Cellular bioenergetics as a target for obesity therapy

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Abstract | Obesity develops when energy intake exceeds energy expenditure. Although most current obesity therapies are focused on reducing calorific intake, recent data suggest that increasing cellular energy expenditure (bioenergetics) may be an attractive alternative approach. This is especially true for adaptive thermogenesis — the physiological process whereby energy is dissipated in mitochondria of brown fat and skeletal muscle in the form of heat in response to external stimuli. There have been significant recent advances in identifying the factors that control the development and function of these tissues, and in techniques to measure brown fat in human adults. In this article, we integrate these developments in relation to the classical understandings of cellular bioenergetics to explore the potential for developing novel anti-obesity therapies that target cellular energy expenditure.

Standard metabolic rate
The steady-state rate of energy utilized by a whole organism that is awake but resting, stress free, not actively digesting food, and is at thermoneutrality.

Basal metabolic rate
(BMR). The energy expended by an individual when physically and mentally at rest 12–18 hours after a meal in a thermoneutral environment. It is similar to the standard metabolic rate, although it is now usually applied to human metabolism only.

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Obesity is occurring at epidemic rates in the United States of America and worldwide. According to the World Health Organization, more than 1 billion adults (~15% of the world population) are overweight (body mass index (BMI)>25) and more than 300 million are classed as truly obese (BMI>30); these numbers are expected to increase by more than half again by the year 2025. Obesity represents a major risk factor for the development of many of the most common medical conditions, including type 2 diabetes mellitus, dyslipidaemias, non-alcoholic fatty liver and gallstones, cardiovascular disease, Alzheimer's disease, and even some cancers[1].

Obesity develops when energy intake exceeds energy expenditure. Of the nutrient energy intake, a small proportion is lost in faeces and urine; a proportion is used for physiological needs (growth, pregnancy or lactation); a variable, and unfortunately decreasing, proportion is used in physical activity; and the majority is used for metabolic processes or is lost in the production of heat (FIG. 1). The standard metabolic rate or basal metabolic rate (BMR) is the rate of energy utilized by an organism in the awake but resting state, not actively digesting food and at thermoneutrality[2]. Every organ of the body contributes to the standard metabolic rate because nearly every enzymatic reaction is thermogenic. Of the remaining energy loss, there is the heat produced during digestion and absorption of food (the thermic effect of food), the thermic effect of exercise, and the energy dissipated in response to environmental changes, such as cold temperature and diet. Heat production caused by responses by the body to cold temperature and diet are referred to as adaptive thermogenesis[3]. These occur primarily in the mitochondria of skeletal muscle and brown fat, which are distinct from other body tissues in that their thermogenesis is finely regulated and therefore has the potential to be manipulated therapeutically to serve as a target for obesity treatment.

Current treatments of obesity

As the laws of thermodynamics must be obeyed, any treatment for obesity must reduce energy intake, increase energy expenditure, or have an effect on both. Despite this simple reality, treatment of obesity remains one of the most important challenges facing the health-care system. Current approved clinical approaches for the treatment of obesity include diet and exercise, medical therapies aimed at reducing calorific intake or absorption (which are of limited effectiveness), and bariatric surgery for extremely obese individuals. Unfortunately, only a small proportion of individuals on dietary and/ or exercise programmes maintain long-term weight loss[4]. Moreover, although bariatric surgery has gained popularity for highly obese patients, it is not without significant risk.

Only three drugs are currently approved by the US Food and Drug Administration specifically for weight loss. They work by decreasing energy intake, either by acting at satiety centres in the brain (sibutramine and

Category	Site	Comments	
Standard metabolic rate	All organs	Energy utilized for basic cellular and organ function	Obligatory thermogenesis
Thermic effect of food	Stomach, intestine, liver, white fat	Heat generated during digestion, absorption, processing and storing of energy	
Cold-induced shivering thermogenesis	Skeletal muscle	Heat generated by shivering to stay warm	
Cold-induced non-shivering thermogenesis	Brown fat, skeletal muscle?	Heat generated to adapt to cold	Adaptive thermogenesis
Diet-induced thermogenesis	Brown fat	Heat generated to dissipate excess energy from food	
Non-exercise activity thermogenesis	Skeletal muscle	Energy utilized by movement other than exercise	Movement-related heat and energy expenditure
Thermic and work effect of exercise	Skeletal muscle	Heat generated by muscle movement	
Growth	All organs	Energy utilized for body growth	Obligatory energy expenditure
Reproduction	Reproductive organs	Energy utilized for pregnancy, lactation	
Urine/faeces			

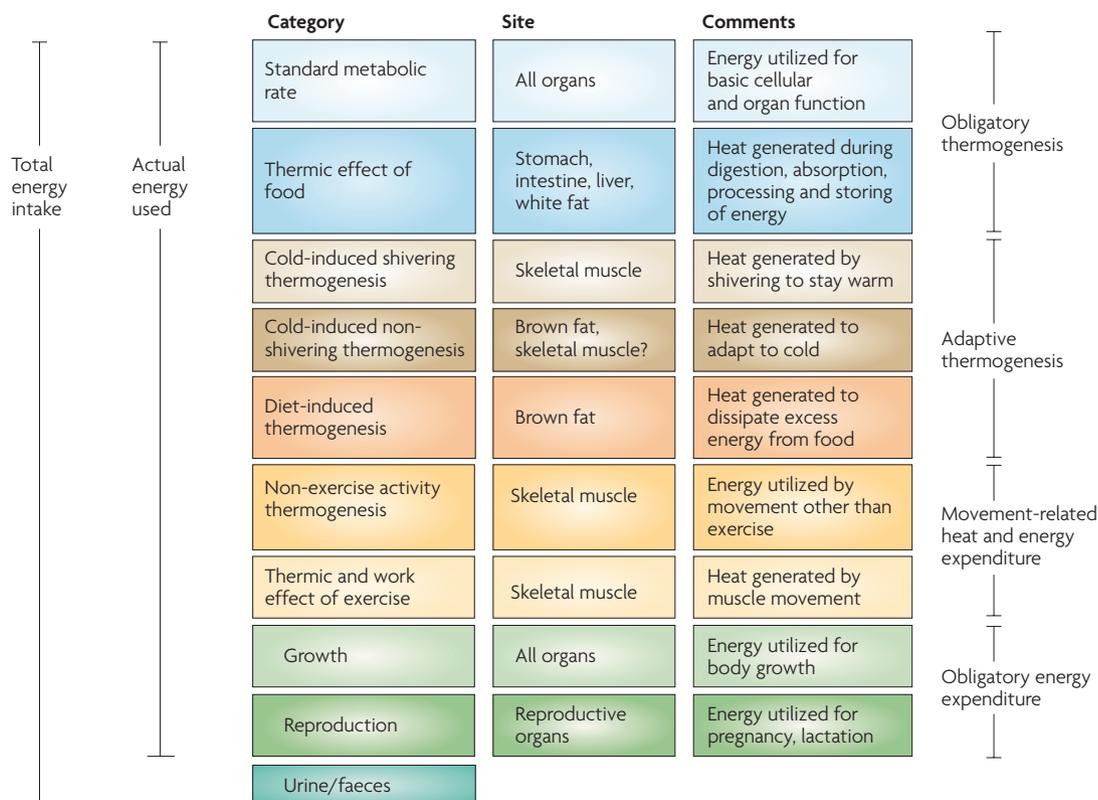(Left brackets labelled: Total energy intake; Actual energy used)

Figure 1 | **Cellular energy utilization.** Of the nutrient energy intake of an individual organism, a small proportion is lost in the faeces and urine; a proportion is used for physiological needs such as growth, pregnancy or lactation; a variable proportion is used in physical activity; and the majority of ingested calories is used for metabolic processes or is lost in the production of heat. Based on the function and tissues of heat production, thermogenesis can be further classified into seven categories: standard metabolic rate, thermic effect of food, cold-induced shivering thermogenesis, cold-induced non-shivering thermogenesis, diet-induced thermogenesis, non-exercise activity thermogenesis, and thermic and work effect of exercise. Adaptive thermogenesis is defined as regulated heat production in response to environmental temperature or diet. There are three subcategories of adaptive thermogenesis. Cold exposure induces shivering thermogenesis in skeletal muscle, and non-shivering thermogenesis in brown fat. Although current evidence does not indicate a role of muscle in non-shivering thermogenesis, indirect evidence suggests that such mechanisms may exist. Overfeeding triggers diet-induced thermogenesis; this is also a function of brown fat. Obligatory thermogenesis refers to the minimal heat produced by all the processes that maintain the body in a basal state (fasting) at thermoneutral temperature, and obligatory energy expenditure is the energy used for special physiological needs, such as growth and reproduction.

Thermoneutrality
The environmental temperature at which heat production is not stimulated, e.g., ~28°C for adult humans. In general, humans usually make the microclimate thermoneutral through clothing choices.

Adaptive thermogenesis
Heat production in response to environmental temperature or diet. It serves the purpose of protecting the organism from cold exposure or regulating energy balance after changes in diet. Brown fat and skeletal muscle are the two principal sites of adaptive thermogenesis.

Bioenergetics
Studies the flow of chemical bond energy within organisms. In a living cell, the principal reactions of fuel metabolism take place in the mitochondria, where food energy is released, oxygen is consumed, and water and carbon dioxide are produced.

phentermine) or by reducing the efficiency of intestinal absorption (orlistat)[5], thereby reducing the elevated energy intake that is crucial in maintaining the obese state[6]. However, because of the unacceptable side effects or inadequate long-term clinical efficacy, these medications have so far met with limited success[7]. There are more than two-dozen treatments in at least Phase I clinical trials, and more in the pipeline (TABLES 1–3), almost all of which are aimed at reducing energy intake.

However, losing weight by only calorific restriction faces three conceptual challenges. First, mammals are designed to guard against starvation. Although there is an active debate surrounding the mechanisms underlying this process[8,9], most agree that the human body is designed to defend against a low level of fat content. Therefore, redundant systems are in place to overcome suppression of any one pathway to appetite, suggesting that a drug acting by one principal mechanism may

be unlikely to have long-term efficacy. Second, as the experience with endocannabinoid receptor antagonists has shown, central satiety centres often interconnect with other core regulators in the brain, causing psychotropic side effects[10]. Third, and probably most importantly, the body has homeostatic mechanisms such that weight loss produces an increase in calorific efficiency, that is, a reduction in BMR, making further weight loss even more difficult[11,12].

Targeting energy expenditure, that is, cellular bioenergetics, is therefore an attractive alternative strategy that could be used alone or in conjunction with other approaches for several reasons. First, few drugs in this class are currently available, so there is significant opportunity for the development of novel treatments. Second, recent studies have shown that adult humans maintain potentially active brown adipose tissue (BAT), making this highly energetic tissue a real therapeutic target[13–17].

Table 1 | **Treatments that decrease energy intake via appetite suppression**

Class	Drug	Principal mechanism of action
Anticonvulsant	Topiramate	Enhances GABA-activated chloride channels; inhibits excitatory neurotransmission through actions on kainate and AMPA receptors[173]
	Zonisamide	Blocks voltage-gated sodium and T-type calcium channels; blocks potassium-evoked glutamate release; modulates central dopaminergic and serotonergic function[174]
Enzyme activator	Metformin	Activates AMP-activated protein kinase[175,176]
Enzyme inhibitor	Trodusquemine	Inhibits protein tyrosine phosphatase 1B[177]
Hormone	Adiponectin	Stimulates fatty acid oxidation [178]
	Exenatide, liraglutide	Agonists of glucagon-like peptide 1 receptors[179]
	Leptin, metreleptin	Reflect size of fat depots[151,180]
	Oleoyl-oestrone	Alters body weight set-point and/or ponderostat[181,182]
	Oxyntomodulin	Probably an agonist of glucagon-like peptide 1 and other receptors[183]
	Pramlintide, AC2307	Agonist of amylin receptors[180]
Neurotransmitter reuptake inhibitor	Phentermine	Blocks reuptake of adrenalin[184]
	Rivastigmine	Inhibits cholinesterase[185]
	Tesofensine	Blocks presynaptic uptake of noradrenaline, dopamine and serotonin[186]
	Sibutramine	Blocks reuptake of monoamines[10]
	Venlafaxine	Blocks reuptake of noradrenaline and serotonin[187]
Neurotransmitter reuptake inhibitor and receptor antagonist	Bupropion	Blocks reuptake of noradrenaline and dopamine, and is an anatagonist of nicotinic receptors[188]
Receptor agonist	CE-326597	Agonist of cholecystokinin receptors[189] (NCT00542009*)
	Lorcaserin	Agonist of 5-hydroxytryptamine 2C receptors[190]
	MK0493	Agonist of melanocortin 4 receptors[191]
	TM30339	Agonist of neuropeptide Y receptor Y4 (REF. 191)
Receptor agonist and antagonist	TTP435	Antagonist of agouti-related protein and agonist of melanocortin 4 receptors[192] (NCT00779519*)
	Betahistine	Agonist of histamine 1 receptors and antagonist of histamine 3 receptors[193]
Receptor antagonist	Naltrexone	Antagonist of opioid receptors[194]
	Rimonabant, taranabant, and others	Antagonists of cannabinoid 1 receptors[10]
	Velneperit, MK0557	Antagonists of neuropeptide Y receptor Y5 (REF. 191) (NCT00748605*)

AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid; GABA, γ-aminobutyric acid. *Denotes ClinicalTrials.gov identifier.

Third, increasing energy expenditure has already proved to be effective in achieving weight loss. For example, 2,4-dinitrophenol (DNP), a non-selective uncoupler of mitochondrial oxidation, effectively increased energy expenditure that could be sustained without tolerance[18]. Unfortunately, DNP produces unwanted side effects that preclude its clinical use as therapy. Last, increasing energy expenditure may be a way to combat the body's own adaptive changes to lose weight. The integrated systems by which the brain and body communicate to regulate body weight may indicate single or multiple set-points for weight that may be difficult to adjust[12,19–21]. Therapeutic interventions designed to increase energy expenditure may be able to reset an obese individual's set-point for body weight back to a lower, healthier range. This raises the possibility that drug treatment might only need to be short-term or intermittent, reducing the risks and costs of lifelong exposure to medications.

Bioenergetics and mitochondrial metabolism

Bioenergetics takes place largely within the mitochondria, where, through the tricarboxylic acid cycle and the electron transport chain, energy from nutrients is released. In addition, oxygen is consumed, and water, carbon dioxide and ATP are produced (BOX 1; FIG. 2a). Under normal circumstances, in most tissues, the release of the energy in chemical bonds is mediated enzymatically[22] as a series of interconnected reactions that allow

Table 2 | **Treatments that decrease energy intake via impaired absorption**

Class	Drug	Principal mechanism of action
Enzyme inhibitor	Cetilistat, orlistat, GT 389-255	Block lumenal intestinal and pancreatic lipases[10]
Transporter inhibitor	JNJ-16269110 (R256918)	Inhibits gut-selective microsomal triglyceride transfer protein[195] (NCT00622765*)
	GW869682, JNJ-28431754	Inhibit low affinity sodium-dependent glucose cotransporter[196] (NCT00297180, NCT00650806*)
Vaccine	Anti-ghrelin vaccine	Blocks ghrelin receptor binding[197]

*Denotes ClinicalTrials.gov identifier.

the minimization of loss as heat and the maximization of production of ATP. The ATP is distributed throughout the cell for maintenance of Na^+/K^+ and Ca^{2+} pumps (30%), for protein synthesis (30%), and for gluconeogenesis, ureagenesis and turnover of carbohydrate and lipid stores (20%)[23].

This interconversion of chemical energy is by nature an inefficient process. Even when reactions are tightly coupled, some energy is lost as heat. However, in the mammalian cell, there are certain processes that are entirely uncoupled, or 'futile', and therefore thermogenic or energy consuming. In all cells, H^+, Na^+, K^+ and Ca^{2+} leak back across membrane channels down their electrochemical gradients. In mitochondria, the proton leak is typically as high as 20%, dissipating free energy substantially and reducing the amount of ATP actually generated for every molecule of oxygen split by the electron transport chain. Muscle tissue has at least four uncoupled, or energy-burning, reactions, including the energy dissipated as heat by the inwardly rectifying Ca^{2+} and Na^+ channels; cycling of actin–myosin during contraction and relaxation; heat dissipated during physical work; and energy lost by triglyceride/fatty-acid cycling[2] (FIG. 2b–d).

The energy lost by triglyceride/fatty-acid cycling is an example of a futile cycle in which esterification of triglycerides is followed by hydrolysis, leading to heat expenditure; a cycle that was originally proposed more than 30 years ago by Newsholme and colleagues[24,25]. This cycling is involved in the thermogenesis associated with burn injuries[26], cancer cachexia[27], and after exercise[28]. Recent evidence has shown that this cycle is also present in human white adipocytes *in vitro* after treatment with the specific peroxisome proliferator-activated receptor-α (PPARα) agonist GW7647 (REF. 29).

An obvious question is why are so many mammalian processes uncoupled? Teleological explanations include the flexibility that inefficiency may offer in the degree of control of cell function, as well as the more rapid kinetics that energy-releasing reactions allow[2]. However, there is one particular uncoupled reaction that occurs clearly by design: the regulated proton leak in BAT by uncoupling protein 1 (UCP1).

UCP1 is a 32 kDa inner mitochondrial transmembrane protein expressed only in brown adipocytes, which allows protons in the mitochondrial intermembrane space to re-enter the mitochondrial matrix without generating ATP, that is, uncoupled. As a result, heat is generated directly by protons rushing down their electrochemical gradient and also indirectly by the subsequent increase in flux through the electron transport chain that follows. UCP1 is unique to BAT and is necessary to mediate BAT thermogenesis[30]. UCP1-deficient mice are sensitive to cold temperatures[31] and exhibit increased susceptibility to diet-induced obesity[32,33]. Conversely, transgenic mice with *UCP1* expression in white fat display a lean phenotype[34,35]. In addition to UCP1, two other uncoupling proteins have been identified: UCP2 and UCP3. *UCP2* is expressed at low levels in many tissues, whereas *UCP3* is expressed preferentially in skeletal muscle. However, there is no convincing data to support their involvement in thermogenesis[18].

In rodents, the thermogenic capacity of BAT is enormous. In a cold-acclimatized rats weighing 350–400 g, oxygen consumption by 3 g of BAT is approximately twice the BMR[36]. Humans, however, are different from rats, with greater body mass (~200-times greater), only moderately greater BAT mass (~10-times greater), and much lower BMR (~6.5-times lower per gram of body weight)[13,36,37]. In this context, it has been estimated that in humans as little as 50 g of BAT (less than 0.1% of body weight) could utilize up to 20% of basal calorific needs if maximally stimulated[38].

Adaptive thermogenesis

Studies in identical twins suggest that genetic factors account for 50–90% of the variance in weight gain[39,40]. Part of this disparity is due to differences in energy expenditure and adaptive thermogenesis. Indeed, more than threefold variation in energy expenditure and weight gain has been observed in response to overfeeding in normally lean individuals[39]. Because of its cumulative nature, very small differences in energy expenditure can result in a large impact on body weight over time. For example, differences in energy balance as little as 71 kJ per day (17 kcal), which is approximately the energy utilized in standing for 1 hour, and only about 0.6% of daily total energy expenditure, could theoretically lead to a weight gain or loss of 1 kg per year[23,41–43].

Categories of adaptive thermogenesis. In mammals, adaptive thermogenesis occurs primarily in brown fat and in skeletal muscle. Adaptive thermogenesis can be divided into three subtypes (FIG. 1). Cold exposure induces shivering thermogenesis, a function of skeletal muscle, and non-shivering thermogenesis, a function of brown fat. Overfeeding triggers diet-induced thermogenesis, which is also a function of brown fat. Although current evidence does not indicate a role of muscle in non-shivering thermogenesis, this may be due to the lack of technologies allowing a direct measurement of muscle non-shivering thermogenesis separately from other muscle thermogenic processes. Nevertheless, indirect evidence from malignant hyperthermia[44] and mild cold exposure (16°C) in humans[45] suggest that such mechanisms may exist, and so further investigation of this area is warranted.

Coupled
Coupled in this article refers to processes in which the energy released by one reaction is directly used to drive another one; e.g., protons rushing into the mitochondrial matrix is coupled to ATP production.

Uncoupled
Uncoupled in this article refers to processes in which the energy released is not used by the cell to drive another process; e.g., ion leak.

Diet-induced thermogenesis
The heat produced in response to diet that allows excessive calorific intake. It primarily occurs in brown fat.

Table 3 | **Treatments that increase energy expenditure**

Class	Drug	Principal mechanism of action
Chemical uncoupler	2,4-Dinitrophenol	Non-selective uncoupler of mitochondrial oxidation[18]
Enzyme inhibitor	INCB13739	Inhibits 11-β hydroxysteroid dehydrogenase type 1 (REF. 198) (NCT00398619*)
Hormone	GC-1, KB-141	Thyroid hormone mimetics[145,146]
	Somatotropin	Agonist of growth hormone receptors[199]
Metabolic target	AICAR, metformin	Activate AMP-activated protein kinase[200]
	Desnutrin	Activates adipose triglyceride lipase[201]
	Resveratrol	Activates sirtuin 1 (REFS 157,196)
Receptor agonist	Bromocriptine	Agonist of dopamine D_2 receptors[202]
	Bile acids, INT-777	Agonists of TGR5 (also known as G protein-coupled bile acid receptor 1)[148,149]
	Ephedrine	Mixed sympathomimetic[134]
	Ephedra (ma huang; herb)	Mixed sympathomimetic[134]
	BRL-26830, L-796568, N-5984	Selective agonists of $β_3$-adrenergic receptor[141]
Transcription factor activator	GW501516	Agonist of peroxisome proliferator-activated receptor-δ[203]
Unknown	Reesterified long-chain n-3 polyunsaturated fatty acids (EPA, DHA)	Modifies gene expression[204] (NCT00760760*)

AICAR; 5-aminoimidazole-4-carboxamide 1-β-ᴅ-ribofuranoside; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid. *Denotes ClinicalTrials.gov identifier.

Thermogenesis is essential for warm-blooded (endothermic/homeothermic) animals, ensuring normal cellular and physiological function under conditions of environmental challenge. During prolonged cold exposure of rodents and humans, even as shivering disappears, energy expenditure remains elevated due to non-shivering thermogenesis[46]. Newborn human infants do not shiver, and thus maintenance of body temperature depends entirely on non-shivering thermogenesis by brown fat[47].

Diet-induced thermogenesis was described over a century ago by Neumann as "luxuskonsumption"; that is, a physiological mechanism exists that permits excessive calorific intake to be dissipated as heat, allowing individuals to eat without gaining weight[48]. Diet-induced thermogenesis was found to be closely associated with the recruitment of BAT by enhanced adrenergic activity[38]. It has been proposed that differential responses to diet-induced thermogenesis may account for the large variance in weight gain between individuals in response to overfeeding and thus contribute to the development of obesity[49]. Studies of pair-fed genetically obese mice have clearly shown a defect in BAT-mediated thermogenesis[50,51]. In addition, nutritional components can influence diet-induced thermogenesis. For example, a diet rich in polyunsaturated fatty acids causes a greater induction of diet-induced thermogenesis than a diet rich in saturated fatty acids[52]. The exact impact of other nutritional components on thermogenesis and systemic energy regulation remains to be elucidated.

Diet-induced thermogenesis and non-shivering thermogenesis share common features in that both occur in brown fat and both are regulated by the sympathetic nervous system (SNS). In healthy young adults, heat production in response to both overfeeding and mild cold exposure correlates closely with increased plasma noradrenaline concentrations[53]. At the molecular level, animal experiments have indicated an indispensable role of UCP1 in mediating both non-shivering and diet-induced thermogenesis. Thus, UCP1-ablated mice are more susceptible to cold temperatures and have to rely on shivering for thermoregulation[31]. When kept at thermoneutrality (30°C for mice), *Ucp1*-knockout mice lack diet-induced thermogenesis and develop obesity[33], indicating that diet-induced thermogenesis is fully dependent on UCP1. Although these data suggest a convergence of non-shivering and diet-induced thermogenesis around brown fat and UCP1, there is an important distinction between them. In non-shivering thermogenesis, heat produced is used to maintain body temperature. By contrast, heat produced by diet-induced thermogenesis is quickly dissipated to the environment to prevent body temperature from rising.

Neuronal and hormonal regulation of adaptive thermogenesis. Thermogenesis is rigorously controlled by the nervous and endocrine systems. Hypothalamic nuclei in the central nervous system (CNS) integrate stimuli from two separate pathways to regulate thermogenesis. One is a feed-forward pathway involving cutaneous thermal receptors acting via thermosensory neurons. These cause GABA (γ-aminobutyric acid)-ergic interneurons in the preoptic area of the hypothalamus to disinhibit thermogenesis-promoting neurons in the dorsomedial hypothalamus and thereby drive non-shivering thermogenesis in BAT[54–56]. The second pathway is a negative feedback pathway involving temperature-sensitive neurons in the brain, which inhibit SNS outflow to BAT. The pathways underlying shivering thermogenesis are less well understood, but involve signals that travel through the pontine parabrachial nucleus[57], which are then integrated in the preoptic area[58] to activate α-motor neurons that stimulate shivering[59,60].

A key question that needs to be answered for developing drugs that target adaptive thermogenesis is whether the central pathways stimulating shivering, non-shivering and diet-induced thermogenesis are necessarily connected or whether they can be selectively activated. Clearly, there are circumstances in which BAT activation occurs independently of shivering. In humans, for example, when cold exposure is not extreme, non-shivering thermogenesis precedes shivering thermogenesis[61,62]. It is not yet known whether this separation between non-shivering and shivering thermogenesis results from separated pathways or merely the magnitude of cold exposure.

Adaptive thermogenesis is regulated primarily by the SNS. In response to cold exposure or overfeeding, noradrenaline released from the SNS regulates brown

Carbohydrates and fats arrive into the cytoplasm for metabolism from two sources: uptake of extracellular substrates (such as glucose and free fatty-acids), and intracellular release of substrates from the catabolism of cellular glycogen and lipid stores. Both processes are controlled by both nutrient availability and the action of hormones such as insulin, glucagon and noradrenaline. In the cytoplasm, several preparatory steps take place before fuel substrates are transported into the mitochondrial matrix for energy production. Activated fatty acids and pyruvate are each metabolized to produce the same common intermediate, acetyl-CoA, which is fed into the tricarboxylic acid cycle. Progressive steps of oxidation yield carbon dioxide and reduced forms of NADH and FADH, which deliver their electrons to the electron transport chain.

The electron transport chain is the principal site for the regulated release and transfer of chemical bond energy in eukaryotes. Its goal is to preserve as much of the electrons' potential energy for generation of ATP — the chemical currency of life — as possible. This is accomplished by passing electrons through macromolecular complexes until they are accepted by molecular oxygen, which is split to generate water. As the electron transport chain shuttles electrons through its machinery, hydrogen ions are drawn from the mitochondrial matrix and deposited in the intermembrane space, creating an electrochemical gradient. The potential energy of this gradient is harnessed by the F_0F_1-ATPase (ATPase synthase), which straddles the inner mitochondrial membrane and converts the proton-motive force into the chemical bond energy of ATP. In the resting state, about 90% of cellular oxygen consumption takes place in the mitochondria, and 80% of this is coupled to ATP synthesis[2].

adipocytes at multiple levels. It promotes proliferation and differentiation of brown preadipocytes, directly regulates the thermogenic programme of BAT by activating *UCP1*, and protects brown adipocytes from tumour necrosis factor-α (TNFα)-induced apoptosis[63].

Adaptive thermogenesis is also modulated by hormones. Type 2 iodothyronine deiodinase plays a vital role in regulating the amount of active thyroid hormone (triiodothyronine) in brown fat, thereby modulating adaptive thermogenesis[64,65]. In addition, leptin, released by white adipocytes, regulates energy balance by effects on the hypothalamus that lead to inhibition of food intake and increased thermogenesis via activation of the SNS. However, so far, there is limited evidence that leptin mediates thermogenesis via the SNS in humans[66-70]. Insulin can affect thermogenesis by increasing substrate uptake by BAT and increasing hypothalamic-mediated sympathetic activity[71], both of which may be connected to the thermic effect of food[72]. Glucagon and adrenaline also increase oxygen consumption, but both may be permissive — rather than regulatory — in thermogenesis, in that they provide fuel for thermogenesis, but do not appear to have a primary role in temperature homeostasis. Similarly, glucocorticoids do not directly increase thermogenesis, but they may have an important role in coordinating the thermogenic response to substrate and food availability[65].

Brown fat in human thermogenesis

Rediscovery of brown fat in humans. Brown fat is important for a thermogenic response and energy balance in small mammals. Induction of BAT in mice promotes energy expenditure, reduces adiposity, and protects mice from diet-induced obesity[73,74]. Conversely, ablation of BAT leads to reduced energy expenditure and increased obesity when fed a high-fat diet[75]. In humans, the role of

BAT has been more controversial. Histological evidence has indicated that brown fat is present, albeit in small amounts, in adults throughout life[76]; however, attempts to find functional BAT[77] or utilize its thermogenic capacity for weight loss[78,79] have been largely unsuccessful. This has led to the widely held belief that there is no functional BAT in normal adult humans[80,81].

However, this dogma has recently been reversed by studies using positron emission tomography (PET) and computed tomography (CT) imaging. PET uses radiotracers such as ^{18}F-fluorodeoxyglucose (^{18}F-FDG) to measure the metabolic activity of different regions of the body. CT provides high-resolution anatomical detail. Fusion of the PET and CT images therefore simultaneously provides both functional and precise structural information, which has been mainly used for the detection and staging of tumours[82]. The possibility that this FDG-avid adipose tissue could represent functional BAT was first noted in the radiological literature[83,84], and a potential physiological role in humans was suggested by Nedergaard and colleagues[80,81]. However, it was only during the past year that PET and CT imaging was used to prove conclusively that adult humans possess physiologically active UCP1-positive BAT[13–17]. This identification of functional BAT in adult humans has led to a rethinking within the medical and scientific communities that BAT may have a role in normal physiology, and therefore could be a target for obesity treatment[81,85].

The location of BAT in adult humans was also unexpected. In rodents and in human babies, BAT is interscapular. In adult humans, on the other hand, the most common location for metabolically active BAT is the cervical–supraclavicular depot, in a distinct fascial plane in the front of the neck, sometimes extending into the thoracic and lumbar region. The percentage of adult humans with functional BAT under normal conditions is unresolved. Retrospective case studies using ^{18}F-FDG PET and CT scans report this to be less than 10%[13,83]. Nevertheless, histological analysis of cervical fat biopsies show rates three times higher[17], and prospective studies using cold stimulation to increase BAT activity and detection via PET and CT show that among younger people, 96% have functional BAT[14]. This discrepancy is in part due to fundamental limitations in PET and CT imaging technology for quantifying BAT mass and activity. CT alone cannot yet distinguish between brown versus white fat. Complicating the PET image is the need to see a concentrated signal above background, and various factors can alter ^{18}F-FDG uptake, including dietary fatty acids and drugs such as β-adrenergic blockers. In rodents, small collections of physiologically relevant BAT have been found in the hindlimb in intermuscular adipose tissue and mixed with white fat[86,87]. Whether humans could have such scattered small collections of BAT remains unknown. Improvements in imaging technology will be essential to identify small or scattered depots, and also essential for studies devoted to exploiting BAT energy expenditure for the treatment of obesity. Given the scanning data and the finding of brown adipocyte precursors in human tissues[17], it is likely that virtually every adult human has the capacity to develop some functional BAT.
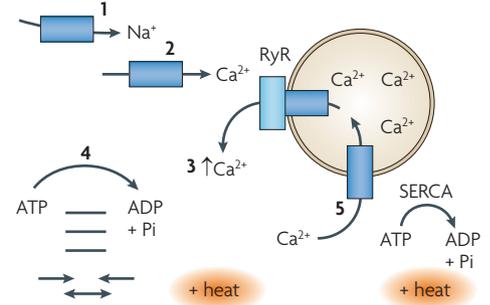
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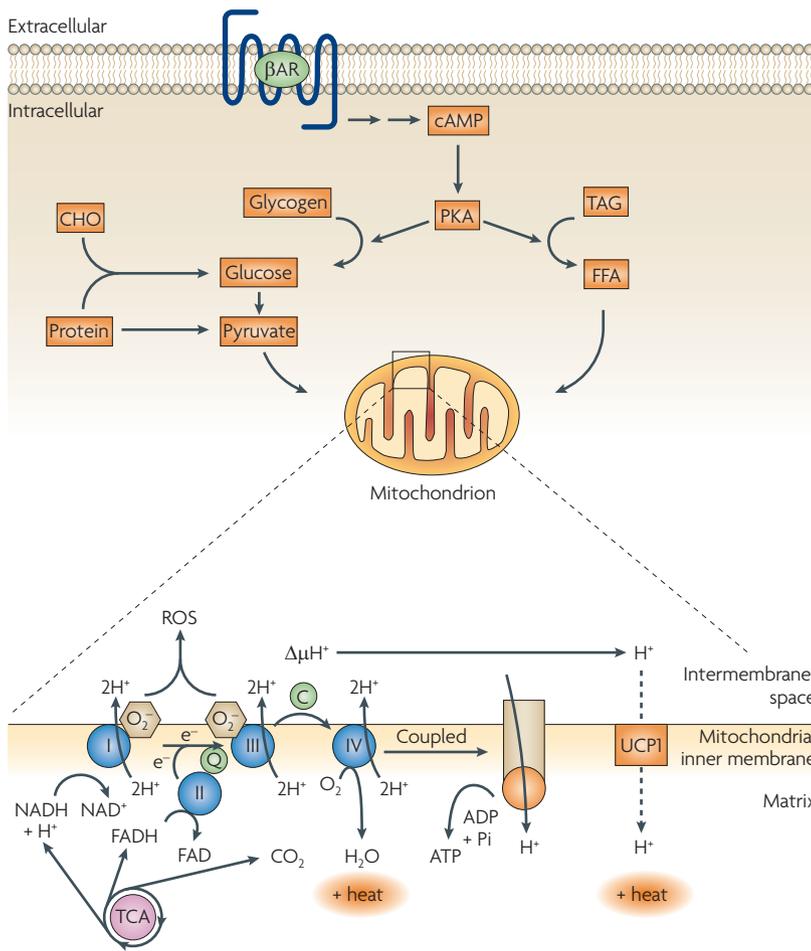
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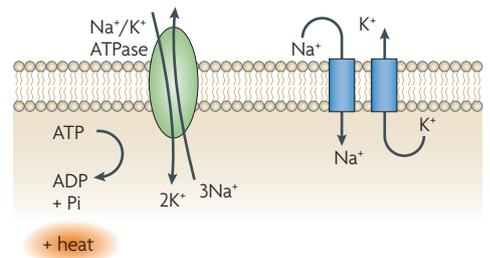
c Muscle/actin–myosin relaxation

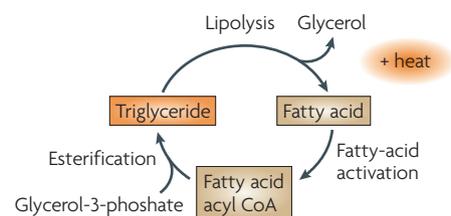**d** Triglyceride/fatty-acid cycling

Figure 2 | **Molecular mechanisms of cellular thermogenesis. a** | Regulated increases in thermogenesis occur in brown adipocytes with the stimulation of β-adrenergic receptors (βARs), initiating a signal transduction cascade that produces cyclic AMP (cAMP) and activates protein kinase A (PKA), which then activates multiple enzymes responsible for converting the catabolic end products of macronutrients (carbohydrates (CHO), fats (triacylglycerols (TAG) and free fatty acids (FFA)) and proteins) into mitochondrial fuel. The tricarboxylic acid (TCA) cycle generates protons (H^+) and electrons (e^-) that are carried by NADH and FADH to the electron transport chain (ETC) where the protons are transported to the mitochondrial intermembrane space, generating an electrochemical gradient ($\Delta\mu H^+$) that is used by the F_0F_1-ATPase (ATPase synthase) to convert that potential energy into the phosphoanhydride bonds in ATP. Meanwhile, the electrons are transported in successive steps through the ETC complexes — complex I, NADH–ubiquinone (Q) oxidoreductase; complex II, succinate–ubiquinone oxidoreductase; complex III, ubiquinone–cytochrome c (C) oxidoreductase; and complex IV, cytochrome-c oxidase — until they are received by O_2 to make H_2O. The highly reactive electrons also lead to the generation of reactive oxygen species (ROS), which can cause significant cellular damage. The TCA also produces CO_2 as a by-product. The respiratory quotient (RQ) is the ratio of CO_2 produced $\div O_2$ consumed and typically ranges between 0.7 for fats and 1.0 for carbohydrates. Thus, RQ can help identify the mitochondrial fuel source. UCP1 is located in the inner mitochondrial transmembrane of brown adipocytes, which allows protons in the mitochondrial intermembrane space to re-enter the mitochondrial matrix without generating ATP, that is, uncoupled, and heat is generated in this process. **b** | Multiple tissues, including muscle, generate heat via uncoupled processes such as leakage of ions (H^+, Na^+, K^+ and Ca^{2+}) through channels back down their electrochemical gradients. Shown here is the ubiquitous Na^+/K^+ ATPase releasing heat energy and Na^+ and K^+ leaking back to perpetuate this 'futile' cycle. **c** | Myocytes can also increase thermogenesis through a series of uncoupled reactions. Neurotransmitter-mediated opening of cell-surface Na^+ channels (**1**) leads to release of Ca^{2+} into the cytoplasm from sources both outside the cell (**2**) and the sarcoplasmic reticulum (**3**) via the ryanodine receptor (RyR). Dysfunction of this receptor leads to uncontrolled Ca^{2+} release, and underlies the thermogenesis in malignant hyperthermia[44]. Ca^{2+} results in heat generation from ATP hydrolysis (**4**) during both muscle relaxation and actin–myosin crossbridge cycling during sustained contraction. Additional heat energy is released when Ca^{2+} ions are pumped back into the sarcoplasmic reticulum by the sarcoplasmic reticulum Ca^{2+} ATPases (SERCAs) (**5**). **d** | Triglyceride/fatty-acid cycling is an example of a 'futile' cycle involving muscle and adipose tissue in which esterification of triglycerides is followed by hydrolysis, leading to increased heat expenditure in processes as diverse as burn injuries, cancer cachexia, and after exercise. UCP1, uncoupling protein 1.

Some parameters of human BAT function have already been defined. PET and CT positivity shows seasonal variation[13,16,88], indicating a role of BAT in normal adaptation to cold. More importantly, BAT activity correlates inversely with BMI[13,14] and percentage body fat[13,14], suggesting a role in energy balance. This inverse correlation between BAT activity and obesity was seen despite the fact that the lean and overweight subjects had similar resting metabolic rates in both thermoneutral and cold exposure scenarios, suggesting that overweight individuals increase their energy expenditure using different physiological mechanisms[14]. Functional BAT also decreases with age, and is rarely observed in non-stimulated (that is, no investigator-controlled stimulation such as cold or pharmacological interventions), overweight adults over 64 years of age[13], suggesting a possible role of decreased BAT activity in the development of age-related obesity. Together, these findings suggest that drugs that can increase BAT activity may be useful in combating obesity, and in older adults may help restore a component of normal human physiology.

Regulation of brown fat development

Cellular lineage specification. Adipose tissue is generally considered to arise from the multipotent mesenchymal stem cells of mesodermal origin[89]. Emerging evidence suggests that brown adipocytes located in different anatomical locations may arise from different developmental origins. *In vivo* fate-mapping has shown that progenitors derived from the central dermomyotome give rise to the interscapular brown fat cells[90], suggesting that the interscapular brown fat and skeletal muscle may share a common developmental ancestry. In support of this notion, cell-cultured brown fat precursors appear to possess a myogenic signature, which includes myogenic factor 5 (MYF5)[91]. Most recently, lineage-tracing studies have indicated that *MYF5*-expressing progenitors can give rise to both skeletal muscle and the preformed BAT in the interscapular and perirenal depots[92] (FIG. 3).

However, not all brown fat cells are derived from *MYF5*-expressing progenitors. For example, the brown fat cells emerging in white fat in response to β_3-adrenergic receptor stimulation are not marked by the MYF5-driven fluorescent protein[92]. When stimulated by a PPARγ agonist, they express molecular characteristics that are distinct from the interscapular brown fat cells[93]. Likewise, we have found that MYF5-negative progenitors isolated from adult mouse skeletal muscle can differentiate into brown fat *in vitro* (Y.-H.T. *et al.*, manuscript in preparation). Thus, there exists a second class of progenitors that serve as a common precursor for white adipocytes and systemic brown adipocytes (FIG. 3).

It is also possible that some UCP1-positive brown fat cells found in white adipose tissue (WAT) or skeletal muscle come from transdifferentiation of white adipocytes[94]. Whatever their origin, these systemic brown fat cells are found in white fat and between muscle bundles, and have distinct features compared with the interscapular brown adipocytes. These systemic brown adipocytes are often found admixed with white fat cells; are more

Resting metabolic rate
The amount of energy expended at rest. It is also similar to the standard metabolic rate, except that the metabolic rate is measured while the organism is still digesting food.
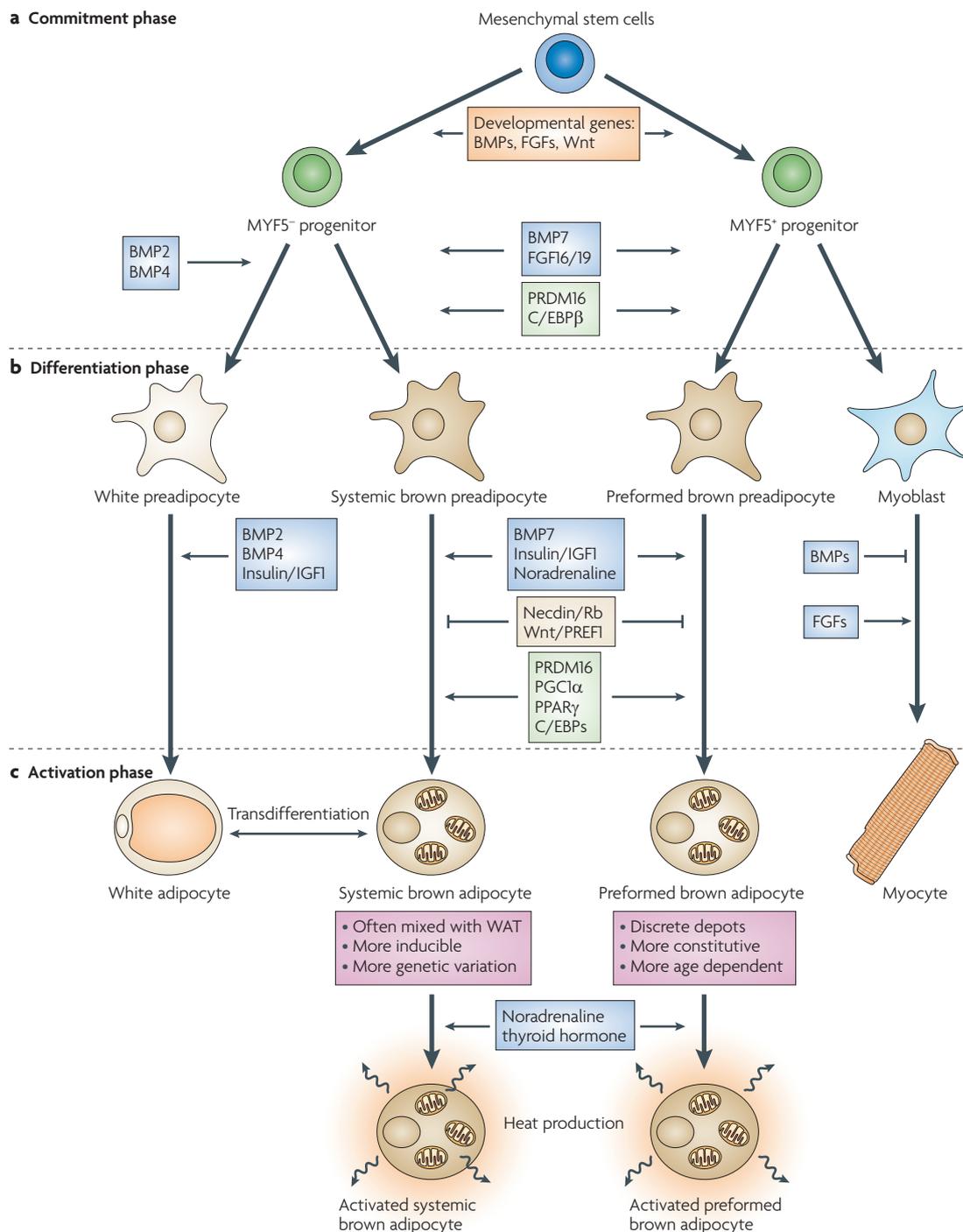
sensitive to β_3-adrenergic receptor stimulation and cold exposure; and their thermogenic capacity seems to be regulated by genetic background[74,86,87].

Interestingly, intermuscular brown adipocytes are more abundant in the obesity-resistant strain of mice[86], and feeding with a high-fat diet does not alter *Ucp1* expression in skeletal muscle[95], suggesting a crucial role of these systemic brown adipocytes in protection against obesity. In humans, interscapular BAT is only a transient phenomenon in newborns[96] and is replaced in adults by BAT in the neck and other anatomical locations. Which, if either, population of progenitors gives rise to this adult human brown fat remains to be determined; however, the brown adipocytes present in the neck are often admixed with white adipocytes and seem to be highly sensitive to activation by cold exposure.

Stages and signals inducing brown fat development. The development of fully functional brown adipocytes can be divided into three phases: a commitment phase, a differentiation phase and an activation phase (FIG. 3). Several developmental signalling molecules that have been implicated in the evolution of mesodermal tissue have been shown to affect early stages of brown fat development. These include nodal; wingless; members of the fibroblast growth factor (FGF), transforming growth factor-β (TGFβ) and bone morphogenetic protein (BMP) families; and others[89]. The exact effects of these factors depend on concentration, stage of differentiation, cell–cell interactions and the nature of the extracellular matrix.

Although TGFβ inhibits adipocyte differentiation *in vitro*[97], TGFβ expression in fat is paradoxically increased with obesity in rodents and humans[98,99]. BMPs are a family of developmental regulators belonging to the TGFβ superfamily that promote adipogenesis at different stages[100]. BMP2 and BMP4 promote white fat differentiation, whereas BMP7 serves as a potent inductive signal for brown adipogenesis[101]. BMP7 activates a full programme of brown adipogenesis, including induction of early regulators of brown fat fate (PPARγ coactivator 1α (PGC1α) and PRDM16); increased expression of adipogenic transcription factors (CCAAT/enhancer-binding proteins (C/EBPs) and PPARγ); mitochondrial biogenesis; and increased expression of *UCP1* (FIG. 3). Moreover, BMP7 triggers commitment of mesenchymal progenitor cells to a brown adipocyte lineage and also plays a crucial role in embryonic brown fat development. This is exemplified by the *Bmp7*-knockout embryo that shows a marked paucity of brown fat and near complete absence of UCP1 (REF. 101). Adenoviral-mediated expression of *Bmp7* in mice results in a significant increase in brown but not white fat mass, and leads to an increase in energy expenditure and reduced weight gain[101]. In addition, mice deficient in growth differentiation factor 3 (GDF3), another member of the TGFβ/BMP superfamily, are protected from diet-induced obesity due to an increased BMR, which is presumably caused by the occurrence of UCP1-positive systemic brown adipocytes within WAT[102].

The other important developmental signalling system guiding brown fat development is the FGF family. Basic FGF (bFGF; also known as FGF2) stimulates the

a Commitment phase

Mesenchymal stem cells

Developmental genes:
BMPs, FGFs, Wnt

MYF5⁻ progenitor

MYF5⁺ progenitor

BMP2
BMP4

BMP7
FGF16/19

PRDM16
C/EBPβ

b Differentiation phase

White preadipocyte

Systemic brown preadipocyte

Preformed brown preadipocyte

Myoblast

BMP2
BMP4
Insulin/IGF1

BMP7
Insulin/IGF1
Noradrenaline

Necdin/Rb
Wnt/PREF1

PRDM16
PGC1α
PPARγ
C/EBPs

BMPs

FGFs

c Activation phase

White adipocyte

Transdifferentiation

Systemic brown adipocyte

Preformed brown adipocyte

Myocyte

• Often mixed with WAT
• More inducible
• More genetic variation

• Discrete depots
• More constitutive
• More age dependent

Noradrenaline
thyroid hormone

Heat production

Activated systemic
brown adipocyte

Activated preformed
brown adipocyte

Figure 3 | **Lineage determination and control of brown adipocyte development.** In this model, we propose that there are distinct progenitors giving rise to the preformed versus systemic brown adipocytes. Although the myogenic factor 5 (*MYF5*)-expressing progenitors give rise to skeletal muscle and interscapular brown fat[92], a distinct MYF5-negative tissue resident progenitor serves as the common precursor for white and systemic brown adipocytes. The development of a fully functional brown adipocyte can be divided into three phases. The commitment phase is when multipotent mesenchymal stem cells become committed to brown adipocyte lineage in response to developmental cues, such as bone morphogenetic proteins (BMPs) and fibroblast growth factors (FGFs) (**a**). The differentiation phase is when committed brown preadipocytes undergo a series of morphological and enzymatic changes to become rounded lipid-containing fat cells (**b**). This process is regulated by a number of growth factors and hormones, and involves the activation of the transcriptional cascade. The activation phase refers to the stage when the maximal thermogenic capacity in matured brown adipocytes is turned on by hormonal or sympathetic stimulations (**c**). C/EBP, CCAAT/ enhancer-binding protein; IGF1, insulin-like growth factor 1; PGC1α, peroxisome proliferation-activated-γ (PPARγ) coactivator 1α; PRDM16, PR domain zinc finger protein 16; Rb, retinoblastoma; PREF1, preadipocyte factor 1 (also known as DLK1); WAT, white adipose tissue.

Box 2 | Molecular controls of brown fat development

At the molecular level, brown fat differentiation involves mechanisms that are common to both brown and white adipocyte differentiation, as well as some specific factors[89,165–167]. Before initiation of the adipogenic transcriptional cascade, both brown and white preadipocytes need to be released from suppression and become committed to terminal differentiation. Among the known inhibitors of preadipocyte to adipocyte transition, proteins of the retinoblastoma (Rb) family and necdin (NDN), a growth repressor functionally resembling Rb, selectively inhibit brown preadipocyte differentiation[132,133,168].

After release from suppression, the committed brown preadipocytes initiate a transcriptional cascade involving the transcription factors CCAAT/enhancer-binding proteins (C/EBPs) and peroxisome proliferator-activated receptor-γ (PPARγ) to turn on lipid synthesis and other adipocyte-specific programmes. Several transcription factors and co-regulators seem to play particularly important roles in the final stages of differentiation of brown adipose tissue and in the modulation of the expression of thermogenic genes, especially uncoupling protein 1 (UCP1). Nuclear co-repressor receptor interacting protein (RIP140; also known as NRIP1) directs histone and DNA methylation to silence UCP1 expression and to suppress mitochondrial biogenesis in white adipocytes[169,170]. The zinc-finger-containing protein PRDM16, which is expressed at higher levels in brown compared with white adipocytes[131], has been shown to drive differentiation of white preadipocytes or myoblasts into functional brown adipocytes. This effect depends on the interaction of PRDM16 with PPARγ coactivator 1α (PGC1α)/PGC1β, PPARγ and C/EBPβ, whereas binding of PRDM16 to C-terminal binding protein 1 (CTBP1) and CTBP2 suppresses expression of white-fat-selective genes[171,172].

growth of brown fat precursors and may contribute to cold-induced enlargement of brown fat via an autocrine mechanism[103]. FGF16 mRNA is expressed predominantly in BAT during embryonic days 17.5–19.5, and thereafter at lower levels into the neonatal period[104]. Transgenic mice overexpressing FGF19 have increased BAT mass and reduced susceptibility to diet-induced obesity[105]. Interestingly, mice that overexpress FGF21, a circulating FGF that is induced by a ketogenic diet[106], are lean and have increased brown fat[107], suggesting that the beneficial metabolic effects induced by a ketogenic diet are brought about, in part, by increases in BAT mass. Recent identification of the klotho (KL) gene family as cofactors essential for FGF function has added complexity to the role of FGF signalling in brown fat development. Disruption of the Kl gene in mice results in almost complete absence of WAT and although BAT is present, its size and UCP1 protein expression are reduced. These mice also have lower core body temperature[108].

At the molecular level, brown fat differentiation involves mechanisms common to both brown and white adipocyte differentiation, as well as some specific factors (BOX 2). Noradrenaline has an important role in regulating proliferation and differentiation of brown preadipocytes, as well as directly modulating the thermogenic function in mature brown adipocytes. In addition, the insulin–insulin-like growth factor 1 (IGF1) signalling system also exerts an essential role in brown fat differentiation and function (FIG. 3). Brown preadipocytes lacking insulin receptor substrate 1 (IRS1) display a severe defect in differentiation[109], and IRS2-null brown adipocytes have impaired glucose uptake function[110]. Both insulin and IGF1 protect these precursor cells from apoptosis, and this effect is mainly mediated by IRS1 (REF. 111).

Muscle as a thermogenic organ

After BAT, skeletal muscle is the other important organ for thermogenesis[46]. Three types of thermogenesis occur in skeletal muscle: exercise-induced thermogenesis, non-exercise activity thermogenesis and cold-induced shivering thermogenesis. There is no doubt that exercise has profound beneficial effects on virtually all biological systems, and is an effective way to burn excess energy. For an 80 kg human, jogging 40 min at 6 miles (10 km) per hour will burn about 535 kcal (480 kcal above BMR). If this is done three times a week and there is no increase in food intake, there would be a negative calorific balance equivalent to 18.3 lbs (8.3 kg) of fat over the course of a year.

Non-exercise activity thermogenesis is energy burned by movement other than exercise, such as fidgeting, maintenance of posture and other activities of daily life, and has been found to play an important part in dissipating excess energy to preserve leanness in adult humans[43]. Non-exercise activity thermogenesis is highly variable and ranges from ~15% of total daily energy expenditure in highly sedentary individuals to more than 50% in highly active people. Non-exercise activity thermogenesis is also generally higher in lean than obese individuals. Indeed, lean and obese individuals are different in the energy dedicated to non-exercise activity thermogenesis — on average 350 kcal per day (equivalent to 30.3 pounds of fat over a year)[112].

Shivering thermogenesis is the physiological response to help protect body temperature from cold exposure. Shivering thermogenesis occurs in muscle and complements non-shivering thermogenesis, which occurs primarily in BAT in response to cold. Up to 250 kcal per hour are consumed by shivering[113], but this response is highly variable, usually of short duration, and not a likely method to be used for therapeutic intervention.

Skeletal muscle contains different types of myofibres that differ in speed of contraction, mitochondrial content and pattern of energy use. Type I (red) myofibres have a slow-twitch speed of contraction, a higher mitochondrial content, and thus a higher rate of oxidative metabolism. Type II (white) myofibres have a more rapid speed of contraction and both oxidative and glycolytic properties. Endurance exercise training triggers a remodelling of skeletal muscle by increasing the expression of genes involved in mitochondrial respiration and fatty-acid oxidation, which helps protect against obesity and related metabolic disorders[114]. Chronic cold exposure may also trigger a switch from fast- to slow-twitch muscle with more oxidative myofibres by inducing the expression of nuclear co-activator PGC1α, the same co-activator that is induced in brown fat in response to cold[115]. Forced overexpression of PGC1α in myotubes can produce the same change[116]. On the other hand, thyroid hormone, which also increases thermogenesis, promotes formation of less oxidative fibres[46], indicating that different physiological stimuli regulate thermogenesis in muscle by different mechanisms.

Another important mechanism for heat production in skeletal muscle involves ATP turnover and maintenance of the Ca^{2+} gradient mediated by the

sarcoplasmic reticulum Ca²⁺ ATPases (SERCAs) (FIG. 2c). Neurotransmitter-mediated opening of cell-surface sodium channels leads to release of Ca²⁺ into the cyto-plasm from sources outside the cell and the sarcoplasmic reticulum via the ryanodine receptor (RyR). Dysfunction of RyR results in uncontrolled Ca²⁺ release, and excessive thermogenesis can lead to malignant hyperthermia[44]. Ca²⁺ leads to heat generation from ATP hydrolysis during both muscle relaxation and actin–myosin cross-bridge cycling during sustained contraction. Additional heat energy is released when Ca²⁺ ions are pumped back into the sarcoplasmic reticulum by SERCA. Cold expo-sure induces the expression of and increases the activity of SERCA1 (also known as ATP2A1) in skeletal mus-cle to increase muscle oxidative metabolism and heat production[117].

Ephedrine is a mixed sympathomimetic capable of directly activating both α-adrenergic and β-adrenergic receptors and enhancing release of noradrenaline from sympathetic neurons[118]. Astrup *et al.* estimated that up to 50% of the increase in metabolism in lean individuals induced by ephedrine is attributable to skeletal muscle, and 24% is contributed by BAT[119]. However, consider-ing the relative mass of these tissues, BAT is 100–200 times more effective as a thermogenic organ per gram of tissue than muscle. Moreover, these calculations were performed focusing only on the minor perirenal BAT depot, suggesting that the contribution of total body BAT to thermogenesis is even greater. Mild cold exposure (16°C) also induces muscle mitochondrial uncoupling and increases energy expenditure via non-shivering thermogenesis[45]. However, the recent identifi-cation of brown adipocytes interspersed between muscle bundles in mice[86] raises the question of whether some of the measured non-shivering thermogenesis energy expenditure in skeletal muscle comes from these inter-spersed brown adipocytes. As the extent of intermus-cular brown fat is determined by genetic factors, these systemic brown adipocytes could play a role in the large variations in energy expenditure between individuals.

Therapeutically targeting bioenergetics

With the recognition that adult humans have BAT, tar-geting cellular bioenergetics has become an increasingly attractive way to dissipate excess energy and provide a potential therapeutic approach for the treatment or prevention of obesity and its associated diseases. Approaches to increase adaptive thermogenesis may include small-molecule pharmaceuticals, growth factors and nutritional factors, as well as cell-based or *ex vivo* therapy (FIG. 4). Based on the current knowledge of bioen-ergetics, four potential therapeutic approaches could be envisioned: increasing brown fat differentiation from progenitor cells; activating brown fat thermogenesis; promoting skeletal muscle thermogenesis; or increasing general mitochondrial uncoupling.

Increasing brown fat differentiation from progenitor cells. Recently, brown fat progenitors have been identi-fied in skeletal muscle and white fat of humans[120,121], sug-gesting that it may be possible to increase the oxidative

capacities in these tissues by targeting these endogenous precursors to differentiate *in vivo*, and therefore produce energy-dissipating BAT. However, factors that regulate differentiation and function of these progenitors remain to be determined. Among the various newly identified factors that control the commitment and differentiation of brown fat progenitors discussed above, secreted pro-teins provide the most directly druggable agents. Indeed, both BMP7 and FGFs are of direct therapeutic potential for obesity and its related metabolic disorders.

Originally identified as a bone inducer, BMP7 is now being recognized as a multifunctional cytokine and has been implicated, mainly using mouse models, as a poten-tial therapeutic agent for cardiovascular, metabolic, and degenerative disorders[122]. Because of its important role in renal development and maintenance of normal kid-ney function in adult life, BMP7 has been proposed as a therapeutic agent for chronic kidney diseases such as diabetic nephropathy[123,124]. In addition, BMP7 has been found to exert a neuroregenerative effect for the treat-ment of ischaemic stroke or Parkinson's disease[125–127]. BMP7 has also been shown to reverse endothelial-to-mesenchymal transition associated with cardiac fibrosis[128], and facilitates liver regeneration[129]. BMP7 is pharmaceutically available and is already in use for orthopaedic procedures in the United States under the US FDA's Humanitarian Device Exemptions. When systemically expressed in mice, BMP7 leads to reduced weight gain[101], suggesting that it may have anti-obesity potential; although more study is needed to determine an optimal therapeutic approach and whether there will be any unwanted side effects. It is possible that tissue-selective or action-selective forms of BMP7 could be developed, eliminating the bone-inducing effect, but retaining its brown adipogenic effects.

Recently, there is a growing interest in exploring the pharmacological potential of the FGF family in the treatment of metabolic diseases[130]. As discussed above, FGF16, FGF19 and FGF21, together with the KL family of cofactors, have been implicated in the regulation of brown fat development. Although the biology of this family of growth factors still needs to be fully explored, development of recombinant FGFs and small-molecule mimetics may hold therapeutic potential for the treat-ment of obesity. In addition to these secreted factors, the development of agents targeting key regulators of brown adipogenesis, such as PRDM16, PGC1α, retinoblas-toma (Rb) or necdin (NDN)[115,131–133], warrants further investigation.

Alternatively, an *ex vivo* approach could be used to create thermogenic cells that could be reimplanted into obese individuals (FIG. 4). Recent advances in stem cell biology open up the possibility of isolating progenitor cells from an individual (by liposuction or muscle biopsy), stimulating them *ex vivo* by factors that promote BAT differentiation (such as BMP7 or FGFs) or transfecting them with genes that specify BAT development (such as *PRDM16* or *PGC1A*). These cells could then poten-tially be transplanted back to the donor to allow *in vivo* expansion and differentiation into functional brown fat that could help burn energy. Because this approach
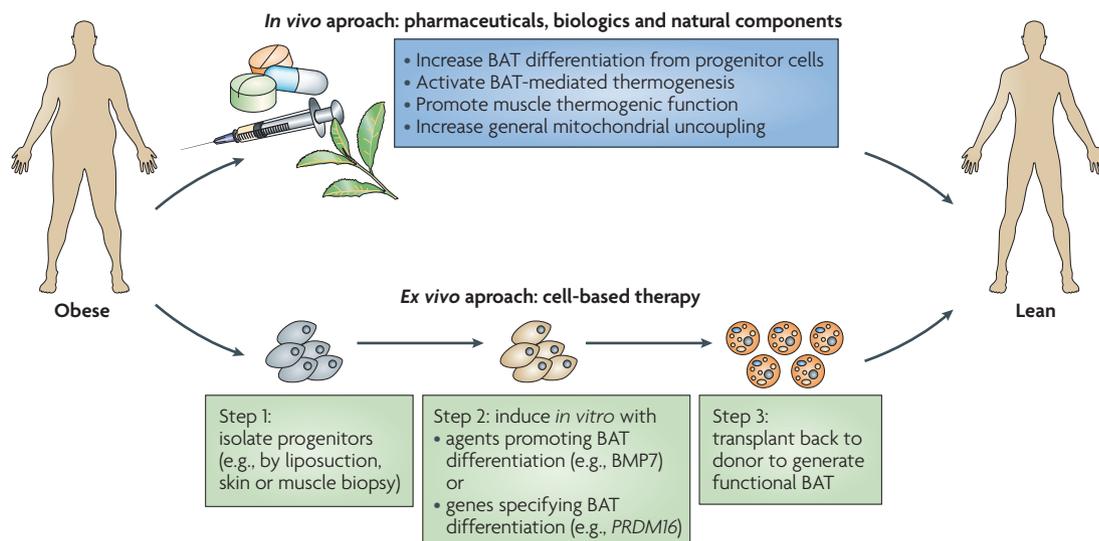
Figure 4 | Approaches to increasing thermogenesis as an anti-obesity therapy. Based on the current knowledge of bioenergetics, four potential therapeutic approaches could be envisioned: increasing brown fat differentiation from progenitor cells; activating brown fat thermogenesis; promoting skeletal muscle thermogenesis; or increasing general mitochondrial uncoupling. For skeletal muscle, there are three types of thermogenesis: exercise-induced thermogenesis, non-exercise activity thermogenesis, and cold-induced shivering thermogenesis. Thus, therapeutic interventions that mimic these mechanisms could potentially increase the thermogenic capacity of muscle and counteract obesity. This is especially beneficial to individuals with physical limitations in exercising or those who are genetically predisposed to obesity. All of these strategies can be applied in the conventional pharmaceutical approaches of developing drugs and/or using natural food components that target key pathways of cellular bioenergetics. Alternatively, a cell-based therapy may be possible, whereby progenitors are isolated from patients during liposuction or biopsy, manipulated *ex vivo* by treating them with factors that promote brown adipose tissue (BAT) differentiation or transfecting them with genes specifying brown adipose tissue differentiation, then transplanting these cells back into the same individual to generate a functional brown fat to help dissipate excess energy. BMP7, bone morphogenetic protein 7; *PRDM16*, PR domain zinc finger protein 16.

involves minimal surgical procedures, it may represent an attractive alternative to those obese individuals who are unwilling to undergo liposuction or bariatric surgery for weight loss. Clearly, additional experiments in animal models will be needed to determine the optimal locations of engineered-cell implantation and assess the efficacy and potential side effects before this approach becomes feasible.

Activating brown fat-mediated thermogenesis. Although it has only recently been proved that adult human BAT is functional and may be involved in protecting against weight loss[13–17], attempts to exploit BAT energy expenditure for treating obesity have been around for decades.

Ephedra spp. is a family of plants comprising more than 40 species, many indigenous to China, known as ma huang. Among its myriad of chemical compounds, ephedra contains the alkaloids ephedrine, pseudoephedrine and other sympathomimetics that can induce CNS stimulation, bronchodilation and vasoconstriction[118]. A meta-analysis of several dozen trials showed that ephedrine promotes modest short-term weight loss, but there are no data on longer-term efficacy[134]. PET and CT imaging in rats shows that ephedrine's effects on metabolism are mediated at least in part by activation

of BAT[135]. Unfortunately, ephedrine is associated with increased risk of adverse psychiatric, autonomic, gastrointestinal and cardiovascular symptoms[134], which will probably preclude the use of ephedrine itself for the treatment of obesity.

Caffeine is the most widely used psychoactive agent in the world[136]. Caffeine is a trimethylxanthine that inhibits adenosine A_{2A} receptors[137], thus stimulating the CNS and reducing the perception of fatigue. By itself, caffeine is not thought to stimulate the SNS enough to activate BAT[138]. However, the combination of ephedrine and caffeine is a potent mediator of short-term weight loss[139] that probably involves activation of BAT by β_3-adrenergic receptors[140].

Targeted approaches to activate BAT by selective sympathetic activation have had limited success so far. In rodents, the β_3-adrenergic receptor is found nearly exclusively on brown adipocytes, and treatment with the β_3-adrenergic receptor-specific agonist CL-316243 substantially increases energy expenditure[18]. Although human brown fat also expresses β_3-adrenergic receptors[15], initial human trials using β_3-adrenergic receptor agonists were not successful, as human β_3-adrenergic receptors have different binding characteristics to those in rodents[23]. A second generation of β_3-adrenergic receptor agonists with improved binding properties had poor

oral bioavailability or unfavourable pharmacokinetics[141]. In addition, these studies were done before PET and CT scanning was known to be useful for specifically measuring BAT function and mass. One β_3-adrenergic receptor agonist, L-796568, acutely increased lipolysis and energy expenditure in overweight individuals[142], but its effect seemed to be lost after 28 days of treatment[79,141]. Given the new ability to quantify human BAT activity, attempts at therapeutically developing β_3-adrenergic receptor agonists and other drugs that activate BAT or stimulate BAT growth requires re-evaluation. If BAT activity can be measured, then whether or not a given intervention has worked as designed can be determined. For example, when testing β_3-adrenergic receptor agonists designed to stimulate adipose tissue activity, quantifying BAT activity will validate whether any increases in energy expenditure were due to changes in WAT, BAT, both or neither.

The principal safety considerations regarding increasing BAT thermogenesis involve the thermodynamic implications of this therapy and raise a number of questions. How easily regulated is BAT thermogenesis? Would stimulated BAT induce necrosis? Could the body temperature rise to dangerous levels as seen with DNP[143]? Will there be such an increased demand for cardiac output that treatment will be contraindicated in the elderly and those with heart disease? Going forward, these considerations must remain in the forefront of efforts designed to induce weight loss through BAT-mediated energy expenditure. Fortunately, BAT is not a simple combustion engine, but an exquisitely regulated biological tissue with internal negative feedback pathways[30].

Early attempts to use thyroid hormone to increase thermogenesis and induce weight loss were confounded by tachycardia, bone loss and muscle catabolism[144]. Current approaches focus on thyroid hormone mimetics that selectively increase energy expenditure, in part by activating specific thyroid hormone receptor isoforms. Indeed, two of the selective thyroid hormone mimetics, GC-1 and KB141, can promote fat loss in rodents without causing unwanted effects on heart or muscle[145,146]. Recently, a new role in thyroid hormone-mediated thermogenesis was observed in response to bile acids[147]. Bile acids are ligands for the nuclear hormone receptor farnesoid X receptor-α, which regulates enterohepatic lipid recycling and causes downregulation of hepatic fatty acid and triglyceride biosynthesis. Bile acids also increase energy expenditure in BAT, preventing obesity and resistance to insulin. This effect is dependent on induction of the type 2 iodothyronine deiodinase and is mediated by the binding of bile acids to a novel G protein-coupled receptor TGR5 (REF. 148). In addition, TGR5 stimulates glucagon-like peptide 1 (GLP1) production in enteroendocrine cells, which may improve glucose metabolism through its insulinotropic effects. TGR5 is therefore a new and attractive target for treating obesity, as it can theoretically protect against obesity and its complications via two distinct and potentially synergistic mechanisms: increased energy expenditure and improved insulin sensitivity[148]. One promising TGR5 agonist, INT-777, has already shown efficacy *in vivo*, increasing energy expenditure and reducing adiposity in mice with diet-induced obesity[148,149].

The adipokine leptin decreases appetite and increases energy expenditure, and is able to completely reverse obesity in the leptin-deficient *ob/ob* mouse[150]. However, leptin deficiency is rare in humans, and most obese individuals suffer from leptin resistance, therefore leptin administration is only effective in a small fraction of patients[151]. Relative leptin deficiency — such as occurs in congenital or acquired lipodystrophies, in thin, very athletic women with hypothalamic amenorrhea, and in anorexia nervosa — does respond to leptin administration[152,153]. A promising approach combines metreleptin (recombinant human methionyl-leptin) treatment with the amylin analogue pramlintide, which has been suggested to act as a leptin sensitizer[154] (TABLE 1).

Promoting the thermogenic function of skeletal muscle. Given the numerous health benefits of exercise, the idea of a pill that mimics the effects of exercise is highly attractive[46], especially in individuals with physical limitations in exercising or those who are genetically predisposed to obesity[114]. Resveratrol (3,5,4′-trihydroxystilbene), a polyphenol found in grape skins, red wine, peanuts and mulberries, can improve exercise endurance and protect mice against diet-induced obesity and insulin resistance[155]. This effect is mediated by increasing mitochondrial biogenesis and oxidative phosphorylation via activation of the NAD+-dependent deacetylase sirtuin 1 (SIRT1)–PGC1α complex (TABLE 3). Resveratrol treatment increases lifespan and improves the metabolic profile and activity levels of mice with high-fat diet-induced obesity[156]. Preclinical observations have suggested that resveratrol and its analogues such as SRT1720 are safe and may have applications in the treatment of obesity and insulin resistance in humans. At this time, resveratrol has not yet been demonstrated to affect BAT directly. However, SRT1720 has been shown in BAT to modify lipid droplet size and gene expression[157,158].

Another enzyme central to cellular bioenergetics is AMP-activated protein kinase (AMPK), which detects the nutrient status of the cell and helps regulate glucose transport, fatty-acid oxidation and metabolic adaptations in skeletal muscle[159]. Chronic activation of AMPK by 5-aminoimidazole-4-carboxamide-1-β-D-ribofuranoside (AICAR; TABLE 3) enhances mitochondrial function in skeletal muscle[159]. Recent studies show that AMPK also enhances SIRT1 activity by increasing cellular NAD+ levels, resulting in the deacetylation and modulation of the activity of downstream SIRT1 targets[160]. Pilot studies are currently underway using resveratrol (ClinicalTrials.gov identifier: NCT00654667) or AICAR (ClinicalTrials.gov identifier: NCT00168519) to treat metabolic diseases. Recently, Narkar *et al.*, showed that treatment of mice with a combination of AICAR and GW1516, an agonist of the muscle-specific transcriptional regulator PPARδ, synergistically increased oxidative myofibres and running endurance[161]. Although these data suggest a potential use of these compounds

in improving skeletal muscle function and increasing energy expenditure, the safety issue of using AICAR or other drugs targeting AMPK needs to be considered[162]. New promising candidates include A769662, a non-nucleoside thienopyridone that has been shown to activate AMPK to stimulate glucose uptake *in vitro*[163].

Increasing general mitochondrial uncoupling. Increased mitochondrial uncoupling leads to energy inefficiency and increased energy expenditure. DNP, a non-selective uncoupler of mitochondrial oxidation, at 3–5 mg per kg in humans led to a 20–30% increase in energy expenditure that could be sustained without tolerance[18]. Unfortunately, DNP itself cannot be used as a therapy because of its narrow therapeutic window and serious adverse effects related to overdose[143]. However, if safety can be proven, increasing mitochondrial uncoupling may represent a potential therapeutic approach. A new class of molecules that includes butylated hydroxytoluene utilizes the mitochondrial adenine nucleotide translocase to induce limited uncoupling at low concentrations and can have a dynamic range of more than a million-fold *in vitro*. Although still years from clinical use, these compounds are attractive because of their small size and favourable pharmacokinetics[164].

Conclusions

With the growing worldwide epidemic of obesity, it is clear that new and effective anti-obesity therapies are needed. Compelling data suggest that targeting cellular bioenergetics may provide an exciting new therapeutic approach for the treatment or prevention of obesity. However, owing to the high safety concerns for anti-obesity treatments, both the conventional pharmaceutical approach and the cell-based therapy approach require intensive benefit–risk assessments.

Many questions remain to be answered before these therapies become possible. First, at the systemic level, it is not known whether chronically increasing peripheral energy expenditure will cause compensatory mechanisms, such as increased appetite, that might overcome its benefit. The exact contributions of brown fat and skeletal muscle in energy expenditure by adaptive thermogenesis in humans, especially in response to overfeeding, remain to be determined. At the cellular and molecular levels, cellular lineage determination and factors determining the developmental fate of energy-dissipating brown fat need to be further elucidated. Together, answers to these questions would help in implementing the idea of targeting cellular bioenergetics to treat obesity and its many associated metabolic disorders.

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REVIEWS

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DATABASES

Entrez Gene:
http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=gene
FGF19 | *FGF21* | *KL*
OMIM:
http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=OMIM
Obesity
UniProtKB: http://ca.expasy.org/sprot
β$_3$-adrenergic receptor | bFGF | BMP2 | BMP4 | BMP7 | CTBP1 | CTBP2 | FGF16 | GDF3 | GLP1 | IGF1 | IRS1 | IRS2 | MYF5 | NDN | nodal | PGC1α | PGC1β | PRDM16 | RIP140 | SERCA1 | SIRT1 | TGR5 | UCP1 | UCP2 | UCP3
ClinicalTrials.gov: http://clinicaltrials.gov/ct2/home
NCT00168519 | NCT00297180 | NCT00398619 | NCT00542009 | NCT00622765 | NCT00650806 | NCT00654667 | NCT00748605 | NCT00760760 | NCT00779519

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