ), but the changes were quantitatively much less than the fold increases observed in the insulin infusion group (Table 3). Likewise, the significant decrease in EGR1 mRNA expression in the control saline infusion ( $-3.57 \pm 2.30$  fold change) was contrary to the significant increase observed during insulin infusion (Table 3).

## DISCUSSION

The present study was undertaken in part to test the hypothesis that short-term exposure (4 h) to physiological hyperinsulinemia in normal, healthy subjects without a family history of diabetes would induce a low-grade inflammatory response independently of glycemic status. To test this hypothesis subjects received an  $80 \text{ mU}\cdot\text{m}^{-2}\cdot\text{min}^{-1}$  euglycemic hyperinsulinemic clamp with vastus lateralis muscle biopsies performed before, during, and at the end of the insulin clamp. The concentration of insulin ( $\sim 560 \text{ pmol/l}$ ) achieved during the insulin clamp was within the high physiological range, as described elsewhere (32), and was similar to that observed during an oral glucose/meal tolerance test, although we infused insulin for 4 h, which is longer than the usual tissue exposure to insulin following a meal in normal, healthy subjects. Therefore, we believe that the results in the present study have relevance to the effects of physiological hyperinsulinemia on muscle mRNA expression and may provide insights into the etiology of insulin resistance in type 2 diabetes and obesity (11).

The uniqueness of the present study compared with previous studies (3, 15, 21, 25, 28, 37, 40, 44) is the inclusion of a control saline infusion group and validation of the results in a separate group of individuals. In our study, we felt that it was important to demonstrate that the observed changes in mRNA expression during the insulin infusion clamp were independent of the biopsy procedure per se, which could have led to increased mRNA



expression of some genes involved in inflammation. The results of the saline infusion study clearly demonstrate that the genes altered during the euglycemic hyperinsulinemic clamp were due to insulin and independent of the muscle biopsy procedure.

The results of the present study demonstrate that a number of genes involved in inflammation were upregulated. Thrombomodulin, which is dysregulated during inflammation, had the largest fold change (7.69) from the microarray analysis. A number of inflammatory chemokines (CCL8, CXCL2, and CCL2) and cytokines (interleukin-1 receptor type I and interferon- $\gamma$ -inducible protein-16) were also upregulated by insulin. Chemokine, cc motif ligand 2 (CCL2) and chemokine, cc motif ligand 2 (CCL8), also known as monocyte chemoattractant protein-1 and -2, are members of the CC chemokine family and promote monocyte chemotaxis to sites of inflammation. These results are consistent with those of a previous study that identified a number of inflammatory genes (including THBD, CCL2, and CXCL2) that were upregulated following insulin treatment (21). Rome et al. (37) also reported that a number of immune-response genes were increased following a euglycemic hyperinsulinemic clamp, but those genes did not overlap with our study (37). QRT-PCR confirmed the increased mRNA expression of inflammation genes originally identified by microarray analysis. Furthermore, the control saline infusion demonstrated that the increased expression of inflammatory genes in response to the insulin infusion was due to the action of insulin on the muscle and was not secondary to the muscle biopsy per se. Taken collectively, these results provide evidence that in skeletal muscle insulin augments the expression of multiple genes that are involved in inflammation. This inflammation response may then provide positive feedback to cause/enhance insulin resistance.

Several candidate gene and microarray studies have identified genes whose expression was altered following an insulin infusion (3, 15, 21, 25, 28, 37, 40, 44). We examined some of these previously published microarray datasets (21, 28, 37) and found a number of genes that changed in parallel with our insulin-regulated genes. The majority of genes whose expression was altered by insulin coded for proteins involved in transcription regulator binding and activity. Other groups have reported similar findings concerning the effect of insulin on the mRNA expression of transcription factors (21, 37). Hansen et al. (21) described seven transcription genes (ATF3, BHLHB2, HES1, KLF10, JUNB, FOS, and FOSB) whose mRNA expression was upregulated and in common with those in the present study. Of particular note, we found that increased mRNA expression of HES1 and BHLHB2 was replicated in the majority of the datasets analyzed (21, 28, 37). Reduced insulin-mediated glucose disposal in skeletal muscle is a common metabolic feature of type 2 diabetes (4, 10, 14). In the present study, we observed an inverse relationship between BHLHB2 expression and whole body (primarily reflects muscle) glucose  $R_d$ . Moreover, we observed that the mRNA expression of HES1 and BHLHB2 was increased in muscle tissue of type 2 diabetic subjects (27, 29). Collectively, the HES1 and BHLHB2 mRNA expression results suggest that these two genes may be involved in the insulin-resistant phenotype observed in type 2 diabetes. However, the exact mechanism of action of the HES1 and BHLHB2 genes in skeletal muscle requires further investigation.

Oxidative stress has been implicated in the development of insulin resistance and microvascular/macrovascular complications in patients with type 2 diabetes (9).



protein (CBP)/p300, TFAP2, Lhx2, and nuclear receptors, such as peroxisome proliferator-activated receptor and estrogen receptor, to function as a transcriptional modulator (41). Another chromosomal region that has been linked with insulin resistance, type 2 diabetes and obesity is 10q24–25 (17). The peroxisome proliferative activated receptor- $\gamma$ , coactivator-related 1 (PPRC1) is located at 10q24.32 and was found to be significantly upregulated by insulin in the present study. PPRC1 was first identified in database searches for sequences with similarities to peroxisome proliferator-activated receptor (PPAR) $\gamma$ , coactivator-1 (PGC1) (2). PGC1 is a transcriptional regulator that activates (in conjunction with nuclear respiratory factor-1 and PPAR $\alpha$ ), PPAR $\gamma$ , hepatocyte nuclear factor 4, and other transcription factors which are key metabolic regulators. PPRC1 is a growth-regulated coactivator that may coordinate the activities of multiple transcription factors required for cell growth (2). The exact role of the CITED2 and PPRC1 genes remains unclear, and further studies will be needed to understand their role in insulin signaling.

In summary, we have demonstrated that a sustained physiological increase in the plasma insulin concentration for as little as 4 h in nonexercised subjects alters the levels of multiple muscle mRNAs, including inflammatory factors, which may be involved in the development of insulin resistance in type 2 diabetes mellitus. Of note, several of these genes are localized to chromosomal regions that previously have been shown to be associated with type 2 diabetes, insulin resistance, obesity, and atherosclerosis.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

## ACKNOWLEDGMENTS

We thank John Kincaid, Jim King, Norma Diaz, and Tricia Wolfe for outstanding nursing assistance, as well as the volunteers who took part in this study. All microarray studies were carried out in the Microarray Core Facility of the University of Texas Health Science Center at San Antonio, San Antonio, TX, under the direction of Drs. Dawn K. Coletta and Christopher P. Jenkinson.

## REFERENCES

1. Adams JM 2nd, Pratipanawat T, Berria R, Wang E, DeFronzo RA, Sullards MC, Mandarino LJ. Ceramide content is increased in skeletal muscle from obese insulin-resistant humans. *Diabetes*. 2004; 53:25–31. [PubMed: 14693694]
2. Andersson U, Scarpulla RC. Pgc-1-related coactivator, a novel, serum-inducible coactivator of nuclear respiratory factor 1-dependent transcription in mammalian cells. *Mol Cell Biol*. 2001; 21:3738–3749. [PubMed: 11340167]
3. Asmann YW, Stump CS, Short KR, Coenen-Schimke JM, Guo Z, Bigelow ML, Nair KS. Skeletal muscle mitochondrial functions, mitochondrial DNA copy numbers, and gene transcript profiles in type 2 diabetic and nondiabetic subjects at equal levels of low or high insulin and euglycemia. *Diabetes*. 2006; 55:3309–3319. [PubMed: 17130474]
4. Bajaj M, DeFronzo RA. Metabolic and molecular basis of insulin resistance. *J Nucl Cardiol*. 2003; 10:311–323. [PubMed: 12794631]
5. Bajaj M, Suraamornkul S, Romanelli A, Cline GW, Mandarino LJ, Shulman GI, DeFronzo RA. Effect of a sustained reduction in plasma free fatty acid concentration on intramuscular long-chain fatty Acyl-CoAs and insulin action in type 2 diabetic patients. *Diabetes*. 2005; 54:3148–3153. [PubMed: 16249438]

6. Beattie JH, Wood AM, Newman AM, Bremner I, Choo KH, Michalska AE, Duncan JS, Trayhurn P. Obesity and hyperleptinemia in metallothionein (-I and -II) null mice. *Proc Natl Acad Sci USA*. 1998; 95:358–363. [PubMed: 9419380]
7. Berria R, Wang L, Richardson DK, Finlayson J, Belfort R, Pratipanawatr T, De Filippis EA, Kashyap S, Mandarino LJ. Increased collagen content in insulin-resistant skeletal muscle. *Am J Physiol Endocrinol Metab*. 2006; 290:E560–E565. [PubMed: 16249255]
8. Bonadonna RC, Del Prato S, Bonora E, Saccomani MP, Gulli G, Natali A, Frascerra S, Pecori N, Ferrannini E, Bier D, Cobelli C, DeFronzo RA. Roles of glucose transport and glucose phosphorylation in muscle insulin resistance of NIDDM. *Diabetes*. 1996; 45:915–925. [PubMed: 8666143]
9. Brownlee M. The pathobiology of diabetic complications: a unifying mechanism. *Diabetes*. 2005; 54:1615–1625. [PubMed: 15919781]
10. Cusi K, Maezono K, Osman A, Pendergrass M, Patti ME, Pratipanawatr T, DeFronzo RA, Kahn CR, Mandarino LJ. Insulin resistance differentially affects the PI 3-kinase- and MAP kinase-mediated signaling in human muscle. *J Clin Invest*. 2000; 105:311–320. [PubMed: 10675357]
11. DeFronzo RA, Ferrannini E. Insulin resistance. A multifaceted syndrome responsible for NIDDM, obesity, hypertension, dyslipidemia, and atherosclerotic cardiovascular disease. *Diabetes Care*. 1991; 14:173–194. [PubMed: 2044434]
12. DeFronzo RA, Gunnarsson R, Bjorkman O, Olsson M, Wahren J. Effects of insulin on peripheral and splanchnic glucose metabolism in non-insulin-dependent (type II) diabetes mellitus. *J Clin Invest*. 1985; 76:149–155. [PubMed: 3894418]
13. DeFronzo RA, Tobin R, Andres R. The glucose clamp technique. A method for quantifying insulin secretion and resistance. *Am J Physiol Endocrinol Metab Gastrointest Physiol*. 1979; 237:E214–E223.
14. DeFronzo RA. Lilly lecture 1987. The triumvirate: beta-cell, muscle, liver. A collusion responsible for NIDDM. *Diabetes*. 1988; 37:667–687. [PubMed: 3289989]
15. Ducluzeau PH, Perretti N, Laville M, Andreelli F, Vega N, Riou JP, Vidal H. Regulation by insulin of gene expression in human skeletal muscle and adipose tissue. Evidence for specific defects in type 2 diabetes. *Diabetes*. 2001; 50:1134–1142. [PubMed: 11334418]
16. Duggirala R, Blangero J, Almasy L, Arya R, Dyer TD, Williams KL, Leach RJ, O'Connell P, Stern MP. A major locus for fasting insulin concentrations and insulin resistance on chromosome 6q with strong pleiotropic effects on obesity-related phenotypes in nondiabetic Mexican Americans. *Am J Hum Genet*. 2001; 68:1149–1164. [PubMed: 11283790]
17. Duggirala R, Blangero J, Almasy L, Dyer TD, Williams KL, Leach RJ, O'Connell P, Stern MP. Linkage of type 2 diabetes mellitus and of age at onset to a genetic location on chromosome 10q in Mexican Americans. *Am J Hum Genet*. 1999; 64:1127–1140. [PubMed: 10090898]
18. Eady JJ, Wortley GM, Wormstone YM, Hughes JC, Astley SB, Foxall RJ, Doleman JF, Elliott RM. Variation in gene expression profiles of peripheral blood mononuclear cells from healthy volunteers. *Physiol Genomics*. 2005; 22:402–411. [PubMed: 16014386]
19. Golay A, Felber JP, Jequier E, DeFronzo RA, Ferrannini E. Metabolic basis of obesity and noninsulin-dependent diabetes mellitus. *Diabetes Metab Rev*. 1988; 4:727–747. [PubMed: 3069401]
20. Goodpaster BH, Kelley DE. Skeletal muscle triglyceride: marker or mediator of obesity-induced insulin resistance in type 2 diabetes mellitus? *Curr Diab Rep*. 2002; 2:216–222. [PubMed: 12643176]
21. Hansen L, Gaster M, Oakeley EJ, Brusgaard K, Damsgaard Nielsen EM, Beck-Nielsen H, Pedersen O, Hemmings BA. Expression profiling of insulin action in human myotubes: induction of inflammatory and pro-angiogenic pathways in relationship with glycogen synthesis and type 2 diabetes. *Biochem Biophys Res Commun*. 2004; 323:685–695. [PubMed: 15369805]
22. Hsueh WA, Law RE. Insulin signaling in the arterial wall. *Am J Cardiol*. 1999; 84:21–24.
23. Krssak M, Falk Petersen K, Dresner A, DiPietro L, Vogel SM, Rothman DL, Roden M, Shulman GI. Intramyocellular lipid concentrations are correlated with insulin sensitivity in humans: a 1H NMR spectroscopy study. *Diabetologia*. 1999; 42:113–116. [PubMed: 10027589]

24. Lang F, Cohen P. Regulation and physiological roles of serum- and glucocorticoid-induced protein kinase isoforms. *Sci STKE*. 2001; 108:RE17. [PubMed: 11707620]
25. Laville M, Auboeuf D, Khalfallah Y, Vega N, Riou JP, Vidal H. Acute regulation by insulin of phosphatidylinositol-3-kinase, Rad, Glut 4, and lipoprotein lipase mRNA levels in human muscle. *J Clin Invest*. 1996; 98:43–49. [PubMed: 8690802]
26. Mandarino LJ, Printz RL, Cusi KA, Kinchington P, O'Doherty RM, Osawa H, Sewell C, Consoli A, Granner DK, DeFronzo RA. Regulation of hexokinase II and glycogen synthase mRNA, protein, and catalytic activity in human skeletal muscle in vivo. *Am J Physiol Endocrinol Metab*. 1995; 269:E701–E708.
27. Mootha VK, Lindgren CM, Eriksson KF, Subramanian A, Sihag S, Lehar J, Puigserver P, Carlsson E, Ridderstråle M, Laurila E, Houstis N, Daly MJ, Patterson N, Mesirov JP, Golub TR, Tamayo P, Spiegelman B, Lander ES, Hirschhorn JN, Altshuler D, Groop LC. PGC-1 $\alpha$ -responsive genes involved in oxidative phosphorylation are coordinately downregulated in human diabetes. *Nat Genet*. 2003; 34:267–273. [PubMed: 12808457]
28. Parikh H, Carlsson E, Chutkow WA, Johansson LE, Storgaard H, Poulsen P, Saxena R, Ladd C, Schulze PC, Mazzini MJ, Jensen CB, Krook A, Björnholm M, Tornqvist H, Zierath JR, Ridderstråle M, Altshuler D, Lee RT, Vaag A, Groop LC, Mootha VK. TXNIP regulates peripheral glucose metabolism in humans. *PLoS Med*. 2007; 4:e158. [PubMed: 17472435]
29. Patti ME, Butte AJ, Crunkhorn S, Cusi K, Berria R, Kashyap S, Miyazaki Y, Kohane I, Costello M, Saccone R, Landaker EJ, Goldfine AB, Mun E, DeFronzo R, Finlayson J, Kahn CR, Mandarino LJ. Coordinated reduction of genes of oxidative metabolism in humans with insulin resistance and diabetes: potential role of PGC1 and NRF1. *Proc Natl Acad Sci USA*. 2003; 100:8466–8471. [PubMed: 12832613]
30. Pendergrass M, Koval J, Vogt C, Yki-Jarvinen H, Iozzo P, Pipek R, Ardehali H, Printz R, Granner D, DeFronzo RA, Mandarino LJ. Insulin-induced hexokinase II expression is reduced in obesity and NIDDM. *Diabetes*. 1998; 47:387–394. [PubMed: 9519744]
31. Perrotti N, He RA, Phillips SA, Haft CR, Taylor SI. Activation of serum- and glucocorticoid-induced protein kinase (Sgk) by cyclic AMP and insulin. *J Biol Chem*. 2001; 276:9406–9412. [PubMed: 11096081]
32. Polonsky KS, Given BD, Van Cauter E. Twenty-four-hour profiles and pulsatile patterns of insulin secretion in normal and obese subjects. *J Clin Invest*. 1988; 81:442–448. [PubMed: 3276730]
33. Radich JP, Mao M, Stepanians S, Biery M, Castle J, Ward T, Schimmack G, Kobayashi S, Carleton M, Lampe J, Linsley PS. Individual-specific variation of gene expression in peripheral blood leukocytes. *Genomics*. 2004; 83:980–988. [PubMed: 15177552]
34. Reaven GM. Pathophysiology of insulin resistance in human disease. *Physiol Rev*. 1995; 75:473–486. [PubMed: 7624391]
35. Reynet C, Kahn CR. Rad: a member of the Ras family overexpressed in muscle of type II diabetic humans. *Science*. 1993; 262:1441–1444. [PubMed: 8248782]
36. Richardson DK, Kashyap S, Bajaj M, Cusi K, Mandarino SJ, Finlayson J, DeFronzo RA, Jenkinson CP, Mandarino LJ. Lipid infusion decreases the expression of nuclear encoded mitochondrial genes and increases the expression of extracellular matrix genes in human skeletal muscle. *J Biol Chem*. 2005; 280:10290–10297. [PubMed: 15598661]
37. Rome S, Clement K, Rabasa-Lhoret R, Loizon E, Poitou C, Barsh GS, Riou JP, Laville M, Vidal H. Microarray profiling of human skeletal muscle reveals that insulin regulates approximately 800 genes during a hyperinsulinemic clamp. *J Biol Chem*. 2003; 278:18063–18068. [PubMed: 12621037]
38. Scheede-Bergdahl C, Penkowa M, Hidalgo J, Olsen DB, Schjerling P, Prats C, Boushel R, Dela F. Metallothionein-mediated antioxidant defense system and its response to exercise training are impaired in human type 2 diabetes. *Diabetes*. 2005; 54:3089–3094. [PubMed: 16249430]
39. Shepherd PR, Kahn BB. Glucose transporters and insulin action - implications for insulin resistance and diabetes mellitus. *N Engl J Med*. 1999; 341:248–257. [PubMed: 10413738]
40. Sreekumar R, Halvatsiotis P, Schimke JC, Nair KS. Gene expression profile in skeletal muscle of type 2 diabetes and the effect of insulin treatment. *Diabetes*. 2002; 51:1913–1920. [PubMed: 12031981]

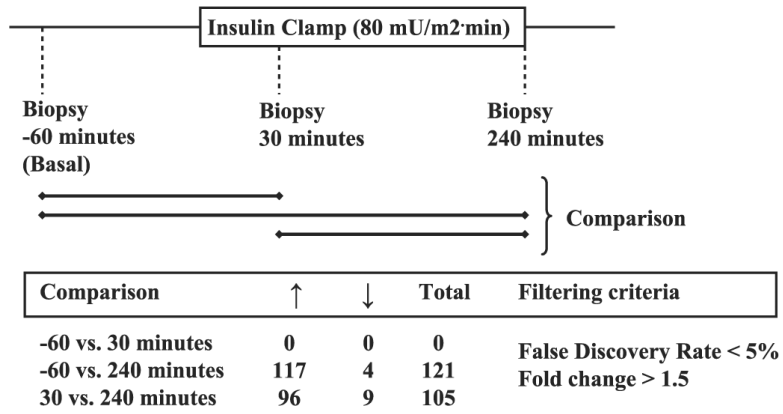
41. Tien ES, Davis JW, Vanden Heuvel JP. Identification of the CREB-binding protein/p300-interacting protein CITED2 as a peroxisome proliferator-activated receptor alpha coregulator. *J Biol Chem.* 2004; 279:24053–24063. [PubMed: 15051727]
42. Vestergaard H, Lund S, Larsen FS, Bjerrum OJ, Pedersen O. Glycogen synthase and phosphofructokinase protein and mRNA levels in skeletal muscle from insulin-resistant patients with non-insulin-dependent diabetes mellitus. *J Clin Invest.* 1993; 91:2342–2350. [PubMed: 8514849]
43. Whitney AR, Diehn M, Popper SJ, Alizadeh AA, Boldrick JC, Relman DA, Brown PO. Individuality and variation in gene expression patterns in human blood. *Proc Natl Acad Sci USA.* 2003; 100:1896–1901. [PubMed: 12578971]
44. Yang X, Pratley RE, Tokraks S, Bogardus C, Permana PA. Microarray profiling of skeletal muscle tissues from equally obese, non-diabetic insulin-sensitive and insulin-resistant Pima Indians. *Diabetologia.* 2002; 45:1584–1593. [PubMed: 12436343]

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript



**Fig. 1.** Number of probe sets upregulated or downregulated following insulin infusion.

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

**Table 1**

Results of functional classification clustering analysis performed in DAVID (<http://david.abcc.ncifcrf.gov>) on the genes that were upregulated ( $n = 93$ ) by insulin

Gene Functional Classification	Gene Name	Gene Symbol
Transcription binding, activity and regulation ( $n = 18$ )	nuclear receptor subfamily 4, group a, member 3	NR4A3
	kruppel-like factor 10	KLF10
	v-maf musculoaponeurotic fibrosarcoma oncogene homolog f (avian)	MAFF
	v-ets erythroblastosis virus e26 oncogene homolog 2 (avian)	ETS2
	nuclear factor, interleukin 3 regulated	NFIL3
	jun b proto-oncogene	JUNB
	interferon, gamma-inducible protein 16	IFI16
	v-myc myelocytomatosis viral oncogene homolog (avian)	MYC
	activating transcription factor 3	ATF3
	btg family, member 2	BTG2
	fbj murine osteosarcoma viral oncogene homolog b	FOSB
	early growth response 1	EGR1
	v-fos fbj murine osteosarcoma viral oncogene homolog	FOS
	basic helix-loop-helix domain containing, class b, 2	BHLHB2
	tgfb-induced factor (tale family homeobox)	TGIF
	hairy and enhancer of split 1, (drosophila)	HES1
	tsc22 domain family, member 2	TSC22D2
	Protein kinase activity ( $n = 4$ )	ccaat/enhancer-binding protein (c/ebp), delta
pim-1 oncogene		PIM1
snf1-like kinase		SNF1LK
serum/glucocorticoid-regulated kinase		SGK
Inflammation and chemokine activity ( $n = 4$ )	tribbles homolog 1	TRIB1
	chemokine (C-C motif) ligand 2	CCL2
	chemokine (C-C motif) ligand 8	CCL8
	chemokine (C-X-C motif) ligand 2	CXCL2
Metal ion binding ( $n = 6$ )	interleukin 1 receptor, type I	IL1R1
	metallothionein 2e	MT2A
	metallothionein 1f	MT1F
	metallothionein 1e	MT1E
	metallothionein 1h	MT1H
	metallothionein 1m	MT1M
	metallothionein 1x	MT1X



**Table 2**

Individual insulin-signaling genes with increased expression following 240 min of insulin infusion

Probe Set ID	Gene Name	Gene Symbol	Basal (mean ± SE)	Insulin (mean ± SE)	P Value
201435_s_at	eukaryotic translation initiation factor 4E	EIF4E	7.7±0.2	8.5±0.3	0.001
201436_at	eukaryotic translation initiation factor 4E	EIF4E	5.2±0.2	5.8±0.2	0.017
201437_s_at	eukaryotic translation initiation factor 4E	EIF4E	6.7±0.2	7.9±0.2	0.00002
203719_at	excision repair cross-complementing rodent repair deficiency, 1	ERCC1	6.6±0.1	6.7±0.1	0.052
209189_at	v-fos FBJ murine osteosarcoma viral oncogene homolog	FOS	3.2±0.1	10.2±0.8	0.00001
215075_s_at	growth factor receptor-bound protein 2	GRB2	7.4±0.1	7.6±0.1	0.032
202934_at	hexokinase II	HK2	5.0±0.4	6.4±0.4	0.001
213792_s_at	insulin receptor	INSR	7.0±0.2	7.4±0.2	0.039
209184_s_at	insulin receptor substrate 2	IRS2	3.3±0.1	4.0±0.3	0.010
209185_s_at	insulin receptor substrate 2	IRS2	8.1±0.3	9.0±0.4	0.023
202393_s_at	Kruppel-like factor 10	KLF10	9.3±0.3	11.6±0.1	0.000001
204009_s_at	v-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog	KRAS	6.1±0.4	6.9±0.2	0.006
214352_s_at	v-Ki-ras2 Kirsten rat sarcoma viral oncogene homolog	KRAS	6.2±0.6	7.9±0.3	0.002
202068_s_at	Low-density lipoprotein receptor	LDLR	2.3±0.1	3.4±0.4	0.013
212688_at	phosphoinositide-3-kinase, catalytic, beta polypeptide	PIK3CB	6.2±0.2	6.6±0.2	0.006
212240_s_at	phosphoinositide-3-kinase, regulatory subunit 1 (p85 alpha)	PIK3R1	7.8±0.2	9.0±0.2	0.002
202743_at	phosphoinositide-3-kinase, regulatory subunit 3 (p55, gamma)	PIK3R3	3.3±0.2	4.7±0.4	0.001
209678_s_at	protein kinase C, iota	PRKCI	7.4±0.3	7.8±0.2	0.030
212610_at	protein tyrosine phosphatase, nonreceptor type 11	PTPN11	10.3±0.2	10.5 ± 0.1	0.040
212590_at	related RAS viral (r-ras) oncogene homolog 2	RRAS2	3.5±0.2	4.0±0.3	0.024
212589_at	Sterol carrier protein 2	SCP2	8.4±0.3	9.0±0.2	0.001
210512_s_at	vascular endothelial growth factor	VEGF	10.2±0.2	10.9±0.1	0.00008
211527_x_at	vascular endothelial growth factor	VEGF	3.8±0.1	4.0±0.1	0.033
212171_x_at	vascular endothelial growth factor	VEGF	8.8±0.1	9.1±0.2	0.040

Significance tested by a Student's paired two-tailed *t*-test (not corrected for multiple testing); *P* < 0.05.

**Table 3**

Results of the microarray with those of qRT-PCR following insulin infusion

Probe Set ID	Gene Symbol	Fold Change		
		Microarray (4-h clamp)	qRT-PCR (4-h clamp)	qRT-PCR (3-h clamp)
216598_s_at	CCL2	3.78	64.3±21.4*	1.7±0.2*
214038_at	CCL8	3.05	116.6±49.5*	4.8±1.5*
202284_s_at	CDKN1A	3.91	39.4±16.6*	2.2±0.4*
209774_x_at	CXCL2	3.80	82.0±39.9*	1.6±0.2*
201693_s_at	EGR1 <sup>a</sup>	6.09	174.2±121.0	3.4±0.8*
201329_s_at	ETS2 <sup>a</sup>	2.56	5.0±1.3*	1.3±0.2
209189_at	FOS	7.00	203.9±93.4	2.7±0.7*
207574_s_at	GADD45B <sup>a</sup>	6.42	70.2±18.2*	2.9±1.8
202948_at	IL1R1	2.30	3.9±0.8*	1.4±0.1*
201473_at	JUNB	4.06	14.4±8.8	2.1±0.3*
202393_s_at	KLF10	2.30	5.4±1.2*	1.7±0.2*
202431_s_at	MYC	4.79	43.1±15.5*	3.5±1.1*
203574_at	NFIL3	5.38	8.7±2.6*	1.7±0.1*
211302_s_at	PDE4B <sup>a</sup>	3.97	8.9±2.6*	1.7±0.2*
204803_s_at	RRAD <sup>a</sup>	3.80	14.3±4.4*	2.5±0.7
201739_at	SGK	1.63	3.6±0.9*	1.5±0.2*
203887_s_at	THBD <sup>a</sup>	7.69	8.4±5.0	1.9±0.3*
201531_at	ZFP36	4.34	54.4±17.4*	1.7±0.3
209357_at	CITED2	-2.25	-2.5±0.1*	-2.1±0.1*
206765_at	KCNJ2	-1.79	-2.0±0.1*	-1.6±0.1*

<sup>a</sup>More than one probe set was significantly altered for this gene on the microarray. The probe set with the greatest fold change in the microarray analysis is represented here (data taken directly from Supplementary Tables 1 and 2). Significance for the genes selected for qRT-PCR was assessed looking at each dataset separately and using Student's paired *t*-test.

\* *P* < 0.05 vs. control 18S ribosomal RNA (used for qRT-PCR analysis).

**Table 4**

mRNA expression determined by qRT-PCR during saline infusion

Probe Set ID	Gene Symbol	Fold Change: Saline Infusion
216598_s_at	CCL2	-1.09±0.22
214038_at	CCL8	1.02±0.67
202284_s_at	CDKN1A	1.10±0.14
209774_x_at	CXCL2	-1.69±0.83
201693_s_at	EGR1	-3.57±2.30*
201329_s_at	ETS2	1.23±0.03*
209189_at	FOS	-2.94±2.60
207574_s_at	GADD45B	-1.28±0.30
202948_at	IL1R1	1.60±0.60
201473_at	JUNB	-1.02±0.40
202393_s_at	KLF10	2.45±0.66
202431_s_at	MYC	1.00±0.41
203574_at	NFIL3	-1.25±0.30
211302_s_at	PDE4B	1.38±0.31
204803_s_at	RRAD	1.78±0.09*
201739_at	SGK	-1.28±0.16
203887_s_at	THBD	2.33±1.68
201531_at	ZFP36	-1.56±0.73
209357_at	CITED2	1.15±0.26
206765_at	KCNJ2	-1.12±0.25

\*  $P < 0.05$  vs. control 18S ribosomal RNA, using Student's paired *t*-test.

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript