A Sustained Increase in Plasma Free Fatty Acids Impairs Insulin Secretion in Nondiabetic Subjects Genetically Predisposed to Develop Type 2 Diabetes

Sangeeta Kashyap,[1] Renata Belfort,[1] Amalia Gastaldelli,[1] Thongchai Pratipanawatr, Rachele Berria,[1] Wilailak Pratipanawatr,[1] Mandeep Bajaj,[1] Lawrence Mandarino,[1] Ralph DeFronzo,[1,2] and Kenneth Cusi[1,2]

Acute elevations in free fatty acids (FFAs) stimulate insulin secretion, but prolonged lipid exposure impairs β-cell function in both in vitro studies and in vivo animal studies. In humans data are limited to short-term (≤48 h) lipid infusion studies and have led to conflicting results. We examined insulin secretion and action during a 4-day lipid infusion in healthy normal glucose tolerant subjects with (FH+ group, $n = 13$) and without (control subjects, $n = 8$) a family history of type 2 diabetes. Volunteers were admitted twice to the clinical research center and received, in random order, a lipid or saline infusion. On days 1 and 2, insulin and C-peptide concentration were measured as part of a metabolic profile after standardized mixed meals. Insulin secretion in response to glucose was assessed with a +125 mg/dl hyperglycemic clamp on day 3. On day 4, glucose turnover was measured with a euglycemic insulin clamp with [3-^{3}H]glucose. Day-long plasma FFA concentrations with lipid infusion were increased within the physiological range, to levels seen in type 2 diabetes (~500–800 μmol/l). Lipid infusion had strikingly opposite effects on insulin secretion in the two groups. After mixed meals, day-long plasma C-peptide levels increased with lipid infusion in control subjects but decreased in the FH+ group (+28 vs. −30%, respectively, $P < 0.01$). During the hyperglycemic clamp, lipid infusion enhanced the insulin secretion rate (ISR) in control subjects but decreased it in the FH+ group (first phase: +75 vs. −60%, $P < 0.001$; second phase: +25 vs. −35%, $P < 0.04$). When the ISR was adjusted for insulin resistance ($ISR_{Rd} = ISR \div [1/R_d]$, where R_d is the rate of insulin-stimulated glucose disposal), the inadequate β-cell response in the FH+ group was even more evident. Although ISR_{Rd} was not different between the two groups before lipid infusion, in the FH+ group, lipid infusion reduced first- and second-phase ISR_{Rd} to 25 and 42% of that in control subjects, respectively (both $P < 0.001$ vs. control subjects). Lipid infusion in the FH+ group (but not in control subjects) also caused severe hepatic insulin resistance with an increase in basal endogenous glucose production (EGP), despite an elevation in fasting insulin levels, and impaired suppression of EGP to insulin. In summary, in individuals who are genetically predisposed to type 2 diabetes, a sustained physiological increase in plasma FFA impairs insulin secretion in response to mixed meals and to intravenous glucose, suggesting that in subjects at high risk of developing type 2 diabetes, β-cell lipotoxicity may play an important role in the progression from normal glucose tolerance to overt hyperglycemia. *Diabetes* 52:2461–2474, 2003

Type 2 diabetes is characterized by defects in both insulin secretion and insulin action (1). Subtle defects in β-cell function and insulin resistance precede the development of hyperglycemia in individuals at high risk of developing type 2 diabetes, including subjects with a strong family history of type 2 diabetes (2–5), obese subjects (6,7), individuals with impaired glucose tolerance (IGT) (2,4,5), and women with polycystic ovary syndrome (PCOS) (8,9) or a history of gestational diabetes (10). In these groups hyperglycemia develops as a result of a progressive decline in β-cell function, a finding reported across multiple ethnic populations (4,10–12).

Adipose tissue insulin resistance is believed to play an important role in the development of type 2 diabetes (1,13–16). Insulin resistance in adipose tissue is characterized by excessive rates of lipolysis, increased plasma free fatty acid (FFA) levels despite hyperinsulinemia, and impaired suppression of plasma FFA levels by insulin (1,14–16). Excessive rates of lipid turnover have been shown to precede the development of type 2 diabetes in subjects with a family history of type 2 diabetes (3–5) and nondiabetic obese individuals (6,7). The negative effect on glucose homeostasis of an elevation in plasma FFA concentration has been referred to as "lipotoxicity" (13). Elevation in the plasma FFA causes hepatic and skeletal muscle insulin resistance in healthy individuals (1,14–18). However, the effect of increased plasma FFA on insulin secretion has been less well studied, and it remains unknown whether a chronic elevation in plasma FFA concentration can impair β-cell function in subjects genetically predisposed to type 2 diabetes.

From the [1]Diabetes Division, Department of Medicine, the University of Texas Health Science Center at San Antonio, San Antonio, Texas; and the [2]Audie L. Murphy Veterans Administration Medical Center, San Antonio, Texas.

Address correspondence and reprint requests to Kenneth Cusi, University of Texas H.S.C. at San Antonio, Diabetes Division, Room 3.380S, 7703 Floyd Curl Dr., San Antonio, TX 78284-3900. E-mail: cusi@uthscsa.edu.

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EGP, endogenous glucose production; FFA, free fatty acid; FPG, fasting plasma glucose; FPI, fasting plasma insulin; IGT, impaired glucose tolerance; ISR, insulin secretion rate; LBM, lean body mass; PCOS, polycystic ovary syndrome; R_d, glucose disposal rate.

Under fasting conditions, mild elevations in plasma FFAs play an important role in sustaining basal insulin secretion and in maintaining a normal insulin secretory response to glucose (16). In contrast, exposure to pharmacological levels of lipids for >24–48 h have been shown to impair β-cell function in vitro (19) and in vivo (20–22).

In humans, an acute elevation in plasma FFA either has no effect (23,24) or enhances (25,26) glucose-induced insulin secretion, but the effect of a more prolonged increase in plasma FFA on glucose-stimulated insulin secretion has yielded more variable results. In lean healthy subjects, a 24- to 48-h lipid infusion has been reported to increase (27,28), not significantly change (26), or decrease (25) insulin secretion. In obese insulin-resistant individuals, a 48-h lipid infusion has been reported to reduce insulin secretion by ~20%, but plasma insulin concentration was increased because of a ~50% reduction in insulin clearance (29). In type 2 diabetes subjects, who already have a severe impairment in β-cell function, an increase in plasma FFA for 2 days with lipid infusion did not further worsen insulin secretion (29). These conflicting results may be explained, in part, by differences in study populations, plasma FFA levels achieved, variable duration of lipid infusion, or concomitant glucose infusion in some studies (27). At present, it remains unclear whether a prolonged (>48 h) physiological increase in plasma FFA concentration (~500–800 μmol/l) impairs insulin secretion in healthy glucose-tolerant subjects, and whether the response to elevated plasma FFA would differ in subjects who are genetically predisposed to develop type 2 diabetes. Of note, a reduction in plasma FFA concentration with the antilipolytic agent acipimox enhanced first-phase insulin secretion in nondiabetic patients with a family history of type 2 diabetes (30).

The aim of the present study was to test the hypothesis that normal glucose-tolerant individuals with a strong family history of type 2 diabetes (genetically predisposed to develop diabetes) might have a defective adaptation to elevated plasma FFA and be more susceptible to lipotoxicity compared with individuals without a family history of type 2 diabetes.

RESEARCH DESIGN AND METHODS

Subjects. A total of 13 healthy subjects with a strong family history of type 2 diabetes (the FH+ group) and 8 control subjects without a family history of type 2 diabetes participated in the study. The two groups of subjects were matched for age, sex, ethnicity, BMI, and lean body mass (Table 1). A strong family history of type 2 diabetes was defined as having both parents with type 2 diabetes (n = 12), or one parent and ≥2 siblings with type 2 diabetes (n = 1). All participants had a normal 75-g oral glucose tolerance test (OGTT). Body weight and physical activity were stable in all subjects for ≥3 months before enrollment. Each subject gave written informed consent before participation. The study protocol was approved by the institutional review board of the University of Texas Health Science Center at San Antonio, San Antonio, Texas.

Study design. After the initial screening visit, subjects were admitted to the clinical research center at 1700 on two occasions for the infusion, in random order, of Liposyn III (20% triglyceride emulsion, largely composed of soybean oil) or normal saline (control study) (Fig. 1). Heparin was not coinfused to avoid a maximal stimulation of lipoprotein lipase, which would artificially alter the physiological handling of lipid by the enzyme. A 20-gauge catheter was placed into an antecubital vein, and lipid or normal saline infusion was started at a rate of 30 ml/h. This rate was kept constant during the entire in-hospital stay. During the next 48 h (days 1 and 2), blood was drawn every 2 h from 0800 to 2400. Subjects were fed a eucaloric standardized diet and allowed to ambulate freely within the clinical research center. After an overnight fast, at 0800 on the morning of day 3, subjects underwent a +125 mg/dl hyperglycemic clamp. After this, subjects were given lunch (1300), dinner (1800), and a bedtime snack (2100), and fasted overnight (except for water). At 0700 on day 4, a 40 $mU \cdot m^{-2} \cdot min^{-1}$ euglycemic insulin clamp was performed with [3-^{3}H]glucose and indirect calorimetry. Subjects were discharged after this study. For both studies, all test substances were infused via the catheter inserted into the antecubital vein. A second catheter was inserted retrogradely into a vein on the dorsum of the hand for collection of blood samples, and the hand was placed in a thermoregulated box at 65°C to achieve arterialization of venous blood. Within 2–6 weeks, individuals were readmitted to the clinical research center and given either lipid or normal saline. During the repeat study, all procedures were performed in an identical fashion, as previously described.

TABLE 1
Clinical and laboratory characteristics of subjects

	Control subjects	FH+	P
n	8	13	—
Age (years)	39 ± 2	41 ± 2	NS
Sex (M/F)	4/4	4/9	—
BMI (kg/m^2)	25.3 ± 1.2	26.5 ± 0.8	NS
LBM (%)	68 ± 3	67 ± 2	NS
FPG (mg/dl)	90 ± 1	92 ± 3	NS
HbA_{1c} (%)	4.8 ± 0.1	5.0 ± 0.1	NS
2-h glucose (mg/dl)	96 ± 6	113 ± 5	NS
FPI (μU/ml)	6 ± 1	13 ± 5	<0.02
2-h insulin (μU/ml)	24 ± 6	82 ± 22	<0.003
Fasting FFA (μmol/l)	607 ± 86	575 ± 47	NS
Systolic BP (mmHg)	120 ± 4	120 ± 3	NS
Diastolic BP (mmHg)	69 ± 3	72 ± 2	NS
Triglyceride (mg/dl)	122 ± 21	113 ± 10	NS
HDL (mg/dl)	46 ± 3	48 ± 4	NS

Data are means ± SE. BP, blood pressure.

Metabolic profile. Subjects were admitted to the clinical research center at 1700 and fasted after a bedtime snack (2100). On the following 2 days (days 1 and 2), they underwent a metabolic profile from 0800 to 2400. Plasma was drawn before and every 2 h after each meal for glucose, insulin, C-peptide, and FFA concentrations. A research dietitian administered a weight-maintaining diet (50% carbohydrate, 30% fat, and 20% protein) with meals given at 0800, 1200, 1800, and 2100. The caloric distribution was 30, 30, 30, and 10% of total daily calories in each meal, respectively. Special attention was given to ensure that the timing and caloric distribution was identical in every subject between days 1 and 2 and during both admissions. Consumption of the entire meal was confirmed by a research nurse.

Hyperglycemic clamp. After an overnight fast, subjects underwent a hyperglycemic clamp on day 3 (31). Briefly, after collection of baseline samples, plasma glucose was acutely raised and maintained (±5%) by 125 mg/dl above baseline for 120 min by periodic adjustment of a 20% dextrose infusion based on the negative feedback principle. Plasma samples were obtained every 2 min from 0 to 10 min (first-phase insulin) and every 5 min from 10 to 120 min (second phase). Subjects voided immediately before and at the end of the study for measurement of urinary glucose loss.

Euglycemic insulin clamp. At 0730 on day 4, a primed (20 μCi/min × FPG/100) continuous (0.2 μCi/min) infusion of [3-^{3}H]glucose (DuPont-New England Nuclear, Boston, MA) was started and continued until the end of the study. After allowing 120 min for isotopic equilibration, a 2-h euglycemic insulin (40 $mU \cdot m^{-2} \cdot min^{-1}$) clamp was performed using a primed-continuous insulin infusion, and the plasma glucose concentration was maintained constant at each subjects' fasting level by periodic adjustment of a 20% dextrose infusion as previously described (31). Continuous indirect calorimetry (Deltatrac; Sensormedics, Anaheim, CA) was performed during the last 40 min of the baseline (−40 to 0 min) and insulin clamp (80–120 min) periods. Patients were fed at the conclusion of the study and discharged from the hospital.

Analytical determinations. The plasma glucose concentration was determined by the glucose oxidase method with a Beckman Glucose Analyzer II (Beckman Instruments, Fullerton, CA). Plasma insulin and C-peptide concentrations were determined by radioimmunoassays. The plasma FFA concentration was measured by standard colorimetric methods. Plasma glucose radioactivity was determined on barium hydroxide/zinc sulfate–precipitated plasma extracts.

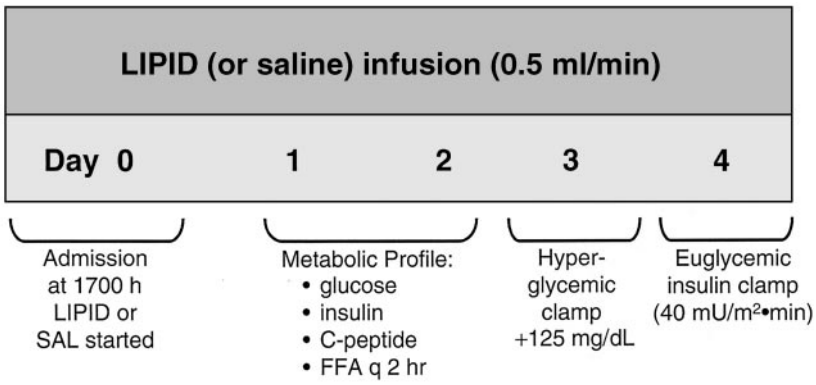

FIG. 1. Study design. LIPID, lipid infusion; SAL, saline infusion.

Calculations. The C-peptide areas under the curve after breakfast, lunch, and dinner were calculated using the trapezoidal method. During the hyperglycemic and insulin clamp studies, basal (−30 to 0 min) and steady-state (80–120 min) plasma glucose, FFA, insulin, and C-peptide represent the mean of values drawn at 10-min intervals. The steady-state glucose infusion rate during the hyperglycemic clamp represents the mean glucose infusion rate from 80 to 120 min, corrected for urinary glucose losses.

Estimation of insulin secretion rates. Insulin secretion rates (ISRs) were estimated from peripheral plasma C-peptide levels by deconvolution analysis and linear regularization using a two-compartment model with standard parameters for C-peptide kinetics (32). As validated by Van Cauter et al. (32), use of standard parameters for C-peptide clearance and distribution results in ISRs that differ only slightly from those obtained with individual parameters. There is no evidence that differences in plasma FFA levels or lipid infusion affect C-peptide kinetics in humans during acute (2 h) (24) or prolonged (24–48 h) lipid infusion (26,27,29). Insulin sensitivity differed significantly between FH+ and control subjects before and after lipid infusion. Diamond et al. (33) and others (10,11,34–37) have shown that the β-cell responds to the presence of insulin resistance with a compensatory increase in insulin secretion. Therefore, to assess ISR between groups with different insulin sensitivities, we related the ISR to the severity of insulin resistance (quantified with the insulin clamp). During the insulin clamp, insulin resistance is the inverse of the glucose disposal rate (R_d), i.e., the lower the R_d, the greater the insulin resistance. Thus, ISR related to peripheral insulin resistance (ISR_{Rd}), was expressed as:

$$ISR_{Rd} = \frac{ISR}{\text{Insulin resistance } (1/R_d)}$$

Estimation of endogenous insulin clearance. Hepatic insulin clearance was calculated by dividing the mean prehepatic ISRs obtained by deconvolution analysis of plasma C-peptide concentration ($pmol \cdot min^{-1} \cdot m^{-2}$) by the mean peripheral plasma insulin concentration ($pmol \cdot ml^{-1}$) during the 20–120 min time period of the hyperglycemic clamp (38).

Endogenous glucose production and insulin-stimulated R_d. During the basal period, the rate of endogenous plasma glucose appearance (which primarily represents hepatic glucose production) (39) equals the rate of plasma glucose disappearance and was calculated by dividing the [3-^{3}H]glucose infusion rate (dpm/min) by the steady-state plasma-tritiated glucose specific activity (dpm/mg) during the last 30 min of tracer equilibration. Because the infusion of insulin results in non–steady-state conditions, the rate of plasma glucose appearance was calculated using Steele's non–steady-state equation (40). During the euglycemic insulin infusion period, endogenous glucose production (EGP) was computed as the difference between the exogenous glucose infusion rate and the isotopically measured rate of plasma glucose appearance. The rate of total-body insulin-mediated glucose disposal was calculated by adding the residual rate of EGP to the rate of exogenous glucose infusion. Net glucose and lipid oxidation rates were calculated from oxygen consumption (Vo_2) and carbon dioxide production (Vco_2) using standard calorimetric equations. Nonoxidative glucose disposal, which primarily represents glycogen synthesis (1), was calculated by subtracting the rate of glucose oxidation (measured by indirect calorimetry) from the rate of total-body glucose disposal. Assessment of lean body mass (LBM) was done by dual-energy X-ray absorptiometry scanning (Hologic Delphi-A; Hologic, Bedford, MA).

Hepatic insulin sensitivity index. To better describe the effect of an elevation in plasma FFA concentration on hepatic insulin sensitivity, we report EGP in relation to the prevailing fasting plasma insulin (FPI) levels (EGP × FPI), using a validated index of hepatic insulin resistance (41). Under basal conditions, the majority (~85–90%) of EGP is derived from the liver (39), and there is a linear relationship between the rise in FPI and the decline in EGP over the range of plasma insulin concentrations from ~5 to 25 μU/ml (42).

Statistical analysis. All values represent the mean ± SE. Within-group differences were determined by the paired two-tailed Student's t test. Differences between basal and insulin clamp periods and between groups (the FH+ group vs. control subjects) were tested by two-way ANOVA for repeated measures. Normal distribution was checked before all analyses, and nonparametric estimates were used when appropriate. Comparisons were considered statistically significant if the P value was <0.05.

RESULTS

Patient characteristics and oral glucose tolerance test (Table 1). Both groups were well matched for clinical and metabolic parameters. In FH+ subjects, the fasting plasma glucose (FPG) concentration was kept within the normal range at the expense of a two- to threefold increase in the plasma insulin concentration.

Metabolic profile (48 h): plasma glucose, hormone, and FFA concentrations. During saline infusion, both groups had similar FPG (93 ± 2 vs. 93 ± 1 mg/dl for control vs. FH+ subjects, respectively; P = NS) and mean 48-h glucose concentrations (95 ± 3 vs. 96 ± 3 mg/dl, P = NS) (Fig. 2). In both groups lipid infusion caused a modest but significant ($P < 0.05$ vs. baseline, NS between groups) rise in FPG (98 ± 3 vs. 100 ± 2 mg/dl for control vs. FH+ subjects, respectively) and mean 48-h plasma glucose levels (101 ± 3 vs. 103 ± 3 mg/dl).

During saline infusion, both groups had similar fasting (492 ± 39 vs. 478 ± 50 μmol/ml for control vs. FH+ subjects, respectively; P = NS) and mean 48-h plasma FFA concentrations (347 ± 49 vs. 386 ± 45 μmol/ml, P = NS) (Fig. 2). Both groups experienced a similar ~1.7-fold rise in FFA levels during lipid infusion ($P < 0.01$ within each group vs. saline infusion). The mean 48-h plasma FFA concentration with lipid infusion (551 ± 64 vs. 686 ± 70 for control vs. FH+ subjects, respectively P = NS) was not significantly different between groups, although there was a trend for FFA to be slightly higher in the FH+ group. Fasting plasma triglycerides on day 4 were similar with saline infusion (97 ± 17 vs. 107 ± 7 mg/dl for control vs. FH+ subjects, respectively; P = NS) and slightly but not significantly higher in the FH+ group on lipid infusion (147 ± 12 vs. 187 ± 28 mg/dl, P = 0.26).

The mean fasting C-peptide level increased with lipid infusion in control subjects from 0.8 ± 0.2 to 1.2 ± 0.3 ng/ml but diminished in the FH+ group from 1.5 ± 0.3 to 1.2 ± 0.3 ng/ml ($P < 0.05$ for the difference from saline

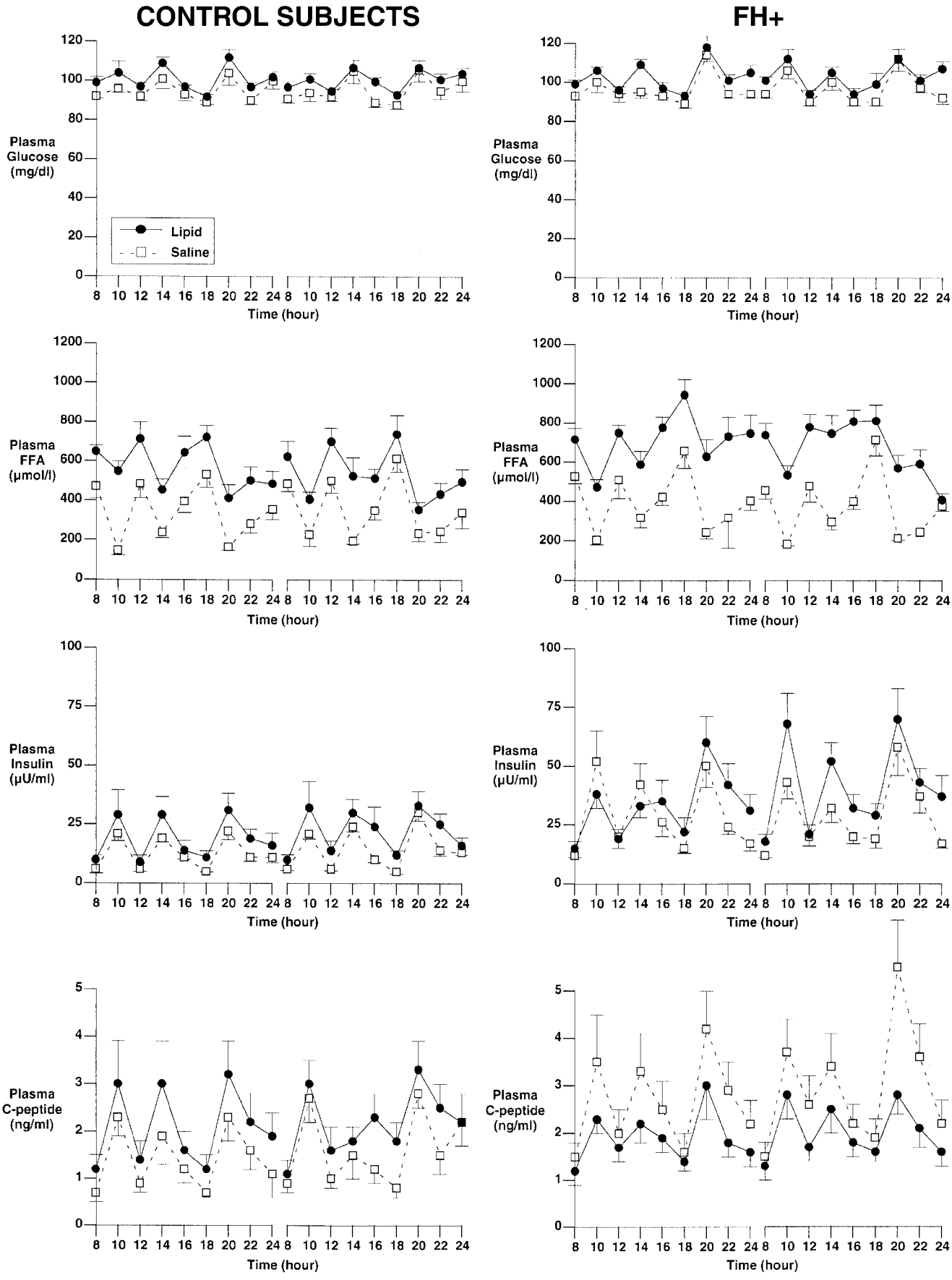

FIG. 2. Plasma glucose, FFA, insulin, and C-peptide concentrations during the 48-h metabolic profile in control subjects (left panels) and in subjects with a strong family history of type 2 diabetes (FH+; right panels). Data are means ± SE.

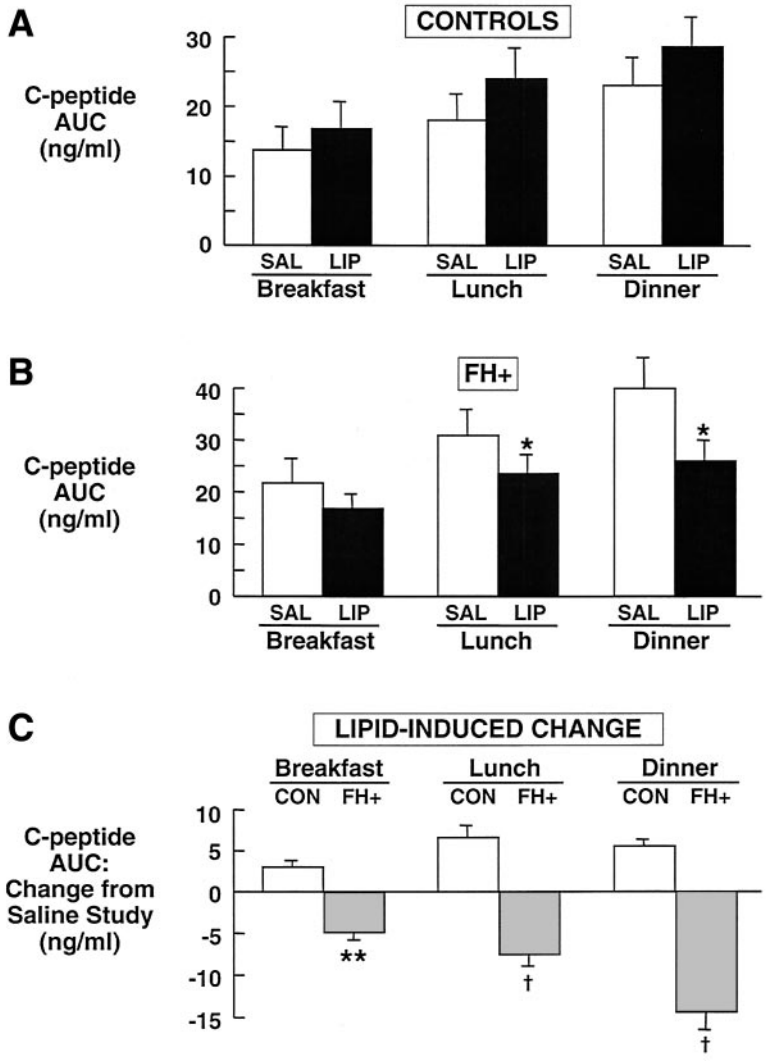

FIG. 3. Area under the curve (AUC) values for plasma C-peptide concentration (ng/ml) after breakfast (0800–1200), lunch (1200–1800), and dinner (1800–2400) during the 48-h metabolic profile in control subjects (CON) (*A*) and in subjects with a strong family history of type 2 diabetes (FH+) (*B*). □, saline infusion; ■, lipid infusion. *C*: Lipid-induced change. This panel summarizes the C-peptide area under the curve *change* induced by a 4-day lipid infusion compared with the respective saline study. LIP, lipid infusion; SAL, saline infusion. Data are means ± SE. **P* < 0.05 vs. saline; ***P* < 0.05 vs. control subjects; †*P* < 0.01 vs. control subjects.

infusion between groups). The mean C-peptide concentration during the 48-h metabolic profile (Fig. 2 and 3) increased 28% with lipid infusion in control subjects (1.7 ± 0.4 vs. 2.2 ± 0.6 ng/ml for saline vs. lipid infusion, respectively; P = NS), whereas it decreased by 30% in the FH+ group (2.8 ± 0.6 vs. 2.0 ± 0.4 ng/ml, $P < 0.05$; $P < 0.01$ control subjects vs. the FH+ group). In the FH+ group, C-peptide concentration was markedly reduced after each meal (Fig. 3). The deleterious effect of lipid on C-peptide secretion was more pronounced with increasing duration of lipid infusion exposure (Fig. 2). The deleterious effect of lipid infusion on β-cell function after each meal in the FH+ group is more clearly appreciated by comparing the change with lipid infusion relative to the saline infusion study, as summarized Fig. 3*C* and for the overall 48-h metabolic profile in Fig. 6.

FH+ subjects were hyperinsulinemic compared with control subjects during both the fasting state (10 ± 2 vs. 6 ± 1 μU/ml for control vs. FH+ subjects, respectively; $P < 0.01$) and the 48-h metabolic profile (29 ± 5 vs. 13 ± 2 μU/ml, $P < 0.01$) (Fig. 2). Lipid infusion caused a ~50% increase in 48-h mean insulin levels in control subjects (13 ± 2 to 18 ± 5 μU/ml, $P < 0.05$) but only a modest 11% increase in the FH+ group (29 ± 5 to 37 ± 7 μU/ml, P = NS). The small increase in the FH+ group resulted from a marked reduction in insulin clearance (−42%, $P < 0.001$), as estimated from the ISR during the hyperglycemic clamp studies (see below). In contrast, in control subjects lipid infusion did not significantly alter insulin clearance (~5%, P = NS).

Hyperglycemic clamp (day 3) (Figs. 4 and 5). Fasting plasma FFA concentrations were not significantly different between groups, although they were slightly higher in the FH+ group (saline infusion: 543 ± 54 vs. 651 ± 32 μmol/l for control vs. FH+ subjects, respectively; $P = 0.15$; lipid infusion: 701 ± 67 vs. 785 ± 57 μmol/l, $P = 0.31$). During the hyperglycemic clamp studies, the decrease from baseline in plasma FFA concentration was similar in both groups during saline infusion (−461 ± 52 vs. −509 ± 64 μmol/l for control vs. FH+ subjects, respectively; P = NS) and lipid infusion (−380 ± 34 vs. −404 ± 29 μmol/l, P = NS) studies.

The increment in plasma glucose concentration was nearly identical between groups during saline infusion (+125 ± 2 vs. +127 ± 1 mg/dl for control vs. FH+ subjects, respectively; P = NS) and lipid infusion (+125 ± 2 vs. +125 ± 1 mg/dl, P = NS) studies. The amount of glucose metabolized (M) per unit of plasma insulin concentration (I), a measure of tissue sensitivity to endogenously secreted insulin, was significantly reduced in the

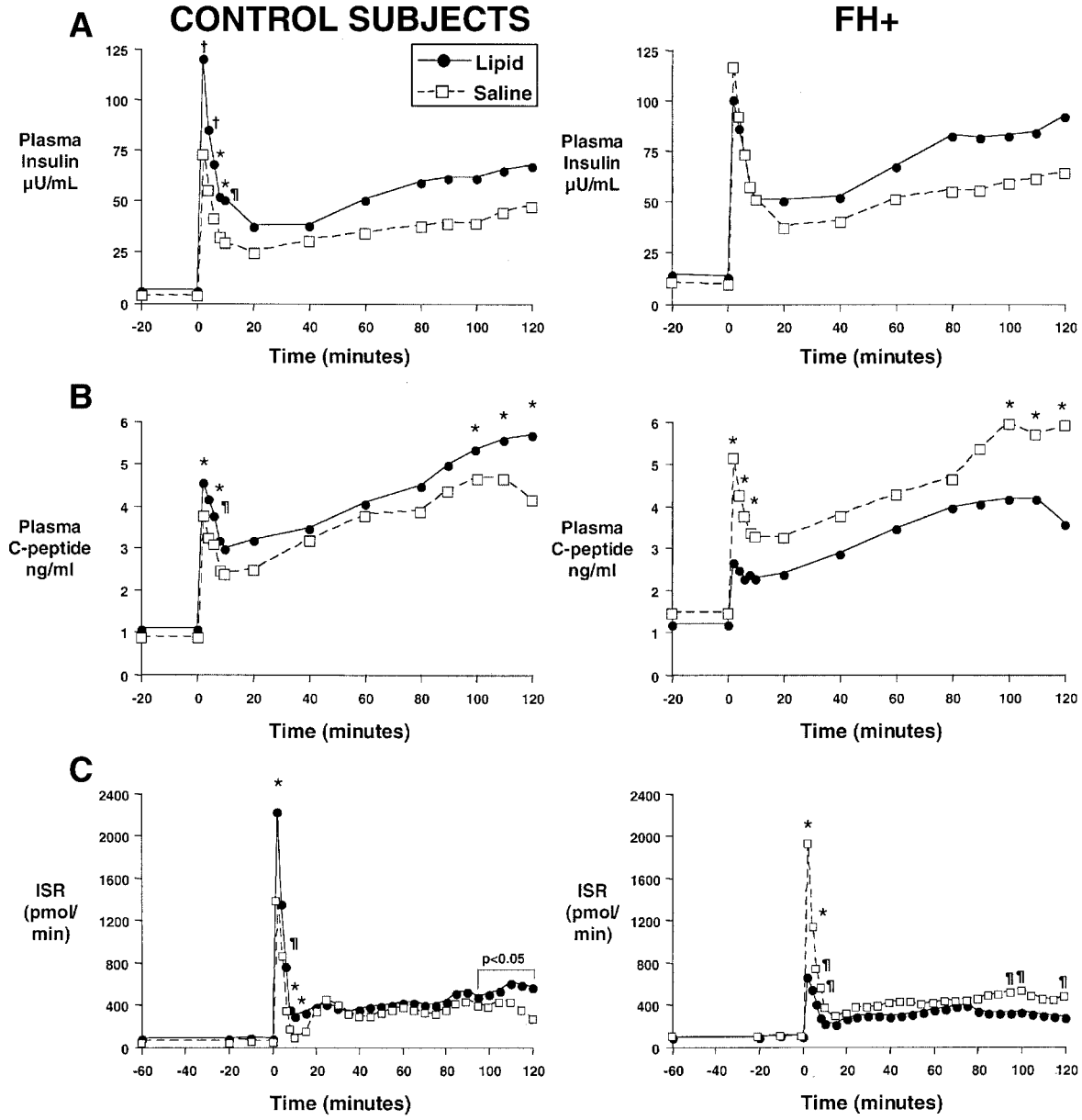

FIG. 4. Plasma insulin (*A*) and C-peptide (*B*) concentration and ISR (*C*) during the hyperglycemic clamp studies (day 3) with saline (□) or lipid (■) infusion in control subjects and in subjects with a strong family history of type 2 diabetes (FH+). †*P* < 0.001 vs. saline; **P* < 0.01 vs. saline; ¶*P* < 0.05 vs. saline.

FH+ group compared with control subjects during saline infusion (6.7 ± 0.8 vs. 15.1 ± 1.4 mg/kg $LBM^{-1} \cdot min^{-1}$ per μU/ml, $P < 0.0001$) and lipid infusion (5.8 ± 0.6 vs. 11.4 ± 1.0 mg/kg $LBM^{-1} \cdot min^{-1}$ per μU/ml, $P < 0.0001$). There was a close correlation between insulin sensitivity during the hyperglycemic (*M/I*) and euglycemic insulin (R_d) clamps ($r = 0.94$, $P < 0.00001$ for saline infusion; $r = 0.82$, $P < 0.001$ for lipid infusion; and $r = 0.90$, $P < 0.0001$ for saline infusion and lipid infusion studies combined).

Plasma insulin and C-peptide concentrations and ISR during the hyperglycemic clamp are shown in Figs. 4 and 5. During saline infusion, the incremental first-phase (Δ72 ± 14 vs. Δ43 ± 6 μU/ml, $P < 0.08$) and second-phase (Δ44 ± 4 vs. Δ33 ± 5 μU/ml, $P < 0.13$) insulin responses to hyperglycemia tended to be higher in FH+ versus control subjects. During lipid infusion, the incremental first-phase insulin response increased by 63% in control subjects (Δ43 ± 6 to Δ70 ± 15 μU/ml, $P < 0.01$) but decreased by 12% in the FH+ group (Δ70 ± 17 to Δ62 ± 14 μU/ml, $P < 0.01$ control vs. FH+ subjects) (Figs. 4 and 5). Lipid infusion in control subjects enhanced the incremental second-phase insulin response by 50% (Δ33 ± 5 to Δ50 ± 8 μU/ml, $P < 0.01$). In the FH+ group, the incremental second-phase insulin response increased by only 38% ($P < 0.05$ vs. control subjects). The plasma insulin response to hyperglycemia represents the composite of both insulin secretion and insulin clearance. It is well established that increased plasma FFA levels inhibit hepatic insulin clearance (29,43–45). Before lipid infusion, the FH+ group had slightly lower insulin clearance compared with control subjects (0.72 ± 0.09 vs. 1.01 ± 0.13 $l \cdot min^{-1} \cdot m^{-2}$, $P < 0.11$), most likely caused by insulin resistance and chronic hyperinsulinemia (8,35,38,46–53). Lipid infusion caused a marked 42% decrease in insulin

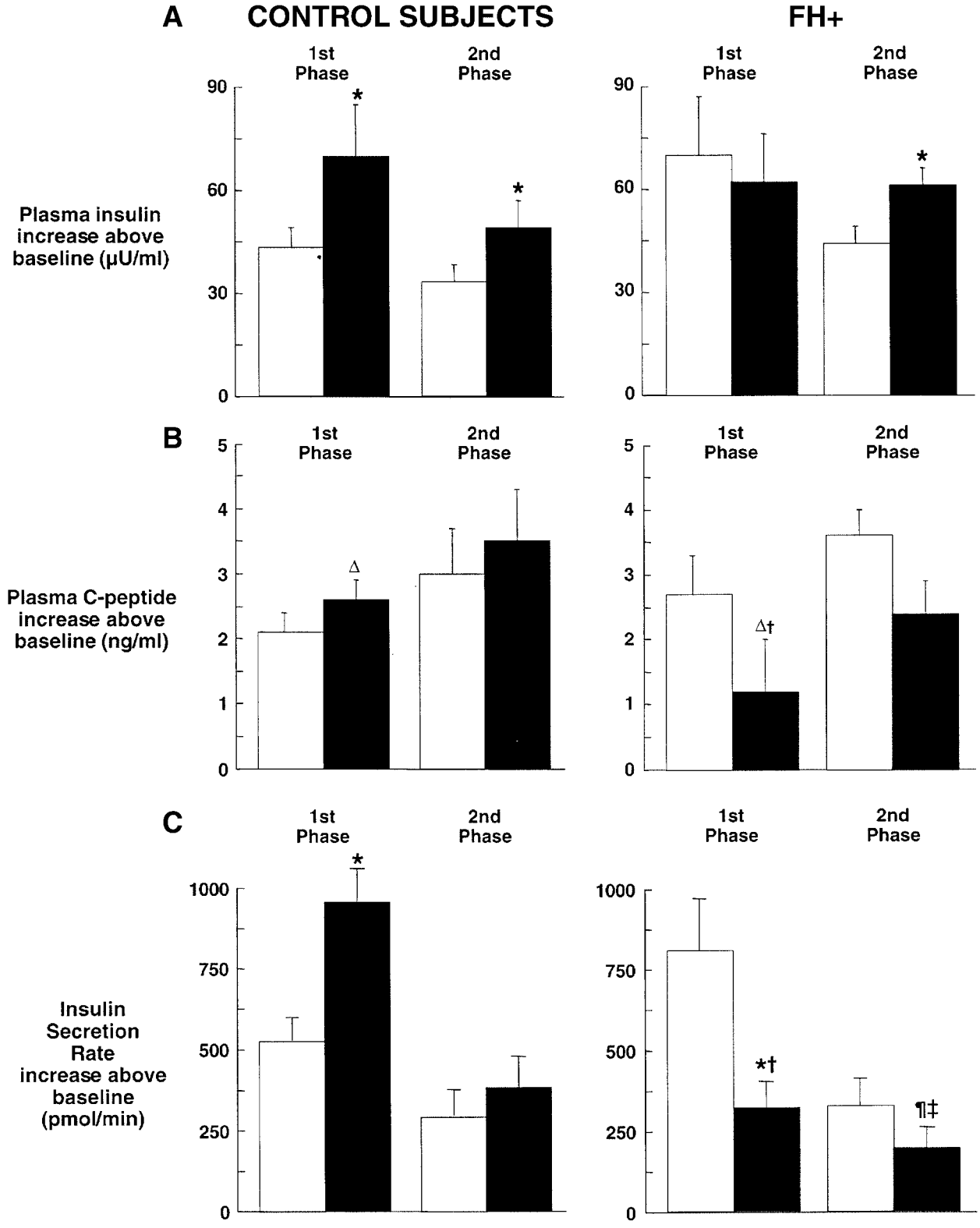

FIG. 5. Incremental plasma insulin (*A*), C-peptide (*B*) concentration, and ISR (*C*) during the hyperglycemic clamp studies (day 3) with saline or lipid infusion in control subjects and in subjects with a strong family history of type 2 diabetes (FH+). □, saline infusion; ■, lipid infusion. **P* < 0.01 vs. saline; Δ*P* < 0.02 vs. saline; ¶*P* < 0.05 vs. saline; †*P* < 0.001 vs. control subjects; ‡*P* < 0.04 vs. control subjects.

clearance in the FH+ group (from 0.72 ± 0.09 to 0.42 ± 0.07 l · min^{-1} · m^{-2}, $P < 0.002$) but had no effect on insulin clearance in control subjects (1.01 ± 0.13 to 0.96 ± 0.08 l · min^{-1} · m^{-2}, NS; $P < 0.01$ vs. the FH+ group). A strong correlation was noted between plasma FFA and insulin concentrations ($r = 0.57$, $P < 0.01$) but not between plasma FFA and C-peptide levels ($r = 0.23$, $P > 0.5$). If the increase in plasma FFA concentration had not markedly

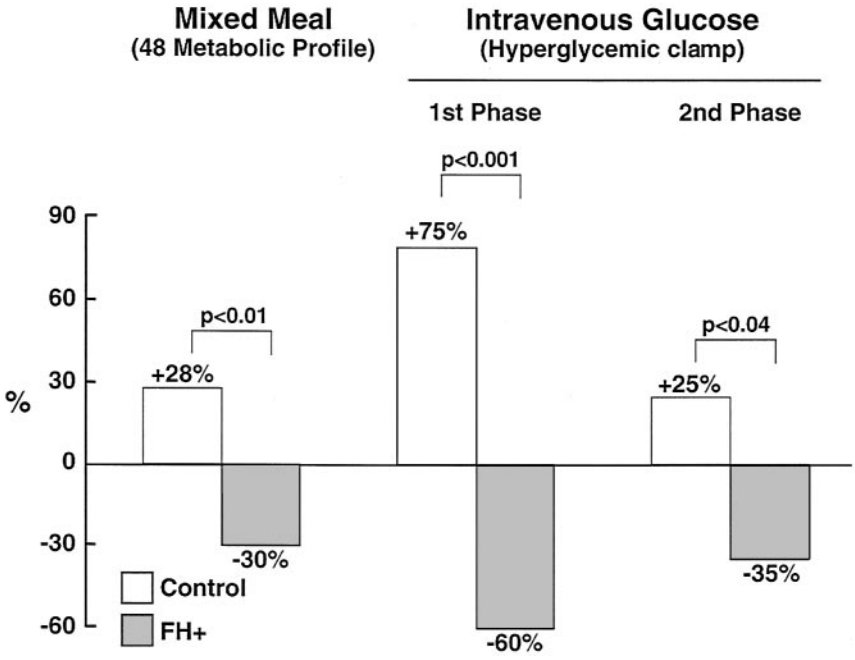

FIG. 6. Effect of lipid infusion compared with saline infusion on C-peptide concentration in response to mixed meals (from 48-h metabolic profile) and ISRs in response to intravenous glucose (from hyperglycemic clamps) in control subjects (□) and in subjects with a strong family history of type 2 diabetes (FH+) (▨). Data are expressed as the percentage difference from the saline study.

reduced insulin degradation in the FH+ group, the plasma insulin concentrations during the hyperglycemic clamp would have been much lower.

In control subjects, lipid infusion caused a significant increase above baseline in first-phase (0–10 min) C-peptide concentration compared with saline infusion (Δ2.1 ± 0.3 to Δ2.6 ± 0.3 ng/ml, $P < 0.02$) (Fig. 4*B* and 5*B*), particularly at 2 min ($P < 0.002$) and 4 min ($P < 0.006$) (Fig. 4*B*). There was also a 17% increase in second-phase (10–120 min) insulin response that did not reach statistical significance but that was progressively greater with the duration of the glucose infusion (90 min: $P < 0.056$; 100 min: $P < 0.008$; 110 min: $P < 0.004$; and 120 min: $P < 0.002$) (Fig. 4*B*). In marked contrast, lipid infusion in the FH+ group led to a 56% reduction in incremental first-phase C-peptide response to hyperglycemia (Δ2.7 ± 0.6 to Δ1.2 ± 0.4 ng/ml, $P < 0.02$; $P < 0.01$ vs. control subjects) and a 33% decrease in incremental second-phase C-peptide response to hyperglycemia (Δ3.6 ± 0.8 to Δ2.4 ± 0.5 ng/ml, $P = 0.06$; $P < 0.05$ vs. control subjects) (Figs. 4 and 5).

In control subjects, the increase above baseline in first-phase ISR with lipid infusion was 75% greater than on saline infusion (Δ512 ± 71 vs. Δ897 ± 174 pmol/min for saline vs. lipid infusion, respectively; $P < 0.01$) (Fig. 4*C* and 5*C*). A similar trend was observed in second-phase ISR (Δ287 ± 64 vs. Δ357 ± 81 pmol/min for saline vs. lipid infusion, respectively; $P = 0.16$). This contrasted with the marked reduction in the FH+ group during lipid infusion versus saline infusion studies: first-phase ISR was reduced 60% (Δ818 ± 162 vs. Δ311 ± 86 pmol/min, $P < 0.003$) and second-phase ISR decreased by 35% (Δ327 ± 63 vs. Δ208 ± 49 pmol/min, $P < 0.04$) (Fig. 5*C*). The reduction in second-phase ISR response in the FH+ group compared with the increase in control subjects was most evident toward the last 40 min of the hyperglycemic clamp, during which time ISR increased by 25% in control subjects ($P < 0.05$ vs. saline infusion), compared with a 50% reduction in the FH+ group ($P < 0.01$ vs. saline infusion; $P < 0.01$ between groups). Figure 6 summarizes the striking differences in β-cell response to lipid infusion between control and FH+ subjects.

Because basal and glucose-stimulated insulin secretion are increased in the presence of insulin resistance (10,11, 33–37), we related the insulin secretory rate to the severity of the prevailing insulin resistance (ISR_{Rd}). As shown in Fig. 7, first-phase ISR ($ISR_{Rd\ 0–10}$) was similar in both groups on saline infusion: 37 ± 5 vs. 35 ± 8 pmol/min per (mg · kg LBM^{-1} · min^{-1}) · 10^{-2} for control vs. FH+ subjects, respectively; P = NS. However, it diverged significantly in response to an elevation in plasma FFA: control subjects experienced a 48% increase in $ISR_{Rd\ 0–10}$ ($P < 0.01$ vs. saline infusion), whereas it decreased by 63% in the FH+ group: from 37 ± 5 to 54 ± 12 vs. from 35 ± 8 to 13 ± 4 pmol/min per (mg · kg LBM^{-1} · min^{-1}) · 10^{-2} for control vs. FH+ subjects, respectively; $P < 0.01$ vs. saline infusion; $P < 0.001$ between groups) (Fig. 7*A*). In control subjects, second-phase ISR ($ISR_{Rd\ 10–120}$) increased in direct proportion to the lipid-induced increase in insulin resistance, and $ISR_{Rd\ 10–120}$ remained constant: 20 ± 5 vs. 22 ± 6 pmol/min per (mg · kg LBM^{-1} · min^{-1}) · 10^{-2} for saline vs. lipid infusion, respectively; P = NS. In contrast, lipid infusion in the FH+ group led to a 32% reduction in $ISR_{Rd\ 10–120}$: 14 ± 3 vs. 9 ± 3 pmol/min per (mg · kg LBM^{-1} · min^{-1}) · 10^{-2} for saline vs. lipid infusion, respectively ($P < 0.05$; $P < 0.05$ between groups) (Fig. 7*B*).

Euglycemic insulin clamp (day 4) (Fig. 8). Plasma glucose concentration during the insulin clamp studies were similar in both groups during saline infusion and lipid infusion (95 ± 1 to 97 ± 1 mg/dl) (Fig. 8). FPI was lower in control subjects versus the FH+ group (4 ± 1 vs. 8 ± 1 μU/ml, $P < 0.01$) and rose in both groups with lipid infusion (6 ± 1 vs. 15 ± 2 μU/ml, $P < 0.01$ vs. baseline and between groups). The increments in plasma insulin concentration during the insulin clamp were similar during saline infusion and lipid infusion in control and FH+ subjects (49 ± 4 to 54 ± 4 μU/ml). Fasting plasma FFA was slightly greater in FH+ (648 ± 61 μmol/l) versus control subjects (501 ± 51 μmol/l, $P = 0.09$) during saline

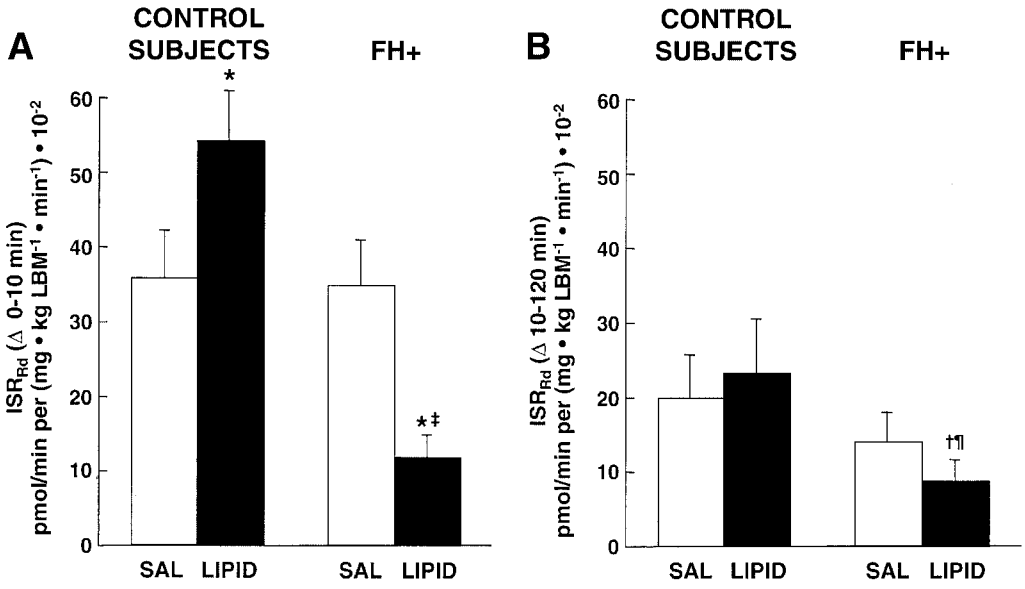

FIG. 7. ISRs during the hyperglycemic clamp studies related to the prevailing severity of insulin resistance (ISR$_{Rd}$). *A*: First-phase ISR$_{Rd\ 0–10}$. *B*: Second-phase ISR$_{Rd\ 10–120}$. Insulin resistance is the inverse of insulin-stimulated glucose disposal (R_d), as determined during the euglycemic insulin clamps ($1/R_d$). When comparing control vs. FH+ subjects, first-phase ISR$_{Rd}$ is similar during the saline studies; with lipid infusion, first-phase ISR$_{Rd}$ deteriorates in FH+ subjects, whereas it increases in control subjects. Second-phase ISR$_{Rd}$ is also reduced by lipid infusion in the FH+ group but is unchanged in control subjects. *$P < 0.01$ vs. saline; †$P < 0.05$ vs. saline; ‡$P < 0.001$ vs. control subjects; ¶$P < 0.05$ vs. control subjects.

infusion, and it rose to similar levels with lipid infusion (691 ± 53 vs. 797 ± 62 μmol/l for control vs. FH+ subjects, respectively; $P = 0.24$ between groups and $P < 0.01$ vs. saline infusion for both groups). Suppression of plasma FFA during insulin infusion was greater in control than FH+ subjects during both saline infusion (86 ± 12 vs. 138 ± 15 μmol/l, $P < 0.02$) and lipid infusion (301 ± 26 vs. 398 ± 30 μmol/l, $P < 0.03$) studies.

Basal EGP was not different between groups during saline infusion (2.7 ± 0.1 vs. 2.4 ± 0.1 mg · kg LBM^{-1} · min^{-1} for control vs. FH+ subjects, respectively; $P = 0.8$). Lipid infusion caused a small but significant increase in basal EGP in the FH+ group (from 2.4 ± 0.1 to 2.9 ± 0.2 mg · kg LBM^{-1} · min^{-1}, $P < 0.001$), whereas basal EGP was unchanged in control subjects during lipid infusion (2.7 ± 0.1 to 2.8 ± 0.1 mg · kg LBM^{-1} · min^{-1}, NS). The hepatic insulin resistance index (basal EGP × FPI) during saline infusion was increased in FH+ versus control subjects (19 ± 2 vs. 11 ± 2 mg · kg LBM^{-1} · min^{-1} × μU/ml, $P < 0.04$). Lipid infusion had no significant effect on the basal hepatic insulin resistance index in control subjects (11 ± 2 vs. 14 ± 2 mg · kg LBM^{-1} · min^{-1} × μU/ml, $P = 0.14$) but increased it significantly in the FH+ group from 19 ± 2 to 44 ± 6 mg · kg LBM^{-1} · min^{-1} × μU/ml ($P < 0.001$ vs. saline infusion; $P < 0.004$ vs. control subjects). EGP was nearly completely suppressed during the insulin clamp on saline infusion in both groups, but lipid infusion significantly impaired the suppression of EGP in the FH+ group (0.22 ± 0.08 vs. 0.71 ± 0.10 mg · kg LBM^{-1} · min^{-1} for saline vs. lipid infusion, respectively; $P < 0.002$) but not in control subjects (0.20 ± 0.03 vs. 0.32 ± 0.03 mg · kg LBM^{-1} · min^{-1}, NS; $P < 0.01$ vs. the FH+ group).

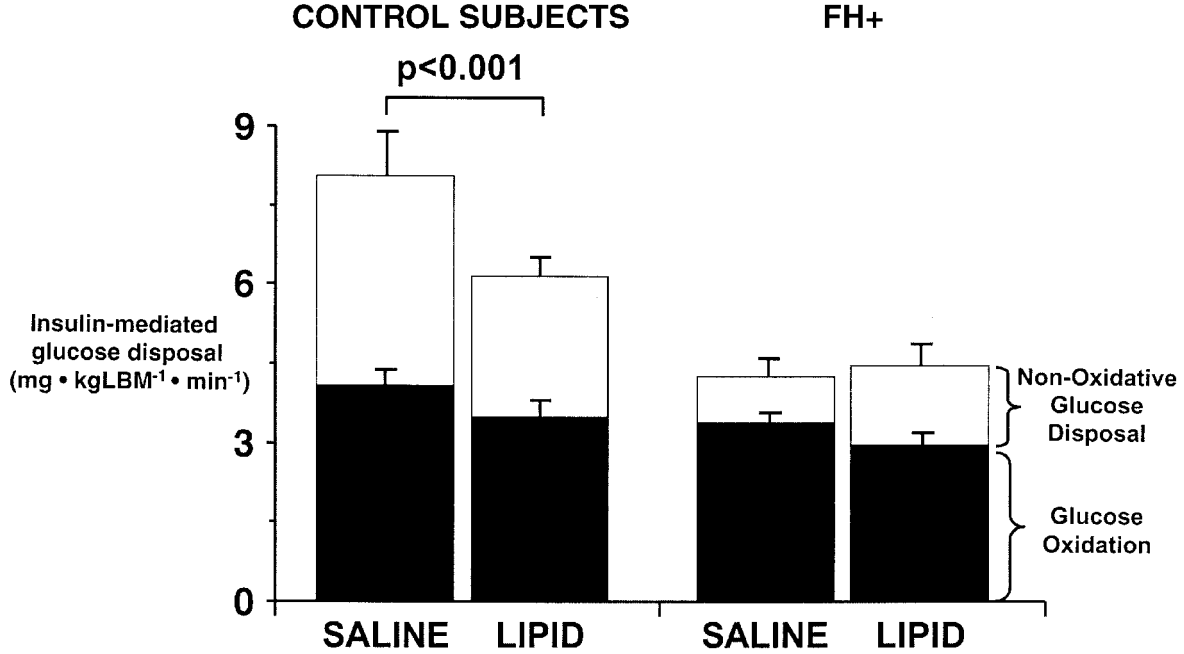

FIG. 8. Insulin-stimulated glucose disposal (R_d), glucose oxidation (black area of bars) and nonoxidative glucose disposal (open area of bars) during the euglycemic insulin clamp (day 4) in control subjects and subjects with a strong family history of type 2 diabetes (FH+). Data are means ± SE.

During saline infusion, insulin-stimulated glucose disposal (R_d) was reduced by 47% in the FH+ group compared with control subjects (4.3 ± 0.3 vs. 8.1 ± 0.8 mg · kg LBM^{-1} · min^{-1}, $P < 0.0001$) (Fig. 8). Lipid infusion decreased R_d by 24% in control subjects ($P < 0.001$), but it caused no further deterioration in the FH+ group ($P < 0.03$ vs. control subjects during lipid infusion) (Fig. 8). Basal glucose oxidation was similar between groups during saline infusion, and it was unchanged by lipid infusion. During the insulin clamp, glucose oxidation increased more in control subjects compared with the FH+ group during saline infusion ($P < 0.05$) (Fig. 8), whereas lipid infusion impaired glucose oxidation similarly in both groups by ~15% ($P < 0.01$ vs. saline infusion) (Fig. 8). Nonoxidative glucose disposal was greatly reduced in response to insulin stimulation in the FH+ group compared with control subjects during saline infusion and lipid infusion studies (both $P < 0.01$) (Fig. 8).

DISCUSSION

Numerous studies have demonstrated that elevated plasma FFA levels cause hepatic and muscle insulin resistance (1,14–18). However, the effect of elevated plasma FFA levels on pancreatic β-cell function remains unclear. In humans, studies have been limited to acute (23,24) or short-term (24–48 h) (25–29) elevations in plasma FFA concentration, usually within the pharmacological range (~1,000–1,500 μmol/l). This study examines, for the first time, the effect of an increase in plasma FFA concentration beyond 48 h on insulin secretion and insulin action. We also used a more physiological increase in the plasma FFA, targeting plasma FFA to levels seen in obesity and type 2 diabetes (~500–800 μmol/l). Heparin was not coinfused with lipids to avoid artificially stimulating lipoprotein lipase. We studied normal glucose-tolerant subjects with a strong family history of type 2 diabetes because we hypothesized that β-cell function might be more susceptible to the deleterious effects of lipotoxicity in this population that is genetically predisposed to develop type 2 diabetes.

The most striking finding is that, in FH+ subjects, a sustained physiological increase in plasma FFA concentration for 3 days markedly impaired both acute (first-phase) and second-phase insulin secretion by 60 and 35%, respectively. To fully appreciate the severity of FFA-induced impairment in β-cell function, ISRs were examined in the context of the prevailing insulin resistance. ISR was assessed with the hyperglycemic clamp (day 3) and insulin resistance (inverse of R_d) was assessed with the euglycemic insulin clamp technique (day 4). These studies were performed on separate days because it is not feasible to perform both tests on the same day. It should be noted that the measurement of insulin sensitivity (M/I) from the hyperglycemic clamp correlated very closely ($r = 0.90$, $P < 0.0001$) with the measure of insulin sensitivity (R_d) from the euglycemic insulin clamp, indicating that lipid-induced insulin resistance was already established by day 3 and was no different between the hyperglycemic clamp and euglycemic insulin clamp days.

Using the euglycemic and hyperglycemic clamp techniques, we previously have documented a strong positive correlation between the severity of insulin resistance and the increase in insulin secretion in healthy subjects without a family history of type 2 diabetes (33). Using the same techniques, a close relationship between increased insulin secretion and decreased insulin sensitivity has been reported in insulin-resistant subjects genetically predisposed to develop type 2 diabetes (2,3,5) and in insulin-resistant states such as puberty (46,47), obesity (7,47), and PCOS (9). Similar results have been reported by Reaven and colleagues (34,35), who evaluated insulin secretion and insulin resistance with the OGTT and steady-state plasma glucose technique, respectively, as well as by other investigators using the minimal model approach (10,36,37).

In healthy control subjects, when the ISR was related to the prevailing insulin resistance (ISR_{Rd}), insulin secretion adapted to FFA-induced insulin resistance with a compensatory increase in first-phase (0–10 min) and second-phase (10–120 min) insulin secretion, and ISR_{Rd} either increased ($ISR_{Rd\ 0–10}$) or remained constant ($ISR_{Rd\ 10–120}$) (Fig. 7). Note that $ISR_{Rd\ 0–10}$ was nearly identical in FH+ and control subjects during saline infusion. However, lipid infusion decreased first-phase $ISR_{Rd\ 0–10}$ in the FH+ group to 25% of the control subjects' response: 13 vs. 54 pmol/min per (mg · kg LBM^{-1} · min^{-1}) · 10^{-2} for FH+ vs. control subjects, respectively; $P < 0.001$ (Fig. 7). It also reduced second-phase $ISR_{Rd\ 10–120}$ in FH+ to just 42% of that in control subjects: 9 vs. 22 pmol/min per (mg · kg LBM^{-1} · min^{-1}) · 10^{-2}, $P < 0.05$ (Fig. 7). It is noteworthy that in control subjects, the ~20% decrease in R_d was closely matched by a 20% increase in second-phase insulin secretion, so that the $ISR_{Rd\ 10–120}$ was unchanged. Thus, enhanced β-cell response in healthy subjects during lipid infusion was tightly coupled with the decrease in insulin sensitivity. This observation is in agreement with recent 48-h lipid infusion studies by Boden et al. (27) and Magnan et al. (28), although this was not seen in the study by Carpentier et al. (26).

McGarry (16) has demonstrated the important role of plasma FFA in the regulation of pancreatic β-cell function. In the fasting state, FFAs sustain basal insulin secretion and assure efficient nutrient-stimulated insulin secretion when the fast is terminated. Elevated plasma FFAs have been reported to play an important role in maintaining chronic hyperinsulinemia in insulin-resistant obese subjects, and removal of this FFA stimulus by overnight reduction of plasma FFAs with nicotinic acid impairs glucose-induced insulin secretion (48). In normal glucose-tolerant obese individuals (1,14,15), fasting plasma FFA levels are comparable to those achieved during lipid infusion in the control subjects, in whom the induction of insulin resistance was offset by an increase in insulin secretion. These results suggest that elevated plasma FFAs in obesity may represent a compensatory response to augment pancreatic β-cell function to offset insulin resistance. Our observations in control subjects stand in contrast to those in insulin-resistant FH+ subjects, who already manifested inadequate β-cell compensation to insulin resistance. During the saline infusion, second-phase $ISR_{Rd\ 10–120}$ was reduced already by 32% compared with control subjects (Fig. 7*B*), although this difference did not reach statistical significance ($P = 0.13$). Adipose tissue insulin resistance was evident in FH+ because the plasma FFA level was "normal" (rather than suppressed) in the

presence of marked hyperinsulinemia; however, insulin secretion was increased sufficiently to maintain normal glucose tolerance and prevent a further deleterious rise in the plasma FFA concentration. These results suggest that β-cell lipotoxicity may develop very early in individuals genetically predisposed to develop type 2 diabetes, although one cannot rule out that β-cell dysfunction was caused by other factors. When the plasma FFA in FH+ subjects was increased by lipid infusion to levels seen in obesity (~600 μmol/l), there was a marked reduction in both first- and second-phase insulin secretion (Figs. 4 and 5). When expressed on a percentage basis (60 vs. 35%) (Fig. 6), the reduction in first-phase insulin secretion was approximately twice that of second-phase insulin secretion. These observations are consistent with previous studies demonstrating that first-phase insulin secretion is highly predictive of the development of type 2 diabetes across all populations studied (1,2,4,5,10,11,37). Our results suggest that FH+ subjects may have a unique genetic susceptibility to the deleterious effect of elevated plasma FFAs on insulin secretion.

Lipid infusion caused a 42% decrease in the metabolic clearance rate of insulin in the FH+ group but not in control subjects. A similar finding has been reported by Carpentier et al. (29) in insulin-resistant nondiabetic subjects using the same methodology as in this study, i.e., by dividing the mean prehepatic ISR obtained by deconvolution analysis of plasma C-peptide concentration by the mean peripheral plasma insulin concentration during the hyperglycemic clamp. Reduced hepatic insulin clearance has been previously reported to contribute to the hyperinsulinemia of insulin-resistant subjects who are genetically predisposed to develop type 2 diabetes (35,50), as well as in subjects who are overweight (38,51) and in individuals with IGT (52) or PCOS (8,53).

In FH+ subjects, hyperinsulinemia caused by a reduction of hepatic insulin clearance during lipid infusion was more evident when the portal plasma insulin levels were higher, i.e., in the postprandial periods and during the last hour of the hyperglycemic clamp studies. However, when insulin clearance was estimated during the euglycemic insulin clamps, no change in the metabolic clearance of insulin was observed. Three potential explanations can be offered. First, lipid infusion may have induced an increase in C-peptide clearance and led to an underestimation of the ISR. This is an unlikely explanation because it has been shown that neither acute (24) nor chronic (48 h) (26,27,29) pharmacological increases in plasma FFA concentration by lipid infusion alter C-peptide kinetics or clearance. Furthermore, because the plasma FFA concentration achieved during lipid infusion was similar in FH+ and control subjects, an alteration in C-peptide clearance by increased plasma FFA levels would have underestimated ISRs similarly in both groups and cannot explain the contrasting effect of FFAs on β-cell function. Second, it is possible that lipid infusion impaired first-pass hepatic insulin clearance of secreted insulin but did not alter the insulin levels during exogenous insulin administration (euglycemic insulin clamp), which measures the posthepatic systemic insulin clearance. Insulin clearance is mediated through receptor-mediated internalization, primarily by the liver (49), but also by the kidney and muscle, which, in contrast to the liver, are not known to be affected by elevated plasma FFA. Posthepatic estimation of insulin clearance by the euglycemic insulin clamp is more likely to reflect insulin clearance by extra-hepatic tissues and is less likely to provide information on first-pass hepatic insulin extraction, in particular at the lower plasma insulin levels observed during the euglycemic insulin clamp compared with the hyperglycemic clamp.

A third explanation for a modest increase in plasma insulin despite a marked reduction in insulin secretion with lipid in FH+ subjects could be the combined effect of a dose-dependent impairment of hepatic insulin clearance mediated by elevated plasma FFAs, in the setting of chronic hyperinsulinemia and very high portal insulin levels after meals or glucose infusion. Exposure to elevated FFAs in vitro (43,54) and in vivo (28,44,45) reduces insulin clearance in a dose-dependent manner with maximal inhibition (~40–50% reduction) within the physiological range. Moreover, insulin clearance in FH+ subjects was already 29% lower during saline control studies, although it did not reach statistical significance ($P = 0.11$). Thus, one could speculate that in insulin-resistant FH+ subjects, chronic exposure to increased plasma insulin levels would saturate, or nearly saturate, insulin uptake and degradation by hepatocytes. An additional insult, either directly from FFA-induced inhibition of hepatic insulin degradation and/or indirectly through FFA-induced insulin resistance with a further stimulation of hyperinsulinemia, would exceed the liver's ability to clear insulin, leading to an "escape" of insulin toward peripheral tissues.

Because prehepatic insulin levels are usually two- to threefold higher than peripheral plasma insulin concentrations (49), we estimated that during lipid infusion, portal insulin levels in FH+ subjects were ~150–250 μU/ml during the last hour of the hyperglycemic clamp (measured peripheral plasma insulin levels were ~80–90 μU/ml during this period) (Fig. 4). This is very different than during the euglycemic insulin clamp, when the portal plasma insulin concentration closely approximates the peripheral insulin levels (50–54 μU/ml) because endogenous insulin secretion is inhibited. Thus, during the euglycemic insulin clamp, one would not expect an inhibition of insulin clearance by a modest increase in the plasma FFA concentration, as in our study, because the portal insulin concentration would be well below the level for insulin clearance saturation.

Under postabsorptive conditions, the liver of FH+ individuals demonstrated marked insulin resistance, as evidenced by a 73% increase in the hepatic insulin resistance index (EGP × FPI) (19 ± 2 vs. 11 ± 2 mg · kg LBM^{-1} · min^{-1} × μU/ml, $P < 0.04$). This observation is consistent with previous reports (1,14–16). No previous study has examined the effect of a chronic physiological increase in plasma FFAs on EGP in humans. In lean healthy volunteers, an acute pharmacological elevation in plasma FFAs (≥1,000 μmol/l) causes hepatic insulin resistance and inhibits insulin-mediated suppression of EGP (17,18,55,56). In the present study, 4 days of lipid infusion in the FH+ group increased the hepatic insulin resistance index by 2.3-fold (19 ± 2 vs. 44 ± 2 mg · kg $LBM^{-1} \cdot min^{-1}$ × μU/ml, $P < 0.001$ vs. saline infusion; $P < 0.004$ vs. control subjects). The worsening hepatic resistance, combined with

β-cell lipotoxicity, resulted in portal insulin levels that were inadequate to prevent a rise in basal EGP (from 2.4 to 2.9 mg · kg LBM^{-1} · min^{-1}, $P < 0.001$). In control subjects, lipid infusion did not significantly increase the hepatic insulin resistance index, and a small increase in the FPI from 4 to 6 μU/ml was sufficient to keep EGP from rising. Taken together, these findings indicate that the liver of FH+ individuals is more susceptible to the "lipotoxic" effect of elevated plasma FFAs compared with control subjects. Consistent with this conclusion, the suppression of EGP during the insulin clamp was significantly impaired by lipid infusion in FH+ individuals but unchanged in control subjects.

In agreement with previous studies from our laboratory (3,57) and others (2,4,5), FH+ individuals were insulin resistant compared with matched control subjects without a family history of diabetes. The 47% reduction in insulin-mediated R_d was accounted for primarily by decreased nonoxidative glucose disposal (glycogen synthesis) and, to a lesser extent, by impaired glucose oxidation. After lipid infusion, R_d was reduced by 24% ($P < 0.01$) in control subjects but not further decreased in the FH+ group (Fig. 8). These results can be interpreted in one of two ways: *1*) the "lipotoxic" effect of elevated plasma FFA on peripheral tissues is fully established in normal glucose-tolerant FH+ subjects, so a further elevation in plasma FFA by lipid infusion causes no further reduction in Rd, or *2*) peripheral insulin resistance in FH+ is unrelated to "lipotoxicity" but is near maximally established in healthy FH+ subjects, thus lipid infusion cannot further impair insulin-stimulated glucose disposal. Whatever the explanation, the lack of worsening in peripheral insulin resistance by lipid infusion indicates that the deterioration in insulin secretion with lipid infusion resulted from a deleterious effect of elevated plasma FFAs on insulin secretion, and not from worsening insulin resistance and increased β-cell demand. Chronic exposure to elevated plasma FFA may impair the conversion of proinsulin to insulin (22) and disrupt the physiological glucose–fatty acid cross-talk by altering β-cell gene expression and signaling pathways (16,58).

The FFA-induced increase in hepatic insulin resistance and impairment in β-cell function contrasts with the lack of worsening of insulin resistance in skeletal muscle. This may be the result of different thresholds to the lipotoxic effect of plasma FFA on these target tissues. Thus, muscle might be particularly susceptible to even a modest chronic increase in plasma FFA concentration, whereas liver and β-cells have better adaptive mechanisms and might be less sensitive. It also is possible that mechanisms unrelated to increased FFA availability are responsible for muscle insulin resistance in FH+ individuals. If so, lipotoxicity could play a key role to induce hepatic insulin resistance and increase basal EGP and to impair insulin secretion in individuals genetically predisposed to develop type 2 diabetes, but it could be of less pathophysiological importance in inducing peripheral insulin resistance in FH+ subjects.

In the present study, we infused Liposyn III, composed largely of unsaturated long-chain fatty acids (55% linoleate, 22% oleate, 11% palmitate, and 4% stearate), which is different from the higher saturated fatty acid composition of plasma (11% linoleate, 38% oleate, 28% palmitate, and 12% stearate). One study in rats (59) indicated that the insulinotropic effect of FFAs increases with chain length and degree of saturation, with the saturated fatty acids palmitate and stearate being more potent to stimulate insulin secretion. However, discrepant results regarding the insulinotropic effects of various fatty acids have been reported by others (60,61). In the only study in humans, Stefan et al. (62) reported no difference in either first- or second-phase insulin secretion after 24-h exposure to lipids containing various proportions of saturated versus unsaturated fatty acids.

In conclusion, we have demonstrated for the first time the deleterious effect of a sustained increase of plasma FFA concentration on insulin secretion in nondiabetic subjects who are genetically predisposed to develop type 2 diabetes. In addition, lipid infusion caused a mild increase in basal EGP and impaired the suppression of EGP by insulin, changes characteristic of the early stages of type 2 diabetes. In contrast, healthy control subjects adapted to FFA-induced insulin resistance by mounting an adequate compensatory β-cell response. We hypothesize that chronically elevated plasma FFA concentrations may contribute to progressive β-cell failure in at least some individuals who are genetically predisposed to develop type 2 diabetes. This hypothesis emphasizes the important role of adipose tissue insulin resistance in the natural history of progressive β-cell failure leading to type 2 diabetes. From a therapeutic perspective, future interventions to prevent the development of type 2 diabetes may target insulin resistance in adipose tissue in individuals genetically predisposed to develop type 2 diabetes.

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