Fatty Acid Inhibition of Glucose-Stimulated Insulin Secretion Is Enhanced in Pancreatic Islets From Insulin-Resistant Rats

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A study was initiated to test two hypotheses. The first was the postulate that glucose-stimulated insulin secretion would be enhanced in pancreatic islets isolated from normal non-obese rats made insulin-resistant by dietary means. The second, related hypothesis was that glucose-stimulated insulin secretion by pancreatic islets isolated from insulin-resistant rats would be more vulnerable to inhibition following culture in the presence of fatty acids. For this purpose, insulin resistance was induced in normal Sprague-Dawley rats by feeding fat-enriched and fructose-enriched diets. The results indicate that islets isolated from either fat-fed or fructose-fed rats secreted significantly more insulin at a glucose concentration of 2.5 to 10.0 mmol/L. In addition, the mean maximal glucose (27 mmol/L)-stimulated insulin secretion rate was significantly lower (15.3 ± 2.5 ng/islet/h) in islets from fructose-fed rats versus chow-fed rats (25.2 ± 3.1 ng/islet/h) following culture for 48 hours in the presence of palmitate (0.125 μmol/L). These results support the view that glucose-stimulated insulin secretion is enhanced in islets from insulin-resistant rats, and that these islets are more vulnerable to the inhibitory effects of free fatty acid (FFA) on insulin secretion.

WE HAVE RECENTLY demonstrated that the resistance to insulin-mediated glucose disposal in nondiabetic subjects is characterized by a leftward shift of the glucose-stimulated insulin secretion dose-response curve.[1] When these individuals lose the ability to compensate for the insulin resistance, plasma glucose increases and type 2 diabetes supervenes.[2,3] A major unresolved issue concerning the pathogenesis of type 2 diabetes is the reason that the compensatory ability of the pancreatic β cell only fails in some insulin-resistant individuals. One possibility is that the greater the degree of insulin resistance, the more insulin secretion must be enhanced to maintain glucose tolerance and the greater the risk of eventual β-cell failure. Although this formulation may serve as a general hypothesis, it does not identify either the precipitating cause of the β-cell failure or the reason that it only happens to a portion of insulin-resistant individuals.

Although we have little insight into the latter issue, there is considerable information as to the factors that may play a mechanistic role in precipitating β-cell failure. For example, the evidence that the plasma insulin response to glucose stimulation increases when glycemic control is improved following either weight loss[4] or insulin treatment[5] in patients with type 2 diabetes emphasizes the untoward effects of hyperglycemia on the insulin secretory response to glucose. More recently, it has been shown that a chronic elevation of free fatty acid (FFA) concentrations will impair glucose-stimulated insulin secretion in vitro and in vivo,[6-8] suggesting that the elevated plasma FFA concentrations characteristic of patients with type 2 diabetes[9,10] may also impair the insulin secretory response.

This study was initiated to test two related hypotheses. Firstly, we postulated that glucose-stimulated insulin secretion will be enhanced in pancreatic islets isolated from insulin-resistant and hyperinsulinemic rats that are not diabetic, obese, or highly inbred. Secondly, we proposed that pancreatic islets isolated from insulin-resistant, hyperinsulinemic nondiabetic rats would be more vulnerable to the inhibitory effect of elevated FFA concentrations on β-cell function than islets from normal animals. The second hypothesis could have been tested using islets from rodent models of insulin resistance known to display the same leftward shift in the glucose-stimulated insulin dose-response curve described in insulin-resistant nondiabetic subjects.[1] On the other hand, these models are generally extremely obese and highly inbred.[11,12] As such, the ability to generalize from the results would be questionable. For this reason, we chose to evaluate the possibility that islets isolated from Sprague-Dawley rats made insulin-resistant by dietary manipulation would secrete more insulin at a given glucose concentration than islets from rats fed conventional chow, as well as being more sensitive to the inhibitory effects of increased FFAs. For this purpose, we used rats fed diets enriched with either fat or fructose. Both of these interventions have been shown to produce insulin resistance and compensatory hyperinsulinemia in non-obese rats of nonunique genetic background.[13,14] By studying glucose-stimulated insulin release by islets isolated from these well-recognized models of insulin resistance, we believed it possible to test our hypothesis as to the untoward effects of elevated FFAs on islets from insulin-resistant animals in a situation likely to be more relevant to the human condition.

MATERIALS AND METHODS

Male Sprague-Dawley rats (Harlan, San Diego, CA) initially weighing 250 to 280 g were used for all experiments. They were maintained in accordance with the *National Institutes of Health Guide for the Care and Use of Laboratory Animals.* The rats were housed at 24°C with a 12-hour light/dark cycle (lights on at 7 AM). Standard chow (Teklad Laboratory Diet 8640; Harlan, Bartonville, IL) and water were provided ad libitum prior to division of the animals into three groups fed subsequently with three different diets (standard, high-fat, or high-fructose) as described in Table 1. The fat in the high-fat diet contains 40% shortening and 20% hydrogenated coconut oil. All of the chemicals and reagents were obtained from Sigma Chemical (St Louis, MO)AU#1 unless otherwise indicated.

The first series of experiments were designed to test the effect of diet-induced insulin resistance on the ability of isolated islets to respond

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Table 1. Composition of the Test Diets (% wt/wt)

Ingredient	Standard	High-Fat	High-Fructose
Fat	4.5	60	5
Protein	23	25	20
Carbohydrate			
Starch	31.9	8.4	—
Fructose	3.7	—	60
Fiber	5.3	6.6	9.4

to increases in glucose throughout the physiological range. For this purpose, islets were isolated from the pancreas after feeding the three test diets for 2 weeks (with six rats in each of the three groups). A batch of 10 to 20 islets were preincubated in 2.5 mmol/L glucose to establish baseline insulin secretion for 30 minutes, followed by exposure to stepwise increases in glucose to construct a glucose-stimulated insulin secretion dose-response curve. The details of the methods used to isolate islets and to quantify the insulin secretory response are subsequently described.

The second series of experiments were performed to compare glucose-stimulated insulin secretion in islets isolated from rats fed for 2 weeks with either standard chow (n = 6) or the high-fructose diet (n = 6) and cultured in the presence of palmitate (0.125 μmol/L). Islets from fat-fed rats were not included in these studies in order to avoid the potential confounding effect of elevated circulating levels of FFA in rats eating a high-fat diet for the preceding 2 weeks. Details of these experiments are also provided subsequently.

At the end of each dietary period, blood was obtained from the tail vein for determination of plasma insulin by radioimmunoassay (Linco Research, St Louis, MO). Afterward, the rats were decapitated and the pancreatic islets of Langerhans were freshly isolated by collagenase digestion (type v, lot 32H6803; Sigma Chemical) using a modification[12] of the method of Lacy and Kostianovsky.[15] After the islets were freed from the pancreatic tissue, they were harvested under a stereoscopic microscope and placed in petri dishes.

Islets for evaluation of the effect of dietary-induced insulin resistance were removed from the petri dishes and washed in Krebs-Ringer bicarbonate (KRB) buffer. The islets were then incubated in KRB buffer and bovine serum albumin ([BSA] 1.0 g/L, fraction IV; Sigma Chemical) at 37°C in the presence of 95% O_2: 5% CO_2 with the addition of increasing amounts of glucose, ie, 2.5, 5, 6, 7, 8, 9, and 10 mmol/L. Aliquots were removed after 20 minutes of incubation, and the insulin concentration was determined.

When not used immediately for incubation studies to determine glucose-stimulated insulin secretion, 25 to 30 islets were cultured in petri dishes containing 6 mL RPMI 1640 supplemented with 100 U/mL penicillin, 0.1 mg/mL streptomycin, 2 mmol/L L-glutamine, and 10% (vol/vol) heat-inactivated fetal calf serum (Sigma Chemical). Palmitate (sodium salt) was dissolved in 95% ethanol before addition to the culture medium. The final concentration of ethanol in the medium was 1% or 2% (vol/vol) at the FFA concentrations used in this study. The concentration of glucose in culture media was 11.1 mmol/L in the presence or absence of palmitate (0.125 μmol/L). Islets were cultured free-floating at 37°C in an atmosphere of 5% CO_2: 95% O_2 in humidified air for 48 hours.

After 48 hours of culture in RPMI 1640, the islets were preincubated for 30 minutes in KRB buffer (pH 7.4) supplemented with 10 mmol/L HEPES, 3.3 mmol/L glucose, and BSA (1.0 g/L). Islets were selected after preincubation in batches of three islets in 200 μL KRB buffer containing either 3.3 or 27 mmol/L glucose at 37°C for 60 minutes. Triplicates were assayed for each experimental condition. Incubation was performed in a water bath with continuous shaking and in an atmosphere of 95% O_2:5% CO_2. At the end of the incubation, aliquots of the incubation media were removed for insulin assay.

All results are presented as the mean ± SEM. Treatments were compared using one-way or two-way ANOVA with Tukey's test as a post hoc comparison. A P level less than .05 is considered statistically significant.

RESULTS

Plasma insulin increased significantly ($P < .05$) in rats eating either the high-fat diet (52 ± 4 μU/mL) or the high-fructose diet (45 ± 3 μU/mL) compared with rats eating conventional rat chow (25 ± 2 μU/mL). These results represent the values for 15 rats in each experimental group. The rate of glucose-stimulated insulin secretion in incubated islets from the three experimental groups was higher in fructose-fed and fat-fed rats as the glucose concentration increased from 2.5 to 10 mmol/L (Fig 1).

To avoid the potential confounding effect of the high-fat diet on overall FFA metabolism, the effects of palmitate on basal or maximal glucose-stimulated insulin secretion were studied only in islets from fructose-fed rats. The results are illustrated in Fig 2. Insulin secretion in response to 3 mmol/L glucose was significantly increased ($P < .05$) in islets from fructose-fed rats incubated in the absence of palmitate. The addition of palmitate to 3.3 mmol/L glucose increased insulin secretion in islets from both groups, but the relative increase was attenuated in islets from fructose-fed rats (71% ± 12%) versus chow-fed rats (282% ± 42%, $P < .05$). As a consequence, insulin secretion at 3.3 mmol/L glucose was lower in islets from fructose-fed rats incubated in the presence of palmitate (Fig 2A).

Figure 2B depicts the comparison of maximal insulin secretion by islets incubated in 27 mmol/L glucose. As under basal conditions, maximal glucose-stimulated insulin secretion was higher in islets from fructose-fed rats in the absence of palmitate. In contrast to the studies performed at 3.3 mmol/L glucose, the addition of palmitate to the islets resulted in a decrease of maximal glucose-stimulated insulin secretion in islets from both chow-fed and fructose-fed rats (21% ± 8% and 62% ± 11%, $P < .01$). However, the palmitate-induced inhibition of insulin secretion was of much greater magnitude in islets from fructose-fed rats. As a result, the maximal rate of glucose-induced insulin secretion, which was higher under control conditions in fructose-fed rats, was lower when palmitate was added to the culture medium.

DISCUSSION

The results of the study provide support for the two hypotheses stated in the Introduction. The first hypothesis was that

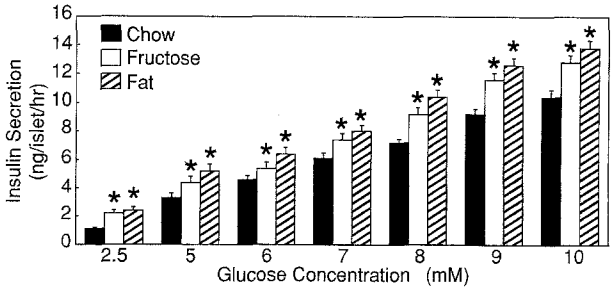

Fig 1. Glucose-stimulated insulin secretion by pancreatic islets isolated from rats fed either conventional chow or diets enriched in fructose or fat. Insulin secretion was compared by 1-way ANOVA at each glucose concentration. *$P < .05$.

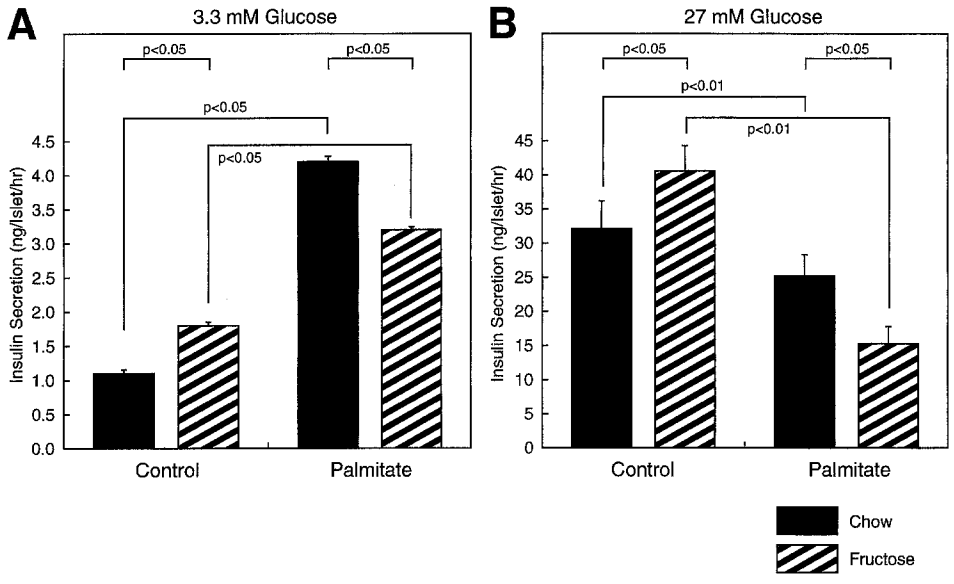

Fig 2. Effect of palmitate on insulin secretion by pancreatic islets isolated from fructose-fed rats and incubated at a glucose concentration of 3.3 and 27 mmol/L.

pancreatic islets from non-obese and outbred rats with dietary-induced insulin resistance would have an exaggerated insulin secretory response to glucose stimulation. The results shown in Figs 1 and 2 indicate that this was the case. More specifically, it was shown that insulin secretion by islets isolated from insulin-resistant rats was significantly increased at both basal and maximal glucose concentrations, and in response to increasing glucose throughout the physiological range. These observations are consistent with previous findings in several strains of rodents with genetic forms of obesity and insulin resistance,[11] as well as our previous results in insulin-resistant humans.[1]

Although they are somewhat more complicated to interpret, the results also support the second hypothesis. Islets from insulin-resistant fructose-fed rats were more sensitive to the untoward effects of elevated FFA concentrations. In the case of the insulin secretory response in the absence of palmitate, both basal (3.3 mmol/L) and maximal (27 mmol/L) glucose-stimulated insulin secretion were greater in islets from fructose-fed rats. However, just the opposite was observed when palmitate was added, and in this instance, the insulin secretory response in islets from fructose-fed rats was significantly lower with incubation at either glucose concentration.

The observation that chronic FFA elevations decrease glucose-stimulated insulin secretion is consistent with previously published data.[6-8] However, the fact that the inhibitory effect of FFA on glucose-stimulated insulin secretion is accentuated in islets from insulin-resistant rats represents an entirely new observation. As such, these results may help to explain the transition from prediabetes to type 2 diabetes in insulin-resistant individuals. Although there seems to be general agreement that glucose intolerance develops when insulin-resistant individuals are no longer able to maintain the necessary degree of compensation, an understanding as to why this relative β-cell failure occurs remains elusive. It has been emphasized that significant increases in plasma FFAs occur concomitantly with the decline in plasma insulin and the increase in glucose.[16,17] These data led to the suggestion that elevated FFA concentrations play a central role in the development of hyperglycemia, both by decreasing peripheral glucose uptake and by impairing the ability of the expanding plasma glucose pool to inhibit hepatic glucose output.[16,17] The current results provide evidence to suggest that insulin resistance, the prediabetic state, may render β cells more susceptible to the inhibitory effects of elevated FFAs on glucose-stimulated insulin secretion. If this is the case, it is easy to visualize how an extremely harmful positive-feedback situation can be created: the elevated FFA concentrations tend to increase in insulin-resistant individuals as the insulin level begins to decline, inhibiting glucose-stimulated insulin secretion, leading to a further decrease in β-cell function, accentuating the concomitant increase in plasma glucose and FFAs, further decreasing insulin secretion, and so on.

Although our results demonstrate that the inhibitory effect of FFAs on glucose-stimulated insulin secretion is exaggerated in islets from insulin-resistant rats, they provide no insight as to why this might be the case. In this context, Zhou and Grill[18] have suggested that the inhibition of glucose-stimulated insulin secretion following long-term exposure of islets to palmitate is due to a decrease in the islets' ability to convert pyruvate to acetyl-coenzyme A, secondary to a decline in islet pyruvate dehydrogenase activity. Assuming that this mechanism accounts for the inhibitory effects of palmitate on glucose-stimulated insulin secretion, one may speculate that pyruvate dehydrogenase activity is lower in islets from insulin-resistant rats, possibly due to an increase in the activity of a non–pyruvate dehydrogenase-bound kinase.

In conclusion, although our results do not provide insight into the underlying mechanisms, they demonstrate a leftward shift in glucose-stimulated insulin secretion in islets isolated from non-obese outbred rats with dietary-induced insulin resistance and hyperinsulinemia. Furthermore, the inhibitory effect of palmitate on insulin secretion was accentuated when islets were isolated from insulin-resistant rats. These data provide support for the view that the untoward effects of elevated plasma FFA concentrations are not confined to a decrease in muscle glucose disposal but also involve inhibition of insulin secretion.

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