Fundació
La Marató de TV3
22nd SYMPOSIUM
Diabetes and Obesity

CARNITINE PALMITOYLTRANSFERASE 1-MEDIATED STRATEGIES TO ACTIVATE BROWN FAT AND REDUCE FOOD INTAKE TO FIGHT AGAINST OBESITY

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1. Abstract

Obesity is characterized by a dysregulation between food intake and energy expenditure. Brown adipose tissue (BAT) and ventromedial hypothalamus (VMH) have emerged as important regulators of energy expenditure in obesity through the control of thermogenesis and food intake, respectively. Interestingly, BAT thermogenesis is reduced in human obesity and diabetes. Carnitine palmitoyltransferase 1A (CPT1A) and CPT1C have been involved in the control of energy homeostasis. While CPT1A is the key enzyme in fatty acid oxidation (FAO), CPT1C has minimal catalytic activity, and their role in the control of BAT thermogenesis is still unknown. Our main objective was to find new approaches to reduce obesity through the modulation of CPT1 enzymes in BAT or in VMH by: a) peripheral enhancement of BAT FAO and b) central reduction of food intake and activation of BAT thermogenesis.

Our results showed that the increase in FAO by the overexpression of the permanently active mutant form of CPT1A (CPT1AM) into the interscapular BAT of NCD or HFD-treated mice was able to reduce body weight and blood glucose levels compared to HFD control mice. At the central level, we have observed that: a) CPT1A deletion specifically in AgRP neurons of the hypothalamus of mice results in a lean phenotype with an increased thermogenesis and a reduced body weight and food intake, and 2) CPT1C might be a sensor of malonyl-CoA levels in VMH activating satiety and BAT thermogenesis. Furthermore, we have found a new product (UB-207) that reduces food intake. Altogether, we conclude that the approaches explored in this project could be new strategies to combat obesity.

2. Results

Peripheral enhancement of BAT FAO

To enhance BAT thermogenic power through an increase in its FAO we expressed, for the first time *in vivo*, the permanently active mutant form of the FAO limiting enzyme, CPT1AM. In collaboration with Dr. Fàtima Bosch (UAB) we generated AAVs carrying the enzyme CPT1AM under the UCP1 promoter to drive the expression specifically to BAT (AAV8-UCP1-CPT1AM-WPRE). We have also produced the control AAVs (AAV8-UCP1-Null-WPRE). These AAVs have been injected intradepot into the interscapular BAT of

NCD or HFD-treated mice. Importantly, CPT1AM-expressing mice under HFD improved the obese phenotype and reduced body weight and blood glucose levels compared to HFD control mice. No significant differences were seen in the interscapular temperature measured with the thermographic camera. These results suggest that an enhancement of BAT's fat-burning power could be a good strategy to fight against obesity. Some of these results have been published in Advanced Science (2017) and the others are being currently prepared for publication.

Central reduction of food intake and activation of BAT thermogenesis

To demonstrate the role of CPT1A and CPT1C in the activation of satiety and BAT thermogenesis and to validate them as potential therapeutic targets, we have performed three strategies: 1) we generated a mouse model with a CPT1A deletion specifically in AgRP neurons of the hypothalamus and analyzed its phenotype. 2) We studied the role of CPT1C as a sensor of malonyl-CoA in the VMH in the activation of satiety and BAT thermogenesis by using VMH-specific CPT1A KO mice and CPT1C KO mice. 3) We designed and synthesized new C75 and etomoxir analogs to test *in vitro* and *in vivo* the inhibitory effect on CPT1A activity to promote satiety and BAT thermogenesis.

We have found that: 1) The CPT1A deletion in AgRP neurons of the hypothalamus results in a lean phenotype. Both male and females showed reduced body weight, an enhanced BAT thermogenesis and reduced adipose depots. While males showed a reduction in food intake, females did not show any change suggesting a sexual dimorphism. All these results confirm that central CPT1A could be a therapeutic target to combat obesity. Some of these results have been published in Molecular Neurobiology (2018) and a review in Biochemical Pharmacology (2018) and others are being currently prepared for publication. 2) We have demonstrated that CPT1C acts as a malonyl-CoA sensor in the VMN of the hypothalamus activating thermogenesis (published in Molecular Metabolism (2019)). In addition we have also discerned the molecular mechanisms that involve CPT1C in the control of BAT thermogenesis and it has been reported in the Journal of Lipid Research (2019) and British Journal Pharmacology (2021). 3) We have synthesized and analysed six new products derivatives from etomoxir and all of them showed less CPT1A inhibitory effect than etomoxir. However, we found that two products derived from C75 (UB183 and UB207) showed higher CPT1A inhibitory effect than C75. *In vivo* analysis of the ICV injection of

UB207 showed a reduction in food intake. Further analysis are necessary to examine their applicability as future anti-obesity drugs.

3. Relevance and future applications

We have confirmed *in vivo* in mice that the activation of BAT fat-burning power by CPT1AM overexpression could be a new strategy to fight against obesity. At the central level in mice, we have demonstrated that CPT1A and CPT1C in the hypothalamus could be new potential targets to activate thermogenesis and to reduce food intake and body weight. In addition, the UB207 product has been tested in mice with an important effect on the reduction of food intake. If the future toxicity experiments are positive, this product could be an interesting new anti-obesity drug with a potential application to human therapy in the treatment of obesity and overweight.

4. Publications

1. Calderon-Dominguez M, Alcalá M, Sebastián D, Zorzano A, Viana M, Serra D, Herrero L
Brown adipose tissue bioenergetics: a new methodological approach
Advanced Science, 2017. 4(4): 1600274. IF = 9.034. Q1.
Published in 37 media. The work received the Ángel Herrera Award (XXI Edition) to the best research work in Experimental sciences. Fundación Universitaria San Pablo CEU. Journal's cover selected.

2. Mir JF, Zagmutt S, Lichtenstein MP, García-Villoria J, Weber M, Gracia A, Fabriàs G, Casas J, López M, Casals N, Ribes A, Suñol C, Herrero L, Serra D
Ghrelin causes a decline in GABA release by reducing fatty acid oxidation in cortex
Molecular Neurobiology, 2018. 55:7216-28. IF=5.076. Q1

3. Zagmutt S, Mera P, Soler-Vázquez MC, Herrero L, Serra D
Targeting AgRP neurons to maintain energy balance: lessons from animal models
Biochemical Pharmacology, 2018. 155: 224-32. Review. IF=4.235. Q1

4. Rodríguez-Rodríguez R*, Miralpeix C, Fosch A, Pozo M, Calderón-Domínguez, M, Perpinyà X, Vellvehí M, López M, Herrero L, Serra D, Casals N* (co-corresponding authors)
CPT1C in the ventromedial nucleus of the hypothalamus is necessary for brown fat thermogenesis activation in obesity
Molecular Metabolism, 2018. pii: S2212-8778(18)30935-9. IF=6.291. Q1

5. Alcalá M, Calderon-Dominguez M, Serra D*, Herrero L*, Viana M* (* co-corresponding authors)
Mechanisms of impaired brown adipose tissue recruitment in obesity
Frontiers in Physiology-Integrative Physiology, 2019. 10:94. Review. IF=3.394. Q1

6. Miralpeix C, Fosch A, Casas J, Baena M, Herrero L, Serra D, Rodríguez-Rodríguez R*, Casals N* (*Co-corresponding authors)
Hypothalamic endocannabinoids inversely correlate with the development of diet-induced obesity in male and female mice
Journal of Lipid Research, 2019. 60:1260-69. IF=4.505. Q1

7. Bastías-Pérez M, Zagmutt S, Soler-Vázquez MC, Serra D*, Mera P*, Herrero L* (*co-corresponding authors)
Impact of adaptive thermogenesis in mice on the treatment of obesity
Cells, 2020. 9, 316; Review. IF=4.366. Q2

8. Alcalá M, Herrero L, Serra D, Viana M*
Brown adipose tissue in obesity and diabetes.
e-book: Faintuch J., Faintuch S. (eds) Obesity and Diabetes. Springer, Cham.
Springer Nature. 2020, 35-54. https://doi.org/10.1007/978-3-030-53370-0_4

9. Miralpeix C, Reguera C, Fosch A, Casas M, Lillo J, Navarro G, Franco G, Casas J, Alexander SPH, Casals N*, Rodríguez-Rodríguez R*
CPT1C negatively regulates the endocannabinoid hydrolase ABHD6 depending on nutritional status. **Br J Pharmacol. 2021**. In press. IF=7.730. D1

PHD Theses

1. Sebastián Zagmutt. 2020
Role of hypothalamic CPT1A in the control of food intake in obesity. Excellent Cum Laude. Universitat de Barcelona
Directors: Dolors Serra and Laura Herrero

2. Cristina Miralpeix Monclus. 2019
CPT1C and encocannabinoids as hypothalamic players in early stages of obesity development. Excellent cum laude. Universitat Internacional de Catalunya
Directors: Núria Casals and Rosalía Rodríguez

3. Maria Casas Prat. 2019
CPT1C-dependent regulation of GluA1 trafficking under metabolic stress. International mention. Universitat Internacional de Catalunya
Directors: Núria Casals and Rut Fadó

4. Marta Palomo Guerrero. 2018
Papel de CPt1C en el desarrollo axonal y en el transporte de los endosomes tardíos. International mention. Universitat Internacional de Catalunya.
Director: Núria Casals