Effect of IL-6 on the insulin sensitivity in patients with type 2 diabetes

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While obesity and type 2 diabetes are increasing global health threats (40), regular physical activity partly protects against these lifestyle-related conditions (2, 24, 36). However, the exact mechanisms by which physical exercise interferes with the development of a specific metabolic phenotype are complex and still not fully understood. Skeletal muscle is capable of producing and secreting several hundred peptides (4, 20, 41). In particular, it has been shown that the cytokine interleukin-6 (IL-6) is produced in, and subsequently released from, the contracting muscle (31). In addition, obesity and type 2 diabetes are conditions associated with chronically elevated plasma IL-6, and increased levels of plasma IL-6 have been shown to be an independent risk factor for the development of type 2 diabetes (28).

IL-6 can be released from a substantial number of various cell types (9), and very high plasma IL-6 levels are typically found in response to sepsis (13). IL-6 has well-established systemic effects; high levels of IL-6 can induce fever and release of catecholamines (33, 35), whereas lesser amounts are required to stimulate C-reactive protein, interleukin-1 receptor antagonist (IL-1ra), and interleukin-10, although without increasing tumor necrosis factor-α (TNF-α) (29). Thus moderate levels, and/or acute administration, of IL-6 appear to evoke an anti-inflammatory rather than an inflammatory response, and the concomitant increase of cortisol by IL-6 contributes to this picture.

However, data regarding the effect of IL-6 on skeletal muscle in vivo are still limited and to some extent different from in vitro data. In vitro, IL-6 increases protein phosphorylation of signal transducer and activator of transcription 3 (STAT3) in myocytes obtained from subjects with or without type 2 diabetes (27). Inhibition of IL-6 and its downstream signaling in mouse myoblasts impairs further differentiation into myotubes, suggesting a role of IL-6 in myogenesis (15).

In vivo, IL-6 infusion reduces availability of amino acids in plasma and protein turnover in skeletal muscle (37). In addition, IL-6 infusion increases systemic fatty acid oxidation and lipolysis in both healthy and those with type 2 diabetes during rest (25, 38). Of note, the source of the increased lipolysis appears to be skeletal muscle rather than adipose tissue (39). Exposure to IL-6 in vitro may directly increase glucose uptake and glycogen synthesis in skeletal muscle (1, 12). More recently, it was reported that IL-6 increases glucose uptake in myotubes from healthy subjects, but not in myotubes from type 2 diabetic patients (16). In the same study, the effect of IL-6 on STAT3 phosphorylation was blunted, whereas baseline expression of the suppressor of cytokine signaling 3 (SOCS3) was higher in myotubes from subjects with type 2 diabetes compared with myotubes from healthy subjects. In vivo, IL-6 infusion has no apparent effect on glucose uptake or endogenous glucose production at rest in humans with or without type 2 diabetes, but plasma insulin levels seem to be lowered in those with type 2 diabetes (30, 38). The latter observation is not easily explained, since IL-6 stimulates insulin secretion by β-cells and pancreatic islets in vitro (6), perhaps mediated by glucagon-like peptide-1 (GLP-1) released from intestinal L cells and pancreatic α-cells (7). In contrast, coadministration of
IL-6 and insulin may increase glucose uptake in mice and resting healthy humans (5, 42). However, the latter is not a consistent finding (18), perhaps due to differences regarding the employed doses of insulin and recombinant human (rh) IL-6. Furthermore, IL-6 administration during exercise appears to increase both endogenous glucose production and metabolic clearance rate (8).

Thus, it is not yet clear whether IL-6 plays a role in regulating glucose metabolism in humans with type 2 diabetes. Accordingly, the aim of this study was to investigate the effect of IL-6 on glucose metabolism during continuous insulin infusion in humans with type 2 diabetes. We hypothesized that an acute, moderate IL-6 elevation would increase the insulin-mediated glucose uptake in patients with type 2 diabetes.

METHODS

Participants. The study participants were given both oral and written information about the study before both parties signed a consent form. The study was approved and followed the guidelines from the Danish Ethics Committees (file no. KF0104804) and was carried out in accordance with the Helsinki declaration. The participants were men with type 2 diabetes treated using only oral medication (n = 9, Table 1). Malignancies, other chronic diseases, and use of insulin were exclusion criteria. Before the first study day, the participants underwent a medical examination with blood screening tests and a standard 2-h oral glucose tolerance test (OGTT; glucose, plasma-glucose at the 2-h time point of the OGTT; HbA1c, glycosylated hemoglobin; LBM, lean body mass by dual-energy X-ray absorptiometry (DXA) scan; FM, fat mass by DXA scan.

Table 1. Baseline characteristics of the participants in the study

<table>
<thead>
<tr>
<th>Variable</th>
<th>Mean ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yr</td>
<td>54.9 ± 9.7</td>
</tr>
<tr>
<td>Height, cm</td>
<td>181.8 ± 4.4</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>115.0 ± 20.0</td>
</tr>
<tr>
<td>BMI, kg/m²</td>
<td>34.8 ± 6.1</td>
</tr>
<tr>
<td>WHR</td>
<td>1.05 ± 0.06</td>
</tr>
<tr>
<td>Total LBM, kg</td>
<td>19.0 ± 1.1</td>
</tr>
<tr>
<td>Total FM, kg</td>
<td>14.2 ± 3.3</td>
</tr>
<tr>
<td>Glucose0h, mmol/l</td>
<td>9.6 ± 3.8</td>
</tr>
<tr>
<td>Glucose2h, mmol/l</td>
<td>15.8 ± 4.5</td>
</tr>
<tr>
<td>HbA1c, %</td>
<td>7.0 ± 1.0</td>
</tr>
</tbody>
</table>

Shown are means ± SD; n = 9 participants. BMI, body-mass index; WHR, waist-to-hip ratio; glucose0h, plasma-glucose before the oral glucose tolerance test (OGTT); glucose2h, plasma-glucose at the 2-h time point of the OGTT; HbA1c, glycosylated hemoglobin; LBM, lean body mass by dual-energy X-ray absorptiometry (DXA) scan; FM, fat mass by DXA scan.

catheters were placed in an antecubital vein on one side for blood sampling, in the contralateral antecubital vein for infusion of glucose, insulin, and stable isotopes, and finally in a dorsal hand vein for blood sampling. The catheterized hand was wrapped in a heating blanket to obtain arterialized venous blood for measurement of glucose and potassium during the subsequent clamp. The experiment was commenced by priming the glucose pool by injection of a bolus of 17.5 μmol/kg of [6,6-D2]glucose (Cambridge Isotopes Laboratories). This was followed by a continuous infusion (rate 0.4 μmol·kg⁻¹·min⁻¹).

Next, the participants received infusion with either rhIL-6 (Sandoz Pharmaceuticals, Basel, Switzerland) or placebo (20% human albumin). The placebo and rhIL-6 infusions (2.4 μg·h⁻¹·m⁻² dissolved in 20% human albumin) were started 1 h after the infusion with stable isotopes. Based on previous pilot experiments, the chosen dose of IL-6 would be expected to increase plasma IL-6 to ~100 pg/ml, which is within the range observed after physical exercise (9).

After one additional hour, the hyperinsulinemic-isoglycemic clamp was started and continued for 3 h. Because of the risk of inducing counterregulatory mechanisms when lowering plasma glucose, maintaining fasting plasma glucose levels (isoglycemia) was considered more appropriate rather than inducing euglycemia (5 mmol/l). The exact, individual value for isoglycemia was defined as fasting plasma glucose during rest on the morning of the first trial day. The same value was used as the target plasma glucose level during the hyperinsulinemic clamp on the second study day. Insulin (100 IE/ml Actrapid; Novo Nordisk Insulin) was infused continuously at a rate of 0.18 mU·min⁻¹·m⁻², and the plasma glucose concentration was kept stable by a coinfusion of glucose (200 g/1,000 ml, enriched with [6,6-D2]glucose to 2.5%) at a variable rate. During the 3-h clamp, the infusion rate of [6,6-D2]glucose was reduced to 0.08 μmol·kg⁻¹·min⁻¹. Arterialized blood was analyzed for glucose and potassium concentrations at intervals of 5 min during all 3 h of the clamp.

Additional blood samples (Fig. 1) were collected in EDTA-containing tubes and immediately centrifuged for 15 min at 3,500 rpm, and the plasma was stored at −80°C until further analysis.

Before and at the end of the insulin clamp, a biopsy from skeletal muscle was taken. Under sterile conditions and after subcutaneous injection of local anesthesia (lidocaine, 20 mg/ml; SAD) at the biopsy site, a 0.5-cm incision was made in the skin. With the use of a Bergström biopsy needle (3), a muscle biopsy from vastus lateralis was obtained. The biopsies were frozen in liquid nitrogen and stored at −80°C until further analysis.

Fig. 1. Overview of the study design. The numbers below the horizontal arrow represent time points (h). The hyperinsulinemic-isoglycemic clamp started at time point 0 h and continued for 3 h. The infusion with stable isotope-labeled glucose started 2 h before the clamp, whereas the infusion with interleukin-6 (IL-6) or placebo started 1 h before the clamp. The smaller, dark gray arrows indicate time points for blood sampling, the smaller, light gray arrows indicate indirect calorimetry, whereas the larger, dark gray arrows indicate time points for muscle biopsies.
Blood flow in the femoral artery was measured using Doppler ultrasonography (CFM 800; Vingmed Sound, Horten, Norway) as previously described (10).

Finally, expired air was sampled through a two-way nonrebreathing valve (Hans Rudolph, Kansas City, MO) for 5 min using an open-circuit indirect calorimetry system (Moxus modular VO2 system; AEI Technologies, Pittsburgh, PA). Time points for sampling of expired air were before the infusion of rhIL-6 or placebo (−1 h), just before start of the hyperinsulinemic-isoglycemic clamp (0 h), and then every 30 min until the end of the clamp (Fig. 1).

Biochemical assays. The measurements of leukocytes, cholesteroles, triglycerides, and HbA1C were performed using standard techniques at the Department of Clinical Biochemistry, Rigshospitalet University Hospital, Copenhagen, Denmark. Free fatty acid (FFA) and glycerol levels were determined using a colorimetric assay (Cobas Fara; Roche, Basel, Switzerland).

Hormone analysis. Commercially available ELISA kits were used for measurements of plasma IL-6 (no. HS600B; R&D Systems, Minneapolis, MN), TNF-α (no. HSTA00C; R&D Systems), soluble tumor necrosis factor receptor 2 (sTNFR2, no. DRT200; R&D Systems), cortisol (no. DSL-10–2000; DSL, Webster, TX), insulin (no. K6219; Dako, Glostrup, Denmark), and C-peptide (no. K6220; Dako). Measured intra-assay coefficient of variation values were 4, 4, 5, 5, 2, and 0.2% for measurements of plasma IL-6 (no. HS600B; R&D Systems, Minneapolis, MN), TNF-α (no. HSTA00C; R&D Systems), soluble tumor necrosis factor receptor 2 (sTNFR2, no. DRT200; R&D Systems), cortisol (no. DSL-10–2000; DSL, Webster, TX), insulin (no. K6219; Dako, Glostrup, Denmark), and C-peptide (no. K6220; Dako).

In addition, plasma samples were assayed for total GLP-1 immuno-reactivity as described previously (22), whereas total gastric inhibitory polypeptide (GIP) was measured using the COOH-terminally directed antiserum R65 (17). Detection limits for the two assays were around 1 pmol/l and intra-assay coefficient of variation below 6%.

Muscle glycogen content was measured fluorometrically as glycosyl units after acid hydrolysis, as previously described (23).

Stable isotopes. The plasma glucose enrichment was measured as previously described (26). The glucose turnover rate of appearance (Rg) and rate of disappearance (Rd) were calculated assuming steady state:

\[ \text{Baseline: } R_g = R_d = \frac{F_{\text{fus}}}{E_{\text{glucose}}} \]

\[ \text{Clamp: } R_{g(\text{endoogenous})} = \frac{F_{\text{total}} - \text{GIR}}{E_{\text{glucose}}} \]

\[ R_d = \frac{F_{\text{total}}}{E_{\text{glucose}}} \]

where \( F_{\text{fus}} \) is the infusion rate of glucose tracer (\( \mu \)mol·kg\(^{-1}\)·min\(^{-1} \)) in terms of lean body mass, \( E_{\text{glucose}} \) is the enrichment of glucose in plasma, \( F_{\text{total}} \) is the sum of \( F_{\text{fus}} \) and the [6,6-D\(_2\)]glucose infused by the clamp, GIR is the glucose infusion rate, and kilograms refers to lean body mass.

Western blotting. Human muscle lysate was prepared by homogenizing muscle tissue in ice-cold lysis buffer (50 mM Tris-HCl, pH 7.4) with phosphatase inhibitor cocktails 1 and 2 (Sigma-Aldrich) and Complete mini protease inhibitor cocktail (Roche, Mannheim, Germany) using a TissueLyser (Qiagen, Hilden, Germany). Protein lysates were centrifuged at 13,000 × g for 15 min at 4°C, and the supernatants were collected. Protein concentration was measured using a protein assay dye reagent (Bio-Rad Laboratories, Hercules, CA). The samples were mixed in a double-blinded system and afterward mixed with 4× Loading NuPAGE LDS Sample buffer (Invitrogen, Carlsbad, CA) and boiled before loading of 20 µg on 4–15% Criterion TGX Precast gels (Bio-Rad Laboratories). The gels were run for 35 min at 200 volts, and the protein was transferred on a membrane (Trans-Blot Turbo Midi Format; Bio-Rad Laboratories) using a semidry blotting system (Trans-Blot Turbo Transfer system; Bio-Rad Laboratories). The membranes were blocked for 1 h at room temperature in either 5% bovine serum albumin for phosphorylated STAT (pSTAT3) or 5% skimmed milk (for STAT3 and SOCS3) and subsequently incubated overnight at 4°C with primary antibodies (pSTAT3 no. 9145, STAT3 no. 9139, and SOCS3 no. 2923 from Cell Signaling Technology). The membranes were washed in TBS-T and incubated with a secondary antibody, anti-rabbit or anti-mouse IgG, for 1 h at room temperature (1:5,000; DakoCytomation, Glostrup, Denmark).

Before incubation with a new primary antibody the membranes were stripped for 2 h in stripping buffer [500 mM Tris-HCl, pH 6.7, 2% SDS, and 0.78% (wt/vol) β-mercaptoethanol; Sigma-Aldrich]. Protein bands were detected using SuperSignal West Femto chemiluminescent Substrate (Thermoscientific, Rockford, IL) and photographed using a charge-coupled device image sensor (ChemidocXR5; Bio-Rad Laboratories). Compared with protein standards (Dual Color and All Blue; Bio-Rad Laboratories), molecular weights of the bands identified as STAT3 and pSTAT3 were estimated to be 80 kDa, thus similar to the expected value. The estimated molecular mass of the bands identified as SOCS3 was 18 kDa, and therefore slightly lower than the expected value of 26 kDa. However, since only one band per lane was present on the gels, and the same Western blotting protocol for SOCS3 has been validated and published previously (27), the deviation between estimated and expected weight was considered tolerable. All membranes were incubated with 0.5% reactive brown 10 (Sigma-Aldrich) to correct for uneven protein loading and transfer. The membranes were analyzed and quantified using software Image Lab (version 3.0; Bio-Rad Laboratories).

Statistical analysis. Subject characteristics (Table 1) are presented as means ± SD, whereas the remaining data were normally distributed after logarithmic transformation, thus presented as geometric means and geometric SE. For variables with repeated measures, e.g., plasma glucose, the area under curve was calculated from time point 0 to 3 h during the hyperinsulinemic-isoglycemic clamps, and subsequently rhIL-6 and placebo trials were compared using paired Student’s t-tests. Variables with only two time points, e.g., STAT3, were evaluated using a two-way mixed model (PROC MIXED). The statistical analyses were performed on \( n = 9 \), with the exception of the biopsy results ( \( n = 7 \) ) because of lacking biopsy in one subject and exclusion of one outlier (visually and statistically) in all Western blot, and significance was accepted as \( P < 0.05 \). Data analysis was performed using SAS statistical software (release 9.3; SAS Institute).

RESULTS

Participants. Five participants received placebo on the first study day and rhIL-6 on the second study day, whereas the remaining four subjects received rhIL-6 first, then placebo. None of the participants experienced adverse side effects of the rhIL-6 infusion. By asking the participants after each study day, it was clear that the participants were unable to distinguish between the placebo and IL-6 study days.

Effects of rhIL-6 on immunological variables. As expected, plasma IL-6 levels increased in response to rhIL-6 infusion compared with placebo ( \( P < 0.001 \), Fig. 2A). Noteworthy, the measured plasma IL-6 levels were quite close to the aimed levels with a mean value of 85 (95% confidence interval: 72–101) pg/ml. Plasma TNF-α levels were not different between rhIL-6 and placebo study days (Fig. 2B). Also, plasma sTNFR2 levels were not changed by infusion with rhIL-6 (data not shown). Circulating levels of neutrophils ( \( P < 0.001 \), Fig. 2C) and monocytes ( \( P < 0.001 \), data not shown) increased markedly, whereas circulating levels of lymphocytes decreased ( \( P < 0.01 \), Fig. 2D) during rhIL-6 infusion compared with placebo infusion.

Effects of rhIL-6 on hormone levels. Also as expected, plasma cortisol levels increased in response to rhIL-6, but not
in response to placebo ($P < 0.001$, Fig. 3A). Plasma glucagon levels decreased during the hyperinsulinemic clamp (Fig. 3B), but similarly when comparing rhIL-6 and placebo trials. During the hyperinsulinemic clamp, plasma levels of insulin increased markedly, but to a lower level during the IL-6 study days ($P < 0.01$ between trials, Fig. 3C). Plasma C-peptide levels decreased during the clamp, but more in response to IL-6 ($P < 0.01$ between trials, Fig. 3D).

The plasma levels of the gastrointestinal hormones GLP-1 (Fig. 3E) and GIP (Fig. 3F) remained unchanged over time and were not affected by the rhIL-6 infusion.

**Effects of rhIL-6 on glucose metabolism.** Plasma glucose levels were maintained stable during the hyperinsulinemic-isoglycemic clamp and were similar during IL-6 and placebo study days (Fig. 4A). Compared with placebo, rhIL-6 did not change either GIR, $R_s$, or $R_d$ during the hyperinsulinemic-isoglycemic clamp (all $P > 0.5$, Fig. 4, B–D).

**Other metabolic effects of rhIL-6.** Plasma FFA (Fig. 4E) and glycerol (Fig. 4F) levels both decreased during the insulin clamp, with only a borderline significant difference between trials for plasma FFA ($P = 0.058$).

Whole body oxygen uptake, $\dot{V}O_2$, was $\sim 15\%$ higher during rhIL-6 infusion compared with placebo ($P < 0.01$, Fig. 5A). Similarly, expired carbon dioxide was higher during rhIL-6 study days compared with placebo ($P < 0.01$, Fig. 5B). The respiratory exchange ratio (RER) increased during the insulin clamp (data not shown), with no difference between trials.

Heart rate was higher during the rhIL-6 infusion than during the placebo infusion ($P < 0.001$, Fig. 5C). The systolic and the diastolic blood pressure both decreased $\sim 10$ mmHg during the first two hours on both trial days, then remained very stable during the hyperinsulinemic-isoglycemic clamp without any differences between placebo and rhIL-6 trials (data not shown).
Femoral blood flow was not different when comparing placebo and IL-6 trials ($P_{\text{H11005}} = 0.654$, Fig. 5D).

Body temperature (Fig. 5E) did not increase significantly in response to rhIL-6.

Effects of rhIL-6 in skeletal muscle. To investigate how downstream IL-6 signaling in skeletal muscle was affected by IL-6 infusion in patients with type 2 diabetes, we measured activation of STAT3 protein signaling (presented as pSTAT3/total STAT3) and SOCS3 in skeletal muscle tissue by Western blotting.

Regarding STAT3 phosphorylation, a significant interaction between trials (rhIL-6 vs. placebo) was observed ($P_{\text{H11005}} = 0.041$, Fig. 6, A and B), so STAT3 phosphorylation appeared to increase in response to rhIL-6 but to decrease in response to placebo during the hyperinsulinemic-isoglycemic clamp. On the other hand, neither SOCS3 protein expression ($P_{\text{H11005}} = 0.958$, Fig. 6, C and D) nor muscle glycogen content ($P_{\text{H11005}} = 0.191$, data not shown) was affected by the hyperinsulinemic clamp or rhIL-6 infusion.

**DISCUSSION**

We report the effect of IL-6 in vivo on insulin-stimulated glucose metabolism in patients with type 2 diabetes.

Although we observed clear immunological effects of IL-6 and an increase of plasma levels of cortisol, there was no effect of IL-6 on insulin-stimulated glucose uptake or endogenous glucose production. The latter finding is in accordance with some (30, 38) but not all (5) studies in healthy humans.

We hypothesized that IL-6 would increase the insulin-stimulated glucose uptake, since it has been reported that acute elevation of plasma IL-6 results in increased insulin-mediated glucose uptake in resting young, healthy men (5). In addition, previous studies using comparable IL-6 doses have reported lipolysis, fat oxidation (38), and decreased muscle protein turnover (37). We attempted to attain plasma IL-6 levels of 100 pg/ml and reached average levels at 85 pg/ml during the IL-6 infusion. This target value was chosen, since it falls within the range reported after prolonged physical exercise (9). Impor-

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**Fig. 3.** Effect of rhIL-6 infusion on plasma hormone concentrations during the hyperinsulinemic-isoglycemic clamp. Response over time is shown as curves, whereas the corresponding AUC are shown as bar graphs for cortisol (A), glucagon (B), insulin (C), C-peptide (D), glucagon-like peptide 1 (GLP-1, E), and gastric inhibitory polypeptide (GIP, F). Shown are geometric means with geometric SE. **$P < 0.01$** and ***$P < 0.001$***.
tantly, the employed dose of IL-6 was considered appropriate to induce effects both in the circulation and within target tissues. All of our participants, without exceptions, demonstrated robust increases in circulating neutrophils, monocytes, and cortisol in response to the IL-6 infusion, which is in accordance with previous findings (29). Although IL-6 autoantibodies are more prevalent in patients with type 2 diabetes compared with the general population (11, 14), the robust changes and the reported prevalence below 3% make it unlikely that autoantibodies interfered with the observed findings in the present study. Aiming for higher plasma IL-6 levels is associated with increased risk of developing symptoms, including fever as well as changes in glucagon and catecholamines (34, 35). These are all factors that potentially could influence glucose metabolism and should be considered during situations with very high IL-6 plasma levels, e.g., sepsis, but appear less relevant when focusing on the possible role of IL-6 per se in glucose metabolism in skeletal muscle of patients with type 2 diabetes.

A recent study reported that glucose uptake in vitro by myotubes from subjects with type 2 diabetes only responds to insulin, but not to IL-6. In contrast, IL-6 stimulates glucose uptake in cultured skeletal muscle cells from healthy subjects, with an additive effect to insulin (16). Also noteworthy, IL-6 increased STAT3 phosphorylation in both groups, whereas SOCS3 was unaffected. Our findings are in agreement with these in vitro findings, although the in vivo IL-6 concentrations were markedly lower than those previously used in vitro. Accordingly, one explanation could be that glucose metabolism in skeletal muscle in our subjects was less sensitive to IL-6 compared with healthy subjects, but a comparison with a group of age-matched subjects without type 2 diabetes would

![Graphs A to F showing glucose metabolism, free fatty acids (FFA), and glycerol during the hyperinsulinemic-isoglycemic clamp response over time.](http://ajpendo.physiology.org/)
add further information to this matter. In both healthy, young men (30) and patients with type 2 diabetes (25), infusion of moderate amounts of IL-6 alone has no effect on glucose metabolism in the resting, fasted state. In vitro, IL-6 increases glucose uptake in strips of skeletal muscle obtained from healthy subjects (12), but the much higher IL-6 concentrations used in vitro may explain this difference from the in vivo studies. It should be noted, however, that following physical exercise, IL-6 is produced and released from the contracting muscle (31), thus the IL-6 concentration within the contracting muscle tissue may be much higher than in the circulation. It is therefore possible that IL-6 within the exercising muscle may increase glucose uptake independent of insulin. In vivo, the increased glucose disposal by IL-6 in healthy subjects looks as if dependent on the presence of insulin (5) or physical exercise (8).

Although there was no difference between trials with regard to GIR, glucose Ra, and Rd, it is not possible to rule out that different results would be seen if different hyperinsulinemic clamp settings were used. We employed an isoglycemic clamp to minimize the effects of changing plasma glucose levels. Euglycemia (5 mmol/l plasma glucose) would implicate a marked reduction in basal plasma glucose, thus increasing the risk of inducing counterregulatory mechanisms as seen with hypoglycemia. Hyperglycemia, e.g., 15 mmol/l plasma glucose, would involve a decrease in basal plasma glucose for some participants, but an increase for other participants. Between 2 and 3 h of the hyperinsulinemic clamp, GIR appeared a bit higher on the IL-6 study days; however, it was still not significantly different when compared only with GIR data within this time interval. It could be speculated that a prolonged hyperinsulinemic clamp would have shown a difference between IL-6 and placebo, but previous data indicate that GIR is enhanced by concomitant IL-6 infusion within the first hour of the hyperinsulinemic clamp (5). The measured plasma insulin levels were higher in our study than in the aforemen-

![Graph A: Whole body oxygen uptake (VO2) vs. Time (h)](A)
![Graph B: Whole body expired CO2 (VCO2) vs. Time (h)](B)
![Graph C: Heart rate (beats/min) vs. Time (h)](C)
![Graph D: Femoral blood flow (ml/min) vs. Time (h)](D)
![Graph E: Temperature (ºC) vs. Time (h)](E)

Fig. 5. Effect of rhIL-6 infusion on physical characteristics during the hyperinsulinemic-isoglycemic clamp. Response over time is shown as curves, whereas the corresponding AUC are shown as bar graphs for whole body oxygen uptake (VO₂, A), whole body expired CO₂ (VCO₂, B), heart rate (C), femoral blood flow (D), and temperature (E). Shown are geometric means with geometric SE. **P < 0.01 and ***P < 0.001.
Fig. 6. Effect of rhIL-6 infusion on IL-6 signaling in skeletal muscle (n = 7) during the hyperinsulinemic-isoglycemic clamp. Representative Western blots for phosphorylated signal transducer and activator of transcription (pSTAT3), signal transducer and activator of transcription (STAT3, A), and suppressor of cytokine signaling 3 (SOCS3, C). The proteins were loaded in a double-blinded fashion, but in pairs of each subject’s first and second biopsy, at 0 and 3 h, respectively. Corresponding arbitrary units (AU) are shown as bar graphs for pSTAT3/STAT3 (B) and SOCS3 (D), and reactive brown (RB) stains for both membranes (E). Shown are geometric means with geometric SE. Corresponding boxes show P values of main effects obtained by 2-way mixed-model analysis. Plc, placebo infusion.
We recognize that a larger number of participants would decrease the risk of statistical type 2 errors; however, our data suggest that, even if a systemic increase of IL-6 does affect insulin-stimulated glucose metabolism in type 2 diabetes, this effect would probably be minimal. Of note, the employed number of participants in our study is comparable to the previous studies reporting effects of IL-6 infusion on glucose (5) and lipid (38) metabolism. Also, the crossover design whereby the participants were used as their own controls limited the disadvantage of a small sample size.

It should also be noted that the effect of an acute elevation of plasma IL-6 may be different from the chronically elevated IL-6 plasma levels in insulin resistance (19). Furthermore, IL-6 receptor α seems to be downregulated in skeletal muscle of subjects with type 2 diabetes (27), suggesting less sensitivity to IL-6 during conditions associated with chronically elevated IL-6 plasma levels.

We observed lower plasma insulin levels during the hyperinsulinemic clamp on IL-6 study days. Similar results have been found in subjects with type 2 diabetes exposed to IL-6 infusion without concurrent insulin infusion (25). Together with the increased plasma cortisol levels, it is intriguing that glucose disposal is maintained unaffected. It could be argued that this suggests increased insulin sensitivity by IL-6; at least, it further argues against circulating IL-6 being involved in skeletal muscle insulin resistance. Although the lower C-peptide levels during IL-6 infusion indicate lower insulin secretion, it is also possible that insulin clearance is affected by IL-6. Further studies comparing glucose disposal with similar insulin and cortisol levels between IL-6 and placebo trials would be required to answer this question.

In response to IL-6, there were no changes in plasma levels of the hormones GIP and GLP-1, neither over time or when compared with placebo. It has been reported that IL-6 stimulates insulin secretion via GLP-1 induction in vitro (7). Instead, we observed a decrease in plasma levels of insulin. Also, despite the marked suppression of plasma levels of FFA due to the infusion of insulin, we observed that the levels appeared a bit higher during IL-6, although not significant (P = 0.058). Of note, increased lipolysis has been observed with rhIL-6 infusion in both healthy and type 2 diabetes subjects without concomitant insulin infusion (25, 38).

As shown before in healthy subjects (5), we found that even moderate doses of IL-6 increase the oxygen consumption in subjects with type 2 diabetes. These findings emphasize that IL-6 has a role in metabolism. Nevertheless, because IL-6 enhances the release of other cytokines, it is not clear which aspects of the metabolic response are caused by IL-6 per se, or by other cytokines or hormones (33). The plasma IL-6 levels in the present study are, however, unlikely to produce major changes in other hormones than cortisol. The increased oxygen consumption was matched by increased amounts of expired carbon dioxide, although RER increased throughout the hyperinsulinemic clamp to reach ~0.9, indicating carbohydrates as the predominant energy source. The RER was similar when comparing IL-6 and placebo, but the overall increase in energy consumption suggests that more carbohydrates were used for oxidation in response to IL-6, which is in accordance with the previous study on healthy subjects (5). We could not, however, detect changes in glucose uptake, muscle glycogen content, or endogenous glucose production. One explanation could be utilization of glycogen stored in liver, which has been observed previously in animals using higher doses of IL-6 (32). In summary, our data show that IL-6 has preserved immunoregulatory effects in humans with type 2 diabetes, but no effect on glucose metabolism in skeletal muscle, despite preserved activation of STAT3. The observed decrease in plasma levels of insulin and increase in cortisol plasma levels producing a more insulin-resistant environment may, however, blunt some of the effect of IL-6. We find no evidence that short-term elevation of IL-6 attenuates insulin sensitivity in skeletal muscle in type 2 diabetes.

In conclusion, we did not find that IL-6 had an effect on glucose metabolism in type 2 diabetic skeletal muscle despite preserved systemic and local effects. Accordingly, our hypothesis was rejected. While different factors may explain our results, one explanation could be an impaired effect of IL-6 on the glucose metabolism within skeletal muscle. Our findings add useful data to previous in vitro studies regarding the effect of IL-6 on the insulin-stimulated glucose metabolism in vivo in type 2 diabetic humans.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS


REFERENCES