Biochemical Journal (2020) **477** 709–725
https://doi.org/10.1042/BCJ20190463

PORTLAND PRESS

Review Article

UCP1-independent thermogenesis

Anna Roesler[1,2] **and Lawrence Kazak**[1,2]

[1]Goodman Cancer Research Centre, McGill University, Montreal, QC H3A 1A3, Canada; [2]Department of Biochemistry, McGill University, Montreal, QC H3G 1Y6, Canada

Correspondence: Lawrence Kazak (lawrence.kazak@mcgill.ca)

Obesity results from energy imbalance, when energy intake exceeds energy expenditure. Brown adipose tissue (BAT) drives non-shivering thermogenesis which represents a powerful mechanism of enhancing the energy expenditure side of the energy balance equation. The best understood thermogenic system in BAT that evolved to protect the body from hypothermia is based on the uncoupling of protonmotive force from oxidative phosphorylation through the actions of uncoupling protein 1 (UCP1), a key regulator of cold-mediated thermogenesis. Similarly, energy expenditure is triggered in response to caloric excess, and animals with reduced thermogenic fat function can succumb to diet-induced obesity. Thus, it was surprising when inactivation of *Ucp1* did not potentiate diet-induced obesity. In recent years, it has become clear that multiple thermogenic mechanisms exist, based on ATP sinks centered on creatine, lipid, or calcium cycling, along with Fatty acid-mediated UCP1-independent leak pathways driven by the ADP/ATP carrier (AAC). With a key difference between cold- and diet-induced thermogenesis being the dynamic changes in purine nucleotide (primarily ATP) levels, ATP-dependent thermogenic pathways may play a key role in diet-induced thermogenesis. Additionally, the ubiquitous expression of AAC may facilitate increased energy expenditure in many cell types, in the face of over feeding. Interest in UCP1-independent energy expenditure has begun to showcase the therapeutic potential that lies in refining our understanding of the diversity of biochemical pathways controlling thermogenic respiration.

Introduction

The significance of brown adipose tissue (BAT) in body weight regulation is under intense investigation. The study of thermogenic mechanisms in mammals may lay the foundation to exploit these biochemical pathways for energy dissipation and mitigation of obesity. Maintenance of body weight requires energy balance. Excess intake, reduced expenditure, or both can lead to obesity. Homeostatic systems exist to match energy expenditure in response to dynamic alterations in energy intake [1] and dysfunction of each or both arms can produce obesity. The precision of this system is demonstrated by the fact that most individuals are not obese, despite the thermodynamic reality that a slight mismatch between intake and expenditure can markedly alter weight. Although components of this energy balance system have been identified [2–9], many questions remain to be answered. We focus on the intracellular mechanisms in thermogenic fat as one part of the energy expenditure arm of energy balance.

Energy expenditure has several major components including physical activity, obligatory energy expenditure (required to perform cellular functions), and adaptive (non-shivering) thermogenesis. This latter process refers to the adaptive dissipation of energy that primarily takes place in brown and beige adipocytes in response to environmental triggers, such as decreased ambient temperature or over feeding. In addition to muscle shivering, non-shivering thermogenesis plays a major role in thermal homeostasis and maintenance of body temperature [10]. The ability to maintain a constant body temperature in sub-thermoneutral environments is an important homeostatic mechanism to mitigate thermal stress, and is key for newborns (including humans) to tolerate the cold environment following birth as well as for rewarming of animals following hibernation [11–13]. The pioneering work of Foster and Frydman identified BAT as the major site of non-shivering thermogenesis by

Received: 13 December 2019
Revised: 20 January 2020
Accepted: 21 January 2020

Version of Record published:
14 February 2020

Biochemical Journal (2020) 477 709–725
https://doi.org/10.1042/BCJ20190463

measuring the arteriovenous difference in blood O_2 across interscapular BAT [14]. BAT-mediated energy expenditure occurs most notably in response to decreased environmental temperature (cold-induced thermogenesis) and over feeding (diet-induced thermogenesis), making thermogenic fat a central regulator of thermal and body weight homeostasis [14,15]. Strong support for impaired energy expenditure in obesity comes from pair-feeding studies of hypothalamic-lesioned animals, *ob/ob, db/db,* and *Mc4r* knockout mice, which all become obese despite identical energy intake to wild-type animals [16–21]. In humans, the classic study with Pima Indians demonstrated that low energy expenditure predicted future weight gain [22]. BAT recruitment increases host energy expenditure and minimizes weight gain, while BAT inactivation reduces energy expenditure and can contribute to obesity [23]. Thus, while adipocyte thermogenesis likely evolved as a defense against hypothermia, the unique capacity for cellular thermogenesis by adipocytes could potentially be leveraged to combat obesity and its associated disorders such as type 2 diabetes, hyperlipidemia, and fatty liver that are associated with these altered metabolic states [24].

Principles of non-shivering thermogenesis

BAT is highly innervated by sympathetic nerves and vascularized to support heat production and the distribution of metabolic heat generation to the rest of the body [25,26]. At the cellular level, brown adipocytes are densely packed with mitochondria and exhibit high metabolic activity upon sympathetic activation. Norepinephrine is the main effector molecule that is released by sympathetic nerves and acts on adrenergic receptors present on the cell surface of brown and white adipocytes. In rodents, the β3-adrenergic receptor is the most abundant adrenoreceptor on the adipocyte surface and has been shown to be a key mediator of metabolic fuel supply to support BAT-mediated thermogenesis following sympathetic stimulation [27–29].

The chemiosmotic theory provides the framework for the understanding of respiratory control [30]. Mitochondria are the major site of cellular respiration and use free energy in the form of reduced substrates (NADH and $FADH_2$), which become re-oxidized in a series of electron transfer reactions in the electron transport chain (ETC). The result of these reactions is the movement of protons out of the mitochondrial matrix into the intermembrane space (IMS) and the generation of an electrochemical gradient across the mitochondrial inner membrane, known as the protonmotive force (Δp). Mitochondrial respiration drives the formation of Δp, and the energy stored in Δp is harnessed to drive the phosphorylation of ADP while concomitantly driving protons back into the mitochondrial matrix by ATP synthase.

Movement of electrons through the ETC (disregarding electron leak/slip) results in a stoichiometric number of protons pumped into the IMS, and a fixed number of protons transverse through ATP synthase to generate a fixed number of ATP molecules. Within these limitations, energy expenditure can occur by increasing ATP turnover (directly or indirectly) to increase ADP availability, or by uncoupling metabolic fuel oxidation from ATP synthesis. ATP consumption can be increased by the performance of work such as growth or physical activity (but these are distinct to adaptive thermogenesis), or by utilizing futile cycles that consume ATP but do not perform any useful work, and essentially act as ATP sinks. Several futile cycles have been reported, with varying levels of evidence. The activity of futile cycles is difficult to examine *in vivo*; however, some molecular components have begun to be identified, enabling the examination of their physiological relevance [31–39]. Mitochondrial oxidative phosphorylation dominates metabolism in mammalian cells, transducing this free energy into the displacement of the [ATP = ADP + Pi] reaction from equilibrium. Importantly, only a fraction of the enthalpy of substrate oxidation is conserved in the free energy of the displaced [ATP = ADP + Pi] equilibrium; the rest is lost as heat. In living cells, ATP hydrolysis has a net change in energy of ∼12 kcal/mol. The major fuel source in adipocytes are lipids, and oxidation of each palmitate molecule (for example) yields a net of 106 ATP molecules. Thus, the energy conserved in ATP from each palmitate oxidation is 1272 kcal/mol (12 kcal × 106). The standard free energy change for oxidation of palmitate is 2380 kcal/mol. Thus, ∼50% of the free energy of palmitate is conserved as ATP. Therefore, in cells where the mitochondrial respiratory chain dominates oxidative metabolism, the rate of mitochondrial respiration is the major determinant of heat production. Since brown and beige adipocyte metabolism is predominantly oxidative, thermogenesis in these cells is effectively controlled by rate limiting steps in the oxidative reaction sequence leading to and involving respiration.

Diet induced thermogenesis

Data from humans and mice suggest that homeostatic mechanisms exist to compensate when either energy intake or expenditure is perturbed to resist either weight gain or loss from an individual's initial set point [40–43]. Indeed, experimentally imposed alterations in body weight (either increased or decreased) results in a change

in energy expenditure above or below that of individuals with a similar body composition, respectively, whose body weight was unaltered [41,44]. Evidence for BAT in adult humans initially came from the groups of Ricquier and Trayhurn [45,46]. Ignition of the field was sparked by observations that obesity in humans is associated with reduced levels of BAT [47–49]. The implication is that individuals with lower levels of BAT have a reduced capacity to burn off excess calorie consumption, leading to their obesity. This phenomenon, known as diet-induced thermogenesis was first demonstrated by Rothwell and Stock, who noted that voluntary overeating in rats induced the activity of the sympathetic nervous system [15]. Similarly, human BAT thermogenesis can be triggered by feeding to the same extent as cold exposure [50]. Of course, BAT function impairment will only result in obesity if energy balance is not re-established, which could occur by reducing food intake [51]. In the last decade, several studies have definitively shown that adult human BAT activity accounts for up to 5% of metabolic rate, which could promote more than 4 kg of fat loss per year [48,52,53]. Thus, the capacity for cellular thermogenesis by adipocytes could potentially be leveraged to combat an obesity-promoting environment. However, the biochemical pathways that trigger diet-induced thermogenesis are not fully understood. Attempts to treat obesity by increasing physical activity or by suppressing energy intake with drugs that suppress appetite or reducing caloric absorption in the gut have had limited success, often due to poor patient adherence [54,55]. Thus, identifying the molecular pathways in thermogenic fat that govern energy expenditure is a key step towards anti-obesogenic therapies.

UCP1-dependent thermogenesis

In most cell types, cellular respiration and ATP synthesis are tightly coupled processes and do not occur independently of one another. However, the ability of BAT to produce heat relies on the dissociation of these processes by proton conductance through a fatty acid/H^+ symport mechanism mediated by uncoupling protein 1 (UCP1), that is selectively expressed in this tissue [56]. UCP1 uncouples electron transport from ATP synthesis by dissipating Δp, which results in increased substrate flux through the ETC and increased respiration, resulting in heat generation (Figure 1a).

The *Ucp1*$^{-/-}$ mouse (with constitutive deletion) was developed by Enerback, Kozak, and co-workers and has become the primary model for studying the function of UCP1 [57]. When bred on a congenic background, *Ucp1*$^{-/-}$ mice become hypothermic upon acute cold exposure, but can survive the cold if gradually adapted to lower environmental temperatures [31,58–60]. The primary argument for UCP1 being the only thermogenic effector is as follows: since *Ucp1*$^{-/-}$ mice do not cease to shiver following chronic cold exposure, no alternative thermogenic pathway(s) can be of sufficient physiological relevance because the requirement for shivering is not abolished [61]. The assumption of this argument is that muscle shivering is the sole mediator of thermal homeostasis in cold-adapted *Ucp1*$^{-/-}$ animals. First, however, there is no experimental evidence to support that shivering is the only mechanism generating heat in these mice. Second, the degree of shivering between *wild-type* and *Ucp1*$^{-/-}$ mice on a congenic background is quantitatively and qualitatively identical [58]. However, *Ucp1*$^{-/-}$ animals succumb to hypothermia upon acute cold challenge, whereas *wild-type* mice typically do not. Thus, because the degree of shivering in *Ucp1*$^{-/-}$mice that are exposed to cold acutely or slowly is similar, shivering is unlikely to fully explain the ability of these mice to adapt to slow cold exposure. Third, *Ucp1*$^{-/-}$ animals on a hybrid background maintain body temperatures indistinguishable from *wild-type* animals even following acute cold exposure [62]. Collectively, these data demonstrate that UCP1 is dispensable for cold-mediated thermogenesis. A study has reported that *Ucp1*$^{-/-}$ mice decrease thermal conductivity to reduce heat loss [63]. It would be prudent to determine if thermal conductivity in hybrid mice is altered in response to environmental cold.

It has recently become apparent that most components of the ETC in BAT of constitutive *Ucp1*$^{-/-}$ mice are significantly reduced compared with *wild-type* BAT. The impairment of the ETC is evident at thermoneutrality and becomes incredibly striking at sub-thermoneutral temperatures (when many phenotypes of *Ucp1*$^{-/-}$ brown adipocytes become apparent) [64,65]. Consistent with this, the respiratory rate of *Ucp1*$^{-/-}$ BAT mitochondria is severely reduced in response to FCCP-mediated chemical uncoupling, particularly following cold exposure [66,67]. With these data, a reasonable conclusion is that the *Ucp1*$^{-/-}$ mouse model exhibits defects in BAT beyond UCP1. Consistent with this supposition, BAT mitochondria of *Ucp1*$^{-/-}$ mice are extraordinarily sensitive to calcium overload induced dysfunction [64], which is an impairment second to loss of UCP1. Of course, *Ucp1*$^{-/-}$ mice can be leveraged to identify *Ucp1*-independent thermogenic pathways, however the extent to which these alternative pathways can be activated will likely be underestimated due to the general impairment of BAT in these animals.

PORTLAND PRESS

Biochemical Journal (2020) **477** 709–725
https://doi.org/10.1042/BCJ20190463

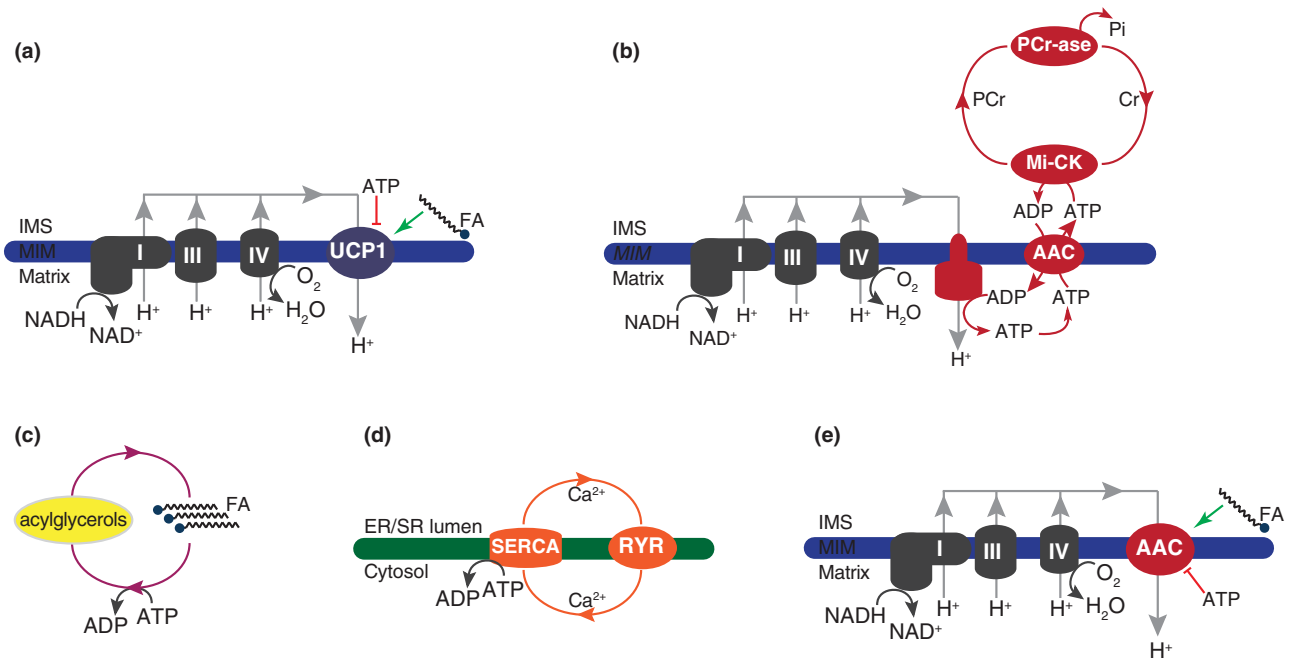

Figure 1. UCP1- and creatine-dependent thermogenesis.

The respiratory chain pumps protons into the intermembrane space (IMS), generating an electrochemical gradient (protonmotive force) across the mitochondrial inner membrane (MIM). Thermogenic mechanisms trigger an increase in the rate of substrate oxidation, which leads to thermogenesis. (**a**) Uncoupling protein 1 (UCP1) dissipates the energy stored in the protonmotive force by mediating proton leak from the IMS to the matrix. Fatty acids (FA) activate UCP1-dependent leak respiration, and purine nucleotides (primarily ATP *in vivo*) inhibit UCP1-dependent leak. (**b**) Creatine dissipates the energy stored in the protonmotive force by stimulating a cycle of ATP turnover, mediated by mitochondrial Creatine Kinase (Mi-CK) and coupled to the turnover of phosphocreatine (PCr). Mitochondrial ATP is used to drive the synthesis of PCr. (**c**) Lipolysis/re-esterification cycling. ATP is used to drive acylglycerol synthesis into triacylglycerols, diacylglycerols, or monoacylglycerols. (**d**) The model of calcium cycling across the ER membrane triggered by Sarcoplasmic/ Endoplasmic Reticulum Calcium ATPase (SERCA)-mediated calcium uptake and efflux by the ryanodine receptor (RYR). ATP is use to drive calcium influx into the endoplasmic reticulum (ER). (**e**) ADP/ATP carrier (AAC) dissipates the energy stored in the protonmotive force by mediating proton leak from the IMS to the matrix. FA activate AAC-dependent leak respiration, and ADP/ATP transport inhibits AAC-dependent leak.

The most unexpected phenotype of *Ucp1*$^{-/-}$ mice is their normal resting energy expenditure and resistance to weight gain on a high-fat diet (HFD) under standard room temperature housing conditions (~20–24°C; assumed temperature unless otherwise noted) [57]. This was an unforeseen observation given the involvement of BAT in diet-induced thermogenesis [68], and the idea that UCP1 was the only key thermogenic effector. Soon after, it was proposed that thermoneutral housing was required to reveal the effect of UCP1 on mitigating obesity [69]; however, this may not be the case, as several recent reports have now demonstrated that obesity is not potentiated in *Ucp1*$^{-/-}$ mice, even when housed at thermoneutrality [70–73]. Adding to the unpredictable nature of these observations was that, prior work had shown that mice with targeted BAT ablation using toxigene expression driven by the Ucp1-promoter (UCP-DTA) develop diet-induced obesity to a greater extent than control mice with functional BAT [23]. Moreover, mice lacking all three β-adrenergic receptors (β-less mice) develop massive obesity on a high-fat diet (HFD) at room temperature [5]. β-adrenoreceptor activation triggers UCP1-mediated leak through lipolysis-induced increases in fatty acid concentrations, primarily from white fat stores [28,29]. How can two distinct models with defective brown fat (UCP-DTA and β-less) reveal such different phenotypes from that of *Ucp1*$^{-/-}$ mice under similar conditions? A reasonable conclusion is that additional thermogenic effectors, downstream of β-adrenergic signaling, are still operational in these animals. These thermogenic mechanisms cannot be fully recruited in the context of BAT diminution itself (UCP-DTA) or inactivation of adrenergic signaling all together (β-less) [5,23,74,75]. Indeed, it has been suggested that *Ucp1*$^{-/-}$ mice are protected from diet-induced obesity when housed at room temperature because, under these conditions, they must induce alternative thermogenic pathways that are less efficient (and thus, require the

dissipation of more calories to produce the same amount of heat) to sustain their body temperature [35]. Consistent with this idea, *Ucp1*$^{-/-}$ mice can activate ∼50% cold-mediated heat production, compared with *wild-type* animals, suggesting that UCP1-independent mechanisms of heat production control half of the cold-mediated increase in energy expenditure [31,59,70,76,77]. However, just as UCP1 is apparently equally thermogenic in beige fat and BAT [78], there is no reason to assume that BAT cannot operate thermogenic pathways in the absence of *Ucp1*. Consistent with this, BAT temperature of *Ucp1*$^{-/-}$ mice has recently been shown to significantly increase following norepinephrine administration, which precedes a rise in rectal temperature, corroborating the idea that UCP1-independent thermogenic pathways may be operational in classical BAT [79]. Moreover, because of the impairment of BAT mitochondrial biology of the *Ucp1*$^{-/-}$ mouse, the physiological relevance of UCP1-independent pathways to thermogenesis may be underestimated. The next sections will look at how a cell can generate heat independently of UCP1.

Shivering thermogenesis

Ucp1$^{-/-}$ mice shiver at sub-thermoneutral temperatures [38,58], and it has been proposed that shivering-based thermogenesis is the only alternative to maintaining thermal homeostasis in *Ucp1*$^{-/-}$ mice [58]. However, when accounting for the following phenotypic characteristics of *Ucp1*$^{-/-}$ mice, it is evident that additional non-shivering based mechanisms exist and are critical for the survival of *Ucp1*$^{-/-}$ mice. First, only congenic *Ucp1*-deficient strains are cold-sensitive [62]. Second, these congenic strains require gradual (∼2 weeks) acclimation to mild sub-thermoneutral (18°C) temperatures before they can tolerate cold (4°C), suggesting that the shivering capacity alone is not sufficient to maintain body temperature upon acute cold exposure [72]. Therefore, the capacity for shivering-based heat distribution must be heightened, or alternate modes of non-shivering capacity must be triggered. Skeletal muscle has been posited to support *Ucp1*-independent thermogenesis through enhanced capacity for shivering thermogenesis. Molecular evidence of such an endurance training effect has been explored in models with impaired BAT. Consistent with a training adaptation in muscle, one study found evidence that hind limb skeletal muscle mitochondria from *Ucp1*$^{-/-}$ mice exhibited increased ADP-dependent and chemically uncoupled respiration, but no change in leak-dependent respiration, compared with *wild-type* controls [80]. In contrast, another study identified increased leak-dependent oxygen consumption, but no change in ADP-dependent respiration [81]. However, later work failed to find any differences in basal, ADP-stimulated, leak, and maximal respiration rates of skeletal muscle mitochondria between *Ucp1*$^{-/-}$ mice compared with controls, despite most of these parameters being induced by cold exposure itself, independent of genotype [59]. A possible discrepancy between these reports may be the use of different respiratory substrates. However, this explanation is not completely satisfying because when studied *in vivo*, the data do not suggest an increased capacity for lipid-fueled oxidation capacity of skeletal muscle of *Ucp1*$^{-/-}$ mice [59]. The switch toward increased capacity for greater lipid utilization was not observed between cold-exposed *wild-type* and *Ucp1*$^{-/-}$ mice *in vivo*, as skeletal muscle triglyceride and glycogen content was shown to be identical between the genotypes [31]. More recently, cold acclimation was shown to similarly increase muscle oxidative capacity of BAT-impaired UCP-DTA transgenic mice compared with *wild-type* animals, without any genotypic differences in basal or ADP-stimulated respiration in purified muscle mitochondria respiring on glucose- or lipid-derived fuels [82]. Taken together, the major thrust of reported data show that shivering-induced adaptations to skeletal muscle occur in mice, but not more so in *Ucp1*$^{-/-}$ mice. Moreover, when studied *in vivo*, there does not seem to be an increased capacity for such metabolic reprogramming in *Ucp1*$^{-/-}$ animals. If shivering was sufficient to protect mice from environmental cold, *Ucp1*$^{-/-}$ mice should be able to tolerate an acute cold challenge, as shivering under this context is quantitatively and qualitatively indistinguishable in *Ucp1*$^{-/-}$ mice whether exposed to cold acutely or gradually [58]. It is likely that the adaptive increase in metabolic heat production of *Ucp1*$^{-/-}$ mice reflects multiple thermogenic pathways that compensate in the absence of UCP1. Future experiments may necessitate exploring the complete removal of shivering with curare in *Ucp1*$^{-/-}$ mice to reveal the extent that shivering sustains body temperature in these animals. However, completely blocking shivering will introduce the requirement for artificial respiration [83], which may make interpretation of findings challenging.

Ucp1-independent thermogenesis

Norepinephrine-dependent energy expenditure increases in response to cold acclimation in wild-type mice. *Ucp1*$^{-/-}$ mice also exhibit norepinephrine-dependent increases in energy expenditure; however, this effect has been reported to not increase in response to cold acclimation. Thus, based on these data, at least in the mouse,

Biochemical Journal (2020) 477 709–725
https://doi.org/10.1042/BCJ20190463

UCP1 has been posited to be the sole mediator of adaptive adrenergic non-shivering thermogenesis. Reflecting upon new data, two issues must be considered. First, it is becoming increasingly clear that thermogenic respiration in BAT can be activated by non-adrenergic means [84–88]. Second, all prior data using *Ucp1*$^{-/-}$ mice (particularly upon cold-adaptation), must be re-examined because BAT of these animals has profound molecular impairments that go well beyond UCP1. Whether non-adrenergic extrinsic signals and nutrients can utilize biochemical pathways that do not rely on UCP1-mediated uncoupling will be an important area of future research.

Creatine-dependent substrate cycling

The influx of creatine into BAT occurs at a rate comparable to that of skeletal muscle, and brown adipocytes exhibit creatine kinase activity in the same order of magnitude as cardiac or nerve tissue [89]. The creatine kinase isoenzyme responsible for this activity is currently unknown. In human BAT, two mitochondrial creatine kinases, *CKMT1* and *CKMT2*, are enriched over WAT at the mRNA and protein level [50,90,91], and correlate well with *UCP1* [92]. Various creatine kinase isoenzymes (*CKM*, *CKMT1*, and *CKMT2*) are expressed at significantly higher levels in multilocular perirenal brown fat from humans, compared with unilocular perirenal brown fat and subcutaneous fat [93].

Unbiased quantitative mitochondrial proteomics first identified creatine metabolism as a signature of beige fat from cold-exposed mice [38]. The creatine pool is regulated by intracellular synthesis and influx from the circulation [36,37], and adipocyte creatine energetics is a key regulator of thermogenesis [36–38,90,91,94–97]. Genetic and pharmacological depletion of creatine levels in fat potentiates diet-induced obesity [36,37]. Similarly, global inactivation of creatine transport causes elevated fat accumulation [98]. Following the initial characterization of creatine cycling in beige adipocytes, new data emerged revealing the significance of this pathway in normal mouse physiology (i.e. creatine plays a role in thermogenesis in animals with intact UCP1 abundance). Mice with adipocyte creatine depletion due to genetic deletion of the rate-limiting step of creatine biosynthesis (glycine amidinotransferase, *Gatm*) or creatine transport (*CrT*) exhibit reduced adrenergic-mediated thermogenesis, diet-induced thermogenesis, and are more prone to diet-induced obesity than age-matched littermate controls [36,37]. Given that these phenotypes are observed at thermoneutrality and standard housing temperatures, creatine-dependent thermogenesis is suggested to be operational in all thermogenic adipocytes (brown and beige).

The forward and reverse phosphotransfer reactions of PCr/creatine in most cells occur in 1:1 stoichiometry with the ATP/ADP couple. However, thermogenic adipocytes seem to release a molar excess of ADP with respect to creatine, as concluded by detecting the respiratory response to creatine under ADP-limited conditions [38,94]. Thus, creatine elicits a substrate cycle of mitochondrial ATP turnover in a sub-stoichiometric fashion (Figure 1b). The cycle is understood to be downstream of canonical β3-adrenergic signaling and to be triggered by the phosphorylation of creatine by creatine kinase-mediated phosphotransferase activity [38,94]. Expert review of this topic has been recently published [24], and so here we will center on recent data exploring the role of creatine in thermogenic fat.

In rodents, most of the effects of the sympathetic nervous system on adipose tissue are understood to be attributed to norepinephrine-mediated activation of G_s-coupled β-adrenoreceptors [99,100]. The activity of adrenoreceptor signaling is terminated by agonist-induced internalization by β-arrestins [101]. The abundance of sarcomeric mitochondrial creatine kinase, CKMT2, is substantially increased in two mouse models that are protected from diet-induced obesity [102,103]. In fat, β-arrestin-2 (*barr2*) is a negative regulator of adipocyte β3-adrenergic signaling. Activation of the β3-adrenoreceptor is followed by its robust *barr2*-mediated internalization [102]. Fat-selective *barr2* knockout mice are protected from diet-induced obesity and exhibit significant activation of mitochondrial creatine kinase activity in adipocytes, associated with powerful increases in mRNA and protein expression of CKMT2 [102]. In the same vein, selective activation of G_s signaling in fat triggers energy expenditure, reduces diet-induced obesity, and increases glucose tolerance and insulin sensitivity [103]. These benefits, driven by adrenergic activation of adipocyte thermogenesis are linked with significant increases in *Ckmt2* expression [103]. Together, these data confirm prior work demonstrating that genes of creatine metabolism are downstream of canonical adrenergic signaling in fat. The interaction of PPARγ (peroxisome proliferator activated receptor gamma) with PGC-1α (PPARγ coactivator 1 alpha) is a key complex regulating adaptive thermogenesis [104]. Acetylation of PGC-1α by N-α-acetyltransferase 10 (Naa10p) blocks the PGC-1α/PPARγ interaction and suppresses thermogenesis [105]. Naa10p acetyltransferase activity inhibits PGC-1α-dependent transcriptional regulation by modulating PGC-1α occupancy on promoters of thermogenic

genes, such as *Ckmt2*. Fat-selective inactivation of *Naa10* in mice increases energy expenditure, mitigates diet-induced obesity, and increases the expression of thermogenic genes such as *Ckmt2* and *Ucp1* [105]. Recently, nitrate-mediated thermogenesis has been linked to creatine metabolism. Dietary nitrate mitigates diet-induced obesity, and supplementation of primary subcutaneous adipocytes by this compound increases basal respiration which is associated with significantly elevated expression of the creatine transporter (*Slc6a8*), and *Ucp1*. These data suggest that nitrite-induced thermogenesis may act partly through triggering creatine-dependent thermogenesis [106].

Mitochondria contact other organelles such as the endoplasmic reticulum (ER) and peroxisomes, and these organelles are present in mitochondrial preparations following standard purification techniques. Creatine and PCr are understood to readily pass through the voltage-dependent anion channel (VDAC). It is conceivable that PCr may be channeled out of the mitochondrial IMS through VDAC towards molecular factors exhibiting PCr phosphatase activity present at mitochondrial contact sites (Figure 1b). This topology and ease of creatine diffusion back to the IMS could drive cyclic substrate flux and energy dissipation, with creatine continuously entering mitochondria to simultaneously trigger PCr formation and ATP turnover. Alternatively, a pool of intra-mitochondrial PCr and creatine may circulate within the IMS. The data in support of a creatine-driven substrate cycle in purified mitochondrial preparations has not precluded either of these possibilities.

Importantly, an enzyme with PCr phosphatase activity would require tight regulation to avoid unnecessary dissipation of cytosolic PCr and energy state (low PCr/ATP). Substrate channeling through mitochondrial/ER contact sites, as well as the formation of these contact sites themselves, could provide such regulation. Considering that the mitochondria divide during thermogenic respiration [107], and that ER tubules command sites of mitochondrial division [108], this prospect seems plausible and merits further investigation.

The creatine kinase isoenzyme and the molecular component(s) mediating PCr hydrolytic activity are likely to be co-ordinately regulated downstream of thermogenic stimuli, such as β-adrenergic receptor signaling, to trigger creatine-dependent thermogenesis. Indeed, CKMT2 seems to fulfill this property [94,102,103], and it is likely that other creatine kinase isoforms will also be regulated by a similar or diverse repertoire of thermogenic stimuli [109]. Environmental cold increases creatine kinase activity in skeletal muscle of wood frogs [110]. How creatine kinase activity is altered in distinct tissues (adipose and non-adipose) in response to thermogenic stimulation will be an interesting future pursuit.

Enzyme kinetic measurements characterized *in vitro* cannot be simply extrapolated to the *in vivo* environment, because biochemical reactions of living systems do not occur in isolation [111]. For example, an enzyme with a high K_m value relative to the physiological concentration of substrate will not be saturated with substrate *in vivo*, and so the rate of formation of product will depend on substrate availability. This type of regulation may be a key requirement for flux through metabolic pathways in intact cells. The sub-stoichiometric action of creatine [38,94] was obtained with concentrations of creatine below the K_m of purified mitochondrial creatine kinase functioning in isolation [112]. However, in a native setting, enzyme activity can be markedly different compared with an isolated environment [113,114]. These differences could be due to intracellular allosteric regulation, binding partners, post-translational modifications, crowding and microcompartments that would be lost from work done with purified proteins in isolation. To our knowledge, the physiological concentration of creatine in thermogenic fat (in culture or *in vivo*) has never been examined using quantitative methods with isotopic internal standards. This will be a key consideration as this area moves forward.

Brown fat mitochondria are capable of normal oxidative phosphorylation [115–117]. Liberation of fatty acids in brown adipocytes can partly uncouple respiration from ATP synthesis. However, a substantial portion of adrenergically stimulated brown adipocyte respiration is mediated by mitochondrial ATP synthesis [118], and BAT mitochondria from cold-adapted rats and rabbits are capable of oxidative phosphorylation with P:O ratios similar to that of other tissues [116]. Thus, an important and often overlooked aspect of brown adipocyte function is that the coupling apparatus is unusually labile *in vitro* as that of other tissues. However, mitochondria isolated under proper conditions from the BAT of cold-adapted rats clearly exhibit oxidative phosphorylation that can be chemically uncoupled [115]. Importantly, coupled and uncoupled respiration is operational in human BAT [90]. Which conditions favor coupled vs uncoupled respiration *in vivo*? Purine nucleotides, primarily ATP *in vivo*, inhibit UCP1 transport activity. BAT takes up circulating fatty acids that are released following cold-mediated activation of the lipolytic cascade in white adipocytes [28,29], or long-chain fatty acids are released by mitochondrial phospholipase 2 (PLA2) within the mitochondrial inner membrane [56]. These fatty acids compete with purine nucleotide binding to UCP1 and support the re-entry of protons into the mitochondrial matrix. Clearly, cold exposure is a powerful stimulus that drives catabolic processes and loose

Biochemical Journal (2020) 477 709–725
https://doi.org/10.1042/BCJ20190463

coupling of oxidative phosphorylation, which is an efficient thermogenic mechanism. In contrast with cold-mediated thermogenesis, BAT also participates in diet-induced thermogenesis, which occurs when energy reserves are replete. ATP levels would not be decreased under these conditions, and would be more prone to be utilized for energy-dissipating biochemical pathways in so-called ATP sinks.

Thermogenic pathways that become activated chiefly in response to the deletion of *Ucp1* are still in fact dependent on *Ucp1* (inactivation). However, not all *Ucp1*-independent thermogenic pathways require *Ucp1* inactivation to reveal their thermogenic role in response cold exposure and caloric excess [36,37,119,120]. Creatine-dependent thermogenesis seems to fall into this category of *Ucp1*-independence. Notwithstanding, creatine metabolism may somehow alter brown adipocyte function to support UCP1-mediated thermogenesis. However, there is no experimental evidence that suggests this, thus far. How might two thermogenic pathways, effectively demanding the extraction of stored energy from the mitochondrial protonmotive force, interact within a BAT depot? One possibility is that these two pathways occur in distinct adipocyte populations within BAT and beige fat, which are known to be heterogenous [93,121,122]. A second prospect is that creatine-dependent thermogenesis is favored in the acute phase (for example following acute transition to environmental cold), until UCP1 levels accumulate to sufficiently high levels to take over thermogenic function. Finally, a third possibility is that both pathways operate in the same cells but the propensity of one pathway dominates under distinct physiological states (UCP1 for cold, creatine for diet).

The molecular mechanisms regulating creatine-dependent thermogenesis downstream of creatine accumulation in adipocytes are not fully understood. Mechanistically, creatine-dependent thermogenesis can trigger mitochondrial ATP turnover, which is understood to depend on the phosphorylation of creatine by creatine kinase-mediated phosphotransferase activity [38,94]. However, whether creatine kinase activity primarily regulates ATP turnover to drive thermogenic respiration [38,94], or whether classical creatine-dependent spatiotemporal buffering of ATP [123,124] is also operational in thermogenic fat is not known. These two pathways are not mutually exclusive. It is reasonable to assume that adipocytes exhibit the well-known ATP buffering functions of the creatine kinase system and PCr shuttling from sites of ATP synthesis to sites of ATP demand. Moreover, the key creatine kinase isoenzyme(s) and proposed PCr phosphatase controlling thermogenic respiration in adipocytes has yet to be identified (Figure 1b). Finally, the extrinsic thermogenic stimuli that regulate creatine metabolism in adipocytes are not completely understood. Clearly, much remains to be discovered.

Lipolysis/re-esterification cycling

A futile cycle of lipolysis/re-esterification has been proposed as a thermogenic process based on the ATP demand of acylglyercol synthesis [125–127]. This thermogenic pathway posits that adipocytes harbor the cellular machinery to simultaneously trigger a process whereby fat is broken down to fatty acid and glycerol with a subsequent re-esterification of the fatty acid by way of glycerol 3-phosphate [39]. Simultaneous operation of these two seemingly opposite metabolic pathways would constitute a futile cycle, as the re-esterification of free fatty acids with glycerol has been proposed as an ATP sink (Figure 1c). The origin of the fatty acids could be from intracellular triacylglycerol (TAG) stores, extracellular-derived fatty acids from the diet or lipoproteins that are delivered by the circulation, or *de novo* synthesis by lipogenesis. The diversity of fatty acid delivery sources suggests that lipolysis and re-esterification do not have to simultaneously occur in the same cell to constitute a thermogenic cycle.

Evidence put forth for such a pathway relies on measurements showing that adrenergic activation simultaneously activates TAG breakdown and re-synthesis, and so a futile cycle is inferred. Consistently, environmental cold or thiazolidinediones trigger the simultaneous expression of genes involved in oxidation and synthesis of fatty acids [128,129]. Early work characterizing this cycle assessed rates of fatty acid and glycerol production in adipose depots using a radiochemical method that depends on incorporation of ^{3}H from tritiated water into fatty acid and glycerol molecules. Applying this technique *in vivo*, triglyceride/fatty acid cycling was shown to be induced in WAT upon feeding, whereas cold exposure stimulated cycling in WAT and to a much greater extent in BAT [130,131]. Importantly, this process has been measured directly using deuterium-labeled water. Monitoring lipid cycling in this way *in vivo* demonstrated that β3-adrenergic agonism and cold exposure trigger fatty acid re-esterification, as indicated by deuterium labeling of glycerol in triglycerides [39,132]. Using substrate flux analysis, evidence for a futile cycle of lipolysis/re-esterification has recently been proposed in brown adipocytes [133]. Corroborating with older data, futile cycling of lipolysis/re-esterification is UCP1-independent and is induced in response to adrenergic stimuli in WAT [39]. However, $Ucp1^{-/-}$ mice

exhibit higher RER than *wild-type* animals upon gradual cooling [59], suggesting that liberated fatty acids do not substantially contribute to fuel delivery in the absence of UCP1, *in vivo*.

The glycerol phosphate pathway is the primary mechanism of TAG synthesis. Sn-glycerol-3-phosphate acyltransferase (GPAT), sn-1-acylglycerol-3-phosphate acyltransferase (AGPAT), and phosphatidate phosphatase-1 activity (conferred by the LIPIN family of proteins) catalyze the first, second, and third steps in diacylglyerol (DAG) synthesis. DAG is then converted to TAG by diacylglycerol acyltransferase (DGAT). A second pathway that controls TAG synthesis, the monoacylglycerol pathway, begins with acylation of monoacylglycerol (MAG) to DAG by monoacylglycerol acyltransferase (MGAT), and plays a predominant role in enterocytes after feeding [134]. The role of both pathways in physiology has been studied using global knockout mice. These enzymes control energy homeostasis, energy portioning, metabolic efficiency, and behavior.

GPAT4 is localized to the ER and is highly expressed in BAT [135]. *Gpat4*$^{-/-}$ mice exhibit decreased body weight, increased energy expenditure without a change in food intake, and are resistant to genetic and diet-induced obesity [136]. AGPAT2 is the dominant isoenzyme of this family expressed in adipose tissue. Its key role in human fat has been established from studies of patients with *AGPAT2* mutations, which causes a type of congenital generalized lipodystrophy [137]. *Agpat2*$^{-/-}$ mice severely lack WAT and BAT with a 90% reduction in body fat compared with control animals [138]. As expected, *Agpat2*$^{-/-}$ mice have abnormal energy metabolism, with massive hyperphagia, possibly secondary to near-absent circulating leptin levels. The precise alteration in energy expenditure is difficult to determine as the body composition of *Agpat2*$^{-/-}$ mice is dramatically different from controls. However, when normalizing for body weight, energy expenditure exhibits a powerful elevation [138]. Despite lack of fat tissue, *Agpat2*$^{-/-}$ mice have normal body weight, but increased lean mass and organomegaly of liver, kidney, spleen and intestine. LIPIN1 is the dominant form in adipose tissue. *Lipin1*$^{-/-}$ mice fail to store TAG in adipocytes despite similar caloric intake as *wild-type* mice [139]. *Lipin1*$^{-/-}$ mice exhibit increased energy expenditure [140]. In contrast, transgenic expression of lipin1 using the Fabp4 promoter enhances weight gain driven by excess fat accretion and driven by decreased energy expenditure and enhanced metabolic efficiency (energy from the diet stored somatically) [140]. Interestingly, transgenic expression of lipin1 using the muscle creatine kinase promoter causes similar, albeit more pronounced, changes in body fat, energy expenditure and metabolic efficiency [140]. Changes in *Ucp1* levels are not associated with these alterations in energy expenditure. DGAT mediates the final step of fatty acid esterification, converting DAG to TAG. *Dgat1*$^{-/-}$ mice are lean, exhibit increased energy expenditure compared with control animals, and are resistant to diet-induced obesity [141,142]. In contrast with global *Dgat1* deletion, *Dgat2* inactivation causes lethal neonatal lipopenia and skin barrier abnormalities [143]. *Mgat2*$^{-/-}$ mice exhibit reduced metabolic efficiency and increased thermogenic energy expenditure. *Mgat2*$^{-/-}$ mice are protected from developing obesity, fatty liver, hyperlipidemia, and glucose intolerance following high-fat feeding [134,144].

The effect of global deletion of acylglycerol synthesis genes on the control of metabolic efficiency by adipocyte energy expenditure is currently difficult to interpret. The altered (typically increased) energy expenditure and resistance to diet-induced obesity observed in the models with impaired whole body acylglycerol synthesis argues against the importance of thermogenic lipid cycling in combating obesity. However, the phenotypes associated with these global knockout mice may result from compensation due to lack of an insulating layer of subdermal fat. In addition, many of these models display atypical circulating dietary fat with altered kinetics of absorption and tissue deposition. Moreover, variations in metabolic fuel partitioning towards energy loss rather than storage may be an important contributor to decreased metabolic efficiency.

The extent to which re-synthesis of acylglycerols in adipocytes is sufficiently energetically costly to drive substantial increases in whole body energy expenditure remains to be determined. WAT lipolysis and subsequent oxidation of liberated fatty acids in BAT is key for sustaining thermogenic respiration [28,29], but is not necessarily dependent on UCP1 activation [145], but unavoidably involves heart function [28]. Interestingly, cold-activated beige adipocytes concomitantly increase the rate of lipolysis and glycerol and palmitate incorporation into lipids, with no change in UCP1-mediated uncoupling [146], indicating that the primary function of cold-activated beige fat is to export and take up fatty acids for ATP-dependent acylglycerol synthesis. Furthermore, palmitate oxidation in beige adipocytes is primarily ATP-dependent, and not controlled by UCP1-mediated uncoupling [146]. Intriguingly, lipolysis is not essential for thermogenesis in the presence of sufficient carbohydrate-rich dietary substrate supply [28]. Thus, glucose can be utilized as fuel to maintain thermal homeostasis in response to environmental cold, and so BAT-mediated thermogenesis is highly flexible depending on nutritional status. The biochemical pathway(s) supporting glucose-mediated thermogenesis remain to be determined, but any thermogenic pathway that does not exclusively rely on lipid could suffice.

Biochemical Journal (2020) 477 709–725
https://doi.org/10.1042/BCJ20190463

The physiological relevance of futile lipid cycling merits further investigation. Using genetically engineered mouse models coupled with *in vivo* labeling methods noted above may specifically test the lipid cycling hypothesis. In addition, altering fuel availability may be another way to modulate thermogenic lipid cycling. Free fatty acids within brown adipocytes increase by lipolysis from intracellular stores or by influx from the circulation. These fatty acids are activated to acyl-CoA, which then can undergo two fates: (1) oxidation in mitochondria, or (2) esterification to a glycerol backbone. The glycerol is provided from glycerol 3-phosphate through phosphorylation by glycerol kinase. Glycerol kinase is a key enzyme controlling the re-esterification and replenishment of the TAG pool, and could be an interesting candidate to selectively manipulate in adipocytes to examine the role of lipid cycling in energy expenditure *in vivo*. However, it is important to note that glycerol 3-phosphate has multiple fates aside from FA esterification, which must be considered in the context of lipid cycling-mediated thermogenesis. In response to thermogenic stimulation, brown adipocytes accumulate cytosolic calcium (Ca^{2+}), which could be taken up extracellularly or could be due to efflux from mitochondria [147,148]. Ca^{2+} activates glycerol 3-phosphate dehydrogenase (GPDH) which can use glycerol 3-phosphate to donate electrons to the ETC from the cytosol. The rise in cytosolic Ca^{2+} would thus cause glycerol 3-phosphate to be diverted from fatty acid re-esterification to promote its use by GPDH. Consequently, substrate will become limiting for acylglycerol re-esterification. Indeed, norepinephrine stimulation impairs fatty acid re-esterification in a Ca^{2+}-dependent manner [147,149]. Interpretation of mechanisms driving putative lipid cycling must consider these data. In addition, the fatty acids generated from lipolytic processes in adipocytes could be re-esterified in other tissues, leading to additional energy expenditure.

Sarcoplasmic/endoplasmic reticulum-based calcium cycling

Skeletal muscle can contribute to heat production using shivering and non-shivering mechanisms [150–154]. Heat produced by skeletal muscle shivering is an important physiological reflex that defends body temperature under various environmental conditions. Shivering involves rapid muscle contraction and relaxation and is highly dependent on intracellular Ca^{2+}. The major storage site of intracellular Ca^{2+} is in the sarcoplasmic reticulum (SR) of striated muscle and the ER in other cell types. Ryanodine receptors (RyRs) are the major Ca^{2+} release channels located along the SR/ER, and muscle contraction is triggered by this Ca^{2+} release. Control over cytosolic Ca^{2+} abundance regulates the strength and frequency of contraction-relaxation cycles. This regulation is primarily achieved by RyR-mediated Ca^{2+} release into the cytosol and subsequent uptake of Ca^{2+} by the sarco/endoplasmic reticulum Ca^{2+} ATPase (SERCA) pump. SERCA harnesses energy from ATP to pump Ca^{2+} into the SR lumen against a steep concentration gradient, in a 2 : 1 stoichiometry of Ca^{2+} : ATP. A single-pass transmembrane peptide, sarcolipin (SLN) interacts with SERCA to decrease the Ca^{2+} : ATP stoichiometry, which results in a potential ATP sink. Sarcolipin negatively regulates SERCA activity *in vitro*, possibly by decreasing Ca^{2+} affinity or by inducing, Ca^{2+} slip [155–157] (Figure 1d). Expert mechanistic reviews have recently been published on this topic [83].

The group of Leslie Kozak first hinted at a possible role for calcium cycling in inguinal adipose tissue [31]. Like calcium cycling in skeletal muscle, SERCA activity has been posited to be regulated by a small homopentameric protein, phospholamban (PLB), instead of SLN [158]. PLB is elevated in $Ucp1^{-/-}$ beige fat, suggesting reciprocal regulation [31]. Interestingly, no difference in the expression of SERCA1 or SERCA2 was found at the protein level between $Ucp1^{-/-}$ and control animals, and *Serca2a* and *Serca2b* were surprisingly reduced with cold exposure in $Ucp1^{-/-}$ mice [31]. Following this, Fabp4 promoter controlled PRDM16 overexpression was shown to overcome acute cold intolerance of $Ucp1^{-/-}$ mice, which was associated with *Serca2b* mRNA induction. It remains to be determined whether endogenous PRDM16 levels can activate SERCA-mediated thermogenesis in fat, and whether this pathway is physiologically relevant in wild-type animals with intact UCP1. Future work should investigate how calcium cycling in fat is regulated at the level of factors that modulate SERCA activity, and through the use of assays that directly examine the stoichiometry of the SERCA-mediated Ca^{2+}/ATPase influx relationship.

Proton leak by the mitochondrial ADP/ATP carrier

Mitochondria can cycle protons (H^+) independent of UCP1 [159]. H^+ leak appears to be a feature common to all mitochondria. This phenomenon has been characterized indirectly (through examination of oxygen consumption rate, which will increase in a non-linear proportion to elevated H^+ leak). The nature of the factor(s) at least partly responsible have recently become clear. The mitochondrial ADP/ATP carrier (AAC) is a key transport protein localized to the mitochondrial inner membrane. Four AAC isoforms (AAC1–AAC4) are

expressed in humans, whereas mice lack AAC3, and mainly express AAC1 and AAC2 [160]. The most well-established function of AAC is the exchange of mitochondrial ATP for cytosolic ADP to control the cellular ATP pool. Two additional functions of the AAC have been proposed: (1) inducible H^+ leak, and (2) control of the permeability transition pore. Importantly both mechanisms can be activated by fatty acids, and so for a long time it was unknown if AAC contributed to fatty acid-dependent mitochondrial uncoupling via selective H^+ leak or via non-selective induction of the permeability transition pore. In any case, the ubiquitous expression of AAC in cells supports the experimental observations that proton leak occurs in most mitochondrial populations.

AAC1 has been shown to control oxygen consumption under conditions where mitochondrial ATP synthesis is blocked [161], suggesting that H^+ leak by AAC is inhibited by its AAC activities. Importantly, using whole mitochondrial inner membrane patch-clamping, the first direct evidence of AAC-mediated H^+ leak was recently demonstrated in non-UCP1 expressing mitochondria, including skeletal muscle, liver, kidney and heart [162] (Figure 1e). Because H^+ leak was absent from patched plasma membrane preparations (but powerful in mitochondrial inner membranes) this work also laid to rest the idea that the protonophoric activity of fatty acids is somehow inherent to these molecules so that H^+ leak could occur through any lipid bilayer. Additionally, these studies revealed that the similarly named uncoupling proteins (UCP2, and UCP3) do not mediate H^+ leak [162]. Like UCP1 [163], potentiation of H^+ leak occurred in association with cysteine oxidation on AAC. The ability to specifically manipulate inducible H^+ leak, through cysteine modification, whilst maintaining ADP/ATP transport function of the AACs will be critical to develop anti-obesogenic therapies surrounding AAC-dependent thermogenesis.

Therapeutics focused on adipocyte thermogenesis

Obesity occurs when there is a chronic imbalance between assimilated energy and energy expenditure. Obesity may be reduced by affecting either side of this energy balance equation. Proof of principle that increases in metabolic rate can cause weight loss in humans comes from clinical data following exposure to the protonophore, 2,4-dinitrophenol (DNP) [164]. With increasing knowledge about brown and beige fat, interest in these tissues as targets for safely increasing thermogenic energy expenditure has re-emerged. Animal models with increased thermogenic fat amounts and activities have shown powerful metabolic improvements with no obvious detriment. These studies suggest that it may be possible to induce thermogenic mechanisms that could influence energy balance. Promising mechanisms relevant to human physiology may lie in the potential for induction of brown adipocytes in human white fat depots [165,166].

The mechanisms limiting excess weight gain are likely a relic of our evolutionary past, where the obese would be susceptible to predation [1]. However, the mechanisms controlling thermogenic respiration that respond to a positive energy balance are poorly defined. In humans, the estimates of the contribution of BAT to whole body energy expenditure are likely underestimated due to the limitations of accurately measuring BAT volume with positron emission tomography coupled to computed tomography with the glucose tracer 18-fluorodeoxyglucose (^{18}FDG PET/CT). Recent data suggest that total BAT volume may be much larger than the typically observed 50–150 ml with ^{18}FDG PET/CT. Therefore, the current estimates of total BAT thermogenesis, largely relying on total BAT volume using ^{18}FDG PET/CT, may underestimate the actual influence of BAT to total energy expenditure [52]. More recently, ^{11}C-acetate tracing has been utilized to measure the rate of the citric acid cycle turnover in BAT by quantifying $^{11}CO_2$ production. Using this method, several studies have reported that BAT CO_2 increases 2 to 3-fold in response to cold [167,168]. As other expert reviews have noted [52], the estimated contribution of BAT to human whole body energy expenditure remains uncertain due to a limited capacity to precisely measure BAT thermogenesis using current imaging modalities. The current estimates with the tools at hand place BAT at the lower end of providing a clinically meaningful contribution towards the treatment of obesity and type 2 diabetes. Nevertheless, novel imaging methods may reveal the true potential of BAT to human metabolism by accurately quantifying its abundance [52,169]. As thermogenic pathways and determination factors are identified, there is no reason to assume that the activity or amount of BAT cannot be altered. New therapeutic treatments for metabolic diseases surrounding adipocyte dysfunction may benefit from a refined understanding of the diversity in biochemical pathways contributing to thermogenic respiration. If BAT quantity or activity can be increased without side effects, there may still be a place for human BAT in treating obesity and obesity-associated disease.

Biochemical Journal (2020) **477** 709–725
https://doi.org/10.1042/BCJ20190463

Abbreviations

AAC, ADP/ATP carrier; BAT, brown adipose tissue; DAG, diacylglyerol; DGAT, diacylglycerol acyltransferase; ER, endoplasmic reticulum; ETC, electron transport chain; GPDH, glycerol 3-phosphate dehydrogenase; HFD, high-fat diet; IMS, intermembrane space; PLB, phospholamban; SERCA, sarco/endoplasmic reticulum Ca^{2+} ATPase; SLN, sarcolipin; SR, sarcoplasmic reticulum; TAG, triacylglycerol; VDAC, voltage-dependent anion channel.

Competing Interests

The authors declare that there are no competing interests associated with the manuscript.

References

1 Speakman, J.R. (2007) A nonadaptive scenario explaining the genetic predisposition to obesity: the "predation release" hypothesis. *Cell Metab.* **6**, 5–12 https://doi.org/10.1016/j.cmet.2007.06.004
2 Zhang, Y., Proenca, R., Maffei, M., Barone, M., Leopold, L. and Friedman, J.M. (1994) Positional cloning of the mouse obese gene and its human homologue. *Nature* **372**, 425–432 https://doi.org/10.1038/372425a0
3 Lee, G.H., Proenca, R., Montez, J.M., Carroll, K.M., Darvishzadeh, J.G., Lee, J.I. et al. (1996) Abnormal splicing of the leptin receptor in diabetic mice. *Nature* **379**, 632–635 https://doi.org/10.1038/379632a0
4 Chen, H., Charlat, O., Tartaglia, L.A., Woolf, E.A., Weng, X., Ellis, S.J. et al. (1996) Evidence that the diabetes gene encodes the leptin receptor: identification of a mutation in the leptin receptor gene in db/db mice. *Cell* **84**, 491–495 https://doi.org/10.1016/S0092-8674(00)81294-5
5 Bachman, E.S., Dhillon, H., Zhang, C.Y., Cinti, S., Bianco, A.C., Kobilka, B.K. et al. (2002) betaAR signaling required for diet-induced thermogenesis and obesity resistance. *Science* **297**, 843–845 https://doi.org/10.1126/science.1073160
6 Krude, H., Biebermann, H., Luck, W., Horn, R., Brabant, G. and Grüters, A. (1998) Severe early-onset obesity, adrenal insufficiency and red hair pigmentation caused by POMC mutations in humans. *Nat. Genet.* **19**, 155–157 https://doi.org/10.1038/509
7 Huszar, D., Lynch, C.A., Fairchild-Huntress, V., Dunmore, J.H., Fang, Q., Berkemeier, L.R. et al. (1997) Targeted disruption of the melanocortin-4 receptor results in obesity in mice. *Cell* **88**, 131–141 https://doi.org/10.1016/S0092-8674(00)81865-6
8 Locke, A.E., Kahali, B., Berndt, S.I., Justice, A.E., Pers, T.H., Day, F.R. et al. (2015) Genetic studies of body mass index yield new insights for obesity biology. *Nature* **518**, 197–206 https://doi.org/10.1038/nature14177
9 Shungin, D., Winkler, T.W., Croteau-Chonka, D.C., Ferreira, T., Locke, A.E., Mägi, R. et al. (2015) New genetic loci link adipose and insulin biology to body fat distribution. *Nature* **518**, 187–196 https://doi.org/10.1038/nature14132
10 Davis, T.R., Johnston, D.R., Bell, F.C. and Cremer, B.J. (1960) Regulation of shivering and non-shivering heat production during acclimation of rats. *Am. J. Physiol.* **198**, 471–475 https://doi.org/10.1152/ajplegacy.1960.198.3.471
11 Smalley, R.L. and Dryer, R.L. (1963) Brown fat: thermogenic effect during arousal from hibernation in the bat. *Science* **140**, 1333–1334 https://doi.org/10.1126/science.140.3573.1333
12 Smith, R.E. and Hock, R.J. (1963) Brown fat: thermogenic effector of arousal in hibernators. *Science* **140**, 199–200 https://doi.org/10.1126/science.140.3563.199
13 Dawkins, M.J. and Hull, D. (1964) Brown adipose tissue and the response of new-born rabbits to cold. *J Physiol* **172**, 216–238 https://doi.org/10.1113/jphysiol.1964.sp007414
14 Foster, D.O. and Frydman, M.L. (1978) Brown adipose tissue: the dominant site of nonshivering thermogenesis in the rat. *Experientia Suppl.* **32**, 147–151 https://doi.org/10.1007/978-3-0348-5559-4_16
15 Rothwell, N.J. and Stock, M.J. (1979) A role for brown adipose tissue in diet-induced thermogenesis. *Nature* **281**, 31–35 https://doi.org/10.1038/281031a0
16 Bray, G.A. and York, D.A. (1979) Hypothalamic and genetic obesity in experimental animals: an autonomic and endocrine hypothesis. *Physiol. Rev.* **59**, 719–809 https://doi.org/10.1152/physrev.1979.59.3.719
17 Coleman, D.L. (1978) Obese and diabetes: two mutant genes causing diabetes-obesity syndromes in mice. *Diabetologia* **14**, 141–148 https://doi.org/10.1007/bf00429772
18 Ste Marie, L., Miura, G.I., Marsh, D.J., Yagaloff, K. and Palmiter, R.D. (2000) A metabolic defect promotes obesity in mice lacking melanocortin-4 receptors. *Proc. Natl Acad. Sci. U.S.A.* **97**, 12339–12344 https://doi.org/10.1073/pnas.220409497
19 Breslow, M.J., Min-Lee, K., Brown, D.R., Chacko, V.P., Palmer, D. and Berkowitz, D.E. (1999) Effect of leptin deficiency on metabolic rate in ob/ob mice. *Am. J. Physiol.* **276**, E443–E449 https://doi.org/10.1152/ajpendo.1999.276.3.E443
20 Cox, J.E. and Powley, T.L. (1977) Development of obesity in diabetic mice pair-fed with lean siblings. *J. Comp. Physiol. Psychol.* **91**, 347–358 https://doi.org/10.1037/h0077322
21 Cox, J.E. and Powley, T.L. (1981) Intragastric pair feeding fails to prevent VMH obesity or hyperinsulinemia. *Am. J. Physiol.* **240**, E566–E572 https://doi.org/10.1152/ajpendo.1981.240.5.E566
22 Ravussin, E., Lillioja, S., Knowler, W.C., Christin, L., Freymond, D., Abbott, W.G.H. et al. (1988) Reduced rate of energy expenditure as a risk factor for body-weight gain. *N. Engl. J. Med.* **318**, 467–472 https://doi.org/10.1056/NEJM198802253180802
23 Lowell, B.B., S-Susulic, V., Hamann, A., Lawitts, J.A., Himms-Hagen, J., Boyer, B.B. et al. (1993) Development of obesity in transgenic mice after genetic ablation of brown adipose tissue. *Nature* **366**, 740–742 https://doi.org/10.1038/366740a0
24 Chouchani, E.T., Kazak, L. and Spiegelman, B.M. (2019) New advances in adaptive thermogenesis: UCP1 and beyond. *Cell Metab.* **29**, 27–37 https://doi.org/10.1016/j.cmet.2018.11.002
25 Wirsen, C. (1964) Adrenergic innervation of adipose tissue examined by fluorescence microscopy. *Nature* **202**, 913 https://doi.org/10.1038/202913a0
26 Engel, B.T., Sato, A. and Sato, Y. (1992) Responses of sympathetic nerves innervating blood vessels in interscapular, brown adipose tissue and skin during cold stimulation in anesthetized C57BL/6J mice. *Jpn. J. Physiol.* **42**, 549–559 https://doi.org/10.2170/jjphysiol.42.549

27 Himms-Hagen, J., Cui, J., Danforth, E., Taatjes, D.J., Lang, S.S., Waters, B.L. et al. (1994) Effect of CL-316,243, a thermogenic beta 3-agonist, on energy balance and brown and white adipose tissues in rats. *Am J Physiol* **266**, R1371–R1382 https://doi.org/10.1152/ajpregu.1994.266.4.R1371
28 Schreiber, R., Diwoky, C., Schoiswohl, G., Feiler, U., Wongsiriroj, N., Abdellatif, M. et al. (2017) Cold-induced thermogenesis depends on ATGL-mediated lipolysis in cardiac muscle, but not brown adipose tissue. *Cell Metab* **26**, 753–763 e757 https://doi.org/10.1016/j.cmet.2017.09.004
29 Shin, H., Ma, Y., Chanturiya, T., Cao, Q., Wang, Y., Kadegowda, A.K.G. et al. (2017) Lipolysis in brown adipocytes is not essential for cold-induced thermogenesis in mice. *Cell Metab.* **26**, 764–777 e765 https://doi.org/10.1016/j.cmet.2017.09.002
30 Mitchell, P. (1961) Coupling of phosphorylation to electron and hydrogen transfer by a chemi-osmotic type of mechanism. *Nature* **191**, 144–148 https://doi.org/10.1038/191144a0
31 Ukropec, J., Anunciado, R.P., Ravussin, Y., Hulver, M.W. and Kozak, L.P. (2006) UCP1-independent thermogenesis in white adipose tissue of cold-acclimated Ucp1$^{-/-}$ mice. *J. Biol. Chem.* **281**, 31894–31908 https://doi.org/10.1074/jbc.M606114200
32 Rowland, L.A., Maurya, S.K., Bal, N.C., Kozak, L. and Periasamy, M. (2016) Sarcolipin and uncoupling protein 1 play distinct roles in diet-induced thermogenesis and do not compensate for one another. *Obesity (Silver Spring)* **24**, 1430–1433 https://doi.org/10.1002/oby.21542
33 Rowland, L.A., Bal, N.C., Kozak, L.P. and Periasamy, M. (2015) Uncoupling protein 1 and sarcolipin are required to maintain optimal thermogenesis, and loss of both systems compromises survival of mice under cold stress. *J. Biol. Chem.* **290**, 12282–12289 https://doi.org/10.1074/jbc.M115.637603
34 Koza, R.A., Hohmann, S.M., Guerra, C., Rossmeisl, M. and Kozak, L.P. (2000) Synergistic gene interactions control the induction of the mitochondrial uncoupling protein (Ucp1) gene in white fat tissue. *J. Biol. Chem.* **275**, 34486–34492 https://doi.org/10.1074/jbc.M002136200
35 Anunciado-Koza, R., Ukropec, J., Koza, R.A. and Kozak, L.P. (2008) Inactivation of UCP1 and the glycerol phosphate cycle synergistically increases energy expenditure to resist diet-induced obesity. *J. Biol. Chem.* **283**, 27688–27697 https://doi.org/10.1074/jbc.M804268200
36 Kazak, L., Rahbani, J.F., Samborska, B., Lu, G.Z., Jedrychowski, M.P., Lajoie, M. et al. (2019) Ablation of adipocyte creatine transport impairs thermogenesis and causes diet-induced obesity. *Nat. Metab.* **1**. https://doi.org/10.1038/s42255-019-0035-x
37 Kazak, L., Chouchani, E.T., Lu, G.Z., Jedrychowski, M.P., Bare, C.J., Mina, A.I. et al. (2017) Genetic depletion of adipocyte creatine metabolism inhibits diet-induced thermogenesis and drives obesity. *Cell Metab.* **26**, 660–671 e663 https://doi.org/10.1016/j.cmet.2017.08.009
38 Kazak, L., Chouchani, E.T., Jedrychowski, M.P., Erickson, B.K., Shinoda, K., Cohen, P. et al. (2015) A creatine-driven substrate cycle enhances energy expenditure and thermogenesis in beige fat. *Cell* **163**, 643–655 https://doi.org/10.1016/j.cell.2015.09.035
39 Mottillo, E.P., Balasubramanian, P., Lee, Y.-H., Weng, C., Kershaw, E.E. and Granneman, J.G. (2014) Coupling of lipolysis and de novo lipogenesis in brown, beige, and white adipose tissues during chronic beta3-adrenergic receptor activation. *J. Lipid Res.* **55**, 2276–2286 https://doi.org/10.1194/jlr.M050005
40 Dulloo, A.G. and Jacquet, J. (1998) Adaptive reduction in basal metabolic rate in response to food deprivation in humans: a role for feedback signals from fat stores. *Am. J. Clin. Nutr.* **68**, 599–606 https://doi.org/10.1093/ajcn/68.3.599
41 Leibel, R.L., Rosenbaum, M. and Hirsch, J. (1995) Changes in energy expenditure resulting from altered body weight. *N. Engl. J. Med.* **332**, 621–628 https://doi.org/10.1056/NEJM199503093321001
42 Luke, A. and Schoeller, D.A. (1992) Basal metabolic rate, fat-free mass, and body cell mass during energy restriction. *Metabolism* **41**, 450–456 https://doi.org/10.1016/0026-0495(92)90083-m
43 Hainer, V., Stunkard, A.J., Kunešová, M., Parízková, J., Štich, V. and Allison, D.B. (2000) Intrapair resemblance in very low calorie diet-induced weight loss in female obese identical twins. *Int. J. Obes. Relat. Metab. Disord.* **24**, 1051–1057 https://doi.org/10.1038/sj.ijo.0801358
44 Bouchard, C., Tremblay, A., Després, J.-P., Nadeau, A., Lupien, P.J., Thériault, G. et al. (1990) The response to long-term overfeeding in identical twins. *N Engl J Med* **322**, 1477–1482 https://doi.org/10.1056/NEJM199005243222101
45 Bouillaud, F., Villarroya, F., Hentz, E., Raimbault, S., Cassard, A.-M. and Ricquier, D. (1988) Detection of brown adipose tissue uncoupling protein mRNA in adult patients by a human genomic probe. *Clin Sci (Lond)* **75**, 21–27 https://doi.org/10.1042/cs0750021
46 Lean, M.E., James, W.P., Jennings, G. and Trayhurn, P. (1986) Brown adipose tissue in patients with phaeochromocytoma. *Int. J. Obes.* **10**, 219–227 PMID:3019909
47 Cypess, A.M., Lehman, S., Williams, G., Tal, I., Rodman, D., Goldfine, A.B. et al. (2009) Identification and importance of brown adipose tissue in adult humans. *N. Engl. J. Med.* **360**, 1509–1517 https://doi.org/10.1056/NEJMoa0810780
48 van Marken Lichtenbelt, W.D., Vanhommerig, J.W., Smulders, N.M., Drossaerts, J.M.A.F.L., Kemerink, G.J., Bouvy, N.D. et al. (2009) Cold-activated brown adipose tissue in healthy men. *N. Engl. J. Med.* **360**, 1500–1508 https://doi.org/10.1056/NEJMoa0808718
49 Virtanen, K.A., Lidell, M.E., Orava, J., Heglind, M., Westergren, R., Niemi, T. et al. (2009) Functional brown adipose tissue in healthy adults. *N. Engl. J. Med.* **360**, 1518–1525 https://doi.org/10.1056/NEJMoa0808949
50 U Din, M., Saari, T., Raiko, J., Kudomi, N., Maurer, S.F., Lahesmaa, M. et al. (2018) Postprandial oxidative metabolism of human brown fat indicates thermogenesis. *Cell Metab.* **28**, 207–216.e3 https://doi.org/10.1016/j.cmet.2018.05.020
51 Fischer, A.W., Schlein, C., Cannon, B., Heeren, J. and Nedergaard, J. (2019) Intact innervation is essential for diet-induced recruitment of brown adipose tissue. *Am. J. Physiol. Endocrinol. Metab* **316**, E487–E503 https://doi.org/10.1152/ajpendo.00443.2018
52 Carpentier, A.C., Blondin, D.P., Virtanen, K.A., Richard, D., Haman, F. and Turcotte, É.E. (2018) Brown adipose tissue energy metabolism in humans. *Front. Endocrinol (Lausanne)* **9**, 447 https://doi.org/10.3389/fendo.2018.00447
53 Leitner, B.P., Huang, S., Brychta, R.J., Duckworth, C.J., Baskin, A.S., McGehee, S. et al. (2017) Mapping of human brown adipose tissue in lean and obese young men. *Proc. Natl Acad. Sci. U.S.A.* **114**, 8649–8654 https://doi.org/10.1073/pnas.1705287114
54 Leibel, R.L. and Hirsch, J. (1984) Diminished energy requirements in reduced-obese patients. *Metabolism* **33**, 164–170 https://doi.org/10.1016/0026-0495(84)90130-6
55 Rosenbaum, M., Hirsch, J., Gallagher, D.A. and Leibel, R.L. (2008) Long-term persistence of adaptive thermogenesis in subjects who have maintained a reduced body weight. *Am. J. Clin. Nutr.* **88**, 906–912 https://doi.org/10.1093/ajcn/88.4.906
56 Fedorenko, A., Lishko, P.V. and Kirichok, Y. (2012) Mechanism of fatty-acid-dependent UCP1 uncoupling in brown fat mitochondria. *Cell* **151**, 400–413 https://doi.org/10.1016/j.cell.2012.09.010
57 Enerbäck, S., Jacobsson, A., Simpson, E.M., Guerra, C., Yamashita, H., Harper, M.-E. et al. (1997) Mice lacking mitochondrial uncoupling protein are cold-sensitive but not obese. *Nature* **387**, 90–94 https://doi.org/10.1038/387090a0
58 Golozoubova, V., Hohtola, E., Matthias, A., Jacobsson, A., Cannon, B. and Nedergaard, J. (2001) Only UCP1 can mediate adaptive nonshivering thermogenesis in the cold. *FASEB J* **15**, 2048–2050 https://doi.org/10.1096/fj.00-0536fje

Biochemical Journal (2020) **477** 709–725
https://doi.org/10.1042/BCJ20190463

59 Meyer, C.W., Willershäuser, M., Jastroch, M., Rourke, B.C., Fromme, T., Oelkrug, R. et al. (2010) Adaptive thermogenesis and thermal conductance in wild-type and UCP1-KO mice. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **299**, R1396–R1406 https://doi.org/10.1152/ajpregu.00021.2009
60 Keipert, S., Kutschke, M., Ost, M., Schwarzmayr, T., van Schothorst, E.M., Lamp, D. et al. (2017) Long-term cold adaptation does not require FGF21 or UCP1. *Cell Metab.* **26**, 437–446 e435 https://doi.org/10.1016/j.cmet.2017.07.016
61 Golozoubova, V., Cannon, B. and Nedergaard, J. (2006) UCP1 is essential for adaptive adrenergic nonshivering thermogenesis. *Am. J. Physiol. Endocrinol. Metab.* **291**, E350–E357 https://doi.org/10.1152/ajpendo.00387.2005
62 Hofmann, W.E., Liu, X., Bearden, C.M., Harper, M.E. and Kozak, L.P. (2001) Effects of genetic background on thermoregulation and fatty acid-induced uncoupling of mitochondria in UCP1-deficient mice. *J. Biol. Chem.* **276**, 12460–12465 https://doi.org/10.1074/jbc.M100466200
63 Wang, Y., Kimura, K., Inokuma, K.I., Saito, M., Kontani, Y., Kobayashi, Y. et al. (2006) Potential contribution of vasoconstriction to suppression of heat loss and homeothermic regulation in UCP1-deficient mice. *Pflugers Arch.* **452**, 363–369 https://doi.org/10.1007/s00424-005-0036-3
64 Kazak, L., Chouchani, E.T., Stavrovskaya, I.G., Lu, G.Z., Jedrychowski, M.P., Egan, D.F. et al. (2017) UCP1 deficiency causes brown fat respiratory chain depletion and sensitizes mitochondria to calcium overload-induced dysfunction. *Proc. Natl Acad. Sci. U.S.A.* **114**, 7981–7986 https://doi.org/10.1073/pnas.1705406114
65 Cannon, B. and Nedergaard, J. (2011) Nonshivering thermogenesis and its adequate measurement in metabolic studies. *J. Exp. Biol.* **214**, 242–253 https://doi.org/10.1242/jeb.050989
66 Matthias, A., Ohlson, K.B.E., Fredriksson, J.M., Jacobsson, A., Nedergaard, J. and Cannon, B. (2000) Thermogenic responses in brown fat cells are fully UCP1-dependent. UCP2 or UCP3 do not substitute for UCP1 in adrenergically or fatty scid-induced thermogenesis. *J. Biol. Chem.* **275**, 25073–25081 https://doi.org/10.1074/jbc.M000547200
67 Oelkrug, R., Kutschke, M., Meyer, C.W., Heldmaier, G. and Jastroch, M. (2010) Uncoupling protein 1 decreases superoxide production in brown adipose tissue mitochondria. *J. Biol. Chem.* **285**, 21961–21968 https://doi.org/10.1074/jbc.M110.122861
68 Rothwell, N.J. and Stock, M.J. (1997) A role for brown adipose tissue in diet-induced thermogenesis. *Obes. Res.* **5**, 650–656 https://doi.org/10.1002/j.1550-8528.1997.tb00591.x
69 Feldmann, H.M., Golozoubova, V., Cannon, B. and Nedergaard, J. (2009) UCP1 ablation induces obesity and abolishes diet-induced thermogenesis in mice exempt from thermal stress by living at thermoneutrality. *Cell Metab.* **9**, 203–209 https://doi.org/10.1016/j.cmet.2008.12.014
70 Maurer, S.F., Fromme, T., Mocek, S., Zimmermann, A. and Klingenspor, M. (2019) Uncoupling protein 1 and the capacity for non-shivering thermogenesis are components of the glucose homeostatic system. *Am. J. Physiol. Endocrinol. Metab.* **318**, E198–E215 https://doi.org/10.1152/ajpendo.00121.2019
71 Fromme, T., Hüttinger, K., Maurer, S., Li, Y., Gantert, T., Fiamoncini, J. et al. (2019) Bile acid supplementation decreases body mass gain in C57BL/6J but not 129S6/SvEvTac mice without increasing energy expenditure. *Sci. Rep.* **9**, 131 https://doi.org/10.1038/s41598-018-37464-z
72 Liu, X., Rossmeisl, M., McClaine, J. and Kozak, L.P. (2003) Paradoxical resistance to diet-induced obesity in UCP1-deficient mice. *J. Clin. Invest.* **111**, 399–407 https://doi.org/10.1172/JCI15737
73 Zietak, M. and Kozak, L.P. (2016) Bile acids induce uncoupling protein 1-dependent thermogenesis and stimulate energy expenditure at thermoneutrality in mice. *Am. J. Physiol. Endocrinol. Metab.* **310**, E346–E354 https://doi.org/10.1152/ajpendo.00485.2015
74 Hamann, A., Flier, J.S. and Lowell, B.B. (1996) Decreased brown fat markedly enhances susceptibility to diet-induced obesity, diabetes, and hyperlipidemia. *Endocrinology* **137**, 21–29 https://doi.org/10.1210/endo.137.1.8536614
75 Douris, N., Desai, B.N., Fisher,.M., Cisu, T., Fowler, A.J., Zarebidaki, E. et al. (2017) Beta-adrenergic receptors are critical for weight loss but not for other metabolic adaptations to the consumption of a ketogenic diet in male mice. *Mol. Metab.* **6**, 854–862 https://doi.org/10.1016/j.molmet.2017.05.017
76 Granneman, J.G., Burnazi, M., Zhu, Z. and Schwamb, L.A. (2003) White adipose tissue contributes to UCP1-independent thermogenesis. *Am. J. Physiol. Endocrinol. Metab.* **285**, E1230–E1236 https://doi.org/10.1152/ajpendo.00197.2003
77 Ukropec, J., Anunciado, R.V., Ravussin, Y. and Kozak, L.P. (2006) Leptin is required for uncoupling protein-1-independent thermogenesis during cold stress. *Endocrinology* **147**, 2468–2480 https://doi.org/10.1210/en.2005-1216
78 Shabalina, I.G., Petrovic, N., de Jong, J.M.A., Kalinovich, A.V., Cannon, B. and Nedergaard, J. (2013) UCP1 in brite/beige adipose tissue mitochondria is functionally thermogenic. *Cell Rep.* **5**, 1196–1203 https://doi.org/10.1016/j.celrep.2013.10.044
79 Antonacci, M.A., McHugh, C., Kelley, M., McCallister, A., Degan, S. and Branca, R.T. (2019) Direct detection of brown adipose tissue thermogenesis in UCP1$^{-/-}$ mice by hyperpolarized (129)Xe MR thermometry. *Sci. Rep.* **9**, 14865 https://doi.org/10.1038/s41598-019-51483-4
80 Shabalina, I.G., Hoeks, J., Kramarova, T.V., Schrauwen, P., Cannon, B. and Nedergaard, J. (2010) Cold tolerance of UCP1-ablated mice: a skeletal muscle mitochondria switch toward lipid oxidation with marked UCP3 up-regulation not associated with increased basal, fatty acid- or ROS-induced uncoupling or enhanced GDP effects. *Biochim. Biophys. Acta* **1797**, 968–980 https://doi.org/10.1016/j.bbabio.2010.02.033
81 Monemdjou, S., Hofmann, W.E., Kozak, L.P. and Harper, M.E. (2000) Increased mitochondrial proton leak in skeletal muscle mitochondria of UCP1-deficient mice. *Am. J. Physiol. Endocrinol. Metab.* **279**, E941–E946 https://doi.org/10.1152/ajpendo.2000.279.4.E941
82 Mineo, P.M., Cassell, E.A., Roberts, M.E. and Schaeffer, P.J. (2012) Chronic cold acclimation increases thermogenic capacity, non-shivering thermogenesis and muscle citrate synthase activity in both wild-type and brown adipose tissue deficient mice. *Comp. Biochem. Physiol. A Mol. Integr. Physiol.* **161**, 395–400 https://doi.org/10.1016/j.cbpa.2011.12.012
83 Campbell, K.L. and Dicke, A.A. (2018) Sarcolipin makes heat, but is it adaptive thermogenesis? *Front. Physiol.* **9**, 714 https://doi.org/10.3389/fphys.2018.00714
84 Mills, E.L., Pierce, K.A., Jedrychowski, M.P., Garrity, R., Winther, S., Vidoni, S. et al. (2018) Accumulation of succinate controls activation of adipose tissue thermogenesis. *Nature* **560**, 102–106 https://doi.org/10.1038/s41586-018-0353-2
85 Yoneshiro, T., Wang, Q., Tajima, K., Matsushita, M., Maki, H., Igarashi, K. et al. (2019) BCAA catabolism in brown fat controls energy homeostasis through SLC25A44. *Nature* **572**, 614–619 https://doi.org/10.1038/s41586-019-1503-x
86 Braun, K., Oeckl, J., Westermeier, J., Li, Y. and Klingenspor, M. (2018) Non-adrenergic control of lipolysis and thermogenesis in adipose tissues. *J. Exp. Biol.* **221**, jeb165381 https://doi.org/10.1242/jeb.165381
87 Cereijo, R., Gavaldà-Navarro, A., Cairó, M., Quesada-López, T., Villarroya, J., Morón-Ros, S. et al. (2018) CXCL14, a brown adipokine that mediates brown-fat-to-macrophage communication in thermogenic adaptation. *Cell Metab.* **28**, 750–763.e6 https://doi.org/10.1016/j.cmet.2018.07.015

88 Liu, D., Ceddia, R.P. and Collins, S. (2018) Cardiac natriuretic peptides promote adipose 'browning' through mTOR complex-1. *Mol. Metab.* **9**, 192–198 https://doi.org/10.1016/j.molmet.2017.12.017
89 Berlet, H.H., Bonsmann, I. and Birringer, H. (1976) Occurrence of free creatine, phosphocreatine and creatine phosphokinase in adipose tissue. *Biochim. Biophys. Acta* **437**, 166–174 https://doi.org/10.1016/0304-4165(76)90358-5
90 Müller, S., Balaz, M., Stefanicka, P., Varga, L., Amri, E.-Z., Ukropec, J. et al. (2016) Proteomic analysis of human brown adipose tissue reveals utilization of coupled and uncoupled energy expenditure pathways. *Sci. Rep.* **6**, 30030 https://doi.org/10.1038/srep30030
91 Svensson, P.A., Jernås, M., Sjöholm, K., Hoffmann, J.M., Nilsson, B.E., Hansson, M. et al. (2011) Gene expression in human brown adipose tissue. *Int. J. Mol. Med.* **27**, 227–232 https://doi.org/10.3892/ijmm.2010.566
92 Jash, S., Banerjee, S., Lee, M.J., Farmer, S.R. and Puri, V. (2019) CIDEA transcriptionally regulates UCP1 for britening and thermogenesis in human fat cells. *iScience* **20**, 73–89 https://doi.org/10.1016/j.isci.2019.09.011
93 Jespersen, N.Z., Feizi, A., Andersen, E.S., Heywood, S., Hattel, H.B., Daugaard, S. et al. (2019) Heterogeneity in the perirenal region of humans suggests presence of dormant brown adipose tissue that contains brown fat precursor cells. *Mol. Metab.* **24**, 30–43 https://doi.org/10.1016/j.molmet.2019.03.005
94 Bertholet, A.M., Kazak, L., Chouchani, E.T., Bogaczyńska, M.G., Paranjpe, I., Wainwright, G.L. et al. (2017) Mitochondrial patch clamp of beige adipocytes reveals UCP1-positive and UCP1-negative cells both exhibiting futile creatine cycling. *Cell Metab.* **25**, 811–822 e814 https://doi.org/10.1016/j.cmet.2017.03.002
95 Yamashita, H., Ohira, Y., Wakatsuki, T., Yamamoto, M., Kizaki, T., Oh-ishi, S. et al. (1995) Increased growth of brown adipose tissue but its reduced thermogenic activity in creatine-depleted rats fed beta-guanidinopropionic acid. *Biochim. Biophys. Acta* **1230**, 69–73 https://doi.org/10.1016/0005-2728(95)00067-S
96 Wakatsuki, T., Hirata, F., Ohno, H., Yamamoto, M., Sato, Y. and Ohira, Y. (1996) Thermogenic responses to high-energy phosphate contents and/or hindlimb suspension in rats. *Jpn. J. Physiol.* **46**, 171–175 https://doi.org/10.2170/jjphysiol.46.171
97 Wada, S., Neinast, M., Jang, C., Ibrahim, Y.H., Lee, G., Babu, A. et al. (2016) The tumor suppressor FLCN mediates an alternate mTOR pathway to regulate browning of adipose tissue. *Genes Dev* **30**, 2551–2564 https://doi.org/10.1101/gad.287953.116
98 Perna, M.K., Kokenge, A.N., Miles, K.N., Udobi, K.C., Clark, J.F., Pyne-Geithman, G.J. et al. (2016) Creatine transporter deficiency leads to increased whole body and cellular metabolism. *Amino Acids* **48**, 2057–2065 https://doi.org/10.1007/s00726-016-2291-3
99 Bartness, T.J., Vaughan, C.H. and Song, C.K. (2010) Sympathetic and sensory innervation of brown adipose tissue. *Int. J. Obes. (Lond.)* **34** (Suppl 1), S36–S42 https://doi.org/10.1038/ijo.2010.182
100 Caron, A., Reynolds, R.P., Castorena, C.M., Michael, N.J., Lee, C.E., Lee, S. et al. (2019) Adipocyte Gs but not Gi signaling regulates whole-body glucose homeostasis. *Mol. Metab.* **27**, 11–21 https://doi.org/10.1016/j.molmet.2019.06.019
101 Pierce, K.L., Premont, R.T. and Lefkowitz, R.J. (2002) Seven-transmembrane receptors. *Nat. Rev. Mol. Cell Biol.* **3**, 639–650 https://doi.org/10.1038/nrm908
102 Pydi, S.P., Jain, S., Tung, W., Cui, Y., Zhu, L., Sakamoto, W. et al. (2019) Adipocyte beta-arrestin-2 is essential for maintaining whole body glucose and energy homeostasis. *Nat. Commun.* **10**, 2936 https://doi.org/10.1038/s41467-019-11003-4
103 Wang, L., Pydi, S.P., Cui, Y., Zhu, L., Meister, J., Gavrilova, O. et al. (2019) Selective activation of Gs signaling in adipocytes causes striking metabolic improvements in mice. *Mol. Metab.* **27**, 83–91 https://doi.org/10.1016/j.molmet.2019.06.018
104 Puigserver, P., Wu, Z., Park, C.W., Graves, R., Wright, M. and Spiegelman, B.M. (1998) A cold-inducible coactivator of nuclear receptors linked to adaptive thermogenesis. *Cell* **92**, 829–839 https://doi.org/10.1016/S0092-8674(00)81410-5
105 Lee, C.C., Shih, Y.C., Kang, M.L., Chang, Y.C., Chuang, L.M., Devaraj, R. et al. (2019) Naa10p inhibits beige adipocyte-mediated thermogenesis through *N*-alpha-acetylation of Pgc1alpha. *Mol. Cell* **76**, 500–515.e8 https://doi.org/10.1016/j.molcel.2019.07.026
106 Peleli, M., Ferreira, D.M.S., Tarnawski, L., McCann Haworth, S., Xuechen, L., Zhuge, Z. et al. (2019) Dietary nitrate attenuates high-fat diet-induced obesity via mechanisms involving higher adipocyte respiration and alterations in inflammatory status. *Redox Biol.* **28**, 101387 https://doi.org/10.1016/j.redox.2019.101387
107 Wikstrom, J.D., Mahdaviani, K., Liesa, M., Sereda, S.B., Si, Y., Las, G. et al. (2014) Hormone-induced mitochondrial fission is utilized by brown adipocytes as an amplification pathway for energy expenditure. *EMBO J.* **33**, 418–436 https://doi.org/10.1002/embj.201385014
108 Friedman, J.R., Lackner, L.L., West, M., DiBenedetto, J.R., Nunnari, J. and Voeltz, G.K. (2011) ER tubules mark sites of mitochondrial division. *Science* **334**, 358–362 https://doi.org/10.1126/science.1207385
109 Watanabe, M., Yamamoto, T., Kakuhata, R., Okada, N., Kajimoto, K., Yamazaki, N. et al. (2008) Synchronized changes in transcript levels of genes activating cold exposure-induced thermogenesis in brown adipose tissue of experimental animals. *Biochim. Biophys. Acta* **1777**, 104–112 https://doi.org/10.1016/j.bbabio.2007.10.014
110 Dieni, C.A. and Storey, K.B. (2009) Creatine kinase regulation by reversible phosphorylation in frog muscle. *Comp. Biochem. Physiol. B. Biochem. Mol. Biol.* **152**, 405–412 https://doi.org/10.1016/j.cbpb.2009.01.012
111 Zotter, A., Bauerle, F., Dey, D., Kiss, V. and Schreiber, G. (2017) Quantifying enzyme activity in living cells. *J. Biol. Chem.* **292**, 15838–15848 https://doi.org/10.1074/jbc.M117.792119
112 Schlattner, U., Eder, M., Dolder, M., Khuchua, Z.A., Strauss, A.W. and Wallimann, T. (2000) Divergent enzyme kinetics and structural properties of the two human mitochondrial creatine kinase isoenzymes. *Biol. Chem.* **381**, 1063–1070 https://doi.org/10.1515/BC.2000.131
113 Menniti, F.S., Knoth, J., Peterson, D.S. and Diliberto, Jr, E.J. (1987) The in situ kinetics of dopamine beta-hydroxylase in bovine adrenomedullary chromaffin cells. Intravesicular compartmentation reduces apparent affinity for the cofactor ascorbate. *J. Biol. Chem.* **262**, 7651–7657 PMID:3584135
114 Nakae, Y. and Stoward, P.J. (1997) Kinetic parameters of lactate dehydrogenase in liver and gastrocnemius determined by three quantitative histochemical methods. *J. Histochem. Cytochem.* **45**, 1427–1431 https://doi.org/10.1177/002215549704501011
115 Joel, C.D., Neaves, W.B. and Rabb, J.M. (1967) Mitochondria of brown fat: oxidative phosphorylation sensitive to 2,4,-dinitrophenol. *Biochem. Biophys. Res. Commun.* **29**, 490–495 https://doi.org/10.1016/0006-291x(67)90510-4
116 Guillory, R.J. and Racker, E. (1968) Oxidative phosphorylation in brown adipose mitochondria. *Biochim. Biophys. Acta* **153**, 490–493 https://doi.org/10.1016/0005-2728(68)90091-1

Biochemical Journal (2020) **477** 709–725
https://doi.org/10.1042/BCJ20190463

117 Hittelman, K.J., Lindberg, O. and Cannon, B. (1969) Oxidative phosphorylation and compartmentation of fatty acid metabolism in brown fat mitochondria. *Eur. J. Biochem.* **11**, 183–192 https://doi.org/10.1111/j.1432-1033.1969.tb00759.x

118 Williamson, J.R. (1970) Control of energy metabolism in hamster brown adipose tissue. *J. Biol. Chem.* **245**, 2043–2050 PMID:4191717

119 Bal, N.C., Maurya, S.K., Sopariwala, D.H., Sahoo, S.K., Gupta, S.C., Shaikh, S.A. et al. (2012) Sarcolipin is a newly identified regulator of muscle-based thermogenesis in mammals. *Nat. Med.* **18**, 1575–1579 https://doi.org/10.1038/nm.2897

120 MacPherson, R.E., Gamu, D., Frendo-Cumbo, S., Castellani, L., Kwon, F., Tupling, A.R. et al. (2016) Sarcolipin knockout mice fed a high-fat diet exhibit altered indices of adipose tissue inflammation and remodeling. *Obesity (Silver Spring)* **24**, 1499–1505 https://doi.org/10.1002/oby.21521

121 Song, A., Dai, W., Jang, M.J., Medrano, L., Li, Z., Zhao, H. et al. (2019) Low- and high-thermogenic brown adipocyte subpopulations coexist in murine adipose tissue. *J. Clin. Invest.* **130**, 247–257 https://doi.org/10.1172/JCI129167

122 Chen, Y., Ikeda, K., Yoneshiro, T., Scaramozza, A., Tajima, K., Wang, Q. et al. (2019) Thermal stress induces glycolytic beige fat formation via a myogenic state. *Nature* **565**, 180–185 https://doi.org/10.1038/s41586-018-0801-z

123 Wyss, M. and Kaddurah-Daouk, R. (2000) Creatine and creatinine metabolism. *Physiol. Rev.* **80**, 1107–1213 https://doi.org/10.1152/physrev.2000.80.3.1107

124 Wallimann, T., Wyss, M., Brdiczka, D., Nicolay, K. and Eppenberger, H.M. (1992) Intracellular compartmentation, structure and function of creatine kinase isoenzymes in tissues with high and fluctuating energy demands: the 'phosphocreatine circuit' for cellular energy homeostasis. *Biochem. J.* **281** (Pt 1), 21–40 https://doi.org/10.1042/bj2810021

125 Steinberg, D. (1963) Fatty acid mobilization–mechanisms of regulation and metabolic consequences. *Biochem. Soc. Symp.* **24**, 111–143 PMID:5335113

126 Wolfe, R.R., Herndon, D.N., Jahoor, F., Miyoshi, H. and Wolfe, M. (1987) Effect of severe burn injury on substrate cycling by glucose and fatty acids. *N. Engl. J. Med.* **317**, 403–408 https://doi.org/10.1056/NEJM198708133170702

127 Wolfe, R.R., Klein, S., Carraro, F. and Weber, J.M. (1990) Role of triglyceride-fatty acid cycle in controlling fat metabolism in humans during and after exercise. *Am. J. Physiol.* **258**, E382–E389 https://doi.org/10.1152/ajpendo.1990.258.2.E382

128 Yu, X.X., Lewin, D.A., Forrest, W. and Adams, S.H. (2002) Cold elicits the simultaneous induction of fatty acid synthesis and beta-oxidation in murine brown adipose tissue: prediction from differential gene expression and confirmation in vivo. *FASEB J.* **16**, 155–168 https://doi.org/10.1096/fj.01-0568com

129 Guan, H.P., Li, Y., Jensen, M.V., Newgard, C.B., Steppan, C.M. and Lazar, M.A. (2002) A futile metabolic cycle activated in adipocytes by antidiabetic agents. *Nat. Med.* **8**, 1122–1128 https://doi.org/10.1038/nm780

130 Newsholme, E.A., Arch, J.R., Brooks, B. and Surholt, B. (1983) The role of substrate cycles in metabolic regulation. *Biochem. Soc. Trans.* **11**, 52–56 https://doi.org/10.1042/bst0110052

131 Brooks, B., Arch, J.R. and Newsholme, E.A. (1982) Effects of hormones on the rate of the triacylglycerol/fatty acid substrate cycle in adipocytes and epididymal fat pads. *FEBS Lett.* **146**, 327–330 https://doi.org/10.1016/0014-5793(82)80945-9

132 Flachs, P., Adamcova, K., Zouhar, P., Marques, C., Janovska, P., Viegas, I. et al. (2017) Induction of lipogenesis in white fat during cold exposure in mice: link to lean phenotype. *Int. J. Obes. (Lond.)* **41**, 997 https://doi.org/10.1038/ijo.2017.61

133 Schweizer, S., Oeckl, J., Klingenspor, M. and Fromme, T. (2018) Substrate fluxes in brown adipocytes upon adrenergic stimulation and uncoupling protein 1 ablation. *Life Sci. Alliance* **1**, e201800136 https://doi.org/10.26508/lsa.201800136

134 Shi, Y. and Cheng, D. (2009) Beyond triglyceride synthesis: the dynamic functional roles of MGAT and DGAT enzymes in energy metabolism. *Am. J. Physiol. Endocrinol. Metab.* **297**, E10–E18 https://doi.org/10.1152/ajpendo.90949.2008

135 Nagle, C.A., Vergnes, L., DeJong, H., Wang, S., Lewin, T.M., Reue, K. et al. (2008) Identification of a novel sn-glycerol-3-phosphate acyltransferase isoform, GPAT4, as the enzyme deficient in Agpat6$^{-/-}$ mice. *J. Lipid Res.* **49**, 831–823 https://doi.org/10.1194/jlr.M700592-JLR200

136 Vergnes, L., Beigneux, A.P., Davis, R., Watkins, S.M., Young, S.G. and Reue, K. (2006) Agpat6 deficiency causes subdermal lipodystrophy and resistance to obesity. *J. Lipid Res.* **47**, 745–754 https://doi.org/10.1194/jlr.M500553-JLR200

137 Agarwal, A.K., Arioglu, E., de Almeida, S., Akkoc, N., Taylor, S.I., Bowcock, A.M. et al. (2002) AGPAT2 is mutated in congenital generalized lipodystrophy linked to chromosome 9q34. *Nat. Genet.* **31**, 21–23 https://doi.org/10.1038/ng880

138 Cortés, V.A., Curtis, D.E., Sukumaran, S., Shao, X., Parameswara, V., Rashid, S. et al. (2009) Molecular mechanisms of hepatic steatosis and insulin resistance in the AGPAT2-deficient mouse model of congenital generalized lipodystrophy. *Cell Metab.* **9**, 165–176 https://doi.org/10.1016/j.cmet.2009.01.002

139 Peterfy, M., Phan, J., Xu, P. and Reue, K. (2001) Lipodystrophy in the fld mouse results from mutation of a new gene encoding a nuclear protein, lipin. *Nat. Genet.* **27**, 121–124 https://doi.org/10.1038/83685

140 Phan, J. and Reue, K. (2005) Lipin, a lipodystrophy and obesity gene. *Cell Metab* **1**, 73–83 https://doi.org/10.1016/j.cmet.2004.12.002

141 Smith, S.J., Cases, S., Jensen, D.R., Chen, H.C., Sande, E., Tow, B. et al. (2000) Obesity resistance and multiple mechanisms of triglyceride synthesis in mice lacking Dgat. *Nat. Genet.* **25**, 87–90 https://doi.org/10.1038/75651

142 Chen, H.C., Ladha, Z., Smith, S.J. and Farese, Jr, R.V. (2003) Analysis of energy expenditure at different ambient temperatures in mice lacking DGAT1. *Am. J. Physiol. Endocrinol. Metab.* **284**, E213–E218 https://doi.org/10.1152/ajpendo.00248.2002

143 Stone, S.J., Myers, H.M., Watkins, S.M., Brown, B.E., Feingold, K.R., Elias, P.M. et al. (2004) Lipopenia and skin barrier abnormalities in DGAT2-deficient mice. *J. Biol. Chem.* **279**, 11767–11776 https://doi.org/10.1074/jbc.M311000200

144 Yen, C.L., Cheong, M.-L., Grueter, C., Zhou, P., Moriwaki, J., Wong, J.S. et al. (2009) Deficiency of the intestinal enzyme acyl CoA:monoacylglycerol acyltransferase-2 protects mice from metabolic disorders induced by high-fat feeding. *Nat. Med.* **15**, 442–446 https://doi.org/10.1038/nm.1937

145 Ellis, J.M., Li, L.O., Wu, P.-C., Koves, T.R., Ilkayeva, O., Stevens, R.D. et al. (2010) Adipose acyl-CoA synthetase-1 directs fatty acids toward beta-oxidation and is required for cold thermogenesis. *Cell Metab.* **12**, 53–64 https://doi.org/10.1016/j.cmet.2010.05.012

146 Sepa-Kishi, D.M., Jani, S., Da Eira, D. and Ceddia, R.B. (2019) Cold acclimation enhances UCP1 content, lipolysis, and triacylglycerol resynthesis, but not mitochondrial uncoupling and fat oxidation, in rat white adipocytes. *Am. J. Physiol. Cell Physiol.* **316**, C365–C376 https://doi.org/10.1152/ajpcell.00122.2018

147 Nedergaard, J. (1981) Effects of cations on brown adipose tissue in relation to possible metabolic consequences of membrane depolarisation. *Eur. J. Biochem.* **114**, 159–167 https://doi.org/10.1111/j.1432-1033.1981.tb06187.x

148 Chen, Y., Zeng, X., Huang, X., Serag, S., Woolf, C.J. and Spiegelman, B.M. (2017) Crosstalk between KCNK3-mediated ion current and adrenergic signaling regulates adipose thermogenesis and obesity. *Cell* **171**, 836–848 e813 https://doi.org/10.1016/j.cell.2017.09.015
149 Al-Shaikhaly, M.H., Nedergaard, J. and Cannon, B. (1979) Sodium-induced calcium release from mitochondria in brown adipose tissue. *Proc. Natl Acad. Sci. U.S.A.* **76**, 2350–2353 https://doi.org/10.1073/pnas.76.5.2350
150 Anunciado-Koza, R.P., Zhang, J., Ukropec, J., Bajpeyi, S., Koza, R.A. and Rogers, R.C. (2011) Inactivation of the mitochondrial carrier SLC25A25 (ATP-Mg^{2+}/Pi transporter) reduces physical endurance and metabolic efficiency in mice. *J. Biol. Chem.* **286**, 11659–11671 https://doi.org/10.1074/jbc.M110.203000
151 Arruda, A.P., Nigro, M., Oliveira, G.M. and de Meis, L. (2007) Thermogenic activity of Ca^{2+}-ATPase from skeletal muscle heavy sarcoplasmic reticulum: the role of ryanodine Ca^{2+} channel. *Biochim. Biophys. Acta* **1768**, 1498–1505 https://doi.org/10.1016/j.bbamem.2007.03.016
152 Kjelstrup, S., Barragan, D. and Bedeaux, D. (2009) Coefficients for active transport and thermogenesis of Ca^{2+}-ATPase isoforms. *Biophys. J.* **96**, 4376–4386 https://doi.org/10.1016/j.bpj.2009.02.070
153 Bal, N.C., Maurya, S.K., Singh, S., Wehrens, X.H. and Periasamy, M. (2016) Increased reliance on muscle-based thermogenesis upon acute minimization of brown adipose tissue function. *J. Biol. Chem.* **291**, 17247–17257 https://doi.org/10.1074/jbc.M116.728188
154 DosSantos, R.A., Alfadda, A., Eto, K., Kadowaki, T. and Silva, J.E. (2003) Evidence for a compensated thermogenic defect in transgenic mice lacking the mitochondrial glycerol-3-phosphate dehydrogenase gene. *Endocrinology* **144**, 5469–5479 https://doi.org/10.1210/en.2003-0687
155 Babu, G.J., Bhupathy, P., Timofeyev, V., Petrashevskaya, N.N., Reiser, P.J., Chiamvimonvat, N. et al. (2007) Ablation of sarcolipin enhances sarcoplasmic reticulum calcium transport and atrial contractility. *Proc. Natl Acad. Sci. U.S.A.* **104**, 17867–17872 https://doi.org/10.1073/pnas.0707722104
156 Odermatt, A., Becker, S., Khanna, V.K., Kurzydlowski, K., Leisner, E., Pette, D. et al. (1998) Sarcolipin regulates the activity of SERCA1, the fast-twitch skeletal muscle sarcoplasmic reticulum Ca^{2+}-ATPase. *J. Biol. Chem.* **273**, 12360–12369 https://doi.org/10.1074/jbc.273.20.12360
157 Smith, W.S., Broadbridge, R., East, J.M. and Lee, A.G. (2002) Sarcolipin uncouples hydrolysis of ATP from accumulation of Ca^{2+} by the Ca^{2+}-ATPase of skeletal-muscle sarcoplasmic reticulum. *Biochem. J.* **361**, 277–286 https://doi.org/10.1042/0264-6021:3610277
158 Fujii, J., Kadoma, M., Tada, M., Toda, H. and Sakiyama, F. (1986) Characterization of structural unit of phospholamban by amino acid sequencing and electrophoretic analysis. *Biochem. Biophys. Res. Commun.* **138**, 1044–1050 https://doi.org/10.1016/s0006-291x(86)80387-4
159 Jastroch, M., Divakaruni, A.S., Mookerjee, S., Treberg, J.R. and Brand, M.D. (2010) Mitochondrial proton and electron leaks. *Essays Biochem.* **47**, 53–67 https://doi.org/10.1042/bse0470053
160 Levy, S.E., Chen, Y.S., Graham, B.H. and Wallace, D.C. (2000) Expression and sequence analysis of the mouse adenine nucleotide translocase 1 and 2 genes. *Gene* **254**, 57–66 https://doi.org/10.1016/s0378-1119(00)00252-3
161 Brand, M.D., Pakay, J.L., Ocloo, A., Kokoszka, J., Wallace, D.C., Brookes, P.S. et al. (2005) The basal proton conductance of mitochondria depends on adenine nucleotide translocase content. *Biochem. J.* **392**, 353–362 https://doi.org/10.1042/BJ20050890
162 Bertholet, A.M., Chouchani, E.T., Kazak, L., Angelin, A., Fedorenko, A., Long, J.Z. et al. (2019) H(+) transport is an integral function of the mitochondrial ADP/ATP carrier. *Nature* **571**, 515–520 https://doi.org/10.1038/s41586-019-1400-3
163 Chouchani, E.T., Kazak, L., Jedrychowski, M.P., Lu, G.Z., Erickson, B.K., Szpyt, J. et al. (2016) Mitochondrial ROS regulate thermogenic energy expenditure and sulfenylation of UCP1. *Nature* **532**, 112–116 https://doi.org/10.1038/nature17399
164 Tainter, M.L., Cutting, W.C. and Stockton, A.B. (1934) Use of dinitrophenol in nutritional disorders : a critical survey of clinical results. *Am. J. Public Health Nations Health* **24**, 1045–1053 https://doi.org/10.2105/ajph.24.10.1045
165 Harms, M. and Seale, P. (2013) Brown and beige fat: development, function and therapeutic potential. *Nat. Med.* **19**, 1252–1263 https://doi.org/10.1038/nm.3361
166 Kozak, L.P., Koza, R.A. and Anunciado-Koza, R. (2010) Brown fat thermogenesis and body weight regulation in mice: relevance to humans. *Int. J. Obes. (Lond.)* **34** (Suppl 1), S23–S27 https://doi.org/10.1038/ijo.2010.179
167 Blondin, D.P., Labbé, S.M., Tingelstad, H.C., Noll, C., Kunach, M., Phoenix, S. et al. (2014) Increased brown adipose tissue oxidative capacity in cold-acclimated humans. *J. Clin. Endocrinol. Metab.* **99**, E438–E446 https://doi.org/10.1210/jc.2013-3901
168 Blondin, D.P., Tingelstad, H.C., Noll, C., Frisch, F., Phoenix, S., Guérin, B. et al. (2017) Dietary fatty acid metabolism of brown adipose tissue in cold-acclimated men. *Nat. Commun.* **8**, 14146 https://doi.org/10.1038/ncomms14146
169 Nedergaard, J., Bengtsson, T. and Cannon, B. (2007) Unexpected evidence for active brown adipose tissue in adult humans. *Am. J. Physiol. Endocrinol. Metab.* **293**, E444–E452 https://doi.org/10.1152/ajpendo.00691.2006