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

Pavel Flachs, Martin Rossmeisl, Ondrej Kuda, Jan Kopecky *

Department of Adipose Tissue Biology, Institute of Physiology Academy of Sciences of the Czech Republic, v.v.i., Prague, Czech Republic

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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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* Corresponding author at: Department of Adipose Tissue Biology, Institute of Physiology of the Academy of Sciences of the Czech Republic, v.v.i., Videnska 1083, 14220 Prague 4, Czech Republic. Tel.: +420 241063701; fax: +420 241062599.

E-mail address: kopecky@biomed.cas.cz (J. Kopecky).

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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

preadipocytes [61], while their content depends on the anatomical location of fat depots. Thus, in rodents, gonadal adipocytes contain more mitochondria and have higher oxidative capacity together with higher content of OXPHOS proteins as compared with adipocytes in subcutaneous (inguinal) depot [62,63]. Similarly, in obese humans, mitochondrial DNA (**mtDNA**) copy number per mg tissue is higher in visceral than in abdominal subcutaneous WAT [64]. Although mitochondrial respiration normalized to cell number or mtDNA is lower in visceral WAT compared to subcutaneous WAT due to smaller adipocytes in the former fat depot, the visceral fat is bioenergetically more active and more sensitive to substrates of electron transport chain than the subcutaneous WAT [64].

It should be stressed that the interpretation of the above data regarding mitochondrial content is not straightforward. Because of the complex nature of mitochondria and their biogenesis, multiple parameters need to be analysed in order to assess mitochondrial content or activity in WAT. The preferred methods of ours are: (i) transmission electron microscopy (e.g. described in [65]); (ii) evaluation of activity of cytochrome c oxidase and cytochrome b content in crude membrane fraction (e.g. used in [27]); (iii) quantification of selected mitochondrial proteins by immunoblotting (e.g. described in [66]); and (iv) measurement of mitochondrial respiration using high-resolution respirometry in digitonin-permeabilized adipocytes [27]. Moreover, mtDNA quantification is frequently used as a marker of mitochondrial content and activity, although each mitochondrion may contain 2–10 copies of mtDNA. The direct relationship between the cychrome c oxidase activity and the mtDNA was found in WAT [62] and in skeletal muscle but not in the liver and other tissues [67].

2.1. Control of mitochondrial biogenesis in adipose tissue

In adipocytes, various properties of mitochondria including the biogenesis, content and specific composition are controlled at different levels. At the transcriptional level, this control involves the key adipogenic transcription factor, PPARγ [68–70], which is predominantly expressed in adipose tissue (namely the PPARγ2 isoform), especially in mature adipocytes [61,71], and in immune cells [72]. However, the dynamic control of biogenesis and respiratory function of mitochondria depends in large on the PPARγ coactivator 1 (**PGC-1**) family of transcriptional regulators, consisting of **PGC-1α**, **PGC-1β** and PGC-1 related coactivator (**PRC**; reviewed in [73]). These coactivators target multiple transcription factors including nuclear respiratory factor 1 (**NRF-1**) and oestrogen-related receptor α, while coordinately inducing mitochondrial biogenesis in all metabolically relevant tissues (reviewed in [73,74]). In addition, (i) PGC-1α interacts with either PPARα [75] or farnesoid X receptor [76] to increase mitochondrial β-oxidation; and (ii) the interaction of PGC-1α with PPARγ is required for the induction of UCP1-mediated thermogenesis in both brown adipocytes and brite cells in response to various signals including thiazolidinedione (**TZD**) antidiabetic drugs [32,33,77], and fibroblast growth factor-21 (**FGF21**; Ref. [34,36]). Importantly, the induction of UCP1-mediated thermogenesis by TZD also requires **PPARα** [78] as well as the presence and accumulation of PRMD16 protein [32], the marker of both brown and brite adipocytes, which is absent in white adipocytes (reviewed in [28,32]). In contrast to the role of PGC-1α in the recruitment of UCP1-mediated thermogenesis, PGC-1β plays a dominant role in the control of general mitochondrial gene expression in white adipocytes [33]. Accordingly, the expression of PGC-1β but not PGC-1α increased along with the in vitro differentiation of preadipocytes isolated from subcutaneous WAT of the rat [79]. Also in the liver and skeletal muscle, PGC-1β was required for normal expression of genes encoding mitochondrial proteins, including OXPHOS components [80]. PPARα is the main transcriptional regulator of the lipid oxidation pathway [81]. Activation of PPARs by endogenous ligands, which are generated during lipolysis of TAG in adipocyte, contributes to the induction of mitochondrial biogenesis and lipid catabolism in response to lipolytic signals (see Section 2.3).

Reflecting the indispensable roles of PPARγ and PGC-1 family members in adipocytes, the activity of these factors is modulated by several mechanisms, depending also on the metabolic status. Thus, PPARγ activity is regulated by classical agonists—various FA and their metabolites, TZD drugs as well as by non-TZD agonists, which all bind to PPARγ and could induce its conformational change (reviewed in [68,82,83]). Furthermore, PPARγ activity is modulated by CDK5-mediated phosphorylation at serine 273 in response to obesity-linked pro-inflammatory signals, which prevents the expression of a set of PPARγ targets with anti-obesity effects. Agonist binding to PPARγ interferes with serine 273 phosphorylation [84]. Association of PPARγ with CDK5 is facilitated by nuclear receptor corepressor (**NCoR**), leading to PPARγ phosphorylation and inactivation [85]. These findings: (i) triggered a search for non-agonist PPARγ ligands blocking the serine 273 phosphorylation, which could elicit antidiabetic action (including the induction of adiponectin gene expression) without having side effects of the TZD therapy [86], and (ii) partially elucidated the link between low-grade WAT inflammation in obesity and insulin resistance [87–89]. PPARγ activity is also repressed by phosphorylation at serine 112, as well as by sumoylation at lysine 107, which is, in turn prevented by FGF21 (reviewed in [90,91]). In mice, FGF21 helps to recruit UCP1-mediated thermogenesis (see Section 1 and [34,36]), while PPARγ-FGF21 interaction in white adipocytes is required for the whole-body antidiabetic effects of TZD (see Section 2.5 and [90]). Importantly, the activation of both PPARα and PPARγ also results in potent anti-inflammatory effects, which are mediated by multiple mechanisms, including transrepression of transcription of inflammatory genes (reviewed in [92]; see Sections 2.5 and 4).

The activity of PGC-1 family members is also regulated by post-translational modification elicited by intracellular sensors of energy status such as AMP-activated protein kinase (**AMPK**) and NAD^+-dependent deacetylase sirtuin1 (**SIRT1**; reviewed in [73]). AMPK is a heteromeric protein, with a dominant expression of the α1-subunit isoform in WAT. AMPK activation aims to suppress ATP-consuming processes (such as lipogenesis; see Section 2.2) and stimulate processes leading to the generation of ATP (e.g. β-oxidation, mitochondrial biogenesis, and glucose uptake; reviewed in [93]), while, in analogy with its role in heart [94], it could probably also stimulate uptake of FA by increasing LPL activity in adipocytes. Moreover, by a complex mechanism, AMPK modulates lipolysis of TAG in adipocytes (see Section 2.3).

SIRT1 closely interacts with AMPK [73,95], and by deacetylating multiple lysine residues in PGC-1α and NF-κB, it promotes lipid catabolism and counteracts WAT inflammation (see Section 2.4 and [96,97]). The stimulation of mitochondrial biogenesis in response to AMPK activation probably depends on the phosphorylation of PGC-1 factors on specific serine and threonine residues by NO- and p38 MAPK-related mechanisms, although this mechanism remains somehow controversial (compare [77] and [98]). Furthermore, it is known that AMPK signalling in white adipocytes is activated by leptin and adiponectin [99]. In contrast, mitochondrial biogenesis is down-regulated by endocannabinoids that decrease AMPK activity [98] through G-protein-coupled cannabinoid type-1 (**CB1**) receptor in WAT [100]. By this mechanism, elevated endocannabinoid tonus in obesity may interfere with mitochondrial biogenesis [98,101] and deteriorate the ability of mitochondria to augment oxidative capacity in face of increased lipid supply (see Section 2.4).

2.2. Major role of mitochondria in white adipocytes

Proper functioning of mitochondria in white adipocytes is essential for metabolism of these cells as well as for the role of WAT in the whole organism. In fact, even adipocyte differentiation and maturation may

be synchronized by initiation of de novo mitochondrial biogenesis [102], and mitochondrial production of ROS maybe a causal factor in promoting adipocyte differentiation [103]. In mature white adipocytes, mitochondrial ATP synthesis is essential for major metabolic pathways, i.e. lipolysis, de novo FA synthesis, TAG synthesis, glyceroneogenesis, and FA re-esterification.

WAT is an important site of de novo FA synthesis from glucose, mainly in rodents, however, even in humans WAT may account for up to 40% of whole-body lipogenesis [104]. The key transcription factor regulating lipogenic gene expression and thus lipid synthesis is sterol regulatory element-binding protein-1c (**SREBP-1c**), and the important enzymes are fatty acid synthase (**FAS**) and acetyl-CoA carboxylase (**ACC**). ACC is regulated by AMPK (see Section 2.1) through phosphorylation, which suppresses formation of malonyl-CoA, while promoting FA oxidation in mitochondria, reflecting the release of malonyl-CoA-mediated inhibition of CPT1 (reviewed in [105]). Furthermore, stearoyl-CoA desaturase 1 (**SCD1**) catalyses the synthesis of monounsaturated fatty acids used to build up TAG, while it also controls lipid partitioning between lipogenesis and FA oxidation [106]. In this respect, mitochondrial ATP production is required for lipogenesis, both for de novo FA synthesis as well as for esterification of FA. Thus, the formation of acetyl-CoA for FA synthesis depends on the cleavage of citrate exported from mitochondria into cytosol, where it is cleaved by ATP-citrate lyase to generate acetyl-CoA and oxalacetate. Cytosolic oxalacetate is reduced to malate by NADH, and the malate is subsequently decarboxylated to pyruvate in a reaction that is catalysed by malic enzyme and that also generates NADPH to be used during the synthesis of FA. Pyruvate returns to the mitochondrion, where it can be converted to oxalacetate in a reaction that requires ATP and is catalysed by pyruvate carboxylase (**PC**). This enzyme is a key component of the 'pyruvate cycle' [107–109] and its activity is about 3-fold higher in WAT than in the liver (reviewed in [110]), while it is also indispensable for the thermogenic function of BAT ([50]; see above). Depending on the activity of citric acid cycle, oxalacetate generated by the action of PC can either enter the cycle to increase its activity and regenerate citrate or enter the pathway of gluconeogenesis (in the liver) or glyceroneogenesis (in WAT).

TAG production requires a continuous supply of glycerol-3-phosphate to esterify FA. Glycerol-3-phosphate can be formed from: (i) glucose via glycolytic pathway; (ii) glycerol, which is converted to glycerol-3-phosphate by glycerol kinase; and (iii) precursors other than glucose and glycerol (such as pyruvate, lactate and amino acids), by glyceroneogenesis, which forms phosphoenolpyruvate via the mitochondrial dicarboxylic shuttle and subsequently produces glycerol-3-phosphate by a partial reversion of glycolysis. While glycerol kinase is essential for glycerol-3-phosphate formation in BAT [111], glyceroneogenesis is the dominant pathway for TAG-glycerol synthesis in WAT ([112]; see Section 2.5). The key enzyme of this metabolic process is the cytosolic isoform of phosphoenolpyruvate carboxykinase (**PEPCK**), which catalyses decarboxylation of oxaloacetate to form phosphoenolpyruvate. Another important player in the process of glyceroneogenesis is mitochondrial pyruvate dehydrogenase that functions as a metabolic switch between glucose and FA utilisation. Its inhibition by pyruvate dehydrogenase kinase 4 (**PDK4**) enables pyruvate to be used for glyceroneogenesis when the supply of glucose is low [113].

2.3. TAG/FA cycle in white adipocytes leads to energy expenditure

Lipolysis of TAG in white adipocytes is associated with re-esterification of a part (30–90% at basal state, and 10–20% at stimulated state) of lipolyzed FA back into adipose TAG, i.e. futile TAG/FA cycling ([5,58,59,114,115]; reviewed in [116–119]). As for the first time suggested by Newsholme and Crabtree (see Ref. [120]), substrate cycles could provide a mechanism of variable sensitivity for controlling the flux through a metabolic pathway (see [121]). Also in WAT, an extensive rate of futile FA recycling probably allows for a fine and fast tuning of these opposite metabolic fluxes in response to metabolic demands [122,123], and it is essential for buffering of plasma FA levels [112,113,117]. Thus, TAG/FA cycle in white adipocytes has the key role in metabolic flexibility of adipocytes, as well as the whole organism. In fact, it has been recently suggested that FA liberated from TAG intracellularly during lipolysis are released from the adipocyte and immediately taken back by fatty acid translocase/CD36 (FAT/CD36)-mediated mechanism to impose an additional level of regulation on lipolysis [124].

In isolated white adipocytes, the activity of TAG/FA cycle is stimulated by all lipolytic agents (reviewed in [125,126]), like β-adrenergic agonists, ACTH, TSH and glucagon, while insulin and triidothyronine have no effect [127]. Injection of β-adrenergic agonist in mice could increase activity of TAG/FA cycle 5-fold in parametrial WAT and 3-fold in interscapular BAT [128]. In accordance with its control by adrenergic system activity, TAG/FA cycle is increased in response to fasting in rats [114], while cold exposure of mice had no effect on TAG/FA cycle activity in WAT, but increased this activity 2-fold in the BAT [128]. Importantly, the rate of TAG/FA cycling in WAT was also increased, when 24-hour-starved mice had eaten for 1 h, as a reflection of increased sympathetic stimulation of the tissue under these conditions [128].

There must be a balance between the rates of lipolysis and the generation of glycerol-3-phosphate in order to support FA re-esterification (see Section 2.1). Thus, under physiological conditions such as short-term fasting, there is a striking correlation between lipolysis and FA re-esterification [114], while the expression and activity of PEPCK are up-regulated to match the rate of glyceroneogenesis with that of lipolysis [118,129]. Recently, both PEPCK gene expression and the content of the enzyme in WAT have been shown to be regulated by FAT/CD36, possibly secondary to the regulation of lipolysis [130]. In response to long-term fasting [114] and other strong hormonal and pharmacological stimuli, the balance between the rates of lipolysis and FA re-esterification within adipocytes may be affected ([116,117]; see also below). Moreover, this balance can be shifted in transgenic mice. Thus, overproduction of PEPCK, the key enzyme of glyceroneogenesis, in WAT (see Section 2.2) induces obesity and insulin resistance [131,132], while mice with adipose-specific PEPCK inactivation display lipodystrophic features [133].

Energy required for TAG/FA cycle was estimated by Baldwin [134] to be 8 molecules of ATP per release and re-esterification of 3 molecules of FA. This demand could by covered either by β-oxidation of FA in mitochondria during fasting and under other circumstances leading to the stimulation of lipolysis in WAT, or by oxidation of glucose in fed state, but also via energy equivalents produced during de-novo lipogenesis. Thus, the synthesis of fatty acid-CoA from glucose is associated with a net phosphorylation of ADP—as some NADPH, needed for the process, is generated in pentose phosphate pathway, the amount of ADP phosphorylation linked to oxidation of glucose to acetyl-CoA is greater than the amount of ATP hydrolyzed during conversion of acetyl-CoA into acyl-CoA. Therefore, excessive amounts of ATP or other reducing equivalents produced during de-novo lipogenesis from glucose could be a rate-limiting step for the anabolic process [121,135,136], but the surplus of energy could be consumed by the TAG/FA cycling (ATP needed for activation of FA into FA-CoA and reducing equivalents needed for glycerol phosphate generation; [121,136]).

Higher activity of TAG/FA cycle in human visceral WAT [137] corresponds with relatively high mitochondrial content and OXPHOS activity in this fat depot as compared with subcutaneous WAT (see Section 2). Moreover, induction of lipolysis in adipocytes is linked to stimulation of mitochondrial β-oxidation ([138]; see below). In turn, a decrease in mitochondrial ATP production results in the inhibition of both FA synthesis and lipolytic action of catecholamines (reviewed in [102,139]; see also [140]). Also the transcriptional activity of

PPARγ is inhibited in 3T3-L1 preadipocytes when mitochondrial activity is impaired by OXPHOS inhibitors [141].

In the absence of the stimulation of TAG/FA cycle, oxidation of FA is relatively slow, which is consistent with the low CPT1 activity in WAT [26,52]. For instance, in normal fed rats only 0.2% of endogenous FA in white adipocytes were oxidized while 50.1% were released and 49.7% re-esterified [58]. However, the amount of FA disposed through oxidative pathway increased about 1.5-fold with the stimulation of lipolysis and TAG/FA cycle by fasting [58]. Based on these numbers, it can be calculated that the amount ATP produced during β-oxidation of FA is fully sufficient to cover the energy costs of the process (not shown). In accordance with the data from the animal studies, direct calorimetry in human adipocytes isolated from subcutaneous WAT revealed that FA re-esterification contributed by 15% and 27% to WAT thermogenesis in the absence and presence of catecholamine, respectively [5]. Although Ball [142] has suggested that as much as 15% of basal metabolism in rats can be provided by TAG/FA cycle in WAT, and based on extrapolation of these data he also assumed that TAG/FA cycle could be responsible for 100% (!) of basal metabolism in humans [142], later experiments and calculations indicated that WAT basal metabolism in mouse, rat and dog contributed less than 1% [121]. While even the rat data seem to be unrealistically high, the extrapolation to humans is apparently skewed by much higher specific metabolic rate in the tissues of the smaller species [143]. Based on data published on re-esterification in lean men fasted for 60 h [144], it could be estimated [121] that the rate of heat production by systemic re-esterification (liver, adipose tissue, skeletal muscle) accounts for about 1.5% of basal metabolic rate, similarly as in the animals (see above). Reflecting these conservative estimates, as well as the contribution of FA re-esterification to thermogenesis in white adipocytes and contribution of WAT to resting metabolism in humans ([5], see also Section 1), it is to be inferred that TAG/FA cycle in WAT could be responsible for about 2–3% of resting metabolic rate in moderately obese humans.

As suggested by Gauthier et al. [145], the main role of AMPK (see Section 2.1) activation in white adipocytes could be to restrain energy depletion and oxidative stress associated with lipolysis. Thus, it is conceivable that AMPK plays a key role in the up-regulation of mitochondrial biogenesis and activation of β-oxidation of FA in mitochondria under various conditions leading to stimulation of lipolysis and TAG/FA cycle. Accordingly, activation of AMPK in white adipocytes was observed during starvation [146], or during physical exercise [105], which was also shown to increase activity of mitochondrial enzymes [147] as well as the expression of both PEPCK and PDK4 genes in WAT [148]. Also hyperleptinemia in rats leads to the stimulation of mitochondrial FA oxidation [149], AMPK activation and induction of TAG/FA cycle in white adipocytes [149]. Forced expression of PGC1α in combination with PPARα increases the activity of TAG/FA cycle in human white adipocytes [150]. However, similarly as in the case of leptin (see above), and all the treatments inducing brite cells (see Section 3.1), this manipulation also resulted in the induction of UCP1 in WAT, which makes it difficult to dissect the impact of TAG/FA cycle activation and mitochondrial uncoupling in WAT on whole-body energy balance.

AMPK also exerts complex modulation of lipolysis in white adipocytes [151–153] by regulating independently activities of the key enzymes involved [125], i.e. the hormone-sensitive lipase (**HSL**), which catalyses the hydrolysis of TAG into diacylglycerols and diacylglycerols into monoacylglycerols, and desnutrin/adipose triglyceride lipase (**ATGL**), an enzyme catalysing the initial step in TAG hydrolysis [154,155] and rate-limiting hydrolysis of TAG [156]. In the interplay with HSL, which also involves protein kinase A, activation of AMPK increases acute lipolytic response [153], while in the long term, HSL activity is inhibited by an AMPK-mediated mechanism [152,153]. On the other hand, AMPK-induced phosphorylation of ATGL leads to a long-term activation of lipolysis [116,151,157]. As suggested by Langin et al. [116], diacylglycerols formed by ATGL-mediated lipolysis could augment FA re-esterification. Based on its influence on lipolysis, AMPK may exert fine control of TAG/FA cycle and it could modulate the coupling between lipolysis and FA re-esterification.

Moreover, as shown in the heart [158], probably also in adipose tissue [159,160] ATGL-mediated lipolysis is important for the generation of ligands or precursors of ligands for nuclear receptors such as PPARα (see Section 2.1) or PPARδ, which control the expression of FA oxidation genes and mitochondrial OXPHOS activity [158,160], as well as expression of UCP1 in BAT [78]. As suggested by the group of Granneman [160], the induction of FA catabolism by this mechanism in WAT is important for limiting pro-inflammatory signalling during chronic lipolytic stimulation, and HSL-mediated lipolysis could be also involved [161]. The Granneman's hypothesis could be extended to suggest that the ATGL-dependent remodelling of WAT is also essential for increasing the mitochondrial OXPHOS activity, as required for covering the energy cost of the activation of TAG/FA cycle in white adipocytes.

2.4. Obesity is linked with altered mitochondrial energy metabolism in white adipocytes

Obesity is associated with a poor performance of mitochondria in WAT (see Section 1). However, mitochondrial function in WAT is not impaired in lean subjects with type 2 diabetes [162]. As reviewed by Kusminski and Scherer [163], it is conceivable that in obese state mitochondria in WAT similarly to mitochondria in other tissues [164] are not able to cope with increasing demands for FA oxidation, resulting in incomplete β-oxidation, which is also associated with a stress of endoplasmic reticulum [163,164]. Altered redox state, which is associated with increased mitochondrial ROS formation and accumulation of the products of incomplete β-oxidation, such as partially oxidized acylcarnitines [165], leads to a deterioration of insulin sensitivity [164]. In addition, high anaplerosis in WAT mitochondria in obesity may result in insufficient oxidation of metabolites arising from degradation of branched-chain amino acids (**BCAA**), supporting further the development of systemic insulin resistance [166,167]. Obesity may also lead to impaired detoxification of xenobiotics, which is linked to oxidative metabolism in WAT [168]. Importantly, the redox imbalance in obesity [164] may promote further lipid accumulation due to an increased supply of NADPH for FA synthesis in white adipocytes (see Section 2.2). As mentioned above (Section 2.1), pro-inflammatory signals in obese state may promote CDK5-mediated phosphorylation of PPARγ on serine residue 273 in white adipocytes, resulting in unfavourable changes in gene expression, including down-regulation of adiponectin [84,86]. In concert with a down-regulation of PGC-1α [169] and increased tonus [100,170] of endocannabinoid system [171] in obesity, the changes in the activity of PPARγ signalling may impair mitochondrial biogenesis in adipocytes. In addition, mitochondrial content and function in adipocytes in obesity could be compromised reflecting the impairment of AMPK-SIRT1 signalling [99]. Indeed, low-grade inflammation of WAT induced by HF diet feeding in mice results in proteolytic cleavage of both PPARγ [172] and SIRT1 [173] in adipocytes, while SIRT1 transcription was decreased in visceral WAT of obese human patients [174].

In dietary-obese mice, but not in lean mice, pharmacological blockage of CB1 receptors resulted in lower accumulation of body fat [175], in accordance with the anti-obesity effect of the cannabinoid antagonists in humans [176] and obesity resistance of the CB1 receptor knockout mice [177]. Importantly, the amelioration of obesity in mice treated with cannabinoid antagonists was associated with the induction of mitochondria, β-oxidation, lipolysis and probably also TAG/FA cycle in white adipocytes, while oxidative capacity of brown fat cells was also increased [175].

2.5. Improvement of impaired energy metabolism in white adipocytes in response to PPARγ activation

Mitochondrial insufficiency in white adipocytes of obese mice could be normalized by the treatment with a PPARγ agonist, a TZD compound rosiglitazone [10]. In addition, TZD could also activate AMPK signalling by a rapid, PPARγ-independent mechanism [178]. TZD supports BCAA catabolism in WAT [179], which may contribute significantly to the insulin-sensitizing effect of TZD in obesity [180]. TZD also induces the expression of genes involved in glyceroneogenesis (see Section 2.2), namely the genes encoding for PDK4 and PEPCK [113] as well as glycerol kinase selectively in adipose tissue [181]. Our unpublished results document similar up-regulation of PEPCK by TZD in both epididymal and subcutaneous WAT, while glycerol kinase gene was induced more in the subcutaneous WAT. Moreover, TZD support both mitochondrial anaplerosis and de novo lipogenesis in WAT by up-regulating PC [182], as well as FA re-uptake for re-esterification by stimulating FAT/CD36 gene expression (see Section 2.3 and [124]). Thus, concomitantly with the induction of mitochondrial oxidative capacity, rosiglitazone stimulates glycerol incorporation into TAG, induces futile FA re-esterification cycle and thus reduces FA release from adipocytes [112]. The complex modulation of adipocyte metabolism by this PPARγ agonist documents further the tight link between mitochondrial oxidative capacity (and OXPHOS) and TAG/FA cycle in white adipocytes.

As mentioned above (see Section 2.1), activation of PPARα and PPARγ in WAT also results in a potent anti-inflammatory effect. It is to be inferred that signalling mediated by PPARs in white adipocytes is the key element in the complex regulation of tissue metabolism and inflammation, and underlies the role of WAT in the whole-body homeostasis [92].

The TZD-induced remodelling of WAT is also associated with the induction of UCP1 [10]. The capability to induce UCP1 in WAT in response to TZD rosiglitazone, that is also regarded as a classical inducer of the formation of brite cells [29], depends strongly on the anatomical location of WAT, with subcutaneous WAT showing relatively high inducibility of the UCP1-harbouring adipocytes, while epididymal fat is much less affected [28]. Apparently, the control of the UCP1 content in adipocytes depends on both the specific structure of regulatory parts of the UCP1 gene and the interplay of intracellular transcriptional and translational modulators. It could be speculated that TZD activates UCP1 gene only in brite cells but not in classical white adipocytes. If this was the case, and UCP1 activity in brite cells is regulated similarly as in BAT, induction of UCP1-mediated thermogenesis in these cells would require adrenergic stimulation [183]. However, it can be also speculated that the induction of UCP1 gene expression by strong pharmacological agonists like TZD, which reflects a direct interaction of PPARγ [184] with the regulatory region of the UCP1 gene [185,186], overrides a fine physiological regulation of both UCP1 gene expression and mitochondrial biogenesis, and could result in UCP1 synthesis in classical white adipocytes. It has been shown that ectopic expression of UCP1 in white adipocytes resulted in mitochondrial uncoupling [187] and increased oxygen consumption by WAT in the absence of adrenergic stimulation [6], while the protonophoric activity of the ectopic UCP1 in WAT mitochondria retained its normal control by free FA and nucleotides [187]. Therefore, TZD-induced synthesis of UCP1 in white adipocytes could compromise mitochondrial ATP production even in the absence of adrenergic stimulation.

3. Modulation of metabolic fluxes in white adipocytes may affect adiposity

It is conceivable that a shift in the balance among activities of the major metabolic pathways contributing to TAG/FA cycle in white adipocytes, i.e. lipolysis of TAG and re-esterification of FA, as well as in the associated metabolic fluxes such as de novo FA synthesis and mitochondrial β-oxidation, could affect cellular lipid content ([58]; see Section 2.3). Thus, in spite of the induction of mitochondrial oxidative capacity (and even UCP1 expression in WAT in the animal experiments; see Section 2.5), pharmacological interventions using TZD in diabetic patients lead to redistribution of body fat, with increased TAG accumulation in the subcutaneous fat depots and decreased accumulation of abdominal fat [188]. Conversely, fasting in mice leads to preferential reduction of subcutaneous WAT, which could reflect fat-depot-specific differences in the rate of PEPCK-dependent FA re-esterification [146].

3.1. Energy expenditure in WAT could compromise accumulation of body fat

Some interventions, which modulate metabolic fluxes in adipocytes, may result in a general decrease of accumulation of body fat. Most of them, e.g. pharmacological stimulation of PPARα [78], forced lipolysis [189,190], leptin treatment [149], or overexpression of PGC-1α [77,191] activate the whole programme of UCP1-mediated thermogenesis and mitochondrial uncoupling within adipocytes, i.e. the induction of brite cells (see Section 1). However, in most cases, the changes in lipid accumulation in WAT are also associated with the induction of UCP1-mediated thermogenesis in classical BAT depots (reviewed in [192]), i.e. the process, which is probably much more relevant with respect to changes of whole body energy metabolism.

Importantly, some interventions may limit accumulation of body fat while inducing lipid catabolism in WAT in the absence of any induction of UCP1. This also includes pharmacological activation of AMPK in rats [193], which was accompanied by increased oxidation of FA [157] and, paradoxically (see Section 2.3), decreased activity of FA re-esterification in adipocytes [193]. As mentioned above (see Sections 2.1 and 2.3), AMPK is implicated in the control of activity of both TAG/FA cycle and mitochondrial biogenesis in white adipocytes. Furthermore, mice with genetic disruption of UCP1 are resistant to obesity when reared under the conditions of a mild cold stress but not at thermoneutral temperature [194,195]. This striking induction of obesity resistance could involve increased energy expenditure in WAT, as suggested by the elevated cytochrome oxidase activity in WAT of the UCP1-ablated but not wild type mice in response to cold exposure [196]. It could be speculated that under conditions of increased sympathetic stimulation and increased lipolysis in WAT, TAG/FA cycle could be activated and contribute to the induction of energy expenditure. However, shivering [19,20], as well as non-shivering thermogenesis in skeletal muscle (for review see [15–17]) can be also involved in the obesity resistance of these mice.

3.2. UCP1-independent induction of energy dissipation in WAT by combined intervention using omega 3 FA and mild calorie restriction

As we have shown recently in mice fed HF diet [27], combined intervention using long-chain *n*-3 polyunsaturated FA (**omega 3**) and mild calorie restriction (**CR**) exerted synergism in the prevention of obesity despite no increases in physical activity. The combined intervention also prevented low-grade obesity-associated inflammation of WAT, exerted hypolipidemic and insulin-sensitizing effects, as well as synergistic induction of mitochondrial OXPHOS in epididymal WAT, which could not be detected either in other fat depots including interscapular BAT or in non-adipose tissues. Importantly, all these metabolic changes occurred in the absence of any induction of UCP1 in WAT, and they were associated with a significant stimulation of palmitate oxidation measured ex vivo in both epididymal fat fragments and collagenase-liberated adipocytes from epididymal WAT of mice subjected to the combined intervention. Thus, the induction of UCP1-independent energy expenditure in WAT could be involved in the lower accumulation of body fat.

Although some previous studies in rats [197–199] showed increased levels of UCP1 mRNA and/or protein in BAT in response to dietary omega 3 supplemented to a diet high in fat [197,199] or sucrose [198], we did not observe any changes in UCP1 mRNA levels in BAT of mice fed omega 3-supplemented diet when compared to HF-fed controls [27]. Moreover, histological analysis of BAT from HF-fed mice suggested an increased lipid deposition in the tissue, which was unchanged by supplementing the HF diet with omega 3 (unpublished data). It could be only speculated whether interspecies differences or, perhaps, different dietary contents and intake of omega 3 could explain differential effects of omega 3 on UCP1 gene expression in the above studies. That the anti-obesity effect of omega 3 in mice fed HF diet was independent of cold-induced thermogenesis is further supported by our recent publication [200], showing reduced accumulation of body fat following a long-term feeding with omega 3-supplemented HF diet in mice maintained at thermoneutral (30 °C) temperature.

Omega 3, namely eicosapentaenoic acid (**EPA**; 20:5 *n*-3) and docosahexaenoic acid (**DHA**; 22:6 *n*-3), exert hypolipidemic effects [201,202], reduce systemic inflammation [203,204] as well as low-grade inflammation of WAT [27,205–207] and limit hepatosteatosis [27,207–210]. In humans, omega 3 lowers cardiovascular morbidity and possibly also the incidence of type 2 diabetes [211–213]. Many animal studies showed anti-obesity effects of omega 3 [27,66,207,208,214], while human studies documenting lower accumulation of body fat in response to omega 3 are scarce and they were mostly performed in the combination with energy restricted diets [215,216].

CR without malnutrition is an essential component in the treatment of obesity and associated diseases [217]. In many aspects, CR and metabolic syndrome could be opposite extremes of the same metabolic spectrum [218]. In organisms ranging from yeast to mammals, CR prolongs life span while inducing SIRT1 and mitochondrial SIRT3 deacetylases, which modify activity of several transcription factors, regulatory proteins and metabolic enzymes (see Sections 2.1 and 2.4 and [218,219]).

Our results suggested that the synergistic induction of FA oxidation in WAT in response to the combined intervention using omega 3 and CR could be explained by the stimulation of TAG/FA cycle in white adipocytes [27]. As documented in Fig. 1A, this idea is strongly supported by additive effects of the combined intervention on the expression of PEPCK in epididymal fat. PEPCK is the rate-limiting enzyme in glyceroneogenesis and it is required for TAG/FA cycle in white adipocytes (see Section 2.2). Accordingly, DHA alone could act as an inducer of PEPCK mRNA expression (Fig. 1A and [220]), and our recent results documented that omega 3 could induce FA re-esterification in epididymal WAT of mice fed a HF diet even at the thermoneutral temperature [200]. Moreover, CR as well as fasting stimulated the expression of PEPCK in rodents fed a low-fat chow diet [221,222] and up-regulated the expression of lipogenic genes while increasing the rate of FA synthesis specifically in WAT [223]. Interestingly, proteome analysis in rats indicated an activation of FA synthesis by CR in both WAT and BAT, while mitochondrial function was enhanced only in WAT [224].

When applied separately, both omega 3 [66] and CR [27,101,222,224,225] induce mitochondrial biogenesis in WAT, and both omega 3 [207,226] and CR [227] can be combined with other interventions to modulate the activity of an integrated circuit of intracellular energy sensors SIRT1 and AMPK (see Section 2.1). The induction of mitochondrial biogenesis down-stream of AMPK and SIRT1 involves transcription factors PPARα/γ, PGC-1α and NRF-1 (see Section 2.1 and Fig. 2).

That the combined intervention using omega 3 and CR results in a relatively strong induction of TAG/FA cycle, mitochondrial OXPHOS activity, and FA oxidation specifically in epididymal WAT, could reflect interplay of the following factors: (i) epididymal WAT is characterised by inherently higher mitochondrial content (see Section 2), relatively high responsiveness of adipocytes to both insulin [228] and β-adrenergic agonists [229], and much higher activation of AMPK in response to energy deprivation as compared with subcutaneous WAT [146]; (ii) omega 3 induce adiponectin [202,230] that may act locally in WAT to stimulate AMPK signalling; (iii) omega 3 may normalise the tonus of the endocannabinoid system [209,231,232], which is dysregulated in obesity (see Section 2.4), especially through their effects on WAT [233], hence counteracting the inhibition of AMPK by CB1 receptor-mediated signalling (see Section 2.1); (iv) both omega 3 and CR augment lipid catabolism through the activation of PPARα signalling ([81]; in fact, only FA released from endogenous TAG in adipocytes represents the available energy substrate when food intake is

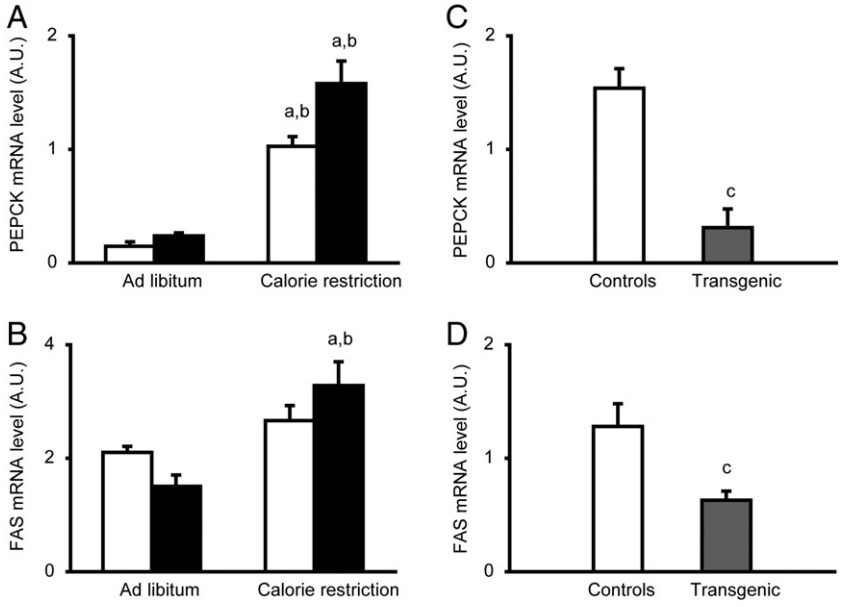

Fig. 1. Expression of genes encoding PEPCK and FAS in WAT. A, B: Quantitative real-time RT-PCR data showing mRNA levels in epididymal fat of male mice (C57BL/6J) after 5 weeks of the combined intervention using omega 3 and mild CR. For experimental details see [27]. C, D: mRNA levels in epididymal fat of control non-transgenic and aP2-*Ucp1* transgenic male mice fed HF diet. For experimental details see [273,277]. Data are means ± SE; $n = 7–10$. In A and B: white bars, HF diet; black bars, HF diet supplemented with omega 3. [a,b]Significant differences (ANOVA) compared with ad libitum HF diet supplemented or not with omega 3; [c]Significant differences (*t*-test) compared to control mice (i.e. non-transgenic littermates).

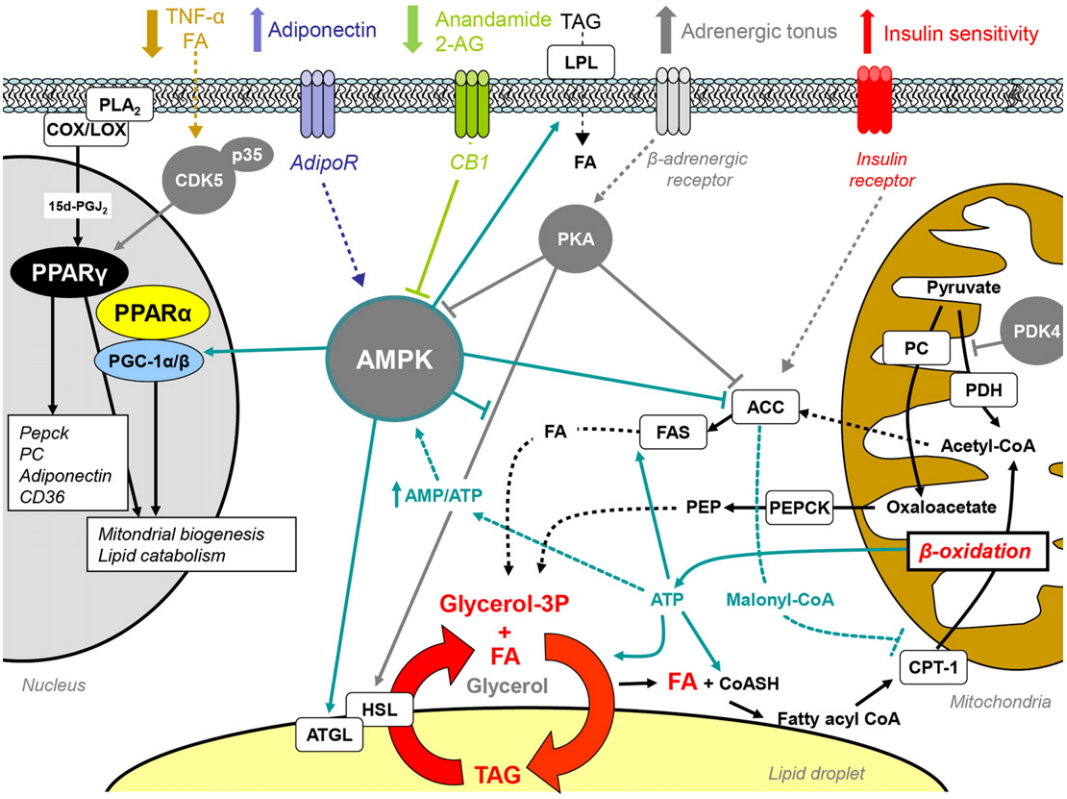

Fig. 2. Proposed scheme of modulation of metabolic fluxes in adipocytes in the epididymal WAT in response to the combined intervention using omega 3 and CR in mice fed HF diet. As explained in the main text, the combined intervention is associated with the reduction of low-grade inflammation of WAT, induction of adiponectin, lowering of the tonus of the endocannabinoid system (lower levels of major endocannabinoids in the tissue in response to omega 3 supplementation), higher adrenergic stimulation of adipocytes (elicited by CR), and amelioration of HF diet-induced insulin resistance. Synergistic induction of the formation of lipid mediators such as 15d-PGJ_2, which are derived from polyunsaturated FA released from membrane phospholipids by phospholipase A2 (**PLA_2**), and decreased activity of the CDK5 in response to the anti-inflammatory effect of the intervention lead to the activation of PPARγ signalling and up-regulation of specific genes engaged in mitochondrial biogenesis and lipid catabolism, as well as FA synthesis and glyceroneogenesis. PPARα and PGC-1 are also involved in the changes of the transcriptional programme in white adipocytes. The changes at the level of gene expression are reflected in the induction of mitochondrial β-oxidation, PEPCK-dependent glyceroneogenesis, and activation of the TAG/FA cycle. De novo FA synthesis is supported by the insulin-sensitizing effect of the intervention, depending possibly in large on the PPARγ activation. The induction of TAG/FA cycle (FA liberated from TAG during lipolysis may be released from the adipocyte and immediately taken back by FAT/CD36-mediated mechanism—not shown) and activation of FA for β-oxidation requires ATP, leading to lowering of cellular energy status (i.e. an increased AMP/ATP ratio) and activation of AMPK. At the early stages of adrenergic stimulation, the activity of AMPK is compromised by protein kinase A (**PKA**)-mediated inhibition that is released at later stages of the stimulation. In addition, AMPK activity is possibly further augmented, reflecting a decrease in the endocannabinoid tonus. The activation of AMPK would support the induction of mitochondrial biogenesis, OXPHOS, and β-oxidation while limiting the lipolytic response, and hence the release of FA from adipocytes. AdipoR, adiponectin receptor; COX, cyclooxygenase; LOX, lipoxygenase.

limited); and (v) both omega 3 [205–207,234] and CR [96] reduce low-grade inflammation of WAT, which, in turn may prevent CDK5-mediated phosphorylation of PPARγ, and therefore, secure a beneficial pattern of PPARγ-mediated changes in gene expression (see Section 2.1).

The anti-inflammatory effects of omega 3 depend in large on the formation of their active metabolites. These lipid mediators originate from either targeted enzymatic synthesis, as in case of resolvins and protectins [235,236], or from non-enzymatic oxidation reactions [234,237]. They can act as ligands for surface receptors, namely the lipid sensor GPR120 [206] or can interact with transcription factors like PPARγ and NF-κB [234]. Notably, resolvins and protectins mediate the anti-inflammatory and protective actions of omega 3 on obesity-induced insulin resistance and hepatic steatosis [235,238]. As we have shown recently [27], the combined intervention using omega 3 and CR induced 15-deoxy-Δ12,14-prostaglandin J2 (**15d-PGJ2**) and protectin D1 (**PD1**) with a surprising synergism observed in epididymal fat, but not in the liver. The induction of these lipid mediators could contribute to the anti-inflammatory effect of this intervention, while activation of PPARγ resulting from the supposed 15d-PGJ2 binding [239,240] could impact on mitochondrial biogenesis, glyceroneogenesis, adiponectin formation and other PPARγ-mediated effects (see Section 2.1).

As summarized in the following section, the levels of various lipid mediators in WAT could largely depend on the presence of various cell types in the tissue and on the changes in the pattern of formation of these lipid mediators in response to local inflammatory status.

4. Role of local lipid mediators in regulation of WAT metabolism

In addition to differentiated adipocytes and their precursors, innate immune cells including macrophages, mast cells, neutrophils, or eosinophils can be found in WAT [87,88]. Among them, adipose-tissue macrophages (**ATM**), major antigen-presenting cells, are functionally and numerically dominant and exert both immune and housekeeping functions. A state of chronic mild inflammation and dysfunction of WAT present in obesity can be linked to the infiltration of pro-inflammatory macrophages (**M1** phenotype), which produce pro-inflammatory cytokines [87–89,241]. ATM in lean mice show an altered, less-inflammatory properties (**M2** phenotype; [242]). Cytokines released from M1 macrophages block insulin action in adipocytes via TNF-α pathway, promote extracellular matrix remodelling and fibrosis of WAT, recruit other immune cells such as mast cells, B-cells or T-cells, and form crown-like-structures that encircle necrotic adipocytes to phagocytose debris [243]. These sub-populations release either anti-inflammatory (IL-4, IL-10, IL-13) or pro-inflammatory (TNF-α,

IFN-γ) cytokines, respectively [244]. Also other molecules like lipopolysaccharide (**LPS**) or palmitic acid promote M1-polarization via TLR4 pathway [245,246], and TLR antagonists including EPA or the adiponectin paralog C1q/TNF-related protein 3 (**CTRP3**; [247]), an endogenous LPS antagonist in adipose tissue, might be slowing down the inflammation. All these mediators affect also adipocytes, which themselves produce all kinds of adipokines with pro- or anti-inflammatory properties (monocyte chemoattractant protein-1, **MCP-1**; resistin, adiponectin, etc.; [87]). Importantly, reduction of WAT expression of SIRT1 in obesity (see Section 2.4) is causally linked to the macrophage infiltration [96]. Recent hypothesis on WAT inflammation proposes that the M1 response increases fuel demand for sterile immune response, thus increasing hyperglycaemia and lipolysis to supply nutrients for activated immune cells, while the M2 response suppresses the clonal expansion of Th1 cells and enhance nutrient storage by potentiating the action of insulin [248].

Besides the cytokines, a large family of endogenous lipid mediators derived mainly from arachidonic acid (**AA**; 22:4 *n*-6), contributes to the inflammatory state in WAT [244]. As mentioned above (see Sections 2.1, 2.4, and 3.2), endocannabinoids deteriorate AMPK signalling in WAT, and omega 3 supplementation results in lowering of their tissue levels, reflecting the replacement of AA in membrane phospholipids by DHA and EPA. Eicosanoids, products of cyclooxygenases, lipoxygenases, and cytochromes P450, are potent local mediators of signal transduction and modulate the inflammatory response. Although immune cells are the main producers of eicosanoids, also adipocytes synthetize prostanoids (PGE2, PGI2, PGF2α) and leukotrienes and express eicosanoid receptors. Thus, members of both adipocyte and immune cell lineages are able to communicate using lipid mediators as membrane or nuclear receptor ligands. Selected lipid mediators produced in adipose tissue are summarized in Table 1. Prostanoids are important for differentiation of adipocytes, and can regulate lipolysis in an autocrine and paracrine manner through PGE2 or even shift the differentiation of defined mesenchymal progenitors toward a brown adipocyte phenotype. PGE2 was shown to induce UCP1 in white adipocytes and stimulate thermogenesis in BAT. Cyclopentenone

Table 1
Lipid mediators and intercellular signalling in WAT.

Model	Species	Tissue	Lipid mediator	Source of mediator	Effect	Reference
Adipocytes, SVF	Human	Subcutaneous	PGE2	Endo	Modulation of adipogenesis	[279]
Adipocytes, SVF	Rat		PGE2, PGI2	Endo + exo	Modulation of lipolysis	[280]
Adipocytes, SVF, 3T3-L1	Mouse	Epididymal	PGE2, PGI2	Endo	Modulation of lipolysis	[190]
Adipose tissue, adipocytes	Human	Subcutaneous	PGE2	Endo	Stimulation of leptin release	[281]
3T3-L1			PGE2	Endo	Modulation of adipogenesis	[282]
Adipose tissue, adipocytes, 3T3-L1	Mouse	Intra-abdominal	cPGI2, PGE2, PGI2	Exo + endo	Shift the differentiation of mesenchymal progenitors toward a brown adipocyte phenotype, induction of UCP1 in WAT	[254,255]
Adipocytes, SVF, BAT	Mouse	Inguinal	PGE2	Exo	Induction of UCP1 in white adipocytes, no effect in BAT	[254]
BAT	Mouse	Interscapular	PGE2	Exo	Stimulation of thermogenesis in BAT	[283]
BAT, adipose tissue	Mouse	Interscapular, subcutaneous	PGD2-related	Endo	Regulation of BAT substrate utilisation, modulation of lipolysis	[256]
3T3-L1			PGF2a	Exo	Increase of glucose transport	[284]
3T3-L1			PGJ2 series	Endo	Modulation of adipogenesis and MCP-1 expression	[285]
Adipocytes, 3T3-L1	Human	Subcutaneous	d15-PGJ2	Exo	Production of macrophage inhibitory cytokine-1	[286]
3T3-L1			d15-PGJ2	Exo	Decrease of leptin production	[287]
NIH-3T3			d15-PGJ2	Exo	Stimulation of adipogenesis	[239]
C3H10T1/2			d15-PGJ2	Exo	Promotes adipocyte differentiation	[240]
Adipocytes, SVF	Mouse	Epididymal	LTB4	Exo	Secretion of MCP-1, IL-6, and TNF-a	[288]
Adipopocytes, 3T3-L1	Human, mouse	Subcutaneous, epididymal	LTB4, CysLTs	Endo	Chemoattractant for macrophages in obesity	[289]
Adipocytes, SVF, 3T3-L1	Rat	Perigonadal	12-HETE, 5-HETE, LTB4	Exo	Upregulation of IL-6, TNF-alpha, MCP-1	[290]
Adipocytes, 3T3-L1	Mouse	Epididymal	12-HETE, 12-HpETE	Endo + exo	Induction of ER stress	[291]
Adipose tissue, adipocytes	Mouse	Visceral	EET, DHET mix	Exo	Modulation of adipocyte differentiation, decreased TNF-alpha, MCP-1, increase of adiponectin	[292]
Adipocytes, SVF, macrophage	Mouse	Epididymal	RvD1	Endo + exo	Downregulation IL-6, MCP-1, and TNF-a, ROS; upregulation of IL-10, CD206, arginase 1, resistin-like molecule a, and chitinase-3 like protein, stimulated nonphlogistic phagocytosis; shift M1 > M2 macrophage phenotype	[249]
Adipose tissue, SVF	Mouse	Epididymal	RvD1	Exo	Increase of adiponectin production and insulin sensitivity; decrease of IL-6 production and macrophage infiltration; shift M1 > M2 macrophage phenotype	[293]
Adipose tissue	Mouse	Epididymal	17-HDHA, PD1, RvD1	Exo	Increase of adiponectin and insulin sensitivity	[235]
Adipose tissue	Mouse	Epididymal	PD1, 18-HEPE, 17-HDHA	Endo	Prevention of obesity-linked inflammation and insulin resistance	[236]
Adipose tissue, adipocytes	Human, mouse	Epididymal	RvD1, RvD2	Exo	Increase of adiponectin; decrease of leptin, TNF-a, MCP-1, IL-6, and IL-1b	[253]
Adipose tissue	Mouse	Epididymal	PD1, d15-PGJ2	Endo	Induction of lipid catabolism, antiinflammatory	[27]
Adipose tissue/leukocytes	Mouse	Perinodal	RvE1, PD1, LXA4	Exo	Resolution of inflammation	[294]
Adipose tissue	Mouse		omega 3 and omega 6 oxylipins	Endo	Modulation of inflammatory status in adipose tissue	[295]
Adipose tissue, adipocytes, SVF	Mouse	Perigonadal	LXA4	Exo	Decrease of IL-6 and increase of IL-10 expression; increase of insulin sensitivity, antiinflammatory	[296]

Adipocytes, collagenase-liberated adipocytes from adipose tissue; SVF, stromal vascular fraction from adipose tissue (containing immune cells); endo, endogenous source of the mediator (produced by the tissue itself); exo, exogenous application of the mediator (artificial stimulation).

prostaglandins (e.g. 15d-PGJ2) act as PPARγ ligands and modulate transcription of genes (see Section 2.1), including those involved in the production of adipokines linked to inflammation. Both PGE2 and 15d-PGJ2 are synthetized within cyclooxygenase pathway, but in different branches using either prostaglandin E or D synthases, potentially leading to either pro- or anti-inflammatory mediators. The possibility of modulation of these synthases adds another layer of complexity. Discovery of pro-resolving and anti-inflammatory omega 3-derived lipid mediators called resolvins (E-resolvins and D-resolvins), protectins, and maresins opened a new field concerning the mechanisms involved in resolution of WAT inflammation [236,249,250].

In this context, our finding (see Section 3.2) of the synergistic induction in WAT of specific lipid mediators, namely 15d-PGJ2 and PD1 (or its isomer PDX with the 11E, 13Z, 15E geometry instead of 11E, 13E, and 15Z in PD1), by a combined intervention using omega 3 and CR is of utmost importance. The observed induction of 15d-PGJ2, a metabolite derived from AA (omega 6 FA), is consistent with the results of another study [251] showing a similar induction of 15d-PGJ2 by dietary DHA, as well as with a non-enzymatic mechanism of its synthesis (see Section 3.2 and [27,251]). These results clearly demonstrate that dietary interventions can be used to increase local levels of lipid mediators in WAT, which can help to resolve inflammation [252] and even modulate metabolic properties of adipocytes by interacting with the major transcriptional regulatory pathways including PPARs (see Sections 2.1 and 2.5). In fact, changes in the levels of these types of lipid mediators may exert stronger effects than those exerted by pharmacological treatments. Thus, e.g., resolvins RvD1 and RvD2 counteract both local adipokine production and monocyte accumulation in inflamed WAT with a 300-fold higher efficiency as compared with rosiglitazone [253].

With respect to the induction of brite cells and UCP1 by PGE2 [254,255], and the induction of energy-dissipating adipocytes by 15d-PGJ2 and PD1 in response to the combined intervention with omega 3 and CR [27], it is unclear whether these lipid mediators could affect the same signalling pathway. PGE2 stimulated adipocytes through EP4 receptors on the plasma membrane while 15d-PGJ2, generated inside the cells, acted probably as a PPARγ ligand (see Section 2.1). Also a precise role of enzymes involved in PGE2, PGD2 and PGF2α metabolism has to be further explored [256–260]. A detailed characterisation of lipid mediators involved in the induction of energy expenditure in adipocytes may contribute to better characterisation of the (distinct) adipocyte cell lineage(s) involved. It could help to clarify, whether adipocytes, in which energy expenditure is activated through TGA/FA cycling, belong to the brite cell lineage or whether typical white adipocytes are involved.

It has been demonstrated that obesity-associated macrophage infiltration of WAT in mice is one order of magnitude higher in epididymal as compared with subcutaneous WAT, and that this inflammatory response is much lower in female as compared with male mice. Furthermore, female mice showed a relatively weak link between adipocyte hypertrophy and various phenotypes such as impaired glucose homeostasis and hepatosteatosis induced by HF feeding [261]. Accordingly, the obesity-associated WAT inflammation resulted in mitochondrial dysfunction in epididymal but not in periovarian WAT in the rat [262]. Therefore, it has to be learned how mouse strain-, fat depot- and gender-specific differences in macrophage infiltration of WAT could affect metabolism of adipocytes.

In addition to various cytokines and lipid mediators, M2 macrophages have been recently shown to produce catecholamines in animals exposed to cold, with a straightforward implication for the control of energy metabolism and thermogenesis in adipocytes [263]. That also adipocytes and cells within stromal vascular fraction could serve as a source of catecholamines has been recently observed [264,265].

It can be expected that the cell–cell interactions mediated by lipid mediators will be affected, reflecting not only the low-grade inflammation associated with obesity, but also changes in the immune cell population in WAT, which could be associated with cancer and, therefore, impact on metabolism of adipocytes and adiposity. Although no direct evidence linking inflammation, lipid mediators, cancer, and WAT is known, results on adipose-related breast cancer and adipocytes show that especially PGE2, its receptors, and cyclooxygenase-2 pathway are involved in increased aromatase activation leading to carcinogenesis [266–269]. On the contrary, growth of breast cancer cells was decreased by omega 3 when incorporated in plasma membranes, thus creating a pool for the synthesis of anti-inflammatory mediators [270].

5. Testing the impact of increased lipid catabolism in white adipocytes—mitochondrial uncoupling induced by transgenic expression of UCP1

As described above (see Sections 3.1 and 3.2), mitochondrial oxidative capacity in white adipocytes could be augmented, in the absence of any changes in the UCP1 levels, e.g. by cold acclimation in UCP1-ablated mice, or more physiologically, in response to the combined intervention using omega 3 and CR. This combined intervention induced a significant increase of FA oxidation in WAT. A question remains, however, how important the induction of energy dissipation in bona fide white adipocytes could be with respect to the regulation of body fat stores. Useful information can be drawn from the phenotype of transgenic mice expressing UCP1 gene from the aP2 gene promoter, with an enhanced expression of UCP1 in BAT and ectopic expression of UCP1 in WAT (**aP2-*Ucp1* mice**; Ref. [25]). These mice are resistant to obesity while showing a modulation of various aspects of WAT metabolism as well as whole-body metabolic responses (see below; for review see [93]). Since UCP1 over-expression in BAT of the aP2-*Ucp1* mice results in BAT atrophy and the impairment of BAT thermogenic functions [6,271], presumably due to a toxic effect of excessive amounts of UCP1, the obesity resistant phenotype of these mice likely results from uncoupled mitochondrial respiration solely in mature white adipocytes.

5.1. Induction of mitochondrial oxidative capacity in WAT in different models

Even the hemizygous aP2-*Ucp1* transgenic mice are strongly protected against obesity when crossed with genetically obese A^{vy} mice or when fed a HF diet [25,272]. In agreement with a higher expression of transgenic UCP1 in subcutaneous as compared with gonadal WAT [65], the transgene-induced metabolic changes were more pronounced in the former fat depot, which also preferentially decreased its size [25,272]. Thus, in the subcutaneous WAT of the aP2-*Ucp1* transgenic mice, 1.6-fold higher mitochondrial content was found as compared with wild-type mice [65], in association with a 3-fold increase in FA oxidation [273] and a 1.5-fold increase in oxygen consumption ([6]; as measured ex vivo in epididymal WAT).

In the UCP1-ablated mice, short-term cold exposure resulted in a 2- and 3.6-fold increase in specific cytochrome oxidase activity (adjusted to protein content of tissue homogenates used for the measurements) detected in epididymal and subcutaneous inguinal WAT, respectively [196]. Furthermore, in epididymal WAT of inbred, wild-type C57BL/6J mice, the intervention using omega 3 combined with CR increased specific cytochrome oxidase activity 1.3-fold, while the specific content of mitochondrial cytochromes as well as palmitate oxidation adjusted to tissue weight increased 1.6-fold. Moreover, both mitochondrial respiratory capacity and OXPHOS activity in digitonine-permeabilised adipocytes from epididymal WAT of mice subjected to the combined intervention were 2-fold higher as compared with the control non-treated group, while mitochondria in both groups of mice were highly coupled [27].

Thus, in all the models mentioned above, obesity resistance was associated with a similar induction of mitochondrial oxidative capacity and activity of FA oxidation in WAT and/or white adipocytes. At the same time, up-regulation of mitochondrial oxidative capacity was relatively high in WAT as compared with skeletal muscle or BAT [27,196]. Importantly, all these experiments (except for those with aP2-*Ucp1* transgene transferred to the genetically obese A^{vy} mice; Ref. [25]) were performed in mice having the same C57BL/6J genetic background. It is to be inferred that the induction of FA oxidation in white adipocytes of mice either using genetic manipulations to induce mitochondrial uncoupling or by activating the TAG/FA cycle (or perhaps also by other physiological mechanisms independent of UCP1) could contribute to obesity resistance.

5.2. Ectopic expression of UCP1 in white adipocytes as a model of brite cells

Since cellular physiology and metabolic properties of UCP1-containing adipocytes in WAT of the aP2-*Ucp1* transgenic mice have been already well characterised (see below), the results obtained with this transgenic model could serve as a lead to understand the physiology of brite adipocytes. In fact, studies of specific metabolic features of brite adipocytes are at their beginnings, and they are complicated by problems regarding the isolation of a homogenous cell population in sufficient quantities that are required for biochemical studies. Some of the consequences arising from the ectopic expression of UCP1 in white adipocytes, which could be of interest with respect to understanding the function of brite adipocytes, are summarized below.

Ectopic UCP1 lowered mitochondrial membrane potential in adipocytes isolated from gonadal fat of aP2-*Ucp1* mice and rendered it sensitive to FA and purine nucleotides. Full uncoupling activity was achieved in the presence of ~15-fold less UCP1 than in BAT mitochondria, resulting in the molar UCP1/respiratory chain ratio ~1, while this ratio is between 5 and 11 in BAT [187]. These results document that ectopic UCP1 was inserted correctly into the inner mitochondrial membrane, and that a relatively very low amount of UCP1 is sufficient for a full release of mitochondrial respiratory control. These data are also consistent with the original results of Lin and Klingenberg [274], suggesting that the protonophoric activity of UCP1 exceeds several-fold the proton-pumping activity of the respiratory chain in the native mitochondrial membrane. Furthermore, despite a decline in the content of transgenic UCP1 in WAT during ageing of the aP2-*Ucp1* mice [65], the robust anti-obesity effect of transgenic UCP1 was observed both in the aged A^{vy} mice expressing aP2-*Ucp1* transgene (3) and in the aP2-*Ucp1* mice fed HF diet during the whole life (our unpublished results). These results suggest that indeed even minute changes in the expression of UCP1 in WAT, as could be the case of the induction of brite adipocytes, may have significant impact on the respiratory control in mitochondria in these adipocytes. With respect to possible metabolic consequences of the induction of brite adipocytes, which takes place preferentially in subcutaneous WAT [28], it is important to note that the ectopic expression of UCP1 in the transgenic mice is also much stronger in subcutaneous as compared with gonadal WAT [65].

6. Key role of AMPK in the metabolic effects associated with the induction of energy expenditure in adipocytes

It is striking that very different mechanisms of the induction of energy expenditure triggered in white adipocytes in vivo, either by the combined intervention using omega 3 and CR or transgenic expression of UCP1, could elicit similar systemic effects, namely the protection against obesity [25,27,272] and hypolipidemic effects [27,275]. With respect to improvement of glucose homeostasis, the combined intervention may be even more efficient [27,272]. It can be speculated that the cellular energy sensor AMPK in adipocytes plays the key unifying role in these metabolic responses (Fig. 3). Thus, in case of the combined intervention, AMPK could be activated in response to increased demands for ATP, reflecting both the induction of FA re-esterification and de novo FA synthesis. Accordingly, starvation [146], omega 3 [226] and PPARγ agonists [178] could stimulate AMPK activity in WAT. However, in the case of combined intervention, the involvement of AMPK remains to be confirmed experimentally. On the other hand, in the aP2-*Ucp1* mice, the activation of AMPK [273] associated with a significant decrease of cellular energy status in WAT [273,276] has been unequivocally demonstrated. Thus, the activation of AMPK in response to mitochondrial uncoupling could explain fat depot-specific depression of lipogenesis [277] and lipolysis [276], as well as the increases in FA oxidation [273] and mitochondrial content [65]. Indeed, an elevated activity of α1 subunit of AMPK [273], a dominant catalytic subunit in adipose tissue [93], was detected in WAT (and especially in subcutaneous WAT) of the aP2-*Ucp1* mice. In analogy with its effects in the heart [94], AMPK activation could also explain the induction of LPL [275]. Therefore, it seems likely that AMPK represents the major regulatory intracellular pathway responsible for the wide spectrum of effects induced by transgenic UCP1 in white adipocytes, while at the same time the activation of FA oxidation and inhibition of lipogenesis in response to AMPK activation triggered by mitochondrial uncoupling would reduce fat accumulation.

Compared with the modulation of WAT metabolism by the transgene-induced mitochondrial uncoupling, which could be explained in full by the AMPK activation, the mechanistic basis of the metabolic effects of the combined intervention with omega 3 and CR in adipocytes is more complex (see Section 3.2 and the legend to Fig. 2). These mechanisms could explain some of the differences between the combined intervention and ectopic UCP1 regarding their effects on WAT and its secretory functions. For instance, PEPCK and FAS genes in epididymal WAT are up-regulated in response to the combined intervention (Fig. 1A,B), and it is conceivable that PPARγ-signalling is activated in WAT of mice subjected to this intervention due to both a synergistic induction of the endogenous PPARγ activator 15d-PGJ2 and the anti-inflammatory effects of omega 3 (Fig. 2). This is reflected by the induction of adiponectin [27] and could be critical for improving whole-body insulin sensitivity [88]. In contrast, a down-regulation of PPARγ and its target genes aP2 [273], PEPCK (Fig. 1C), and adiponectin (our unpublished results) has been observed in the aP2-*Ucp1* mice. Also the expression of FAS has been down-regulated in response to mitochondrial uncoupling (Fig. 1D and [277]). In addition, down-regulation of PC, another PPARγ target [182], together with decreased mitochondrial ATP production may augment the AMPK-mediated inhibition of de novo FA synthesis in WAT of the aP2-*Ucp1* mice.

It is to be stressed that under both conditions, i.e. in response to the combined intervention and mitochondrial uncoupling in WAT, activation of AMPK could support the metabolic effects as well as the induction of energy expenditure by augmenting the oxidative capacity in WAT. However, activation of AMPK alone, without concomitant activation of futile substrate cycling or mitochondrial uncoupling, respectively, for thermodynamic reasons, could not lead to any increase in energy expenditure [93,278]. In any case, independent of energy expenditure, AMPK-mediated activation of FA oxidation in adipocytes in obesity (even at the expense of decreased glucose oxidation) could help to counteract adipose tissue inflammation, as well as lipotoxicity resulting from excessive accumulation of fat in other tissues.

7. Conclusions, future research and new therapeutic strategies

In face of the renewed interest in mitochondrial uncoupling and the induction of UCP1-mediated thermogenesis in adipose tissue as

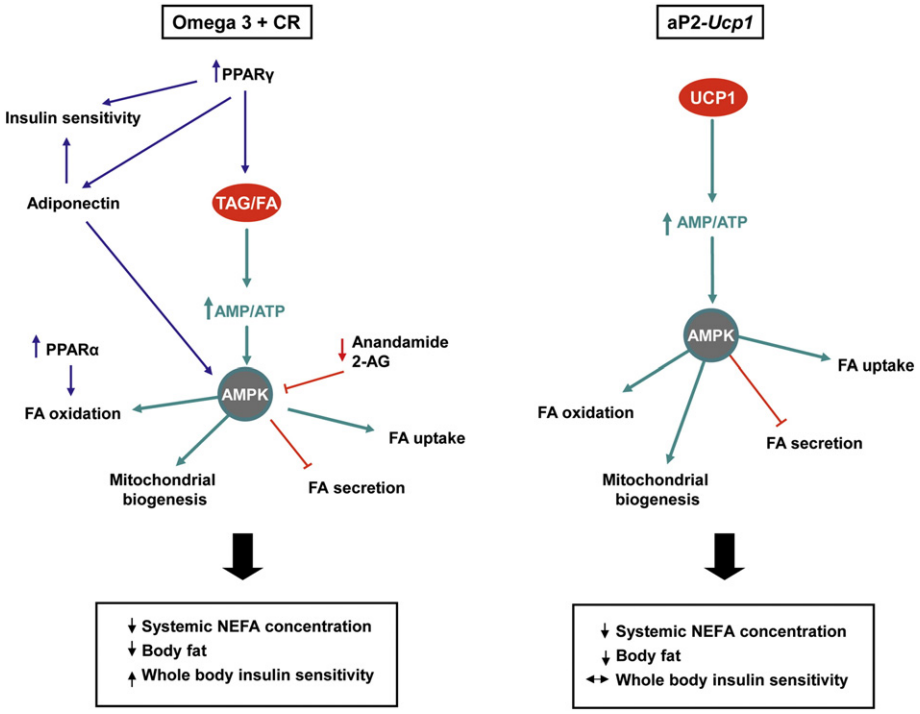

Fig. 3. Putative involvement of AMPK in white adipocytes in the metabolic effects either elicited by the combined intervention using omega 3 and CR or by mitochondrial uncoupling in the aP2-*Ucp1* transgenic mice. For further details, see the main text and Fig. 2.

potential therapeutic tools for obesity, increasing energy expenditure in WAT independently of UCP1 should be also considered. As demonstrated in mice, combined intervention based on the use of specific micronutrients, e.g. omega 3, and very mild CR could result in additive increases of FA oxidation in WAT, which is based on the activation of TAG/FA cycle and synergistic anti-inflammatory effects of the intervention. The mechanistic aspects of such changes in the phenotype of adipocytes should be further studied. That the above metabolic effects were observed in the epididymal but not subcutaneous WAT depot suggests an involvement of bona fide white adipocytes rather than newly differentiated cells belonging to another cell lineage, e.g. the one that gives rise to brite adipocytes. Further studies in this respect should be performed in order to characterise the expression of cell lineage markers in the cells with augmented UCP1-independent thermogenesis.

In spite of the widely neglected contribution of WAT to whole-body energy metabolism, previous studies in mice with ectopic expression of UCP1 in WAT support the notion that modulation of WAT metabolism could contribute to reduction of adiposity. The comparison of phenotypic changes observed in adipocytes from epididymal fat of mice subjected to the combined intervention with those seen in the transgenic mice strongly suggests that by combining physiological stimuli it should be possible to modify metabolic properties of white adipocytes to the extent that would elicit systemic effects. As compared with the induction of energy expenditure by the robust transgenic modification resulting in mitochondrial uncoupling, physiological modifications based on the combined intervention using omega 3 and CR may have additional health benefits, namely those, which are linked to the anti-inflammatory effects and to the suppression of the endocannabinoid system activity. With respect to the insulin-sensitizing effect of the combined intervention, the induction of adiponectin and stimulation of PPARγ signalling in WAT may be important for glucose homeostasis at the whole body level, reflecting the key role of PPARγ signalling in adipocytes for insulin sensitivity of the organism. High capacity of mitochondrial OXPHOS linked to inducible TAG/FA cycling activity is essential for metabolic flexibility of WAT and represent the markers of healthy adipocytes.

In this review, we better characterised the intracellular handles, which could be targeted to improve metabolic properties of adipocytes in WAT. With respect to novel therapeutic strategies, it remains to be established whether the mechanism triggered by the combined intervention, which modulates metabolic fluxes specifically in epididymal WAT of mice, could be also elicited in human WAT. This may be possible given a relatively high mitochondrial content and pronounced activity of the TAG/FA cycle in visceral fat. New combination treatment strategies may be designed including naturally occurring micronutrients like omega 3 and plant polyphenols, reduced calorie intake, and pharmacological modulators of key signalling pathways in fat cells. Involvement of the mutual interactions between adipocytes and immune cells contained in WAT may be essential for the induction of lipid catabolism in white adipocytes and the lean phenotype.

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