



Review

Hypothalamic malonyl-CoA and the control of food intake

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HIGHLIGHTS

- Changes in hypothalamic malonyl-CoA level impact food intake.
- Arcuate nucleus malonyl-CoA is involved in leptin's effect on feeding.
- CPT-1a may not act downstream of malonyl-CoA effect on feeding in the Arc nucleus.
- CPT-1c and ceramide are potential downstream effectors in malonyl-CoA action of feeding control.

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ABSTRACT

Fatty acid metabolism is implicated in the hypothalamic control of food intake. In this regard, malonyl-CoA, an intermediate in fatty acid synthesis, is emerging as a key player. Malonyl-CoA in the hypothalamus has been proposed as an anorectic mediator in the central control of feeding. A large body of evidence demonstrates that modulating hypothalamic activities of malonyl-CoA metabolic enzymes impacts food intake. Malonyl-CoA action appears to play a significant role in the intracellular signaling pathways underlying leptin anorectic effect in the arcuate nucleus. Ghrelin's hypothalamic effect on feeding may also involve the change in malonyl-CoA metabolism. Hypothalamic malonyl-CoA levels are altered in response to fasting and refeeding, suggesting physiological relevance of the changes in malonyl-CoA level in the controls of feeding and energy balance. Malonyl-CoA inhibits the acyltransferase activity of carnitine palmitoyltransferase-1 (CPT-1), and CPT-1 was considered as a downstream effector in hypothalamic malonyl-CoA effect on feeding. However, recent evidence has not been entirely consistent with this notion. In the arcuate nucleus, the inhibition of CPT-1 acyltransferase activity does not play an important role in the feeding effect of either leptin or cerulenin (a fatty acid synthase inhibitor) that requires the increase in malonyl-CoA level. Alternatively, the brain isoform of CPT-1 (CPT-1c) may act as a downstream target in the malonyl-CoA signaling pathways. CPT-1c does not possess a typical acyltransferase activity, and the exact molecular function of this protein is currently unknown. Recent data indicate it is involved in ceramide metabolism. Of relevance, in the arcuate nucleus, CPT-1c may link malonyl-CoA to ceramide metabolism to affect food intake.

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1. Introduction

Maintaining a healthy body weight requires balancing food intake, energy expenditure and energy deposition [7,46]. Dysregulation of energy homeostasis leads to overweight and obesity, a major health issue in developed countries. Obesity is the major cause of insulin resistance and type 2 diabetes in developed countries, with the associated costs exceeding 200 billion dollars [101]. Control of food intake is a fundamental aspect in maintaining normal body weight, and it has been known for over a century that the hypothalamus in the central nervous system (CNS) plays an essential role [81]. The hypothalamus integrates neural, humoral and nutritional cues to control feeding, as well as govern peripheral metabolic processes to maintain energy homeostasis [81]. The intracellular signaling mechanisms underlying hypothalamic controls of feeding and energy balance have been studied intensively. A growing body of evidence shows that intermediary metabolism in the hypothalamus plays a significant role in controlling feeding [54]. In this regard, fatty acid metabolism has been implicated in the hypothalamic intracellular signaling pathways [19,52,54]. In particular, malonyl-CoA, an intermediate in fatty acid biosynthetic pathway, is emerging as a key player [19,52,54]. The levels of hypothalamic malonyl-CoA are altered in response to different energy balance conditions, and modulation of malonyl-CoA metabolism in the hypothalamus impacts food intake [19,52,54]. The aim of this review is to summarize the roles of hypothalamic malonyl-CoA in the central control of food intake, and to shed light on the intracellular signaling mechanisms mediating the effect of malonyl-CoA on food intake.

2. Effects of modulating hypothalamic malonyl-CoA metabolism on food intake

Malonyl-CoA is derived mainly from glucose metabolism. Glucose is an essential fuel substrate of oxidative metabolism in the production of ATP, and the primary source of energy for brain [85]. Circulating glucose is routed into brain cells through a series of facilitated transports by glucose transporters [85]. Glucose is converted to pyruvate via glycolysis, and pyruvate is then oxidized to form acetyl-CoA inside mitochondria. Acetyl-CoA can then be fed into the tricarboxylic acid (TCA) cycle by condensation with oxaloacetate to form citrate. When citrate accumulates in the mitochondria, it is shuttled to the cytosol for conversion to acetyl-CoA. Cytosolic acetyl-CoA is a direct and major substrate in the generation of malonyl-CoA, and it is carboxylated by the action of acetyl-CoA carboxylase (ACC) [19,52,94] (Fig. 1). In addition to ACC,

fatty acid synthase (FAS) and malonyl-CoA decarboxylase (MCD) are the other important enzymes involved in malonyl-CoA metabolism [19,52]. Malonyl-CoA can be either incorporated into long-chain fatty acids by FAS during fatty acid de novo synthesis [19,26,52,58], or decarboxylated to acetyl-CoA by MCD [19,49,52] (Fig. 1). Pharmacological and molecular genetic studies have demonstrated that modulating hypothalamic malonyl-CoA level by altering the activities of these enzymes (ACC, FAS and MCD) impacts food intake [10,19,52]. A summary of the major findings of the effects of altering hypothalamic malonyl-CoA metabolism on food intake is shown in Fig. 2.

2.1. Fatty acid synthase (FAS)

The model of hypothalamic malonyl-CoA action on food intake stems from the study of compound C75 [53], a potent inhibitor of FAS [51]. In this prototypical study, intracerebroventricular (ICV) or peripheral treatment of mice with C75 reduced food intake [53]. The feeding inhibition following the ICV injection indicates a central effect, with the hypothalamus being considered as a potential target site [53].

By inhibition of FAS activity, treatment of C75 causes two direct changes including [1] the increase in malonyl-CoA level and [2] the decrease in fatty acid synthesis. Either change might mediate the C75-induced feeding inhibition. Another compound 5-(tetradecyloxy)-2-furoic Acid (TOFA), administered by ICV injection prior to C75, blocks the anorectic effect of C75 [53]. TOFA is an inhibitor of ACC, and TOFA treatment reduces cellular level of malonyl-CoA [72], as well as inhibiting fatty acid synthesis [75]. While both TOFA and C75 inhibit fatty acid synthesis, TOFA antagonizes the anorectic effect of C75. This suggests that inhibition of fatty acid synthesis per se is unlikely to mediate the feeding inhibition exerted by C75. Since TOFA treatment can reduce malonyl-CoA level (an opposing action of C75), the increase in malonyl-CoA level was then hypothesized to play the primary role in the C75-induced anorectic action [53]. Subsequent studies demonstrated that ICV injection of C75 increases hypothalamic malonyl-CoA level, while central pretreatment with TOFA prevents this from occurring [43]. These data show that the increase in hypothalamic malonyl-CoA level may lead to reduction of food intake.

Importantly, these findings established a framework for developing a model for the control of food intake by hypothalamic malonyl-CoA. Further evidence supporting this model came from studies of the effect of tamoxifen on feeding [56]. Tamoxifen, a modulator of the estrogen receptor, exerts an anorectic action [56]. Along with the decrease in food intake, tamoxifen treatment downregulates FAS in the hypothalamus,

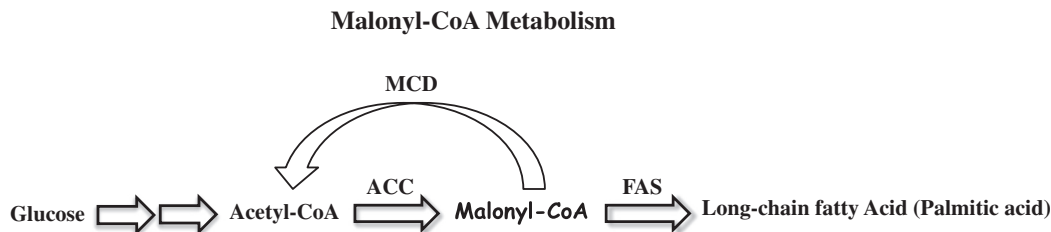


Fig. 1. Acetyl-CoA, derived from glucose metabolism, is converted to malonyl-CoA by acetyl-CoA carboxylase (ACC). Malonyl-CoA is incorporated by fatty acid synthase (FAS) into long-chain fatty acid such as palmitic acid, or is degraded to acetyl-CoA by malonyl-CoA decarboxylase (MCD).

Malonyl-CoA Model in Hypothalamic Control of Food Intake

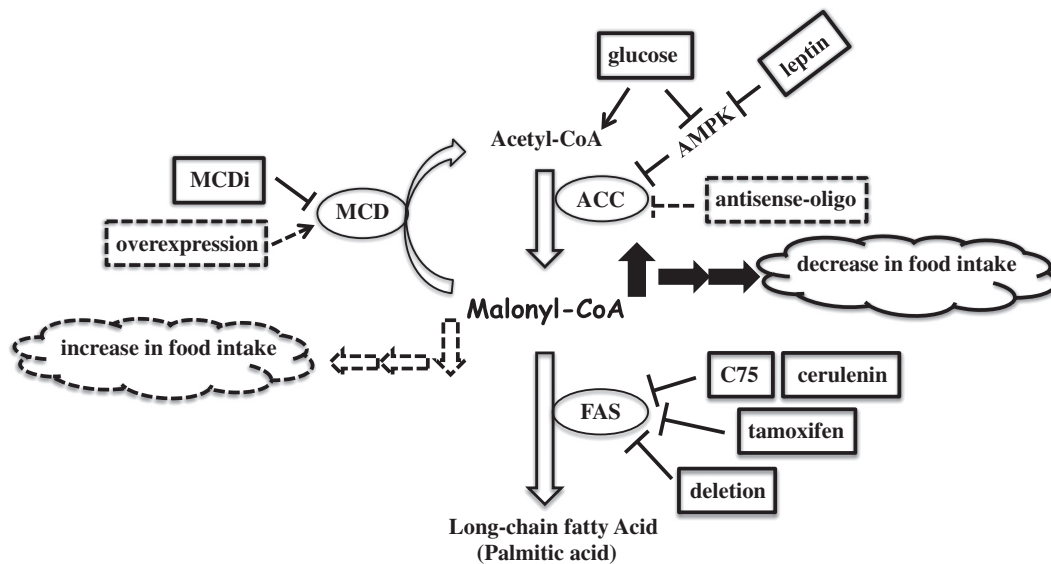


Fig. 2. Malonyl-CoA in the hypothalamus is proposed as an anorectic mediator in the CNS control of food intake. Leptin inhibits AMP-activated kinase (AMPK) that inhibits ACC. This leads to activation of ACC resulting in increase in malonyl-CoA level. Glucose activates ACC by inhibiting AMPK, and increases availability of acetyl-CoA. Both effects result in increases in malonyl-CoA level. FAS inhibitors such as C75 and cerulenin, deletion of FAS protein, and tamoxifen that downregulates FAS level, increase malonyl-CoA levels. MCD inhibitor (MCDi) reduces MCD activity resulting in increase in malonyl-CoA level. The increases in malonyl-CoA level lead to decrease in food intake. Overexpression of MCD reduces malonyl-CoA level, which leads to increase in food intake. Antisense oligonucleotide reduces ACC protein level decreasing malonyl-CoA level, which increases food intake.

thereby increasing malonyl-CoA level [56]. As in the C75-study, central administration of TOFA (ACC inhibitor) also prevents the feeding inhibition (by tamoxifen), suggesting that the increase in hypothalamic malonyl-CoA plays a role in mediating the anorectic action of tamoxifen [56]. Evidence from mouse knockout model also supports a potential role of hypothalamic malonyl-CoA in the control of food intake. It has been shown that mice with deletion of FAS in the mediobasal hypothalamus exhibit an elevated level of hypothalamic malonyl-CoA, and have decreased food intake [10]. Considering that illness and nonspecific effects may underlie the anorectic response of C75 treatment [15,88], the genetic evidence indicates that FAS inhibition increasing malonyl-CoA level can indeed induce anorectic effects. Thus, evidence from the pharmacological and genetic studies suggests that malonyl-CoA in the hypothalamus acts as an anorectic mediator in the CNS control of food intake.

2.2. Acetyl-CoA carboxylase (ACC)

Malonyl-CoA is produced primarily by the action of ACC, which catalyzes the carboxylation of acetyl-CoA to malonyl-CoA [19,52,94]. ACC has two distinct isoforms, ACC- α and ACC- β [90]. In the periphery, the α -isoform predominates in tissues having high levels of fatty acid synthesis such as liver, and the β -isoform is present in tissues with a high oxidative capacity such as heart [90]. In the hypothalamus, both isoforms are expressed [30,47], and at least the α -isoform has been shown to play a role in hypothalamic control of food intake [29].

The role of ACC in malonyl-CoA-mediated control of food intake has been demonstrated in the leptin-induced anorectic effect in rats [29]. Central leptin treatment increases ACC activity in the hypothalamus, which involves the action of AMP-activated protein kinase (AMPK) [29]. AMPK is a kinase of ACC, and phosphorylation (by AMPK) of a serine residue (Ser79 in ACC- α) inhibits ACC activity [8,37]. Leptin treatment inhibits AMPK activity in the hypothalamus, which is required in the anorectic effect induced by leptin [66]. By reducing the activity of AMPK, central leptin further induces activation of ACC in the hypothalamus [29]. Blockade of ACC activation by TOFA prevents

leptin's anorectic action, suggesting that ACC activation is a necessary step in leptin's hypothalamic anorectic signaling pathway [29]. Through activating ACC, central leptin increases the level of malonyl-CoA in hypothalamic arcuate nucleus [29]. As the prevention of the increase by overexpression of MCD blocks leptin-induced reduction of food intake [28,41], the increase in malonyl-CoA level is required in leptin's feeding effect. Thus, ACC links AMPK and malonyl-CoA to mediate leptin's hypothalamic effect on food intake.

The AMPK–ACC–malonyl-CoA axis is also involved in the anorectic response to glucose. Glucose treatment can inhibit AMPK activity [66] and increase malonyl-CoA level [96] in the hypothalamus. Central infusion of TOFA abrogates the anorectic effect by glucose [34], suggesting that hypothalamic ACC activation mediates glucose-induced reduction in food intake. These data [34,96] also show that the increase in hypothalamic malonyl-CoA may constitute an important step in glucose-induced anorectic effect.

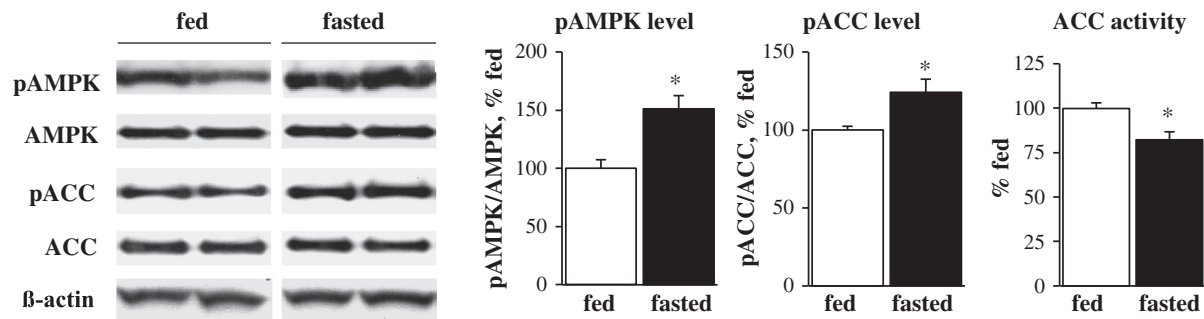
In support of the malonyl-CoA signaling model, the effect of primary disruption of hypothalamic ACC activity on feeding has been demonstrated. It has been shown that antisense inhibition of hypothalamic ACC induces increase in food intake [77].

2.3. Malonyl-CoA decarboxylase (MCD)

Studies of hypothalamic MCD have provided further evidence supporting a role for the malonyl-CoA model. MCD is an important enzyme in malonyl-CoA degradation [19,49,52]. Overexpression of MCD in the mediobasal hypothalamus of rats reduces malonyl-CoA levels, and increases food intake [28,41]. In contrast, central infusion of MCD inhibitors increases hypothalamic malonyl-CoA level, and induces hypophagia [54]. In addition to these animal studies, a clinical case report has observed a marked reduction of appetite in a girl harboring brain MCD deficiency, which would produce an increased level of malonyl-CoA in the hypothalamus [16]. Taken together, these data show that modulating MCD activity thereby altering malonyl-CoA level can impact the central control of food intake.

ACC activities in the Arc nucleus under fasting and refeeding conditions

(A) Fasting (negative energy balance state)



(B) Refeeding (positive energy balance state)

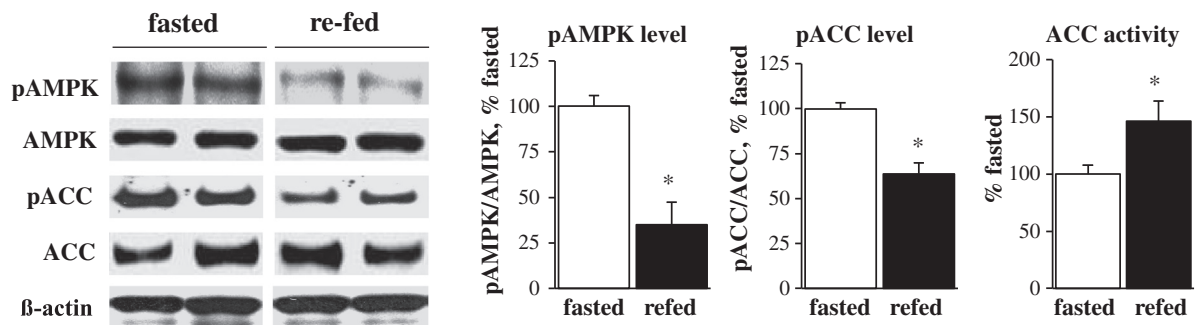


Fig. 3. (A) Fasting: Some rats were fasted for around 24 h (fasted, $n = 10$). The other rats were fed ad lib. (fed, $n = 12$). (B) Refeeding: Some rats were fasted for around 48 h (fasted, $n = 12$). The other rats were presented with food at the end of fasting (refed, $n = 11$). The mediobasal hypothalamus consisting mainly of the Arc nucleus was dissected. Phospho-AMPK (pThr 172), phospho-ACC (pSer79), AMPK (α -subunit) and ACC (α -isoform) were measured by Western blotting analysis. The level of phospho-AMPK (pAMPK) or phospho-ACC (pACC) was normalized to that of AMPK or ACC. Actin was used as the loading control. ACC activity assay was performed based on the method of $^{14}\text{CO}_2$ fixation to acid-stable products as described in Reference [29].

3. Regulation of hypothalamic malonyl-CoA levels in fasting and refeeding states

The levels of hypothalamic malonyl-CoA vary under different energy balance conditions such as fasting and refeeding. Following fasting, a negative energy balance state, malonyl-CoA level drops [43,55]. Since malonyl-CoA acts as an anorectic mediator, the decrease in its level would stimulate food intake when food is available. Following refeeding, hypothalamic malonyl-CoA level increases [43,55], which would limit the rebound feeding response. These changes were initially demonstrated using malonyl-CoA assayed in whole hypothalamic tissue extracts [43,55], and later studies have identified the Arc nucleus as a site where these changes occur [28]. In the Arc, AMPK is likely an upstream mediator, resulting in the changes in malonyl-CoA level through ACC. In fasting state, AMPK activity in the Arc increases [66]. This would increase the phosphorylation level of ACC inhibiting ACC activity, thereby reducing malonyl-CoA level. In contrast, in re-fed state, AMPK activity in the Arc decreases [66]. This would increase the activity of ACC, thereby increasing malonyl-CoA level. Indeed, the phosphorylation level and activity of ACC in the Arc change as expected in fasting and refeeding states (Gao, S. and Moran, T.H.; unpublished data; Fig. 3). Thus, AMPK, ACC and malonyl-CoA appear to act as an intracellular signaling module in different nutritional states. In addition to ACC, MCD may also be involved in regulating malonyl-CoA level under fasting or refeeding condition. There has been evidence showing the activation of hypothalamic MCD following fasting, which would contribute to the reduction of malonyl-CoA level [54].

4. Malonyl-CoA metabolism and hypothalamic neuropeptides controlling food intake

The arcuate nucleus (Arc) is a key site mediating hypothalamic controls of food intake and energy balance [23,81]. In the Arc, there are two well-known populations of neurons with opposing effects on food intake and energy balance [23,36,57,81,100]. One subset of neurons express neuropeptide Y (NPY) and Agouti-related peptide (AgRP) that are orexigenic and promote positive energy balance [1,6,13,14,19,35,38,81,83,86], while the other subset of neurons express proopiomelanocortin (POMC) and cocaine- and amphetamine-regulated transcript (CART) that are anorexigenic and promote negative energy balance [4,13,17,20,21,24,45,50,71,81]. The arcuate neuropeptide system mediates aspects of the hypothalamic mechanisms underlying the controls of feeding and energy balance. Changes in hypothalamic malonyl-CoA levels following the modulations of malonyl-CoA metabolic enzymes (ACC, FAS and MCD) are associated with alterations of the neuropeptide levels [19,54]. Of physiological relevance, accompanying the alterations of malonyl-CoA levels in fasting and refeeding states are the expressions of the Arc neuropeptides [19,43,82]. Thus, increases in malonyl-CoA levels (when food intake is reduced) are associated with downregulations of NPY and AgRP [10,29,43,82,96], and upregulations of POMC and CART [43,56,82,87,96]. Conversely, decreases in malonyl-CoA levels (when food intake is augmented) are associated with upregulations of NPY and AgRP [41]. The correlations indicate that malonyl-CoA may regulate the expressions of the Arc neuropeptides.

5. Hormonal regulation of hypothalamic malonyl-CoA metabolism

The hypothalamus receives and integrates hormonal, neuronal and nutritional cues to control food intake [54,81]. A number of hormones exert effects on the control of feeding via interaction with hypothalamic receptors [54]. A growing body of evidence has shown that the hypothalamic intracellular signaling pathways of these hormones involve changes in intermediary metabolic pathways [54]. In this regard, the roles of malonyl-CoA action have received much attention.

5.1. Leptin and hypothalamic malonyl-CoA

As mentioned above, malonyl-CoA metabolism in the hypothalamus plays an important role in mediating aspects of leptin-associated feeding effect. Leptin, an adipose-derived hormone, is a critical humoral factor involved in the CNS control of feeding and regulation of energy homeostasis. Leptin receptors are abundantly expressed in the hypothalamic nuclei, such as the Arc nucleus containing NPY/AgRP and POMC/CART neurons [12,22,25,39,40,44,63,64,79,95]. The Arc nucleus mediates aspects of leptin's anorectic actions [67,93]. Leptin's feeding actions involve the downregulation of orexigenic NPY and AgRP, and upregulation of anorexigenic POMC/CART [50,78–80,91,95]. The Arc nucleus is a major site in mediating leptin's effects on malonyl-CoA metabolism [29]. In the Arc, leptin impacts malonyl-CoA metabolism by altering the activities of AMPK and ACC [29]. Leptin treatment inhibits AMPK activity, which results in reduced phosphorylation level (of Ser79) of ACC [29]. Consistent with the change in phosphorylation state, an enzyme activity assay demonstrates that exogenous leptin increases the ACC activity in the Arc [29]. The activation of ACC further leads to the increase in malonyl-CoA level in the Arc, which is required in leptin's anorectic effect [28,29,41]. In addition, prevention of leptin-induced increase in Arc malonyl-CoA level is associated with the blockade of leptin-mediated upregulation of Arc NPY [29,41]. This suggests malonyl-CoA metabolism in the Arc is implicated in leptin-mediated regulation of hypothalamic neuropeptide. Leptin's effect on hypothalamic malonyl-CoA level appears to be site-specific, as the change is confined to the Arc [29].

5.2. Ghrelin and hypothalamic malonyl-CoA

Ghrelin, a stomach-derived hormone, has been implicated in the central control of food intake [18,68,98,99]. Pharmacological studies have demonstrated that administration of ghrelin induces feeding [18,68,98,99]. The hypothalamus is a target site in mediating ghrelin's orexigenic effect [11,33,68,89,92,99]. Ghrelin treatment increases the level of phosphorylated AMPK indicating activation, and the level of phosphorylated ACC indicating inhibition [2,3,55]. It is then predicted that the inhibition of ACC would reduce the level of malonyl-CoA. Indeed, it has been reported that ghrelin treatment decreases the malonyl-CoA level in the hypothalamus [55]. However, the role of this decrease in ghrelin's hypothalamic feeding action is less clear. It is expected that the decrease in malonyl-CoA level is an important step in the hypothalamic intracellular signaling pathways underlying ghrelin's central orexigenic effect. The target site in mediating ghrelin's effect on hypothalamic malonyl-CoA metabolism appears to be the ventromedial nucleus (VMN), but not the Arc [27].

6. Downstream mediators of the feeding effect of hypothalamic malonyl-CoA

6.1. Roles of the classical isoforms of carnitine palmitoyltransferase-1

Three isoforms of carnitine palmitoyltransferase-1 (CPT-1) have been identified. Among them, CPT-1-liver type (CPT-1a) and CPT-1-muscle type (CPT-1b) are the ones that possess the typical or "classical" acyltransferase activity. The third CPT-1 isoform (CPT-1c or the "novel"

CPT-1) does not possess typical acyltransferase activity [74,84,97], and the exact function of this protein is currently unknown. The CPT-1a and CPT-1b are mitochondrial enzymes that play an essential role in the β -oxidation pathway of fatty acid [62]. In the mitochondria, the acyltransferase activity of CPT-1 catalyzes the conversion of long-chain fatty acyl-CoA (the esterified fatty acid) to long-chain acylcarnitine, which is translocated across the mitochondrial membrane and into the matrix for oxidative metabolism [61,62].

Malonyl-CoA acts as a potent inhibitor of CPT-1 acyltransferase activity [61,62,76]. In the hypothalamus, the predominant isoform possessing the typical acyltransferase activity is CPT-1a [69]. Of relevance, inhibition of CPT-1a in the hypothalamic Arc nucleus is associated with anorectic effect, indicating that Arc CPT-1a may have a role in the central control of feeding [69]. Furthermore, given that malonyl-CoA acts as an anorectic mediator in the Arc, CPT-1a was considered to mediate the intracellular downstream action of Arc malonyl-CoA feeding effect [69]. In this regard, however, several lines of evidence have now strongly challenged this hypothesis. First, although it induces a robust increase in malonyl-CoA level in the Arc, leptin treatment does not alter the activity of Arc CPT-1a acyltransferase activity [28,29]. This suggests that changes in malonyl-CoA do not necessarily impact CPT-1a activity. Second, Arc overexpression of a malonyl-CoA insensitive mutant CPT-1a fails to alter leptin or cerulenin's central anorectic effect, in which the increase in Arc malonyl-CoA level is required [28,29]. These results indicate that the potential inhibitory action (by malonyl-CoA) on CPT-1a is not significant in Arc malonyl-CoA-mediated feeding effect. Finally, the CPT-1 acyltransferase activity in the Arc is not altered in fasting or refeeding state, although Arc malonyl-CoA level is [28,29], which shows a dissociation of malonyl-CoA action from CPT-1a. Taken together, these data suggest that CPT-1a is not a critical component of the intracellular signaling pathway underlying Arc malonyl-CoA effect on food intake. Several potential mechanisms underlie this conclusion. First, as the hypothalamus is comprised of a heterogeneous population of highly specialized neurons, the enzymes involved in malonyl-CoA metabolism and CPT-1a may not be expressed in the same cell. Thus, in the Arc, the alteration of malonyl-CoA level may have taken place in the cells that do not express CPT-1a. Second, the two isoforms of ACC that produce malonyl-CoA, i.e., ACC- α and ACC- β , may exert different effects on regulating CPT-1a activity [60]. It has been reported that the ACC- α -associated malonyl-CoA does not significantly affect CPT-1a-mediated fatty acid β -oxidation, as compared to the ACC- β -derived malonyl-CoA [60]. Since leptin specifically activates ACC- α isoform to increase the malonyl-CoA level [29], the differential effect between ACC isoforms may be a cause for the lack of change in Arc CPT-1a activity following leptin treatment. Finally, the activity of CPT-1 acyltransferase in the brain is known to be low as compared to that in the peripheral tissues [5]. It follows that CPT-1a in the hypothalamic Arc nucleus may not be subject to the regulation of malonyl-CoA. This potential mechanism becomes particularly significant under the conditions that malonyl-CoA levels are increased, such as following leptin and cerulenin treatments. Thus, the already low activity of CPT-1a in the Arc might be resistant to a further inhibition by malonyl-CoA.

Increases in the level of hypothalamic long-chain fatty acyl-CoA (LCFA-CoA), the substrate of CPT-1 acyltransferase activity, have been proposed to exert anorectic effect [69,70]. As malonyl-CoA-mediated inhibition of CPT-1 can increase the cytosolic levels of LCFA-CoA, LCFA-CoA was considered as an effector downstream of CPT-1a in mediating malonyl-CoA effect on food intake [69,70]. Since the current data strongly suggest that Arc malonyl-CoA-mediated control of feeding does not involve the action of CPT-1a, LCFA-CoA appears not to be implicated in malonyl-CoA anorectic signaling action, at least in the Arc nucleus. In addition, change in CPT-1 acyltransferase activity might impact downstream fatty acid oxidation (FAO), and the alteration of FAO activity might play a role in hypothalamic control of feeding [3,69]. Because the change in FAO is secondary to that of CPT-1 acyltransferase activity, and CPT-1a activity does not seem to be relevant in Arc

malonyl-CoA feeding effect, FAO may not act as intracellular downstream effector in Arc malonyl-CoA-mediated control of feeding.

It should be emphasized that there has been no published evidence excluding the potential role of Arc CPT-1a per se in the hypothalamic control of food intake. In the Arc, CPT-1a may act to regulate food intake, independent of malonyl-CoA action. The hypothalamic ventromedial nucleus (VMN) is another important site in the control of feeding [48], and malonyl-CoA feeding action is also implicated in this nucleus [55,56]. It is possible that CPT-1a plays significant roles in the malonyl-CoA-mediated control of feeding in the VMN. Recent evidence has demonstrated that VMN overexpression of the malonyl-CoA insensitive mutant CPT-1a is able to block the anorectic effect by central cerulenin, suggesting that malonyl-CoA inhibition of CPT-1a is implicated in malonyl-CoA anorectic signaling action in the VMN [31].

6.2. The roles of novel carnitine palmitoyltransferase-1 in the malonyl-CoA control of food intake

The novel isoform of CPT-1 or CPT-1c, with a sequence similar to the classical CPT-1 members, is predominantly expressed in brain [74,84,97]. The exact function of CPT-1c is currently less clear. Initial studies showed that CPT-1c did not possess acyltransferase activity [74,97]. A later study using a more sensitive assay identified a very weak acyltransferase activity associated with CPT-1c (20–300 times lower when compared to CPT-1a or CPT-1b) [84]. Unlike CPT-1a or CPT-1b that uses LCFA-CoA's (such as palmitoyl-CoA, stearoyl-CoA and oleoyl-CoA) as the substrates, CPT-1c preferentially uses palmitoyl-CoA as the substrate [84]. In addition, unlike the classical CPT-1 members, a significant portion of CPT-1c is localized in the endoplasmic reticulum (ER) [84]. Recent evidence demonstrates that CPT-1c has a role in the ceramide metabolism [9,32]. In particular, due to the substrate preference and subcellular localization, it has been hypothesized [32] that CPT-1c is involved in de novo biosynthesis of ceramide that uses palmitoyl-CoA as an initial substrate and starts in the ER [59,65,73]. Indeed, in the Arc nucleus, overexpression of CPT-1c increases the level of ceramide, while CPT-1c knockout animals exhibit a reduced level [32]. Thus, CPT-1c regulates ceramide level in the Arc, possibly by promoting the de novo biosynthesis of ceramide.

CPT-1c knockout mice fed with regular chow diet have reduced food intake [97], while Arc-overexpression of CPT-1c induces an increase in rebound intake of chow diet following fasting [32]. These results suggest a role of CPT-1c in the central control of food intake. In addition, CPT-1c has a similar binding affinity for malonyl-CoA as the classical CPT-1 [74,84,97]. It follows that CPT-1c acts as a downstream target in

hypothalamic malonyl-CoA-mediated feeding control [32,97]. Since the deletion of CPT-1c produces an anorectic effect, malonyl-CoA likely inhibits the function of CPT-1c to exert the anorectic effect. In support, Arc overexpression of CPT-1c was reported to block leptin or cerulenin-induced anorectic effect, in which the increase in Arc malonyl-CoA is required [32]. It was further predicted that CPT-1c related ceramide metabolism would be a target of intracellular malonyl-CoA action [32]. Consistent with this prediction, Arc overexpression of MCD reducing the malonyl-CoA level increases the ceramide level, and leptin treatment that increases Arc malonyl-CoA level reduces ceramide level [32]. Thus, in the Arc, malonyl-CoA may regulate ceramide metabolism. Importantly, the inhibition of ceramide de novo biosynthesis prevents the development of the orexigenic effect by MCD overexpression [32]. This suggests that ceramide de novo biosynthesis is necessary for and mediates the downstream action of Arc malonyl-CoA on feeding. Furthermore, inhibition of ceramide de novo biosynthesis also prevents the development of the orexigenic effect of the overexpression of CPT-1c in the Arc nucleus [32], which suggests that ceramide de novo biosynthesis acts downstream of the Arc CPT-1c effect on feeding. Finally, Arc pretreatment with a ceramide analog compound blocks leptin-induced feeding inhibition [32], suggesting the decrease in ceramide level is an important step in leptin-induced anorectic effect. Collectively, these results indicate that in the Arc, CPT-1c and ceramide de novo biosynthesis may mediate the downstream action in malonyl-CoA control of food intake (Fig. 4).

7. Future directions

7.1. Downstream mediators of hypothalamic malonyl-CoA feeding effect

CPT-1c and ceramide metabolism have been hypothesized to act downstream of hypothalamic malonyl-CoA in the control of feeding. In this model, two critical issues should be addressed in future studies. First, the exact molecular function of CPT-1c is currently unknown. As a result, the inhibitory effect of malonyl-CoA on CPT-1c has been speculative. Elucidation of the molecular function of CPT-1c will clarify the nature of the potential interaction of malonyl-CoA with CPT-1c. Furthermore, this will also facilitate the identification of the molecular mechanism(s) underlying the CPT-1c-mediated regulation of ceramide metabolism. Second, the mechanisms of how ceramide modulates feeding should be investigated. Identification of the underlying signaling pathway would contribute to bridging the gap between malonyl-CoA metabolism and its action on feeding.

Intracellular Downstream Mediators of Arc Malonyl-CoA Action on Feeding

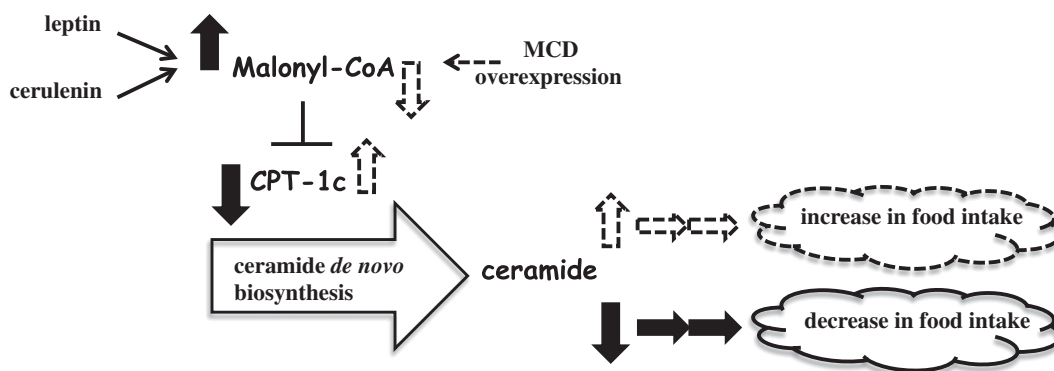


Fig. 4. In the Arc nucleus, CPT-1c and ceramide de novo biosynthesis mediates downstream effect of malonyl-CoA action on feeding. Malonyl-CoA may inhibit CPT-1c function, and CPT-1c regulates ceramide level, possibly by enhancing de novo biosynthesis. Leptin and cerulenin that increase the levels of malonyl-CoA would inhibit CPT-1c, which leads to decreases in ceramide level. The reduction of ceramide level is involved in the decrease in food intake. In contrast, MCD overexpression reducing the malonyl-CoA level would activate CPT-1c, which leads to increase in ceramide level. The upregulation of ceramide level contributes to the increase in food intake.

7.2. Neuron-specific actions of malonyl-CoA control of feeding

The hypothalamic nuclei consist of neurons with different functions. For example, the Arc nucleus contains two distinct neuronal populations, NPY/AGRP neurons and POMC neurons, with opposing roles in the controls of feeding and energy balance. The potential neuron-specific effects of malonyl-CoA on feeding are unknown. It has been shown that FAS is present in NPY/AGRP neurons [47], which indicates active malonyl-CoA metabolism in these neurons. Yet, it is not known whether malonyl-CoA metabolic enzymes are expressed in POMC neurons. Furthermore, recent evidence suggests a functional heterogeneity across the POMC neuronal population [42]. Thus, future studies should aim at addressing malonyl-CoA actions in the specific subsets of neuronal populations.

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