Lipid rather than glucose metabolism is implicated in altered insulin secretion caused by oleate in INS-1 cells

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1Molecular Nutrition Unit, Department of Nutrition, University of Montreal, and the Centre de Recherche du Centre Hospitalier de l’Université de Montréal and Institut du Cancer, Montreal, Quebec, Canada H2L 4M1; 2Department of Medical Biochemistry, Centre Médical Universitaire, University of Geneva, Geneva, Switzerland 1211; and 3Center for Obesity and Metabolism at Boston University Medical Center, Boston University Medical School, Boston, Massachusetts 02118

Segall, Laura, Nathalie Lameloise, Françoise Assimacopoulos-J eannet, Enrique Roche, Pamela Corkey, Stéphane Thumelin, Barbara E. Corkey, and Marc Prentki. Lipid rather than glucose metabolism is implicated in altered insulin secretion caused by oleate in INS-1 cells. Am. J. Physiol. 277 (Endocrinol. Metab. 40): E521–E528, 1999.—A comprehensive metabolic study was carried out to understand how chronic exposure of pancreatic β-cells to fatty acids causes high basal secretion and impairs glucose-induced insulin release. INS-1 β-cells were exposed to 0.4 mM oleate for 3 days and subsequently incubated at 5 or 25 mM glucose, after which various parameters were measured. Chronic oleate promoted triglyceride deposition, increased fatty acid oxidation and esterification, and reduced malonyl-CoA at low glucose in association with elevated basal O2 consumption and redox state. Oleate caused a modest (25%) reduction in glucose oxidation but did not affect glucose usage, the glucose 6-phosphate and citrate contents, and the activity of pyruvate dehydrogenase of INS-1 cells. Thus changes in glucose metabolism and a Randle-glucose/fatty acid cycle do not explain the altered secretory properties of β-cells exposed to fatty acids. The main response of INS-1 cells to chronic oleate, which is to increase the oxidation and esterification of fatty acids, may contribute to cause high basal insulin secretion via increased production of reducing equivalents and/or the generation of complex lipid messenger molecul(e)s.

fatty acids; insulin secretion; obesity; type 2 diabetes

OBESE PATIENTS with type 2 diabetes show altered insulin release with respect to prevailing blood glucose characterized by elevated circulating insulin in the fasting state and relatively low insulin levels postprandially when glucose is high (29, 30). The biochemical basis of these alterations is not known, but the emerging evidence suggests that elevated circulating free fatty acids (FFA) as well as long chain acyl-CoA esters (Lc-CoA) and triglyceride deposition in the β-cell may be causally implicated (25, 32).

FFA are important nutrients for β-cell function because they are used as fuels (23) and play a permissive role for glucose-induced insulin release, as evidenced with a pharmacological approach (44) and leptin gene transfer experiments (13). In addition, their Lc-CoA or complex lipid derivatives may act as coupling factors in nutrient-stimulated insulin secretion (8, 31).

Acute exposure of β-cells to fatty acids potentiates glucose-induced insulin secretion by mechanisms that apparently do not involve K ATP channels but may involve C-kinase activation by Lc-CoA and Ca2+ channels (33, 45). By contrast, over longer time ranges (>24 h) fatty acids such as palmitate and oleate cause both in vitro and in vivo high basal insulin secretion characterized by a lowered glucose set point and markedly reduced secretion in response to a further elevation of the sugar (4, 12, 17, 38, 48, 49). At relatively high concentrations, fatty acids also activate the nitric oxide transduction system of islet tissue, a process that may induce cell apoptosis and be implicated in final β-cell decompensation (42, 43).

Regarding secretion, several studies have suggested that the long-term action of fatty acids is caused by changes in lipid and glucose metabolism, possibly as a consequence of variations in the expression of genes encoding key enzymes implicated in fuel signaling. In this respect, a complex picture with conflicting results has emerged. Thus palmitate and oleate not only increase the total activity of hexokinase in rat islets (12) and HC9 β-cell (17) but also decrease the expression of glucokinase and GLUT-2, possibly via reduced expression of the transcription factor IDX-1 (11). In addition, fatty acids induce the carnitine palmitoyltransferase I gene (CPT-I; Ref. 3) and reduce acetyl-CoA carboxylase expression (4) in INS-1 β-cells, thus causing enhanced fat oxidation (4). An enhanced rate of oxidation of fatty acids might reduce glucose metabolism and consequently insulin secretion if a so-called Randle cycle (34) were operative in the β-cell after fat exposure. In this respect, FFA were shown to cause a modest (~20%) reduction of glucose oxidation in islets (49) and HC9 β-cells (17) or to increase glucose oxidation in another islet study (12). A reduced islet pyruvate dehydrogenase (PDH) activity predicted by the Randle cycle (34) was observed in the β-cell after fat exposure. In this respect, FFA were shown to cause a modest (~20%) reduction of glucose oxidation in islets (49) and HC9 β-cells (17) or to increase glucose oxidation in another islet study (12). A reduced islet pyruvate dehydrogenase (PDH) activity predicted by the Randle cycle (34) was observed in the β-cell after fat exposure.

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explanation for the long-term action of fatty acids on insulin secretion.

As reviewed previously (34), the following sequence of events associated with predicted alterations in metabolite levels characterize a Randle cycle. Accelerated fat oxidation results in exaggerated NADH and acetyl-CoA production, thus causing PDH inhibition and reduced glucose oxidation. Citrate also rises via a mass effect in the mitochondrion and, after its export to the cytosolic compartment, allosterically inhibits 6-phosphofructo-1-kinase, thus causing reduced glycolytic flux and a rise in glucose 6-phosphate. To better understand how fatty acids increase insulin secretion at low glucose and to determine whether altered glucose metabolism via a Randle glucose-fatty acid cycle may account for the lack of glucose response in cells chronically exposed to FFA, we have carried out a comprehensive study of glucose and fatty acid metabolism in INS-1 β-cells after long-term exposure to olate.

**METHODS**

Cell culture and incubation conditions. The experiments have been carried out with the β-cell line INS-1, which responds to glucose at physiological concentrations of the sugar to an extent similar to that of pancreatic islets in culture (2, 6, 39). All experiments have been carried out with cell passages below 85 because we noticed that at higher passage numbers INS-1 cells lose their response to glucose (∼5 mM). INS-1 cells were seeded in 21-cm² petri dishes (1.4 × 10⁶ cells/dish) or in 24-well plates (10⁵ cells/well) and grown as described previously (2) in RPMI 1640 medium at 11 mM glucose supplemented with 10 mM HEPES, 10% heat-inactivated fetal calf serum, 2 mM L-glutamine, 1 mM sodium pyruvate, 50 µM β-mercaptoethanol, 100 U/ml penicillin, and 100 µg/ml streptomycin in a humidified atmosphere (5% CO₂, 95% air). When cells reached 80% confluence after ∼7 days, they were washed twice with PBS and preincubated at 37°C for 2 days in culture medium containing 5 mM glucose. Cells were then washed with PBS and incubated for 3 days in culture medium at 5 mM glucose in the absence or presence of olate (0.4 mM). Albumin-bound olate was prepared by stirring the fatty acid Na salt at 45°C with defatted BSA (fraction V, Sigma). After adjustment of the pH to 7.4, the solution was filtered through a 0.22-µm filter and the fatty acid concentration was measured with a NEFA C kit (Wako Chemicals). For incubations longer than 24 h, the medium was changed daily to maintain a constant concentration of fatty acid. The final concentration of BSA in the culture medium was 0.5%.

Metabolites and insulin secretion measurements. Citrate and malate were determined as follows. After a culture period of 3 days at 5 mM glucose with or without olate, cells were washed with PBS and preincubated at 37°C for 30 min in Krebs-Ringer bicarbonate buffer (KRBB) with 25 mM HEPES, pH 7.4 (35), at 5 mM glucose, containing 0.07% BSA (fraction V). Cells were then incubated for 30 min in fresh KRBB-HEPES at 5 or 25 mM glucose. Incubation media were discarded or collected for insulin measurement, and 0.5 ml of 13% perchloric acid was added to the cells. Precipitated proteins were removed by centrifugation, and the supernatants were neutralized by adding 2 N KH₂CO₃. The precipitated potassium perchlorate salt was eliminated by centrifugation, and the resulting supernatants were used for metabolite measurements. Total cellular proteins were measured by the Bradford (Bio-Rad) assay after dissolution of the protein pellets in 0.5 N NaOH. Malate was determined with the malate dehydrogenase (Boehringer) reaction (46). Citrate was measured by coupling the citrate lyase-malate dehydrogenase (Boehringer) reactions according to Williamson and Corkey (46). Malonyl-CoA and glucose 6-phosphate were extracted from cells with 10% trichloroacetic acid. After centrifugation of precipitated proteins, cell extracts were brought to pH 5–6 by successive ether extractions. Samples were lyophilized and stored at −70°C. Malonyl-CoA was assayed with a radioactive method with fatty acid synthase (27). Glucose 6-phosphate was measured by a fluorometric method with glucose 6-phosphate dehydrogenase (6). ATP and ADP were measured by a bioluminescence assay (40). For insulin secretion determinations, INS-1 cells were plated (10⁵ cells/well) into 24-well plates. They were cultured and incubated as described previously for metabolite measurements. The insulin concentration in the medium was determined by RIA with rat insulin as standard (2). Total cellular insulin content was measured after acid-ethanol (1.5% HCl, 75% ethanol) extraction. DNA was measured according to Labarca and Paigen (14).

Fatty acid metabolism and triglyceride measurements. Fatty acid oxidation was measured in INS-1 cells cultured in 21-cm² petri dishes. After a preexposure for 3 days at 5 mM glucose in the absence or presence of olate (0.4 mM), cells were washed with PBS and preincubated at 37°C for 30 min in KRBB-HEPES (pH 7.4) medium, at 5 mM glucose, containing 0.07% BSA. Cells were then incubated for 1 h at 37°C in 5 ml of fresh KRBB-HEPES at 5 or 25 mM glucose in the presence of 0.1 mM palmitate, 0.5% defatted BSA, 1 mM carnitine, and 0.11 µCi of [1-14C]palmitate (55 mCi/mmol; Amersham). At the end of the incubations, media were collected and transferred to 25-ml Erlenmeyer flasks covered with septa caps. Media were acidified by injecting perchloric acid (6% final concentration) with a syringe. The liberated CO₂ was trapped in a plastic well, suspended from the septa caps, containing 0.4 ml of methanolic benzethonium hydroxide. After 1 h of incubation at 37°C, the wells were removed and the trapped ¹⁴CO₂ was measured by liquid scintillation counting. Cells were scraped in cold PBS, pelleted by centrifugation, and resuspended in 4 ml of Folch reagent (10). Total lipids were extracted by chloroform-methanol (2:1, v/v). Triacylglycerol was measured radiochemically by measuring the incorporation of labeled palmitate into triglycerides and phospholipids (36). An aliquot of the chloroform phase was dried, and total cell triglycerides were measured with a commercial kit (PAP 150, bioMérieux, Marcy l’Etoile, France). Triglyceride recovery assessed with triolein was 94 ± 5% (n = 4).

Glucose metabolism, cell respiration, pyridine nucleotide fluorescence, and enzymatic activity measurements. Glucose usage was determined radio metrically as the production of ³H₂O from [⁵⁻H]glucose (16). Cells were cultured in 24-well plates with or without olate as described in Cell culture and incubation conditions and subsequently incubated for 1 h at 5 or 25 mM glucose in KRBB-HEPES medium containing 0.15 µCi/µmol of [⁵⁻H]glucose (Amersham). Glucose oxidation was measured as ¹⁴CO₂ production from [U-¹³C]glucose (0.1 µCi/µmol) with the same experimental design as for the measurement of palmitate oxidation, except that cells were incubated for 1 h with [U-¹³C]glucose in the absence of fatty acids (3). In some experiments, enzymatic activity measurements were carried out at the end of the 1-h incubation period. Lactate dehydrogenase activity was determined according to Schuit et al. (39). PDH activity measurements were made according to a published procedure (47). Total PDH activity was assayed after conversion of inactive PDH complex with PDH phosphatase extracted from minipig heart (47).
50). For cell respiration and pyridine nucleotide measurements, cells were cultured as described Cell culture and incubation conditions for 3 days in 21-cm² petri dishes in the absence or presence of oleate. Cells were detached from culture flasks by incubation in the presence of EDTA without trypsin for 5 min at 37°C (7). They were subsequently washed twice with KRBB-HEPES medium and incubated as a suspension at 37°C in acrylic cuvettes as described before (7). After 10 min of incubation at 5 mM glucose to observe basal O₂ consumption, glucose was added to the cuvette to reach 25 mM. O₂ consumption was measured with a Clark-type electrode in a stirred thermostatized chamber designed by the Bio-Instrumentation Group of the University of Pennsylvania, which allows simultaneous measurements of O₂ and fluorescence. The pyridine nucleotide fluorescence was measured with an MB-2 air turbine fluorescence spectrophotometer set at wavelengths of 340 nm (excitation) and 460 nm (emission) (7).

Statistical analysis. All results are expressed per milligrams of protein or micrograms of DNA as means ± SE of the indicated number of experiments. Statistical significance was calculated with the Student’s t-test.

RESULTS

Insulin secretion, glucose metabolism, and anaplerosis in INS-1 β-cells after long-term exposure to oleate. Figure 1 shows that the acute addition of oleate stimulated insulin release at low glucose and amplified the action of elevated glucose. By contrast, a 3-day preexposure of INS-1 cells to oleate caused elevated secretion at low glucose and suppressed the action of elevated glucose on insulin release. Similar effects were noted either in the absence or in the presence of oleate during the 30-min incubation period when insulin measure-

ments were made. These short- and long-term actions of oleate reproduce previous observations made with pancreatic islets (12, 38, 48, 49) and HC9 β-cells (17) and therefore indicate that INS-1 cells are an appropriate cell model for studying the biochemical basis of the action of FFA on β-cell metabolic signaling.

To better understand the mechanism of the chronic action of fatty acids, we endeavored to carry on a detailed metabolic study of INS-1 cells after oleate exposure in which many metabolic pathways, metabolites, and enzymatic activities are measured. For feasibility reasons, we studied INS-1 cells after a 3-day exposure to the fatty acid, a time that is long enough to establish the secretory defect described previously, and after incubation at 5 and 25 mM glucose only. The results indicated that oleate did not alter glucose usage either at low or high glucose (Fig. 2). Chronic exposure of INS-1 cells to oleate caused a modest (∼28%) nonsignificant (P < 0.075) decrease in glucose oxidation at low (5 mM) glucose and reduced by 25% (P < 0.01) the oxidation of the sugar at 25 mM glucose. However, the differences in the rate of oxidation of glucose between 5 and 25 mM were similar in oleate and control cells.

The aforementioned results of glucose usage and oxidation do not favor the view that fatty acids inhibit the action of glucose through a Randle cycle. This is further supported by the data shown in Fig. 3 indicating that oleate did not affect basal and glucose-stimulated levels of citrate and glucose 6-phosphate in INS-1 β-cells. The apparent slight decrease in glucose 6-phosphate at high glucose in oleate-treated cells was not significantly different [24.2 ± 3.0 (n = 9) vs. 17.1 ± 1.2 (n = 7); P < 0.075]. Glucose oxidation and glucose 6-phosphate measurements were similar at either low or high glucose whether oleate was present or absent during the preincubation and incubation periods (not

Fig. 1. Effect of short- and long-term exposure of INS-1 cells to oleate on insulin secretion. Data are means ± SE of 12 experiments. INS-1 cells were cultured in RPMI medium containing 10% serum with 0.5% BSA for 3 days at 5 mM glucose (G) in absence or presence of oleate (Ol.; 0.4 mM). Cells were subsequently incubated in Krebs-Ringer bicarbonate buffer-HEPES medium at 5 or 25 mM glucose in absence (open bars) or presence (solid bars) of oleate (0.4 mM). Insulin release during 30-min incubation period is expressed as percentage of total cellular insulin content. Insulin content of cells cultured in absence or presence of oleate was 1,862 ± 12 and 1,725 ± 5 ng/mg protein, respectively. IRI, immunoreactive insulin.
shown). The acute addition of oleate did not affect glucose oxidation and the glucose 6-phosphate content of INS-1 cells at low or high glucose (not shown). In addition, chronic oleate treatment of INS-1 cells did not alter total PDH activity of INS-1 cells (measured after activation by PDH phosphatase) nor did it change its active form (measured in the absence PDH phosphatase treatment). The following values were obtained for total PDH activity in control and oleate-treated cells: 0.40 ± 0.01 and 0.38 ± 0.01 μM/mg protein, respectively (means ± SE of 6 experiments). The proportion of active PDH of control and oleate-treated cells was 64 ± 3 and 58 ± 4%, respectively (not significantly different).

Low lactate dehydrogenase is a characteristic feature of normal β-cells and INS-1 cells (39, 41). Dedifferentiated cell lines showing a left shift in the dose dependence of glucose-stimulated insulin secretion express high amounts of the enzyme (41). To determine whether oleate alters insulin secretion by changing the expression of LDH, we measured the activity of the enzyme in control and oleate-treated cells. The LDH activity of cells chronically exposed to oleate was 40.8 ± 3.8 μM/mg protein, a value not different from that of control cells, which was 40.5 ± 4.8 μM/mg protein (means ± SE of 3 experiments). This is consistent with the lack of action of fatty acids on the glucose usage of INS-1 cells.

Increased anaplerotic input into the citric acid cycle is thought to be implicated in the mechanism whereby nutrients activate the metabolic stimulus secretion coupling of the β-cell (5, 39). To assess whether this parameter of β-cell activation is altered by oleate, malate measurements were carried out. A rise in glucose from 5 to 25 mM caused an ∼10-fold increase in the malate content of INS-1 cells (0.25 ± 0.05 vs. 3.26 ± 0.13 nmol/mg protein). Oleate treatment barely affected the malate content of INS-1 cells either at low or high glucose (0.35 ± 0.05 vs. 2.77 ± 0.17 nmol/mg protein, means ± SE of 6 experiments). In view of the fact that citrate levels also remained unaffected by oleate (Fig. 3), the data indicate that chronic exposure of INS-1 cells to FFA does not affect anaplerosis in the β-cell.

Lipid metabolism in INS-1 β-cells chronically exposed to oleate. Malonyl-CoA is known to rise in the β-cell in response to glucose stimulation and may act as a signaling molecule for the short- and/or long-term control of insulin secretion (8). This intracellular signal of glucose abundance is a key regulator of fuel partitioning in various tissues (19, 26, 32, 37). Chronic exposure of INS-1 β-cells to oleate decreased the malonyl-CoA content at low glucose by 30% (P < 0.001) without affecting the glucose-induced rise of this metabolic switch molecule (Fig. 4). Because malonyl-CoA controls fatty oxidation and esterification, an associated rise in the rate of oxidation of fatty acids measured at low glucose occurred as expected in oleate-treated cells (P < 0.05; Fig. 4). Likewise, an increased incorporation of FFA into total lipids was also noted at low glucose (P < 0.05; Fig. 5). Consistent with the malonyl-CoA measurements made at high glucose, oleate did not affect the action of glucose to reduce fat oxidation (Fig. 4) or to promote the esterification of FFA into total lipids (Fig. 5). Similar observations were made with respect to the incorporation of FFA into phospholipids (data not shown). It should be mentioned that due to tracer palmitate dilution in the large intracellular pool of fatty acids in cells chronically treated with oleate, the increase in palmitate oxidation and esterification is likely to be much underestimated. Figure 5 also indicates that oleate caused a pronounced deposition of triglycerides in INS-1 cells (P < 0.001). The results are consistent with the view that the reduced malonyl-CoA content at low glucose associated with an increased fat oxidation may contribute to cause high basal secretion. By contrast, the lack of glucose-stimulated insulin
release cannot be ascribed to altered levels of malonyl-CoA or changes in fat oxidation and esterification processes.

Energy metabolism in INS-1 β-cells chronically exposed to oleate. The rates of reducing equivalent and ATP production in response to fuel stimuli are believed to be key components of the β-cell metabolic signal transduction cascade (9, 20, 21, 24, 31). We therefore wished to determine whether the altered secretion caused by oleate correlates with changes in the INS-1 β-cell NAD(P)H-to-NAD(P) ratio and in oxygen consumption, which reflect the overall cellular fuel oxidation and ATP production.

Simultaneous measurements of the pyridine nucleotide oxidation state, with native fluorescence at 340 nm (λ-excitation) and 540 nm (λ-emission), and of medium O₂ showed a rise in the β-cell redox state and an increase in O₂ consumption in response to elevated glucose in control cells. When cells were cultured for 3 days in the presence of oleate, basal redox state (Fig. 6) and respiration (Fig. 7) were higher, achieving levels reached by glucose-stimulated control cells. Furthermore, the responses to glucose in both respiration and redox were severely dampened. Interestingly, Fig. 7 also shows that oleate increased by ~2.5-fold the maximal rate of respiration measured at saturating substrate concentrations (25 mM exogenous glucose + 0.4 mM oleate) in the presence of the uncoupler carbonyl cyanide-p-trifluoromethoxyphenylhydrazone. The precise biochemical nature of this phenomenon is uncertain. It shows that oleate enhances the total oxidative capacity of INS-1 β-cells. This observation is possibly explained by an induction of limiting enzyme(s) of the respiratory chain.

Finally, the cellular content of ATP, ADP as well as the ATP-to-ADP ratio at low glucose, was found not to be affected by oleate. The values for ATP at 5 mM glucose were for control and chronic oleate 29.8 ± 1.5 and 33.3 ± 1.2 nmol/mg protein, respectively (n = 6). The ATP-to-ADP ratios of control and oleate-treated cells at low glucose were 11.3 ± 0.3 and 10.3 ± 1.1, respectively. This indicates that changes in the ATP level or the ATP-to-ADP ratio do not account for high basal secretion at low glucose in oleate-treated cells.

**DISCUSSION**

The results indicate that the cellular levels of glucose 6-phosphate, malate, citrate, as well as glucose usage, remain unaltered at either high or low glucose after a 3-day preexposure of INS-1 cells to oleate. A modest (~25%) inhibition of glucose oxidation, which is quantitatively similar to previous observations made in rat islets and the β-cell line HC9, occurs in oleate-treated cells. However, the difference in the rate of glucose oxidation between 5 and 25 mM glucose remains unchanged by the FFA. In addition, PDH activity is similar in both oleate-treated and control cells. By contrast, long-term exposure of INS-1 cells to oleate causes a massive triglyceride (TG) deposition that is associated at low glucose with the following changes: a decrease in the cellular content of malonyl-CoA, an increase in the rate of fat oxidation, and a promotion of fatty acid esterification processes, all of which finally result in considerable increases in the cellular redox state and the rate of oxygen consumption.

Together these observations allow the following conclusions. 1) Long-term exposure of INS-1 β-cells to FFA...

![Fig. 5. Effect of chronic oleate treatment on esterification of palmitate and triglyceride (TG) content of INS-1 cells. Means ± SE of 3 experiments. Cont., control. Cells were cultured with or without oleate (Ol.) for 3 days and incubated for 1 h at 5 or 25 mM glucose as described in Fig. 2. Left panel, measurements of palmitate incorporation into total lipids. Right panel, triglyceride content of INS-1 cells at 5 mM glucose after culture with or without oleate.](http://ajpendo.physiology.org/)

![Fig. 6. Chronic oleate treatment alters oxidation state of pyridine nucleotides in INS-1 cells. Data are means ± SE of 6 (control) and 4 (oleate) experiments. Fluorescence measurements of cellular NAD(P)H were carried out at 5 or 25 mM glucose after a 3-day culture period with or without oleate (0.4 mM). Percentage of reduced NAD(P)H at 5 mM glucose vs. 5 mM glucose plus oleate (P < 0.001).](http://ajpendo.physiology.org/)
does not induce a Randle cycle in the β-cell because the predictions that characterize this inverse relationship between fat and glucose oxidation in some tissues (34) have not been verified. 2) The rate of fatty acid oxidation, either in control cells or cells chronically exposed to oleate, barely affects glucose metabolism. 3) The lack of response to elevated glucose in oleate-treated cells cannot be explained by the metabolic hypothesis, which predicts that reduced glucose metabolism and PDH activity account for this effect. 4) It is, however, attractive to believe that enhanced fat oxidation caused by chronic oleate in association with increased intracellular FFA availability due to TG deposition may contribute to cause high basal insulin secretion. In accordance with this possibility, the modifications of insulin secretion caused by oleate were found to correlate with changes in NAD(P)H fluorescence and O2-consumption measurements. In this respect, FFA induction of the CPT-I gene (3) and repression of the acetyl-CoA carboxylase gene (4) may be instrumental because CPT-I catalyzes the limiting step of fat oxidation and acetyl-CoA carboxylase catalyzes the formation of the CPT-I inhibitor malonyl-CoA. Increased esterification processes may also contribute to high basal secretion by providing complex lipid messenger molecules such as diacylglycerol and phosphatidic acid (28, 31).

Our comprehensive metabolic study by assessing in addition to glucose metabolism, lipid metabolism, and anaplerosis, the redox state, PDH activity, and the Randle cycle, extends the results of a recent publication concerning HC9 β-cells (17) showing that ATP production at different glucose concentrations (calculated on the basis of the rates of glucose usage, lactate production, and glucose oxidation) is not modified by chronic cell exposure to oleate. In accordance with our results, these authors concluded that altered glucose metabolism is unlikely to explain the lack of glucose response of FFA-treated cells (17). They are, however, at variance with other reports carried out in human and rat islets (38, 48, 49), which proposed that a Randle cycle accounts for the lack of glucose responsiveness of β-cells after long-term exposure to FFA. It should be stated that fatty acid inhibition of glucose metabolism was modest in these studies (~20%) and only observed at 27 mM glucose and not at 11 or 3 mM. In addition, the fatty acids were added to cells in ethanol solutions and not bound to BSA, which might have resulted in very high nonphysiologically free concentrations of the fatty acids. It should be underlined that when one considers the relative capacities of β-cells to metabolize fatty acids vs. glucose it appears unlikely that accelerated fat oxidation may more than marginally influence glucose metabolism. Accordingly, the ratio of glucose oxidation (at ~16–20 mM) vs. that of palmitate (at ~0.3 mM) has been found to be 80 (1) and 60 (22) in two rat islets studies and > 85 in INS-1 cells (3). Thus a twofold rise in fat oxidation rate caused by chronic oleate should not affect in a major way glucose metabolism in the β-cell. Irrespective of whether Randle effects have or have not been previously demonstrated in islets (38, 48, 49), those studies do not prove a causative relationship between the glucose-fatty acid cycle and the accompanying secretory alterations of fatty acid-treated cells; in contrast, the current study shows that an active Randle cycle is not necessary for observed alterations in secretory response in lipid-treated cells. Furthermore, some previous islets studies have also shown metabolic changes incompatible with a Randle cycle, i.e., increased glucose oxidation in palmitate-treated islets (12) and decreased glucose 6-phosphate content after treatment with oleate (18). The dissociation between the action of chronic oleate on glucose-induced insulin release on the one hand and on glucose metabolism, anaplerosis, and malonyl-CoA formation on the other indicates that other factors account for the inhibitory action of FFA on insulin secretion promoted by the sugar. Several possibilities may be considered. 1) If the redox state acts as a
metabolic coupling factor in secretion not only via ATP production, for example by controlling sulfhydryl groups of transducing proteins in the β-cell, it may be that a rise in glucose from 5 to 25 mM cannot further enhance secretion because the reduction state of pyridine nucleotides is already maximal at low (5 mM) glucose and consequently secretion cannot be further enhanced. 2) The elevated TG content of the β-cell is expected to result, after lipolysis, in a rise in cytosolic FFA and Lc-CoA. FFA and Lc-CoA stimulate C-kinase enzymes (1, 28), which might be downregulated after long-term exposure to FFA with a resulting loss of a possible important component implicated in secretion. 3) Lc-CoA directly stimulate the exocytotic release of insulin in permeabilized HIT-β-cells (J. Deeney, B. Corkey, C. Rhodes, M. Prentki, and P.O. Berggren, unpublished observations). Thus glucose might not be active because secretion is already maximally promoted by this candidate metabolic coupling factor. 4) Lc-CoA, which are known to be elevated in FFA-treated β-cells, are very effective openers of K_{ATP} channels (15). Thus the β-cell might resist depolarization by glucose after chronic oleate treatment.

In addition to these direct effects, pretreatment with FFA may alter the expression of various proteins, including acetyl-CoA carboxylase, CPT-I, uncoupling protein 2, and enzymes of fat oxidation. Our data support a major alteration in fat oxidation, most likely due to changes in enzyme expression, in particular elevated CPT-I activity (3). This is supported by the increase in basal respiration, the more than doubling in elevated CPT-I activity (3). This is supported by the rise in glucose from 5 to 25 mM cannot further enhance secretion because the reduction state of pyridine nucleotides is already maximal at low (5 mM) glucose and consequently secretion cannot be further enhanced. 2) The elevated TG content of the β-cell is expected to result, after lipolysis, in a rise in cytosolic FFA and Lc-CoA. FFA and Lc-CoA stimulate C-kinase enzymes (1, 28), which might be downregulated after long-term exposure to FFA with a resulting loss of a possible important component implicated in secretion. 3) Lc-CoA directly stimulate the exocytotic release of insulin in permeabilized HIT-β-cells (J. Deeney, B. Corkey, C. Rhodes, M. Prentki, and P.O. Berggren, unpublished observations). Thus glucose might not be active because secretion is already maximally promoted by this candidate metabolic coupling factor. 4) Lc-CoA, which are known to be elevated in FFA-treated β-cells, are very effective openers of K_{ATP} channels (15). Thus the β-cell might resist depolarization by glucose after chronic oleate treatment.

In conclusion, chronic FFA treatment markedly alters the energy metabolism of the β-cell. The main response of INS-1 β-cells to long-term FFA treatment is to increase the mitochondrial capacity to oxidize and esterify FFA rather than altering glucose metabolism.

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