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Beta-cell hypersensitivity to glucose following 24-h exposure of rat islets to fatty acids

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Summary Prolonged exposure of islets to fatty acids results in a lowered glucose set-point for insulin secretion. We examined the mechanism in islets cultured for 24 h with 0.25 mmol/l palmitate. As expected, insulin secretion at 2.8 and 8.3 mmol/l glucose was increased in the palmitate-treated islets as opposed to no change at 27.7 mmol/l glucose. Co-culturing with 0.05 μg/ml Triacsin C, an inhibitor of long chain acyl-CoA synthetase, blocked this effect. Glucose utilization and oxidation showed the same pattern as insulin secretion, with the step-up for both measurements being fully manifest at 2.8 mmol/l glucose. Glucokinase K_m and V_{max} measured in islet extracts were unaffected by the palmitate. In contrast, hexokinase V_{max} was increased by 25–35 % in both the cytoplasmic and mitochondrial-bound pools. Our data suggest prolonged exposure to fatty acids increased beta-cell hexokinase activity, thereby modifying the kinetics of glucose entry into the metabolic pathway and glucose-induced insulin secretion. The cellular mediator is likely an increased level of long chain fatty acyl-CoA esters. [Diabetologia (1997) 40: 392–397]

Keywords Palmitate, long chain acyl-CoA esters, Triacsin C, glucose phosphorylation, glucose utilization, islets of Langerhans, glucokinase, hexokinase.

Long chain fatty acids acutely stimulate insulin secretion [1–3], with the magnitude depending on the chain length and degree of unsaturation [4]. Changes in the cellular level of malonyl-CoA and long-chain acyl-CoA esters have been proposed as key mechanistic factors [5]. Islet culture studies have shown additional changes in beta-cell function associated with long-term exposure to fatty acids: a lowered glucose set-point for insulin secretion, and inhibited maximal glucose-induced insulin secretion [6, 7]. These findings are of interest because plasma levels of non-esterified fatty acids are raised in obesity and non-insulin-dependent diabetes mellitus in association with similar patterns of beta-cell dysfunction [5, 8].

More than one mechanism is presumed to underlie the long-term effects, since a lowered glucose set-point was noted after 6 h exposure to 0.125 mmol/l palmitate vs the impaired glucose-induced insulin secretion which took 48 h [6]. Reduced activity of pyruvate dehydrogenase has been reported to cause the suppressed glucose-induced insulin secretion [9]. In contrast, the mechanism of the glucose hypersensitivity is unknown. An upregulated activity of hexokinase has been hypothesized [7], in part because of the suggestion by us and others [7, 10, 11] this mechanism underlies the heightened beta-cell sensitivity to glucose that is found in diabetic animal models [12–15].

The current study investigated the mechanism of the beta-cell hypersensitivity to glucose in islets exposed to fatty acids. The experimental protocol entailed culturing rat islets for 24 h with the fatty acid

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Abbreviations: BSA, Bovine serum albumin; HEPES, 4-(2-hydroxyethyl)-1-piperazineethane sulphonic acid; DMSO, dimethylsulphoxide; KRB, Kreb's Ringer buffer.

palmitate, which is insufficient time for the suppressed glucose-induced insulin secretion [6]. Thus, the only functional change in our system was a left-shifted glucose concentration – insulin secretion curve.

Materials and methods

Islet isolation and culture. Islets were isolated from 200 g male Sprague-Dawley rats (Taconic, Germantown, N.Y., USA) using an adaptation of the method of Gotoh et al. [16]: pancreatic duct infiltration with collagenase (Serva, Heidelberg, Germany), Histopaque gradient separation (Sigma, St. Louis, Mo., USA), and hand picking. They were cultured overnight in RPMI 1640 medium containing 5.5 mmol/l glucose, 2 mmol/l glutamine, 10% newborn calf serum, 100 units/ml penicillin, and 0.1 mg/ml streptomycin (all from Gibco, Grand Island, N.Y., USA), followed by an additional 24 h of culture with 0.25 mmol/l palmitate (sodium salt) in 2% ethanol or 2% ethanol alone. The insulin secretion study incorporated Triacsin C in the culture protocol which is an inhibitor of long chain acyl-CoA synthetase (Biomol, Plymouth Meeting, Pa., USA) [17–19]. Thus, four groups of additives were used during the 24-h culture: 0.25 mmol/l palmitate in 2% ethanol plus 0.05 μg/ml Triacsin C in 0.5% dimethyl sulphoxide (DMSO); 0.25% palmitate in 2% ethanol plus 0.5% DMSO; 2% ethanol plus 0.05 μg/ml Triacsin C in 0.5% DMSO; 2% ethanol plus 0.5% DMSO. Post-culture, all experiments were preceded by a 30-min preincubation at 2.8 mmol/l glucose with no additives.

Insulin secretion. Triplicate batches of 10 islets in glass vials containing 1 ml Kreb's Ringer Buffer (KRB), 10 mmol/l 4-(2-hydroxyethyl)-1-piperazineethanesulphonic acid (HEPES), 0.5% bovine serum albumin (BSA), glucose (2.8, 8.3, 27.7 mmol/l), were incubated for 60 min in a 37 °C shaking water bath. The medium was separated from the islets by gentle centrifugation (500 rev/min 5 min at 10 °C) and stored at –20 °C pending insulin measurement by radioimmunoassay (RIA) [20].

Glucose utilization. Islet glucose utilization was measured by the method of Ashcroft et al. [21]. Triplicate groups of 20 islets were incubated for 90 min at 37 °C in 100 μl KRB, glucose (2.8, 8.3, 27.7 mmol/l), 1.7 μCi D-[5-^{3}H] glucose (Amersham, Arlington Heights, Ill., USA). The reaction was carried out in a 1-ml cup in a rubber-stoppered 20 ml scintillation vial with 500 μl of distilled water surrounding the cup. Islet glucose metabolism was stopped with 100 μl 1 mol/l HCl injected through the stopper into the cup. Following overnight incubation at 37 °C to allow equilibration of the [^{3}H]-H_2O in the reaction cup and the distilled water, the ^3H_2O in the distilled water was quantified in a liquid scintillation counter. Tubes containing [^{3}H]-H_2O and no islets were used to estimate the recovery of ^3H_2O; it averaged approximately 50%.

Glucose oxidation. Triplicate groups of 20 islets were incubated 90 min at 37 °C in 100 μl KRB, glucose (2.8, 8.3, 27.7 mmol/l), 1.7 μCi [U-^{14}C] glucose (NEN, Cambridge, Mass., USA). The reaction was carried out in a 1-ml cup in a 20 ml scintillation vial capped by a rubber stopper with a centre well that contained filter paper (Kontes, Vineland, N.J., USA). Islet metabolism was stopped and the CO_2 liberated with 300 μl 1 mol/l HCl injected through the stopper into the cup containing the islets. CO_2 was trapped in the filter paper by injecting 100 μl 1 mol/l KOH into the centre well, followed 2 h later by liquid scintillation counting. Tubes containing [^{14}C] $NaHCO_3$ and no islets were used to estimate the recovery of $^{14}CO_2$ in the filter paper which was routinely close to 100%.

Glucokinase and hexokinase activity. Glucose phosphorylation was measured in islet extracts using the method of Liang et al. [22] that measures NAD^+ conversion to NADH by exogenous glucose 6-phosphate dehydrogenase [22]. All chemicals were from Sigma unless otherwise stated. Three hundred islets per individual rat were homogenized at 5 °C in 1 μl/islet of buffer (1 mmol/l EDTA, 20 mmol/l K_2HPO_4, 110 mmol/l KCl, 5 mmol/l dithiothreitol) by 25 strokes of a machine-driven Teflon pestle in a Kontes glass homogenizer (0.004–0.006 inch). Aliquots (20 μl × 3) were used to measure DNA content [23]. Following a 10-min centrifugation at 12000 *g* to remove the mitochondrial-bound hexokinase [24], 5-μl aliquots were added to 100 μl of reaction buffer consisting of 50 mmol/l HEPES/HCl pH 7.6, 5 mmol/l ATP, 100 mmol/l KCl, 7.4 mmol/l $MgCl_2$, 15 mmol/l β-mercaptoethanol, 0.5 mmol/l NAD^+, 0.05% BSA, glucose (0.03, 0.06, 0.125, 0.25, 0.5, 6, 12, 24, 60, 100 mmol/l), and 0.7 unit/ml glucose 6-phosphate dehydrogenase from *Leuconostoc mesenteroides* (Boerhinger Mannheim, Indianapolis, Ind., USA). After 90 min at 30 °C, the reaction was stopped with 1 ml 500 mmol/l $NaHCO_3$ pH 9.4. Triplicate samples were performed at each glucose concentration in parallel with reagent blanks (no homogenate) and a tissue blank (islet homogenate in reaction buffer that contained 0 mmol/l glucose). The standard curve was glucose 6-phosphate (0.3–3 nmol) in reaction buffer that contained 100 mmol/l glucose. The mean of replicate results for each glucose concentration was obtained to give a single data point. Glucokinase and hexokinase V_{max} and K_m were calculated by linear regression from an Eadie-Scatchard plot after extrapolating the data to 37 °C assuming a Q_{10} of 2 [25], followed by 10 cycles of the method of Spears et al. [26] to separate hexokinase and glucokinase activity.

The method was adapted to measure cytoplasmic and mitochondrial-bound hexokinase activity. Islet homogenization was carried out as described with a portion undergoing the 12000 *g* centrifugation. Glucose phosphorylation was measured as described using glucose concentrations of 0.03, 0.06, 0.125, 0.25, 0.5 mmol/l in equivalent amounts of the uncentrifuged extract (whole) and the post-centrifugation supernatant (cytoplasm) and pellet (mitochondria/microsomes). Hexokinase V_{max} and K_m were calculated by linear regression.

Statistical analysis

All data are expressed as mean ± SEM with the "*n*" values being the number of experiments performed. Statistical significance was determined by unpaired Student's *t*-test or one-way analysis of variance (ANOVA).

Results

Insulin secretion. As expected, exposing islets for 24 h to 0.25 mmol/l palmitate caused a "left-shifted" glucose concentration-insulin secretion curve: insulin release was raised at 2.8 and 8.3 mmol/l glucose ($p < 0.006$ for both) as opposed to no change at

Table 1. Effect of Triacsin C on insulin secretion in 24 h cultured islets

	Insulin secretion (pmol · 10 islets^{-1} · 60 min^{-1}) Glucose (mmol/l)		
Culture conditions	2.8	8.3	27.7
Control	0.15 ± 0.02	0.32 ± 0.02	1.58 ± 0.14
Triacsin C	0.11 ± 0.01	0.29 ± 0.05	1.15 ± 0.07[e]
Palmitate	0.28 ± 0.03[a]	0.53 ± 0.05[a, c]	1.33 ± 0.11
Palmitate + Triacsin C	0.20 ± 0.03[b]	0.36 ± 0.04[d]	1.27 ± 0.14

Rat islets were cultured for 24 h with the following additives: control = 2% ethanol + 0.5% DMSO; Triacsin C = 0.05 mg/ml Triacsin C in 0.05% DMSO + 2% ethanol; Palmitate = 0.25 mmol/l palmitate in 2% ethanol + 5% DMSO; Palmitate + Triacsin C = 0.25 mmol/l palmitate in 2% ethanol + 0.05 mg/ml Triacsin C in 0.5% DMSO. A total of five experiments were performed. [a]$p < 0.006$ vs control islets; [b]$p < 0.018$ vs Triacsin C islets; [c]$p < 0.01$ vs Triacsin C islets; [d]$p < 0.027$ vs Palmitate islets; [e]$p < 0.024$ vs control islets

27.7 mmol/l glucose (Table 1). Adding Triacsin C (0.05 μg/ml), an inhibitor of long chain acyl-CoA synthetase [17–19], to the 24-h culture prevented the glucose hypersensitivity so that the insulin responses at 2.8 and 8.3 mmol/l glucose were no longer raised compared to the control islets. Interpretation of the result at 2.8 mmol/l glucose was complicated since the islets given palmitate and Triacsin C had higher insulin output than the Triacsin C-treated controls (0.20 ± 0.03 vs 0.11 ± 0.01 pmol, $p < 0.018$). In contrast, the results at 8.3 mmol/l glucose were clear-cut. Triacsin C significantly reduced insulin output in the palmitate-treated islets (0.36 ± 0.04 palmitate + Triacsin C islets vs 0.53 ± 0.05 pmol palmitate islets, $p < 0.027$) to a value that was not significantly different from either control group. A final observation of note was the effect of Triacsin C to inhibit insulin secretion at 27.7 mmol/l glucose in the control islets ($p < 0.024$), but not at the lower glucose concentrations.

Glucose utilization and oxidation. To investigate the mechanism of the lowered glucose set-point for insulin secretion, islet glucose utilization ($n = 6$) and oxidation ($n = 5$) were measured (Fig. 1). Both results showed the same pattern as insulin secretion: significantly augmented at 2.8 and 8.3 mmol/l glucose as opposed to no change at 27.7 mmol/l. Note the "step-up" for both measures was fully present at 2.8 mmol/l glucose as shown by there being no additional increase in the delta values at the higher glucose concentrations for either measurement. Thus, the causative factor was a regulatory process for basal islet glucose usage rather than being active at physiologic glucose concentrations. Furthermore, the parallel utilization and oxidation patterns with palmitate

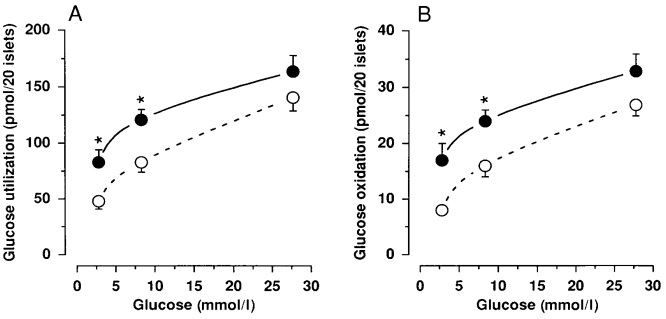

Fig. 1. Glucose utilization (panel **A**, $n = 6$) and glucose oxidation (panel **B**, $n = 5$) in islets cultured 24 h in 0.25 mmol/l palmitate (●) or 2% ethanol alone (○). Twenty islets per tube were measured at each of the glucose concentrations shown. *$p < 0.025$

localized the upregulated site to early glycolysis, up to phosphofructokinase.

Glucose phosphorylation. We next investigated glucokinase and hexokinase activities in the supernatant of islet extracts that had undergone a 12000 *g* centrifugation (Table 2). Islet DNA was unaffected by the palmitate. Hexokinase V_{max} was increased in the palmitate-exposed islets ($p < 0.047$) with no change in K_m. In contrast, glucokinase activity was unchanged.

Fractionated hexokinase activity. A general characteristic of hexokinase is that it exists in two pools, free in the cytoplasm and bound to mitochondria [27]. The previous measurement included only the cytoplasmic fraction. To investigate whether redistribution of the enzyme between the pools accounted for the raised cytoplasmic activity with palmitate, hexokinase kinetics were measured in whole islet extracts and in the post-centrifugation cytoplasm (supernatant) and mitochondrial (pellet) fractions (Fig. 2). Hexokinase V_{max} was increased by 25% in whole extracts of palmitate-exposed islets ($p < 0.013$). The cytoplasm and mitochondrial fractions showed similar increases although only the former value was significant; K_m values were unchanged in all groups.

Discussion

In this study, the heightened glucose sensitivity for insulin secretion with long-term exposure of islets to fatty acids was investigated. The important findings were: comparably increased islet glucose utilization and oxidation, with the step-up being fully manifest at 2.8 mmol/l glucose; increased activity (V_{max}) of hexokinase in islet extracts as opposed to no change in glucokinase; Triacsin C averted the beta-cell glucose hypersensitivity. Based on these data we propose that an upregulated activity of hexokinase causes the

Table 2. Kinetic parameters for glucokinase and hexokinase in 24-h palmitate-cultured islets

Culture conditions	Hexokinase		Glucokinase		Islet DNA content (ng)
	V_{max} (mol glucose/kg DNA^{-1} 60 min^{-1})	K_m (mmol/l glucose)	V_{max} (mol glucose/kg DNA/60 min)	K_m (mmol/l glucose)	
Control	2.8 ± 0.3	0.05 ± 0.02	3.6 ± 0.3	16.5 ± 4.7	17 ± 2
0.25 mmol/l palmitate	3.9 ± 0.4	0.04 ± 0.02	3.8 ± 0.2	16.1 ± 2.8	16 ± 1
P value	0.047	NS	NS	NS	NS

The data are expressed as mean ± SEM for six experiments. The culture protocol and measurement of glucose phosphorylation were carried out as described in the text

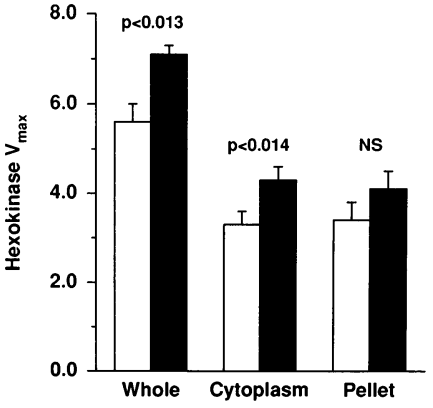

Fig. 2. Hexokinase V_{max} ($n = 6$) expressed as mol glucose kg DNA^{-1} 60 min^{-1} in whole extracts, the cytoplasm fraction, and mitochondrial fraction of islets cultured for 24 h in 0.25 mmol/l palmitate (■) or 2% ethanol alone (□)

beta-cell glucose hypersensitivity, and a raised cellular level of long chain acyl-CoA esters is an essential factor. A similar conclusion regarding the causative role of hexokinase was recently reported in abstract form based on the use of oleate in transformed beta-cells [28]. Possible mechanisms based on known modulatory effects of fatty acids include acylation [29], increased gene transcription [30], increased production of long chain-CoA products, or a direct effect of fatty acid metabolites on the enzyme activity. Regarding the latter, an inhibitory effect of long chain acyl-CoA esters has been reported for glucokinase [31, 32], phosphofructokinase [33], and glucose 6-phosphatase [34]: we are not aware of any reports of stimulation of a glycolytic enzyme such as that noted in this study. No attempt to address these different possibilities was made in this study. The term *hexokinase* is used to represent a family of three low K_m isoforms with different tissue distribution and regulation [27]. The beta-cell isoform(s) has not been delineated. Insight into this issue is needed before investigating the basis for the increased hexokinase activity with fatty acids.

When considering our results, an important issue is the normal beta-cell sensor for glucose metabolism and insulin secretion is glucokinase, the high K_m glucose phosphorylation enzyme [35, 36]. Hexokinase is present in beta-cells in amounts comparable to glucokinase [37], but it normally has a minimal regulatory role over glucose metabolism because of insensitivity to physiological levels of glucose and end-product inhibition by glucose 6-phosphate [38, 39]. As such, a shift in the glucose set-point for insulin secretion would be predicted to reflect a variation in glucokinase activity. The possibility that glucokinase was inhibited by long chain acyl-CoA esters thereby lowering glucose 6-phosphate levels and deinhibiting hexokinase was excluded in the palmitate-treated islets by the lack of change in glucokinase activity on direct measurement, and the fact that upregulation of glucose metabolism was fully evident at 2.8 mmol/l glucose (glucokinase $K_m \approx 15$ mmol/l glucose). Instead, the latter finding is more compatible with the postulated hexokinase regulatory effect in keeping with the observed increase in its activity.

A noteworthy finding was the dichotomy between the islet functional parameters at 2.8 mmol/l glucose (insulin secretion 190% of control, glucose usage 170%, and glucose oxidation 210%) and the considerably smaller increase (25–35%) in hexokinase activity measured in islet extracts. This result suggests a complex process in the intact beta-cell. Islets of diabetic rats are known to have increased glucose 6-phosphatase activity [40, 41]. One possibility is that fatty acids induce a similar effect, resulting in lowered glucose 6-phosphate levels and augmented hexokinase activity on that basis. In keeping with that idea, Clore et al. [42] recently suggested a regulatory role for fatty acyl-CoA over glucose 6-phosphatase gene expression in liver. An alternate hypothesis is that elevated long chain acyl-CoAs enhanced exocytosis as suggested previously [5]. The energy use from insulin secretion would activate phosphofructokinase and increase flux through hexokinase because of lowered glucose 6-phosphate levels. Both of these effects depend on the allosteric regulation of hexokinase and thus would not be evident in islet extracts. Our studies do not differentiate among these possibilities; however, they emphasize the potentially important role of hexokinase in regulating glucose entry to the glycolytic pathway with long-term exposure of beta-cells to fatty acids.

The approach taken to localize the step-up in beta-cell glucose metabolism was to compare the patterns

for glucose utilization and glucose oxidation in the palmitate-treated islets. Glucose utilization was measured as the conversion of [5-^{3}H] glucose to [^{3}H] H_2O. The labelled water is given off at the triose phosphate isomerase reaction [39] so this technique assessed glycolysis up to the generation of 3 carbon fragments. Glucose oxidation is dependent on all the steps in glucose metabolism through mitochondrial oxidation. Comparing the two curves allowed step-ups in glucose metabolism to be localized to before or after the triose phosphate isomerase reaction. Finding identical curves localized the site of upregulation to the former, with the major candidates being GLUT2, glucose phosphorylation by glucokinase or hexokinase, and phosphofructokinase. Finding the step-up in glucose usage was fully present at 2.8 mmol/l glucose eliminated GLUT2 and glucokinase since both are high K_m processes with values of 10–15 mmol/l glucose, narrowing the choice to the low K_m enzyme, hexokinase, or activation of phosphofructokinase via an alteration in the cellular energy state.

A particularly interesting finding was the effect of Triacsin C to inhibit insulin output to 27.7 mmol/l glucose in the control islets. Substantial data have implicated long chain acyl-CoA esters as second messengers for glucose-induced insulin secretion, with the coupling to glucose metabolism being an inhibitory regulation of malonyl CoA on long chain acyl-CoA ester metabolism by carnitine palmitoyl transferase I [5, 43, 44]. The current finding supports that idea by showing that inhibiting the production of long chain acyl-CoA esters with Triacsin C impaired glucose-induced insulin secretion. At first glance, the effect appears weak (30% reduction in insulin output at 27.7 mmol/l glucose). However, this study was not designed to test that question, and the experimental protocol probably underestimated the inhibitory effect of Triacsin C since it was administered during the 24-h culture period, but then the insulin secretion study was performed in the absence of Triacsin C after a 30-min preincubation also without Triacsin C. The inference is that Triacsin C may be a potent inhibitor of glucose-induced insulin secretion. Note that the same effect was not present in the palmitate-cultured islets. Whether that result reflects higher long chain acyl-CoA ester cellular levels that are insufficiently inhibited by the Triacsin C rather than another explanation is unknown.

In summary, we speculate that the lowered glucose set-point for insulin secretion with long-term exposure of islets to non-esterified fatty acid (in this case, palmitate) stems from augmented regulation by hexokinase of the flux of glucose metabolism in beta-cells. Important for this suggestion is the recognition that only a small increase of active hexokinase activity is needed to "left shift" glucose concentration-insulin secretion relationship because of the very low K_m of this enzyme. Indeed, our results clearly showed that glucokinase continued to be the dominant regulator of beta-cell glucose sensitivity as shown by the multifold increase in insulin secretion and glucose utilization from 2.8 to 27.7 mmol/l glucose. Therefore, palmitate did not alter the beta-cell "glucose sensing" mechanism per se as reflected in normal glucokinase activity. Instead, the heightened beta-cell glucose sensitivity was secondary to a raised basal level of insulin secretion.

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References

1. Malaisse WJ, Malaisse-Lagae F (1968) Stimulation of insulin secretion by noncarbohydrate metabolites. J Lab Clin Med 72: 438–448
2. Campillo JE, Luyckx AS, Torres MD, Lefebvre PJ (1979) Effect of oleic acid on insulin secretion by the isolated perfused rat pancreas. Diabetologia 16: 267–273
3. Opara EC, Hubbard VS, Burch WM, Akwari OE (1992) Characterization of the insulinotropic potency of polyunsaturated fatty acids. Endocrinology 130: 657–662
4. Opara EC, Garfinkel M, Hubbard VS, Burch WM, Akwari OE (1994) Effect of fatty acids on insulin release: role of chain length and degree of unsaturation. Am J Physiol 266:E635–E639
5. Prentki M, Corkey BE (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
6. Zhou Y-P, Grill VE (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
7. Milburn JL Jr, Hirose H, Lee YH et al. (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
8. Unger RH (1995) Lipotoxicity in the pathogenesis of obesity-dependent NIDDM. Genetic and clinical implications. Diabetes 44: 863–870
9. Zhou Y-P, Grill VE (1995) Palmitate-induced ß-cell insensitivity to glucose is coupled to decreased pyruvate dehydrogenase activity and enhanced kinase activity in rat pancreatic islets. Diabetes 44: 394–399
10. Hosokawa H, Hosokawa YA, Leahy JL (1995) Upregulated hexokinase activity in isolated islets from diabetic 90% pancreatectomy rats. Diabetes 44: 1328–1333
11. Chan CB, MacPhail RM, Kibenge MT, Russell JC (1995) Increased glucose phosphorylation activity correlates with insulin secretory capacity of male JCR:LA-corpulent rat islets. Can J Physiol Pharmacol 73: 501–508
12. Leahy JL, Bumbalo LM, Chen C (1993) Beta-cell hypersensitivity for glucose precedes loss of glucose-induced insulin secretion in 90% pancreatectomized rats. Diabetologia 36: 1238–1244
13. Thibault C, Guettel C, Lowry MC et al. (1993) In vivo and in vitro increased pancreatic beta-cell sensitivity to glucose in normal rats submitted to a 48-h hyperglycaemic period. Diabetologia 36: 589–595

14. Chen N-G, Tassava TM, Romsos DR (1993) Threshold for glucose-stimulated insulin secretion in pancreatic islets of genetically obese (ob/ob) mice is abnormally low. J Nutr 123: 1567–1574
15. Jia X, Elliot R, Kwok YN, Pederson RA, McIntosh CHS (1995) Altered glucose dependence of glucagon-like peptide I(7–36)-induced insulin secretion from the Zucker (*fa/fa*) rat pancreas. Diabetes 44: 495–500
16. Gotoh M, Maki T, Satomi S, Porter J, Bonner-Weir S, O'Hara CJ, Monaco AP (1987) Reproducible high yield of rat islets by stationary in vitro digestion following pancreatic ductal or portal venous collagenase injection. Transplantation 43: 725–730
17. Tomoda H, Igarashi K, Cyong J-C, Omura S (1987) Inhibition of acyl-CoA synthetase by triacsins. Biochim Biophys Acta 921: 595–598
18. Tomada H, Igarashi K, Cyong J-C, Omura S (1990) Evidence for an essential role of long chain acyl-CoA synthetase in animal cell proliferation. Inhibition of long chain acyl-CoA synthetase by triacsins caused inhibition of Raji cell proliferation. J Biol Chem 286: 4214–4219
19. Korchak HM, Kane LH, Rossi MW, Corkey BE (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
20. Albano JDM, Ekins RP, Maritz G, Turner RC (1972) A sensitive, precise radioimmunoassay of serum insulin relying on charcoal separation of bound and free hormone moieties. Acta Endocrinol 70: 487–509
21. Ashcroft SJH, Weerasinghe LCC, Bassett JM, Randle PJ (1972) The pentose cycle and insulin release in mouse pancreatic islets. Biochem J 126: 525–532
22. Liang Y, Najafi H, Matschinsky FM (1990) Glucose regulates glucokinase activity in cultured islets from rat pancreas. J Biol Chem 265: 16863–16866
23. Labarca C, Paigen KD (1980) A simple, rapid, and sensitive DNA assay procedure. Analytical Biochem 102: 344–352
24. Meglasson MD, Matschinsky FM (1984) Purification of the putative islet cell glucose sensor glucokinase from isolated pancreatic islets and insulinoma tissue. In: Larner J, Pohl S (eds) Methods in diabetes research. Vol 1: Laboratory Methods. Wiley, New York, part A pp 213–225
25. Salas J, Salas M, Vinuela E, Sols A (1965) Glucokinase of rabbit liver: purification and properties. J Biol Chem 240: 1014–1018
26. Spears G, Sneyd GT, Loten EG (1971) A method for deriving kinetic constants for two enzymes acting on the same substrate. Biochem J 125: 1149–1151
27. Wilson JE (1994) Hexokinases. Revs Physiol Biochem Pharm 126: 65–198
28. Liang Y, Buettger C, Berner DK, Matschinsky FM (1996) Oleic acid alters glucose metabolism and glucose-induced insulin release in pancreatic β-cells. Diabetes 45 [Suppl 2]: 314A (Abstract)
29. Schmidt MFG (1989) Fatty acylation of proteins. Biochim Biophys Acta 988: 411–426
30. Clark SD, Jump DB (1994) Dietary polyunsaturated fatty acid regulation of gene transcription. Annu Rev Nutr 14: 83–98
31. Tippett PS, Neet KE (1982) Specific inhibition of glucokinase by long chain acyl coenzymes A below the critical micelle concentration. J Biol Chem 257: 12839–12845
32. Capito K, Hansen SE, Hedeskov CJ, Islin H, Thams P (1992) Fat-induced changes in mouse pancreatic islet insulin secretion, insulin biosynthesis, and glucose metabolism. Acta Diabetol 28: 193–198
33. Weber G, Hird Convery HJ, Lea MA, Stamm NB (1966) Feedback inhibition of key glycolytic enzymes in liver: action of free fatty acids. Science 154: 1357–1360
34. Fulceri R, Gamberucci A, Scott UU, Giunti R, Burchell A, Benedetti A (1995) Fatty acyl coA esters inhibit glucose 6-phosphatase in rat liver microsomes. Biochem J 307: 391–397
35. Matschinsky FM (1990) Glucokinase as glucose sensor and metabolic generator in pancreatic beta-cells and hepatocytes. Diabetes 39: 647–652
36. Newgard CB, McGarry JD (1995) Metabolic coupling factors in pancreatic β-cell signal transduction. Annu Rev Biochem 64: 689–719
37. Heimberg H, De Vos A, Vandercammen A, Van Schaftingen E, Pipeleers D, Schuit F (1993) Heterogeneity in glucose sensitivity among pancreatic β-cells is correlated to differences in glucose phosphorylation rather than glucose transport. EMBO J 12: 2873–2879
38. Giroix M-H, Sener A, Pipeleers DG, Malaisse WJ (1984) Hexose metabolism in pancreatic islets. Inhibition of hexokinase. Biochem J 223: 447–453
39. Meglasson MD, Matschinsky FM (1986) Pancreatic islet glucose metabolism and regulation of insulin secretion. Diabetes Metab Revs 2: 163–214
40. Khan A, Chandramouli V, Östenson C-G, Berggren P-O, Löw H, Landau BR, Efendic S (1990) Glucose cycling is markedly enhanced in pancreatic islets of obese hyperglycemic mice. Endocrinology 126: 2413–2416
41. Khan A, Hong-Lie C, Landau BR (1995) Glucose-6-phosphatase activity in islets from ob/ob and lean mice and the effect of dexamethasone. Endocrinology 136: 1934–1938
42. Clore JN, Blackard WG, Stillman JS, Levy JR (1996) Evidence for a regulatory role of FFA on hepatic glucose-6-phosphatase expression. Diabetes 45 [Suppl 2]: 35A (Abstract)
43. Corkey BE, Glennon MC, Chen KS, Deeney JT, Matschinsky FM, Prentki M (1989) A role for malonyl CoA in glucose-stimulated insulin secretion from clonal pancreatic β-cells. J Biol Chem 264: 21608–21612
44. Prentki M, Vischer S, Glennon MC, Regazzi R, Deeney JT, Corkey BE (1992) Malonyl-CoA and long chain acyl-CoA esters as metabolic coupling factors in nutrient-induced insulin secretion. J Biol Chem 267: 5802–5810