Symposium: The Role of Long Chain Fatty Acyl-CoAs as Signaling Molecules in Cellular Metabolism

The Role of Long-Chain Fatty Acyl-CoA Esters in β-Cell Signal Transduction[1,2]

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ABSTRACT Glucose-induced insulin secretion is associated with inhibition of free fatty acid (FFA) oxidation, increased esterification and complex lipid formation by pancreatic β-cells. Abundant evidence favors a role for cytosolic long-chain acyl-CoA (LC-CoA), including the rapid rise in malonyl CoA, the inhibitory effect of hydroxycitrate or acetyl CoA carboxylase knockout, both of which prevent malonyl CoA formation, and the stimulatory effect of exogenous FFA. On the other hand, some evidence opposes the concept, including the fall in total LC-CoA levels in response to glucose, the stimulatory effect of LC-CoA on K_{ATP} channels and the lack of inhibition of glucose-stimulated secretion either by overexpression of malonyl CoA decarboxylase, which markedly lowers malonyl CoA levels, or by triacsin C, which blocks FFA conversion to LC-CoA. Alternative explanations for these data are presented. A revised model of nutrient-stimulated secretion involving two arms of signal transduction that occur simultaneously is proposed. One arm depends on modulation of the K_{ATP} channel evoked by changes in the ATP/ADP ratio. The other arm depends upon anaplerotic input into the tricarboxylic acid cycle, generation of excess citrate, and increases in cytosolic malonyl-CoA. Input from this arm is increased LC-CoA. Signaling through both arms would be required for normal secretion. LC-CoA esters and products formed from them are potent regulators of enzymes and channels. It is hypothesized that their elevations directly modulate the activity of enzymes, genes and various β-cell functions or modify the acylation state of key proteins involved in regulation of ion channels and exocytosis. J. Nutr. 130: 299S–304S, 2000.

KEY WORDS: • *long chain acyl CoA* • *insulin secretion* • *malonyl CoA* • *ATP-sensitive K^+-channel* • *triascin C* • *free fatty acids* • *ATP/ADP ratio* • *B-cell*

The consensus model of nutrient-stimulated secretion consists of several steps. First, increased glycolysis and respiration due to glucose metabolism lead to an increase in the ATP/ADP ratio. Next, this closes the K_{ATP} channel, depolarizes the cell, increases the open time of voltage-dependent Ca^{2+} channels and raises intracellular Ca^{2+}. Finally, the increased Ca^{2+}, directly or through its receptor, calmodulin, modulates kinases or other effector systems. This model, which explains Ca^{2+} changes, is insufficient because K^+-induced secretion, which maximally increases Ca^{2+}, stimulates secretion only transiently (Hedeskov 1980). Although an increase in cytosolic free Ca^{2+} is a necessary component of normal insulin secretion (Prentki and Corkey 1996), it is not sufficient because the concentration dependence of glucose-induced insulin release remains intact under conditions in which Ca^{2+} is elevated maximally (in the presence of 30 mmol/L K^+) and in which K_{ATP} channels are bypassed (in the presence of diazoxide) (Gembal et al. 1992). Thus, the concept is emerging that glucose also controls insulin release independently of its action on K_{ATP} channels and that Ca^{2+} plays only a permissive role in glucose-induced insulin secretion (Berggren and Larsson 1994).

A more inclusive model of nutrient-stimulated secretion involves two arms of signal transduction that occur simultaneously. One arm is dependent upon modulation of the K_{ATP} channel, evoked by changes in the ATP/ADP ratio as outlined above. Input from this arm would be an increase in cytosolic Ca^{2+} and, secondary to this, changes in cAMP and phospholipids. The other arm is dependent upon anaplerotic input into the tricarboxylic acid cycle, generation of excess citrate and

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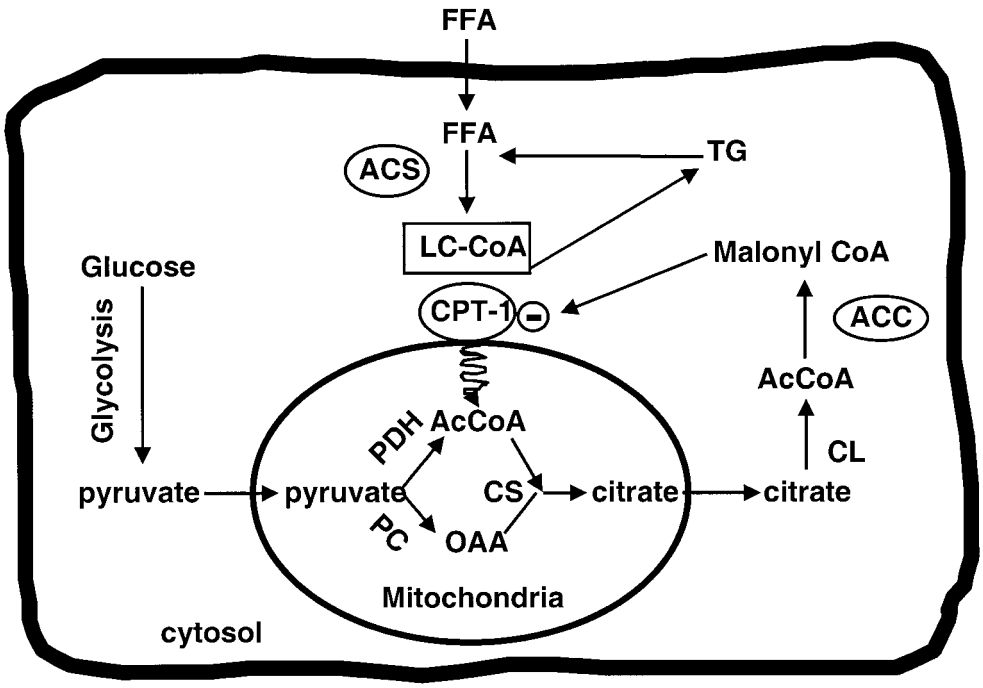

FIGURE 1 Pathways for formation of cytosolic long-chain acyl-CoA (LC-CoA). Abbreviations: FFA, free fatty acids; TG, triglyceride; ACS, acyl-CoA synthetase; CPTI; carnitine palmitoyltransferase I; PDH, pyruvate dehydrogenase; PC, pyruvate carboxylase; CS, citrate synthase; CL, citrate lyase; OAA, oxaloacetate.

increases in cytosolic malonyl-CoA (Prentki and Corkey 1996, Roche et al. 1998). Input from this arm is increased cytosolic long-chain acyl-CoA (LC-CoA)[4] and, secondary to this, the synthesis of complex lipids. We have proposed that a key coupling factor is cytosolic LC-CoA, the activated, energy-rich, intracellular form of free fatty acids (FFA) and actual substrate for FFA metabolizing enzymes, which rises as a consequence of glucose metabolism (Prentki and Corkey 1996). Signaling through both arms would be required for a normal secretory response to glucose. Therefore, the anaplerosis-dependent arm of the model is K_{ATP} channel independent. The action of glucose in the absence of Ca^{2+} can be mimicked by FFA (Komatsu and Sharp 1998), consistent with the notion that both glucose and FFA are signaling through cytosolic LC-CoA.

It should also be noted that insulin secretion is oscillatory as observed in humans and animals in vivo and from the perfused pancreas and perifused islets in vitro (Tornheim 1997). The physiologic importance of the oscillatory mode is suggested by its loss in patients with NIDDM and their near relatives. We have suggested (Tornheim 1997) that oscillations characterize most steps of stimulus-secretion coupling, starting with oscillatory glucose metabolism and associated rises in the ATP/ADP ratio, causing closure of K_{ATP} channels, and thus leading to the oscillations in membrane potential and intracellular free Ca^{2+} that have been observed in glucose-stimulated single β-cells and islets. Oscillatory metabolism would also characterize the anaplerotic steps and LC-CoA.

FFA appear to be a major source of energy for islets (Malaisse et al. 1983). Glucose stimulation of β-cells diminishes fatty acid oxidation and increases total respiration (Prentki and Corkey 1996). Thus, one of the metabolic events induced by glucose stimulation appears to be a relative shift from FFA to glucose as an oxidative fuel. We have hypothesized that this occurs through glucose conversion to the "switch" compound, malonyl-CoA, which in turn inhibits carnitine palmitoyltransferase (CPTI) and thus blocks LC-CoA transport into the mitochondria (McGarry and Foster 1980).

It has been demonstrated (Corkey et al. 1989, Liang and Matschinsky 1991, Prentki et al. 1992) that stimulation of insulin secretion by glucose causes marked alterations in the CoA profile of clonal pancreatic β-cells, with the largest and earliest (by 2 min) change occurring in malonyl-CoA and LC-CoA (Prentki et al. 1992). Anaplerosis is essential for the production of malonyl-CoA because efflux of citrate, its mitochondrial precursor, does not occur unless there has been compensatory input into the citric acid cycle. Inhibition of mitochondrial fatty acid (LC-CoA) oxidation presumably leads to an elevation of LC-CoA in the cytosol, which is the precursor for triglyceride, diacylglycerol and phospholipids (Prentki and Corkey 1996).

Glucose-induced insulin secretion is associated with inhibition of FFA oxidation, increased FFA esterification and complex lipid formation by pancreatic β-cells (Prentki and Corkey 1996). Significant increases occur in the total mass of diacylglycerol (DAG) (Peter-Riesch et al. 1988), triglyceride (Berne 1975) and phosphatidic acid (PA) (Farese et al. 1986) in glucose-stimulated β-cells. Indeed, islets contain high levels of triglyceride similar to liver (Malaisse et al. 1983). Glucose and endogenous LC-CoA are the main sources of glycerol and lipid components, respectively, of DAG and PA. In addition, exogenous FFA acutely potentiate glucose-stimulated secretion (Prentki and Corkey 1996), possibly by providing additional acyl groups for LC-CoA formation or complex lipid synthesis.

The steps involved in the glucose-induced increase in LC-CoA are shown in **Figure 1**. It should be noted that acetyl CoA carboxylase (ACC) in β-cells appears to act as a regulatory signal generator rather than as a step in FFA biosynthesis because fatty acid synthase is very low in the islet,

[4] Abbreviations used: ACC, acetyl CoA carboxylase; ACS, acyl-CoA synthetase; ANT, adenine-nuceotide transferase; CL, citrate lyase; CPTI, carnitine palmitoyltransferase I; CS, citrate synthase; DAG, diacylglycerol; FFA, free fatty acids; GK, glucokinase; LC-CoA, long-chain acyl-CoA; MCD, malonyl CoA decarboxylase; OAA, oxaloacetate; PA, phosphatidic acid; PC, pyruvate carboxylase; UCP, uncoupling protein.

whereas ACC protein and activity are expressed at appreciable levels (Brun et al. 1996). The reason why FFA alone do not stimulate secretion in the absence of glucose is probably due to their rapid entry into the mitochondria when malonyl-CoA levels are low. It is noteworthy that CPTI is abundant in islets and that an enzyme of the fatty acid oxidation pathway, β-hydroxyacyl-CoA dehydrogenase, is expressed in the islet at a level that is among the highest in all tissues (Hammar and Berne 1970).

The identification of LC-CoA rather than malonyl-CoA as the effector signal is based on the finding that pharmacologic inhibition of CPTI, which bypasses malonyl-CoA, enhances glucose-induced secretion as do exogenous FFA (McGarry and Dobbins 1999, Prentki and Corkey 1996). However, this does not diminish the physiologic importance of malonyl-CoA which, by regulating CPTI and the level of cytosolic LC-CoA, determines fuel partitioning (the relative rates of glucose and FFA oxidation in the β-cell) and the fate of LC-CoA (oxidation, esterification or acylation). Hence, malonyl-CoA can be considered a *regulatory signaling molecule* in insulin secretion, whereas LC-CoA acts as an *effector signal*. ACC, which controls the synthesis of malonyl-CoA, the "signal of plenty," and CPTI, which is regulated by it, should be considered integrators of the concentrations of all circulating fuel stimuli. Indeed, the metabolism of various classes of nutrient stimuli (carbohydrate, ketoacids and amino acids) converge to form malonyl-CoA and increase LC-CoA esters. Thus, we propose that ACC and CPTI are "fuel sensors" in the β-cell, in comparison to the "glucose sensor," glucokinase, which senses only glucose.

Cytosolic concentrations of LC-CoA esters are controlled by feedback inhibition of acyl-CoA synthetase (ACS) and are buffered by fatty acid and LC-CoA binding proteins (Boylan and Hamilton 1992). LC-CoA in the micromolar range modulates the activity of enzymes, receptors and transporters, including the adenine nucleotide translocase, CPTI, the tricarboxylic acid carrier, the nuclear thyroid hormone receptor, the ATP-sensitive K^+-channel and several ATPases. Of particular interest is the finding that LC-CoA esters modulate the activity of proteins that contain adenine or guanine nucleotide binding sites, possibly as a consequence of the similarities in structure with coenzyme A (Prentki and Corkey 1996). In the case of the ATP-sensitive K^+-channel, LC-CoA opens the channel, whereas the corresponding FFA closes the channel (Bränström et al. 1997, Larsson et al. 1996). LC-CoA has also been shown to be essential for vesicular processing through the Golgi (Glick and Rothman 1987). Furthermore, LC-CoA has been shown to be the major cytosolic component required to induce complex formation of VIP21-caveolin (Monier et al. 1996), one of the components that form the cytoplasmic surface of caveolae and are hypothesized to play a role in vesicle or lipid trafficking. This is believed to be a consequence of acylation of a vesicular protein.

The total CoA pool is fixed, over short intervals, and distributed unevenly between mitochondrial and cytosolic pools that are not interchangeable (Corkey 1988). Thus, the maximum LC-CoA concentration is limited by the total CoA pool and compartmental distribution. There is a reciprocal relationship between cytosolic and mitochondrial pools of LC-CoA such that when CPTI is inhibited, the cytosolic pool rises and the mitochondrial pool diminishes. In response to CPTI inhibition, measured changes in total cellular LC-CoA may increase or decrease depending on the percentage of the pool in mitochondria in which the CoA concentration tends to be higher. High fat and certain drugs or steroids have the potential to increase this pool over a period of hours to days. The actual cytosolic free concentration of LC-CoA is not known in any cell type, but the total concentration is 94 and 219 nmol/g dry weight in the livers of fed and food-deprived rats, respectively (Corkey 1988). On the basis of Scatchard analysis of palmitoyl CoA binding in permeabilized β-cells, there is an estimated half-maximal free cytosolic concentration of ~1 μmol/L (Deeney et al. 1992).

Evidence that a rise in cytosolic LC-CoA plays a role in signaling is indirect and based on the following findings. First, addition of FFA increases total LC-CoA (Prentki and Corkey 1996). Second, although glucose acutely lowers total LC-CoA, due to consumption of mitochondrial levels, their replenishment through CPTI is inhibited (Prentki and Corkey 1996); it must increase the cytosolic pool because complex lipid synthesis, which is regulated by LC-CoA availability, is stimulated (Prentki and Corkey 1996). Third, 30 min stimulation of islets with glucose increases total LC-CoA (Liang and Matschinsky 1991). Fourth, pharmacologic inhibition of LC-CoA transport into the mitochondria, which should elevate cytosolic LC-CoA, enhances glucose-induced secretion (Chen et al. 1994, Vara and Tamarit-Rodriguez 1986). Fifth, inhibition of malonyl CoA production from glucose, which should prevent the rise in cytosolic LC-CoA, blocks glucose-induced insulin secretion (Chen et al. 1994).

Abundant evidence favors a role for LC-CoA in secretion, including the rapid rise in malonyl CoA induced by fuels, the inhibitory effect of hydroxycitrate (Chen et al. 1994) or ACC knockout (Zhang and Kim 1998), both of which prevent malonyl CoA formation, and the stimulatory effect of exogenous FFA addition (Prentki and Corkey 1996). On the other hand, four pieces of evidence that might seem to oppose the concept are the fall in total LC-CoA levels in response to glucose (Prentki et al. 1992), the stimulatory effect of LC-CoA on K_{ATP} channels (Bränström et al. 1997, Larsson et al. 1996), and the lack of inhibition of glucose-stimulated secretion either by overexpression of malonyl CoA decarboxylase (MCD) (Antinozzi et al. 1998), which markedly lowers malonyl CoA levels, or by triacsin C (Antinozzi et al. 1998), which blocks FFA conversion to LC-CoA. However, there are alternative explanations for each of the four disparate data sets, such as the following.

1) A likely explanation for the fall in LC-CoA is compartmentation, such that LC-CoA falls in the larger mitochondrial compartment and rises in the smaller cytosolic compartment. Support for this possibility is derived from observations in β-cells and the fact that liver lipid synthesis, for which cytosolic LC-CoA is the precursor, is associated with decreases in total cellular LC-CoA (McGarry and Dobbins 1999, Prentki and Corkey 1996).

2) The quandary of the stimulatory effect of LC-CoA on K_{ATP} channels could be due to LC-CoA playing a major role in the "off" response (repolarization), rather than the "on" response (depolarization), and changing like the ATP/ADP ratio in an oscillatory manner but out of phase with the ATP/ADP ratio. There are no data yet for or against this hypothesis but we predict, if this is correct, that LC-CoA levels will oscillate out of phase with the ATP/ADP ratio.

3) The lack of effect of overexpression of MCD (Antinozzi et al. 1998) could be due to its localization in a noncytosolic compartment. MCD from goose is targeted to the mitochondria but the construct used in these experiments was engineered to remove its mitochondrial signal sequence; however, it retained a peroxisomal localization signal (Courchesne-Smith et al. 1992), *Ser-Lys-Leu* in the C-terminus of the molecule (Voilley et al. 1999). The distribution of malonyl CoA is not known, but the fact that the percentage of change

in malonyl CoA levels in glucose-stimulated cells is not impaired in the MCD overexpressing cells suggests compartmentation. Overexpression of MCD in peroxisomes may then decrease only the malonyl CoA transferred to or stored in that compartment and have little effect on the rapid changes in the cytosolic pool. It is predicted that if MCD were able to prevent the threefold rise in malonyl CoA in response to glucose, secretion would be blocked.

4) The failure of triacsin C to inhibit insulin secretion despite its ability to inhibit FFA oxidation and lipid synthesis could be due to different LC-CoA synthetases that vary in their sensitivity to triacsin C or selective channeling of LC-CoA to specific sites (Igal et al. 1997). Consistent with that idea, Antinozzi et al. (1998) showed variations in the ability of triacsin C to block various processes. Exogenous FFA oxidation was inhibited by almost 80%, whereas inhibition of glucose conversion to lipid varied from 25 to 60%, depending on the concentration of glucose, and LC-CoA levels were decreased by only ~45%. In a similar vein, triacsin C blocked de novo synthesis of glycerolipids and cholesterol esters but not recycling of FFA into phospholipids in human fibroblasts (Igal et al. 1997). If such channeling occurs, endogenous FFA oxidation and lipid turnover might not be greatly affected by triacsin C. Alternatively, triacsin C, like other inhibitors of lipid metabolism such as bromopalmitate or tetradecylglycidic acid, may perhaps be activated to a CoA ester (Lieu et al. 1997, McGarry and Dobbins 1999, Prentki and Corkey 1996), block CPTI activity and could actually elevate cytosolic LC-CoA levels.

It should be noted that what is at issue here is whether LC-CoA plays an important role in glucose-induced insulin secretion. It is clear that FFA, presumably via LC-CoA, generate important signals for insulin secretion because secretion is dramatically stimulated by exogenous or endogenous FFA (Prentki et al. 1992). Furthermore, depletion of lipid stores together with deprivation of FFA prevents secretion (McGarry and Dobbins 1999).

Most models of exocytosis include Ca^{2+} as the trigger for the final stage of vesicle fusion (Jahn and Hanson 1998). In contrast, insulin release is not regulated by Ca^{2+} alone. Islets incubated with diazoxide and KCl, to maintain elevated Ca^{2+} and avoid metabolic regulation through the K_{ATP} channel, exhibit concentration-dependent glucose-stimulated insulin secretion (Gembal et al. 1992). Furthermore, oscillatory insulin secretion also occurs under these conditions and others in which Ca^{2+} is not changing (Cunningham et al. 1996). This suggests that Ca^{2+} may have more of a permissive role in the regulation of insulin exocytosis from the β-cell. In this regard, insulin release has not always coincided with elevated Ca^{2+}. A dissociation between Ca^{2+} and insulin release has been demonstrated in pancreatic islets subjected to increased phosphorylation (Zaitsev et al. 1995). In that study, glucose-induced insulin release continually increased even as Ca^{2+} fell. A number of signals derived from glucose metabolism may play a role in modulating Ca^{2+}-induced insulin release, including an increase in DAG or acylation by LC-CoA. Indeed, insulin release from pancreatic islets is enhanced even in the absence of extracellular Ca^{2+} under conditions of increased phosphorylation (Komatsu et al. 1995). Addition of FFA stimulates insulin secretion even further under these conditions (Komatsu et al. 1995).

The link between metabolism and exocytosis has received less attention than the earlier steps in the β-cell stimulus-coupling cascade. Exocytosis is a complex multistep process, involving vesicle movement, docking, priming and finally membrane fusion (Wollheim et al. 1996). Some exocytotic proteins such as Munc-18, and SNAP-25 are subject to phosphorylation by protein kinase C (Fujita et al. 1996). Others, such as SNAP-25 (Hess et al. 1992) and CSP (Braun and Scheller 1995), are acylated to enhance association to their target membranes and thus are potentially sensitive to changes in LC-CoA. These types of post-translational modification of proteins involved in the exocytotic process may play a role in the modulation of Ca^{2+}-induced secretion of insulin.

LC-CoA esters and products formed from them are potent regulators of enzymes and channels. High circulating FFA and certain drugs or steroids have the potential to increase the total CoA pool over a period of hours to days (Chen et al. 1992, Corkey 1988, Woldegiorgis et al. 1985). It is hypothesized that the elevations in LC-CoA, PA and DAG resulting from glucose stimulation could directly modulate the activity of enzymes, including protein kinase C isoforms, or modify the acylation state of key proteins involved in regulation of ion channel activity and exocytosis. **Figure 2** illustrates several potential sites of action of LC-CoA as key regulators of enzymes, genes and various β-cell functions (Prentki and Corkey 1996). They inhibit the activities of glucokinase, glucose-6-phosphatase, ACC and certain protein kinase C isoforms. They stimulate the activities of other protein kinase C isoforms, and the endoplasmic reticulum Ca^{2+}-ATPase (Deeney et al. 1992); activate peroxisome proliferation (Keller and Wahli 1993); and also overcome malonyl-CoA inhibition of CPTI activity (Mills et al. 1983). LC-CoA esters accelerate the transfer of proteins from the *cis-* to the *trans*-Golgi by increasing the budding of vesicles from the *cis*-Golgi and their fusion to the *trans*-Golgi compartment. Our current work shows LC-CoA stimulation of exocytosis from permeabilized clonal β-cells (Deeney et al., unpublished data) and potent stimulation of the ATP-sensitive K^+-channel (Bränström et al. 1997 and 1998, Gribble et al. 1998, Larsson et al. 1996). Not shown is the LC-CoA inhibition of adenine nucleotide translocase (Woldegiorgis et al. 1982), which plays an important role in controlling the cytosolic ATP/ADP ratio, and the sodium pump, which is stimulated by LC-CoA in some cells (Prentki and Corkey 1996).

Protein acylation appears essential for the process of signaling through GTP-binding proteins (G-proteins), possibly as a means of targeting these proteins to appropriate membrane sites (Schmidt 1989). All α-subunits are modified by saturated fatty acyl chains, either by a myristoyl or a palmitoyl moiety. Mutation in palmitoylation sites of α-subunits impairs their regulatory function (Bouvier et al. 1995). It is interesting to note in this regard that several G-proteins have been implicated in exocytosis (Olszewski et al. 1994). Thus, LC-CoA are proposed to exert multiple potent effects on diverse β-cell functions from glycolysis and energy metabolism, to signal transduction, exocytosis and gene expression.

A key question as insulin resistance escalates to diabetes, is whether signaling abnormalities occur in β-cells, insulin target tissues, and possibly, the hypothalamic-pituitary-adrenal axis, concurrently or sequentially in response to increased circulating lipids, or whether FFA may produce insulin resistance and obesity even when they do not produce hyperinsulinemia or altered patterns of insulin secretion. It should be noted that the altered responsiveness to FFA refers mainly to studies in which either palmitate or oleate, the most prevalent FFA in the circulation and in the LC-CoA pools (Corkey 1988, Woldegiorgis et al. 1985), were used. The influence of polyunsaturated, *trans* or other less prevalent fatty acids has not been evaluated extensively and may differ from the more common FFA. Animal studies have documented a strong correlation between LC-CoA content in liver and skeletal

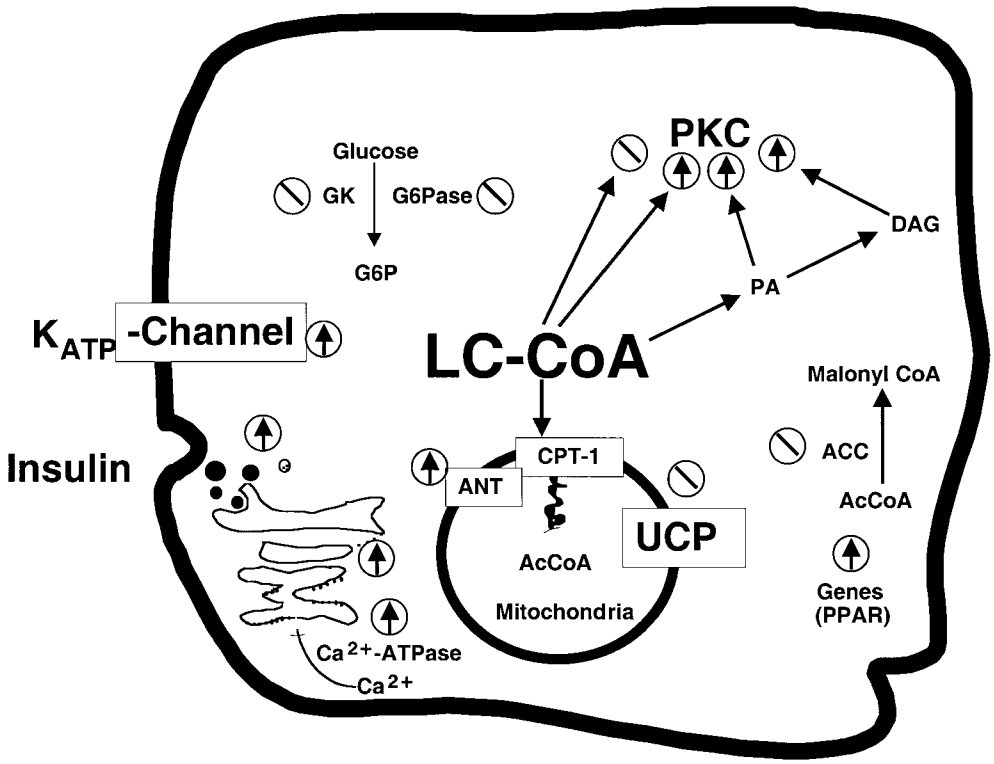

FIGURE 2 Long-chain acyl-CoA (LC-CoA) as an effector of numerous cellular processes. Abbreviations: PKC, protein kinase C; DAG, diacylglycerol; CPTI, carnitine palmitoyltransferase I; ACC, acetyl CoA carboxylase; PPAR, peroxisome proliferator-activated receptor; ANT, adenine-nucleotide transferase; UCP, uncoupling protein; GK, glucokinase.

muscle, and plasma insulin levels in rats fed a diet high in saturated fat. In the same study, a positive correlation between tissue LC-CoA content and body weight or weight gain was also found (Chen et al. 1992). Also noteworthy is the observation that exposure of pancreatic islets to palmitate or oleate for several days leads to basal hypersecretion of insulin (Milburn et al. 1995) and that FFA-treated β-cells have increased LC-CoA content (Prentki et al. 1992). Thus, we present the concept that altered cell content of LC-CoA is an early common feature shared by several cell types exposed to elevated FFA.

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