Carnitine palmitoyltransferase 1C: From cognition to cancer

Núria Casals, Victor Zammit, Laura Herrero, Rut Fadó, Rosalía Rodríguez-Rodríguez, Dolors Serra

PII: S0163-7827(15)30007-2
DOI: doi: 10.1016/j.plipres.2015.11.004
Reference: JPLR 900

To appear in:

Received date: 29 July 2015
Revised date: 18 November 2015
Accepted date: 23 November 2015

Please cite this article as: Casals Núria, Zammit Victor, Herrero Laura, Fadó Rut, Rodríguez-Rodríguez Rosalía, Serra Dolors, Carnitine palmitoyltransferase 1C: From cognition to cancer, (2015), doi: 10.1016/j.plipres.2015.11.004

Carnitine palmitoyltransferase 1C: from Cognition to Cancer

Núria Casals[1,2,*], Victor Zammit[3,*], Laura Herrero[1,4], Rut Fadó[1,2], Rosalía Rodríguez-Rodríguez[1,2], Dolors Serra[1,4]

[1]CIBER Fisiopatología de la Obesidad y la Nutrición (CIBEROBN), Instituto de Salud Carlos III, Madrid, Spain

[2]Basic Sciences Department, Faculty of Medicine and Health Sciences, Universitat Internacional de Catalunya, E-08195 Sant Cugat del Vallés, Barcelona, Spain

[3]Metabolic and Vascular Health, Warwick Medical School, University of Warwick, Coventry, KA4 7AL, UK

[4]Department of Biochemistry and Molecular Biology, Institut de Biomedicina de la Universitat de Barcelona (IBUB), Universitat de Barcelona, E-08028 Barcelona, Spain

***Address correspondence to:**

Núria Casals, PhD
Basic Sciences Department
Faculty of Medicine and Health Sciences
Universitat Internacional de Catalunya
E-08195 Sant Cugat del Vallés, SPAIN
Tel: (34) 932 541 800
E-mail: ncasals@uic.es

Victor A. Zammit, PhD
Warwick Medical School
Gibbet Hill Campus
University of Warwick
Coventry, CV4 7AL, UK
Tel: 02 476 522 798
E-mail: V.A.Zammit@warwick.ac.uk

Running title

Carnitine palmitoyltransferase 1C

ABSTRACT

Carnitine palmitoyltransferase 1 (CPT1) C was the last member of the CPT1 family of genes to be discovered. CPT1A and CPT1B were identified as the gate-keeper enzymes for the entry of long-chain fatty acids (as carnitine esters) into mitochondria and their further oxidation, and they show differences in their kinetics and tissue expression. Although CPT1C exhibits high sequence similarity to CPT1A and CPT1B, it is specifically expressed in neurons (a cell-type that does not use fatty acids as fuel to any major extent), it is localized in the endoplasmic reticulum of cells, and it has minimal CPT1 catalytic activity with L-carnitine and acyl-CoA esters. The lack of an easily measurable biological activity has hampered attempts to elucidate the cellular and physiological role of CPT1C but has not diminished the interest of the biomedical research community in this CPT1 isoform. The observations that CPT1C binds malonyl-CoA and long-chain acyl-CoA suggest that it is a sensor of lipid metabolism in neurons, where it appears to impact ceramide and triacylglycerol (TAG) metabolism. CPT1C global knock-out mice show a wide range of brain disorders, including impaired cognition and spatial learning, motor deficits, and a deregulation in food intake and energy homeostasis. The first disease-causing CPT1C mutation was recently described in humans, with *Cpt1c* being identified as the gene causing hereditary spastic paraplegia. The putative role of CPT1C in the regulation of complex-lipid metabolism is supported by the observation that it is highly expressed in certain virulent tumor cells, conferring them resistance to glucose- and oxygen-deprivation. Therefore, CPT1C may be a promising target in the treatment of cancer. Here we review the molecular, biochemical, and structural properties of CPT1C and discuss its potential roles in brain function, and cancer.

Keywords (max 6)

Carnitine palmitoyltransferase 1C, lipid metabolism, cognition, energy homeostasis, hereditary spastic paraplegia, cancer

Abbreviations

ACC, acetyl-CoA carboxylase; ACO, aconitase; AICAR, 5-aminoimidazole-4-1-b-D-ribofuranoside; AMPK, AMP-dependent protein kinase; Arc, Arcuate; BSX, brain-specific homeobox; cAMP, cyclic AMP; CNS, central nervous system; COT, carnitine octanoyltransferase; CPT, carnitine palmitoyltransferase; CrAT, carnitine acetyltransferase; CREB, cAMP response element-binding protein; DG, deoxyglucose; ER, endoplasmic reticulum; ERRα, estrogen-related receptor α; FA, fatty acid; FAO, fatty acid oxidation; FAS, fatty acid synthase; HFD, high-fat diet; i.c.v., intracerebroventricular; HSP, hereditary spastic paraplegia; KO, knock-out; LD, lipid droplet; MBH, mediobasal hypothalamus; MCAD, malonyl-CoA dehydrogenase; MCD, malonyl-CoA decarboxylase; PGC-1β, peroxisome proliferator-activated receptor gamma coactivator-1β; PPARα, peroxisome proliferator-activated receptor α; SPG, spastic paraplegia genes; TAG, triacylglycerol; VMN, ventromedial nucleus; WT, wild-type.

Contents

1. Introduction

Carnitine palmitoyltransferase 1C was the last of the family of three carnitine long-chain acyltransferases (CPTs) to be identified [1] (the others being CPT1A and CPT1B and CPT2), and in many respects it has proved to be the most enigmatic. In addition to CPTs, other carnitine acyltransferases also include the short-chain acyl-CoA-specific carnitine acetyltransferase (CrAT) and the medium chain-specific carnitine octanoyltransferase (COT). These molecules catalyze the reversible transesterification of acyl-CoA esters and carnitine to form acylcarnitine esters and coenzyme A. Therefore, these reactions result in the formation of molecular species (acylcarnitines) that can be transported through membranes by specific carnitine-acylcarnitine translocases, in contrast to the membrane-impermeant acyl-CoA esters from which they are derived (Fig. 1). Classically, it is considered that CPTs facilitate the inter-compartmental transfer of acyl moieties between cellular compartments, with the retention of the particular characteristics of the acyl-CoA pools within individual compartments (*e.g.* mitochondrial matrix, peroxisomes, nucleus); however, they are also central to the control of esterified/non-esterified CoA levels in individual cellular compartments [2].

1.1. CPT1A and CPT1B enzymes

Membrane-impermeant long chain acyl-CoAs require CPT1A and CPT1B in order to enter mitochondria and undergo β-oxidation. Malonyl-CoA, the product of the first committed step in FA synthesis and usually derived from glucose, is the physiological inhibitor of CPT1A and CPT1B and thus turns them into enzymes exhibiting strong flux control on fatty acid oxidation (FAO). CPT1A (present in the liver and other tissues capable of high rates of FA synthesis) and CPT1B (expressed

mainly in tissues characterized by high rates of FAO, such as muscle and brown adipose tissue) [3] were initially identified as separate proteins on the basis of distinct kinetic characteristics. In particular, the sensitivity of these two enzymes to their inhibitor malonyl-CoA differs greatly (CPT1A has a 10-fold higher K_i for malonyl-CoA). Subsequently, cloning experiments and sequencing revealed that they show considerable sequence similarity [4]. No crystal structures are available for these two enzymes owing to the fact that they are integral membrane proteins and they lose catalytic function when solubilized. However, homology analysis of their sequences compared to those of the globular, soluble members of the carnitine acyltransferase family (CrAT, COT, CPT2) for which crystal structures have been obtained at a high resolution as well as homology modelling and docking using highly similar sequence motifs identified in other proteins have allowed *in silico* models of CPT1A tertiary structure [5–7]. Moreover, extensive structure-function relationship studies of CPT1A have provided evidence of intricate interactions between a relatively small (47 residues) regulatory N-terminal domain and a large (approx. 610 residues) catalytic C-terminal domain, which are separated by two transmembrane domains and a short connecting loop. This tertiary structure is thought to be adopted by all three isoforms of CPT1 (Fig. 2). In CPT1A, it provides an intricate molecular mechanism for the modulation of malonyl-CoA sensitivity. Key residues within the N-terminal regulatory domain act as positive or negative determinants of the sensitivity of CPT1A to this metabolite [8–15]. In addition, changes in the malonyl-CoA sensitivity of the enzyme have also been associated with homo-oligomerization [16,17] or heteroassociation with other proteins [18], and they also depend on the composition and curvature of the membrane in which CPT1A lies [15,19].

The molecular properties of CPT1A allow it to adapt its catalytic function to the pathophysiological cellular and subcellular requirements, *e.g.* in the liver, of the normal fed state and the fasted or diabetic condition [20–22]. Although CPT1B is also an integral protein of the mitochondrial outer membrane, it does not undergo physiologically induced changes in malonyl-CoA sensitivity and has a permanently low K_i for this enzyme [19].

1.2. The discovery of CPT1C

CPT1C is both similar and different from CPT1A and CPT1B. It was discovered when searches of expressed sequence tag (EST) data using the human CPT1A cDNA nucleotide of protein sequences were performed using BLASTn and tBLAST searches, respectively [1]. Partial *Cpt1c* cDNA was assembled into contiguous sequences, and a particular mouse sequence yielded the full-length *Cpt1c* code [1]. It immediately became apparent that the sequences were restricted predominantly to brain- and tumor-derived ESTs. Although CPT1A was known to be expressed in the brain, and its distribution and sensitivity to malonyl-CoA in the hypothalamus had been implicated in the control of food intake [23,24], CPT1C was the first CPT1 found to be expressed exclusively in neurons, in addition to tumor cell lines [1].

The primary nucleotide sequence for the protein-coding region of *Cpt1c* shares 86% or 85% identity with those of CPT1A or CPT1B, respectively, with a short C-terminal extension in the translated protein [1]. Indeed, the CPT1C sequence contains all the motifs necessary for binding acyl-CoA and carnitine and for the catalysis of the carnitine acyltransferase reaction [1]. However, surprisingly, using a range of acyl-CoA esters and carnitine as substrates, when the protein was expressed in *Pichia pastoris* it had no detectable acyltransferase activity [1,25]. Moreover, it was localized exclusively

to a fraction that comprised mostly microsomes, with no protein being detected in the 'heavy' mitochondrial fraction [1].. This initial characterization made it evident that the function of CPT1C was not likely to be the same as that of CPT1A and CPT1B in controlling the rate of FAO.

Recently, a human CPT1C point-mutation in the N-terminal domain has been associated with spastic paraplegia [26]. Importantly, it is the first CPT1C mutation-associated pathology described in humans. The unique features of CPT1C are not only scientifically intriguing, but may also provide the biological basis for the experimental and clinical evidence of its relevance in brain functions such as appetite control, motor function, and cognition, and also in cancer cell survival, which will be discussed below.

2. Molecular and biochemical properties of CPT1C

The distinct molecular and biochemical properties of CPT1C compared to the other CPT1 isoforms are important clues to its distinctive cellular function. A detailed description of these characteristics is presented in this section.

2.1. Tissue expression

The initial data from EST suggested that CPT1C was expressed predominantly in mammalian brain, which was later confirmed by a detailed study of its expression in a range of human and mouse tissues [1].

A detailed analysis of CPT1C expression by *in situ* hybridization and immunohistochemistry on coronal mouse sections showed widespread expression of this protein in the central nervous system (CNS), with a major concentration in discrete areas like the hippocampus (involved in learning and cognition), hypothalamic nuclei (involved in feeding behavior and body energy expenditure), and amygdala (a center

that coordinates the autonomic and endocrine responses to emotional state) [1,27]. Recently, it has been demonstrated that CPT1C is expressed in mouse motor neurons and dorsal root ganglia, thereby indicating that it is also present in the peripheral nervous system [26] (Table 1).

Interestingly, co-localization studies with cell-specific markers demonstrated that CPT1C is expressed only in neurons, and no co-localization of this protein with endothelial or glial cells was detected [27,28]. Neurons do not use FAs as fuels to any significant extent, thus indicating that CPT1C may have functions other than the canonical carnitine acyltransfrase activity. The observation that CPT1C is expressed only in mammalian neurons points to it having a specific role in mammalian brain.

2.2. Physiological regulation of CPT1C expression

Few studies have focused on the regulation of CPT1C expression in neuronal tissues. Lavrentyey *et al*. found no differences in CPT1C mRNA expression in fasted or diabetic mice compared to controls in any of the brain regions analyzed [29]. However, CPT1C expression is regulated during development in various brain regions of the mouse (cerebellum, motor cortex and striatum) [30] (Table 1). CPT1C protein levels were very low at birth but increased progressively with development, peaking at postnatal day 21, just before weaning. After weaning, the expression of this protein remained high compared to birth values. The physiological meaning of this increase towards a peak in CPT1C at weaning is unknown, but the hormonal (e.g. low insulin/glucagon ratio pre-weaning) and nutritional changes (high fat to high carbohydrate diet) that occur at this age may be key factors in its regulation.

Interestingly, Zaugg's group has demonstrated that CPT1C expression in embryonic tissues is induced by different metabolic stress factors such as glucose

deprivation or hypoxia [31,32], and also by other kinds of stress stimuli such as ionizing irradiation [32] (Table 1). They found that mouse embryonic fibroblasts maintained under glucose deprivation and/or hypoxia had a 3-4 fold upregulation of CPT1C mRNA levels mediated by the energy sensor AMP-activated protein kinase (AMPK). They also found that ionizing radiation of 12.5 *post coitum* mouse embryos raised CPT1C mRNA levels in neuronal tissues and non-neuronal tissues such as heart, and that this upregulation was mediated by the transcription factor p53. It remains to be determined whether this regulation also occurs in postnatal tissues.

In mammalian cell lines, it has been demonstrated that CPT1C is post-transcriptionally regulated [33]. CPT1C mRNA has a long 5' untranslated region (5'UTR) that contains an upstream open reading frame (uORF). uORFs codify for short peptides lacking known physiological activity. The presence of an uORF in the 5'UTR usually inhibits the translation of the main ORF because eukaryotic ribosomes normally only initiate once per mRNA. This is the case of the uORF of CPT1C mRNA, which, therefore, appears to act as translation repressor. Interestingly, the activity of the CPT1C uORF is regulated by various cellular energy stress stimuli. Specifically, glucose depletion, and exposure of cells to palmitate relieve the repression of translation exerted by CPT1C uORF, resulting in a 3-fold increase in protein levels. However, other FAs, like oleate and octanoate, do not have any effect on the regulation of CPT1C translation, perhaps because, unlike palmitate [34], they do not induce cellular stress. Given that these studies were performed *in vitro* (using CPT1C 5'UTR/luciferase construct in mammalian cell lines) confirmation of the translational regulation of CPT1C *in vivo* is required.

In summary, it seems that CPT1C expression is induced by different cellular stress stimuli, suggesting a role of CPT1C in the adaptation of neuronal and non-neuronal tissues to metabolic stress (Table 1).

2.3. Subcellular localization

In the first article published on CPT1C [1], Price *et al*. reported its presence in the microsomal fraction obtained from mouse brain and from yeast overexpressing CPT1C, with no protein being detected in pure mitochondria. Later, overexpression of CPT1C fused to GFP in cultured mammalian cells and co/localization with specific markers confirmed the distribution of this protein in the ER and its absence in mitochondria and peroxisomes [28]. Moreover, the interchange of the first 153 amino acids of CPT1C (containing both the N-regulatory domain and the two transmembrane domains) between CPT1A and CPT1C made these proteins switch location, confirming that the N-terminal region of CPT1 protein is responsible for the subcellular location [28]. The presence of CPT1C in the ER and not in mitochondria is the clearest indication that this protein has a cellular function that differs from that of the two other isoforms.

2.4. Catalytic activity

Much research effort has been channeled into determining CPT1C catalytic activity. The protein was first expressed in the yeast *Pichia pastoris*, which lacks endogenous CPT1 activity [1]. This system has been extensively used to study the catalytic properties of CPT1 enzymes. Although CPT1C has all the motifs required for catalytic activity (the catalytic histidine and the putative carnitine and fatty acyl-CoA binding sites), it has very low catalytic efficiency with carnitine and acyl-CoA as

substrates. Several acyl-CoA esters known to be substrates for CPT1A and CPT1B isoforms (palmitoyl-CoA, arachidonoyl-CoA, or linoleoyl-CoA) or to be abundant in brain (nervanoyl-CoA and lignoceryl-CoA) were tested. CPT1C showed no activity with shorter-chain FAs such as octanoyl-CoA or decanoyl-CoA [1].

In order to perform experiments in a more physiological cellular environment, CPT1C activity was assayed in eukaryotic HEK293T cells overexpressing CPT1C, and the analysis was extended to acyl-CoA esters of various chain lengths and saturations, and modifications (like hydroxyl-acyl-CoAs or methyl-acyl-CoAs). Several acceptor substrates other than carnitine, such as ethanolamine, serine, choline and sphingosine, were also tested; however, none gave rise to catalytic activity *in vitro* [35,36]. Of note, these activity measurements were performed in crude mitochondria which may not have expressed CPT1C (see above). Therefore, Sierra *et al*. measured CPT1C activity in microsomes of CPT1C-overexpressing PC12 cells and used the HPLC-MS/MS method to measure the acyl-carnitine species formed in the reaction. Although they found an increase of 40% in CPT1 activity in cells over-expressing CPT1C compared to control cells [28], when this is compared with the 20-fold increase in expression of the heterologous CPT1C protein it illustrates the very low catalytic activity of the heterologously expressed protein. As PC12 cells highly express CPT1A, results should be interpreted with caution because of possible residual contamination of microsomal fractions with mitochondria.. Later, similar results were obtained by Hada *et al*. [37]. They over-expressed the three CPT1 isoforms in COS7 cells and obtained values of specific CPT1 activity normalized by expression levels. The CPT1C specific activity was 2% of CPT1A and 5% of CPT1B specific activities, using palmitoyl-CoA and carnitine as substrates. All together, these data indicate that CPT1C has minimal activity which is probably of minor physiological relevance, certainly with respect to

acylcarnitine formation in neurons. It could be hypothesised that CPT1C requires a neuron-specific modification for its activity as carnitine acyltransferase or that an allosteric activator is absent in the heterologous expression system. However, the observation that no relevant differences in acyl-CoA and acyl-carnitine levels were found in brain regions of fed and fast CPT1C knock-out (KO) and wild-type (WT) mice [36,38] does not support this concept.

However, another possible interpretation of the results is that CPT1C uses a unique acyl donor or acceptor substrate that has not been tested in any of the assays performed to date. Other classes of lipids (e.g. the newly described fatty acid esters of hydroxy fatty acids [39]), remain to be tested.

2.5. Malonyl-CoA binding

Malonyl-CoA is an intermediate in the FA biosynthetic pathway that is able to bind to CPT1 enzymes (and inhibit the activities of CPT1A and 1B, thus down-regulating FAO). There has been great interest in the role of malonyl-CoA as a regulator of metabolism, in addition to its reciprocal regulation of FAO and synthesis. In fact, malonyl-CoA expression in various brain regions (hippocampus, cortex, hypothalamus, etc.) in fed mice is 3–4 times higher than that found in the same regions in fasted counterparts [40]. There is physiological, pharmacological, and genetic evidence that hypothalamic malonyl-CoA is a major regulator of food intake and energy homeostasis (see [41] for a review), and CPT1C has been hypothesized to be its downstream target. Malonyl-CoA binding affinity assays were performed both in microsomal fractions of yeast expressing CPT1C and in crude mitochondria (subcellular fraction encompassing mitochondria and large microsomes) of HEK293T cells overexpressing the same protein. These assays demonstrated that CPT1C binds to malonyl-CoA with the same

affinity as CPT1A [1,35]. Importantly, the K_d for CPT1C in HEK293T cells was 0.3 μM, which is within the dynamic range of neuronal malonyl-CoA. Thus, fluctuation in malonyl-CoA content in neurons is likely to play an important role in CPT1C function.

2.6. Spatial structure of the N-terminal domain

The N-terminal domain of the CPT1A isoform makes a critical contribution towards the integration of the response of the protein to cytosolic malonyl-CoA levels, and membrane curvature and composition into one regulatory signal. The three-dimensional structure of the CPT1C N-terminal domain encompassing Met1-Phe50 shows crucial sequence differences from that of CPT1A, and was studied by Ulmer *et al*. using NMR spectroscopy and micellar folding scaffolds [42]. The authors performed the experiments under the same conditions as those used in a previous study on the three-dimensional structure of the CPT1A N-terminal domain [15]. They observed that the CPT1C N-terminal domain adopted an inhibitory Nα state that structurally matches that observed for CPT1A. The Nα state is characterized by the formation of two α-helix secondary structures, and it promotes malonyl-CoA inhibition of CPT1A enzyme. In contrast, the authors found that the N-terminus of CPT1C cannot attain the Nβ conformation, also called the malonyl-CoA non-inhibitory state (previously described for CPT1A). These authors proposed that Nβ structural destabilization makes its association with the C-terminal domain unlikely, and that this may contribute to the low catalytic activity of CPT1C (Fig. 2). They concluded that while the switch between Nα and Nβ states or the Nα/Nβ ratio confers CPT1A with a highly sophisticated regulatory mechanism that determines its sensitivity to malonyl-CoA and the enzymatic response to the properties of the outer mitochondrial membrane in different metabolic states, this mechanism appears to be missing in CPT1C because of the lack of the Nβ state. This

interpretation implies that the protein is constitutively inactive even in the absence of malonyl-CoA. Further studies are required to answer the many questions still surrounding the regulatory mechanisms of CPT1C by the N-terminal domain and malonyl-CoA.

2.7. Regulation of long-chain fatty acid oxidation

Although CPT1C has only minimal CPT1 activity *in vitro*, a number of authors have tested the capacity of CPT1C to facilitate long-chain FAO in a cellular context [3,9]. In this regard, they measured the formation of ^{14}C-CO_2 from ^{14}C-palmitate or from ^{14}C-oleate in PC12 and in COS-1 cells overexpressing CPT1C. They proved that CPT1C is unable to increase FAO, even when activators such as 8-Br-cAMP or AICAR, known to induce CPT1A activity through the lowering of cellular malonyl-CoA levels, were added to the media. Moreover, FAO was measured in cortical and hypothalamic explants from CPT1C KO and WT mice, and no differences were found between genotypes. The inability of CPT1C to enhance FAO in intact cells is consistent with the previous observation that this protein is present in the ER, not in the mitochondria, and that it lacks catalytic activity.

2.8. Alternative roles for CPT1C in lipid metabolism

2.8.1. Metabolism of TAGs

An interesting potential new role for CPT1C in lipid metabolism has recently come to light, namely its involvement in lipid droplet (LD) synthesis [26]. LDs are the main organelles storing FAs in the form of TAG, the ER network being a major regulator in LD generation and expansion [43,44]. The size of LDs varies in response to changes in nutrient availability, increasing with nutrient overload and decreasing during

starvation. In cortical cultured neurons, the enhanced LD synthesis induced by oleic acid treatment was attenuated in CPT1C KO cells. Moreover, overexpression of human CPT1C in COS7 cells increased the number and size of LDs, while human CPT1C with the mutation Arg37Cys in the N-terminal region did not. These results indicate that CPT1C facilitates the storage of acyl-CoAs in TAGs rather than facilitating their mitochondrial oxidation. As TAG synthesis occurs on the ER the subcellular location of CPT1C may be relevant. It is also plausible that the CPT1C effect on TAG metabolism is indirect, *i.e.* that CPT1C affects the expression, mobility or specific location of TAG metabolizing enzymes in the ER membrane. It is noteworthy that brain neurons, in contrast to *in vitro* cultured neurons, do not usually show LDs in their cytoplasm although they have all the enzymes for their synthesis. In fact, the only case of LD being visualized in brain slices was in the DDHD2$^{-/-}$ mouse, which was deficient in a protein with TAG hydrolase activity [45]. Therefore, the physiological relevance of CPT1C involvement in LD synthesis in neurons needs to be interpreted with caution.

It has also been proposed that CPT1C participates in the elongation of unsaturated long-chain fatty acids (FAs). The cerebral cortex of transgenic mice overexpressing CPT1C in neurons showed a depletion of total saponified very long-chain FAs, mainly unsaturated C24, C20, C22 and C26 species. Notably, these differences were rescued when animals received a high-fat diet (HFD) [46]. These results suggest that CPT1C attenuates the elongation of unsaturated very long-chain species, although the mechanism involved is completely unknown.

2.8.2. Metabolism of ceramides

When ceramide levels were analyzed in various regions of the brain (hippocampus, motor cortex, cerebellum, and striatum), the content of the most

abundant ceramide in brain (C18:0), and its derivative sphingosine was lowered in fasted CPT1C KO mice [30,47]. Moreover, overexpression of CPT1C in the arcuate (Arc) nucleus increased ceramide levels in this nucleus, while CPT1C KO mice did not show the expected increase in ceramide in response to fasting or to ghrelin treatment. These findings thus established a relationship between CPT1C expression and ceramide levels [48,49].

To confirm that CPT1C-induced ceramide modulation occurs in neurons and not in other brain cells, an *in vitro* model of pure neurons was used in following experiments [47]. CPT1C overexpression in cultured hippocampal neurons increased ceramide almost two-fold, while cultured neurons from CPT1C KO mice showed a notable reduction. Given that the *de novo* synthesis of ceramide takes place in the ER, cultured neurons were treated with labelled serine, a precursor of ceramide, and labelled ceramide was measured along time. The results showed no effect of CPT1C overexpression on serine utilization for *de novo* ceramide synthesis, thus suggesting that it may affects another ceramide metabolic pathway, such as sphingomyelin hydrolysis, glycosphingolipid hydrolysis, or the salvage pathway from sphingosine. Although results clearly demonstrate that CPT1C is involved in ceramide metabolism, the acting molecular mechanism is completely unknown. Proteomic studies might be useful to explore the possibility that CPT1C is interacting with some enzyme or some regulator of the ceramide metabolism.

Taking into account that CPT1C is able to modulate TAG and ceramide content in neurons, as well as the chain length of unsaturated FA (Fig. 3), a more extensive lipidomic analysis would be of great value to elucidate whether other lipid species are also influenced by CPT1C expression.

2.9. Metabolomics of brain extracts obtained from CPT1C KO mice

An unbiased metabolomic profile of CPT1C KO brains extracts compared to those from WT mice revealed the following: a) a small decrease in carnitine and its precursors betaine and glutaroylcarnitine; b) an increase in oxidized glutathione; and c) a decrease in the endogenous endocannabinoids palmitoylethanolamine and eicosapentaenoate [38]. However, the authors did not find any significant variation in any species of acyl-carnitines or acyl-CoAs, thus confirming the insubstantial role of CPT1C in the formation of acylcarnitine esters from acyl-CoA.

Glutathione is the major endogenous anti-oxidant compound produced by cells, preventing damage caused by reactive oxygen species such as free radicals, peroxides, lipid peroxides, and heavy metals [50]. Under normal conditions, more than 90% of the total cellular glutathione pool is in the reduced form (GSH), while the rest is present in the oxidized form (GSSG). An increased GSSG-to-GSH ratio is considered indicative of oxidative stress and has been associated with neurological diseases [50]. The increase in GSSG in CPT1C KO brains suggests an impaired redox homeostasis system, resulting in an increased oxidative environment and a reduced capacity to protect cells from oxidative damage. Moreover, betaine, in addition to being a precursor in carnitine biosynthesis, also has anti-oxidant properties in the brain [51]. All together, these data suggest that CPT1C may participates in neural oxidative metabolism and that CPT1C KO cells are more prone to oxidative stress (Fig. 3).

The reduction in endogenous endocannabinoids is also interesting because these molecules are involved in the hypothalamic control of food intake. Thus, it cannot be ruled out that the reduction of endocannabinoids in CPT1C KO brains contributes to the decrease in food intake observed in these mice (see section 3 below).

3. CPT1C involvement in the physiological functions of the brain

Although the molecular function of CPT1C is not completely understood, numerous lines of experimental evidence demonstrate that it is involved in various physiological functions of the brain, such as energy homeostasis, cognition, and motor function (Fig. 3). This diversity of functions is consistent with the widespread expression of CPT1C in the nervous system.

3.1. Control of appetite and body weight

When discovered in 2002, CPT1C generated much interest in the research field addressing the control of food intake because this protein is highly expressed in appetite regulation nuclei, such as the Arc nucleus, the paraventricular nucleus, and the ventromedial hypothalamus [1], and it also binds malonyl-CoA [2,3], a key indicator of energy status in hypothalamic neurons. An increase in malonyl-CoA in the hypothalamus caused by pharmacological inhibition or genetic knock-down of FAS (fatty acid synthase) expression suppresses food intake and adiposity. Conversely, a decrease by means of ectopic expression of malonyl-CoA decarboxylase (MCD) is sufficient to promote feeding and adiposity (see [52] for a review). Since CPT1C retains the ability to bind malonyl-CoA, it has been proposed to act as a malonyl-CoA sensor in hypothalamic neurons [41] and, therefore, in appetite control.

In order to elucidate the role of CPT1C in food intake, a CPT1C KO mouse was developed by Wolfgang *et al*. in 2006 [35]. These mice showed no apparent developmental abnormalities but did manifest a reduction in body weight and a decrease in daily food intake, thus pointing to an involvement of CPT1C in appetite control. However, no changes were detected in malonyl-CoA levels in the hypothalamus of both genotypes [35]. Subsequently, the involvement of CPT1C in the orexigenic signaling

pathway of ghrelin was reported. Intracerebroventricular (i.c.v.) injection of pharmacological doses of ghrelin to satiated mice increased food intake and promoted food-seeking behavior, both effects being blunted in CPT1C KO mice [49]. Moreover, ghrelin injection failed to increase the mRNA levels of the orexigenic neuropeptides agouti-related protein (AgRP) and neuropeptide Y (NPY) in these animals, thereby indicating that CPT1C is necessary for ghrelin-induced expression of these two peptides. Interestingly, further experiments on ghrelin showed that CPT1C modulates ceramide levels in the mediobasal hypothalamus (MBH). Thus, i.c.v. injection of ghrelin induced a CPT1C-dependent transitory increase in ceramide. CPT1C KO mice or mice pre-treated with an inhibitor of ceramide synthesis did not respond to the orexigenic effect of ghrelin.

The opposite trends were observed in the anorectic effects of leptin [48]. In wild-type animals, a decrease in ceramide levels in the Arc nucleus was necessary for leptin to induce satiating effects and to down-regulate the expression of NPY and its transcription factor, *brain-specific homeobox* (BSX). Arc overexpression of CPT1C and also i.c.v. injection of soluble ceramide (c6-ceramide) attenuated the leptin-induced reduction of BSX and NPY expression and diminished the anorectic action of leptin [48]. All these data demonstrate that the CPT1C/ceramide axis is involved in the regulation of orexigenic neuropeptide expression in response to hormonal cues (Fig. 4). Although the mechanisms by which ceramide regulates BSX expression are unclear, two sets of observations are relevant: i) ceramide forms gel-phase platforms in the plasma membrane and recruits target proteins for specific signaling that can regulate gene expression [53,54]; and ii) ceramides or their soluble derivatives, such as ceramide-1-phosphate and sphingosine, bind transcription factors and modulate target gene expression [55–58].

Of note, neither CPT1C overexpression in the Arc nucleus nor i.c.v. injection of ceramide into *fed* rats was able to induce food intake or to up-regulate NPY and AgRP expression [48,49]. Such effects were triggered only in fasted rats. Therefore, the putative CPT1C/ceramide axis in the Arc nucleus is necessary but not sufficient to induce food intake. This observation suggests that other hypothalamic changes induced by fasting or ghrelin, concomitantly to the CPT1C/ceramide signal, are required to promote feeding. One of these other ghrelin-induced pathways is the AMPK/ACC/CPT1A-mediated modulation of FAO in the ventromedial nucleus (VMN) [59]. It is well known that pharmacological or genetic inhibition of hypothalamic CPT1A reduces food intake while CPT1A overexpression in the VMN induces hyperphagia [23,24,60]. We conclude that both brain isoforms of CPT1 (CPT1A and CPT1C) have combined or synergistic roles in hypothalamic control of food intake.

3.2. Regulation of peripheral lipid metabolism

Although CPT1C KO mice show a reduced food intake, when fed a HFD they are more susceptible to obesity [35,36]. After two weeks on a HFD, these animals have a higher rate of body weight gain than WT mice. Consistent with a decrease in the expression of FAO genes and CPT1A or CPT1B activities, oleic acid oxidation in liver and muscle was markedly decreased in CPT1C KO mice on a HFD compared to WT mice. As a result, TAG content in these tissues was increased [61]. Moreover, CPT1C KO mice exhibited more severe insulin resistance, with elevated hepatic gluconeogenesis and decreased glucose uptake in skeletal muscle [61]. In contrast, adenovirus-induced overexpression of CPT1C in the ventral hypothalamus was sufficient to attenuate body weight gain in HFD mice [27], a finding that confirms the protective role of CPT1C against obesity induced by fats. Later, a novel mouse model

with exogenous expression of CPT1C in the brain was generated [22]. Interestingly, when fed a HFD, mice were protected from weight gain and adiposity [22], thus demonstrating the potential beneficial effects of CPT1C in the control of body weight. These observations suggest that CPT1C is involved in hypothalamus-peripheral tissue communication to regulate FAO in liver and muscle in response to a HFD (Fig. 5).

The molecular hypothalamic mechanisms that regulate FAO in peripheral tissues are not well understood. Some data suggest that hypothalamic malonyl-CoA plays a significant role in the regulation of muscle and liver FAO [62–67], although the downstream factor remains elusive. In this context, we would like to suggest that CPT1C could be that factor, and act as a malonyl-CoA sensor in the hypothalamus to regulate FAO in peripheral tissues.

3.3. Cognition

As mentioned above, CPT1C expression is restricted to the CNS. *In situ* hybridization on mouse coronal brain sections showed high expression of this protein in the hippocampus [1], thereby suggesting that it is likely to play a role in this brain region. Therefore, the repercussion of the CPT1C KO genotype on memory consolidation and learning processes was evaluated [47]. In the hippocampal-dependent Morris water maze test, CPT1C KO mice showed significantly higher escape latency (delayed learning) during the acquisition period. This poorer performance was not associated with motor deficits because swimming speed remained unaltered in 3-month old animals. Platform removal (to test visuospatial memory) and platform relocation (to test cognitive flexibility) revealed that CPT1C KO deficits were limited to the learning phase [47].

Furthermore, brain-specific exogenous over-expression of CPT1C displayed a reduction in the brain weight and severe growth retardation in the postnatal period [46]. Histological examination of these brains showed that all of the major brain structures were present, but were smaller. Moreover, the conditional ubiquitous expression of CPT1C in adult mice, a model that bypasses the developmental impact of CPT1C overexpression, did not result in a remarkable phenotype at least with respect to the regulation of cellular bioenergetics and FA metabolism. Further research is needed to analyze the effect of CPT1C overexpression on cognition.

The behavioral impairment observed in CPT1C KO mice has been associated with poor maturation of dendritic spines [47]. In fact, CPT1C is located in the ER of hippocampal pyramidal neurons, including the ER inside the dendritic spines. Morphological analysis demonstrated that CPT1C KO neurons have a strong increase in immature filopodia number and a marked reduction of mature mushroom and stubby spines. However, the spine-head area in mature spines in these animals was the same as in WT mice. The requirement of CPT1C for efficient spine maturation was related to its ability to regulate ceramide levels, because exogenous ceramide treatment rescued the CPT1C KO phenotype on spine morphology, while treatment of cultured neurons with an inhibitor of ceramide biosynthesis resulted in the same phenotype as that observed in CPT1C KO cells. However, this novel role of CPT1C in the maturation of dendritic spines and spatial learning could also be associated with its capacity to interact directly with α-amino-3-hydroxyl-5-methyl-4-isoxazole-propionate-type glutamate receptors (AMPARs) [68–70].

During synaptogenesis, AMPARs are recruited to dendritic sites of contact with axons to promote synaptic formation and maturation [71,72]. In established synapses, AMPARs mediate fast excitatory synaptic transmission, activity-dependent plasticity

and maintenance of synapsis, underlying memory, and learning processes [73,74]. At the molecular level, the complexity of AMPARs lies in the number of distinct protein constituents present in different locations and times. A high-resolution proteomic analysis identified CPT1C as one of the 34 proteins that form the native AMPAR complexes in the rodent brain [68]. Recently, it has been described that CPT1C is abundant in the periphery of native AMPARs and displays a very similar distribution across all brain regions [69,70]. Moreover, as an ER-resident protein, CPT1C co-localized with AMPARs only at the ER, not at the Golgi apparatus or at the plasma membrane [75].

Unlike other auxiliary proteins of AMPAR complexes, which have been extensively demonstrated to influence AMPAR kinetics and gating properties [76], CPT1C controls AMPAR synthesis and trafficking to the postsynaptic membrane [75,77]. CPT1C deficiency causes a decrease in total levels of GluA1 and GluA2, the most abundant AMPAR subunits in hippocampal neurons, due to a reduction in the rate of their translation. As a result, AMPAR levels at synaptic puncta are decreased and synaptic transmission reduced [77]. Moreover, CPT1C overexpression in an heterologous system increased whole-cell currents of GluA1-containing AMPARs as a consequence of increased trafficking of GluA1 to the surface [75], revealing that CPT1C not only controls the synthesis of AMPAR but also their export from the ER. It is well known that GluA1 trafficking to the plasma membrane is regulated by palmitoylation at two conserved cysteine residues (C585 and C811) [78]. Given that palmitoyl-CoA is a putative CPT1C substrate, the potential involvement of CPT1C in the post-translational modification of AMPARs was considered, but no evidence for its implication were found [75]. The high abundance of CPT1C in hippocampal AMPAR complexes suggests that this protein operates as a chaperone during the synthesis and

mobility of AMPARs through the ER and in their export from the ER to the cell surface (Fig. 6).

Further studies are needed to elucidate the mechanism by which CPT1C controls AMPAR protein synthesis and trafficking and to determine whether the suggested role of CPT1C as a regulator of ceramide metabolism is involved in this function. It is unknown whether ceramide itself modulates AMPAR trafficking; however, the disruption of GM1 ganglioside biosynthesis (a sialic acid-containing oligosaccharide attached to a ceramide lipid) reduces the synaptic expression of GluA2-containing AMPARs [79]. Moreover, ceramide regulates the activity of signaling proteins, such as kinases like PI3K and some isoforms of PKC, and also phosphatases, like PP2A [80], which are involved in AMPAR phosphorylation and synaptic trafficking (reviewed by Bassani *et al.*) [76]. Furthermore, metabolic hormones such as ghrelin and leptin also contribute to the synaptic incorporation of AMPARs and learning processes. It has been demonstrated that ghrelin increases memory retention in rodents [81], enhances long-term potentiation in the hippocampus [82], and increases the delivery of AMPARs to synapses [83]. In addition, leptin regulates AMPAR trafficking [84] and synaptic plasticity [85]. We propose that CPT1C, which has been proved to be a downstream factor of leptin and ghrelin in the hypothalamus [48,49], could be a key link between hippocampal energy metabolism and learning through the direct regulation of AMPAR synthesis and trafficking. Since the disruption of AMPAR function is a major causative agent of synaptic dysfunction and cognitive decline in neurodegenerative diseases [86], CPT1C-enhanced AMPAR synthesis and trafficking may provide a therapeutic strategy to prevent the AMPAR decline and learning deficits associated with aging processes and neurodegeneration.

3.4. Motor function

In addition to the involvement of CPT1C in the hypothalamic control of energy homeostasis and in hippocampus-dependent spatial learning, deficiency in this protein has recently been associated with motor function impairment and hypoactivity [30]. A battery of neurological tests on CPT1C KO mice revealed impaired coordination and gait, severe muscle weakness, and reduced daily locomotor activity [30]. Although observational tests did not show significant differences in general health, sensory reflexes, or autonomous function, these mice presented significant hypoactivity and delayed touch scape compared to WT counterparts. A detailed analysis of motor function indicated impairment in all parameters measured in CPT1C KO mice. The key observations were: i) A shorter latency-to-fall in the rotarod test at fixed rotational speeds and in the accelerating test, indicating impaired motor coordination and therefore disturbances in cerebellar function; ii) significant reduction in stride- length when evaluating the walking pattern, indicative sign of ataxia; iii) shorter latency-to-fall in the bar-hang test, and (iv) greater time required to climb the bar using hind limbs, both results suggesting reduced muscle strength.

Interestingly, this study also revealed that motor deficiencies in CPT1C KO mice are already present in young animals (6 weeks old) and that this impairment progressively increased with age. The authors suggested a potential association between CPT1C deletion and progressive neurodegeneration. It is important to mention that incoordination and hypoactivity appeared at earlier ages than muscle weakness, suggesting that neuronal deterioration developed in a specific timeframe that varied depending on the type of neurons. In addition, analysis of CPT1C expression in brain motor regions during development revealed that CPT1C levels were low at birth and then rapidly increased, peaking at postnatal day 21, at weaning [30]. This observation

suggests that CPT1C plays a key role in motor function during and after weaning. On the basis of these data, the authors hypothesized that the onset of motor disorders observed in CPT1C KO mice occurred between 3 and 7 weeks of life.

The impaired motor function and hypoactivity caused by CPT1C deficiency has been associated with the role of the protein in ceramide metabolism in neurons [30]. Ceramide and its metabolite sphingosine are lipidic factors necessary for the development and survival of neurons [87–89]. The findings that ceramide and sphingosine levels were reduced in brain motor regions of CPT1C KO mice [47] led the authors to propose this alteration as a potential cause of motor impairment in these animals. These deficits in CPT1C KO mice are consistent with the symptoms associated with human CPT1C mutation recently observed in hereditary spastic paraplegia, a human disorder affecting motor function [90].

4. A novel human *Cpt1c* mutation: hereditary spastic paraplegia

Hereditary spastic paraplegias (HSPs) are a group of inherited neurological disorders characterized by length-dependent axonopathy of corticospinal motor neurons, resulting in lower-extremity spasticity and weakness [91]. These disorders have been traditionally classified as pure or complicated, based on the absence (pure) or the presence (complicated) of associated features (*i.e.* cognitive dysfunction, distal amyotrophy, retinopathy, thin *corpus callosum*, and neuropathy) [92,93].

More recently, a genetic classification scheme has been considered, with HSPs commonly identified by their spastic gait *loci*. To date, nearly 75 distinct *loci* and more than 50 spastic paraplegia genes (SPGs) have been identified [93]. Despite this extensive genetic heterogeneity, the functions of the encoded proteins are converging on a small number of common aspects, such as alterations in ER morphogenesis, lipid

metabolism disturbances, mitochondrial regulation, myelination, and endosomal trafficking (Fig. 7) [94].

A recent study by Rinaldi *et al*. [90] identified a mutation in *Cpt1c* as the genetic cause of a pure form of autosomal dominant HSP (AD-HSP), termed *hereditary spastic paraplegia type 73* (SPG73). This is the first disease-causing *Cpt1c* mutation described in humans to date. Researchers found three generations of a family from southern Italy with dominantly inherited, adult onset, and pure spastic paraplegia of unknown genetic cause. Whole-exome sequencing on genomic DNA from the affected individuals allowed identification of a single-nucleotide substitution c.109C>T (p.Arg37Cys) in exon 3 of CPT1C, not present in unaffected individuals, which was, therefore, considered to be the cause of SPG73. The authors hypothesized that the Arg37Cys mutation alters the interaction between the N-terminus and 'catalytic' C-terminal domain of CPT1C.

This study also confirmed the expression of CPT1C in soma and dendritic and axonal projections of motor neurons, as well as its localization to the ER and not to mitochondria. A relevant finding from this study was the interaction between CPT1C and atlastin-1 (SPG3A), one of the most frequently mutated proteins causing HSP [95]. Given that atlastin-1 is a GTPase involved in ER morphogenesis and microtubule dynamics [96,97], it has been suggested that CPT1C may participate in a network of ER proteins involved in ER morphology. In addition to atlastin-1, CPT1C has been also identified as a potential protein associated with protrudin (SPG33) [98], another ER protein that interacts with atlastin-1 and whose gene is mutated in several HSP patients [99].

The *Cpt1c* mutation in HSPs was related to changes in LD biogenesis [90]. LDs are the main organelle for storing FAs in the form of TAG and are thought to contribute

to cell repair. LD-associated proteins are abundantly expressed in the brain and neurons and are especially susceptible to oxidative stress induced by lipid peroxidation [100]. In recent years, several ER-resident proteins mutated in HSPs were found to be involved in the regulation of LD size and formation [101–103]. For instance, depletion of atlastin-1 or expression of a dominant-negative mutant resulted in LD size reduction, whereas atlastin-1 overexpression had the opposite effect [101]. Therefore, it may be particularly relevant that Rinaldi *et al.* [90] found that mutated *Cpt1c* notably reduced the number and size of LDs in transfected COS7 cells and also in primary cortical neurons isolated from CPT1C KO mice compared to WT counterparts. These results were in line with those previously described by Carrasco *et al.* [30], who found that motor function abnormalities in CPT1C KO mice were related to alterations in lipid metabolism (specifically, ceramide levels) in brain motor regions.

Therefore, the association of *Cpt1C* with a HSP phenotype highlights the relevance of lipid metabolism in the pathogenesis of HSPs and possibly also in other motor neuron disorders (Fig. 7). These findings are consistent with the emerging view that ER lipid metabolism is critical for long-term axonal maintenance. Despite these strong lines of evidence, further studies are required to describe the exact mechanisms underlying the interaction between CPT1C with other ER-associated proteins, such as atlastin-1 and protruding [90,104], and to determine how CPT1C alters LD expansion and size.

5. CPT1C in tumor cells

The metabolic demands of the neoplastic cell are significantly higher than those of other tissues, and cancer cells adapt their energy metabolism to the requirement for increased growth and proliferation. This adaptive process includes the following: i) an

increased rate of glucose uptake and glycolysis to compensate the diminished efficiency of production of ATP caused by defective oxidative phosphorylation (Warburg effect) [105,106]. This increased glycolysis also allows the diversion of glucose into the pentose phosphate pathway to produce NADPH and regenerate the reduced anti-oxidant glutathione [107] and to promote the diversion of glycolytic intermediates into various biosynthetic pathways, including those generating nucleosides and amino acids; this facilitates, in turn, the biosynthesis of the macromolecules and organelles required for the assembly of new cells; ii) a change of glutamine metabolism by redirecting glutamine carbon to also support biosynthetic pathways such as nucleosides and amino acids and to maintain redox homeostasis [108]; and iii) a change in lipid metabolism (for general reviews see [109–111]). The cancer cell develops a lipogenic phenotype that increases *de novo* FA synthesis [109,112,113]. In addition, under conditions of metabolic stress, some tumors scavenge lipids from their environment to maintain viability and growth [114,115]. Both *de novo* and FA scavenging pathways are sources of FAs, which are required for the production of phosphoglycerides, which, together with other complex lipids and cholesterol, can be used not only for building cell membranes but also signaling pathways [116]. However, some cancer types develop a lipolytic phenotype. To maintain cellular energy homeostasis, these cancer cell increases FA catabolism through FAO either from *de novo* synthesis or from monoglyceride reserves [117]. In fact, certain types of tumors, including prostate tumors, leukemia, and large B-cell lymphomas, display increased dependence on the FAO of FAs as their main source of energy for proliferation and survival [118–120].

Given that FA entry into mitochondria is regulated by CPT1A or CPT1B, these long-chain acylcarnitine acyltransferases have emerged as new potential therapeutic targets in types of cancer that depend on continued FAO for proliferation. The liver

isoform, CPT1A, is overexpressed mainly in cancers affecting blood cells, such as chronic myeloid leukemia and acute myeloid leukemia [121]. It has been proposed that CPT1A contributes to cell survival, not only by increasing FAO [122] but also stimulating histone acetylase activity in the nucleus [123]. Interestingly, *Cpt1c* has also emerged as a key gene in tumor cell survival in certain types of cancer [124]. Zaugg *et al.* [31] first showed that the expression of CPT1C is unusually increased in many human breast and lung cancers. Furthermore, the study performed by Reilly and Mak [124] in a wide array of human tumor types revealed an increased expression of CPT1C in brain cancers such as neuroblastoma and an unusual expression in several sarcomas of soft-tissues and lung. Interestingly, cells with unlimited availability of nutrients, *e.g.* malignant blood cells, do not overexpress CPT1C.

The specific role of CPT1C in cancer cells remains unknown. Since this protein is located in the ER of normal neuronal cells and it shows very low carnitine acyltransferase activity [28], it is not thought to participate in the regulation of mitochondrial FAO. However, Zaugg *et al.* showed that MCF-7 breast cancer cells constitutively overexpressing CPT1C increased FAO, ATP production, and resistance to glucose deprivation or hypoxia [31]. Conversely, the depletion of CPT1C by *Cpt1c*-specific shRNA had the opposite effect [32]. These findings suggest that CPT1C may be a regulator of FA homeostasis and might be involved in the modulation of bioenergetics that occurs in tumor cells under metabolic stress. The fact that CPT1C is involved in ceramide metabolism in normal neuronal cells [47] and that CPT1C deficiency in embryonic stem cells causes the accumulation of specific species of long-chain FAs, among them arachidonic acid [31], suggest that CPT1C affects additional, as yet undefined, pathways. Moreover, recent studies performed by Wakamiya *et al.* [125] showed that a truncated form of CPT1C is located in the nuclei of diffuse gliomas of

surgical human specimens. Confirmation of these new data would provide new perspectives on the role and regulation of CPT1C expression in cancer cells.

5.1 Regulators of CPT1C expression in response to metabolic stress

CPT1C expression is induced in conditions of extra ATP requirements and under metabolic stress factors such as hypoxia and glucose deprivation. Tumor cells treated with metformin, an inhibitor of electron transport chain, and therefore with an increased AMP/ATP ratio, overexpress CPT1C [31]. This expression might be induced by mechanisms that include direct and indirect effects of AMPK activation (Fig. 8). Zaugg *et al.* proposed that AMPK de-represses the CPT1C uORF resulting in increased CPT1C expression [126]. Under glucose deprivation, AMPK might also activate CPT1C expression in a p53-dependent manner [32]. AMPK is known to link glucose availability to the p53 pathway, a central regulator of cell proliferation and survival [126]. The tumor suppressor gene *p53* is a transcriptional factor that directly activates *Cpt1c* transcription *in vitro* and *in vivo* through the p53-consensus motif present in the first intron of *Cpt1c* [32]. A recent study also indicates that CPT1C might be induced by the indirect action of activated AMPK in the MCF-7 breast cancer cell line [127]. This study showed that 2-deoxyglucose (2-DG), a glycolytic inhibitor, decreased ATP levels, leading to AMPK activation, thus contributing to intracellular ATP recovery in MCF-7 cells. AMPK activation stimulated cAMP response element-binding protein (CREB) phosphorylation and activity and promoted nuclear peroxisome proliferator-activated receptor gamma coactivator-1β (PGC-1β) and estrogen-related receptor α (ERRα) protein expression, leading to augmented mitochondrial biogenesis and expression of genes related with FAO, including peroxisome proliferator-activated receptor α (PPARα), malonyl-CoA dehydrogenase (MCAD), and aconitase (ACO), in addition to

increased CPT1C expression. Conversely, the inhibition of AMPK and PGC-1β by genetic or pharmaceutical approaches attenuated 2-DG-stimulated increases in *PPARα*, *MCAD*, *ACO*, and *CPT1C* expression. These results demonstrated that *CPT1C* is a downstream target of ERRα activated by 2-DG via the AMPK/PGC-1β pathway. Although AMPK emerges as the major contributor to the increase in CPT1C expression in cancer cells, other less explored mechanisms, such as epigenetic alterations by changes in DNA methylation, cannot be disregarded [128].

5.2 CPT1C as a potential target in cancer therapy

Several genetic and pharmacological strategies have been designed to decrease cancer cell viability. The capacity of inhibitors of glycolysis, the electron transport chain, and FAO to reduce intracellular ATP have been tested. Approaches aimed at inhibiting the PI3K/Akt/mTOR pathway have also been assayed. Furthermore, many Phase III clinical trials are underway, including mTOR inhibitors and electron transport chain inhibitors such as metformin. Nevertheless, new strategies based on the inhibition of FAO have recently emerged as a new potential strategy in the pharmacological treatment of cancer.

Several drug development programs have targeted the inhibition of CPT1A and CPT1B *in vitro* and *in vivo* [129]. These inhibitors impair the viability of tumor cells [119,130]. However, given the wide tissue distribution of these two isoforms, the major drawback of their inhibition is the undesired effects in non-tumor cells. In contrast, suppressing CPT1C in peripheral cancers, which show increased CPT1C expression, provides a remarkable advantage over the use of other CPT1 isoforms because neuronal cells are protected by the blood-brain barrier. Unfortunately, to date, none of the small organic molecules used as CPT1 inhibitors have shown significant selectivity towards

the different CPT1 isoforms. Neither is it known whether these inhibitors also act on CPT1C. Given the similarity in sequence and probably tertiary structure of the three CPT1 isoforms, the synthesis of selective CPT1C inhibitors is an exciting possibility.

Genetic tools have become an alternative strategy to treat peripheral cancers. The use of shRNA to decrease CPT1C expression is effective in reducing breast cancer xenografts [31]. However, this strategy cannot be applied to neuronal tumors because shRNA will decrease CPT1C protein in the ER, thus producing alterations in normal brain function. The confirmation that CPT1C may be involved in FA homeostatic pathways in tumor cells has the potential to result in novel therapeutic opportunities. The identification and understanding of these mechanisms will lead to the future design of strategies to fight neuronal tumors by means of CPT1C silencing.

6. Concluding remarks and future perspectives

CPT1C was the last member of the CPT1 family of proteins to be identified and have its gene cloned. It has also proved to be the most difficult to characterize functionally, primarily because it does not display a measurable catalytic activity, in spite of its similarity in primary structure to CPT1A and CPT1B. However, as evidence builds for its major role in many different physiological processes (appetite and motor control, cognition, and the response of certain cancer cell types to hypoxia and hypoglycaemia), it is apparent that, from its intracellular location in the endoplasmic reticulum, it is capable of exerting a wide range of influences, and to interact with different proteins, including those involved in the maintenance of endoplasmic reticular integrity (*e.g.* Atlasin-1). It would be sensible, therefore, not to overlook the importance of the one biological activity which it clearly demonstrates, namely its binding of malonyl-CoA (with the same affinity as CPT1A). This was the property that

was demonstrated in the first publication on the protein [1], and suggested at the time to constitute its functional *raison d'être*.

The binding of malonyl-CoA by CPT1C might regulate the local concentration of the metabolite in specific microenvironments within the cell; and it could explain many of the observations that have been discussed in this review. For example, the stimulation of FAO after overexpression in cancer cells, while unlikely to be related to its intracellular location (not mitochondrial) or catalytic activity (it has minimal carnitine palmitoyltransferase activity) may be due to its ability to sequester malonyl-CoA and lower its cytosolic concentrations, thus de-inhibiting the endogenous CPT1A or CPT1B expressed by the cells.

Similarly, in light of the observation that malonyl-CoA is a direct and specific inducer of FapR, a conserved and transcriptional repressor that regulates expression of genes involved in de novo lipogenesis and phospholipid synthesis in bacteria [131,132], it is plausible that sequestration of malonyl-CoA by CPT1C may affect gene expression, and therefore, alter ceramide synthesis and/or fatty acid composition in neurons. Thus, some effects of CPT1C may be related to its putative role of regulating malonyl-CoA concentration, which is not only the product of the first reaction of fatty acid synthesis, but may also be a modulator of gene expression.

As discussed above, CPT1C also interacts directly with other proteins, notably Atlastin-1, which is important in the formation and stabilization of the endoplasmic reticulum, and in axon function in neurons. It is possible that this interaction is affected by the binding of malonyl-CoA to CPT1C (which would change its conformational state), thus linking cellular metabolism to cell structure and function. Similarly, the observation that CPT1C affects AMPAR trafficking in neurons suggests that it may also achieve this through protein-protein interactions, which would be modulated by its

(malonyl-CoA-dependent) conformational state. Evolutionarily, CPT1C could have retained the core property of malonyl-CoA-binding, which it shares with the other members of the CPT1 family of proteins, as a specific trait to sense the metabolic status of neurons, and regulate specific functions, including synaptic activity (through AMPAR interaction) or ER-mediated axon transport (through Atlastin-1 interaction).

Therefore, at a time when the first disease shown to be specifically caused by a CPT1C point mutation has been described [90], a role for CPT1C in the integration of acute metabolic status and longer-term gene transcription/protein interaction effects, especially in neurons, will need to be considered in future studies.

7. Disclosure

The authors have no conflict of interest to declare.

8. Acknowledgement

We thank Servier Medical Art Webmaster for its contribution to drawing the figures. This work was supported by the Ministry of Spain (Grant SAF2013-45887-R to LH, SAF2014-52223-C2-1-R to DS and SAF2014-52223-C2-2-R to NC), by the *Centro de Investigación Biomédica en Red Fisiopatología de la Obesidad y la Nutrición* (CIBEROBN) (Grant CB06/03/0001 to DS), by the *Generalitat de Catalunya* (2014SGR465 to DS), by the *Fundació La Marató de TV3* to NC and RF, by the European Foundation for the Study of Diabetes (EFSD)/Janssen-Rising Star and L'Oréal-UNESCO "For Women in Science" research fellowships to LH and by Projectes de Recerca per a investigadors novells, Convocatòria 2015 to RR-R. Work in the laboratory of VAZ was supported by Diabetes UK and the British Heart Foundation.

9. References

[1] Price N, van der Leij F, Jackson V, Corstorphine C, Thomson R, Sorensen A, et al. A novel brain-expressed protein related to carnitine palmitoyltransferase I. Genomics 2002;80:433–42. doi:10.1006/geno.2002.6845.

[2] Ramsay RR, Zammit VA. Carnitine acyltransferases and their influence on CoA pools in health and disease. Mol Aspects Med 2004;25:475–93. doi:10.1016/j.mam.2004.06.002.

[3] McGarry JD, Brown NF. The mitochondrial carnitine palmitoyltransferase system. From concept to molecular analysis. Eur J Biochem 1997;244:1–14.

[4] McGarry JD. Travels with carnitine palmitoyltransferase I: from liver to germ cell with stops in between. Biochem Soc Trans 2001;29:241–5. doi:10.1042/BST0290241.

[5] Fraser F, Corstorphine CG, Zammit VA. Topology of carnitine palmitoyltransferase I in the mitochondrial outer membrane. Biochem J 1997;323 (Pt 3:711–8.

[6] Morillas M, Gómez-Puertas P, Roca R, Serra D, Asins G, Valencia A, et al. Structural model of the catalytic core of carnitine palmitoyltransferase I and carnitine octanoyltransferase (COT): Mutation of CPT I histidine 473 and alanine 381 and COT alanine 238 impairs the catalytic activity. J Biol Chem 2001;276:45001–8. doi:10.1074/jbc.M106920200.

[7] Morillas M, López-Viñas E, Valencia A, Serra D, Gómez-Puertas P, Hegardt FG, et al. Structural model of carnitine palmitoyltransferase I based on the carnitine acetyltransferase crystal. Biochem J 2004;379:777–84. doi:10.1042/BJ20031373.

[8] Cohen I, Kohl C, McGarry JD, Girard J, Prip-Buus C. The N-terminal domain of rat liver carnitine palmitoyltransferase 1 mediates import into the outer mitochondrial membrane and is essential for activity and malonyl-CoA sensitivity. J Biol Chem 1998;273:29896–904. doi:10.1074/jbc.273.45.29896.

[9] Jackson VN, Zammit VA, Price NT. Identification of positive and negative determinants of malonyl-CoA sensitivity and carnitine affinity within the amino termini of rat liver- and muscle-type carnitine palmitoyltransferase I. J Biol Chem 2000;275:38410–6. doi:10.1074/jbc.M007722200.

[10] Shi J, Zhu H, Arvidson DN, Woldegiorgis G. A single amino acid change

(substitution of glutamate 3 with alanine) in the N-terminal region of rat liver carnitine palmitoyltransferase I abolishes malonyl-CoA inhibition and high affinity binding. J Biol Chem 1999;274:9421–6.

[11] Jackson VN, Price NT, Zammit VA. Specificity of the interactions between Glu-3, Ser-24, and Gln-30 within the N-terminal segment of rat liver mitochondrial overt carnitine palmitoyltransferase (L-CPT I) in determining the malonyl-CoA sensitivity of the enzyme. Biochemistry 2001;40:14629–34.

[12] Swanson ST, Foster DW, McGarry JD, Brown NF. Roles of the N- and C-terminal domains of carnitine palmitoyltransferase I isoforms in malonyl-CoA sensitivity of the enzymes: insights from expression of chimaeric proteins and mutation of conserved histidine residues. Biochem J 1998;335 (Pt 3:513–9.

[13] Pan Y, Cohen I, Guillerault F, Fève B, Girard J, Prip-Buus C. The extreme C terminus of rat liver carnitine palmitoyltransferase I is not involved in malonyl-CoA sensitivity but in initial protein folding. J Biol Chem 2002;277:47184–9. doi:10.1074/jbc.M208055200.

[14] Faye A, Borthwick K, Esnous C, Price NT, Gobin S, Jackson VN, et al. Demonstration of N- and C-terminal domain intramolecular interactions in rat liver carnitine palmitoyltransferase 1 that determine its degree of malonyl-CoA sensitivity. Biochem J 2005;387:67–76. doi:10.1042/BJ20041533.

[15] Rao JN, Warren GZL, Estolt-Povedano S, Zammit VA, Ulmer TS. An environment-dependent structural switch underlies the regulation of carnitine palmitoyltransferase 1A. J Biol Chem 2011;286:42545–54. doi:10.1074/jbc.M111.306951.

[16] Faye A, Esnous C, Price NT, Onfray MA, Girard J, Prip-Buus C. Rat liver carnitine palmitoyltransferase 1 forms an oligomeric complex within the outer mitochondrial membrane. J Biol Chem 2007;282:26908–16. doi:10.1074/jbc.M705418200.

[17] Jenei ZA, Warren GZL, Hasan M, Zammit VA, Dixon AM. Packing of transmembrane domain 2 of carnitine palmitoyltransferase-1A affects oligomerization and malonyl-CoA sensitivity of the mitochondrial outer membrane protein. FASEB J 2011;25:4522–30. doi:10.1096/fj.11-192005.

[18] Brdiczka D. Contact sites between mitochondrial envelope membranes. Structure and function in energy- and protein-transfer. Biochim Biophys Acta - Rev Biomembr 1991;1071:291–312. doi:10.1016/0304-4157(91)90018-R.

[19] Zammit VA. Carnitine palmitoyltransferase 1: Central to cell function. IUBMB

Life 2008;60:347–54. doi:10.1002/iub.78.

[20] Zammit VA. Hepatic triacylglycerol synthesis and secretion: DGAT2 as the link between glycaemia and triglyceridaemia. Biochem J 2013;451:1–12. doi:10.1042/BJ20121689.

[21] Zammit VA, Corstorphine CG, Kolodziej MP, Fraser F. Lipid molecular order in liver mitochondrial outer membranes, and sensitivity of carnitine palmitoyltransferase I to malonyl-CoA. Lipids 1998;33:371–6. doi:10.1007/s11745-998-0217-7.

[22] Zammit VA, Price NT, Fraser F, Jackson VN. Structure-function relationships of the liver and muscle isoforms of carnitine palmitoyltransferase I. Biochem Soc Trans 2001;29:287–92.

[23] Obici S, Feng Z, Arduini A, Conti R, Rossetti L. Inhibition of hypothalamic carnitine palmitoyltransferase-1 decreases food intake and glucose production. Nat Med 2003;9:756–61. doi:10.1038/nm873.

[24] Mera P, Mir JF, Fabriàs G, Casas J, Costa ASH, Malandrino MI, et al. Long-term increased carnitine palmitoyltransferase 1A expression in ventromedial hypotalamus causes hyperphagia and alters the hypothalamic lipidomic profile. PLoS One 2014;9:e97195. doi:10.1371/journal.pone.0097195.

[25] Dai Y, Wolfgang MJ, Cha SH, Lane MD. Localization and effect of ectopic expression of CPT1c in CNS feeding centers. Biochem Biophys Res Commun 2007;359:469–74. doi:10.1016/j.bbrc.2007.05.161.

[26] Rinaldi C, Schmidt T, Situ AJ, Johnson JO, Lee PR, Chen KL, et al. Mutation in CPT1C Associated With Pure Autosomal Dominant Spastic Paraplegia. JAMA Neurol 2015;72:561–70.

[27] Dai Y, Wolfgang MJ, Cha SH, Lane MD. Localization and effect of ectopic expression of CPT1c in CNS feeding centers. Biochem Biophys Res Commun 2007;359:469–74.

[28] Sierra AY, Gratacós E, Carrasco P, Clotet J, Ureña J, Serra D, et al. CPT1c is localized in endoplasmic reticulum of neurons and has carnitine palmitoyltransferase activity. J Biol Chem 2008;283:6878–85. doi:10.1074/jbc.M707965200.

[29] Lavrentyev EN, Matta SG, Cook GA. Expression of three carnitine palmitoyltransferase-I isoforms in 10 regions of the rat brain during feeding,

fasting, and diabetes. Biochem Biophys Res Commun 2004;315:174–8.

[30] Carrasco P, Jacas J, Sahún I, Muley H, Ramírez S, Puisac B, et al. Carnitine palmitoyltransferase 1C deficiency causes motor impairment and hypoactivity. Behav Brain Res 2013;256:291–7.

[31] Zaugg K, Yao Y, Reilly PT, Kannan K, Kiarash R, Mason J, et al. Carnitine palmitoyltransferase 1C promotes cell survival and tumor growth under conditions of metabolic stress. Genes Dev 2011;25:1041–51. doi:10.1101/gad.1987211.

[32] Sanchez-Macedo N, Feng J, Faubert B, Chang N, Elia A, Rushing EJ, et al. Depletion of the novel p53-target gene carnitine palmitoyltransferase 1C delays tumor growth in the neurofibromatosis type I tumor model. Cell Death Differ 2013;20:659–68. doi:10.1038/cdd.2012.168.

[33] Lohse I, Reilly P, Zaugg K. The CPT1C 5'UTR contains a repressing upstream open reading frame that is regulated by cellular energy availability and AMPK. PLoS One 2011;6:e21486. doi:10.1371/journal.pone.0021486.

[34] Diakogiannaki E, Welters HJ, Morgan NG. Differential regulation of the endoplasmic reticulum stress response in pancreatic beta-cells exposed to long-chain saturated and monounsaturated fatty acids. J Endocrinol 2008;197:553–63. doi:10.1677/JOE-08-0041.

[35] Wolfgang MJ, Kurama T, Dai Y, Suwa A, Asaumi M, Matsumoto S-I, et al. The brain-specific carnitine palmitoyltransferase-1c regulates energy homeostasis. Proc Natl Acad Sci U S A 2006;103:7282–7. doi:10.1073/pnas.0602205103.

[36] Wolfgang MJ, Cha SH, Millington DS, Cline G, Shulman GI, Suwa A, et al. Brain-specific carnitine palmitoyl-transferase-1c: Role in CNS fatty acid metabolism, food intake, and body weight. J Neurochem 2008;105:1550–9. doi:10.1111/j.1471-4159.2008.05255.x.

[37] Hada T, Yamamoto T, Yamamoto A, Ohkura K, Yamazaki N, Takiguchi Y, et al. Comparison of the catalytic activities of three isozymes of carnitine palmitoyltransferase 1 expressed in COS7 cells. Appl Biochem Biotechnol 2014;172:1486–96.

[38] Lee J, Wolfgang MJ. Metabolomic profiling reveals a role for CPT1c in neuronal oxidative metabolism. BMC Biochem 2012;13:23. doi:10.1186/1471-2091-13-23.

[39] Yore MM, Syed I, Moraes-Vieira PM, Zhang T, Herman MA, Homan EA, et al. Discovery of a class of endogenous mammalian lipids with anti-diabetic and anti-inflammatory effects. Cell 2014;159:318–32.

[40] Tokutake Y, Onizawa N, Katoh H, Toyoda A, Chohnan S. Coenzyme A and its thioester pools in fasted and fed rat tissues. Biochem Biophys Res Commun 2010;402:158–62.

[41] Wolfgang MJ, Lane MD. Hypothalamic malonyl-CoA and CPT1c in the treatment of obesity. FEBS J 2011;278:552–8.

[42] Samanta S, Situ AJ, Ulmer TS. Structural characterization of the regulatory domain of brain carnitine palmitoyltransferase 1. Biopolymers 2014;101:398–405. doi:10.1002/bip.22396.

[43] Murphy DJ, Vance J. Mechanisms of lipid-body formation. Trends Biochem Sci 1999;24:109–15.

[44] Wilfling F, Wang H, Haas JT, Krahmer N, Gould TJ, Uchida A, et al. Triacylglycerol synthesis enzymes mediate lipid droplet growth by relocalizing from the ER to lipid droplets. Dev Cell 2013;24:384–99. doi:10.1016/j.devcel.2013.01.013.

[45] Inloes JM, Hsu K-L, Dix MM, Viader A, Masuda K, Takei T, et al. The hereditary spastic paraplegia-related enzyme DDHD2 is a principal brain triglyceride lipase. Proc Natl Acad Sci U S A 2014;111:14924–9. doi:10.1073/pnas.1413706111.

[46] Reamy AA, Wolfgang MJ. Carnitine palmitoyltransferase-1c gain-of-function in the brain results in postnatal microencephaly. J Neurochem 2011;118:388–98. doi:10.1111/j.1471-4159.2011.07312.x.

[47] Carrasco P, Sahún I, McDonald J, Ramírez S, Jacas J, Gratacós E, et al. Ceramide levels regulated by carnitine palmitoyltransferase 1C control dendritic spine maturation and cognition. J Biol Chem 2012;287:21224–32.

[48] Gao S, Zhu G, Gao X, Wu D, Carrasco P, Casals N, et al. Important roles of brain-specific carnitine palmitoyltransferase and ceramide metabolism in leptin hypothalamic control of feeding. Proc Natl Acad Sci U S A 2011;108:9691–6.

[49] Ramírez S, Martins L, Jacas J, Carrasco P, Pozo M, Clotet J, et al. Hypothalamic ceramide levels regulated by cpt1c mediate the orexigenic effect of ghrelin. Diabetes 2013;62:2329–37.

[50] Dringen R, Hirrlinger J. Glutathione pathways in the brain. Biol Chem 2003;384:505–16. doi:10.1515/BC.2003.059.

[51] Alirezaei M, Khoshdel Z, Dezfoulian O, Rashidipour M, Taghadosi V. Beneficial antioxidant properties of betaine against oxidative stress mediated by levodopa/benserazide in the brain of rats. J Physiol Sci 2015;65:243–52. doi:10.1007/s12576-015-0360-0.

[52] Lane MD, Wolfgang M, Cha S-H, Dai Y. Regulation of food intake and energy expenditure by hypothalamic malonyl-CoA. Int J Obes (Lond) 2008;32 Suppl 4:S49–54. doi:10.1038/ijo.2008.123.

[53] Castro BM, Prieto M, Silva LC. Ceramide: a simple sphingolipid with unique biophysical properties. Prog Lipid Res 2014;54:53–67.

[54] Zhang Y, Li X, Becker KA, Gulbins E. Ceramide-enriched membrane domains--structure and function. Biochim Biophys Acta 2009;1788:178–83.

[55] Wooten-Blanks LG, Song P, Senkal CE, Ogretmen B. Mechanisms of ceramide-mediated repression of the human telomerase reverse transcriptase promoter via deacetylation of Sp3 by histone deacetylase 1. FASEB J 2007;21:3386–97.

[56] Hait NC, Allegood J, Maceyka M, Strub GM, Harikumar KB, Singh SK, et al. Regulation of histone acetylation in the nucleus by sphingosine-1-phosphate. Science 2009;325:1254–7.

[57] Patwardhan GA, Liu YY. Sphingolipids and expression regulation of genes in cancer. Prog Lipid Res 2011;50:104–14.

[58] Zou X, Gao Y, Ruvolo VR, Gardner TL, Ruvolo PP, Brown RE. Human glycolipid transfer protein gene (GLTP) expression is regulated by Sp1 and Sp3: involvement of the bioactive sphingolipid ceramide. J Biol Chem 2011;286:1301–11.

[59] López M, Lage R, Saha AK, Pérez-Tilve D, Vázquez MJ, Varela L, et al. Hypothalamic Fatty Acid Metabolism Mediates the Orexigenic Action of Ghrelin. Cell Metab 2008;7:389–99. doi:10.1016/j.cmet.2008.03.006.

[60] Mera P, Bentebibel A, López-Viñas E, Cordente AG, Gurunathan C, Sebastián D, et al. C75 is converted to C75-CoA in the hypothalamus, where it inhibits carnitine palmitoyltransferase 1 and decreases food intake and body weight.

Biochem Pharmacol 2009;77:1084–95. doi:10.1016/j.bcp.2008.11.020.

[61] Gao XF, Chen W, Kong XP, Xu AM, Wang ZG, Sweeney G, et al. Enhanced susceptibility of Cpt1c knockout mice to glucose intolerance induced by a high-fat diet involves elevated hepatic gluconeogenesis and decreased skeletal muscle glucose uptake. Diabetologia 2009;52:912–20.

[62] Minokoshi Y, Kim YB, Peroni OD, Fryer LG, Muller C, Carling D, et al. Leptin stimulates fatty-acid oxidation by activating AMP-activated protein kinase. Nature 2002;415:339–43.

[63] Minokoshi Y, Kahn BB. Role of AMP-activated protein kinase in leptin-induced fatty acid oxidation in muscle. Biochem Soc Trans 2003;31:196–201.

[64] Xue B, Pulinilkunnil T, Murano I, Bence KK, He H, Minokoshi Y, et al. Neuronal protein tyrosine phosphatase 1B deficiency results in inhibition of hypothalamic AMPK and isoform-specific activation of AMPK in peripheral tissues. Mol Cell Biol 2009;29:4563–73.

[65] Gallardo N, Bonzón-Kulichenko E, Fernández-Agulló T, Moltó E, Gómez-Alonso S, Blanco P, et al. Tissue-specific effects of central leptin on the expression of genes involved in lipid metabolism in liver and white adipose tissue. Endocrinology 2007;148:5604–10. doi:10.1210/en.2007-0933.

[66] Cha SH, Hu Z, Chohnan S, Lane MD. Inhibition of hypothalamic fatty acid synthase triggers rapid activation of fatty acid oxidation in skeletal muscle. Proc Natl Acad Sci U S A 2005;102:14557–62.

[67] Vázquez MJ, González CR, Varela L, Lage R, Tovar S, Sangiao-Alvarellos S, et al. Central resistin regulates hypothalamic and peripheral lipid metabolism in a nutritional-dependent fashion. Endocrinology 2008;149:4534–43. doi:10.1210/en.2007-1708.

[68] Schwenk J, Harmel N, Brechet A, Zolles G, Berkefeld H, Müller CS, et al. High-Resolution Proteomics Unravel Architecture and Molecular Diversity of Native AMPA Receptor Complexes. Neuron 2012;74:621–33. doi:10.1016/j.neuron.2012.03.034.

[69] Chen N, Pandya NJ, Koopmans F, Castelo-Székelv V, van der Schors RC, Smit AB, et al. Interaction proteomics reveals brain region-specific AMPA receptor complexes. J Proteome Res 2014;13:5695–706. doi:10.1021/pr500697b.

[70] Schwenk J, Baehrens D, Haupt A, Bildl W, Boudkkazi S, Roeper J.

NeuroResource Regional Diversity and Developmental Dynamics of the AMPA-Receptor Proteome in the Mammalian Brain. Neuron 2014. doi:10.1016/j.neuron.2014.08.044.

[71] McAllister AK. Dynamic aspects of CNS synapse formation. Annu Rev Neurosci 2007;30:425–50. doi:10.1146/annurev.neuro.29.051605.112830.

[72] McKinney RA. Excitatory amino acid involvement in dendritic spine formation, maintenance and remodelling. J Physiol 2010;588:107–16. doi:10.1113/jphysiol.2009.178905.

[73] Malenka RC, Nicoll RA. Long-term potentiation--a decade of progress? Science 1999;285:1870–4.

[74] Ehlers MD. Reinsertion or degradation of AMPA receptors determined by activity-dependent endocytic sorting. Neuron 2000;28:511–25.

[75] Gratacòs-Batlle E, Yefimenko N, Cascos-García H, Soto D. AMPAR interacting protein CPT1C enhances surface expression of GluA1-containing receptors. Front Cell Neurosci 2014;8:469. doi:10.3389/fncel.2014.00469.

[76] Bassani S, Folci A, Zapata J, Passafaro M. AMPAR trafficking in synapse maturation and plasticity. Cell Mol Life Sci 2013;70:4411–30. doi:10.1007/s00018-013-1309-1.

[77] Fadó R, Soto D, Miñano-Molina AJ, Pozo M, Carrasco P, Yefimenko N, et al. Novel Regulation of the Synthesis of α-Amino-3-hydroxy-5-methyl-4-isoxazolepropionic Acid (AMPA) Receptor Subunit GluA1 by Carnitine Palmitoyltransferase 1C (CPT1C) in the Hippocampus. J Biol Chem 2015;290:25548–60. doi:10.1074/jbc.M115.681064.

[78] Hayashi T, Rumbaugh G, Huganir RL. Differential regulation of AMPA receptor subunit trafficking by palmitoylation of two distinct sites. Neuron 2005;47:709–23. doi:10.1016/j.neuron.2005.06.035.

[79] Prendergast J, Umanah GKE, Yoo S-W, Lagerlof O, Motari MG, Cole RN, et al. Ganglioside Regulation of AMPA Receptor Trafficking. J Neurosci 2014;34:13246–58. doi:10.1523/JNEUROSCI.1149-14.2014.

[80] Ruvolo PP. Intracellular signal transduction pathways activated by ceramide and its metabolites. Pharmacol Res 2003;47:383–92.

[81] Carlini VP, Monzón ME, Varas MM, Cragnolini AB, Schiöth HB, Scimonelli TN, et al. Ghrelin increases anxiety-like behavior and memory retention in rats. Biochem Biophys Res Commun 2002;299:739–43.

[82] Chen L, Xing T, Wang M, Miao Y, Tang M, Chen J, et al. Local infusion of ghrelin enhanced hippocampal synaptic plasticity and spatial memory through activation of phosphoinositide 3-kinase in the dentate gyrus of adult rats. Eur J Neurosci 2011;33:266–75. doi:10.1111/j.1460-9568.2010.07491.x.

[83] Ribeiro LF, Catarino T, Santos SD, Benoist M, van Leeuwen JF, Esteban J a, et al. Ghrelin triggers the synaptic incorporation of AMPA receptors in the hippocampus. Proc Natl Acad Sci U S A 2014;111:E149–58. doi:10.1073/pnas.1313798111.

[84] Moult PR, Cross A, Santos SD, Carvalho A-L, Lindsay Y, Connolly CN, et al. Leptin regulates AMPA receptor trafficking via PTEN inhibition. J Neurosci 2010;30:4088–101. doi:10.1523/JNEUROSCI.3614-09.2010.

[85] Shanley LJ, Irving AJ, Harvey J. Leptin enhances NMDA receptor function and modulates hippocampal synaptic plasticity. J Neurosci 2001;21:RC186.

[86] Henley JM, Wilkinson K a. AMPA receptor trafficking and the mechanisms underlying synaptic plasticity and cognitive aging. Dialogues Clin Neurosci 2013;15:11–27.

[87] Zhao L, Spassieva SD, Jucius TJ, Shultz LD, Shick HE, Macklin WB, et al. A deficiency of ceramide biosynthesis causes cerebellar purkinje cell neurodegeneration and lipofuscin accumulation. PLoS Genet 2011;7:e1002063. doi:10.1371/journal.pgen.1002063.

[88] Furuya S, Mitoma J, Makino A, Hirabayashi Y. Ceramide and its interconvertible metabolite sphingosine function as indispensable lipid factors involved in survival and dendritic differentiation of cerebellar Purkinje cells. J Neurochem 1998;71:366–77.

[89] Irie F, Hirabayashi Y. Ceramide prevents motoneuronal cell death through inhibition of oxidative signal. Neurosci Res 1999;35:135–44.

[90] Rinaldi C, Schmidt T, Situ AJ, Johnson JO, Lee PR, Chen K, et al. Mutation in *CPT1C* Associated With Pure Autosomal Dominant Spastic Paraplegia. JAMA Neurol 2015;72:561. doi:10.1001/jamaneurol.2014.4769.

[91] Fink JK. Hereditary spastic paraplegia: Clinico-pathologic features and emerging

molecular mechanisms. Acta Neuropathol 2013;126:307–28. doi:10.1007/s00401-013-1115-8.

[92] Harding AE. Classification of the hereditary ataxias and paraplegias. Lancet (London, England) 1983;1:1151–5.

[93] Lo Giudice T, Lombardi F, Santorelli FM, Kawarai T, Orlacchio A. Hereditary spastic paraplegia: Clinical-genetic characteristics and evolving molecular mechanisms. Exp Neurol 2014;261:518–39. doi:10.1016/j.expneurol.2014.06.011.

[94] Noreau A, Dion PA, Rouleau GA. Molecular aspects of hereditary spastic paraplegia. Exp Cell Res 2014;325:18–26. doi:10.1016/j.yexcr.2014.02.021.

[95] Zhao X, Alvarado D, Rainier S, Lemons R, Hedera P, Weber CH, et al. Mutations in a newly identified GTPase gene cause autosomal dominant hereditary spastic paraplegia. Nat Genet 2001;29:326–31. doi:10.1038/ng758\rng758 [pii].

[96] Park SH, Zhu P-P, Parker RL, Blackstone C. Hereditary spastic paraplegia proteins REEP1, spastin, and atlastin-1 coordinate microtubule interactions with the tubular ER network. J Clin Invest 2010;120:1097–110. doi:10.1172/JCI40979.

[97] Blackstone C, O'Kane CJ, Reid E. Hereditary spastic paraplegias: membrane traffic and the motor pathway. Nat Rev Neurosci 2011;12:31–42. doi:10.1038/nrn2990.

[98] Hashimoto Y, Shirane M, Matsuzaki F, Saita S, Ohnishi T, Nakayama KI. Protrudin regulates endoplasmic reticulum morphology and function associated with the pathogenesis of hereditary spastic paraplegia. J Biol Chem 2014;289:12946–61. doi:10.1074/jbc.M113.528687.

[99] Mannan AU, Krawen P, Sauter SM, Boehm J, Chronowska A, Paulus W, et al. ZFYVE27 (SPG33), a novel spastin-binding protein, is mutated in hereditary spastic paraplegia. Am J Hum Genet 2006;79:351–7. doi:10.1086/504927.

[100] Welte MA. Expanding Roles for Lipid Droplets. Curr Biol 2015;25:R470–81. doi:10.1016/j.cub.2015.04.004.

[101] Klemm RW, Norton JP, Cole R a., Li CS, Park SH, Crane MM, et al. A Conserved Role for Atlastin GTPases in Regulating Lipid Droplet Size. Cell Rep 2013;3:1465–75. doi:10.1016/j.celrep.2013.04.015.

[102] Falk J, Rohde M, Bekhite MM, Neugebauer S, Hemmerich P, Kiehntopf M, et al. Functional Mutation Analysis Provides Evidence for a Role of REEP1 in Lipid Droplet Biology. Hum Mutat 2014;35:497–504. doi:10.1002/humu.22521.

[103] Windpassinger C, Auer-Grumbach M, Irobi J, Patel H, Petek E, Hörl G, et al. Heterozygous missense mutations in BSCL2 are associated with distal hereditary motor neuropathy and Silver syndrome. Nat Genet 2004;36:271–6. doi:10.1038/ng1313.

[104] Hashimoto Y, Shirane M, Matsuzaki F, Saita S, Ohnishi T, Nakayama KI. Protrudin regulates endoplasmic reticulum morphology and function associated with the pathogenesis of hereditary spastic paraplegia. J Biol Chem 2014;289:12946–61. doi:10.1074/jbc.M113.528687.

[105] Warburg O. Injuring of Respiration the Origin of Cancer Cells. Science (80-) 1956;123:309–14. doi:10.1126/science.123.3191.309.

[106] Warburg O. What is flux balance analysis? Science 1956;124:269–70.

[107] Backos DS, Franklin CC, Reigan P. The role of glutathione in brain tumor drug resistance. Biochem Pharmacol 2012;83:1005–12. doi:10.1016/j.bcp.2011.11.016.

[108] Son J, Lyssiotis CA, Ying H, Wang X, Hua S, Ligorio M, et al. Glutamine supports pancreatic cancer growth through a KRAS-regulated metabolic pathway. Nature 2013;496:101–5. doi:10.1038/nature12040.

[109] Swinnen J V, Brusselmans K, Verhoeven G. Increased lipogenesis in cancer cells: new players, novel targets. Curr Opin Clin Nutr Metab Care 2006;9:358–65. doi:10.1097/01.mco.0000232894.28674.30.

[110] Santos CR, Schulze A. Lipid metabolism in cancer. FEBS J 2012;279:2610–23. doi:10.1111/j.1742-4658.2012.08644.x.

[111] Currie E, Schulze A, Zechner R, Walther TC, Farese R V. Cellular fatty acid metabolism and cancer. Cell Metab 2013;18:153–61. doi:10.1016/j.cmet.2013.05.017.

[112] Menendez JA, Lupu R. Fatty acid synthase and the lipogenic phenotype in cancer pathogenesis. Nat Rev Cancer 2007;7:763–77. doi:10.1038/nrc2222.

[113] Pandey PR, Liu W, Xing F, Fukuda K, Watabe K. Anti-cancer drugs targeting fatty acid synthase (FAS). Recent Pat Anticancer Drug Discov 2012;7:185–97.

[114] Kamphorst JJ, Cross JR, Fan J, de Stanchina E, Mathew R, White EP, et al. Hypoxic and Ras-transformed cells support growth by scavenging unsaturated fatty acids from lysophospholipids. Proc Natl Acad Sci U S A 2013;110:8882–7. doi:10.1073/pnas.1307237110.

[115] Zaidi N, Lupien L, Kuemmerle NB, Kinlaw WB, Swinnen J V, Smans K. Lipogenesis and lipolysis: the pathways exploited by the cancer cells to acquire fatty acids. Prog Lipid Res 2013;52:585–9. doi:10.1016/j.plipres.2013.08.005.

[116] Louie SM, Roberts LS, Mulvihill MM, Luo K, Nomura DK. Cancer cells incorporate and remodel exogenous palmitate into structural and oncogenic signaling lipids. Biochim Biophys Acta 2013;1831:1566–72. doi:10.1016/j.bbalip.2013.07.008.

[117] Nomura DK, Long JZ, Niessen S, Hoover HS, Ng S-W, Cravatt BF. Monoacylglycerol lipase regulates a fatty acid network that promotes cancer pathogenesis. Cell 2010;140:49–61. doi:10.1016/j.cell.2009.11.027.

[118] Zha S, Ferdinandusse S, Hicks JL, Denis S, Dunn TA, Wanders RJ, et al. Peroxisomal branched chain fatty acid beta-oxidation pathway is upregulated in prostate cancer. Prostate 2005;63:316–23. doi:10.1002/pros.20177.

[119] Samudio I, Harmancey R, Fiegl M, Kantarjian H, Konopleva M, Korchin B, et al. Pharmacologic inhibition of fatty acid oxidation sensitizes human leukemia cells to apoptosis induction. J Clin Invest 2010;120:142–56. doi:10.1172/JCI38942.

[120] Caro P, Kishan AU, Norberg E, Stanley IA, Chapuy B, Ficarro SB, et al. Metabolic signatures uncover distinct targets in molecular subsets of diffuse large B cell lymphoma. Cancer Cell 2012;22:547–60. doi:10.1016/j.ccr.2012.08.014.

[121] Novartis cancer cell Line Encyclopedia. www.broadinstitute.org/ccle. Broad Inst (Http//www.broadinstitute.org/); Novartis Institutes Biomed Res (https//www.nibr.com/); Genomics Inst Novartis Res Found□ ;□ ; n.d.

[122] Linher-Melville K, Zantinge S, Sanli T, Gerstein H, Tsakiridis T, Singh G. Establishing a relationship between prolactin and altered fatty acid β-oxidation via carnitine palmitoyl transferase 1 in breast cancer cells. BMC Cancer 2011;11:56. doi:10.1186/1471-2407-11-56.

[123] Mazzarelli P, Pucci S, Bonanno E, Sesti F, Calvani M, Spagnoli LG. Carnitine

palmitoyltransferase I in human carcinomas: a novel role in histone deacetylation? Cancer Biol Ther 2007;6:1606–13. doi:10.4161/cbt.6.10.4742.

[124] Reilly PT, Mak TW. Molecular pathways: Tumor cells Co-opt the brain-specific metabolism gene CPT1C to promote survival. Clin Cancer Res 2012;18:5850–5. doi:10.1158/1078-0432.CCR-11-3281.

[125] Wakamiya T, Suzuki SO, Hamasaki H, Honda H, Mizoguchi M, Yoshimoto K, et al. Elevated expression of fatty acid synthase and nuclear localization of carnitine palmitoyltransferase 1C are common among human gliomas. Neuropathology 2014;34:465–74. doi:10.1111/neup.12132.

[126] Jones RG, Plas DR, Kubek S, Buzzai M, Mu J, Xu Y, et al. AMP-activated protein kinase induces a p53-dependent metabolic checkpoint. Mol Cell 2005;18:283–93. doi:10.1016/j.molcel.2005.03.027.

[127] Wu Y, Sarkissyan M, Mcghee @bullet Eva, Lee S, Vadgama J V. Combined inhibition of glycolysis and AMPK induces synergistic breast cancer cell killing n.d.

[128] Maeda O, Ando T, Ohmiya N, Ishiguro K, Watanabe O, Miyahara R, et al. Alteration of gene expression and DNA methylation in drug-resistant gastric cancer. Oncol Rep 2014;31:1883–90. doi:10.3892/or.2014.3014.

[129] Ceccarelli SM, Chomienne O, Gubler M, Arduini A. Carnitine palmitoyltransferase (CPT) modulators: A medicinal chemistry perspective on 35 years of research. J Med Chem 2011;54:3109–52. doi:10.1021/jm100809g.

[130] Pike LS, Smift AL, Croteau NJ, Ferrick DA, Wu M. Inhibition of fatty acid oxidation by etomoxir impairs NADPH production and increases reactive oxygen species resulting in ATP depletion and cell death in human glioblastoma cells. Biochim Biophys Acta - Bioenerg 2011;1807:726–31. doi:10.1016/j.bbabio.2010.10.022.

[131] Schujman GE, Altabe S, de Mendoza D. A malonyl-CoA-dependent switch in the bacterial response to a dysfunction of lipid metabolism. Mol Microbiol 2008;68:987–96. doi:10.1111/j.1365-2958.2008.06202.x.

[132] Schujman GE, Guerin M, Buschiazzo A, Schaeffer F, Llarrull LI, Reh G, et al. Structural basis of lipid biosynthesis regulation in Gram-positive bacteria. EMBO J 2006;25:4074–83. doi:10.1038/sj.emboj.7601284.

Figure legends

Fig. 1. Carnitine acyltranferases. The carnitine palmitoyltransferases (CPTs) CPT1 and CPT2, carnitine acetyltransferase (CrAT) and carnitine octanoyltransferase (COT) catalyze the reversible transesterification of acyl-CoA esters and carnitine to form acylcarnitine esters and coenzyme A. CPT1A and CPT1B are located in the outer mitochondrial membrane and are specific for long-chain fatty acids (LCFA). Peroxisomal COT is specific for medium-chain FA, and CrAT (located in the mitochondria, peroxisomes and the ER) is specific for short-chain FA. COT and CrAT are soluble enzymes located in the lumen of the above mentioned organelles. The acylcarnitines formed are transported through membranes by specific carnitine-acylcarnitine translocases and finally, in the mitochondria, they undergo β-oxidation. Malonyl-CoA, usually derived from glucose metabolism and the product of the first committed step in the FA biosynthetic pathway, regulates FAO by inhibiting CPT1A and CPT1B. This makes CPT1A and CPT1B the gate-keepers in mitochondrial FAO. CPT1C is located in the ER membrane and has minimal activity.

Fig 2. CPT1A and CPT1C structures. CPT1 proteins have a short N-terminal domain and a long C-terminal domain separated by two transmembrane domains and a short connecting loop. The C-terminal domain encompasses the catalytic core and the interaction with malonyl-CoA. A) The N-terminal domain of CPT1A is mobile and switches between the Nα (malonyl-CoA sensitive) and the Nβ (malonyl-CoA insensitive) conformations. CPT1A can form oligomeric complexes (hexamers and trimers) that may be involved in its sensitivity to malonyl-CoA [16,17]. B) CPT1C N-terminal domain is always in the Nα conformation. The C-terminal domain of CPT1C is around 30 residues longer that the other isoforms.

Fig. 3. CPT1C roles at the molecular, cellular, and physiological levels. At the molecular level, CPT1C is located in the ER and is able to bind malonyl-CoA. At the cellular level, CPT1C is involved in various lipid metabolic pathways and the redox homeostasis system. At the physiological level, CPT1C is involved in several brain functions.

Fig. 4. CPT1C involvement in the control of food intake by ghrelin and leptin. When the AMPK/ACC pathway is activated and malonyl-CoA expression decreases, CPT1C induces a transitory increase in ceramide levels that regulate the expression of the transcription factor BSX, which triggers an increase in the orexigenic neuropeptides agouti-related protein (AgRP) and neuropeptide Y (NPY).

Fig. 5. CPT1C involvement in the regulation of peripheral metabolism. CPT1C KO mice fed a HFD show reduced FAO in muscle and liver and impaired glucose homeostasis, resulting in an obese phenotype with insulin resistance.

Fig. 6. CPT1C and cognition. Correlation between CPT1C deficiency, immature filopodia in hippocampal neurons, and impaired cognition (left). Requirement of CPT1C expression for efficient spine maturation, alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor (AMPAR) subunits GluA1 and GluA2 synthesis, GluA1 trafficking to the plasma membrane, and enhanced cognition (right). CPT1C is not involved in N-methyl-D-aspartate receptor (NMDAR) synthesis.

Fig. 7. Pathogenic mechanisms involved in hereditary spastic paraplegia caused by *Cpt1c* human mutation: ER morphogenesis (throughout interaction with atlastin-1 and protrudin) and LD formation.

Fig. 8. Regulators of CPT1C expression in cancer cells**.** Glucose deprivation induces metabolic stress and activation of AMPK. CPT1C expression might be activated via the AMPK/PGC-1β pathway and in a p53-dependent manner, thus contributing to metabolic adaptation and cancer cell survival. cAMP response element-binding protein (CREB); Peroxisome proliferator-activated receptor gamma, coactivator 1 beta (PGC-1β), Estrogen receptor-related receptor alpha (ERRα); Peroxisome proliferator-activated receptor alfa (PPARα).

Table 1

Regions in the nervous system that express CPT1C	**Regulators of CPT1C expression**
Hippocampus Cortex Hypothalamic nuclei Amygdala Cerebellum Striatum Motor neurons Dorsal ganglia	Postnatal development Glucose depletion Palmitate Hypoxia Ionizing radiation AMPK P53

Fig. 1

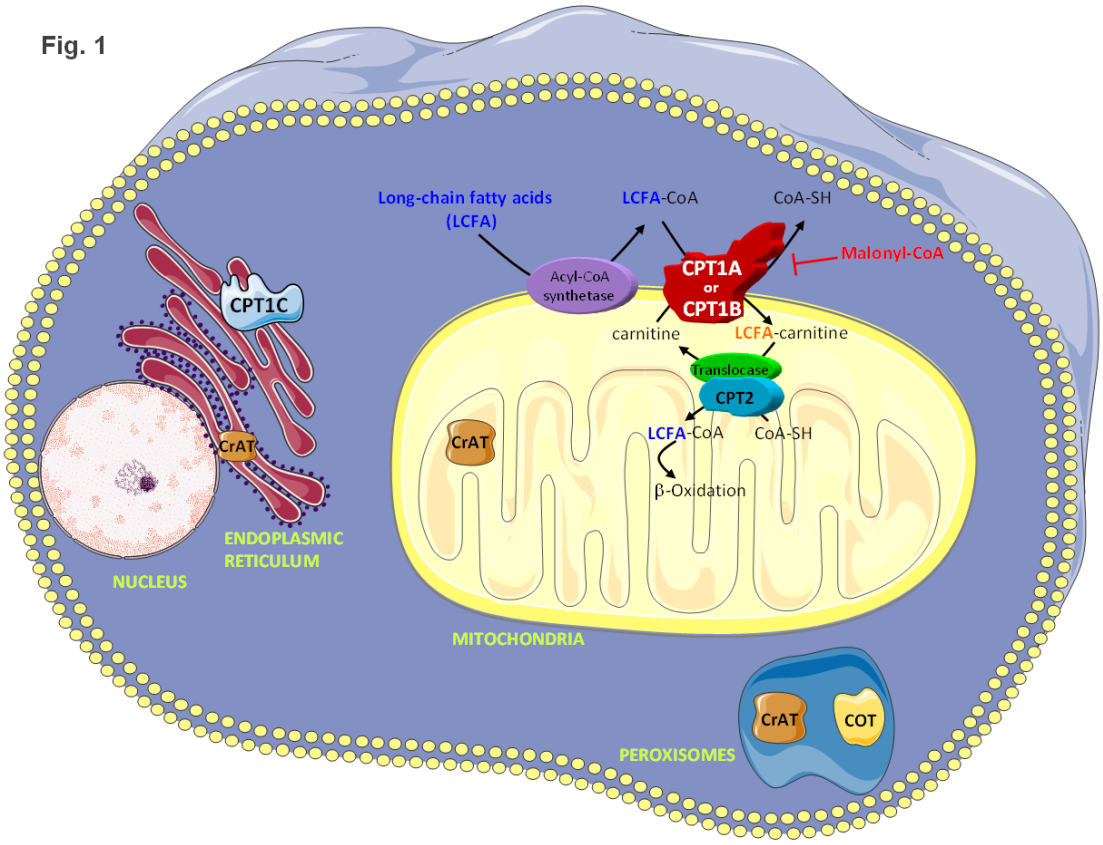

Long-chain fatty acids
(LCFA)
LCFA-CoA
CoA-SH
Malonyl-CoA
Acyl-CoA
synthetase
CPT1A
or
CPT1B
CPT1C
carnitine
LCFA-carnitine
Translocase
CPT2
CrAT
LCFA-CoA
CoA-SH
β-Oxidation
CrAT
ENDOPLASMIC
RETICULUM
NUCLEUS
MITOCHONDRIA
CrAT
COT
PEROXISOMES

Fig. 2

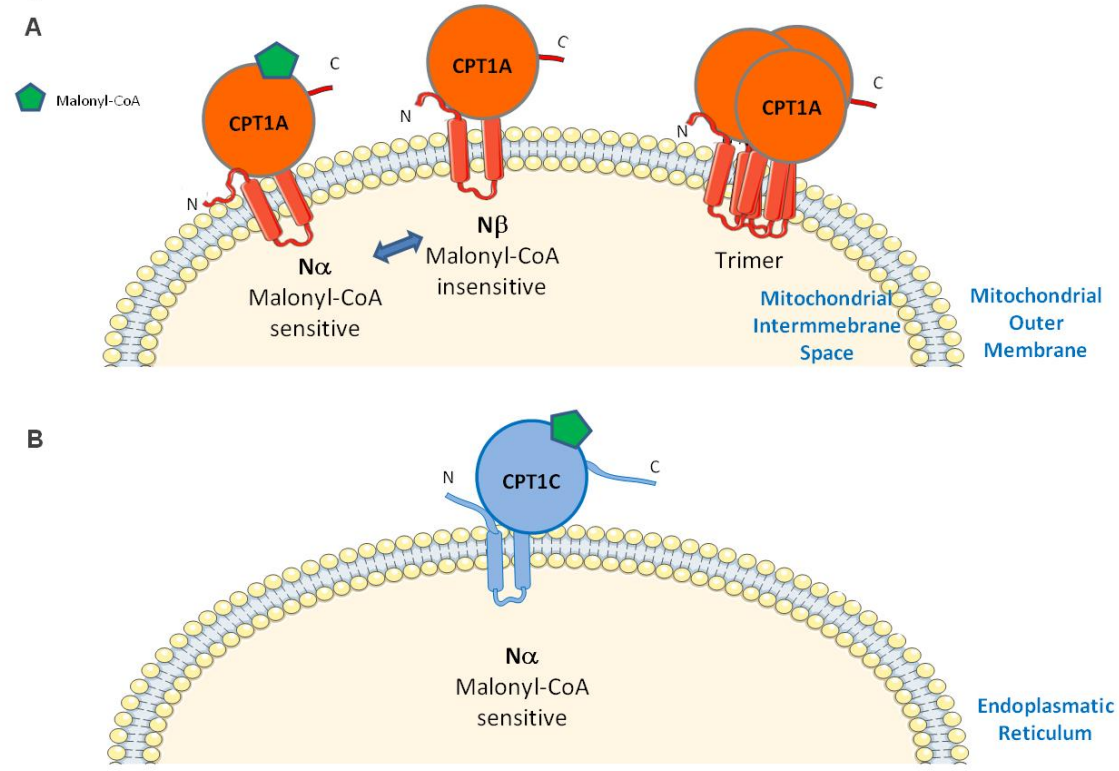

A
Malonyl-CoA
CPT1A
C
N
CPT1A
C
N
CPT1A
C
N
Nβ
Malonyl-CoA
insensitive
Nα
Malonyl-CoA
sensitive
Trimer
Mitochondrial
Intermmebrane
Space
Mitochondrial
Outer
Membrane
B
CPT1C
N
C
Nα
Malonyl-CoA
sensitive
Endoplasmatic
Reticulum

Fig. 3

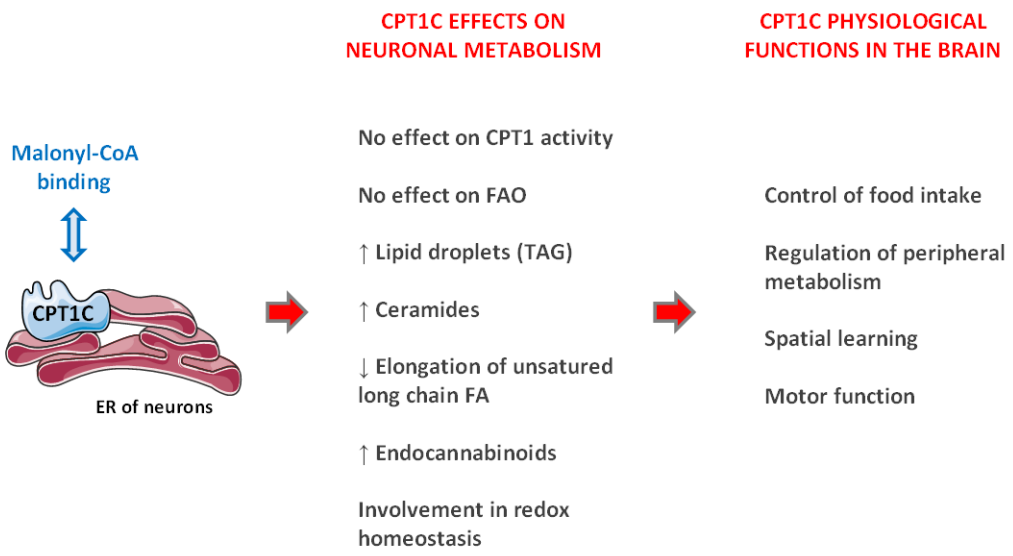

CPT1C EFFECTS ON NEURONAL METABOLISM
CPT1C PHYSIOLOGICAL FUNCTIONS IN THE BRAIN
Malonyl-CoA binding
CPT1C
ER of neurons
No effect on CPT1 activity
No effect on FAO
↑ Lipid droplets (TAG)
↑ Ceramides
↓ Elongation of unsatured long chain FA
↑ Endocannabinoids
Involvement in redox homeostasis
Control of food intake
Regulation of peripheral metabolism
Spatial learning
Motor function

Fig. 4

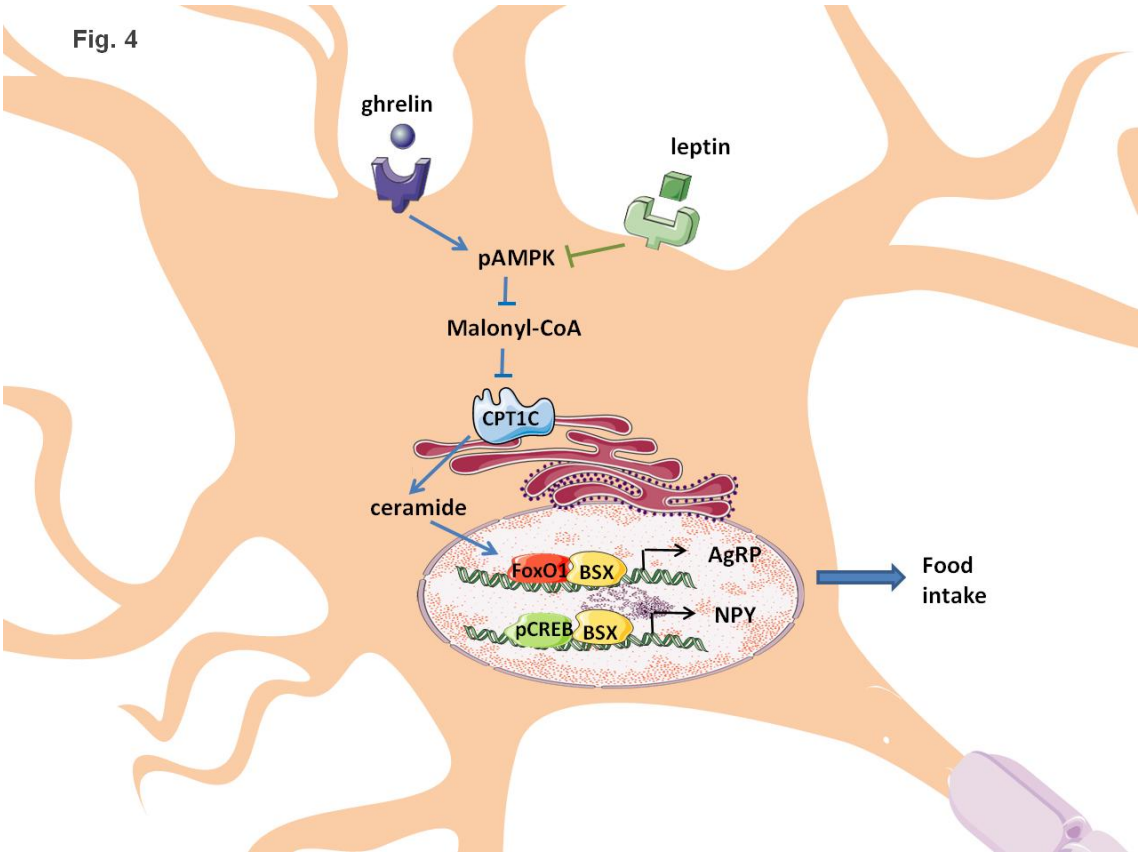

ghrelin
leptin
pAMPK
Malonyl-CoA
CPT1C
ceramide
FoxO1
BSX
AgRP
pCREB
BSX
NPY
Food
intake

Fig. 5

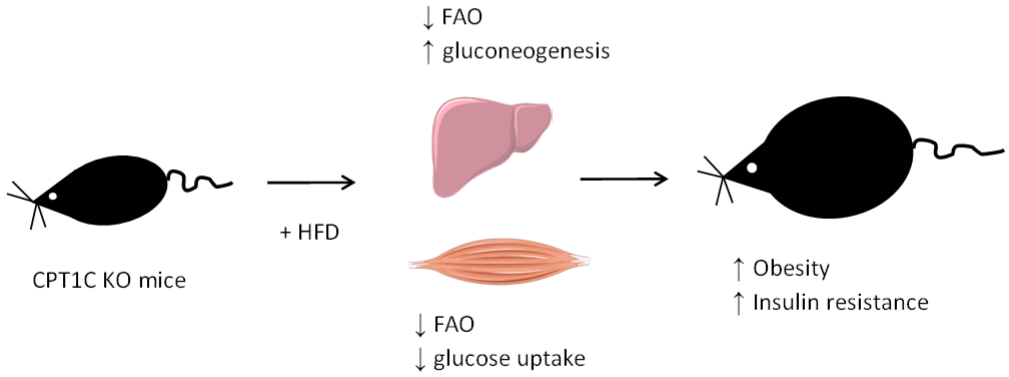

↓ FAO
↑ gluconeogenesis
+ HFD
CPT1C KO mice
↓ FAO
↓ glucose uptake
↑ Obesity
↑ Insulin resistance

Fig. 6

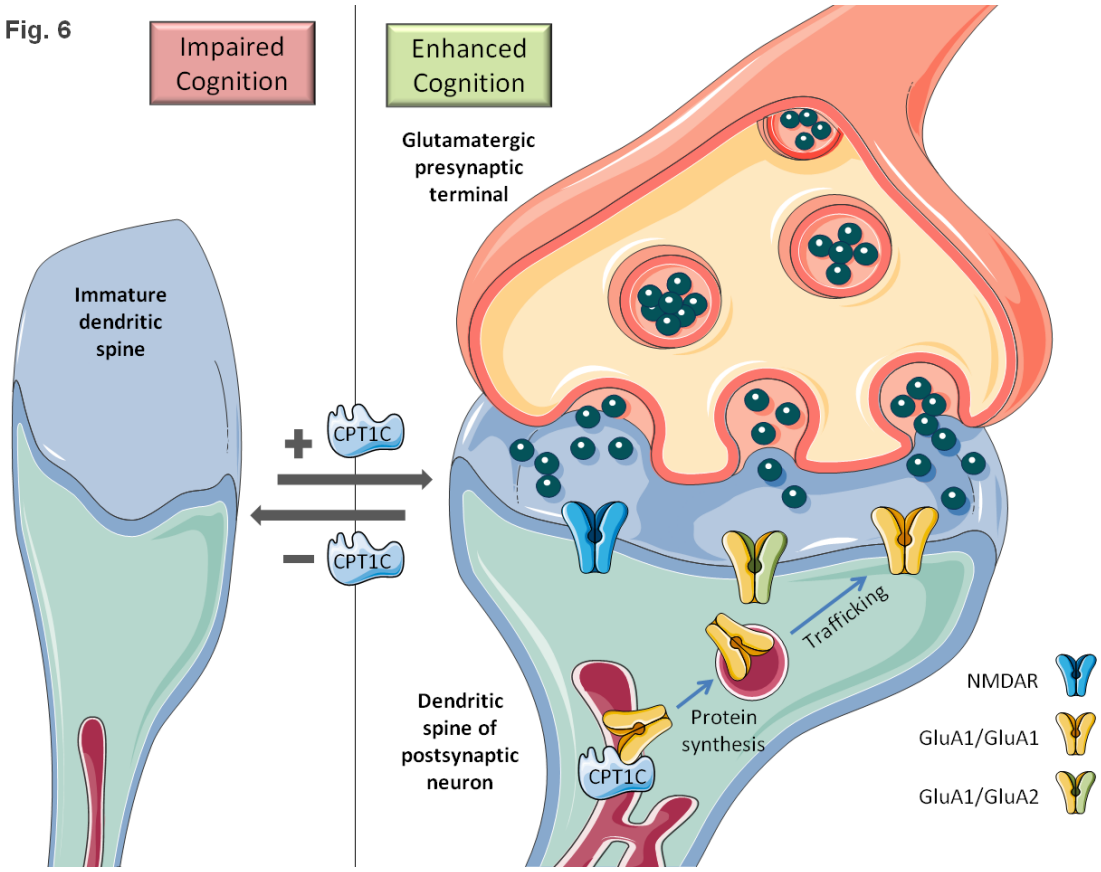

Impaired Cognition
Enhanced Cognition
Glutamatergic presynaptic terminal
Immature dendritic spine
CPT1C
CPT1C
Trafficking
Protein synthesis
Dendritic spine of postsynaptic neuron
CPT1C
NMDAR
GluA1/GluA1
GluA1/GluA2

Fig. 7

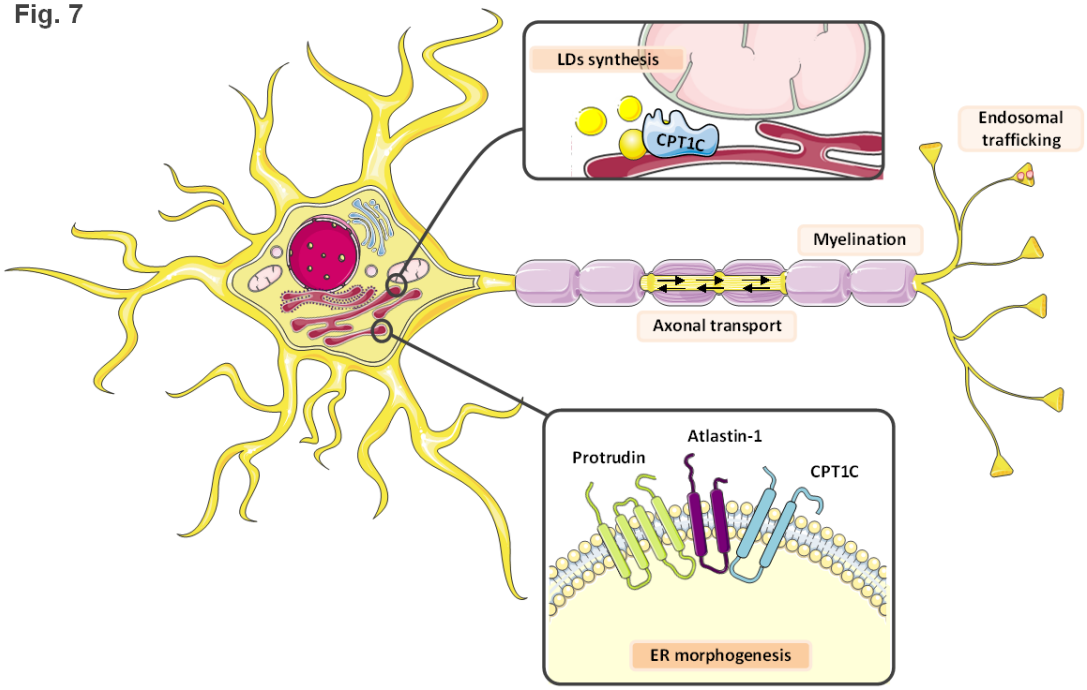
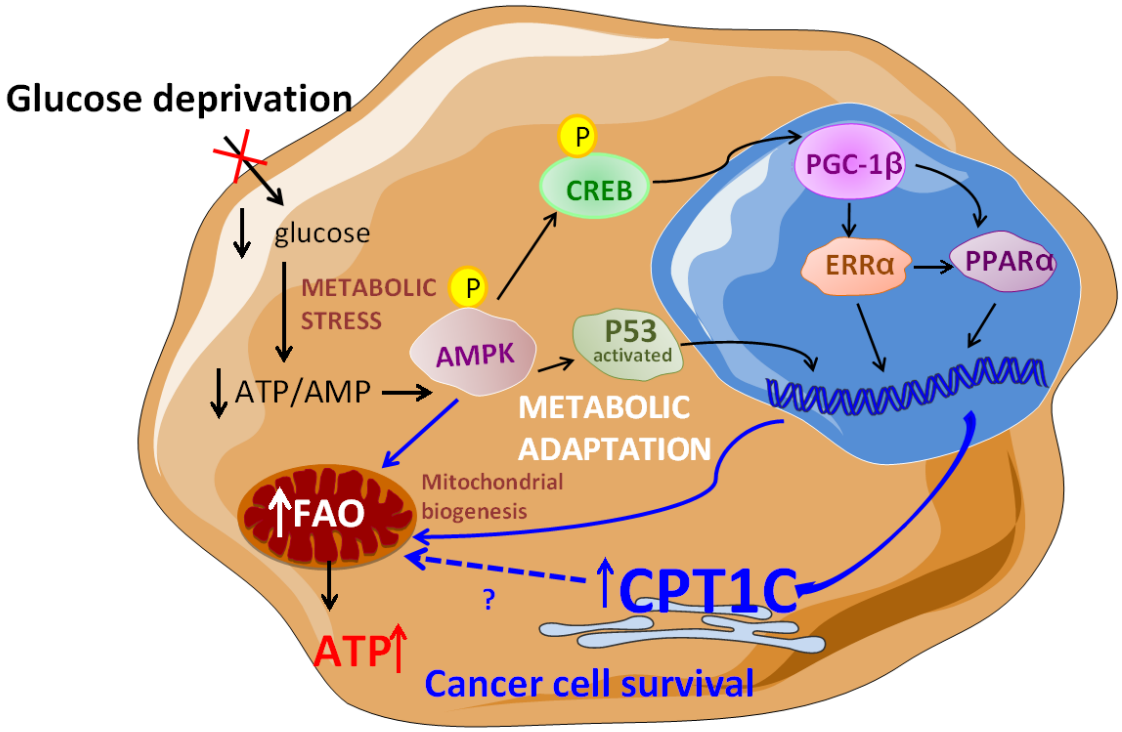

LDs synthesis
CPT1C
Endosomal trafficking
Myelination
Axonal transport
Atlastin-1
Protrudin
CPT1C
ER morphogenesis

Fig. 8


Glucose deprivation
glucose
METABOLIC STRESS
ATP/AMP
P
AMPK
CREB
P
P53
activated
PGC-1β
ERRα
PPARα
METABOLIC ADAPTATION
Mitochondrial biogenesis
FAO
CPT1C
?
ATP
Cancer cell survival