The Composition of Dietary Fat Directly Influences Glucose-Stimulated Insulin Secretion in Rats

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Acute elevations of plasma free fatty acid (FFA) levels augment glucose-stimulated insulin secretion (GSIS). Prolonged elevations of FFA levels reportedly impair GSIS, but no one has previously compared GSIS after prolonged exposure to saturated or unsaturated fat. Rats received a low-fat diet (Low-Fat) or one enriched with either saturated (Lard) or unsaturated fat (Soy) for 4 weeks. Insulin responses during hyperglycemic clamps were augmented by saturated but not unsaturated fat (580 ± 25, 325 ± 30, and 380 ± 50 pmol · l^{-1} · min^{-1} in Lard, Soy, and Low-Fat groups, respectively). Despite hyperinsulinemia, the amount of glucose infused was lower in the Lard compared with the Low-Fat group. Separate studies measured GSIS from the perfused pancreas. Without fatty acids in the perfusate, insulin output in the Lard group (135 ± 22 ng/30 min) matched that of Low-Fat rats (115 ± 13 ng/30 min), but exceeded that of Soy rats (80 ± 7 ng/30 min). When FFAs in the perfusate mimicked the quantity and composition of plasma FFAs in intact animals, in vivo insulin secretory patterns were restored. Because the GSIS of rats consuming Lard diets consistently exceeded that of the Soy group, we also assessed responses after 48-h infusions of lard or soy oil. Again, lard oil exhibited greater insulinotropic potency. These data indicate that prolonged exposure to saturated fat enhances GSIS (but this does not entirely compensate for insulin resistance), whereas unsaturated fat, given in the diet or by infusion, impairs GSIS. Inferences regarding the impact of fatty acids on GSIS that are based on models using unsaturated fat may not reflect the effects of saturated fat. *Diabetes* 51:1825–1833, 2002

Type 2 diabetes is characterized by impaired insulin action coupled with defective glucose-stimulated insulin secretion (GSIS). Plasma free fatty acid (FFA) concentrations are elevated in obesity/type 2 diabetes syndromes (1–4), and evidence links these elevations to an increased risk for future progression to glucose intolerance and type 2 diabetes in humans (5,6). The contribution of fatty acids to impaired insulin action in the liver and skeletal muscle has been confirmed by numerous investigators (7–10), but the role of these lipid substrates as modulators of β-cell function is less clearly defined (11).

Acute stimulation of GSIS by fatty acids has been described both in vitro (12,13) and in vivo (14–22), but the impact of fat on insulin secretion appears to be a time-dependent phenomenon. Although exposing islets to FFAs for short periods of time stimulates insulin release, rat or human islets cultured with elevated concentrations of fatty acids for >24 h became refractory to glucose stimulation (23–25). An analogous pattern is evident in some, but not all, in vivo experiments in which lipid emulsions and heparin have been infused to elevate plasma FFA levels (8,16,19,26,27). In a classic clinical trial, Paolisso et al. (16) administered Intralipid and heparin to raise plasma FFA concentrations threefold above normal for 24 h. This maneuver resulted in an enhanced insulin response to intravenous glucose 6 h into the infusion period, but a markedly suppressed response after 24 h. Similarly, enhanced insulin and C-peptide responses to glucose were demonstrated in rats after 90 min of Intralipid-heparin infusion, but the stimulatory effect waned after 48 h of lipid infusion (26). High-fat diets provide a more physiological model for evaluating how prolonged exposure to fatty acids alters insulin secretion, but the results from diet studies are mixed, reporting both enhanced and suppressed secretory responses (28–31).

One limitation of the aforementioned in vivo experiments is that none of them have demonstrated whether the source of fat alters β-cell function. Epidemiological evidence suggests that insulin resistance and hyperinsulinemia are closely linked to the ingestion of saturated rather than unsaturated fat (32–34). Furthermore, the insulinotropic potency of fatty acids during acute pancreas perfusion experiments is influenced profoundly by their chain length and degree of saturation as follows: stearate > palmitate > oleate > linoleate > linolenate > octanoate (20). Therefore, the current experiments were undertaken

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Received for publication 26 June 2001 and accepted in revised form 4 March 2002.

FFA, free fatty acid; GSIS, glucose-stimulated insulin secretion; Lard, saturated fat protocol; PPAR, peroxisome proliferator–activated receptor; Saline, saline protocol; Soy, unsaturated fat protocol; TG, triglyceride; UCP2, uncoupling protein-2.

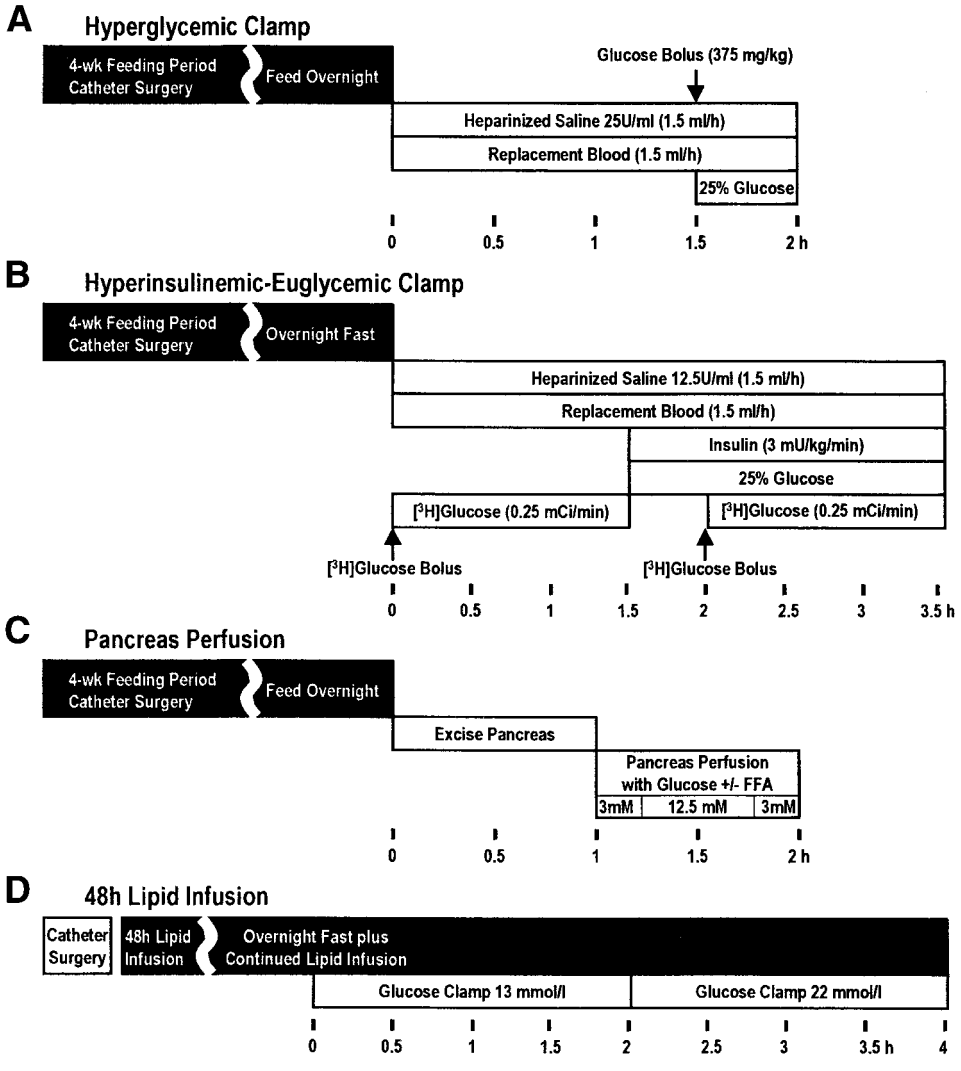

FIG. 1. Schematic diagrams illustrating the design of the experimental protocols discussed in the RESEARCH DESIGN AND METHODS section.

to compare physiological responses in rats after prolonged provision of either saturated or unsaturated fatty acids.

RESEARCH DESIGN AND METHODS

Animals. Male Sprague-Dawley rats weighing 125–150 g were obtained from Harlan Sprague Dawley (Indianapolis, IN). They were placed in metabolic cages at 22°C and maintained on a 12-h light-dark cycle (lights on from 1000 to 2200) with ad libitum access to diets prepared by Harlan Teklad (Madison, WI). Two groups of rats were fed diets enriched with highly saturated lard oil (Lard group) or unsaturated soy oil (Soy group) for 4 weeks and compared with animals consuming a diet containing only essential fatty acids (Low-Fat group). The low-fat diet (TD94128) contained primarily cornstarch and had a caloric density of 3.5 kcal/g, with a distribution of 0.6% saturated fat, 3.4% unsaturated fat, 75% carbohydrate, and 21% protein. The soy diet (TD97072) had a caloric density of 4.4 kcal/g and contained 6% saturated fat, 35% unsaturated fat, 40% carbohydrate, and 19% protein. The lard diet (TD96001) had the same macronutrient distribution, but it contained 14% saturated and 27% unsaturated fat. The animals used for in vivo studies were fitted with carotid artery and jugular vein catheters (17) 3 weeks into the feeding period and were then allowed 6–8 days to recover. Hyperinsulinemic-euglycemic clamps were completed in conscious, unrestrained rats after an overnight fast (18–24 h), whereas animals used for hyperglycemic clamps were studied in the postabsorptive state.

Hyperglycemic clamps. On the morning of experiments (1000), catheters were flushed with heparinized saline. The venous line was connected for infusion of replacement blood and glucose, whereas the arterial line was used for obtaining blood samples. After a 90-min equilibration period, an intravenous glucose bolus was administered, and 25% glucose was subsequently infused to maintain plasma glucose concentrations of 11 mmol/l (17). Blood samples were obtained before the glucose bolus and at 5-min intervals thereafter. Plasma glucose concentrations were immediately determined, and the remaining sample was frozen in liquid N_2 and stored at −20°C for additional assays of FFA, triglyceride (TG), and insulin concentrations (Fig. 1*A*).

Hyperinsulinemic-euglycemic clamps. Animals were fasted starting at 1600, and the studies began at ~1000 the following day. The venous line was connected for infusion of 25% glucose, 3-[^{3}H]glucose tracer, insulin, and replacement blood. The arterial line was used for blood sampling and was kept patent by the infusion of heparinized saline. Studies were conducted according to a modification of the procedure outlined by Rossetti et al. (35) as previously described (36) (Fig. 1*B*).

Pancreas perfusions. After consuming an experimental diet for 4 weeks, 6–8 rats from each group were anesthetized during the postprandial period, and the pancreas was excised and perfused as described by Chen et al. (12) and others (37) (Fig. 1*C*). The basal perfusate, maintained at 37°C under an atmosphere of 95% O_2/5% CO_2, contained Krebs-Ringer bicarbonate buffer at pH = 7.4, 3 mmol/l glucose, 4.5% wt/vol dextran, and 1% fatty acid–free BSA (catalog no. A-6003; Sigma, St. Louis, MO). Studies began after a 15-min equilibration period. After completion of a 10-min basal period, glucose was infused to raise its concentration in the perfusate to 12.5 mmol/l. The glucose infusion was stopped after 40 min, and perfusion was continued for an additional 10 min. The flow rate was 1.5 ml/min throughout. The effluent was collected in 2-min fractions in chilled tubes and, after storage for 1–7 days at −20°C, assayed for insulin. In a separate set of studies, sodium salts of palmitic, oleic, and linoleic acid were added to the medium to simulate the plasma fatty acid profile measured at the outset of the hyperglycemic clamp studies. The medium for the Low-Fat studies contained 0.05 mmol/l palmitate, 0.05 mmol/l oleate, and 0.025 mmol/l linoleate complexed with 1% BSA. These concentrations were doubled in preparing the perfusate for the Lard experiments, whereas the concentrations of palmitate, oleate, and linoleate were 0.1, 0.05, and 0.1 mmol/l, respectively, for the Soy studies to reflect the more

unsaturated plasma FFA profile in this group. The additions were selected so that levels of unbound fatty acids in the context of 1% BSA would approximate in vivo levels (38).

Body composition measured by ^{1}H mass resonance spectroscopy. Separate groups of 6–8 rats maintained on the three experimental diets were sedated with methoxyflurane gas and received an intraperitoneal injection of sodium pentobarbital (50 mg/kg). Whole-body proton spectra were obtained using a 4.7T Omega system, and fat mass was computed from the ratio of fat to water peak areas corrected for known proton densities of fatty acid chains and water, assuming that water comprises 72% of fat-free mass (36).

Total insulin content and histologic evaluation of the pancreas. To determine total insulin content, the pancreas was dissected out from fed rats that had received the experimental diets for 4 weeks. The pancreas was homogenized for 2 min by ultrasonic disintegration at 4°C in acid-alcohol solution, and the insulin was extracted with a modification of the method described by Portha et al. (39). Insulin in the extract was assayed using a specific double-antibody radioimmunoassay kit obtained from Linco Research (St. Charles, MO). In separate animals, the pancreas was dissected out, and Bouin-fixed paraffin-embedded serial sections were evaluated for islet size and structure.

Isolated islet TG content. Islets were isolated by a modification (40) of the method of Lacy and colleagues (41) for determination of TG content. The islet cells were place in hypotonic buffer (33 mmol/l NaCl, 22 mmol/l NaH_2PO_4, and 0.4 mmol/l EDTA at pH 7.4) and freeze-thawed three times to fracture cell membranes. Subsequently, they were sonicated for 15 s, vortexed, and cooled in an ice water bath for 15 s. The sonication procedure was repeated twice. Then, 15 ul of the solution was diluted to a final volume of 200 μl with assay buffer (2 mol/l NaCl, 50 mmol/l sodium phosphate, and 1 mmol/l EDTA at pH 7.4) for determination of protein and DNA concentration. An additional 150 μl was pipetted into glass test tubes for measurement of TGs after chloroform-methanol extraction (42,43). Values for islet TG content were corrected for both DNA and protein content. The fatty acid composition of islet, liver, and muscle TGs was analyzed by gas-liquid chromatography after chloroform-methanol extraction and fractionation over silica Sep-Pak columns obtained from Waters (Milford, MA) (44,45).

48-h lipid infusions. We also used 48-h lipid infusions to assess the importance of prolonged exposure to saturated versus unsaturated fatty acids in modifying insulin secretion (Fig. 1*D*). Female Wistar rats (Charles River, Wilmington, MA) weighing 250–300 g were used for these experiments. They were housed in the Department of Comparative Medicine of the University of Toronto on a 12-h light/dark cycle and fed rat chow (Purina 5001, 4.5% fat; Ralston Purina, St. Louis, MO) and water ad libitum. Indwelling catheters were inserted into the right internal jugular vein and the left carotid artery and then tunneled through a subcutaneous interscapular implant and exteriorized. The rats were randomized to one of the following protocols: soy oil plus heparin (Soy), lard oil plus heparin (Lard), saline (Sal). At least 2–3 days after the surgery, PE-50 infusion tubing was connected to each of the catheters. The infusion lines ran inside a tether that was fitted to the subcutaneous implant. Each rat was placed in a circular cage and the infusion lines were run through a swivel, which was suspended on top of the cage. The infusates contained of 20% soy oil, 20% lard oil, or saline plus 20 units of heparin per ml and were given through the jugular catheter at 5.8 μl/min. Both the soy and lard oil emulsions contained 1.2 g of lecithin and 2.2 g of glycerol per 100 ml (20). Throughout the 48-h infusion period, the rats had free access to water and food until the food was removed at 1900 on the second day. At 1000 the following morning, glucose-induced insulin secretion was determined by measuring the insulin and C-peptide response to a two-step (~13 and ~22 mmol/l) hyperglycemic clamp, as described previously (26). The venous infusion of Soy, Lard, or Sal was continued throughout the experiment. However, soy and lard oil were given without added heparin because the same total amount of heparin (0.1 unit/min) as that infused during the preclamp period was used to flush the arterial catheter when sampling during the experiment.

Analytical procedures and materials. Plasma and perfusate glucose concentrations were measured by the glucose oxidase method on a Beckman Glucose Analyzer II machine (Fullerton, CA). Plasma [^{3}H]glucose specific activity and content of ^3H_2O were determined by scintillation counting after precipitation with BaOH and $ZnSO_4$. Glucose utilization was calculated using the Steele equation, whereas glucose oxidation was estimated from the rate of ^3H_2O production. Plasma FFA concentrations were determined by a colorimetric assay incorporating fatty acyl-CoA synthetase, acyl-CoA oxidase, and a peroxidase-linked reagent (catalog no. 1381375; Boehringer Mannheim, Indianapolis, IN). Plasma TG concentrations were also measured using a colorimetric assay baseline-corrected for glycerol concentration (procedure no. 337; Sigma). Insulin and C-peptide levels in plasma were determined by radioimmunoassays using kits specific for rat insulin and C-peptide that were from Linco Research. The coefficients of variation were <9 and <10.5% for insulin and C-peptide, respectively. Palmitic acid, the sodium salts of oleic and linoleic acid, and lard oil were obtained from Sigma.

Statistical analyses. Experimental results were analyzed using multiple ANOVA with a probability for type 1 error set at $P < 0.05$. All calculations were made with SigmaStat software for Windows (SPSS, Chicago, IL).

RESULTS

Food intake and body composition. Food intake was similar in all three diet groups (69.6 ± 1.9, 65.3 ± 1.7, and 69.7 ± 1.9 kcal/day in the Low-Fat, Soy, and Lard groups, respectively). Animals consuming the Low-Fat diet gained 149 ± 6 g after 4 weeks, whereas the Soy and Lard animals gained 143 ± 4 and 150 ± 9 g, respectively. Despite similar food intake, weight gain, and final body weights in each of the three diet groups, whole-body fat mass as determined by proton mass resonance spectroscopy was increased in the rats consuming high-fat diets. The fat mass was 23.5 ± 0.7 g in the Soy group and 29.9 ± 4.5 g in the Lard group, values twofold greater than that of the Low-Fat rats (12.1 ± 0.7 g, $P < 0.01$).

Hyperglycemic clamps. The impact of dietary fat intake on GSIS was assessed using hyperglycemic clamp studies. Figure 2 displays plasma FFA, glucose, and insulin concentrations measured during the experiments. The postprandial FFA concentrations in the Soy and Lard diet groups were increased twofold in comparison to the Low-Fat animals and remained elevated during the hyperglycemic period, despite suppression by insulin. In contrast, plasma glucose values were similar for each of the three diet treatments during both the postprandial baseline and the hyperglycemic clamp periods. The concentration and composition of fat in the diet did not significantly change baseline plasma insulin concentrations, but glucose-stimulated insulin responses were altered (Fig. 2*C*). Compared with the Low-Fat group, the rats receiving the Lard diet had a greater incremental area under the insulin curve (580 ± 25 vs. 380 ± 50 pmol · l^{-1} · min^{-1}), whereas in the Soy group there was actually a trend toward suppressed insulin secretion (325 ± 30 pmol · l^{-1} · min^{-1}).

Although the high-fat diets altered insulin secretion, variations in insulin sensitivity were also apparent. The glucose infused during the clamp studies in the Low-Fat rats (602 ± 24 μmol/100 g) was significantly greater than that required by the Lard group (476 ± 18 μmol/100 g), which in turn required a greater infusion than Soy animals (389 ± 27 μmol/100 g). Dividing the glucose infusion rate by the average insulin concentration achieved during the hyperglycemic period provided a rough index of insulin sensitivity that had units of μmol glucose · min^{-1} · 100 g^{-1} per μmol insulin/l. By this index, the Low-Fat group (31.5 ± 1.3) was considerably more insulin sensitive than the Soy or Lard groups (25.2 ± 1.7 and 20.4 ± 1.4, respectively). Hyperinsulinemic-euglycemic clamp studies provided additional insights into the impact of the two high-fat diets on insulin clearance and insulin sensitivity.

Hyperinsulinemic-euglycemic clamps. Table 1 lists the plasma concentrations of glucose, insulin, FFAs, and TGs during the clamp studies. No significant differences were observed between the three study groups for plasma glucose or insulin concentrations. An insulin infusion of 3 mU · kg^{-1} · min^{-1} raised plasma insulin levels ~300 pmol/l in all groups, whereas plasma glucose was clamped at

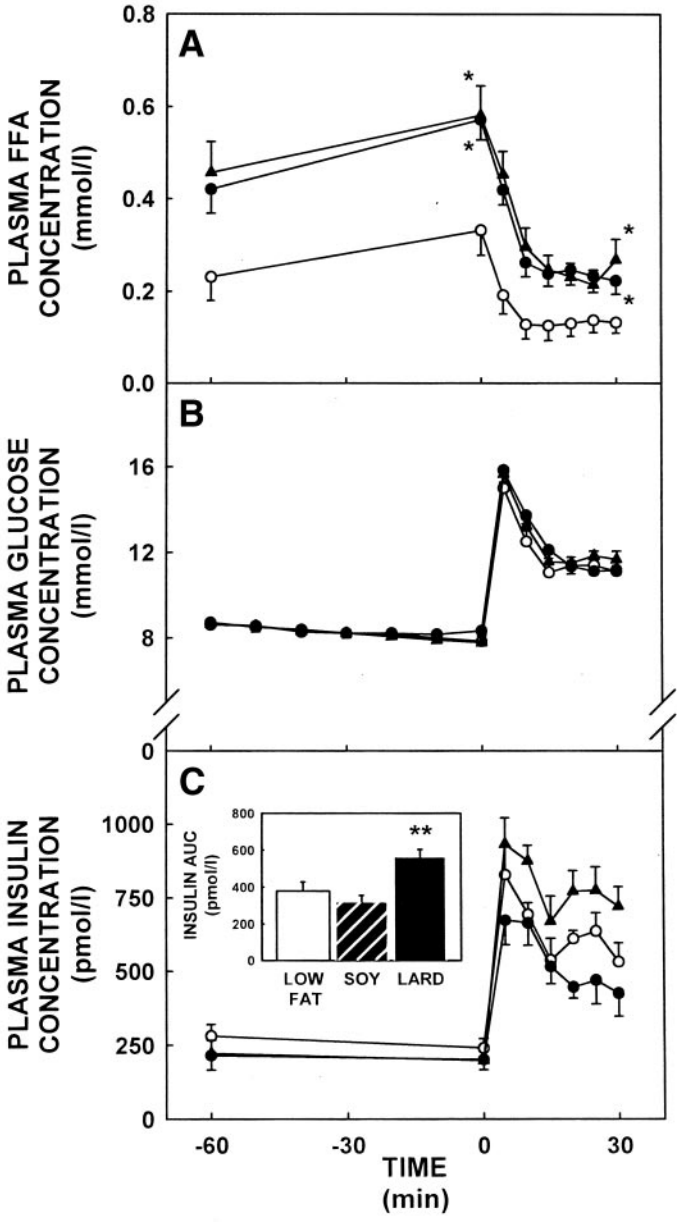

FIG. 2. Plasma FFA (*A*), glucose (*B*), and insulin (*C*) concentrations measured during hyperglycemic clamp studies completed in fed rats. The inset figure in panel C shows stimulated insulin output reported as incremental area under the curve of plasma insulin concentrations during the 30-min hyperglycemic (11 mmol/l) period. Animals received low-fat (○, $n = 7$), soybean oil (●, $n = 7$), or lard (▲, $n = 6$) diets for 4 weeks before the studies were performed. Values are means ± SE. *$P < 0.05$ vs. steady-state values achieved in the Low-Fat group; **$P < 0.05$ vs. the Low-Fat and Soy groups.

basal levels. The elevation in plasma insulin concentrations during insulin infusion suppressed lipolysis and resulted in a fall in FFA concentrations in all animals, although to a lesser extent in the Soy and Lard groups. No significant differences were found in plasma TG levels. Whole-body resistance to insulin action was associated with increased intake of either saturated or unsaturated fat (Fig. 3). The glucose infusion rate required to maintain euglycemia was diminished to 5.9 ± 1.0 and 7.9 ± 0.8 μmol · min^{-1} · 100 g^{-1} in the Lard and Soy rats, respectively, compared with 11.9 ± 0.9 μmol · min^{-1} · 100 g^{-1} for the Low-Fat group. However, steady-state analysis of

TABLE 1
Influence of dietary fat content on plasma parameters before and during hyperinsulinemic-euglycemic clamp studies performed in overnight-fasted rats

	Low-Fat	Soy	Lard
n	6	7	6
Glucose (mmol/l)			
Basal	6.07 ± 0.13	5.88 ± 0.23	6.11 ± 0.22
Clamp	6.28 ± 0.15	6.08 ± 0.14	6.28 ± 0.06
Insulin (pmol/l)			
Basal	30 ± 5	20 ± 5	35 ± 10
Clamp	300 ± 30	320 ± 15	335 ± 15
FFA (mmol/l)			
Basal	0.65 ± 0.06	0.62 ± 0.03	0.65 ± 0.04
Clamp	0.16 ± 0.03	0.29 ± 0.03*	0.35 ± 0.04*
TG (mmol/l)			
Basal	0.13 ± 0.02	0.12 ± 0.01	0.13 ± 0.01
Clamp	0.12 ± 0.02	0.11 ± 0.01	0.12 ± 0.01

Data are means ± SE. *$P < 0.05$ vs. rats receiving the low-fat diet.

3-[^{3}H]glucose tracer data revealed the importance of the fatty acid composition of the diet on the mechanisms involved in the evolution of insulin resistance. Hyperinsulinemia failed to suppress endogenous glucose production in both high-fat diet groups, suggesting that a similar degree of hepatic insulin resistance developed in each

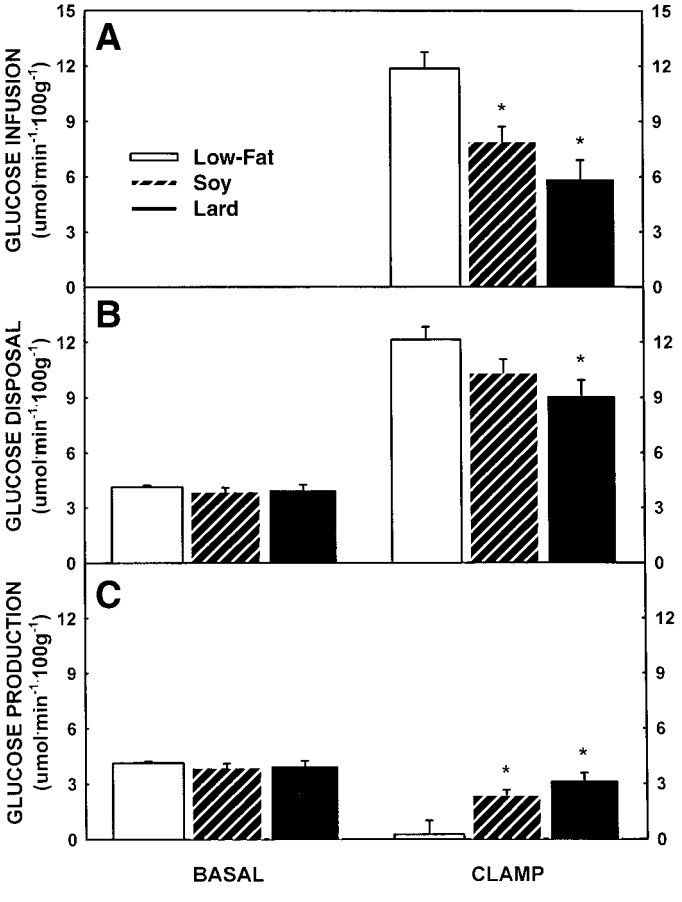

FIG. 3. Glucose infusion rate (*A*), tracer-determined glucose disposal (*B*), and endogenous glucose production (*C*) measured during hyperinsulinemic-euglycemic clamp studies in overnight-fasted rats. The animals received low-fat ($n = 6$), soy oil ($n = 7$), or lard ($n = 6$) diets for 4 weeks before the studies were performed. Values are the means ± SE. *$P < 0.05$ vs. steady-state values achieved in the Low-Fat group.

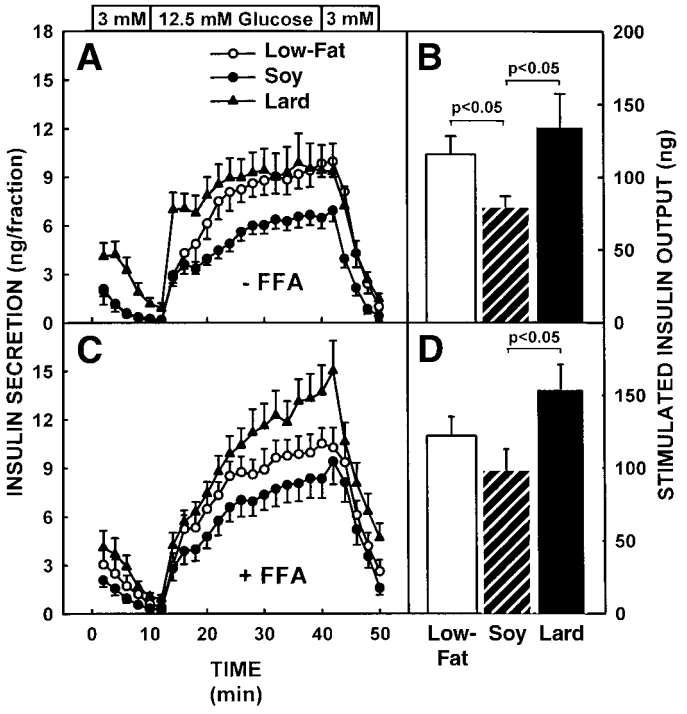

FIG. 4. Insulin secretion (*A* and *C*) from the isolated, perfused pancreas and stimulated insulin output (*B* and *D*) reported as the incremental area under the curve of perfusate insulin concentrations when the glucose concentration of the medium was increased from 3 to 12.5 mmol/l. The animals received low-fat, soybean oil, or lard diets for 4 weeks before the studies were performed. The studies were conducted with fed animals, and the perfusate was either devoid of any fatty acids (*A* and *B*) or contained fatty acids at concentrations listed in the text in the context of 1% albumin. Values are the means ± SE of 7–8 determinations for each group.

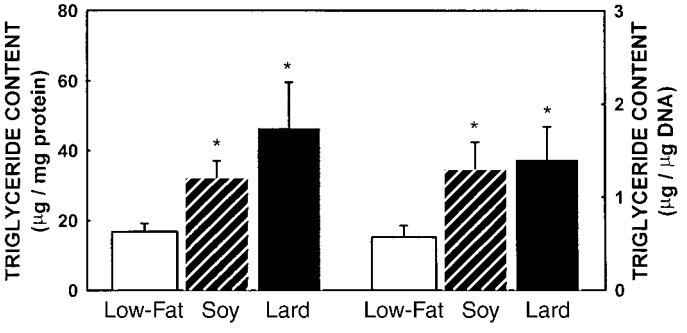

FIG. 5. TG content of the islets of Langerhans isolated from animals in the postprandial state after they had been maintained on low-fat (n = 6), soybean oil (n = 4), or lard (n = 6) diets for 4 weeks. Values are the means ± SE. *P < 0.05 vs. the Low-Fat group.

case. In contrast, only the LARD group exhibited significantly impaired insulin-mediated glucose disposal with the relatively modest degree of hyperinsulinemia used in these studies, the values being 9.1 ± 0.8, 10.4 ± 0.7, and 12.1 ± 0.7 μmol · min^{-1} · 100 g^{-1} in the Lard, Soy, and Low-Fat groups, respectively. Because oxidative glucose disposal rates were similar after all treatments, the high-fat diets primarily affected nonoxidative glucose disposal.

Pancreas perfusion studies. Additional experiments were undertaken to delineate the important determinants of insulin secretion in high-fat fed rats. We evaluated insulin release from the isolated, perfused pancreas to eliminate any acute influences that autonomic tone, gastrointestinal incretins, adipose tissue lipolysis, or hepatic insulin clearance might have on insulin responses. A trend toward increased insulin output during the basal period was observed in the Lard group, but this did not achieve statistical significance. When the glucose concentration was raised to 12.5 mmol/l (Fig. 4*A* and *B*), insulin output for the Soy group (80 ± 7 ng) was not increased to the same extent as that seen in the Low-Fat and Lard groups (115 ± 13 and 135 ± 22 ng, respectively). Thus, the pattern of insulin secretory responses in the ex vivo pancreas preparation differed from that observed in vivo (Fig. 2) because pancreatic insulin output in the Lard group was similar to that of the Low-Fat group, whereas insulin output in the Soy group was reduced. Given that plasma FFA concentrations were elevated twofold by the high-fat diets, we explored the effects of adding fatty acids to the perfusion medium to simulate the physiological levels measured during the clamp experiments. This maneuver enhanced insulin secretion (Fig. 4*C*) and restored the insulin secretion relationships to those observed in vivo, with stimulated values of 122 ± 13, 99 ± 14, and 155 ± 17 ng in the Low-Fat, Soy, and Lard animals, respectively (Fig. 4*D*).

Whole-pancreas insulin content and islet studies. The fact that animals receiving the Soy diet demonstrated suppressed insulin output relative to the Lard group both in vivo and in the perfusion experiments suggested that unsaturated fatty acids directly or indirectly suppressed β-cell function. Therefore, sets of rats were killed during the postprandial period, and the pancreas was removed for biochemical and histologic investigations. Examination of hematoxylin-eosin–stained sections of the pancreas revealed no marked differences in islet size or appearance after 4 weeks of diet treatment, nor were there any

TABLE 2
Influence of dietary fat content on the fatty acid composition of tissue TGs in the pancreatic islets, liver, and skeletal muscle

Fatty acid	Diet		Pancreatic islets		Liver		Skeletal muscle	
	Soy	Lard	Soy	Lard	Soy	Lard	Soy	Lard
16:0	11.0	24.8	22.0	36.7*	12.7	24.5*	22.1	24.6
16:1	—	3.1	1.1	6.7	0.4	2.9*	2.9	3.2
18:0	4.0	12.3	15.2	33.3*	1.9	2.5	8.3	7.4
18:1	23.4	45.1	23.4	19.4	16.5	46.8*	26.8	45.5*
18:2	53.2	9.9	37.1	2.0*	48.0	14.3*	32.8	15.1*
18:3	7.8	—	—	—	1.3	0.3	1.5	0.3

The data are % total fatty acid content and represent the mean of determinations from 3–6 animals in each group. *P < 0.05 between the soy and lard oil diets. The manufacturer provided the values for the diets, whereas the tissue TGs were determined by gas chromatograpy.

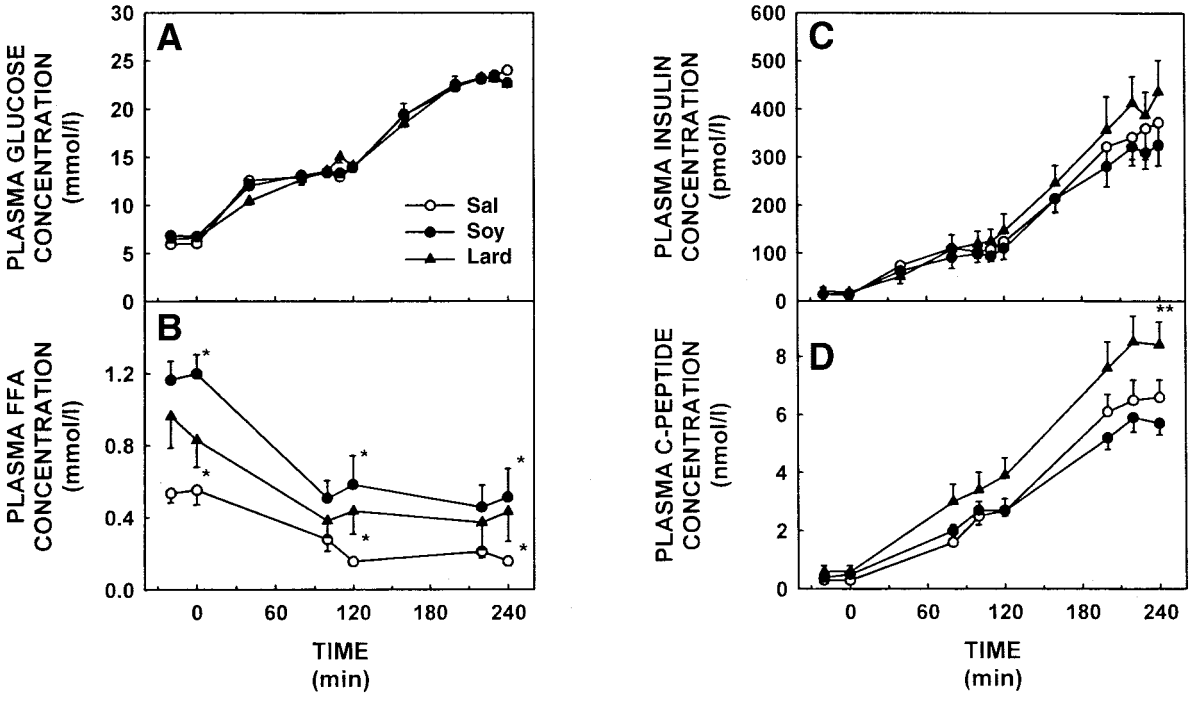

FIG. 6. Plasma glucose (*A*), FFA (*B*), insulin (*C*), and C-peptide (*D*) concentrations during two-step hyperglycemic clamp studies in overnight-fasted rats. The animals had received infusions of saline ($n = 9$), soybean oil ($n = 9$), or lard oil ($n = 9$) diets for 48 h before the studies were performed. Values are the means ± SE. *$P < 0.05$ vs. steady-state values achieved in the Low-Fat group; **$P < 0.05$ vs. steady-state values achieved in the Soy group.

significant changes in pancreatic insulin content (49 ± 7, 54 ± 6, and 58 ± 7 μg per pancreas in the Low-Fat, Soy, and Lard rats, respectively). On the other hand, Fig. 5 shows that islet TG stores were increased by both high-fat diets, regardless of whether values were corrected for either islet protein or DNA content. The influence of dietary fat content on the fatty acid composition of islet TGs is listed in Table 2. Linoleic acid (18:2) is the primary fatty acid moiety found in the Soy diet, and it also predominates in the islets of animals ingesting soy oil. In contrast, islet TGs in the Lard group contain considerably more palmitic (16:0) and stearic acid (18:0) and a paucity of linoleic acid.

For comparison to values obtained from isolated pancreatic islets, the fatty acid composition in skeletal muscle and liver were included in Table 2. Tissue TGs were elevated in the soleus muscle (0.77 ± 0.20, 1.45 + 0.48, and 1.22 + 0.13 mg/g in Low-Fat, Soy, and Lard groups, respectively) and liver (9.3 ± 1.0, 38.3 ± 10.0, and 25.5 ± 1.1 mg/g) as well. The composition of fatty acids in these tissues closely reflected modifications induced by dietary fat.

48-h lipid infusion. We turned to a second experimental model that has previously been used to investigate insulin responses after prolonged exposure to elevated concentrations of fatty acids (26). In this case, normal rats received an infusion of saline, lard oil plus heparin, or soy oil plus heparin for 48 h before a two-step hyperglycemic clamp. The lipid infusions increased the average plasma FFA concentrations measured at the 18, 24, and 48 h time points from 0.51 ± 0.06 mmol/l in the Sal group to 1.17 ± 0.13 and 1.18 ± 0.14 mmol/l in the Lard and Soy groups, respectively, even though plasma glucose concentrations remained similar in all groups. Changes in plasma glucose, FFA, insulin, and C-peptide levels occurring during the clamp are shown in Fig. 6. Plasma glucose concentrations were matched in all three groups, but again the total glucose infused during the clamps tended to be lower in both of the lipid infusion groups than in the Sal group, consistent with impaired insulin sensitivity. Plasma FFA concentrations, though falling from preclamp values, remained elevated in the animals infused with lard or soy oil. Although there were no significant differences in plasma insulin concentrations in the three groups during either the 13 or the 22 mmol/l steps of the hyperglycemic clamp, insulin levels in the Lard group tended to be greater than those of the saline controls, whereas values in the Soy group tended to be suppressed (Fig. 6*C*). Variations in plasma C-peptide concentrations followed the same qualitative trend, but the separation between groups was quantitatively more pronounced (Fig. 6*D*). The differences for C-peptide values at 13 mmol/l did not reach statistical significance ($P = 0.07$), but they were significantly greater in the Lard group than in the Soy animals at 22 mmol/l glucose (8.1 ± 0.8 vs. 5.6 ± 0.4 nmol/l, $P < 0.05$). Therefore, even though changes in insulin clearance tended to mute variations of plasma insulin concentrations, it was apparent that a prolonged infusion of saturated fatty acids elicited a greater β-cell response to glucose than unsaturated fatty acids.

DISCUSSION

The current experiments were designed to assess how prolonged exposure to highly saturated animal fat or unsaturated vegetable oil impacts insulin secretion. A series of protocols were completed in rats that closely characterized alterations in glucose metabolism and insu-

lin secretion after prolonged elevations of plasma FFA levels elicited by either lard or soy oil. Plasma insulin concentrations measured during a hyperglycemic clamp were augmented by 53% in the rats maintained on a lard diet for 4 weeks, in comparison to animals receiving low-fat diets or soy oil diets. When insulin output from the isolated, perfused pancreas was examined in the absence of fatty acids in the perfusate, it was reduced in the Soy group compared with the other two diet treatments. The addition of fatty acids to the perfusate at levels simulating plasma concentrations in vivo restored the insulin secretion pattern to that of intact animals. To provide additional evidence that the composition of fat alters β-cell responses, insulin and C-peptide secretion was measured after 48-h infusions of saline, lard oil, or soy oil. The results coincided with the findings of the diet experiments, with saturated fat again having the greatest insulinotropic potency.

A number of factors might have been responsible for the enhanced insulin response to glucose in the rats consuming the lard diet versus their counterparts receiving the soy diet. One possibility was that elevated saturated plasma FFA concentrations inhibited the clearance of insulin from plasma (18,27,46) without having any significant effect at the level of the β-cell. A second possible interpretation was that altered insulin responses simply reflected compensatory adaptations to differences in insulin sensitivity. Finally, fatty acids could have acted directly at the level of the β-cell to modify insulin responsiveness to glucose. The results of hyperinsulinemic-euglycemic clamp studies suggest that possible indirect actions mediated through differences in insulin clearance or insulin resistance cannot entirely explain the difference in insulin secretion between the two diets. The clearance of peripherally infused insulin was measured from the clamp data by dividing the insulin infusion rate by the steady-state insulin concentration. Values of 16.6 ± 1.6, 18.7 ± 0.8, and 18.0 ± 0.8 $ml \cdot kg^{-1} \cdot min^{-1}$ were obtained in the Low-Fat, Soy, and Lard groups, respectively, so impaired insulin clearance from peripheral plasma could not explain the hyperinsulinemia in the LARD group, although first-pass hepatic insulin clearance was not assessed. Importantly, both of the high-fat diets prompted whole-body insulin resistance, which tended to be greater in the Lard than in the Soy group, but although hyperinsulinemia in the Lard group tended to compensate for impaired insulin action, the Soy group actually inclined toward suppressed GSIS. The pancreas perfusion data provided even stronger evidence favoring disparate effects of saturated and unsaturated fat diets directly on the β-cell over indirect mechanisms mediated through differences in insulin clearance or insulin resistance, because hormone secretion in this model remained isolated from events in other tissues. Insulin output from the perfused pancreas was similar in animals ingesting low-fat and lard diets, whereas the group receiving unsaturated fat had a diminished insulin response.

Additional studies are needed to investigate the cellular mechanism underlying the FFA-induced changes in β-cell function. Previous studies with isolated islets and insulin-secreting cell lines have shown that prolonged exposure to elevated FFAs can have a myriad of effects on metabolism and gene expression in the islet, and these effects have been extensively reviewed (47–50). The current studies serve to focus attention on particular mechanisms that could be differentially impacted by saturated and unsaturated fats. Activation of gene expression through peroxisome proliferator–activated receptor-α (PPAR-α) or PPAR-γ is an example of such a mechanism (51). Palmitate and stearate do not activate PPAR-α at physiological concentrations, whereas more unsaturated fatty acids, such as oleate and linoleate, bind to this nuclear transcription factor, with dissociation constants in the nanomolar range (52,53). One gene that is upregulated by PPAR-γ in islets is uncoupling protein-2 (UCP2) (54). UCP2 overexpression decreases GSIS (55), and UCP2 is induced by prolonged exposure of β-cell lines to fatty acids (56). Alternatively, the saturation of cellular fatty acids would influence the fluidity of the β-cell membrane (57,58) and the acylation of proteins. Protein acylation preferentially utilizes palmitoyl-CoA and is required for both vesicular fusion and budding at the level of the Golgi complex (59,60). The inhibition of protein acylation in the pancreatic islets with cerulenin impairs nutrient stimulation of insulin release (61). Unsaturated fatty acids also induce a greater degree of oxidative stress than saturated fatty acids (62). Oxidative stress has heretofore been implicated in β-cell glucotoxicity (63,64), and preliminary data suggest that it may be implicated in lipotoxicity as well. Finally, all unsaturated fatty acids may not be similar in their degree of lipotoxicity, and further studies are required to compare the effects of diets or infusions enriched with individual monounsaturated or polyunsaturated fatty acids.

The data in rats consuming a lard diet closely parallel the majority of published results (28–31) because their GSIS exceeded that of rats receiving a Low-Fat diet or a standard chow diet containing 4% fat (data not shown). In particular, Holness and Sugden (28,30) showed that the provision of rats with dietary saturated fat significantly enhanced insulin secretion during an intravenous glucose challenge. Ahren et al. (31) examined responses in rats receiving a diet supplemented with 58% of calories from lard. As an exception to the other listed studies, they found insulin secretion during intravenous glucose infusions of 15 and 25 mg/min to be significantly diminished after 4 or 8 weeks of dietary treatment. Two key differences exist between the "Surwit diet" used by Ahren et al. and that provided in the current study. The total and saturated fat content of our Lard diet lies at the upper end of the typical fat intake for people in the U.S. (32–34), whereas the fat intake in the Surwit diet exceeds that normally consumed by humans and may elicit greater lipotoxicity within the β-cell. More importantly, the diets in the current study, and that reported by Holness and Sugden (28,30), provide significant amounts of carbohydrates in the form of complex carbohydrates, whereas the diabetogenic Surwit diet contains only the simple sugars maltose and sucrose. Therefore, it is quite likely that an interaction with the carbohydrate component of the diet modifies the impact of fat intake on GSIS.

Importantly, our findings in rats are entirely consistent with epidemiological evidence in humans linking hyperinsulinemia to increased intake of saturated fat. In the

Normative Aging Study, a high–saturated fat diet independently predicted fasting and postprandial hyperinsulinemia (33). The study estimated that increasing dietary saturated fat from 8 to 14% of total energy would lead to an 18% increase in fasting insulin and a 25% increase in postprandial insulin levels (33). In a separate investigation, saturated fat intake was associated with an increase of plasma insulin values 2 h after an oral glucose load (32).

Although a number of studies in animals and humans link increased dietary saturated fat with hyperinsulinemia, it is widely perceived that fatty acids have a lipotoxic effect on the β-cell. Much of the data supporting this concept comes from infusion studies in which Intralipid, a soy oil–based product, and heparin were infused to raise plasma FFA levels (8,16,19,26,27). We performed experiments using a 48-h infusion of lard and soy-based lipid emulsions. The current studies reproduced previous work showing that a 48-h infusion of soy oil and heparin suppresses insulin secretion during a two-step hyperglycemic clamp (26). Herein, we demonstrate that insulin secretion was not suppressed when a lard oil emulsion was infused to elicit hyperlipacidemia. Instead, insulin and C-peptide levels were highest in the Lard group. These data have important implications for clinical studies of β-cell lipotoxicity. Prolonged Intralipid/heparin infusions inhibit insulin responses in humans (16,19). If a more saturated lipid emulsion was available to reproduce the composition of circulating FFAs observed in obesity and insulin-resistant states, its use would conceivably augment plasma insulin concentrations, as was seen in rats, instead of having a "lipotoxic" effect on insulin secretion.

In conclusion, these data obtained from studies in normal rats do not favor the concept that prolonged exposure to excess saturated fatty acids, as occurs in obesity and insulin-resistant states, impairs GSIS in animal models or people that lack a predisposition to β-cell failure. Insulin concentrations actually increase in the lard infusion and lard diet groups, although they do not reach sufficient levels to compensate for fat-induced insulin resistance. However, the impact of fatty acids on insulin secretion may be modulated by other factors, including chronic hyperglycemia (as in the diabetic state) or positive energy balance (ultimately resulting in obesity). Thus, it is still possible that chronic elevations of saturated fatty acids precipitate or accelerate overt β-cell failure (65,66) when a second environmental or genetic factor is present.

ACKNOWLEDGMENTS

This work is supported by the National Institutes of Health (NIH) (DK-57558) and a Career Development Award from the American Diabetes Association. Additional support was received from NIH Grant DK-18573, the Forrest C. Lattner Foundation, and the Canadian Institute for Health Research (CIHR Grant MOP-37961).

We appreciate the excellent technical assistance provided by Brandon Bentley, Ben Alexander, and Anh Nguyen.

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