Insulin Secretion After Short- and Long-Term Low-Grade Free Fatty Acid Infusion in Men With Increased Risk of Developing Type 2 Diabetes

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We studied the effect of a low-grade short- and long-term 20% Intralipid infusion (0.4 $mL^{-1} \cdot kg^{-1} \cdot h^{-1}$) on insulin secretion and insulin action in 15 elderly obese men; 7 glucose intolerant first-degree relatives of type 2 diabetic patients (impaired glucose tolerance [IGT] relatives) and 8 healthy controls of similar age and body mass index (BMI). Intravenous glucose tolerance test (IVGTT) and a graded glucose infusion (dose-response test [DORE]) were performed to determine first phase insulin response and to explore the dose response relationship between glucose concentration and insulin secretion rates (ISR). ISR were calculated by deconvolution of plasma C-peptide concentrations. Insulin action was determined by performing a 120-minute hyperinsulinemic euglycemic clamp. All tests were performed 3 times, preceded by 0, 2, or 24 hours Intralipid infusion. Disposition indices (DI) were calculated for the IVGTT. Insulin action was reduced 25% after 2 and 24 hours Intralipid infusion in both groups. In IGT relatives, the β-cell responsiveness to glucose (measured during DORE) decreased after 2 and 24 hours Intralipid infusion ($P = .02$), whereas first phase insulin response (measured during IVGTT) decreased after 24 hours Intralipid infusion. Insulin secretion measured during DORE and IVGTT was not affected by Intralipid infusion in controls. DI decreased after 2 and 24 hours Intralipid infusion in the total study population. In conclusion, insulin resistance induced by low-grade short- and long-term Intralipid infusion is not balanced by an adequate compensatory increase in insulin secretion in IGT relatives or in matched controls. IGT relatives appear to be more sensitive to the deleterious effects of low-grade fat infusion on insulin secretion than normal glucose tolerant control subjects.

PATIENTS WITH TYPE 2 diabetes are characterized by elevated fasting and postprandial plasma free fatty acid (FFA) and triglyceride concentrations,[1-4] and changes in lipid metabolism are considered to play an important role in the development of type 2 diabetes. Defects in both insulin secretion and insulin action are known to be present in individuals predisposed to type 2 diabetes already in the prediabetic stage,[5-8] and it is well established that elevated plasma FFA deteriorates insulin action in both healthy subjects[9-12] and in type 2 diabetic patients.[13,14] Most previous studies investigating the effects of elevated plasma FFA on insulin secretion and action were performed applying supraphysiologic amounts of FFA inducing a 2- to 3-fold increase in fasting plasma FFA.[10-18]

The effect of elevated plasma FFA on insulin secretion is controversial. Both in vitro[19,20] and in vivo[16,21] studies have shown that acutely elevated plasma FFA levels had a stimulatory effect on glucose-stimulated insulin secretion (GSIS), whereas prolonged exposure of rat[22,23] and human islets[24] to FFA decreased GSIS. Prolonged (24 to 48 hours) elevation of FFA in vivo decreased GSIS in rats[25] and in humans,[16,21] although one study reported increased GSIS in healthy subjects after prolonged FFA exposure.[26] Carpentier et al[17] showed that 48-hour fat infusion decreased GSIS during graded glucose infusion in obese 50-year-old subjects, whereas no effect on insulin secretion was found in patients with type 2 diabetes,[17] indicating that no further deterioration of insulin secretion can be induced by FFA in patients with overt type 2 diabetes.

Insulin secretion is inversely related to insulin action in subjects with normal glucose tolerance (NGT) in a hyperbolic manner.[27,28] Thus, individuals with NGT exhibit a high insulin response to glucose when insulin sensitivity is low and vice versa. This means that insulin secretion must be related to the concomitant insulin sensitivity to obtain a correct estimate of the β-cell function.

The present study was designed to establish whether a physiologic elevation of plasma FFA causes changes in β-cell function in potentially prediabetic elderly obese men without or with impaired glucose tolerance (IGT) combined with a family history of type 2 diabetes when insulin action is taken into account. In particular, we wanted to study whether IGT relatives were more sensitive to the lipotoxic effects of elevated plasma FFA than control subjects. β-cell function was evaluated by performing 2 different glucose challenge tests addressing different aspects of the β-cell function: first phase responsiveness (IVGTT) and dose-response relationship between plasma glucose concentration and insulin secretion rate (ISR) during a prolonged graded intravenous glucose infusion. The first reflects the acute response and the second the ability of the β cell to detect and respond appropriately to gradually increased plasma glucose concentrations.

In vivo insulin action was also measured in each subject using the hyperinsulinemic euglycemic clamp technique before and after both short- (2-hour) and long-term (24-hour) low-grade Intralipid infusion.

SUBJECTS AND METHODS

Subjects

Seventy-one Caucasian men recruited through local newspaper advertisements underwent an oral glucose (75 g) tolerance test (OGTT).

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Table 1. Clincal Characteristics of the 56 Subjects Not Included

	No.	Age (yr)	BMI (kg/m^2)	Fasting Glucose	2-h Glucose	HbA_{1c} (%)
NGT, relative	36	52 ± 2	29.9 ± 0.8	5.9 ± 0.1	6.3 ± 0.2	5.8 ± 0.1
NGT, not relative*	9	46 ± 2	28.6 ± 1.1	5.8 ± 0.3	6.4 ± 0.4	6.0 ± 0.2
IGT, not relative	5	50 ± 3	33.4 ± 2.2	6.4 ± 0.2	9.4 ± 0.6	6.0 ± 0.1
Type 2 diabetes	6	52 ± 2	33.0 ± 1.5	7.6 ± 0.3	12.1 ± 1.0	6.6 ± 0.2

NOTE. Values are mean ± SEM. Glucose is given in (mol · L^{-1}).

*These subjects were not included as their weight and/or age did not match the IGT relatives.

IGT was diagnosed according to the World Health Organization (WHO) criteria (ie, IGT: 2-hour plasma glucose during OGTTs $>$ 7.8 and $<$ 11.1 mmol · L^{-1}). From this group we were able to include 7 first-degree relatives of type 2 diabetic patients with IGT (IGT relatives) and 8 age- and body mass index (BMI)-matched control subjects with NGT and no family history of diabetes. Fifty-six subjects were not included in the rest of the study due to their combination of glucose tolerance and/or relative status (IGT, but not relative; NGT but relative, type 2 diabetes) or they were too young and/or too lean to match the IGT relatives (Table 1)

The 15 included subjects were not undertaking arduous exercise on a regular basis, and they were instructed to avoid excessive physical exercise and alcohol intake for at least 2 days before the studies. The subjects were also instructed not to change their body weight, eating, drinking, smoking, or exercise habits during their participation in the study. No significant changes were observed in weight, BMI, fasting glucose, insulin, or C-peptide values in any of the groups during the study period. All subjects agreed to participate after oral and written information. The study was approved by the Copenhagen County Ethical Committee, and the study was conducted according to the principles of the Helsinki Declaration.

Body Fat Determinations

A dual-energy x-ray absorptiometry (DEXA) scan was performed to measure total body fat and fat-free body mass (FFM). A Norland XR-36 scanner was used.[29]

Experimental Protocol

All subjects underwent 2 different protocols on 2 different days. Protocol A consisted of an IVGTT followed by a hyperinsulinemic euglycemic clamp. Protocol B was a graded intravenous glucose infusion. Protocol A and B were performed 3 times preceded by 0 (2-hour saline infusion), 2-, or 24-hour Intralipid infusion, respectively. Saline or Intralipid infusion was continued throughout the studies. The 6 studies were performed in random order 4 weeks apart. All studies were initiated at 8 AM after a 10-hour overnight fast. A polyethylene catheter was inserted into an antecubital vein for infusion of test substances. Another polyethylene catheter was inserted into a contralateral wrist vein for blood sampling. This hand was kept in a heated Plexiglas box throughout the test to obtain arterialized venous blood.[30]

Protocol A. A priming tracer bolus of 3-[^{3}H]-glucose was given and a constant rate continuous tracer infusion was initiated at the time point −160 minutes (Fig 1). The period from −70 to −40 minutes was defined as the "basal steady state period" where the basal glucose disposal rate, Rd_{basal}, was estimated. From −40 to −30 minutes, a muscle biopsy was performed. From −30 to 0 minutes, the IVGTT was performed. From 0 to +120 minutes, a hyperinsulinemic euglycemic clamp was performed and at +120 minutes, a second muscle biopsy was performed. The results of the muscle biopsies have been published separately.[31]

IVGTT. A bolus of 18% glucose solution (300 mg · kg^{-1}) was infused over 1 minute. Blood samples were collected at times −10, −5, 0, 2, 4, 6, 8, 10, 15, and 30 minutes. All samples were analyzed for plasma glucose, insulin, and C-peptide.

Hyperinsulinemic euglycemic clamp. A primed infusion of insulin (Actrapid; Novo Nordisk, Bagsvaerd, Denmark) was performed from 0 to +9 minutes. The infusion rate was reduced every third minute from 100 to 80, to 60, and to 40 mU · m^{-2} · min^{-1}. Thereafter, the insulin infusion rate was fixed at 40 mU · m^{-2} · min^{-1} from +9 to +120 minutes. Plasma glucose concentration was maintained constant at euglycemia, 5 mmol · L^{-1}, using a variable 18% glucose infusion[32] spiked with 3-[^{3}H]-glucose. Plasma glucose concentration was monitored every 5 minutes during the clamp. Rd_{ins} was estimated during the insulin-stimulated steady state period from +90 to +120 minutes as described previously.[33,34] Blood samples were drawn in fluoride-treated tubes in the beginning and at the end of the 30-minute basal steady state period and every 10 minutes during the 30-minute insulin-stimulated steady state period for the determination of 3-[^{3}H]-glucose activity. During the rest of the study, plasma 3-[^{3}H]-glucose was measured every 30 minutes. Plasma 3-[^{3}H]-glucose activity was determined from counts from an evaporated 0.5-mL plasma sample.[35]

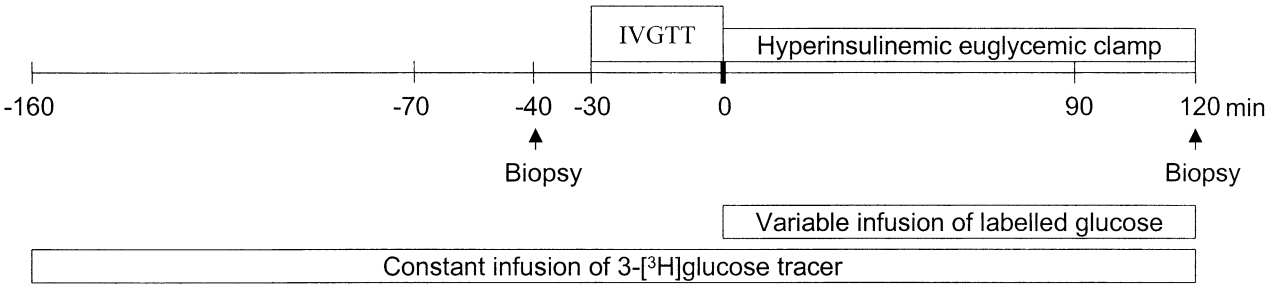

Fig 1. Protocol A is described in detail in the text. It comprised an IVGTT and a hyperinsulinemic euglycemic clamp. During the control study (0 fat), saline infusion was initiated at −160 minutes. For the 2-hour fat infusion study, fat infusion was initiated at −160 minutes. For the 24-hour fat infusion, fat infusion was initiated 24 hours prior to −40 minutes. The fat infusions were stopped after the last biopsy.

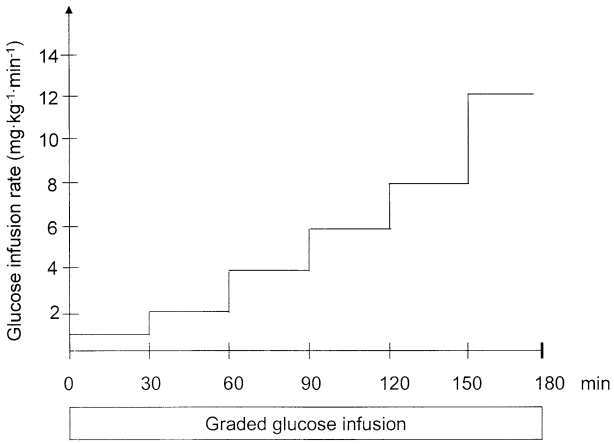

Fig 2. Protocol B is described in detail in the text. A graded glucose infusion test was performed. During the control study (0 fat), saline infusion was initiated 120 minutes prior to the graded glucose infusion. For the 2-hour fat infusion study, fat infusion was initiated 120 minutes prior to the graded glucose infusion. For the 24-hour fat infusion, fat infusion was initiated 24 hours prior to 0 minute. The fat infusions were stopped after 180 minutes.

Protocol B, graded intravenous glucose infusion (dose response test [DORE]). Basal blood samples were collected at the time points −15, −10, and 0 minutes (Fig 2). An intravenous infusion of 18% glucose was started at 0 minute at a rate of 1 mg · kg^{-1} · min^{-1}, followed by infusions of 2, 4, 6, 8, and 12 mg · kg^{-1} · min^{-1}. Each infusion rate was administered for 30 minutes. Plasma insulin, C-peptide, glucose, and FFA were measured every 10 minutes during the 180-minute study.

Intralipid infusion. Intralipid is a fat emulsion consisting of triglycerides; 12% palmitic acid (C16:0), 4% stearic acid (C18:0), 21% oleic acid (C18:1 n-9), 53% linoleic acid (C18:2 n-6), 7% α-linolenic acid (C18:3 n-3), and 3% others. An intended 10% to 30% elevation of the fasting plasma FFA was achieved by infusing 20% Intralipid at a rate of 0.4 mL · kg^{-1} · h^{-1}. The "short-term" (2 hours) Intralipid infusion was given continuously from −160 minutes to +120 minutes during protocol A, and from −120 minutes to 180 minutes on study day B. On the control study day (0 hour Intralipid), saline infusion was given from −160 minutes to +120 minutes on study day A and from −120 minutes to +180 minutes on study day B. For the "long-term" (24 hours) fat infusion, the subjects were hospitalized and the Intralipid infusion was initiated 24 hours before the time point 0 minute (for protocol B and −40 minutes for protocol A) and continued throughout the study period. Blood samples were collected at the time points; −10, −5, and 0 minutes before and at 1:45, 2:45, 4:45, 8:30, 10:30, 15:00, and 19:00 h:min after initiation of Intralipid infusion. All samples were analyzed for plasma glucose, insulin, C-peptide, and FFA. An isocaloric diet with standardized meals was served at 0:15, 3:15, 9:00 and 12:30 h:min after initiation of Intralipid infusion. Light exercise (50 W work load) was performed on a bicycle ergometer for 15 minutes at the time points 2:00 and 5:00 h:min. Short- and long-term Intralipid infusion is referred to as "2 h" and "24 h" fat infusion, respectively.

Twenty-four hour saline infusion. A study consisting of 24-hour saline infusion was performed to obtain 24 h "no fat" plasma glucose, insulin, C-peptide, and FFA profiles as described above. Due to the large number of study days, only 4 controls and 5 IGT relatives underwent the saline infusion.

Blood chemistry. Plasma glucose was determined using an automated glucose oxidase method (Glucose Analyzer 2; Beckman Instruments. Fullerton, CA). Tritiated glucose activity was measured as described by Hother-Nielsen and Beck Nielsen.[35] Plasma insulin and C-peptide concentrations were determined using the 1234 AutoDELFIA immunoassay system (Wallac Oy, Turku, Finland). Plasma FFA was quantified using an enzymatic colometric method (Wako, Richmond, VA).

Calculations

Glucose disposal rate (Rd), insulin action, was calculated during the basal and insulin-stimulated 30-minute steady state periods using Steele's nonsteady state equation.[36] In the calculations the distribution volume of glucose was taken as 200 mL · kg^{-1} and the pool fraction was 0.65.[37] Rd was expressed as mg · FFM^{-1} · min^{-1}.

Assessment of prehepatic insulin secretion rates. It has been shown that elevation of plasma FFA impairs the hepatic insulin clearance,[16] and prehepatic insulin secretion rate (ISR) was therefore calculated for each individual during the β-cell tests by deconvolution of C-peptide concentrations using a 2-compartment model of C-peptide kinetics[38,39] and population-based C-peptide kinetic parameters.[40] The prehepatic secretion rates were expressed as pmol · kg^{-1} · min^{-1}.

For the 2 tests and the 24-hour profile, areas under the curves (AUC) were calculated for ISR and plasma insulin, C-peptide, glucose, and FFA concentration using the trapezoidal method. The 24-hour profile study, however, was not designed for calculation of ISR. Plasma FFA was only measured right before and after the IVGTT test resulting in a mean FFA value for these tests. Insulin, C-peptide, and ISR derived from the IVGTT test were calculated as incremental area above baseline, AUC_{ISR}, from 0 to 6 minutes after glucose injection.

Glucose-stimulated insulin secretion during graded glucose infusion. Baseline glucose, insulin, and C-peptide, as well as ISR were calculated as the mean of the values of the −10, −5, and 0 samples. Mean ISR for the last 20 minutes for each glucose infusion rate was plotted against the corresponding mean glucose level to establish the dose-response relationship. Generally, the relationship was linear. Cross-correlation analyses were made to establish the dose response relationship between ISRs and glucose concentrations expressed as the regression coefficient or slope of the dose-response curve, referred to as the β-cell responsiveness to glucose, β.[41] To obtain comparable β values, the slope was calculated for the glucose range represented by all subjects: 7 to 13 mmol · L^{-1}.

Disposition index. To achieve a measure of β-cell function where the inverse relationship between insulin secretion and insulin action was taken into consideration, the first phase insulin secretion from the IVGTT ($AUC_{0\text{-}6\ min}$ above basal) was multiplied with the insulin action parameter, Rd_{ins}, from the clamp study, to give the so-called disposition index (DI) for each subject.

Insulin clearance. The average insulin clearance during the dose response test was calculated as $AUC_{ISR}/AUC_{insulin}$. For the 24-hour profile, the insulin clearance was calculated as $AUC_{C\text{-}peptide}/AUC_{insulin}$.

Basal values. Basal values were calculated as mean values for test day A and B with 0, 2, and 24 hours fat infusion, respectively. Each test day was represented by mean values derived from 3 basal blood samples.

Statistical analysis. The results are represented as mean ± SEM. The effects of fat infusion and subgroup status (IGT relative or control subject) were analyzed by 2-way analysis of variance (ANOVA) with subjects as a random factor. The effect of fat infusion was analyzed as an effect of duration, ie, the contrast corresponding to the difference between the 2- and 24-hour infusion and as an overall effect of fat compared with saline infusion, ie, the contrast corresponding to the difference between fat and saline infusion. The latter analysis was only considered appropriate if the effect of duration was not statistically significant. When the effect of duration was significant, values after 2 and 24 hours infusion were separately compared with saline infusion tests of the appropriate contrasts. The ANOVA also provided a test for

Table 2. Clinical Characteristics of Subjects

	IGT Relatives	Controls	*P* Value
No.	7	8	
Age (yr)	57 ± 2	53 ± 2	NS
Weight (kg)	101 ± 5	108 ± 6	NS
Height (cm)	177 ± 3	181 ± 1	NS
BMI (kg/m^2)	32.0 ± 1.2	32.7 ± 1.4	NS
Waist/hip ratio	1.00 ± 0.01	1.00 ± 0.02	NS
FFM (kg)	67.8 ± 2.3	67.8 ± 3.3	NS
Glucose (mmol · L^{-1})	6.6 ± 0.3	6.0 ± 0.1	NS
2-h glucose (mmol · L^{-1})	9.0 ± 0.3	5.6 ± 0.3	.003
FFA (mmol · L^{-1})	0.42 ± 0.01	0.46 ± 0.05	NS
Triglyceride (mmol · L^{-1})	1.6 ± 0.4	1.3 ± 0.3	NS
Cholesterol (mmol · L^{-1})	5.6 ± 0.2	5.6 ± 0.4	NS
HbA_{1c} (%)	5.8 ± 0.2	6.0 ± 0.1	NS

NOTE. Values are mean ± SEM.

Abbreviations: BMI, body mass index; FFM, fat free mass; HbA_{1c}, glycated hemoglobin; NS, not significant, $P > .05$.

similarity of the effects of fat infusion in the 2 groups, ie, an interaction effect. Only if this effect is insignificant will it be appropriate to perform the test of an overall difference between the 2 groups. Statistical significance was accepted at $P < .05$.

The assumption of normally distributed data with a common intrasubject variance was evaluated by inspection of summary statistics for each parameter and subgroup. Means and medians were generally not much different, and the quartiles also indicated symmetric distributions. Moreover, the ANOVA is known to be quite robust to deviations from normality and to have higher power for detection of effects than simple *t* tests or corresponding nonparametric tests.

RESULTS

Clinical Characteristics

Apart from the 2-hour plasma glucose concentrations following the OGTT, there were no significant differences between the 2 groups (Table 2).

Twenty-four–hour profiles. Four control subjects and 5 IGT relatives underwent the 24-hour saline infusion. Due to the low number of subjects in each group, statistics were performed only on the combined group (n = 9). However, plasma glucose, insulin, C-peptide, and FFA concentrations measured as AUC increased in all 9 individuals during fat infusion ($P < .03$) (Fig 3). Insulin clearance was reduced during fat infusion in the 24-hour profile study (no fat *v* fat 14.1 ± 1.2 *v* 10.4 ± 0.9, $P = .03$).

Basal values. The values shown in Table 3 are mean values for study day A and B, since no significant differences were found between the 2 days. Within the group of IGT relatives, the plasma FFA and triglyceride concentration increased from 0- to 2- and 24-hour fat infusion. The basal glucose concentration increased from 0- and 2- to 24-hour fat infusion, but plasma insulin, C-peptide, and ISR were not affected by fat infusion (Table 3). In the control subjects, no significant effects on basal plasma glucose and FFA were seen. The plasma

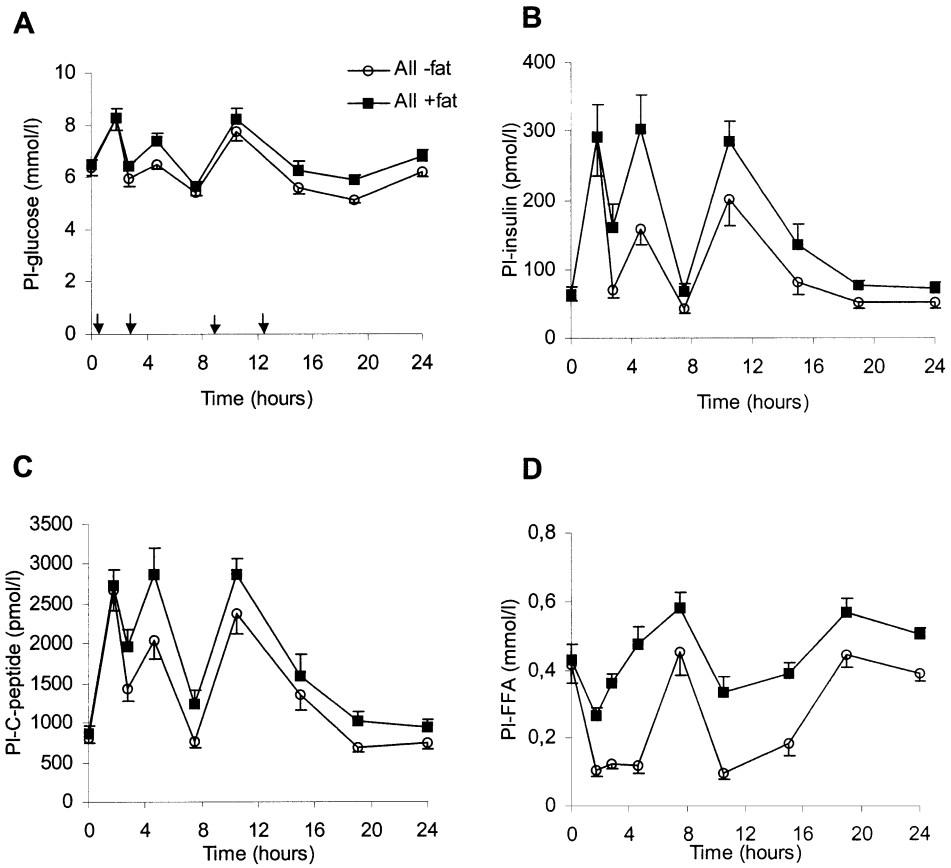

Fig 3. Mean ± SEM 24-hour profiles of (A) glucose, (B) insulin, (C) C-peptide, and (D) FFA in the study population (n = 9). ○, profile during saline infusion (control) study; ■, profile during Intralipid infusion study. Glucose, insulin, C-peptide, and FFA levels were higher in Intralipid *v* control study ($P < .01$); ↓, indicates meal.

Table 3. Basal Values

IGT relatives	Duration of Fat Infusion (h) 0	2	24	ANOVA *P* Value Effect of Duration	Fat
Glucose (mmol · L^{-1})	6.6 ± 0.3	6.5 ± 0.2*	6.8 ± 0.3†	.004	-
Insulin (pmol · L^{-1})	89.4 ± 19.9	87.7 ± 19.1	89.5 ± 15.2	NS	NS
C-peptide (pmol · L^{-1})	1,179 ± 156*	1,205 ± 184*	1,242 ± 139*	NS	NS
ISR (pmol · kg^{-1} · min^{-1})	3.05 ± 0.40†	3.16 ± 0.48†	3.26 ± 0.36†	NS	NS
FFA (mmol · L^{-1})	0.42 ± 0.01	0.55 ± 0.04	0.57 ± 0.05*	NS	.0002
Triglycerides (mmol · L^{-1})	1.6 ± 0.4	2.6 ± 0.4	2.2 ± 0.5	NS	<.0001
Controls					
Glucose (mmol · L^{-1})	6.0 ± 0.1	5.9 ± 0.1	6.1 ± 0.2	NS	NS
Insulin (pmol · L^{-1})	54.3 ± 8.6	45.4 ± 7.1	69.0 ± 10.1‡	.02	-
C-peptide (pmol · L^{-1})	639 ± 83	644 ± 72	847 ± 71‡	.02	-
ISR (pmol · kg^{-1} · min^{-1})	1.60 ± 0.18	1.64 ± 0.13	2.20 ± 0.15‡	.02	-
FFA (mmol · L^{-1})	0.46 ± 0.05	0.52 ± 0.03	0.46 ± 0.02	NS	NS
Triglycerides (mmol · L^{-1})	1.3 ± 0.3	2.0 ± 0.4	1.8 ± 0.3	NS	<.0001

NOTE. Values are mean ± SEM.

Abbreviations: NS, $P > .05$; ISR, insulin secretion rate.

*$P < .05$ *v* controls; †$P < .01$ *v* controls. ‡$P < .05$ *v* 0 fat.

triglycerides, however, showed a significant increase from both 0- to 2-hour and 24-hour fat infusion. The control subjects displayed increases in insulin, C-peptide, and ISR after 24-hour infusion relative to 0 and 2-hour infusion. The IGT relatives had higher plasma FFA concentrations than the control subjects after 24-hour fat infusion (ANOVA, $P = .02$) suggesting an impaired "FFA clearance" in the IGT relatives. The IGT relatives tended to have higher basal plasma glucose concentrations and had significantly higher plasma C-peptide concentration and ISR than the control subjects both with and without fat infusion (Table 3).

Glucose disposal rate. The euglycemic clamp glucose disposal rate (Rd_{ins}) decreased approximately 25% from 0 to 2 hours and from 0 to 24 hours fat infusion in both groups (Rd_{ins} IGT relatives, 0, 2, and 24 hours; 5.10 ± 0.48 *v* 3.69 ± 0.39 and 4.20 ± 0.48, $P = .04$ for 0 *v* 2 and 24 hours) (Rd_{ins} controls, 0, 2, and 24 hours; 8.82 ± 0.76 *v* 6.52 ± 0.42 and 6.11 ± 0.47, $P < .0001$ for 0 *v* 2 and 24 hours). The IGT relatives had significantly lower Rd_{ins} than the control subjects both with and without fat infusion ($P = .01$).

The mean plasma glucose concentrations during the clamp steady state period showed a slight deviation from the target value of 5.0 mmol · L^{-1} between the 0- to 24-hour fat infusion (4.9 ± 0.0 *v* 5.2 ± 0.1 mmol · L^{-1}, $P < .05$). No effects of fat, time, or group status were found on plasma insulin, C-peptide, or FFA during the basal or clamp steady state period in any of the groups (data not shown).

Table 4. IVGTT

	Duration of Fat Infusion (h) 0	2	24	ANOVA *P* Value Effect of Duration	Fat
IGT relatives					
$AUC_{insulin}$ (pmol · L^{-1} · min)	2,891 ± 1,115	4,353 ± 1,965	2,242 ± 728	.009	-
$AUC_{C\text{-}peptide}$ (pmol · L^{-1} · min)	10,300 ± 3,296	13,892 ± 4,844	7,493 ± 2,293	.001	-
AUC_{ISR} (pmol · kg^{-1})	74.1 ± 26.3	89.8 ± 33.8	51.1 ± 16.7*	.01	-
$AUC_{glucose\ 6\ min}$ (mmol · L^{-1} · min)	59.8 ± 2.8	61.9 ± 2.9	63.3 ± 2.4	NS	NS
DI (pmol · kg^{-1} · mg $kgFFM^{-1}$ · min^{-1})	355 ± 104	280 ± 84	241 ± 102	NS	NS
Controls					
$AUC_{insulin}$ (pmol · L^{-1} · min)	1,831 ± 493	1,871 ± 364	1,805 ± 315	NS	NS
$AUC_{C\text{-}peptide}$ (pmol · L^{-1} · min)	7,226 ± 1,722	7,542 ± 1,430	8,489 ± 1107	NS	NS
AUC_{ISR} (pmol · kg^{-1})	51.6 ± 14.0	49.7 ± 11.7	61.8 ± 9.3	NS	NS
$AUC_{glucose\ 6\ min}$ (mmol · L^{-1} · min)	48.6 ± 5.3	54.7 ± 5.4	65.1 ± 2.8*	.004	-
DI (pmol · kg^{-1} mg $kgFFM^{-1}$ · min^{-1})	455 ± 123	306 ± 60	386 ± 69	NS	NS
All					
D_{IVGTT} (pmol · kg^{-1} mg $kgFFM^{-1}$ · min^{-1})	408 ± 80	294 ± 49	319 ± 61	NS	.017

NOTE. Values are mean ± SEM.

Abbreviations: NS, $P > .05$; $AUC_{insulin}$; $AUC_{C\text{-}peptide}$, incremental area above baseline for the curve for insulin/C-peptide concentration (0–10 min); AUC_{ISR}, $AUC_{glucose\ 6\ min}$, incremental area under the curve for insulin secretion rate and glucose concentration (0–6 min); DI, disposition index (AUC_{ISR} Rd_{ina});

*$P < .05$ *v* 0 fat.

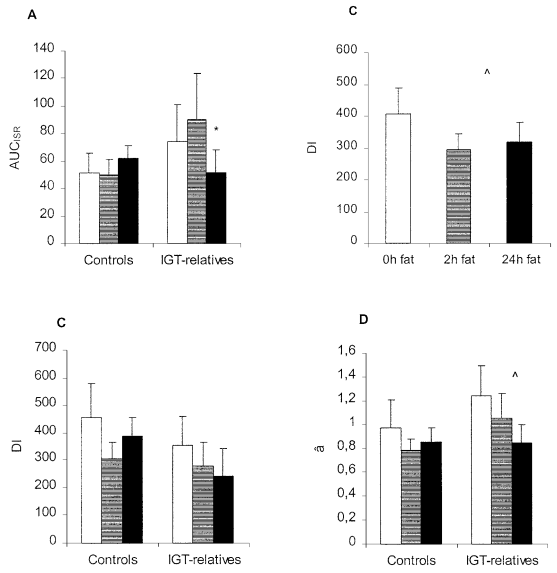

Fig 4. First phase insulin secretion during IVGTT expressed as the incremental area above basal from 0 to 6 minutes (A) (AUC_{ISR}), (B) DI during IVGTT for the 2 groups and (C) the total study population, and (D) glucose responsiveness (β) during 0- (□), 2- (▨), and 24-hour (■) fat infusion. AUC_{ISR} unit; (pmol · kg^{-1}), DI unit; (pmol · kg^{-1} · mg · FFM^{-1} · min^{-1}), β unit; (pmol · kg^{-1} · min^{-1}/$mmol^{-1}$ · L^{-1}). Data are the mean ± SEM for 7 IGT relatives and 8 control subjects. ^$P < .05$ *v* 2- and 24-hour fat infusion. *$P < .05$ *v* 2-hour fat infusion.

IVGTT. The $AUC_{insulin}$, $AUC_{c\text{-}peptide}$, and AUC_{ISR} in the IGT relatives all tended to increase from 0 to 2 hours fat infusion followed by a significant decline from 2 to 24 hours fat infusion (Table 4 and Fig 4A). The DI showed a tendency towards a decrease from 0 to 2 and 24 hours fat infusion (Table 4 and Fig 4B). Within the control group, the incremental AUC for plasma insulin, C-peptide, and ISR were all unaffected by fat infusion (Table 4 and Fig 4A). The DIs displayed a similar pattern as within the group of IGT relatives (Table 4, Fig 4B). When calculated for the total study population, DI decreased significantly after 2 and 24 hours fat infusion ($P = .017$) (Table 4 and Fig 4C). The IGT relatives tended to have lower DI than the control subjects both with and without fat infusion.

Insulin secretion during graded glucose infusion/dose response test. Within the group of IGT relatives AUC_{FFA} increased significantly from 0 to 2 and 24 hours fat infusion ($P < .0001$) (Table 5). $AUC_{insulin}$ increased after both 2 and 24 hours fat infusion, whereas $AUC_{C\text{-}peptide}$ and AUC_{ISR} were unaffected by fat infusion (Table 5). This may be explained by reduced insulin clearance in the IGT relatives after both 2 and 24 hours fat infusion. The $AUC_{glucose}$ increased from 0 to 24 hours fat infusion ($P < .05$), whereas the glucose responsiveness, β, decreased from 0 to 2 and 24 hours fat infusion (0-, 2-, and 24-hour fat infusion; 1.24 ± 0.26 *v* 1.05 ± 0.21 *v* 0.84 ± 0.16 (pmol · kg^{-1} · min^{-1}/mmol · L^{-1}), $P = .02$) (Table 5 and Fig 4D) illustrated by a rightward and downward shift in the dose response curves (Fig 5B). The AUC_{FFA} in the control group increased from 0 to 2 and 24 hours fat infusion (Table 5). The $AUC_{insulin}$, $AUC_{C\text{-}peptide}$ AUC_{ISR}, and $AUC_{glucose}$ were unaffected by short- and long-term fat infusion (Table 5). The β values were similar with and without fat infusion (Table 5 and Fig 4D). The IGT relatives had higher AUC_{ISR} compared with the controls after 0 and 2 hours fat infusion. After 24 hours

Table 5. Graded Glucose Infusion (dose-response) Test

	Duration of Fat Infusion (h)			ANOVA *P* Value: Effect of	
	0	2	24	Duration	Fat
IGT relatives					
$AUC_{insulin}$ (pmol · L^{-1} · min)	58,731 ± 15,402	72,302 ± 17,663	73,220 ± 15,304	NS	.02
$AUC_{C\text{-}peptide}$ (pmol · L^{-1} · min)	449,697 ± 59,059	478,048 ± 81,396	486,895 ± 61,632	NS	NS
AUC_{ISR} (pmol · kg^{-1})	1,526 ± 180*	1,587 ± 273*	1,576 ± 201	NS	NS
$AUC_{glucose}$ (mmol · L^{-1} · min)	2,007 ± 107	2,057 ± 61	2,299 ± 109†	<.0001	-
AUC_{FFA} (mmol · L^{-1} · min)	40.5 ± 3.4	84.4 ± 8.2	83.6 ± 7.4*	NS	<.0001
β (pmol · kg^{-1} · min^{-1}/mmol · L^{-1})	1.24 ± 0.26	1.05 ± 0.21	0.84 ± 0.16	NS	.02
Insulin clearance ($AUC_{ISR}/AUC_{insulin}$)	0.036 ± 0.009	0.026 ± 0.004	0.025 ± 0.003	NS	.004
Controls					
$AUC_{insulin}$ (pmol · L^{-1} · min)	41,276 ± 8,978	41,508 ± 7,814	53,674 ± 8,584	NS	NS
$AUC_{C\text{-}peptide}$ (pmol · L^{-1} · min)	308,407 ± 39,416	342,500 ± 43,102	367,756 ± 36,049	NS	NS
AUC_{ISR} (pmol · kg^{-1})	1,010 ± 135	1,058 ± 107	1,206 ± 120	NS	NS
$AUC_{glucose}$ (mmol · L^{-1} · min)	1,887 ± 68	1,905 ± 54	1,983 ± 79	NS	NS
AUC_{FFA} (mmol · L^{-1} · min)	37.7 ± 4.6	62.7 ± 4.2	52.0 ± 2.2	NS	.004
β (pmol · kg^{-1} · min^{-1}/mmol · L^{-1})	0.97 ± 0.24	0.78 ± 0.10	0.85 ± 0.12	NS	NS
Insulin clearance ($AUC_{ISR}/AUC_{insulin}$)	0.029 ± 0.003	0.029 ± 0.003	0.025 ± 0.002	NS	NS

NOTE. Values are mean ± SEM.

Abbreviations: NS, $P > .05$, $AUC_{insulin}$, $AUC_{C\text{-}peptide}$, AUC_{ISR}, $AUC_{glucose}$, AUC_{FFA}, area under the curve for insulin, C-peptide concentration; insulin secretion rate, glucose and FFA from 0 to 180 min.

*$P < .05$ *v* control subjects.

†$P < .05$ *v* 0 fat.

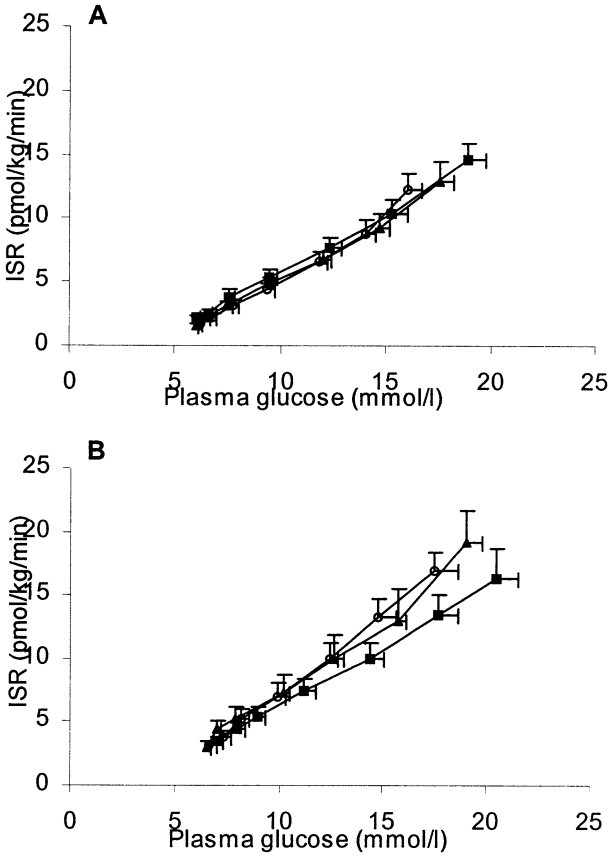

Fig 5. Dose-response relationship between glucose and ISR in (A) control subjects (n = 8) and (B) IGT relatives (n = 7) without (○) and after short- (▲) and long-term (■) Intralipid infusion. The slope of the line for glucose levels between 7 and 13 mmol · L^{-1}, β, decreased after 2- and 24-hour Intralipid infusion in the IGT relatives (P = .02).

fat infusion, the IGT relatives had higher AUC_{FFA} than the controls (Table 5).

DISCUSSION

We found that the first phase insulin response after the IVGTT decreased after long-term fat infusion in glucose intolerant first-degree relatives of type 2 diabetic patients. In addition, when expressed as a DI, first phase insulin secretion tended to decrease in both IGT relatives and controls after both short- and long-term fat infusion. In other words, the approximately 25% reduction of insulin action induced by low-grade short- and long-term fat infusion was not balanced by an adequate increase in first phase insulin secretion in IGT relatives or in matched controls. The glucose responsiveness of the β cell to a graded intravenous glucose infusion, expressed as the slope of the relationship between ISR and plasma glucose values, β, decreased after short- and long-term fat infusion in the IGT relatives, whereas β was unaffected by fat infusion in the controls, despite the fat-induced reduction of insulin action.

Short- and long-term Intralipid infusion decreased insulin clearance in the IGT relatives in agreement with the reduced extraction of insulin by the liver during FFA infusion reported by Wiesenthal et al.[42] Our findings are dependent on the accuracy of the estimation of ISRs by deconvolution of C-peptide concentrations.[40] To our knowledge, there is, however, no evidence that low-grade Intralipid infusion affects the kinetics of C-peptide.

Effect of Intralipid Infusion on Basal Insulin Secretion

The IGT relatives displayed higher basal insulin secretion rates than the controls both with and without fat infusion. The relatively higher basal ISR in the IGT relatives could simply be interpreted as an attempt to compensate for their insulin resistance. Basal ISR was not affected by fat infusion in the IGT relatives, resulting in an increase in basal plasma glucose concentration, whereas 24-hour fat infusion induced increased basal ISR in the control subjects without a concomitant elevation of basal plasma glucose concentration. Zhou et al[22] showed that 48-hour exposure of cultured rat islets to FFA at a constant glucose concentration (3.3 mmol · L^{-1}) increased basal insulin secretion several fold. Hirose et al studied the effect of long-term (7 days) elevated FFA (2 mmol · L^{-1}) on islets from healthy Wistar rats and lean (heterozygous) and obese (homozygous) ZDF rats and found that basal insulin secretion (glucose 3 mmol · L^{-1}) was increased in the healthy islets while basal insulin secretion was reduced in the islets from lean and obese ZDF rats predisposed to develop diabetes.[43,44] These observations support the notion that the increased basal insulin secretion observed in the controls might be a physiologic relevant time-dependent β-cell adaptation to elevated plasma FFA. The lack of increase in basal insulin secretion in the IGT relatives despite a minor increase in plasma glucose concentration might be due to a relative β-cell impairment, referred to as "lipid blindness."[45]

Effect of Intralipid Infusion on First Phase Insulin Response

The first phase insulin response to an intravenous glucose bolus is a sensitive test for even subtle β-cell defects and is thought to represent the release of granules stored in close proximity to the β-cell plasma membrane.[46,47] Furthermore, diminished first phase insulin response is an independent predictor of the risk of type 2 diabetes.[48] The IGT relatives showed a tendency towards increased first phase response after 2 hours fat infusion, whereas first phase insulin response decreased after 24 hours fat infusion. The first phase insulin response was not affected by Intralipid infusion in the control subjects. However, when the changes in insulin action were taken into consideration, the DI tended to decrease after short- and long-term fat infusion in both groups. Mean DI for the total study population decreased significantly after short- and long-term fat infusion. Thus, low-grade fat infusion appears to have the same effect on first phase insulin response in IGT relatives and controls when expressed as DI.

Chalkey et al[18] found no effect of acute elevation of plasma FFA on first phase insulin secretion in young non-obese first-degree relatives or matched controls after 30 minutes Intralipid infusion (fasting FFA levels 3 to 4 mmol · L^{-1}). DI was not calculated. The lack of effect of FFA on insulin release reported in Chalkey et al[18] was most likely explained by the very short-lasting FFA exposure. In contrast, Paolisso et al[21] re-

ported that the first phase insulin response increased after 6 hours 3-fold increase of plasma FFA (to approximately 1.5 mmol · L^{-1}), but decreased after 24 hours fat infusion in healthy, lean, young subjects. Insulin action decreased after both 6 and 24 hours fat infusion, but the DI was not calculated. These discrepancies between our data and their results might simply be ascribed to our much lower fat infusion rate, or perhaps more likely, be interpreted as the occurrence of a latent β-cell defect that is revealed during fat infusion in our subjects who were older, more obese and therefore at increased risk for developing type 2 diabetes. Several in vitro studies showing acute stimulatory effects of FFA on insulin secretion support the latter notion.[19,20]

Effect of Intralipid Infusion on Insulin Secretion During Graded Glucose Infusion

The ability of the β cell to respond to glucose over a wide glucose concentration range was examined during graded glucose infusion. The insulin release during the continuous glucose infusion is thought to originate from both de novo synthesis and stored secretory granules.[46,47] The IGT relatives displayed a fat-induced reduction of the insulin release during the graded glucose infusion resulting in a down- and rightward shift of the dose-response curve, expressed as a decrease in the slope β. Intralipid infusion did not affect ISR or glucose concentrations during the graded glucose infusion in the controls. In accordance with our findings, short- and long-term doubling of plasma FFA (approximately 1 mmol · L^{-1}) lipid exposure did not affect ISR during graded glucose infusion in young non-obese subjects[16] or first-degree relatives of diabetic patients.[15] However, Carpentier et al[17] showed that long-term (48 hours) doubling of fasting plasma FFA (to approximately 1 mmol · L^{-1}), in contrast to our findings, decreased insulin secretion during graded glucose infusion in elderly, obese controls, whereas no further fat-induced deterioration of the β-cell function was found in elderly, obese, diabetic patients. The discrepancies between our data and their findings might be explained by the higher, and perhaps to some extent, also by the more prolonged FFA infusion applied by Carpentier et al[17] and by the fact that their control group included individuals with IGT.

Carpentier et al[16] also studied the effect of short- (90 minutes) and long-term (48 hours) doubling of plasma FFA (to approximately 1 mmol · L^{-1}) on insulin secretion in young, lean, healthy subjects during a 2-step hyperglycemic clamp.[16] They found that short-term fat infusion decreased insulin action and increased insulin secretion appropriately resulting in an unchanged DI. Long-term fat infusion caused no change in insulin secretion resulting in decreased DI. The increased insulin secretion and thereby unchanged DI in response to short-time fat infusion is in accordance with the findings of Paolisso et al[21] during IVGTT, but in contrast to our findings in older, obese subjects.

In the IGT relatives, the β-cell response to the graded glucose infusion, β, decreased after short- and long-term fat infusion indicating an inability of the β cell to compensate for the fat-induced insulin resistance and thereby increasing glucose levels. The acute insulin response to an IVGTT decreased after long-term fat infusion in the IGT relatives. When expressed as DI, the first phase insulin response tended to decrease after acute and prolonged fat exposure in both IGT relatives and control subjects, however, statistical significance was reached when data were analyzed for the total study population. The results from both the acute stimulation and the graded glucose infusion suggest that several steps of the insulin secretory apparatus in the β cell react abnormally to glucose stimulation during FFA exposure in IGT relatives. Whether this is explained by an abnormal glucose sensing in the β cell or a direct effect of FFA on the insulin processing and secretion cannot be concluded from the present study.

Lipotoxicity in Predisposed Individuals

The β-cell responsiveness to graded glucose infusion and the first phase insulin response was more sensitive to low-grade fat exposure in the IGT relatives compared with controls. Despite the tendency towards higher insulin secretion rates in the IGT relatives, they tended to have lower DI than the controls during the IVGTT both with and without fat infusion. Furthermore, the IGT relatives had lower insulin action than the control subjects both with and without fat infusion. Lipotoxicity could therefore be considered relatively more harmful to prediabetics than controls, as low-grade elevation of plasma FFA seemed to bring the IGT relatives closer to the development of type 2 diabetes.

Carpentier et al.[17] showed that 48-hour fat infusion decreased glucose-stimulated insulin secretion during graded glucose infusion in obese 50-year-old subjects, whereas no effect on insulin secretion was found in age- and sex-matched patients with type 2 diabetes, indicating that no further deterioration can be induced by FFA in patients with type 2 diabetes. Nevertheless, our findings demonstrate that individuals genetically predisposed to type 2 diabetes with IGT are as least as sensitive to the deleterious effects of fat infusion on insulin secretion and action as control subjects without genetic predisposition and NGT.

Because IGT relatives of type 2 diabetic patients have an estimated more than 50% risk of progression to type 2 diabetes,[49] our findings underscores the importance of fat restriction to avoid deterioration of β-cell function and insulin action in genetically predisposed glucose intolerant subjects.

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