High Plasma Free Fatty Acids Decrease Splanchnic Glucose Uptake in Patients with Non-Insulin-Dependent Diabetes Mellitus

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Abstract. It has been proposed that high plasma free fatty acid (FFA) levels observed in patients with non-insulin dependent diabetes mellitus (NIDDM) contribute to the development of their insulin resistance. We examined patients with NIDDM to find whether maintaining plasma FFA levels in the fasting range with a euglycemic hyperinsulinemic clamp combined with an oral glucose load (clamp OGL) would affect insulin-mediated peripheral glucose uptake (PGU) and splanchnic glucose uptake (SGU). Nine NIDDM subjects (age, 55 ± 3 years; duration of diabetes, 11 ± 2 years; body mass index, 21.0 ± 0.4 kg/m²; hemoglobin A1c, 9.0 ± 0.3%; fasting plasma glucose, 9.4 ± 3.0 mmol/l, means ± SEM) were hospitalized and treated with diet, oral hypoglycemic agents or insulin for at least 2 weeks to maintain fasting plasma glucose < 8 mmol/l. All the patients were subjected to two different protocols in a random order. On one protocol, under the hyperinsulinemic condition, FFAs were maintained at their fasting levels (1.19 ± 0.08) by triglyceride emulsion infusion (Lipid infusion study, L), and on the other protocol, FFAs were made to fall (0.26 ± 0.06 mmol/l) with saline instead of triglyceride emulsion infusion (Saline infusion study, S). During euglycemic (L, 5.4 ± 0.2; S, 5.1 ± 0.2 mmol/l) hyperinsulinemic (L, 1377 ± 108; S, 1328 ± 67 pmol/l) clamp, high FFA levels significantly reduced PGU (L, 26.7 ± 3.6; S, 32.1 ± 3.4 μmol·kg⁻¹·min⁻¹, P<0.05) and SGU (L, 12.1 ± 4.2; S, 27.5 ± 5.6%, P<0.05). In conclusion, high FFA levels in patients with NIDDM impaired insulin-mediated glucose uptake in the splanchnic as well as peripheral tissues.

Key words: Free fatty acid, Insulin resistance, Splanchnic glucose uptake, Peripheral glucose uptake

NON-insulin-dependent diabetes mellitus (NIDDM) is a heterogeneous disorder characterized by both insulin resistance and defective insulin secretion [1−3]. In patients with NIDDM, although the skeletal muscle is the major site of impaired insulin action [4], the liver also plays an important role in insulin resistance. Insulin-mediated suppression of hepatic glucose production (HGP) is impaired, and accelerated hepatic glucose output correlates well with the degree of fasting hyperglycemia in NIDDM [5, 6]. Moreover, Kawamori et al. [7] have demonstrated that splanchnic glucose uptake is also impaired under euglycemic hyperinsulinemic condition after oral glucose load in patients with NIDDM. Such an impaired splanchnic glucose uptake may contribute in part to postprandial hyperglycemia in NIDDM patients.
Although the precise mechanism of insulin resistance has not been clarified yet, several factors, such as genetics, hyperglycemia, and dyslipidemia may contribute to insulin resistance in NIDDM. Among them, high free fatty acid (FFA) levels could partially account for insulin resistance in NIDDM patients [8]. It has recently been reported that healthy subjects increased plasma FFA levels induced by lipid-heparin administration impair insulin-mediated increase in peripheral glucose uptake [9-13] and suppression of HGP under euglycemic hyperinsulinemic conditions [13, 14]. In patients with NIDDM, circulating plasma FFA concentrations are increased in postabsorptive and postprandial states [15]. High FFA levels were shown to diminish insulin-stimulated peripheral glucose uptake in NIDDM [16, 17], but it has not been clarified whether high plasma FFA levels diminish insulin-mediated splanchnic glucose uptake.

To determine splanchnic glucose uptake, the hepatic venous catheterization method [18] or the dual tracer method [19] has been used in human studies, but these methods require invasive procedures or radioactive materials. As an alternative, the non-invasive euglycemic hyperinsulinemic clamp combined with an oral glucose load (clamp OGL) has recently been successfully used to determine insulin-mediated glucose disposal by the splanchnic and peripheral tissues in obese subjects [20], and patients with NIDDM [7] or with glucokinase gene mutation [21]. In the present study, we investigated the influence of high FFA levels on insulin-mediated splanchnic and peripheral glucose disposal using clamp OGL in patients with NIDDM.

**Subjects and Methods**

**Subjects**

Nine non-obese subjects with NIDDM aged 52–58 years (6 males and 3 females) participated in this study. Their clinical characteristics are presented in Table 1. The definition of NIDDM was based on World Health Organization criteria. Each patient who fulfilled the following inclusion criteria was considered for the study: 1) no episodes of ketoacidosis and absence of ketonuria; 2) diagnosis of diabetes after 30 years of age; 3) insulin therapy (if any) started after at least 5 years of known disease; 4) body mass index less than 30 kg/m²; 5) absence of overt diabetic nephropathy or other renal tract disease and 6) no evidence of cardiac failure. The purpose and protocol of the study were explained to each subject, and all gave their informed consent.

All patients were hospitalized and treated with diet alone or oral hypoglycemic agents or insulin in combination with a standard diet regimen (25 kcal/kg body weight) in order to stabilize the metabolic state before the practice of clamp studies. Of the nine patients, one was on diet alone, five were being treated with oral hypoglycemic agents and three were being treated with insulin.

**Experimental protocol**

Mean fasting plasma glucose levels were maintained < 8 mmol/l in all subjects for 2 to 3 weeks before the studies to stabilize the metabolic state. Euglycemic hyperinsulinemic clamp studies were performed at a seven-day interval with and without lipid infusion in a randomized order. In the lipid infusion study (L), to prevent decrease in plasma FFA levels during the euglycemic hyperinsulinemic clamp, 20% triglyceride emulsion (Intralipid®, Pharmacia A.B., Stockholm, Sweden) was administered at a rate of 1 ml/min with primed and constant infusion of heparin (200 U and 0.4 U·kg⁻¹·min⁻¹, respectively) throughout the experiments. In the saline infusion study (S), saline was infused at a rate of 1 ml/min with primed and constant infusion of heparin (200 U and 0.4 U·kg⁻¹·min⁻¹, respectively) throughout the experiments.

**Table 1. Clinical profiles of the patients**

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<thead>
<tr>
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<th>(M/F)</th>
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<tbody>
<tr>
<td>Gender</td>
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<tr>
<td>Age (years)</td>
<td>55.0 ± 2.9</td>
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<tr>
<td>Duration of diabetes (years)</td>
<td>11.3 ± 2.0</td>
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<tr>
<td>Height (cm)</td>
<td>163.0 ± 2.1</td>
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<tr>
<td>Weight (kg)</td>
<td>57.4 ± 1.9</td>
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<td>BMI (kg/m²)</td>
<td>21.0 ± 0.4</td>
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<td>HbA1C (%)</td>
<td>9.0 ± 0.3</td>
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<tr>
<td>Type of treatment</td>
<td>(Diet/Oral hypoglycemic agents/Insulin)</td>
<td>1/5/3</td>
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Values are expressed as means ± SEM.
Euglycemic hyperinsulinemic clamp combined with an oral glucose load

All studies were started at 0800 h after a 10–12 h overnight fast. An antecubital vein was cannulated to administer infusates, and a dorsal vein was cannulated and kept in a warming device to facilitate venous sampling and provide arterialized venous blood. Each study consisted of three experiment periods (period 1, from −15 to 0 min; period 2, from 0 to 105 min; period 3, from 105 to 120 min) following an equilibration period (from −120 to −15 min). By using an artificial endocrine pancreas (STG 22: Nikkiso, Shizuoka, Japan), primed-constant infusion of insulin (15.6 pmol·kg\(^{-1}\)·min\(^{-1}\)) and computer-controlled exogenous infusion of 10% glucose solution [22] were started to achieve the desired steady-state plasma insulin concentrations and to maintain blood glucose levels within the euglycemic range (from 5 to 5.5 mM) throughout the experiments. After the equilibration period, plasma glucose and insulin levels reached steady levels during period 1, and a fixed amount of glucose (0.2 g/kg) was administered orally to each subject at subject time 0 min. The euglycemic hyperinsulinemic clamp was continued during periods 2 and 3. Blood samples were taken for plasma glucose measurement at 15-min intervals throughout the study. Blood samples were taken at −120, −60, −30, −15, 0, 15, 30, 60, 90, 120 min for measurement of plasma insulin, C-peptide, FFA, triglyceride, glycerol and β-hydroxybutyrate concentrations. Samples for FFA, triglyceride and glycerol were collected in prechilled tubes containing EDTA and Paroxon\textsuperscript® (diethyl p-nitrophenyl phosphate, Sigma, St. Louis, Montana, USA, a lipoprotein lipase inhibitor, 0.275 mg/ml of blood).

Calculation of insulin-mediated glucose disposal by the liver and peripheral tissues

Under euglycemic hyperinsulinemic conditions before an oral glucose load, hepatic glucose production (HGP) has been shown to be suppressed by more than 90% in response to the increase in plasma insulin in NIDDM subjects with moderate fasting hyperglycemia [23]. In addition, most of the glucose infused peripherally is taken up by the peripheral tissues, mainly by the muscle, under these conditions [24], so that the GIR required to maintain euglycemia prior to oral glucose administration during period 1 is determined as the peripheral glucose uptake (PGU).

After oral glucose load during euglycemic hyperinsulinemic clamp, the glucose appearance rate (Ra(t)) is calculated with the equation:

\[ Ra(t) = [OGL(t) - SGU(t)] + GIR(t) \] .................. (1),

where OGL(t), the rate of absorption of oral glucose load by the intestine at time t; SGU(t), the rate of splanchnic glucose uptake of oral glucose load at time t; and GIR(t), the glucose infusion rate at time t. The rates of glucose disappearance rate from the systemic circulation (RdT(t)) is calculated with

\[ RdT(t) = RdP(t) + RdS(t) \] ............................ (2),

where RdP(t) and RdS(t) are the rates of extra-splanchnic and splanchnic glucose disappearance from the systemic circulation at time t, respectively. Under euglycemic hyperinsulinemic conditions, Ra(t) is equal to RdT(t), therefore

\[ RdP(t) + RdS(t) = [OGL(t) - SGU(t)] + GIR(t) \] ....(3).

Splanchnic glucose uptake can be calculated from the difference between the amount of glucose ingested and the sum of GIR decreases after glucose ingestion.

\[ \Sigma(SGU(t) + RdS(t)) = \Sigma OGL(t) - \Sigma (average GIR - GIR(t)) \] ........... (4).

Whole-body glucose utilization has been shown to increase gradually during prolonged euglycemic hyperinsulinemic clamping [25]. Since plasma glucose and insulin levels were constant throughout the experiments, RdP(t) during period 2 was estimated as the averaged GIR between periods 1 and 3 according to a previously established protocol [26].

\[ \Sigma(SGU(t) + RdS(t)) = \Sigma OGL(t) - \Sigma (average GIR - GIR(t)) \] ....(5).

Total splanchnic glucose disposal during period 2 was expressed as the percentage of the oral glucose load.

Laboratory methods

Plasma glucose was measured by a glucose oxidase method using a Beckman Glucose Analyzer 2 (Beckman, Fullerton, CA, USA). Plasma insulin and C-peptide were analyzed by radioimmunoassays. Plasma FFA and β-hydroxybutyrate levels were determined by an enzymatic method.
Triglyceride and glycerol levels were determined by an enzymatic microfluorometric method. Hemoglobin A1c levels were determined by HPLC techniques.

Statistical analysis

Data are represented as the means ± SEM. Statistical comparisons were assessed by analysis of variance (ANOVA) for repeated measurement and Student’s two-tailed paired t-test where appropriate. Statistical significance was accepted at P<0.05.

Results

Fasting levels of metabolites and hormones

There were no significant differences between the saline and lipid infusion studies in fasting plasma glucose, insulin, C-peptide, triglyceride and β-hydroxybutyrate levels (Table 2). Fasting levels of plasma FFA and glycerol in the lipid infusion study were significantly higher than those in the saline infusion study.

Glucose, insulin, C-peptide and GIR during clamp OGL (Fig. 1)

Stable and comparable plasma glucose levels were achieved by euglycemic hyperinsulinemic clamp throughout periods 1 to 3 in both the saline (5.1 ± 0.2) and lipid (5.4 ± 0.2 mmol/l) infusion studies. Plasma insulin and C-peptide levels were constant and comparable during the experimental periods in both the saline and lipid infusion studies (S, 1328 ± 67, 320 ± 63; L, 1377 ± 108 pmol/l, 507 ± 96 pmol/l, respectively). In the lipid infusion study, the averaged GIRs during periods 1 and 3 (26.7 ±
3.6 and 27.3 ± 3.8 μmol·kg⁻¹·min⁻¹, respectively) were significantly lower than those in the saline infusion study (32.1 ± 3.4 and 39.0 ± 3.4 μmol·kg⁻¹·min⁻¹, respectively, P<0.05). During period 2, GIR decreased until 45 min and 60 min in the saline and lipid infusion studies, respectively, and then increased again. In both studies, GIR reached a steady-state level during period 3.

**FFA, triglyceride, glycerol and β-hydroxybutyrate during clamp OGL (Fig. 2)**

In the lipid infusion study, plasma FFA levels were maintained at fasting levels during periods 1 to 3 (1.19 ± 0.08 mmol/l), with no significant differences among the three periods and at fast. In the saline infusion study, FFA decreased to 0.26 ± 0.06 mmol/l and plasma FFA levels were stable during periods 1 to 3. Averaged FFA levels in the lipid infusion study were significantly higher than those in the saline infusion study (P<0.05). Plasma triglyceride, glycerol and β-hydroxybutyrate were stable during periods 1 to 3 in both the saline and lipid infusion studies. Averaged plasma triglyceride, glycerol and β-hydroxybutyrate levels in the lipid infusion study were significantly higher than those in the saline infusion study (4.38 ± 1.21 vs. 0.57 ± 0.14 mmol/l, 0.54 ± 0.05 vs. 0.11 ± 0.02 mmol/l, 526 ± 129 vs. 30.7 ± 7.6 μmol/l, respectively, P<0.05).

**PGU and SGU (Fig. 3)**

PGU in the lipid infusion study was significantly lower than that in the saline infusion study (26.7 ± 3.6 vs. 32.1 ± 3.4 μmol·kg⁻¹·min⁻¹, P<0.05). SGU in the lipid infusion study was significantly lower than that in the saline infusion study (12.1 ± 4.2 vs. 27.5 ± 5.6%, P<0.05).

**Discussion**

In the present study, we used a euglycemic hyperinsulinemic clamp with an oral glucose load.
to investigate the effect of high FFA concentrations on insulin-mediated splanchnic and peripheral glucose disposal. When plasma FFA concentrations were maintained at fasting levels by lipid-plus-heparin infusion in patients with NIDDM, glucose disposal declined 15% and 56% in the peripheral and splanchnic tissues, respectively.

In this method, there are several assumptions to make in calculating SGU: complete absorption of oral glucose load during period 2, complete suppression of hepatic glucose production, no influence of oral glucose load on peripheral glucose utilization, no effect of oral glucose load on endogenous insulin secretion. Although it was possible that a small oral glucose load caused a bigger error than a larger one, 0.2 g/kg glucose was administered orally to prevent a temporary increase in plasma glucose after oral glucose load. It has been reported that absorption of oral loading glucose (12.5 ~ 25 g), which is a similar amount to the glucose load in the present study, from the gut is completed within 2 h in healthy subjects [27] and dogs [28]. The rate of absorption of the oral glucose load in NIDDM subjects was shown to be comparable to that in normal subjects [29]. In the present study, because GIR returned to the level observed before oral glucose load within 90 min in both the saline and lipid infusion studies, the oral glucose load was assumed to be absorbed completely during period 2.

In patients with NIDDM, the ability of the liver to suppress endogenous glucose production in response to an increase in plasma insulin is impaired [5, 6]. Moreover, high FFA levels could accelerate endogenous glucose production in the liver under the physiological insulin concentration [13, 14]. High glycerol level, a substrate for gluconeogenesis [14], also might impair insulin-mediated suppression of HGP. There should therefore have been an effect of endogenous HGP on splanchnic glucose disposal under the present experimental conditions, but we could not determine HGP due to the limitation of using radioactive tracers in human studies in Japan. Bevilacqua et al. [16] demonstrated that HGP in NIDDM subjects was completely suppressed during an infusion of triglyceride emulsion and heparin for 2 h with euglycemic hyperinsulinemic clamp, but Boden et al. [17] have recently reported a conflicting finding: that endogenous glucose production could not be suppressed completely under high FFA levels with euglycemic hyperinsulinemic clamp. The discrepancy between these reports might be caused by the different characteristics of the subjects: those in the study of Boden et al. had higher fasting glucose levels and body mass index than those in that by Bevilacqua et al. Moreover, in our study, the insulin levels were two fold higher but the FFA and glycerol levels were lower than those previously reported.

Fig. 4. Correlation between plasma free fatty acid levels and peripheral (left panel) or splanchnic (right panel) glucose uptake. Open and filled schemas represent the results during triglyceride emulsion and saline infusion, in patients treated with diet alone (△—△), oral hypoglycemic agents (○—○), and insulin (□—□), respectively.
so that under FFA levels observed in fasting NIDDM patients in the present study, hyperinsulinemia could suppress HGP sufficiently so as not to interfere with the calculation of SGU. Nevertheless, assuming that residual glucose production did not change substantially during oral glucose load, endogenous glucose production would simply add to the right side of Eq. (3). In this equation, because RdP(t) - HGP represents average GIR, the calculated splanchnic glucose uptake still should be reliable. It should also be noted that incomplete suppression of hepatic glucose production might underestimate peripheral glucose uptake in the lipid infusion study.

A previous report demonstrated that prior oral glucose load enhanced whole-body glucose utilization under the euglycemic hyperinsulinemic (240 pmol/l) condition [30]. And Ludvik et al. demonstrated that nearly maximal insulin-stimulated peripheral glucose uptake was constant before and after oral glucose load under the euglycemic hyperinsulinemic (1800 pmol/l) condition [20]. The discrepancy in these findings might be caused by the difference in the insulin concentration. Therefore, in our experimental conditions, peripheral glucose utilization was assumed to be constant before and after oral glucose load in marked hyperinsulinemia (1300–1400 pmol/l). In the saline infusion, however, GIR during period 3 increased significantly greater than during period 1. Whole-body glucose utilization has been shown to increase gradually during prolonged euglycemic hyperinsulinemic clamp [25], so that the increase in the GIR in our saline infusion study may have been due to the prolonged clamping. In the lipid infusion study, GIR was comparable in periods 1 and 3. Because high FFA levels have been shown to gradually reduce insulin-stimulated peripheral glucose uptake in a time-dependent manner [12, 13], in the lipid infusion study the time-related decrease in peripheral glucose uptake might suppress the increase in GIR during period 3.

To determine SGU, PGU during period 2 was estimated as the average GIR between period by means of 1 and 3 because of the time-related increase in GIR. PGU during period 2 was also able to be estimated a regression curve to fit GIR in periods 1 and 3. SGU determined by the last method was 30.5 ± 9.3 and 12.4 ± 4.4% in the saline and lipid infusion studies, respectively. The SGU obtained by these two methods were quiet similar. SGU calculated from the average GIR has been shown to be well-correlated with hepatic glucose uptake determined by the arterial-venous difference method in alloxan diabetic dogs [26]. We therefore measured SGU by the former method in the present study.

High FFA levels were reported to increase insulin secretion [31] and decrease insulin clearance [32], but because in the present study plasma C-peptide and insulin levels were stable and comparable throughout the experiments with or without triglyceride emulsion infusion, neither high FFA levels nor oral glucose load affected insulin secretion or clearance.

Several studies demonstrated that high FFA levels reduced insulin-mediated peripheral glucose uptake in healthy subjects [9–13] and NIDDM patients [16, 17]. The present study confirmed these findings. The mechanism responsible for the inhibitory effect of fatty acids on peripheral glucose uptake may be that an increased rate of FFA oxidation with high FFA inhibits glucose oxidation at the level of pyruvate dehydrogenase [33, 34] and glycolysis at the level of phosphofructokinase [35, 36]. Decreased glycolysis consequently increases the glucose-6-phosphate (G-6-P) concentration, inhibits hexokinase activity and leads to impairment of insulin-stimulated glucose uptake.

In the saline infusion study, SGU determined by clamp OGL was decreased by 56%. Averaged B-hydroxybutyrate levels were significantly higher than those in the saline study, thereby documenting enhanced lipid oxidation in the liver. Previous studies suggested that high FFA levels impaired glycolysis [37] probably by inhibiting enzymatic activities, such as glucokinase [38] and pyruvate dehydrogenase [33]. Our present study and previous ones suggested that increased FFA with enhanced lipid oxidation might inhibit glycolysis in the liver as well as peripheral tissues, and such metabolic deterioration might decrease splanchnic glucose uptake.

The correlation between plasma FFA levels and SGU or PGU and the type of treatment in each subject are shown in Fig. 4. Interestingly, in the saline infusion study, SGU decreased with the treatment. SGU in the patients with diet alone, oral hypoglycemic agents (OHA), and insulin was 46.4, 30.4 ± 4.6 and 16.9 ± 10.2%, respectively. These findings suggest that the endogenous insulin
secretory capacity may affect hepatic glucose uptake.

In the present study, fasting FFA and glycerol levels in the lipid infusion study were significantly higher than those in the saline infusion study. All subjects were hospitalized at the Osaka University Hospital, and were treated with a fixed diet, exercise and medication during the experiment. The reasons for these differences in fasting FFA and glycerol levels were unclear. The differences in plasma FFA levels between the saline and lipid infusion studies were 4 fold higher in the experimental period than in the fasting state. Because the fasting glycerol level was within the normal range in each study, impaired glucose disposal in the liver and peripheral tissues in the lipid infusion study was mainly caused by high FFA levels during the clamp period. Furthermore, the influence of a significant increase in blood glycerol, triglyceride and ketone bodies levels on PGU and SGU should be considered in the lipid infusion study. Glycerol [17] or ketone body [40] infusion did not impair insulin-mediated glucose utilization and hepatic glucose production. A high triglyceride level with high FFA levels impaired insulin-suppressed HGP, but did not affect whole-body glucose utilization under mild hyperinsulinemia (200 pmol/l) compared with high FFA levels alone [39], so that increases in blood glycerol, triglyceride or ketone bodies levels probably did not contribute to impaired SGU and PGU.

In conclusion, the present study indicated that high FFA levels in patients with NIDDM suppressed insulin-mediated splanchnic glucose uptake in the postprandial condition. These facts suggested that high circulating FFA levels in NIDDM induce insulin resistance not only in peripheral tissues but also in splanchnic tissues. When NIDDM subjects also have hyperglycemia and/or visceral fat type obesity, the increase in free fatty acid flux into the liver through the portal circulation might lead to hepatic insulin resistance and worsen the metabolic derangement.

References


