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5. New strategies in the modulation of fatty acid oxidation as a treatment for obesity

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Abstract. Strategies that enhance fat degradation or reduce caloric food intake could be considered therapeutic interventions to reduce not only obesity, but also its associated disorders. The enzyme carnitine palmitoyltransferase 1 (CPT1) is the critical rate-determining regulator of fatty acid oxidation (FAO) and might play a key role in increasing energy expenditure and controlling food intake. Our group has shown that mice overexpressing CPT1 in liver are protected from weight gain, the development of obesity and insulin resistance. Regarding food intake control, we observed that the pharmacological inhibition of CPT1 in rat hypothalamus decreased food intake and body weight. This suggests that modulation of CPT1 activity and the oxidation of fatty acids in various tissues can be crucial for the potential treatment of obesity and associated pathologies.

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Introduction

Obesity and its associated metabolic disorders, such as insulin resistance, type 2 diabetes, cardiovascular disease and other severe pathologies, are one of the most serious public health concerns of the twenty-first century. There are more than 500 million obese people worldwide and, more importantly, overweight and obesity are the fifth leading risk for global deaths [1]. In the last few years, a concerted effort has been made to understand the pathophysiology of obesity and particularly its association with insulin resistance. Various mechanisms have emerged in the past two decades to elucidate this causal relationship. i) Ectopic fat deposition: during obesity the capacity of adipose tissue to expand in order to store excess fat fails, resulting in lipid spillover to peripheral organs such as liver, skeletal muscle and pancreas [2,3]. This incorrect lipid accumulation creates a lipotoxic environment, mainly mediated by diacylglycerols (DAG), that blocks correct glucose transport and insulin signaling [4]. ii) Inflammation: the excess of lipids accumulated in the adipose tissue during obesity causes adipocyte hypoxia [5], endoplasmic reticulum (ER) stress [6], cell death, and finally FA spillover [4]. These events lead to the recruitment and activation of immune cells in the adipose tissue. Obese adipocytes and infiltrated immune cells secrete many inflammatory cytokines that promote a proinflammatory state that contributes to local and systemic insulin resistance [7,8]. iii) Food intake: the central nervous system, specifically the hypothalamus, is extremely important in obesity-induced pathologies, since it plays a major role in the control of food intake and the regulation of body weight. In fact, leptin, an adipocyte-secreted hormone, acts on the hypothalamus to inhibit food intake and control body weight and is essential in the interaction between the brain and other organs in obesity-related disorders [9,10].

Every therapeutic strategy to combat obesity is focused on increasing energy expenditure through regular exercise and/or reducing energy intake. However, the maintenance of lifestyle modifications for long periods is difficult and challenging. All the anti-obesity drugs on the market approach obesity by aiming to limit energy intake. Nonetheless, the list of food intake-limiting drugs withdrawn from the market for safety reasons seems to be constantly increasing: fenfluramine, desfenfluramine, sibutramine and rimonabant [11]. Currently, only orlistat and lorcaserin have an approved clinical indication for obesity treatment, but not worldwide, since the EMA is still studying the approval of the latter [12]. Owing to the pandemic of obesity-related diseases, there is a need for a wider range of drugs with different mechanisms and a safer profile to optimize and individualize obesity management therapies. The development of different strategies, such as those designed to increase lipid mobilization and oxidation, has become an imperative.

Long chain fatty acid oxidation plays a key role in the development of obesity and occurs in mitochondria. Lipid transport into the mitochondria is mediated by the carnitine palmitoyltransferase (CPT) system [13], which is comprised of CPT1, acylcarnitine translocase and CPT2. The first protein, CPT1, catalyzes the rate-limiting step in mitochondrial fatty acid oxidation (FAO), since it can be regulated by changes in malonyl-CoA levels. These levels are controlled by acetyl-CoA carboxylase (ACC), which controls malonyl-CoA synthesis, and malonyl-CoA decarboxylase (MCD), which catalyzes malonyl-CoA degradation [13]. Together, these components act as a metabolic network that senses the cell's energy state. Once long chain fatty acids have been degraded to acetyl-CoA in the mitochondria, they are transformed into ATP through the Krebs cycle and oxidative phosphorylation, in which ATP molecules are made up from the derived NADH and $FADH_2$ energy used to create the electrochemical gradient needed by ATP synthase [14].

Mammal tissues express three isoforms of CPT1: CPT1A, which was originally discovered in liver but is present almost ubiquitously [15], CPT1B that is present in muscle and heart [16], and CPT1C, the latest isoform to be discovered, which is expressed mainly in the brain [17].

Our research indicates that the modulation of mitochondrial bioenergetics, and especially FAO, is a good target for anti-obesity therapy. Our strategy is based on two main interventions: one in peripheral tissues such as muscle and liver, and the other at central level in the hypothalamus. In peripheral tissues, overexpression of the CPT1A gene seemed to be a suitable approach, because presumably the effects of this intervention would reduce the intracellular lipid content, and lead to a general improvement in cellular metabolism. Furthermore, we have produced a M593S CPT1A cDNA rat mutant (CPT1AM), whose translated protein is active, but totally insensitive to malonyl-CoA inhibition [18]. Therefore, overexpression of CPT1AM would disconnect glucose metabolism, which increases malonyl-CoA levels, from accelerated fatty acid catalysis. Thus, the reduction in cellular lipids would be produced much more efficiently. At central level, our approach is based on the inhibition of CPT1 activity, which is involved in the signaling pathway that controls food intake.

1. The enhancement of mitochondrial FAO in liver ameliorates insulin resistance and prevents obesity induced by a high-fat diet

In liver, conditions associated with prolonged energy excess or impaired FA metabolism lead to the accumulation of considerable amounts of lipids. This triggers the development of non-alcoholic fatty liver disease (NAFLD),

which involves a series of liver abnormalities. NAFLD produces an abnormal accumulation of lipids and inflammation. It also renders insulin unable to control hepatic gluconeogenesis. This is usually the beginning of diabetes, but episodes of steatohepatitis, cirrhosis and hepatocellular carcinoma are common at later stages of development [19-21].

Considering that NAFLD is ultimately the result of an imbalance between lipid inputs and outputs in the hepatocytes, any intervention that stimulates hepatic FAO should result in reduced hepatic fat accumulation. Several pharmacological approaches that activate FAO have been reported using PPAR [20,22] and AMPK [23,24] agonists and ACC antagonists [25,26]. More interesting are studies in rodents to decrease steatosis by increasing FAO specifically in liver. In these studies, short-term modulation of ACC [27] and MCD [28] gene expression produced a reduction in malonyl-CoA levels and an increase in FAO. In addition, a decrease in hepatic TAG content and insulin resistance was observed in obese animals. However, the notion that these target genes are implicated in other metabolic pathways raises the question of their effectiveness in long-term treatments.

Taking into account that the main factor that increases FAO is the mitochondrial membrane enzyme CPT1A, which is the critical rate-determining regulator of beta-oxidation, overexpression of the CPT1A gene seemed to be a suitable approach, because presumably the effects of this intervention would reduce the lipid content, and lead to a general improvement in hepatic metabolism. Hepatic overexpression of CPT1A mediated by adenovirus has been performed by Stefanovic-Racic *et al.* [29] in obese rats. They observed a slight increase in FAO that led to a reduction in hepatic TAG levels, but did not reveal any improvement in insulin sensitivity. The moderate effect might be due to the increased malonyl-CoA levels induced by a high-fat diet (HFD), which could limit *in vivo* CPT1A activity, in spite of the CPT1A overexpression.

To avoid malonyl-CoA inhibition, we generated a mutant CPT1A isoform, CPT1AM [18], that is insensitive to malonyl-CoA. Results published by our group confirm in the pancreatic cell line INS-1 [30] and muscle cell line L6E9 [31] that adenovirus-mediated overexpression of CPT1AM is more efficient at increasing mitochondrial FAO. Furthermore, overexpression of CPT1AM in L6E9 cells produces a 2-fold increase in palmitate oxidation, and decreases its esterification into cellular lipids. Similar results were obtained in hepatocyte primary culture by [32] and our group (Fig. 1). Adenovirus-mediated CPT1AM overexpression produced higher oleate oxidation than the CPT1A wild type isoform and a higher reduction in intracellular TAG content when hepatocytes where incubated in the presence of palmitate (Fig. 1).

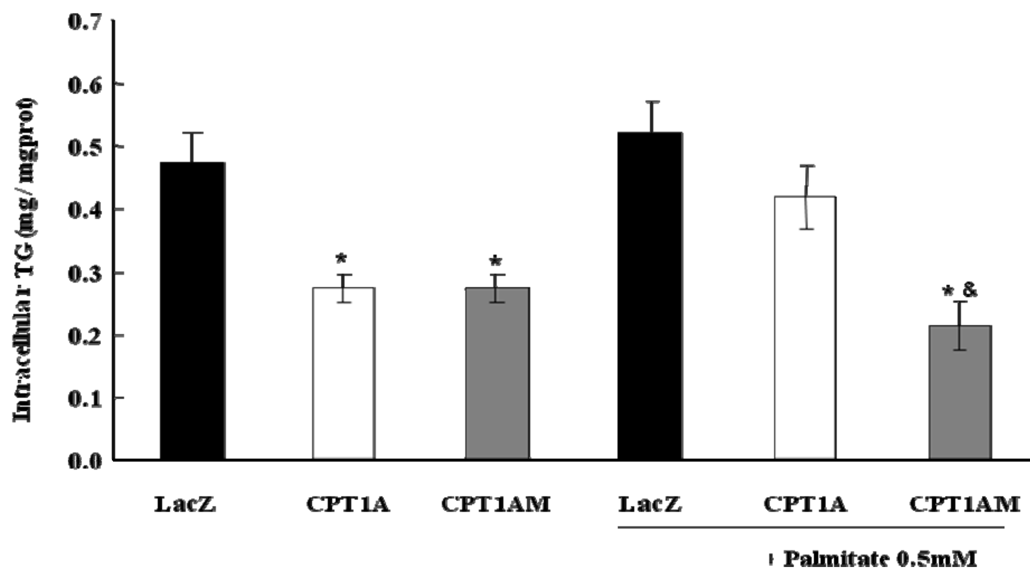

Figure 1. CPT1 overexpression reduced intracellular TAG content. Rat hepatocytes were transduced for 24 h. After this time, cells were treated with palmitate for 4 h. Lipids were extracted and TAG were measured using the Cromatest Triglyceride kit. *P < 0.05 vs. Lac Z – Palmitate, $^{\&}P$ < 0.05 vs. Lac Z + Palmitate.

In addition to *in vitro* studies, we performed *in vivo* experiments in mice fed with HFD [33]. We achieved hepatic gene transfer of CPT1A and the permanent active form CPT1AM to obese mice by injecting adeno-associated viruses (AAV) into the tail vein. The use of AAV-CPT1A and AAV-CPT1AM led to long-term liver-selective gene transfer that allowed us to evaluate the metabolic impact and underlying mechanisms of increased FAO in diet-induced and genetically obese mice. In these studies, we observed that HFD CPT1A- and CPT1AM-expressing mice showed a general improvement in hepatic glucose and lipid metabolism as a consequence of increased hepatic fatty acid flux through mitochondria. In turn, this prevented intracellular lipid accumulation in liver (Fig. 2) and ROS production and rescued the impaired hepatic insulin signal, especially in CPT1AM-expressing mice (Fig. 3). It seems that liver can deal with an increased flux of FA into the mitochondria, thus escaping from liver injury. This is due to the ability of liver to flip the balance from complete oxidation to ketone body production [34]. The ketone bodies produced by enhanced FAO are easily consumed by other tissues. This increases the flux of carbons from liver to other organs (Fig. 3). In addition, enhanced mitochondrial FAO in liver reduced the lipid accumulation in white adipose tissue and prevented the increase of body weight in these HFD mice. The impaired insulin signaling in tissues such as muscle and white adipose tissue was also ameliorated. These results have been further confirmed by Monsenego *et al.* [35].

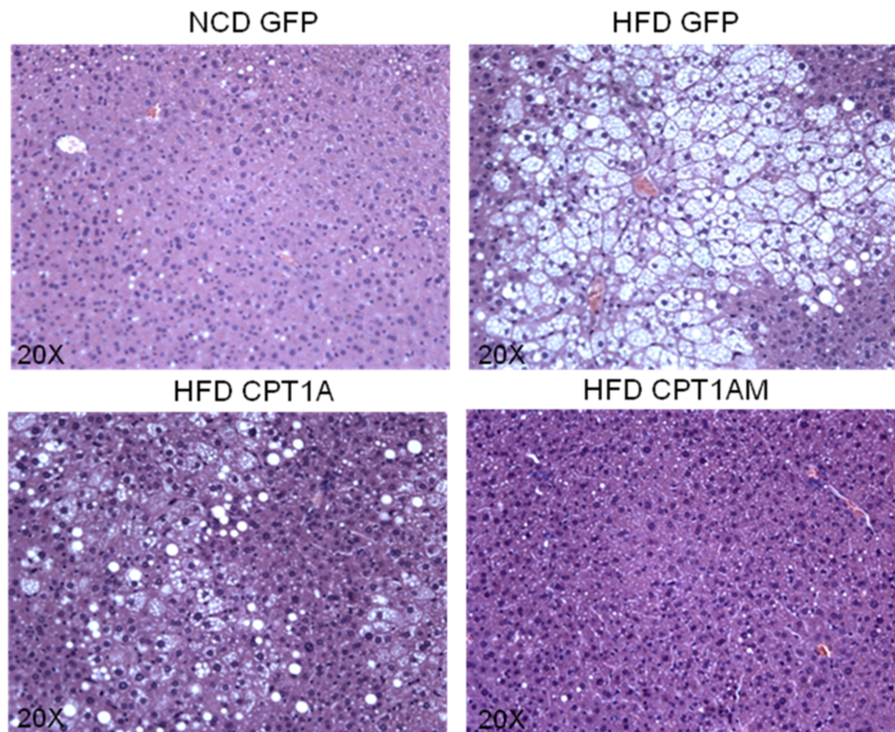

Figure 2. Liver histology in GFP, CPT1A- and CPT1AM-expressing mice. Liver histological sections (hematoxylin and eosin staining) from representative 29-week-old GFP control mice and GFP-, CPT1A- and CPT1AM-expressing HFD littermates.

In genetically obese *db/db* mice expressing CPT1AM we also observed an improvement in the hyperglycemia and hyperinsulinemia that these animals suffer. Taken together, these results highlight an increase in hepatic CPT1AM as a new strategy for the treatment of NAFLD/NASH pathologies and obesity (Fig. 3).

Another strategy has been proposed to improve mainly glucose homeostasis in HFD-treated mice. This approach is based on the administration of CPT1 inhibitors. Although this treatment reduced hepatic gluconeogenesis, it also caused hepatic steatosis in HFD-treated mice [36,37]. This side-effect has discarded the development of other CPT1 systemic inhibitors, such as etomoxir and 2-tetradecylglycidic acid, as a therapeutic tool. Taken together, these data support the idea that any strategy that can switch liver FA's fate from esterification towards oxidation has a beneficial effect on liver and on the whole body.

Recently, a new hepatic factor, fibroblast growth factor 21 (FGF21), has emerged as a key regulator in FAO and ketogenic activation. FGF21 is upregulated in liver during fasting [38,39] and has been increasingly indicated as a potential therapeutic agent in obesity-induced insulin-resistant states [40].

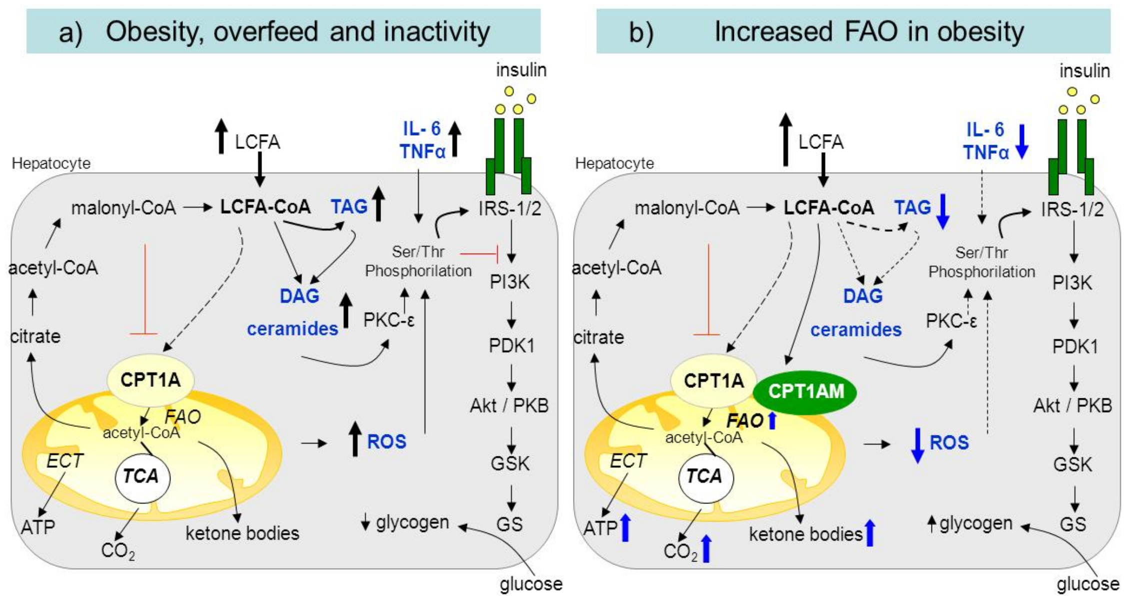

Figure 3. Effects of enhanced FAO in fatty liver. (a) Obesity increases fatty acid (FA) uptake, TAG (triacylglycerides), DAG (diacylglycerol), ceramides and other lipid derivatives that may inhibit insulin signaling. FA accumulation induces mitochondrial dysfunction and increased ROS production, oxidative stress and inflammation that could also disrupt insulin signaling. (b) Enhancing FAO by the overexpression of CPT1AM increases the production of ketone bodies, ATP and CO_2. The reduction of lipid content reestablishes lipid metabolism and insulin signaling. It also decreases inflammation and ROS production. FAO: fatty acid oxidation; GSK: glycogen synthase kinase-3; GS: glycogen synthase; IRS-1/2: insulin receptor substrate; LCFA: long chain fatty acids; PKC: protein kinase C; PI3K: phosphoinositide 3-kinase; PDK1: phosphoinositide-dependent kinase-1; PKB: protein kinase B; ROS: reactive oxygen species; TCA: tricarboxylic acid cycle; TNFα: tumor necrosis factor; NCD normal control diet.

2. Inhibition of CPT1 in the hypothalamus reduces food intake and body weight

The central nervous system (CNS) plays a major role in the evaluation and control of energy homeostasis. Blood concentrations of glucose and fatty acids are sensed by neurons of the hypothalamus, which adjusts feeding behavior by altering the expression of specific neuropeptides and neurotransmitters. It is now well established that hypothalamic lipid metabolism participates in this action of the hypothalamus and is linked to molecular mechanisms by which hormones and nutrients exert their central effect on food intake [41]. Recently, much effort has been invested in designing new anti-obesity drugs and modulating fatty acid metabolism to inhibit food intake.

Both malonyl-CoA, the first intermediate in *de novo* lipogenesis, and LCFA-CoAs have been proposed as satiety molecular signals in the hypothalamus [42-45]. For this reason, drugs designed to raise hypothalamic concentrations of these molecules are good candidates for the treatment of food intake disorders. The enzyme fatty acid synthase (FAS) catalyzes the synthesis of palmitate from acetyl-CoA and malonyl-CoA. It has been reported that FAS inhibition in the hypothalamus suppresses food intake through the accumulation of malonyl-CoA [46,47]. C75 is a synthetic inhibitor of FAS and has been proposed as an anti-obesity agent, since its administration decreases appetite and body weight in rodents [48]. However, the exact molecular mechanism underlying C75-derived anorexia is not completely understood. Firstly, some evidence showed a disconnection between C75-induced hypophagia and FAS inhibition in the hypothalamus [49]. Secondly, the inhibition of FAS by C75 produces an accumulation of malonyl-CoA that is difficult to reconcile with the activation of CPT1 reported by others authors [50,51]. Finally, our group previously reported that the Coenzyme A adduct of C75 (C75-CoA) is a potent inhibitor of CPT1 [52] (Fig. 4). Taking these data into account, further research is needed to clarify whether C75-induced hypophagia is directly related to hypothalamic inhibition of FAS, CPT1 or both enzymes.

It has been stated that pharmacological and genetic inhibition of CPT1 in the hypothalamus reduces food intake [53,54]. Rossetti and co-workers demonstrated that genetic inhibition of the isoform CPT1A in the mediobasal hypothalamus not only reduced feeding in rats, but also diminished hepatic gluconeogenesis. Similar results were obtained with tetradecylglycidic acid

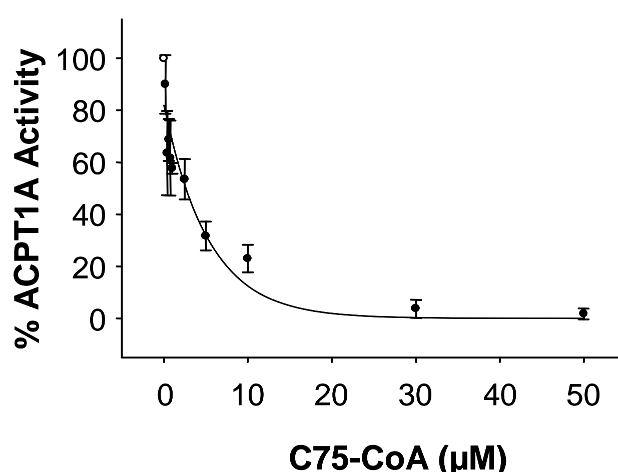

Figure 4. Effect of C75-CoA on the activity of yeast-expressed CPT1A. Mitochondrial extracts from yeast expressing CPT1A were preincubated with increasing concentrations of C75-CoA. CPT1A activity was measured, and data are expressed relative to control values in the absence of C75-CoA (100%).

and the compound ST1326, a synthetic CPT1 inhibitor. The observed anorexia was accompanied by a significant increase of LCFA-CoAs in the hypothalamus. The same authors demonstrated that central injection of oleic acid produced a reduction in food consumption, due to the downregulation of orexigenic neuropeptides in the hypothalamus [44]. Based on these results, our group attempted to explain the anorectic effects of C75 in terms of its inhibitory action on hypothalamic CPT1. We first demonstrated that, following its central administration, C75 is converted to C75-CoA in the hypothalamus [55]. In addition, central C75 administration produced a significant inhibition of CPT1 activity in the hypothalamus [55] (Fig. 5a). Furthermore, central injection of etomoxir, a well-known CPT1 inhibitor, also decreased feeding and reduced body weight in rats (Fig. 5b and c). Together, these data suggested that hypothalamic CPT1 inhibition could, at least partly, mediate the anorectic effect of C75.

We propose that the direct *in vivo* effect of C75-CoA on hypothalamic CPT1 explains the inhibition of CPT1 activity and the subsequent reduction in food intake. However, FAS could also be inhibited by C75 in the hypothalamus. Therefore, malonyl-CoA could be in excess and inhibit CPT1, together with C75-CoA (Fig. 6).

Despite the aforementioned results, further research is needed to unravel the exact mechanism by which CPT1 inhibition affects the expression of hypothalamic neuropeptides and, consequently, feeding behavior. Our group is currently working on two main research lines: the clarification of the exact role of hypothalamic CPT1 in the regulation of appetite and C75-derived

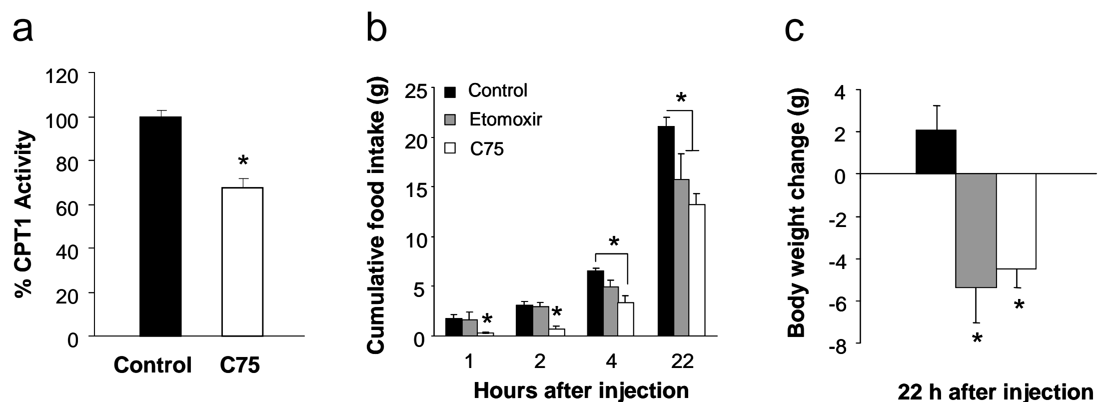

Figure 5. Central nervous system administration of C75 inhibits CPT1 activity and decreases food intake and body weight. (a) CPT1 activity after intracerebroventricular (i.c.v.) injection of C75 (control animals, RPMI medium). (b) Food intake measured in rats at 1, 2, 4 and 22 h after i.c.v. injection of C75, etomoxir and control (RPMI medium). (c) Body weight measured in rats at 22 h after i.c.v. injection of C75, etomoxir and control (RPMI medium). * $P < 0.05$.

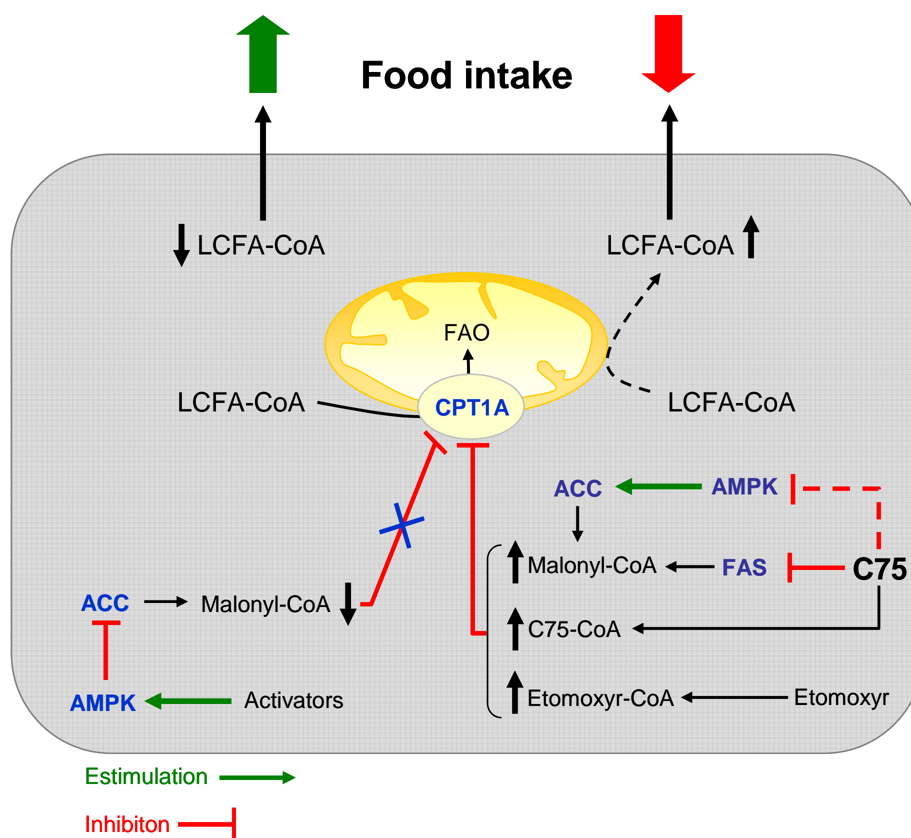

Figure 6. Model of action of C75 on hypothalamic fatty acid metabolism. The energy balance is monitored by the hypothalamus. The accumulation of malonyl-CoA due to FAS-inhibition by C75 leads to a reduction in CPT1 activity. This would increase the cytosolic pool of LCFA-CoA and thus decrease food intake. In addition, C75 may inhibit AMPK, resulting in activation of ACC and a consequent increase in malonyl-CoA levels [56]. Here, we have shown that C75 is converted into C75-CoA in the hypothalamus, which contributes to the direct inhibition of CPT1. Our experiments also demonstrate that other CPT1 inhibitors such as etomoxir produce similar effects of decreasing food intake. However, AMPK-activators lead to ACC inactivation, which reduces malonyl-CoA levels and CPT1 inhibition. Thus, increased FAO reduces LCFA-CoAs and promotes food intake.

anorexia, and the design and synthesis of new CPT1 inhibitors as a first step in the development of new anti-obesity drugs.

3. Conclusion

There is general agreement that we need to look for new treatments to fight against the current epidemic of obesity and related diseases. Dietary modification and regular exercise are two classical and very effective strategies for decreasing nutrient overload. However, long-term maintenance of these strategies is difficult and challenging. Thus, new therapeutic strategies are

needed to reduce the lipid burden and increase energy expenditure. Alternatively, new drugs should be developed to modulate eating behavior.

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References

1. World Health Organization. Fact sheet: obesity and overweight. Internet: http://www.who.int/mediacentre/factsheets/fs311/en/ (accessed 21 June 2013).
2. Carobbio, S., Rodriguez-Cuenca, S., Vidal-Puig, A. 2011, *Curr. Opin. Clin. Nutr. Metab. Care,* 14, 520.
3. Virtue, S., Vidal-Puig, A. 2010, *Biochim. Biophys. Acta,* 1801, 338-349.
4. Samuel, V. T., Shulman, G. I. 2012, *Cell,* 148, 852.
5. Hosogai, N., Fukuhara, A., Oshima, K., Miyata, Y., Tanaka, S., Segawa, K., Furukawa, S., Tochino, Y., Komuro, R., Matsuda, M., Shimomura, I. 2007, *Diabetes,* 56, 901.
6. Hotamisligil, G. S. 2010, *Cell,* 140, 900.
7. Guilherme, A., Virbasius, J. V., Puri, V., Czech, M. P. 2008, *Nat. Rev. Mol. Cell Biol.,* 9, 367.
8. Schenk, S., Saberi, M., Olefsky, J. M. 2008, *J Clin Invest,* 118, 2992.
9. Yue, J. T., Lam, T. K. 2012, *Cell Metab.,* 15, 646.
10. Gautron, L., Elmquist, J. K. 2011, *J. Clin. Invest.,* 121, 2087.
11. Derosa, G., Maffioli, P. 2012, *Expert. Opin. Drug Saf.,* 11, 459.
12. Pedersen, S. D., Astrup, A. 2012, *Endocrinol. Nutr.,*59, 521.
13. McGarry, J. D., Brown, N. F. 1997, *Eur. J. Biochem.,* 244, 1.
14. Tseng, Y. H., Cypess, A. M., Kahn, C. R. 2010, *Nat. Rev. Drug Discov.,* 9, 465.
15. Esser, V., Britton, C. H., Weis, B. C., Foster, D. W., McGarry, J. D. 1993, *J. Biol. Chem.,* 268, 5817.
16. Yamazaki, N., Shinohara, Y., Shima, A., Terada, H. 1995, *FEBS Lett.,* 363, 41.
17. Price, N., van der Leij, F., Jackson, V., Corstorphine, C., Thomson, R., Sorensen, A., Zammit, V. 2002, *Genomics,* 80, 433.
18. Morillas, M., Gomez-Puertas, P., Bentebibel, A., Selles, E., Casals, N., Valencia, A., Hegardt, F. G., Asins, G., Serra, D. 2003, *J. Biol. Chem.,* 278, 9058.
19. Day, C. P., James, O. F. 1998, *Hepatology,* 27, 1463.
20. Starley, B. Q., Calcagno, C. J., Harrison, S. A. 2010, *Hepatology,* 51, 1820.
21. Gambino, R., Musso, G., Cassader, M. 2011, *Antioxid. Redox Signal.,* 15, 1325.

22. Barroso, E., Rodriguez-Calvo, R., Serrano-Marco, L., Astudillo, A. M., Balsinde, J., Palomer, X., Vazquez-Carrera, M. 2011, *Endocrinology,* 152, 1848.
23. Zhou, G., Myers, R., Li, Y., Chen, Y., Shen, X., Fenyk-Melody, J., Wu, M., Ventre, J., Doebber, T., Fujii, N., Musi, N., Hirshman, M. F., Goodyear, L. J., Moller, D. E. 2001, *J. Clin. Invest.,* 108, 1167.
24. Velasco, G., Geelen, M. J., Guzman, M. 1997, *Arch. Biochem. Biophys.,* 337, 169.
25. McCune, S. A., Harris, R. A. 1979, *J Biol Chem,* 254, 10095-10101.
26. Harwood, H. J., Jr., Petras, S. F., Shelly, L. D., Zaccaro, L. M., Perry, D. A., Makowski, M. R., Hargrove, D. M., Martin, K. A., Tracey, W. R., Chapman, J. G., Magee, W. P., Dalvie, D. K., Soliman, V. F., Martin, W. H., Mularski, C. J., Eisenbeis, S. A. 2003, *J. Biol. Chem.,* 278, 37099.
27. Savage, D. B., Choi, C. S., Samuel, V. T., Liu, Z. X., Zhang, D., Wang, A., Zhang, X. M., Cline, G. W., Yu, X. X., Geisler, J. G., Bhanot, S., Monia, B. P., Shulman, G. I. 2006, *J. Clin. Invest.,* 116, 817.
28. An, J., Muoio, D. M., Shiota, M., Fujimoto, Y., Cline, G. W., Shulman, G. I., Koves, T. R., Stevens, R., Millington, D., Newgard, C. B. 2004, *Nat. Med.,* 10, 268.
29. Stefanovic-Racic, M., Perdomo, G., Mantell, B. S., Sipula, I. J., Brown, N. F., O'Doherty, R. M. 2008, *Am. J. Physiol. Endocrinol. Metab.,* 294, E969.
30. Herrero, L., Rubi, B., Sebastian, D., Serra, D., Asins, G., Maechler, P., Prentki, M., Hegardt, F. G. 2005, *Diabetes,* 54, 462.
31. Sebastian, D., Herrero, L., Serra, D., Asins, G., Hegardt, F. G. 2007, *Am. J. Physiol. Endocrinol. Metab.,* 292, E677.
32. Akkaoui, M., Cohen, I., Esnous, C., Lenoir, V., Sournac, M., Girard, J., Prip-Buus, C. 2009, *Biochem. J.,* 420, 429.
33. Orellana-Gavalda, J. M., Herrero, L., Malandrino, M. I., Paneda, A., Sol Rodriguez-Pena, M., Petry, H., Asins, G., Van Deventer, S., Hegardt, F. G., Serra, D. 2011, *Hepatology,* 53, 821.
34. Kotronen, A., Seppala-Lindroos, A., Vehkavaara, S., Bergholm, R., Frayn, K. N., Fielding, B. A., Yki-Jarvinen, H. 2009, *Liver Int.,* 29, 1439.
35. Monsenego, J., Mansouri, A., Akkaoui, M., Lenoir, V., Esnous, C., Fauveau, V., Tavernier, V., Girard, J., Prip-Buus, C. 2012, *J. Hepatol.,* 56, 632.
36. Conti, R., Mannucci, E., Pessotto, P., Tassoni, E., Carminati, P., Giannessi, F., Arduini, A. 2011, *Diabetes,* 60, 644.
37. Giannessi, F., Pessotto, P., Tassoni, E., Chiodi, P., Conti, R., De Angelis, F., Dell'Uomo, N., Catini, R., Deias, R., Tinti, M. O., Carminati, P., Arduini, A. 2003, *J. Med. Chem.,* 46, 303.
38. Pissios, P., Maratos-Flier, E. 2007, *Cell Metab.,* 6, 345.
39. De Sousa-Coelho, A. L., Marrero, P. F., Haro, D. 2013, *Biochem. J.,* 443, 165.
40. Iglesias, P., Selgas, R., Romero, S., Diez, J. J. 2012, *Eur. J. Endocrinol.,* 167, 301.
41. Lopez, M., Lelliott, C. J., Vidal-Puig, A. 2007, *Bioessays,* 29, 248.
42. Hu, Z., Cha, S. H., Chohnan, S., Lane, M. D. 2003, *Proc. Natl. Acad. Sci. U S A,* 100, 12624.
43. Hu, Z., Dai, Y., Prentki, M., Chohnan, S., Lane, M. D. 2005, *J. Biol. Chem.,* 280, 39681.
44. Obici, S., Feng, Z., Morgan, K., Stein, D., Karkanias, G., Rossetti, L. 2002, *Diabetes,* 51, 271.

45. Lam, T. K., Pocai, A., Gutierrez-Juarez, R., Obici, S., Bryan, J., Aguilar-Bryan, L., Schwartz, G. J., Rossetti, L. 2005, *Nat. Med.,* 11, 320.
46. Lopez, M., Lelliott, C. J., Tovar, S., Kimber, W., Gallego, R., Virtue, S., Blount, M., Vazquez, M. J., Finer, N., Powles, T. J., O'Rahilly, S., Saha, A. K., Dieguez, C., Vidal-Puig, A. J. 2006, *Diabetes,* 55, 1327.
47. Cha, S. H., Hu, Z., Lane, M. D. 2004, *Biochem. Biophys. Res. Commun.,* 317, 301.
48. Loftus, T. M., Jaworsky, D. E., Frehywot, G. L., Townsend, C. A., Ronnett, G. V., Lane, M. D., Kuhajda, F. P. 2000, *Science,* 288, 2379.
49. Rohrbach, K. W., Han, S., Gan, J., O'Tanyi, E. J., Zhang, H., Chi, C. L., Taub, R., Largent, B. L., Cheng, D. 2005, *Eur. J. Pharmacol.,* 511, 31.
50. Yang, N., Kays, J. S., Skillman, T. R., Burris, L., Seng, T. W., Hammond, C. 2005, *J. Pharmacol. Exp. Ther.,* 312, 127.
51. Nicot, C., Napal, L., Relat, J., Gonzalez, S., Llebaria, A., Woldegiorgis, G., Marrero, P. F., Haro, D. 2004, *Biochem. Biophys. Res. Commun.,* 325, 660.
52. Bentebibel, A., Sebastian, D., Herrero, L., Lopez-Vinas, E., Serra, D., Asins, G., Gomez-Puertas, P., Hegardt, F. G. 2006, *Biochemistry,* 45, 4339.
53. Obici, S., Feng, Z., Arduini, A., Conti, R., Rossetti, L. 2003, *Nat. Med.,* 9, 756.
54. Pocai, A., Lam, T. K., Obici, S., Gutierrez-Juarez, R., Muse, E. D., Arduini, A., Rossetti, L. 2006, *J. Clin. Invest.,* 116, 1081.
55. Mera, P., Bentebibel, A., Lopez-Vinas, E., Cordente, A. G., Gurunathan, C., Sebastian, D., Vazquez, I., Herrero, L., Ariza, X., Gomez-Puertas, P., Asins, G., Serra, D., Garcia, J., Hegardt, F. G. 2009, *Biochem. Pharmacol.,* 77, 1084.
56. Kuhajda, F. P., Landree, L. E., Ronnett, G. V. 2005, *Trends Pharmacol. Sci.,* 26, 541.