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CONTROL OF ENERGY HOMEOSTASIS: Role of Enzymes and Intermediates of Fatty Acid Metabolism in the Central Nervous System

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■ **Abstract** The regulation of energy homeostasis is critical for normal physiology and survival. Energy flux must be rigorously monitored and adjusted to ensure that fuel intake and expenditure remain within acceptable limits. The central nervous system (CNS) is, in large part, responsible for conducting this energy-monitoring function and for integrating the numerous inputs. It has become evident that neurons of the CNS monitor and respond to levels of metabolic intermediates that reflect peripheral energy status. Intermediates in the fatty acid biosynthetic pathway have been implicated as hypothalamic signaling mediators that sense and respond to changes in circulating fuels. Genetic and pharmacologic manipulation of the enzymes of fatty acid metabolism have led to the hypothesis that neuronal metabolic intermediates affect neural outputs that modify both feeding behavior and energy expenditure. This review focuses on the regulatory roles of these enzymes and intermediates in the regulation of food intake and energy balance.

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INTRODUCTION

The regulation and maintenance of energy homeostasis is critical for an organism's health and survival. Disruption of this balance often leads to a chronic disease state. Skewing energy balance toward surfeit leads to concomitant increases in adipose cell size and number (43, 73). The resulting increased adiposity frequently leads to complications ranging from type 2 diabetes and cardiovascular dysfunction to an increased risk of cancer (10, 87). Despite extensive investigation, the mechanism by which increased adiposity predisposes these disorders has not been elucidated. Energy deficit, brought about by prolonged fasting, extreme exercise, or medical-psychiatric disorders, such as anorexia nervosa, can reduce adipose depots to such low levels that insulin resistance and infertility can manifest (63). The regulation of energy homeostasis is therefore of great interest to biochemists and physiologists as well as to clinicians who seek to intervene to restore normal energy balance to rectify these conditions.

Animals are well adapted to maintain energy stores within normal limits even when caloric intake exceeds expenditure. Control systems have evolved to maintain energy homeostasis by increasing energy expenditure during periods of energy excess and to decrease energy expenditure during times of energy deficit. Historically, metabolic research has focused on peripheral energy depots and major centers of energy metabolism/utilization, notably the liver, adipose tissue, and skeletal muscle. More recently, the central nervous system (CNS) has received greater attention as a site of importance, not only with regard to behavior associated with hunger, pleasure, and appetite, but also in sensing of energy needs and communicating this need directly via neural circuitry to peripheral energy centers to mediate fuel utilization. This model places the CNS at the forefront of energy sensing and integration, for which it is well equipped (71, 72). However, there is still little consensus on the relative contribution of the nervous system to peripheral metabolism. The intracerebroventricular administration (i.c.v.) of leptin reverses both the adiposity and hyperglycemia of obese *ob/ob* mice (11, 24) and insulin resistance and hepatic steatosis in lipodystrophic mice (5), and also rapidly down-regulates the expression of key hepatic genes involved in energy storage (5, 17). Likewise, the i.c.v. administration of agents that increase the level of malonyl-CoA (indicating energy surplus) in the hypothalamus of *ob/ob* or lean mice rapidly up-regulates fatty acid oxidation in skeletal muscle (12). These findings show definitively that the brain has a rapid and profound effect on peripheral energy expenditure. Neither the nature of the efferent signal nor the neural circuitry involved has been identified.

Afferent endocrine-neuroendocrine signaling to the brain is perhaps best illustrated by the leptin signaling pathway, which projects from the adipocyte to the hypothalamus. Leptin, as well as other endocrine molecules (e.g., insulin, CCK, IL-6, and ghrelin), are not the sole sensors, however, that transmit energy status information from the periphery to the brain. Another class of molecules that has received increasing attention recently is the enzymes and intermediates of the fatty acid biosynthetic pathway within the CNS, in particular malonyl-CoA and

long-chain (C_{16}-C_{18}) fatty acids and their acyl-CoA derivatives. Malonyl-CoA, an intermediate in fatty acid biosynthesis, is a classic effector that blocks entry of fatty acyl-CoAs (the end point of the fatty acid biosynthetic pathway) into mitochondria to prevent fatty acid oxidation concurrent with fatty acid synthesis (47–49). There is now substantial pharmacologic, genetic, and physiological evidence to indicate that fluctuations in the level of malonyl-CoA in the CNS act to inhibit food intake.

In addition to its effect on food intake, malonyl-CoA is thought to "sense" energy surplus in the CNS by responding to the level of circulating glucose and to signal via neuronal efferents and neuroendocrine molecules to increase energy expenditure by peripheral tissues, e.g., fatty oxidation by skeletal muscle (18). In this model, the cellular metabolic machinery of neurons in the CNS becomes engaged to signal energy expenditure. This review focuses on the energy-sensing functions of the CNS mediated by the enzymes and intermediates of the fatty acid biosynthetic pathway, and the ways in which these effects alter energy homeostasis in higher animals.

CELLULAR FATTY ACID METABOLISM AND REGULATION

Fatty acids either can be imported into cells from the circulation or synthesized de novo from acetyl-CoA. Once in the cell, a decision must be made whether to direct the fatty acid into mitochondrial oxidation for energy production or into glycerolipid synthesis for energy storage or membrane fabrication. This partitioning into oxidative versus synthetic pathways is critical for cell function and therefore must be tightly regulated. In addition to serving as substrate in oxidative and synthetic pathways, fatty acids also function in the propagation of a myriad of biological signals.

The pathways and the enzymatic machinery for the biosynthesis (80) and oxidation (47) of fatty acids are well characterized. Acetyl-CoA, the cytoplasmic precursor for de novo fatty acid synthesis, is poised at the branch point for entry of multiple metabolic pathways. Thus, it would be expected that the initial and committed step of fatty acid synthesis from acetyl-CoA at this branch point would be catalyzed by a highly regulated enzyme, i.e., acetyl-CoA carboxylase (ACC). ACC converts acetyl-CoA to malonyl-CoA, which serves as the basic chain-elongating unit for fatty acid synthesis by fatty acid synthase (FAS). FAS generates saturated fatty acids of C_{16} to C_{18} chain length (80) that can be further modified by the addition of a double bond catalyzed by acyl-CoA desaturases. This reaction sequence is illustrated in Figure 1. The most abundant unsaturated fatty acid in stored triglyceride is oleic acid, which possesses a single *cis*-double bond at the Δ^9 position that is introduced by stearoyl-CoA desaturase (SCD), a highly regulated enzyme of which there are four encoded genes (55, 56). Some tissues, notably muscle, lack FAS and do not carry out fatty acid synthesis. In this case, another enzyme, malonyl-CoA decarboxylase (MCD), removes malonyl-CoA by

decarboxylation to regenerate acetyl-CoA. In skeletal muscle, the ACC-MCD system serves primarily a regulatory role.

Two isoforms of ACC (ACC1 and ACC2), encoded by independent genes, are expressed in different cell types and have unique functions (2, 3, 27, 28). ACC1 is cytosolic, expressed primarily in lipogenic cell types, and functions in fatty acid synthesis. ACC2 is anchored in the outer mitochondrial membrane and is expressed in cell types, e.g., muscle, where its product, malonyl-CoA, governs the entry of fatty acids into mitochondria. Both ACCs are subject to regulation by citrate, a feed-forward allosteric activator (7, 22, 68) and by phosphorylation catalyzed by 5′-AMP kinase (AMPK) (28, 82). AMPK acts as a cellular sensor that is activated as the energy status of a cell decreases, i.e., as 5′AMP increases relative to ATP. Both ACC isoforms can be phosphorylated/inactivated by AMPK, which lowers malonyl-CoA and suppresses fatty acid synthesis. Lowering malonyl-CoA leads to an increased rate of fatty acid oxidation by a mechanism described below. Thus, fluctuations in malonyl-CoA determine whether fatty acids undergo oxidation in mitochondria or are converted into glycerolipids, e.g., triglycerides or phospholipids.

To undergo β-oxidation, fatty acids taken up from the bloodstream must cross both the inner and outer mitochondrial membranes. The initial and regulated rate-limiting step in this process is catalyzed by carnitine palmitoyltransferase-1 (CPT1), a transmembrane protein bound to the outer membrane (47). CPT1 transfers the fatty acyl group from CoA to carnitine, producing a fatty acyl-carnitine that then translocates through a dedicated channel in the inner membrane (Figure 2). CPT2, an inner mitochondrial enzyme, catalyzes the transfer of the fatty acyl group to CoA for β-oxidation within the mitochondrial matrix. There is clear metabolic logic for the role of malonyl-CoA in the regulation of the translocation process. Malonyl-CoA, an indicator of active fatty acid synthesis, is a potent allosteric inhibitor of CPT1 (47–49). During periods of nutritional abundance, when surplus metabolic intermediates funnel into fatty acid synthesis and energy storage, the steady-state level of malonyl-CoA rises (32, 47–49). This in turn prevents entry of fatty acids into mitochondria and oxidation. Conversely, in the fasted state, when malonyl-CoA is low, CPT1 is activated and fatty acids are translocated into the β-oxidation compartment. Although the role of malonyl-CoA in the regulation of fatty acid oxidation in tissues such as the liver and skeletal muscle is well documented (27, 28, 47–49), only recently has the regulatory role of malonyl-CoA in the hypothalamic control of energy balance been appreciated (32, 34, 45).

The metabolic environment in the liver, where fatty acid synthesis is a dominant factor, differs markedly from that in muscle, where there is little, if any, FAS, and therefore little de novo synthesis of fatty acids. Skeletal muscle, where fatty acids are the major physiological fuel, possesses ACC2 (1, 3) and a specialized CPT, i.e., CPT1b (8, 28, 66), both of which are anchored to the outer mitochondrial membrane. The basis for the unusual localization of ACC2 appears to be regulatory, i.e., to produce malonyl-CoA in close proximity to CPT1b. In addition,

skeletal muscle expresses MCD, an enzyme whose sole function is to decarboxylate malonyl-CoA, returning its substrate to acetyl-CoA. Like ACC, MCD appears to be regulated by AMPK, phosphorylation activating the enzyme. Together these opposing enzymes constitute a highly responsive control system in which AMPK exerts reciprocal effects, inactivating ACC2 while activating MCD (69, 70). It follows that the primary role of ACC2 and MCD in skeletal muscle is to modulate the level of malonyl-CoA and thereby the activity of CPT1 and the rate of fatty acid oxidation. The discussion that follows shows that malonyl-CoA also functions in the hypothalamic system that monitors global energy balance and regulates food intake and energy expenditure.

FATTY ACID SYNTHASE AND ENERGY BALANCE

Effect on Food Intake

The hypothalamus receives and processes hormonal and other afferent signals that reflect the energy status of the animal (71, 72). These signals trigger the expression and secretion of the orexigenic and anorexigenic neuropeptides that regulate food intake and energy expenditure as well as mediate synaptic plasticity (31, 64). Most notable among these neuropeptides are the orexigens, NPY and AgRP, and the anorexigens, POMC/αMSH and CART. These neuropeptides are expressed by NPY/AgRP or POMC/CART neurons in the arcuate nucleus (Arc) of the hypothalamus and send projections to other regions of the hypothalamus, including the paraventricular nucleus, lateral hypothalamic area, ventral medial nucleus, and dorsal medial hypothalamus. Second-order neurons from these regions project to higher brain centers, where this information is integrated and behavioral responses are formulated.

The concept that an intermediate in fatty acid metabolism might play a role in the regulation of food intake and energy expenditure originates from the serendipitous discovery that FAS inhibitors, i.e., fungal-derived cerulenin or synthetic C75 (39), cause dramatic weight loss (13, 16, 40, 45, 46). These inhibitors rapidly provoke a reduction in food intake and loss of body weight and when administered repetitively produce profound long-term weight reduction in obese animals (13, 45). Indeed, C75 can reverse the weight gain caused by diet-induced obesity (40, 78) or mutations in leptin (*ob/ob*) or its receptor (*db/db*) (45) (13). Weight loss is achieved without a concomitant activation of STAT3, the major signaling molecule activated by the long form of the leptin receptor or the anorexogenic IL-6 family of cytokines (ciliary neurotrophic factor, oncostatin M, etc.). These observations showed that the action of the FAS inhibitors is independent of leptin via a hitherto undescribed parallel pathway. Elucidation of the mechanism of action of FAS inhibitors is of great interest as it may be a relevant pharmacologic target to counter weight gain in individuals exhibiting leptin resistance.

The effect of C75 is rapid ($<$20 min) and robust, with food intake being suppressed by $>$90% in Balb/c mice (40, 45). Long-term (30 days) low-dose treatment

of *ob/ob* mice results in an almost complete reversal of obesity (13). This is remarkable in view of the fact that *ob/ob* mice can reach weights >twofold that of littermate heterozygotes and exhibit many health complications associated with human obesity.

The question of whether FAS inhibitors act centrally or peripherally was addressed by administering low levels of C75 by i.c.v. injection. I.c.v. administration of C75 rapidly suppressed food intake in a dose-dependent manner (12, 32, 33, 45). Consistent with its action in the CNS, i.c.v. administration of C75 produces the same pattern of neuropeptide expression as occurs by i.p. administration (32).

Central or i.p. administration of C75 rapidly alters the levels of hypothalamic neuropeptides that regulate feeding behavior (32, 45, 74). Concomitant with a decrease in food intake is a decrease in NPY/AGRP levels and an increase in POMC/CART levels (32, 74). C75 was found to rapidly inhibit NPY production in the arcuate nucleus of mice even after 23 hours of fasting (32, 45). This is a time when NPY is normally up-regulated to stimulate food intake. In addition, administration of NPY (45) or ghrelin (33), an NPY-activating peptide, overcomes the inhibitory effects of C75.

The site of the blockade by C75 appears to lie upstream of NPY (and ghrelin) in the signal transmission pathway, as its effect on food intake could be reversed by the i.c.v. administration of NPY (33, 45). It should be noted that NPY and AgRP, both orexigenic neuropeptides, are produced by the same neuron type in the hypothalamus and raise the possibility of the involvement these neuron in the C75 response. Recent studies lend credence to this viewpoint. Genetic deletion of NPY (19) or AgRP alone (67) or in combination (67) results in little overt effect on feeding behavior. However, deletion of this population of neurons, i.e., NPY/AgRP neurons, results in a decrease in food intake and body weight, stressing the importance of these neurons and validating the notion that compensation of food intake occurs in the NPY or AgRP knockout (KO) animals (23). FAS and ACC are present in the CNS and have been shown to be enriched in hypothalamic centers critical for feeding behavior (21, 38). FAS has been shown to colocalize with NPY neurons (38). The hypothalamus was originally shown to be a critical mediator of feeding behavior through classic studies of nuclei ablation, both chemically and mechanically.

Hormones also play a role in the modulation of feeding. One previously unrecognized role of insulin is the down-regulation of FAS activity. It is well known that chronic insulin stimulation and type 2 diseases such as diabetes increase the transcription of lipogenic genes such as ACC and FAS, and this leads to adverse effects such as nonalcoholic fatty liver disease and increased adiposity. Insulin is clearly involved in the suppression of food intake. Neural-specific insulin receptor KO mice have an increased food intake and are susceptible to diet-induced obesity (9). In the liver, insulin inhibits FAS activity acutely (15 min.) via a carcinoembryonic-related cell adhesion molecule (CEACAM1)-dependent mechanism (53). It is unclear whether insulin inhibits FAS activity in neurons, but if it does, it provides a provocative link between insulin-mediated suppression of food

intake and FAS-mediated inhibition of food intake. In this paradigm, insulin would lead to a decrease in FAS activity and an increase in malonyl-CoA (in the short term), which would serve to inhibit food intake. Since neurons have constitutive rather than insulin-stimulated glucose transporters, this mechanism need not be dependent on glucose uptake.

Effect on Energy Expenditure

Pair feeding experiments in which control mice were restricted to the intake of C75-treated mice showed that C75 does more than merely inhibit food intake, since C75-treated mice lost more weight more rapidly than did pair-fed controls (13, 40, 79). Similar results were obtained with lean, ob/ob, or diet-induced obese mice. C75, therefore, must increase energy expenditure in peripheral tissues. C75-treated diet-induced obese mice exhibit an increase in O_2 consumption as measured by whole-body calorimetry (75, 78). It should be noted that whole-body O_2 consumption can be inhibited by treating mice with a peripheral CPT1 inhibitor (79). This finding suggests that C75 may lead to the activation of CPT1 in peripheral tissues. Although it has been reported that C75 administered peripherally (by intraperitoneal injection) can directly activate CPT1 in peripheral tissues, recent evidence shows that most of the effect may have actually been exerted centrally. Thus, C75 administered via the central route (i.c.v.) at a level too low to exert direct peripheral effects rapidly (<2 hours) strongly activated energy expenditure in skeletal muscle (12). It was found that central administration of C75 to either obese or lean mice rapidly activated fatty acid oxidation in skeletal muscle (as measured by the conversion of 1–14C oleic acid to $^{14}CO_2$) both in the intact animal and in muscle explants from C75-treated animals. Since skeletal muscle is the most abundant fatty acid-metabolizing tissue in the animal and fatty acids are the major physiological fuel for muscle, an increase in the rate of fatty acid oxidation in this tissue would be expected to have a major impact on whole-body energy expenditure (12).

Although the mechanism by which the malonyl-CoA signal, initiated by centrally administered C75, is transmitted from the CNS to muscle tissue is presently unknown, the signal appears to be mediated through the SNS to α-adrenergic receptors in skeletal muscle. This is indicated by the fact that phentolamine, a potent α-blocking agent, prevented both the C75-induced expression of UCP3 and a reduction of malonyl-CoA in skeletal muscle and of whole-body fatty acid oxidation (12). Like the central action of leptin through the hypothalamic-SNS axis, which activates the AMPK-catalyzed phosphorylation of ACC and fatty acid oxidation in skeletal muscle, i.c.v. C75 causes phosphorylation of ACC (on serine 79, a target site of AMPK) and a decrease of malonyl-CoA in skeletal muscle (12).

The probable explanation for the increased energy expenditure induced by C75 is increased thermogenesis caused by up-regulation of skeletal muscle UCP3 (12, 13). Like the other UCPs, UCP3 is believed to dissipate the proton gradient across the inner mitochondrial membrane producing heat, rather than ATP. This thermogenic response may contribute to whole-body energy expenditure. Since UCP3 is

expressed primarily in skeletal muscle, which constitutes a major tissue mass in higher animals, up-regulation of UCP3 may be responsible for the C75-induced increase in energy expenditure. Fatty acids most likely fuel this thermogenic response. Closely correlated to the up-regulation of UCP3 provoked by centrally administered C75 is an increase of fatty acid oxidation by skeletal muscle. Presumably, this increase is the result of an accompanying decrease in malonyl-CoA concentration caused by the phosphorylation-induced inactivation of ACC. The decrease in malonyl-CoA level in skeletal muscle normally leads to activation of CPT1b, allowing translocation of fatty acids into the β-oxidation compartment of mitochondria. In this connection, recent studies (S.H. Cha and M.D. Lane, unpublished results) reveal that repetitive i.p. or i.c.v. administration of C75 promotes an increase in the number of mitochondria in both white and red skeletal muscle.

THE MALONYL-CoA HYPOTHESIS

The evidence described above along with that presented below led to the hypothesis that malonyl-CoA serves as a signaling molecule in hypothalamic neurons to mediate satiety (Figure 3). There is a precedent for malonyl-CoA as a signaling molecule in peripheral cells. Malonyl-CoA, an intermediate in the biosynthesis of fatty acids, is a potent allosteric inhibitor of CPT1 that determines whether fatty acyl-CoAs will enter an energy storage or oxidative pathway. Thus, an increase in malonyl-CoA concentration inhibits fatty acid β-oxidation and diverts fatty acyl-CoAs into the triglyceride or phospholipid biosynthetic pathway for energy storage or membrane assembly. Conversely, a decrease in malonyl-CoA level directs fatty acyl-CoAs into the oxidative pathway. This is consistent with the finding that forced overexpression of glycerol-3-phosphate acyltransferase leads to the diversion of fatty acyl-CoAs into triglyceride synthesis, i.e., energy storage, thereby suppressing entry into mitochondria and β-oxidation (44). Since ACC2 and MCD, but not FAS, are expressed in skeletal and cardiac muscle that does not synthesize fatty acids, the sole function of these enzymes in this context appears to be the control of malonyl-CoA levels for the regulation of fatty acid oxidation (1, 69, 70). These studies are consistent with a role for malonyl-CoA as a mediator of energy expenditure. It is known that the cellular concentration of malonyl-CoA fluctuates widely in muscle in different physiological states and is dependent upon the actions of two highly regulated enzymes, ACC and MCD (see above). Since ACC, MCD, and FAS are known to be expressed in subsets of hypothalamic neurons, the enzymes needed for regulation by malonyl-CoA are present in the cells that have the potential to regulate food intake and energy expenditure.

Moreover, the levels of certain intermediates in the fatty acid biosynthetic pathway that are thought to modulate feeding behavior, most notably malonyl-CoA and fatty acyl-CoA, can be altered by physiological or pharmacological means. Importantly, the concentration of malonyl-CoA in the hypothalamus falls during fasting and rises after refeeding in concert with changes in appetite (32).

The central (i.c.v.) administration of C75 rapidly causes an increase (>fourfold) in hypothalamic malonyl-CoA correlated with an immediate suppression of food intake. The malonyl-CoA hypothesis (Figure 3) predicts that the C75-induced suppression of food intake by inhibiting FAS, which increases hypothalamic malonyl-CoA, should be reversed by inhibition of malonyl-CoA formation with an ACC inhibitor, e.g., TOFA. This indeed is the case, since TOFA, administered i.c.v. before C75, prevents both the C75-induced increase in hypothalamic malonyl-CoA and the closely correlated decrease in food intake (32). Together these findings strongly support the hypothesis that the level of hypothalamic malonyl-CoA is an indicator of energy status that mediates feeding behavior and depends on the relative activities of ACC and FAS.

Use of another approach verified these findings. To lower the malonyl-CoA level in neurons in the critical feeding centers, a malonyl-CoA decarboxylase (MCD) viral expression vector was delivered by stereotactic injection into the ventral region of the hypothalamus (34). Immunocytochemical staining of brain sections verified similar delivery of a control viral expression vector that encodes β-galactosidase. Injections of the MCD vector caused a small but consistent increase of food intake and body weight. Importantly, the MCD vector totally reversed the reduction of food intake caused by i.c.v. C75, which (in other experiments) increases hypothalamic malonyl-CoA. It was also shown that use of the MCD vector to infect a hypothalamic neuronal cell line decreased cellular malonyl-CoA. These findings provide strong evidence that lowering malonyl-CoA in the ventral hypothalamus mice can reverse the effect of C75 on food intake caused by C75.

Recent evidence also shows that cellular hypothalamic malonyl-CoA concentration is regulated through the action of 5′-AMP kinase (4, 34, 37, 41, 50). Consistent with a linkage between malonyl-CoA and 5′-AMP kinase in the mediation of feeding behavior, central administration of AICAR, an activator of 5′-AMP kinase, lowers hypothalamic malonyl-CoA and stimulates food intake (34). Moreover, AICAR activates the phosphorylation/inhibition of ACC and lowers malonyl-CoA concentration in cultured cells (34).

Although several downstream effects of the "malonyl-CoA signal" have been established, i.e., changes in the expression of NPY, AgRP, POMC, and CART (32), the direct target of malonyl-CoA has not been identified. However, one potential enzyme target currently being given strong consideration is CPT1 (59), which is known to bind malonyl-CoA. One candidate CPT of great interest is CPT1c, a brain-specific enzyme that binds malonyl-CoA but whose enzymatic activity is not known (65).

ACETYL-CoA CARBOXYLASE AND 5′-AMP KINASE

ACC catalyzes the key regulatory step of fatty acid biosynthesis in lipogenic tissues (42, 52) and also has a regulatory role in the hypothalamic control of feeding behavior and energy expenditure (18). Although there are two isoforms of ACC,

ACC1 and ACC2, it is uncertain whether one or both function in the hypothalamic system that monitors energy status and governs food intake and energy metabolism. It has been established that disruption of the ACC2 gene results in increased food intake and energy expenditure (1). Knockout of the ACC1 gene is embryonic lethal (2), and a tissue-specific hypothalamic disruption of ACC1 has not yet been reported. Both ACC isoforms are subject to regulation through phosphorylation by 5′-AMP kinase (AMPK; discussed below) and undergo allosteric activation by citrate (28, 82). It is noteworthy that AMPK is coexpressed in ACC-containing hypothalamic neurons (37).

The ability to regulate energy metabolism is required for survival and has been conserved throughout evolution. Critical to this regulation is the ability to assess global energy status and to respond either by diverting metabolites into energy storage pathways, e.g., fatty acid synthesis, or by mobilizing energy reserves to produce ATP. Most cell types (including hypothalamic neurons) express AMPK, which is activated by 5′-AMP and serves as an "energy sensor" by monitoring the AMP/ATP ratio. When ATP is consumed, its major products, ADP and AMP, undergo rapid equilibration catalyzed by adenylate kinase (shown below):

$$\mathrm{ADP} + \mathrm{ADP} \Leftrightarrow \mathrm{ATP} + \mathrm{AMP}.$$

Thus, as ATP falls, AMP rises. AMPK regulates catalytic activity by phosphorylating serine or threonine hydroxyls of target enzymes, ACC1 and ACC2 being classic AMPK substrates (Met-Arg-Pro-Ser^{P}-Met-Ser^{P}-Gly-Leu). AMPK itself is phosphorylated/activated by another kinase, i.e., AMPKK. As cellular AMP rises and binds to AMPK, it becomes a primed substrate for AMPKK, apparently through an allosteric conformational change.

Consistent with a role in regulating feeding behavior, hypothalamic AMPK appears to be activated in the fasting state and inactivated upon refeeding in response to increased AMP and decreased AMP, respectively. It has been shown that AICAR, a pharmacologic activator of AMPK, increases the phosphorylation of ACC, which decreases malonyl-CoA both in hypothalamic neurons in cell culture and in the hypothalamus in vivo (4, 34). These findings correlate well with the effect of AICAR on feeding behavior since the central (i.c.v.) administration of AICAR to mice increases food intake (34). Like the effect of AICAR, expression of an adenoviral cytosolic MCD vector (Ad-cMCD) in hypothalamic GT1-7 cells decreases malonyl-CoA. When delivered by bilateral stereotaxic injection into the ventral hypothalamus (encompassing the arcuate nucleus) of mice, Ad-cMCD causes a modest increase of food intake and body weight (34). Ad-MCD delivered into the ventral hypothalamus also reverses the rapid suppression of food intake caused by i.c.v.-administered C75, which increases hypothalamic malonyl-CoA (34).

In this connection, leptin, an anorexigenic hormone, also appears to modulate hypothalamic AMPK activity (50). Thus, leptin reduces AMPK activity in the

arcuate nucleus and paraventricular nucleus of the hypothalamus, which would be expected to cause activation of ACC and thereby to increase malonyl-CoA concentration. C75, the anorexigenic FAS inhibitor, also inhibits the phosphorylation and thereby activation of hypothalamic AMPK (41). These and other findings indicate that inhibition of hypothalamic AMPK activity is required to elicit the anorexigenic effects of leptin and C75. There is now substantial evidence for the following sequence of events: Activation of hypothalamic AMPK causes phosphorylation/inactivation of ACC, which lowers malonyl-CoA (34) and thereby increases food intake (see Figure 4). Leptin and C75, on the other hand, provoke an increase in hypothalamic malonyl-CoA, which leads to decreased food intake.

Several laboratories have shown that AICAR, a pharmacologic activator of AMPK, can moderately raise food intake in mice (4, 34, 37, 50). Further, a constitutively active adenoviral expression vector can similarly increase food intake, whereas a dominant-negative decreases food intake. Although the effects are not dramatic, there is a clear relationship between the activation of AMPK and changes in food intake (34). These data indicate that AMPK may be the upstream regulator that senses changes in cellular energy and mediates changes in malonyl-CoA levels (34). AMPK appears to sense metabolic changes in hypothalamic neurons and possibly in glucose-sensitive neurons and to regulate the activity of ACC. This, in turn, would alter the level of malonyl-CoA, which would serve as a regulator of CPT1. Complicating issues are that AMPKα1 (36) and AMPKγ3 (6) KO mice have no apparent metabolic phenotype while AMPKα2 KO mice do not have any obvious feeding deficits (36).

The identification of the upstream AMPKK has long been sought and could offer insight into how AMPK is regulated in neurons or responds to changes in circulating energy substrates. AMPKKs recently have been deduced in budding yeast. Three kinases can phosphorylate the putative AMPK homologue (Snf1), Pak1p, Tos3p, and Elm1p. One mammalian homologue to these kinases, LKB1, was indeed identified as a constitutive AMPKK (29, 85). There is evidence that AMP binding and subsequent activation by LKB1 is not the only means to activate AMPK, as there are instances where AMPK is activated without a concomitant change in the AMP/ATP ratio and in cells lacking LKB1. Recently, another kinase has been implicated as a non-AMP-mediated AMPKK, CamKK (30, 35, 84). The Ca^{2+}/calmodulin-dependent kinase kinase has been shown to be a true AMPKK that is activated via changes in Ca^{2+} concentration in neurons. This is an exciting discovery since this may link food intake, neuron excitability, and changes in [malonyl-CoA]. This allows integration of not only circulating energy stores but also higher brain activity such as pleasure or reward.

Many details remain to be elucidated in the hypothalamic signaling pathway that governs feeding behavior and energy expenditure. Nevertheless, a large body of evidence from several laboratories employing different approaches now allows us to include three intermediaries in this signaling pathway: AMPK, ACC, and malonyl-CoA.

CARNITINE PALMITOYL TRANSFERASE-1 AND THE ROLE OF FATTY ACYL-CoAs

Several reports have implicated hypothalamic CPT1 and long-chain fatty acyl-CoAs in the hypothalamic control of feeding behavior. A recent report showed that long-chain fatty acids, particularly oleic acid, administered by i.c.v. injection provoked a decrease of food intake (60). Since fatty acids are quickly converted to their corresponding CoA derivatives when taken up by cells, it is likely that the active species responsible for this effect is the corresponding fatty acyl-CoA or a metabolite derived from the CoA derivative. It was found that inhibitors of CPT1 administered by i.c.v. injection also decrease food intake (59). This response is consistent with the effect of centrally administered fatty acids, since inhibitors of CPT1, which initiates the translocation of fatty acyl-CoAs into mitochondria, would be expected to cause the accumulation of fatty acyl-CoA in the cytoplasm. Another approach, i.e., the i.c.v. administration of a CPT1a-ribozyme to reduce the expression of CPT1 in the hypothalamus, led to a reduction of food intake (59). These findings appear consistent with the malonyl-CoA hypothesis. In this connection, physiological and pharmacological perturbations that increase malonyl-CoA also suppress food intake. Malonyl-CoA is a bona fide inhibitor of CPT1 that would be expected to produce an effect similar to that of CPT1 inhibitors, i.e., it would cause the accumulation of long-chain fatty acyl-CoAs in the cytoplasmic compartment. Two recently studied perturbations, refeeding following fasting and i.c.v. administration of C75, increase hypothalamic malonyl-CoA concentration and lead to the suppression of food intake (32). Taken together, these findings suggest that CPT1 may be a focal point in the control of feeding behavior through changes in the cytoplasmic fatty acyl-CoA level in the hypothalamus.

However, there are obvious inconsistencies in this hypothesis. C75 is an anorexic agent that inhibits FAS, causing the accumulation of cytoplasmic malonyl-CoA; however, C75 also blocks fatty acid synthesis. Therefore, despite the C75-induced increase in hypothalamic malonyl-CoA, there would be a concurrent decrease in cytoplasmic fatty acyl-CoA, assuming that hypothalamic fatty acid is primarily of endogenous origin. These facts raise the question of whether the hypothalamic fatty acids are of endogenous or exogenous origin. If hypothalamic fatty acyl-CoAs originate primarily from endogenous biosynthesis, their concentration would not increase upon C75 administration and therefore could not be responsible for the associated suppression of food intake. If, however, brain fatty acids are of exogenous origin, i.e., are imported from the bloodstream, it follows that fasting should increase, not decrease, food intake. Obviously, appetite and thus food intake is increased when food is offered to a fasted animal. It should be noted that in the fasted state, adipose fat reserves are mobilized, producing an increase in blood fatty acids for delivery to peripheral tissues, whereas in the fed state, blood fatty acids are maintained at a relatively low level. Increased delivery of fatty acids to peripheral tissues, e.g., liver, leads to increased cellular fatty acyl-CoA. In this situation, fatty acyl-CoA levels in the brain in the fasted state would also be

expected to rise. In the fasted state, hypothalamic orexigenic neuropeptide (NPY and AgRP) levels increase while anorexigenic neuropeptide (αMSH and CART) levels decrease, causing an increase—rather than a decrease—of food intake (32, 74). Obici et al. (60) noted that circulating fatty acids can gain rapid access to the brain. This access may be limited to certain regions of the brain. It is known, for example, that the arcuate nucleus/median eminence regions of the hypothalamus have leaky blood-brain barriers that would presumably facilitate the entry of blood-borne circulating molecules. Thus, it might be expected that in the fasting state, as blood fatty acid levels rise, fatty acyl-CoA in the arcuate nucleus would also rise. This scenario would be inconsistent with the thesis that elevated hypothalamic fatty acyl-CoA has an anorexic effect.

This enigma may be explained by the recent finding that the brain expresses an unorthodox CPT1, i.e., CPT1c (65). This brain-specific CPT1 possesses unique characteristics that do not fit the pattern of the classical CPT1s, i.e., liver-type CPT1a and muscle-type CPT1b (65). Unlike its counterpart CPT1s, when tested in vitro CPT1c fails to catalyze the transfer of the fatty acyl group from the fatty acyl-CoA donor to the acceptor, carnitine. However, possible involvement of CPT1c in the regulation of feeding behavior is suggested by its capacity to bind malonyl-CoA. Moreover, recent immunocytochemical studies (83) indicate that CPT1c is expressed primarily in the arcuate nucleus and the ventral medial regions of the hypothalamus that are known to play an important role in the regulation of food intake. Moreover, CPT1c, like CPT1a and b, appears to localize to mitochondria. It is conceivable that CPT1c has a different substrate(s) specificity and/or is regulated differently from CPT1a and CPT1b. Indeed, a mouse knockout of CPT1c results in reduced body weight and decreased food intake, consistent with the idea of CPT1c as the physiologic target of CNS malonyl-CoA (83).

GENETIC MODELS OF FATTY ACID METABOLISM

Allelic variations from gene-targeted embryonic stem cells or natural mutations often reveal a great deal about gene function (see Table 1 for a tabulation of genetic models of relevant metabolic pathways). For example, the positional cloning of the OB gene (89) led to the discovery of leptin and ultimately to human therapies based on leptin deficiency, such as natural human leptin mutants (20) or lipodystrophic individuals (62, 63). Because most of the fatty acid biosynthetic enzymes are known, their function can be tested in knockouts or conditional knockouts. FAS (1) and ACC1 (2) are indispensable for embryonic development, and both are lethal very early in development. Even FAS heterozygotes show a greater propensity to die in utero (1). It is unclear at this stage of development whether there is an embryonic or placental defect that mediates the lethality, as the embryos do not survive far beyond what would be expected for the embryonic genome to be activated since these are long-lived molecules. It is unclear why de novo synthesis is so critical for development since fatty acids can be acquired through dietary

TABLE 1 Relevant genetic models of the fatty acid metabolic pathway

Genetic models	Phenotype	Reference
ACC1	Early embryonic lethal	(2)
ACC2	Lean with increased food intake	(1, 3, 61)
FAS	Heterozygous embryonic lethal	(15)
SCD1	Lean with increased food intake	(17, 57)
SCD2	Skin abnormalities; reduced thrift	(51)
CPT1a	Embryonic lethal	(58)
CPT1c	Reduced body weight; reduced food intake	(83)
Insulin receptor-brain specific	Increased food intake DIO	(9)
mGPAT	Reduced body weight and TG	(25, 26, 54)
AMPKα1	Limited metabolic phenotype	(36)
AMPKα2	Insulin resistance	(36)
AMPKγ3	Limited metabolic phenotype	(6)
Lipoic acid synthase	Embryonic lethal	(88)

DIO, diet-induced obese; TG, triacylglycerol.

means. The conditional knockout of FAS in hepatocytes does not result in lethality, but reveals a regulatory role for FAS in lipogenesis, glucose, and cholesterol metabolism as well as a direct role in signaling (14). FAS-liver-specific knockout mice fed a fat-free diet or subjected to a prolonged fast exhibit PPARα-dependent hypoglycemia and fatty liver disease that can be reversed by the addition of dietary fat or a PPARα agonist (14). These experiments demonstrate a unique, yet unidentified, PPAR ligand that is de novo synthesized. In this way, FAS plays a critical signaling role by providing a signaling ligand. This presents an intriguing set of questions for how FAS expression may play a role in neuronal signaling. Consistent with an important role for central nervous system malonyl-CoA in the regulation of body weight, mice with hypothalamic disruption of the fatty acid synthase gene exhibit an increased hypothalamic malonyl-CoA level (14a) and decreased adiposity.

Although ACC1 is early embryonic lethal, the knockout of the mitochondrial-anchored muscle isoform ACC2 is not. ACC2 knockout mice have an increase in β-oxidation of fatty acids due presumably to a decrease in muscle malonyl-CoA and are therefore lean (3). Also, they have an increased food intake, which would be consistent with the idea that fatty acids or a fatty acid–derived endocrine signal inhibits food intake. A similar phenotype is seen in the *asebia* mutant mouse, which is a natural deletion mutant in the SCD1 gene (77). These mice exhibit hypoplastic sebaceous glands and hair follicle abnormalities. The mutant animals have low levels of monounsaturated fatty acids, which would be predicted. *Asebia* mice and a targeted KO of SCD1 (57) have a phenotype

similar to ACC2 KO mice; that is, they are lean and have an increase in food intake. When bred to an *ob/ob* background, the SCD1 deletion leads to a resolution of fatty liver and ameliorates the weight gain of *ob/ob* animals (17). This weight loss is associated with an increase in energy expenditure but does not resolve the hyperphagia. This suggests that the hyperphagia associated with leptin deficiency cannot be reversed by SCD1 deletion, and leptin may dominate this interaction.

SCD2, which is more widely expressed, includes expression in the brain. SCD2 has recently been knocked out and shows hepatic and skin defects (51). The skin defects are not unexpected since SCD1 KO animals also have defects in skin. However, SCD2 is not expressed in adult liver, but SCD2 KO animals have liver defects. It was revealed from these studies that SCD2 and not SCD1 is expressed in the fetal liver and then in adulthood, when the predominant SCD gene expressed turns to SCD1. There is a high degree of perinatal morbidity in SCD2 KO mice, which rapidly lose weight after birth, possibly due to water loss from hyperpermeable skin (51). The effect on food intake and energy expenditure in these mice is complicated by their lack of thrift, and is therefore unknown.

After the synthesis or absorption of fatty acids into the cell, they are rapidly converted into their CoA esters via an acyl-CoA synthetase. Acyl-CoA synthetases are important for normal brain physiology and are reviewed extensively elsewhere (76, 81). A branch point exists between the utilization of fatty acyl-CoAs as fuels or for the acylation of glycerol to produce lysophosphatidic acid and ultimately other acyl-glycerol species by the enzyme glycerol-3-phosphate acyltransferase, GPAT. There are two known isoforms of GPAT: one that is localized to mitochondria and one that is ER localized. The mitochondrial GPAT comprises only about 10% of the total activity, but 30%–50% in liver. A knockout of mGPAT results in a lower body weight and lower TG levels with improved insulin resistance and a diminished high-fat-induced fatty liver disease (25), but does not mediate resistance to body weight changes as a result of a high-fat diet (26).

GPAT can compete with CPT 1 for acyl-CoAs; CPT1 is the limiting step in β-oxidation. Several CPT1a missense, nonsense, and splicing mutations have been identified in humans. Individuals with decreased or absent CPT1a function have a hypoketotic/hypoglycemia phenotype. The hypoketotic state is brought about due to the inability of fatty acids to undergo β-oxidization in the liver and provide the mitochondrial substrate for ketogenesis, acetyl-CoA. The hypoglycemia results from the inefficient gluconeogenesis due to a decreased acetyl-CoA and NADH/NAD ratio resulting from reduced β-oxidation; both decreases are needed to support gluconeogenesis. Surprisingly, CPT1a KO in mice results in embryonic lethality (58). This raises the possibility that there is another unrecognized function of CPT1a in mice or that another CPT1, such as CPT1b, can compensate in humans but not mice. However, rodents and primates are fundamentally disparate in embryonic and placental development. CPT2 mutations are more commonly found and have the above phenotype as well as an associated cardiomyopathy.

Since CPT2 is obligatory for the transfer of fatty acyl-carnitines into the matrix of mitochondria, both CPT1a and CPT1b would be affected. Muscles, including cardiomyocytes, are the largest consumers of fatty acids for energy production, and loss of this energy source causes damage to muscle. We are unaware of any feeding studies done on individuals with either of these genetic disorders. This would be of great interest to determine if CPT is involved in feeding behavior or energy expenditure.

CONCLUSIONS AND FUTURE DIRECTIONS

A large and growing body of evidence now links the pathway of fatty acid synthesis in the CNS to the system that regulates food intake and peripheral energy expenditure. Although many of the major players (AMPK, FAS, ACC, SCD, and CPT) and intermediates of this regulatory system have been identified, the mechanism(s) by which this system connects to the neuropeptide/neural circuitry that alters behavioral and energy balance remains to be elucidated. How do changes in the activities and levels of these enzymes and intermediates alter the responses that produce complex behavior and changes in energy balance, and what are the modes of efferent and afferent signaling? We propose several areas requiring further investigation:

(a) **Determine the role of malonyl-CoA.** A common feature of these pathways is that they either alter malonyl-CoA or are allosterically modified by malonyl-CoA. Since malonyl-CoA is a biochemical intermediate, the enzymes that synthesize or break down malonyl-CoA must be the centers of regulation. This has been addressed pharmacologically, but bystander effects cannot be ruled out. There are several genetic means by which malonyl-CoA activity can be altered. A cytoplasmic-localized malonyl-CoA decarboxylase can be used to down-regulate malonyl-CoA in neurons. Alternatively, a phosphorylation mutant (constitutively active) ACC expressed in hypothalamic neurons to increase malonyl-CoA could be used. This model could further address the question of whether AMPK acts via the phosphorylation of ACC or another target.

(b) **Determine the role of CPT1a, CPT1c, and CPT2 in neurons.** It has been shown via pharmacologic blockade that CPT1 inhibition blocks food intake. However, it would be useful to make neuron-specific knockouts of CPT1a because its localization in the brain does not correlate with its proposed function. CPT2 has localized focal expression in the CNS as determined by in situ hybridization (GENSAT GeneID: 12896) and is obligatory for entry of fatty acids into the mitochondrial matrix. Can CPT2 be disrupted in hypothalamic neurons?

(c) **Are fatty acids and ketones involved?** It appears unlikely that circulating free fatty acids play a major role in signaling satiety because they are at

their highest level when animals are most hungry, during a fast. However, it is clear from the conditional knockout of FAS that de novo synthesized fatty acids have special properties above that of dietary fatty acids (14). Do circulating or de novo synthesized fatty acids play different roles? How/why are fatty acids involved? Do ketones have an anorectic role in signaling? It was shown that animals on a ketogenic diet do not respond to FAS inhibitors (86). Ketones, like fatty acids, are produced at high levels upon fasting, with a well-described role as an energy substrate for the CNS. Could they act as signaling molecules or cofactors?

(d) **Identify the neuronal cell type responsible.** The wide array of neural cell types in the hypothalamus makes it difficult to define mechanistically the role of these enzymes, since the effect is likely to be limited to a particular cell type. Do glucose-responsive neurons play a role? It is critical to determine which cell types are responsible so that the regulatory pathways involved can be understood.

Finally, it is our hope that this review will help to identify potential targets for intervention to provide therapies to control and reverse obese-related pathologies as alternatives to cytokine-mediated methodologies. From the basic scientist's viewpoint, the ways in which regulatory enzymes and intermediates in lipid metabolic pathways of the CNS affect whole-body energy homeostasis represents a new and exciting area of research.

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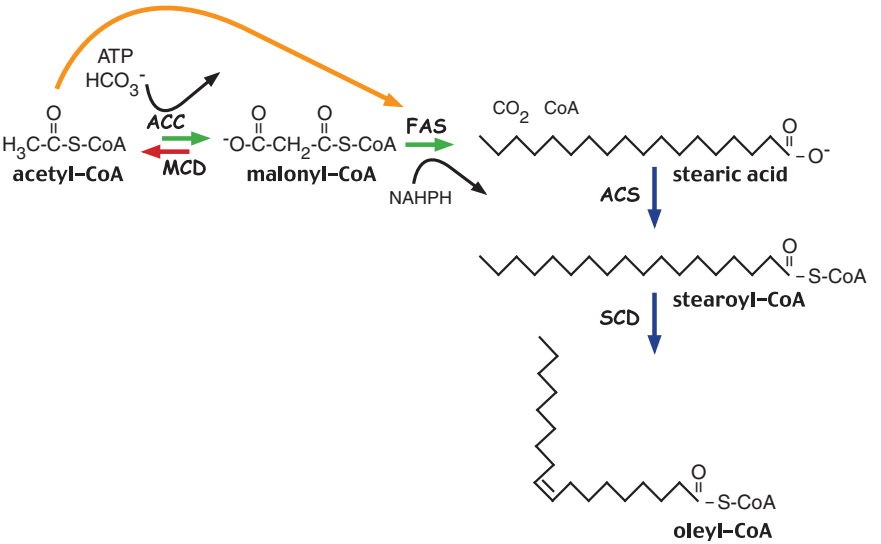

Figure 1 The enzymes of fatty acid biosynthesis. Acetyl-CoA carboxylase (ACC) catalyzes the ATP-dependent carboxylation of cytoplasmic acetyl-CoA to form malonyl-CoA. In some cell types, notably skeletal muscle cells, malonyl-CoA can be decarboxylated by malonyl-CoA decarboxylase (MCD). Fatty acid synthase (FAS) catalyzes reductive chain elongation, adding multiple malonyl-CoAs to an acetyl-CoA primer to produce saturated C_{16} to C_{18} (stearic acid) fatty acids. Acyl-CoA synthetase (ACS) catalyzes the conversion of free fatty acids into their corresponding acyl-CoA thioesters. Stearoyl-CoA desaturase (SCD) catalyzes the insertion of a *cis*-double bond into the Δ^9 position of stearoyl-CoA to produce oleyl-CoA.

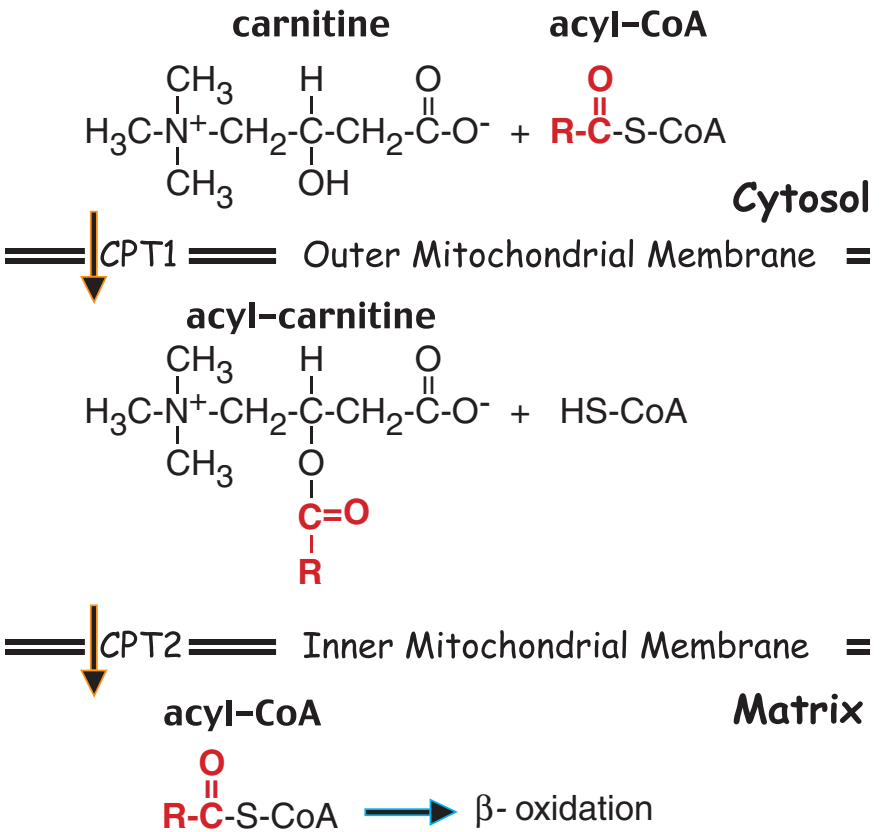

Figure 2 The translocation of fatty acyl-CoAs into mitochondria. Long-chain fatty acyl-CoAs require a specialized regulated system to enter mitochondria. This system determines whether the acyl-CoA will enter the mitochondrial β-oxidation pathway or will enter the triglyceride energy-storage or membrane phospholipid pathways. Fatty acyl-CoAs traverse the mitochondrial membranes by a two-step enzymatic sequence. The first step, catalyzed by carnitine palmitoyltransferase 1 (CPT1), catalyzes the transfer of the fatty acyl group to carnitine, forming a fatty acyl-carnitine. The fatty acyl-carnitine translocates across the inner membrane, where the acyl group is transferred to CoA by carnitine palmitoyltransferase 2 (CPT2) to facilitate transfer in the mitochondrial matrix for β-oxidation.

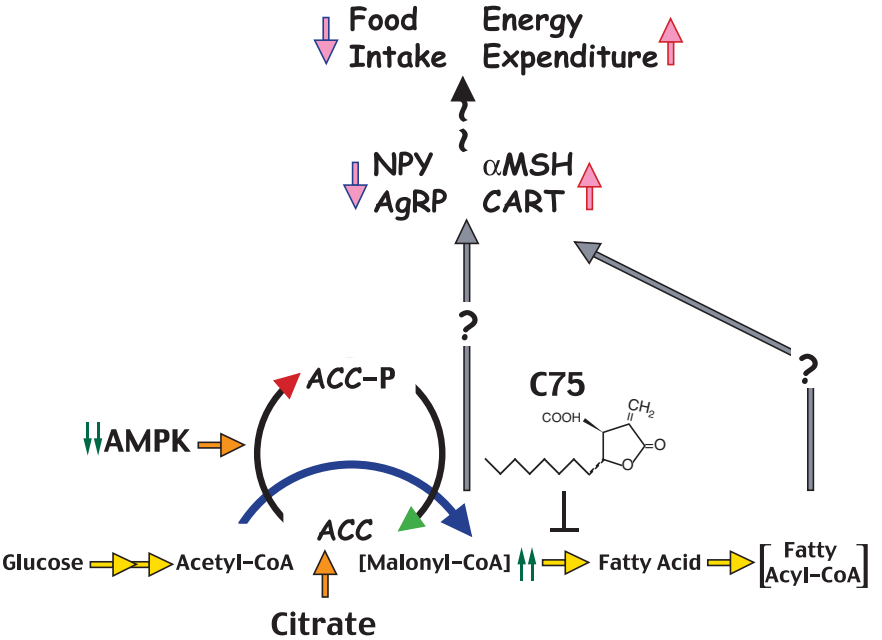

Figure 3 The malonyl-CoA hypothesis. Positive energy balance causes a decreased AMP/ATP ratio, lowered AMP, and inactivation of AMPK, a condition that allows dephosphorylation and activation of ACC. As a consequence, malonyl-CoA is increased. Likewise, the FAS inhibitor C75 leads to an increased malonyl-CoA. An increased malonyl-CoA and/or fatty acyl (FA)-CoA promotes decreased expression of the orexigenic neuropeptides (NPY and AgRP) and increased expression of anorexigenic neuropeptides (POMC/αMSH and CART). These effects combine to reduce food intake and increase energy expenditure.

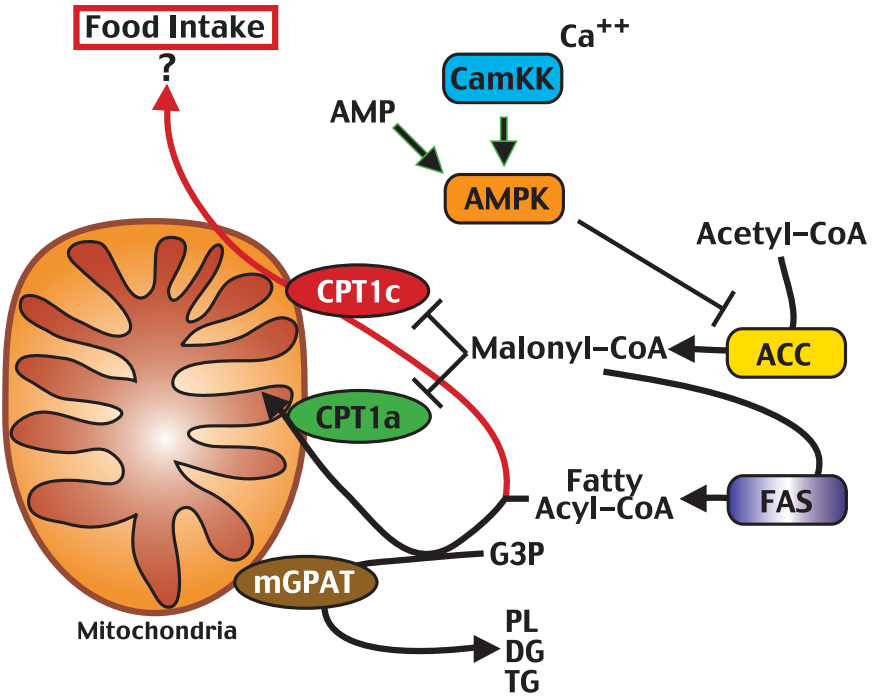

Figure 4 Global model of the regulation of malonyl-CoA and fatty acyl-CoA in hypothalamic neurons regulation of fatty acids and intermediates. AMPK, regulated by the level of AMP and via activation by an AMPKK, possibly CamKK, inactivates ACC, lowering malonyl-CoA, which derepresses CPT1s that utilize fatty acyl-CoAs. Alternatively, GPAT can partition fatty acids into TG. Abbreviations: acetyl-CoA carboxylase, ACC; 5′AMP kinase, AMPK; calmodulin kinase kinase, CamKK; carnitine palmitoyltransferase, CPT; diacylglycerol, DG; fatty acid synthase, FAS; glycerol-3-phosphate, G3P; mitochondrial glycerol phosphate acyltransferase, mGPAT; phospholipid, PL; triacylglycerol, TG.

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