Altered Response of Skeletal Muscle to IL-6 in Type 2 Diabetic Patients

Lake Q. Jiang, Daniella E. Duque-Guimaraes, Ubiratan F. Machado, Juleen R. Zierath, and Anna Krook

Interleukin-6 (IL-6) has a dual role in modulating insulin sensitivity, with evidence for this cytokine as both an enhancer and inhibitor of insulin action. We determined the effect of IL-6 exposure on glucose and lipid metabolism in cultured myotubes established from people with normal glucose tolerance or type 2 diabetes. Acute IL-6 exposure increased glycogen synthesis, glucose uptake, and signal transducer and activator of transcription 3 (STAT3) phosphorylation in cultured myotubes from normal glucose-tolerant subjects. However, in type 2 diabetic patients, IL-6 was without effect on glucose metabolism and STAT3 signaling, concomitant with increased suppressor of cytokine signaling 3 (SOCS3) expression. IL-6 increased fatty acid oxidation in myotubes from type 2 diabetic and normal glucose-tolerant subjects. Expression of IL-6, IL-6 receptor (IL-6R), or glycoprotein 130, as well as IL-6 secretion, was unaltered between cultured myotubes from normal glucose-tolerant or type 2 diabetic subjects. Circulating serum IL-6 concentration was unaltered between normal glucose-tolerant and type 2 diabetic subjects. In summary, skeletal muscle cells from type 2 diabetic patients display selective IL-6 resistance for glucose rather than lipid metabolism. In conclusion, IL-6 appears to play a differential role in regulating metabolism in type 2 diabetic patients compared with normal glucose-tolerant subjects.

The role of interleukin-6 (IL-6) in the development of peripheral insulin resistance in type 2 diabetes is a matter of debate (1,2). Elevated serum concentrations of proinflammatory cytokines such as resistin, tumor necrosis factor-α, and IL-6 are linked to obesity and subsequent progression to diabetes (3). Adipose tissue is a major source of circulating IL-6, and with obesity, the expanded fat mass and subsequent IL-6 secretion are implicated in the development of insulin resistance (3). In obese subjects with or without type 2 diabetes, adipose tissue IL-6 content correlates with impaired whole-body insulin-mediated glucose uptake and glucose tolerance (4,5). However, several other cell types also express and secrete IL-6, including endothelial cells, pancreatic β-cells, hepatocytes, and skeletal and smooth muscle (5–7).

In healthy normal glucose-tolerant subjects, plasma IL-6 is inversely related to insulin sensitivity (6). IL-6 has a number of tissue-specific, metabolically relevant actions. Although epidemiological data confirm a relationship between the serum IL-6 concentration and insulin resistance (7), careful examination of clinical cohorts indicates that this relationship may directly reflect adipose mass rather than insulin resistance (8,9).

Skeletal muscle contraction during exercise improves skeletal muscle insulin sensitivity (10,11) and also increases IL-6 mRNA expression and subsequently the circulating IL-6 concentration (12,13). This observation has led to the proposition that IL-6 may promote nutrient availability and improve whole-body insulin sensitivity during exercise (1,2). Recent evidence suggests IL-6 is a crucial exercise-dependent signal to increase circulating glucagon-like peptide 1 (GLP-1) and enhance β-cell function (14). In this respect, tissue-specific actions of IL-6 may be important to consider. Acute IL-6 exposure has a metabolic effect by increasing insulin action in cultured human skeletal muscle (15–17). Furthermore, chronic IL-6 exposure induces proliferation of muscle satellite cells, thus promoting muscle regeneration and muscle hypertrophy (18). In humans, infusion of a physiological IL-6 concentration in healthy subjects, as well as type 2 diabetic patients, increases lipolysis and enhances glucose infusion rates during euglycemic–hyperinsulinemic clamp (17,19,20).

We hypothesized that IL-6 exposure would improve insulin action in skeletal muscle derived from insulin-resistant type 2 diabetic subjects. We determined insulin and IL-6 effects on glucose and lipid metabolism in primary human skeletal muscle cultures from type 2 diabetic patients and age- and BMI-matched healthy normal glucose-tolerant subjects.

RESEARCH DESIGN AND METHODS

Human studies. Studies were performed with approval from the local ethical committee and in accordance with the Declaration of Helsinki. Informed written consent was obtained from all participants before testing was initiated. Anthropometric data are presented in Table 1. Type 2 diabetic subjects were treated with the following antidiabetic medication: metformin (n = 5); sulfonylurea (n = 1); combination of metformin, sulfonylurea, and thiazolidinedione (n = 1). Type 2 diabetic subjects also received statin treatment (n = 4). One normal glucose-tolerant subject was treated with thyroid hormone replacement.

In vitro contraction of mouse skeletal muscle. Animal experiments were approved by the local animal ethics committee. Male C57BL/6J mice (16 weeks old) from Charles River Laboratories were anesthetized by intraperitoneal injection of 2.5% Avertin (20 μL/g body weight). Extensor digitorum longus (EDL) muscles were removed and incubated in Krebs-Henseleit bicarbonate buffer with 0.1% BSA, 18 mmol/L NaCl, and 2 mmol/L pyruvate for 20 min at 37°C. Muscles were then mounted in an in vitro contraction apparatus (Myograph System DMT, Aarhus, Denmark) and incubated in the same buffer with or without 120 ng/mL mouse IL-6 for 40 min. Thereafter, the buffer was changed to Krebs-Henseleit bicarbonate buffer with 0.1% BSA, 19 mmol/L [1-14C]-mannitol (0.7 μCi/mL), 1 mmol/L (1,2-3H(N))-2-deoxy-D-glucose (2.5 μCi/mL), and 1 mmol/L 2-deoxy-D-glucose (2.5 μCi/mL). We hypothesized that IL-6 exposure would improve insulin action in skeletal muscle derived from insulin-resistant type 2 diabetic subjects. We determined insulin and IL-6 effects on glucose and lipid metabolism in primary human skeletal muscle cultures from type 2 diabetic patients and age- and BMI-matched healthy normal glucose-tolerant subjects.
TABLE 1
General characteristics of the subjects

<table>
<thead>
<tr>
<th>Clinical characteristics</th>
<th>NGT (n = 10)</th>
<th>T2DM (n = 10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>59 ± 1</td>
<td>61 ± 1</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>27.5 ± 0.55</td>
<td>29.77 ± 1.0*</td>
</tr>
<tr>
<td>Waist (cm)</td>
<td>102 ± 1</td>
<td>105 ± 2</td>
</tr>
<tr>
<td>F-plasma glucose (mmol/L)</td>
<td>5.2 ± 0.1</td>
<td>7.8 ± 0.5***</td>
</tr>
<tr>
<td>2-h Plasma glucose (mmol/L)</td>
<td>6.5 ± 0.2</td>
<td>14.8 ± 1.1***</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>4.6 ± 0.1</td>
<td>6.6 ± 0.3***</td>
</tr>
<tr>
<td>Insulin (pmol/L)</td>
<td>49.3 ± 5.6</td>
<td>51.7 ± 12.7</td>
</tr>
<tr>
<td>Total cholesterol (mg/dL)</td>
<td>5.62 ± 0.35</td>
<td>4.69 ± 0.30</td>
</tr>
<tr>
<td>LDL (mg/dL)</td>
<td>3.64 ± 0.27</td>
<td>2.59 ± 0.18**</td>
</tr>
<tr>
<td>HDL (mg/dL)</td>
<td>1.69 ± 0.10</td>
<td>1.25 ± 0.08**</td>
</tr>
<tr>
<td>Triglyceride (mg/dL)</td>
<td>1.44 ± 0.31</td>
<td>1.67 ± 0.33</td>
</tr>
<tr>
<td>IL-6 (pg/mL)</td>
<td>2.28 ± 0.45</td>
<td>2.51 ± 0.52</td>
</tr>
</tbody>
</table>

Data are mean ± SEM (BMI). F, fasting; NGT, normal glucose tolerant; T2DM, type 2 diabetes mellitus. *P < 0.05, **P < 0.01, ***P < 0.001.

RESULTS

Subject characteristics. Subject characteristics and clinical parameters are summarized in Table 1. Type 2 diabetic patients attending a primary health care clinic and normal glucose-tolerant volunteers were matched for age and BMI. As expected, fasting plasma glucose, 2-h glucose, and HbA1c were elevated in the type 2 diabetic patients. Fasting plasma glucose, 2-h glucose, and HbA1c were elevated in the type 2 diabetic patients.

Secretion of IL-6 from cultured skeletal muscle cells. Media appearance of IL-6 was measured in the muscle cell cultures established from subjects with normal glucose tolerance or type 2 diabetes. IL-6 content was measured in media from established normal glucose-tolerant or type 2 diabetic subjects, both in the presence or absence of insulin (Fig. 2A and B). In contrast, IL-6 exposure was without effect on glycogen synthesis or glucose uptake in skeletal muscle cells from type 2 diabetic patients (Fig. 2A and B). Acute exposure of skeletal muscle cells to IL-6 induced a significant increase in fatty acid oxidation, with a similar response noted between the groups (Fig. 3).

IL-6 effects on glucose and lipid metabolism. Acute (1 h) exposure to 25 ng/mL IL-6 increased glycogen synthesis and glucose uptake in skeletal muscle cells from normal glucose-tolerant subjects, both in the presence or absence of insulin (Fig. 2A and B). In contrast, IL-6 exposure was without effect on glycogen synthesis or glucose uptake in skeletal muscle cells from type 2 diabetic patients (Fig. 2A and B). Acute exposure of skeletal muscle cells to IL-6 induced a significant increase in fatty acid oxidation, with a similar response noted between the groups (Fig. 3).

IL-6 activation of intracellular signaling pathways. To assess whether the reduced IL-6-mediated increase in skeletal muscle glucose metabolism was also evident for other effects of IL-6, we assessed acute effects of IL-6-mediated signaling in skeletal muscle cells. In cultures established from normal glucose-tolerant subjects, an acute (20 min) IL-6 exposure increased signal transducer...
and activator of transcription 3 (STAT3) phosphorylation (Fig. 4A). Interestingly, in muscle cultures established from type 2 diabetic patients, this effect was markedly blunted (Fig. 4A). IL-6 increased JAK2 phosphorylation in cultured myotubes from individuals with normal glucose tolerance, but not type 2 diabetes (Fig. 4B).

In myotubes from type 2 diabetic patients, insulin increased Akt Ser473 phosphorylation to a similar extent in myotubes from normal glucose-tolerant subjects and type 2 diabetic patients. Addition of IL-6 was without effect on insulin-induced Akt Ser473 phosphorylation in myotubes from normal glucose-tolerant subjects and type 2 diabetic patients to a similar extent (Fig. 4D).

To assess the effects of IL-6 at time points equivalent to the metabolic assays (Figs. 2 and 3), cultured myotubes were incubated with or without IL-6 (25 ng/mL) for 1 h in the presence or absence of insulin (120 nmol/L) for the final 30 min. Similar to what we observed following a 20-min IL-6 exposure, a 1-h IL-6 exposure increased STAT3 phosphorylation to a greater extent in myotubes from normal glucose-tolerant subjects as compared with type 2 diabetic patients (Fig. 5A). Interestingly, whereas insulin alone was without effect on STAT3 phosphorylation, in muscle cultures established from type 2 diabetic patients, insulin potentiated the effect of IL-6 on signal transduction. In myotubes from type 2 diabetic patients, insulin-induced Akt Ser473 phosphorylation was blunted compared with myotubes from normal glucose-tolerant subjects, suggesting a more rapid dephosphorylation of Akt (Fig. 5B). Addition of IL-6 was without effect on insulin-induced Akt Ser473 phosphorylation (Fig. 5B). To explore signaling events downstream of Akt, phosphorylation of glycogen synthase kinase 3 (GSK3) α and β was determined. IL-6 exposure did not alter GSK3 α/β phosphorylation. Furthermore, insulin-induced phosphorylated GSK3 α or β was not impaired in myotubes derived from type 2 diabetic subjects (Fig. 5C). Finally, IL-6 exposure did not induce any change in the phosphorylation status of glycogen synthase (data not shown).

Expression of intracellular signaling mediators. Because IL-6 signaling and metabolic responses were impaired in myotubes from type 2 diabetic patients, we determined whether expression of key signaling components is altered. Protein abundance of IL-6R or coreceptor gp130 was unaltered in myotubes from people with normal glucose tolerance or type 2 diabetes (Fig. 5D and E). Protein abundance of SOCS3 was elevated in myotubes from type 2 diabetic patients (Fig. 5F). mRNA expression of IL-6, IL-6R, and the gp130 coreceptor were unaltered in cultured myotubes and skeletal muscle biopsies obtained from people with normal glucose tolerance or type 2 diabetes (Table 2).

IL-6 does not enhance contraction-stimulated glucose uptake. To determine whether IL-6 exposure potentiates the effects of muscle contraction on glucose uptake, mouse EDL muscles were electrically stimulated to contract in the presence or absence of 120 ng/mL mouse IL-6. Contraction increased glucose uptake twofold compared with rested muscle (P < 0.05). IL-6 plus contraction did
not have a greater effect on muscle glucose transport than contractile activity alone (Fig. 6). This finding is compatible with the hypothesis that IL-6 and contraction increase glucose transport by a similar pathway.

**DISCUSSION**

In this study, we determined the acute effect of IL-6 on glucose and lipid metabolism in primary cultured skeletal muscle cells from normal glucose-tolerant and type 2 diabetic subjects. IL-6 exposure enhances glucose uptake and glycogen synthesis in skeletal muscle cells from people with normal glucose tolerance at basal and under insulin-stimulated conditions (15–17,23). Thus, our initial hypothesis was that IL-6 exposure may potentiate glucose uptake in myotubes from type 2 diabetic patients and provide an alternative strategy to enhance glucose metabolism in insulin-resistant cells. Contrary to our expectations, myotubes from type 2 diabetic patients are resistant to the metabolic action of IL-6 on glucose metabolism.

To dissect the mechanism by which IL-6 signaling enhances metabolism in skeletal muscle, we assessed phosphorylation of several proteins implicated in the canonical
IL-6 cascade. Phosphorylation of JAK2 and STAT3 was increased significantly in response to IL-6 exposure in myotubes from people with normal glucose tolerance. However, this signaling response was blunted in myotubes from type 2 diabetic patients, possibly attributed to elevated expression of SOCS3. SOCS3 has been shown to interfere with post–receptor insulin or leptin signal transduction (24, 25), as well as tempering cytokine responses (26). Thus, increased skeletal muscle expression of SOCS3 may contribute to insulin resistance in type 2 diabetes. In obese people, circulating mononuclear cells are characterized by reduced insulin receptor tyrosine phosphorylation, which is inversely related to increased SOCS3 expression (27). It is noteworthy that SOCS3 expression is increased in mononuclear cells from healthy people in response to a high-fat, high-carbohydrate meal (28) or following ingestion of glucose or cream (29).

In healthy young males, the IL-6 infusion enhances lipid metabolism in skeletal muscle but not adipose tissue (30). Although we noted that IL-6–mediated glucose metabolism was impaired in myotubes from type 2 diabetic patients, the effect of IL-6 on lipid oxidation was unaltered between people with type 2 diabetes or normal glucose tolerance. IL-6–mediated effects on lipid metabolism involve AMPK signaling (15, 17, 31, 32). Consistent with our finding that IL-6–induced lipid oxidation was preserved in myotubes from type 2 diabetic patients, IL-6–dependent AMPK phosphorylation was also similar between glucose-tolerant and type 2 diabetic subjects. Thus, the JAK–STAT pathway may be dispensable for IL-6 signaling to AMPK; however, this requires further molecular interrogation. In contrast to the increased protein expression of SOCS3 in cells derived from type 2 diabetic patients, mRNA expression of components of the IL-6R was unaltered between myotubes derived from normal glucose-tolerant and type 2 diabetic subjects.

Akt is critical for insulin signaling to glucose metabolism (33, 34). In this study, we report that myotubes from type 2 diabetic patients displayed impaired insulin, as well as IL-6–mediated glucose metabolism. Interestingly, insulin action on Akt phosphorylation was not impaired in myotubes from type 2 diabetic patients following a 20-min insulin...
stimulation. Indeed, insulin-stimulated Akt phosphorylation in skeletal muscle is unaltered in type 2 diabetic patients studied in vivo, despite profound impairments in glucose disposal (35,36). However, in the current study, insulin-stimulated Akt phosphorylation was blunted in myotubes from type 2 diabetic patients following a 30-min insulin exposure, suggesting a more rapid dephosphorylation of Akt.

There is growing appreciation that inflammatory pathways, including those engaged by IL-6, play an endocrine role in the regulation of metabolism (1). However, the role of IL-6 in the pathophysiology of obesity and diabetes has been vigorously debated. Although the serum level of IL-6 often correlates with insulin resistance (7), this is not a universal finding (8,9). Administration of IL-6 to rodents has an antiobesity (37) as well as antidiabetic (14) effect. Mice lacking IL-6 protein develop obesity and insulin resistance (38). In this study, we observed a trend toward increased secretion of IL-6 from myotubes derived from type 2 diabetic patients compared with normal glucose-tolerant subjects. Because IL-6 action on glucose metabolism was reduced in myotubes from type 2 diabetic patients, the increase in IL-6 secretion from these cells may be a compensatory mechanism to maintain glucose uptake.

Skeletal muscle IL-6 mRNA and circulatory IL-6 levels are increased after acute exercise (15,17,22). In this study, we provide evidence that IL-6 plus contraction did not have a greater effect on muscle glucose transport than contractile activity alone, compatible with the hypothesis that IL-6 and contraction increase glucose transport by a similar pathway. Recent evidence suggests that IL-6 is required for the exercise-mediated increase in GLP-1 (17), leading to improved insulin secretion and glucose homeostasis. Thus, intestinal L cells and pancreatic a cells are now considered metabolically relevant IL-6 target tissues. However, in light of the results presented in this paper highlighting IL-6 resistance in subjects with type 2 diabetes, whether exercise-induced IL-6 has a similar effect on GLP-1 secretion in type 2 diabetic patients warrants further investigation.

Several lines of evidence suggest that skeletal muscle cultured cells have a metabolic memory consistent with the host condition. For example, cultured skeletal muscle cells from type 2 diabetic patients retain an insulin-resistant phenotype in culture (39–41). Similarly, elevated nuclear factor-kB activation has been noted in cultured muscle from obese type 2 diabetic patients (42). In addition, the metabolic response endothelin is attenuated in cultured myotubes from type 2 diabetic patients (43). This metabolic memory persists after many passages in culture and underscores the utility of satellite cell cultures to elucidate molecular mechanisms for impaired intracellular signaling in the context of human disease. Whether this metabolic memory is a reflection of disease-specific gene variants or an epigenetically acquired trait following in vivo exposure to altered milieu remains to be investigated.

In conclusion, we present evidence that skeletal muscle cells from subjects with type 2 diabetes are partially IL-6-resistant. IL-6 effects on glucose metabolism through the JAK–STAT pathway are impaired, whereas IL-6-mediated effects on lipid oxidation are not affected. Thus, increased circulatory IL-6 noted in subjects with insulin resistance may be a compensatory mechanism to overcome partial IL-6 resistance in skeletal muscle; alternatively, the increase in IL-6 may serve as a compensatory mechanism mediated by inflammatory response, including the activation of nuclear factor-kB, which in turn may be responsible for the induction of insulin resistance.

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