Human transcriptome analysis of acute responses to glucose ingestion reveals the role of leukocytes in hyperglycemia-induced inflammation

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Choi HJ, Yun HS, Kang HJ, Ban HJ, Kim Y, Nam HY, Hong EJ, Jung SY, Jung SE, Jeon JP, Han BG. Human transcriptome analysis of acute responses to glucose ingestion reveals the role of leukocytes in hyperglycemia-induced inflammation. *Physiol Genomics* 44: 1179–1187, 2012. First published October 16, 2012; doi:10.1152/physiolgenomics.00179.2011.— Glucose ingestion-induced hyperglycemia has been known to induce inflammation, which is related to the pathogenesis of diabetic complications. To examine acute gene expression responses to physiological oral glucose ingestion in human circulating leukocytes, we conducted a microarray study of human circulating leukocytes sampled before, 1 h after, and 2 h after glucose ingestion in community-based participants without previous histories of diabetes (n = 60). Ingestion of 75 g glucose successfully induced acute hyperglycemia (glucose concentration 91.6 ± 5.3 mg/dl for fasting and 180.7 ± 48.5 mg/dl for 1 h after glucose ingestion). Oral glucose ingestion significantly increased the expression of 23 genes and decreased the expressions of 13 genes [false discovery rate (FDR) P value <0.05]. These genes are significantly involved in immunity by way of natural killer cell-mediated immunity, granulocyte-mediated immunity, and the cytokine-mediated signaling pathway (FDR P value <0.05). The present study demonstrated 36 genes that showed acute gene expression change in human leukocytes within 1 h after glucose ingestion, suggesting that leukocytes participate in the inflammatory process induced by acute hyperglycemia. We believe that these results will provide some basic insight into the role of leukocytes in hyperglycemia-induced inflammation and the pathogenesis of diabetic complications.

diabetes; oral glucose tolerance test (OGTT), hyperglycemia; microarray; leukocyte; inflammation

HYPERGLYCEMIA, THE HALLMARK of diabetes, plays a major role in the pathogenesis of diabetic complications, such as myocardial infarction, stroke, and peripheral vascular disease (17). Inflammation and oxidative stress have been suggested as the major mechanisms governing these diabetic complications. Most of these studies were focused on the effects of chronic hyperglycemia, providing an important insight into the roles of acutely induced inflammatory processes as diabetes-related host responses.

Several studies have investigated differences in gene expression between diabetes patients and normal subjects (14, 21). Most of these studies were focused on the effects of chronic hyperglycemia. However, the effects of acute hyperglycemia and chronic hyperglycemia on gene expression may be different, and some studies have reported the acute effect of glucose ingestion-induced hyperglycemia on gene expression and reactive oxygen species (ROS) generation of white blood cells, especially regarding inflammation and oxidative stress (4, 16, 20). These studies have shown that white blood cells contribute to the pathogenesis of diabetic complications, and that hyperglycemia-induced change in white blood cells might be important for governing this process. However, the limiting factors of these previous studies are that they investigated only some of the selected inflammation mediators and oxidative stress markers and that none of thoroughly observed whole genome expression profile changes induced by glucose ingestion in human white blood cells in vivo.

Therefore, we conducted a microarray study to examine acute gene expression alteration in human leukocytes induced by oral glucose ingestion. We assessed participants without histories of diabetes (n = 60) drawn from a community-based cohort to compare transcriptome changes before and after physiological oral glucose ingestion stimulation (60 participants × 3 time points). Our results revealed that some of the genes involved in inflammatory response are induced by acute hyperglycemia, providing an important insight into the roles of acutely induced inflammatory processes as diabetes-related host responses.

**MATERIALS AND METHODS**

**Subjects and blood samples.** Subjects were recruited from the Korean Genome Epidemiology Study, a longitudinal community-based prospective study consisting of participants aged 40–69 yr (2). Subjects with previous histories of diabetes or any malignant disease were excluded. A total of 60 subjects was included in the present study and all underwent oral glucose tolerance test (OGTT). The biospecimens for this study were provided by the National Biobank of Korea, supported by the Ministry of Health Welfare. All samples from the National Biobank of Korea were obtained with informed consent and the study received institutional review board approval. Subjects were given 75 g glucose dissolved in 300 ml water (Glucoda; Alle-giance Healthcare, McGaw Park, IL) to drink within a period of 5 min. Blood samples were obtained at 0, 1, and 2 h after glucose ingestion. Isolation of total RNA. Total RNA was extracted from whole blood using LeukoLOCK Total RNA Isolation Kit (Ambion). The mRNA were assessed for concentration by NanoDrop (ND-1000 spectropho-

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### Table 1. Primers of the 3 genes used for real-time PCR

<table>
<thead>
<tr>
<th>Name</th>
<th>Forward Primer (5’-3’)</th>
<th>Reverse Primer (5’-3’)</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL8</td>
<td>AGGTGCAAGTTTGTGAGAGAA</td>
<td>TTTCTTGTTGCTGCAAGGTT</td>
</tr>
<tr>
<td>PTGS2</td>
<td>CCAACACTTCCACCATCAGT</td>
<td>ACGCTGTCTAAGCCAGAGTT</td>
</tr>
<tr>
<td>EGR1</td>
<td>AAGCCACAGAGGAGGAGATT</td>
<td>TACCAAGAAGGATG6</td>
</tr>
</tbody>
</table>

Statistical analysis. Statistically significant gene (probe) expression change was defined when both of the following criteria were met: 1) a 1 h signal / 0 h signal fold change higher than 1.3 or lower than 1/1.3 and 2) false discovery rate (FDR) P value < 0.05 from paired t-test of 0 h signal and 1 h signal. Functional classification of the selected gene list was performed using the DAVID functional annotation tool with a QC filtered gene list as the background gene list. PANTHER classifications with multiple testing of corrected FDR P value < 0.05 were selected.

### RESULTS

Baseline characteristics and effect of glucose ingestion of studied subjects. The subjects included 45 males and 15 females with an age range of 46–73 yr (Table 2). Ingestion of 75 g glucose successfully induced acute hyperglycemia. The mean (± SD) value of glucose concentration was 91.6 ± 5.3 mg/dl for fasting status, 180.7 ± 48.5 mg/dl for 1 h, and 143.9 ± 42.4 mg/dl for 2 h, and the mean (± SD) value of insulin was 7.6 ± 3.2 μU/ml for fasting status, 52.6 ± 36.7 μU/ml for 1 h, and 52.5 ± 45.6 μU/ml for 2 h.

Analysis of gene expression changes in leukocytes during the acute phase of glucose ingestion. Transcriptional profiles of leukocytes from 60 participants were measured on microarrays that contained 48,803 probes at time point 0 h, 1 h, and 2 h after glucose ingestion. We identified 36 differentially expressed genes after induction of acute hyperglycemia using cut-off criteria described in MATERIALS AND METHODS (Table 3).

#### Table 2. Age, sex, serum glucose, and serum insulin of the participants

<table>
<thead>
<tr>
<th>Total number</th>
<th>60</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yr</td>
<td>54.0 ± 7.2</td>
</tr>
<tr>
<td>Sex, male/female</td>
<td>45/15</td>
</tr>
<tr>
<td>Fasting glucose, mg/dl</td>
<td>91.6 ± 5.3</td>
</tr>
<tr>
<td>1 h glucose, mg/dl</td>
<td>180.7 ± 48.5</td>
</tr>
<tr>
<td>2 h glucose, mg/dl</td>
<td>143.9 ± 42.4</td>
</tr>
<tr>
<td>Fasting insulin, μU/ml</td>
<td>7.6 ± 3.2</td>
</tr>
<tr>
<td>1 h insulin, μU/ml</td>
<td>52.6 ± 36.7</td>
</tr>
<tr>
<td>2 h insulin, μU/ml</td>
<td>52.5 ± 45.6</td>
</tr>
</tbody>
</table>

Values are means ± SD.
natural killer cell group 7 sequence (NKG7); oncostatin M (OSM); perforin 1 (PRF1); prostaglandin-endoperoxide synthase 2 (cylooxygenase-2) (PTGS2); and transforming growth factor, beta receptor III (TGFBR3) (Table 4). The glucose ingestion-induced expression changes of these genes are shown in Fig. 1.

**Real-time PCR validation.** To quantitatively confirm microarray data and examine their reliability, we selected three genes (IL8, PTGS2, EGR1) for RT-PCR validation using the RNA samples used for the microarray analysis. The results of RT-PCR were consistent for all genes (Fig. 2).

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**In vitro replication of gene expression changes in primary leukocyte culture.** To replicate these findings in vitro, a total of 20 genes were investigated by primary culture of human leukocytes. According to the results of real-time PCR experiments, the fold changes of gene expression observed in in vitro primary leukocyte culture were significantly associated with those fold changes observed from the microarray experiments in physiological conditions, which was positively correlated in the same direction ($R = 0.681, P < 0.001$, Fig. 3). For 18 genes out of 20 genes (90%), there was an agreement in the direction of effects. Only two genes (10%) showed the oppo-
site direction of effect (OSM and CCL3). In addition, the gene expression of two genes (GZMA and KLRD1) was significantly increased in vitro by high glucose treatment (paired t-test \( P < 0.05 \)), which replicates the results from our microarray experiments. However, for the other genes, the effect of high glucose did not reach statistical significance.

**DISCUSSION**

We investigated acute changes in whole genome transcription in circulating leukocytes after glucose ingestion in humans and identified 36 genes that showed acute expression changes within 1 h. Among the differentially expressed genes, more than half of the genes were immunity-related genes. To our knowledge, this is the first study to investigate the whole genome transcription change in circulating leukocytes after glucose ingestion in humans. These results support the proposed role of leukocytes in the pathogenesis of diabetes-related inflammation (17).

In addition to diabetic vascular complications, hyperglycemia is an independent risk factor of morbidity and mortality in critical illness (11, 24). Disturbance of the innate immune system has been proposed as the mechanism underlying this harmful effect of hyperglycemia (23). There are accumulating lines of evidence that acute hyperglycemia influences all major components of innate immunity and impairs the capability of the immune system to combat infections (23). Specifically, acute hyperglycemia has been reported to impair phagocytosis by leukocytes and increase apoptosis of neutrophils (23). This evidence indicates that acute, as well as chronic, hyperglycemia can induce a mixed alteration of the immune system to activate inflammation and inhibit immunity at the same time (23). Consistent with these previous results, we identified genes that are involved in the acute proinflammatory action of leukocytes in response to hyperglycemia.

Glucose ingestion increased expression of perforin complex members (PRF1, GZMA, GZMB, GZMH, and GNLY), natural killer cell-related genes (KIR2DL4, KLRD1, KLRF1, and NKG7), chemokine receptors (CX3CR1 and IL2RB), and TGFBR3 and decreased expression of cytokine ligands (CCL3, IL12A, and OSM), PTGS2 (COX-2; a well-known enzyme participating in diabetes-related inflammation) (5), and FOS (Fig. 4). The perforin complex consists of perforin (PRF1), granzymes (GZMA, GZMB, GZMH, GZMK, and GZMM) and granulysin (GNLY), which is found in the granules of leukocytes (6). When secreted from leukocytes, perforin and granulysin create holes in the target cell, allowing granzymes to enter the target cell and induce apoptosis (Fig. 4) (6, 15). Importantly, perforin complexes have been well known to play a dominant role in beta-cell destruction in Type 1 diabetes and in the inflammatory pathogenesis of Type 2 diabetes (22). We demonstrated that almost all members of perforin complexes (PRF1, GZMA, GZMB, GZMH, and GNLY) showed markedly increased expression in response to glucose ingestion. We speculate that the increased expression of perforin complex members in leukocytes could be one of the pathogenic mechanisms that contribute to beta-cell destruction by leukocytes. This model is in accordance with the proposed role of granzymes in diabetes-related inflammation (22).

Natural killer cells, a subtype of leukocyte, are known to play an important role in beta-cell destruction and the patho-
Fig. 1. Microarray results for glucose ingestion-induced expression of immunity-related genes. Multiple lines represent different probe sets for each gene. Results are means ± SE of fold variations vs. baseline ($n = 60$).
genesis of diabetes (18). There was a significant increase in natural killer-related genes (KIR2DL4, KLRD1, KLRF1, and NKG7) in response to glucose ingestion, indicating their roles in the pathogenesis of diabetes (Fig. 4).

A recent study has demonstrated that fractalkine and its receptor (CX3CR1) are novel members of the inflammatory adipose chemokine system that is associated with Type 2 diabetes (19). Consistent with this finding, our results showed upregulation of CX3CR1 expression, supporting the previous results. Chemokine/interleukin family receptors (CX3CR1 and IL2RB) are increased, and ligands (CCL3, IL8 and OSM) are decreased. TGFBR3 is increased, while PTGS2 and FOS are decreased. These results may be related to increased inflammation and consequent feedback downregulation (Fig. 4).

Several studies have performed genome-wide transcriptome analysis in leukocytes from diabetes patients (14, 21). PTGS2 and the Tribbles homolog 1 (TRIB1) gene showed acute expression changes after glucose ingestion in the present study, as also reported in previous studies. This suggests the presence of a shared mechanism underlying both chronic hyperglycemia (diabetes) and acute hyperglycemia. However, it should be noted that these previous studies were focused on the effects of chronic hyperglycemia or diabetes state (comparing between normal control and diabetes patients), whereas the present study was focused on the effect of acute hyperglycemia induced within 1 h after glucose ingestion (comparing before and after glucose ingestion). Therefore, there are substantial distinctions between the present study and these previous studies due to this difference in study objectives.

Previous studies have demonstrated induction of p47phox (16), TNF-α (3), IL-1β (8), and SOCS3 (8) in leukocytes after glucose ingestion. Increased expression of these genes has
Pathophysiological importance since these genes are related to the pathophysiology of hyperglycemia, insulin resistance, and diabetic complications. SOCS3 has been reported to interfere with insulin signal transduction and is induced by proinflammatory stimuli (e.g., TNF-α and IL-1ß). However, in the present study, gene expression of p47phox (fold change = 1.07, paired t-test FDR \( P = 0.70 \)) and TNF-α (fold change = 1.06, paired t-test FDR \( P = 0.12 \)) were not changed, while gene expression of IL-1ß (fold change = 0.93, paired t-test FDR \( P = 0.02 \)) and SOCS3 (fold change = 0.84, paired t-test FDR \( P < 0.01 \)) were slightly decreased 1 h after glucose ingestion. The reason for this discrepancy is not clear. We speculate that difference in cell type (whole leukocytes in the present study vs. mononuclear cells in the previous studies), different subject characteristics (older in the present study, 54 ± 7.2 yr vs. 25–47 yr), and degree of hyperglycemia (higher in the present study, 180.7 vs. 108–128 mg/dl) induced by glucose ingestion may have contributed to these discrepancies.

Previous studies have demonstrated increased inflammation by glucose ingestion in humans (7, 9, 10), as supported by increased levels of serum C-reactive protein, IL-6, IL-1ß, TNF-α, nitrotyrosine, intercellular adhesion molecule-1 (7, 10), and increased activity of nuclear factor-κB in mononuclear cells (9) after hyperglycemia induced by glucose ingestion, which were not observed in the present study. The reason for this discrepancy could be differences in study design. The inflammation-related serum markers measured in the previous studies (7, 10) reflect the whole body response to hyperglycemia, whereas the present study focused on the gene expression change only in the leukocytes. The aim of the other study (9) was to investigate a few target responses (ROS and nuclear factor-κB activity), whereas the aim of the present study was to measure global gene expression changes. We reported acute transcription changes of human circulating leukocytes in response to oral glucose ingestion, which could indirectly contribute to the systemic inflammatory response found in these previous studies (7, 9, 10).

By investigating the effects of oral glucose ingestion on the gene expression profiles of circulating leukocytes, we postulate that acute hyperglycemia induced by glucose ingestion (from 91.6 to 180.7 mg/dl) accounts for most of the links between glucose ingestion and gene expression change in leukocytes. Hyperglycemia is associated with changes in the expression of genes, especially immunity-related genes, inflammatory cytokines, and chemokines (20). However, considering the in vivo nature of the present study, some of our findings could be a result of secondary systemic changes by glucose ingestion such as hyperinsulinemia (from 7.6 ± 3.2 to 52.6 ± 36.7 μU/ml) or increased inflammation, rather than simple direct effects of hyperglycemia. Insulin is known to induce numerous gene expression changes through insulin receptors. In addition, the increased systemic inflammation by glucose ingestion may have considerable influence on gene expression in circulating leukocytes. Therefore, the gene expression changes observed in our study are likely to be the integrated results of various physiological systemic changes (a complex mixture of hyperglycemia, hyperinsulinemia, and other systemic changes) induced by glucose ingestion. Likewise, some genes, such as IL-8, PTGS2, FOSB, FOS, and EGR1, which are generally known to be proinflammatory, showed decreased expressions in response to glucose ingestion. These results could be the compensatory feedback downregulation initiated by other proinflammatory responses or other systemic changes, which is in concordance with the concept of “compensatory anti-inflammatory response syndrome” (1) (e.g., suppression of IL-8 found in leukocytes in response to systemic inflammation).

In vitro replication of gene expression changes showed that most of the fold change values observed in the in vitro
real-time PCR experiments were closely correlated to the fold change values observed in the in vivo microarray experiments in the same direction. Only two genes (10%) showed the opposite direction of effect (OSM and CCL3). The reasons for the replication failure (opposite direction) of these two genes could be explained by following reasons. First, it should be noted that these two genes (OSM and CCL3) were genes with the smallest effect size in the microarray experiments (fold change of 0.71 and 0.73, respectively) among the nine downward genes included in the in vitro replication experiment. It is difficult to replicate findings with a small effect size, and a much larger sample size may be required to replicate the small effect size, considering the substantial technical variabilities. Second, as discussed above, due to the in vivo nature of the present microarray experiments, some of the present findings could be a result of secondary systemic changes (such as hyperinsulinemia or inflammation) rather than the direct effect of high glucose. Therefore, there could be considerable differences between in vitro high glucose treatment and in vivo oral glucose ingestion. Further experiments with high insulin treatment or other stimuli may uncover some of these differences. Third, the primary culture process itself could have disrupted some of the gene expression profile in leukocytes.

From the individual gene analysis of in vitro replication, high glucose treatment significantly increased gene expression for two genes (GZMA and KLRD1), which replicates the results from our in vivo microarray experiments. However, for other genes, the effect of high glucose did not reach statistical significance, probably due to the following reasons. First, the expected effect size were rather small (fold change in microarray experiment were between 0.57 and ~1.60), which attenuates statistical power. Secondly, there was substantial technical variability (viable cell number, RNA quality, etc.) derived from difficulties in maintaining and stabilizing primary cell culture samples even though in vitro replication experiments were performed in duplicate three times. Third and most importantly, the limited sample size (n = 3) of in vitro replication experiments attenuated statistical power in contrast to the microarray experiments (n = 60). Although the individual gene analysis had some limitations, we believe that the statistically significant gene expression changes observed for two genes (GZMA and KLRD1) and the overall agreement (R = 0.681, P < 0.001, n = 20) in fold change values between in vivo and in vitro experiments provide sufficient evidences for an in vitro replication.

One of the strengths of our study is that we conducted a relatively large-scale genome-wide transcriptome study (n = 60) using in vivo human OGTT specimens that were physiologically stimulated by 75 g glucose. Compared with the small sample sizes of previous studies, which were mostly performed in vitro, as well as a few nonphysiological treatment studies, the present study investigated comprehensive gene expression changes induced by physiological stimuli in humans with greater statistical power. Nonetheless, one of the limitations of our study is the heterogeneity of samples, which were a mixture of several subtypes of leukocytes. Isolation of a certain population of leukocytes (for example, mononuclear cells) may have resulted in more informative data. However, preparation of such homogenous samples is difficult, considering the sample volume required for preparation. The second limiting factor is that, as noted above, the present study cannot discern the direct effects of glucose ingestion from the secondary or indirect effects. In addition, the role of hyperinsulinemia in addition to (or aside from) hyperglycemia remains to be further investigated. The third limiting factor is that the magnitude of gene expression changes was rather modest, and only a limited number of genes showed significant expression changes. Therefore, caution should be taken when interpreting these results as a generalized immune response.

In conclusion, we found 36 genes that showed acute gene expression changes in human leukocytes within 1 h after glucose ingestion. Our findings suggest that leukocytes participate in inflammation induced by acute hyperglycemia through altered expression of these genes. We believe that these results will provide basic insight into the roles of leukocytes in hyperglycemia-induced inflammation and contribute to a better understanding of the pathogenesis of diabetic complications.

GRANTS

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS


REFERENCES


