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Convertible visceral fat as a therapeutic target to curb obesity

Antonio Giordano¹, Andrea Frontini² and Saverio Cinti^{1,3}

Abstract | New therapeutic and preventative strategies are needed to address the growing obesity epidemic. In animal models, brown adipose tissue activation and the associated heat produced contribute to countering obesity and the accompanying metabolic abnormalities. Adult humans also have functional brown fat. Here, we present and discuss the concepts of murine and human white adipose tissue plasticity and the transdifferentiation of white adipocytes into brown adipocytes. Human visceral adipocytes — which are crucial contributors to the burden of obesity and its complications — are particularly susceptible to such transdifferentiation. Therefore, we propose that this process should be a focus of anti-obesity research [Au: Edits ok?]. Approved drugs that have browning properties as well as future drugs that target molecular pathways involved in white-to-brown visceral adipocyte transdifferentiation may provide new perspectives for obesity therapy.

Brown adipose tissue (BAT). The mammalian tissue composed of either brown or beige/brite adipocytes. BAT is involved in non-shivering thermogenesis to maintain body temperature homeostasis.

Obesity results from a sustained imbalance between calorie intake and energy expenditure. Obesity is associated with an increased risk of developing various medical conditions that are widespread in industrialized countries; such medical conditions include type 2 diabetes mellitus (T2DM), dyslipidaemia, non-alcoholic fatty liver disease, cardiovascular disease and even some cancers^{1–3}. The metabolic abnormalities associated with obesity form a cluster referred to as metabolic syndrome. Currently, the only effective therapeutic option available to morbidly obese individuals is bariatric surgery⁴. Pharmacological treatments are therefore urgently needed to help these patients and to reduce the enormous cost of obesity and associated diseases to the community and national health services.

In small mammals, brown adipose tissue (BAT) has a key role in both the thermogenic response and in the regulation of energy balance⁵. In mice, induction of BAT heat production helps to prevent fluctuations in body temperature upon cold exposure. Moreover, BAT activation promotes energy expenditure, reduces adiposity and protects against diet-induced obesity^{6,7}. The presence and role of BAT in humans were controversial until physiologically active BAT was unequivocally identified in adults^{8–12}. Such findings have generated substantial interest in BAT activation as a promising target for the treatment of obesity. Indeed, enhancing constitutive BAT activity or stimulating the recruitment of new thermogenic adipocytes in fat depots could provide new therapeutic strategies to curb human obesity.

However, the amount of BAT in humans is inversely correlated with body mass index and age^{8–10}, and is barely detectable in obese patients compared with normal-weight individuals of the same age¹³. Therefore, activating BAT that already exists in obese patients may be of limited therapeutic value. In this Review, we shift the focus from BAT to white adipose tissue (WAT) that can be converted to BAT¹⁴ [Au: ok?]. In particular, we focus on visceral convertible WAT, which is highly inflamed in obese individuals (metainflammation) and is closely involved in the onset of some of the more severe medical complications of obesity^{15–19}. Such an approach is different from current strategies, as it hinges on the distinctive susceptibility of visceral WAT to turn into BAT. Promoting white-to-brown transdifferentiation of visceral adipocytes has the potential to provide an effective anti-obesity treatment.

The adipose organ

Obesity treatments necessarily involve major changes in body fat distribution, adipose tissue cell composition [Au: OK?] and adipocyte biology. Adipocytes are the parenchymal cells of the adipose organ, an organ that has recently been recognized to have distinctive anatomical and functional features^{20,21} (FIG. 1). A thorough knowledge of this organ is a prerequisite to devising strategies to combat obesity and metabolic syndrome.

Adipocytes have traditionally been divided into white and brown adipocytes. White adipocytes are large spherical cells that store energy-providing lipids (triglycerides) in the form of a unilocular droplet that

¹Department of Experimental and Clinical Medicine, University of Ancona (Marche Polytechnic University [Au: ok?]), Via Tronto, 10/A 60020 Ancona, Italy.

²Department of Public Health Experimental and Forensic Medicine, University of Pavia, Pavia, Italy. [Au: post code?]

³Center of Obesity, University of Ancona (Marche Polytechnic University [Au: ok?])-United Hospitals, Ancona, Italy. [Au: post code?]

Correspondence to S.C. s.cinti@univpm.it

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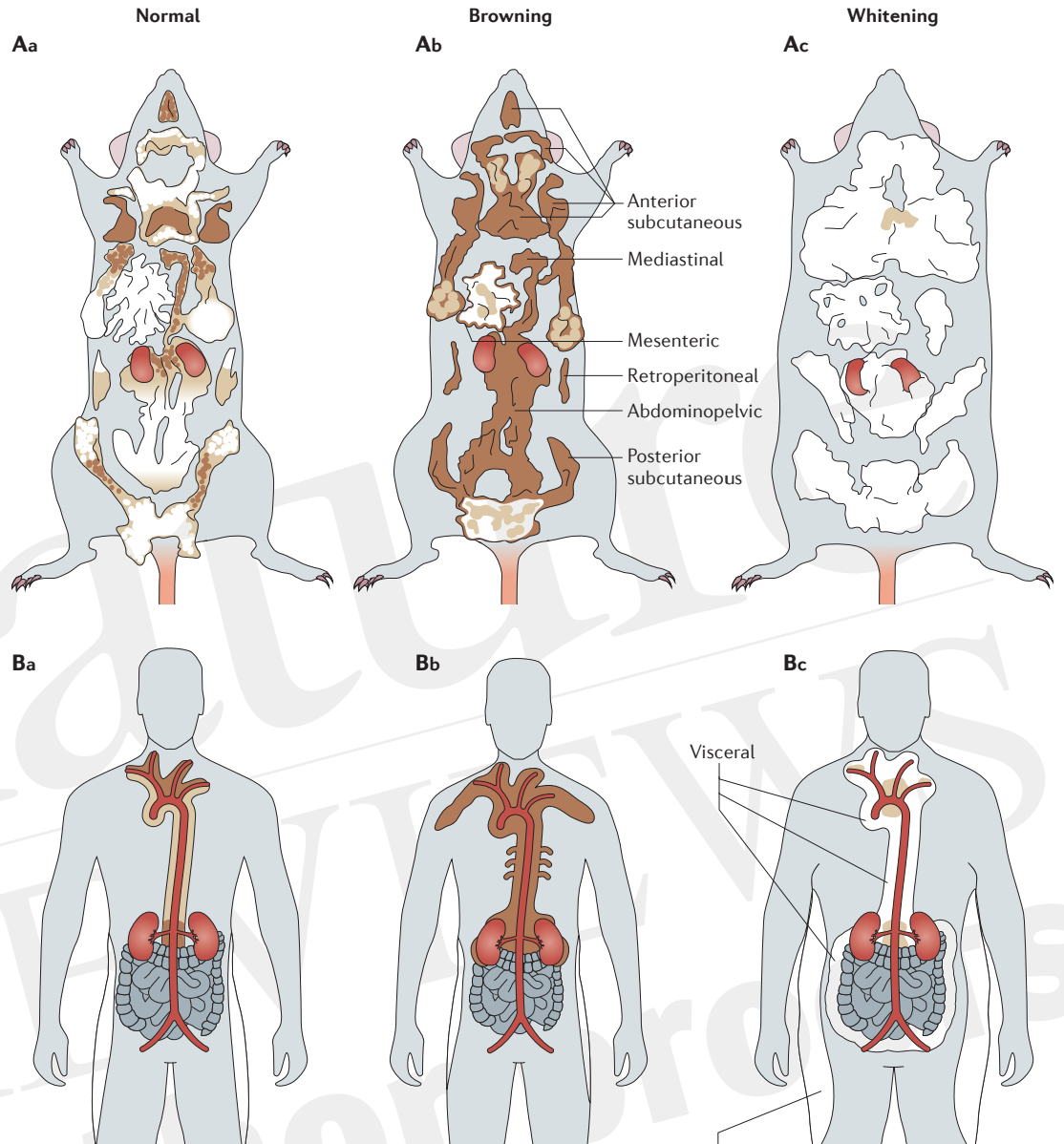


Figure 1 | The adipose organ is highly plastic in both rodents and humans. The upper panels show schematics of the adipose organ of an adult mouse kept at 28 °C (normal phenotype; part **Aa**), a mouse kept at 6 °C for 10 days (browning phenotype; part **Ab**) and a leptin-deficient *db/db* obese mouse (whitening phenotype; part **Ac**). The different physiological requirements triggered by environmental or dietary challenges involve a phenotypic switch that increases the proportion of brown adipose tissue (BAT) in hypothermic conditions [Au: ok?] for heat generation (browning) or increases the proportion of white adipose tissue (WAT) in obesogenic conditions to store lipids (whitening). This transformation is visually evident in every single fat depot forming the adipose organ. In humans, the localization of BAT in normal conditions is restricted to primarily around the large blood vessels, especially in the supraclavicular region (part **Ba**). As in rodents, the human adipose organ can be remodelled: adrenergic stimulation (browning) leads to an increase of BAT (part **Bb**), whereas obesity (whitening) involves an increase of subcutaneous and, importantly, visceral WAT (part **Bc**).

White-to-brown transdifferentiation
The direct and progressive conversion of fully differentiated white adipocytes into heat-producing brown adipocytes without passing through an undifferentiated, stem-like state.

occupies ~90% of the cell volume (FIG. 2). White adipocytes form WAT, giving rise to several fat depots that are distributed in two anatomical compartments of the body: subcutaneous and visceral. In small animals, the two compartments have discrete brown areas that contain brown adipocytes and form BAT. Brown adipocytes have different morphological and functional features from white adipocytes: they are smaller, polygonal cells with ~50% of the volume occupied by

lipids partitioned into several droplets (FIG. 2). Brown adipocytes do not accumulate energy but rather dissipate it by producing heat. Brown adipocyte activity is closely regulated by the sympathetic nervous system²². Indeed, BAT is densely innervated by noradrenergic parenchymal fibres (FIG. 2) that are in direct contact with brown adipocytes through synaptoid junctions²⁰; such junctions are responsible for the functional activation of brown adipocytes [Au:OK?]. Gap junctions

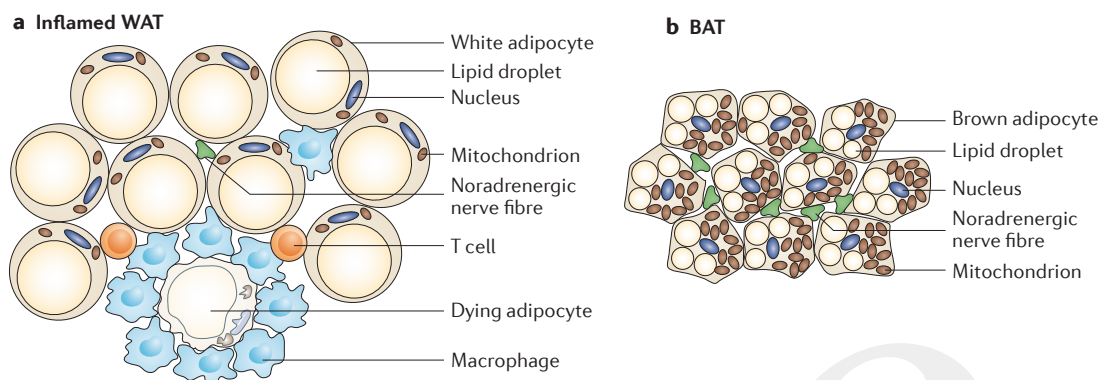


Figure 2 | Cellular complexity of the adipose organ. The parenchyma of white adipose tissue (WAT) is formed primarily by unilocular, lipid-laden white adipocytes, which are endowed with few and small mitochondria and reached by a few sympathetic noradrenergic nerve fibres. Immune cells are also present, such as T cells and macrophages. In obesogenic conditions (part **a**), WAT is massively infiltrated by macrophages that form crown-like structures around dying adipocytes. This phenomenon is highly pro-inflammatory and is one of the pivotal events leading to insulin resistance and type 2 diabetes. The parenchyma of brown adipose tissue (BAT) (part **b**) is formed by multilocular brown adipocytes containing numerous and small lipid droplets and a high number of large mitochondria expressing the mitochondrial protein uncoupling protein 1 (UCP1). BAT is densely innervated by the sympathetic nervous system: tonic noradrenergic stimulation is necessary to maintain the 'brown' phenotype of the tissue, whereas its phasic activation triggers heat production. The energy-dissipating activity of thermogenic BAT counteracts obesity and related diseases.

among adjacent brown adipocytes probably amplify the noradrenergic stimulus that is selectively delivered to a few cells, enabling simultaneous, syncytial-like activation of large numbers of brown adipocytes, including those not directly reached by noradrenergic nerves²³. Noradrenaline acts on brown adipocytes through specific membrane receptors, the β_3 -adrenoceptors, to induce lipolysis, mitochondriogenesis and synthesis of uncoupling protein 1 (UCP1). UCP1 is a protein uniquely expressed by brown adipocyte mitochondria, which are numerous, large and packed with cristae. UCP1 uncouples oxidative phosphorylation from ATP synthesis and dissipates the energy provided by triglycerides in the form of heat. Importantly, the presence of UCP1 in an adipocyte indicates its potential to perform a thermogenic function²⁴. Exposure to temperatures below thermoneutrality and low levels of food intake [Au: ok?] are the main physiological stimuli for BAT activation²⁴.

In the adipose organ, WAT and BAT areas are not distinct, and mixed areas are visible between them. These mixed areas contain adipocytes that exhibit variable UCP1 expression and morphological features that are intermediate between typical white and typical brown adipocytes^{25,26}. Interestingly, the total number of adipocytes in the adipose organ is relatively constant among mouse strains, but the relative numbers of white and brown adipocytes are different even when measured at near-thermoneutral conditions. Notably, obesity-resistant mice have five times more brown adipocytes in their adipose organ than obesity-prone mice do²⁶. Although the reason for such an unequal distribution remains unclear, it could be required by the plasticity of the adipose organ. That is, BAT and WAT have distinctive roles but act cooperatively, through reciprocal conversion, to satisfy the different physiological roles required for optimal adaptation to environmental changes^{27–29}.

This capacity for conversion is key to the development of therapies to increase the amount of BAT in obese patients.

The convertible adipose organ

Browning and whitening. In obese and aged animals, the adipose organ undergoes whitening and WAT is increased at the expense of BAT^{30–32} (FIG. 1). By contrast, after chronic cold exposure or β_3 -adrenoceptor stimulation, the adipose organ becomes visibly browner. Browning is due to an increase of BAT and a concomitant reduction of WAT in the organ²⁶ (FIG. 1). This phenomenon is caused by the appearance of UCP1-expressing, multilocular adipocytes in white (or predominantly white) fat depots^{14,33,34}. Such adipocytes, the development of which is also induced by exercise^{35–37}, are considered to be a novel type of brown fat cell and have variously been designated as beige, brite (brown in white), inducible or recruitable brown-like adipocytes^{38–42}. Here, such adipocytes will be referred to as beige/brite adipocytes. Beige/brite adipocytes can originate from both white-to-brown adipocyte transdifferentiation and *de novo* differentiation of precursor cells, and their characteristic features are currently being defined. So far, it is thought that these cells are not derived from the same myogenic factor 5 (MYF5) [Au: ok?]-positive precursors that have been suggested to give rise to the typical interscapular and perirenal brown adipocytes⁴³. Moreover, at least a subset of beige/brite adipocytes originate from smooth muscle-like cells⁴⁴ and have distinctive gene signatures^{38,41,45,46}. In addition, beige/brite adipocytes are highly sensitive to selected stimuli, such as bone morphogenetic protein 7 (BMP7)⁴⁰, irisin^{36,45}, fibroblast growth factor 21 (REF. 47) and BMP4 (REF. 48).

If adult human BAT is indeed primarily composed of beige/brite cells, as recently hypothesized^{45,49–51}, it might be possible to recruit brown adipocytes selectively to treat

Endothelial–mesenchymal transition

The process by which endothelial cells lose their cell–cell adhesion and gain migratory properties to differentiate into a mesenchymal cell type

Visceral fat

The fat found in the trunk in close contact with the viscera.

obesity. The identification of physiological adipocyte progenitors is therefore therapeutically relevant. However, adipocyte development is a complicated process, and recent reports have shown that even the MYF5 lineage, which had previously been claimed to be a defining feature of brown adipocytes⁵², can also drive the differentiation of a subset of white adipocyte precursors⁵³. In addition, *in vitro* and *in vivo* gene expression and transcriptome experiments that have documented differences between typical brown adipocytes and beige/brite cells do not provide definitive results. For example, the artificial induction of brown adipocyte differentiation *in vitro* may not precisely reproduce *in vivo* conditions, and extracts of whole fat tissue may include the presence of several other cell types, which precludes the conclusion that these results reflect intrinsic differences between adipocytes⁵⁴ [Au: ok? To split up long sentence]. Importantly, a distinct function of beige/brite cells — different from those served by typical brown adipocytes such as those found in the interscapular depot (where densely packed brown adipocytes are also found in aged animals³⁰) — has yet to be described. Indeed, the functional role of beige/brite adipocytes seems to be similar to that of typical brown adipocytes^{28,55}. Therefore, the beige/brite adipocyte might not be a novel thermogenic cell type. At least some of the molecular signatures detected in browning fat that have been attributed to beige/brite cells and that are different from those found in classic BAT could in fact reflect still unknown molecular features that allow white-to-brown adipocyte transdifferentiation. Data from our group^{26,27,56,57} and others^{29,58} indeed suggest that browning of the adipose organ is largely due to white-to-brown transdifferentiation.

In rodents, subcutaneous inguinal adipose tissue has been suggested to be the largest and physiologically most important fat depot that is capable of recruiting beige/brite adipocytes^{26,59}; however, browning is also induced by *de novo* adipogenesis. In the AdipoChaser mouse, mature white adipocytes are permanently labelled following a brief doxycycline pulse. Cold exposure or β_3 -adrenoceptor treatment of these animals give rise to two brown adipocyte populations in the subcutaneous fat: labelled brown adipocytes (derived from white adipocyte transdifferentiation) and unlabelled brown adipocytes (derived from proliferation and/or differentiation of precursor cells)⁶⁰. Clearly, the two browning mechanisms — adipocyte transdifferentiation and precursor differentiation — are both involved in the plasticity of the mammalian adipose organ, with possible differences among depots⁶¹. In addition, if a mature adipocyte phenotype is required to respond to environmental stimuli, it is likely that epigenetic phenomena are involved in browning, as recently reported by some researchers^{62,63}.

Adipocytes therefore have the distinctive ability to physiologically and reversibly undergo phenotype reprogramming to meet the body's requirements for thermogenesis (white-to-brown transdifferentiation following chronic cold exposure) or energy storage (brown to white transdifferentiation to store excess energy when challenged by a positive energy balance). Additionally, during pregnancy, some white adipocytes (which we propose should be called 'pink adipocytes') in the subcutaneous depot of pregnant female mice progressively transdifferentiate into milk-producing and milk-secreting glandular cells, and the reverse process takes place during mammary gland involution (BOX 1).

Box 1 | Pinking of mammary gland adipocytes during pregnancy

White-to-brown and brown-to-white conversion do not seem to be the only physiological, reversible cell-reprogramming phenomena occurring in the adipose organ. Morphological studies have documented that in the second part [Au: trimester?] of pregnancy some white adipocytes in the subcutaneous depot of pregnant female mice progressively transdifferentiate into milk-producing and milk-secreting glandular cells and that the reverse process takes place during mammary gland involution^{185,186}. Lineage studies using the Cre-loxP recombination system have confirmed that aP2-Cre/R26R mice, in which adipocytes are labelled before pregnancy, contained labelled secretory epithelial cells during pregnancy, whereas the mammary gland of whey acidic protein-Cre/R26R mice, whose secretory epithelial cells express the lacZ gene during pregnancy, contained labelled adipocytes during mammary gland involution¹⁸⁵. Finally, the transdifferentiation capacity of white adipocytes has been confirmed in explant experiments, in which both adipose tissue and isolated adipocytes from genetically identifiable mice implanted into pregnant wild-type females gave rise to donor-derived glands¹⁸⁷. The milk-secreting, alveolar mammary epithelial cells derived from white adipocyte transdifferentiation have different morphological features than those of alveolar mammary epithelial cells derived from ductal stem cells. In particular, the white adipose transdifferentiation-derived mammary epithelial cells precociously exhibit large, adipocyte-derived cytoplasmic lipid droplets that make them adipocytes — parenchymal cells of the adipose organ containing abundant cytoplasmic lipids — irrespective of their functional role. We propose that these cells are called 'pink adipocytes' because they arise exclusively in female subcutaneous depots during pregnancy and lactation, and the pregnant mammary gland is visibly pink¹⁸⁸. The purpose of the adipose organ therefore seems to be energy repartition for thermogenesis and metabolism not only for short-term homeostasis (that is, the individual's survival), but also for mid- to long-term homeostasis (that is, litter survival).

Adipocyte precursors. The physiological identity of the adipocyte precursor is heavily debated. Early ultrastructural studies of rat tissues indicated that the growing capillaries of developing fat depots are the site of adipocyte progenitors^{64,65}. Subsequent mouse studies, mainly based on lineage tracing approaches, confirmed that both white and brown adipocyte progenitor cells reside in the blood vessel wall⁶⁶ and may be pericytes⁶⁷ and/or endothelial cells^{67,68} of adipose tissue capillaries. In particular, specialized endothelial cells are programmed to become preadipocytes⁶⁷. These cells may undergo endothelial–mesenchymal transition upon activation of the BMP–transforming growth factor- β signalling pathway, the downstream effectors of which have a role in the developmental fate of adipocytes⁶⁹.

Other *in vivo* fate-mapping studies have suggested that only brown adipocytes (not white adipocytes) are derived from precursors expressing MYF5, a gene previously believed to be exclusive to the myogenic lineage⁴³. Fully differentiated adipocytes may also arise from mesothelial cells in the visceral fat⁷⁰ or neural crest cells (in mice), cephalic adipose depots (in quail)⁷¹, and bone marrow cells (in rodents and humans)^{72–74}. The possibility that adipocytes originate from endothelial cells and bone marrow cells has been recently questioned⁷⁵ in favour of CD24⁺ [Au:OK?] adipose precursors, which are not preferentially localized to the vasculature but instead are scattered throughout WAT⁷⁶.

Numerous, conflicting hypotheses exist largely because of the lack of specific adipocyte stem cell markers. Collectively, however, these hypotheses suggest the astonishing possibility that adipocyte precursors may arise from diverse developmental pathways, possibly depending on the age of the animal (pre- and postnatal developing animals or adults), the species (rodents or humans), metabolic condition (raised on a normal or a high-fat diet) and the location of the fat deposit (subcutaneous or visceral). As noted in other studies^{77,78}, the molecular determinants of the adipogenic niche or niches — rather than the precursor phenotype — might have a primary, yet-unappreciated role in determining the capacity of differentiating precursors to become adipocytes. However, it should be stressed that the origin of mature adipocytes in many cases may be considered to be transdifferentiation, as many putative precursor cells (endothelial, pericytic and mesothelial cells) are themselves differentiated. A possible common precursor cell for white and brown adipocytes has also been suggested^{67,68}, a hypothesis that could partially account for the reciprocal transdifferentiation potential of the mature phenotypes.

Visceral fat is pathogenic in obesity

The recognition that a given distribution of adiposity is associated with metabolic disease dates back to the 1950s, when upper body (android) obesity was reported to occur more frequently in individuals suffering from diabetes, gout and atherosclerosis than in individuals with lower body (gynoid) obesity⁷⁹. In the early 1980s, cross-sectional clinical studies showed a higher incidence of hypertension, hypertriglyceridaemia, hyperinsulinaemia and glucose intolerance in individuals with a high waist/hip ratio^{80,81}. This parameter was subsequently demonstrated to be a reliable predictor of a future diagnosis of diabetes, myocardial infarction, angina pectoris or stroke in both genders^{82,83}.

Analysis of abdominal soft-tissue composition by computed tomography and magnetic resonance imaging has documented that the visceral, but not the subcutaneous, component of abdominal fat is more closely linked to the adverse metabolic outcomes of visceral obesity^{84,85}. It is now well established that increased visceral, intra-abdominal fat (central or apple-shaped obesity, most commonly observed in males) correlates with a high risk of metabolic syndrome and morbidity and mortality from coronary heart disease, certain cancers and diabetes⁸⁶. By contrast, increased subcutaneous fat in the thighs and hips (peripheral or pear-shaped obesity, most common in females) poses little or no risk⁸⁶ (FIG. 1). However, the reason for such a difference remains unclear. One explanation is that intraperitoneal fat, which primarily consists of the greater and lesser omentum and of mesenteric fat, drains directly into the liver through the portal vein. The resulting hepatic overload of adipose-derived secretory products damages the organ, which has a crucial role in carbohydrate and lipid homeostasis. Another explanation may lie in intrinsic properties of visceral fat. For example, hypertrophic visceral adipocytes are characterized by a hyperlipolytic state that is resistant to the anti-lipolytic effect

of insulin^{87,88}. The resulting overload of free fatty acids, glycerol and lactate in the liver adversely affects hepatic metabolism, leading to increased liver glucose and triacylglycerol-rich lipoprotein production. Moreover, white adipocytes are endocrine cells that release hormones such as leptin and adiponectin, as well as pro-inflammatory cytokines, including interleukin-6 (IL-6) and tumour necrosis factor [Au:OK? House style uses TNF not TNF α]. The greater production and secretion of IL-6, IL-8, plasminogen activator inhibitor 1 and angiotensinogen by visceral fat relative to subcutaneous fat may also account for the harmful effects of visceral obesity^{89,90}. By contrast, adiponectin, which counteracts insulin resistance, dyslipidaemia and atherosclerosis, is reduced in obese individuals, especially in those with visceral obesity⁹¹.

The limited expandability of visceral adipocytes may further explain the pathogenicity of visceral obesity. In obese individuals, lipid overload leads to white adipocyte hypertrophy and hyperplasia. Hypertrophic adipocytes cannot grow indefinitely, and after reaching a certain size they become dysfunctional and die^{92–94} (FIG. 2). Dysfunctional and distressed hypertrophic adipocytes produce abnormal amounts of adipokines and release chemoattractants that recruit resident and circulating macrophages^{95,96}, which probably resorb the dead adipocyte remnants^{92,95,97}. This process leads to the formation of typical crown-like structures, in which clusters of activated macrophages surround dead adipocytes⁹². Comparisons of different visceral and subcutaneous fat depots from obese mice indicate that visceral depots are the main sites of adipocyte death and macrophage infiltration^{17,18}. Interestingly, visceral adipocytes die at a smaller size than subcutaneous adipocytes do¹⁸; consequently, the visceral fat of obese mice and humans⁹⁸ usually has more inflammation [Au: than subcutaneous fat does?]. Inflammation is strongly linked to insulin resistance and T2DM^{15,16}, which may further explain why visceral obesity is more closely associated with adverse outcomes than is subcutaneous obesity^{98,99}.

Finally, a substantial fraction of visceral adipose tissue is composed of perivascular fat, which surrounds the thoracic and abdominal aorta and its branches. The perivascular fat around the coronary arteries, which includes epicardial fat, and the fat surrounding the renal arteries are particularly interesting from a pathological perspective. In healthy individuals, perivascular fat not only provides mechanical protection to vessels but also controls vascular function and remodelling by exerting vasodilatory and anti-inflammatory actions^{100,101}. In obese individuals, perivascular fat inflammation and the resulting adipokine dysregulation (in particular, reduced adiponectin levels and increased leptin, tumour necrosis factor and IL-6 levels) adversely affect vascular biology by promoting vasoconstriction¹⁰², medial smooth muscle cell proliferation¹⁰³ and endothelial dysfunction¹⁰⁴. Consequently, several investigators have suggested that the major cardiovascular consequences of obesity, including hypertension and atherosclerosis, predominantly derive from dysregulated and inflamed perivascular fat.

Visceral WAT can be converted to BAT

If the main target of anti-obesity strategies is the conversion of white-to-brown adipocytes, it is essential to identify which white adipocytes are most susceptible to transdifferentiation. Quantitative analyses of the adult murine adipose organ²⁶ and positron emission tomography (PET) analyses of the adult human 'browned' adipose organ¹⁰⁵ suggest that noradrenergic stimuli can promote the conversion of adipocytes in the fat surrounding the thoracic and abdominal aorta and its main branches, especially in the upper part of the trunk, to metabolically active brown adipocytes. Moreover, in line with these data, mediastinal fat that surrounds the aortic arch and thoracic aorta and its branches in mice contains the highest density of noradrenergic parenchymal fibres. Mediastinal fat is the only type of fat that does not require additional innervation after cold exposure to become browned, which is in contrast to what is observed in other fat depots²⁵ [Au: edits ok?]. Notably, mediastinal fat is the only adipose depot that contains putative parasympathetic cholinergic nerves in rats¹⁰⁶ and humans¹⁰⁷. The tight anatomical relationship between BAT and the aorta is teleologically easy to understand as this physical proximity enables the rapid transport of heat to the entire body. In small mammals, in which the high body surface/volume ratio promotes thermodispersion, an additional, sizeable amount of BAT is found in the dorsal–cervical area (interscapular, subscapular and cervical BAT); draining the blood from this fat depot requires a large vessel, the Sulzer vein¹⁰⁸.

In adult humans, the remnants of active BAT have been found in the supraclavicular region, probably because this region contains large branches (subclavians) originating from the aortic arch. Thus, most of these sites consist of visceral fat. Notably, results from PET studies^{105,109} have suggested that mediastinal fat at the root of the neck is anatomically continuous with the deep subcutaneous fat of the supraclavicular region (surrounding subclavian vessels) and the visceral fat of the neck (surrounding the carotid arteries and internal jugular vein)¹¹⁰. This tissue therefore seems to be a transition area between visceral and subcutaneous fat.

It is widely accepted that subcutaneous fat (but not visceral fat) is highly susceptible to browning in small mammals. However, this notion may be incorrect because most studies only investigated epididymal fat, which is considered to be typical visceral fat [Au: edits ok?]. In our experience, epididymal fat is indeed highly resistant to browning, but other visceral fat depots, particularly those in contact with the aorta and its main branches, have a substantial capacity to transdifferentiate²⁶.

PET signals of metabolically active BAT are particularly strong at periaortic sites both in patients with pheochromocytoma, a benign tumour characterized by elevated noradrenaline secretion, and in individuals exposed to cold^{10,105,109,111}. Notably, the surgical excision of the pheochromocytoma results in the disappearance of metabolically active BAT in the visceral depots, which is due either to reduced brown adipocyte activity or perhaps to the reconversion of brown to white adipocytes¹⁰⁹.

Among the visceral depots that are responsive to browning, the most interesting from a pathological perspective are the omental depot (a pure white visceral depot) and the mesenteric depot^{86,112}. Histological analyses of the omentum from patients with pheochromocytoma demonstrate that the omental fat also responds to noradrenergic stimulation¹¹³. In specimens from 12 patients with pheochromocytoma, omental adipocytes were reduced in size in all patients, and several UCP1-positive brown adipocytes were detected among unilocular cells in half of them. These data demonstrate that a visceral depot at a distance from the aorta, but endowed with a rich vascular supply, can respond to circulating noradrenaline. Detailed morphological analyses of these omental samples support the notion that in humans the main phenomenon underlying browning may be direct white-to-brown adipocyte conversion. Indeed, these specimens exhibited all intermediate forms between white and brown adipocytes, including paucilocular adipocytes with different degrees of UCP1 expression, a progressive increase in the number and differentiation of mitochondria and clear remodeling of the tissue. Such tissue remodelling included an increase in the density of capillaries and parenchymal noradrenergic nerve fibres; however, no morphological features suggesting preadipocyte development (obtained by electron microscopy) or proliferation (by Ki67 immunostaining) were detected¹¹³. Together, these data suggest the occurrence of brown-like transdifferentiation. Accordingly, metabolically active BAT was detected by PET in the omentum and mesenteric fat of some patients with pheochromocytoma^{114,115}.

Taking into account the above considerations and the scarcity or absence of BAT in obese individuals¹³, anti-obesity strategies based on WAT to BAT conversion should primarily target visceral WAT derived from BAT whitening. The conversion of visceral fat to energy-dissipating fat would also attenuate the harmful effects of perivascular fat and restore its role in vessel protection. Although there may be adverse effects associated with white-to-brown perivascular fat conversion, possibly owing to the disruption of some of the secretory roles of perivascular white adipocytes, the abundant and preferential distribution of BAT around large blood vessels in small animals, neonates and cold-exposed adult humans suggests that adverse effects are unlikely. Finally, even the induction of the first steps of white-to-brown transdifferentiation — involving the conversion of large, dysfunctional adipocytes that tend to become hypertrophic and die to small, mitochondria-rich adipocytes with less lipid — has the potential to provide substantial metabolic benefit^{16,18}. Such an effect would be particularly beneficial in obesity treatment given the vast amount of WAT found in obese patients, its pathogenic role and the strong positive correlation between adipocyte size and WAT inflammation [Au: ok? To split up long sentence].

In conclusion, BAT induction in the human adipose organ would be therapeutically relevant to the treatment of obesity. Drugs able to increase the amount of functional BAT in obese patients would have to fit into the current landscape of obesity treatments, for which there is still considerable unmet medical need.

The pharmacotherapy of obesity

Over the past few years, several molecules have been tested and marketed as anti-obesity drugs. Their mechanisms of action broadly fall into two categories: drugs that reduce nutrient absorption and anorectic agents that act centrally to enhance satiety, thus reducing food intake (TABLE 1). However, all such compounds have shown limited efficacy and/or substantial adverse effects. Fenfluramine and dexfenfluramine¹¹⁶, sibutramine¹¹⁷ and rimonabant^{118–121} are exemplars of promising anti-obesity compounds that were subsequently found to induce adverse effects that were so substantial as to warrant their withdrawal from the market. Currently, a small number of drugs exerting modest effects are available (TABLE 1). Dietary changes, exercise and, when recommended, cognitive behavioural therapy have been found to be more effective than drugs in the prevention and treatment of obesity. However, motivation and self-discipline are critical, and gains are slow and difficult to maintain. Although anti-obesity drugs can help, their side effects can be severe, and benefits last only as long as the treatment, as the weight that is lost is regained as soon as the drug treatment is stopped. Novel and more selective approaches are clearly needed.

The mammalian energy balance is an extraordinarily complex, integrated and redundant homeostatic system in which alterations induced by anti-obesity agents induce compensatory physiological and behavioural responses that tend to counteract weight loss. To overcome such compensatory responses, pharmacological research now also focuses on molecules that elicit both anorectic and energy expenditure effects, thus targeting both sides of the energy balance equation. Such drugs should have greater and longer term effectiveness. Glucagon-like peptide 1 (GLP1) analogues fall into this category. GLP1 and glucose-dependent insulinotropic polypeptide (GIP) are incretin hormones released by intestinal cells in response to nutrient ingestion, and their primary effect is to enhance glucose-stimulated insulin release by pancreatic β -cells and to reduce blood sugar levels¹²². However, long-acting GLP1 analogues, including liraglutide and exenatide, not only ameliorate hyperglycaemia in patients with T2DM but also cause weight loss, primarily through a central anorectic effect¹²³. Importantly, recent data from studies of rats and mice suggest that GLP1 analogues also increase energy expenditure through BAT activation and mild epididymal visceral WAT browning by activating AMPK in the hypothalamic ventromedial nucleus¹²⁴. Thus, in humans, some of the weight-reducing effects of these agents may also be related to BAT activation. Interestingly, obese patients with T2DM who were treated with these drugs for 52 weeks had significant weight loss as well as increased resting energy expenditure¹²⁴. Very recently, liraglutide, which was approved by the US Food and Drug Administration in 2010 to treat T2DM, has also been approved to treat obesity (TABLE 1). In early 2015, a monomeric peptide (GIP) that acts as both a GLP1 analogue and a glucagon receptor agonist was synthesized and tested in mice; GIP demonstrated efficacy in reducing obesity and ameliorating T2DM by promoting both thermogenesis and satiety¹²⁵.

Among the novel molecules that are being assessed for their potential as anti-obesity drugs (TABLE 2), some seem to increase energy expenditure via brown remodelling of WAT in addition to their other weight-reducing effects; however, whether they can exert a direct effect on white-to-brown adipocyte transdifferentiation remains to be determined.

Methionine aminopeptidase 2 (METAP2) is an enzyme involved in the post-translational modification of several proteins and may also have a critical role in the growth of different types of tumours by promoting angiogenesis. Originally developed as anticancer agents, METAP2 antagonists are able to induce significant and sustained weight reduction at low doses in both animal models and humans^{126–128}. Although the mechanism through which these agents reduce obesity has not been fully elucidated, beloranib, the prototype METAP2 inhibitor, reduces the production of new fatty acids in the liver and helps to convert stored fat into useful energy in the adipose organ¹²⁹. However, whether these drugs have browning effects remains unknown. Beloranib is currently in Phase II trials to establish its safety and efficacy¹³⁰.

Sirtuin 1 (SIRT1), similar to all members of the sirtuin family, requires NAD⁺ to exert its deacetylating activity. The dependence of SIRT1 on NAD⁺ bioavailability crucially links its activity to cellular energy levels. SIRT1 expression is induced by both caloric restriction and exercise¹³¹. Among other effects, SIRT1 activation promotes fat mobilization in white adipocytes¹³² and, importantly, promotes brown remodelling of subcutaneous WAT by interacting with peroxisome proliferator-activated receptor- γ (PPAR γ) coactivator 1 α (PGC1 α)¹³³. Resveratrol, a natural polyphenol found in several plants, is a SIRT1 allosteric activator and possesses calorie-restriction-like effects, including reducing insulin resistance and increasing oxidative metabolism and fatty acid oxidation in BAT and skeletal muscle¹³⁴. Resveratrol and several other sirtuin activators are now in the early development phase as anti-obesity drugs.

Drug repurposing for WAT browning

Several molecules are now recognized to drive adipose organ browning (TABLE 3). The most obvious molecular target for inducing white-to-brown transdifferentiation is the β_3 -adrenoceptor. In experimental animals, administration of selective β_3 -adrenoceptor agonists induced white-to-brown transdifferentiation in both subcutaneous and visceral WAT areas^{27,57,58} [Au: ok?], effectively counteracting diet-induced obesity^{135–137}. However, β_3 -adrenoceptors are expressed in several other organs and tissues, including the gastrointestinal tract, prostate and bladder¹³⁸, and highly selective agonists are not available. To date, administration of a β_3 -adrenoceptor agonist in humans has failed to substantially influence body weight and energy balance, although trials have involved only severely obese patients for short periods^{139,140}. None of these early [Au: investigational?] agonists have been approved for clinical use.

Recently, mirabegron, a latest generation β_3 -adrenoceptor agonist with a higher *in vitro* binding affinity for human β_3 -adrenoceptor than other members of

Table 1 | The pharmacotherapy of obesity: past and current drugs*

Drug	Mechanism of action	Effects in obesity	Advantages and limitations	Regulatory status	
				FDA	EMA
Lorcaserin (Belviq; Arena Pharmaceuticals)	5-HT _{2c} receptor agonist, inhibits 5-HT reuptake in the hypothalamus, cortex and midbrain brainstem, and stimulates POMC neurons	<ul style="list-style-type: none"> Reduces appetite but has limited weight loss efficacy Mean body weight loss was 5.8 ± 0.2 kg for lorcaserin vs 2.2 ± 0.1 kg for placebo 	<ul style="list-style-type: none"> Limited long-term data[†] Expensive Limited efficacy Potential risk of cancer 	Approved in 2012	Withdrawn in 2013
Desvenlafaxine (Pristiq; Wyeth)	5-HT, dopamine and noradrenaline reuptake inhibitor in the CNS	<ul style="list-style-type: none"> Induces anorexia Mean body weight loss was 0.22–1.41 kg greater than placebo 	<ul style="list-style-type: none"> Effect on body weight unclear Severe side effects (such as suicide risk and hypertension) 	Off-label usage for obesity; approved for depression in 2008	Withdrawn in 2008
Phentermine	Noradrenaline-releasing agent in the hypothalamus, brainstem and hippocampus	<ul style="list-style-type: none"> Suppresses appetite and induces weight loss Weight loss was ~3.6 kg greater than placebo 	<ul style="list-style-type: none"> Inexpensive Substantial weight loss Side effects include uncontrolled hypertension No long-term data on safety or efficacy 	Approved in 1956 for short-term use (3 months); not approved for long-term use	Withdrawn in 2000
Diethylpropion (also known as amfepramone)	Noradrenaline-releasing agent in the hypothalamus and brainstem	<ul style="list-style-type: none"> Weight loss was ~3.0 kg [Au: more details? i.e., greater than placebo?] 	<ul style="list-style-type: none"> Contraindicated in patients with anxiety disorder, history of heart disease or uncontrolled hypertension 	Approved in 1959 for short-term use (3 months)	Withdrawn in 2000
Topiramate	Promotes anorexia by enhancing GABA transmission, and inhibits voltage-gated channels and AMPA receptors in hypothalamic orexigenic neurons	<ul style="list-style-type: none"> Weight loss was 6.5 kg greater than placebo (95% CI: 4.8–8.3 kg) 	<ul style="list-style-type: none"> Anticonvulsant with unclear effects on the energy balance Causes paresthesia and somnolence Impairs concentration, memory and attention 	Off-label usage for obesity; approved for epilepsy in 1996	Off-label usage for obesity; approved for epilepsy in 2000
Phentermine plus topiramate (Qsymia; Vivus)	See the mechanisms of action of the individual drugs	<ul style="list-style-type: none"> Weight loss was 8–10% of initial body weight 	<ul style="list-style-type: none"> Robust weight loss Limited long-term data[†] Extended release formulation Expensive Teratogenic Possible adverse cardiovascular effects and CNS toxicity 	Approved in 2012	Rejected in 2012
D-fenfluramine	5-HT receptor agonist in the hypothalamus and brainstem (amphetamine analogue)	<ul style="list-style-type: none"> Has potent anorexigenic effects in humans 	<ul style="list-style-type: none"> Addictive Cardiovascular complications In association with phentermine, caused pulmonary hypertension and heart valve disease 	Withdrawn in 1997	Not marketed
Sibutramine	5-HT, dopamine and noradrenaline reuptake inhibitor in the hypothalamus and brainstem (amphetamine analogue)	<ul style="list-style-type: none"> Has limited weight loss efficacy 	<ul style="list-style-type: none"> Addiction Severe cardiovascular effects 	Approved in 1997 and withdrawn in 2010	Approved in 1999 and withdrawn in 2010
Orlistat	Reversible inhibitor of gastric and pancreatic lipases	<ul style="list-style-type: none"> Mean body weight loss was ~4.2 kg greater than placebo 	<ul style="list-style-type: none"> No systemic effects Limited long-term data[†] Minimal reduction of appetite due to lack of effect on the brain Reduced absorption of fat-soluble vitamins Adverse gastrointestinal and hepatic events 	Approved for prescription use in 1999 and in 2007 as an OTC drug	Approved in 1998 for prescription use and in 2007 as an OTC drug
Liraglutide	GLP1 receptor agonist in the hypothalamus	<ul style="list-style-type: none"> Induces sustained weight loss of 5–10% of initial body weight in obese patients and reduces food intake Suggested for use in obese patients with T2DM in combination with insulin 	<ul style="list-style-type: none"> Well tolerated Limited long-term data[†] Expensive Only available as an injectable formulation Possible risk of pancreatic cancer and cardiovascular disease Contraindicated for patients with history of medullary thyroid cancer 	Approved in 2012	Approved in 2015

Table 1 (cont.) | The pharmacotherapy of obesity: past and current drugs*

Drug	Mechanism of action	Effects in obesity	Advantages and limitations	Regulatory status	
				FDA	EMA
Exenatide	GLP1 receptor agonist in the hypothalamus	<ul style="list-style-type: none"> Reduces fasting and post-prandial glucose levels Slows gastric emptying and reduces food intake by 19% Suggested for obese patients with T2DM in combination with insulin 	<ul style="list-style-type: none"> Weight loss occurred without prescribing lifestyle modifications Only available as an injectable formulation Limited long-term data 	Off-label usage for obesity; approved for T2DM in 2005	Off-label usage for obesity; approved for T2DM in 2006
Pramlintide (Symilin; Amylin)	Pancreatic-derived small peptide (amylin analogue) that activates amylin receptors in the hypothalamus and brainstem	<ul style="list-style-type: none"> Weight loss was 2.2 ± 0.7% greater than placebo Indicated for obese patients with T2DM in combination with insulin Proposed combination with phentermine 	<ul style="list-style-type: none"> Reduces blood glucose and body weight Glucose regulation is not well documented Only available as an injectable formulation 	Approved in 2005	Not marketed
Bupropion	Inhibits the neuronal uptake of dopamine, noradrenaline and 5-HT, and increases POMC neuron activation	<ul style="list-style-type: none"> Weight loss was greater than placebo 5.1% [Au: of initial body weight?] for 400 mg per day dosage and 2.2% for 300 mg per day dosage 	<ul style="list-style-type: none"> Modest weight loss 	Off-label usage for obesity; approved as an antidepressant in 1996	Approved through the Mutual Recognition Procedure in 1999
Bupropion plus naltrexone (Contrave; Takeda) [Au: ok?]	See above for information on bupropion; naltrexone is an opioid receptor antagonist of POMC neurons	<ul style="list-style-type: none"> Weight loss was 8–10% of initial body weight 	<ul style="list-style-type: none"> Substantial weight loss Food addiction Limited long-term data[†] Side effect includes uncontrolled hypertension Mid-level price range 	Approved in 2014	Approved in 2015
Rimonabant	CB ₁ receptor antagonist of hypothalamic, striatal and brainstem neurons	<ul style="list-style-type: none"> Highly effective in reducing food intake and body weight 	<ul style="list-style-type: none"> Severe adverse gastrointestinal and psychiatric effects (suicide risk) 	Rejected	Approved in 2006 and withdrawn in 2008
Metformin	Reduces hepatic glucose production by suppressing the gluconeogenesis pathway	<ul style="list-style-type: none"> Weight loss was 2.9 kg (2.5%) with drug vs 1.04 kg with placebo Indicated for obese patients with T2DM in combination with insulin 	<ul style="list-style-type: none"> Insufficient evidence for efficacy in overweight patients without T2DM Slightly affects appetite due to a minor effect on the brain 	Off-label usage for obesity; approved for T2DM in 1995	Off-label usage for obesity; approved for T2DM [Au: year?]

5-HT, 5-hydroxytryptamine (serotonin); CB₁ receptor, type I cannabinoid receptor; CI, confidence interval; EMA, European Medicines Agency; FDA, US Food and Drug Administration; GABA, γ -aminobutyric acid; GLP1, glucagon-like peptide 1; OTC, over the counter; POMC, anorexigenic proopiomelanocortin; T2DM, type 2 diabetes mellitus. *Data from REFS 196–198 **[Au: ok? Ref citations not allowed in titles]**. [†]Long-term data means 1–2 years. **[Au: should the double-dagger symbol be applied to all mentions of 'Limited long-term data?']**

its class¹⁴¹, was approved in several countries to treat hyperactive bladder. In a PET study, a single, high oral dose of mirabegron activated BAT and increased resting energy expenditure in normal **[Au: healthy?]** young volunteers; interestingly, the level of BAT activation was similar to that found in the same individuals exposed to cold (14 °C) for 2 hours¹⁰⁵. In both scenarios, metabolically active BAT was detected in visceral fat around the aorta and its main branches, including the subclavian and axillary regions, the sites where BAT persists in adult humans. As mirabegron is widely prescribed, a multicentre study involving a suitable number of overweight and/or obese patients with urological

disease who have received long-term treatment of mirabegron would enable assessment of its potential as an anti-obesity agent. Adverse cardiovascular effects have been reported in a small percentage of patients receiving mirabegron; the adverse effect was attributed to weak activation of the β_1 -adrenoceptor, a β -adrenoceptor variant that is expressed predominantly in the heart and kidney¹⁴². A combination of mirabegron and nebivolol, a specific β_1 -adrenoceptor antagonist with β_3 -adrenoceptor agonist properties¹⁴³, might have the desired effects on the energy balance without the adverse cardiovascular effects. Notably, nebivolol has recently been shown to induce white-to-brown

Table 2 | Selected anti-obesity drugs in the pipeline*

Drug	Mechanism of action	Status	Effects
Metreleptin (Myalept; Amylin Pharmaceuticals)	<ul style="list-style-type: none"> Leptin receptor agonist (leptin analogue) Activates leptin receptors in the hypothalamus, inducing increased POMC expression and signalling and decreased NPY/AgRP expression and signalling Suppresses appetite 	Approved in 2014 by the FDA for patients with congenital generalized or acquired generalized lipodystrophy	<ul style="list-style-type: none"> As leptin monotherapy does not lower food intake or body weight in obese patients (who have leptin resistance) Metreleptin is contraindicated in patients with general obesity Combination therapy with amylin analogues could restore leptin receptor responsiveness²⁰⁰
RM-493	<ul style="list-style-type: none"> MC receptor agonist Increases MC₃ and MC₄ receptor signalling in hypothalamic and brainstem neurons 	Phase II trials in genetic obesity	<ul style="list-style-type: none"> Induces weight loss Minimal effect on cardiovascular function²⁰¹ [Au: URL moved to refs list.]
MK-0493	<ul style="list-style-type: none"> MC receptor agonist Increases MC₃ and MC₄ receptor signalling in hypothalamus and brainstem 	Phase II trial completed	<ul style="list-style-type: none"> Induces a small reduction in body weight²⁰²
MOD-6030	<ul style="list-style-type: none"> GLP1 receptor agonist (oxyntomodulin analogue) Activates central GLP1 receptors by stimulating POMC neurons 	Preclinical	<ul style="list-style-type: none"> Causes a significant reduction in weight and appetite²⁰³ [Au: URL moved to refs list.]
GLP1-oestrogen [Au: do you perhaps mean GLP1 and glucagon co-agonist? Can you please check the reference and update this row? There seem to be some inconsistencies]	<ul style="list-style-type: none"> Co-agonistic strategy consisting of delivering peptide agonists with an attached small molecule, thus allowing selective delivery of a complex molecule to specific cells 	Preclinical studies	<ul style="list-style-type: none"> Improves the metabolic parameters of obesity and T2DM and reduces body weight more effectively than GLP1 alone²⁰⁴
Dapagliflozin and canagliflozin	<ul style="list-style-type: none"> SGLT2 inhibitors Prevent the absorption of glucose and water in the renal tubules 	Clinical trials [Au: specify phase?] Currently used to treat diabetes [Au: OK? When were they approved for this indication?]	<ul style="list-style-type: none"> Reduces renal glucose reabsorption in the proximal convoluted tubule, which increases urinary glucose excretion Reduce body weight²⁰⁵ Suggested to treat obese patients with T2DM
Beloranib	METAP2 inhibitor	Phase III trials (Prader-Willi syndrome); Phase IIb (severe obesity with T2DM)	<ul style="list-style-type: none"> Reduces liver production of fatty acids and promotes lipid metabolism in adipose tissue²⁰⁶ [Au: URL moved to refs list.]
Resveratrol	SIRT1 allosteric activator	Phase II trials	<ul style="list-style-type: none"> Reduces insulin resistance and increases oxidative metabolism in brown fat and skeletal muscle²⁰⁷ [Au: URL moved to refs list.]

AgRP, agouti-related peptide; FDA, US Food and Drug Administration; GLP1, glucagon-like peptide 1; MC, melanocortin; METAP2, methionine aminopeptidase 2; NPY, orexigenic neuropeptide Y; POMC, anorexigenic pro-opiomelanocortin; SGLT, sodium-glucose-linked transporter 2; SIRT1, sirtuin 1; T2DM, type 2 diabetes mellitus. *See REFS 197–199 for more details.

transdifferentiation of human visceral and subcutaneous adipocytes *in vitro*¹⁴⁴.

An anti-obesity drug whose primary mode of action is to induce browning should act predominantly on visceral fat, thereby directly counteracting the major cause of obesity-associated metabolic disorders. Accumulation of abdominal visceral fat is, to some extent, linked to increased local levels and/or activity of androgen and glucocorticoid steroid hormones^{145,146}. These hormones are also ligands of the mineralocorticoid receptors, which are found on white and brown adipocytes and could have a role in abdominal visceral fat accumulation and BAT to WAT conversion^{147–151}. As visceral adipocytes are considerably more prone than subcutaneous adipocytes to die by pyroptosis^{18,93}, inflammation caused by fat accumulation is more common in visceral than in subcutaneous adipose tissue. Inflammation itself could

increase the activity of 11-β hydroxysteroid dehydrogenase type 1 (REF. 152), an enzyme that can regenerate active cortisol from inactive cortisone, thus establishing a vicious circle. In this context, mineralocorticoid receptor antagonism has been shown to protect mice from the adverse obesogenic and metabolic effects of a high-fat diet via conversion of a substantial amount of visceral and subcutaneous WAT into BAT¹⁵³. Given that mineralocorticoid receptor antagonists are widely prescribed diuretics, used to manage chronic heart failure, hyperaldosteronism and female hirsutism¹⁵⁴, patients receiving such drugs should also be assessed for weight loss and metabolic parameters to establish whether these compounds have anti-obesity properties.

Thiazolidinediones activate the nuclear transcription factor PPARγ and induce transcription of PPARγ-responsive genes, resulting in increased

Pyroptosis

A highly inflammatory type of cell death involving caspase 1 activation, DNA fragmentation, cell membrane pore formation and cellular lysis.

insulin-dependent glucose disposal and decreased insulin resistance. Some thiazolidinediones, such as pioglitazone and rosiglitazone, are approved for the treatment of T2DM. Thiazolidinediones are associated with adverse effects, including weight gain, oedema and an increased risk of bone fracture; recent reports documenting the risk of congestive heart failure and bladder cancer have substantially restricted their use in several countries. Notably, PPAR γ agonists also induce visceral fat browning¹⁵⁵. SIRT1-dependent deacetylation of PPAR γ is required to induce browning and repress the transcription of visceral WAT genes associated with insulin resistance¹³³. Combined thiazolidinedione and SIRT1 agonist treatment could enable lower doses of both drugs to be used, thus avoiding some of the adverse effects associated with these drugs while still ensuring visceral WAT browning. A recent paper has shown that the transcription factor Kruppel-like factor 11 (KLF11) is required for rosiglitazone-induced browning of human white adipocytes *in vitro*¹⁵⁶.

Obesity is associated with a chronic low-grade inflammatory state, and inhibition of inflammatory signalling is anticipated to ameliorate metabolic syndrome and decrease obesity. The inflammatory state is, to some extent, caused by the activation of nuclear factor- κ B (NF- κ B) in WAT, liver and muscle¹⁵⁷, where this transcription factor markedly impairs insulin signalling, thereby leading to insulin resistance and T2DM. NF- κ B activation in high-fat-diet-induced obese mice is accompanied by the sustained activation of downstream effectors, such as the protein kinases TBK1 and inhibitor- κ B kinase ϵ (IKK ϵ ; encoded by *IKBKE*) in liver, adipocytes and adipose tissue macrophages¹⁵⁸. Notably, *Ikk ϵ* knockout mice are protected against diet-induced obesity and insulin resistance by increased energy expenditure and thermogenesis due to BAT activation¹⁵⁸. Amlexanox is an anti-inflammatory drug that acts by inhibiting the IKK ϵ -TBK1 pathway. It has been used topically since 1994 to treat recurrent aphthous ulcers both in Europe and the United States. In Japan, systemic amlexanox is also used to treat allergic conditions such as bronchial asthma and rhinitis. Treatment of genetically and diet-induced obese mice with amlexanox reduced body weight and ameliorated insulin resistance and liver steatosis^{159,160}. This effect was associated with a substantial increase in energy expenditure owing to activation of BAT and browning of subcutaneous WAT. The WAT browning and, possibly, the weight-loss effects mainly depend on an increased catecholamine sensitivity of adipocytes, which results in increased cAMP levels. Thus, inhibitors of NF- κ B downstream factors, such as IKK ϵ , may constitute novel anti-obesity targets through which BAT activation and, potentially, recruitment promote energy expenditure.

The characteristic inflammatory state of obesity is driven by resident and blood-derived immune cells that infiltrate dysregulated fat and activate the type 1 immune response. However, adipose tissue normally contains resident leukocytes, which have recently been shown to support physiological functions through type 2 innate immune responses¹⁶¹ (BOX 2).

Drugs with proven or presumed browning effects have already been used for some time in various therapeutic regimens and patient populations. Clinical and metabolic assessments, radiological investigations of BAT quantities and activity, and retrospective studies of these patients may identify unrecognized anti-obesity effects of molecules that are already clinically [Au: OK?] available, and provide further insights into the possible role of BAT activation and recruitment and weight control in humans.

Current research into BAT-inducing drugs

The induction of even small amounts of functional BAT in the human adipose organ could be a new, valuable approach to the treatment and/or prevention of obesity and its metabolic complications. This notion is spurring leading research groups and drug manufacturers to search for novel browning molecules. Both molecular target-based and high-throughput phenotypic screening approaches are proving successful in this regard. Several molecules and cellular pathways that regulate browning have recently been discovered. However, it should be noted that these studies are often performed in human or mouse pluripotent stem cell models, which are not fully characterized, and that the browning effects obtained *in vitro* are validated in mouse models, in which browning is usually tested in subcutaneous fat and not in visceral (especially periaortic and mesenteric) adipose tissue. The role of these molecules and pathways could therefore be confined to brown adipocyte precursors and/or be important only in mice, in which BAT is much more abundant than in humans and in which subcutaneous fat is easier to recruit. By contrast, data from our group and others suggest that browning of fully differentiated fat is due more to white-to-brown transdifferentiation than to the proliferation and differentiation of immature cells, and that browning of visceral fat could be more therapeutically relevant than browning of subcutaneous fat to the treatment of obesity and metabolic syndrome.

Another substantial concern is specificity. Unfortunately, none of the targets of browning identified to date is exclusive to adipocytes. Their modulation is therefore complicated by their involvement in the development and/or function of other organs or systems. Consequently, to target fat, the drug would almost certainly need to be delivered locally and prevented from spreading systemically, or carried selectively to visceral white adipocytes. Thus, the ability to hit one of these targets to induce browning in obese humans requires technological advances that enable selective drug delivery to specific cell types.

Selected recently discovered molecules and pathways that seem best suited to target adipocytes to induce browning are discussed below. However, findings on the role of these molecules and pathways in visceral fat are scant, and no data are available on their potential to induce white-to-brown visceral adipocyte transdifferentiation.

Target-based approach. Several molecules have recently been identified as potential targets for therapies based on WAT browning (TABLE 3). As many of these molecules

Table 3 | Selected potential molecular targets to induce browning of WAT

Molecular target (gene symbol)	Comments	Refs
Adenosine A _{2A} receptor (<i>ADORA2A</i>)	Adenosine is co-released with noradrenaline by BAT sympathetic nerves and activates murine and human brown adipocytes to induce browning of subcutaneous WAT	208
β ₃ -adrenergic receptor (<i>ADRB3</i>)	A specific adrenoceptor also found in human WAT, it is required for brown adipocyte activation and physiological browning of the adipose organ	209
Bone morphogenetic protein receptor 8 (BMP/8-r) [Au: do you mean Bone morphogenetic protein 7 and 8B (BMP7, BMP8B)?]	Capable of enhancing brown adipocyte activity by acting directly on the sympathetic nervous system leading to subcutaneous WAT browning	210,211
Casein kinase 2 (<i>CSNK2</i>)	An evolutionarily conserved serine/threonine kinase preferentially activated in white fat by noradrenaline and under obesity conditions; its genetic or pharmacological inhibition in WAT promotes browning of subcutaneous and visceral fat and increases thermogenesis <i>in vivo</i>	174
Fibroblast growth factor 21 (<i>FGF21</i>) [Au:OK, or <i>FGF21R?</i>]	Capable of activating the adipocyte thermogenic programme by interacting with FGF-β-Klotho [Au: <i>FGFR-β-Klotho?</i>] complexes and triggering subcutaneous and visceral WAT browning	47,212
Natriuretic peptides A and B (<i>NPPA</i> and <i>NPPB</i>)	Produced by the heart, their interaction with natriuretic peptide receptors activate cGMP-dependent protein kinase, leading to browning of subcutaneous and visceral WAT [Au: ok?]	213
Thyroid hormone [Au:OK, or the receptor?]	Thyroid hormone signalling induces the local formation of BMP8B and further sensitizes adipocytes to adrenergic stimuli to acquire the brown phenotype	214
Eukaryotic translation initiation factor 4E-binding protein 1 (<i>EIF4EBP1</i>)	Inhibition of this enzyme allows translation of <i>PPARGC1A</i> mRNA in white adipocytes and triggers the browning of subcutaneous WAT	173,215
Arrestin domain containing 3 (<i>ARRDC3</i>)	Loss of <i>ARRDC3</i> [Au: gene OK?] enhances the response to adrenergic stimulation, induces resistance to obesity and promotes BAT thermogenesis and visceral as well as subcutaneous WAT browning	216
Brain-derived neurotrophic factor (<i>BDNF</i>)	Levels of this secreted hypothalamic factor are increased by exercise; it drives the adrenergic outflow to WAT to induce browning in visceral and subcutaneous compartments	35
Cardiotrophin 1 (<i>CTF1</i>)	A heart- and muscle-derived protein able to induce browning of visceral WAT	217
CCAAT/enhancer-binding protein-β (<i>CEBPB</i>)	A key transcription factor <i>in vivo</i> that promotes expression of the brown gene programme in cell culture models [Au: ok?]	166
Cyclooxygenase 2 (<i>COX2</i> ; also known as <i>PTGS2</i>)	Overexpression of this enzyme determines resistance to weight gain and demonstrates the role of prostaglandins in the browning of WAT	218,219
Euchromatic histone-lysine N-methyltransferase 1 (<i>EHMT1</i>)	Forms a transcriptional complex with <i>PRDM16</i> and determines browning by activating <i>PRDM16</i> in subcutaneous WAT	220
Early B cell factor 2 (<i>EBF2</i>)	Regulates <i>PRDM16</i> function and induces the acquisition of BAT features <i>in vitro</i>	169
Forkhead box protein C2 (<i>FOXC2</i>)	Sensitizes adipocytes to cAMP signalling via the β-adrenergic pathway, and its overexpression triggers the acquisition of the brown phenotype in visceral WAT	221
Liver X receptor (<i>LXR</i> ; also known as <i>NR1H</i>)	<i>Nr1h3^{-/-}</i> ; <i>Nr1h2^{-/-}</i> [Au: ok?] mice exhibit enhanced energy dissipation due to browning of WAT	222
Fibronectin type III domain-containing protein 5 (<i>FNDC5</i> ; also known as irisin)	A myokine secreted during endurance exercise and shivering that is capable of inducing browning of subcutaneous WAT	36
Meteorin-like peptide (<i>METRNL</i>)	A myokine that acts through eosinophils and macrophages within WAT and capable of inducing browning indirectly in subcutaneous and visceral fat	223
miR-133	miR-133 acts on <i>PRDM16</i> by blocking its signalling; its downregulation after cold exposure allows increased expression of genes associated with browning in subcutaneous WAT	170
miR-155	<i>miR155</i> expression is positively regulated by TGFβ1 and suppresses <i>CEBPB</i> translation; knockout mice showed impaired browning of subcutaneous WAT following cold exposure	224

Table 3 (cont.) | Selected potential molecular targets to induce browning of WAT

Molecular target (gene symbol)	Comments	Refs
miR-193b-365	RUNX1T1 is a brown adipogenesis inhibitor; miR-193b-365 upregulation during adipogenesis directly inhibits RUNX1T1, thus raising the levels of PRDM16 and PPAR α in subcutaneous WAT	225
miR-196a	miR-196a is upregulated after cold exposure, leading to HOXC8 suppression and enhancing CEBP β expression, which in turn leads to browning of subcutaneous WAT	164
Nitric oxide	A short-lived gaseous signalling molecule capable of inducing the acquisition of the brown phenotype in WAT via cGMP and PKG	226
PPAR γ coactivator 1 α (PPARGC1A)	The master regulator of the brown fat cell phenotype; several molecules (for example, p107, RB and RIP140) act through it; SRC and TWIST1 are PGC1 α repressors [Au: edits correct?]	227
Peroxisome proliferator-activated receptor alfa (PPAR α) [Au: PPAR α or PPAR γ meant?]	Induces PGC1 α activation and stabilizes PRDM16, leading to browning of WAT	133,228
Retinoblastoma family (RB)	Loss of p107 causes loss of RB and upregulation of thermogenic genes in subcutaneous WAT	229
PR domain containing 16 (PRDM16)	PRDM16 increases the transcriptional activities of PGC1 α , PPAR γ and C/EBPs through direct interactions; mice selectively transgenic for PRDM16 in fat show increased browning of the adipose organ	230
Phosphatase and tensin homologue (PTEN)	Increased PTEN levels inhibit PI3K, which drives a thermogenic programme in subcutaneous WAT	231
Receptor-interacting protein 140 (RIP140)	A nuclear factor that modulates the transcriptional activity of various transcription factors; is a PGC1 α repressor; knockout mice exhibit enhanced browning of subcutaneous WAT	232
Small heterodimer partner (SHP; also known as NROB2)	Inhibits PPARGC1A expression; its suppression induces browning of WAT	233
Dual serine/threonine kinase receptors and transcription factors (SMADs) [Au: italics?]	SMAD3 represses PPARGC1A expression; systemic SMAD3 blockade downregulates white adipocyte genes, leading to browning of WAT	234
Transducin-like enhancer of split 3 (TLE3)	A white fat-selective cofactor that antagonizes PRDM16 and suppresses brown fat differentiation; knockout mice showed enhanced browning of subcutaneous WAT	235
TLQP-21, a VGF-derived peptide	A small neuropeptide capable of inducing specific adrenergic outflow to WAT, thus stimulating browning of both visceral and subcutaneous WAT	236
TWIK-related acid-sensitive K ⁺ channel 1 (TASK1; also known as KCNK3)	A pH-sensitive potassium channel that controls the thermogenic activity in brown adipocytes through modulation of β -adrenergic receptor signalling; its activity is also involved in blocking autophagy and white phenotype acquisition in subcutaneous WAT	237
Transient receptor potential channel subfamily M member 8 (TRPM8)	Treatment with TRPM8 agonists (for example, menthol) promotes energy expenditure and protects against obesity through subcutaneous WAT browning	238
Transient receptor potential vanilloid family members 1 and 4 (TRPV1 and TRPV4)	Trpv-knockout mice are resistant to weight gain and exhibit thermogenic gene upregulation in subcutaneous WAT	239
Twist-related protein 1 (TWIST1)	Binds to and inhibits PGC1 α at target genes; blocking its activity leads to browning of WAT	240
Vitamin A derivatives (for example, retinoic acid and retinaldehyde)	Increased energy expenditure through WAT browning of subcutaneous and visceral depots has been described in retinoic acid-treated mice	241

BAT, brown adipose tissue; cGMP, cycling GMP; HOXC8, homeobox protein Hox-C8; p107, also known as retinoblastoma (RB)-like protein 1; PGC1 α , PPAR γ coactivator 1 α ; PI3K, phosphoinositide 3-kinase; PKG, protein kinase G [Au: ok?]; PPAR, peroxisome proliferator-activated receptor; SRC, steroid receptor coactivator [Au: ok?]; TGF β , transforming growth factor- β ; WAT, white adipose tissue.

have been shown to be downstream to the activation of β_3 -adrenoceptors, which are expressed by mature white adipocytes^{27,144,162}, they may be involved in white-to-brown conversion (FIG. 3). Here, we focus on the master molecular targets CCAAT/enhancer-binding

protein- β (C/EBP β) and PR domain zinc finger protein 16 (PRDM16). Both agonists and antagonists of their inhibitors may be useful for drug development.

The transcription factor C/EBP β is considered a master regulator of the thermogenic gene expression

[Au: do you mean 'Transforming growth factor- β (TGF β) and SMAD family member 3 (SMAD3)?]

Box 2 | The type 2 immune response promotes browning [AU: Title ok?]

In lean white adipose tissue (WAT), resident leukocytes enhance insulin sensitivity through the following functions: secreting interleukin-10 (IL-10)¹⁸⁹; sequestering and processing excess iron¹⁹⁰; safely disposing of dead cells and debris; and promoting vascularization and tissue matrix remodelling¹⁹¹. Type 2 immune responses are also crucial for brown adipose tissue (BAT) thermogenesis and adipose organ browning. Resident type 2 macrophages of BAT respond to cold-induced sympathetic stimulation by synthesizing and releasing catecholamines, which enhance brown adipocyte thermogenic activation and induce lipolysis of adjacent white adipocytes, supplying the free fatty acids that are required by activated brown cells¹⁹². In white areas of the adipose organ, leukocytes are involved in cold-induced browning via at least two different pathways. First, IL-4 production by eosinophils and IL-13 production by group 2 innate lymphoid cells drive adipocyte precursor proliferation and promote their brown differentiation in mouse subcutaneous fat^{193,194}. Second, resident leukocytes promote differentiation both in subcutaneous inguinal fat and in visceral epididymal fat by directly affecting adipocyte precursors¹⁹⁵. Therefore, existing immunomodulatory biological agents, which elicit type 2 immune responses, may promote a 'browned', healthy phenotype in the adipose organ and exert anti-obesity effects. Future studies could assess whether any metabolic benefit induced by these drugs might also stem from white-to-brown adipocyte transdifferentiation.

profile responsible for the brown phenotype¹⁶³. During development, inhibition of C/EBP β forces adipocyte precursors to acquire a white phenotype¹⁶⁴. Molecules with browning properties might act by countering such inhibition, thus promoting brown adipocyte differentiation. However, C/EBP β is a widespread transcription factor that has key roles in the development and function of a variety of cell types, including adipocytes, keratinocytes, hepatocytes, mammary epithelial cells, ovarian luteal cells, B cells, osteoclasts and macrophages¹⁶⁵. Thus, C/EBP β should be specifically targeted in adipocytes without disrupting physiological processes in other cells.

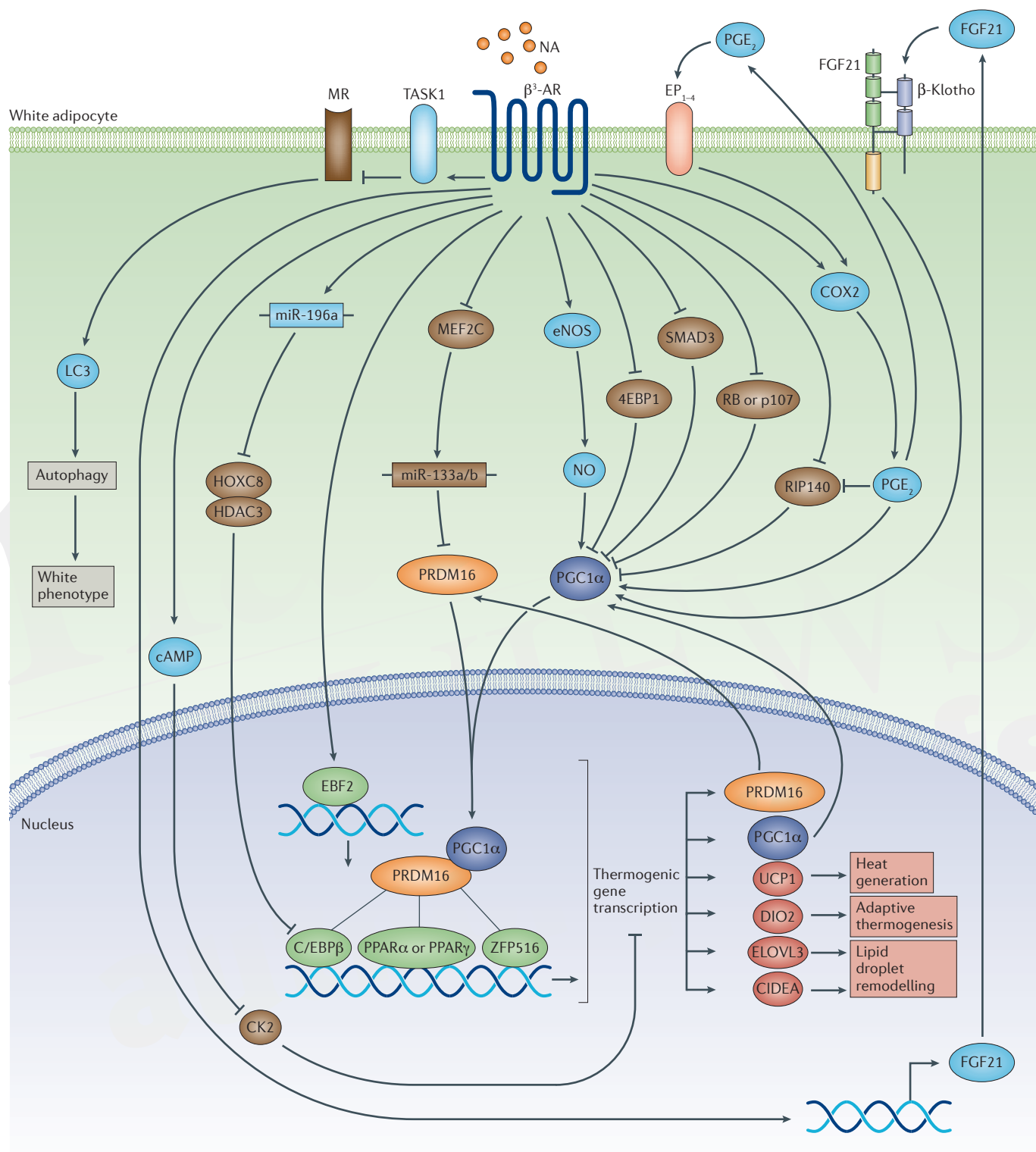
PRDM16, a 140 kDa zinc-finger protein, is highly expressed in BAT and is an important factor for normal brown adipocyte development⁴³. It acts in synergy with C/EBP β , and its transgenic expression in WAT induces browning¹⁶⁶. Mice lacking *Prdm16* expression in WAT are more prone to meta-inflammation¹⁶⁷, and PRDM16 is required for the maintenance of BAT identity and function in adult mice^{43,168}. Importantly, PRDM16 staining has been detected in the nucleus of paucilocular adipocytes in browned omentum from patients with pheochromocytoma, suggesting a key role for PRDM16 in white-to-brown conversion in human visceral fat¹¹³. Notably, *PRDM16* expression in WAT can be induced by the activation of early B cell factor 2 (EBF2)¹⁶⁹ as well as by the inhibition of microRNA-133 (miR-133)¹⁷⁰. miR-133 directly targets PRDM16 and therefore reduces its expression and browning effects. Furthermore, PRDM16 and C/EBP β transfection can induce the transdifferentiation of mature skin fibroblasts into brown adipocytes, and the thermogenic capacity of these cells was preserved in explants¹⁶⁶. PRDM16 appears to be the most effective molecular target to induce white-to-brown adipocyte transdifferentiation. However, further data on how these factors act and interact in adipocytes to promote an energy-dispersing phenotype are required and, to our knowledge, no agents targeting EBF2, PRDM16, C/EBP β or miR-133 are currently sufficiently advanced to be tested in humans.

Figure 3 | Potential β_3 -adrenoceptor-dependent molecular mechanisms driving white-to-brown adipocyte transdifferentiation.

The transcription factor PR domain zinc finger protein 16 (PRDM16) is the key control point in acquiring the brown phenotype. Following β_3 -adrenoceptor (β_3 -AR) stimulation, some pathways (for example, cyclooxygenase 2 (COX2) and endothelial nitric oxide (NO) synthase (eNOS)) induce the thermogenic gene programme typical of brown adipocytes by activating peroxisome proliferator-activated receptor- γ (PPAR γ) coactivator 1 α (PGC1 α). Other molecules (for example, CCAAT/enhancer-binding protein- β (C/EBP β), receptor-interacting protein 140 (RIP140), eukaryotic translation initiation factor 4E-binding protein 1 (4EBP1), SMAD family member 3 (SMAD3), homeobox protein Hox-C8 (HOXC8)) inhibit the thermogenic gene programme, and blocking them through β_3 -AR stimulation removes this inhibition. PGC1 α is a transcription factor involved in mitochondrial biogenesis and a co-activator of browning genes. COX2 also regulates the formation of prostaglandins, particularly prostaglandin E2 (PGE $_2$), which increases PGC1 α expression, blocks RIP140 and leads to a phenotypic shift towards browning in mature adipocytes. NO interacts directly with PGC1 α transcription to modulate mitochondrial biogenesis. Adipose-derived fibroblast growth factor 21 (FGF21) acts in an autocrine and paracrine manner to increase the expression of protein uncoupling protein 1 (UCP1) and other thermogenic genes in fat tissue, in part by increasing PGC1 α protein levels through post-transcriptional mechanisms. Defective autophagy in adipose tissues reduces white adipose tissue mass and promotes a brown-like phenotype, enhancing insulin sensitivity. The thermogenic gene programme also includes type 2 iodothyronine deiodinase (DIO2), fatty acyl chain elongase 3 (ELOVL3) and cell death activator A (CIDEA). DIO2 converts thyroxine to triiodothyronine, thus activating the sympathetic nervous system and promoting adaptive thermogenesis in brown adipose tissue (BAT). ELOVL3 regulates the endogenous synthesis of saturated very long chain fatty acids and triglyceride formation and has a potential role in lipid droplet remodelling during the white-to-brown transition. CIDEA is a lipid droplet-protein enriched in brown adipocytes that promotes the enlargement of lipid droplets. Some of the transcriptional mechanisms highlighted in the figure (that is, early B cell factor 2 (EBF2)) have been characterized during the development and differentiation of adipocyte precursors, but might be recapitulated during the white-to-brown phenotypic switch occurring in the convertible adult fat depots. cAMP, cyclic AMP; CK2, casein kinase 2; EP $_{1-4}$, PGE $_2$ receptor 1–4 subtypes; HDAC3, histone deacetylase 3; LC3, microtubule-associated protein 1A/1B-light chain 3; MEF2C, myocyte-specific enhancer factor 2C; miR, microRNA; MR, mineralocorticoid receptor; NA, noradrenaline; p107, also known as retinoblastoma (RB)-like protein 1; TASK1, TWIK-related acid-sensitive K $^+$ channel 1; ZFP516, zinc finger protein 516.

[Au: please check that all abbreviations in this figure have been defined correctly]

PRDM16 stability is closely controlled by PPAR γ agonists such as thiazolidinediones, which are well known to induce WAT browning^{38,133,155,171}. The mechanism by which thiazolidinediones induce the transcription of brown fat genes involves the activation of SIRT1, which



deacetylates two residues in PPAR γ . The deacetylated form of PPAR γ binds more efficiently to PRDM16. Increased SIRT1 activity levels in adipose tissue promote WAT browning and alleviate obesity¹³³. Through an apparently unrelated mechanism, thiazolidinediones also stabilize the PRDM16 protein, raising its level in adipocytes¹⁷¹.

Notably, several lines of evidence seem to support the notion that a large proportion of white adipocytes in some depots are ‘forced’ into a white phenotype but can regain their natural propensity to differentiate into brown adipocytes. Some molecules involved in this process have already been identified: receptor-interacting protein 140 (RIP140)¹⁷², eukaryotic translation

initiation factor 4E-binding protein 1 (4EBP1)¹⁷³, homeobox protein Hox-C8 (HOXC8)¹⁶⁴ and casein kinase 2 (CK2)¹⁷⁴ inhibit the brown phenotype. Interestingly, the expression of these molecules is downregulated by the β_3 -adrenoceptor signalling pathway, which in turn upregulates browning master regulators such as C/EBP β and PRDM16, thereby inducing the brown phenotype¹⁶⁶. Thus, inhibiting RIP140, 4EBP1, HOXC8 and CK2 [Au: ok?] could be another interesting therapeutic strategy. The most interesting of these molecules is HOXC8, which is highly and specifically expressed by differentiating white adipocytes¹⁷⁵. Moreover, HOXC8 can repress C/EBP β expression to force precursors to differentiate into white adipocytes¹⁶⁴. Notably, β_3 -adrenoceptor stimulation and/or cold exposure of human WAT progenitor cells and mouse subcutaneous and visceral fat result in the upregulation of miR-196a in adipocyte precursors; in turn, miR-196a post-transcriptionally suppresses HOXC8. Blocking the inhibitory action of HOXC8 allows the cell to differentiate into a brown adipocyte¹⁶⁴. Notably, fat-specific *mir-196a* overexpression in transgenic mice induces browning, making these mice more resistant to high-fat-diet-induced obesity¹⁶⁴. Although it remains to be determined whether miR-196a can induce the brown phenotype in fully differentiated white adipocytes, these findings highlight the role of microRNAs in adipocyte phenotype commitment and pave the way for their therapeutic use to control adipocyte transdifferentiation and to treat human obesity and associated diseases¹⁷⁶.

Phenotypic screen approach. Phenotypic screening in drug discovery has been making a comeback after the target-focused approach, which dominated the industry over the past decades, failed to provide a larger number of new first-in-class small-molecule medications¹⁷⁷. Recent advances in chemical proteomics, genomics and informatics also often enable rapid identification of the molecular targets of bioactive compounds that have been shown to induce the desired phenotype^{178,179}. Importantly, phenotypic screening approaches may allow the testing of molecules with browning properties directly on human adipocytes, thus enabling the identification of compounds with a high probability of exerting therapeutic effects in obese patients. However, studies specifically aimed at converting visceral human fat-storing adipocytes into metabolically active, potentially thermogenic cells have not yet been performed.

A screening platform to identify small molecules involved in white-to-brown adipocyte conversion has recently been implemented¹⁸⁰. The tool enabled the identification of two Janus kinase (JAK) inhibitors that can confer brown-like metabolic activity (defined by high mitochondrial content, multilocular arrangement of the cellular lipid content and *UCP1* expression) on human pluripotent stem cells during their differentiation into mature white adipocytes. One of these compounds, tofacitinib, is a JAK inhibitor that is currently approved in the United States for the treatment of rheumatoid arthritis; retrospective studies of patients receiving chronic tofacitinib could therefore document its anti-obesity potential. Both compounds inhibit the JAK-signal transducer

and activator of transcription 1 (STAT1) pathway and/or JAK-STAT3 pathway [Au: ok?] in cultured adipocytes and have a long-lasting effect¹⁸⁰ [Au: ok to cite 180 here?]. The observation that inhibiting the JAK-STAT3 pathway promotes a brown phenotype was unexpected, given recent *in vivo* data suggesting a stimulatory role for this signalling pathway in mouse BAT development¹⁸¹. There are currently no data supporting a role for STATs in white-to-brown adipose conversion.

High-throughput screening of putative transcription factors acting on the proximal -5.5 kb region of the *UCP1* promoter has recently identified a previously uncharacterized Kruppel-like zinc finger transcription factor, ZFP516, as a novel BAT-enriched and cold-inducible regulator of BAT development and function¹⁸². In brown adipocytes, ZFP516 interacts with PRDM16 to promote the transcription of key BAT-specific genes including *UCP1*. Importantly, mice overexpressing *Zfp516* show browning of inguinal WAT and increased body temperature and are resistant to diet-induced obesity. Conversely, *Zfp516* ablation is associated with massive impairment of BAT development. *Zfp516* knockout mice die soon after birth, suggesting that the gene is required for the correct development of other tissues or organs.

A phenotypic screen evaluating the metabolite profiles of a large number of normoglycaemic individuals who were followed for 12 years identified some branched-chain and aromatic amino acids, in particular valine, whose levels predicted the future development of obesity and T2DM¹⁸³. β -aminoisobutyric acid (BAIBA) is a non-protein amino acid generated by valine catabolism. Increased BAIBA secretion by skeletal muscle during exercise increases the expression of brown adipocyte genes (including *UCP1*, cell death activator A (*CIDEA*) and *PRDM16*) in mouse adipose precursors and human pluripotent stem cells *in vitro*, induces WAT browning in mouse subcutaneous fat, and increases fat oxidation in the liver¹⁸⁴. Testing BAIBA in obese patients to assess its therapeutic potential is likely to provide interesting data. These findings could open the way to the identification of novel metabolites and other small molecules — secreted from skeletal muscle, browned WAT or other organs that function as paracrine and endocrine signals to regulate adipose tissue plasticity and systemic metabolism — that could be harnessed as novel drugs to manage body weight.

Conclusion and prospects

Over the past few decades, substantial experimental evidence has increased our understanding of the central and peripheral mechanisms regulating the energy balance and the distinctive roles of the adipose organ, skeletal muscle and liver in normal and pathological body metabolism. Viewed in terms of the adipose organ, morbid obesity reflects a massive increase in WAT, especially visceral WAT, at the expense of BAT. In mice, tilting the WAT-BAT balance in favour of BAT — thus increasing energy expenditure through non-shivering thermogenesis — unequivocally prevents and treats diet-induced and genetic obesity. Hopefully,

these findings will be relevant to humans. However, the mouse adipose organ has a large amount of BAT and also has a large capacity for browning. Humans, especially obese patients, have smaller amounts of functional BAT, and the precise role of BAT in the body energy balance remains to be determined.

Intense basic and pharmacological research work is under way to discover targets and molecules that can stimulate mature brown adipocytes and/or induce the brown phenotype in differentiating adipocytes. In our opinion, white-to-brown transdifferentiation of visceral adipocytes — a currently neglected topic in anti-obesity drug research — should be a primary area

of investigation. Visceral white adipocytes are particularly vulnerable to lipid overload and prone to develop a stressed, dysfunctional state that leads to death, meta-inflammation and the major complications of metabolic syndrome. Reversing this state and promoting an energy-expenditure-prone phenotype would be a crucial therapeutic strategy to treat obesity. Exploring the biology of visceral adipocytes through basic research and dissecting molecules and pathways relevant for their conversion into brown adipocytes is anticipated to provide crucial insights and to identify specific targets for drugs that can make visceral fat healthier and more geared to consuming than storing energy.

- Lloyd-Jones, D. *et al.* Heart disease and stroke statistics — 2009 update: a report from the American Heart Association Statistics Committee and Stroke Statistics Subcommittee. *Circulation* **119**, e21–e181 (2009).
- Bornfeldt, K. E. & Tabas, I. Insulin resistance, hyperglycemia, and atherosclerosis. *Cell Metab.* **14**, 575–585 (2011).
- Hauner, D. & Hauner, H. Metabolic syndrome and breast cancer: is there a link? *Breast Care* **9**, 277–281 (2014).
- Dixon, J. B., le Roux, C. W., Rubino, F. & Zimmet, P. Bariatric surgery for type 2 diabetes. *Lancet* **379**, 2300–2311 (2012).
- Rothwell, N. J. & Stock, M. J. A role for brown adipose tissue in diet-induced thermogenesis. *Nature* **281**, 31–35 (1979).
- Bachman, E. S. *et al.* β AR signaling required for diet-induced thermogenesis and obesity resistance. *Science* **297**, 843–845 (2002).
- Feldmann, H. M., Golozoubova, V., Cannon, B. & Nedergaard, J. UCP1 ablation induces obesity and abolishes diet-induced thermogenesis in mice exempt from thermal stress by living at thermoneutrality. *Cell Metab.* **9**, 203–209 (2009).
- Saito, M. *et al.* High incidence of metabolically active brown adipose tissue in healthy adult humans: effects of cold exposure and adiposity. *Diabetes* **58**, 1526–1531 (2009).
- Virtanen, K. A. *et al.* Functional brown adipose tissue in healthy adults. *N. Engl. J. Med.* **360**, 1518–1525 (2009).
- van Marken Lichtenbelt, W. D. *et al.* Cold-activated brown adipose tissue in healthy men. *N. Engl. J. Med.* **360**, 1500–1508 (2009).
- Cypess, A. M. *et al.* Identification and importance of brown adipose tissue in adult humans. *N. Engl. J. Med.* **360**, 1509–1517 (2009).
- Zingaretti, M. C. *et al.* The presence of UCP1 demonstrates that metabolically active adipose tissue in the neck of adult humans truly represents brown adipose tissue. *FASEB J.* **23**, 3113–3120 (2009). **References 8–12 independently confirm the presence of metabolically active and functional BAT in adult humans.**
- Vijgen, G. H. *et al.* Brown adipose tissue in morbidly obese subjects. *PLoS ONE* **6**, e17247 (2011).
- Loncar, D. Convertible adipose tissue in mice. *Cell Tissue Res.* **266**, 149–161 (1991).
- Xu, H. *et al.* Chronic inflammation in fat plays a crucial role in the development of obesity-related insulin resistance. *J. Clin. Invest.* **112**, 1821–1830 (2003).
- Weisberg, S. P. *et al.* Obesity is associated with macrophage accumulation in adipose tissue. *J. Clin. Invest.* **112**, 1796–1808 (2003).
- Strissel, K. J. *et al.* Adipocyte death, adipose tissue remodeling and obesity complications. *Diabetes* **56**, 2910–2918 (2007).
- Murano, I. *et al.* Dead adipocytes, detected as crown-like structures, are prevalent in visceral fat depots of genetically obese mice. *J. Lipid Res.* **49**, 1562–1568 (2008).
- Eckel, R. H., Grundy, S. M. & Zimmet, P. Z. The metabolic syndrome. *Lancet* **365**, 1415–1428 (2005).
- Cinti, S. *The Adipose Organ* (Kurtis, 1999).
- de Jong, J. M., Larsson, O., Cannon, B. & Nedergaard, J. A stringent validation of mouse adipose tissue identity markers. *Am. J. Physiol. Endocrinol. Metab.* **308**, E1085–E1105 (2015).
- Morrison, S. F., Madden, C. J. & Tupone, D. Central neural regulation of brown adipose tissue thermogenesis and energy expenditure. *Cell Metab.* **19**, 741–756 (2014).
- Barbatelli, G., Heinzlmann, M., Ferrara, P., Morroni, M. & Cinti, S. Quantitative evaluations of gap junctions in old rat brown adipose tissue after cold acclimation: a freeze-fracture and ultra-structural study. *Tissue Cell* **26**, 667–676 (1994).
- Cannon, B. & Nedergaard, J. Brown adipose tissue: function and physiological significance. *Physiol. Rev.* **84**, 277–359 (2004).
- Murano, I., Barbatelli, G., Giordano, A. & Cinti, S. Noradrenergic parenchymal nerve fiber branching after cold acclimatization correlates with brown adipocyte density in mouse adipose organ. *J. Anat.* **214**, 171–178 (2009).
- Vitali, A. *et al.* The adipose organ of obesity-prone C57BL/6J mice is composed of mixed white and brown adipocytes. *J. Lipid Res.* **53**, 619–629 (2012). **This study uses two different strains of mice to demonstrate that WAT and BAT both occur in the majority of fat depots and are composed of white, brown and paucilocular adipocytes with variable UCP1 expression and intermediate morphological features, even in warm-acclimated mice. [Au: ok?]**
- Barbatelli, G. *et al.* The emergence of cold-induced brown adipocytes in mouse white fat depots is determined predominantly by white to brown adipocyte transdifferentiation. *Am. J. Physiol. Endocrinol. Metab.* **298**, E1244–E1253 (2010). **A transmission electron microscopy study of the progressive steps through which white adipocytes transdifferentiate into brown adipocytes in mice under several experimental conditions.**
- Rosenwald, M., Perdikari, A., Rulicke, T. & Wolfrum, C. Bi-directional interconversion of brite and white adipocytes. *Nat. Cell Biol.* **15**, 659–667 (2013). **This paper unequivocally demonstrates the conversion of adipocytes with a white-like morphological and molecular phenotype into bona fide brown fat cells.**
- Lee, Y. H., Petkova, A. P., Konkar, A. A. & Granneman, J. G. Cellular origins of cold-induced brown adipocytes in adult mice. *FASEB J.* **29**, 286–299 (2015). **Using adipocytes tagged by adiponectin-CreER²-mediated recombination, this study shows that all newly observed brown adipocytes in mouse subcutaneous WAT following cold exposure originate from unilocular adipocytes. [Au: ok?]**
- Sbarbati, A., Morroni, M., Zancanaro, C. & Cinti, S. Rat interscapular brown adipose tissue at different ages: a morphometric study. *Int. J. Obes.* **15**, 581–587 (1991).
- Cinti, S. *et al.* Immunohistochemical localization of leptin and uncoupling protein in white and brown adipose tissue. *Endocrinology* **138**, 797–804 (1997).
- Himms-Hagen, J. Defective brown adipose tissue thermogenesis in obese mice. *Int. J. Obes.* **9** (Suppl. 2), 17–24 (1985).
- Young, P., Arch, J. R. & Ashwell, M. Brown adipose tissue in the parametrical fat pad of the mouse. *FEBS Lett.* **167**, 10–14 (1984).
- Cousin, B. *et al.* Occurrence of brown adipocytes in rat white adipose tissue: molecular and morphological characterization. *J. Cell Sci.* **103**, 931–942 (1992).
- Cao, L. *et al.* White to brown fat phenotypic switch induced by genetic and environmental activation of a hypothalamic–adipocyte axis. *Cell Metab.* **14**, 324–338 (2011).
- Bostrom, P. *et al.* A PGC1- α -dependent myokine that drives brown-fat-like development of white fat and thermogenesis. *Nature* **481**, 463–468 (2012).
- De Matteis, R. *et al.* Exercise as a new physiological stimulus for brown adipose tissue activity. *Nutr. Metab. Cardiovasc. Dis.* **23**, 582–590 (2013).
- Petrovic, N. *et al.* Chronic peroxisome proliferator-activated receptor γ (PPAR γ) activation of epididymally derived white adipocyte cultures reveals a population of thermogenically competent, UCP1-containing adipocytes molecularly distinct from classic brown adipocytes. *J. Biol. Chem.* **285**, 7153–7164 (2009).
- Schulz, T. J. *et al.* Brown-fat paucity due to impaired BMP signalling induces compensatory browning of white fat. *Nature* **495**, 379–383 (2013).
- Schulz, T. J. *et al.* Identification of inducible brown adipocyte progenitors residing in skeletal muscle and white fat. *Proc. Natl Acad. Sci. USA* **108**, 143–148 (2011).
- Harms, M. & Seale, P. Brown and beige fat: development, function and therapeutic potential. *Nat. Med.* **19**, 1252–1263 (2013).
- Wu, J., Cohen, P. & Spiegelman, B. M. Adaptive thermogenesis in adipocytes: is beige the new brown? *Genes Dev.* **27**, 234–250 (2013).
- Seale, P. *et al.* PRDM16 controls a brown fat/skeletal muscle switch. *Nature* **454**, 961–967 (2008). **Using a fate-mapping approach, the authors identify the transcriptional regulator PRDM16 as a switch that specifies the classic brown phenotype fate of developing adipocytes.**
- Long, J. Z. *et al.* A smooth muscle-like origin for beige adipocytes. *Cell Metab.* **19**, 810–820 (2014).
- Wu, J. *et al.* Beige adipocytes are a distinct type of thermogenic fat cell in mouse and human. *Cell* **150**, 366–376 (2012). **This study investigates the phenotype of isolated white, brown and beige/brite adipocytes in mice and uses gene expression analyses to compare adipocytes in the mouse with human supraclavicular BAT. [Au: OK?]**
- Kazak, L. *et al.* A creatine-driven substrate cycle enhances energy expenditure and thermogenesis in beige fat. *Cell* **163**, 643–655 (2015).
- Fisher, F. M. *et al.* FGF21 regulates PGC-1 α and browning of white adipose tissues in adaptive thermogenesis. *Genes Dev.* **26**, 271–281 (2012).
- Qian, S. W. *et al.* BMP4-mediated brown fat-like changes in white adipose tissue alter glucose and energy homeostasis. *Proc. Natl Acad. Sci. USA* **110**, E798–E807 (2013).
- Sharp, L. Z. *et al.* Human BAT possesses molecular signatures that resemble beige/brite cells. *PLoS ONE* **7**, e49452 (2012).
- Shinoda, K. *et al.* Genetic and functional characterization of clonally derived adult human brown adipocytes. *Nat. Med.* **21**, 389–394 (2015).
- Sacks, H. S. *et al.* Adult epicardial fat exhibits beige features. *J. Clin. Endocrinol. Metab.* **98**, E1448–E1455 (2013).
- Timmons, J. A. *et al.* Myogenic gene expression signature establishes that brown and white adipocytes originate from distinct cell lineages. *Proc. Natl Acad. Sci. USA* **110**, 143–148 (2011).

- Sci. USA* **104**, 4401–4406 (2007).
53. Sanchez-Gurmaches, J. *et al.* PTEN loss in the Myf5 lineage redistributes body fat and reveals subsets of white adipocytes that arise from Myf5 precursors. *Cell Metab.* **16**, 348–362 (2012).
This paper identifies subsets of white adipocytes originating from both MYF5-positive and MYF5-negative precursors in response to β_3 -adrenoceptor stimulation, thus suggesting that beige/brite adipocytes may have multiple origins.
54. Rosenwald, M. & Wolfrum, C. The origin and definition of brite versus white and classical brown adipocytes. *Adipocyte* **3**, 4–9 (2014).
55. Seale, P. *et al.* Prdm16 determines the thermogenic program of subcutaneous white adipose tissue in mice. *J. Clin. Invest.* **121**, 96–105 (2011).
56. Murano, I. *et al.* Morphology of ferret subcutaneous adipose tissue after 6-month daily supplementation with oral β -carotene. *Biochim. Biophys. Acta* **1740**, 305–312 (2005).
57. Himmis-Hagen, J. *et al.* Multilocular fat cells in WAT of CL-316243-treated rats derive directly from white adipocytes. *Am. J. Physiol. Cell Physiol.* **279**, C670–C681 (2000).
58. Granneman, J. G., Li, P., Zhu, Z. & Lu, Y. Metabolic and cellular plasticity in white adipose tissue I: effects of β_3 -adrenergic receptor activation. *Am. J. Physiol. Endocrinol. Metab.* **289**, E608–E616 (2005).
59. Walden, T. B., Hansen, I. R., Timmons, J. A., Cannon, B. & Nedergaard, J. Recruited versus nonrecruited molecular signatures of brown, “brite”, and white adipose tissues. *Am. J. Physiol. Endocrinol. Metab.* **302**, E19–E31 (2012).
60. Wang, Q. A., Tao, C., Gupta, R. K. & Scherer, P. E. Tracking adipogenesis during white adipose tissue development, expansion and regeneration. *Nat. Med.* **19**, 1338–1344 (2013).
61. Lee, Y. H., Mottillo, E. P. & Granneman, J. G. Adipose tissue plasticity from WAT to BAT and in between. *Biochim. Biophys. Acta* **1842**, 358–369 (2014).
62. Tateishi, K., Okada, Y., Kallin, E. M. & Zhang, Y. Role of Jhdm2a in regulating metabolic gene expression and obesity resistance. *Nature* **458**, 757–761 (2009).
63. Mikkelsen, T. S. *et al.* Comparative epigenomic analysis of murine and human adipogenesis. *Cell* **143**, 156–169 (2010).
64. Iyama, K., Ohzono, K. & Usuku, G. Electron microscopic studies on the genesis of white adipocytes: differentiation of immature pericytes into adipocytes in transplanted preadipose tissue. *Virchows Archiv. B Cell Pathol. Incl. Mol. Pathol.* **31**, 143–155 (1979).
65. Cinti, S., Cigolini, M., Bosello, O. & Bjorntorp, P. A morphological study of the adipocyte precursor. *J. Submicroscop. Cytol.* **16**, 243–251 (1984).
66. Tang, W. *et al.* White fat progenitor cells reside in the adipose vasculature. *Science* **322**, 583–586 (2008).
67. Gupta, R. K. *et al.* Zfp423 expression identifies committed preadipocytes and localizes to adipose endothelial and perivascular cells. *Cell Metab.* **15**, 230–239 (2012).
68. Tran, K. V. *et al.* The vascular endothelium of the adipose tissue gives rise to both white and brown fat cells. *Cell Metab.* **15**, 222–229 (2012).
69. McDonald, M. E. *et al.* Myocardin-related transcription factor a regulates conversion of progenitors to beige adipocytes. *Cell* **160**, 105–188 (2015).
70. Chau, Y. Y. *et al.* Visceral and subcutaneous fat have different origins and evidence supports a mesothelial source. *Nat. Cell Biol.* **16**, 367–375 (2014).
71. Billon, N. *et al.* The generation of adipocytes by the neural crest. *Development* **134**, 2283–2292 (2007).
72. Crossno, J. T. *et al.* Rosiglitazone promotes development of a novel adipocyte population from bone marrow-derived circulating progenitor cells. *J. Clin. Invest.* **116**, 3220–3228 (2006).
73. Tomiyama, K. *et al.* Characterization of transplanted green fluorescent protein⁺ bone marrow cells into adipose tissue. *Stem Cells* **26**, 330–338 (2008).
74. Ryden, M. *et al.* Transplanted bone marrow-derived cells contribute to human adipogenesis. *Cell Metab.* **22**, 408–417 (2015).
75. Berry, R. & Rodeheffer, M. S. Characterization of the adipocyte cellular lineage *in vivo*. *Nat. Cell Biol.* **15**, 302–308 (2013).
76. Rodeheffer, M. S., Birsoy, K. & Friedman, J. M. Identification of white adipocyte progenitor cells *in vivo*. *Cell* **135**, 240–249 (2008).
77. Morrison, S. J. & Spradling, A. C. Stem cells and niches: mechanisms that promote stem cell maintenance throughout life. *Cell* **132**, 598–611 (2008).
78. Blanpain, C. & Simons, B. D. Unravelling stem cell dynamics by lineage tracing. *Nat. Rev. Mol. Cell Biol.* **14**, 489–502 (2013).
79. Vague, J. The degree of masculine differentiation of obesities: a factor determining predisposition to diabetes, atherosclerosis, gout, and uric calculous disease. *Am. J. Clin. Nutr.* **4**, 20–34 (1956).
80. Kissebah, A. H. *et al.* Relation of body fat distribution to metabolic complications of obesity. *J. Clin. Endocrinol. Metab.* **54**, 254–260 (1982).
81. Krotkiewski, M., Bjorntorp, P., Sjostrom, L. & Smith, U. Impact of obesity on metabolism in men and women. Importance of regional adipose tissue distribution. *J. Clin. Invest.* **72**, 1150–1162 (1983).
82. Lapidus, L. *et al.* Distribution of adipose tissue and risk of cardiovascular disease and death: a 12 year follow up of participants in the population study of women in Gothenburg, Sweden. *BMJ* **289**, 1257–1261 (1984).
83. Ohlson, L. O. *et al.* The influence of body fat distribution on the incidence of diabetes mellitus. 13.5 years of follow-up of the participants in the study of men born in 1913. *Diabetes* **34**, 1055–1058 (1985).
84. Peiris, A. N. *et al.* Relationship of anthropometric measurements of body fat distribution to metabolic profile in premenopausal women. *Acta Med. Scand. Suppl.* **723**, 179–188 (1988).
85. Fujioka, S. *et al.* Treatment of visceral fat obesity. *Int. J. Obes.* **15** (Suppl. 2), 59–65 (1991).
86. Despres, J. P. & Lemieux, I. Abdominal obesity and metabolic syndrome. *Nature* **444**, 881–887 (2006).
87. Mittelman, S. D., Van Citters, G. W., Kirkman, E. L. & Bergman, R. N. Extreme insulin resistance of the central adipose depot *in vivo*. *Diabetes* **51**, 755–761 (2002).
88. Mauriege, P. *et al.* Regional variation in adipose tissue metabolism of severely obese premenopausal women. *J. Lipid Res.* **36**, 672–684 (1995).
89. White, U. A. & Tchoukalova, Y. D. Sex dimorphism and depot differences in adipose tissue function. *Biochim. Biophys. Acta* **1842**, 377–392 (2014).
90. Smith, U. Abdominal obesity: a marker of ectopic fat accumulation. *J. Clin. Invest.* **125**, 1790–1792 (2015).
91. Cote, M. *et al.* Adiponectinemia in visceral obesity: impact on glucose tolerance and plasma lipoprotein and lipid levels in men. *J. Clin. Endocrinol. Metab.* **90**, 1434–1439 (2005).
92. Cinti, S. *et al.* Adipocyte death defines macrophage localization and function in adipose tissue of obese mice and humans. *J. Lipid Res.* **46**, 2347–2355 (2005).
93. Giordano, A. *et al.* Obese adipocytes show ultrastructural features of stressed cells and die of pyroptosis. *J. Lipid Res.* **54**, 2423–2436 (2013).
94. Cotillard, A. *et al.* Adipocyte size threshold matters: link with risk of type 2 diabetes and improved insulin resistance after gastric bypass. *J. Clin. Endocrinol. Metab.* **99**, E1466–E1470 (2014).
95. Trayhurn, P. Hypoxia and adipose tissue function and dysfunction in obesity. *Physiol. Rev.* **93**, 1–21 (2013).
96. Guilherme, A., Virbasius, J. V., Puri, V. & Czech, M. P. Adipocyte dysfunctions linking obesity to insulin resistance and type 2 diabetes. *Nat. Rev. Mol. Cell Biol.* **9**, 367–377 (2008).
97. Murano, I. *et al.* Time course of histomorphological changes in adipose tissue upon acute lipotrophy. *Nutr. Metab. Cardiovasc. Dis.* **23**, 723–731 (2013).
98. Harman-Boehm, I. *et al.* Macrophage infiltration into omental versus subcutaneous fat across different populations: effect of regional adiposity and the comorbidities of obesity. *J. Clin. Endocrinol. Metab.* **92**, 2240–2247 (2007).
99. Bjorntorp, P. Metabolic abnormalities in visceral obesity. *Ann. Med.* **24**, 3–5 (1992).
100. Fernandez-Alfonso, M. S. *et al.* Mechanisms of perivascular adipose tissue dysfunction in obesity. *Int. J. Endocrinol.* **2013**, 402053 (2013).
101. Lastra, G. & Manrique, C. Perivascular adipose tissue, inflammation and insulin resistance: link to vascular dysfunction and cardiovascular disease. *Horm. Mol. Biol. Clin. Investig.* **22**, 19–26 (2015).
102. Villacorta, L. & Chang, L. The role of perivascular adipose tissue in vasoconstriction, arterial stiffness, and aneurysm. *Horm. Mol. Biol. Clin. Investig.* **21**, 137–147 (2015).
103. Miao, C. Y. & Li, Z. Y. The role of perivascular adipose tissue in vascular smooth muscle cell growth. *Br. J. Pharmacol.* **165**, 643–658 (2012).
104. Iantorno, M. *et al.* Obesity, inflammation and endothelial dysfunction. *J. Biol. Regul. Homeost. Agents* **28**, 169–176 (2014).
105. Cypess, A. M. *et al.* Activation of human brown adipose tissue by a β_3 -adrenergic receptor agonist. *Cell Metab.* **21**, 33–38 (2015).
106. Giordano, A., Frontini, A., Castellucci, M. & Cinti, S. Presence and distribution of cholinergic nerves in rat mediastinal brown adipose tissue. *J. Histochem. Cytochem.* **52**, 923–930 (2004).
107. Wei, H. *et al.* A clinical approach to brown adipose tissue in the para-aortic area of the human thorax. *PLoS ONE* **10**, e0122594 (2015).
108. Smith, R. E. & Roberts, J. C. Thermogenesis of brown adipose tissue in cold-acclimated rats. *Am. J. Physiol.* **206**, 143–148 (1964).
109. Kuji, I., Imabayashi, E., Minagawa, A., Matsuda, H. & Miyauchi, T. Brown adipose tissue demonstrating intense FDG uptake in a patient with mediastinal pheochromocytoma. *Ann. Nucl. Med.* **22**, 231–235 (2008).
110. Cypess, A. M. *et al.* Anatomical localization, gene expression profiling and functional characterization of adult human neck brown fat. *Nat. Med.* **19**, 635–639 (2013).
This clinical study shows that mirabegron activates BAT and increases energy expenditure in healthy young volunteers; the level of BAT activation was similar to that found in the same subjects exposed to cold. [Au: ok? Edits made to shorten blurb]
111. van der Lans, A. A. *et al.* Cold acclimation recruits human brown fat and increases nonshivering thermogenesis. *J. Clin. Invest.* **123**, 3395–3403 (2013).
112. Smith, J. D. *et al.* Visceral adipose tissue indicates the severity of cardiometabolic risk in patients with and without type 2 diabetes: results from the INSPIRE ME IAA study. *J. Clin. Endocrinol. Metab.* **97**, 1517–1525 (2012).
113. Frontini, A. *et al.* White-to-brown transdifferentiation of omental adipocytes in patients affected by pheochromocytoma. *Biochim. Biophys. Acta* **1831**, 950–959 (2013).
This paper shows white-to-brown adipocyte trans-differentiation in human omental fat from subjects with adrenergic hyperstimulation due to pheochromocytoma. [Au: ok? Edits made to shorten blurb]
114. Cheng, W. *et al.* Intense FDG activity in the brown adipose tissue in omental and mesenteric regions in a patient with malignant pheochromocytoma. *Clin. Nucl. Med.* **37**, 514–515 (2012).
115. Dong, A., Wang, Y., Lu, J. & Zuo, C. Hypermetabolic mesenteric brown adipose tissue on dual-time point FDG PET/CT in a patient with benign retroperitoneal pheochromocytoma. *Clin. Nucl. Med.* **39**, e229–e232 (2014).
References 114–115 report that metabolically active BAT is detected by PET in the omental and mesenteric fat of patients with pheochromocytoma.
116. Connolly, H. M. *et al.* Valvular heart disease associated with fenfluramine-phentermine. *N. Engl. J. Med.* **337**, 581–588 (1997).
117. James, W. P. *et al.* Effect of sibutramine on cardiovascular outcomes in overweight and obese subjects. *N. Engl. J. Med.* **363**, 905–917 (2010).
118. Despres, J. P., Golay, A. & Sjostrom, L. Effects of rimonabant on metabolic risk factors in overweight patients with dyslipidemia. *N. Engl. J. Med.* **353**, 2121–2134 (2005).
119. Van Gaal, L. F. *et al.* Effects of the cannabinoid-1 receptor blocker rimonabant on weight reduction and cardiovascular risk factors in overweight patients: 1-year experience from the RIO-Europe study. *Lancet* **365**, 1389–1397 (2005).
120. Pi-Sunyer, F. X. *et al.* Effect of rimonabant, a cannabinoid-1 receptor blocker, on weight and cardiometabolic risk factors in overweight or obese patients: RIO-North America: a randomized controlled trial. *JAMA* **295**, 761–775 (2006).
121. Scheen, A. J. *et al.* Efficacy and tolerability of rimonabant in overweight or obese patients with type 2 diabetes: a randomised controlled study. *Lancet* **368**, 1660–1672 (2006).
122. Ezcurra, M., Reimann, F., Gribble, F. M. & Emery, E. Molecular mechanisms of incretin hormone secretion. *Curr. Opin. Pharmacol.* **13**, 922–927 (2013).
123. Astrup, A. *et al.* Effects of liraglutide in the treatment of obesity: a randomised, double-blind, placebo-controlled study. *Lancet* **374**, 1606–1616 (2009).

124. Beiroa, D. *et al.* GLP-1 agonism stimulates brown adipose tissue thermogenesis and browning through hypothalamic AMPK. *Diabetes* **63**, 3346–3358 (2014).
125. Finan, B. *et al.* A rationally designed monomeric peptide triagonist corrects obesity and diabetes in rodents. *Nat. Med.* **21**, 27–36 (2015).
126. Ruppnick, M. A. *et al.* Adipose tissue mass can be regulated through the vasculature. *Proc. Natl Acad. Sci. USA* **99**, 10730–10735 (2002).
127. Kim, Y. M. *et al.* Assessment of the anti-obesity effects of the TNP-470 analog, CKD-732. *J. Mol. Endocrinol.* **38**, 455–465 (2007).
128. Brakenhielm, E. *et al.* Angiogenesis inhibitor, TNP-470, prevents diet-induced and genetic obesity in mice. *Circul. Res.* **94**, 1579–1588 (2004).
129. Joharapurkar, A. A., Dhanesha, N. A. & Jain, M. R. Inhibition of the methionine aminopeptidase 2 enzyme for the treatment of obesity. *Diabetes Metab. Syndr. Obes.* **7**, 73–84 (2014).
130. Kim, D. D. *et al.* Efficacy and safety of beloranib for weight loss in obese adults: a randomized controlled trial. *Diabetes Obes. Metab.* **17**, 566–572 (2015).
131. Ruderman, N. B. *et al.* AMPK and SIRT1: a long-standing partnership? *Am. J. Physiol. Endocrinol. Metab.* **298**, E751–E760 (2010).
132. Picard, F. *et al.* Sirt1 promotes fat mobilization in white adipocytes by repressing PPAR- γ . *Nature* **429**, 771–776 (2004).
133. Qiang, L. *et al.* Brown remodeling of white adipose tissue by SIRT1-dependent deacetylation of Ppar γ . *Cell* **150**, 620–632 (2012).
134. de Ligt, M., Timmers, S. & Schrauwen, P. Resveratrol and obesity: can resveratrol relieve metabolic disturbances? *Biochim. Biophys. Acta* **1852**, 1137–1144 (2015).
135. Collins, S., Daniel, K. W., Petro, A. E. & Surwit, R. S. Strain-specific response to β -adrenergic receptor agonist treatment of diet-induced obesity in mice. *Endocrinology* **138**, 405–413 (1997).
136. Ghorbani, M., Claus, T. H. & Himms-Hagen, J. Hypertrophy of brown adipocytes in brown and white adipose tissues and reversal of diet-induced obesity in rats treated with a β -adrenoceptor agonist. *Biochem. Pharmacol.* **54**, 121–131 (1997).
137. Ghorbani, M. & Himms-Hagen, J. Appearance of brown adipocytes in white adipose tissue during CL 316,243-induced reversal of obesity and diabetes in Zucker fa/fa rats. *Int. J. Obes. Relat. Metab. Disord.* **21**, 465–475 (1997).
138. Berkowitz, D. E. *et al.* Distribution of β 3-adrenoceptor mRNA in human tissues. *Eur. J. Pharmacol.* **289**, 223–228 (1995).
139. van Baak, M. A. *et al.* Acute effect of L-796568, a novel β 3-adrenergic receptor agonist, on energy expenditure in obese men. *Clin. Pharmacol. Ther.* **71**, 272–279 (2002).
140. Larsen, T. M. *et al.* Effect of a 28-d treatment with L-796568, a novel β (3)-adrenergic receptor agonist, on energy expenditure and body composition in obese men. *Am. J. Clin. Nutr.* **76**, 780–788 (2002).
141. Takasu, T. *et al.* Effect of (R)-2-[2-aminothiazol-4-yl]-4'-[2-[[2-hydroxy-2-phenylethyl]amino]ethyl] acetamide (YM178), a novel selective β 3-adrenoceptor agonist, on bladder function. *J. Pharmacol. Exp. Ther.* **321**, 642–647 (2007).
142. Arch, J. R. Challenges in β (3)-adrenoceptor agonist drug development. *Ther. Adv. Endocrinol. Metab.* **2**, 59–64 (2011).
143. Karter, Y. Nebivolol: more than a highly selective Beta blocker. *Recent Pat. Cardiovasc. Drug Discov.* **2**, 152–155 (2007).
144. Bordicchia, M. *et al.* Nebivolol induces, via β 3 adrenergic receptor, lipolysis, uncoupling protein 1, and reduction of lipid droplet size in human adipocytes. *J. Hypertension* **32**, 389–396 (2014).
145. Elbers, J. M., Asscheman, H., Seidell, J. C., Megens, J. A. & Gooren, L. J. Long-term testosterone administration increases visceral fat in female to male transsexuals. *J. Clin. Endocrinol. Metab.* **82**, 2044–2047 (1997).
146. Masuzaki, H. *et al.* A transgenic model of visceral obesity and the metabolic syndrome. *Science* **294**, 2166–2170 (2001).
147. Viengchareun, S., Penforis, P., Zennaro, M. C. & Lombes, M. Mineralocorticoid and glucocorticoid receptors inhibit UCP expression and function in brown adipocytes. *Am. J. Physiol. Endocrinol. Metab.* **280**, E640–E649 (2001).
148. Zennaro, M. C. *et al.* Hibernoma development in transgenic mice identifies brown adipose tissue as a novel target of aldosterone action. *J. Clin. Invest.* **101**, 1254–1260 (1998).
149. Caprio, M. *et al.* Pivotal role of the mineralocorticoid receptor in corticosteroid-induced adipogenesis. *FASEB J.* **21**, 2185–2194 (2007). **[Au/Ed: title and journal correct?]**
150. Ehrhart-Bornstein, M. *et al.* Human adipocytes secrete mineralocorticoid-releasing factors. *Proc. Natl Acad. Sci. USA* **100**, 14211–14216 (2003).
151. Fallo, F. *et al.* Prevalence and characteristics of the metabolic syndrome in primary aldosteronism. *J. Clin. Endocrinol. Metab.* **91**, 454–459 (2006).
152. Esteves, C. L. *et al.* Proinflammatory cytokine induction of 11 β -hydroxysteroid dehydrogenase type 1 (11 β -HSD1) in human adipocytes is mediated by MEK, C/EBP β , and NF- κ B/RelA. *J. Clin. Endocrinol. Metab.* **99**, E160–E168 (2014).
153. Armani, A. *et al.* Mineralocorticoid receptor antagonism induces browning of white adipose tissue through impairment of autophagy and prevents adipocyte dysfunction in high-fat-diet-fed mice. *FASEB J.* **28**, 3745–3757 (2014).
154. Parviz, Y. *et al.* Emerging cardiovascular indications of mineralocorticoid receptor antagonists. *Trends Endocrinol. Metab.* **26**, 201–211 (2015).
155. Fukui, Y., Masui, S., Osada, S., Umesono, K. & Motojima, K. A new thiazolidinedione, NC-2100, which is a weak PPAR- γ activator, exhibits potent antidiabetic effects and induces uncoupling protein 1 in white adipose tissue of KKAY obese mice. *Diabetes* **49**, 759–767 (2000).
156. Loft, A. *et al.* Browning of human adipocytes requires KLF11 and reprogramming of PPAR γ superenhancers. *Genes Dev.* **29**, 7–22 (2015).
157. Wunderlich, F. T. *et al.* Hepatic NF- κ B essential modulator deficiency prevents obesity-induced insulin resistance but synergizes with high-fat feeding in tumorigenesis. *Proc. Natl Acad. Sci. USA* **105**, 1297–1302 (2008).
158. Chiang, S. H. *et al.* The protein kinase IKK ϵ regulates energy balance in obese mice. *Cell* **138**, 961–975 (2009).
159. Reilly, S. M. *et al.* A subcutaneous adipose tissue-liver signalling axis controls hepatic gluconeogenesis. *Nat. Commun.* **6**, 6047 (2015).
160. Reilly, S. M. *et al.* An inhibitor of the protein kinases TBK1 and IKK ϵ improves obesity-related metabolic dysfunctions in mice. *Nat. Med.* **19**, 313–321 (2013).
161. Brestoff, J. R. & Artis, D. Immune regulation of metabolic homeostasis in health and disease. *Cell* **161**, 146–160 (2015).
162. De Matteis, R. *et al.* Immunohistochemical identification of the β (3)-adrenoceptor in intact human adipocytes and ventricular myocardium: effect of obesity and treatment with ephedrine and caffeine. *Int. J. Obes. Relat. Metab. Disord.* **26**, 1442–1450 (2002).
163. Seale, P., Kajimura, S. & Spiegelman, B. M. Transcriptional control of brown adipocyte development and physiological function — of mice and men. *Genes Dev.* **23**, 788–797 (2009).
164. Mori, M., Nakagami, H., Rodriguez-Araujo, G., Nimura, K. & Kaneda, Y. Essential role for miR-196a in brown adipogenesis of white fat progenitor cells. *PLoS Biol.* **10**, e1001314 (2012).
165. Smink, J. J. & Leutz, A. Instruction of mesenchymal cell fate by the transcription factor C/EBP β . *Gene* **497**, 10–17 (2012).
166. Kajimura, S. *et al.* Initiation of myoblast to brown fat switch by a PRDM16-C/EBP- β transcriptional complex. *Nature* **460**, 1154–1158 (2009). **This study identifies the PRDM16–C/EBP β complex as a potent initiator of brown fat formation.**
167. Cohen, P. A. Ablation of PRDM16 and beige adipose causes metabolic dysfunction and a subcutaneous to visceral fat switch. *Cell* **156**, 304–316 (2014).
168. Harms, M. J. *et al.* Prdm16 is required for the maintenance of brown adipocyte identity and function in adult mice. *Cell Metab.* **19**, 593–604 (2014).
169. Rajakumari, S. *et al.* EBF2 determines and maintains brown adipocyte identity. *Cell Metab.* **17**, 562–574 (2013).
170. Trajkovski, M., Ahmed, K., Esau, C. C. & Stoffel, M. Myomir-133 regulates brown fat differentiation through Prdm16. *Nat. Cell Biol.* **14**, 1330–1335 (2012).
171. Ohno, H., Shinoda, K., Spiegelman, B. M. & Kajimura, S. PPAR γ agonists induce a white-to-brown fat conversion through stabilization of PRDM16 protein. *Cell Metab.* **15**, 395–404 (2012).
172. Christian, M. *et al.* RIP140-targeted repression of gene expression in adipocytes. *Mol. Cell Biol.* **25**, 9383–9391 (2005).
173. Tsukiyama-Kohara, K. *et al.* Adipose tissue reduction in mice lacking the translational inhibitor 4E-BP1. *Nat. Med.* **7**, 1128–1132 (2001).
174. Shinoda, K. *et al.* Phosphoproteomics identifies CK2 as a negative regulator of beige adipocyte thermogenesis and energy expenditure. *Cell Metab.* **22**, 997–1008 (2015).
175. Cantile, M., Prociño, A., D'Armiendo, M., Cindolo, L. & Cillo, C. HOX gene network is involved in the transcriptional regulation of *in vivo* human adipogenesis. *J. Cell Physiol.* **194**, 225–236 (2003).
176. Trajkovski, M. & Lodish, H. MicroRNA networks regulate development of brown adipocytes. *Trends Endocrinol. Metab.* **24**, 442–450 (2013).
177. Swinney, D. C. & Anthony, J. How were new medicines discovered? *Nat. Rev. Drug Discov.* **10**, 507–519 (2011).
178. Rix, U. & Superti-Furga, G. Target profiling of small molecules by chemical proteomics. *Nat. Chem. Biol.* **5**, 616–624 (2009).
179. Moellering, R. E. & Cravatt, B. F. How chemoproteomics can enable drug discovery and development. *Chem. Biol.* **19**, 11–22 (2012).
180. Moisan, A. *et al.* White-to-brown metabolic conversion of human adipocytes by JAK inhibition. *Nat. Cell Biol.* **17**, 57–67 (2015).
181. Derecka, M. *et al.* Tyk2 and Stat3 regulate brown adipose tissue differentiation and obesity. *Cell Metab.* **16**, 814–824 (2012).
182. Dempersmier, J. *et al.* Cold-inducible Zfp516 activates UCP1 transcription to promote browning of white fat and development of brown fat. *Mol. Cell.* **57**, 235–246 (2015).
183. Wang, T. J. *et al.* Metabolite profiles and the risk of developing diabetes. *Nat. Med.* **17**, 448–453 (2011). **Results obtained in human trials underscore the potential key role of amino acid metabolism in the early pathogenesis of diabetes, and suggest that amino acid profile could aid in diabetes risk assessment. [Au: is this blurb relevant to this article? Also, this blurb is extremely similar to the final sentence of the abstract of ref. 183: "These findings underscore the potential key role of amino acid metabolism early in the pathogenesis of diabetes and suggest that amino acid profiles could aid in diabetes risk assessment". Could you please re-word?]**
184. Roberts, L. D. *et al.* β -aminoisobutyric acid induces browning of white fat and hepatic β -oxidation and is inversely correlated with cardiometabolic risk factors. *Cell Metab.* **19**, 96–108 (2014).
185. Morroni, M. *et al.* Reversible transdifferentiation of secretory epithelial cells into adipocytes in the mammary gland. *Proc. Natl Acad. Sci. USA* **101**, 16801–16806 (2004).
186. Prokesch, A. *et al.* Molecular aspects of adipoeithelial transdifferentiation in mouse mammary gland. *Stem Cells* **32**, 2756–2766 (2014).
187. De Matteis, R. *et al.* *In vivo* physiological transdifferentiation of adult adipose cells. *Stem Cells* **27**, 2761–2768 (2009).
188. Giordano, A., Smorlesi, A., Frontini, A., Barbatelli, G. & Cinti, S. White, brown and pink adipocytes: the extraordinary plasticity of the adipose organ. *Eur. J. Endocrinol.* **170**, R159–R171 (2014).
189. Lumeng, C. N., Bodzin, J. L. & Saltiel, A. R. Obesity induces a phenotypic switch in adipose tissue macrophage polarization. *J. Clin. Invest.* **117**, 175–184 (2007).
190. Orr, J. S. *et al.* Obesity alters adipose tissue macrophage iron content and tissue iron distribution. *Diabetes* **63**, 421–432 (2014).
191. Sun, K., Tordjman, J., Clement, K. & Scherer, P. E. Fibrosis and adipose tissue dysfunction. *Cell Metab.* **18**, 470–477 (2013).
192. Nguyen, K. D. *et al.* Alternatively activated macrophages produce catecholamines to sustain adaptive thermogenesis. *Nature* **480**, 104–108 (2011).
193. Qiu, Y. *et al.* Eosinophils and type 2 cytokine signaling in macrophages orchestrate development of functional beige fat. *Cell* **157**, 1292–1308 (2014).
194. Lee, M. W. *et al.* Activated type 2 innate lymphoid cells regulate beige fat biogenesis. *Cell* **160**, 74–87 (2015).
195. Brestoff, J. R. *et al.* Group 2 innate lymphoid cells promote beiging of white adipose tissue and limit obesity. *Nature* **519**, 242–246 (2015).

196. Apovian, C. M. *et al.* Pharmacological management of obesity: an endocrine society clinical practice guideline. *J. Clin. Endocrinol. Metab.* **100**, 342–362 (2015).
197. Bray, G. A. Medical treatment of obesity: the past, the present and the future. *Best Pract. Res. Clin. Gastroenterol.* **28**, 665–684 (2014).
198. Kim, G. W., Lin, J. E., Blomain, E. S. & Waldman, S. A. Antiobesity pharmacotherapy: new drugs and emerging targets. *Clin. Pharmacol. Ther.* **95**, 53–66 (2014).
199. Gautron, L., Elmquist, J. K. & Williams, K. W. Neural control of energy balance: translating circuits to therapies. *Cell* **161**, 133–145 (2015).
200. Muller, T. D. *et al.* Restoration of leptin responsiveness in diet-induced obese mice using an optimized leptin analog in combination with exendin-4 or FGF2. *J. Pept. Sci.* **18**, 383–393 (2012).
201. Chen, K. Y. *et al.* RM-493, a melanocortin-4 receptor (MC4R) agonist, increases resting energy expenditure in obese individuals. *J. Clin. Endocrinol. Metab.* **100**, 1639–1645 (2015). **[Au: the original url is broken (leads to an error). Is this an acceptable alternative? If not, please provide an alternative link/reference.]**
202. Krishna, R. *et al.* Potent and selective agonism of the melanocortin receptor 4 with MK-0493 does not induce weight loss in obese human subjects: energy intake predicts lack of weight loss efficacy. *Clin. Pharmacol. Ther.* **86**, 659–666 (2009).
203. Opko Health. OPKO announces presentation of data on long-acting oxyntomodulin at diabetes conference. *Opko* [online], <http://investor.opko.com/releasedetail.cfm?releaseid=841731> (2014).
204. Day, J. W. *et al.* A new glucagon and GLP-1 co-agonist eliminates obesity in rodents. *Nat. Chem. Biol.* **5**, 749–757 (2009).
205. Rajeev, S. P., Cuthbertson, D. J. & Wilding, J. P. Energy balance and metabolic changes with SGLT2 inhibition. *Diabetes Obes. Metab.* **18**, 125–134 (2015).
206. Zafgen. Zafgen announces beloranib program update. *Zafgen* [online], <http://ir.zafgen.com/releasedetail.cfm?ReleaseID=937850> (2015).
207. US National Library of Medicine. Resveratrol and the metabolic syndrome. *ClinicalTrials.gov* [online], <https://clinicaltrials.gov/ct2/show/NCT01714102?term=resveratrol+AND+obesity&rank=7> (2016).
208. Gnad, T. *et al.* Adenosine activates brown adipose tissue and recruits beige adipocytes via A2A receptors. *Nature* **516**, 395–399 (2014).
209. Emorine, L. J. *et al.* Molecular characterization of the human β_3 -adrenergic receptor. *Science* **245**, 1118–1121 (1989).
210. Tseng, Y. H. *et al.* New role of bone morphogenetic protein 7 in brown adipogenesis and energy expenditure. *Nature* **454**, 1000–1004 (2008).
211. Whittle, A. J. *et al.* BMP8B increases brown adipose tissue thermogenesis through both central and peripheral actions. *Cell* **149**, 871–885 (2012).
212. Hondares, E. *et al.* Hepatic FGF21 expression is induced at birth via PPAR α in response to milk intake and contributes to thermogenic activation of neonatal brown fat. *Cell Metab.* **11**, 206–212 (2010).
213. Bordicchia, M. *et al.* Cardiac natriuretic peptides act via p38 MAPK to induce the brown fat thermogenic program in mouse and human adipocytes. *J. Clin. Invest.* **122**, 1022–1036 (2012).
214. Lopez, M. *et al.* Hypothalamic AMPK and fatty acid metabolism mediate thyroid regulation of energy balance. *Nat. Med.* **16**, 1001–1008 (2010).
215. Lehr, L. *et al.* Control of 4E-BP1 expression in mouse brown adipose tissue by the β_3 -adrenoceptor. *FEBS Lett.* **576**, 179–182 (2004).
216. Patwari, P. *et al.* The arrestin domain-containing 3 protein regulates body mass and energy expenditure. *Cell Metab.* **14**, 671–683 (2011).
217. Moreno-Aliaga, M. J. *et al.* Cardiotrophin-1 is a key regulator of glucose and lipid metabolism. *Cell Metab.* **14**, 242–253 (2011).
218. Vegiopoulos, A. *et al.* Cyclooxygenase-2 controls energy homeostasis in mice by *de novo* recruitment of brown adipocytes. *Science* **328**, 1158–1161 (2010).
219. Madsen, L. *et al.* UCP1 induction during recruitment of brown adipocytes in white adipose tissue is dependent on cyclooxygenase activity. *PLoS ONE* **5**, e11391 (2010).
220. Ohno, H., Shinoda, K., Ohyama, K., Sharp, L. Z. & Kajimura, S. EHMT1 controls brown adipose cell fate and thermogenesis through the PRDM16 complex. *Nature* **504**, 163–167 (2013).
221. Cederberg, A. *et al.* FOXC2 is a winged helix gene that counteracts obesity, hypertriglyceridemia, and diet-induced insulin resistance. *Cell* **106**, 563–573 (2001).
222. Kalaany, N. Y. *et al.* LXRs regulate the balance between fat storage and oxidation. *Cell Metab.* **1**, 231–244 (2005).
223. Rao, R. R. *et al.* Meteorin-like is a hormone that regulates immune–adipose interactions to increase beige fat thermogenesis. *Cell* **157**, 1279–1291 (2014).
224. Chen, Y. *et al.* miR-155 regulates differentiation of brown and beige adipocytes via a bistable circuit. *Nat. Commun.* **4**, 1769 (2013).
225. Sun, L. *et al.* Mir193b-365 is essential for brown fat differentiation. *Nat. Cell Biol.* **13**, 958–965 (2011).
226. Nisoli, E. *et al.* Mitochondrial biogenesis in mammals: the role of endogenous nitric oxide. *Science* **299**, 896–899 (2003).
227. Puigserver, P. *et al.* A cold-inducible coactivator of nuclear receptors linked to adaptive thermogenesis. *Cell* **92**, 829–839 (1998).
228. Hondares, E. *et al.* Peroxisome proliferator-activated receptor α (PPAR α) induces PPAR γ coactivator 1 α (PGC-1 α) gene expression and contributes to thermogenic activation of brown fat: involvement of PRDM16. *J. Biol. Chem.* **286**, 43112–43122 (2011).
229. Scime, A. *et al.* Rb and p107 regulate preadipocyte differentiation into white versus brown fat through repression of PGC-1 α . *Cell Metab.* **2**, 283–295 (2005).
230. Seale, P. *et al.* Transcriptional control of brown fat determination by PRDM16. *Cell Metab.* **6**, 38–54 (2007).
231. Ortega-Molina, A. *et al.* PTEN positively regulates brown adipose function, energy expenditure, and longevity. *Cell Metab.* **15**, 382–394 (2012).
232. Leonardsson, G. *et al.* Nuclear receptor corepressor RIP140 regulates fat accumulation. *Proc. Natl Acad. Sci. USA* **101**, 8437–8442 (2004).
233. Wang, L. *et al.* The orphan nuclear receptor SHP regulates PGC-1 α expression and energy production in brown adipocytes. *Cell Metab.* **2**, 227–238 (2005).
234. Yadav, H. *et al.* Protection from obesity and diabetes by blockade of TGF- β /Smad3 signaling. *Cell Metab.* **14**, 67–79 (2011).
235. Villanueva, C. J. *et al.* Adipose subtype-selective recruitment of TLE3 or Prdm16 by PPAR γ specifies lipid storage versus thermogenic gene programs. *Cell Metab.* **17**, 423–435 (2013).
236. Bartolomucci, A. *et al.* TLQP-21, a VGF-derived peptide, increases energy expenditure and prevents the early phase of diet-induced obesity. *Proc. Natl Acad. Sci. USA* **103**, 14584–14589 (2006).
237. Pisani, D. F. *et al.* The K⁺ channel TASK1 modulates β -adrenergic response in brown adipose tissue through the mineralocorticoid receptor pathway. *FASEB J.* <http://dx.doi.org/10.1096/fj.15-277475> (2015).
238. Ma, S. *et al.* Activation of the cold-sensing TRPM8 channel triggers UCP1-dependent thermogenesis and prevents obesity. *J. Mol. Cell Biol.* **4**, 88–96 (2012).
239. Ye, L. *et al.* TRPV4 is a regulator of adipose oxidative metabolism, inflammation, and energy homeostasis. *Cell* **151**, 96–110 (2012).
240. Pan, D., Fujimoto, M., Lopes, A. & Wang, Y. X. Twist-1 is a PPAR δ -inducible, negative-feedback regulator of PGC-1 α in brown fat metabolism. *Cell* **137**, 73–86 (2009).
241. Kiefer, F. W. *et al.* Retinaldehyde dehydrogenase 1 regulates a thermogenic program in white adipose tissue. *Nat. Med.* **18**, 918–925 (2012).

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Competing interests statement

The authors declare no competing interests.

Biographies

Antonio Giordano obtained his medical degree from the University of Ancona, Italy, in 1991, where he subsequently specialized in psychiatry in 1995 and obtained a Ph.D. in neuroscience in 1999. From 1999 to 2006 he was an assistant professor at the School of Medicine, Ancona, Italy. Since 2006 he has been Associate Professor of Human Anatomy at the School of Medicine, Marche Polytechnic University, Ancona, Italy. His research interests span the functional anatomy of the mammalian adipose tissue, its involvement in obesity and the central mechanisms regulating energy balance. He has authored 43 peer-reviewed articles published in international journals.

Andrea Frontini graduated in biological science from the University of Ancona, Italy, in 1998. He obtained an M.Sc. at the University of Windsor, Ontario, Canada, in 2003, in the field of neurobiology. He returned to Italy to join Saverio Cinti's research group and complete his Ph.D. programme in neuroscience (awarded in 2006 [Au: ok?]). After several years as a postdoctoral researcher and as an assistant professor at the Department of Experimental and Clinical Medicine, Marche Polytechnic University, Ancona, Italy, he became Associate Professor of Human Anatomy at the University of Pavia, Italy, in September 2015. He has authored 45 peer-reviewed articles published in international journals.

Saverio Cinti is a medical doctor and a specialist in internal medicine and anatomical pathology. He has been Full Professor of Human Anatomy at the School of Medicine of Marche Polytechnic University, Ancona, Italy, since 1984. He began working in the field of adipose tissue in the laboratory of Per Bjorntorp (Gothenburg University, Sweden) in 1980. In 2006, he was invited to the 134th Nobel Symposium, held in Gothenburg. He received the Blaise Pascal Medal of the European Academy of Science for Biology in 2008, and the Friedrich Wasserman Award of the European Society for the Study of Obesity (a lifetime award for senior researchers) in 2013 [Au: ok?]. He has authored more than 250 peer-reviewed papers.

Key points

- Obesity is a pathological enlargement of the adipose organ; this process involves the whitening and functional impairment of thermogenic brown adipose tissue and the inflammation of hypertrophied adipose depots.
- The occurrence of these two phenomena at visceral fat sites causes the most dangerous outcomes of obesity [Au: specify what these outcomes are?]. Visceral adipocytes are particularly vulnerable to lipid overload, possibly because of their developmental route and the reduced size at which they die, thereby promoting inflammation [Au:OK?].
- Human and rodent visceral adipocytes exhibit remarkable cell plasticity. Indeed, such cells are particularly prone to re-convert into metabolically healthy, energy-dissipating adipocytes.
- Molecular targets and pathways that are involved in white-to-brown visceral adipocyte transdifferentiation are potential novel targets of anti-obesity drugs.
- Even the early steps of white-to-brown adipocyte transdifferentiation, which include adipocyte size reduction and mitochondriogenesis, could promote a healthy adipose phenotype and achieve effective therapeutic outcomes [Au: OK?].

Subject categories

Biological sciences / Physiology / Metabolism / Metabolic diseases / Obesity [URI /631/443/319/1642/393]
 Biological sciences / Drug discovery [URI /631/154]
 Biological sciences / Developmental biology / Differentiation [URI /631/136/142]

Toc blurb

000 Convertible visceral fat as a therapeutic target to curb obesity

Antonio Giordano, Andrea Frontini and Saverio Cinti
 Therapies for obesity are limited, and existing therapies have substantial adverse effects. Cinti and colleagues discuss the conversion of white fat to brown, thermogenic fat as a potential strategy to curb obesity. Adipocyte conversion could be particularly important in the visceral compartment, as fat in this region is associated with morbidity but is also particularly prone to transdifferentiation.