

# C75, a Fatty Acid Synthase (FAS) Inhibitor

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**Abstract:** Recent data has demonstrated that fatty acid metabolism plays a critical role in the hypothalamic regulation of food intake and the evidence is as follows. Circulating long chain fatty acids act as nutrient surplus signals in the hypothalamus. On addition, fatty acid synthesis pathway enzymes, such as fatty acid synthase (FAS) and acetyl-CoA carboxylase (ACC) and its upstream regulator, AMP-activated protein kinase (AMPK) are regulated by nutritional and hormonal stimuli. Very importantly, current evidence also indicates that fatty acid metabolism pathway may be a potential target for obesity treatment. In this sense, it has been demonstrated that pharmacological inhibition of FAS results in profound decrease in food intake and body weight in rodents. These anorectic actions are mediated by the modulation of hypothalamic neuropeptide systems, through a malonyl-CoA dependent mechanism.

In this review, we recapitulate what is known about hypothalamic fatty acid metabolism and the regulation of feeding, with particular interest in a specific FAS inhibitor, C75, which has been recently patented as a potential drug for adipose mass reduction.

**Keywords:** AMP-activated protein kinase (AMPK), C75, cerulenin, fatty acid synthase (FAS), FAS inhibitors, food intake, hypothalamus, lipid sensing, tamoxifen.

## INTRODUCTION

In both the developed and developing world, levels of obesity and its related disorders are increasing at a rate that could be considered as epidemic. The major alarm is its association with insulin resistance, type 2 diabetes, fatty liver (steatosis) and range of other disorders generally known as Metabolic Syndrome [1-5], as well as sleep apnea, musculoskeletal disorders and several types of cancer [6]. For this cause, much effort is focus on recognizing the basic molecular mechanisms governing energy balance and food intake [4,5,7,8].

Energy balance depends on the effectiveness of firmly regulated mechanisms of energy intake and consumption. Despite energy balance being affected by many modulatory factors, obesity is at last the result of a positive imbalance between energy acquirement and energy expenditure. At biochemical and physiological level, there is a noteworthy amount of cross-regulation between the various parts of the energy balance equation, such that changes in one part of the equation leads to counter-regulation of the other [1-3,8].

In this precise homeostatic network, the central nervous system (CNS) and more specifically the hypothalamus play a major role. Discrete nuclei within the hypothalamus respond to changes in energy status by modifying the expression of specific neuropeptides which cause changes in energy intake and expenditure [3,8-10]. Once the information is processed in discrete hypothalamic nuclei, specific signals are transmitted to other brain centers, in the cerebral cortex and the brainstem, to produce an appropriate feeding response [3,8-10].

Hypothalamic neurons respond to changes in energy status by modulating the synthesis of neuropeptides that results in appropriate changes in energy intake and expenditure. Thus, when energy intake exceeds expenditure, expression of the orexigenic (feeding-promoting) neuropeptides i.e., agouti-related protein (AgRP) and neuropeptide Y (NPY) decreases. Conversely, the expression of anorexigenic (feeding-inhibitors) neuropeptides, i.e., cocaine and amphetamine-regulated transcript (CART) and proopiomelanocortin (POMC) increases. On the other hand, when energy expenditure exceeds intake the opposite response occurs, with increase in orexigenic and decrease in anorexigenic neuropeptides [3,8-10].

## MACRONUTRIENTS AS SIGNALING MOLECULES: LIPID SENSING

The role of lipids on the regulation of feeding has been proposed since long time ago. In fact, one of the first hypotheses proposed to explain the periphery-brain interaction in the regulation of food intake was the Lipostatic Hypothesis. This model suggested that circulating lipids, produced in proportion to body fat stores and/or nutritional status, acted as signals to the brain, eliciting changes in energy intake and expenditure [11]. During the 1980s, some convincing evidence demonstrated the anorectic effect of peripheral lipid emulsion treatments [12]. However, in spite of these data, the specific molecular nature of the signal molecule remained unidentified until four years ago when, in a series of smart experiments, Rossetti and colleagues demonstrated that central administration of LCFAs exerted a signaling role within specific hypothalamic energy centers. For instance, intracerebroventricular (ICV) administration of oleic acid (OA, C18:1) inhibited food intake [13,14], an effect that was not reproduced by medium chain fatty acids (MCFAs), such as octanoic acid (C8) [13]. Of note, octanoic

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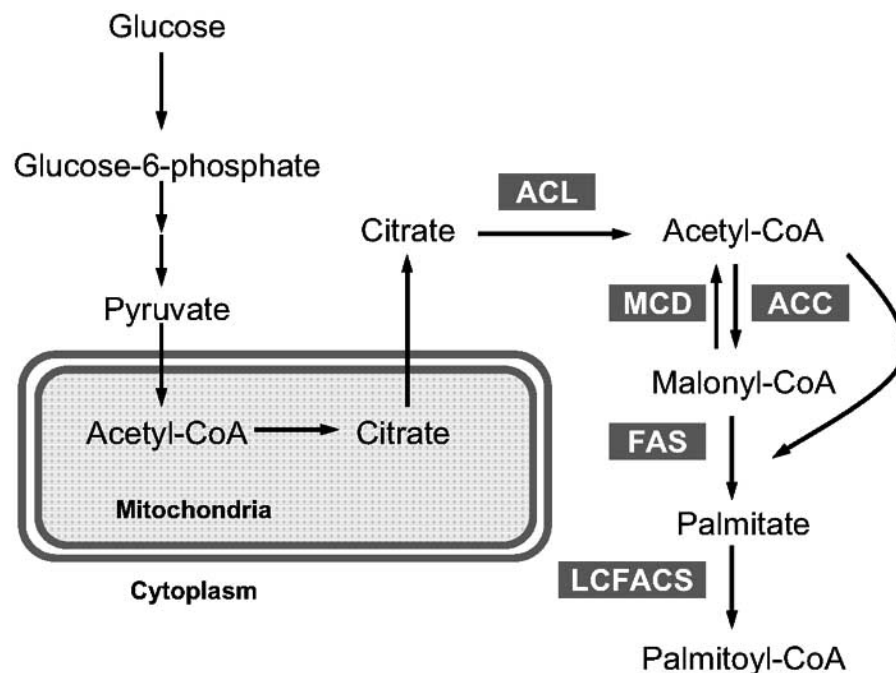
acid does not require carnitine palmitoyltransferase 1 (CPT1) for entry in the mitochondria for  $\beta$ -oxidation, (see below), indicating the importance of this step in LCFA-mediated hypothalamic regulation of feeding [13]. Equally important, the esterification of LCFAs to LCFA-CoA by the enzyme long-chain fatty acyl-CoA synthetase (LCFACS) is an obligatory step for the hypothalamic action of LCFAs in the hypothalamus [15,16]. The anorectic effects of OA are mainly exerted on the arcuate nucleus of the hypothalamus (ARC) nucleus where AgRP and NPY mRNA expression was decreased after OA treatment [13,14]. Conversely, POMC expression was not affected by OA, indicating that the anorectic action of LCFAs is mediated by a specific inhibition of those orexigenic neuropeptides [14].

The major implication of this finding is the possibility that physiological oscillations of circulating levels of LCFAs may directly affect the LCFA-CoA levels in the hypothalamus proportionally to the energy surplus. As expected, peripheral administration of LCFAs induced a rapid elevation in the hypothalamic content of LCFA-CoA [15], in addition the simultaneous hypothalamic administration of triascin C, a pharmacological inhibitor of LCF-ACS, prevented the effect of peripheral LCFAs on the hypothalamus [15]. Taken these data together with the powerful anorectic action of central administration of OA, these findings suggest that an increase in the hypothalamic levels of LCFA-CoAs may also be important for the physiological control of food intake.

## FATTY ACID SYNTHESIS PATHWAY IN THE HYPOTHALAMUS

In situations where total energy intake exceeds energy expenditure, fatty acids and triacylglycerols are synthesized and triacylglycerols are stored in adipose tissue. Under these lipogenic conditions, excess glucose in the cell is first converted to pyruvate via glycolysis in the cytoplasm. Pyruvate is converted to acetyl coenzyme A (acetyl-CoA) and transported as citrate from mitochondria into cytoplasm. ATP citrate lyase (ACL) then converts citrate back to acetyl-CoA. Acetyl-CoA carboxylase (ACC) catalyzes the carboxylation of acetyl-CoA to malonyl-CoA in an ATP-dependent manner. The synthesis step of malonyl-CoA is a reversible regulated mechanism and malonyl-CoA decarboxylase (MCD) converts malonyl-CoA back to acetyl-CoA. Acetyl-CoA and malonyl-CoA are then used as the substrates for the production of palmitate by the seven-enzymatic reactions catalyzed by fatty acid synthase (FAS) Fig. (1). The fatty acids produced are then used for the synthesis of triacylglycerol [16-19].

Neurons and glial cells require lipid synthesis to preserve their metabolic homeostasis. Fatty acid biosynthetic enzymes and CPT1 are constitutively expressed in the brain. ACC, FAS, MCD and AMP-activated protein kinase (AMPK) mRNA and protein expression have been detected at extremely elevated levels in the ARC, dorsomedial (DMH) and ventromedial (VMH) hypothalamic nuclei in rodents



**Fig. (1).** Fatty acid synthesis pathway. Under lipogenic conditions, surplus glucose in the cell is first converted to pyruvate via glycolysis in the cytoplasm. Pyruvate is converted to acetyl-CoA and transported as citrate from mitochondria into cytoplasm. ATP citrate lyase (ACL) then converts citrate back to acetyl-CoA. Acetyl-CoA carboxylase (ACC) catalyzes the carboxylation of acetyl-CoA to malonyl-CoA in an ATP-dependent manner. Acetyl-CoA and malonyl-CoA are then used as the substrates for the production of palmitate by the seven-enzymatic reactions catalyzed by fatty acid synthase (FAS). The synthesis step of malonyl-CoA is a reversible regulated mechanism and malonyl-CoA decarboxylase (MCD) converts malonyl-CoA back to acetyl-CoA. The resulting saturated fatty acid molecule produced by FAS can be further metabolised depending on requirements; desaturated to form unsaturated fatty acids; to triglyceride molecules for storage; or to a range of phospholipids and derivatives for membrane and signaling functions.

and humans [13,20-24], suggesting that the FA synthesis pathway is particularly important in this system.

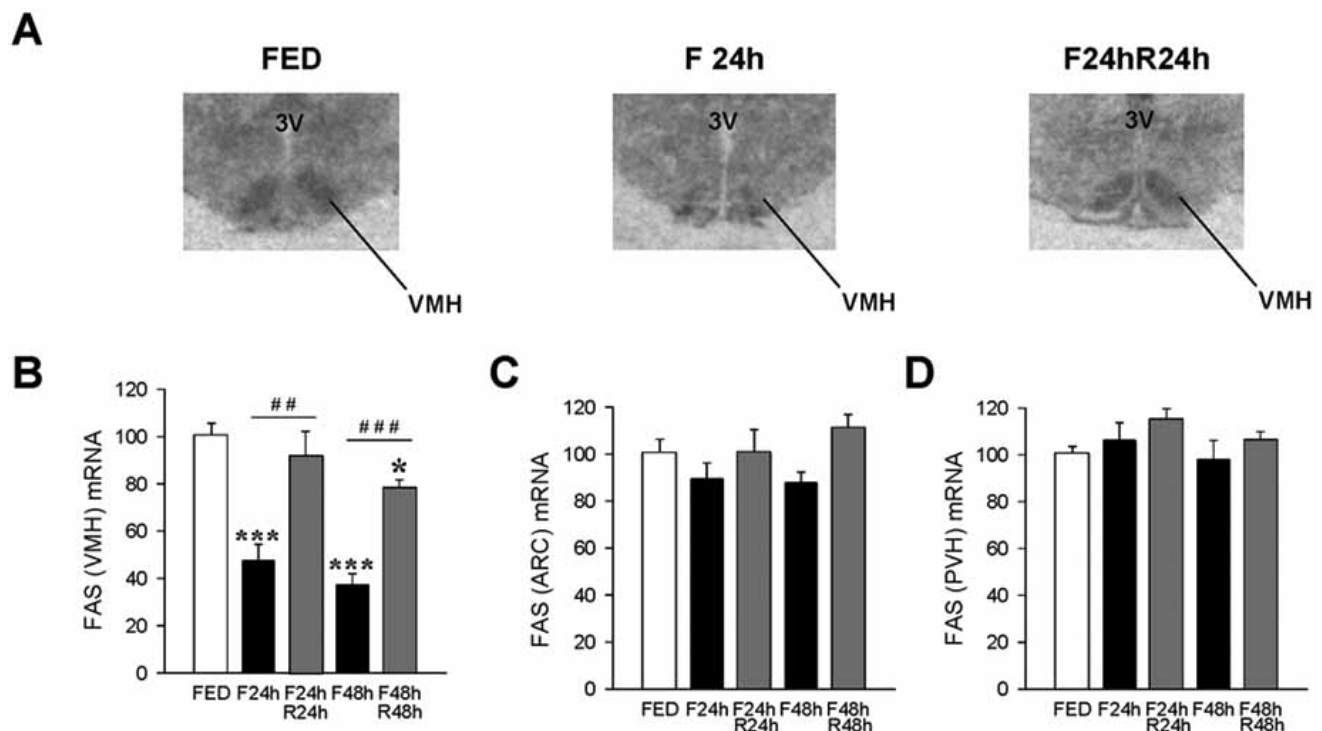
The regulation of hypothalamic fatty acid synthesis enzymes has been extensively studied during the last three years, with special focus on FAS and AMPK. Nutritional status has a remarkable effect on the expression of both enzymes. Within 24 h of starvation, FAS mRNA and protein levels in liver are highly reduced [20,25-27]. However, the effect of starvation on brain FAS expression is more complex. Total levels of FAS mRNA and protein are essentially unaffected by 24 h of fasting when analyzing the whole brain or the whole hypothalamus in mammals [20,27]. However, recent data obtained by our group have challenged, at least in part, this idea. We have recently demonstrated that nutritional status regulates hypothalamic malonyl-CoA levels and FAS expression in a nucleus-specific manner, with FAS mRNA levels being downregulated by fasting and upregulated by refeeding specifically in the VMH Fig. (2) [24]. Although FAS is ubiquitously expressed in the brain, FAS expression was unchanged in other hypothalamic nuclei, such as the ARC or PVH, and in other brain areas (e.g. cortex, thalamus, and hippocampus) Fig. (2). Our data suggests that besides its metabolic housekeeping roles, FAS regulation in the VMH may have a specific function as nutritional sensor. The exact mechanism regulating FAS expression in the hypothalamus remains unknown. In this sense, sterol response element-binding protein-1 (SREBP-1), one of the main transcription factor regulating lipogenic enzymes, has been detected in the hypothalamus of rodents and humans [28-31] (and our

unpublished observations) but it has been reported that fasting, refeeding, leptin and insulin do not affect SREBP-1 expression in the whole rat hypothalamus [30](and our unpublished observations). Whether nuclei specific changes in FAS expression are related to SREBP-1 modifications in the same nuclei will merit further investigation.

On addition to decreasing expression of FAS in the VMH, fasting also induces a marked increase in the phosphorylation levels of hypothalamic AMPK, activating it. Phosphorylated AMPK (pAMPK) phosphorylates and inhibits ACC [22,32-34]. The final result of modifying the expression and activities of ACC, FAS and AMPK is the change in the cytoplasmatic pool of malonyl-CoA in hypothalamic neurons [24,35]. Increased malonyl-CoA concentration (i.e. after FAS inhibition) inhibits CPT1 which is the enzyme that translocates LCFAs into mitochondria and makes their oxidation possible [16-19]. Under physiological conditions, the inhibition of CPT1 activity occurs when animals are fed and thus the levels of malonyl-CoA are increased due to an increased flux of glucose into the lipogenic pathway. For these reasons, it has been hypothesized that the increased levels of malonyl-CoA might act by signaling the fed state in several cell types, this has been called "Malonyl-CoA hypothesis" [16-18,24,35].

#### FAS INHIBITORS: INSTRUMENTS FOR INVESTIGATING THE ROLES OF FATTY ACID METABOLISM IN FEEDING AND ENERGY BALANCE

The development of drugs to treat specific diseases occasionally reveals unexpected side effects of potential



**Fig. (2A).** Autoradiographic images (20X) showing FAS mRNA levels in the ARC and in the VMH in the described experimental groups. FAS mRNA levels in the VMH (**B**), in the ARC (**C**) and in the PVH (**D**) in the described groups. 3V: third ventricle; \*: P<0.05 vs. fed; \*\*\*: P<0.001 vs. fed; ##: P<0.01 fast 24 hours (F24h) vs. fast 24 hours/refed 24 hours (F24hR24h); ###: P<0.001 fast 48 hours (F48h) vs. fast 48 hours/refed 48 hours (F48hR48h).

significance. The interest in the role of FAS in feeding and energy balance was originated in the field of cancer biology [36]. The finding that a lot of tumors expressed elevated levels of FAS [37,38] raised the possibility that inhibition of this enzyme might be a therapeutic target for cancer treatment. The first compound to be studied was cerulenin [37]. Cerulenin ([2*S*,3*R*]2,3-epoxy-4-oxo-7*E*,10*E*-dodecadienamide) Fig. (3A) is a natural antibiotic product of the fungus *Cephalosporium ceruleans* and a broad-spectrum FAS inhibitor [39-41]. Cerulenin induced apoptosis in cancer cells, with non-effect on non-transformed cells [37]. Very interestingly, cerulenin treatment induced a remarkable weight loss in rodents. Further evidence reported that cerulenin exerted a potent anorectic action in rodents, which was leptin independent [42,43] and chickens [44]. Despite these promising actions, cerulenin had two main inconveniences: low solubility in aqueous solutions (it should be dissolved in DMSO for *in vivo* treatments) and a very reactive epoxide group, which blocked protein acylation [45-47].

In an attempt to investigate the anorectic effects of FAS inhibition, several synthetic inhibitors were developed. The selected strategy was to design compounds that resembled malonyl-CoA, the natural substrate for FAS [37]. Of the seven enzyme activities on FAS, the  $\beta$ -ketoacyl synthase, which covalently joins acetate and malonate together, is exclusive to FAS. The  $\beta$ -ketoacyl synthase activity was thus, chosen for the design of several families of inhibitors [36,37]. C75, a  $\beta$ -methylene- $\gamma$ -butyrolactone Fig. (3B), is a competitive irreversible inhibitor of the overall reaction with regard to all three substrates (i.e. acetyl-CoA, malonyl-CoA and NADPH), exhibiting pseudo-first-order kinetics of the complexing type (a weak non-covalent enzyme-inhibitor complex is formed before irreversible enzyme modification) [48]. C75 inactivates the  $\beta$ -ketoacyl synthase partial activity of FAS. Unexpectedly, C75 also inactivates the enoyl reductase and thioesterase partial activities of FAS with about the same rates as for inactivation of the  $\beta$ -ketoacyl synthase. In contrast with the overall reaction, the  $\beta$ -ketoacyl synthase activity and the enoyl reductase activity, substrates do not protect the thioesterase activity of rat liver FAS from inactivation by C75. These results differentiate inactivation by C75 from that by cerulenin, which only inactivates the  $\beta$ -ketoacyl synthase activity of FAS, by forming an adduct with an active-site cysteine [48]. The most likely explanation for the multiple effects observed with C75 on rat liver FAS and its partial reactions is that there are multiple sites of interaction between C75 and FAS.

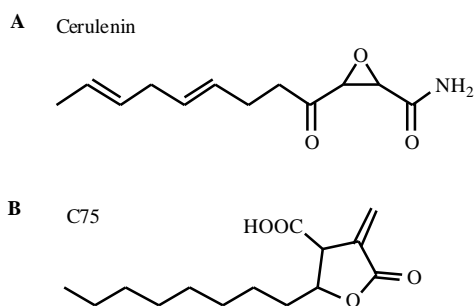


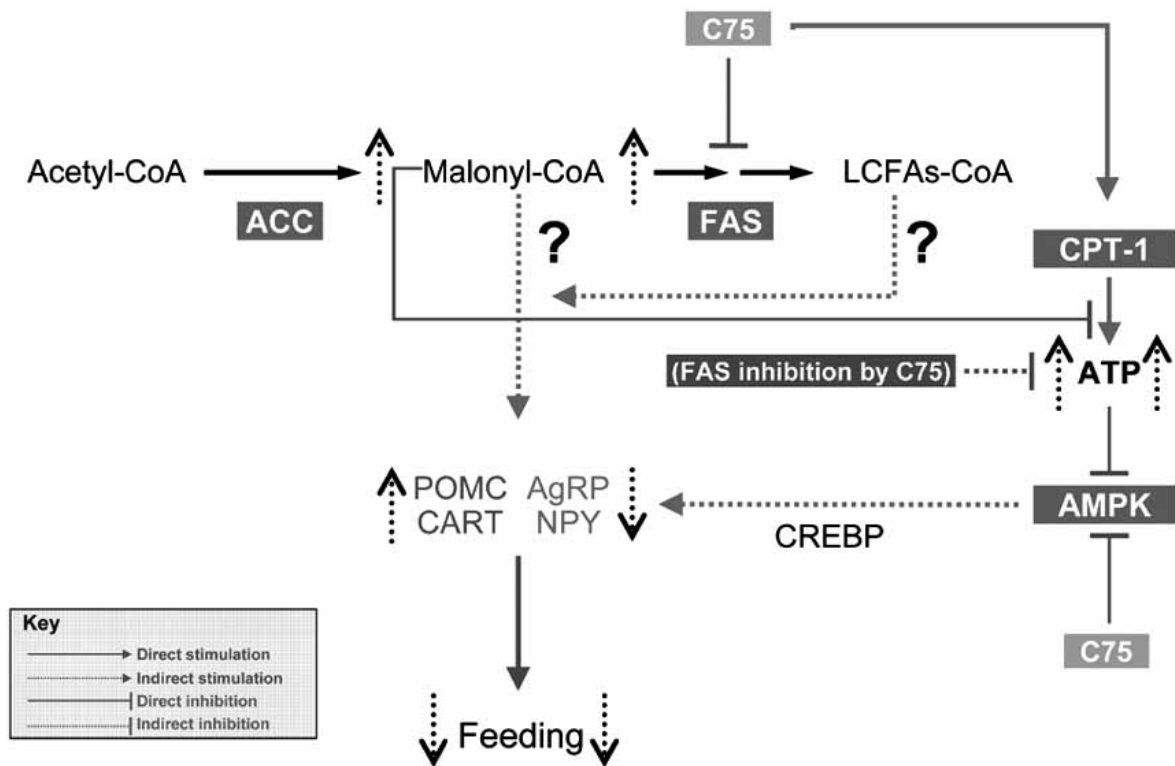
Fig. (3). Chemical structures for cerulenin (A) and C75 (B).

As predicted, peripheral administration of the C75 reduces food intake and body weight [42,43,49,50]. This effect was exerted through the CNS since ICV administration also caused marked effects on feeding and body weight [42,49,50]. The anorectic effect of C75 requires the accumulation of malonyl-CoA in the hypothalamus, which may be sensed as a signal of nutrient abundance by critical neurons regulating food intake (“Malonyl-CoA Hypothesis”) [24,35]. Simultaneous inhibition of FAS (by C75) and ACC (by 5-(tetradecyloxy)-2-furoic acid, TOFA), prevents malonyl-CoA accumulation and consequently does not result in decreased food consumption [35,42], and effect also reported for other FAS inhibitors [24] (see below).

The anorectic action of C75 is linked to decreased expression of orexigenic and elevated expression of anorexigenic neuropeptides in the ARC Fig. (4). Thus, one single administration of C75 prevents fasting-induced up-regulation of AgRP and NPY and down-regulation of POMC and CART [42,49-52]. Of interest, in *ob/ob* mice C75 prevents the normal fasting-induced increase in expression of AgRP and NPY but not CART and POMC, suggesting that an intact leptin-signaling system is necessary for the effect of C75 on POMC/CART neurons. Despite these differences, the suppressive effect of C75 on food intake is the same in both *ob/ob* and wildtype lean mice, indicating that the C75 anorectic action (as it happened with cerulenin) is leptin-independent [42,49,51,53,54]. Of note, although lean mice become resistant to the effect of C75, long term treatment of diet-induced obesity (DIO) mice and *ob/ob* mice induces a continuous suppression of food intake and body weight, and effect which correlates to decreased expression of orexigenic neuropeptides and increased expression of anorexigenic neuropeptides [53,54].

It is interesting to note that the effect of cerulenin on hypothalamic neuropeptides is more controversial. One report demonstrated that, in contrast to C75, cerulenin did not prevent the effects of fasting on hypothalamic mRNA levels of AgRP, NPY, CART and POMC [43]. Also, in this study cerulenin was highly effective at reducing body weight in agouti (*A<sup>y</sup>*) mice, in which obesity is caused by blockade of the melanocortin receptor, suggesting melanocortin-independence [43]. Conversely, a more recent paper suggests that the actions of cerulenin, as in the case of C75, are mediated by activation of hypothalamic POMC neurons [55]. The reasons for these discrepancies are not clear but they could be related with the doses used and the toxic effects of cerulenin [56-58].

The molecular mechanisms of the FAS inhibitors actions on neuropeptides are not completely understood. Two possible mechanisms have been postulated Fig. (4): 1) malonyl-CoA or a derivative interacts directly with a signaling protein that regulates expression of neuropeptides, or 2) that malonyl-CoA acts indirectly by inhibiting CPT1, therefore preventing the access of long-chain fatty acyl-CoAs to the mitochondria. Accumulation of cytoplasmic fatty acyl-CoA in this context could interact with signaling proteins that regulates expression of the orexigenic and anorexigenic neuropeptides. Both hypotheses are based on indirect supporting evidence. For instance pharmacological inhibition or genetic ablation of hypothalamic CPT1 activity



**Fig. (4).** Mechanisms of C75 and cerulenin on fatty acid metabolism. C75 inhibits FAS, activates CPT1 and inhibits AMPK. As a result of these actions C75 decreases feeding and body weight.

reduces food intake [59,60] suggesting that accumulation of fatty acyl-CoA is the mediator in the signaling pathway controlling feeding behavior. Distant from the brain of mammals, there is evidence that malonyl-CoA may directly act as signaling molecule in bacteria. The FapR (also known as FabR) transcription factor in *Bacillus subtilis* modulates the expression of several genes involved in fatty acid metabolism. Interestingly, decreasing bacterial cellular malonyl-CoA inhibits expression of FapR-regulated genes by a FapR-dependent mechanism [61]. Therefore in this model, malonyl-CoA affects the activity of DNA-binding transcription factors. It stays to be determined whether the effect of malonyl-CoA on FapR is direct or indirect, nonetheless, it is conceivable that malonyl-CoA binds directly to FapR and functions as a ligand to control FapR activity [61].

Despite of this compelling evidence, there has been some controversy about the anorectic effects of C75. Several papers have reported aversive and non-specific neuronal effects of C75 [49,58,62]. However, the effects of much lower ICV doses, as well as the specific c-FOS activation patterns [50,63,64], suggest that the effect of C75 on feeding is specific and non-aversive. In this sense, during a wash-out period following C75 treatment, feeding returns to normal. [20,42].

Finally, we have recently demonstrated that tamoxifen (TMX), a type of drug widely used for the treatment of estrogen-receptor positive breast cancers [65,66], displays a very potent FAS inhibitory effect in the liver [67] and in the hypothalamus [24]. In fact, TMX-induced anorexia [67] is associated to specific FAS inhibition in the VMH and a

marked increase in the hypothalamic levels of malonyl-CoA [24].

#### C75 ACTIONS ON CPT1

The integrated nature of lipid metabolism is exemplified by the cooperative modulation of fatty acid synthesis and oxidation Fig. (4). Levels of malonyl-CoA play a key role in this system by acting both as a substrate for FAS and as inhibitor of CPT1, which is the enzyme that translocates LCFAs into mitochondria and makes their oxidation possible [16-19]. During anabolic situations, when fatty acid synthesis intermediates are present, high levels of malonyl-CoA inhibit the ability of CPT1 to import fatty acyl-CoAs into mitochondria for oxidation. Conversely, during situations where fatty acid oxidation is desired, flux through the *de novo* lipogenesis pathway is reduced so that malonyl-CoA mediated repression of CPT1 activity is decreased [16-19].

Pharmacological inhibition of hypothalamic CPT1 in the CNS inhibits food intake [59], suggesting malonyl-CoA may suppress food intake through a CNS-expressed CPT1. In this sense, it has been recently shown that mammals express another CPT1, i.e., CPT1c, which is brain-specific and has high homology to liver CPT1a and muscle CPT1b [60,68]. CPT1c binds malonyl-CoA but, unlike CPT1a, is unable to catalyze the acyltransferase activity from fatty acyl-CoAs to carnitine [60,68]. Disruption of the CPT1c gene leads to decreased food intake and body weight. CPT1c KO mice also exhibit decreased rates of fatty acid oxidation, which may contribute to their paradoxical increased susceptibility to diet-induced obesity [60].

If C75 increased malonyl-CoA levels, turning off fatty acid oxidation, it would be expected that C75 would induce an increase of fat in the liver [67,69], however, C75 treatment resulted in a decrease of fat in liver and white adipose tissue (WAT) of diet-induced obesity (DIO) mice [70]. The reason of this discrepancy was that C75 stimulated, CPT1 [70]. Further studies demonstrated that C75-treated DIO mice had a greater weight loss and an elevated production of energy related to fatty acid oxidation compared to pairfed animals [71,72]. Treatment with etomoxir, a potent inhibitor of CPT1 inverted the increased energy expenditure and reduced the C75-induced weight loss by inhibiting fatty acid oxidation [71,72]. Similar results were obtained by using several alternative models, such as rodent neurons, adipocytes, hepatocytes, human breast cancer cells and, very remarkably, isolated mitochondria: all displayed C75-induced increased fatty acid oxidation and elevated ATP levels due to increased CPT1 activity, even in the presence of high concentrations of malonyl-CoA [41,71,73,74].

Overall, these data indicated that C75 may cause weight loss not only centrally by decreasing food intake, but also peripherally by stimulating CPT1 and rising fatty acid oxidation, leading to a loss of WAT and decrease of steatosis, in addition to profound weight loss. Besides this direct effect of C75 on peripheral tissues, recent evidence has also suggested that central FAS inhibition may play a role in increasing peripheral fatty acid oxidation. Thus, central administration of cerulenin and C75 increased peripheral CPT1 activity in isolated soleus muscle and liver and elevated core temperature [75,76]. Also correlated with these events are C75-induced increases in the expression of skeletal muscle peroxisomal proliferator-activated receptor alpha (PPRA), a transcriptional activator of fatty acid oxidizing enzymes, uncoupling protein 3 (UCP3), a thermogenic mitochondrial uncoupling protein, estrogen receptor-related receptor alpha (ERR) and key oxidative mitochondrial enzymes, including pyruvate dehydrogenase kinase, medium-chain length fatty acyl-CoA dehydrogenase, ubiquinone-cytochrome C reductase, cytochrome oxidase, as well as ATP synthase [76,77]. Consistent with the up-regulation of UCP3 and PPAR is the concomitant increase in the expression of peroxisomal proliferator activator regulator gamma coactivator 1 alpha (PGC-1), transcriptional coactivator of the UCP3 and PPAR-activated genes [76,77]. The final result of PGC-1 up-regulation is an increase in the mitochondrial biogenesis in the skeletal muscle [77].

The mechanism under the peripheral effects of central ICV administration of cerulenin and C75 is probably related to increased sympathetic activity [75-77]. Centrally administered C75 rapidly ( $\leq 2$  h) increased the expression (in skeletal muscle) of the beta-adrenergic signaling molecules, i.e., noradrenaline, beta3-adrenergic receptor, and cAMP [77]. In this sense, in the middle 90's, before the effects of FAS inhibitors on feeding were studied, it was demonstrated that the inhibition of fatty acid oxidation using mercaptoacetate stimulated the release of noradrenaline from the sympathetic system, but did not induce a release of adrenaline from the adrenal medulla [78,79].

## C75 ACTIONS ON AMPK PATHWAY

AMP-activated protein kinase (AMPK) is the downstream component of a protein kinase cascade that acts as an intracellular energy sensor, sustaining the energy balance within the cell [17,34,80]. AMPK is activated by an increased cellular AMP:ATP ratio. Activation of AMPK requires phosphorylation of threonine 172 (pAMPK Thr<sup>172</sup>) of the catalytic subunit (AMPK is a heterotrimer) by LKB1 kinase [17,34,80]. Activated AMPK is a counter-regulatory mechanism in many tissues to switch off ATP-consuming processes, such as fatty acid synthesis, while switching on catabolic processes, such as fatty acid oxidation, that produce ATP and rebalance the AMP:ATP ratio [17,34,80]. One of the best characterised target metabolic pathways for AMPK activity is the fatty acid synthesis pathway. The activities of ACC and MCD are allosterically regulated by phosphorylation by AMPK [17,34,34,80]. Activated AMPK phosphorylates and inhibits ACC whilst activating MCD, thereby reducing flux of substrates in the fatty acid biosynthetic pathway [17,34,34,80]. By altering the amount of flux through the pathway, levels of the CPT1-inhibitor malonyl-CoA can be regulated to activate or inhibit entry of fatty acids into the mitochondria for  $\beta$ -oxidation.

AMPK has also been implicated in the hypothalamic regulation of food intake. AMPK is expressed in several hypothalamic nuclei, such as the ARC, LHA, PNV and VMH [22-24,81,82], in the ARC, AMPK 2 subunit is expressed in AgRP/NPY neurons [23]. Modulation of hypothalamic AMPK activity is part of the adaptive changes observed during physiological regulation of food intake. Fasting increases AMPK activity in multiple hypothalamic regions, while refeeding inhibits it [22]. Alterations in hypothalamic AMPK activity are associated with changes of neuropeptide expression. Overexpression of a dominant negative AMPK in the mediobasal hypothalamus represses mRNA expression of NPY and AgRP in the ARC whilst conversely, overexpression of a constitutively active AMPK increased the fasting-induced increase in expression of NPY and AgRP in the ARC and MCH in the LHA [22]. The actions of AMPK activation on NPY expression are mediated by cAMP response element-binding protein (CREB) Fig. (4) [23]. The effects of fasting and refeeding on hypothalamic AMPK can be related to changes in circulating nutrients and hormones involved in energy control. Activation of AMPK in several hypothalamic nuclei such as VMH, ARC and PVH, appear to play a prominent role in the hypoglycaemia sensing [81], mediating counter-regulatory responses [82,83]. Hormonal signals involved in feeding control also modulate hypothalamic AMPK function. For example, leptin and insulin decrease the phosphorylation and inhibit the activity of hypothalamic AMPK [22,32], an effect mediated by the melanocortin system within the PVH [22]. In contrast, the orexigenic canna-binoids and ghrelin increase hypothalamic AMPK phosphorylation levels, activating it [32,84].

Two recent reports have demonstrated that C75 inhibits AMPK in the hypothalamus. In a first study Ronnet and colleagues demonstrated that in primary cortical neurons,

**Table 1. List of Patents Involving FAS Inhibitors. (\*: Most Important Patents)**

| Patent number   | Patent Title  | Country/Organization                            |
|-----------------|---|---|
| CN1728994A      | Method for inhibiting cancer development by fatty acid inhibitors   | China   |
| EP0869784B1*    | Inhibition of fatty acid synthase as a means reduce adipocyte mass  | European Patent Office (EPO)                    |
| EP1565180A2*    | A method for inhibiting cancer development using fatty acid synthase inhibitors   | European Patent Office (EPO)                    |
| MX5004390A      | A method for inhibiting cancer development using fatty acid synthase inhibitors   | Mexico  |
| US20050053631A1 | Method of decreasing sebum production   | USA   |
| WO05023207A1    | Method of decreasing sebum production   | World Intellectual Property Organization (WIPO) |
| WO05107801A2*   | Systems and methods for treating human inflammatory and proliferate diseases, with a combination of fatty acid metabolism inhibitors and glycolytic inhibitors and/or UCP and/or FAS antibodies | World Intellectual Property Organization (WIPO) |
| WO05070126A3*   | Methods and compositions for treating human diseases and wounds with UCP and FAS inhibitors   | World Intellectual Property Organization (WIPO) |

C75 and cerulenin alter neuronal ATP levels and AMPK phosphorylation levels and activity [73]. Treatment with TOFA, an inhibitor of ACC, increased ATP levels, but did not affect AMPK activity [73]. In a second and, elegant study, the same group translated their findings to an *in vivo* model, demonstrating that C75 regulates feeding by modulation of AMPK [23]. AICAR (5-aminoimidazole-4-carboxamide-1- $\beta$ -D-ribofuranoside) is a compound that is taken up into cells and phosphorylated, which mimics the effects of AMP on AMPK activation, thus stimulating AMPK activity [17,34,80]. AICAR stimulates feeding, but C75 and compound C, an inhibitor of AMPK, inhibit food intake. The C75-induced food intake inhibition is associated to a marked reduction in the levels of the phosphorylation of hypothalamic AMPK (pAMPK) subunit in the hypothalamus, an effect still observed in fasted mice that had elevated hypothalamic pAMPK levels. AICAR is able to reverse both the C75-induced hypophagia and the reduction in hypothalamic pAMPK levels. Very interestingly, C75 increases the neuronal ATP levels in the hypothalamus, which may contribute to the C75-induced decrease in AMPK activity. Of note, C75 also decreased the levels of cAMP response element-binding protein (CREB) in the ARC, suggesting a mechanism for the reduction in NPY expression seen with C75 treatment [42,49-51].

Finally, an interesting study hypothesized that the anorexic action of C75 could be mediated by a change in glucose uptake or utilization in the CNS [85]. As a result, feeding rats with an essentially carbohydrate-free diet inhibited the effects of C75 on food intake. The effect of C75 was re-established by the addition of other nutrient sources [85]. Further work will be necessary to address this issue.

#### **OTHER PERIPHERAL AND CENTRAL EFFECTS OF C75**

Besides its actions on liver and skeletal muscle, FAS inhibitors have been demonstrated to have a strong effect on preadipocyte differentiation *in vitro*. Both, C75 and ceru-

lenin reduced the differentiation of 3T3-L1 cells completely preventing lipid accumulation. This effect of cerulenin is associated to reduction of key adipocyte differentiation genes, such as CCAAT/enhancer-binding protein alpha (C/EBPalpha) and proliferator-activated receptor gamma (PPRA) [86,87]. Overall, these results suggest that FAS-generated signals may be involved to support preadipocyte differentiation. Further work will be necessary to demonstrate the relevance of this action *in vivo*.

Finally, it is also interesting to note that central administration of C75 inhibits the ghrelin secretion by hypothalamic explants *ex vivo* and by the stomach *in vivo*. In fact, the anorexigenic action of C75 is reversed by ghrelin administration through an AgRP/NPY-mediated mechanism [64].

#### **CURRENT & FUTURE DEVELOPMENTS**

Compelling data indicate that FAS, a housekeeping metabolic enzyme, plays a critical role in the hypothalamic regulation of feeding. On addition, a bulk of pharmacological data unequivocally demonstrates that FAS inhibition reduces food intake and weight loss in rodents. These results suggest that FAS inhibition may be an appropriate target for the development of antiobesity drugs in humans. Nevertheless, some important questions must be addressed before recommending FAS inhibition as a potential obesity target. 1) Which is the real contribution of FAS inhibition to the weight-reducing effects of C75? 2) Does C75 inhibit FAS in all hypothalamic neurons or just in the VMH, as it happens with TMX? 3) Which are the long-term consequences of FAS inhibition on neurons? 4) Is it possible to inhibit neuronal FAS without any deleterious consequences in neuron function and survival? To address these issues it will be critical the generation of FAS knockout models in specific hypothalamic nuclei and studying their feeding behavior and neuronal viability.

Currently, the use of FAS inhibitors is under patent as potential treatment for reduction of adipose mass and related disorders, such as diabetes (EP0869784B1, WO05070126

A3), cancer development (CN1728994A, EP1565180A2, MX5004390A), immune and proliferate diseases (WO 05107801A2, WO05070126A3) and sebum production (US20050053631A1, WO05023207A1) (Table 1) [88-95]. However, there is a critical common question for all these patents: is it possible to develop a specific FAS inhibitor without effect on CPT1, AMPK or other molecules involved in fatty acid metabolism?

Given the broad spectrum molecules affected by C75 and its controversial toxic effects in rats, whether C75 will achieve the status of compounds used in the clinical setting is yet unclear. However, there is little doubt that it has helped to uncover a potential drug-target for obesity treatment, namely fatty acid metabolism. Further studies are needed to identify the molecular mechanism under FAS inhibition in the hypothalamus and, very importantly, to link this effect to the classical pathways involved on feeding control.

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#### DISCLOSURE STATEMENT

None of the authors of this manuscript have any financial interest that has influenced the results or interpretation of this manuscript.

#### ABBREVIATIONS

|            |   |   |
|------------|---|---|
| ACC        | = | Acetyl-CoA carboxylase                              |
| Acetyl-CoA | = | Acetyl-coenzyme A                                   |
| ACL        | = | ATP citrate lyase                                   |
| ACS        | = | Acyl-CoA synthetase                                 |
| AgRP       | = | Agouti-related protein                              |
| AICAR      | = | 5-Aminoimidazole-4-carboxamide-1-β-D-ribofuranoside |
| AMPK       | = | AMP-activated protein kinase                        |
| ARC        | = | Arcuate nucleus of the hypothalamus                 |
| C/EBPα     | = | CCAAT/enhancer-binding protein alpha                |
| CAC        | = | Citric acid cycle                                   |
| CART       | = | Cocaine and amphetamine-regulated transcript        |
| CNS        | = | Central nervous system                              |
| CPT1       | = | Carnitine palmitoyltransferase 1                    |
| CREB       | = | cAMP response element-binding protein               |
| DIO        | = | Diet-induced obesity                                |
| DMH        | = | Dorsomedial nucleus of the hypothalamus             |

|                    |   |  |
|--------------------|---|--|
| ERR                | = | Estrogen receptor-related receptor alpha                               |
| FA(s)              | = | Fatty acid(s)  |
| FAS                | = | Fatty acid synthase  |
| FFAs               | = | Free fatty acids   |
| ICV                | = | Intracerebroventricular  |
| IP                 | = | Intraperitoneal  |
| LCFA               | = | Long chain fatty acid  |
| LCFACS             | = | long-chain fatty acyl-CoA synthetase                                   |
| LHA                | = | Lateral hypothalamic area  |
| LKB1               | = | LKB1 serine/threonin kinase  |
| Malonyl-CoA        | = | Malonyl-coenzyme A   |
| MCD                | = | Malonyl-CoA decarboxylase  |
| MCFAs              | = | Medium chain fatty acids   |
| MCH                | = | Melanin-concentrating hormone  |
| NPY                | = | Neuropeptide Y   |
| OA                 | = | Oleic acid   |
| pAMPK              | = | Phosphorylated AMP-activated protein kinase                            |
| PGC-1 <sub>α</sub> | = | Peroxisomal proliferator activator regulator gamma coactivator 1 alpha |
| POMC               | = | Proopiomelanocortin  |
| PPRA <sub>α</sub>  | = | Peroxisomal proliferator-activated receptor alpha                      |
| PPRA <sub>γ</sub>  | = | Peroxisomal proliferator-activated receptor gamma                      |
| PVH                | = | Paraventricular nucleus of the hypothalamus                            |
| SC                 | = | Subcutaneous   |
| SERM               | = | Selective estrogen receptor modulator                                  |
| SREBP              | = | Sterol response element-binding protein                                |
| TMX                | = | Tamoxifen  |
| TOFA               | = | 5-(Tetradecyloxy)-2-furoic acid  |
| UCP3               | = | Uncoupling protein 3   |
| VMH                | = | Ventromedial nucleus of the hypothalamus                               |
| WAT                | = | White adipose tissue   |

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