# Targeting adipose tissue in the treatment of obesity-associated diabetes

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Abstract | Adipose tissue regulates numerous physiological processes, and its dysfunction in obese humans is associated with disrupted metabolic homeostasis, insulin resistance and type 2 diabetes mellitus (T2DM). Although several US-approved treatments for obesity and T2DM exist, these are limited by adverse effects and a lack of effective long-term glucose control. In this Review, we provide an overview of the role of adipose tissue in metabolic homeostasis and assess emerging novel therapeutic strategies targeting adipose tissue, including adipokine-based strategies, promotion of white adipose tissue beiging as well as reduction of inflammation and fibrosis.

Lipotoxicity
Impaired cellular functionality (insulin resistance) that is due to exposure to elevated levels of endogenous or exogenous lipids.

Adipokine
An adipocyte-derived secretory product that is either exclusively produced in adipocytes or highly enriched in adipocytes.

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Obesity is a highly complex multifaceted disease and one of the strongest risk factors for the development of type 2 diabetes mellitus (T2DM). Obesity is associated with an increased risk for other metabolic, cardiovascular and chronic inflammatory diseases, such as dyslipidaemia, non-alcoholic fatty liver disease (NAFLD), hypertension, coronary heart disease and stroke[1]. The primary cause of T2DM is obesity-driven insulin resistance in liver, white adipose tissue (WAT) and skeletal muscle, combined with insufficient secretion of insulin by pancreatic β-cells to overcome this resistance[2].

Dietary and lifestyle modifications can be effective for the treatment of obesity and the prevention of diabetes; however, these approaches are difficult to maintain in the long term. Existing pharmacological approaches for treating obesity largely act by suppressing food or caloric intake, whereas current antidiabetic therapies typically enhance insulin sensitivity and/or secretion. Although these agents have proved beneficial, there is still a huge unmet need for effective pharmacological interventions that are capable of more permanently reversing the diabetic phenotype with minimal side effects. An improved understanding of the cellular and molecular mechanisms underlying obesity and inter-organ crosstalk is essential for the development of novel therapeutic interventions.

Although at first glance there seems to be a limited number of pharmacological targets being investigated in diabetes and obesity, a recent report by Global Business Intelligence Research estimated that nearly 250 products are under active development in the context of obesity alone (see Further information), and we can assume a similar level of diversity in the diabetes arena. A key process for new discoveries is abandoning established drug targets, for which further improvements can be only marginal at best, and instead adopting an 'out of the box' approach with respect to new targets and mechanisms in tissues that have not received sufficient attention to date. One such tissue is adipose tissue, which has recently gained substantial interest with regard to its potential to be therapeutically targeted.

WAT is a multifactorial organ, performing intricate metabolic functions under physiological conditions[3,4]. However, during obesity, WAT may become severely dysfunctional and fail to appropriately expand to store the surplus energy. This results in ectopic fat deposition in other tissues that regulate metabolic homeostasis, progressive insulin resistance and an increased risk of T2DM, a phenomenon generally referred to as 'lipotoxicity' (REFS 2,4,5). 'Unhealthy' WAT expansion has been associated with numerous deleterious consequences, including inflammation, fibrosis, hypoxia, dysregulated adipokine secretion and disrupted mitochondrial function[4,6], each of which may present potential novel therapeutic avenues.

This Review highlights some of the most effective current anti-obesity and antidiabetic pharmacological interventions and addresses their key limitations and the current unmet needs in disease management. We focus on emerging therapeutic strategies and associated challenges for the development of new pharmacotherapies that exploit recent discoveries about adipose tissue biology, including adipokine-based therapeutics, promotion of white fat beiging (also known as browning), and modulation of inflammation and fibrosis.

## Whole-body energy homeostasis

Under physiological conditions, the metabolic functions of WAT, the different WAT depots, developmental and adipogenic processes, cellular turnover and adipokine secretory profiles are all tightly regulated. During obesity, key events disrupt WAT architecture and function, and several molecular pathways have now been associated with unhealthy WAT expansion, such as fibrosis, hypoxia and a dysregulated adipokine milieu[4,7]. Below, we discuss the roles of WAT and the consequences of WAT dysfunction at the whole-body level.

***Adipose tissue function, development, turnover and homeostasis.*** Originally, WAT was regarded simply as a sink for excess calories in the fed state and a reservoir from which free fatty acids (FFA) were released during fasting to fuel the energy demands of the organism. Research over the past two decades has revealed WAT to be a remarkably complex endocrine organ that serves as a 'master regulator' of systemic energy homeostasis[3,8] (FIG. 1). In addition to secreting a plethora of adipocyte-derived factors known as adipokines, WAT plays a major part in inflammation, mechanical organ protection and thermoregulation[9]. The physical location of WAT determines its metabolic identity and core functions. In humans, WAT is located in many distinct depots, one of which is the intra-abdominal visceral WAT (vWAT) depot, the dysregulation of which is associated with metabolic disease risk[10]. By contrast, the subcutaneous WAT (sWAT) depot can convey protective effects on energy homeostasis[11] (BOX 1). Each depot has a unique developmental window during which a distinct gene signature, adipokine profile and lipid storage capacity emerges[12]. This is largely due to the different depots displaying tissue-autonomous characteristics, including the presence of different sets of progenitor cell populations[13].

Adipocytes arise from an existing pool of precursor cells that reside along a perivascular niche[14]. A pool of committed pre-adipose fibroblasts is maintained by self-replication[15]. Upon activation of the transcriptional pathways that control adipocyte differentiation[16–18], these committed pre-adipocytes complete the differentiation programme to become mature adipocytes[19]. Adipogenesis is driven by an intrinsic network of transcription factors, with the nuclear hormone receptor peroxisome proliferator-activated receptor-γ (PPARγ) and CCAAT/enhancer binding protein-α (C/EBPα)[17,20] at the epicentre. We recently reported that terminal embryonic adipogenesis is independent of C/EBPα but requires PPARγ[21]. However, C/EBPα is crucial for all adult adipogenic stages, such as WAT regeneration and expansion[21]. The pre-adipocyte-enriched C2H2 zinc-finger protein ZFP423 is one of the newest contributors to the adipogenic transcriptional cascade[22]. Initially identified as a key transcriptional mediator of pre-adipocyte determination, ZFP423 robustly activates PPARγ to drive adipocyte differentiation[22]. Recently, a population of ZFP423-expressing mural cells was identified as a unique subset of physiologically mediated adipose precursors, which are highly committed to the adipose lineage[23]. This study provides genetic evidence for adipose perivascular cells as a developmental origin of the white adipocytes that are formed in obesity[23]. Future studies identifying the key mechanisms by which ZFP423 (as well as other transcription factors) regulates pre-adipocyte competency in an *in vivo* fat-depot-specific manner, in addition to its role in the mature white fat cell during obesity, should prove illuminating.

Adipocyte turnover is sustained throughout life by a delicate balance between adipogenesis and apoptosis, which requires a constant source of adipocyte progenitor cells. Total adipocyte numbers are static during adulthood, even in the context of obesity. The total number of adipocytes is set during childhood and adolescence,

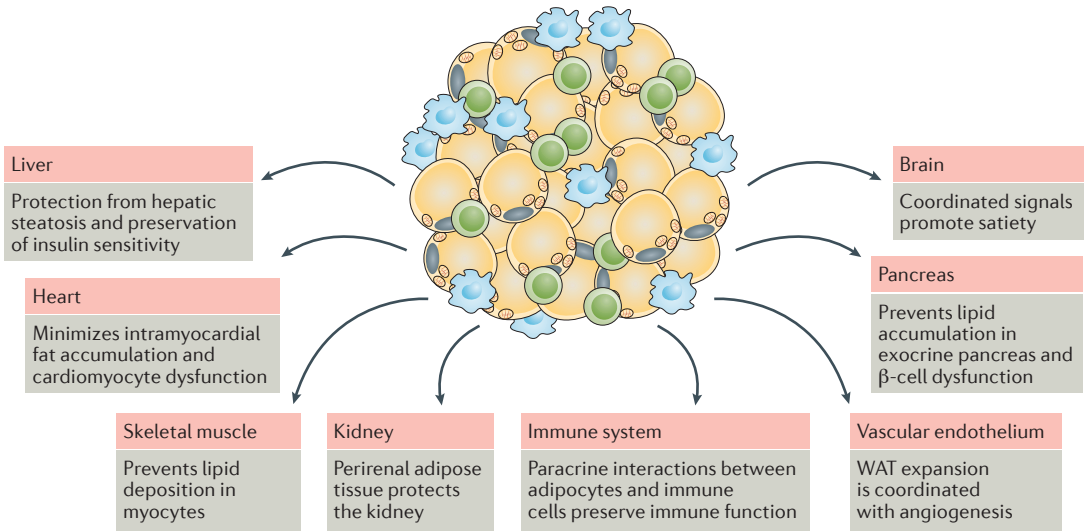


Figure 1 | **Crosstalk between white adipose tissue and other tissues in the body.** Signalling events between white adipose tissue (WAT) and multiple tissues in the body (liver, heart, brain, pancreas, skeletal muscle, kidney and vascular endothelium), as well as the immune system, promote physiological and cellular changes that ultimately confer protection from lipotoxicity and metabolic dysfunction.

Adipogenesis
The process of adipocyte differentiation.

Box 1 | **Healthy versus unhealthy adipose tissue expansion**

The location of white adipose tissue (WAT) expansion during obesity is a key factor determining metabolic outcome. 'Metabolically unhealthy' expansion of the visceral fat depots in obese individuals places them in a high-risk category for the development of insulin resistance, diabetes and cardiovascular-related disorders[305]. Conversely, the propensity to safely accumulate surplus lipids in the subcutaneous depot, typically termed 'metabolically healthy' expansion, can yield enhanced insulin sensitivity and positive metabolic outcomes[306]. Such patients have been termed metabolically healthy obese (MHO) individuals[307]. Two mouse model equivalents of MHO patients — that involve adipocyte-specific transgenic overexpression of adiponectin or the mitochondrial protein mitoNEET (also known as CDGSH iron–sulfur domain 1) — exhibit severe obesity that is due to massive expansion of the subcutaneous fat depots[54,303]. Remarkably, the latter model achieved the highest body weight ever recorded for a mouse[303]. Both models are potently protected from the detrimental effects of a high-fat diet or an *ob/ob* mutation, as seen by vast improvements in glucose and lipid metabolism, protection from hepatic steatosis and negligible signs of WAT fibrosis and inflammation[54,303].

and the mean lifespan of an adipocyte in adult humans is ~10 years, with ~10% of human subcutaneous adipocytes being renewed annually[24]. Given that adipocyte numbers are determined during childhood, this seems to be the only window of opportunity to alter the number of adipocytes available for the system.

Adipocytes communicate with the rest of the body by orchestrating nutritional and hormonal signals, and by secreting an array of factors known as adipokines[25]. Adipocyte-derived lipids, such as ceramides and sphingosines, can also serve as signalling molecules, targeting extra-adipose tissues under various metabolic circumstances[26]. Over the past two decades, the WAT field has seen major developments in adipokine identification and function. Early studies entailed the description of WAT-derived secretory factors, such as adipsin and the cytokine tumour necrosis factor (TNF)[27,28], which provided a direct link between WAT biology and the inflammatory system. However, it was the discoveries of leptin[29] and adiponectin[30] that firmly positioned the adipocyte in the spotlight as a dynamic endocrine cell, capturing the imagination of the obesity field and raising many hopes for drug intervention. Leptin is produced by adipocytes, and circulating leptin levels are directly proportional to body fat mass[31,32]. Through specific receptors in the hypothalamus and the periphery, leptin signals mediate numerous biological processes, such as satiety, food intake, energy expenditure, reproductive function, atherogenesis and blood pressure[33,34]. Leptin binds to the long form of its receptor in several neuronal populations to activate the Janus kinase 2–signal transducer and activator of transcription 3 (JAK2–STAT3) pathway, which in turn increases energy expenditure, promotes satiety and reduces body weight[33]. In rodents, leptin deficiency yields the metabolically unhealthy obese *ob/ob* mouse[29].

The second major milestone in adipokine history was the discovery of adiponectin[30]. Adiponectin exhibits insulin-sensitizing, anti-apoptotic and anti-inflammatory properties[35], and correlations between adiponectin and many human physiological processes and disease states have been reported[35,36]. Almost exclusively secreted from adipocytes, adiponectin circulates as trimers, hexamers and high molecular weight (HMW) structures[35,37]; with the latter representing its most biologically active form[38]. There are two adiponectin receptors: adiponectin receptor 1 (ADIPOR1), which is expressed primarily in skeletal muscle, and ADIPOR2, which signals preferentially in the liver[35,39,40]. T-cadherin, a glycosylphosphatidylinositol-anchored cadherin cell-surface glycoprotein, is an additional non-signalling receptor for adiponectin hexamers and HMW structures[41], and these interactions mediate some of its cardioprotective effects[42]. T-cadherin-deficient mice exhibit elevated plasma levels of HMW adiponectin[43], and adiponectin itself is recruited by T-cadherin to the cell surface of macrophages[44].

Adiponectin harbours an interesting mode of regulation, such that circulating levels are enhanced during metabolically favourable conditions, and are reduced during metabolically adverse conditions[35,45,46]. Adiponectin exerts largely beneficial effects, as mice that constitutively overexpress adiponectin are protected against HFD-induced glucose intolerance and dyslipidaemia[47,48]. Our early studies revealed that administration of adiponectin improves insulin sensitivity and glucose metabolism[35,38,49], a phenotype that has been confirmed in many recent *in vivo* studies[39,50,51]. Conversely, chronic treatment of diabetic muscle insulin-like growth factor 1 receptor (IGF1R)–lysine–arginine (MKR) mice with full-length adiponectin yields no marked improvements in insulin sensitivity[52], indicating a case of 'adiponectin resistance'. Similar phenomena may be observed in the vasculature and in the heart[53]. Nevertheless, WAT-specific induction of adiponectin can effectively rescue the profoundly diabetic phenotype of the leptin-deficient *ob/ob* mouse[54]. We also recently demonstrated that adiponectin improves survival in several mouse models of cell-type-specific apoptosis[40]. Collectively, the diversity of the beneficial effects of adiponectin on energy metabolism that it orchestrates from WAT makes it an excellent central integrator of metabolic signals.

***Obesity-induced adipose tissue dysfunction.*** A unique characteristic of WAT is its extraordinary capacity to change its dimensions in response to nutritional status by remodelling through a series of mechanisms. At the tissue level, WAT alters its size, function, inflammatory state and whole-body distribution. From a cellular perspective, WAT changes its extracellular matrix (ECM) composition, vascularization, adipocyte size and number, oxidative stress levels, adipokine secretory profile and the inflammatory state of infiltrating immune cells[4]. WAT expansion is achieved through enlargement of pre-existing adipocytes by lipid accumulation (hypertrophy) or by increasing adipocyte numbers through recruitment of pre-adipocytes from resident pools of progenitor cells (hyperplasia); the latter process embodies *de novo* adipocyte formation (adipogenesis)[9]. In obesity, WAT may become severely dysfunctional and thereby fail to appropriately expand to store surplus energy. At the whole-body level, this dysfunction results in ectopic fat deposition in other tissues that regulate

Ceramides and sphingosines
Subclasses of lipids consisting of a serine and a fatty acid. They are implicated in cell death, inflammation and insulin resistance (ceramides) or proliferation and survival (sphingosines).

Extracellular matrix
A fibrotic milieu on the outside of cells that makes up an extracellular scaffold. It can be upregulated in pathological states.

metabolic homeostasis (such as the liver, skeletal muscle and the endocrine pancreas). This results in progressive insulin resistance and an increased risk of T2DM.

Although obesity drives the pathological expansion of dysfunctional WAT through adipocyte hypertrophy[4], both hypertrophy and hyperplasia play a part in tissue expansion[55]. Generation of the AdipoChaser mouse[56], in which all mature adipocytes are permanently and uniformly labelled upon administration of doxycycline, revealed that mouse subcutaneous and epididymal depots undergo inherently different rates of adipogenesis during development and expansion[56]. Epididymal WAT develops postnatally and sustains high adipogenic capacity during HFD exposure; by contrast, subcutaneous WAT develops between embryonic days 14–18 and maintains a low rate of adipogenesis[56]. Our findings emphasize the remarkable plasticity of WAT and how different adipose depots display vastly different adipogenic potential. Reflective of our studies, human data show that overfeeding promotes adipocyte hypertrophy in upper subcutaneous depots, but hyperplasia in lower depots[57]. Recently, transplanted human bone marrow-derived cells were reported to serve as reservoirs of adipocyte progenitor cells and to contribute ~10% to adipogenesis, a contribution that was more prominent in an obesity setting[58].

Obesity-induced WAT expansion through adipocyte hypertrophy is accompanied by a shift to an adverse adipokine secretory profile, which typically includes an elevated array of pro-inflammatory factors, such as TNF, interleukin-1β (IL-1β), IL-6, IL-8, leptin, resistin and monocyte chemoattractant protein 1 (MCP1), with a parallel reduction in anti-inflammatory factors, such as IL-10 and adiponectin[4,59]. As obesity progresses, chemotactic signals such as MCP1 recruit circulating monocytes, which infiltrate obese WAT to fuel an inflammatory milieu[60,61]. Resident WAT immune cells and newly recruited monocytes become polarized to pro-inflammatory M1 'classically activated' macrophages, as opposed to anti-inflammatory M2 'alternatively activated' macrophages[62,63]. M1 macrophages dysregulate local adipocyte signalling and impair whole-body insulin sensitivity[4,59], primarily by secreting pro-inflammatory cytokines and by encircling apoptotic fat cells to create 'crown-like' structures[62–64]. However, local pro-inflammatory responses in the adipocyte also appear to have an important beneficial role with respect to adipogenesis and remodelling[65], as is discussed later in this Review.

Dysfunctional WAT harbours a higher degree of fibrosis and hypoxia. The ECM provides a scaffold that is intertwined with adipocytes, and its rigidity, composition and remodelling is a crucial determinant of the expandability of WAT as obesity progresses[7]. Fibrosis is a pathological consequence of ECM dysregulation, characterized by an abundance of ECM proteins, particularly collagens[66]. The higher the flexibility of the ECM, the more room WAT is allowed to freely expand in a healthy stress-free manner. However, if the ECM is inflexible, embedded adipocytes cannot appropriately store surplus lipids, which hinders WAT growth and promotes lipotoxicity[4,7] (FIG. 2). Collagen VI is a highly enriched ECM constituent of WAT[67] that we identified to play a crucial part in the fibrotic component of obesity[68]. In mouse models of dietary and genetic obesity, type VI collagen deficiency results in uninhibited expansion of adipocytes, which increases the adipose tissue storage capacity and minimizes ectopic lipid accumulation in non-adipose tissues. This beneficial partitioning of excess fat in adipose tissue is associated with improvements in whole-body glucose and lipid homeostasis[68].

Hypoxia is an early determinant of WAT dysfunction and a major initiating factor for causing ECM stress[7]. Grossly expanding WAT rapidly outpaces its own neo-vascularization system and, as such, a state of hypoxia arises[7,69]. Coined as the 'master regulator of oxygen homeostasis' (REF. 69), the transcription factor hypoxia-inducible factor 1α (HIF1α) displays enhanced activity in obese WAT[70]. In mice, HIF1α overexpression in WAT initiates fibrosis and local inflammatory events specifically through a lysyl oxidase-dependent pathway[71]. In contrast, HIF1α ablation in WAT leads to metabolic improvements in diet-induced obesity[72].

## Current treatments for obesity-associated T2DM

Although dietary and lifestyle modifications can be effective for the treatment of obesity and prevention of diabetes[73], these lifestyle changes are difficult to maintain and regain of weight is typical. Therefore, several US Food and Drug Administration (FDA)-approved anti-obesity and antidiabetic treatments are available, with many other potential therapies currently undergoing investigation.

***Dietary and exercise interventions.*** Lifestyle interventions aimed at lowering body weight through dietary alterations and increasing physical exercise (as used successfully by the Diabetes Prevention Program[74]) reduce adiposity and alleviate hyperglycaemia. In particular, lifestyle intervention in adults lowers the progression of diabetes by 58%[74], with tissue-specific effects including increased mitochondrial biogenesis, reduced adipocyte size and lipid content and improved WAT inflammation[75]. With respect to childhood obesity, the level of weight loss achieved with lifestyle intervention is only moderate, and the success rate 2 years following the onset of an intervention is surprisingly low[76]. Current strategies must therefore focus on sustainable interventions that prevent future risk of T2DM.

Although the benefits of exercise have been largely documented in studies using skeletal muscle, a novel role for sWAT in exercise-induced improvements in metabolism was recently determined[77]. Transplantation of sWAT from exercise-trained mice into the visceral cavity of sedentary HFD-fed mice enhances insulin sensitivity and reverses the metabolic complications of recipient mice[77]. These findings suggest that exercise elicits beneficial changes in WAT plasticity, and furthermore, they highlight a previously unrecognized role for WAT in exercise-driven energy homeostasis.

***Current and emerging drug treatments and their efficacy.*** Given the strong association of obesity with several serious comorbidities[78], developing safe, effective and affordable anti-obesity pharmacotherapies is a global

Hypertrophy
Tissue growth through an increase in cell size.

Hyperplasia
Tissue growth through an increase in cell number.

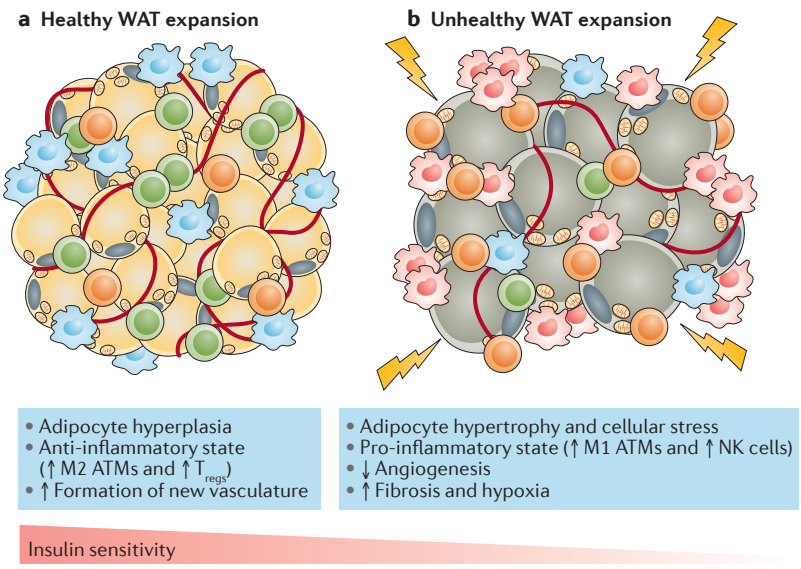


Figure 2 | **Metabolically healthy white adipose tissue expansion versus unhealthy pathological expansion during the progression of obesity. a** | During healthy white adipose tissue (WAT) expansion the tissue is in an anti-inflammatory state, there is sufficient vasculature to support the expansion and the adipocytes undergo hyperplasia. The tissue is characterized by elevated levels of regulatory T cells ($T_{reg}$ cells) and M2 adipose tissue macrophages (ATMs; light blue cells) and displays high insulin sensitivity. **b** | Unhealthy WAT harbours enlarged hypertrophic adipocytes (grey cells). Unhealthy WAT also displays a heightened state of inflammation: it has a higher ratio of infiltrating pro-inflammatory M1 ATMs (red cells) to anti-inflammatory M2 ATMs and natural killer (NK) cells (orange cells), as well as a reduction in $T_{reg}$ cell (green) numbers. The formation of new vasculature to support WAT growth (red lines) is also impaired, and enhanced fibrosis and hypoxia are evident. Such events contribute to the development of insulin resistance.

priority. Anti-obesity pharmacotherapy is considered for white individuals with a body mass index of 30 kg per $m^2$ or greater (obese individuals) or 27 kg per $m^2$ or greater (overweight individuals) — with adjustment of these values for other ethnicities — with weight-related comorbidities that have insufficiently responded to dietary and exercise interventions. There are currently five FDA-approved anti-obesity drugs[79], including two combination therapies (TABLE 1), allowing physicians to tailor obesity treatment for each individual. These include: the glucagon-like peptide 1 receptor (GLP1R) agonist liraglutide[80,81], the lipase inhibitor orlistat[82], the 5-hydroxytryptamine receptor 2C (5-$HT_{2C}$) agonist lorcaserin[83] and dual noradrenaline agonist–carbonic anhydrase inhibitor combinations. Phentermine–topiramate extended-release[84] and naltrexone–bupropion sustained-release[85] are two such combinations that activate hypothalamic pro-opiomelanocortin (POMC) neurons through the melanocortin system and antagonize the opioid system, respectively[86] (TABLE 1). The effectiveness of an anti-obesity drug is based on its ability to achieve a 3–5% reduction in body weight after 3 months.

Obesity represents one of the strongest risk factors for the development of T2DM. The management strategy for T2DM aims to achieve long-term glycaemic control and reduce cardiovascular risk[87]. The number of anti-hyperglycaemic agents has increased markedly over the past decades, and the use of combination therapies is now a well-recognized T2DM therapeutic strategy[88]. The FDA-approved antidiabetic drugs most commonly used to treat T2DM include the biguanide metformin[89], insulin therapy[90], GLP1R agonists[91,92], PPARγ agonists (such as thiazolidinediones (TZDs))[93,94], sodium–glucose co-transporter 2 (SGLT2) inhibitors[95,96], sulfonylureas[90], meglitinides[97], dipeptidyl peptidase 4 (DPP4) inhibitors[98] and α-glucosidase inhibitors[99] (TABLE 2).

Of particular interest are the SGLT2 inhibitors, which promote glycosuria by blocking glucose reabsorption in the proximal renal tubule[95], thereby increasing urinary glucose excretion and ameliorating hyperglycaemia[100]. The ability to 'lose' 300–400 kcals per day in urine is certainly an attractive feature of this drug class, although individuals treated with SGLT2 inhibitors frequently compensate with increased food intake. Additional concerns are rare instances of euglycaemic diabetic ketoacidosis, particularly in people with T1DM. However, a recent landmark study highlighted that patients with T2DM at high risk for cardiovascular events who are treated with an SGLT2 inhibitor (plus standard care) display a surprisingly large reduction in cardiovascular events. Elucidation of the detailed mechanisms mediating these effects may provide insight into the connection between hyperglycaemia, cardiovascular complications and T2DM. However, it must be noted that this study involved an 'at risk' population that had experienced a previous cardiovascular event, were older and had suffered from diabetes for many years.

The potential of co-activation of multiple receptor systems to achieve optimal therapeutic efficacy is gaining interest in the T2DM field. Indeed, co-agonism of the major incretin hormone receptors — GLP1R and glucose-dependent insulinotropic polypeptide (GIP) — was shown to elicit enhanced antihyperglycaemic and insulinotropic efficacy relative to GLP1 agonists alone[101]. These metabolic effects in obese and diabetic rodents translated very effectively to cynomolgus monkeys and early clinical studies[101]. More recently, a novel monomeric peptide tri-agonist was shown to reduce body weight, enhance glycaemic control and ameliorate hepatic steatosis in diet-induced obese (DIO) rodents through targeted activation of GLP1, GIP and glucagon receptors[102]. By synergizing the individual metabolic benefits of each receptor into a single molecule, enhanced metabolic improvements are achieved compared to existing mono-agonists and co-agonists[102].

With respect to adipose tissue, receptors for GLP1 are found on adipocytes; therefore, it is likely that GLP1R agonists directly impact adipocytes, as well as other cell types. By binding to GLP1R, compounds such as liraglutide can promote pre-adipocyte proliferation and the inhibition of apoptosis[103]. Loss of GLP1R expression reduces adipogenesis through induction of apoptosis in pre-adipocytes[103]. In addition, liraglutide reduces lipogenic signals in visceral adipose tissue of obese mice, prompting activation of 5′ AMP-activated protein kinase (AMPK) and suppression of AKT. This leads to reduced body weight gain and reduced visceral adipose tissue, as well as decreased expression of the lipogenic transcription factors PPARγ and C/EBPα[104].

Table 1 | **Current US-approved anti-obesity therapeutics**

| Drug name | Brand (manufacturer) | Mechanism of action in weight loss | Body weight loss (%) | Adverse effects | Refs |
|---|---|---|---|---|---|
| GLP1R agonists Liraglutide | Saxenda (Novo Nordisk) | Reduces appetite and food intake, partly through regulation of soluble leptin receptors | 5–12 | Transient gastrointestinal effects, nausea, hypoglycaemia and gallstones | 80,81 |
| Orlistat | Xenical (Roche) and Alli (GlaxoSmithKline) | Inhibits gastric and pancreatic lipases, reduces intestinal fat digestion to reduce caloric intake and improves glycaemic parameters and endothelial function | 5–11 | Blockade of triglyceride digestion in the intestine, gastrointestinal symptoms, liver failure and body weight re-gain (~35%) | 82 |
| Lorcaserin | Belviq (Arena Pharmaceuticals) | 5-$HT_{2c}$ receptor agonist. Activates hypothalamic POMC neurons through 5-$HT_{2c}$ to promote satiety. Lowers $HbA_{1C}$ levels and CVD risk factors | 3–5 | Headache, respiratory tract infection and depression. A 'pregnancy category X' drug | 83 |
| Phenteramine–topiramate ER | Qsymia (Vivus) | Suppresses food intake through antagonism of AMPA and kainite receptors. Enhances noradrenaline and GABA receptor activity. Increases energy expenditure | 9–11 | Paraesthesia, dizziness, dysgeusia, insomnia, glaucoma, creatine elevation and kidney stones | 84 |
| Naltrexone–bupropion XR | Contrave (Takeda Pharmaceuticals) and Mysimba (Orexigen) | Activates POMC neurons by binding to MC4Rs and antagonizes the opioid system. Increases energy expenditure and reduces food intake | <5 | Seizures, glaucoma and potential CVD risk owing to elevations in heart rate | 85 |

5-$HT_{2c}$, 5-hydroxytryptamine receptor 2C; AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; CVD, cardiovascular disease; ER, endoplasmic reticulum; GABA, γ-aminobutyric acid; GLP1R, glucagon-like peptide 1 receptor; $HbA_{1C}$, glycated haemoglobin; MC4R, melanocortin receptor 4; POMC, pro-opiomelanocortin.

Given its central role in metabolic health and disease, the potential of targeting WAT in the treatment of obesity and diabetes is gaining substantial interest. There have been major recent advances in understanding the intricate mechanisms of appetite control and energy expenditure, as well as in the understanding of how the physiology of WAT impacts these mechanisms during obesity[4]. This knowledge has helped to shape current drug development. In particular, in light of the difficulty of interfering with these pathways centrally within the brain owing to unwanted side effects (such as those reported for the cannabinoid receptor 1 (CNR1) antagonist rimonabant[105]), strategies targeting the peripheral organs have gained momentum.

## Adipokine-based therapeutics

As discussed above, adipokines are mostly peptide hormones that reflect various aspects of the metabolic status of WAT and communicate this signal to target tissues in the body. Therefore, they play an instrumental part in regulating a variety of processes, such as insulin sensitivity, energy expenditure, satiety, inflammation and endothelial function[25,35]. Adipokine biology has been advanced by proteomic approaches, including an effort to define the complete WAT secretome[106]. More than 600 potential secretory proteins have been identified[106], including many well-studied circulating factors, such as leptin, adiponectin, retinol-binding protein 4 (RBP4), adipsin, fibroblast growth factor 21 (FGF21), apelin, vaspin, visfatin, hepcidin and numerous inflammatory cytokines (such as TNF and IL-6). Adipokine secretion is altered as obesity progresses and adipocytes become dysfunctional[4]. Indeed, secretome analysis has revealed that gene expression levels of 60% of adipokines are regulated by obesity[107].

Adipokines are therefore regarded as promising tools for novel pharmacological strategies and in diagnosis (FIG. 3; TABLE 3), providing that their mode of action is fully delineated and the key molecular target is identified. Unravelling the signalling mechanisms of adipokines is therefore an ongoing focus of metabolic research. Small molecules that either promote or inhibit adipokine actions may prove useful as future novel pharmacological agents. Below, we discuss the most promising adipokine-based therapeutic strategies.

***Leptin in combination pharmacotherapy.*** Following the discovery of leptin as a central negative regulator of body weight, it seemed obvious to use it as an anti-obesity agent in human patients. However, obese individuals did not lack leptin as predicted, but rather they exhibited enhanced circulating leptin levels that did not function adequately to improve metabolic homeostasis owing to central leptin-resistance[108], which is characterized by disrupted leptin signalling in the hypothalamus and impaired leptin transport across the blood–brain barrier[109]. This phenomenon cannot be corrected by treating obese individuals with exogenous leptin, as marginal effects on food intake, body weight or insulin sensitivity were evident following injections of recombinant methionyl leptin[110,111], or PEGylated recombinant native leptin[112]. Despite these setbacks, ongoing studies still aim to take advantage of leptin as an anti-obesity and/or an antidiabetic drug. Recombinant leptin treatment diminished atherosclerotic lesions and enhanced adiponectin production in a mouse model of hypercholesterolaemia[113]. Moreover, co-intracerebroventricular infusion of leptin with insulin was reported to enhance WAT beiging and decrease adiposity; these effects occurred through both hormones acting

Table 2 | **US-approved antidiabetic drugs most commonly used to treat T2DM**

| Drug type (examples) | Primary antidiabetic effects | $HbA_{1c}$ lowering capacity (%) | Adverse effects | Refs |
|---|---|---|---|---|
| Biguanides (metformin) | Alleviate hyperglycaemia and enhance insulin sensitivity by suppressing gluconeogenesis through inhibition of the mitochondrial ETC, glucagon signalling or the glycerophosphate shuttle enzyme | ~1–1.5 | Lactic acidosis and diarrhoea | 89 |
| Insulin therapy | Improves insulin secretion capacity and predictably stabilizes glycaemia | >1.5 | Hypoglycaemia and weight gain | 90 |
| GLP1R agonists (liraglutide) and exenatide | Increases cAMP to enhance insulin secretion and alleviate hyperglycaemia. Delays gastric emptying. Suppresses secretion of glucagon | 1.5 | Nausea, transient gastrointestinal effects and pulse increases | 91,92 |
| TZDs (pioglitazone and rosiglitazone) | Activate PPARγ–RXR heterodimerization. Block CDK5-mediated PPARγ phosphorylation. Increase insulin sensitivity and glucose disposal | 1 | Bone fracture, CVD risk, fluid retention, weight gain and congestive heart failure | 93,94 |
| SGLT2 inhibitors (canagliflozen and empagliflozin) | Alleviate hyperglycaemia by blocking kidney glucose reabsorption. Increase urinary glucose excretion | ~0.6–0.8 | Diabetic ketoacidosis and urinary tract infections | 95,96, 100 |
| Sulfonylureas (glyburide, glipizide and glimepiride) | Enhance insulin secretion by binding to $K_{ATP}$ channels on pancreatic β-cells. Suppress hepatic gluconeogenesis | ~1–1.5 | Hypoglycaemia and weight gain | 90 |
| Meglitinides (repaglinide and nateglinide) | Increase insulin secretion by binding to $K_{ATP}$ channels on β-cells | ~1 | Hypoglycaemia and weight gain | 97 |
| DPP4 inhibitors (sitagliptin and saxagliptin) | Enhance endogenous GLP1 levels and insulin secretion. Suppresses glucagon secretion | ~0.6–0.8 | Upper respiratory infections | 98 |
| α-glucosidase inhibitors (acarbose and miglitol) | Reduce blood glucose levels by inhibiting the enzymes that digest carbohydrates | ~0.8 | Gastrointestinal symptoms and diarrhoea | 99 |

cAMP, cyclic AMP; CDK, cyclin-dependent kinase; CVD, cardiovascular disease; DPP4, dipeptidyl peptidase 4; ETC, electron transport chain; GLP1, glucagon-like peptide 1; GLP1R, GLP1 receptor; $HbA_{1c}$, glycated haemoglobin; $K_{ATP}$ channels, ATP-sensitive potassium channels; PPAR, peroxisome proliferator-activated receptor; RXR, retinoid X receptor; SGLT2, sodium–glucose cotransporter 2; T2DM, type 2 diabetes mellitus.

synergistically on POMC neurons[114]. Currently, however, human recombinant leptin (metreleptin, a synthetic analogue of leptin) is only FDA-approved for rare cases of monogenetically inherited leptin deficiency, in which body weight loss is observed upon leptin restoration[115], and for patients with generalized lipodystrophy, in whom metreleptin improves hyperglycaemia and hypertriglyceridaemia[116] (TABLE 3). Nevertheless, AstraZeneca-sponsored clinical trials in obese patients using metreleptin in combination with the synthetic amylin analogue pramlintide (see below) to reduce body weight have advanced to Phase II[117,118]. Similarly, another clinical trial explored the effects of leptin injection in obese and overweight patients that are actively losing body weight, as weight loss could potentially 'sensitize' these patients to the effects of leptin[119]. The major finding from this clinical trial is that leptin administration to patients who have lost weight markedly increased satiation, indicating that under states of physiologically reduced leptin signalling (that is, after weight loss), pharmacological supplementation with leptin can be effective[120,121]. In a National Institute of Diabetes and Digestive and Kidney Diseases (NIDDK)-sponsored Phase II clinical trial in patients with leptin deficiency, lipodystrophy and T2DM[122], metreleptin treatment was shown to improve glycaemic control, lower glycated haemoglobin ($HbA_{1C}$) and decrease triglyceride levels[123].

Although the outcomes of recombinant leptin monotherapy are disheartening[110,111], the use of leptin in combination therapy still holds therapeutic potential. Recent clinical trials have focused on the co-administration of leptin with other weight loss-inducing drugs, with the hypothesis that resolving leptin resistance would restore leptin action. Amylin is a peptide hormone co-secreted with insulin from pancreatic β-cells[124] that improves glycaemic regulation by slowing gastric emptying, inducing satiety and restoring leptin responsiveness[125]. Clinical studies show that 24-week co-administration of human recombinant leptin and pramlintide restores leptin sensitivity at least partially and increases mean body weight loss in obese individuals[125].

In a non-obese diabetic mouse model of uncontrolled T1DM, co-administration of recombinant leptin with low-dose insulin achieved superior glycaemic stability without an increase in body fat or an upregulation in cholesterologenic or lipogenic genes typically observed with insulin monotherapy[126]. Leptin also effectively suppresses pancreatic α-cell glucagon production to prevent diet-induced hyperinsulinaemia and sterol regulatory element-binding protein 1c (SREBP1c)-driven lipogenesis[127]. Of note, an interesting hypothesis for an antilipotoxic role for leptin as a physiological suppressor of toxic lipid accumulation in pancreatic β-cells has been proposed[127]. Ceramide is a lipid moiety that induces β-cell lipoapoptosis[128] and promotes insulin resistance. As hyperleptinaemia downregulates the rate-limiting enzyme for ceramide biosynthesis, serine palmitoyl transferase[128], leptin may play a crucial part in reducing ceramide production in the endocrine pancreas, thus preventing the ceramide-induced lipotoxic effects on insulin and potentially glucagon secretion. Future studies examining the role of ceramide signalling in the endocrine

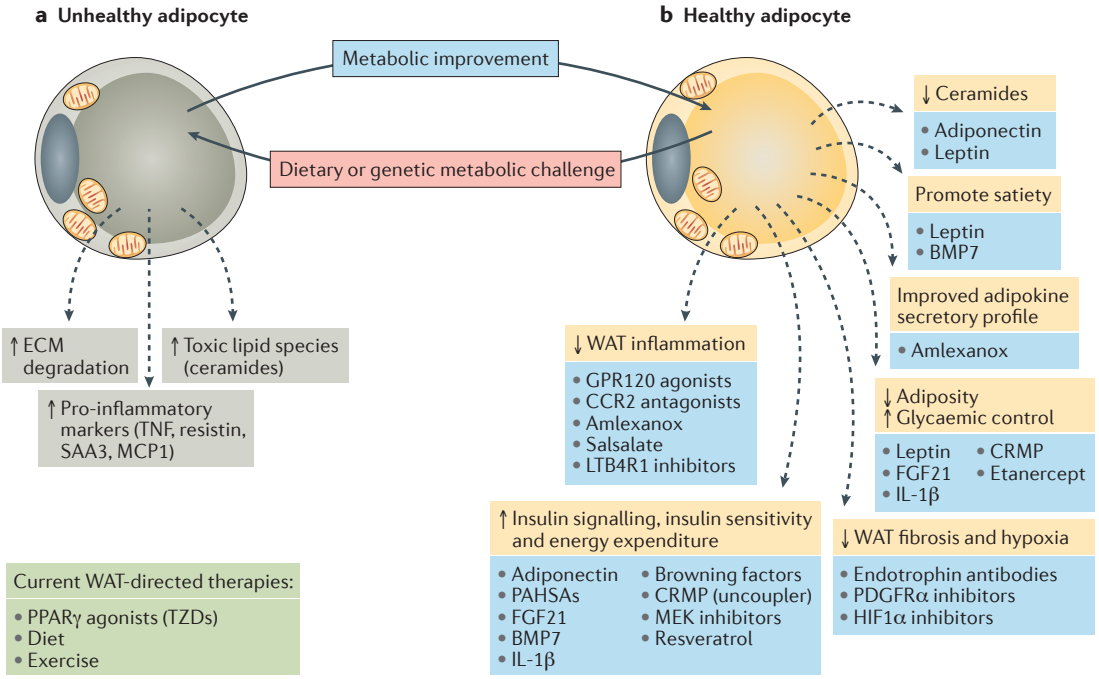


Figure 3 | **Targeting white adipose tissue for the treatment of obesity-associated diabetes. a** | Unhealthy adipocytes are characterized by increased pro-inflammatory marker production, enhanced extracellular matrix (ECM) degradation and the accumulation of toxic lipid species. The currently approved white adipose tissue (WAT)-directed therapies are thiazolidinediones (TZDs), dietary interventions and exercise (green box). **b** | Strategies to promote healthy adipocytes (by improving adipokine secretory profiles to reduce inflammation, fibrosis and hypoxia) and emerging therapies that target WAT for the treatment of obesity-associated T2DM are shown (yellow boxes). Promising candidates (blue boxes) to turn unhealthy adipocytes into healthy ones are located adjacent to their primary target mechanism of action and key positive metabolic outcomes. BMP7, bone morphogenetic protein 7; CCR2, C-C chemokine receptor 2; CRMP, controlled-release mitochondrial protonophore; FGF21, fibroblast growth factor 21; GPR120, G protein-coupled receptor 120; HIF1α, hypoxia-inducible factor 1α; IL-1β, interleukin-1β; LTB4R1, leukotriene $B_4$ receptor 1; MCP1, monocyte chemoattractant protein 1; MEK, MAPK/ERK kinase 1; PAHSA, palmitic-acid-9-hydroxy-stearic-acid; PDGFRα, platelet-derived growth factor receptor-α; PPARγ, peroxisome proliferator-activated receptor-γ; SAA3, serum amyloid A-3; TNF, tumour necrosis factor.

pancreas should therefore prove illuminating. Finally, novel monopegylated leptin antagonists have been developed that increase food intake to promote weight gain in rodents[129,130]. Such potent orexigenic agents will have great therapeutic importance for the treatment of cachexia and immune-regulatory diseases. Ongoing research in using leptin in a variety of clinical settings indicates a pharmacological 'rebirth' of this protein as a therapeutic after numerous years with limited success in this area.

***Adiponectin as a diagnostic tool.*** Adiponectin is a potent antidiabetic adipokine that is regarded as an excellent correlative marker of systemic insulin sensitivity and functionality of the adipocyte[35,45]. Adiponectin exerts insulin-sensitizing, antilipotoxic, anti-apoptotic, anti-atherogenic and anti-inflammatory effects on multiple cell types[35].

Stimulation of the adiponectin receptors ADIPOR1 or ADIPOR2 results in activation of AMPK and PPARγ, respectively[131]. Adiponectin also triggers extracellular $Ca^{2+}$ influx by ADIPOR1, which results in enhanced PPARγ co-activator 1α (PPARGC1α; referred to as PGC1α) activity and increased mitochondrial biogenesis[50]. By signalling through its cognate receptors ADIPOR1 and ADIPOR2 (REF. 40), adiponectin stimulates ceramidase activity. This leads to reduced levels of metabolically detrimental ceramide[26], as well as the formation of the anti-apoptotic metabolites sphingosine and sphingosine-1-phosphate, which promote cellular survival and mitochondrial proliferation independently of AMPK[40]. Adiponectin also signals through a crucial FGF21–adiponectin–ceramide axis to enhance energy expenditure and insulin action[132]. In accordance with this, inducible expression of acid ceramidase in either the liver or in adipocytes rapidly improves insulin sensitivity[133]. Therefore, several lines of evidence point to a common downstream signalling model in which adiponectin upregulates mitochondrial biogenesis and signalling through specific mediators[40,50,54,134].

There are two possible strategies to therapeutically restore adiponectin levels. One approach is to increase the levels of adiponectin itself, through injection of the protein. However, direct administration of adiponectin is problematic owing to relatively high baseline plasma concentrations and the fact that adiponectin is most active as a complex quaternary structure, which requires proper post-translational modifications for multimerization[35].

In addition, adiponectin has a short half-life (serum half-life of 40–60 min in a mouse[135]) and therefore administering substantial amounts of adiponectin that can withstand immediate degradation may not be a cost-effective option. The tendency of adiponectin to promote weight gain in mice when delivered in excess, as part of a complex crosstalk with the leptin axis, would also need to be addressed[54], although this could potentially be resolved through the use of combination therapies. An alternative approach to induce adiponectin signalling is to activate its receptors. A compound that activates both adiponectin receptors through AMPK and PPARα-mediated pathways was recently identified in a small-molecule screen[136]. The molecule, named AdipoRon, is an orally active, synthetic adiponectin receptor agonist that improves glucose intolerance and insulin resistance, increases exercise endurance capacity and even extends longevity in HFD-fed leptin-receptor-deficient *db/db* mice[136]. Notably, the adiponectin receptors ADIPOR1 and ADIPOR2 are conserved in evolution from mammals to plants and yeasts, indicating that they have crucial biological roles. The unique crystal structures of human ADIPOR1 and ADIPOR2 were recently determined[137], with both receptors harbouring a zinc-binding site, a putative adiponectin-binding surface and a unique seven-transmembrane architecture that encircles a cavity holding three conserved histidine residues and a zinc ion[137]. This structural study should contribute to the development and optimization of highly specific adiponectin receptor agonists.

There are a few concerns associated with enhancing adiponectin signalling. Notably, mice overexpressing adiponectin exhibit reduced bone mineral density[138] or harbour some degree of heart damage in the form of left-ventricular hypertrophy. Chronically high adiponectin levels may also affect fertility[47]. Despite this, adiponectin is serving as a valuable biomarker for insulin resistance and metabolic dysfunction, and it is one of the 'elite adipokines' that functionally increases insulin sensitivity. Further understanding of pathways downstream of adiponectin receptor activation is essential for the development of effective and safe therapeutic strategies.

***Adipose-derived fatty acid derivatives.*** Although most described adipokines are peptides, adipose-derived fatty acid derivatives with signalling characteristics have recently garnered much attention. A novel class of endogenous mammalian lipids that harbour anti-diabetic and anti-inflammatory properties — fatty acid esters of hydroxyl fatty acids (FAHFAs) — are significantly upregulated in mice that selectively overexpress glucose transporter 4 (GLUT4) in WAT[139]. FAHFA isomers (such as palmitic-acid-9-hydroxy-stearic-acid (PAHSA)) that correlate highly with insulin sensitivity have also been identifed[139]. Treatment of DIO mice with PAHSA augments glucose tolerance by lowering WAT inflammation and enhancing insulin and GLP1 secretion. Mechanistically, PAHSAs may signal through G protein-coupled receptor 120 (GPR120, also known as FFAR4) in an adipocyte-specific manner to promote insulin-stimulated glucose uptake[139]. However, many questions remain unanswered. More careful dose–response studies with respect to receptor activation are awaited. In addition, it is not yet understood which structural features set these specific lipids apart from other related lipids that fail to display metabolically beneficial effects. It will be interesting to determine whether these lipid species are superior to the classical omega-3 fatty acid supplements. Nevertheless, PAHSAs may hold promise as candidates for future treatment approaches for obesity and T2DM (FIG. 3; TABLE 3). Because PAHSAs promote endogenous GLP1 secretion, combining them with DPP4 inhibition may provide synergistic benefits.

***FGF21.*** FGF21 is a catabolic hormone that is largely synthesized in the liver[140], with expression also evident in WAT, brown adipose tissue (BAT), skeletal muscle and the pancreas[141]. FGF21 signals through its cognate receptor FGFR1 and cofactor β-klotho to elicit a plethora of positive effects on glucose and lipid metabolism[141–144]. In mouse models of obesity[145,146] and in diabetic primates[147], administration of FGF21 enhances β-oxidation[148] and increases energy expenditure[149] to lower glucose levels, improve hepatic and peripheral insulin sensitivity[145], improve triglyceride and cholesterol profiles and promote weight loss. This weight reduction can be quite profound, as DIO mice lose up to 20% of their body weight in just 2 weeks of FGF21 treatment[146]. Very recently, FGF21 administration was shown to lower sweet and alcohol preference in mice by signalling centrally through β-klotho; this effect correlates with central dopamine levels[150]. These findings raise the likelihood that FGF21 could alter nutrient preferences and reward behaviour systems in obese patients with diabetes who were treated with FGF21 analogues. From an adipocentric perspective, the insulin-sensitizing actions of FGF21 induction are abolished in mice harbouring a WAT-specific deletion of either *Fgf21* (REF. 151) or *Klb* (the gene encoding β-klotho[143]), thus pinpointing adipose as one of the major target tissues for the pleiotropic actions of FGF21. Similarly, FGF21 directly enhances the secretion of adiponectin from WAT, and vice versa, with adiponectin mediating the insulin sensitizing and glucose-lowering actions of FGF21, but not its effects on body weight[132,152]. Recent studies in mice have revealed that the anti-obesogenic effect of FGF21 may be partly due to the promotion of 'beiging' of WAT by induction of mitochondrial brown fat uncoupling protein 1 (UCP1)[153].

Considerable effort has been placed on generating drugs that mimic native FGF21 (TABLE 3). Despite the limitations in developing an optimized FGF21 analogue — which are primarily due to its short half-life *in vivo*, immunogenicity and self-aggregation[154] — two FGF21 analogues have advanced to clinical trials: LY2405319 (Eli Lilly)[155] and PF-05231023 (Pfizer)[156]. LY2405319 is a human FGF21 variant that has resistance to self-aggregation, enhanced biophysical stability and exerts lipid-, glucose- and body weight-reducing properties that are fundamentally identical to native FGF21 in diabetic mice and primates[157–159]. LY2405319 reached Phase I clinical trials[155], in which it lowered body weight, enhanced adiponectin levels and substantially improved

Table 3 | **Potential therapeutic strategies and agents under development for the treatment of obesity-associated T2DM**

| Agent class | Compound name (company) | Mechanism of action or key metabolic effect | Developmental status | Refs |
|---|---|---|---|---|
| ***Adipokines*** | | | | |
| Leptin analogues | Metreleptin (Aegerion Pharmaceuticals) | Increase energy expenditure via JAK–STAT3 | Approved for congenital or acquired lipodystrophy. Phase II for obesity | 115–126 |
| Adiponectin and adiponectin receptor agonists | AdipoRon (University of Tokyo) | Stimulate ceramidase activity via ADIPOR1 and/or ADIPOR2 | Pre-clinical | 131–137 |
| PAHSAs | NA (Harvard Medical School) | May enhance gut GLP1 secretion through GPR120 | Pre-clinical | 139 |
| FGF21 Analogues | LY2405319 (Eli Lilly) and PF-05231023 (Pfizer) | Lower glucose and lipids | Phase I, discontinued owing to adverse effects | 155,156 |
| Human recombinant BMP7 | OP1 (Olympus Biotech) | Suppress appetite through mTOR pathway | OP1 is approved for bone surgery. Pre-clinical for obesity and T2DM | 167–170 |
| IL-1R antagonists and anti-IL-1β antibodies | Anakinra (Amgen) and canakinumab (Novartis) | Improve glycaemia and β-cell function | Approved for RA (Anakinra), CAPS, arthritis and SJIA. Pre-clinical for obesity and T2DM | 171–176 |
| ***BAT activation or WAT beiging*** | | | | |
| $\beta_3$-adrenergic receptor agonists | Myrbetriq (Astellas Pharmaceuticals) | Activate BAT and/or WAT to stimulate thermogenesis | Phase I: halted as CVD risk. Approved for overactive bladder | 197–202 |
| Mitochondrial uncouplers | DNPME and CRMP (both Yale University) | Improve insulin sensitivity | DNP is banned for weight loss. DNPME and CRMP are pre-clinical | 203–205 |
| WAT beiging factors | Fexaramine (Salk Institute) and PF-05231023 (Pfizer) | Increase energy expenditure | Pre-clinical | 206–225 |
| ***PPAR signalling*** | | | | |
| MEK inhibitors | Trametinib and dabrafenib (both GlaxoSmithKline) | Signal through PPARγ in WAT | Approved for metastatic melanoma | 229,230 |
| SIRT1 activators | Resveratrol, SRT1720 (Sirtris Pharmaceuticals) and SRT501 (Sirtris Pharmaceuticals) | Increase SIRT1-mediated deacetylation | Phase II | 240–243 |
| FGF21 mimetics | LY2405319 (Eli Lilly) and PF-05231023 (Pfizer) | Enhance PPARγ activity | Phase I, discontinued | 244–246 |
| ***Inflammation*** | | | | |
| IKKε and/or TBK1 inhibitors | Amlexanox (Meda AB/ULURU Inc.) | Reduce WAT inflammation | Approved for ulcers (in the United States) and inflammatory conditions (Japan). Phase II in obesity and T2DM. | 254–256 |
| TNF inhibitors | Etanercept (Immunex) and Infliximab (Pfizer) | Improve insulin resistance | Phase I for obese patients or patients with T2DM. Approved for RA and psoriasis | 258–261 |
| CRP, TNF and IL-6 suppressors | Salsalate and aspirin (NIH- and NIDDK-sponsored) | Increase adiponectin and lower $HbA_{1C}$ | Phase IV for salsalate. Phase I for aspirin | 262–268 |
| GPR120 agonists | Compound A (Merck) and GSK-137647 (GlaxoSmithKline) | Reduce WAT infiltration of macrophages | Pre-clinical | 269,270 |
| CCR2 antagonists | CCX140-B (Chemocentryx), PF-4136309 (Pfizer), MK-0812 (Merck) and BMS-741672 (Bristol-Myers Squibb) | Block macrophage chemotaxis into WAT | Phase II | 271–273, 276 |
| LTB4R1 Inhibitors | CP-105696 (Pfizer) | Promote insulin sensitization | Phase I | 277,279 |
| ***WAT fibrosis and hypoxia*** | | | | |
| Anti-endotrophin antibodies | NA (University of Texas Southwestern Medical Center) | Reverse obesity-induced WAT fibrosis | Pre-clinical | 281,282 |
| PDGFRα signalling inhibitors | NA | Alleviate WAT fibrosis | Pre-clinical | 283 |

Table 3 (cont.) | **Potential therapeutic strategies and agents under development for the treatment of obesity-associated T2DM**

| Agent class | Compound name (company) | Mechanism of action or key metabolic effect | Developmental status | Refs |
|---|---|---|---|---|
| MRTFA and/or ROCK inhibitors | CCG-203971 (University of Michigan) and fasudil (Asahi Pharmaceuticals) | Target myofibroblast production of ECM | Pre-clinical for obesity and T2DM. Phase II/III for vascular function | 225,284, 285 |
| HIF1α inhibitors | NSC-134754 (NCI), EZN-2968 (Enzon Pharmaceuticals) and PX-478 (Oncothyreon Inc.) | Prevent HFD-induced WAT fibrosis | Phase I for cancer studies. Pre-clinical for obesity and T2DM | 68,71,72, 291,292 |

ADIPOR1, adiponectin receptor 1; BMP7, bone morphogenetic protein 7; CAPS, cryopyrin-associated periodic syndrome; CCR2, CC-chemokine receptor 2; CRMP, controlled-release mitochondrial protonophore; CRP, C-reactive protein; CVD, cardiovascular disease; DNP, 2,4-dinitrophenol; DNPME, DNP-methyl ether; ECM, extracellular matrix; FGF21, fibroblast growth factor 21; GLP1, glucagon-like peptide 1; GPR120, G protein-coupled receptor 120; $HbA_{1C}$, glycated haemoglobin; HFD, high-fat diet; HIF1α, hypoxia-inducible factor 1α; IKKε, inhibitor of nuclear factor-κB kinase subunit ε; IL-1β, interleukin-1β; IL-1R, IL-1β receptor; JAK, Janus kinase; LTB4R1, leukotriene B4 receptor 1; mTOR, mechanistic target of rapamycin; MEK, MAPK/ERK kinase 1; MRTFA, myocardin-related transcription factor A; NA, not applicable; NCI, National Cancer Institute; NIH, National Institutes of Health; NIDDK, National Institute of Diabetes and Digestive and Kidney Diseases; PAHSA, palmitic-acid 9 hydroxy-stearic-acid; PPAR, peroxisome proliferator-activated receptor; RA, rheumatoid arthritis; ROCK, RHO-associated protein kinase; SIRT1, NAD-dependent protein deacetylase sirtuin 1; SJIA, systemic juvenile idiopathic arthritis; STAT, signal transducer and activator of transcription; T2DM, type 2 diabetes mellitus; TBK1, TANK-binding kinase 1; TNF, tumour necrosis factor; WAT, white adipose tissue.

dyslipidaemia, cholesterol and apolipoprotein profiles in patients with T2DM; however, no significant reduction in fasting glucose levels was apparent, and the therapy was discontinued owing to adverse effects[155]. The second FGF21-based protein mimetic, PF-05231023, has an extended serum half-life[156]. Although PF-05231023 corrected dyslipidaemia in patients with T2DM, robustly reducing triglyceride levels, no marked improvements in glucose levels were reported[156]. The lipid-lowering effects by PF-05231023 were confirmed recently in obese cynomolgus monkeys and obese patients with T2DM[160]. Although no significant effects of PF-05231023 on glycaemic control were evident, the treatment altered multiple markers of bone formation and reabsorption and elevated levels of IGF1 (REF. 160). Given that diabetic rodent studies clearly demonstrate improvements in glycaemic end points with these agents[158,159,161], future studies will have to carefully dissect the key mechanisms that result in such a translational disconnect.

Finally, there are several safety considerations that must be addressed before future clinical trials of FGF21 analogues. In mice, FGF21 directly inhibits the growth hormone stimulatory effects on chondrogenesis[162], suggesting potential restriction of skeletal growth. Moreover, mice that were administered native FGF21 exhibit decreased bone mass[163], a decline in female fertility[164] and elevated systemic corticosterone levels[165]. Interestingly however, plasma FGF21 levels positively correlate with bone mineral density in women[166]. Nevertheless, detailed evaluation of the effect of FGF21 on bone turnover is clearly warranted.

***Bone morphogenetic protein 7.*** Bone morphogenetic protein 7 (BMP7) is a member of the transforming growth factor-β (TGFβ) superfamily that mediates numerous processes such as organogenesis, brown adipogenesis, energy expenditure and satiety[167,168]. Rodent studies demonstrate that systemic treatment of DIO mice with BMP7 increases energy expenditure and suppresses appetite, with consequent reduction in body weight through a central mechanistic target of rapamycin (mTOR) pathway[168]. A positive correlation between BMP7 and insulin resistance is also evident in obese patients[169]. Therapeutically, although human recombinant BMP7 is used in orthopedic surgical procedures under the brand name of OP1 (Olympus Biotech)[170] (TABLE 3), there are currently no clinical trials examining the actions of BMP7 in patients who are obese or who have T2DM.

***IL-1β.*** IL-1β is a major pro-inflammatory cytokine, produced primarily by macrophages, that has been implicated in causing insulin resistance[171]. In human primary adipocytes and mouse 3T3-L1 adipocytes, IL-1β regulates macrophage-induced impairment in insulin signalling[172], glucose transport and fatty acid uptake[173]; such effects are neutralized through use of IL-1β monoclonal antibodies[172]. IL-1β binds to IL-1 receptor (IL-1R), and blockade of IL-1R signalling has therapeutic potential, as several IL-1R antagonists have reached Phase I clinical trials (TABLE 3). For instance, treatment of patients with T2DM with the recombinant human IL-1R antagonist anakinra improves glycaemia and β-cell function and lowers markers of inflammation[174]; enhanced insulin sensitivity was also observed in patients with T1DM[175]. In another clinical trial, the anti-IL-1β antibody canakinumab improved glucose tolerance and insulin secretion in patients with T2DM[176]. Taken together, inhibition of IL-1β signalling could be a viable therapeutic strategy in the treatment of obesity and diabetes.

## BAT activation and WAT beiging

An alternative emerging strategy to promote weight loss is the induction of beiging of WAT. BAT dissipates chemical energy by uncoupling mitochondrial respiration to generate heat, and thus it has an important role in cold- and diet-induced thermogenesis[177]. Recent BAT studies have revealed a new distinct type of thermogenic adipocyte intermingled within sWAT, named beige cells (also known as brite cells). Beige cells are UCP1-positive multilocular adipocytes that are induced within WAT by cold exposure, specific factors or exercise training[178].

Although BAT was first discovered during autopsy in human adults in 1972 (REF. 179), it was not until 2009 that metabolically active BAT and beige adipocytes

were identified in humans within the supraclavicular regions[180–182], a finding which was recently confirmed using contrast-enhanced ultrasound[183]. These studies raised the possibility of therapeutically activating beige cells. Humans have on average 50–80 g of BAT[184], and quantitatively, 50 g of BAT could burn ~20% of an individual's daily energy intake if fully activated[185]. This is encouraging, as even though the BAT depot is present in relatively small amounts, the activated tissue has the potential to contribute significantly to energy expenditure[186]. However, whether this approach will translate into the clinic remains unknown. Indeed, it is possible that the relatively modest increase in energy expenditure might be counterbalanced by a homeostatic increase in appetite or reduction in involuntary movements.

BAT and beige cell activation offer numerous additional benefits beyond promoting a negative energy balance. BAT consumes substantial amounts of lipid and glucose, which may evoke cardioprotective and antidiabetic effects[187]. Indeed, there is evidence that active BAT may exert positive effects on insulin sensitivity. Human BAT expresses high levels of GLUT4, and insulin stimulates glucose uptake into this tissue to the same extent as into muscle. BAT activation also exerts improvements in dyslipidaemia and insulin secretion, as well as reducing insulin resistance in a diabetic setting[188]. Notably, in a mouse model of 'human-like' lipoprotein metabolism, metformin increased BAT mitochondrial content and activity and enhanced very low-density lipoprotein (VLDL) clearance into BAT, implicating this depot in the actions of this widely used antidiabetic drug[189].

***Emerging compounds and candidates.*** The list of promising candidates that activate BAT and/or induce the beiging of WAT is continuing to grow (TABLE 3). The simplest way to stimulate BAT is through the use of classical inducers: catecholamine derivatives and thyroid hormone. However, noradrenaline cannot be used therapeutically owing to cardiovascular side effects, and supraphysiological doses of thyroid hormone cause substantial morbidity, therefore prohibiting therapeutic use[190].

Cold is one of the strongest activators of BAT, and chronic cold acclimation increases active BAT volume and its contribution to cold-induced thermogenesis in humans[191]. Cold itself could be used as a therapy, as repeated intermittent exposure to mild cold (2 hours per day at 19 °C for 6 weeks) expanded the volume of BAT and caused a significant reduction in body fat mass in humans[192]. Similarly, lowering the ambient temperature from 24 °C to 19 °C increases an individual's energy expenditure by 5% and increases BAT activity by 10%[193]. BAT also serves as an important independent determinant of plasma glucose and $HbA_{1C}$ levels[194]. A short-term mild cold stimulus enhances insulin sensitivity in healthy individuals with active supraclavicular BAT[195]. In contrast, cold- and insulin-stimulated glucose uptake into BAT is markedly reduced in obese individuals[196].

The effects of cold exposure may be mimicked by pharmacological activation of the β-adrenergic pathway through the use of $\beta_3$-adrenergic receptor agonists, which activate BAT, recruit beige cells and stimulate thermogenesis[197]. Chronic $\beta_3$-adrenergic stimulation in rodents promotes brown adipocyte development in WAT depots, increases energy expenditure and ameliorates obesity and insulin resistance[177,198]. In humans, treatment with ephedrine[199] or isoprenaline[200] enhances energy expenditure but not BAT activity. Doubling the dose of ephedrine appears to modestly increase BAT activity, albeit at the expense of significantly elevating blood pressure[201]. The $\beta_3$-adrenergic receptor-selective agonist mirabegron (currently FDA-approved for the treatment of overactive bladder) promotes higher BAT metabolic activity, which increases energy expenditure in young individuals to a similar degree as cold exposure, but significantly increases blood pressure and heart rate[202]. These serious issues of cardiovascular safety therefore warrant further investigation and have halted the progression of $\beta_3$-adrenergic pharmacotherapy. Achieving absolute $\beta_3$-adrenergic receptor selectivity for these compounds has also been problematic, and their oral bioavailability is suboptimal.

Mitochondrial energy dissipation through 'chemical uncoupling' was once considered a target for obesity treatment. The nonspecific mitochondrial uncoupling agent 2,4-dinitrophenol (DNP) is a small lipophilic protonophore that dissipates the mitochondrial proton gradient, and the energy derived from substrate oxidation is converted to heat. DNP was widely used as a weight loss therapy in the 1930s; however, it was removed from the market in 1938 owing to uncontrolled use and the occurrence of fatal hyperthermia, owing to its very narrow therapeutic window. Recent studies using DNP have also not been encouraging and report adverse effects, such as rash, cataracts, hyperpyrexia and even fatalities[203]. However, in 2013, the generation of a functionally active derivative of DNP was reported. DNP-methyl ether (DNPME) specifically targets the liver[204], thereby bypassing potentially fatal systemic DNP-associated toxicities. Treatment with DNPME effectively ameliorates hypertriglyceridaemia, NAFLD and insulin resistance in HFD-fed rats[204]; DNPME treatment also lowers hyperglycaemia and augments glucose tolerance in a rat model of T2DM[204]. Importantly, no adverse DNP-linked systemic or hepatic toxicities are evident in animals treated with DNPME. Furthermore, a sustained-release oral formulation of DNP, controlled-release mitochondrial protonophore (CRMP), has been developed[205]. CRMP improves glucose tolerance and insulin sensitivity and lowers hepatic triglyceride content by 90% in rodent models of obesity and T2DM, without signs of toxicity[205]. These findings have provided proof of concept that mild hepatic mitochondrial uncoupling has promising therapeutic potential for the future.

Over recent years, several factors have been identified as endogenous promoters of WAT beiging. Muscle-specific induction of PGC1α led to the discovery of a novel secreted myokine called irisin that promotes brown or beige adipocyte formation in murine sWAT depots and elicits protection against diet-induced obesity and diabetes[206]. Although the existence of irisin as a circulating protein in humans was initially controversial[207,208],

studies using quantitative mass spectroscopy have firmly validated the presence of human irisin in plasma[209] and its endogenous upregulation with aerobic exercise training[210]. However, it remains to be seen whether this molecule or portions thereof have the potential to be developed therapeutically. Meteorin-like protein, a muscle-derived cold- and exercise-induced peptide, has also been shown to upregulate a transcriptional beige fat thermogenic programme to increase energy expenditure and augment glucose tolerance[211]. In addition, adipocyte-specific deletion of *PRDM16*, the gene encoding co-regulatory protein PR domain zinc-finger protein 16, blunts cold- and $\beta_3$-adrenergic receptor agonist-induced beige adipocyte formation in sWAT and promotes a subcutaneous-to-visceral fat switch, resulting in obesity and insulin resistance[212]. Interferon regulatory factor 4 has also been identified as a cold- and cyclic AMP-induced transcriptional driver of thermogenesis that enhances energy expenditure in HFD-fed mice; such effects occur through direct interaction with PGC1α[213]. Recently, arginine and creatine metabolism have been identified as being crucial to enhancing energy expenditure and thermogenesis in beige fat[214].

The list of brown and/or beige-inducing factors seems to be vast. Other candidates known to influence brown and/or beige fat formation include the antidiabetic TZDs, developmental regulators such as BMP7 and BMP8b[167,215], the hormone FGF21 (REF. 209), intestinal agonism of the bile acid sensor farnesoid X receptor (FXR)[216], vascular endothelial growth factor (VEGF)[217], adiponectin[47], the outer mitochondrial protein mitoNEET (also known as CDGSH iron–sulfur domain 1)[218] and alternatively-activated macrophages and eosinophils[211,219] that induce beiging through enhanced IL-4 release[220]. In terms of therapeutics developed in relation to targeting WAT beiging, the majority of these beiging factors are still in the preclinical stage (TABLE 3). With regard to FGF21, despite two human clinical trials reporting no significant differences in glycaemic endpoints[155,156] (see above), FGF21 analogues still hold great therapeutic potential, as recent studies reveal that FGF21 promotes beiging of WAT, by inducing UCP1 in mice[153] and in human primary adipocytes[209]. For instance, in mice, a humanized bi-specific antibody that activates the FGFR1–β-klotho complex[221] increases BAT energy expenditure and beiging of WAT, promotes weight loss and improves insulin resistance, hyperglycaemia, dyslipidaemia and hepatosteatosis[221]. The proposed mechanisms by which FGF21 induces WAT beiging entail both adipose-derived FGF21 upregulating UCP1 expression locally through PGC1α[153] and central regulation through activation of the sympathetic nervous system[222]. However, two recent studies challenged the view that WAT beiging underlies the FGF21-mediated pharmacological effects, proposing UCP1- and beiging-independent antidiabetic and anti-obesogenic effects of FGF21-based treatments[223,224]. Future studies with FGF21 analogues should address the relative contribution of central versus peripheral WAT beiging on mediating appetite, energy expenditure and glucose-lowering effects. Furthermore, examining whether the antidiabetic effects of FGF21 are more evident in patients with greater BAT content will be of great interest.

Intestinal-restricted agonism of the bile acid sensor FXR also evokes WAT beiging and has been suggested as a new gut-based strategy to treat obesity and insulin resistance through nuclear receptor FXR activation[216]. The FXR agonist fexaramine effectively lowers body weight gain, systemic inflammation and hepatic glucose output and promotes thermogenesis and WAT beiging[216]. However, how fexaramine performs in human clinical trials is yet be determined. Recent studies have also identified a role for immune cells, specifically alternatively-activated macrophages and eosinophils, in beiging WAT. Alternatively-activated macrophages enhance the release of catecholamines and eosinophils to promote the secretion of IL-4 and IL-13, which together activate β-adrenergic signalling and thermogenesis[219]. However, how such adipose–immune crosstalk applies to obese human patients with T2DM has not been investigated.

Finally, a novel BMP7–RHO-associated protein kinase (BMP7–ROCK) signalling axis was recently identified that controls the development of beige adipocytes in WAT through regulation of a globular actin-regulated transcriptional co-activator circuit, referred to as myocardin-related transcription factor A–serum response factor (MRTFA–SRF)[225]. MRTFA-deficient mice exhibit WAT beiging and are protected from diet-induced obesity and insulin resistance[225]. The availability of MRTFA- and ROCK-specific inhibitors (such as CCG-203971 and fasudil, respectively) may offer a possible pharmacological approach, although their specificity and side effects have not been investigated in detail. Future challenges in developing therapies entail identifying pharmacological agents that achieve positive metabolic outcomes in a sWAT- or BAT-specific manner without side effects on the cardiovascular system or stimulation of metabolic compensation.

## Modulation of PPARγ signalling

The PPARs (PPARα, PPARδ (also known as PPARβ) and PPARγ) are members of the nuclear receptor superfamily of transcription factors that regulate multiple gene networks, including those of lipid metabolism, adipogenesis, adipokine production and inflammation[226]. PPARγ2 in particular is abundant in WAT and BAT depots and functions as a dominant regulator of adipogenesis and a powerful modulator of lipogenic pathways and insulin sensitivity[18,226].

Although TZDs are potent antidiabetic drugs, their adverse effects (TABLE 2) have promoted research towards developing the next generation of PPARγ-specific therapeutics. Recent studies have shed light on the complexities of PPARγ signalling and have provided new molecules and post-translational modifications to target for pharmacological intervention (TABLE 3). Current efforts are focused on elucidating crucial downstream mediators of PPARγ activation to avoid global activation of the pathway, thereby avoiding some of the negative side effects.

***SIRT1–CDK5–ERK–PPARγ axis.*** PPARγ is phosphorylated at Ser273 by cyclin-dependent-like kinase 5 (CDK5)[227], a protein kinase induced by pro-inflammatory signals[228]. This phosphorylation is activated in fat-depots of HFD-fed mice, where it causes the dysregulation of a subset of genes that are instrumental to metabolic homeostasis, such as adiponectin (*Adipoq*) and adipsin (*Cfd*)[227]. The CDK5-induced modification of PPARγ is blocked by antidiabetic PPARγ ligands[227], highlighting an alternative strategy for addressing the transcriptional dysregulation in obesity. Expanding on this, enhanced compensatory extracellular signal-regulated kinase (ERK)-mediated PPARγ phosphorylation at Ser273 in adipose-specific *Cdk5*-knockout mice has been reported, resulting in glucose intolerance and severe insulin resistance[229]. Importantly, CDK5 can block ERK action through the ERK kinase MEK. Pharmacological inhibition of ERK and MEK corrected the metabolically unhealthy phenotype of *Cdk5*-knockout mice and improved insulin sensitivity in diabetic *ob/ob* mice[229]. This study identifies MEK inhibitors as novel insulin-sensitizing agents that signal through PPARγ in WAT. Although MEK inhibitors are yet to be used in human clinical trials in obese patients with diabetes, the FDA has approved the GlaxoSmithKline-developed MEK inhibitor trametinib in combination with dabrafenib (an inhibitor against the oncogene *BRAF*) for the treatment of patients with metastatic $BRAF^{V600}$ melanoma (with $BRAF^{V600E}$ and $BRAF^{V600K}$ mutations)[230].

SIRT1 is a nuclear $NAD^+$-dependent class III histone deacetylase that directly interacts with PGC1α[231] and oestrogen-related receptor-α (ERRα)[232]. In adipose, SIRT1 hinders adipocyte differentiation, mediates lipolysis and dampens pro-inflammatory events through direct suppression of PPARγ and nuclear factor-κB (NF-κB) activity[233]. Selective adipocyte deletion of *SIRT1* exerts protective metabolic effects during chronic HFD feeding, specifically through reduction of CDK5 activity and a decrease in Ser273 PPARγ phosphorylation[234]. Consequently, *Sirt1*-knockout mice exhibit enhanced PPARγ-driven adipogenesis and improved insulin sensitivity[234]. SIRT1 downregulation in WAT may play a key part in PPARγ-mediated resolution of obesity-induced inflammation and serve as a promising candidate for therapeutic intervention. Furthermore, a SIRT1-dependent PPARγ-mediated mechanism of WAT beiging has been reported[235]. SIRT1 promoted deacetylation of PPARγ on Lys268 and Lys293, which recruited the BAT programme co-activator PRDM16 to PPARγ, resulting in upregulation of BAT-specific genes[235].

Sirtris Pharmaceuticals and GlaxoSmithKline developed a proprietary formulation of the SIRT1 activator resveratrol termed SRT-501, but clinical trials were terminated owing to safety concerns[236]. However, resveratrol has advanced to Phase II clinical trials[237–239], with high doses of resveratrol improving hypertriglyceridaemia[240] and suppressing postprandial glucagon levels[241] in obese patients. Although these studies reported no marked augmentation in insulin sensitivity or glycaemic index[240,241], others have demonstrated significant improvements in insulin resistance[242] and urinary oxidative stress in patients with T2DM[243].

***PPARγ–FGF21 signalling pathway.*** In the fed state, PPARγ-activating TZDs upregulate FGF21 in the WAT of *db/db* mice[142,244] and in cultured adipocytes[245]. Conversely, adipose-derived FGF21 acts locally in a feed-forward loop to increase PPARγ transcriptional activity by suppressing its sumoylation at Lys107, enhancing adipogenesis and insulin sensitivity[246]. The recently identified PPARγ–FGF21 connection pinpoints FGFs as important new mediators of the beneficial actions of TZDs.

***PPARγ in WAT immune cells.*** PPARγ also has a crucial role in macrophage functions, particularly with regard to anti-inflammation and maintenance of lipid homeostasis[247]. This role was established in mice harbouring a macrophage-specific ablation of *Pparg* that exhibited impaired maturation of anti-inflammatory M2 macrophages and whole-body insulin resistance[248]. Invasion of vWAT by M1 pro-inflammatory macrophages is a crucial event driving WAT inflammation and insulin resistance. A unique population of vWAT-resident $CD4^+$ $FOXP3^+$ T regulatory ($T_{reg}$) cells regulate the degree of WAT inflammation and thus affect insulin sensitivity[249]. These vWAT-based $T_{reg}$ cells exhibit anti-inflammatory properties and express high levels of PPARγ[250]. A mouse model of $T_{reg}$ cell-specific ablation of *Pparg* displays a compromised response to the TZD pioglitazone[250], thus pinpointing PPARγ, among many other functions, as a crucial orchestrator of vWAT $T_{reg}$ cell function. Beyond the direct effects of TZDs on these cells, these findings highlight $T_{reg}$ cells as worthwhile targets to further explore antidiabetic therapeutic approaches.

## Adipose tissue inflammation, fibrosis and hypoxia

Obesity-induced activation of low-grade inflammation contributes to insulin resistance and exacerbates the progression of T2DM[59]. Key components of the innate immune signalling cascade inhibit multiple steps of the insulin signalling pathway. In obesity, enhanced activation of NF-κB and specific protein kinases (inhibitor of NF-κB kinase subunit-β (IKKβ), IKKε, Jun *N*-terminal kinase (JNK) and protein kinase Cγ (PKCγ)) suppress insulin signal transduction directly at the level of insulin receptor substrate 1 (IRS1), AKT or GLUT4 (REF. 251), or indirectly through induction of the toxic lipid ceramide, which inactivates AKT[252]. The net effect results in insulin resistance, and as such, these inflammatory factors are considered attractive therapeutic candidates for obesity. Recent studies also pinpoint a novel role for the insulin gene (*INS*) in impairing insulin sensitivity, by specifically driving obesity-related WAT inflammation[253]. A streptozotocin-induced reduction in systemic insulin levels in DIO mice and genetically obese mice was shown to markedly lower adipose tissue macrophage content and promote insulin-stimulated AKT phosphorylation[253]. Recent studies have also highlighted the use of adipose-derived stem cells in regenerative medicine, given their anti-inflammatory and antifibrotic potential (BOX 2). In contrast to the notion that chronic inflammation induces insulin resistance, some degree of initial inflammation exerts a beneficial impact on WAT

Box 2 | **Use of adipose-derived stem cells for regenerative interventions**

The characterization and clinical use of adipose-derived stem cells (ASCs) in regenerative medicine is currently gaining substantial interest. White adipose tissue (WAT) is enriched with multipotent ASCs that possess the capacity to proliferate, self-renew and differentiate along multiple lineage pathways, such as the osteogenic, adipogenic and chondrogenic lineages[308]. For example, during wound healing, ASCs at an ischaemic site can robustly secrete an array of cytokines, angiogenic factors, anti-apoptotic factors, growth factors and antioxidants in a paracrine manner. Such ASCs can also exert immune-modulatory and re-vascularization properties that enhance recovery of the injured tissue[309]. Interestingly, direct intravenous injection of syngeneic ASCs effectively lowers blood glucose levels and enhances glucose tolerance in diet-induced obese (DIO) mice[310]. Similarly, the delivery of ASCs derived from lean mice to DIO mice attenuates WAT inflammation and improves insulin action[311]. Recent studies also show an antifibrotic role for ASCs in an innovative anti-scarring therapy, by ameliorating collagen deposition to reduce hypertrophic scar formation[312], in addition to a role in ischaemic heart disease[313]. Therefore, ASCs hold tremendous cell-based reparative therapeutic potential and are prime candidates for regenerative intervention aimed at repairing and regenerating tissues.

homeostasis. During the onset of obesity, local adipocyte pro-inflammatory signalling is essential to sustain the adipogenic programme *in vivo* and allow the remodelling and expansion of healthy WAT[65].

***Pro-inflammatory-driven insulin resistance.*** In rodents, knockout models of innate immune factors or anti-inflammatory treatments have been demonstrated to dampen inflammation-driven insulin resistance. However, successful use of anti-inflammatory agents to ameliorate insulin resistance in obese patients with diabetes in clinical trials is yet to be fully confirmed. Several avenues currently under investigation hold promise for the use of anti-inflammatories to treat obesity-driven T2DM (TABLE 3).

Amlexanox is a selective small-molecule inhibitor of the innate immune system proteins noncanonical IKKε and TANK-binding protein 1 (REF. 254) , which has previously been used to treat patients with asthma and allergic rhinitis[255]. Administration of amlexanox to DIO mice or genetically obese mice improves insulin sensitivity, promotes weight loss through enhanced energy expenditure and ameliorates WAT inflammation and hepatic steatosis[254]. More recently, amlexanox treatment was shown to promote IL-6 secretion from adipocytes (FIG. 3) by increasing cAMP levels in the sWAT depot of obese mice; consequently, IL-6 stimulates phosphorylation of hepatic STAT3 to suppress gluconeogenesis[256]. Because of its long history of use and good safety profile in patients with asthma, further clinical investigation into the use of amlexanox as a therapeutic agent for obesity and T2DM seems warranted. Indeed, the efficacy of amlexanox is currently being investigated in a Phase II clinical trial in obese patients with T2DM[257] (TABLE 3) and preliminary data show a significant correlation between IL-6 levels and improvements in the homeostasis model assessment–insulin resistance (HOMA–IR) score[256].

Neutralizing antibodies or antagonizing compounds that target pro-inflammatory cytokine signalling have also provided some therapeutic benefit. Immunoneutralization of TNF with the monoclonal antibody etanercept significantly reduces fasting glucose levels in obese patients[258] and lowers systemic inflammatory markers in obese patients with diabetes[259]. Similarly, administration of the anti-TNF monoclonal antibody infliximab to patients with rheumatoid arthritis improves insulin resistance[260]. However, it seems unlikely that these therapeutic avenues will be applicable to all people with diabetes, as these beneficial effects have not been reported in all studies. For instance, both short-term and chronic neutralization of TNF may lead to a lowering of systemic inflammatory markers, but, importantly, both neutralization methods failed to improve insulin sensitivity across a range of different patient populations[259,261].

Another anti-inflammatory approach being investigated is the use of salsalate, an analogue of salicylate that suppresses the production of TNF, IL-6 and C-reactive protein[262]. Salsalate reached Phase III in the Targeting Inflammation Using Salsalate for Type 2 Diabetes-stage II (TINSAL-T2D-II) clinical trial, which reported improved glycaemia in patients with T2DM[263], and Phase IV in a clinical trial in obese patients[264] (TABLE 3). Treatment of obese non-diabetic patients with salsalate improves glycemia[264] and insulin clearance[265], increases systemic concentrations of adiponectin and lowers $HbA_{1C}$ levels[266]. However, no marked effects on insulin sensitivity or secretion have been reported in people treated with salsalate[267], nor have any statistically significant differences in the incidence of T2DM in individuals treated with aspirin been reported[268]. Collectively, the results obtained so far in this area indicate that blockade of the innate immune pathway is not a viable therapeutic approach.

Dietary fish oil supplements — specifically the major natural constituents in fish oil, the omega-3 fatty acids docosahexaenoic acid and eicosapentaenoic acid — exert potent anti-inflammatory actions by signalling through the lipid-sensing GPR120 (REF. 59). Treatment of DIO mice with an omega-3 fatty acid-enriched diet reduces GPR120-mediated infiltration of pro-inflammatory adipose tissue macrophages into WAT and enhances insulin sensitivity[269], indicating that fish oil supplements provide substantial metabolic health benefits in rodents. However, to reach a substantial and beneficial GPR-induced anti-inflammatory effect, fish oil supplements would need to be consumed in large quantities in humans. A promising alternative to fish oil treatment is a high-affinity, GPR120-selective small-molecule agonist (cpdA). CpdA administration to obese mice yields robust anti-inflammatory and insulin-sensitizing effects[270]. Although murine studies show great therapeutic potential, there are no GPR120 agonists in human clinical trials.

Pharmaceutical interventions that block macrophage chemotaxis into WAT have also garnered attention based on their promising experimental and clinical antihyperglycaemic and insulin-sensitizing effects[271,272]. C-C chemokine receptor 2 (CCR2) is essential for the migration of macrophages into inflamed tissues. The orally administered novel CCR2 antagonist CCX140-B, which is currently in Phase II clinical trials, effectively lowers $HbA_{1C}$ levels, improves glycaemic parameters and exerts

renoprotective effects in patients with T2DM[273] (TABLE 3). Similarly, treatment of patients with T2DM with the CCR2 antagonist JNJ-41443532 modestly improved glycaemic parameters[272]. Phase II clinical trials have also been performed using the CCR2 antagonist BMS-741672 in patients with T2DM[274] and the Pfizer-sponsored CCR2 antagonist PF-04634817 in patients with diabetic nephropathy[275] (TABLE 3). However, no results are currently available for these two trials. Furthermore, Pfizer also generated the CCR2 antagonist PF-4136309 (REF. 276), but results from human clinical trials with patients who are obese or have diabetes have not been reported.

Another promising chemokine-promoting target is the pro-inflammatory chemotactic eicosanoid leukotriene $B_4$ ($LTB_4$), which signals through its receptor LTB4R1 (REF. 277). Pharmacological treatment of obese mice with the LTB4R1 inhibitor CP-105696 results in an anti-inflammatory phenotype, robust insulin-sensitizing effects and protection from hepatic steatosis[278]. This compound has been successfully validated in one human clinical trial in healthy male volunteers[279]; however, it has not been validated in the context of obesity and T2DM. Taken together, these results suggest that altering chronic WAT inflammation to alleviate obesity-driven insulin resistance and perhaps other sequelae of overnutrition seems to be a potentially viable strategy in rodents, which warrants further investigation in humans. Whether therapeutically disrupting specific inflammatory signalling pathways will be complicated by increased risk for additional infections, as has been observed with anti-TNF monoclonal antibodies[280], remains to be determined.

***Adipose tissue fibrosis and hypoxia.*** As previously discussed, the ECM is crucial to the architecture and function of WAT expandability[4,7]. Fibrosis, a disease process that results from disturbance of the ECM owing to an imbalance in collagen synthesis and degradation, is a hallmark of dysfunctional WAT in the obese state[7]. Although there is currently no drug treatment to reverse WAT fibrosis in obesity, novel potential pro-fibrotic targets have emerged (FIG. 3; TABLE 3).

Endotrophin, a carboxy-terminal cleavage product with a C5 domain of collagen VIα3, functions as a novel obesity-associated adipokine to promote malignant tumour progression[281]. Endotrophin is also a powerful co-stimulator of WAT fibrosis and metabolic dysfunction[282]. Mice overexpressing endotrophin specifically in adipocytes exhibit enhanced WAT fibrosis, inflammation and insulin resistance[282]. Conversely, blockade of endotrophin by neutralizing antibodies protects mice from metabolic dysfunction by reversing WAT fibrosis and improving insulin sensitivity[282]. Anti-endotrophin antibodies therefore harbour therapeutic potential for drug intervention.

Platelet-derived growth factor receptor-α (PDGFRα) signalling also triggers WAT fibrosis by targeting progenitor cell plasticity[283]. In particular, enhancing PDGFRα activity in perivascular cells through an mTOR-dependent pathway transforms these cells into ECM-producing pro-fibrotic cells, which ultimately leads to fibrotic WAT[283]. Future studies using genetic or pharmacological models of PDGFRα signalling inhibition in perivascular cells should prove to be exciting.

As discussed above, specific inhibitors for MRTFA and ROCK promote WAT beiging[225]. In addition, MRTFA and ROCK inhibitors (such as CCG-203971 and fasudil, respectively) may target myofibroblasts in the context of ECM production, thereby potentially acting as effective antifibrotic agents. In fact, recent studies have demonstrated potent antifibrotic actions of these inhibitors in murine models of skin injury[284] and lung fibrosis[285]. Although ROCK1 inhibition is at a preclinical stage for the treatment of obesity and T2DM[286], fasudil has reached Phase II in clinical trials focused on vascular function in patients with atherosclerosis and hyperlipidaemia[287] and Phase III as a vasodilator to treat Raynaud disease[288]. Delineating the role of MRFTA and ROCK in WAT fibrosis and whole-body insulin sensitivity under metabolic challenges warrants further attention.

Finally, a potential role for Toll-like receptor 4 (TLR4) in obesity-associated WAT fibrosis has been indicated[289]. Lipopolysaccharide- or fatty acid-activated macrophages were shown to secrete TGFβ, which potentially affects myofibroblasts to result in ECM deposition[289]. These results were based on bone marrow transplants. However, recent data using macrophage-specific *Tlr4*-knockout mice failed to reveal such a phenotype[290], suggesting the involvement of other immune-related cell types in this process.

Similar to tumour growth, obese WAT quickly outgrows its vasculature to form a state of hypoxia, which, as an adaptive response, upregulates levels of the transcription factor HIF1α[4,71]. In WAT, HIF1α induction triggers a transcriptional programme that enhances local ECM constituents, fibrosis and inflammation[68,71]. Treatment of DIO mice with PX-478, a selective HIF1α inhibitor established in tumour models, effectively suppresses HIF1α to increase energy expenditure, reduce body weight gain and alleviate WAT fibrosis and inflammation[72]. These effects were confirmed using WAT-specific, inducible dominant-negative *Hif1a* mutant mice challenged with a HFD[72]. As tumour hypoxia is a recognized hallmark of solid tumours, therapeutic cancer studies have targeted direct HIF1α inhibition in tissue culture models, rodents and Phase I clinical trials in patients[291]. For instance, PX-478 hinders the growth of multiple types of tumours[292]. Similarly, the HIF1α inhibitor NXC-124754 displays antitumour activity in human prostate cancer cells and in orthotopic tumour-bearing mice[293]. In fact, there are numerous inhibitors for HIF1α that target HIF1α expression, dimerization and transcriptional activity[294]. Targeting HIF1α in WAT harbours promising therapeutic potential. However, it will be important to achieve adipose tissue selectivity, as secondary consequences of HIF1α systemic inhibition include altered haematocrits, although these effects may be transient in nature[292]. For these reasons, no HIF1α inhibitors have been used in human clinical trials in the context of obesity and T2DM to date. Given that fibrosis is difficult to target specifically, further understanding of the key mechanisms that drive WAT fibrosis is vital for the future development of this therapeutic approach.

### Adipose tissue mitochondrial dysfunction

Mitochondrial dysfunction includes reduced mitochondrial mass and ATP production, and overproduction of reactive oxygen species (ROS), and it is a substantial contributor to insulin resistance[295]. The role for mitochondria in WAT has long been underappreciated, partly owing to the relatively low abundance of this organelle in WAT compared with that in the heart tissue and BAT. However, the rationale for targeting WAT mitochondria with anti-obesogenic drugs for therapeutic benefit lies in the fact that mitochondria are essential for maintaining metabolic homeostasis in adipocytes, given their crucial roles in adipogenesis, fatty acid synthesis and esterification, and lipolysis[6]. Indeed, loss of mitochondrial DNA (mtDNA), reduction in the number of mitochondria and impaired mitochondrial respiration have been reported in adipocytes from *db/db* mice[296]. In humans, sWAT from obese individuals displays a downregulation in mtDNA, mitochondrial oxidative genes, the oxidative phosphorylation machinery and fatty acid oxidation, with the latter inversely correlating with insulin resistance[297]. In obese individuals with T2DM, sWAT exhibits enhanced ROS production and oxidative damage, which are associated with a reduction in the mitochondrial antioxidant enzymes superoxide dismutase and glutathione peroxidase[298]. Therefore, drugs that target mitochondrial biogenesis and prevent mitochondrial dysfunction and oxidative damage in WAT are conceptually viable approaches to treat insulin resistance, although the challenge is, as in many other cases, how to achieve this in a selective, WAT-specific way.

Several drugs and nutritional compounds can directly or indirectly target mitochondrial function by stimulating mitochondrial biogenesis. TZDs (such as pioglitazone or rosiglitazone) upregulate PGC1α expression levels, resulting in an increased mtDNA copy number, enhanced mitochondrial biogenesis and increased oxidative capacity in adipocytes, leading to enhanced insulin sensitization[299]. Several natural compounds such as (*R*)-α-lipoic acid, acetyl-L-carnitine and coenzyme $Q_{10}$ enhance mitochondrial biogenesis[300,301]. In 3T3-L1 adipocytes, treatment with (*R*)-α-lipoic acid and acetyl-L-carnitine increases mitochondrial mass, expression of mtDNA and mitochondrial protein complexes and oxygen consumption, as well as stimulating fatty acid oxidation[302]. Obesity-related insulin resistance can be treated with mitochondrial antioxidant drugs or mitochondrial ROS scavengers. Clinical data suggest that antioxidant treatments, such as vitamin E, vitamin C, *N*-acetylcysteine and glutathione, improve insulin sensitivity in patients with diabetes[6,300,301]. Chemical uncouplers generate energy as heat and reduce ATP production by increasing proton leaks. As discussed above, controlled and specifically targeted mitochondrial uncoupling is another attractive pharmacological approach[205].

Several recently identified mitochondrial proteins may represent future therapeutic targets. For instance, mitoNEET, when overexpressed in adipocytes, compromises mitochondrial iron homeostasis and function, which forces the cell to unleash a compensatory mechanism that upregulates PGC1α, PPARγ and adiponectin. This compensatory mechanism enhances mitochondrial biogenesis and improves whole-body insulin sensitivity[218,303]. Furthermore, a WAT-specific deletion of *Tfam* (the gene encoding mitochondrial transcription factor A) in mice increases mitochondrial fatty acid oxidation and whole-body energy expenditure, which protects mice from diet-induced obesity, hepatosteatosis and insulin resistance[304]. Although there are currently no drugs that specifically target WAT mitochondria in human clinical trials, this approach holds promise for the future treatment of obesity and T2DM.

### Summary and conclusions

We live in exciting times with respect to target discoveries in the field of obesity and diabetes. The ability to analyse ever-larger 'omics' datasets with increasing levels of sophistication has led to an unprecedented number of new targets that are being investigated in the diabetes field. The realization that central brain-derived signals play a major part in energy homeostasis has found widespread acceptance, and targeted manipulation of crucial signalling pathways in specific neurons represents a powerful approach to control obesity. However, the tight coupling of central processes with hedonic pathways reflects our 'hard-wired' behavioural drives that — when interrupted — lead to depression and other unwanted neurological side effects. This makes this area of research particularly difficult to translate into patients. How to effectively manipulate the central 'lipostat' without affecting mood remains a key challenge. Peripheral targets, such as adipose tissue, therefore represent promising alternatives to the manipulation of central pathways. The growing appreciation for adipose tissue expansion as a protective response to store lipids in a tissue specialized to do so, rather than depositing excess calories in other tissues that are much more subject to lipotoxic side effects, effectively alters how we think about therapeutic approaches. Although the removal of adipose tissue across the board through apoptosis or targeted liposuction does not appear to be a wise approach, increasing energy expenditure by activating futile cycles in the periphery seems more tangible. However, if these futile cycles result in the production of heat, we must learn how to avoid excessive local heat production to allow for a broader therapeutic dose of such compounds.

An alternative antidiabetic approach may be to use excess energy for the biosynthesis of molecules that are non-toxic endogenous substances, which can easily be disposed of through release through the gut or in urine. The SGLT2 inhibitors are in that sense the proof of principle, but additional metabolites may be identified that may be suitable for such an approach. The possibility of directly activating a futile biosynthetic cycle at the level of the adipocyte warrants investigation. Although we may therefore be able to dispose of excess energy in the periphery by exercise or by stimulating heat production, excess glucose disposal or futile biosynthetic cycles, we must be prepared for the fact that any increase in peripheral energy expenditure will likely be compensated with enhanced food intake or (minimally) with increased metabolic efficacy.

Lipostat
A mechanism by which food intake and energy expenditure are regulated, such that an organism's existing fat mass is maintained.

At present, the most promising areas for intervention with regard to adipose tissue seem to be adipokine related, whereby low molecular-weight agonists for beneficial pathways stimulated by adiponectin, and potentially even leptin, may change the way we approach anti-diabetic treatments. Opportunities remain in the area of insulin-sensitizing downstream targets of PPARγ. Furthermore, attempts to induce additional beiging transitions in WAT bear great promise for future antidiabetic regimens. Finally, antihypoxic approaches that ultimately aim to reduce fibrosis display great potential. In light of the important role the adipocyte exerts on all processes related to systemic metabolic homeostasis, adipose tissue remains a prominent target for new antidiabetic therapeutics.


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### Competing interests statement
The authors declare no competing interests.

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