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Metabolic adaptation and maladaptation in adipose tissue

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Adipose tissue possesses the remarkable capacity to control its size and function in response to a variety of internal and external cues, such as nutritional status and temperature. The regulatory circuits of fuel storage and oxidation in white adipocytes and thermogenic adipocytes (brown and beige adipocytes) play a central role in systemic energy homeostasis, whereas dysregulation of the pathways is closely associated with metabolic disorders and adipose tissue malfunction, including obesity, insulin resistance, chronic inflammation, mitochondrial dysfunction, and fibrosis. Recent studies have uncovered new regulatory elements that control the above parameters and provide new mechanistic opportunities to reprogram fat cell fate and function. In this Review, we provide an overview of the current understanding of adipocyte metabolism in physiology and disease and also discuss possible strategies to alter fuel utilization in fat cells to improve metabolic health.

The human body has the remarkable ability to adapt to internal and external changes, including nutritional status, environmental temperature, infection, circadian rhythm, ageing, and more. These adaptations involve dynamic reprogramming of cellular metabolism in the peripheral tissues. For example, caloric restriction promotes mitochondrial biogenesis and fatty acid oxidation, thereby shifting cellular metabolism preferentially toward fatty acid oxidation rather than glucose oxidation in the mitochondria[1,2]. Similarly, cold exposure is associated with metabolic adaptation in skeletal muscle and adipose tissue by inducing a switch from carbohydrate metabolism to fatty acid oxidation[3,4]. From an evolutionary viewpoint, such metabolic shifts are considered to be protective mechanisms to secure enough glucose for the brain, while other fuels, such as fatty acids and amino acids, are allocated to the peripheral metabolic organs.

A main player during such adaptive processes, adipose tissue is a highly dynamic organ that can rapidly remodel in response to environmental inputs. White adipose tissue quickly expands by increasing lipid storage (hypertrophy) and by increasing the number of adipocytes (hyperplasia) that can be observed within days after initiation of an obesogenic diet, whereas involution of adipose tissue occurs within 24 hours of fasting[5,6]. On the other hand, thermogenic fat cells, such as brown adipocytes and beige adipocytes, swiftly adapt to utilize systemic fuels to drive thermogenesis. Systemic cues, such as norepinephrine released from the sympathetic nerve system (SNS), rapidly activate thermogenic fuel utilization. Moreover, beige adipocytes can transform into energy-storing white adipocytes within days after external stimuli are withdrawn[7,8]. These observations indicate that adipocytes are extraordinarily adaptive to the environment, as would be expected of cells that coordinate fuel storage and futile oxidation of stored energy.

By contrast, maladaptation in adipose tissue is tightly associated with the development of metabolic disorders, including insulin resistance, dyslipidaemia, hepatic steatosis, and type 2 diabetes. Chronic inflammation in white adipose tissue is induced by excess caloric intake, leading to insulin resistance and subsequent fat cell dysfunction[9,10]. Dysregulation of mitochondrial biogenesis and oxidative phosphorylation (OXPHOS), excess accumulation of extracellular matrix (ECM), and altered adipokines and lipid profile are strongly associated with adipose tissue malfunction and metabolic disorders. Here we review the current understanding of adipocyte metabolism and discuss strategies to reprogram fat cell fate and metabolism.

Regulatory pathways of adipocyte metabolism

Regulation of lipogenesis. Adipocytes are integrated into systemic lipid metabolism with factors that control the uptake of free fatty acids from the circulation. Circulating chylomicron- and very-low-density lipoprotein (VLDL)-bound triacylglycerols (TAG) interact with lipoprotein lipase (LPL), which hydrolyses circulating TAG to free fatty acids and monoacylglycerols that are taken up by adipocytes. CD36 (also known as fatty acid translocase (FAT)) is the principal fatty acid transporter in adipocytes that facilitates uptake, which depending on circumstance, can be either catabolized or stored[11]. Fat cells are also equipped with machinery capable of synthesizing new lipids from circulating carbohydrates through a process known as de novo lipogenesis (DNL). DNL relies on two key enzymes that are abundant in adipocytes: fatty acid synthase (FAS) and acetyl-CoA carboxylase (ACC), which are transcriptionally regulated by sterol response element binding protein 1c (SREBP1c) and carbohydrate response element binding protein (ChREBP)[12,13] (Fig. 1a).

Compared with lipid accumulation that relies on dietary fat, DNL is energetically costly. The adipocyte relies on two tracks of glucose metabolism to accomplish DNL. Glucose is oxidized to generate mitochondrial acetyl-CoA to fuel mitochondrial citrate synthesis (although citrate can also be synthesized using acetyl-CoA from other carbon sources). Mitochondrial citrate is then exported to the cytosol, where adenosine triphosphate (ATP)-citrate lyase and ACC drive malonyl-CoA production. In parallel, glucose-6-phosphate is also shunted to the pentose phosphate pathway to generate reducing equivalents in the form of NADPH[14]. This energy is required to

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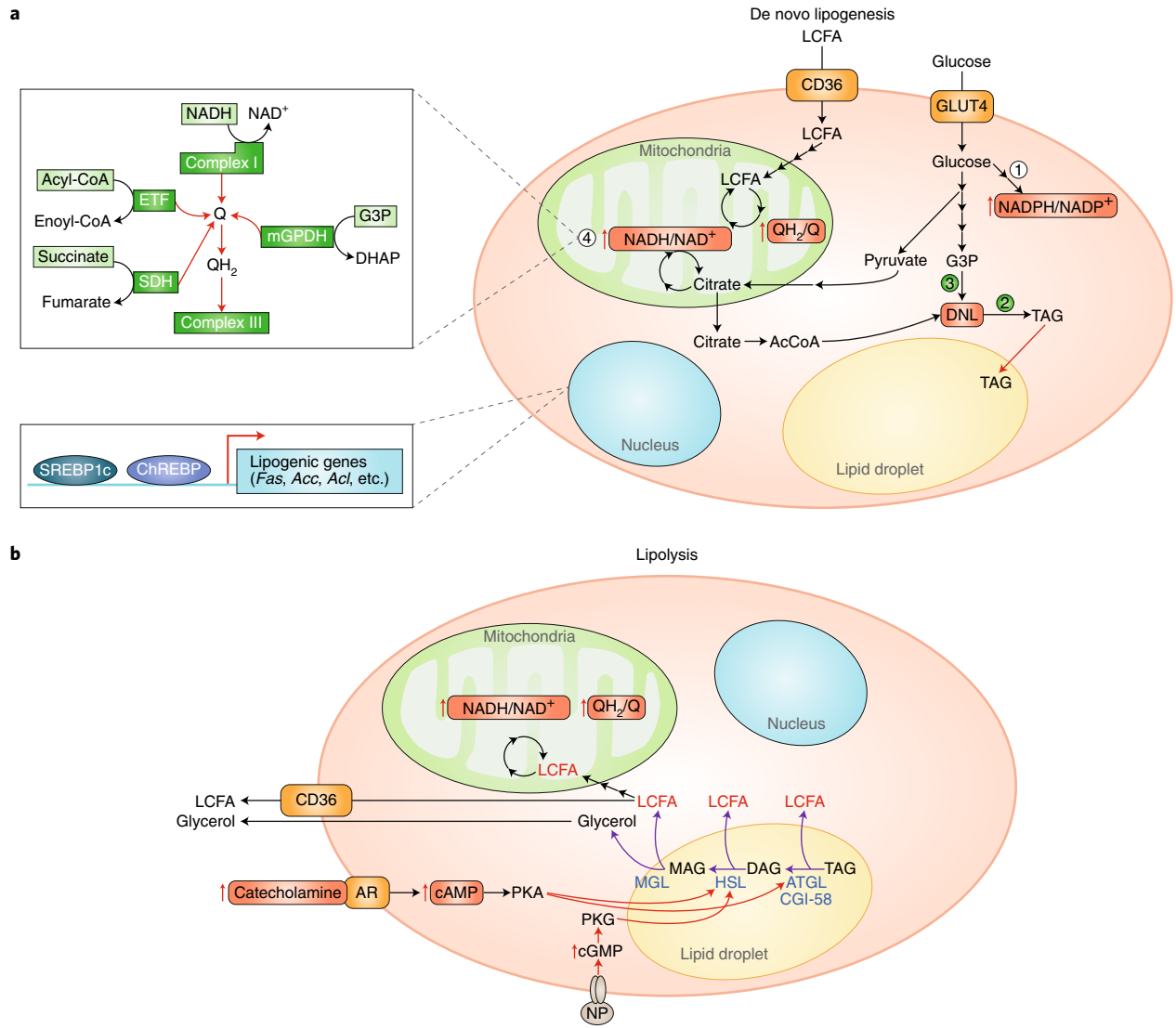

Fig. 1 | Control of fatty acid storage and oxidation. a, Adipocytes access long-chain fatty acids (LCFAs) and glucose from the circulation. Fatty acids can be oxidized by mitochondrial beta-oxidation, which elevates the reduction state of the mitochondrial ubiquinone and NADH/NAD$^+$ pool. Imported glucose drives maintenance of an elevated NADPH/NADP$^+$ ratio (1), which is essential for supporting both TAG synthesis (2) and thiol redox state in the cell. Oxidized glucose also generates G3P, which is required to initiate DNL as the glycerol backbone (3). Citrate, generated by either glucose or fatty-acid-linked metabolism is exported by mitochondria to form cytosolic Acetyl-CoA, which is the building block of the elongated acyl chains of TAG upon DNL. Importantly, high mitochondrial Q and NADH/NAD$^+$ reduction will feedback to inhibit mitochondrial citrate metabolism, and these may act as a metabolic signal to promote citrate export to the cytosol and DNL. Additionally, a high Q reduction state will inhibit oxidation of cytosolic G3P by the mitochondrial glycerophosphate dehydrogenase and similarly may promote DNL (2). An expanded schematic on the left (4) summarizes the major metabolic pathways that share electrons with the mitochondrial Q pool and that therefore are subject to feedback regulation on the basis of its reduction state (that is, the QH_2/Q ratio). These include mitochondrial complex I-mediated oxidation of mitochondrial NADH; mitochondrial oxidation of fatty acids through the electron transferring flavoprotein (ETF); succinate oxidation by succinate dehydrogenase (SDH); and oxidation of cytosolic G3P by the mitochondrial glycerol-phosphate dehydrogenase (mGPDH). In the nucleus, the transcription factors ChREBP and SREBP1c control genes involved in DNL. **b**, Adipocytes are engaged to generate high levels of LCFAs locally via triglyceride lipolysis. This process is initiated by external stimulation, for example by catecholamines followed by elevation in cytosolic cAMP and PKA activation, or by NP followed by elevation in cGMP and PKG signalling. PKA or PKG signalling stimulates liberation of free fatty acids from TAG through endogenous lipases, such as ATGL, HSL, and MGL. Upon liberation, LCFAs are either oxidized locally or released into the circulation to supply fuel to other cells.

fuel the fatty acid synthesis reactions that utilize malonyl-CoA to generate long-chain fatty acids for TAG synthesis. The metabolic intermediates of DNL act as acute regulators of fatty acid utilization in the adipocyte. Cytoplasmic malonyl-CoA is a potent inhibitor of mitochondrial β-oxidation via its inhibition of carnitine palmitoyltransferase 1 (CPT1)[15], and citrate allosterically activates ACC to stimulate malonyl-CoA formation[16]. The NADPH/NADP$^+$ ratio in the adipocyte likely plays a vital role in controlling fatty acid utilization, as will be discussed below.

Fatty acids taken up from the circulation or synthesized de novo are most often directed toward TAG synthesis for storage. This process requires a series of acyltransferases that facilitate stepwise esterification of a glycerol molecule to three free fatty acid molecules. TAG synthesis rates depend on both the levels of free fatty

acids and glycerol-3-phosphate, which acts as the precursor for the TAG glycerol backbone[17]. As such, adipocyte TAG synthesis critically relies on enzymes that produce glycerol-3-phosphate (G3P). To this end, G3P can be generated by glycerol kinase, whereby free glycerol from the circulation or TAG hydrolysis is phosphorylated. However, the dominant pathway in adipocytes is to shunt glucose to produce G3P, which depends on the cytosolic glycerophosphate dehydrogenase (GPD1)[18]. Interestingly, G3P production by this enzyme is controlled by the cytosolic NADH/NAD^+ ratio, while its consumption is controlled by competition between TAG synthesis and mitochondrial oxidation by the mitochondrial glycerophosphate dehydrogenase (GPD2). Since GPD2-mediated oxidation of G3P is inhibited by a reduced mitochondrial respiratory chain, that is, a high ubiquinol/ubiquinone (QH_2/Q) ratio, it is reasonable to speculate that rates of mitochondrial fuel utilization regulate G3P levels and thereby promote TAG synthesis under conditions of nutrient excess.

An essential regulator of DNL is the mammalian target of rapamycin mTORC2, which controls ChREBP-β-dependent transcription of the major enzymes that coordinate fatty acid synthesis[19,20]. This transcriptional program is initiated by mTORC2-dependent glucose flux into adipocytes, which can elevate ChREBP-β expression via glucose-dependent activation of ChREBP-α. This systemic integration between glucose metabolism and adipocyte DNL appears to be a key nutrient-sensing mechanism for coordination of fuel storage and release.

Regulation of lipolysis. On the opposing side of DNL and TAG synthesis is the cellular machinery for fatty acid liberation and oxidation. The dominant mode of free fatty acid liberation in adipocytes is through PKA-dependent lipolysis that is triggered by fasting or cold exposure via increased release of norepinephrine from the SNS[21,22]. Cold stimulus engages cold-sensitive thermoreceptors that transmit afferent signals to the hypothalamus and brain stem, leading to the release of norepinephrine from postganglionic sympathetic nerves that innervate adipocytes[23,24]. Norepinephrine acts on adrenoreceptors on the adipocyte plasma membrane, which ultimately results in the release of free fatty acids from stored triglycerides. The best-characterized effectors of this cascade are β-adrenergic receptors (β-ARs), which elevate intracellular cAMP to drive PKA-dependent phosphorylation of lipases within the adipocyte. The sequential hydrolysis of TAG by critical lipases, including adipose triglyceride lipase (ATGL), hormone-sensitive lipase (HSL), and monoacylglycerol lipase (MGL), mediates the conversion of TAGs to diacylglycerols (DAG), monoacylglycerols (MAG), and eventually fully liberated glycerol and free fatty acids[25]. Following liberation, the fate of free fatty acids depends on fat cell types. From white adipocytes, liberated fatty acids enter the bloodstream and supply peripheral tissues with reducing equivalents for mitochondrial respiration (Fig. 1b). In contrast, thermogenic brown and beige adipocytes can utilize proximal liberated fatty acids to drive uncoupled respiration, as discussed in the next section.

Recent studies also demonstrate that activation of lipolysis by catecholamine release from the SNS can be antagonized by competitive uptake and consumption of the activating molecules. Sympathetic-neuron-associated macrophages (SAMs) possess specialized transporters and monoamine oxidase A (MAOA), which effectively sequester and catabolize local extracellular norepinephrine[26,27]. These negative regulators of adipocyte lipolysis increase in content and activity with age and obesity, while genetic ablation of this pathway is sufficient to elevate adipocyte lipolysis and thermogenic activity.

In addition to cold temperature, other environmental perturbations that elevate local catecholamine levels can affect adipocyte lipolysis and have substantial effects on systemic metabolism. For instance, elevated energy expenditure in response to high-calorie diets (diet-induced thermogenesis) appears to involve local adrenergic signalling in thermogenic adipocytes. In fact, genetically engineered mouse models with defects in β-ARs are predisposed to obesity and exhibit reduced metabolic rate[28]. Moreover, individuals who are predisposed to obesity exhibit decreased adrenergic dependent thermogenic capacity[29], suggesting that this impairment may be relevant in the context of human weight gain.

Regulation of adipose tissue thermogenesis

In contrast to white adipocytes, thermogenic fat cells—brown adipocytes and beige adipocytes— house mitochondria with the distinct capacity to rapidly oxidize fatty acids at extraordinary rates. Their highly active cellular metabolism is the result of unique protein machinery that allows thermogenic adipocytes to engage in futile cycling of metabolites that would typically be stored. The thermogenic oxidation machinery in brown and beige adipocytes is under the regulation of transcriptional factors and co-factors, such as PR domain containing 16 (PRDM16) and peroxisome proliferator-activated receptor gamma coactivator-1α (PGC-1α), which cooperate with DNA-binding transcription factors to coordinately induce mitochondrial biogenesis and fatty acid oxidation that require thermogenesis (Fig. 2a). Transcriptional regulation of brown and beige fat thermogenesis has been recently reviewed[30,31].

Mechanisms of canonical thermogenesis. In response to cold exposure, norepinephrine from the SNS primarily acts on β1-AR and β3-AR, leading to the activation of PKA and p38MAPK signalling. Natriuretic peptides (NP; for example, atrial NP (ANP) and brain NP (BNP)) also activate adipose tissue thermogenesis through PKG signalling[32]. A new study showed that acetylcholine acts on the cholinergic receptor (CHRNA2) and activates beige fat biogenesis via the cAMP–PKA pathway[33]. The PKA–cAMP signalling pathway is known to activate many thermogenic genes in the nucleus, including *Ucp1* and *Pgc1a*[34].

The best-characterized mechanism of adipose thermogenesis is via uncoupling protein 1 (UCP1), a mitochondrial inner membrane protein that can dissipate the proton gradient across the mitochondrial lipid bilayer. The result is that the mitochondrial membrane potential can no longer be used for ATP synthesis. This uncoupling alleviates the inhibition of respiration that typically results from an elevation of the ATP/adenosine diphosphate (ADP) ratio in most cells. The result is a substantial elevation in the rate of respiration, substrate oxidation, and release of heat.

A well-characterized regulatory circuit for UCP1-dependent respiration is the competitive interaction between fatty acids and purine nucleotides. UCP1 is bound by purine nucleotides, the dominant species in the adipocytes being ATP. At concentrations that exist in the cell, this interaction appears to be sufficient to inhibit UCP1 proton current substantially. However, elevated local concentrations of long-chain fatty acids (longer than eight carbon atoms) overcome purine nucleotide inhibition and drive H^+ leak through a fatty acid/H^+ symport mechanism[35]. This competitive interaction between long-chain fatty acids and purine nucleotides occurs upon adrenergic stimulation and elevation of free fatty acid levels in thermogenic adipocytes, although structural insight into this competition remains unclear. Of note, a recent study has found that purine nucleotide pools in thermogenic adipocytes are dynamic, and modulation of these levels could additionally modify UCP1 activity[36]. This study showed that pharmacological inhibitors of the purine-nucleotide-breakdown pathway significantly inhibit β-adrenergic signal-induced respiration in brown adipocytes and that overexpression of guanosine monophosphate reductase (GMPR) depletes guanosine triphosphate pools and potentiates fatty-acid-dependent respiration through UCP1. These findings are consistent with the fact that purine nucleotides competitively inhibit fatty-acid-dependent leak through UCP1; depleting ATP and ADP pools may alleviate

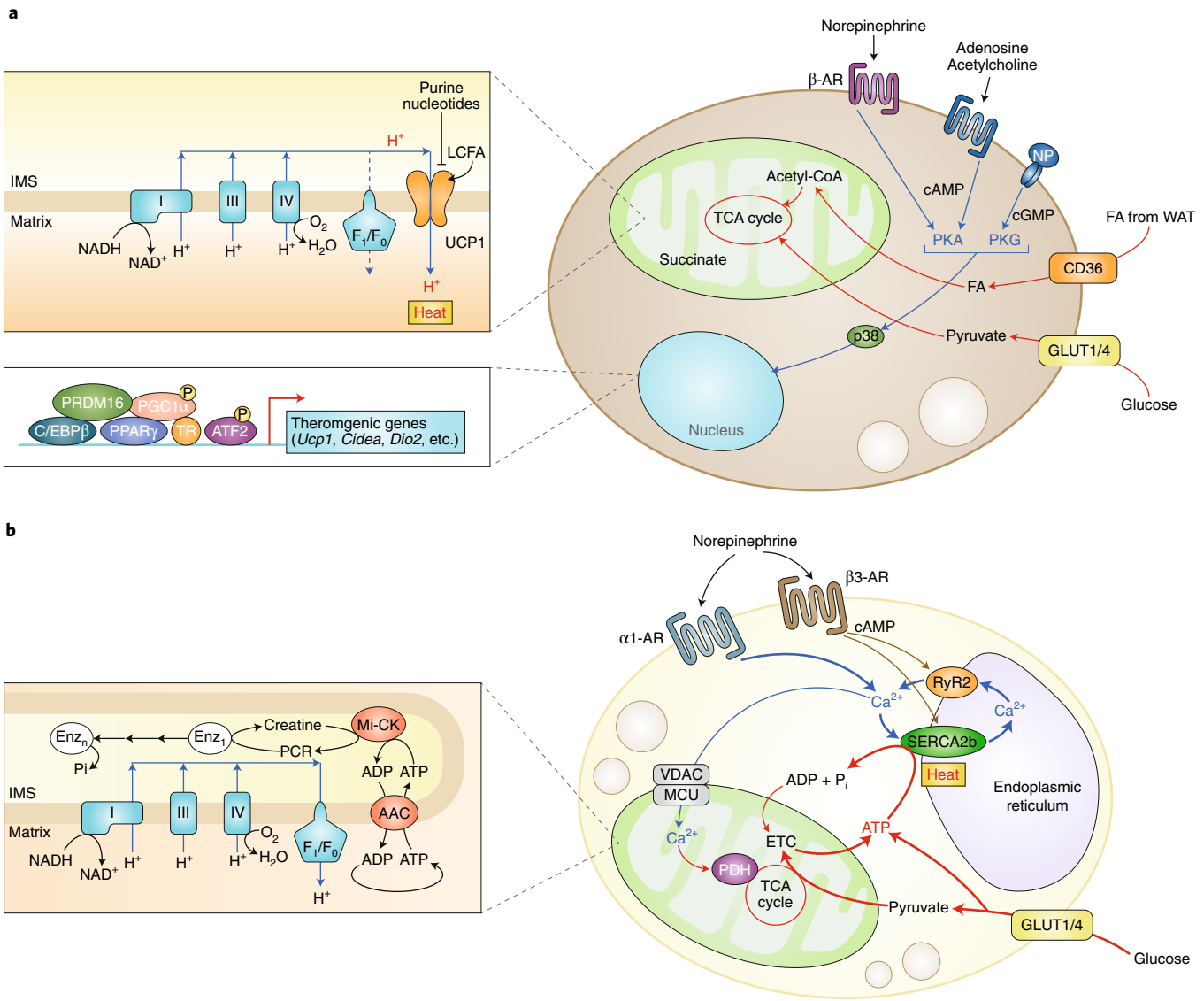

Fig. 2 | Cellular metabolism in thermogenic fat cells. a, UCP1-dependent thermogenesis in brown and beige adipocytes involves active free fatty acid and glucose oxidation. In response to thermogenic stimuli, such as norepinephrine, adenosine, acetylcholine, or NP, the intracellular cAMP or cGMP level is elevated, which triggers PKA or PKG signalling, respectively. p38MAPK (p38) phosphorylates transcriptional regulators, including PGC1a and ATF2, and promote the expression of BAT-specific thermogenic genes (*Ucp1*, *Cidea*, and *Dio2*) and mitochondrial biogenesis. Free fatty acid and glucose are actively imported from the circulation through CD36 and glucose transporter 1 or 4 (Glut1/4), respectively, and are oxidized in the mitochondria for UCP1-dependent thermogenesis. UCP1 activity is inhibited by purine nucleotides, while long-chain free fatty acids bind to UCP1 and stimulate proton uncoupling. **b**, UCP1-independent thermogenesis in beige adipocytes involves Ca^{2+} cycling through the SERCA2b–RyR2 pathway in the sarco/endoplasmic reticulum and subsequent activation of PDH in the mitochondria. In the mitochondria, creatine substrate cycling also generates heat independently of UCP1. Both pathways require active ATP synthesis in the mitochondria (ATP-dependent thermogenesis) and glucose oxidation. FA, fatty acid; P, phosphorylation; P_i, inorganic phosphate.

this inhibition. These observations also suggest that modulation of purine nucleotide pool size in thermogenic adipocytes is an important mode of regulation of UCP1-dependent thermogenesis.

Another mode of regulation of adipocyte metabolism that has emerged more recently is through modification of cellular redox status. Genetic or pharmacological elevation of adipocyte reactive oxygen species (ROS) levels or resulting oxidation of cellular thiols (the major sensitive targets to ROS) can drive elevated thermogenesis[37]. Moreover, activation of thermogenesis in mouse BAT by applying either thermal stress (for example, cold exposure) or β-adrenergic stimulus results in elevated levels of multiple forms of ROS that play a role in supporting maximal thermogenesis, as evidenced by the fact that pharmacological depletion of these species compromises acute induction of thermogenic respiration[37]. A thermogenic action of mitochondrial ROS is likely mediated through cysteine modification of target proteins, including a regulatory cysteine residue (Cys253) on UCP1 itself[38]. More generally, the role of reversible thiol oxidation as an effector of thermogenic ROS signalling is important in the context of triggering thermogenic gene expression and mitochondrial biogenesis, as well as in acute control of thermogenic respiration. Continued investigation of protein cysteine targets of redox signalling in the context of thermogenesis is likely to yield additional modes of regulation of thermogenic gene expression and thermogenic function. Redox metabolism is

also a noteworthy link between adipocyte DNL and thermogenesis, considering that two major adipocyte processes compete for the same NADPH pool: thiol antioxidant machinery, which maintains a reduced state for cellular thiols, and the above-described FAS-dependent DNL. Interestingly, genetic and pharmacological perturbations of thiol antioxidant systems and the NADPH/$NADP^+$ ratio have substantial effects on both lipid accumulation and activation of thermogenesis in adipocytes. Elevation of the NADPH/$NADP^+$ ratio thus appears to drive the adipocyte to a lipogenic phenotype, while oxidation of the NADPH/$NADP^+$ drives cellular thiol oxidation and enhanced thermogenesis in BAT.

Mechanisms of non-canonical fat thermogenesis in beige fat. As described above, UCP1 plays a central role in non-shivering thermogenesis via BAT. Because beige fat residing within WAT expresses lower levels of UCP1 than BAT, the contribution of beige fat to the regulation of whole-body energy homeostasis was thought to be marginal[39]. However, this view has been revised with the discovery of non-canonical (UCP1-independent) thermogenic mechanisms in beige fat involving Ca^{2+} cycling and creatine-dependent substrate cycling[40,41] (Fig. 2b).

In the mitochondria-associated membrane (MAM) of beige adipocytes, ATP-dependent Ca^{2+} cycling by sarco/endoplasmic reticulum Ca^{2+}-ATPase 2b (SERCA2b) and ryanodine receptor 2 (RyR2) is enhanced in the absence of UCP1, serving as a robust thermogenic mechanism in beige fat[40]. Furthermore, creatine and creatine-mediated ATP hydrolysis stimulate the futile cycling of ATP production and consumption within the mitochondria[41]. Notably, UCP1-negative beige adipocytes exist under physiological conditions; murine subcutaneous adipose tissue may contain ~10% or more of multilocular adipocytes that lack UCP1 expression[42]. Importantly, these UCP1-independent thermogenic mechanisms are active in humans, at least in cultured adipocytes[40,41].

A particular interest related to Ca^{2+} cycling thermogenesis is fuel utilization in beige adipocytes. In contrast to thermogenesis driven by free fatty acid oxidation, Ca^{2+} cycling thermogenesis actively utilizes glucose as a primary substrate in the absence of UCP1. UCP1-deficient mice with increased beige fat mass (fat-specific PRDM16 transgenic mice in a *Ucp1* KO background) display high respiratory exchange ratio (~0.9) and are protected from diet-induced obesity and glucose intolerance[40]. Mechanistically, this preferential glucose oxidation by UCP1-negative beige fat is due to increased activity of the pyruvate dehydrogenase (PDH) complex, the gatekeeper of glucose oxidation by transforming pyruvate into acetyl-CoA. As PDH activity is potentiated by Ca^{2+} entry into the mitochondria[43], enhanced Ca^{2+} influx in the mitochondria likely leads to increased PDH activity, thereby promoting glucose oxidation in the absence of UCP1. It remains unknown, however, if enhanced Ca^{2+} flux in fat cells is sufficient to improve glucose homeostasis.

Besides Ca^{2+} and creatine cycling, a triacylglycerol (TG) futile cycle has been proposed as a thermogenic process in fat cells. In this context, glycerol derived from lipolysis is recycled back to TG by glycerol kinase (GyK). Intriguingly, this pathway is highly induced by thiazolidinediones (TZDs) or genetic knockout of NRIP1 (also known as receptor-interacting protein 140 (RIP140)), a transcriptional repressor of thermogenic genes[44,45]. However, the biological significance of TG futile cycling in humans is unclear[46]. The metabolic adaptation that occurs in conjunction with the futile cycle requires future investigation.

Fuel source of fat thermogenesis. For many years, fatty acids generated within brown adipocytes were thought to be primarily used for BAT thermogenesis, given that brown adipocytes actively engage in de novo lipolysis[47]. However, recent studies demonstrate that adipocyte-specific deletion of adipose triglyceride lipase (ATGL) by a tamoxifen-inducible Cre recombinase under the control of the *Adipoq* gene promoter (*Adiponectin*-Cre) causes a defect in BAT thermogenesis, whereas BAT-specific deletion of ATGL or the ATGL-activating protein comparative gene identification-58 (CGI-58) with a *Ucp1*-Cre driver does not compromise cold-induced BAT thermogenesis in vivo[48,49]. This suggests that, within brown adipocytes, fatty acids derived from DNL are dispensable for BAT thermogenesis. Instead, circulating fatty acids generated through WAT lipolysis appear to be taken up by brown adipocytes via CD36 and are subsequently used as fuel by mitochondria and for activation of UCP1.

At a cellular level, mitochondrial free fatty acid oxidation is required for thermogenesis because fat-specific loss of carnitine palmitoyltransferase 2 (CPT2), essential for long-chain fatty acid oxidation in the mitochondria, leads to a substantial decline in BAT thermogenesis in mice[50]. Furthermore, genetic deletion of CD36, an essential membrane transporter of long-chain fatty acids, causes hypothermia in mice[51,52]. These results align well with the notion that thermogenic fat cell metabolism generally depends on free fatty acid oxidation in the mitochondria and subsequent activation of UCP1-mediated proton uncoupling. As discussed above, however, beige adipocytes have high metabolic plasticity, enabling them to shift between fuel sources depending on their availability and on internal thermogenic mechanisms, such as glucose-driven thermogenesis in the absence of UCP1.

Are all beige adipocytes the same? Thermogenic adipocytes respond to β-AR stimulation by the SNS. When β-AR signalling is impaired (for example, as a result of aging) or pharmacologically blocked (for example, with β-blockers), brown fat thermogenesis is highly suppressed. Genetic studies have also demonstrated that mice lacking all the three forms of β-adrenergic receptors ($\beta1^{-/-}\beta2^{-/-}\beta3^{-/-}$; also known as 'β-less mice') exhibit a defect in BAT thermogenesis and quickly develop hypothermia upon acute cold exposure[28]. However, if slowly acclimated to a cold environment, β-less mice can tolerate it for over 1 month because of enhanced beige fat biogenesis and thermogenesis. This observation suggests that compensatory pathways must exist that stimulate beige fat thermogenesis in the absence of β-AR signalling.

How is fat thermogenesis induced in the absence of β-AR signalling? This question is important because extensive efforts to develop selective β3-AR agonists for human obesity have so far been unsuccessful. This failure is partly because of poor bioavailability of available agonists and the relatively low expression levels of β3-AR in human adipocytes compared with murine adipocytes[53]. A potential side effect that β3-AR agonists will have to circumvent in the clinic is that pharmacological activation of β3-AR potently increases heart rate and blood pressure in humans[54], which constitutes a major risk factor for the development of cardiovascular diseases.

One potential mechanism to activate brown and beige fat thermogenesis in the absence of β-AR signalling is mediated by adenosine[55,56]. An alternative mechanism, which does not conflict with the earlier studies, is the compensatory activation of a previously uncharacterized form of beige fat, the development and thermogenic regulation of which is independent of the β-AR pathway. A recent study[57] identified a subset of adipose progenitors that express MyoD, which emerges in subcutaneous WAT when β-AR signalling is blocked. Unexpectedly, $MyoD^+$ progenitors in WAT retain cellular plasticity and give rise to a unique population of beige adipocytes that express not only UCP1 but also key enzymes involved in glucose oxidation, such as enolase (ENO1) and an isoenzyme of the glycolytic enzyme pyruvate kinase (PKM2). Their gene expression profile and cellular metabolism are distinct from that of the 'conventional' beige adipocytes that emerge in response to β-AR activation (for example, synthetic β3-AR agonists) in that they possess high glucose uptake and oxidation capacities; accordingly, these thermogenic fat cells are termed 'glycolytic beige fat' (g-beige fat)[57].

Although these glycolytic beige adipocytes represent only a relatively small amount (~15%) of all UCP1$^+$ beige adipocytes, glucose uptake and oxidation of g-beige fat makes a significant contribution to systemic glucose homeostasis because depletion of this cell type, either by selectively deleting peroxisome proliferator-activated receptor-γ (PPARγ) or expressing *Diphtheria* toxin receptor in MyoD$^+$ progenitors, leads to glucose intolerance when β-AR signalling is blocked[57]. These results suggest that cellular metabolism and fuel utilization within adipose tissue are heterogeneous and that highly specialized subtypes of adipocytes exist.

Regulation of metabolite flux in the adipose tissue

The extraordinary metabolic plasticity of adipocytes extends to their integration into systemic metabolism. Brown, beige, and white adipocytes rapidly alter fuel uptake and consumption and the production of various metabolite classes in response to systemic cues, including the SNS-driven stimuli. In many cases, these acute changes in metabolite flux are critical upstream regulators of adipocyte function. As discussed below in detail, recent studies have demonstrated that exposure to cold temperatures has profound effects on metabolite flux in adipocytes. Notably, manipulation of some metabolites sufficiently alters the functional properties of adipocytes.

Cold-induced cellular metabolites. An example of cold-induced changes in metabolite flux is purine nucleotide metabolism in brown adipocytes, where the total purine nucleotide pool size is dynamic, and ATP and ADP pools are substantially depleted upon β-adrenergic stimulation[36]. Depletion of guanosine triphosphate pools is sufficient to potentiate fatty-acid-dependent respiration through UCP1, suggesting that modulation of purine nucleotide pool size in thermogenic adipocytes by rapid catabolism is an essential mode of regulation of UCP1-dependent thermogenesis. Because additional thermogenic cycles rely on futile utilization of ATP, for example, via SERCA and creatine metabolism, it will be interesting to determine the extent to which β-AR-dependent effects on purine nucleotide pools modulate futile cycling of ATP in adipocytes.

In addition to the unusual dynamics of high-energy purine nucleotide phosphates, the utilization of creatine and creatine phosphate in thermogenic adipocytes differs from other cells in the body. The phosphorylation and dephosphorylation of creatine in most cells occur in 1:1 stoichiometry with the ATP/ADP couple. However, mitochondria in thermogenic adipocytes appear to liberate a molar excess of ADP with respect to creatine, as inferred by observing the respiratory response to creatine under ADP-limited conditions[41,58]. This observation suggests that creatine phosphorylation and dephosphorylation cycles futilely in these mitochondria to drive thermogenic respiration. Alternatively, creatine could stimulate spontaneous hydrolysis of newly generated ATP without cycling itself. In either case, this highly unusual type of creatine utilization suggests that thermogenic adipocytes possess the cellular machinery for atypical phosphotransfer reactions involving ATP and creatine, or perhaps a novel class of metabolite phosphatase that facilitates this thermogenic process. Detailed examination of the metabolites and proteins involved in this process will likely reveal novel mechanisms of thermogenesis in these cells.

Recent unbiased investigations of the adipocyte metabolome have also revealed additional distinct metabolic cascades that have substantial effects on thermogenic adipocyte function. Upon cold exposure, thermogenic adipocytes can selectively accumulate the mitochondrial tricarboxylic acid (TCA) cycle metabolite succinate, which drives activation of thermogenic respiration in these cells[59]. This accumulation can be achieved by sequestering succinate from the circulation, which suggests that this metabolite can coordinate thermogenic adipocyte function via a systemic release of succinate from other tissues or the diet. Mechanistically, succinate stimulates mitochondrial thermogenic respiration via the production of ROS, which is essential for its thermogenic role, as discussed above. Importantly, systemic administration of succinate can be potently thermogenic, which requires competent thermogenic adipose tissue[59]. The study further supports the notion that manipulation of cellular metabolites sufficiently alters adipose tissue function in vivo.

Analysis of metabolomic changes in thermogenic fat cells has identified rapid adaptations in metabolites that control redox tone. Rapid oxidation and depletion of the reduced glutathione pool, concomitant with a rapid elevation in numerous reactive metabolite intermediates and ROS, occurs early upon activation of adipocyte thermogenesis by exposure to cold[38,60,61]. Succinate sequestration and oxidation appears to be a critical ROS-producing pathway in this context, as it has been demonstrated that the production of thermogenic ROS depends on succinate metabolism by succinate dehydrogenase, while the adrenergic cascade may also play a potentiating role[38,59]. The production of these reactive molecules and consequent oxidation of cellular thiols appear to be a mode of thermogenic regulation via post-translational modification of key thermogenic proteins, including UCP1. The extent to which succinate–ROS metabolism initiates thermogenesis via UCP1 compared with other effectors such as creatine and Ca^{2+} metabolism will be an interesting area of future research.

Thermogenic fat as a metabolic sink. Positron emission tomography-computed tomography (PET/CT) with ^{18}F-fluorodeoxyglucose (^{18}FDG) and the fatty acid tracer ^{18}F-fluoro-thiaheptadecanoic acid (^{18}FTHA)[62–68] have demonstrated the remarkable capacity of BAT to take up fuels—thus BAT is often referred to as a 'metabolic sink' for glucose and free fatty acids. BAT fuel uptake is tightly coupled with its ability to increase whole-body energy expenditure and with improvement in systemic glucose homeostasis, lipid metabolism, and insulin sensitivity, both in rodents and adult humans. For example, cold promotes the uptake of triglyceride-rich lipoproteins in BAT, thereby contributing to triglyceride clearance and improvement in systemic lipid metabolism[69,70]. Cold acclimation also improves systemic glucose homeostasis and insulin sensitivity in adult humans[71–73]. Of note, glucose uptake per volume or mg of BAT is substantially higher than that of other metabolic organs; however, total glucose uptake and utilization by skeletal muscle tissue contributes to a greater extent to whole-body glucose homeostasis than BAT when the relative tissue mass is taken into consideration[74].

It is worth mentioning that active glucose uptake by brown and beige fat is independent of UCP1. This somewhat unexpected observation is based on the studies showing that glucose uptake in brown and beige fat, as assessed by ^{18}FDG-PET scans, remains unaffected in *Ucp1* KO mice[40,75]. Because fuel uptake into fat cells can be uncoupled from its fuel oxidation capacity under certain conditions, measuring both fuel uptake and oxidation is essential for the critical assessment of thermogenic function in adipose tissue. Recent findings also suggest that creatine energetics play an important role in human adipose tissue thermogenesis. ^{18}FDG-PET studies in humans have demonstrated that renal creatinine clearance is a significant predictor of total activated human BAT[76]. Since creatinine is a direct product of phosphocreatine metabolism, these results are consistent with the activation of UCP1-independent thermogenesis by creatine substrate cycling in human BAT.

Going forward, it will be interesting to determine whether thermogenic adipocytes can sequester and utilize any circulating metabolites other than glucose, fatty acids, and succinate. Such investigations may reveal that thermogenic fat is systemically integrated to metabolize many classes of metabolites. While the contribution of the human BAT to diet-induced thermogenesis was once in question[77], recent studies clearly demonstrate that human BAT is metabolically active even under normal or warm ambient

temperatures with higher blood flow than WAT[78] and that glucose uptake in BAT is highly activated after food intake, a phenomenon known as diet-induced thermogenesis[79,80]. Hence, BAT might control the clearance of specific metabolites in the circulation, and such metabolites could serve as predictors of BAT activity.

Metabolic maladaptation in the adipose tissue

Cellular mechanisms of adipose tissue expansion. In response to excess caloric intake, adipose tissue has the incredible capacity to expand its tissue mass by increasing adipose cell size (hypertrophy) and cell number (hyperplasia). In humans, adipose cell number is reportedly the primary determinant for the adipose tissue mass; adipocyte number is determined during childhood and adolescence, and maintained in adults even after substantial weight loss[81].

Recent advances in tracing of fat-cell lineage in mice have provided precise information about the regulation of adipose tissue expansion in a location- and gender-selective manner (Fig. 3a). For instance, expansion of epididymal WAT, a major visceral WAT depot in mice, involves both hypertrophy and hyperplasia[5,82]. Adipocyte precursors in the visceral WAT that are marked by Lin^-:$CD29^+$:$CD34^+$:$Sca1^+$ cells actively proliferate within 1 week after onset of high-fat diet feeding and undergo adipogenesis[5]. Moreover, perivascular preadipocytes that express zinc finger protein 423 (ZFP423) and platelet derived growth factor receptor-β (PDGFRβ) contribute to WAT hyperplasia in diet-induced obesity by increasing the cell number and by de novo differentiation[83]. On the other hand, inguinal WAT, a subcutaneous adipose depot in mice, expands exclusively through hypertrophy in male mice, although high-fat diet feeding in female mice can promote de novo adipogenesis and hyperplasia in the inguinal WAT[84]. Intriguingly, diet-induced hyperplasia depends on the activation of Akt2 signalling because high-fat diet feeding rapidly increases phosphorylation of Akt2 (S474) in adipocyte progenitors, and Akt2-null mice fail to stimulate diet-induced adipocyte progenitor proliferation. Since these observations were made in mice on the C57BL/6 genetic background, it would be intriguing to examine possible variations among mouse strains.

Mechanisms of adipose tissue remodelling. Besides hypertrophy and hyperplasia, emerging evidence suggests that ECM proteins regulate adipose tissue expansion in response to excess caloric intake. ECM proteins—including collagens (for example, collagen I, III, IV, VI, etc.), fibronectin, and elastin—ensure physical integrity of adipose tissue during expansion. On the other hand, excessive deposition of the ECM proteins is associated with infiltration of proinflammatory immune cells, such as M1 macrophages. Clinical studies show a strong correlation between increased adipose tissue fibrosis in the subcutaneous WAT and insulin resistance and type 2 diabetes[85–89]. Furthermore, the degree of adipose tissue fibrosis in the subcutaneous WAT is inversely correlated with the effectiveness of weight loss following gastric bypass surgery in humans[90]. These observations suggest that adipose tissue fibrosis is a morphological hallmark of adipose tissue malfunction that is coupled with increased pro-inflammatory responses and reduced angiogenesis (Fig. 3b).

Which cell types within the adipose tissue contribute to the accumulation of ECM proteins? A recent study suggests that myofibroblasts expressing PDGFRα and high levels of CD9 are profibrogenic and drive adipose tissue fibrosis[91]. It has also been suggested that the majority of ECM-producing myofibroblasts in the dermal WAT originate from *Adiponectin*-expressing adipocytes that go through adipocyte–myofibroblast transition[92]. Additionally, macrophages and mature adipocytes produce pro-fibrotic collagens, fibronectin, and tenascin-C in WAT that contribute to the accumulation of ECM[93,94]. Hence, multiple cell types likely contribute to the accumulation of ECM in the adipose tissue. Of note, the degree of adipose tissue fibrosis

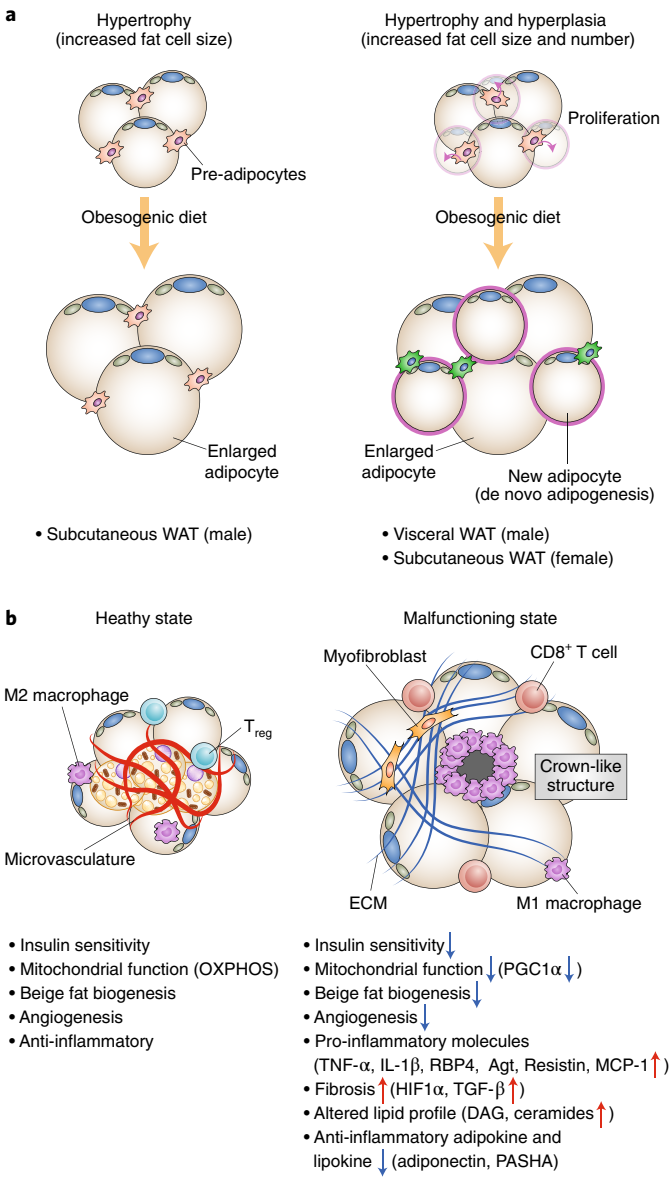

Fig. 3 | Adaptation and maladaptation in adipose tissue. a, Adipose tissue expansion occurs through hypertrophy and hyperplasia. Left, Adipose tissue hypertrophy involves increased cell size of existing adipocytes. Subcutaneous WAT in mice is increased in mass by hypertrophy. Right, Adipose tissue hyperplasia involves an increase in the number of adipocytes by adipocyte precursor proliferation and de novo adipogenesis. Visceral WAT of mice and subcutaneous WAT of female mice increase in mass through the combination of hypertrophy and hyperplasia. **b**, Hallmarks of healthy adipose tissue (left) and malfunctioning adipose tissue (right). Healthy adipose tissues are insulin sensitive with active mitochondrial biogenesis and OXPHOS. They also possess thermogenic beige adipocytes, increased angiogenesis, and anti-inflammatory M2 macrophages and regulatory T cells (T_{reg}s). By contrast, malfunctioning 'unhealthy' adipose tissues are insulin resistant, and mitochondrial biogenesis and OXPHOS, beige adipocyte biogenesis, and angiogenesis are impaired. Adipose tissue expresses many pro-inflammatory cytokines (M1 macrophages and $CD8^+$ T cells) and produces excess ECM from myofibroblasts, leading to the recruitment of pro-inflammatory immune cells and the formation of adipose tissue fibrosis and crown-like structures. Adipocytes also display altered lipid and adipokine and lipokine profiles, including increased DAG and ceramides as well as reduced secretion of adiponectin and palmitic acid hydroxystearic acids (PASHA).

and its relationship to metabolic disorders appears to be dependent on adipose tissue depots and may be different between rodents and humans. In rodents, visceral WAT fibrosis is prominent in obese mice, whereas subcutaneous WAT fibrosis correlates well with metabolic dysfunction in humans. Therefore, the cell types responsible for the accumulation of ECM proteins and the development of adipose tissue fibrosis in humans warrant further investigation.

A key player in the initiation of adipose tissue fibrosis is transforming growth factor-β (TGF-β). TGF-β levels in the circulation and adipose tissues are highly elevated in obese humans and mice[95,96]. Adipose tissue hypoxia and subsequent accumulation of hypoxia-inducible factor 1α (HIF1α) lead to the activation of HIF1α-dependent gene transcription of pro-fibrotic genes in the adipose tissue[97–99]. Whereas mouse models with ectopic activation of HIF1α in adipose tissues induce fibrosis and systemic glucose intolerance[100], treatment with PX-478, the HIF1α-selective inhibitor, potently ameliorates adipose tissue fibrosis, inflammation, and glucose intolerance[101]. Intriguingly, cold acclimation potently represses adipose tissue fibrosis—such repression in adipose tissue fibrosis is independent of UCP1-dependent thermogenesis and body-weight loss because cold acclimation inhibits adipose tissue fibrosis even in *Ucp1* KO mice[94].

Is excess ECM accumulation merely an epiphenomenon of adipose tissue malfunction? Recent work suggests that adipose tissue fibrosis is more than an epiphenomenon and functionally contributes to the regulation of adipose tissue homeostasis and systemic glucose metabolism. *Col6*-deficient mice, which lack the major adipose tissue ECM protein collagen VI (Col6), can expand adipose tissue with reduced levels of inflammation and exhibit high glucose tolerance and insulin sensitivity on a high-fat diet[102]. In another study, transgenic mice expressing general transcription factor II-I repeat domain-containing protein (GTF2IRD1), a cold-inducible transcription factor, have repressed pro-fibrotic gene expression in the adipose tissue[94]. The adipose tissue of GTF2IRD1 transgenic mice contains significantly less fibrosis relative to control mice on a high-fat diet, and these mice are protected from diet-induced insulin resistance and glucose intolerance although their body weight remains unaffected[94]. Furthermore, antibody-based blockade of endotrophin, a C-terminal cleavage product of Col6α3 that drives adipose tissue fibrosis, prevents diet-induced adipose tissue fibrosis and inflammation and ameliorates diet-induced insulin resistance[103]. These results hint at the exciting possibility that adipose tissue fibrosis can be a therapeutic target for the improvement of systemic glucose homeostasis and insulin sensitivity, suggesting adipose tissue fibrosis is not simply a consequence of an unhealthy metabolic state.

Mitochondrial dysfunction in adipose tissue. Independent lines of evidence in mice and humans suggest that obesity is associated with reduced mitochondrial function in the adipose tissue. For example, mitochondrial OXPHOS and biogenesis in human subcutaneous WAT are compromised under obese and diabetic states[104–106]. Such a decline occurs independently of genetic background; comparisons between monozygotic twin pairs with discordant body mass index (BMI) found that mitochondrial biogenesis and OXPHOS in the subcutaneous WAT are downregulated in acquired obesity and associated with metabolic abnormalities[107,108]. Conversely, therapeutic interventions that improve insulin sensitivity, such as exercise and treatment with synthetic PPARγ agonists, potently increase mitochondrial biogenesis and OXPHOS in adipocytes[109–113]. Intriguingly, expression of genes that control mitochondrial function—such as OXPHOS, the TCA cycle, and free fatty acid oxidation pathways—is significantly higher in the subcutaneous WAT of individuals who successfully lost weight than in those who re-gained weight after a dietary-based weight loss intervention, suggesting that mitochondrial function is associated with success in weight loss[114].

Is reduced mitochondrial function simply a metabolic consequence of obesity, or is it involved in the development of adipose tissue malfunction? Genetic studies in mice indicate a causal link between reduced mitochondrial function in adipocytes and the development of insulin resistance. For instance, fat-specific deletion of mitochondrial transcription factor A (TFAM) causes adipocyte death, adipose tissue inflammation, and systemic insulin resistance[115]. Similarly, fat-specific deletion of PGC1α leads to a decline in mitochondrial biogenesis in the subcutaneous WAT and systemic insulin resistance[116]. Conversely, improvement of mitochondrial function in adipocytes sufficiently prevents adipose tissue inflammation, fibrosis, and insulin resistance. Fat-specific overexpression of mitoNEET (CDGSH iron sulfur domain 1), an outer mitochondrial membrane containing iron-sulfur (Fe-S) clusters, ameliorates obesity-associated adipose tissue dysfunction, and thereby mice are protected from insulin resistance and display a 'healthy obese' phenotype[117,118]. Similarly, fat-specific overexpression of mitochondrial thiosulfate sulfurtransferase, a mitochondrial protein that controls Fe-S clusters, enhances insulin sensitivity and glucose tolerance[119]. These results suggest that impaired mitochondrial function, that is, mitochondrial OXPHOS and biogenesis, is an index of adipose tissue health and that reduced mitochondrial mass and/or function is an indicator of adipose tissue malfunction (Fig. 3b).

Altered adipokine and lipid profiles. Adipocytes constitute a significant source of secretory cytokines, also known as adipokines, and adipokine profile is dynamically changed in response to nutritional cues. Generally speaking, expression and secretion of many pro-inflammatory adipokines, such as tumour necrosis factor-α (TNF-α), interleukin-6 (IL-6), angiotensinogen, monocyte chemoattractant protein-1 (MCP-1, also known as CCL2), and resistin, are elevated in obesity, and each factor contributes to the activation of chronic inflammatory state in the adipose tissue through interaction with pro-inflammatory immune cells[120]. By contrast, adiponectin expression is closely correlated with anti-inflammatory states of adipose tissue in rodents and humans. Administration of adiponectin increases free fatty acid oxidation and insulin sensitivity in metabolic organs, including liver and skeletal muscle, through its receptors Adipo-R1 and Adipo-R2 (ref. [121]). It is also notable that lipidomics studies have identified unique lipid species that are secreted from brown and white adipocytes, often referred to as lipokines. For instance, C16:1n7-palmitoleate is a lipokine that stimulates insulin sensitivity in skeletal muscle, while it suppresses hepatosteatosis in mice[122]. Palmitic acid hydroxystearic acids (PAHSAs) are recently identified lipokines with levels in circulation that are significantly lowered in the insulin-resistant state in mice and humans[123]. PAHSA treatment reduces adipose tissue inflammation and enhances insulin-stimulated glucose uptake through G-protein-coupled receptor 120 (GPR120). PAHSAs also stimulate intracellular Ca^{2+} flux through GPR40 and augment insulin and glucagon-like peptide (GLP-1) secretion[124], although a recent paper has challenged this observation[125]. Circulating levels of 12,13-dihydroxy-9Z-octadecenoic acid (12,13-diHOME) are increased in response to cold exposure in mice and humans, which enhances fatty acid uptake by thermogenic adipocytes[126], while 12,13-diHOME is also synthesized in nervous tissue and causes thermal pain hypersensitivity via transient receptor potential cation channel subfamily V member 1 (TRPV1) in the sensory neurons[127]. Genetic manipulation of the biosynthesis enzymes will determine the physiological roles of these lipokines in the regulation of systemic energy homeostasis and insulin sensitivity in vivo. Given the recent advances in lipidomic profiling technology, new classes of lipokines will likely be identified, some of which may mediate inter-organ communication between adipose tissue and other metabolic organs.

Another change in fat cells that is associated with adipose tissue maladaptation is increased free fatty acid flux as well as increased

levels of diacylglycerol (DAG) and ceramides. The 'spillover' of lipids from adipocytes and ectopic deposition in liver and skeletal muscle are considered to cause mitochondrial dysfunction, insulin resistance, and cardiometabolic diseases (that is, lipotoxicity)[128]. Recently, sphingolipids (for example, ceramide and its metabolites) have gained much attention as prominent lipotoxic lipids that induce insulin resistance. Circulating levels of many ceramide species are elevated in insulin-resistant subjects[129], and pharmacological inhibitors of ceramide biosynthesis effectively alleviate obesity-induced inflammation, increase energy expenditure, and improve insulin sensitivity in rodent models[130–134]. Importantly, identification of key enzymes responsible for ceramide biosynthesis (CerS1-6) has allowed for genetic manipulation of de novo ceramide synthesis and establishment of a causal relationship between ceramides and metabolic disorders in vivo. For instance, CerS6 expression and C16:0 ceramides are elevated in adipose tissue of obese humans, whereas CerS6-knockout mice displayed reduced C16:0 ceramides and are protected from diet-induced obesity and glucose intolerance[135]. Furthermore, fat-specific deletion of serine palmitoyltransferase (Sptlc), the first enzyme in the sphingolipid biosynthesis pathway, induces beige adipocyte biogenesis and mitochondrial function that is associated with reduced inflammation and increased insulin sensitivity[136]. Notably, adiponectin potently reduces ceramide levels through its receptors AdipoR1 and AdipoR2, which possess ceramidase activity[137,138]. These observations are exciting because the ceramide biosynthesis pathway may form a part of the underlying mechanism by which dysregulated lipids causes adipose tissue malfunction and insulin resistance.

Future perspectives

One may presume that metabolites are equally distributed within cells, but some metabolites are compartmentalized, and the locally produced metabolites have unique cellular functions. A recent study shows that NAD^+ accumulates in the nucleus of preadipocytes and inhibits the transcriptional activity of CCAAT/enhancer binding protein-β (C/EBPβ), a critical initiation factor of adipogenesis. When the expression of nicotinamide nucleotide adenylyltransferase 2 (NMNAT2), the cytosolic form of the NAD^+ synthesis enzyme, is elevated, cytosolic NMNAT2 depletes the supply of NAD precursors to the nucleus, leading to the activation of CEBPβ and adipogenesis[139]. This study elegantly explains how a compartmentalized metabolite NAD^+ controls the signal-dependent regulation of adipogenesis. Although stable chemical or protein indicators that allow accurate monitoring of intracellular metabolite flux are currently limited (for example, GCaMP6 for intracellular Ca^{2+}), many metabolites other than NAD^+ might be similarly compartmentalized in specific organelles.

At the tissue level, it will be intriguing to explore the mechanisms by which metabolic tissues communicate with each other to coordinate their fuel utilization. For instance, promoting glycolysis in hepatocytes by overexpressing glucokinase in the liver attenuates BAT thermogenesis and increases adiposity by suppressing sympathetic nerve activity[140]. Also, certain cancer cells, such as those in triple-negative breast cancers, actively utilize fatty acids to maximize cell growth and proliferation, and their metabolic activity heavily depends on the nutritional microenvironment[141]. A better understanding of the upstream regulators of fuel utilization will eventually allow for selective manipulation of cellular metabolism and function.

Lastly, cellular heterogeneity within adipose tissues needs further exploration. Although functional and developmental differences between visceral WAT and subcutaneous WAT have been recognized, more subtypes of adipocytes likely exist. Even among thermogenic adipocytes, multiple subtypes of these cells of distinct developmental origin exist, and each subtype likely has a unique biological role, depending on the nature of external stimuli, such as β-AR activation, exercise, bariatric surgery, cancer cachexia, bile acids, or PPARγ agonists. One case in point is g-beige fat, a new subtype of beige adipocytes whose developmental regulation is distinct from that of conventional beige adipocytes and whose thermogenic program can be induced even in the absence of β-AR signalling[57]. Another example is that pharmacological inhibition of cyclin-dependent kinase5 (CDK5) promotes the formation of beige adipocytes that exhibit a distinct molecular profile that is different from β3-AR agonist-induced beige adipocytes[142]. We envision rapid advances in cellular mapping studies because single-cell RNA sequencing analyses of adipose tissues indicate the existence of diverse progenitor cell populations[143–145]. In addition, a FACS-based approach for the isolation of mature adipocytes for single-cell analyses has become available[146]. Studies at this single-cell resolution will provide new insights into functional fat-cell heterogeneity in physiology and disease.

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E.T.C and S.K. conceived the project and wrote the manuscript.

Competing interests

The authors declare no competing interests.

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