

# Effects of Oral Fat Load on Insulin Output and Glucose Tolerance in Healthy Control Subjects and Obese Patients Without Diabetes

OLIVER WUESTEN, MD<sup>1,2</sup>  
CRISTOPH H. BALZ, MD<sup>2,3</sup>  
REINHARD G. BRETZEL, MD<sup>2</sup>

HANS-ULRICH KLOER, MD<sup>2</sup>  
PHILIP D. HARDT, MD<sup>2</sup>

**OBJECTIVE** — Elevated plasma nonesterified fatty acid (NEFA) concentrations cause peripheral and hepatic insulin resistance and may play an important role in regulating glucose-induced insulin secretion. The aim of our study was to investigate the influence of physiologically elevated NEFA levels on glucose-stimulated insulin secretion in order to find evidence that NEFAs are a potential factor predisposing for type 2 diabetes and related metabolic disorders, which are known risk factors for cardiovascular disease.

**RESEARCH DESIGN AND METHODS** — We combined an orally administered fat emulsion with an intravenous glucose tolerance test and measured the time course of NEFA, insulin, and glucose. In order to find pathological conditions we applied the experiment to healthy and obese subjects.

**RESULTS** — The main findings are a significant increase in glucose-stimulated insulin secretion after oral fat load in both groups compared with the condition without preceding fat ingestion and a prolonged insulin secretion after fat load in obese patients compared with control subjects.

**CONCLUSIONS** — The results provide evidence that fat ingestion modulates  $\beta$ -cell function and that NEFA is a plausible mediator that acts as a link between fat and glucose metabolism by modulating glucose-stimulated insulin secretion. Under the condition of elevated plasma levels of NEFA, this mechanism may be responsible for hyperinsulinemia in obese patients and a potential target of type 2 diabetes prevention strategies.

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**E**levated levels of plasma nonesterified fatty acid (NEFA) cause peripheral insulin resistance by inhibiting insulin-stimulated glucose transport, phosphorylation, and glycogen synthesis (1–5). They further cause hepatic insulin

resistance resulting in increased endogenous glucose production in relation to the degree of hyperinsulinemia (6–9). NEFAs also support 30–50% of basal insulin secretion and potentiate glucose-stimulated insulin secretion (10–13). It has been hy-

pothesized that this stimulatory action on glucose-induced insulin secretion is the mechanism by which obese people compensate peripheral insulin resistance mediated by elevated NEFA levels without developing hyperglycemia (14). It has been shown that this compensatory mechanism does not work in patients predisposed to type 2 diabetes; while NEFAs mediate peripheral insulin resistance in diabetic or pre-diabetic subjects, there is no potentiating effect on the glucose-induced insulin output (15). Most of the fundamental studies elucidating the impact of NEFA on glucose metabolism have been performed by means of intravenous application of fat solutions. Because the parenteral application of NEFA is artificial and there might be independent effects of fat digestion and enteral absorption on the glucose metabolism, in the present study we investigated the effects of oral fat load on insulin secretion and glucose tolerance in healthy control subjects and obese patients.

## RESEARCH DESIGN AND METHODS

— Sixteen male obese patients (BMI >25 kg/m<sup>2</sup>) with a history of hypertriglyceridemia were included after informed consent. All patients had known coronary heart disease. Subjects with coronary bypass surgery within the last 6 weeks, under lipid-lowering therapy (medication had to be interrupted 4 weeks ago), or with the diagnosis of diabetes (postprandial blood glucose above 140 mg/dl, fasting blood glucose above 126 mg/dl) were excluded.

Control subjects consisted of 17 healthy volunteers (8 men, 9 women) without clinical evidence of cardiovascular, renal, or endocrine disorder. Subjects were excluded in case of severe illness in the history, medication affecting metabolism, or BMI above 26 kg/m<sup>2</sup>. Diabetes and hypertriglyceridemia were ruled out by measurement of fasting blood concentrations of glucose and triglycerides.

From the <sup>1</sup>Department of Diagnostic Radiology, Justus-Liebig-University, Giessen, Germany; the <sup>2</sup>Section of Gastroenterology, Third Medical Department and Policlinic, Justus-Liebig-University, Giessen, Germany; and the <sup>3</sup>Department of Dermatology, University of Erlangen, Erlangen, Germany.

Address correspondence and reprint requests to Philip D. Hardt, MD, Third Medical Department and Policlinic, Rodthohl 6, D-35392 Giessen, Germany. E-mail: philip.hardt@innere.med.uni-giessen.de.

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**Abbreviations:** AUC, area under the curve; FSIVGTT, frequently sampled intravenous glucose tolerance test; NEFA, nonesterified fatty acid.

A table elsewhere in this issue shows conventional and Système International (SI) units and conversion factors for many substances.

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**Table 1—Characteristics of patients and control subjects**

|  | Control subjects | P value | Patients    |
|--|------------------|---------|-------------|
| n  | 17               |         | 16          |
| Age (years)  | 29 ± 9           | <0.001  | 53 ± 3      |
| Body weight (kg)                                   | 63.5 ± 11.8      | <0.001  | 81.8 ± 10.1 |
| Height (m)   | 1.71 ± 0.10      | NS*     | 1.72 ± 0.06 |
| BMI (kg/m <sup>2</sup> )                           | 21.6 ± 2.4       | <0.001  | 27.6 ± 3.5  |
| Waist-to-hip ratio                                 | 0.78 ± 0.09      | <0.001  | 1.00 ± 0.05 |
| Relative body fat content, BIA (%) <sup>†</sup>    | 21.7 ± 6.6       | NS*     | 24.3 ± 7.4  |
| Relative lean body mass, BIA (%) <sup>†</sup>      | 78.3 ± 6.6       | NS*     | 75.7 ± 7.4  |
| Relative body water content, BIA (%) <sup>†</sup>  | 58.5 ± 5.0       | NS*     | 55.2 ± 4.6  |
| Absolute body fat content, BIA (kg) <sup>†</sup>   | 13.6 ± 4.6       | <0.01   | 20.2 ± 7.2  |
| Absolute lean body mass, BIA (kg) <sup>†</sup>     | 49.9 ± 11.1      | <0.01   | 61.7 ± 7.8  |
| Absolute body water content, BIA (kg) <sup>†</sup> | 37.2 ± 7.7       | <0.01   | 45.0 ± 4.7  |
| Fasting blood concentration                        |                  |         |             |
| Glucose (mg/dl)                                    | 90 ± 6           | <0.01   | 96 ± 6      |
| Insulin (μU/ml)                                    | 5.1 ± 1.7        | NS*     | 6.5 ± 2.5   |
| Triglycerides (mg/dl)                              | 94 ± 53          | <0.05   | 147 ± 88    |
| NEFA (mmol/l)                                      | 0.54 ± 0.25      | NS*     | 0.46 ± 0.15 |
| Total Cholesterol (mg/dl)                          | 183 ± 24         | NS*     | 182 ± 22    |

Data are mean ± SE. Control subjects are compared with patients. \*NS,  $P > 0.05$ . <sup>†</sup>BIA, bioelectric impedance analysis.

There were no ethnical differences between both groups, which consisted of Caucasian subjects. Baseline characteristics are presented in Table 1.

Two corresponding experiments were performed on each subject within a period of 140 days on average. The experiments began at 0800 after an overnight fast of 10 h (Fig. 1).

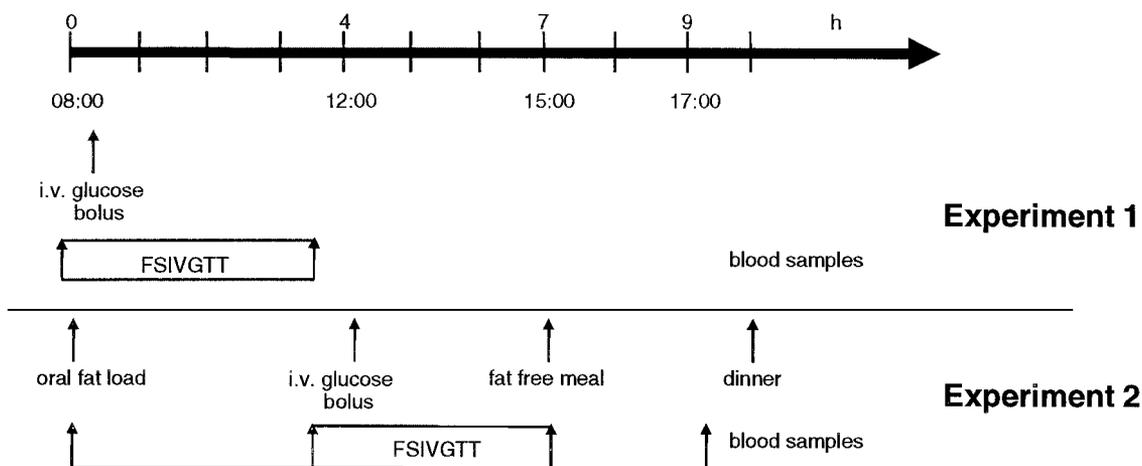
Experiment 1 was a frequently sampled intravenous glucose tolerance test (FSIVGTT) without fat consumption and lasted 3.5 h. After inserting an intravenous catheter (18 gauge) in an antecubital

vein and flushing it with 0.9% saline, a bolus of 40% glucose (0.3 g/kg) was injected at time 0 over 60 s. Venous blood for measurements of plasma glucose and immunoreactive insulin were obtained with a total of 20 samples using a modified protocol suggested by Bergman et al. (16). Blood samples of glucose concentration were collected at -20, 0, 4, 6, 8, 10, 12, 14, 16, 19, 20, 25, 30, 40, 50, 60, 90, 120, 140, and 180 min. Insulin was measured at the same time points except 8, 12, 16, and 19 min. Blood samples for determination of NEFA concentration

were collected at -20, 0, 20, 40, 60, 90, and 120 min.

Experiment 2 is a repetition of experiment 1 after previous oral fat ingestion. Therefore, it was started with ingestion of 1.5 g/kg (lean body mass) of a triglyceride emulsion followed by FSIVGTT (according to experiment 1) 4 h later because studies had shown that NEFA concentration was highest and stable at that time. This experiment lasted 9 h altogether, and subjects were allowed to drink fruit tea without sugar or caffeine and to move without physical strain. Differences in the metabolic reaction to intravenous glucose administration were calculated only for the corresponding time period in both experiments starting with the glucose injection and lasting until 120 min after the bolus. This allowed us to compare the metabolic situations with and without oral fat ingestion in the same individual. A total of 60 ml of blood was obtained per single experiment. The study protocol was approved by the local ethical committee.

The triglyceride emulsion consisted of 50% sunflower oil (60.2% linolic acid, 21.9% oleic acid, 6.2% palmitic acid, and 4.8% stearic acid) supplemented with vitamin A (Nutricia, Zoetermeer, the Netherlands). Forty percent glucose solution and 0.9% saline solution were from Braun (Melsungen, Germany). Lean body mass was determined by bioimpedance analysis (17). Two-milliliter sample tubes coated with sodium fluoride (Kabe Laboratories, Nuembrecht-Elsenroth, Germany) were used for blood sampling of glucose and EDTA-coated tubes (Eppen-



**Figure 1—Time course of experiment 1 (intravenous glucose tolerance test) and experiment 2 (intravenous glucose tolerance test after oral fat load).**

dorf Laboratories, Hamburg, Germany) for insulin and NEFA measurements.

Laboratory measurements of plasma glucose concentrations were determined by the hexokinase method using an analyzer (Hitachi System 717; Roche Diagnostics, Basel, Switzerland). Insulin was measured by micro-particle enzyme immunoassay (Abbott, Wiesbaden, Germany). The cross-reactivity to proinsulin of this assay was  $<0.005\%$ , and the inter-individual coefficient of variation (CV) was 4–6%. NEFA concentration was determined by an enzymatic colorimetric method (NEFA C; Wako Chemicals, Neuss, Germany) in serum. The CV of this test was 1–3%.

### Statistical evaluation

Differences in the time course of glucose, insulin, and NEFA concentrations for both groups and experiments were calculated comparing the area under the curve (AUC) of the time concentration curves statistically. AUC was calculated from the time interval between the beginning of the intravenous glucose bolus (time 0) and the following 120 min. In order to detect differences between early secretion and prolonged elevation of insulin concentration, the time interval of insulin and glucose was divided into an early (0–30 min) and late (30–120 min) phase. Effects of insulin on NEFA concentration were investigated by comparison of the suppression area of the NEFA curves, which are calculated from the difference between the AUC of the NEFA concentration curve between 0–120 min and the fictive rectangular area, which would have been measured if NEFA concentration was constant at the level of time 0.

Statistical analyses were made using the SPSS software package (release 6.1.3; SPSS, Chicago, IL). Significance of differences between the AUC of each parameter was determined by Student's *t* test or non-parametrical tests depending on the results of Kolmogorow-Smirnow test for normal distribution. The time course of NEFA concentrations during both experiments were analyzed using the MANOVA procedure of the SPSS package and comparing consecutive time points pairwise.

**RESULTS** — Patients and control subjects showed significant differences in certain clinical parameters and laboratory findings (Table 1). Patients were older,

had a higher BMI and waist-to-hip ratio as well as higher fasting concentrations of glucose and triglycerides.

Oral fat load (experiment 2) did not influence the time course of glucose concentrations following intravenous glucose application as shown in Fig. 2. In the patient group as well as in control subjects, glucose and insulin concentrations did not differ significantly between experiment 1 and 2 before the FSIVGTT. Separating the glucose tolerance test into an early phase of 30 min and a late phase of 30–120 min after glucose injection, we saw no statistical differences in the AUC of glucose concentration during the early phase for the corresponding periods during experiment 1 vs. experiment 2 (control subjects  $6,080 \pm 586$  vs.  $6,035 \pm 628$  mg/dl  $\cdot$  min,  $P > 0.05$ ; patients  $7,552 \pm 1,093$  vs.  $7,466 \pm 950$  mg/dl  $\cdot$  min,  $P > 0.05$ ). The same result was noticed for the late phase except a slight decrease of glucose concentration in the control group (control subjects  $9,293 \pm 1,024$  vs.  $8,383 \pm 1,170$  mg/dl  $\cdot$  min,  $P < 0.05$ ; patients  $12,764 \pm 2,252$  vs.  $12,288 \pm 1,453$  mg/dl  $\cdot$  min,  $P > 0.05$ ).

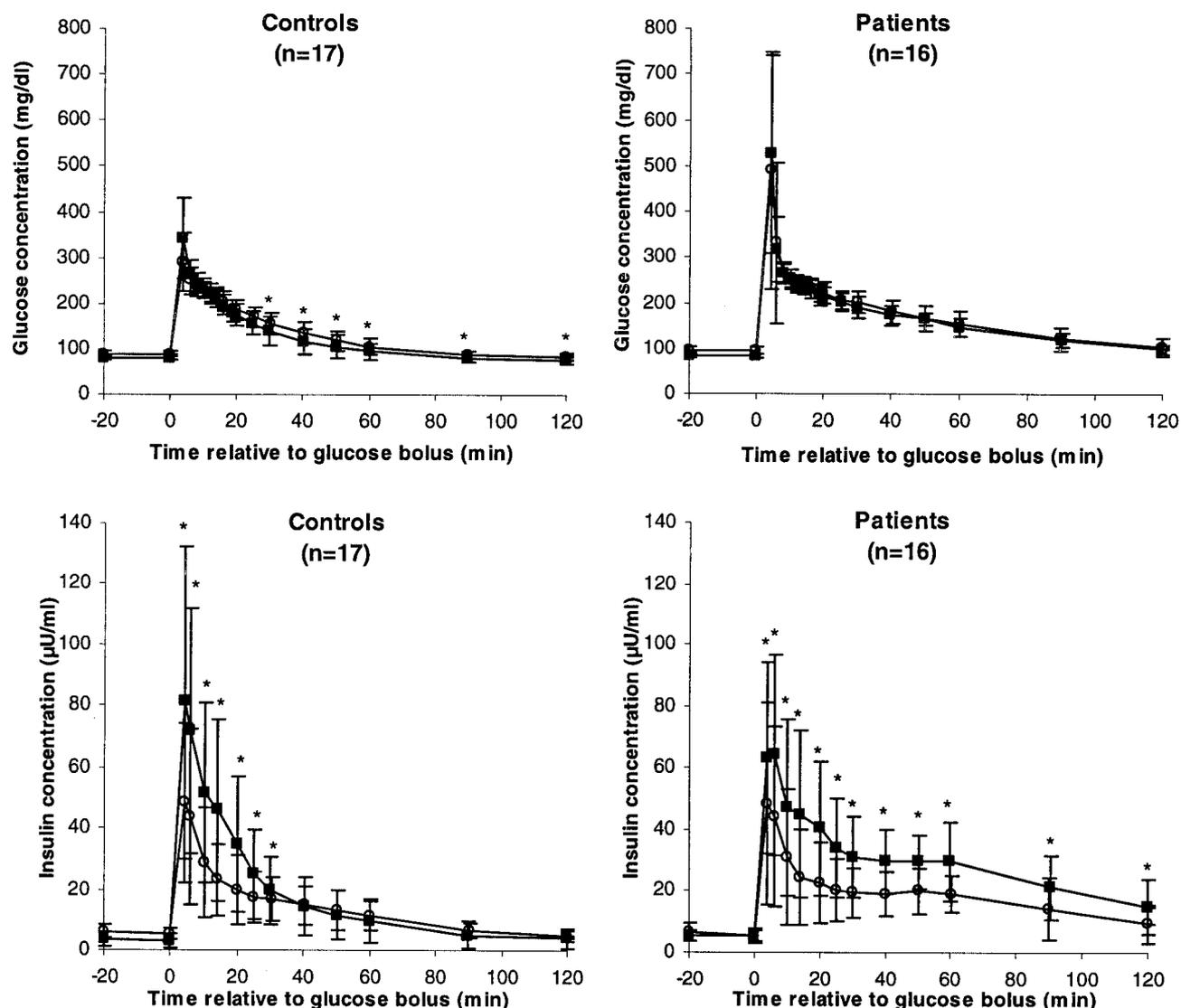
Insulin output was increased following oral fat load during the first 30 min (control subjects  $758 \pm 397$  vs.  $1,264 \pm 704$   $\mu$ U/ml  $\cdot$  min,  $P < 0.001$ ; patients  $810 \pm 480$  vs.  $1,288 \pm 604$   $\mu$ U/ml  $\cdot$  min,  $P < 0.001$ ) maintaining a stable glucose concentration in obese patients and control subjects (Fig. 2). The peak value of glucose-induced insulin secretion during experiment 2 was lower in obese patients than in control subjects ( $64 \pm 32$  vs.  $81 \pm 51$   $\mu$ U/ml,  $P < 0.05$ ). Whereas insulin output after oral fat load normalized after 30 min in control subjects (30–90 min, experiment 1 vs. experiment 2:  $854 \pm 318$  vs.  $763 \pm 510$   $\mu$ U/ml  $\cdot$  min,  $P > 0.05$ ), insulin levels remained elevated in obese patients during the following 90 min compared with glucose tolerance test without the oral fat load (30–90 min, experiment 1 vs. experiment 2:  $1,436 \pm 594$  vs.  $2,186 \pm 882$   $\mu$ U/ml  $\cdot$  min,  $P < 0.001$ ). NEFA concentration raised after oral fat load and was suppressed once insulin was secreted without significant differences between patients and control subjects (suppression AUC experiment 1 control subjects vs. patients:  $27.6 \pm 12.0$  vs.  $29.4 \pm 9.3$  mmol/l  $\cdot$  min,  $P > 0.05$ ; experiment 2 control subjects versus patients:  $38.0 \pm 15.9$  vs.  $28.6 \pm 12.2$  mmol/l  $\cdot$  min,  $P > 0.05$ ) (Fig. 3). The

amount of suppressed NEFA during the insulin secretion phase expressed as suppression AUC between 0 and 120 min was higher after previous fat intake only in the control group (control subjects: experiment 1 vs. experiment 2:  $27.6 \pm 12.0$  vs.  $38.0 \pm 15.9$  mmol/l  $\cdot$  min,  $P < 0.05$ ; patients:  $29.4 \pm 9.3$  vs.  $28.6 \pm 12.2$  mmol/l  $\cdot$  min,  $P > 0.05$ ).

**CONCLUSIONS** — Elevated levels of plasma NEFA have been shown to cause peripheral and hepatic insulin resistance (1–9). NEFAs also support 30–50% of basal insulin secretion and potentiate glucose-stimulated insulin secretion (10–13). This mechanism seems to allow obese people to compensate peripheral insulin resistance mediated by elevated NEFA levels without developing hyperglycemia (14). This loss of compensatory action might be a key event in the pathogenesis of type 2 diabetes (15). Most studies about the impact of NEFA on glucose metabolism have been performed by means of intravenous application of fat solutions (18,19).

In the present study we could show similar effects after the more physiological oral fat load. Following oral fat load, healthy control and obese subjects showed an increase of insulin secretion after glucose challenge. The peak of insulin secretion was higher in control subjects, returning to normal levels after 30 min, whereas insulin levels remained elevated in obese patients during the entire follow up of 120 min. Both findings, less accentuated peak value and prolonged insulin secretion after oral fat load, show a reduction of insulin effectiveness in obese patients and demonstrate the existence of a NEFA-mediated effect on insulin action. The counterregulatory effect of insulin on NEFA suppression after oral fat load is also reduced in the patient group. We conclude that studying insulin characteristics following oral fat administration may be useful as a test for prediabetic states and may be more sensitive than glucose tolerance testing. It might be possible to predict the development of type 2 diabetes using a comparable test protocol.

The differences in clinical characteristics and fasting blood concentrations between our patient and control groups (Table 1) show that patients were obese without significant changes in relative body composition. Patients also presented with elevated fasting glucose and triglycerides.



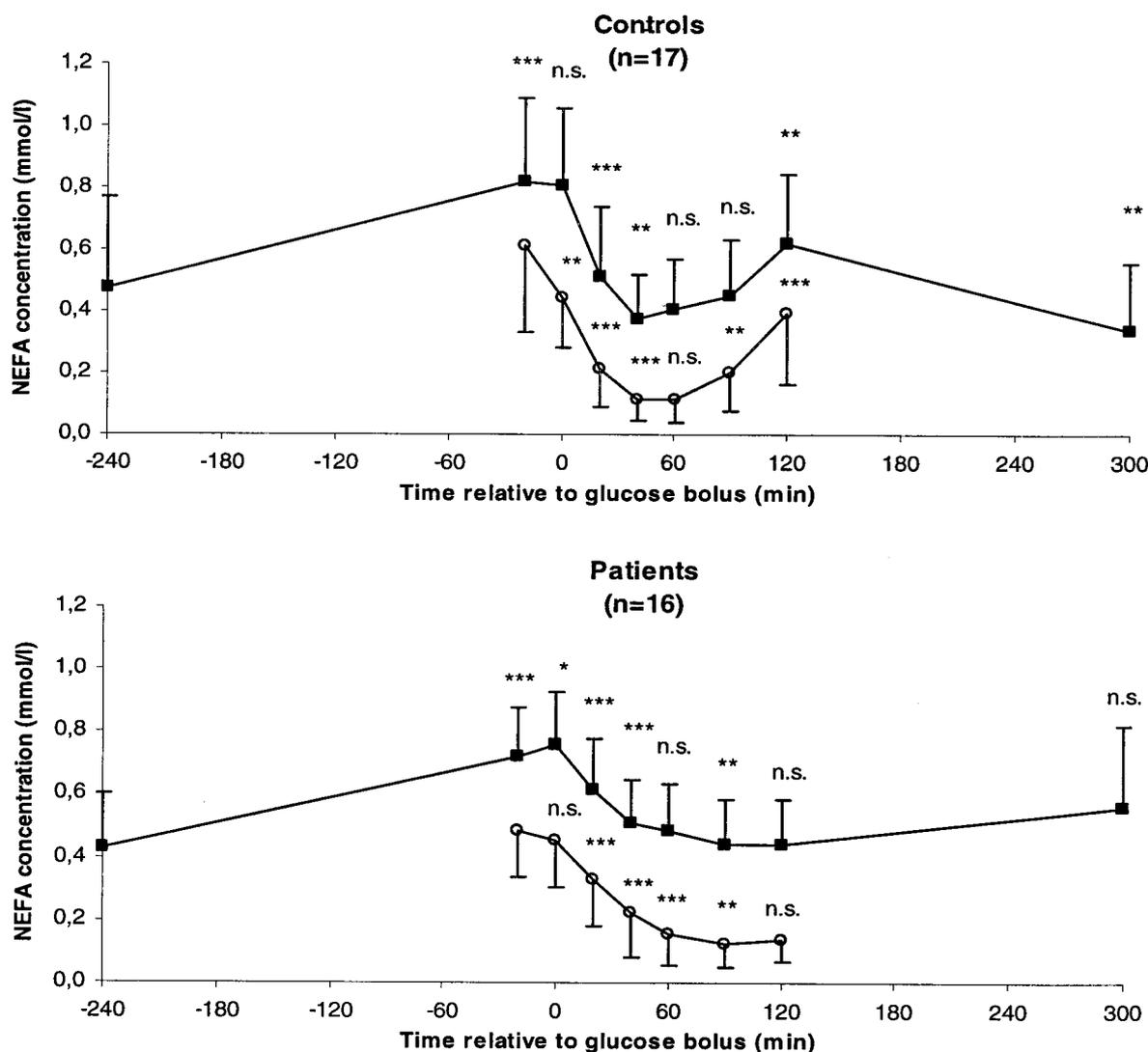
**Figure 2**—Plasma glucose and insulin concentrations during experiment 1 and 2 for the control and patient groups. Only comparable blood samples during the intravenous glucose tolerance tests for both experiments are shown. Statistically significant differences between the corresponding values of test 1 and 2 are marked (\* $P < 0.05$ ). Differences that are not significant are not indicated. ○, experiment 1; ■, experiment 2.

Compared with control subjects, no differences were found concerning NEFA and insulin concentrations. The group differences do not characterize a specific metabolic disorder, but the detection of reduced insulin effectiveness after oral fat load during our experiments may be the key finding. The responsible pathogenesis could be described by the metabolic syndrome X representing insulin resistance (20).

Our test combines physiologically administered oral fat load with intravenous application of glucose. This design was chosen to represent all potential consequences of fat administration, including effects of fat digestion and uptake as well as secretion of gastrointestinal hormones,

which may affect  $\beta$ -cell function. Studies have demonstrated a stimulation of the  $\beta$ -cell 90 min after oral fat load due to a release of intestinal hormones (e.g., secretin-pancreozymin) (21,22). The time course of our study provides an intravenous glucose challenge 4 h after oral fat load, leading to the assumption that there was no direct impact on insulin concentration by gastrointestinal factors at this time. On the other hand, modulatory effects on  $\beta$ -cell function may have influenced the response to glucose administration. Studies have shown that lipid infusion may also increase glucose-stimulated insulin secretion (10,18,23). Opposite results of studies that could not

find this stimulatory effect of fat infusion (24) may be due to short time administration of fat. NEFA elevation as the potential link between fat metabolism and  $\beta$ -cell function has shown to have different time-dependent effects (18,25). Elevation of NEFA for 6 h increased insulin secretion, whereas an inhibition was noted after 24–48 h. Therefore, it is important to recognize the time course of NEFA elevation in our experiment. Following oral fat load, the maximum of serum triglyceride and NEFA concentration has to be expected after 4–6 h (21). In our protocol, this is the time point chosen to perform an intravenous glucose tolerance test in order to see maximum effects of NEFA on



**Figure 3**—Time course of NEFA concentration (mean  $\pm$  SE) during the entire experiment 1 (intravenous glucose tolerance test alone) and experiment 2 (intravenous glucose tolerance test 4 h after oral fat load). The levels of significance (NS,  $P > 0.05$ ; \* $P < 0.05$ ; \*\* $P < 0.01$ ; \*\*\* $P < 0.001$ ) demonstrate the change in NEFA concentration for consecutive time points comparing with the preceding time point in the same experiment.  $\circ$ , experiment 1;  $\blacksquare$ , experiment 2.

glucose utilization and insulin secretion. The interpretation of the study might be limited due to the different time points when FSIVGTT was started in both experiments. However, both tests were initiated at the same time in the morning when patients are in a comparable fasting state. The FSIVGTT had to be postponed 4 h during test 2 due to the previous fat ingestion. The alternative way to start test 2 during the night or delay test 1 for 4 h would also have changed physiological conditions. Because in both experiments individuals were treated in the same way, the different pattern of insulin release with and without fat allows us to con-

clude that the oral fat load influenced insulin release and effectiveness.

There were preexisting differences between obese patients and control subjects concerning some parameters. These differences were caused by the selection of the study populations. However, there were no gender-related differences, and none of the individuals was taking drugs affecting metabolism. Therefore, a comparison between the two groups appears to be justified.

In the present study we could show that oral fat load can potentiate insulin secretion after glucose challenge. The  $\beta$ -cell reaction in obese patients differs

from control subjects, with peak values of insulin being less high and insulin elevation lasting longer in obese patients. The exact mechanisms subsequently leading to the development of overt diabetes are not known. If there is an important influence of oral fat feeding on  $\beta$ -cell function, this might have interesting implications. It has been observed that several patients with diabetes show exocrine pancreatic insufficiency (26). A relevant number of diabetic patients even suffer from steatorrhea (27). The fat maldigestion in patients with exocrine pancreatic insufficiency might result in a loss or modification of dietary fat impact on  $\beta$ -cell function. Cer-

tain fatty acids might be useful as a dietary tool to increase  $\beta$ -cell function in type 2 diabetes. Studies on possible effects of different fatty acids should be performed as well as studies on the impact of oral fat load in type 2 diabetic patients with exocrine pancreatic insufficiency with and without enzyme supplementation.

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