Overexpression of a Modified Human Malonyl-CoA Decarboxylase Blocks the Glucose-induced Increase in Malonyl-CoA Level but Has No Impact on Insulin Secretion in INS-1-derived (832/13) β-Cells*

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The long-chain acyl-CoA (LC-CoA) model of glucose-stimulated insulin secretion (GSIS) holds that secretion is linked to a glucose-induced increase in malonyl-CoA level and accumulation of LC-CoA in the cytosol. We have previously tested the validity of this proposal by overexpressing goose malonyl-CoA decarboxylase (MCD) in INS-1 cells, but these studies have been criticized due to: 1) the small insulin secretion response (2–4-fold) of the INS-1 cells used; 2) unknown contribution of the ATP-sensitive K⁺ (K\text{ATP}) channel-independent pathway of GSIS in INS-1 cells, which has been implicated as the site at which lipids regulate insulin granule exocytosis; and 3) deletion of the N-terminal mitochondrial targeting sequence, but not the C-terminal peroxisomal targeting sequence in the goose MCD construct, raising the possibility that a significant fraction of the overexpressed enzyme was localized to peroxisomes. To address these outstanding concerns, INS-1-derived 832/13 cells, which exhibit robust K\text{ATP}-dependent and -independent pathways of GSIS, were treated with a new adenovirus encoding human MCD lacking both its mitochondrial and peroxisomal targeting sequences (AdCMV-MCDΔ5), resulting in large increases in cytosolic MCD activity. Treatment of 832/13 cells with AdCMV-MCDΔ5 completely blocked the glucose-induced rise in malonyl-CoA and attenuated the inhibitory effect of glucose on fatty acid oxidation. However, MCD overexpression had no effect on K\text{ATP}-dependent or -independent GSIS in 832/13 cells. Furthermore, combined treatment of 832/13 cells with AdCMV-MCDΔ5 and triacsin C, an inhibitor of long chain acyl-CoA synthetase that reduces LC-CoA levels, did not impair GSIS. These findings extend our previous observations and are not consistent with the LC-CoA hypothesis as originally set forth.

The regulation of insulin secretion by glucose is mediated by metabolism of the sugar in pancreatic islet β-cells, resulting in an increase in the ATP:ADP ratio and closure of ATP-dependant K⁺ (K\text{ATP}) channels. Subsequently, voltage-gated Ca²⁺ channels open, intracellular Ca²⁺ rises, and insulin exocytosis is initiated. However, it has become clear that this is a minimal model of glucose sensing, because glucose-stimulated insulin secretion (GSIS) still occurs when closure of the K\text{ATP} channel or the rise in intracellular Ca²⁺ is prevented (2, 3). It has been suggested that factors that complement changes in ATP:ADP ratios in regulation of insulin secretion may arise from post-mitochondrial metabolism of glucose, and interaction of glucose and lipid metabolism. The long-chain acyl-CoA (LC-CoA) model of GSIS holds that malonyl-CoA levels increase in response to increasing glucose concentrations, resulting in inhibition of carnitine palmitoyltransferase I and fatty acid oxidation (4–6). This could lead to accumulation of LC-CoA in the cytosol, which may act as a coupling factor in stimulation of insulin secretion (7).

Our laboratory has recently addressed the LC-CoA model using adenovirus-mediated overexpression of goose malonyl-CoA decarboxylase (MCD) in INS-1 cells (8). In these studies, we were able to partially block the glucose-induced increase in malonyl-CoA levels and lessen glucose-mediated inhibition of fatty acid oxidation. Despite this perturbation of the link between glucose and lipid metabolism, GSIS remained unchanged. However, the findings and conclusions of this paper have been called into question at several levels (9, 10). Concerns raised include: 1) the INS-1 cell line used in our studies exhibited only a 2–4-fold stimulation of insulin secretion as glucose was raised from 3 to 15 mM, as opposed to freshly isolated rat islets, which can exhibit a 10–15-fold response. 2) It is unclear if the so-called K\text{ATP}-channel-independent pathway of GSIS is operative in INS-1 cells. This pathway, which is revealed when β-cells are exposed to depolarizing K⁺, has been implicated as the site at which lipids regulate insulin granule exocytosis (10, 11). 3) The goose MCD cDNA used in our previous study had its N-terminal mitochondrial targeting sequence deleted, but contained an intact C-terminal SKL peroxisomal targeting motif, raising the possibility that a significant fraction of the overexpressed enzyme failed to localize to the cytosol.

The current study was undertaken to address these outstanding concerns. This has been facilitated by our recent development of INS-1-derived cell lines (e.g. 832/13) with robust K\text{ATP}-channel-dependent and -independent GSIS (12). Un-

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The abbreviations used are: K\text{ATP}, ATP-sensitive K⁺; GSIS, glucose-stimulated insulin secretion; LC-CoA, long-chain acyl-CoA; MCD, malonyl-CoA decarboxylase; HBSS, Heps balanced salt solution.
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Under normal ionic conditions, 832/13 cells exhibit a 10-fold increase in insulin secretion when glucose is raised from 3 to 15 mM, and are also responsive to glucose when the KATP channel is bypassed by application of depolarizing K+ and diazoxide. Thus, these cells appear to be an improved model for evaluation of secretory mechanisms relative to parental INS-1 cells, which comprised a mixture of glucose-responsive and -unresponsive cells (12). In addition, since publication of our prior study, the human MCD cDNA has been cloned (13), and mutant forms of the cDNA lacking the mitochondrial and peroxisomal localization sequences have been inserted into adenovirus vectors. With this cadre of improved reagents, we have re-examined the effect of MCD overexpression on GSIS, and find no impairment of robust GSIS in 832/13 cells, despite complete blockade of the glucose-induced rise in malonyl-CoA level. These findings extend our previous observations (8) and are not consistent with the LC-CoA hypothesis as originally set forth.

MATERIALS AND METHODS

All materials were from Sigma, unless otherwise stated.

Cell Culture—The INS-1-derived cell line 832/13 was used throughout these studies. Of interest, these cells were cultured in 3 mM glucose, supplemented with 10% fetal bovine serum, 100 units/ml penicillin, 100 μM streptomycin, 10 mM HEPES, 2 mM glutamine, 1 mM sodium pyruvate, and 50 μM β-mercaptoethanol, at 37 °C in a humidified atmosphere containing 5% CO2.

Preparation and Use of Recombinant Adenoviruses—Fragments of the cDNA encoding human MCD lacking the mitochondrial localization sequence (amino acids 1–39) or the mitochondrial and peroxisomal localization sequences (amino acids 1–39 and 490–493; see Ref. 13 for complete sequence of human MCD) were cloned into the adenovirus vector pACCMV-pLpa (14) and used to prepare recombinant adenoviruses (AdCMV-MCD6 and AdCMV-MCD5, respectively) as described previously (15). The resulting viruses were plaque-purified (15) and used to treat confluent 832/13 cells at varying titers as described in the figure legends. A virus containing the bacterial β-galactosidase gene (AdCMV-JGAL) was used as a control (16). After a 1-h incubation with viruses, cells were washed once in phosphate-buffered saline, culture medium was added, and assays and analyses were undertaken 24 h later.

Malonyl-CoA Decarboxylase Activity Assay—MCD activity was determined as the rate of decarboxylation of malonyl-CoA to acetyl-CoA as previously described (8). In brief, the rate of acetyl-CoA formation was monitored by cleavage of its thioester bond by carnitine acetyltransferase. The thiol group of CoA was colorimetrically measured at 412 nm monitored by cleavage of its thioester bond by carnitine acetyltransferase. The thiol group of CoA was colorimetrically measured at 412 nm.

Malonyl-CoA Assay—832/13 cells were cultured in RPMI containing 3 mM glucose for 24 h following viral treatment. Cells were washed with HEPES-balanced salt solution (HBSS: 114 mM NaCl, 4.7 mM KCl, 1.2 mM KH2PO4, 1.16 mM MgSO4, 20 mM HEPES, 2.5 mM CaCl2, 5.5 mM NaHCO3, pH 7.2) containing 3 mM glucose and incubated in the same buffer for 2 h. Following this preincubation, cells were washed into HBSS containing 3 or 15 mM glucose for 30 min. Cells were collected into 2-ml isopropylene tubes by adding 1.5 ml of ice-cold 3.5% (v/v) HClO4 to each plate and centrifugation of the collected extract at 12,000 rpm for 5 min at 4 °C. The supernatant containing acid-soluble metabolites was neutralized to pH 2–3 with 5% KOH and to pH 6 with 2.5 mM KHCO3. After centrifugation at 2000 rpm for 5 min, the supernatant volume was measured, prior to its transfer to a 15-ml tube. For each assay, sample was divided into two 0.2-ml aliquots. 50 pmol of malonyl-CoA was added to one of the two samples, and malonyl-CoA levels were assayed in both samples according to the method of McGarry et al. (17), using purified fatty acid synthase.

14C-Palmitate Oxidation—832/13 cells in 6-well plates were harvested by light trypsinization and kept in HBSS containing 3 mM glucose for 30 min at 37 °C. The cells were then treated in cell wells (Kontes, Vineland, NJ) suspended from a rubber sleeve stopper (Fisher, Pittsburgh, PA) inserted into a glass scintillation vial. A reaction mixture consisting of 0.5 mM palmitic acid complexed to 0.5% bovine serum albumin (essentially fatty acid free), with 1 × 10−6 cpm/mg of [1-14C]-palmitic acid (PerkinElmer Life Sciences, Boston, MA) as tracer, 0.5 mM t-carnitine, and glucose, at a final concentration of 3 or 15 mM, was added and the vials sealed. After 2 h the reaction was terminated by injection of 100 μl of 7% perchloric acid into the center well. The rate of [1-14C]palmitate oxidation was measured as released 14CO2, which was trapped by adding 300 μl of benzenthonium hydroxide to the bottom of the sealed vials, followed by an additional 2-h incubation at 37 °C. Then, the center wells and rubber stoppers were discarded, scintillation mixture was added, and 14CO2 counted.

Insulin Secretion Studies—For assay of insulin secretion, 832/13 cells were grown to confluence in 12-well plates and treated with the various recombinant adenoviruses. After 24 h, cells were washed in HBSS with 0.2% bovine serum albumin and 3 mM glucose followed by preincubation in 3 ml of the same buffer for 2 h. Insulin secretion was then measured by static incubation of the cells for 2 h in 1.5 mM of HBSS containing glucose, triascin C, and oleate/palmitate (2:1 molar ratio) concentrations indicated in figure legends. When ATP-sensitive K+ channel-independent GSIS was examined, the K+ concentration in the HBSS buffer was increased to 35 mM, while the Na+ concentration was lowered to 89.8 mM, and 250 μM diazoxide was added. Because the human proinsulin gene is stably expressed in the 832/13 cell line (12), insulin was measured using the Coat-a-Count kit (DPC, Los Angeles, CA). The antibody in this assay recognizes human insulin and cross-reacts ~20% with rat insulin.

Statistical Analysis—Data represent mean ± S.E. and different experimental groups were compared either with a one-tailed Student’s t test or a one-way ANOVA followed by Newman-Keuls’ test for comparisons post-hoc. A probability level of p < 0.05 was considered to be statistically significant.

RESULTS

MCD Activities of the Different Recombinant Adenoviruses in 832/13 Cells—In our previous work, a recombinant adenovirus containing the cDNA encoding malonyl-CoA decarboxylase from the goose uropygial gland (AdCMV-MCDgoose) was used to partially attenuate increases in malonyl-CoA levels during glucose stimulation of INS-1 cells (8). The cDNA used in this virus encoded a protein that lacked its N-terminal mitochondrial, but not the C-terminal peroxisomal localization sequence. These features, coupled with its non-mammalian origin, may have limited the ability of the modified goose MCD to degrade malonyl-CoA formed in the cytosol of rat cells, which is the critical pool for modulation of carnitine palmitoyltransferase I enzyme activity. To deal with this concern, we have constructed three new adenoviruses containing modified human MCD cDNAs. The first of these is analogous to the goose MCD construct in that it lacks its N-terminal mitochondrial localization sequence (AdCMV-MCD6), and the second lacks both the mitochondrial and SKL targeting sequences (AdCMV-MCDJ5). Finally, a control virus was constructed containing the MCD cDNA of a patient with two point mutations in the coding sequence that render the enzyme catalytically inactive (AdCMV-MCDmut) (13).

To assess the capacity of the different recombinant adenoviruses to increase MCD activity, 832/13 cells were treated with increasing doses of the viruses. Twenty-four h after viral treatment, total MCD activity was determined in unfraccionated cell extracts. As is shown in Fig. 1, each of the adenoviruses encoding functional enzymes (AdCMV-MCDgoose, AdCMV-MCD6, AdCMV-MCDJ5) increased enzyme activity in a dose-dependent manner, with maximal values ~20-fold higher than in either of the control groups (AdCMV-JGAL-treated or AdCMV-MCDmut-treated cells). Interestingly, the maximal MCD activity achieved was 10-fold higher in this study than in our previous report (8). The reasons for this increased efficiency of expression of MCD in the current study are not known, but could

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related to the fact that we treated the cells with a higher viral titer over a shorter time period than in our previous study. Alternatively, expression of the construct may have been more efficient in the novel 832/13 cells than in the parental INS-1 cells used previously (8).

More relevant than total MCD activity in whole cell extracts is the amount of activity residing in the cytosolic compartment of cells. To estimate this, different batches of 832/13 cells were treated with the same titer of AdCMV-MCDgoose, AdCMVMCD6, or AdCMV-MCDΔ5 adenoviruses, and used for preparation of cytosolic cell fractions 24 h after viral treatment. Fig. 2 shows that the MCD activity in the cytosolic fraction of AdCMV-MCDΔ5 was increased by 120 and 50% compared with cells treated with AdCMV-MCDgoose or AdCMV-MCD6, respectively. Thus, while the three MCD viruses caused similar increases in MCD activity in whole cell extracts, AdCMV-MCDΔ5 containing the cDNA that lacks both its mitochondrial and peroxisomal localization sequences caused a larger increase in cytosolic activity than the other two constructs. In theory, AdCMV-MCDΔ5 may therefore be a more specific tool for perturbing the link between metabolism of glucose and lipids, because the pool of malonyl-CoA that is implicated in β-cell stimulus-secretion coupling is formed in the cytosol (9).

Effect of MCD Overexpression on Malonyl-CoA Levels—In our previous study in which goose MCD was overexpressed in INS-1 cells, cellular malonyl-CoA levels were increased in response to changes in external glucose concentrations, although total malonyl-CoA levels were significantly lower in MCD overexpressing cells than in controls (8). The incomplete blockade in malonyl-CoA formation could have led to an erroneous conclusion about its role in regulation of insulin secretion. We therefore investigated the ability of our new MCD adenoviruses to lower malonyl-CoA levels in 832/13 cells. As shown in Fig. 3, malonyl-CoA levels were increased by 4.8-fold in AdCMV-βGAL-treated 832/13 cells as glucose was raised from 3 to 15 mM. This glucose-induced rise in malonyl-CoA levels was completely blocked in 832/13 cells treated with any of the three MCD viruses, AdCMV-MCDgoose, AdCMV-MCD6, or AdCMV-MCDΔ5. The more effective blockade of malonyl-CoA production is consistent with the higher levels of enzyme activity achieved in this study compared with the previous one (8). Given the similar performance of the three viral constructs, all subsequent experiments were carried out exclusively with AdCMV-MCDΔ5.

Impact of Recombinant MCD Adenoviruses on Fatty Acid Oxidation in 832/13 Cells—We next proceeded to evaluate the metabolic effects of the AdCMV-MCDΔ5 virus. [1-14C]Palmiitate oxidation was measured in 832/13 cells treated with a titer of AdCMV-MCDΔ5 that raised MCD activity to ~1.8 μmol/mg/min. As shown in Fig. 4, incubation of AdCMV-βGAL-treated 832/13 cells at 15 mM glucose reduced the rate of [1-14C]palmi-
were treated with AdCMV-MCD6. 63% of that in cells incubated in 3 mM glucose. Thus, glucose regulates fatty acid oxidation in 832/13 cells in a fashion similar to what has been reported for rat islets (18) and INS-1 cells (8). In contrast, the high glucose concentration was much less effective at suppressing fatty acid oxidation in cells treated with AdCMV-MCD6, such that the rate remained at 63% of that in cells incubated in 3 mM glucose.

There is a possibility that the most pronounced metabolic effects of MCD overexpression occur at higher viral titers than used in the experiment of Fig. 4. Alternatively, high viral titers could have compromised certain aspects of metabolic function, so that the effects on fatty acid oxidation may have been more pronounced at lower viral titers. To address this, we determined the titer dependence of the effect of AdCMV-MCD5 on fatty acid oxidation. At one-fourth the viral titer used in the foregoing experiments (5 x 10⁷ pfu/well corresponding to an MCD activity of 0.85 μmol/mg/min) the effect of MCD overexpression to impair glucose-mediated inhibition of fatty acid oxidation was already maximal. Moreover, it was neither increased nor decreased when the titer was increased stepwise over a 4-fold range (data not shown). This incomplete reversal of glucose suppression of fatty acid oxidation in AdCMV-MCD5 expressing cells, despite the complete blockade of glucose-induced malonyl-CoA accumulation, suggests that factors other than the malonyl-CoA level are involved in regulation of fatty acid oxidation by high glucose.

Glucose-stimulated Insulin Secretion in AdCMV-MCD5-transduced and/or Triacsin-treated 832/13 Cells—Having established that AdCMV-MCD5 blocks glucose-induced malonyl-CoA production and attenuates the inhibitory effect of glucose on fatty acid oxidation, we proceeded to evaluate the effects of this new reagent on insulin secretion. In 832/13 cells treated with the control virus AdMV-βGAL, 15 mM glucose caused a 10-fold increase in insulin secretion relative to secretion at 3 mM glucose (Fig. 5A). Cells treated with AdCMV-MCD5 exhibited the same robust response to stimulatory glucose, providing clear evidence that a rise in malonyl-CoA and complete blockade of fatty acid oxidation are not required events in GSIS. This lack of effect of MCD was not specific to AdCMV-MCD5, as cells treated with AdCMV-MCD6 exhibited the same potent response to stimulatory glucose (data not shown).

Insulin secretion was also studied in the presence of 35 mM 

**Fig. 4. [1-14C]Palmitate oxidation in 832/13 cells.** 832/13 cells were treated with AdCMV-MCDΔ5 or AdCMV-βGAL. 24 h after viral treatment, [1-14C]palmitate oxidation was measured as described under "Materials and Methods." Results represent the mean ± S.E. of four independent experiments. The double asterisk symbol (***) (p < 0.01) indicates that at 15 mM glucose, cells treated with AdCMV-MCDΔ5 oxidized fatty acids at a significantly higher rate than cells treated with AdCMV-βGAL.

**Fig. 5. Glucose-stimulated insulin secretion in 832/13 cells.** 832/13 cells were treated with the indicated adenoviruses and cultured for 24 h in medium containing 3 mM glucose. Insulin secretion was then measured by incubating cells in HBSS containing either 3 or 15 mM glucose for 2 h. Panel A, experiments conducted in the presence of normal K⁺ concentrations. Panel B, experiments conducted in the presence of depolarizing K⁺ (35 mM) and 250 μM diazoxide to measure K<sub>ATP</sub> channel-independent glucose sensing. Results represent the mean ± S.E. for eight independent experiments for panel A and four independent experiments for panel B. Insulin secretion was compared with basal secretion measured at 3 mM glucose for cells treated with each virus, using a one-way ANOVA followed by Newman-Keul’s test post-hoc; *, p < 0.05; **, p < 0.01; ***, p < 0.001.

K⁺ and 250 μM diazoxide, which measures the K<sub>ATP</sub> channel-independent pathway of glucose sensing (2). It has been suggested that his pathway is responsible for the LC-CoA-mediated potentiation of GSIS (10, 11). However, in the presence of the high K⁺ concentration and diazoxide, 15 mM glucose had the same stimulatory effect (2.3-fold) on insulin secretion in AdCMV-βGAL or AdCMV-MCDΔ5-treated 832/13 cells (Fig. 5B), demonstrating that blockade of malonyl-CoA production has no effect on glucose sensing via the K<sub>ATP</sub> channel-independent pathway.

We have previously demonstrated that treatment of INS-1 cells with triacsin C, an inhibitor of LC-CoA synthetase, results in a 50% decrease in LC-CoA levels, with no effect on GSIS (8). We therefore examined the effects of combined treatment of 832/13 cells with the AdCMV-MCDΔ5 virus, which blocks malonyl-CoA accumulation, and triacsin C, which lowers LC-CoA levels. The combined addition of AdCMV-MCDΔ5 and triacsin C to 832/13 cells had no effect on the 10-fold stimulation of insulin secretion by 15 mM glucose under normal culture conditions (Fig. 5A) or on the stimulation by glucose of insulin secretion via the K<sub>ATP</sub> channel-independent pathway (Fig. 5B). Thus, complete blockade of malonyl-CoA accumulation and simultaneous lowering of the cellular LC-CoA pool has no effect on GSIS.

**Preincubation of AdCMV-MCDΔ5-treated 832/13 Cells with...**
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Discussion

The idea that malonyl-CoA could be a coupling factor in β-cell stimulus-secretion originated from the observation that the levels of this intermediate rise prior to insulin secretion when HIT-T15 hamster insulinoma cells are exposed to glucose (4–6). Furthermore, the glucose-induced rise in malonyl-CoA was coincident with inhibition of fatty acid oxidation and was assumed to result in an increase in cytosolic levels of LC-CoA. That LC-CoA could be an effector molecule in β-cell stimulus-secretion coupling was further supported by the fact that perfusion of permeabilized HIT-T15 cells with LC-CoA-esters induced insulin secretion (7). Additional supporting evidence for the LC-CoA model comes from the use of hydroxycitrate, which inhibits ATP-citrate lyase and consequently malonyl-CoA formation. Use of this agent to disrupt the link between glucose and malonyl-CoA formation was shown to inhibit GSIS from the perfused rat pancreas (18). Conversely, inhibition of carnitine palmitoyltransferase I and fatty acid oxidation with agents such as etomoxir-stimulated GSIS from the perfused pancreas (18). Finally, stable expression of an acetyl-CoA carboxylase-antisense construct in INS-1 cells resulted in lowering of malonyl-CoA levels, increased fatty acid oxidation, and inhibition of GSIS (21).

In contrast to these results, we have previously demonstrated that adenovirus-mediated overexpression of goose MCD in INS-1 cells reduced malonyl-CoA levels and partially alleviated inhibition of fatty acid oxidation by glucose (8). Triacsin C, an inhibitor of LC-CoA synthetase was also shown to block glucose incorporation into lipids and lower LC-CoA levels, but neither the molecular nor the pharmacologic approaches to impairing LC-CoA formation had any effect on GSIS (8). The current study has attempted to address perceived limitations of the prior work that have come to light (9, 10), and to resolve the apparent discrepancy between our work and that of others. Key improvements in our experimental approach reported herein include. 1) The use of a new INS-1-derived cell line with robust KATP channel-dependent or -independent pathways of glucose sensing (12). 2) The construction of new recombinant adenoviruses containing the cDNAs encoding human forms of MCD, including one in which both the mitochondrial and peroxisomal localization sequences were removed to enhance expression of the enzyme in the cytosolic compartment. 3) New studies involving preincubation of cells in a mixture of oleate and palmitate to boost cellular lipid stores. 4) Co-application of the molecular (AdCMV-MCD∆5) and pharmacologic (triacsin C) reagents to maximally perturb malonyl-CoA and LC-CoA synthesis. These improvements have allowed us to demonstrate in a clear and unequivocal fashion that complete blockade of malonyl-CoA accumulation has no impact on the KATP channel-dependent or independent pathways of GSIS. The co-application of AdCMV-MCD∆5 and triacsin C also has no effect on insulin secretion, suggesting that robust glucose sensing is not linked to changes in total LC-CoA levels. Furthermore, preincubation of 832/13 cells for 3 days in 0.5 mM oleate/palmitate impaired glucose sensing, as has been described extensively in islets (19, 20). However, culture of cells in fatty acids does not uncover an effect of AdCMV-MCD∆5 on GSIS. Finally, treatment of 832/13 cells with AdCMV-MCD∆5 does not affect fatty acid potentiation of GSIS, supporting the view that this action of fatty acids is not related to their oxidation (18).

How can our findings be reconciled with those supporting the malonyl-CoA/LC-CoA model of glucose sensing? First, the rise in malonyl-CoA levels that occurs prior to insulin release is not proof of a causal relationship between the two phenomena. Also, the recently reported effect of palmitoyl-CoA on insulin secretion is modest, and the use of permeabilized cells in these studies raises questions about the physiological relevance of the experiment (7). Moreover, in view of the proposed critical role of mitochondrial metabolism in GSIS (22), abrogation of GSIS conferred by hydroxycitrate may be due in part to perturbed mitochondrial metabolism. It should also be noted that the inhibitory effect of hydroxycitrate on GSIS is not observed in isolated rat islets (23). The enhanced GSIS that accompanies inhibition of fatty acid oxidation by carnitine palmitoyltransferase I inhibitors may be explained by the fact that these compounds, e.g. 2-bromopalmitate or etomoxir, are modified fatty acids, which may act like native lipids to potentiate GSIS independent of their effects on fatty acid oxidation (18). Finally, chronic lowering of malonyl-CoA levels in INS-1 cells with a
stable transfection strategy may cause profound alterations in β-cell metabolism and function (21). Of particular concern here is that chronic lowering of malonyl-CoA levels could result in depletion of stored lipids. It has been shown that depletion of β-cell lipids in nicotinamide-treated animals or humans (24, 25) or in hyperleptinemic rats (26) blocks insulin secretion in response to glucose and many other secretagogues. Interestingly, in both cases, GSIS is immediately restored by provision of free fatty acids. These findings clearly support an essential role for lipids in regulation of insulin secretion, possibly at the level of membrane lipid turnover or acylation of regulatory proteins. The latter possibility is also supported by recent studies in which insulin secretion was inhibited by addition of cerulenin, an inhibitor of protein acylation (11). However, while a minimal pool of lipids may be essential for normal regulation of insulin secretion, the current study provides strong evidence that glucose sensing can occur in the absence of a rise in malonyl-CoA and despite acute perturbation of lipid metabolism.

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