

Differential Effects of Hyperlipidemia on Insulin Secretion in Islets of Langerhans From Hyperglycemic Versus Normoglycemic Rats

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Chronic elevations in plasma levels of fatty acids (FAs) adversely affect pancreatic β -cell function in type 2 diabetes. In vitro, we have previously shown that deleterious effects of prolonged exposure of isolated islets to FAs were dependent on the presence of elevated glucose concentration. This led us to hypothesize that both hyperlipidemia and hyperglycemia must be present simultaneously for FAs to affect β -cell function. To test this hypothesis in vivo, we administered a high-fat diet for 6 weeks to Goto-Kakizaki (GK) rats. High-fat feeding had no effect on insulin secretion, insulin content, or insulin mRNA levels in islets from normoglycemic Wistar rats. In contrast, high-fat feeding markedly impaired glucose-induced insulin secretion in islets from GK rats. High-fat feeding did not affect triglyceride (TG) content or the rate of glucose oxidation in islets. It was, however, accompanied by a twofold increase in uncoupling protein (UCP)-2 levels in GK rat islets. Insulin treatment completely normalized glucose-induced insulin secretion and prevented the increase in UCP-2 expression in islets from high-fat-fed GK rats. We conclude that hyperlipidemia induced by high-fat feeding affects insulin secretion in islets from hyperglycemic GK rats only, by a mechanism which may involve, at least in part, modulation of UCP-2 expression. *Diabetes* 51:662–668, 2002

Chronic elevations in plasma levels of fatty acids (FAs) have been postulated to aggravate pancreatic β -cell dysfunction in type 2 diabetes, a hypothesis referred to as lipotoxicity (1). In vivo, chronic hyperlipidemia has been proposed as the major deleterious factor in Zucker diabetic fatty (ZDF) rats (2), via a mechanism involving increased triglyceride (TG) accumulation in islets (3). However, the confounding effects of chronic hyperglycemia have not been clearly distinguished from those of hyperlipidemia in this model. Selective modulation of plasma lipid or glucose levels in ZDF rats has demonstrated that chronic hyperglycemia, rather than hyperlipidemia, is a determining factor for TG

accumulation and decreased insulin gene expression in islets (4). The ZDF rat is massively obese and insulin-resistant because of a mutation in the leptin receptor gene (5,6), and observations made in this model are unlikely to be directly applicable to the mechanisms of lipotoxicity in leptin-sensitive β -cells. In fact, ectopic accumulation of TG in islets from ZDF rats is likely caused by defective leptin signaling (7).

In vitro, chronic exposure to elevated FA levels decreases glucose-stimulated insulin secretion in isolated rat islets (rev. in 8), but the mechanisms of these effects remain elusive. We (9) and others (10,11) have shown that prolonged (>1 day) culture of normal islets in the presence of supraphysiological concentrations of FAs decreases insulin content and insulin gene expression only in the presence of elevated glucose levels, consistent with the notion that chronic effects of FAs on β -cell function are glucose-dependent. We also showed that the decrease in insulin gene expression was associated with a glucose-dependent increase in islet TG content (12), although we have not provided any evidence for a causal relationship. Indeed, several other potential mechanisms have been proposed, including changes in glucose oxidation (7) and, more recently, modulation of expression of uncoupling protein (UCP)-2 in islets (13,14).

Based on the hypothesis that hyperglycemia plays a permissive role in the deleterious effects of hyperlipidemia on β -cell function, this study was designed to determine whether high-fat feeding affects insulin secretion and gene expression in islets from hyperglycemic Goto-Kakizaki (GK) rats and whether these effects are prevented by insulin treatment. To elucidate the mechanisms whereby high-fat diet impairs β -cell function in GK rats, we ascertained whether accumulation of TG, changes in glucose oxidation, or modulation of UCP-2 levels in islets are involved.

RESEARCH DESIGN AND METHODS

Animal care and diet. The spontaneously diabetic GK rat is a lean model of type 2 diabetes characterized by deficient insulin response to glucose in vivo and in vitro (15). Our colony originates from that of Dr. R. Farese (Tampa, FL) and is maintained at the Pacific Northwest Research Institute (PNRI). Age-matched male Wistar rats were purchased from Harlan Teklad. GK and Wistar rats 6 weeks of age were caged with free access to water and fed either a high-fat diet (Harlan Teklad, Madison, WI) or a regular diet for 6 or 10 weeks ad libitum. The composition of both diets is given in Table 1. Both were adequately supplemented with vitamins and minerals. In the high-fat diet, the relative proportions of FAs were 39.1% saturated, 48.9% monounsaturated, and 12.1% polyunsaturated. Animals were housed on a 12-h light/dark cycle (9:00 P.M. to 9:00 A.M. dark cycle). All procedures using animals were approved by the PNRI Institutional Animal Care and Use Committee.

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AUC, area under the curve; FA, fatty acid; KRB, Krebs-Ringer buffer; PBS-T, PBS with 0.05% Tween; PNRI, Pacific Northwest Research Institute; RIA, radioimmunoassay; TG, triglyceride; UCP, uncoupling protein.

TABLE 1
Composition of the high-fat and regular diets

	Regular diet	High-fat diet
Proteins (%)	24.5	22.5
Carbohydrates (%)	46.6	28.4
Fat (%)	4.4	31.1
kcal from fat (%)	12	58
Energy (kcal/g)	3.2	4.8

Oral glucose tolerance tests. At the end of the diet, overnight-fasted GK and Wistar rats were given 2 g/kg glucose orally by gavage using a 60% glucose solution. Blood was collected from the tail vein at 0, 30, 60, 90, and 120 min after dosing. Plasma glucose levels were determined using a Glucose Analyzer 2 (Beckman Instruments, Fullerton, CA), and plasma C-peptide levels were measured by a C-peptide radioimmunoassay (RIA) kit (Linco Research, St. Louis, MO).

Epididymal fat, plasma TG, FA, insulin, and leptin levels. Epididymal fat was excised and weighed. Intracardiac blood samples were collected into EDTA-containing vials. Plasma was immediately separated by 3,000-rpm centrifugation at 4°C for 10 min. Plasma TG and FA levels were measured immediately using GPO-Trinder reagent (Sigma Diagnostics, St. Louis, MO) and the Half-Micro test (Boehringer-Mannheim, Roche Diagnostics, Mannheim, Germany), respectively. Plasma insulin and leptin levels were measured using the Sensitive Rat Insulin RIA kit and the Rat Leptin RIA kit, respectively (Linco Research).

Insulin secretion in static incubations. Rat islets were isolated from nonfasted animals by collagenase digestion as described (16). After isolation, islets were cultured for 1 h in RPMI 1640 containing 10% fetal bovine serum and 11.1 mmol/l glucose. Batches of 10 islets each were washed twice in Krebs-Ringer buffer (KRB) containing 0.1% BSA and 2.8 mmol/l glucose for 20 min at 37°C, and then they were incubated for 60 min in the presence of 2.8, 5.6, 8.3, 11.1, or 16.7 mmol/l glucose; 40 mmol/l KCl; 10 mmol/l DL-glyceraldehyde; or 20 mmol/l succinic acid monomethyl ester, as indicated in the figure legends. Each condition was run in duplicate. Insulin levels in samples collected from the static incubations were measured using the Sensitive Rat Insulin RIA kit (Linco Research).

Insulin mRNA levels, insulin content, and TG content in isolated islets. Comparative analysis of insulin mRNA levels was performed using the Gold RT-PCR kit from Perkin-Elmer and an ABI Prism 7700 sequence detector equipped with a thermocycler (Taqman technology), as previously described (12). β -Actin was used as an internal control. Primer and probe sequences for insulin and β -actin have been described elsewhere (12). Insulin content was measured in duplicate batches of 20 islets after acid-alcohol extraction. TG content was measured in batches of 100 islets after chloroform:methanol extraction as previously described (12).

Glucose oxidation in isolated islets. Batches of 100 isolated islets each were preincubated twice for 20 min at 37°C in KRB containing 0.1% BSA, and then they were incubated in KRB containing 0.1% BSA, either 2.8 or 16.7 mmol/l unlabeled glucose, and 0.1 μ Ci/ μ mol [14 C]glucose (NEN, Boston, MA) for 1 h at 37°C. A volume of 1 ml incubation buffer was then transferred to an Erlenmeyer flask with a rubber cap and acidified with 100 μ l of 7% perchloric acid. Benzethonium hydroxide (400 μ l) was then injected into small wells suspended under the rubber caps, and after 16 h at room temperature, the trapped 14 CO₂ was measured by liquid scintillation counting. Background counts obtained from a control batch treated side by side with the samples but without islets was subtracted from the counts.

TABLE 2
Characteristics of GK and Wistar rats after 6 weeks of high-fat diet or regular diet

	Wistar		GK	
	RD	HFD	RD	HFD
Food intake (g/day)	23.6 \pm 0.5 (8)	23.2 \pm 0.6 (8)	21.2 \pm 0.1 (8) [†]	16.8 \pm 0.8 (8)*
Body weight (g)	407 \pm 10 (10)	443 \pm 14 (10)*	287 \pm 9 (10) [†]	300 \pm 9 (12)
Epididymal fat (g)	5.3 \pm 0.4 (6)	12.6 \pm 0.8 (6)*	3.2 \pm 0.2 (8) [†]	6.7 \pm 0.2 (8)*
Plasma TGs (mg/dl)	104 \pm 15 (6)	196 \pm 22 (5)*	50 \pm 7 (8) [†]	154 \pm 24 (8)*
Plasma FAs (mmol/l)	0.07 \pm 0.01 (6)	0.14 \pm 0.01 (6)*	0.08 \pm 0.02 (8)	0.14 \pm 0.02 (8)*
Fasting plasma glucose (mg/dl)	118 \pm 3 (10)	133 \pm 8 (10)	189 \pm 9 (12) [†]	201 \pm 10 (12)
Fed plasma insulin (mU/ml)	118 \pm 3 (19)	127.2 \pm 16.7 (22)*	76.1 \pm 5.1 (28)	88.7 \pm 7.3 (22)

Data are means \pm SE (*n* replicate measurements in each group). HFD, high-fat diet; RD, regular diet. **P* < 0.05 GK vs. Wistar within diet treatment; [†]*P* < 0.05 HFD vs. RD within animal species.

Western blot analysis of UCP-2 protein levels. Batches of 100 islets each were harvested in 75 μ l lysis buffer (140 mmol/l NaCl, 10 mmol/l Tris-HCl, pH 7.4, 1 mmol/l CaCl₂, 1 mmol/l MgCl₂, 10% glycerol, 1% Nonidet P-40, 1 mmol/l Na₃VO₄, 0.5 mmol/l phenylmethylsulfonyl fluoride, 0.5 mmol/l dithiothreitol, and 1 μ g/ml each of leupeptin, aprotinin, antipain, and pepstatin A), frozen and thawed three times, and cell debris was removed by centrifugation. A volume of 30 μ g protein were separated by SDS-PAGE with 12% acrylamide gels. Resolved proteins were transferred to nitrocellulose membranes (Bio-Rad, Hercules, CA) by electroblotting. For immunoblotting, the membranes were blocked with 1% nonfat dry milk in PBS with 0.05% Tween (PBS-T) for 1 h and then incubated for 1 h at room temperature with a rabbit polyclonal antibody raised against the COOH terminus of mouse UCP-2 (Alpha Diagnostic International, San Antonio, TX) as previously described (13). Membranes were then washed in PBS-T and incubated with horseradish peroxidase-coupled rabbit IgG antibody (Santa Cruz Biotechnologies, Santa Cruz, CA) for 1 h at room temperature. The bands were visualized by enhanced chemiluminescence detection reagents (Amersham, U.K.) and exposed to X-Omat Blue Kodak films for 1–5 min. Membranes were stripped and reprobed with an anti-bovine cytochrome oxidase subunit IV mouse monoclonal antibody (Molecular Probes, Eugene, OR).

Insulin treatment. A group of GK rats was treated for 6 weeks, during the diet period, with either subcutaneous insulin implants releasing 2 units/24 h of insulin (Linplant, Linshin Canada, ON, Canada) or blank implants. Additional insulin pellets were implanted as needed over the course of the study to maintain normal plasma glucose levels. Fed glucose levels from plasma samples collected from the tail vein were determined once a week using an Accu-Chek device (Roche).

Expression of data and statistics. Data are expressed as the means \pm SE. Intergroup comparisons were performed using Student's unpaired *t* test or ANOVA with post hoc Bonferroni adjustment, where appropriate. *P* < 0.05 was considered significant.

RESULTS

Metabolic changes in GK and Wistar rats fed high-fat diet. Metabolic parameters measured after 6 weeks of diet are shown in Table 2. GK rats fed a regular diet had higher plasma glucose (*P* < 0.05, *n* = 12), lower body weight (*P* < 0.05, *n* = 12), lower epididymal fat weight (*P* < 0.05, *n* = 8), and lower plasma TG levels (*P* < 0.05, *n* = 8) than Wistar rats. Plasma FA levels were similar in GK and Wistar rats (NS, *n* = 8). As expected, epididymal fat weight, plasma TG, and FA levels were increased after 6 weeks of high-fat diet in both GK and Wistar rats. Fasting plasma glucose levels were slightly increased by the high-fat diet in both groups, but the difference was not statistically significant. Fed insulin levels were significantly increased by high-fat diet in Wistar (*P* < 0.05, *n* = 19–22) but not in GK rats.

To further explore the effects of a high-fat diet on glucose tolerance and insulin secretion, oral glucose tolerance tests were performed after 6 weeks of diet (Fig. 1). As previously reported (17), glucose tolerance was decreased in GK rats compared with Wistar rats (Fig. 1A) and

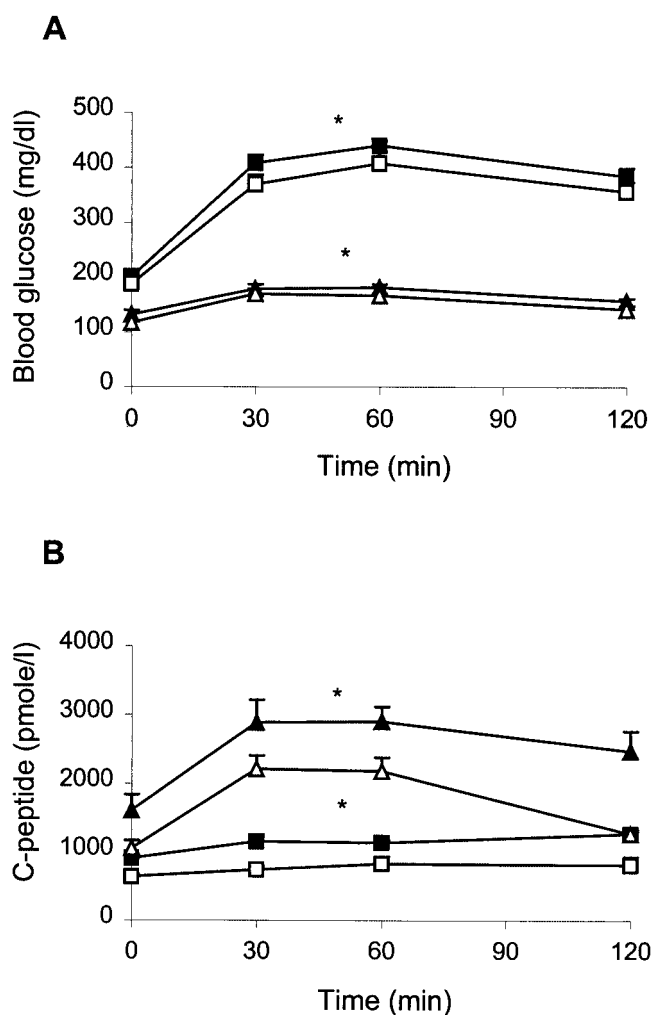


FIG. 1. Oral glucose tolerance test in GK and Wistar rats fed a high-fat or regular diet for 6 weeks. Overnight-fasted animals were administered 2 g/kg glucose by oral gavage. Tail vein blood samples were taken at 0, 30, 60, and 120 min postdose, and blood glucose (A) and C-peptide (B) levels were measured. Results are mean \pm SE of 6–12 rats in each group. □, GK rats fed regular diet; ■, GK rats fed high-fat diet; △, Wistar rats fed regular diet; ▲, Wistar rats fed high-fat diet. *ANOVA, $P < 0.05$.

was accompanied by markedly decreased circulating C-peptide levels (area under the curve [AUC] $16,572 \pm 7,025$ vs. $91,472 \pm 13,519$ pmol \cdot l $^{-1}$ \cdot 120 min $^{-1}$, $n = 6$, $P < 0.001$) (Fig. 1B). Glucose tolerance was slightly but significantly decreased by high-fat diet in both Wistar ($n = 10$, $P < 0.05$, ANOVA) and GK ($n = 12$, $P < 0.05$, ANOVA) rats (Fig. 1A). The C-peptide response was not significantly increased by high-fat diet in either Wistar (AUC $122,762 \pm 16,723$ vs. $91,472 \pm 13,518$ pmol \cdot l $^{-1}$ \cdot 120 min $^{-1}$, $n = 6$, NS) or GK ($27,872 \pm 5,151$ vs. $16,572 \pm 7,025$ pmol \cdot l $^{-1}$ \cdot 120 min $^{-1}$, NS, $n = 6$) rats (Fig. 1B). Similar effects of the diet on glucose tolerance were observed after 10 weeks (data not shown).

Glucose-induced insulin secretion in islets from high fat-fed GK and Wistar rats. To determine the effects of high-fat diet on insulin secretion in vitro, glucose-induced insulin release was measured in static incubation in islets isolated after 6 weeks of diet (Fig. 2A). As previously reported (15), glucose-induced insulin secretion was impaired in GK rat islets as compared with Wistar rat islets.

Insulin secretion was essentially unchanged in islets from Wistar rats fed a high-fat diet compared with those fed a regular diet. In contrast, insulin secretion induced by 16.7 mmol/l glucose was decreased by $\sim 50\%$ in islets from high fat-fed GK rats compared with regular diet-fed GK rats (36 ± 7 vs. 69 ± 7 μ U/islet, $n = 6$, $P < 0.05$). Similar effects were observed after 10 weeks of diet (data not shown).

Insulin secretion induced by KCl, glyceraldehyde, and methyl-succinate in islets from high fat-fed GK and Wistar rats. To further characterize the insulin secretory defect observed in islets from high fat-fed GK rats, we measured insulin secretion induced by secretagogues acting at different steps of the stimulus/secretion-coupling pathway. The response to glucose was decreased after 6 weeks of high-fat diet in islets from GK rats (26 ± 3 vs. 52 ± 12 μ U/islet, $n = 7$, $P < 0.05$) (Fig. 2B), confirming the results shown in Fig. 2A. Insulin secretion induced by either glyceraldehyde (a glycolytic intermediate) or succinic acid monomethyl ester (an intermediate of the Krebs cycle) was decreased in islets from GK rats compared with Wistar rats (glyceraldehyde: 20 ± 5 vs. 65 ± 11 μ U/islet; $n = 6$, $P < 0.05$; succinic acid monomethyl ester: 17 ± 5 vs. 52 ± 12 μ U/islet, $n = 6$, $P < 0.05$) (Fig. 2B). Insulin secretion induced by KCl was not statistically decreased in GK rat islets compared with Wistar rat islets (23 ± 5 vs. 36 ± 7 μ U/islet, $n = 6$, NS) (Fig. 2B). None of the responses to nonglucose secretagogues was affected by high-fat feeding (Fig. 2B).

Insulin content and insulin mRNA levels in islets from high fat-fed GK and Wistar rats. To determine whether the impairment of glucose-induced insulin secretion was caused by a decrease in intracellular insulin stores, we measured insulin content and insulin mRNA levels in islets isolated after 6 weeks of high-fat diet. As previously reported in the same colony of GK rats (18), insulin content in islets from GK rats was similar to that of control rats ($1,676 \pm 124$ vs. $1,831 \pm 158$ μ U/islet, $n = 6$, NS). Insulin content was not significantly modified by the high-fat diet in islets from GK rats ($1,386 \pm 149$ vs. $1,676 \pm 124$ μ U/islet, $n = 6$, NS) or Wistar rats ($1,884 \pm 238$ vs. $1,831 \pm 158$ μ U/islet, $n = 6$, NS). Insulin mRNA levels were markedly decreased in islets from GK rats compared with Wistar rats (1.7 ± 0.5 vs. 7.8 ± 2.1 , $P < 0.05$, $n = 6$ –8) but were not affected by high-fat diet in either Wistar (7.8 ± 2.2 vs. 7.8 ± 2.1 , $n = 8$, NS) or GK (2.2 ± 0.7 vs. 1.7 ± 0.5 , $n = 6$, NS) rat islets. Similar results were observed after 10 weeks of diet (data not shown).

Insulin treatment in high fat-fed GK rats. Insulin was administered in the form of subcutaneously implanted pellets to high fat-fed and regular diet-fed GK rats for 6 weeks. Metabolic parameters measured after diet and insulin treatment are shown in Table 3. In contrast to observations made with the first group of rats (Table 2), high-fat feeding significantly increased fasting plasma glucose levels in GK rats ($n = 6$, $P < 0.05$) (Table 3). As expected, insulin treatment lowered plasma glucose levels in GK rats to levels comparable to those of Wistar rats. In insulin-treated GK rats, high-fat feeding did not increase plasma FA levels, whereas plasma TG levels were significantly increased. Plasma leptin levels were significantly increased by high-fat feeding ($n = 6$, $P < 0.0001$, ANOVA) (Table 3) but were not affected by insulin treatment.

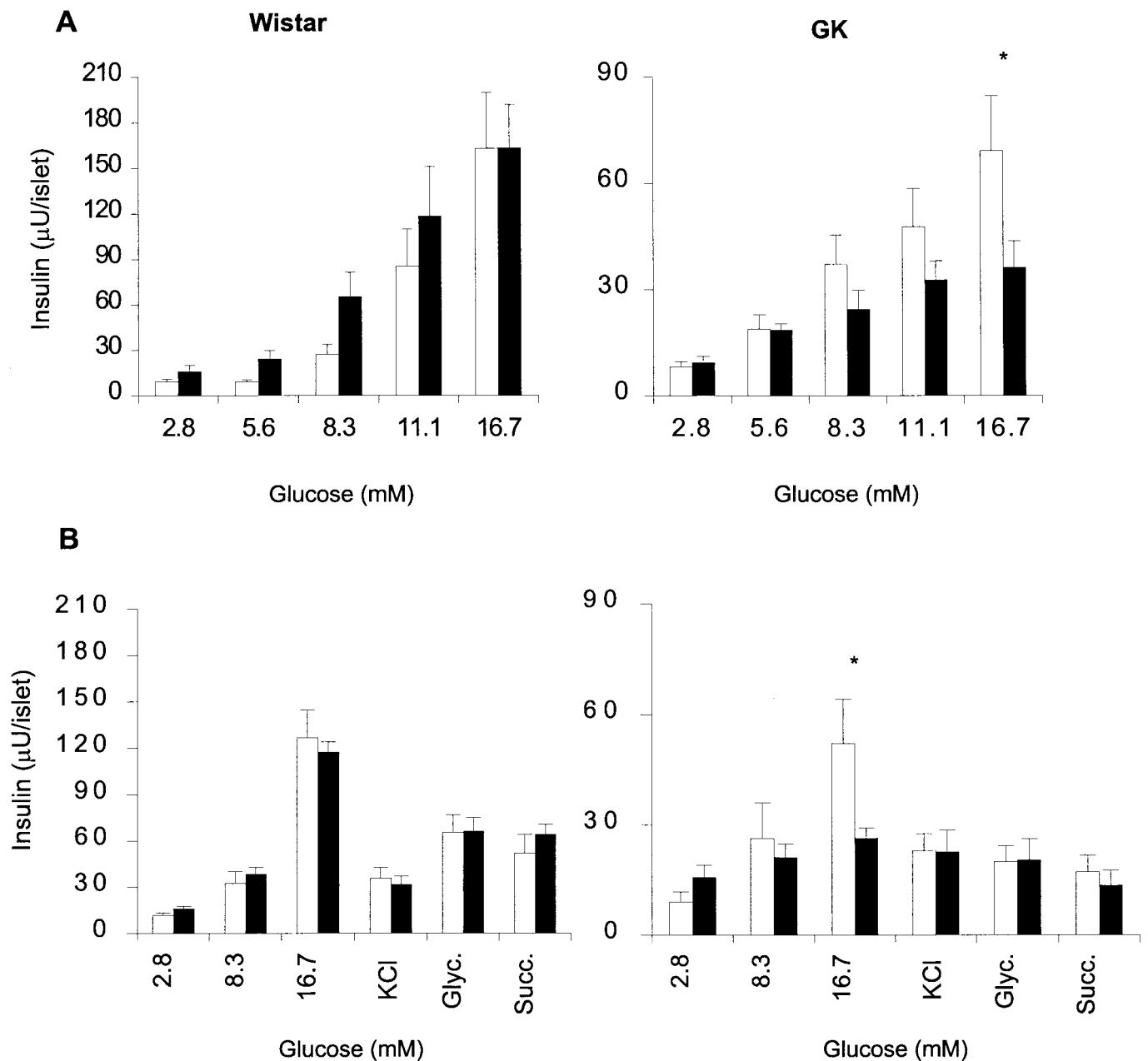


FIG. 2. Effect of 6 weeks of high-fat diet (■) or regular diet (□) on insulin secretion from isolated Wistar and GK rat islets. Insulin secretion induced by glucose (A) or by nonglucose secretagogues (B) was measured. Duplicate batches of 10 islets each were incubated for 60 min in the presence of 2.8, 5.6, 8.3, 11.1, or 16.7 mmol/l glucose; 40 mmol/l KCl; 10 mmol/l glyceraldehyde; or 20 mmol/l succinate methyl ester. Results are the means \pm SE of 6–12 replicate experiments in each group. * $P < 0.05$ vs. regular diet-fed animals.

Glucose tolerance was increased by insulin treatment, but the effect was significant only in high fat-fed rats (AUC $12,412 \pm 2,049$ vs. $25,710 \pm 3,095$ $\text{mg} \cdot \text{dl}^{-1} \cdot 120 \text{ min}^{-1}$, $n = 6$, $P < 0.01$). In isolated islets, insulin secretion induced by 16.7 mmol/l glucose was markedly decreased in the high fat-fed group (23 ± 3 vs. 65 ± 7 $\mu\text{U}/\text{islet}$, $n = 9$, $P < 0.05$) (Fig. 3), confirming our results shown in Fig. 2. Additionally, potentiation of glucose-induced insulin secretion by palmitate was decreased in islets from high fat-fed GK rats (Fig. 3). Importantly, glucose and palmitate stimulation of insulin secretion was fully restored by insulin treatment (Fig. 3). Insulin content was not significantly different between the four groups of animals (data not shown).

TG content in islets from high fat-fed GK and Wistar rats. To investigate the mechanisms of high-fat diet-induced impairment of insulin secretion in GK rat islets, we measured TG content in islets after 6 weeks of high-fat feeding. Islet TG content was similar between GK and Wistar rats and was not affected by the diet in either group (Wistar rats: 128 ± 24 ng/islet in high fat-fed animals vs. 150 ± 43 ng/islets in regular diet-fed animals, $n = 6$, NS; GK rats: 127 ± 33 ng/islet in high fat-fed animals vs. 194 ± 35 in regular diet-fed animals, $n = 6$, NS).

Effects of high-fat feeding on glucose oxidation and UCP-2 expression in islets from GK rats. Neither high-fat feeding nor insulin treatment modified the rate of glucose oxidation at either 2.8 or 16.7 mmol/l glucose in

TABLE 3
Characteristics of GK rats after 6 weeks of high-fat diet or regular diet and insulin treatment

	Nontreated		Insulin-treated	
	GK-RD	GK-HFD	GK-RD	GK-HFD
Body weight (g)	296 \pm 9	330 \pm 6*	310 \pm 5	336 \pm 8
Epididymal fat (g)	3.8 \pm 0.3	7.3 \pm 0.3*	3.4 \pm 0.1	7.4 \pm 0.4*
Plasma TGs (mg/dl)	54.0 \pm 6.7	135.7 \pm 20.2*	43.0 \pm 2.9	99.7 \pm 14.0*
Plasma FAs (mM)	0.16 \pm 0.02	0.42 \pm 0.07*	0.14 \pm 0.03	0.18 \pm 0.03†
Fasting plasma glucose (mg/dl)	169.5 \pm 2.6	224.3 \pm 5.9*	129.8 \pm 27.6	138.5 \pm 15.9†
Plasma leptin (ng/ml)	7.4 \pm 0.4	16.7 \pm 0.7‡	7.0 \pm 0.5	18.5 \pm 1.5‡

Data are means \pm SE. HFD, high-fat diet, RD, regular diet. * P < 0.05; † P < 0.05 (insulin-treated vs. untreated within diet treatment); ‡ P < 0.0001 (HFD vs. RD within treatment). n = 4–11 replicate measurements in each group.

islets from GK rats (Table 4). We compared UCP-2 protein levels by Western analysis in islets isolated from GK rats after 6 weeks of high-fat feeding with or without concurrent insulin treatment (Fig. 4). UCP-2 protein levels were increased 2.0 \pm 0.4-fold (P < 0.05, n = 3) after high-fat feeding in islets from untreated GK rats. In marked contrast, UCP-2 protein levels did not increase in islets from high fat-fed rats treated with insulin (0.4 \pm 0.2-fold as compared with islets from insulin-treated, regular diet-fed GK rats, n = 3, NS). These changes were specific for UCP-2 because expression of the mitochondrial protein cytochrome oxidase was not affected by high-fat feeding (1.0 \pm 0.1-fold increase, n = 3, NS) or insulin treatment (1.0 \pm 0.1-fold increase, n = 3, NS). These results indicate that the deleterious effects of high-fat feeding on insulin secretion in GK rat islets are associated with increased UCP-2 expression.

DISCUSSION

This study was designed to assess whether hyperlipidemia induced by high-fat feeding differentially affects β -cell function in hyperglycemic versus normoglycemic rats, and to determine whether the mechanisms of these effects

involve TG accumulation, changes in glucose oxidation, or increased UCP-2 expression in islets. Our results show that high-fat diet impairs glucose-induced insulin secretion in islets from GK rats but not from Wistar rats and that this is associated with increased UCP-2 expression. Both the decrease in glucose-induced insulin secretion and the increase in UCP-2 protein levels are prevented by insulin treatment.

Variable effects of a high-fat diet on insulin secretion have been reported in the literature (19–22). Ahren et al. (19) have shown that glucose-induced insulin secretion in islets from normal rats was decreased after 4 weeks of high-fat diet. This was associated with a decreased insulin response to glucose in vivo. Others (20,21) have observed an increase in plasma insulin levels attributed to insulin resistance in normal rats fed a high-fat diet, whereas in another study, Kahn and Pedersen (22) showed that high-fat diet decreases plasma insulin levels. These discrepancies may be explained by variations in the composition of the diet and in the balance between the effects of lipids on insulin sensitivity and on insulin secretion. In the present study, high-fat feeding for up to 10 weeks increased circulating insulin levels but did not impair glucose-induced insulin secretion in vivo or in vitro in Wistar rats. In contrast, the maximal response to glucose was markedly impaired in islets from high fat-fed GK rats. Although we did not directly measure the effects of high-fat feeding on β -cell mass, the fact that basal insulin release, insulin content, and insulin mRNA levels were not affected by the diet indicates that the decreased insulin response to glucose is a true functional impairment rather than an indirect effect caused by changes in β -cell mass. Insulin secretion in response to glyceraldehyde, succinate methyl ester, and KCl were unchanged after 6 weeks of high-fat diet, suggesting that high-fat diet-induced hyperlipidemia selectively affects the response to glucose, a characteristic also observed in human type 2 diabetes (23). An alternative explanation is that any impairment in the response to glyceraldehyde, succinate methyl ester, and KCl in islets from high fat-fed GK rats may not be detectable, given the already small amplitude of the response observed in islets from normal diet-fed GK rats. Potentiation of glucose response in the presence of the FA palmitate was also decreased by high-fat feeding in GK islets. Insulin treatment did not modify the response to glucose or palmitate in islets from regular diet-fed rats, but it completely prevented the inhibitory effect of high-fat diet on insulin secretion. Because both glucose and FA levels were nor-

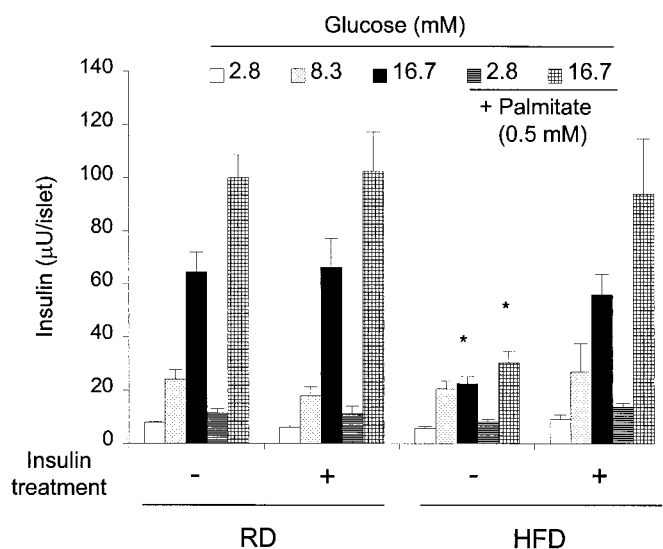


FIG. 3. Effect of insulin treatment on insulin secretion induced by glucose or palmitate in islets isolated from GK rats fed a high-fat (HFD) or regular diet (RD). Duplicate batches of 10 islets each were incubated for 60 min in the presence of 2.8, 8.3, or 16.7 mmol/l glucose alone, or 2.8 or 16.7 mmol/l glucose with 0.5 mmol/l palmitate. Results are the means \pm SE of 7–9 replicate experiments in each group * P < 0.05 vs. nontreated rats.

TABLE 4
Glucose oxidation rates in islets isolated from GK rats after 6 weeks of high-fat diet or regular diet

Glucose oxidation (pmol · islet ⁻¹ · h ⁻¹)	Nontreated		Insulin-treated	
	GK-RD	GK-HFD	GK-RD	GK-HFD
At 2.8 mmol/l glucose (<i>n</i> = 4)	6.3 ± 0.2	6.1 ± 0.2	5.6 ± 0.3	5.4 ± 0.2
At 16.7 mmol/l glucose (<i>n</i> = 4)	30.1 ± 2.2	29.6 ± 1.2	28.7 ± 0.7	27.3 ± 0.3

Data are means ± SE. HFD, high-fat diet; RD, regular diet.

malized by insulin treatment, we cannot unequivocally conclude that the restoration of insulin secretion was due to normalization of glycemia. However, it is noteworthy that prevention of the high-fat diet-induced insulin secretory defect in GK rat islets by insulin treatment was not associated with decreased adiposity (as assessed by epididymal fat weight), circulating TG levels, or plasma leptin concentrations, confirming that effects of high-fat feeding are caused by the synergistically deleterious association of hyperglycemia and hyperlipidemia.

In ZDF rats, the marked increase in islet TG content associated with hyperlipidemia has been suggested to play a mechanistic role in β -cell dysfunction and loss (2), mainly by inducing apoptosis, the primary cause of decreased β -cell mass in this model (24). Marked elevation in islet TG content and deposition of fat droplets has also been observed in OLETF rats, a model of hypertriglyceridemia-associated diabetes (25). Here we show that impairment of glucose-induced insulin secretion by high-fat diet in hyperglycemic GK rats is not associated with a detectable increase in islet TG content. Recently, Lee et al. (7) observed that leptin deficiency in ZDF rats results in the ectopic accumulation of TG in nonadipose tissues, and they have suggested that overnutrition-induced hyperleptinemia protects nonadipose tissues from lipid accumulation. This antisteatotic role of hyperleptinemia may account for the fact that in leptin-sensitive GK as well as Wistar rats, high-fat diet did not lead to islet TG accumulation. The present results contrast with our previous observations in isolated rat islets that prolonged exposure to elevated FAs in the presence of high glucose is associated with increased TG content and decreased insulin gene expression (9,12). We speculate that these discrepancies might be caused by differences between *in vitro* and *in vivo* levels of FAs, as well as a different sensitivity of isolated islets *in vitro* and *in situ* islets *in vivo* to the effects of FAs. Regardless of the cause for this discrepancy, the

present results indicate that the concomitant presence of hyperglycemia and hyperlipidemia can impair glucose-induced insulin secretion without significant changes in insulin gene expression.

Several studies have reported that chronic exposure to elevated levels of FAs induces an increase in FA oxidation and a decrease in glucose oxidation (26–30), suggesting that a glucose-FA cycle, as described in the muscle (31), may be operative in the β -cell. In contrast, other groups have found minimal, if any, changes in glucose oxidation after culture of β -cell lines (32,33) or islets (34) in the presence of FAs. Similarly, in the present study, we did not find any effect of high-fat feeding on glucose oxidation, suggesting that the site of action of FAs lies downstream of mitochondrial oxidation.

UCPs are located in the inner mitochondrial membrane, and they uncouple mitochondrial respiration from oxidative phosphorylation, resulting in decreased ATP production (35). UCP-2 is a ubiquitously expressed UCP family member, and its expression is increased by high-fat feeding or FAs in various tissues (35,36). Adenovirus-mediated overexpression of UCP-2 in islets decreases glucose-stimulated insulin secretion (14,37). Recently, it has been suggested that an increase in UCP-2 expression might mediate deleterious effects of FAs on β -cell function *in vitro* (13) and *in vivo* (38). We found increased UCP-2 protein levels in islets from GK rats fed a high-fat diet, and this increase was prevented by insulin treatment. Whether these changes are due to translational or transcriptional regulation of UCP-2 remains to be determined. Although these results do not prove that the impairment in insulin secretion was caused by changes in UCP-2 expression, they are consistent with a model in which elevated plasma levels of FAs increase UCP-2 levels and mitochondrial uncoupling, thereby decreasing ATP generation and thus insulin secretion, without changes in glucose oxidation. This model does not exclude the possibility that the expression of other metabolic genes may be modulated by the association of hyperglycemia and hyperlipidemia. Indeed, both of these abnormalities are known to modify the expression of a number of genes and metabolic pathways implicated in glucose-stimulated insulin secretion (rev. in 8). Understanding the relative contribution of changes in UCP-2 expression and other potential targets requires further studies.

In conclusion, our results demonstrate that high-fat feeding impairs glucose-induced insulin secretion only in the context of chronic hyperglycemia in GK rat islets. These effects are not mediated by TG accumulation in islets but are associated with changes in UCP-2 expression. Whether similar mechanisms prevail in obesity-associated diabetes in humans remains to be established.

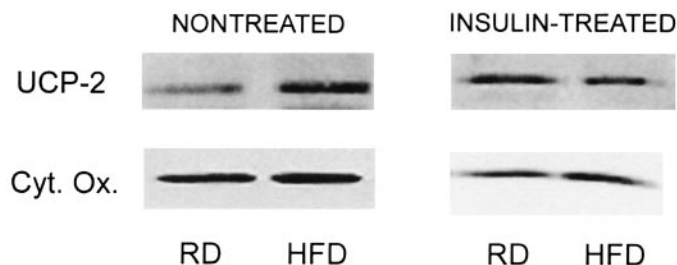


FIG. 4. Effects of 6 weeks of high-fat diet (HFD) and insulin treatment on UCP-2 protein levels in islets from GK rats. Lysates from freshly isolated islets were analyzed for UCP-2 expression by Western analysis, as described in RESEARCH DESIGN AND METHODS. Blots were stripped and reprobed with the mitochondrial protein cytochrome oxidase subunit IV (Cyt. Ox.) to control for loading differences. A: Untreated animals. B: Insulin-treated animals. Blots from one representative experiment are shown. RD, regular diet.

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