

Fatty Acid-induced β cell Hypersensitivity to Glucose

Increased Phosphofructokinase Activity and Lowered Glucose-6-phosphate Content

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Abstract

Diabetic states are characterized by a raised serum/islet level of long chain fatty acids and a lowered ED_{50} for glucose-induced insulin secretion. Prolonged culture (> 6 h) of islets with long chain fatty acids replicates the basal insulin hypersecretion. We examined this effect in rat islets cultured for 24 h with 0.25 mM oleate. Insulin secretion at 2.8 mM glucose was doubled in combination with a 60% lowered islet content of glucose-6-phosphate (G6P). Investigation of the lowered G6P showed: (a) increased glucose usage from 0.5 to 100 mM glucose with identical values measured by $[2-^3H]$ glucose and $[5-^3H]$ glucose, (c) indicating little glucose-6-phosphatase activity, (b) unchanged low pentose phosphate shunt activity, (c) 50% increased phosphofructokinase (PFK) V_{max} , (d) a normal ATP/ADP ratio, and (e) unchanged fructose 2,6 biphosphate content. Triacsin C, an inhibitor of fatty acyl-CoA synthetase, prevented the increase in PFK activity and the lowered G6P content. These results suggest that long chain acyl-CoA mediates the rise in PFK activity, which in turn lowers the G6P level. We speculate that the inhibition of hexokinase by G6P is thus attenuated, thereby causing the basal insulin hypersecretion. (*J. Clin Invest.* 1998. 101:1870–1875.) Key words: islets of Langerhans • glucose metabolism • pentose phosphate shunt • long chain fatty acyl-CoA • Triacsin C

Introduction

Islet β cells secrete insulin in response to circulating nutrients and cellular fuels. Best studied is the plasma glucose concentration: a rise in the level of glycemia induces insulin secretion through a process that is dependent on increased glucose metabolism. The flux through glycolysis is the sensed process that makes β cells glucose-responsive (1).

β cell glucose metabolism is a multienzyme-regulated process. Glucokinase determines the kinetics of glucose entry into glycolysis (2, 3). Hexokinase is present in β cells (4), but its activity at physiologic glucose levels is minimal because of allosteric inhibition by glucose-6-phosphate (G6P)¹ (5). G6P is in

equilibrium with fructose-6-phosphate so that the steady-state level of G6P is governed by control of the outflow by phosphofructokinase (PFK). PFK activity is modulated by multiple positive and negative allosteric factors including ATP, ADP, AMP, fructose-2, 6-bisphosphate (Fru-2,6- P_2), and citrate (6). Thus, altered kinetics of glucose-induced insulin secretion could result from a changed intrinsic activity, cellular level, or allosteric regulation of hexokinase, glucokinase, or PFK.

Long chain fatty acids (FA) stimulate insulin secretion (7, 8) with the postulated mediator being long-chain acyl-CoA esters (LC-CoA) (9). With chronic FA exposure, the glucose concentration/insulin secretion relationship is left-shifted such that basal insulin release is increased (10, 11). This finding is of interest since plasma levels of FA are raised in type II diabetes mellitus (12, 13), and we have proposed that a lowered ED_{50} for glucose-induced insulin secretion is an early, causative step of the β cell failure of type II diabetes through β cell overwork (14).

In a previous study, we investigated islets cultured with palmitate for 24 h (15). The expected increase in glucose sensitivity was present as reflected by a doubled insulin secretion and glucose use at 2.8 mM glucose as opposed to the maximal values being unchanged. This result suggested a low K_m process such as increased hexokinase activity. However, hexokinase V_{max} was increased only 30% (glucokinase was unchanged) which was insufficient to cause the functional changes. An additional finding was that Triacsin C, an inhibitor of fatty acyl-CoA synthetase (16–18), attenuated the basal insulin hypersecretion, implying an increased level of LC-CoA was the cellular mediator. This study tested the hypothesis that G6P inhibition of hexokinase was altered in FA cultured islets.

Methods

Reagents. Reagents were obtained from Sigma (St. Louis, MO) unless otherwise stated.

Islet isolation and culture. Islets were isolated from Sprague-Dawley rats (Taconic, Germantown, NY) using an adaptation of the Gotoh method (19): pancreatic duct infiltration with collagenase (Serva, Heidelberg, Germany), histopaque gradient separation, and hand picking. The culture medium was RPMI 1640 supplemented with 5.5 mM glucose, 2 mM glutamine, 10% FCS, 100 U/ml penicillin, 0.1 mg/ml streptomycin (GIBCO BRL, Grand Island, NY). Islets were cultured overnight at 37°C in humidified air and 5% CO_2 followed by an additional 24 h with 0.25 mM oleate (sodium salt) in 2% ethanol or 2% ethanol alone added to the culture medium. Diazoxide or Triacsin C (Biomol Research Laboratories, Inc., Plymouth Meeting, PA) in DMSO was included during the 24-h culture as stated in

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1. **Abbreviations used in this paper:** FA, long chain fatty acids; Fru-2,6- P_2 , fructose 2,6-bisphosphate; G6P, glucose-6-phosphate; G6Pase, glucose-6-phosphatase; LC-CoA, long chain acyl-CoA esters; PFK, phosphofructokinase.

the text. Islet protein was measured by a commercial kit (Bio-Rad, Hercules, CA) with BSA as standard.

Insulin secretion. Islets were preincubated in KRB, 10 mM Hepes, 0.5% BSA, and 2.8 mM glucose for 30 min at 37°C. Triplicate batches of 10 islets in glass vials were incubated in KRB/2.8 or KRB/16.7 mM glucose for 60 min in a 37°C shaking water bath. The medium was separated from the islets by gentle centrifugation (500 rpm, 5 min at 10°C) and stored at -20°C pending measurement of the insulin concentration by an RIA that used rat insulin standards (Eli Lilly, Indianapolis, IN) and charcoal separation (20).

Islet G6P content. G6P was measured using an adaptation of the method of Lowry and Passonneau (21). Islets (20 per tube) were preincubated for 30 min at 37°C in KRB/5.5 mM glucose with or without 0.25 mM oleate as in the previous 24-h culture. They were lysed by 10 μ l 40 mM NaOH and placed on ice for 10 min, followed by addition of 3 μ l 0.15 M HCl and incubated at 75°C for 20 min to destroy the cellular enzymes. A second protocol included a 60-min incubation at 2.8, 8.3, and 27.7 mM glucose in KRB with or without 0.25 mM oleate before lysis of the islets. The reaction consisted of adding 15 μ l of reaction buffer that contained 20 μ M NADP, 0.15 M Tris/HCl (pH 8.1), 0.02 U/ml grade 1 G6P dehydrogenase from yeast (Boehringer Mannheim, Indianapolis, IN), and incubating 30 min at 28°C in a shaking water bath followed by addition of 4 μ l 1 M NaOH and incubation at 75°C for 20 min to destroy any remaining NADP. The NADPH formed was amplified by the cycling method by adding 100 μ l of reagent that contained 9 U/ml of type II glutamate dehydrogenase, 5 mM α -ketoglutaric acid, 1 mM G6P, and 6 U/ml G6P dehydrogenase for 60 min at 38°C, followed by heating to 100°C for 10 min to stop the reaction. Sample (100 μ l) was transferred to a UV cuvette containing 1 ml 0.006 U/ml 6-phosphogluconate dehydrogenase in 0.15 M Tris/HCl, (pH 8.1), 30 μ M NADP, 0.1 mM EDTA, 30 mM ammonium acetate, and 5 mM MgCl₂ and stood at room temperature for 30 min. The 6-phosphogluconate formed was measured by a fluorometer at 340 nm excitation and 420 nm emission, and the islet G6P content was determined from G6P standards (1–20 pmol) handled in the same fashion. The blank was islet extract added to reaction buffer that did not contain G6P dehydrogenase.

Islet glucose use. Glucose use was measured by the method of Ashcroft (22). Triplicate batches of 20 islets underwent a 30-min preincubation in KRB/5.5 mM glucose with or without 0.25 mM oleate followed by a 90-min incubation at 37°C in 100 μ l KRB/glucose (0.03–100 mM) plus 2 μ Ci D-[2-³H]glucose or D-[5-³H]glucose (Amersham Corp., Arlington Heights, IL). The reaction was carried out in a 1-ml cup inside a rubber-stoppered 20-ml scintillation vial with 500 μ l of distilled water surrounding the cup. Glucose metabolism was stopped with 100 μ l 1 M HCl injected through the stopper into the cup. After overnight incubation at 37°C to allow equilibration of the [³H]H₂O in the reaction cup with the distilled water, the radioactivity in the distilled water was determined in a liquid scintillation counter. Islet glucose use consists of high affinity and low affinity components (23). Kinetics were estimated by linear regression of the Eadie-Scatchard plot followed by 10 cycles of the method of Spears (24).

Islet pentose phosphate shunt activity. The proportion of glucose metabolism attributable to the pentose phosphate shunt was calculated by the method of Verspohl (25). Islet glucose oxidation was measured by a previously published method (15) using [1-¹⁴C] and [6-¹⁴C]glucose for 90 min at 2.8 and 16.7 mM glucose. The values were stratified against glucose use measured with D-[5-³H]glucose to yield the number of picomoles of glucose oxidized/used for each tracer (glucose oxidation specific yield), and the percent contribution of the pentose shunt to glucose metabolism was calculated (25).

Islet PFK activity. Islets (300) were washed in KRB and sonicated on ice in 0.15-ml extraction buffer containing 15 mM K₂PO₄ (pH 7.0), 100 mM KCl, 2 mM EDTA, 2 mM PMSF, 0.2 mg/ml leupeptin, and 50 μ g/ml aprotinin (26). After centrifugation at 12,000 g for 15 min at 4°C, PFK activity in the supernatant was measured by the method of Narabayashi et al. (27). Extract (20 μ l) was added to 1 ml of reaction buffer containing 50 mM Tris/HCl (pH 8.0), 1 mM EDTA,

2.5 mM DTT, 5 mM ammonium sulfate, 2 mM MgCl₂, 1 mM ATP, 1 mM fructose-6-phosphate, 0.16 mM NADH, 0.4 U/ml aldolase, and a 2.4 U/ml triosephosphate isomerase/0.8 U/ml glycerophosphate dehydrogenase mixture (last two from Boehringer Mannheim) in a quartz cuvette, and the NADH metabolized over 15 min and assessed at 340 nm by spectrophotometer. PFK activity was calculated with the equation 1 μ mol fructose-1, 6-bisphosphate = 2 μ mol NADH consumed.

Islet Fru-2,6-P₂ content. Islets (350) were washed in KRB and extracted in 100 μ l of preheated 1 mM Hepes (pH 8.5) for 10 min at 75°C and then placed on ice. The extract was centrifuged, and Fru-2,6-P₂ was measured in the supernatant using a method which is based on its ability to overcome ATP inhibition of muscle PFK activity (28, 29). The reaction buffer contained 50 mM Hepes (pH 7.25), 0.2 mM EDTA, 5 mM MgCl₂, 1 mM NH₄Cl, 0.16 mM NADH, 2.5 mM DTT, 1 mM fructose-6-phosphate, 0.4 U aldolase, and a 2.4 U triosephosphate isomerase/0.8 U glycerophosphate dehydrogenase mixture (last two from Boehringer Mannheim) in a final volume of 1 ml. All enzymes were desalted by the method of Burch (30). Type III rabbit muscle PFK (15 mU) was added to the reaction buffer in a cuvette followed by 20- μ l Fru-2,6-P₂ (0.5–10 pmol) standards or islet extract. The reaction was initiated by addition of an amount of ATP that near maximally inhibited PFK activity in the absence of Fru-2,6-P₂, and the decrease in NADH was read at 340 nm. Islet Fru-2,6-P₂ content was determined from the standard curve.

Islet ATP/ADP ratio. Islets (100) were preincubated for 30 min at 37°C in 100 μ l KRB/5.5 mM glucose with or without 0.25 mM oleate, followed by addition of 100 μ l 2% TCA, vortexed, and incubated 15 min on ice. After centrifugation at 12,000 g for 5 min, supernatants were cleared of the TCA by 0.2 ml ethyl ether addition/aspiration five times, followed by lyophilization and storage at -70°C. The ATP/ADP ratio was determined as previously described (31).

Data presentation and statistical methods. All data are expressed as mean $\pm$ SEM. Unless stated otherwise, *n* values represent the number of experiments performed using islets from separate isolation and culture days. Statistical significance was determined by the unpaired Student's *t* test or one-way ANOVA.

Results

Insulin secretion. Protein levels were equal in the oleate and control islets (0.70 $\pm$ 0.02 μ g/islet in controls vs. 0.75 $\pm$ 0.05 μ g/islet oleate-treated, *n* = 4). Insulin secretion results are shown in Table I. Insulin secretion from the oleate islets was doubled at 2.8 mM glucose (*P* < 0.015), reflecting the basal insulin hypersecretion that characterizes chronic exposure of islets to FA (10, 11, 15). There also was an increase at 16.7 mM glucose,

Table I. Islet Insulin Secretion

	Insulin secretion	
	2.8 mM glucose	16.7 mM glucose
	ng/10 islets/60 min	
Control islets	4.6 $\pm$ 0.6	12.1 $\pm$ 0.9
0.25 mM oleate	11.9 $\pm$ 2.0	16.4 $\pm$ 0.9

The data are expressed as mean $\pm$ SEM. Nonpooled islets from eight rats were separately cultured with 0.25 mM oleate in 2% ethanol (*n* = 4) or 2% ethanol alone (*n* = 4) for 24 h as described in Methods. Islets were preincubated for 30 min in KRB/2.8 mM glucose, followed by a 60-min incubation at 2.8 or 16.7 mM glucose. Statistical significance was determined by the Student's *t* test (*P* < 0.015 for 2.8 and 16.7 mM glucose).

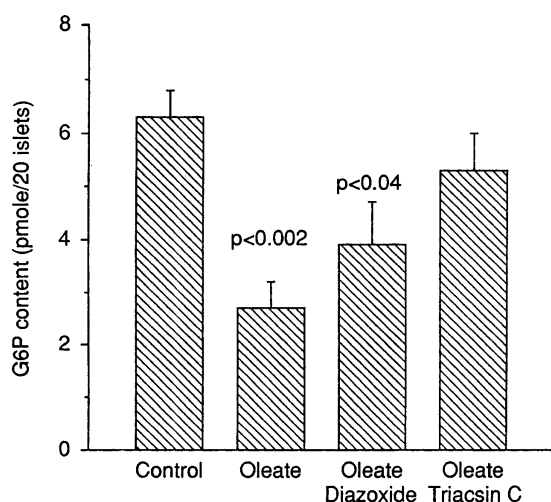


Figure 1. G6P content in 24-h cultured islets ($n = 4$). Rat islets were cultured for 24 h with 5.5 mM glucose and 0.25 mM oleate in 2% ethanol or ethanol alone as stated in Methods. Additional islets were cultured with oleate plus diazoxide (75 $\mu\text{g/ml}$) or Triacsin C (0.5 $\mu\text{g/ml}$). G6P was measured after a 30 min-incubation in KRB/5.5 mM glucose with or without 0.25 mM oleate. The listed significance values refer to comparisons with the control islets.

although the fold change was smaller than at 2.8 mM glucose (135% of control).

Islet G6P content. The G6P content was lowered 60% in the islets cultured with oleate (Fig. 1). Adding diazoxide (75 $\mu\text{g/ml}$), an inhibitor of insulin secretion, to the oleate culture failed to prevent the subnormal G6P level ($P < 0.04$ versus controls). In contrast, 0.5 $\mu\text{g/ml}$ of Triacsin C, an inhibitor of long chain fatty acyl-CoA synthetase, normalized the G6P content ($P = \text{NS}$ versus control islets and $P < 0.02$ versus islets that received oleate alone).

The previous protocol was performed in islets that were cultured and KRB-incubated at 5.5 mM glucose. We expanded the results by performing the usual 24-h culture protocol at 5.5 mM glucose followed by a 60-min KRB incubation at 2.8, 8.3, or 27.7 mM glucose (Fig. 2). The G6P level was reduced 30–50% at all glucose concentrations in the oleate islets although only the lowest glucose concentration reached statistical significance ($P < 0.01$, < 0.07 , and > 0.1 at 2.8, 8.3 and 27.7 mM glucose, respectively).

Islet glucose use and glucose-6-phosphatase (G6Pase) activity. Glucose use was assessed at multiple glucose concentrations to estimate the V_{max} and ED_{50} values for the high affinity and low affinity components (23). Two tracers were used, [$2\text{-}^3\text{H}$] and [$5\text{-}^3\text{H}$]glucose, because the labilization to $^3\text{H}_2\text{O}$ occurs at different steps in glycolysis, the two tracers at phosphoglucose isomerase and the five tracer at triose phosphate isomerase (32). Thus, the release of [$2\text{-}^3\text{H}$] is a measure of the glucose phosphorylation rate versus [$5\text{-}^3\text{H}$] which is a measure of the PFK rate. Comparison of the results provided an indirect measure of G6Pase activity such that an increase would be manifest as a higher usage rate with the two tracers over the five tracer, and lower maximal values with both.

Glucose use was increased in the oleate islets as determined by both tracers: there was no difference in the measurements of the tracers at any glucose concentration (Fig. 3). The

kinetics of the low and high affinity components are shown in Table II. Oleate doubled the high affinity V_{max} ($P < 0.03$ for [$2\text{-}^3\text{H}$]glucose and $P < 0.01$ for [$5\text{-}^3\text{H}$]glucose) without changing the ED_{50} . The low affinity kinetics were largely unchanged, although there appeared to be a tendency for a reduced V_{max} with oleate. These results indicate activation or de/inhibition of hexokinase. Furthermore, increased G6Pase activity was eliminated as the cause of the lowered G6P content of the oleate-treated islets.

Pentose phosphate shunt. Table III shows the absolute and percentage rates of glucose use through the pentose phosphate shunt at 2.8 and 16.7 mM glucose. Its activity is normally minimal in β cells (1, 22) which was confirmed here in the control islets, with the proportion of glucose use being 1.2–1.4%. Oleate had no significant effect on either value.

PFK activity in islet extracts. PFK V_{max} was increased 50% in the oleate islets (15.5 ± 0.6 pmol fructose-1, 6-bisphosphate islet $^{-1}$ min $^{-1}$ in oleate-treated vs. 10.1 ± 0.7 islet $^{-1}$ min $^{-1}$ in controls, $n = 7$, $P < 0.001$). A second set of experiments showed that adding Triacsin C to the 24-h culture fully blocked the oleate-induced increase in PFK activity (Fig. 4).

Allosteric regulators of PFK activity. PFK is regulated by several allosteric factors, including the ATP/ADP ratio and Fru-2,6- P_2 (9). Neither of these was altered in the oleate islets (ATP/ADP 3.6 ± 0.3 for controls vs. 4.3 ± 0.3 oleate, $n = 5$; Fru-2,6- P_2 23.1 ± 3.1 fmol/islet for controls vs. 24.9 ± 2.7 fmol/islet oleate, $n = 4$).

Discussion

This study has provided important insight into the left-shifted glucose concentration/insulin secretion curve that characterizes long-term exposure of β cells to FA. The critical finding

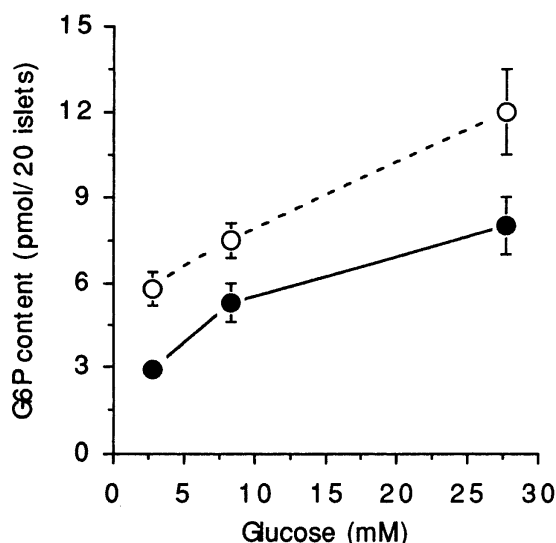


Figure 2. G6P content in 24-h cultured islets ($n = 3$). Rat islets were cultured for 24 h with 5.5 mM glucose and 0.25 mM oleate in 2% ethanol (closed circles) or ethanol alone (open circles), followed by a 60-min incubation in KRB containing 2.8, 8.3, or 27.7 mM glucose with or without 0.25 mM oleate. The G6P level was measured as stated in Methods. It was reduced 30–50% in the oleate islets although only the lowest glucose concentration reached statistical significance ($P < 0.01$ at 2.8, $P < 0.07$ at 8.3, and $P > 0.1$ at 27.7 mM glucose).

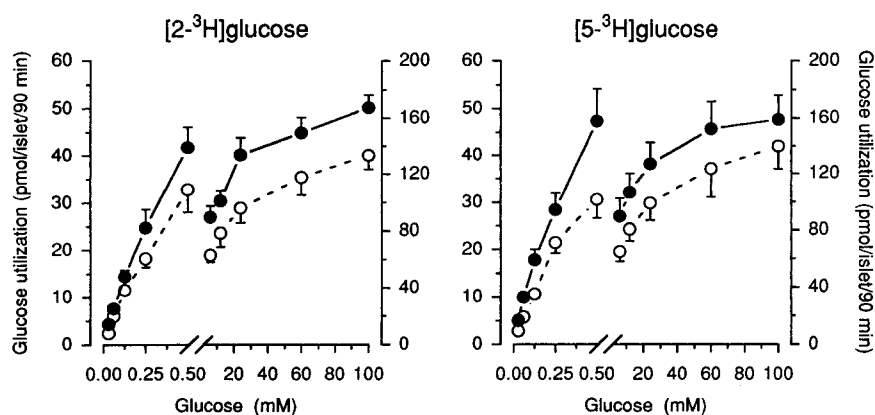


Figure 3. Islet glucose use measured by conversion of $[2\text{-}^3\text{H}]\text{glucose}$ and $[5\text{-}^3\text{H}]\text{glucose}$ to $[^3\text{H}]\text{H}_2\text{O}$ ($n = 7$). Rat islets were cultured for 24 h with 5.5 mM glucose and 0.25 mM oleate in 2% ethanol (closed circles) or ethanol alone (open circles) followed by a 90-min incubation at the glucose concentrations shown.

was a lowered cellular level of G6P especially at low normal glucose concentrations. This finding is even more impressive in that the reduced G6P level was found in tandem with the rate of basal glucose use, and thus production of G6P, being doubled. We speculate the inhibition of hexokinase by G6P was attenuated (5), causing the increased basal glucose usage/insulin secretion. This finding clarifies the dichotomy in insulin secretion and glucose metabolism at 2.8 mM glucose in our previous study (doubled) versus the activity of hexokinase (30% increased $V_{\max}$), since activity was measured in islet extracts which eliminated the regulatory influences of endogenous G6P (15). Thus, our results suggest a complex effect of FA on hexokinase, an attenuation of the G6P inhibitory effect, plus an increased $V_{\max}$. The latter is presumed to reflect upregulated hexokinase gene expression as shown for other β cell genes (33). A technical issue concerns our use of islets and the possibility that non- β cells contributed to the metabolic changes. Liang et al. reported similar functional effects as those found in this study in 4-d FA-cultured clonal β cells (βHC9) in terms of increased basal insulin secretion and glucose usage plus a small increase in hexokinase $V_{\max}$ and unchanged glucokinase activity (34), indicating the relevance of our islet findings to β cells. Also, this study focused on basal insulin secretion. A longer exposure to FA (48 h) than in this study inhibits maximal glucose-induced insulin secretion (10, 11) so that FA have both stimulatory and inhibitory effects on β cell function.

We investigated the basis for the subnormal G6P content. G6P levels are a function of several metabolic processes. Most obvious is production and metabolism through glycolysis.

Others with varying significance depending on the tissue studied are glycogen production and metabolism, G6Pase, and the pentose phosphate shunt. Glycogen is present in small amounts in β cells (35) except with diabetes where substantial accumulations occur (36). We attempted to quantify islet glycogen for this study: the oleate and control islets were both below the level of detectability which we interpret as excluding glycogen buildup in the oleate islets. The pentose phosphate shunt is a minor metabolic pathway in β cells (22) which was confirmed here. We did note a nonsignificant threefold increase in the activity at 16.7 mM glucose with oleate which may have become statistically significant with additional experiments. Regardless, the absolute value was so small that the functional significance in terms of the lowered G6P is minor. A potentially important issue was G6Pase since FA infusion was shown recently to upregulate G6Pase gene expression in the liver of rats (37). Also, islets of diabetic rodents are known to have increased activity (38, 39) and gene expression (40) of G6Pase. Nevertheless, G6Pase activity was not changed in the oleate islets as determined by $[2\text{-}^3\text{H}]$ and $[5\text{-}^3\text{H}]\text{glucose}$ assessment of glucose usage (32). This conclusion is based on indirect evidence, and there could be concern as to the sensitivity for identifying changes in G6Pase. However, the sizable reduction in G6P level in the oleate islets would require a large increase in G6Pase activity that should easily have been observed. Finally, the possibility of lowered G6P production was excluded by direct measure of glucose use. It should be noted that there was not perfect agreement between the glucose usage findings in Fig. 3 and the insulin secretion data in Table I in that secretion at 2.8 mM glucose was doubled in the oleate

Table II. Kinetics of Low and High Affinity Components of Islet Glucose Use

Tracer	Islets	$V_{\max}$	ED ₅₀ for glucose	$V_{\max}$	ED ₅₀ for glucose
		pmol/islet/90 min	mM	pmol/islet/90 min	mM
$[2\text{-}^3\text{H}]\text{glucose}$	control	37±6	0.3±0.1	94±24	15.6±1.9
	0.25 mM oleate	76±14*	0.4±0.1	77±24	16.0±3.4
$[5\text{-}^3\text{H}]\text{glucose}$	control	37±5	0.3±0.0	112±17	22.2±4.0
	0.25 mM oleate	86±14‡	0.5±0.1	69±15	21.2±3.7

Data are mean±SEM from seven experiments. The islet glucose use data from Fig. 1 were analyzed by the method of Spears (24) to estimate the kinetics of the high affinity and low affinity components of islet glucose use. Statistical significance was determined by one-way ANOVA. $P < 0.026$ and $P < 0.007$ versus tracer-matched control islets.

Table III. Pentose Phosphate Shunt

	Control islets		Oleate islets	
	2.8 mM glucose	16.7 mM glucose	2.8 mM glucose	16.7 mM glucose
[1- ¹⁴ C]glucose oxidation (<i>pmol/islet/90 min</i>)	9.3±1.7	17.3±3.9	22.6±3.8	41.6±11.5
[6- ¹⁴ C]glucose oxidation (<i>pmol/islet/90 min</i>)	6.7±1.7	11.8±3.7	16.3±3.8	25.4±4.2
[5- ³ H]glucose utilization (<i>pmol/islet/90 min</i>)	65.2±7.3	161±23.1	95.2±3.5	191±25.3
Glucose oxidation via pentose phosphate shunt (<i>pmol/islet/90 min</i>)	0.9±0.4	1.9±0.5	2.2±1.0	7.6±2.1
Pentose phosphate shunt (%)	1.4±0.7	1.2±0.3	2.3±1.0	4.2±1.0

Data are mean±SEM from five experiments and are calculated as stated in Methods.

islets as opposed to the increase in glucose use being somewhat less. The significance of this dichotomy is currently uncertain.

Thus, the decrease in G6P was due to enhanced removal. Insight was provided by the increase in assayed PFK activity, plus its reversal with Triacsin C which paralleled its effect to normalize the G6P level (current study) and attenuate the basal insulin hypersecretion (15). We conclude an upregulatory effect of LC-CoA on PFK activity is the basis for the lowered G6P level.

Mammalian PFK is a family of three isoforms from different genes with varying tissue distribution and allosteric regulation (41). Islets and clonal β cells contain all three although the muscle isoform (called both M and A) appears to be dominant (26, 42). Our working hypothesis is the increased PFK $V_{\max}$ is due to an upregulated gene expression/cellular level of one or multiple PFK isoforms. This idea comes from the well known FA stimulatory effect on gene expression (43), plus published results with chronic exposure of β cells to high glucose (44). The relevance of these results is based on glucose's effect to raise cytosolic LC-CoA levels in β cells through its conversion

to malonyl-CoA which inhibits carnitine palmitoyltransferase I activity, and, thus, LC-CoA metabolism (45, 46). Moreover, chronic exposure to high glucose potentiates the effect by increasing acetyl-CoA carboxylase expression, the enzyme that catalyses malonyl-CoA production (47). Thus, chronic high glucose and FA both raise cytosolic LC-CoA which would be expected to have the same effect on PFK. It is thus notable that PFK gene expression/cell content is increased in high glucose cultured INS-1 cells (44).

An alternate hypothesis for the β cell glucose hypersensitivity with FA has been proposed based on a direct effect of FA to promote insulin secretion, thereby depleting the cellular stores of ATP and activating PFK on that basis (13). Our data are contrary to that idea since the ATP/ADP ratio was not altered in the oleate islets, nor was there a beneficial effect of diazoxide, an inhibitor of insulin secretion, on the cellular G6P content.

In summary, oleate lowered the cellular level of G6P secondary to an effect of cytosolic LC-CoA to increase the activity of PFK. We speculate that β cell glucose metabolism was changed from a primarily glucokinase-driven process to one that incorporated hexokinase such that basal glucose usage and insulin secretion were augmented. These results emphasize the important roles G6P and PFK play in regulating β cell glucose metabolism, and, thus, glucose sensing for insulin secretion. Diabetic states are characterized by a basal hypersecretion of insulin and raised serum/islet levels of FA (48). We speculate that comparable biochemical changes to those identified in this study underlie the β cell dysfunction. Also, our results may have relevance for the impaired pulsatile insulin release which is found in diabetic states (49). Previously, there has been no mechanistic basis on which to interrelate the β cell glucose hypersensitivity and impaired insulin pulsatility in diabetes. However, pulsatile secretion is believed to result from the oscillatory pattern of glycolysis in β cells, and PFK is proposed to be the key regulatory element (50, 51). It is tempting to speculate that altered PFK activity underlies both forms of β cell dysfunction.

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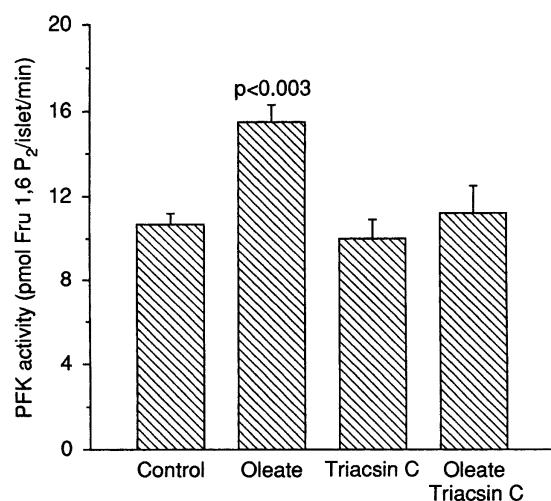


Figure 4. Phosphofructokinase activity in 24-h cultured islets ($n = 4$). Rat islets were cultured for 24 h with 5.5 mM glucose and 0.25 mM oleate in 2% ethanol or ethanol alone with or without 0.5 μ g/ml Triacsin C. PFK activity was measured as stated in Methods. The listed significance values refer to comparisons with the control islets.

References

- Meglasson, M.D., and F.M. Matschinsky. 1986. Pancreatic islet glucose metabolism and regulation of insulin secretion. *Diabetes Metab. Rev.* 2:163–214.
- Matschinsky, F.M. 1990. Glucokinase as glucose sensor and metabolic generator in pancreatic β cells and hepatocytes. *Diabetes*. 39:647–652.
- Newgard, C.B., and J.D. McGarry. 1995. Metabolic coupling factors in pancreatic β cell signal transduction. *Annu. Rev. Biochem.* 64:689–719.
- Heimberg, H., A. De Vos, A. Vandercammen, E. Van Schaftingen, D. Pipeleers, and F. Schuit. 1993. Heterogeneity in glucose sensitivity among pancreatic β cells is correlated to differences in glucose phosphorylation rather than glucose transport. *EMBO (Eur. Mol. Biol. Organ.) J.* 12:2873–2879.
- Giroix, M.-H., A. Sener, D.G. Pipeleers, and W.J. Malaisse. 1984. Hexose metabolism in pancreatic islets. Inhibition of hexokinase. *Biochem. J.* 223:447–453.
- Kemp, R.G., and L.G. Foe. 1983. Allosteric regulatory properties of muscle phosphofructokinase. *Mol. Cell. Biochem.* 57:147–154.
- Malaisse, W.J., and F. Malaisse-Lagae. 1968. Stimulation of insulin secretion by noncarbohydrate metabolites. *J. Lab. Clin. Med.* 72:438–448.
- Campillo, J.E., A.S. Luyckx, M.D. Torres, and P.J. Lefebvre. 1979. Effect of oleic acid on insulin secretion by the isolated perfused rat pancreas. *Diabetologia*. 16:267–273.
- Prentki, M., S. Vischer, M.C. Glennon, R. Regazzi, J.T. Deeney, and B.E. Corkey. 1992. Malonyl-CoA and long chain acyl-CoA esters as metabolic coupling factors in nutrient-induced insulin secretion. *J. Biol. Chem.* 267:5802–5810.
- Zhou, Y.-P., and V.E. Grill. 1994. Long-term exposure of rat pancreatic islets to fatty acids inhibits glucose-induced insulin secretion and biosynthesis through a glucose fatty acid cycle. *J. Clin. Invest.* 93:870–876.
- Milburn, J.L., Jr., H. Hirose, Y.H. Lee, Y. Nagasawa, A. Ogawa, M. Ohneda, H. Beltran del Rio, C.B. Newgard, J.H. Johnson, and R.H. Unger. 1995. Pancreatic β cells in obesity. Evidence for induction of functional, morphologic, and metabolic abnormalities by increased long chain fatty acids. *J. Biol. Chem.* 270:1295–1299.
- Unger, R.H. 1995. Lipotoxicity in the pathogenesis of obesity-dependent NIDDM. Genetic and clinical implications. *Diabetes*. 44:863–870.
- Prentki, M., and B.E. Corkey. 1996. Are the β cell signaling molecules malonyl-CoA and cytosolic long-chain acyl-CoA implicated in multiple tissue defects of obesity and NIDDM? *Diabetes*. 45:273–283.
- Leahy, J.L. 1996. Impaired β cell function with chronic hyperglycemia: “overworked β cell” hypothesis. *Diabetes Rev.* 4:298–319.
- Hosokawa, H., B.E. Corkey, and J.L. Leahy. 1997. 24-hour islet exposure to fatty acids is associated with a β cell hypersensitivity to glucose. *Diabetologia*. 40:392–397.
- Tomoda, H., K. Igarashi, J.-C. Cyong, and S. Omura. 1987. Inhibition of acyl-CoA synthetase by triacins. *Biochim. Biophys. Acta*. 921:595–598.
- Tomoda, H., K. Igarashi, J.-C. Cyong, and S. Omura. 1990. Evidence for an essential role of long chain acyl-CoA synthetase in animal cell proliferation. Inhibition of long chain acyl-CoA synthetase by triacins caused inhibition of Raji cell proliferation. *J. Biol. Chem.* 265:4214–4219.
- Korchak, H.M., L.H. Kane, M.W. Rossi, and B.E. Corkey. 1994. Long chain acyl CoA and signaling in neutrophils: an inhibitor of acyl CoA synthetase, Triacsin C, inhibits superoxide anion generation and degranulation by human neutrophils. *J. Biol. Chem.* 269:30281–30287.
- Gotoh, M., T. Maki, S. Satomi, J. Porter, S. Bonner-Weir, C.J. O'Hara, and A.P. Monaco. 1987. Reproducible high yield of rat islets by stationary in vitro digestion following pancreatic ductal or portal venous collagenase injection. *Transplantation*. 43:725–730.
- Albano, J.D.M., R.P. Ekins, G. Maritz, and R.C. Turner. 1972. A sensitive, precise radioimmunoassay of serum insulin relying on charcoal separation of bound and free hormone moieties. *Acta Endocrinol.* 70:487–509.
- Lowry, O.H., and J.V. Passonneau. 1972. A Flexible System of Enzymatic Analysis. Academic Press, New York. 291 pp.
- Ascroft, S.J.H., L.C.C. Weerasinghe, J.M. Bassett, and P.J. Randle. 1972. The pentose cycle and insulin release in mouse pancreatic islets. *Biochem. J.* 126:525–532.
- Trus, M.D., W.S. Zawulich, P.T. Burch, D.K. Berner, V.A. Weill, and F.M. Matschinsky. 1981. Regulation of glucose metabolism in pancreatic islets. *Diabetes*. 30:911–922.
- Spears, G., G.T. Sneyd, and E.G. Loten. 1971. A method for deriving kinetic constants for two enzymes acting on the same substrate. *Biochem. J.* 125:1149–1151.
- Verspohl, E.J., I. Breuning, and H.P.T. Ammon. 1989. Effect of CCK-8 on pentose phosphate shunt activity, pyridine nucleotides, and glucokinase of rat islets. *Am. J. Physiol.* 256:E68–E73.
- Yaney, G.C., V. Schultz, B.A. Cunningham, G.A. Dunaway, B.E. Corkey, and K. Tornheim. 1995. Phosphofructokinase isozymes in pancreatic islets and clonal β cells (INS-1). *Diabetes*. 44:1285–1289.
- Narabayashi, H., J.W.R. Lawson, and K. Uyeda. 1985. Regulation of phosphofructokinase in perfused rat heart: requirement for fructose 2,6-bisphosphate and a covalent modification. *J. Biol. Chem.* 260:9750–9758.
- Thomas, H., and K. Uyeda. 1986. A competitive binding assay for fructose 2,6-bisphosphate. *Anal. Biochem.* 154:50–56.
- Liu, Y.Q., and K. Uyeda. 1996. A mechanism for fatty acid inhibition of glucose utilization in liver: role of xylulose 5-P. *J. Biol. Chem.* 271:8824–8830.
- Burch, P.T., D.K. Berner, M.D. Meglasson, and F.M. Matschinsky. 1985. Regulatory role of fructose-2,6-bisphosphate in pancreatic islet glucose metabolism remains unsettled. *Diabetes*. 34:1014–1018.
- Schultz, V., I. Sussman, E. Bokvist, and K. Tornheim. 1993. Bioluminometric assay of ADP and ATP at high ATP/ADP ratios: assay of ADP after enzymatic removal of ATP. *Anal. Biochem.* 215:302–304.
- Wolfe, R.R. 1990. Isotopic measurement of glucose and lactate kinetics. *Ann. Med.* 22:163–170.
- Assimakopoulos-Jeannet, F., S. Thumelin, E. Roche, V. Esser, J.D. McGarry, and M. Prentki. 1997. Fatty acids rapidly induce the carnitine palmitoyl-transferase I gene in the pancreatic β cell line INS-1. *J. Biol. Chem.* 272:1659–1664.
- Liang, Y., C. Buettger, D.K. Berner, and F.M. Matschinsky. 1997. Chronic effect of fatty acids on insulin release is not through the alteration of glucose metabolism in a pancreatic beta-cell line (β HC9). *Diabetologia*. 40:1018–1027.
- Malaisse, W.J., A. Sener, M. Koser, M. Ravazzola, and F. Malaisse-Lagae. 1977. The stimulus-secretion coupling of glucose-induced insulin release: insulin release due to glycogenolysis in glucose-deprived islets. *Biochem. J.* 164:447–454.
- Marynissen, G., V. Leclercq-Meyer, A. Sener, and W.J. Malaisse. 1990. Perturbation of pancreatic islet function in glucose-infused rats. *Metabolism*. 39:87–95.
- Massillon, D., N. Barzilai, M. Hawkins, D. Prus-Wertheimer, and L. Rossetti. 1997. Induction of hepatic glucose-6-phosphatase gene expression by lipid infusion. *Diabetes*. 46:153–157.
- Khan, A., V. Chandramouli, C.-G. Östenson, P.-O. Berggren, H. Löw, B.R. Landau, and S. Efendic. 1990. Glucose cycling is markedly enhanced in pancreatic islets of obese hyperglycemic mice. *Endocrinology*. 126:2413–2416.
- Khan, A., C. Hong-Lie, and B.R. Landau. 1995. Glucose-6-phosphatase activity in islets from ob/ob and lean mice and the effect of dexamethasone. *Endocrinology*. 136:1934–1938.
- Tokuyama, T., J. Sturis, A.M. DePaoli, J. Takeda, M. Stoffel, J. Tang, X. Sun, K.S. Polonsky, and G.I. Bell. 1995. Evolution of β -cell dysfunction in the male Zucker diabetic fatty rat. *Diabetes*. 44:1447–1457.
- Dunaway, G.A., and T.P. Kasten. 1987. Nature of the subunits of the 6-phosphofructo-1-kinase isozymes from rat tissues. *Biochem. J.* 242:667–671.
- Ma, Z., S. Ramanadham, K. Kempe, Z. Hu, J. Ladenson, and J. Turk. 1996. Characterization of expression of phosphofructokinase isoforms in isolated pancreatic islets and purified beta cells and cloning and expression of the rat phosphofructokinase-A isoform. *Biochim. Biophys. Acta*. 1308:151–163.
- Clark, S.D., and D.B. Jump. 1994. Dietary polyunsaturated fatty acid regulation of gene transcription. *Annu. Rev. Nutr.* 14:83–98.
- Roche, E., F. Assimakopoulos-Jennet, L.A. Witters, B. Perruchoud, G. Yaney, B. Corkey, M. Asfari, and M. Prentki. 1997. Induction by glucose of genes coding for glycolytic enzymes in a pancreatic β cell line (INS-1). *J. Biol. Chem.* 272:3091–3098.
- Chen, S., A. Ogawa, M. Ohneda, R.H. Unger, D.W. Foster, and J.D. McGarry. 1994. More direct evidence for a malonyl-CoA-carnitine palmitoyl-transferase I interaction as a key event in pancreatic β cell signaling. *Diabetes*. 43:878–883.
- Brun, T., E. Roche, F. Assimakopoulos-Jeannet, B.E. Corkey, K.-H. Kim, and M. Prentki. 1996. Evidence for an anaplerotic/malonyl-CoA pathway in pancreatic β cell nutrient signaling. *Diabetes*. 45:190–198.
- Brun, T., E. Roche, K.-H. Kim, and M. Prentki. 1993. Glucose regulates acetyl-CoA carboxylase gene expression in a pancreatic β cell line (INS-1). *J. Biol. Chem.* 268:18905–18911.
- Lee, Y., H. Hirose, M. Ohneda, J.H. Johnson, J.D. McGarry, and R.H. Unger. 1994. β cell lipotoxicity in the pathogenesis of non-insulin-dependent diabetes mellitus of obese rats: impairment in adipocyte- β -cell relationships. *Proc. Natl. Acad. Sci. USA*. 91:10878–10882.
- Sturis, J., W.L. Pugh, J. Tang, D.M. Ostrega, J.S. Polonsky, and K.S. Polonsky. 1994. Alterations in pulsatile insulin secretion in the Zucker diabetic fatty rat. *Am. J. Physiol.* 267:E250–E259.
- Cunningham, B.A., J.T. Deeney, C.R. Bliss, B.E. Corkey, and K. Tornheim. 1996. Glucose-induced oscillatory insulin secretion in perfused rat pancreatic islets and clonal β cells (HIT). *Am. J. Physiol.* 34:E702–E710.
- Tornheim, K. 1997. Are metabolic oscillations responsible for normal oscillatory insulin secretion? *Diabetes*. 46:1375–1380.