Glucagon regulates ACC activity in adipocytes through the CAMKKβ/AMPK pathway

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Energy homeostasis at the whole body level is tightly controlled by a myriad of biochemical signaling in tissues mediating metabolism, i.e., adipose tissue, muscle, liver, and hypothalamus. Regulating multiple metabolic processes, AMP-activated protein kinase (AMPK) is widely recognized as a cellular energy gauge (15). Activated AMPK upregulates catabolic pathways and downregulates anabolic pathways to promote ATP generation in peripheral tissues (27, 34, 35). Furthermore, orexigenic and anorexigenic signals regulate hypothalamic AMPK to modulate food intake (28). Upon energy deficiency, AMPK is phosphorylated at Thr172 in its catalytic α-subunit by one of two AMPK kinases (AMPKK): Ca2+/calmodulin-dependent protein kinase kinase-β (CaMKKβ or CaMKK2) or tumor suppressor LKB1. Expressed abundantly in the brain (41), CaMKKβ controls food intake by regulating hypothalamic AMPK, leading to the production of the orexigenic hormone neuropeptide Y (4). Although many studies show that LKB1 acts as an AMPKK in the periphery (8, 20, 26), CaMKKβ may still be involved in AMPK activation, which is shown by experiments using cultured adipocytes (12).

Among the pantheon of hormones regulating energy mobilization, glucagon is widely thought to exert its major effects on regulating glycogenolysis and gluconeogenesis in the liver (42). However, the metabolic effects of glucagon may also involve tissues other than the liver because the glucagon receptor is expressed in the heart, lymphoblasts, brain, retina, adrenal gland, gastrointestinal tract, and adipose tissue (39). During fasting or starvation, the increased level of glucagon together with decreased insulin result in attenuated lipogenesis in white adipose tissue (WAT). In the liver, the action of glucagon is linked to the activation of both AMPK and cAMP-dependent protein kinase A (PKA), which attenuates the activity of acetyl-CoA carboxylase (ACC) (18, 37). There are two major isoforms of ACC, namely ACC1 or ACCα (265 kDa) and ACC2 or ACCβ (280 kDa). ACC1 is the major isoform in lipogenic tissues, including WAT, mammary gland, and liver, and it converts acetyl-CoA to malonyl-CoA, the rate-limiting step in fatty acid synthesis (3, 21). ACC2, preferentially expressed in skeletal muscle, heart, and liver, governs the transfer of fatty acyl-CoA to the mitochondria for fatty acid oxidation through its regulation of malonyl-CoA (1, 2). ACC activity is regulated mainly by phosphorylation (23), and AMPK has been shown to inactivate both ACC1 and ACC2 through the phosphorylation of residues Ser79 and Ser212, respectively (9, 13, 24, 29). In addition, PKA phosphorylates ACC1 at Ser77 and Ser212 in vitro (9, 13, 29).

Although WAT is a major organ for energy storage (32), the molecular basis for glucagon action in this tissue remains elusive. Results from this study show that AMPK, but not PKA, regulates ACC1 and ACC2 in adipocytes under glucagon stimulation. Furthermore, CaMKKβ, but not LKB1, is the upstream AMPKK regulating this pathway.
MATERIALS AND METHODS

Antibodies and reagents. Antibodies against phospho-AMPKα Thr172, AMPKα, phospho-ACC Ser212, phospho-CAMP response element-binding (CREB) Ser133, PKA, ACC, LKB1, α-tubulin, gluta-thione-S-transferase (GST)-AMPKα, GST-CaMKKβ, and PKAc were from Cell Signaling Technology. Anti-CaMKK was purchased from BD Biosciences Pharmingen. Anti-ACC, anti-phospho-CaMKI Thr172, and anti-β-actin were from Santa Cruz Biotechnology. Compound C, bovine brain calmodulin (CaM), and myristoylated PKA inhibitor amide 14–22 (PKI) were from Calbiochem. Glucagon, STO-609, 5-aminoimidazole-4-carboxamide 1-ribosuranoside (AICAR), Fura-2-AM, EGTA, and ATP were from Sigma. The siRNA targeting AMPKα, PKA, CaMKKβ, LKB1, and scrambled RNA were from Qiagen.

CaMKKβ−/− mice. A targeting construct was designed to replace exons 2–5 of the mouse CaMKKβ gene with a neomycin gene cassette. RW4 embryonic stem (ES) cells were transfected and then subjected to positive (neomycin analog G418) and negative (ganciclovir) selection. Doubly resistant clones were screened for homology to EcoRI genomic fragment overlapping the targeted exons (Fig. 1A). Heterozygous CaMKKβ-deficient ES cells were injected into C57BL/6 blastocysts, which were subsequently bred to generate homozygous CaMKKβ−/− mice. Complete CaMKKβ deficiency was confirmed by immunoblotting with anti-CaMKK antibodies, whereas CaMKKα was largely expressed (Fig. 1C). The CaMKKβ−/−/− mutant mice were further bred with C57BL/6 strain to obtain CaMKKβ−/−/−, CaMKKβ+/−, and CaMKKβ+/+/+ littermates.

Cell culture and transient transfection. Murine 3T3-L1 preadipocytes were cultured, maintained, and differentiated as described previously (7). Briefly, cells were plated and grown for 2 days postconfluence in DMEM supplemented with 10% calf serum. Differentiation was then induced by changing the medium to DMEM supplemented with 10% fetal bovine serum (FBS), 0.5 mM 3-isobutyl-1-methylxanthine, 1 μM dexamethasone, and 1.7 μM insulin. After 48 h, the differentiation medium was replaced with a maintenance medium containing DMEM supplemented with 10% FBS. The maintenance medium was changed every 48 h. The induction lasted for 8 days until the cells were utilized for experiments. Wild-type and CaMKKβ−/− murine embryonic fibroblasts (MEFs) were isolated from the wild-type C57BL/6 and CaMKKβ−/− mouse embryos (E13) and cultured in vitro using a standard protocol (16). LKB1−/− and AMPK−/− MEFs were gifts from Dr. Reuben Shaw at the Salk Institute. Induction of adipogenesis in MEFs was carried out as described elsewhere (40). Transient transfection was performed with Lipofectamine RNAiMAX (Invitrogen). 3T3-L1 adipocytes were transfected with scramble siRNA, AMPKα1 siRNA (CAGGAGTGACGG-GACATAAA), AMPKα2 siRNA (CAGAGAGGCTTAAATATTTA), PKCoa siRNA (CAGTTGCTGTGTAAACATCT), PKAcβ siRNA (CTCGTGTGGTGAAGATCTCA), CaMKKβ siRNA (CAGAGAGTGTATCCTTCAAA), or LKB1 siRNA (CAGGCGGTCAAGATCCTCAA; 10 nM) in Opti-MEM (Gibco). Four hours after transfection, the medium was changed to fresh DMEM supplemented with 10% FBS. Seventy-two hours posttransfection, the cells were exposed to 100 nM glucagon for 2 h. For the inhibition experiments, the cells were pretreated with 20 μM compound C, 10 μM PKI, or 5.3 μM STO-609 for 30 min prior to glucagon exposure. For the activation of AMPK with AICAR, cells were treated with 1 mM AICAR for 30 min.

Western blotting. After treatment, cells were harvested and lysed. After the protein quantification, an equal amount of total protein was resolved by 8% SDS-PAGE and then transferred to a polyvinylidene difluoride membrane. The samples were immunoblotted with primary antibodies as indicated, followed by secondary antibodies conjugated with horseradish peroxidase. The recognized bands were visualized by an enhanced chemiluminesence detection kit (Amersham Biosciences) and quantified by Image J.

Nano liquid chromatography tandem mass spectrometry. ACC was immunoprecipitated from 4 mg of glucagon-treated 3T3-L1 adipocyte lysates by the use of 8 μg anti-ACC antibody. The phosphorylation of immunoprecipitated ACC was detected by nano liquid chromatography tandem mass spectrometry (nano-LC-MS-MS) analysis, which consists of Q-TOF Premier mass spectrometer, nano-Aquity UPLC (ultraperformance liquid chromatography), using a BEH130 C18 analytical column (Waters). The immunoprecipitated ACC was first trypsin treated and then purified by the titanium dioxide-coated magnetic beads provided with the Phos-Trap phosphopeptide enrichment kit (PN no. PRT302001KT; PerkinElmer, Waltham, MA). The bound phosphopeptides were collected and lyophilized. The freeze-dried pellet was dissolved in 0.1% trifluoroacetic acid and subjected to nano-LC-MS-MS analyses. The phosphorylation sites within ACC1 were determined by a characteristic neutral loss of the phosphate group in the process of collision-induced dissociation.

Fig. 1. Targeting disruption of the mouse Ca2+/calmodulin (CaM)-dependent protein kinase kinase-β (CaMKKβ) gene. A: targeting strategy. Exons 2–5 of the CaMKKβ gene were replaced with a neomycin cassette, which resulted in a change from 6 to 4 kb in size of an EcoRI genomic fragment overlapping with the targeted exons. B: Southern blot analysis of control and successfully targeted embryonic stem cells demonstrating the presence of wild-type (WT) and mutant alleles (+/−) in the latter cells. C: Western blotting analysis of the epididymal fat pad extracts obtained from the WT and CaMKKβ−/− mice.
Lipogenesis assay. 3T3-L1 adipocytes cultured in a 35-mm culture dish were incubated with 2 μCi [1,2-14C]acetate (PerkinElmer) and 0.5 mM unlabeled acetate for 16 h. The cells were either untreated or pretreated with 20 μM compound C, 10 μM PKI, or 5.3 μM STO-609 for 30 min and then exposed to 100 nM glucagon for 2 h. Cellular total lipids were extracted by collecting cells into 500 μl of PBS containing 1 mM PMSF and protease inhibitor, which were added to 4 ml of chloroform-methanol (2:1, vol/vol). The solution was centrifuged at 1,300 g, and the organic phase collected. Solvents were dried with nitrogen gas, and the pellets were resuspended in chloroform. Incorporation of [1,2-14C]acetate into lipid phase was assessed by scintillation counting. All experiments were performed in triplicate and normalized by protein concentrations.

In vitro kinase assay. GST-AMPKα1 (44 nM) or PKA (95 nM) was incubated with a synthetic fragment of ACC1 (HMRSSMSGL-HLVKQG) or SAMS (HMRSAMSGLHLVKRR) at 1 mM in 25 μl of HEPES buffer (50 mM, pH 7.4) containing 5 mM MgCl2, 0.2 mM ATP, and 0.2 mM AMP. The kinase activity of AMPK or PKA, in the presence of GST-CaMKKB (46 nM) and/or Ca2+ (2 mM)/CaM (2 μM), was determined by ACC1 peptide or SAMS peptide phosphorylation. The phosphorylation site of either ACC1 peptide or SAMS peptide was determined by nano-LC-MS-MS analyses. The phosphorylation level changes of ACC1 and SAMS peptides were quantified by the selected ion monitoring mode in the LC chromatogram.

Animal experiments. The animal experimental protocols were approved by the University of California Riverside (UCR) Institutional Animal Care and Use Committee. Twelve-week-old male C57BL/6, CaMKKB+/+, CaMKKB−/−, AMPKα2+/+, or AMPKα2−/− mice were used. The mice were housed in a 12:12-h light-dark cycle with ad libitum access to Lab Diet 5001 unless otherwise described. For glucagon experiments, mice were administered glucagon (5 μg/kg) or saline as a vehicle control by intraperitoneal injection for 30 min, 1 h, 2 h, and 4 h. Epididymal fat pads and livers were collected for Western blotting. For fasting experiments, mice were fasted for 30 h. The water was provided ad libitum during food deprivation. At the end of fasting, the mice were euthanized, and the epididymal fat pads were treated with compound C (Comp C; 20 μM) or myristolated PKA inhibitor amide 14–22 (PKI; 10 μM) for 30 min. The cells in C and D were then treated with Glu (100 nM) for 2 h. Western blotting was performed on cell lysates collected from A, C, and D to detect the phosphorylation of AMPKα Thr172, ACC1 Ser79, ACC2 Ser212, and CREB Ser133. The bar graphs at the bottom represent the densitometry analyses of the ratios of phospho-(p)-ACC1 Ser79 or p-ACC2 Ser212 to α-tubulin. Data represent means ± SE from 3 independent experiments. *P < 0.05 between indicated groups.

Fig. 2. Glucagon (Glu)-induced acetyl-CoA carboxylase (ACC) phosphorylation in adipocytes is mediated by AMP-activated protein kinase (AMPK). A: 3T3-L1 and murine embryonic fibroblast (MEF)-derived adipocytes were treated with Glu (100 nM) for the time indicated or various concentrations for 2 h. B: ACC immunoprecipitated from Glu-treated 3T3-L1 adipocytes was trypsin digested, and the phosphopeptides were concentrated by using titanium dioxide-coated magnetic beads. Nano liquid chromatography tandem mass spectrometry (nano LC-MS-MS) was performed to map the phosphorylation site(s) within the peptide containing ACC1 Ser77 and Ser79. C: 3T3-L1 adipocytes were transfected with AMPKα1/α2 siRNA, PKAα/β siRNA, or control (Ctrl) RNA (10 nM). D: 3T3-L1 adipocytes were treated with compound C (Comp C; 20 μM) or myristolated PKA inhibitor amide 14–22 (PKI; 10 μM) for 30 min. The cells in C and D were then treated with Glu (100 nM) for 2 h. Western blotting was performed on cell lysates collected from A, C, and D to detect the phosphorylation of AMPKα Thr172, ACC1 Ser79, ACC2 Ser212, and CREB Ser133. The bar graphs at the bottom represent the densitometry analyses of the ratios of phospho-(p)-ACC1 Ser79 or p-ACC2 Ser212 to α-tubulin. Data represent means ± SE from 3 independent experiments. *P < 0.05 between indicated groups.
were collected for Western blotting. The serum glucagon and insulin levels were detected by Milliplex Map mouse endocrine panel (Millipore).

Statistical analyses. Unless indicated, results are expressed as means ± SE from three independent experiments. Experiments comparing two groups were analyzed with the Student t-test or Mann-Whitney U-test. Differences among multiple groups were evaluated initially by ANOVA followed by the Dunnett or Newman-Keuls post hoc test with GraphPad Prism 4 for Windows (GraphPad Software, San Diego, CA). Unless otherwise indicated, P < 0.05 was considered statistically significant.

RESULTS

AMPK mediates glucagon-induced ACC phosphorylation in adipocytes. To lay a foundation to explore the role of CaMKKβ in glucagon-induced molecular pathways in adipocytes, we initially examined the time and dose dependence of glucagon-induced phosphorylation of AMPKα, ACC1, and ACC2 in cultured adipocytes. As illustrated in Fig. 2A, glucagon treatment of 3T3-L1 and MEF-derived adipocytes increased the phosphorylation of ACC1 at Ser79 and ACC2 at Ser212 in a time- and dose-dependent manner similar to that reported for hepatocytes (37). Concurrently, the phosphorylation of AMPKα at Thr172, the putative target of CaMKKβ, was increased by glucagon treatment. Although previous studies suggested that PKA phosphorylates ACC1 at Ser77 (9, 29), LC-MS-MS analysis illustrates that glucagon induced the phosphorylation of ACC1 only at Ser79 (Fig. 2B). A trypsin-digested fragment containing Ser1200 was not phosphorylated following glucagon treatment (data not shown) despite it being a presumed PKA phosphorylation site (13).

To provide additional evidence that AMPK, not PKA, mediates glucagon-induced regulation of the two ACC isoforms, AMPKα1/α2

![Figure 3](http://ajpendo.physiology.org/)
and PKACα/Cβ in 3T3-L1 adipocytes were knocked down by siRNA, and the effects of glucagon were examined. As shown in Fig. 2C, the glucagon-induced phosphorylation of ACC1 and ACC2 was dampened in AMPKα1/α2 siRNA- but not in PKACα/Cβ siRNA-transfected adipocytes despite the observation that glucagon activates both AMPK and PKA, as evidenced by the increased phosphorylation of AMPKα and CREB protein. Similarly, compound C, an AMPK inhibitor, but not myristoylated PKI, a membrane-permeable PKA inhibitor, impaired the phosphorylation of ACC1 and ACC2 (Fig. 2D).

CaMKKβ mediates glucagon activation of AMPK. The ability of glucagon to activate AMPK in the wild-type, CaMKKβ−/−, LKB1−/−, and AMPKα−/− MEF-derived adipocytes was next studied to determine whether CaMKKβ or LKB1 mediates the glucagon activation of AMPK. As illustrated in Fig. 3A, the glucagon-induced AMPKα, ACC1, and ACC2 phosphorylation was attenuated in both CaMKKβ−/− and AMPKα−/− but not in LKB1−/− adipocytes. In control experiments, AICAR, an AMPK activator, increased AMPK phosphorylation of ACC1 at Ser79, ACC1 at Ser77, and SAMS and CaMKK (Fig. 3D). Further evidence that CaMKKβ mediates glucagon-induced AMPK activation was observed when STO-609, a CaMKK inhibitor, attenuated glucagon-induced phosphorylation of AMPKα, ACC1, and ACC2 (Fig. 3C). Consistent with ACC phosphorylation by the CaMKKβ/AMPK pathway, STO-609 and compound C, but not PKI, reversed the negative effect of glucagon on de novo lipogenesis in Fig. 3D. Therefore, CaMKKβ and AMPK are required for glucagon-reduced de novo lipogenesis in 3T3-L1 adipocytes.

An in vitro kinase assay was then used to investigate whether CaMKKβ can directly activate the AMPK-ACC axis. As illustrated in Fig. 4A, purified GST-AMPKα1 in the presence of CaMKKβ phosphorylated a synthetic fragment of ACC1 that corresponds to the sequence around residue Ser79, and the inclusion of Ca2+/CaM dramatically enhanced the phosphorylation of this site by AMPK. PKA, on the other hand, failed to phosphorylate the homologous Ser79 of the ACC1 fragment in either the presence or absence of Ca2+/CaM and CaMKKβ. However, PKA phosphorylated the ACC1 fragment at the residue corresponding to Ser77, but this effect was only marginally enhanced by the addition of Ca2+/CaM and CaMKKβ. Similar results were obtained using SAMS peptide, which is derived from ACC sequence with Ala substituted at a residue homologous to Ser77 (Fig. 4B).

CaMKKβ and AMPK are necessary for glucagon-increased ACC phosphorylation in vivo. To verify that the glucagon-elicited CaMKKβ-AMPK-ACC axis is functional in WAT in vivo, glucagon was administered to C57BL/6 mice. Glu-

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Fig. 4. Effects of CaMKKβ alone and with Ca2+/CaM on AMPK and PKA-mediated phosphorylation of ACC1 at Ser79, ACC1 at Ser77, and SAMS peptides. The kinase activity of purified glutathione-S-transferase (GST)-AMPKα1 (44 nM) or PKA (95 nM) was determined by mixing 1 mM ACC1 peptide (HMRSSMSGHLVKQG; A) or SAMS peptide (B) with GST-CaMKKβ (46 nM) in the presence or absence of Ca2+ (2 mM)/CaM (2 μM). Nano-LC-MS-MS spectrometry was used to quantify the relative amount of phosphorylation. The fold change in the level of phosphorylated peptide was normalized to that of control (without GST-CaMKKβ) set as 1. The bar graphs represent means ± SD from 3 independent experiments. *P < 0.05 between indicated groups.
Glucagon administration caused an increase in the phosphorylation of AMPKα, ACC1, and ACC2 in epididymal fat pads, which lasted for 2 h (Fig. 5A). The phosphorylation of CaMKI, the canonical downstream target of CaMKK, was also increased. The increased phosphorylation of AMPKα, ACC1, ACC2, and CaMKI was also found in the liver of these animals (Fig. 6A). Importantly, ablation of AMPKα2 and CaMKKβ in mice abolished the glucagon induction of ACC1 and ACC2 phosphorylation in both WAT (Fig. 5, B and C) and liver (Fig. 6, B and C) when compared with their respective wild-type littermates. However, glucagon still activated PKA even in the absence of either AMPKα2 or CaMKKβ. These data indicate that the CaMKKβ/AMPK, but not PKA, pathway is necessary for glucagon-induced phosphorylation of ACC1 at Ser79 and ACC2 at Ser212 in both adipocyte and hepatocyte at the organ level.

CaMKKβ is necessary for fasting-induced ACC phosphorylation. To explore further the role of CaMKKβ in adipose tissue under physiological conditions, 12-wk-old CaMKKβ−/− and their CaMKKβ+/+ littermates were divided into two groups: fed ad libitum (control) and fasted for 30 h prior to euthanization. Fasting increased serum level of glucagon but decreased insulin in both CaMKKβ−/− and CaMKKβ+/+ mice (Fig. 7A). Fasting increased the phosphorylation of AMPKα, ACC1, and ACC2 in WAT of CaMKKβ−/− mice when compared with those under ad libitum (Fig. 7B). For CaMKKβ−/− mice, the extent of phosphorylation of these three proteins was essentially the same for the two metabolic conditions. These results indicate that fasting with attendant glucagon surge increases the AMPK and ACC phosphorylation, which is mediated by CaMKKβ.

DISCUSSION

Ample evidence indicates that AMPK regulation of energy metabolism at the whole body level is an integration of its functions in both the central nervous system and peripheral tissues (5, 33). Because AMPK activity is tightly regulated by upstream kinases, investigation of the mechanism by which environmental cues such as nutritional status, circadian cycle, and hormonal actions control an AMPKK to regulate energy metabolism becomes an important research topic. In this study, the principal finding is that glucagon regulates ACC activity through the CaMKKβ/AMPK pathway in adipocytes in vitro and in vivo, which contributes to the energy-mobilizing effect of glucagon.

Fig. 5. Glu-induced ACC phosphorylation is mediated by CaMKKβ/AMPK in mouse adipose tissue. A: Glu (5 μg/kg) was administered intraperitoneally to C57BL/6 mice for the indicated time. B and C: AMPKα2+/+, AMPKα2−/−, CaMKKβ+/+, and CaMKKβ−/− mice received the same dose of glucagon or 0.2 ml of saline for 2 h. After euthanization, the tissue extracts of the epididymal fat pads were subjected to Western blotting to reveal the phosphorylation of AMPKα Thr172, ACC1 Ser79, ACC2 Ser212, cAMP response element-binding (CREB) Ser133, and CaMKI Thr177. The bar graphs below represent the densitometry analyses of the ratios of p-AMPKα Thr172 to AMPKα, p-ACC1 Ser79 or p-ACC2 Ser212 to β-actin or α-tubulin. Data represent means ± SE from 3 animals. *P < 0.05 between indicated groups.

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Here, we demonstrate that CaMKKβ/H9252 is the upstream AMPKK that mediates the glucagon activation of AMPK in adipocytes. Glucagon rapidly and transiently increases intracellular calcium ([Ca^{2+}]) in hepatocytes, human embryonic kidney-293, and baby hamster kidney cells (14, 38, 43). However, imaging of Fura 2-loaded 3T3-L1 adipocytes revealed that glucagon treatment did not produce a detectable increase in [Ca^{2+}] (data not shown). Thus, the mechanism underlying glucagon activation of CaMKKβ/H9252 in adipocytes appears to be distinct from hepatocytes and other cell types. Glucagon binding to its receptor also increases the intracellular level of cAMP. The ensuing activated PKA has been suggested to phosphorylate and hence, inhibit ACC in isolated rat hepatocytes and adipocytes based solely on the phosphorylation of proteolytic ACC fragments containing both Ser77 and Ser79 (17, 18). Whereas residues Ser77 and Ser1200 are the PKA phosphorylation sites, Ser 79 is phosphorylated exclusively by AMPK (9, 13, 29). Our results from LC-MS-MS analysis indicate that Ser79, but not Ser77, is phosphorylated in glucagon-treated adipocytes (Fig. 2B) and an in vitro kinase assay system (Fig. 4), suggesting that AMPK is the major kinase to regulate ACC activity in response to glucagon. Park et al. (30) reported recently that resveratrol activation of the CaMKKβ/AMPK pathway in C2C12 myotubes is mediated through competitive inhibition of cAMP-degrading phosphodiesterases. The resulting elevation of cAMP activates Epac1, which increases [Ca^{2+}], and then in turn activates CaMKKβ. Depending on the stimuli, AMPK and PKA can affect each other (10, 11, 19). However, our results show that glucagon activates AMPK and PKA independently (Fig. 2, C and D), which is similar to that exerted by resveratrol in myotubes (30). But resveratrol increases [Ca^{2+}] in myotubes, and we found no detectable calcium response in the glucagon-treated adipocytes. In all, the glucagon activation of CaMKKβ in adipocytes seems to be [Ca^{2+}] and PKA independent. The exact mechanism requires further study.

Glucagon-activated PKA triggers several acute effects, including gluconeogenesis and glycogen breakdown in the liver, which is believed to be the canonical function of glucagon (22). Although both ACC1 and ACC2 catalyze the conversion of acetyl-CoA to malonyl-CoA, ACC1 and ACC2 are responsible for enhanced fatty acid synthesis and decreased β-oxidation, respectively. Because ACC1/ACC2 phosphorylation leads to their deactivation, our results suggest that glucagon...
also inhibits fatty acid synthesis and enhances fatty acid oxidation through AMPK phosphorylation of ACC1/ACC2. The phylogeny of human kinome (25) suggests that PKA and AMPK belong to different kinase superfamilies. In eukaryotes, the glucagon-regulated glucose and lipid metabolism are likely to be controlled by PKA and AMPK, respectively. In humans, glucagon-regulated glucose metabolism appears to be mediated through the cAMP/PKA pathway, resulting in a rapid change of blood glucose. On the other hand, glucagon-regulated lipid metabolism is likely to be regulated through the CaMKKβ/AMPK pathway, leading to reduced fat accumulation. Although both AMPK and PKA are activated by glucagon, ACC1 Ser79 seems to be a better substrate of AMPK. Structural modeling demonstrates that Ser79 of ACC1 is positioned with the lowest free energy near the catalytic residue Asp139 of AMPK, whereas Ser77 of ACC1 is optimally positioned near the catalytic residue Asp166 of PKA (data not shown). It is reasonable to hypothesize that AMPK has a higher affinity for ACC1 Ser79 and, in turn, lower Km.

Glucagon appears to increase AMPK/ACC phosphorylation through CaMKKβ in both adipocytes (Fig. 3, A–C, and Fig. 5, A–C) and hepatocytes (Fig. 6, A–C). Our results are consistent with previous findings that, in the liver, glucagon increases AMPK phosphorylation independent of LKB1 (6). The decreased phosphorylation of AMPK, ACC1, and ACC2 in the liver of glucagon-administered AMPKo2−/− and CaMKKβ−/− mice reveals that glucagon also regulates ACC activity in the liver via the CaMKKβ/AMPK pathway. Similarly to adipocytes, glucagon administration should result in decreased fatty acid synthesis and increased β-oxidation in the liver. During the fasting phase, the elevated glucagon in circulation diminishes lipogenesis in the liver (31), which in turn reduces transport and storage of triglycerides in adipose tissue. Glucagon also inhibits de novo lipogenesis in adipocytes (17) via AMPK, as illustrated in Fig. 3D. The sum of these effects exerted by glucagon would be “catabolic” in the context of whole body lipid metabolism. Indeed, glucagon administration in humans results in weight loss (36). Because the activation of CaMKKβ/AMPK by glucagon or fasting is one of the major pathways controlling ACC activity in adipose tissue, a translational implication of the current study is to target this pathway in WAT for a possible drug intervention to dysregulate energy storage.

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DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

GLUCAGON REGULATES ACC ACTIVITY THROUGH CAMKKβ/AMPK

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