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Stimulation of mitochondrial oxidative capacity in white fat independent of UCP1: A key to lean phenotype☆

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ABSTRACT

We are facing a revival of the strategy to counteract obesity and associated metabolic disorders by inducing thermogenesis mediated by mitochondrial uncoupling protein-1 (UCP1). Thus, the main focus is on the adaptive non-shivering thermogenesis occurring both in the typical depots of brown adipose tissue (BAT) and in UCP1-containing cells that could be induced in white adipose tissue (WAT). Because contribution of WAT to resting metabolic rate is relatively small, the possibility to reduce adiposity by enhancing energy expenditure in classical white adipocytes is largely neglected. However, several pieces of evidence support a notion that induction of energy expenditure based on oxidation of fatty acids (FA) in WAT may be beneficial for health, namely: (i) studies in both humans and rodents document negative association between oxidative capacity of mitochondria in WAT and obesity; (ii) pharmacological activation of AMPK in rats as well as cold-acclimation of UCP1-ablated mice results in obesity resistance associated with increased oxidative capacity in WAT; and (iii) combined intervention using long-chain *n*-3 polyunsaturated FA (omega 3) and mild calorie restriction exerted synergism in the prevention of obesity in mice fed a high-fat diet; this was associated with strong hypolipidemic and insulin-sensitizing effects, as well as prevention of inflammation, and synergistic induction of mitochondrial oxidative phosphorylation (OXPHOS) and FA oxidation, specifically in epididymal WAT. Importantly, these changes occurred without induction of UCP1 and suggested the involvement of: (i) futile substrate cycle in white adipocytes, which is based on lipolysis of intracellular triacylglycerols and re-esterification of FA, in association with the induction of mitochondrial OXPHOS capacity, β-oxidation, and energy expenditure; (ii) endogenous lipid mediators (namely endocannabinoids, eicosanoids, prostanoids, resolvins, and protectins) and their cognate receptors; and (iii) AMP-activated protein kinase in WAT. Quantitatively, the strong induction of FA oxidation in WAT in response to the combined intervention is similar to that observed in the transgenic mice rendered resistant to obesity by ectopic expression of UCP1 in WAT. The induction of UCP1-independent FA oxidation and energy expenditure in WAT in response to the above physiological stimuli could underlie the amelioration of obesity and low-grade WAT inflammation, and it could reduce the release of FA from adipose tissue and counteract harmful consequences of lipid accumulation in other tissues. In this respect, new combination treatments may be designed using naturally occurring micronutrients (e.g. omega 3), reduced calorie intake or pharmaceuticals, exerting synergism in the induction of the mitochondrial OXPHOS capacity and stimulation of lipid catabolism in white adipocytes, and improving metabolic flexibility of WAT. The role of mutual interactions between adipocytes and immune cells contained in WAT in tissue metabolism should be better characterised. This article is part of a Special Issue entitled Brown and White Fat: From Signaling to Disease.

Abbreviations: ACC, acetyl-CoA carboxylase; AMPK, AMP-activated protein kinase; aP2-*Ucp1* mice, transgenic mice expressing UCP1 gene from the aP2 gene promoter; AA, arachidonic acid; ATM, adipose tissue macrophages; BAT, brown adipose tissue; BCAA, branched-chain amino acids; CB1, cannabinoid type-1 receptor; CR, calorie restriction; CTRP3, adiponectin paralog C1q/TNF-related protein 3; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; FAT/CD36, fatty acid translocase/CD36; FA, fatty acids; FAS, fatty acid synthase; FGF-21, fibroblast growth factor-21; HF, high-fat; HSL, hormone-sensitive lipase; LPS, lipopolysaccharide; omega 3, long-chain *n*-3 polyunsaturated fatty acids; mtDNA, mitochondrial DNA; MCP-1, monocyte chemoattractant protein-1; OXPHOS, oxidative phosphorylation; PC, pyruvate carboxylase; PDK4, pyruvate dehydrogenase kinase 4; PEPCK, phosphoenolpyruvate carboxykinase; PD1, protectin D1; PGC-1, the PPARγ coactivator 1; PPAR, peroxisome proliferator-activated receptor; SIRT1, NAD^+-dependent deacetylase sirtuin 1; SREBP-1c, sterol regulatory element-binding protein-1c; TAG, triacylglycerols; TAG/FA cycle, futile substrate cycle based on lipolysis of intracellular triacylglycerols and re-esterification of fatty acids; TZD, thiazolidinedione; UCP1, mitochondrial uncoupling protein 1; WAT, white adipose tissue; 15d-PGJ2, 15-deoxy-Δ12,14-prostaglandin J2

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1. Introduction

White adipose tissue (**WAT**), the most plastic among the metabolically relevant tissues in the body, is essential for storing metabolic energy, and through its endocrine functions, it is also involved in the regulation of glucose as well as energy homeostasis. WAT has also a major role in the control of systemic fatty acids' (**FA**) levels. Accordingly, both hypertrophy and atrophy [1] of WAT are associated with lipotoxic damage of insulin signalling in other tissues. The key role of WAT in glucose homeostasis [2,3] is supported by the recent experimental evidence of anti-diabetic effects of WAT-specific up-regulation of peroxisome proliferator-activated receptor-γ (**PPARγ**; [4]). Concerning energy homeostasis, the role of WAT metabolism is usually neglected. However, it is not insignificant. Thus, the contribution of WAT to resting metabolic rate in lean human subjects is close to 5% and it doubles in obesity (reviewed in [5]), while in adult mice reared at 20 °C the total oxidative capacity of WAT represents ~30–50% of that in brown adipose tissue (**BAT**; [6]).

In both humans [7] and rodents [8], oxygen consumption in WAT cells declines with age and it is negatively correlated with obesity in humans [7]. Decreased white fat cell thermogenesis in obese humans was also found using direct microcalorimetry [5]. Accordingly, mitochondrial content in WAT is relatively low in genetically obese and insulin-resistant mice [9–12]. Moreover, in monozygotic twins discordant for obesity, copy number of mitochondrial DNA in subcutaneous WAT was reduced by 47% in obese co-twins, in association with reduced expression of the genes for mitochondrial proteins [13]. In turn, induction of mitochondria and activation of FA oxidation, specifically in WAT, was observed under the conditions promoting loss of adiposity (see Section 2.4). Very recently, it has been reported [14] that systemic nonselective β-adrenergic stimulation in humans that increases energy expenditure to the same extent as cold exposure does not activate brown adipose tissue (**BAT**; see below), and could be explained only in part by the activation of muscle non-shivering thermogenesis (for review on muscle thermogenesis, see [15–17]). These data support the notion that also adrenergically-stimulated energy expenditure in WAT may, though to a relatively small extent, influence total energy balance (see also [16] and Section 2.3).

In contrast to energy expenditure in WAT, major role of mitochondrial uncoupling protein-1 (**UCP1**) in BAT with respect to cold- and diet-induced adrenergically regulated thermogenesis is well appreciated [18–20], although examination of several older studies [15,21] casts some doubts on the unique role of the UCP1-mediated thermogenesis. As described in other articles of this issue, recent discovery of functional BAT in adult humans [22] has led to a revival of the strategy [23–25] to counteract obesity and associated metabolic disorders by inducing UCP1-mediated thermogenesis. In rodents, the existence of several fat cell lineages was uncovered, which underlie the differentiation of precursor cells into: (i) classical multilocular *brown adipocytes*, which are closely related to myocytes, and which are responsible for the bulk of the adaptive UCP1-mediated thermogenesis; (ii) classical unilocular *white adipocytes* lacking UCP1, which are fully competent for ATP synthesis by oxidative phosphorylation (**OXPHOS**; [13,26,27]); and (iii) *brite adipocytes* [28,29] named also beige cells (reviewed in [30]). The last cell type is of special interest, since these cells are interspersed in some depots of WAT, they show high inducibility of the UCP1-linked thermogenic programme, and their abundance correlates negatively with the propensity to obesity while being under genetic control [31]. Besides the sympathetic stimulation, synthetic ligands of **PPARγ** (reviewed in [10,32,33]), as well as several hormonal factors could induce brite cells in rodents ([34–38]; see Section 3.1). However, in spite of the fact that markers discriminating BAT from brite cells have been identified in rodents [28,30,38,39], the existence of brite cells in humans remains to be unequivocally established [30,40], and typical white adipocyte markers also remain to be defined. In fact, inducibility of at least some of the UCP1-containing cells in WAT depots probably reflects transdifferentiation of white adipocytes [41], bringing another level of complexity to the identification of markers characteristic of cell lineages engaged in adipose tissue plasticity.

Energy-dissipating function of UCP1 depends on its protonophoric activity in the inner mitochondrial membrane, which is activated by FA in response to β-adrenergic stimulation and allows for a full unmasking of mitochondrial oxidative capacity without concomitant synthesis of ATP (for review, see [42]). In fact, the content of mitochondrial ATP synthase in classical brown adipocytes is extremely low [43,44], and this feature is observed already at the time of perinatal recruitment of BAT thermogenesis both in rodents [45,46] and humans [47]. Adrenergic stimulation of BAT not only activates UCP1-mediated proton leak but also leads to complex metabolic adaptations (for reviews on BAT metabolism see [20]), including mitochondrial biogenesis, aimed at increasing fuel supply and oxidation. Thus, lipolysis of intracellular triacylglycerols (**TAG**) stores is accelerated and the uptake of FA derived from blood-born lipoproteins is increased due to the action of lipoprotein lipase (**LPL**) that is rapidly recruited during cold exposure [48]. Although glucose is not a major substrate for BAT thermogenesis, glucose utilisation by activated BAT increases dramatically [49] as does lactate production. Glycolysis supplies ATP when its production via OXPHOS is attenuated, and also makes pyruvate available for the synthesis of oxaloacetate, the condensing partner for acetyl-CoA formed from β-oxidation of FA, thus ensuring a continuous supply of citric acid cycle intermediates (anaplerosis; see Section 2.4 and [50]). High expression of the muscle type carnitine palmitoyl transferase-1 (**CPT1**; [51]) allows for rapid β-oxidation of FA [52], while high activity of mitochondrial glycerol-3-phosphate dehydrogenase supports TAG synthesis and controls cytoplasmic NADH levels [53]. Longer exposure to cold (days) leads to increased lipogenesis, which is necessary for sustained thermogenesis. Triiodothyronine locally produced from thyroxine in brown adipocytes [54] amplifies adrenergic stimulation of UCP1 gene transcription [55], helps to ensure an optimal balance between lipolysis and lipogenesis [54], and contributes to systemic triiodothyronine levels [56].

Here, reflecting the well accepted potential of UCP1-mediated thermogenesis to reduce obesity, we provide a complementary information on adipose tissue biology, while focusing on: (i) the possibility to reduce body fat stores by inducing energy expenditure in white adipocytes based on a futile substrate cycle [57], namely lipolysis of intracellular triacylglycerols and FA re-esterification (**TAG/FA cycle**; [5,58,59]), in association with the induction of mitochondrial OXPHOS capacity and β-oxidation; (ii) the role of local mediators in WAT in the regulation of adipocyte metabolism; (iii) the comparison of local/systemic metabolic effects of TAG/FA cycle vs. mitochondrial uncoupling in white adipocytes; and (iv) possible interventions, which could counteract obesity while stimulating activity of TAG/FA cycle, inducing capacity of mitochondrial OXPHOS and enhancing FA oxidation in white adipocytes. We provide a concept, that white adipocyte endowed with high capacity of mitochondrial OXPHOS represents a metabolically flexible healthy adipocyte.

2. Physiology of mitochondria in white adipocytes

In white adipocytes, similarly as in most other cells, mitochondria represent the main site of ATP production. Fully coupled mitochondria could be isolated from adipocytes liberated by the use of collagenase from epididymal WAT of rats [26], and OXPHOS activity could be also demonstrated using respirometry in isolated digitonin-permeabilized murine white adipocytes [27]. Especially in fully differentiated white adipocytes, mitochondria must generate sufficient ATP to support various energy-consuming processes (see Sections 2.2 and 2.3). They are located in the thin cytoplasmic rim of the cells, and they show similar morphology as in other tissues [60]. Mitochondrial content of mature white adipocytes is several-fold higher as compared with