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Regulation of lipolytic activity by long-chain acyl-coenzyme A in islets and adipocytes

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Hu, Liping, Jude T. Deeney, Christopher J. Nolan, Marie-Line Peyot, Ada Ao, Ann Marie Richard, Esthere Luc, Nils J. Faergeman, Jens Knudsen, Wen Guo, Maria Sorhede-Winzell, Marc Prentki, and Barbara E. Corkey. Regulation of lipolytic activity by long-chain acyl-coenzyme A in islets and adipocytes. *Am J Physiol Endocrinol Metab* 289: E1085–E1092, 2005. First published August 9, 2005; doi:10.1152/ajpendo.00210.2005.—Intracellular lipolysis is a major pathway of lipid metabolism that has roles, not only in the provision of free fatty acids as energy substrate, but also in intracellular signal transduction. The latter is likely to be particularly important in the regulation of insulin secretion from islet β-cells. The mechanisms by which lipolysis is regulated in different tissues is, therefore, of considerable interest. Here, the effects of long-chain acyl-CoA esters (LC-CoA) on lipase activity in islets and adipocytes were compared. Palmitoyl-CoA (Pal-CoA, 1–10 μM) stimulated lipase activity in islets from both normal and hormone-sensitive lipase (HSL)-null mice and in phosphatase-treated islets, indicating that the stimulatory effect was neither on HSL nor phosphorylation dependent. In contrast, we reproduced the previously published observations showing inhibition of HSL activity by LC-CoA in adipocytes. The inhibitory effect of LC-CoA on adipocyte HSL was dependent on phosphorylation and enhanced by acyl-CoA-binding protein (ACBP). In contrast, the stimulatory effect on islet lipase activity was blocked by ACBP, presumably due to binding and sequestration of LC-CoA. These data suggest the following intertissue relationship between islets and adipocytes with respect to fatty acid metabolism, LC-CoA signaling, and lipolysis. Elevated LC-CoA in islets stimulates lipolysis to generate a signal to increase insulin secretion, whereas elevated LC-CoA in adipocytes inhibits lipolysis. Together, these opposite actions of LC-CoA lower circulating fat by inhibiting its release from adipocytes and promoting fat storage via insulin action.

acyl-coenzyme A-binding protein; free fatty acid; hormone-sensitive lipase; lipid signaling

INTRACELLULAR LIPOLYSIS IS A MAJOR PATHWAY of lipid metabolism, the function of which is highly tissue specific. Lipolysis in the adipocyte has received the most attention, and it serves the important role of releasing free fatty acids (FFA) from adipose triglyceride (TG) stores into the circulation for whole body energy provision at times of need such as during the fasted state and exercise (20). Lipolysis in the pancreatic β-cell, however, has been proposed to have an important role in providing FFA for the production of lipid-signaling molecules important in the regulation of normal insulin secretion (29, 33, 46). With such divergent roles between tissues, it is likely that the lipase enzymes involved and the mechanisms regulating lipolysis will be highly tissue specific.

Adipocyte lipolysis is highly regulated according to nutritional status. Hormone-sensitive lipase (HSL, EC 3.1.1.3), an acylglycerol lipase with broad substrate specificity, is believed to be the key enzyme involved (16, 20), although there is evidence that other lipases are also present in fat cells (30). HSL in adipose tissue is activated by hormones such as catecholamines, adrenocorticotropic hormone, and glucagon via stimulating adenylate cyclase activity, which increases cAMP (16, 20). The increased cAMP activates protein kinase A (PKA) which, in turn, activates HSL activity by phosphorylation (16, 20). Insulin inhibits HSL through phosphodiesterase-3-dependent cAMP degradation (16, 20). It has also been proposed that long-chain acyl-CoA esters (LC-CoA) may have a role in regulating lipases (18, 27, 39) and, in particular, adipocyte HSL activity (18, 39).

The role of islet lipolysis in the regulation of normal insulin secretion and the lipases involved is poorly understood. However, it is well established that FFA have important permissive (9) and amplification roles (38) in insulin secretion and, if chronically elevated, particularly in association with hyperglycemia, can induce β-cell failure (11, 34). Both exogenous and endogenous (from TG hydrolysis) sources of FFA are believed to be involved. Of relevance to endogenous sources, it has been demonstrated that inhibition of islet lipolysis by pharmacological agents (26, 29, 46) and enhanced lipolysis by overexpression of HSL (22) both impair insulin secretion. Interestingly, a β-cell-specific isoform of HSL has been determined (22), but experiments in $HSL^{-/-}$ mice show that this enzyme is not important (28), or important in only very specific circumstances (33), for normal glucose-stimulated insulin secretion. Clearly, alternative lipase/s may have much more critical roles in the islet (33). Elevated glucose has been shown to stimulate

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lipolysis in both rat (29) and mouse (33) islets, including $HSL^{-/-}$ mouse (33) islets. It is unknown whether LC-CoA can modulate lipase activity in islets.

LC-CoA and their derivatives are regarded not only as intermediates of lipid biosynthesis but also as potent regulators of enzymes, ion channels, and various signal-transducing effectors (12). In β-cells, LC-CoA esters activate ATP-sensitive K^+ (K_{ATP}) channels (2, 3, 15, 21), modulate intracellular Ca^{2+} (8), activate atypical PKC isoforms (47), and directly stimulate exocytosis resulting in insulin secretion (7). Of relevance to lipolysis, previous studies document that 25 μmol/l palmitoyl-CoA (Pal-CoA) and 40 μmol/l oleoyl-CoA inactivate lipase activity half-maximally in pigeon adipose tissue, whereas 100 μmol/l Pal-CoA reduces lipase activity by 90% (39). Perfusion of rat hearts with 100 and 200 μmol/l Pal-CoA reduces lipase activity by 17 and 67%, respectively (27). HSL from bovine perirenal adipose tissue is inhibited 50% by 0.1 μmol/l oleoyl-CoA (18).

It has been suggested that fatty acid-binding protein (FABP) and acyl-CoA-binding protein (ACBP), with their high affinity for LC-CoA, effectively buffer acyl-CoA concentrations. This may play an important role in the metabolism and partitioning of LC-CoA esters (43). It is well established that ACBP/LC-CoA interactions can modulate activity of several microsomal acyl-CoA-utilizing enzymes (36). ACBP can create an intracellular acyl-CoA pool and shield acyl-CoA esters from acyl-CoA hydrolases (19, 24). Furthermore, ACBP can protect acetyl-CoA carboxylase from the inhibitory effects of acyl-CoA esters (36). Of relevance to lipolysis, Bernlohr and Kraemer recently documented a protein-protein interaction between HSL and FABP in vitro and in vivo and an increased HSL activity with FABP addition (40, 42).

To better understand the role of LC-CoA in the regulation of lipolysis, including HSL and non-HSL lipase activities, and in signal transduction in adipocytes and islets, we assessed lipase enzyme activities in various preparations of these tissues, including $HSL^{-/-}$ tissues, in the presence and absence of Pal-CoA. The effects of interaction between Pal-CoA and ACBP on lipase activities were also assessed. We found that Pal-CoA markedly stimulated islet non-HSL lipase activity, whereas it inhibited HSL in adipose tissue. ACBP enhanced the inhibitory effect of Pal-CoA on HSL in adipocytes while diminishing the stimulatory effect of Pal-CoA on islet lipase.

MATERIALS AND METHODS

Animals. Sprague-Dawley rats and C57BL/6 mice were purchased from Charles River Laboratories. The animals were housed in the Laboratory Animal Science Center at Boston University Medical Center. $HSL^{+/+}$ and $HSL^{-/-}$ islets were obtained from mice generated by Mitchell and Prentki (33, 37, 44). The experimental protocols were approved by the Institutional Animal Care and Use Committees at Boston University Medical Center and the Centre Hospitalier de l'Université de Montréal.

Materials. Purified bovine ACBP was a gift from Faergeman and Knudsen (23). Collagenase *P* was from Roche. Pal-CoA, was from Pharmacia. Palmitate, oleate, Phenyl-Sepharose CL-4B, protease inhibitor cocktail, phosphatase inhibitor cocktail, *p*-nitrophenyl butyrate (PNPB) (31), *p*-nitrophenol standard solution, ethylene glycol, Hanks' balanced salts, PKA, and protein phosphatase 2A (PP2A) were from Sigma. 3-[(3-Cholamidopropyl)dimethylammonio]-1-propanesulfonate (CHAPS) was from Fluka. Amicon ultrafilters were from Millipore.

Isolation of mouse pancreatic islets and subcellular fractionation. Islets from fed 3- to 5-mo-old male mice were isolated by collagenase digestion of the total pancreas, as previously described (33). Islets were handpicked under a stereomicroscope. At the end of the isolation step, islets were cultured overnight at 37°C in a humidified atmosphere (5% CO_2-95% air) in RPMI 1640 medium supplemented with 24 mmol/l $NaHCO_3$, 50 U/ml penicillin, 50 μg/ml streptomycin, 0.03 mmol/l glutathione, 0.1 μmol/l selenious acid, and 10% fetal calf serum, pH 7.2. Islets were then washed and frozen in liquid nitrogen for the lipase activity assay. For subcellular fractionation, frozen islets were resuspended in 6 volumes of homogenization buffer [0.25 mol/l sucrose, 1 mmol/l dithiothreitol, 1 mmol/l EDTA, 1% phosphatase inhibitor cocktail (Sigma P5726), 1% protease inhibitor cocktail (Sigma P8340), pH 7.4] and homogenized by being passed through a 27-gauge needle five times on ice (45). The homogenate was centrifuged at 16,000 *g*, and the resulting supernatant was then centrifuged at 160,000 *g* for 1 h at 4°C, yielding cytosol. Because phosphorylated HSL has higher hydrolytic activity than dephosphorylated protein (31), protein phosphatase inhibitors were used to maintain protein in the phosphorylated state. Homogenization buffer was used throughout the entire fractionation and centrifugation procedures.

Fat tissue preparation and subcellular fractionation. Cytosol was prepared from perirenal and epididymal fat pads from 200- to 220-g rats. Whole perirenal and epididymal fat pads were removed, minced, and then homogenized with 10 strokes in homogenization buffer containing 20 mmol/l Tris·HCl, 0.25 mol/l sucrose, 1 mmol/l EDTA, 1 mmol/l benzamidine, 1% protease inhibitor cocktail, and 1% phosphatase inhibitor cocktail, pH 7.4. Postmicrosomal supernatant was prepared as described above.

Purification of HSL from rat adipose tissue. HSL protein was purified according to the acid precipitation method of Egan (10). After chromatography with Phenyl-Sepharose and elution with ethylene glycol, most hydrolytic activity was in the 45–65% ethylene glycol elution fractions. A protein of 84 kDa was observed in SDS-PAGE. About 10% of the final fraction was HSL (data not shown). Peak activity fractions were pooled and diluted 3.5-fold with the eluting solution containing 0.05% CHAPS and concentrated in a 15-ml Amicon ultrafilter using a PM30 membrane. The final solution was ~30 times more concentrated than the initial pooled column eluate. Fresh or once-frozen (−70°C) concentrated protein from three separate preparations was used for lipase activity experiments. After purification, >90% of cytosolic HSL activity remained for up to 2 days at 4°C, whereas ~20–30% activity was lost after freeze-thawing.

Lipase activity assay and protein determination: lipase activity assay in cell extracts. PNPB, which is a water-soluble lipase substrate, can be digested by both esterases and lipases. TG is a natural substrate of lipases. Compared with other substrates, hydrolytic activity against PNPB is ~20-fold greater (20). To differentiate the possible regulatory role of LC-CoA role on lipase and esterase activities, both PNPB and TG were used as substrates. Lipase/esterase activity against PNPB was determined by measuring the rate of release of *p*-nitrophenol by the procedure described by Osterlund et al. (31). LC-CoA esters were dissolved in H_2O. About 6 μg of the partially purified protein or 30 μg of islet cytosolic protein (representing 150 islets) were added to 1 ml of reaction mixture containing 50 mmol/l NaH_2PO_4 buffer, 0.5 mmol/l PNPB, 1% (vol/vol) acetonitrile, 10% glycerol, and 0.1 mol/l NaCl, pH 7.25, with or without PP2A. PP2A dephosphorylates HSL. The reaction was then incubated for 20 min at 30°C. After addition of 10 μmol/l Pal-CoA, incubation continued for another 10 min at 37°C. Hydrolysis was stopped by addition of 3.25 ml of methanol-chloroform-heptane (10:9:7 vol/vol/vol), followed by vigorous shaking and centrifugation at 1,000 *g* for 1 min. Thereafter, the solution was incubated at 42°C for 3 min. Activity was measured as absorbance at 400 nm of the supernatant containing *p*-nitrophenol. One unit of enzyme activity is equivalent to 1 μmol of fatty acid released per minute at 37°C. Lipase activity against TG was determined by measuring glycerol in islets homogenate with a commercial kit

(Sigma). Briefly, partially purified HSL or islet homogenate was incubated with an emusified mixture of soybean oil at 37°C for 30 min. Islet homogenate without TG addition was blank. The mixture was then incubated with glycerol measurement reagent at 30°C for another 15 min. In the reaction, glycerol is phosphorylated by adenosine-5′-triphosphate (ATP) forming glycerol 1-phosphate (G-1-*P*) and adenosine-5′-diphosphate (ADP) in a reaction catalyzed by glycerol kinase (GK). G-1-*P* is then oxidized by glycerol phosphate oxidase (GPO) to dihydroxyacetone phosphate (DAP) and hydrogen peroxide (H_2O_2). Peroxidase (POD) catalyzes the coupling of H_2O_2 with 4-aminoantipyrine (4-AAP) and sodium *N*-ethyl-*N*-(3-sulfopropyl)-m-anisidine (ESPA) to produce a quinoneimine dye that has absorbance maximum at 540 nm (4).

Lipase activity assay in intact islets. Mouse islets were prepared and recovered in culture medium overnight as described above. On the next day, 100 islets were incubated in Krebs-Ringer bicarbonate buffer (KRB) containing (in mmol/l) 119 NaCl, 4.6 KCl, 5 $NaHCO_3$, 2 $CaCl_2$, 1 $MgSO_4$, 0.15 Na_2HPO_4, 0.4 KH_2PO_4, and 20 HEPES and 0.5% BSA, pH 7.4, with different glucose concentrations in the presence or absence of 0.25 mmol/l FFA (palmitate/oleate 1:1 ratio) overnight, and KRB buffer was then collected and concentrated to 50 μl. Released glycerol was measured using the commercial kit as described above. Protein was determined by the Lowry method (1), using bovine serum albumin as standard.

Statistical analysis. Means and standard errors (SE) were calculated. Student's *t*-test for unpaired data or, for multiple comparisons, one-way ANOVA with Dunnett post testing was used. Differences were considered significant at $P < 0.05$.

RESULTS

Pal-CoA-stimulated islet lipase activity. Although an inhibitory effect of LC-CoA has been reported on adipocyte lipase activity, its capacity to regulate lipase activity in islets has not been assessed. To evaluate this, islet cytosol was prepared from ~1,500 mouse islets (~200 islets were obtained from each mouse). Lipase activity was measured in the presence of 0–20 μmol/l Pal-CoA. Surprisingly, rather than inhibition, Pal-CoA caused an increase in hydrolytic activity in mouse islets against PNPB in a concentration-dependent manner (Fig. 1*A*) and against TG (Fig. 1*B*). In addition, islets incubated with 0.25 mmol/l FFA (palmitate/oleate 1:1 ratio) overnight exhibited increased glycerol release (Fig. 1*C*).

The stimulatory lipolytic response to LC-CoA in islets, as opposed to the inhibitory response in fat, could be due to the expression of different lipases or alternate regulation of the same lipases. Of note, islet and adipose tissue have different HSL isoforms, which could vary in response to regulating factors (22). To further assess this, lipase activities against PNPB and TG, in the absence and presence of Pal-CoA, were determined in cytosolic extracts from $HSL^{+/+}$ and $HSL^{-/-}$ mice islets. Although basal TG lipolysis (Fig. 2*B*) was reduced in the $HSL^{-/-}$ islets, LC-CoA activation of lipase activity against both the PNPB (Fig. 2*B*) and TG (Fig. 2*A*) substrates was robust and unaltered in both $HSL^{+/+}$ and $HSL^{-/-}$ islets. Clearly, alternate lipase/s contribute, but are unable to completely normalize, basal TG lipase, as we have previously reported. Furthermore, it is these alternate lipase/s that are activated by Pal-CoA in islets.

Pal-CoA inhibits both purified HSL and cytosolic lipase activities from rat adipose tissue. Considering the potent effect of Pal-CoA to activate lipase activities in islets, we thought it was important to confirm the previously reported inhibitory effect of LC-CoA on lipase activities of adipocytes (18, 39)

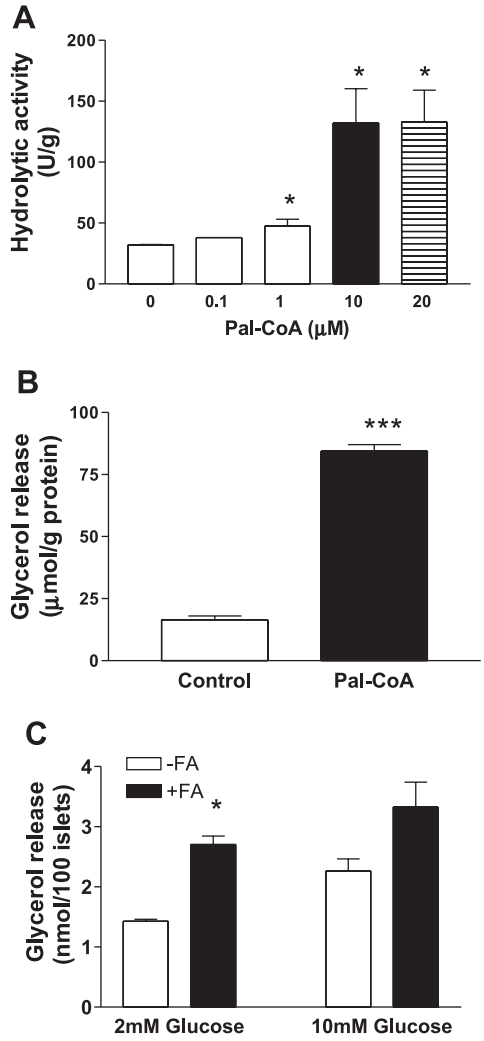

Fig. 1. Palmitoyl-CoA (Pal-CoA) stimulated islet hydrolytic activity. Activity assays with mouse islet cytosol against *p*-nitrophenyl butyrate (PNPB; *A*) or triglyceride (TG; *B*) were performed in the absence or presence of various concentrations of Pal-CoA (10 μM Pal-CoA in *B*). *C*: glycerol release from isolated islets after incubation with 2 or 10 mM glucose in the presence or absence of 0.25 mmol/l free fatty acid (FA; palmitate/oleate 1:1 ratio). Data are means ± SE; $n = 3$. $^*P < 0.05$, $^{***}P < 0.001$.

using our systems. Lipase activity, in the presence and absence of Pal-CoA (0–50 μmol/l), against PNPB [by partially purified adipocyte HSL (Fig. 3*A*) and the adipocyte cytosolic fraction (Fig. 3*C*)] and against TG [partially purified adipocyte HSL only (Fig. 3*B*)] were assessed. Pal-CoA caused a concentration-dependent decrease in lipase activity against PNPB in both preparations (Fig. 3, *A* and *C*). For partially purified HSL, Pal-CoA inhibited hydrolytic activity against both PNPB (Fig. 3*A*) and TG (Fig. 3*B*) substrates. Half-maximal inhibition occurred between 1 and 10 μmol/l, and maximum inhibition was achieved at 50 μmol/l Pal-CoA, at which concentration lipase activity was 70% inhibited (Fig. 3*A*). For the adipocyte cytosolic fraction, 1 μmol/l Pal-CoA caused a half-maximal inhibition and 10 μmol/l Pal-CoA caused a maximum inhibition of 50% of total lipase activity (Fig. 3*C*). Pal-CoA at 10 μmol/l was used for further experiments. The results to date are therefore consistent with a markedly divergent stimulatory effect of LC-CoA on islet lipase activity and an inhibitory

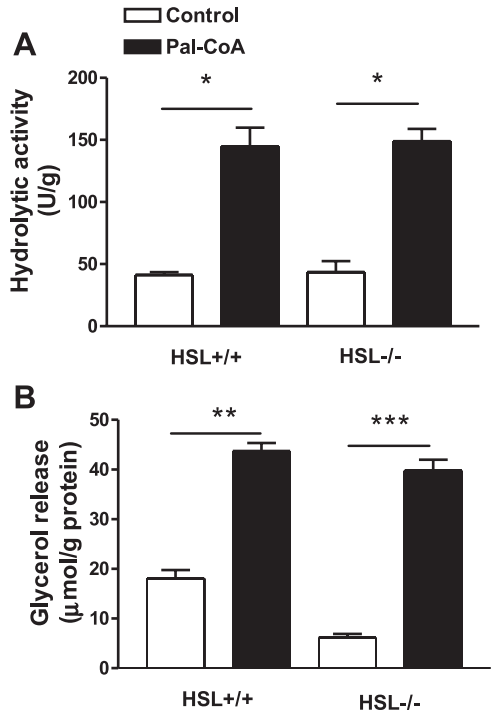

Fig. 2. Pal-CoA (10 μM) stimulated islet lipase activity in $HSL^{+/+}$ and $HSL^{-/-}$ mice against PNPB (*A*) or TG (*B*). Data are means ± SE; $n = 3$. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

effect on adipocyte lipase. Furthermore, the stimulatory effect in islets is not via HSL; whereas the inhibitory effect in adipocytes occurs at least partly via inhibition of HSL. This latter finding is consistent with previous reports, which indicate that LC-CoA inhibits partially purified adipocyte HSL activity (18, 27, 39).

To determine the effect of LC-CoA on non-HSL lipases in adipocytes, activities of adipocyte cytosolic fractions from $HSL^{+/+}$ and $HSL^{-/-}$ mice adipocytes were assessed. Basal hydrolytic activity from $HSL^{-/-}$ adipocytes against PNPB was decreased by approximately one-half (Fig. 4*A*) and against TG was reduced by ~35% (Fig. 4*B*). Interestingly, Pal-CoA inhibited hydrolytic activity against PNPB in both $HSL^{+/+}$ and $HSL^{-/-}$ adipose tissue (Fig. 4*A*) but inhibited lipase activity against TG lipase only in wild-type adipose tissue (Fig. 4*B*). Thus Pal-CoA, in addition to inhibiting esterase and lipase activities of HSL, can also inhibit activity of alternate esterase/s, but not alternate TG lipase/s.

Pal-CoA regulation and phosphorylation state of the enzyme. To determine whether Pal-CoA regulation of lipolysis in adipose tissue and islets is dependent on the phosphorylation state of the lipase enzymes, activity of semipurified HSL from rat adipocytes and the cytosol of islets was measured in the presence and absence of phosphatase inhibitors PP2A or PP2A together with PKA. Results demonstrated that preparation of adipose tissue HSL in the presence of phosphatase inhibitors increased basal lipase activity more than twofold (Fig. 5*A*). Pal-CoA at 10 μmol/l reduced lipase activity of the phosphorylated HSL by 60% but had no significant effect on the dephosphorylated enzyme (Fig. 5*A*). Consistent with these findings, the phosphatase PP2A decreased lipase activity by 70%, and 10 μmol/l Pal-CoA again had no effect on the resultant dephosphylated protein (Fig. 5*B*). Moreover, lipase activity inhibited by PP2A treatment was partially restored by exogenous PKA plus ATP following the PP2A treatment (Fig. 5*B*). The partly rephosphorylated lipase/esterase could then be inhibited by Pal-CoA (Fig. 5*B*). Thus Pal-CoA inhibition of adipose tissue HSL requires the enzyme to be in the most active phosphorylated state.

PP2A inhibited lipase activity by ~50% in islets (Fig. 5*C*). Pal-CoA robustly stimulated lipase activity in both mouse (Fig. 5*C*) and rat (data not shown) islet extracts. Although stimulation was greater in the phosphorylated state, marked stimulation occurred in both the phosphorylated and dephosphorylated islet extracts (Fig. 5*C*). Thus lipase enzyme phosphorylation is not essential for the stimulatory effect of LC-CoA on islet lipolysis.

ACBP modulation of lipase activity. Protein-protein interaction may enhance enzyme activity of some proteins (25). In particular HSL activity increases when bound to FABP (17). To determine whether ACBP affected lipolysis in adipocytes and islets or was essential for LC-CoA regulation, 1 μmol/l ACBP was added to the hydrolytic activity assay. It was found

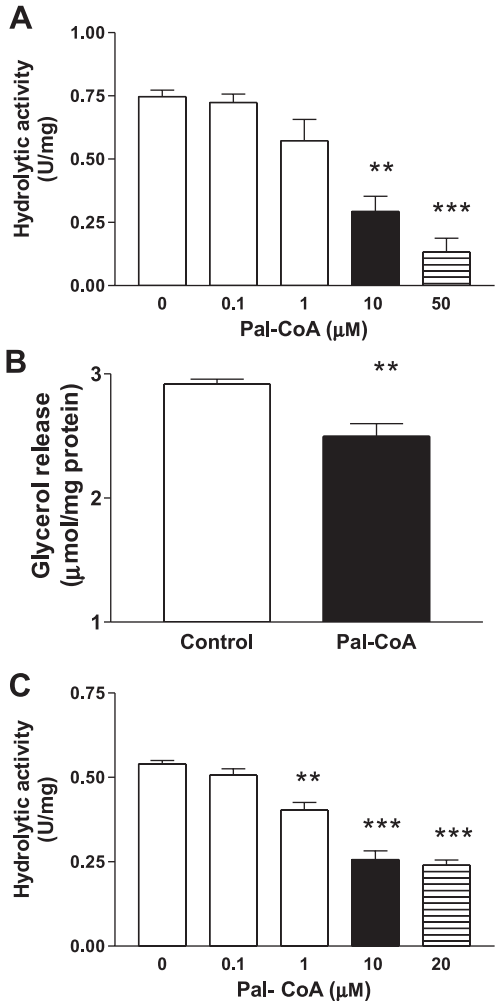

Fig. 3. Pal-CoA inhibited rat adipocyte hormone-sensitive lipase (HSL) activity in partially purified HSL (*A* and *B*) and adipocyte cytosolic extracts (*C*) against PNPB (*A* and *C*) or TG (*B*). Data are means ± SE; $n = 3$. $^{**}P < 0.01$, $^{***}P < 0.001$.

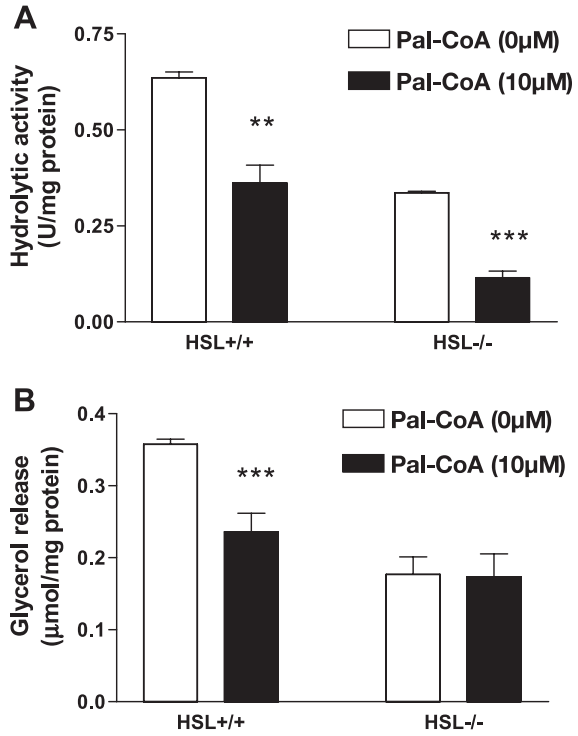

Fig. 4. Hydrolytic activity measurement in the absence or presence of Pal-CoA (10 μM) in adipose tissue of $HSL^{+/+}$ and $HSL^{-/-}$ mice against PNPB (*A*) or TG (*B*). Data are means ± SE; $n = 3$. $^{**}P < 0.01$, $^{***}P < 0.001$.

that ACBP had no effect on adipose tissue HSL/esterase activity in the absence of added LC-CoA but enhanced the inhibitory effect of a minimally inhibitory concentration of Pal-CoA (1 μmol/l; Fig. 6*A*). FABP is reported to bind HSL (40, 42). This enhanced effect may due to binding HSL and presenting LC-CoA to regulate lipolysis. In contrast, the stimulatory effect of Pal-CoA (1 μmol/l) on islet lipase was blocked by 1 μmol/l ACBP (Fig. 6*B*). This reversal of LC-CoA stimulation of lipase activity may have been due to its binding and removal by ACBP.

DISCUSSION

This investigation into the role of LC-CoA in the regulation of lipolysis in both adipose and islet tissue not only confirms the importance of this molecule as a regulator of metabolic enzymes but also exposes some of the complexity of the mechanisms involved, which are highly tissue specific. Remarkably, Pal-CoA inhibited lipase enzyme activity in adipose tissue, consistent with previous reports (18, 39) but, in contrast, robustly stimulated it in islets. From experiments utilizing the $HSL^{-/-}$ mouse, it became evident that Pal-CoA inhibition of TG lipase activity in adipocytes was predominantly due to its effect on HSL activity but that its stimulatory effect in islets was via an alternative lipase. Furthermore, its inhibitory effect was on phosphorylated rather than dephosphorylated HSL, whereas its stimulatory effect was predominantly independent of phosphorylation status. ACBP-enhanced effectiveness of low concentrations of Pal-CoA to inhibit lipase activity could be due to a protein-protein interaction between ACBP and adipocyte HSL. In contrast, ACBP-diminished effectiveness of Pal-CoA to activate lipase enzyme activity in islet cytosolic fractions may be due to sequestration of free Pal-CoA.

These findings and those of previous studies allow us to develop an islet β-cell/adipocyte model (Fig. 7) that incorporates the disparate effects of LC-CoA on lipolysis between the two tissues. In the islet β-cell (Fig. 7), elevated glucose increases intracellular LC-CoA levels by *1*) malonyl-CoA inhibition of FFA oxidation via allosteric inhibition of carnitine palmitoyltransferase I (5, 35, 38) and *2*) increased exogenous FFA supply to the β-cell by glucose and insulin-stimulated islet lipoprotein lipase (LPL) (6, 32). Furthermore, LC-CoA enhances its own formation by activating intracellular lipolysis

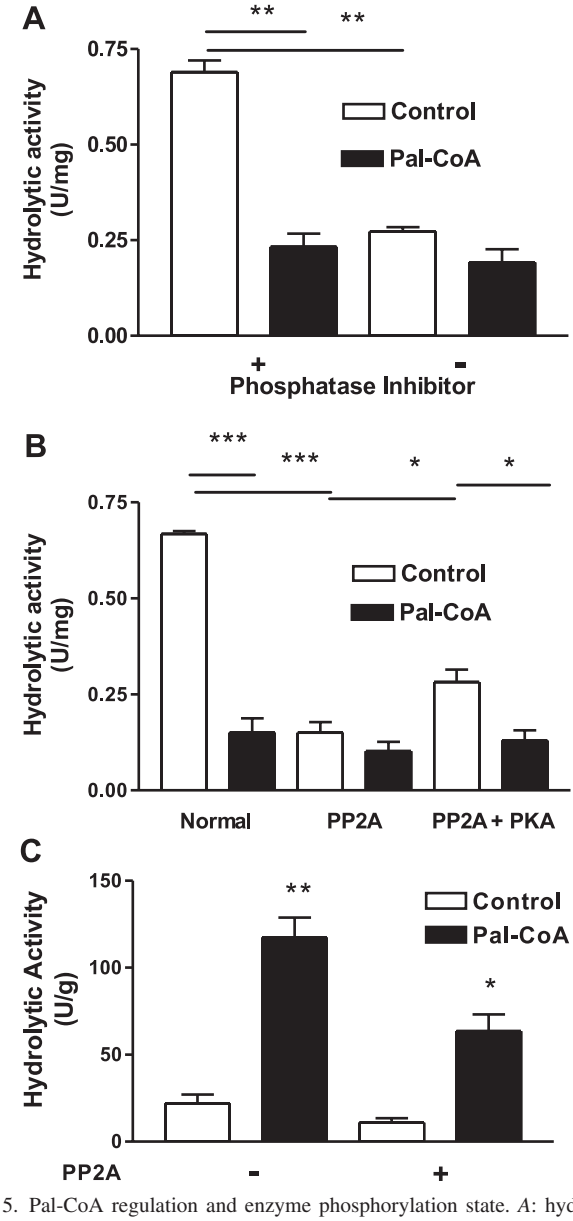

Fig. 5. Pal-CoA regulation and enzyme phosphorylation state. *A*: hydrolytic activity against PNPB of partially purified adipocyte HSL prepared in the presence or absence of a cocktail of phosphatase inhibitors and incubated in the presence and absence of Pal-CoA (10 μM). *B*: hydrolytic activity against PNPB in the presence or absence of Pal-CoA (10 μM) of partially purified adipocyte HSL incubated in the presence or absence of 30 nM protein phosphatase 2A (PP2A) or 30 nM PP2A followed by 5 nM PKA. *C*: hydrolytic activity in the absence or presence of Pal-CoA (10 μM) of islet cytosol incubated with or without 30 nM PP2A. Values represent means ± SE; $n = 3$. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

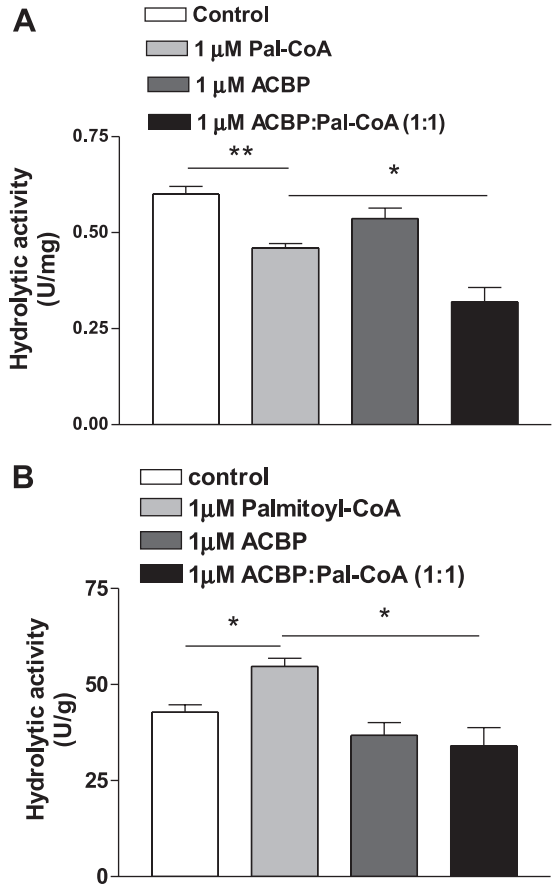

Fig. 6. Influence of acyl-CoA-binding protein (ACBP) on lipase activity in adipocytes and islets. Partially purified fat HSL (*A*) or islet cytosolic extract (*B*) activity against PNPB was measured in the absence or presence of 1 μM Pal-CoA, 1 μM ACBP, or 1 μM ACBP + 1 μM Pal-CoA. Values represent means ± SE; $n = 3$. *$P < 0.05$, **$P < 0.01$.

of complex lipids via non-HSL lipases, thus enhancing FFA supply for LC-CoA synthesis from endogenous sources. The resultant amplification of β-cell LC-CoA levels by elevated glucose augments the effect of the K_{ATP}-dependent pathway of glucose-stimulated insulin secretion to induce insulin exocytosis. In the adipocyte (Fig. 7), glucose and insulin also contribute to elevated intracellular LC-CoA by activation of adipose tissue LPL, which provides increased exogenous FFA supply (14). Inhibition of FFA oxidation by malonyl-CoA may also contribute. Recent data support a major role for cytosolic LC-CoA in explaining the regulation of oscillatory lipolysis (13). The increased LC-CoA together with insulin inhibit adipocyte lipolysis. Insulin prevents the HSL activity by preventing its phosphorylation (20), and LC-CoA inhibits HSL that is already phosphorylated. Thus these disparate tissue effects of LC-CoA work in concert, promoting highly effective inhibition of adipocyte FFA release.

Considering the well-established effect of feeding to inhibit lipolysis in adipose tissue, at first it seems counterintuitive that such conditions should enhance lipolysis in islets. It is not difficult to reconcile these findings, however, when it is remembered that the role of lipolysis is very different between the two tissues. In support of the findings in islet tissue, it has previously been shown that elevated glucose increases lipolysis rates in whole rat (29) and mouse (33) islets. Furthermore, this effect is evident in both wild-type and $HSL^{-/-}$ mouse islets (33), consistent with the finding that HSL is not necessary for LC-CoA activation of lipolysis in islets. The finding that elevated glucose also induces the expression of HSL in clonal β-cells (45) may be important, but it is still unclear whether HSL has a significant role in the islet. The novel islet lipase/s in islets still needs to be identified and will allow further analysis of the mechanisms by which LC-CoA is stimulatory in this tissue.

LC-CoA inhibition of HSL activity was clearly shown to depend on HSL phosphorylation. Previous data demonstrated that lipase preparations activated by PKA phosphorylation were four to six times more sensitive to inhibition by oleoyl-CoA than were nonactivated preparations (39). Our data are therefore consistent with this conclusion.

Protein-protein interaction of HSL with FABP in vitro and in vivo has also been documented (17, 41). It is therefore possible that LC-CoA via ACBP/HSL/LC-CoA or FABP/HSL/LC-CoA complex is involved in the inhibitory effect on HSL. In this

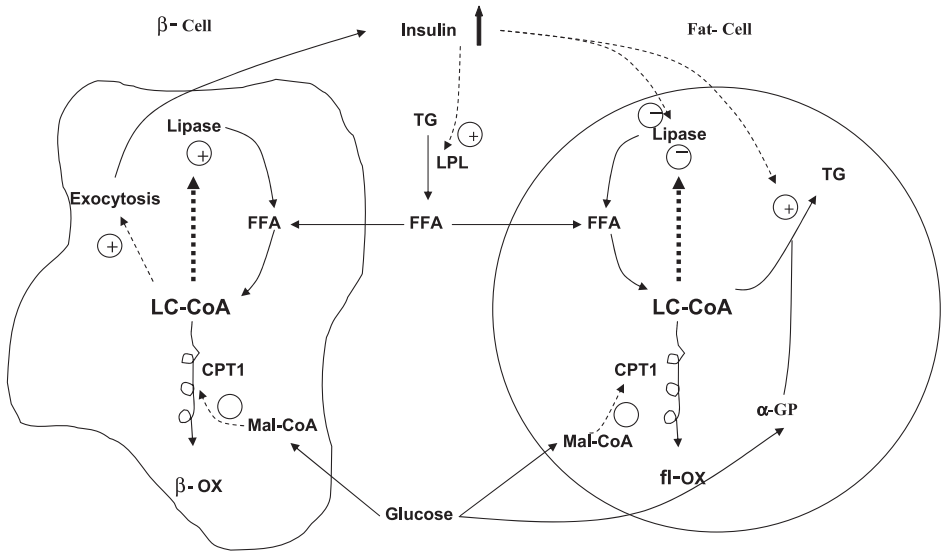

Fig. 7. Integrative model showing the synergistic end effect of disparate actions of long-chain CoA (LC-CoA) on lipolysis regulation in β-cells and fat cells. High glucose and exogenous fatty acids (FFA) increase cytosolic LC-CoA accumulation in β-cells. LC-CoA stimulates lipolysis, which essentially amplifies its own levels and its capacity to signal for enhanced insulin secretion. Insulin, in turn, potently inhibits adipocyte lipolysis by *1*) preventing PKA phosphorylation, which activates HSL and *2*) LC-CoA inhibition of already phosphorylated HSL. Insulin increases LC-CoA via its effect to increase adipocyte LPL activity, thus increasing the exogenous FFA supply to the cell. The end effect of the initiating event of elevated glucose is promotion of adipocyte TG storage. Mal-CoA, malonyl-CoA; LPL, lipoprotein lipase; β-OX, β-oxidation; CPT1, carnitine palmitoyltransferase I; α-GP, α-glycerolphosphate.

study, ACBP enhanced the inhibitory effect of Pal-CoA, suggesting that ACBP, at least in part, could be involved in the inhibitory regulation of LC-CoA on HSL in fat tissue. The stimulatory effect of LC-CoA was blocked by ACBP in islets, suggesting that ACBP and non-HSL lipase interaction is unlikely to be involved in the LC-CoA activation of these lipase/s. In islets, ACBP presumably removed LC-CoA from lipases, preventing activation by the LC-CoA.

In conclusion, we have demonstrated important, but divergent, effects of LC-CoA on the regulation of lipolysis in adipocytes and islets. LC-CoA inhibits adipocyte lipolysis and activates lipolysis in islets. These results increase the evidence implicating LC-CoA not only as an important intermediate of lipid metabolism but also as a major regulator of cellular function. The disparate tissue effects may be due to differences in the dominant lipase enzymes (isoenzymes) present. Furthermore, the inhibitory and/or stimulatory effects of LC-CoA can be influenced by enzyme phosphorylation status and the presence of acyl-CoA-binding proteins such as ACBP and FABP. Finally, the results add to the increasing evidence indicating that lipolysis is a highly regulated and important process in islet β-cell function.

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