Assessment of Hepatic Insulin Action in Obese Type 2 Diabetic Patients

Peter Staehr, Ole Hother-Nielsen, Klaus Levin, Jens Juul Holst, and Henning Beck-Nielsen

Defects in hepatic insulin action in type 2 diabetes and its possible underlying mechanisms were assessed in euglycemic-hyperinsulinemic clamp studies, using improved tracer methods (constant specific activity technique). Ten obese diabetic patients (age 54 years, BMI 29 ± 0.5 kg/m²) and ten matched control subjects were studied at baseline (after an overnight fast) and during insulin infusions of 20- and 40-mU·m⁻²·min⁻¹. In the diabetic patients, plasma glucose levels were normalized overnight before the studies by low-dose insulin infusion. Hepatic sinusoidal insulin levels were estimated, and plasma levels of free fatty acids (FFAs) and glucagon were determined to assess the direct and indirect effects of insulin on hepatic glucose production (HGP) in type 2 diabetes. Baseline rates of HGP (86 ± 3 vs. 76 ± 3 mg·m⁻²·min⁻¹, P < 0.05) were slightly elevated in the diabetic patients compared with control subjects, despite much higher hepatic sinusoidal insulin levels (26 ± 3 vs. 12 ± 2 mU/l, P < 0.001). Consequently, a marked defect in the direct (hepatic) effect of insulin on HGP appeared to be present at low insulin levels. However, in response to a small increase in baseline hepatic sinusoidal insulin levels of 11 mU/l (26 ± 3 to 37 ± 3 mU/l, P < 0.05) in the 20-mU clamp, a marked suppression of HGP was observed in the diabetic patients (86 ± 3 to 32 ± 5 mg·m⁻²·min⁻¹, P < 0.001), despite only minimal changes in FFAs (0.33 ± 0.05 to 0.25 ± 0.05 mmol/l, NS) and glucagon (14 ± 1 to 11 ± 2 pmol/l, P < 0.05) levels, suggesting that the impairment in the direct effect of insulin can be overcome by a small increase in insulin levels. Compared with control subjects, suppression of HGP in the diabetic patients was slightly impaired in the 20-mU clamp (32 ± 5 vs. 22 ± 4 mg·m⁻²·min⁻¹, P < 0.05) but not in the 40-mU clamp (25 ± 2 vs. 21 ± 3 mg·m⁻²·min⁻¹, NS). In the 20-mU clamp, hepatic sinusoidal insulin levels in the diabetic patients were comparable with control subjects (37 ± 3 vs. 36 ± 3 mU/l, NS), whereas both FFA and glucagon levels were higher (i.e., less suppressed) and correlated with the rates of HGP (R = 0.71, P < 0.02; and R = 0.69, P < 0.05, respectively). Thus, at this insulin level impaired indirect (extrahepatic) effects of insulin seemed to prevail. In conclusion, hepatic insulin resistance is present in obese type 2 diabetic patients but is of quantitative significance only at low physiological insulin levels. Defects in both the direct and indirect effects of insulin on HGP appear to contribute to this resistance. Diabetes 50:1363–1370, 2001

Hepatic insulin resistance is considered to play an important role in the pathophysiology of type 2 diabetes (1–4). In studies on this issue, the euglycemic-hyperinsulinemic clamp combined with ³H-glucose infusions has played an important role. Such studies have suggested that insulin-mediated suppression of HGP is impaired at both low and high physiological plasma insulin levels in lean (2,5,6) as well as in obese (7–13) type 2 diabetic patients. Furthermore, at basal and low physiological insulin levels (<30–40 mU/l), the impairment in suppression of HGP has appeared to be quantitatively similar to or even larger than the defect in the stimulation of peripheral glucose disposal (Rₚ) (2,3,7). Consequently, hepatic insulin resistance has been suggested to be a major determinant of fasting hyperglycemia in type 2 diabetes (1–3).

It has become apparent, however, that the conventional tracer-infusion method, which has been used to quantify glucose metabolism in the majority of previous clamp studies, may overestimate defects in the suppression of HGP and underestimate defects in the stimulation of Rₚ in type 2 diabetic patients (14–16). This methodological problem may have biased evaluation of the relative importance of hepatic versus peripheral insulin resistance in type 2 diabetes (16). Assessment of insulin action in clamp studies is markedly improved, though, when plasma specific activity is maintained constant using labeled glucose infusates (14–17).

The aim of the present study was therefore to quantify defects in insulin action on HGP and Rₚ in obese type 2 diabetic patients, using improved tracer methods. Furthermore, hepatic sinusoidal insulin levels were estimated and plasma levels of free fatty acids (FFAs) and glucagon were determined to characterize impairments in the direct (hepatic) and indirect (extrahepatic) effects of insulin, respectively, on HGP in these patients.

RESEARCH DESIGN AND METHODS
Ten moderately obese type 2 diabetic patients and ten control subjects matched for age and BMI participated in the studies. Clinical characteristics are given in Table 1. None of the diabetic patients had received insulin treatment. Four were treated with diet alone, whereas six patients were on oral treatment with metformin or sulfonylurea, which was withdrawn at least 1 week before each study. The patients were without signs of diabetic retinopathy, nephropathy, neuropathy, or macrovascular complications. The control subjects had normal glucose tolerance and no family history of diabetes. All subjects had normal results on screening blood tests of hepatic
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TABLE 1
Clinical characteristics of type 2 diabetic patients and control subjects

<table>
<thead>
<tr>
<th></th>
<th>Diabetic patients</th>
<th>Control subjects</th>
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<tbody>
<tr>
<td>n (M/F)</td>
<td>8/2</td>
<td>8/2</td>
</tr>
<tr>
<td>Age (years)</td>
<td>54 ± 3</td>
<td>55 ± 3</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>29 ± 0.5</td>
<td>29 ± 1</td>
</tr>
<tr>
<td>Waist-to-hip ratio</td>
<td>0.94 ± 0.02</td>
<td>0.96 ± 0.02</td>
</tr>
<tr>
<td>Body surface area (m²)</td>
<td>1.98 ± 0.04</td>
<td>1.96 ± 0.06</td>
</tr>
<tr>
<td>HbA1c (%)</td>
<td>8.0 ± 0.4*</td>
<td>5.4 ± 1.1</td>
</tr>
<tr>
<td>Fasting plasma glucose (mmol/l)</td>
<td>9.3 ± 0.6*</td>
<td>5.2 ± 0.1</td>
</tr>
<tr>
<td>Fasting plasma insulin (mU/l)</td>
<td>16 ± 2*</td>
<td>6 ± 1</td>
</tr>
<tr>
<td>Fasting plasma C-peptide (pmol/l)</td>
<td>0.85 ± 0.11*</td>
<td>0.62 ± 0.07</td>
</tr>
<tr>
<td>Fasting plasma C-peptide (pmol/l)</td>
<td>1.013 ± 134*</td>
<td>509 ± 50</td>
</tr>
<tr>
<td>Diabetes duration (years)</td>
<td>7 ± 2</td>
<td>—</td>
</tr>
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</table>

Data are means ± SE. *P < 0.05 vs. control subjects.

and renal function. The purpose and risks of the study were carefully explained before informed consent to participate was obtained, and the protocol of the study was reviewed and approved by the regional scientific ethical committee.

Oral glucose tolerance test. Oral glucose tolerance tests (OGTTs) were performed after a 14 h overnight fast. All subjects ingested 75 g glucose dissolved in 300 ml water, and blood samples were drawn at timed intervals from 20 min before until 180 min after the glucose load for determination of plasma glucose, insulin, C-peptide, and FFA levels.

Euglycemic clamp studies. All clamp studies were performed on separate days, with a 4-week interval. The diabetic patients were admitted in the evening before each clamp study. Their plasma glucose concentration was normalized (to 5 mmol/l) overnight by a low-dose insulin infusion, which was adjusted based on frequent plasma glucose measurements. The insulin infusion was started at 21:00 h and continued until the end of the baseline period at 10:00 h the next day. The rate of infusion was not altered after 07:00 h.

The studies were initiated at 08:00 h, after a 14 h overnight fast. Two catheters were inserted: one in an antecubital vein for infusion of insulin, glucose, and tracer and another in a contralateral dorsal hand vein for collection of blood samples. This hand was placed in a heated Plexiglass box to obtain arterialized venous blood. At 08:00 h a primed-constant intravenous infusion of [3-3H]glucose (DuPont-New England Nuclear, Boston, MA) was initiated and continued throughout the 5-h study using a precision syringe pump (Harvard Apparatus, Natick, MA). The ratio between priming dose and constant infusion was 100:1. To achieve a common level of basal specific activity, the tracer infusion was adjusted for surface area by adjustment of the infusate volume as previously described (15–17). [3-3H]Glucose was added to the glucose infusates to maintain plasma specific activity constant at baseline levels during the clamp. Optimal labeling of the glucose infusate was calculated as previously described (15–17).

After a 120-min baseline tracer equilibration period, insulin (Actrapid; Novo Nordisk, Bagsvaerd, Denmark) was infused at rates of either 20 or 40 mU·m⁻²·min⁻¹ to obtain insulin levels comparable with those observed at baseline in the diabetic patients. The clamp period lasted 180 min, during which plasma glucose was clamped at baseline levels using a variable infusion of 20% glucose, based on bedside plasma glucose measurements (Glucose Analyzer II; Beckmann Instruments, Fullerton, CA) every 20–20 min.

Blood samples were collected at timed intervals for determination of FFA levels (EDTA-treated tubes), for determination of plasma glucose and plasma [3-3H]glucose activity (heparin fluoride–treated tubes), and for determination of plasma insulin, C-peptide, and glucagon (heparin-trasylol–treated tubes). Blood samples were immediately centrifuged, and plasma was stored at −20°C for later analysis.

As a minus exogenous glucose infusion rates.

Portail vein insulin concentration in the last 30 min of the basal period (P0) in control subjects was calculated as P0 = 2.4 × A0, where A0 denotes arterial (arterialized venous) insulin concentration at time 0, assuming a portal venous/peripheral venous insulin gradient of 2.4 in the postabsorptive state (19). Portail vein insulin concentration at time t was calculated as PIT = [A(t) - [(P0-A0) × (Ct/CP0)], where CPt and CP0 are arterial C-peptide concentrations at time t and during the last 20 min of the baseline period, respectively. However, because the diabetic patients were infused with insulin overnight, the values for P0, A0, and CP0 observed on the OGTT day were used, assuming that insulin and C-peptide clearance was unaffected by overnight insulin infusion. Hepatic sinusoidal insulin (HSI) levels were estimated as: HSI = (0.5 × A0) + (0.5 × PI) assuming that the hepatic artery accounts for 20% and the portal vein accounts for 80% of hepatic blood flow (19).

Statistical analysis. Data are presented as means ± SE. Differences were evaluated using Wilcoxon’s matched-pairs signed-rank test for paired data and the Mann-Whitney U test for unpaired data. Statistical tests and least-squares regression analysis were performed using Stata 6 software (Stata Corporation, Chicago). A significance level of 5% was chosen.

RESULTS
OGTTs. Both fasting plasma glucose (9.3 ± 0.6 vs. 5.2 ± 0.1 mmol/l, P < 0.01), insulin (16 ± 2 vs. 6 ± 1 mU/l, P < 0.01), and C-peptide (1,013 ± 134 vs. 509 ± 50 pmol/l, P < 0.01) concentrations were higher in the diabetic patients than in control subjects. At 120 min, the glucose concentration was still markedly increased in diabetic patients (15 ± 2.3 mmol/l), whereas in control subjects it had returned to basal values. The insulin response to glucose was delayed in the diabetic patients, but with the same peak insulin concentration (54 ± 12 mU/l at 100 min) as in the control group (56 ± 7 mU/l at 50 min). These peak insulin concentrations were similar to the steady-state insulin concentrations observed in the 40-mU clamp in both groups, indicating that the clamp studies were performed within physiologically relevant insulin levels in these subjects.

Plasma glucose and glucose specific activity. Baseline plasma glucose concentration in control subjects was 5.2 ± 0.1 mmol/l and was maintained constant throughout the clamp period (Fig. 1). In the diabetic patients, euglycemia was present from 01:00 h in the night. At 08:00 h plasma glucose was 5.2 ± 0.2 mmol/l (NS vs. control subjects) and was maintained constant during clamp studies (Fig. 1).

Plasma specific activity remained constant in both control subjects and diabetic patients (Fig. 1). During the last 30 min of the baseline and the clamp period, plasma specific activity averaged 1,138 ± 44 and 1,246 ± 80, 1,160 ± 59 and 1,138 ± 47, and 1,179 ± 37 and 1,158 ± 20 cpm/mg in 10, 20, and 40-mU clamps, respectively, in control subjects, and 1,011 ± 27 and 1,062 ± 71, and 1,110 ± 48 and 1,175 ± 41 cpm/mg during 20- and 40-mU clamps, respectively, in diabetic patients (NS, baseline versus clamp).

Insulin, C-peptide and estimated hepatic sinusoidal insulin (HSI) levels. In control subjects, plasma insulin concentrations increased from 6 ± 1 mU/l at baseline to 18 ± 1, 30 ± 2, and 64 ± 5 mU/l during the 10, 20, and 40-mU clamp-studies, respectively (Fig. 1). In diabetic patients, the baseline insulin level (after overnight insulin infusions) was slightly higher than the fasting insulin level on the OGTT day (21 ± 3 vs. 16 ± 2 mU/l, P < 0.05), but similar to the insulin level in the 10 mU clamp in control...
subjects (21 ± 3 vs. 18 ± 6 mU/l, NS). In the 20- and 40-mU clamps, insulin levels were similar in diabetic patients and control subjects (31 ± 6 vs. 30 ± 2 and 59 ± 6 vs. 64 ± 3 mU/l, NS) (Fig. 1).

In control subjects, fasting C-peptide levels were suppressed from 509 ± 50 to 375 ± 60, 334 ± 44, and 378 ± 51 pmol/l during insulin infusion in the 10-, 20-, and 40-mU clamps, respectively (all \( P < 0.05 \), basal vs. clamp).

Fasting C-peptide in the diabetic patients was markedly lower after overnight insulin infusion than on the OGTT day (270 ± 43 vs. 1,013 ± 134 pmol/l, \( P < 0.001 \)), whereas there was no significant further reduction in C-peptide levels during either 20- or 40-mU clamps (267 ± 47 to 255 ± 43 and 273 ± 40 to 206 ± 51 pmol/l, NS).

Estimated HSI levels are shown in Fig. 2. Fasting HSI in the control subjects increased from 12 ± 2 to 23 ± 2, 36 ± 3, and 60 ± 4 mU/l in the 10-, 20-, and 40-mU clamps, respectively (all \( P < 0.01 \) vs. previous level). On the OGTT day, the fasting HSI level in the diabetic patients was 35 ± 3 mU/l, which was reduced by overnight insulin infusion to a baseline level of 26 ± 3 mU/l (\( P < 0.05 \) vs. OGTT day). The baseline HSI level in the diabetic patients was twice as high as baseline HSI in the control subjects (26 ± 3 vs. 12 ± 2 mU/l, \( P < 0.01 \)) but was similar to HSI in the 10-mU clamp (26 ± 3 vs. 23 ± 2 mU/l, NS) (Fig. 2). In the 20- and 40-mU clamps, HSI levels were similar in the diabetic and control group (37 ± 3 vs. 36 ± 3 and 64 ± 3 vs. 69 ± 4 mU/l, NS).

**Plasma FFAs and glucagon concentrations.** Dose-response curves for FFA and glucagon concentrations versus peripheral insulin levels are shown in Fig. 3.

In control subjects, baseline FFA concentrations were suppressed from 0.64 ± 0.08 to 0.13 ± 0.05, 0.10 ± 0.05, and 0.05 ± 0.02 mmol/l in the 10-, 20- and 40-mU clamps, respectively.

In the diabetic patients, the fasting FFA level was 0.85 ± 0.12 mmol/l on the OGTT day, which was higher than baseline FFA in the control subjects (\( P < 0.05 \)). At baseline, following overnight insulin infusion, FFA levels were markedly reduced to 0.33 ± 0.05 mmol/l and were further reduced in the 20- and 40-mU clamps to 0.25 ± 0.05 and 0.11 ± 0.02 mmol/l, respectively, with only the latter reduction being significant compared with baseline (\( P < 0.05 \)). Compared with control subjects, FFA levels were higher in the diabetic patients in the 20-mU clamp (0.25 ± 0.05 vs. 0.10 ± 0.05, \( P < 0.01 \)) but not in the 40-mU clamp (0.11 ± 0.02 vs. 0.05 ± 0.02, NS).

The fasting glucagon concentration of 11 ± 1 pmol/l in control subjects was suppressed to 5 ± 1, 4 ± 1, and 5 ± 1 pmol/l in the 10-, 20- and 40-mU clamps, respectively (all \( P < 0.01 \) vs. baseline).

Despite overnight insulin infusion, the baseline glucagon level in the diabetic patients remained slightly higher than in control subjects (14 ± 1 vs. 11 ± 1 pmol/l, \( P < 0.05 \)). Glucagon levels were suppressed to 11 ± 2 and 9 ± 2 pmol/l (both \( P < 0.05 \) vs. baseline) in the 20- and 40-mU clamps, respectively. Glucagon levels were therefore higher in diabetic patients than in control subjects in both the 20- and the 40-mU clamp studies (\( P < 0.05 \)).
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The dose-response curves for HGP and \( R_d \) versus peripheral insulin levels during the last 30 min of the baseline and clamp periods are given in Fig. 4.

Baseline rates of HGP in the diabetic patients were 13% higher than in the control subjects (86 ± 3 vs. 76 ± 3 mg·m\(^{-2}\)·min\(^{-1}\), \( P < 0.05 \)). HGP in the control subjects was suppressed to 25 ± 3, 22 ± 4, and 21 ± 3 mg·m\(^{-2}\)·min\(^{-1}\) in the 10-, 20- and 40-mU clamps, respectively (all \( P < 0.01 \) vs. baseline). Suppression of HGP in the diabetic patients was impaired in the 20-mU clamp compared with control subjects (32 ± 5 vs. 22 ± 4 mg·m\(^{-2}\)·min\(^{-1}\), \( P < 0.05 \)), whereas there was no significant difference in the 40-mU clamp (25 ± 2 vs. 21 ± 3 mg·m\(^{-2}\)·min\(^{-1}\), NS). Of note, the ability of a small increase in baseline insulin to suppress HGP was similar in the groups. Thus, the 10-mU clamp in the control subjects and the 20-mU clamp in the diabetic patients resulted in comparable increments in peripheral insulin levels during the last 30 min of the clamp periods.

In the 40-mU clamp, the increase in \( R_d \) above baseline of +58 ± 8 mg·m\(^{-2}\)·min\(^{-1}\) was quantitatively similar to the suppression of HGP of −51 ± 3 (NS), while at higher insulin levels in the 20- and 40-mU clamps, the effect on \( R_d \) prevailed (+128 ± 12 vs. −54 ± 2 and +219 ± 14 vs. −55 ± 3 mg·m\(^{-2}\)·min\(^{-1}\), both \( P < 0.05 \)).

Comparing the groups at similar peripheral as well as hepatic insulin levels (baseline and 20-mU and 40-mU clamps in the diabetic patients vs. 10-, 20-, and 40-mU clamps, respectively, in the control subjects), glucose \( R_d \) was lower in the diabetic patients than in control subjects at all three levels (86 ± 4 vs. 134 ± 14, 123 ± 18 vs. 204 ± 16, and 171 ± 28 vs. 295 ± 17 mg·m\(^{-2}\)·min\(^{-1}\), all \( P < 0.05 \)). In contrast, impaired suppression of HGP was present at the two low insulin levels (86 ± 3 vs. 25 ± 3 and 32 ± 5 vs. 22 ± 4 mg·m\(^{-2}\)·min\(^{-1}\), both \( P < 0.05 \)) but not at the high insulin level in the 40-mU clamp (25 ± 3 vs. 21 ± 3 mg·m\(^{-2}\)·min\(^{-1}\), NS). Therefore, the quantitative defect in the insulin-mediated stimulation of \( R_d \) of 48, 81, and 124 mg·m\(^{-2}\)·min\(^{-1}\) appeared comparable with or larger than the defect in suppression of HGP of 61, 10, and 4 mg·m\(^{-2}\)·min\(^{-1}\).

**DISCUSSION**

In the present study, labeled glucose infusates were applied in euglycemic clamp studies of insulin action in type 2 diabetic patients (Fig. 5), whereas, as expected, there was no such relationship between HGP and either peripheral or HSI levels (data not shown). In the 40-mU clamp, the relationship between HGP and plasma glucagon levels in the diabetic patients persisted (\( R = 0.83, P < 0.01 \)), while HGP and FFAs were not related (\( R = 0.19, P = 0.64 \) (Fig. 5).

**Peripheral glucose disposal (\( R_d \)).** Similar to HGP, baseline rates of \( R_d \) were higher in the diabetic patients than in control subjects (86 ± 4 vs. 77 ± 3 mg·m\(^{-2}\)·min\(^{-1}\), \( P < 0.05 \)). \( R_d \) in the control subjects increased to 134 ± 14, 204 ± 16, and 295 ± 17 mg·m\(^{-2}\)·min\(^{-1}\) in the 10-, 20- and 40-mU clamps, respectively (all \( P < 0.01 \) vs. previous insulin level). In both the 20- and the 40-mU clamps, \( R_d \) was lower in the diabetic patients than in control subjects (123 ± 18 vs. 204 ± 16 and 171 ± 28 vs. 295 ± 17 mg·m\(^{-2}\)·min\(^{-1}\), both \( P < 0.01 \)).

**Comparison of the quantitative effect of insulin on HGP and \( R_d \).** In the control subjects in the 10-mU clamp, the increase in \( R_d \) above baseline of +58 ± 8 mg·m\(^{-2}\)·min\(^{-1}\) was quantitatively similar to the suppression of HGP of −51 ± 3 (NS), while at higher insulin levels in the 20- and 40-mU clamps, the effect on \( R_d \) prevailed (+128 ± 12 vs. −54 ± 2 and +219 ± 14 vs. −55 ± 3 mg·m\(^{-2}\)·min\(^{-1}\), both \( P < 0.05 \)). In both the 20- and the 40-mU clamps, \( R_d \) was lower in the diabetic patients than in control subjects (123 ± 18 vs. 204 ± 16 and 171 ± 28 vs. 295 ± 17 mg·m\(^{-2}\)·min\(^{-1}\), both \( P < 0.01 \)).
This tracer method has been shown to improve assessment of glucose metabolism by maintaining a constant level of plasma specific activity, and as shown in Fig. 1, specific activity was in fact maintained essentially constant. Small deviations (up to 10% from baseline) did occur during insulin infusion, but we have previously shown (17) that a deviation of this magnitude does not affect the quantification of HGP and $R_d$ to any appreciable extent.

Using this improved tracer method, Pigon et al. (20) recently demonstrated that hepatic insulin action is normal in lean type 2 diabetic patients with modest peripheral insulin resistance and normal fasting insulin levels. In the present study, on the other hand, we studied moderately obese diabetic patients with marked peripheral insulin resistance and fasting hyperinsulinemia, and the data indicate the presence of hepatic insulin resistance in these patients since suppression of HGP was impaired in the 20-mU clamp. However, contrary to the majority of previous studies (6–11), suppression of HGP was not impaired at high physiological insulin levels in the 40-mU clamp. Furthermore, the quantitative defect in suppression of HGP in type 2 diabetic patients in response to insulin infusions of ~20 mU·m$^{-2}$·min$^{-1}$ has frequently appeared to be similar to or even larger than the quantitative defect in the stimulation of $R_d$ (7–11). In contrast, the defect in suppression of HGP of 10 mg·m$^{-2}$·min$^{-1}$ (32 vs. 22 mg·m$^{-2}$·min$^{-1}$) in the diabetic patients versus the control subjects in the present study was minimal compared with the large defect in the stimulation of $R_d$ of 81 mg·m$^{-2}$·min$^{-1}$ (123 vs. 204 mg·m$^{-2}$·min$^{-1}$). Hence, even though suppression of HGP by a small increase in basal insulin levels is attenuated in obese type 2 diabetic patients, this defect seems of limited quantitative importance. Turk et al. (21) also reported impaired suppression of HGP in type 2 diabetic patients in response to an insulin infusion of ~20 mU·m$^{-2}$·min$^{-1}$ (using the constant specific activity method), but in that study the defect was larger, with threefold higher (i.e., less suppressed) rates of HGP in diabetic patients than in control subjects. This difference, however, may be attributable to the fact that the control subjects in the Turk et al. study were only slightly obese (BMI 26 kg/m$^2$) and displayed a more marked suppression of HGP (84% of basal) than our obese control subjects (71% of basal), since suppression of HGP in the diabetic patients was similar in the two studies (~60% of basal).

The impression of a quantitatively less important role of hepatic insulin resistance than generally assumed did not seem to be due to a more pronounced suppression of HGP in the diabetic patients than observed in studies with conventional tracer methods (5–13). Rather, it was the result of a less pronounced suppression of HGP in the obese control subjects than previously reported (5–13). This view is consistent with the observation from methodological studies that the conventional tracer method overestimates suppression of HGP by insulin in proportion to the rate of glucose infusion during clamp studies (16). Therefore, clamp studies in control subjects who require high rates of glucose infusion are affected more than that shown in studies of insulin-resistant diabetic patients who require low rates of glucose infusion. In fact, when healthy subjects are studied with conventional tracer methods, HGP tends to be almost completely suppressed by insulin infusions of ~20 mU·m$^{-2}$·min$^{-1}$, and at high physiological insulin levels, negative rates of HGP are often observed (5–13,15,16). In contrast, in the present study, HGP was only suppressed by 73% of basal even in the 40-mU clamp, and no negative rates were encountered, suggesting that labeled glucose infusates improved the assessment of HGP. Nevertheless, although hepatic insulin action was only marginally impaired at the two clamp levels, the baseline determinations provide evidence of a marked defect in insulin-mediated suppression of HGP in the diabetic patients. Baseline HGP was 13% higher than in control subjects, despite the fact that baseline plasma insulin levels were much higher in the diabetic patients than in control subjects.

![FIG. 5. Relationship between HGP and plasma levels of FFA (left panels) and glucagon (right panels) in the 20-mU clamp (A and B) and the 40-mU clamp (C and D) in type 2 diabetic patients.](image-url)
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subjects (21 vs. 6 mU/l). Based on the dose-response curve in the control subjects, a 65% reduction in HGP would be expected in the diabetic patients if hepatic insulin sensitivity had been normal. The 13% elevation is in good agreement with the 0–20% increase in basal HGP in type 2 diabetic patients studied at their prevailing hyperglycemic level (5,22–26). This defect of insulin action has generally been considered to be of major importance in the pathophysiology of fasting hyperglycemia in type 2 diabetes (1–3,7,27). In contrast, it has been argued that fasting hyperinsulinemia would not be expected to increase fasting rates of $R_d$ to any appreciable extent in these patients, because insulin levels below ~30 mU/l have not seemed to be able to increase $R_d$ significantly in either lean (7) or obese (12,28) nondiabetic subjects. Recently, however, reevaluation of insulin action in young, lean healthy subjects by use of improved tracer methods have suggested that stimulation of $R_d$ plays a more important role at low insulin levels than previously thought (16). Thus, even a small increase in plasma insulin from 6 to 12 mU/l was shown to stimulate $R_d$. Furthermore, the increment in $R_d$ was quantitatively similar to the suppression of HGP (16). The present study extends these observations to middle-aged, moderately obese control subjects. At an insulin level of 18 mU/l (10-mU clamp), $R_d$ was markedly increased by 57 mg·m⁻²·min⁻¹ (from 77 to 134 mg·m⁻²·min⁻¹), which was quantitatively similar to the suppression of HGP by 51 mg·m⁻²·min⁻¹ (from 76 to 25 mg·m⁻²·min⁻¹). In the diabetic patients, baseline peripheral insulin level was comparable with the 10-mU clamp in control subjects (21 vs. 18 mU/l). Hence, given the observed near-normal baseline rates of HGP and $R_d$ (86 mg·m⁻²·min⁻¹), it appears that impairments not only in the suppression of HGP but also in the stimulation of $R_d$ by the prevailing fasting insulin level are present in these patients. Our data therefore suggest that defects in both hepatic and peripheral insulin action may be of importance at low insulin levels in moderately obese insulin-resistant type 2 diabetic patients. Furthermore, the defect in peripheral insulin action seems to be quantitatively comparable to the defect in suppression of HGP (48 vs. 61 mg·m⁻²·min⁻¹), which may be of relevance to the discussion on the relative importance of hepatic and peripheral insulin resistance in the pathophysiology of fasting hyperglycemia in type 2 diabetes (1–4).

It is well established that insulin regulates HGP through both direct and indirect effects (19,29), and defects in both of these pathways may have contributed to the observed hepatic insulin resistance. The direct effect is considered to represent the impact of HSI on the hepatocytes, mainly to suppress hepatic glycogenolysis (19). The indirect effect, on the other hand, is mediated through reduction of plasma FFA by inhibiting lipolysis (30), thereby reducing rates of gluconeogenesis (31), and through reduction of plasma glucagon levels by inhibition of its secretion from the pancreatic α-cells (29). In the present study, overnight insulin infusion appeared to affect the baseline levels of mediators of both the indirect and direct effects in the diabetic patients. Baseline FFA was 61% lower than in the hyperglycemic state (as determined on the OGTT day), and, consequently, baseline FFA levels were markedly lower in the diabetic patients than in control subjects (Fig. 3). In contrast, even though baseline HSI levels were 25% lower than on the OGTT day, HSI in the diabetic patients remained twice as high as in the control subjects (Fig. 2). The inability of such high HSI levels to suppress baseline HGP even in the presence of low FFA levels strongly indicate an impaired direct suppressive effect of insulin on HGP. On the other hand, in response to a small (10 mU/l) increase in baseline HSI in the 20-mU clamp, a substantial direct effect appeared to be present in the diabetic patients, since HGP was markedly suppressed despite virtually no change in FFA or glucagon levels (i.e., no indirect effects of insulin). Taken together, these findings would seem to suggest a quite steep dose-response curve for the direct effect of HSI on hepatic glycogenolysis in the diabetic patients. In this respect it is interesting that, from elegant studies in nondiabetic dogs, Cherrington et al. (19) have suggested glycogenolysis to be highly insulin-sensitive, and have shown that fasting rates of glycogenolysis are markedly suppressed by even small increments in basal HSI (19). The current data may thus be interpreted as indicating that the steepness of the dose-response effect of insulin on glycogenolysis is preserved in type 2 diabetic patients, but with a marked rightward shift. Consequently, a reduced sensitivity at the level of the hepatocyte appears to be present, whereas the maximal response within physiological insulin concentration limits (in the 40-mU clamp) seems to be intact.

The above considerations may also, at least partly, explain why fasting rates of HGP remained nonsuppressed despite overnight insulin infusion. Thus, the 25% reduction in baseline HSI after overnight insulin infusion represents a decrease in the direct effect of insulin, whereas the 61% lower FFA level represents an increase in the indirect effects of insulin. An increased indirect effect of insulin may therefore have counterbalanced a reduced direct effect, leaving total HGP essentially unchanged. In theory, this would result in increased rates of glycogenolysis but decreased rates of gluconeogenesis. In support of this notion, fasting rates of glycogenolysis after overnight insulin infusion have been suggested to be higher in type 2 diabetic patients than in healthy subjects (21), in contrast to studies of the hyperglycemic fasted state, in which rates of gluconeogenesis appear to be elevated (32,33). Thus, overnight insulin infusion may have altered the relative contribution of glycogenolysis and gluconeogenesis to overall glucose production.

An impaired direct effect of insulin may also have been present at the clamp levels in the diabetic patients. Several lines of evidence, though, may indicate the coexistence of defects in the indirect effects of insulin on HGP. The plasma FFA levels appeared to closely predict the higher (i.e., less suppressed) steady-state rates of HGP in the diabetic patients. Thus, while FFA levels in the 20-mU clamp were 2.5-fold higher in the diabetic patients than in the control subjects (21), in contrast to studies of the hyperglycemic fasted state, in which rates of gluconeogenesis appear to be elevated (32,33). Thus, overnight insulin infusion may have altered the relative contribution of glycogenolysis and gluconeogenesis to overall glucose production.
nondiabetic humans (34) and dogs (30). Furthermore, FFAs have been shown to increase HGP by stimulating gluconeogenesis (31), and qualitative assessment of glucose production in clamp studies at peripheral insulin levels similar to those in the 20-mU clamp in the present study has indicated that suppression of gluconeogenesis by insulin is impaired in type 2 diabetic patients (32).

Also, suppression of plasma glucagon levels was impaired in the diabetic patients, since glucagon levels were two- to threefold higher than in control subjects at steady-state in both clamp studies. Glucagon was also well correlated to HGP in both the 20- and 40-mU clamp, even if suppression of HGP was not impaired in the 40-mU clamp. Nevertheless, even though no significant difference in HGP was present in the 40-mU clamp, HGP tended to be less suppressed in diabetic patients than in control subjects. It may therefore be speculated that plasma glucagon was an important determinant of rates of HGP in the diabetic patients, but that hepatic insulin action was only significantly impaired in the presence of a concomitant defect in suppression of FFA (as in the 20-mU clamp). In support of this view, the effects of insulin to suppress FFA and glucagon levels seem to be additive in the control of HGP (35). In fact, from clamp studies of healthy subjects (at similar insulin levels as observed in the diabetic patients in the 20-mU clamp), the maintenance of FFA and glucagon levels comparable to those observed in the diabetic patients in the present study (by lipid-heparin and glucagon infusions) (35) have been shown to induce a comparable degree of hepatic insulin resistance. Therefore, it appears likely that impaired indirect effects of insulin may explain a considerable part of the observed hepatic insulin resistance at the clamp levels in the present study.

This conclusion may seem at variance with a recent elegant study by Lewis et al. (36), who suggested that hepatic insulin resistance (at a similar plasma insulin level of ~30 mU/L) in obese type 2 diabetic patients is due to an impaired direct effect of HISI levels on the liver. In the Lewis et al. study, the impact of increased peripheral insulin delivery on HGP was compared with the effect of increased portal insulin delivery (achieved by tolbutamide infusion), and the ability of increased HISI levels to suppress HGP was suggested to be markedly reduced in type 2 diabetic patients compared with healthy subjects. However, the diabetic patients in the study by Lewis et al. were studied at their prevailing level of fasting hyperglycaemia (~9 mmol/L), whereas the present study was carried out at euglycaemia. This may be of importance since recent studies in depancreatized dogs have suggested that acute restoration of normal plasma glucose levels may improve the direct effect of insulin (37). Thus, the relative importance of defects in the direct and indirect effects of insulin may differ, depending on the level of glycaemia. This may also be of clinical relevance when the clamp method is used in the evaluation of the impact of, for example, new drug therapies or treatment regimens on glucose metabolism in type 2 diabetes.

In conclusion, through the use of state-of-the-art tracer methods, the present study confirms the presence of hepatic insulin resistance in moderately obese type 2 diabetic patients. This hepatic insulin resistance is primarily expressed at low insulin levels and is overcome by high physiological insulin levels. Our data suggest that both the direct and the indirect pathways of insulin action on the liver may be insulin-resistant. Quantitatively, however, impaired stimulation of peripheral glucose disposal appears to be an at least equally important defect of insulin action at both low and high insulin levels in type 2 diabetes.

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REFERENCES