Towards a molecular understanding of adaptive thermogenesis

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Obesity results when energy intake exceeds energy expenditure. Naturally occurring genetic mutations, as well as ablative lesions, have shown that the brain regulates both aspects of energy balance and that abnormalities in energy expenditure contribute to the development of obesity. Energy can be expended by performing work or producing heat (thermogenesis). Adaptive thermogenesis, or the regulated production of heat, is influenced by environmental temperature and diet. Mitochondria, the organelles that convert food to carbon dioxide, water and ATP, are fundamental in mediating effects on energy dissipation. Recently, there have been significant advances in understanding the molecular regulation of energy expenditure in mitochondria and the mechanisms of transcriptional control of mitochondrial genes. Here we explore these developments in relation to classical physiological views of adaptive thermogenesis.

I t is useful to analyse energy expenditure from a thermodynamic perspective. Such assessment treats the organism as a black box, with energy entering as food and exiting as heat and work (Fig. 1). Obesity is the result of energy imbalance over time and, owing to its cumulative nature, it can develop when energy intake exceeds energy expenditure by only a small margin. Total body energy expenditure represents the conversion of oxygen and food (or stored forms of energy such as fat, glycogen and protein) to carbon dioxide, water, heat and work on the environment. The generation of heat is due to the fact that many reactions in energy metabolism, such as those catalysed by the mitochondrial respiratory chain, and those that consume ATP (for example, Na^+/K^+ ATPase, Ca^{2+} ATPase, and actinomyosin ATPase), are exothermic in the forward direction. Work performed on the environment by the organism plus heat released during biological combustion of food equals the amount of energy given off as heat, measured as calories, during 'physical combustion' of food. When the organism is at rest, and therefore not performing work on the environment, energy expenditure can be measured directly as heat produced (direct calorimetry), hence the term thermogenesis, or indirectly as the amount of oxygen consumed (indirect calorimetry) (Box 1).

Adaptive thermogenesis, also referred to as facultative thermogenesis, is defined operationally as heat production in response to environmental temperature or diet, and serves the purpose of protecting the organism from cold exposure or regulating energy balance after changes in diet. The term usually refers to adaptations of an individual but could also be viewed in the context of adaptations by different phyla or even different species within a phylum to organism–environment interactions (Box 1). In rodents, brown adipose tissue is a major site of adaptive thermogenesis (see later).

Factors influencing adaptive thermogenesis
Cold-induced thermogenesis

Energy expenditure at rest changes markedly in response to environmental temperature. Oxygen consumption increases nearly two- to fourfold in rodents after both acute and chronic cold exposure (4 °C)[1,2]. A portion of the acute response is due to shivering. However, with adaptation, shivering disappears[1,2] and other mechanisms become prominent, including increased adaptive thermogenesis in

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Box 1
Standard metabolic rate

Standard metabolic rate (SMR) is the amount of energy expended when an adult organism is awake but resting, not actively digesting food and is at thermoneutrality (an environmental temperature where heat production is not stimulated, ~28 °C for adult humans). Because work is not performed on the environment, all energy expended is released as heat. Metabolic rate decreases below SMR by ~10% during sleep and by as much as 40% with starvation, and increases above SMR by as much as 10–20-fold for short periods during vigorous exercise[23]. Metabolic rate in sedentary, adult humans tends to be approximately 150–200% of SMR, with the increase above SMR being due to physical activity and the thermic effect of food. SMR varies greatly between phyla, with endotherms having a fivefold greater metabolic rate than ectotherms of the same size[23], indicating a phylogenetic adaptation necessary for the warm-blooded state. In addition, SMR per gram of body weight varies inversely with body size between species within a phylum[83], indicating a scaling adaptation necessary for smaller animals which have a larger surface area (site of heat loss) relative to their volume. Indeed, the metabolic rate of a mouse on a per weight basis is approximately ten times greater than that of a horse[83].
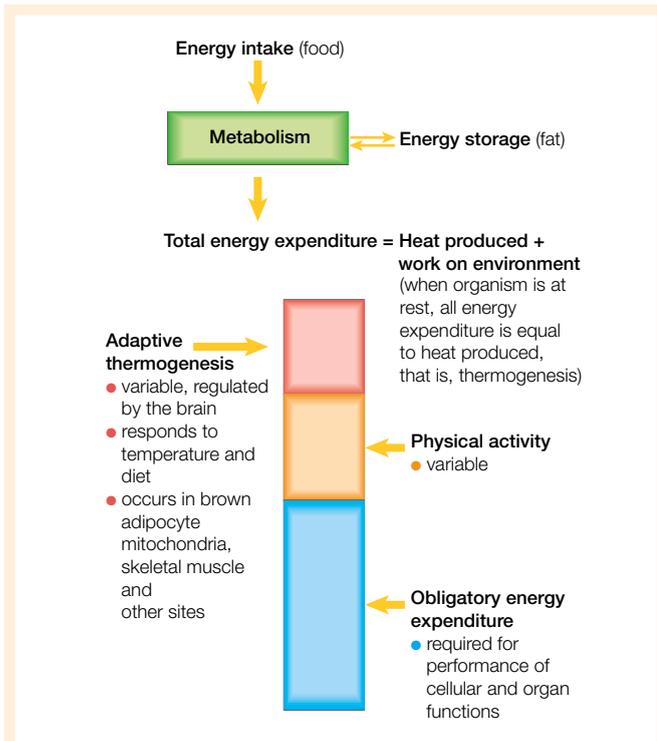
Figure 1 Thermodynamic perspective of energy expenditure. Energy enters an organism as food and exits as heat and work. Energy can also be mobilized from adipose stores. Total energy expenditure can be subdivided into three principal components: obligatory energy expenditure required for normal functioning of cells and organs; energy expenditure resulting from physical activity; and expenditure attributed to adaptive thermogenesis, which is defined as heat production in response to environmental temperature or diet.

brown adipose and possibly other tissues[3]. Energy expenditure in humans is also sensitive to environmental temperature, but the effect on metabolic rate is smaller. Lowering temperature from 28 to 22 °C has been reported to cause a 7% increase in heat production in identically clothed humans[4]. In general, humans, as opposed to rodents, have a broad thermoneutral zone with relatively small changes in metabolic rate occurring over relatively wide temperature changes. This difference is due, in part, to behavioural responses such as adjustments in the amount of clothing.

Diet-induced thermogenesis

Diet is also a potent regulator of adaptive thermogenesis. Starvation can decrease resting metabolic rate by as much as 40% (ref. 5). Similarly, food restriction sufficient to maintain a 10% reduction in body weight is associated with decreased energy expenditure[6]. The adaptive value of decreasing energy expenditure when food intake is limited is obvious. However, this response is counter-productive during dieting, contributing importantly to the poor long-term efficacy of this treatment for obesity.

Feeding, on the other hand, increases energy expenditure, having both acute and chronic effects on metabolic rate. Feeding acutely increases metabolic rate by ~25–40% in humans and rodents, a phenomenon referred to as the thermic effect of food[7,8]. Long-term overfeeding also increases energy expenditure[9]. The consequence of increased energy expenditure with overfeeding is a relative protection against the development of obesity. Of interest, this protective adaptation is influenced by genetic background[10], and abnormal responses could contribute to the development of obesity.

Diet-induced thermogenesis is particularly apparent during ingestion of diets that are low in protein. Food serves two important functions: provision of calories to meet energy demands and provision of amino acids to maintain rates of protein synthesis. If the diet is low in protein, then food intake must be increased to obtain enough protein to sustain protein biosynthesis. This would lead to obesity if the organism lacked the capacity to 'waste' excess calories. Indeed, metabolic efficiency, or the ability to store ingested calories, is decreased by as much as 40% when rodents are fed low-protein diets[11,12]. This effect may be mediated, at least in part, by stimulation of thermogenesis in brown adipose tissue[11,12].

The brain regulates adaptive thermogenesis

Exposure to cold is detected by the brain, leading to activation of efferent pathways controlling energy dissipation. The main effector component of this response is the sympathetic nervous system, which heavily innervates thermogenic targets such as brown adipose tissue and skeletal muscle. Indeed, animals treated with various blockers of the sympathetic nervous system, as well as mice lacking noradrenaline and adrenaline as a result of knockout of the dopamine β-hydroxylase gene, are unable to maintain body temperature during cold exposure[13,14]. In addition, administration of sympathomimetic agents, such as β-adrenergic-receptor agonists, cause an increase in energy expenditure which is comparable in magnitude to that induced by cold[13].

Further evidence for central control of adaptive thermogenesis comes from experimental animals with hypothalamic lesions. Destruction of neurons in the hypothalamus either by physical or chemical means results in obesity (reviewed in ref. 15). Typically, the obesity is associated with increased food intake. However, if food intake is restricted so that it equals that observed in controls, obesity still develops[16]. This increased storage of calories, during normal caloric intake, indicates that energy expenditure is decreased. These studies show that the brain has the capacity to control adaptive thermogenesis. The presence of the adipocyte-derived hormone leptin and neuropeptides, both of which regulate energy balance in the hypothalamus, is further evidence for regulation of thermogenesis by the brain and is discussed in detail in the review by Schwartz *et al.*, pp. 661–671.

The brain also affects energy expenditure by means of the hypothalamic–pituitary–thyroid axis. It is clear that increases or decreases in thyroid hormone are associated with parallel changes in energy expenditure and that relatively small changes in hormone produce significant effects[17]. The mechanism by which thyroid hormone stimulates thermogenesis is not established, but it seems to be due to multiple effects on various aspects of energy metabolism such as substrate cycling, ion cycling and mitochondrial proton leaks[18] (reviewed in ref. 19). Most researchers have viewed thyroid hormone as having, for the most part, a permissive role in adaptive thermogenesis. This is because thyroid hormone levels seem not to be modulated during cold exposure or consumption of high-calorie diets. However, this view may not be entirely correct as thyroid hormone levels have been found to rise in some models of increased caloric intake[20] and, importantly, to drop during starvation, an effect which is dependent upon falling leptin levels and mediated by decreased expression of hypothalamic thyrotropin-releasing hormone[21,22]. This indicates that falling thyroid hormone levels may contribute to starvation-induced decreases in thermogenesis.

Mitochondria convert food to ATP, CO_2 and H_2O

As explained in Box 2 and shown in Figs 2 and 3, fuel metabolism, the electron transport chain, ATP synthesis and ATP use represent coupled reactions in that fixed amounts of reactants produce stoichiometric amounts of products at each step. For example, conversion of glucose to CO_2 generates fixed amounts of NADH and $FADH_2$, oxidation of NADH and $FADH_2$ results in a fixed number of protons being pumped across the mitochondrial inner membrane, re-entry of protons by means of ATP synthase generates fixed amounts of ATP, and enzymatic steps performing cellular work use fixed amounts of ATP. For thermogenesis to increase, the degree of 'coupling' at one or more of these sites must change[23]. Alternatively,

the consequences of cellular work resulting from reactions using ATP would need to be 'undone' at an increasing rate, in essence wasting ATP as part of a futile cycle. Such reactions completing futile cycles include muscle relaxation (as part of shivering), ion leaks (Na^+ in and K^+ out across the plasma membrane and Ca^{2+} into the cytosol from intracellular stores) and protein degradation, to name a few (Fig. 3).

Prevailing evidence indicates that coupling of most reactions, however, does not change[23]. In mammalian cells, reactions that seem to be completely fixed include the amount of NADH and $FADH_2$ generated by fuel metabolism, the number of protons pumped during NADH and $FADH_2$ oxidation (with possible exceptions noted below), the number of protons used by ATP synthase to make ATP, and the amount of ATP used to perform cellular work. One firmly established site of uncoupling is the leakage of protons back across the mitochondrial inner membrane, thus bypassing ATP synthase, and converting energy stored within the protonmotive force directly to heat. Mitochondrial proton leaks are a biophysical property of proteolipid bilayers juxtaposed between a strong protonmotive force[18]. They are also catalysed by specific inner-membrane proteins such as uncoupling protein (UCP)-1, UCP-2 and UCP-3. These proteins will be discussed in more detail in the following section.

Other sites of 'uncoupling' may also exist, although evidence in support of their role is less compelling. These include decreased proton pumping by cytochrome oxidase (complex IV), mediated by an allosterically regulated heart- and muscle-specific isoform of cytochrome oxidase subunit VIa[24], or increased activity of the glycerol–phosphate shuttle, which competes with the more efficient aspartate–malate shuttle for transfer of NADH generated during glycolysis into the mitochondria for oxidation. The glycerol–phosphate shuttle is less efficient because, unlike the aspartate–malate shuttle, it converts cytosolic NADH to mitochondrial $FADH_2$, which, in contrast to NADH, bypasses the first proton pumping site in the electron transport chain (Fig. 2). It is of interest that transgenic mice overexpressing glycerol-3-phosphate dehydrogenase are lean and have increased thermogenesis[25]. Finally, the contribution of ion and substrate cycles that consume ATP — such as Na^+, K^+ and Ca^{2+} ion leaks and protein turnover — to adaptive thermogenesis in mammals is presently unknown, but could be significant. The potential importance of Ca^{2+} ion cycling in muscle is evident from examples presented in Box 3.

Regulation of UCP-1 in brown adipose tissue
Cold-induced adaptive thermogenesis

Brown adipose tissue is heavily innervated by sympathetic nerves, and is responsible for a major portion of thermogenesis during cold exposure in rodents. The primary molecule involved in cold-induced thermogenesis in brown fat is UCP-1, a mitochondrial inner-membrane protein that uncouples proton entry from ATP synthesis (Fig. 2)[26,27]. Indeed, gene-knockout mice lacking UCP-1 have decreased body temperature during cold exposure[28].

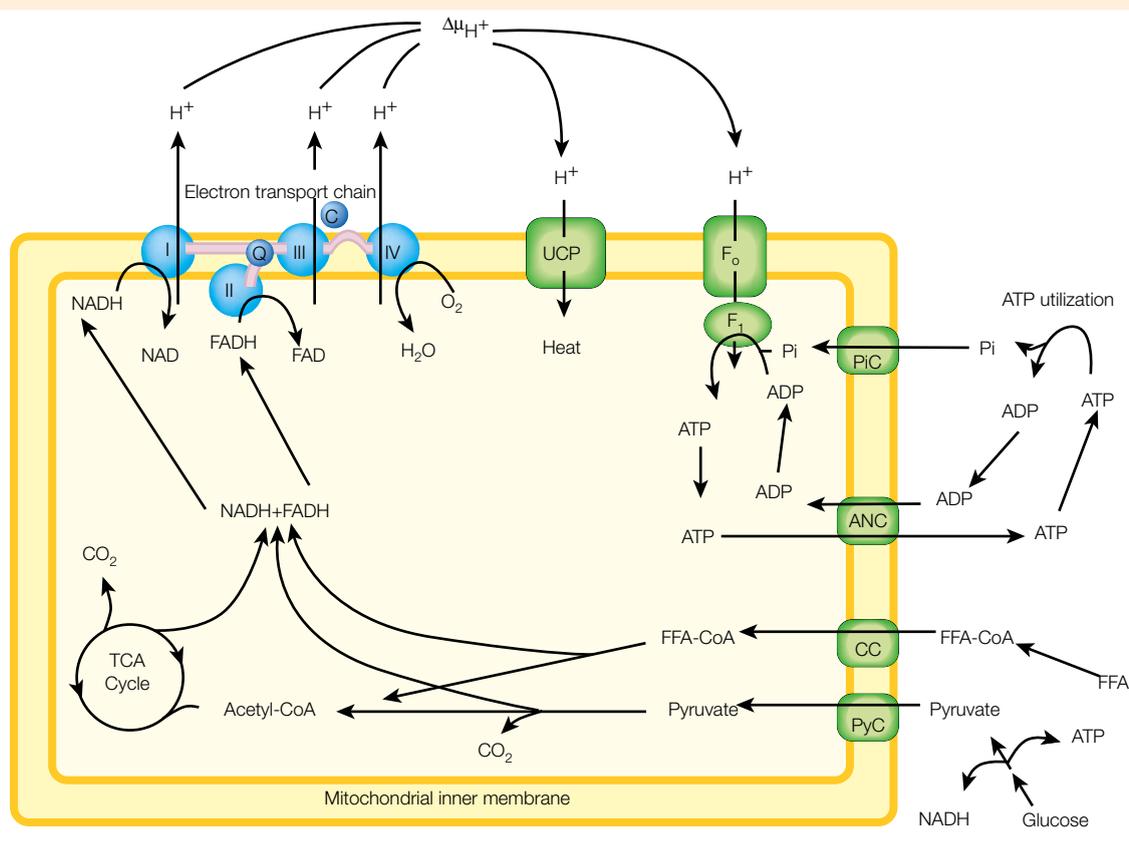Two homologues of UCP-1 have been identified. UCP-2 and

Figure 2 Mitochondrial energy metabolism. Free fatty acids (FFAs) and glucose are oxidized to generate NADH and $FADH_2$ which donate electrons to the electron transport chain. Ubiquinone (Q) shuttles electrons from both complexes I and II to complex III, whereas cytochrome *c* (C) shuttles electrons from complex III to complex IV. Molecular oxygen (O_2) is the terminal electron acceptor. Protons are pumped out by complexes I, III and IV of the electron transport chain, which creates a proton electrochemical potential gradient ($\Delta\mu_{H^+}$). Protons may re-enter the mitochondrial matrix through the F_0/F_1-ATPase, with energy being used to generate ATP from ADP and Pi. Protons may also re-enter through an uncoupling protein (UCP), with energy being released in the form of heat. Proton re-entry by means of ATP synthase depends upon the availability of ADP, which is generated in the cytosol from reactions using ATP. Abbreviations: ANC, adenine nucleotide carrier; CC, carnitine carrier; complex I, NADH–ubiquinone oxidoreductase; complex II, succinate–ubiquinone oxidoreductase; complex III, ubiquinone–cytochrome-*c* oxidoreductase; complex IV, cytochrome-*c* oxidase; PiC, phosphate carrier; PyC, pyruvate carrier.
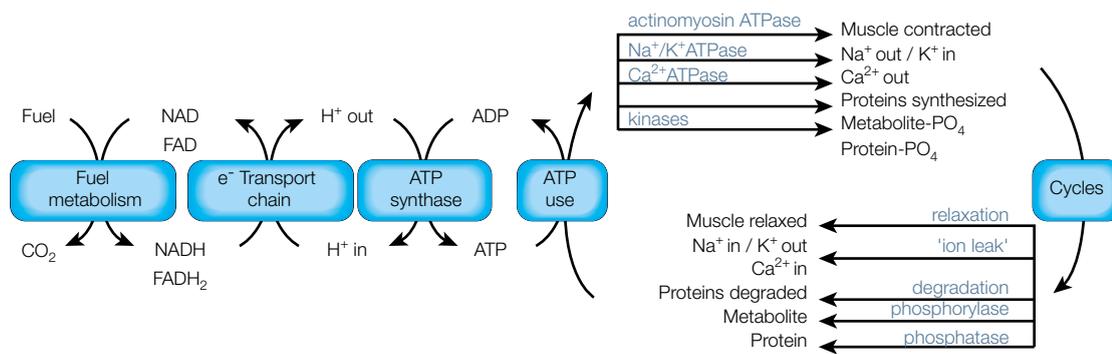
Figure 3 Coupling of reactions in energy metabolism. Metabolism of fuel generates a stoichiometric amount of NADH and FADH$_2$. Oxidation of NADH and FADH$_2$ results in ten and six protons, respectively, being pumped out of the mitochondrial matrix. Three protons enter by means of ATP synthase to synthesize one molecule of ATP from ADP and Pi. One additional proton enters the matrix as it is co-transported with Pi through the phosphate carrier. ATP is then used to perform a fixed amount of work. The major consumers of ATP are shown above. Muscle relaxation, ion leaks, protein degradation and dephosphorylation create the possibility for 'futile cycles'. See ref. 23 for a complete analysis of the concept of coupling with respect to reactions in energy metabolism.

UCP-3 are 73% identical to each other and both are 56% identical to UCP-1 (refs 29–33). UCP-2 is expressed in most tissues at varying levels, whereas UCP-3 is expressed predominantly in skeletal muscle and brown adipose tissue (all three uncoupling proteins are expressed abundantly in brown adipose tissue). Several studies indicate that these UCPs also have proton transport activity[29,30,33–35], including those studies using reconstituted proteoliposomes[36]. In addition, proteins with lower homology to UCP-1 also exist and these too may have proton transport activity[37,38]. Given that UCP-2 and UCP-3 have uncoupling activity and are expressed widely, it has been hypothesized that they could contribute significantly to adaptive thermogenesis. Arguing against this view, however, are observations that the expression of UCP-2 and UCP-3 messenger RNA increases with starvation[39,40], a state known to be associated with decreased energy expenditure. Thus, the function of these UCP homologues, with respect to thermogenesis and regulation of mitochondrial energy metabolism, is presently uncertain and is an active area of investigation.

β-Adrenergic-receptor stimulation, due to cold exposure or pharmacological agents, has both acute and chronic effects on brown adipose tissue (Fig. 4). UCP-1 activity increases within seconds of stimulation, while chronic stimulation over hours and days results in increased amounts of UCP-1 protein, mitochondrial biogenesis, and both hyperplasia and hypertrophy of brown adipose tissue. The coordination of mitochondrial biogenesis with uncoupling is crucial both in allowing for an increased capacity to generate heat, and in providing the means to maintain appropriate cellular ATP levels in the presence of a mitochondrial proton leak. Acute stimulation of UCP-1 activity is due to increased amounts of cyclic AMP, which activates lipolysis[41,42]. The resulting increase in free fatty acids is thought to stimulate UCP-1 activity by one of two possible mechanisms. Either fatty acid carboxyl groups function as H$^+$ donors to the UCP-1

Box 2

Fuel metabolism and oxidative phosphorylation

Energy is released when food is combusted to carbon dioxide and water. The organism controls this combustion such that energy can be channelled to perform work within the cell. This is accomplished by enzymatically controlled fuel metabolism and mitochondrial oxidative phosphorylation, step-by-step processes in which a portion of the energy content of fuels (fat, carbohydrate and protein) is converted to ATP. Energy stored in the form of ATP is then used to perform biological work within the cell.

Fuel metabolism and oxidative phosphorylation represent a series of reactions that are shown schematically in Fig. 2. The key task of the cell is to match rates of ATP production to rates of ATP consumption. This is made more complex by the fact that sites of ATP production and ATP use are spatially distinct within cells. It was suggested many years ago that ADP, the by-product of ATP use, controls rates of ATP production[84]. This was based upon the observation that addition of ADP to isolated mitochondria stimulates fuel oxidation, mitochondrial oxygen consumption and ATP synthesis. Such regulation provided a mechanism whereby rates of ATP production might be matched to rates of ATP utilization. The molecular mechanism for this regulation was explained by the chemiosmotic hypothesis of Peter Mitchell, to whom the Nobel prize in chemistry was awarded in 1978[85].

Metabolism of fuel leads to the production of NADH and FADH$_2$, which in turn donate electrons to the electron transport chain (Fig. 2). As electrons move down through the complexes of the electron transport chain, protons are pumped outside of the mitochondrial inner membrane, creating an electrochemical potential gradient ($\Delta\mu_{H^+}$). Protons then re-enter the mitochondrial matrix through F$_0$/F$_1$-ATP synthase in a reaction that is linked tightly to the synthesis of ATP from ADP. If ADP is unavailable, protons are unable to enter through ATP synthase. The three key features of the chemiosmotic hypothesis are as follows: (1) energy derived from fuel oxidation and mitochondrial respiration is converted to $\Delta\mu_{H^+}$; (2) when ADP is available, protons enter via ATP synthase, converting ADP to ATP; and (3) elevated $\Delta\mu_{H^+}$ puts 'backpressure' on proton pumps in the electron transport chain, inhibiting further fuel oxidation. Thus, when ADP is unavailable, fuel oxidation and mitochondrial respiration decreases.

Although the model has many compelling features, several observations indicate that the chemiosmotic hypothesis, when applied to the *in vivo* state, is an oversimplification and that additional layers of regulation must exist. Perhaps the strongest argument comes from observations of increased mitochondrial respiration in the absence of proportional increases in ADP[86,87]. Such observations have led to the proposal that rates of ATP production increase simultaneously with rates of ATP utilization, possibly as a result of a common activator such as intracellular calcium levels[88,89]. The mechanism by which such regulation allows precise matching of ATP production and utilization rates, however, is presently unknown.

proton translocation channel[27] or UCP-1 transports free fatty acid anions, not protons, from inside to outside of the mitochondrial matrix[43]. Once outside, the free fatty acids become re-protonated, then flip-flop back across the inner membrane bilayer, creating a protonophore cycle with a net transfer of protons into the mitochondrial matrix.

β_3-Adrenergic receptors are expressed abundantly and predominantly on brown adipocytes (and also white adipocytes in rodents), and selective agonists of this receptor have been synthesized[44,45]. Treatment of mice with such agonists doubles oxygen consumption, demonstrating the remarkable capacity of this thermogenic mechanism[46]. In larger mammals, including dogs, cows and primates, discrete deposits of brown fat are present at birth, but become relatively sparse during later development. Chronic treatment with β_3-adrenergic agonists markedly increases the amount of brown fat in adult dogs[47] and primates[48], and brown adipose tissue is abundant in adult humans with catecholamine (that is, adrenaline or noradrenaline)-secreting pheochromocytomas[49]. These data show that a latent source of catecholamine-inducible brown adipocytes exists. Indeed, even in rodents, the efficacy of β_3-adrenergic agonists to prevent or reverse obesity seems to depend on the ability to expand numbers of brown adipocytes in typical white fat depots[50,51], a response influenced by genetic background[51,52]. Although the precise molecular and cellular basis for recruitment of brown adipocytes induced through stimulation of β-adrenergic receptors is not yet established, it is likely to involve signalling and transcriptional pathways outlined later in this review.

Diet-induced adaptive thermogenesis
There is also evidence that brown adipose tissue is important in diet-induced thermogenesis. Sympathetic nerve activity to brown adipose tissue is reduced in many models of obesity, including leptin-deficient *ob/ob* mice[16]. Leptin administration, either centrally or peripherally, increases sympathetic nerve activity to brown fat[53,54] and, as expected, increases UCP-1 mRNA and protein levels[55–57]. During starvation, which reduces leptin expression, sympathetic nerve activity to brown fat declines[13], and this is associated with decreased UCP-1 expression[58]. A role for brown fat in controlling body weight *per se* is illustrated by the fact that transgenic mice with toxigene-mediated reduction of brown fat (UCP-DTA mice) develop obesity[59]. This is in contrast to gene-knockout mice lacking UCP-1, which are cold-sensitive but not obese[28]. The presence of obesity in UCP-DTA transgenic mice, but not UCP-1-knockout mice, could be due to the existence of alternative thermogenic effectors in brown fat, such as UCP-2 or UCP-3, or possibly an appetite regulatory function of brown fat[60]. Alternatively, UCP-DTA mice could have another, as yet unidentified toxin-
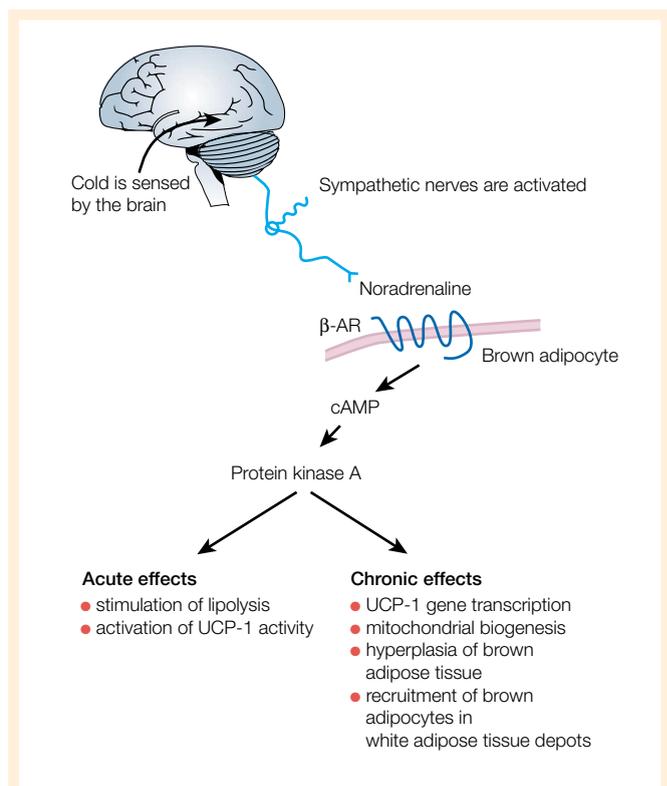

Figure 4 Mechanism of cold-induced adaptive thermogenesis.

induced lesion which causes their obesity. Despite these apparent ambiguities, the evidence for brown fat being important in diet-induced thermogenesis in rodents is strong.

Skeletal muscle as a site for adaptive thermogenesis
Adult humans, unlike rodents, do not have large, distinct depots of brown adipose tissue. But both rodents and humans have varying numbers of brown adipose cells dispersed in white fat depots. Nevertheless, there is considerable suspicion that, in the absence of pharmacological stimulation, brown adipose tissue is important in mediating adaptive thermogenesis in adult humans. Skeletal muscle, on the other hand, represents up to 40% of total body weight and is endowed with significant mitochondrial capacity, causing many researchers to investigate its contribution to adaptive thermogenesis. It has been observed that resting energy expenditure is variable in humans and that low energy expenditure is predictive of future

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Box 3
Ca²⁺ ion cycling and thermogenesis

Two examples highlight the potential for effects of ion cycling on energy expenditure: the heater organ of fish and the pathological condition known as malignant hyperthermia.

In certain species of fish, there is a well characterized mechanism for thermogenesis resulting from regulated ion cycling[90,91]. The 'heater organ' is a derivative of muscle that is relatively devoid of contractile elements. These specialized cells make up most of the superior rectus muscle in the orbit and generate heat for the brain and eyes during cold-water dives. Like typical muscle cells, heater cells possess abundant acetylcholine receptors and have an extensive network of sarcoplasmic reticulum and T-tubules. Mitochondria are also extremely abundant in heater cells, comprising over 60% of total cell volume. Thermogenesis in heater cells is initiated by depolarization, which causes Ca^{2+} release by the sarcoplasmic reticulum. ATP is then consumed by Ca^{2+}-ATPase, which returns Ca^{2+} to the sarcoplasmic reticulum. The increased demand for ATP required to sustain this futile cycle drives fuel oxidation. Thus, depolarization-induced Ca^{2+} entry into the cytoplasm causes ion cycling-mediated thermogenesis.

The thermogenic potential of Ca^{2+} cycling in mammals is evident from the pathological syndrome of malignant hyperthermia, a dominant genetic disorder of humans and pigs which in many cases is due to a mutation in the skeletal muscle ryanodine receptor, the Ca^{2+}-release channel of the sarcoplasmic reticulum[92]. Abnormal Ca^{2+} release, triggered by anaesthesia and/or stress, causes intense thermogenesis, which leads to hyperthermia. Ca^{2+}-ATPase and ryanodine-receptor content increase in the sarcoplasmic reticulum during cold acclimation in birds[93], which are notable for their lack of brown adipose tissue, raising the possibility that Ca^{2+} cycling in muscle could contribute to adaptive thermogenesis.

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weight gain[61,62]. A significant portion of the variation in metabolic rate between humans can be accounted for by differences in skeletal muscle energy expenditure[63], and further support for a probable role of skeletal muscle in mediating adaptive thermogenesis comes from the demonstration that adrenaline infusion, which causes a 25% increase in whole body energy expenditure in humans, stimulates forearm muscle oxygen consumption by as much as 90% (ref. 64). Assuming that forearm muscle is representative of total body musculature, skeletal muscle then accounts for ~40% of adrenaline infusion-induced thermogenesis in humans. The mechanism for this effect is unknown but could include effects on mitochondrial function and uncoupling, Ca^{2+} cycling, or both. Also unknown is the contribution of other tissues, such as liver and white adipose tissue, to adaptive thermogenesis in humans.

Transcriptional control of mitochondrial genes

A thorough understanding of the transcriptional basis of adaptive thermogenesis must account for the regulation and temporal coordination of mitochondrial biogenesis, expression of uncoupling proteins in a tissue-selective manner, and sensitivity to key hormones such as β-adrenergic agents and thyroid hormone. At best, all of these aspects are only partly understood. As described above, the therapeutic potential for modulation of these systems has provided a strong incentive to develop a detailed understanding of the relevant gene regulatory programmes.

The transcriptional regulation of gene expression related to mitochondrial proliferation is beginning to be unravelled. Through analysis of the promoters of mitochondrial genes encoded in the cell nucleus, the nuclear respiratory factors (NRF)-1 and -2 have been identified as key components. NRF-1 and -2 bind to and activate the promoters of many genes of the mitochondrial electron transport system such as cytochrome *c*, cytochrome *c* oxidase subunits II and IV, and subunits of the F_o/F_1-ATP synthase (reviewed in ref. 65). Another target of the NRFs is mitochondrial transcription factor (mtTF)-A, a gene encoded in the nucleus, whose protein product translocates into the mitochondria and stimulates transcription and replication of the mitochondrial genome[66]. Somewhat surprisingly, little quantitative regulation of these components has been demonstrated directly in adaptive thermogenesis *per se*, although altered activity of the ATP synthase promoter through an NRF-2 binding site has been shown during brown fat cell differentiation[67].

There are many studies that have pointed to thyroid hormone as a major regulator of mitochondrial biogenesis and mitochondrial function *in vivo*. In addition, mitochondrial gene expression is reduced in hypothyroid animals and stimulated upon administration of thyroid hormone. Certain genes of mitochondrial structure
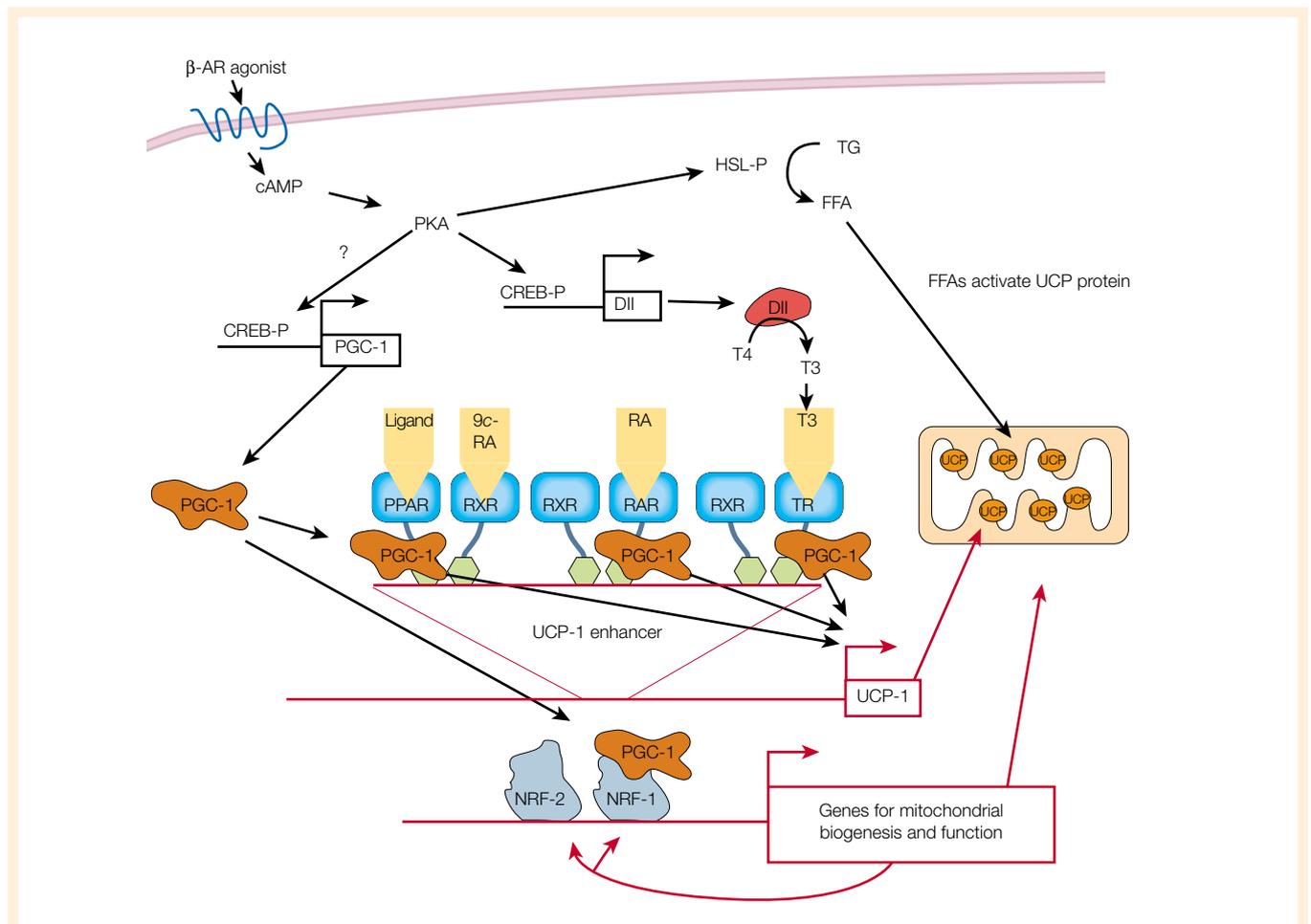

Figure 5 Pathway for β-adrenergic activation of thermogenesis in brown adipocytes. β-adrenergic-receptor (β-AR) agonists stimulate generation of cAMP, which in turn activates protein kinase A (PKA). PKA phosphorylates CREB, which leads to increased gene transcription. It is hypothesized that activated CREB directly induces expression of PGC-1 and the DII. PGC-1 co-activates transcription factors assembled on the UCP-1 enhancer, thus increasing UCP-1 gene expression. In addition, DII increases synthesis of triiodothyronine (T3), the ligand for the thyroid hormone receptor, further increasing UCP-1 gene expression. PKA also activates hormone-sensitive lipase (HSL), increasing the concentration of free fatty acids (FFAs) which in turn activate UCP-1 protein activity. PGC-1 also co-activates the transcription factor NRF-1, which leads to an increase in genes required for mitochondrial biogenesis, including NRF-1 and NRF-2. This results in marked stimulation of mitochondrial biogenesis. Abbreviations: RXR, retinoid X receptor; RAR, retinoic acid receptor; 9c-RA; 9-*cis*-retinoic acid; RA, retinoic acid; TG, triglyceride.

and function encoded in the cell nucleus have thyroid-hormone response elements, indicating that this hormone is working directly on these genes through thyroid hormone receptors. In addition to these effects on nuclear genes, there have also been several reports suggesting that thyroid hormone and its receptor can translocate into mitochondria to affect transcription patterns[68]; these studies must be considered preliminary until further details become available.

Regulation of UCP-1 gene expression

As a prototypical thermogenic molecule, the promoter of the gene encoding UCP-1 has received extensive attention and analysis. Studies from several laboratories have identified a 220-base-pair enhancer element, located approximately 2.4 kilobases upstream of the mouse and rat UCP-1 genes, which promotes transcription that is both brown fat-selective and responsive to β-adrenergic stimulation (through cAMP)[69,70]. This complex enhancer element has putative binding sites for the thyroid hormone receptor, retinoic acid receptor and peroxisome proliferator-activated receptor-γ (PPAR-γ), a nuclear receptor expressed in both white and brown fat. UCP-1 in brown fat can be strongly induced by the addition of cAMP, or by constitutive activation of protein kinase A[71]. But so far there have been no reports showing a direct role for CREB (cAMP-responsive element binding protein) in the UCP-1 enhancer. Hence, although a role for CREB is possible, or even likely in adaptive thermogenesis and UCP-1 expression, cAMP may work indirectly (see later discussion).

The study of gene expression in brown fat is of special interest because this is the only tissue in the mammalian body that functions exclusively as a thermogenic organ. Although brown fat-selective transcription factors have not been identified, the binding of PPAR-γ is essential for the function of the UCP-1 enhancer[72]. Furthermore, PPAR-γ can activate the UCP-1 enhancer only in brown fat preadipocytes and not other fibroblasts, indicating that molecules interacting with PPAR-γ could represent key components of the selectivity and thermogenic response of brown adipose tissue. A regulatory role for the PPAR-γ system in brown fat development and UCP-1 expression was also shown through administration of PPAR-γ ligands, the synthetic thiazolidinedione (TZD) drugs, to brown fat cells *in vitro* and to mice[73,74]. These studies showed that activation of PPAR-γ promoted brown fat cell differentiation and UCP-1 expression in culture, and hypertrophy of brown fat tissue *in vivo*.

Although studies of the transcriptional control of the UCP-2 and UCP-3 genes are just beginning, the PPARs also seem to regulate the expression of these proteins. As mentioned above, UCP-2 and UCP-3 are regulated by various nutritional perturbations in a tissue-selective manner. Present insights into the transcriptional regulation of UCP-2 and UCP-3 have come from *in vivo* administration to animals or *in vitro* treatment of cells with PPAR ligands such as TZDs (selective for PPAR-γ) and fibrates (selective for PPAR-α)[75,76]. These studies have indicated that PPAR-α and PPAR-γ are positive regulators of UCP-2 and UCP-3, with specificity defined by the tissue or cell-type being examined. Given that fatty acids and/or their derivatives are ligands for these receptors, the PPARs may account for much of the nutritional regulation of UCP-2 and UCP-3.

PGC-1: a co-activator linked to thermogenesis

Most biological programmes that have been studied show dominant regulation at the level of DNA-binding transcriptional factors. However, two lines of evidence indicated that a central regulatory component of the adaptive thermogenic programme could be at the level of a transcriptional co-activator. First, the observation that PPAR-γ binding was essential for UCP-1-enhancer functioning[72] suggested that an important determinant of the brown fat expression of UCP-1 could be a modulator of PPAR-γ function rather than PPAR-γ itself. Second, administration of the TZD ligands for PPAR-γ *in vivo* indicated that this transcriptional system was simultaneously involved in energy storage and energy dissipation through

increased respiration. This paradox suggested that there was tissue-specific regulation of PPAR-γ function.

A potential answer emerged when a PPAR-γ co-activator, PGC-1, was cloned from a brown fat library using a yeast two-hybrid system with PPAR-γ as bait[77]. This factor is highly expressed in brown but not white fat, and is also expressed in heart, kidney, brain and skeletal muscle. Its connection to adaptive thermogenesis was first shown by its marked and rapid induction in brown fat and skeletal muscle upon cold exposure of mice. This cold induction of PGC-1 is largely due to sympathetic nervous system input through β-adrenergic receptors and cAMP action[77,78].

In addition to PPAR-γ, PGC-1 also binds to a variety of other nuclear receptors including the retinoic acid and thyroid hormone receptors, both of which positively regulate expression of UCP-1. Interestingly, the docking of PGC-1 to PPAR-γ is not strongly ligand-dependent, whereas its binding to other nuclear receptors, such as the oestrogen receptor, is almost totally ligand-dependent.

Ectopic expression of PGC-1 in cultured cells activates and coordinates multiple aspects of the adaptive thermogenesis programme. Mitochondrial biogenesis is induced, as are many genes of the electron transport system[77]. The expression of uncoupling proteins is also increased in a cell-selective manner[79]. UCP-1 but not UCP-2 or UCP-3 is induced when PGC-1 is introduced into white fat cells, whereas UCP-2 but not UCP-1 or UCP-3 is induced when PGC-1 is expressed in muscle cells. In both fat and muscle cells, these PGC-1-mediated changes in gene expression are reflected in increased respiration, both coupled and uncoupled[79]. These data also indicate that expression of PGC-1 in the adipose lineage could be important in the developmental bifurcation between white and brown fat cells.

PGC-1 also regulates the NRF system

The detailed mechanisms by which PGC-1 induces mitochondrial biogenesis have been examined. Although this co-activator could, in principle, work through a new set of transcription factors, recent data indicate that it is a powerful regulator of the NRF system. PGC-1 expression in muscle cells stimulates a large induction of both NRF-1 and -2 (ref. 79). In addition, PGC-1 binds to and co-activates the action of NRF-1 on the mtTFA promoter, leading to increased mtTFA expression. Presumably, this protein is the direct effector of transcription and replication of the mitochondrial genome. Taken together, these data suggest a direct linkage whereby changes in environmental condition such as temperature or diet can coordinate multiple factors to execute mitochondrial biogenesis and partly uncoupled respiration.

As previously discussed, β-adrenergic-receptor stimulation and the second messenger cAMP potently increase UCP-1 gene expression in brown fat. Surprisingly, the UCP-1 gene enhancer that mediates this effect lacks a well defined CREB binding site, which indicates that an indirect mechanism may be involved. Although the proximal promoter of UCP-1 does contain CREB binding sites, these may not be relevant as they are located outside of the enhancer that mediates effects of cold exposure and β-adrenergic-receptor stimulation in transgenic mice[69].

With the discovery of PGC-1, a potential mechanism for catecholamine-induced activation of UCP-1 gene expression, uncoupling activity and mitochondrial biogenesis can be proposed (Fig. 5). In this model, the UCP-1 enhancer binds PPAR-γ, the retinoic acid receptor and the thyroid receptor, each complexed with the retinoid X receptor. β-Adrenergic-receptor stimulation leads to increased expression of PGC-1, mediated potentially by CREB binding to the PGC-1 promoter. Indeed, acute stimulation *in vivo* by either cold exposure or β3-adrenergic agonists rapidly increases PGC-1 mRNA levels by more than 50-fold in brown fat tissue[77,78]. PGC-1, in turn, co-activates each of the three nuclear receptors assembled on the UCP-1 enhancer. PGC-1 induces NRF-1 and NRF-2, and co-activates NRF-1 as well, thereby simultaneously

stimulating mitochondrial biogenesis and respiratory capacity. β-Adrenergic-receptor stimulation also markedly increases expression of type II thyroxine deiodinase (DII)[80,81], an effect that is probably mediated directly by CREB[82]. DII in turn generates active ligand for the thyroid hormone receptor. Finally, increased free fatty acids resulting from lipolysis stimulate the activity of UCP-1 protein. In summary, increased protein kinase A activity initiates a cascade of actions, all leading to increased adaptive thermogenesis in brown fat and presumably skeletal muscle. The model presented in Fig. 5 predicts that PGC-1 is crucial in this response. Although the effectors of uncoupling in muscle are less clear, the effects of PGC-1 on muscle-cell respiration suggests an analogous process occurs in this tissue as well.

Future directions

Adaptive thermogenesis is an increasingly attractive target for the development of antiobesity therapies. As the key molecular components become defined, screening for drugs that increase energy dissipation is becoming a more attainable goal. Over the next few years we are likely to learn whether β_3-adrenergic agents can be developed with the selectivity and potency to increase adaptive thermogenesis in humans. Because of the multifaceted nature of its actions, PGC-1 may also be an interesting target for the development of new therapeutics, although the ease with which compounds that increase the activity of PGC-1 can be developed is completely unknown.

Several key questions still await answers in basic research. What are the physiological roles of UCP-2 and -3? Do they have a protective role against obesity? What are the dominant thermogenic mechanisms in human muscle: uncoupling protein function, Ca^{2+} cycling or as yet undiscovered pathways? What other tissues besides muscle contribute to adaptive thermogenesis in humans? Is PGC-1 involved only in the execution of the oxidative metabolic programme of brown fat and muscle, or is it also involved in the cellular determination of white versus brown fat, and fast twitch (glycolytic) versus slow twitch (oxidative) muscle fibres? Our ability to answer these questions adequately requires a close synthesis of cellular and molecular approaches with whole-animal physiology. □

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