CB2 stimulation inhibits fat storage in lipid droplets and induces adipocyte browning

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The primary function of adipocytes (ADPs) that forms the white adipose tissue (WAT) is to control the energy balance by storing triacylglycerol in lipid droplets (LD) in energy excess state and mobilizing it during energy deprivation. Perilipin-1 is one of the ADPs-specific LD-associated proteins that allow this mechanism of triacylglycerol storage and mobilization. During adipogenesis and in a state of energetic overload, perilipin expression is enhanced by PPARγ, resulting in LD formation, growth and fusion in large unilocular vessels. Perilipin depletion, or genetic ablation of the perilipin encoding for gene, impairs LD growth (Rexford et al, 2006).

Adipose tissue is not exclusively white. A brown one (BAT) is present in newborn and small mammals and acts as a thermogenic tissue. Indeed, brown ADPs are able to rapidly produce large amounts of heat through activation of uncoupling protein (UCP) 1. In WAT exist a kind of cells that share with white ADPs the cellular precursors but can be induced into brown ADPs. These cells, referred to as beige or brite ADPs, express low basal levels of UCP1, that allow them to act as simply energy storage or to activate a thermogenic program in response to different stimuli, further recruiting UCP1. Obesity is known to decrease beige ADP number and UCP1 basal levels (Kristy Townsend et al, 2012).

The endocannabinoid (EC) system plays a crucial role in regulating food intake and energy metabolism. The EC system comprises two metabotropic receptors, CB1 and CB2, their endogenous ligands and the enzymes for the EC metabolism (Di Marzo et al, 2005).

It has been shown that the CB2 Q63R functional variant is associated with eating disorders in human and that CB2 ligands modulate food intake in mice (Ishiguro et al, 2010).

In order to evaluate the influence of CB2 in the modulation of human body fat mass *in vivo* and the effect of CB2 pharmacological manipulation on ADP activity and morphology *in vitro*, we conduct a case-control association analysis of the Q63R CB2 variant in a cohort of 548 obese Italian children and adolescent versus a cohort 600 Italian healthy children and performed molecular and biochemical study on *in vitro* ADPs differentiated from stem cells of healthy donors or derived from subcutaneous adipose tissue of non-obese, obese and weight-loss subjects, treated or not with drugs selectively acting on CB2 receptor.

We find that the less-functional CB2 R63 variant is significantly associated with a higher z-score BMI and that, accordingly, the pharmacological blockade of the CB2 receptor in lean subjects-derived ADPs with AM630 leads to the formation of unilocular LDs, characteristic of the obese state, together with a lower perilipin and PPARγ expression. We also show that all the obesity-related effects on LDs number and size, adipocytic perilipin amount and adipocytic PPARγ expression are reverted by weight-loss or by the pharmacological stimulation of CB2 receptor with JWH-133.

Moreover, we find that obese-derived ADPs express significant lower levels of UCP1 mRNA and that the pharmacological treatment with the CB2 agonist JWH-133 significantly increases UCP1 expression.

Accordingly to the pharmacological effect of CB2 modulation on UCP1, we find that the ADPs derived from obese patients homozygous for the more functional variant, Q63, express significantly lower levels of perilipin and higher levels of UCP1 with respect to RR-derived ADPs.

Collectively, these data suggest the CB2 receptor as a new molecular determinant of LD growth and unilocular LD formation, possibly through the modulation of the PPARγ-mediated perilipin expression and then as a new pharmacological target for the modulation of WAT ADPs browning, through the modulation of beige ADPs UCP1 levels.

Di Marzo et al. (2005) *Nat Neurosci.* 8,585-589.
Ishiguro et al. (2010) *Synapse*. 64, 92-96.
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