Adipogenesis and metabolic health

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Abstract | Obesity is characterized by increased adipose tissue mass and has been associated with a strong predisposition towards metabolic diseases and cancer. Thus, it constitutes a public health issue of major proportion. The expansion of adipose depots can be driven either by the increase in adipocyte size (hypertrophy) or by the formation of new adipocytes from precursor differentiation in the process of adipogenesis (hyperplasia). Notably, adipocyte expansion through adipogenesis can offset the negative metabolic effects of obesity, and the mechanisms and regulators of this adaptive process are now emerging. Over the past several years, we have learned a considerable amount about how adipocyte fate is determined and how adipogenesis is regulated by signalling and systemic factors. We have also gained appreciation that the adipogenic niche can influence tissue adipogenic capability. Approaches aimed at increasing adipogenesis over adipocyte hypertrophy can now be explored as a means to treat metabolic diseases.

Overnutrition is currently one of the most costly challenges for public health. The increasing prevalence of obesity sparked great interest in understanding the physiological mechanisms promoting effective calorie storage while minimizing the adverse metabolic consequences of obesity such as insulin resistance, dyslipidaemia, hepatic steatosis, coagulopathies and hypertension. Although the role of adipocytes in energy storage is firmly established, we have only recently begun to appreciate the profound influence of adipocytes on other aspects of systemic metabolic homeostasis. Indeed, the ability to sequester lipid effectively inside adipocytes prevents toxic lipid accumulation (lipotoxicity) in other tissues, such as muscle, liver and heart, and firmly correlates with preserved metabolic function at all levels of pathology associated with obesity. The question remains: what factors govern the expansion of adipose depots and how are they regulated?

Adipose tissue can increase in size in one of two main ways: hypertrophy (increase in size of existing adipocytes) or hyperplasia (formation of new adipocytes through differentiation of resident precursors known as preadipocytes) (Fig. 1). Differentiated adipocytes have remarkable hypertrophic potential, being able to increase in size to several hundred micrometres in diameter. The number of adipocytes in a given depot is primarily determined early in life and is mostly stable through adulthood. However, recent lineage-tracing models in rodents have demonstrated that, during prolonged caloric excess, new adipocytes can emerge from the differentiation of preadipocytes and can contribute to adipose tissue expansion. Generally, these observations have led to the appreciation that these adult preadipocytes are fibroblast-like in nature and located in the perivasculature.

The balance of hypertrophic expansion of existing adipocytes and adipogenesis within an individual has a profound impact on metabolic health. As early as the mid-20th century, increased adipocyte size was correlated with increased systemic insulin resistance. Additional studies have suggested that small adipocytes are especially important for counteracting obesity-associated metabolic decline and have shown that small adipocytes are often correlated with decreased susceptibility to developing diabetes and hepatic steatosis, coagulopathies and hypertension. Thus, it constitutes a public health issue of major proportion. The expansion of adipose depots can be driven either by the increase in adipocyte size (hypertrophy) or by the formation of new adipocytes from precursor differentiation in the process of adipogenesis (hyperplasia). Notably, adipocyte expansion through adipogenesis can offset the negative metabolic effects of obesity, and the mechanisms and regulators of this adaptive process are now emerging. Over the past several years, we have learned a considerable amount about how adipocyte fate is determined and how adipogenesis is regulated by signalling and systemic factors. We have also gained appreciation that the adipogenic niche can influence tissue adipogenic capability. Approaches aimed at increasing adipogenesis over adipocyte hypertrophy can now be explored as a means to treat metabolic diseases.

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**Physiological roles of white adipocytes**

The defining feature of adipocytes is their ability to store excess calories in the form of triglycerides, which are packaged into large lipid droplets that take up most of the cell. Storing energy as triglycerides is evolutionarily advantageous; however, the presence of adipocytes, which are specialized for this purpose, is limited to vertebrates. In addition to their energy-storing role, adipocytes provide several additional physiological functions. Animals in cold climates have increased adiposity, which endows them with increased insulation and thermogenesis. Adipocytes also provide mechanical cushioning and are abundant in anatomical regions experiencing high mechanical stress such as the palm, buttocks and heel. Internally, adipose tissue provides cushioning to organs such as the heart, adrenal glands, kidneys and ovaries. Additionally, there is a second class of adipocytes found in euthermic mammals, referred to as brown or beige adipocytes, that are specialized in energy and heat dissipation (Box 1). For the purposes of this Review, we focus on white adipocytes, and the reader is referred to other articles focused on brown and beige adipogenesis.19–22

Classically, adipocytes respond to systemic signals to either store or mobilize nutrients as needed by the organism. In times of nutrient deprivation, blood glucose drops and adipocytes are stimulated by hormones such as glucagon or noradrenaline to activate lipases, such as hormone-sensitive lipase (HSL) and adipose triglyceride lipase (ATGL; also known as PNPLA2) to release non-esterified fatty acids and glycerol from the lipid droplets into circulation for use by organs, such as the liver and skeletal muscle. In times of nutrient excess, blood glucose levels rise, and the pancreatic hormone insulin suppresses HSL and ATGL and promotes glucose uptake and de novo lipogenesis to package lipids into lipid droplets and store the excess nutrients. During obesity-associated metabolic decline, the adipocyte fails to respond properly to almost all these extracellular signals, most notably insulin, leading to elevated plasma glucose and lipids (reviewed elsewhere23,24). There are additional characteristics of adipocyte dysfunction during obesity-associated metabolic decline, including an altered transcriptional programme with an increased rate of collagen synthesis and increased rates of adipocyte necrosis, which are associated with increased levels of pro-inflammatory cytokines.25

The past 20 years have expanded the role of adipose tissue to include its dynamic function as an endocrine organ. Initiated by the discovery of leptin,26 followed closely by adiponectin,27 we now appreciate that white adipose tissue secretes many signalling proteins, known as adipokines. Many of these adipokines help to coordinate the systemic metabolic state. For example, adiponectin suppresses hepatic glucose output and reduces

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**Box 1**

**Lipotoxicity**

A cellular dysfunction arising from accumulation of lipid intermediates in cells other than adipocytes. In liver, accumulation of lipids contributes to the pathogenesis of non-alcoholic fatty liver disease and to insulin resistance. Lipid accumulation in skeletal muscle can contribute to insulin resistance, whereas in cardiac muscle, it can cause apoptotic cell death. Pancreatic lipid accumulation can lead to dysregulation of β-cell insulin secretion, and ultimately to apoptotic cell death.

**Adipokines**

Signaling molecules (proteins or lipids) secreted from adipose tissue.

**Euthermic**

A term referring to an animal that maintains a constant body temperature.
sympathetic output refers to through activation of neurotransmitter noradrenaline primarily with the volume, of total brown adipose tissue.

Sympathetic output refers to central signals that increase the activity of these nerves.

**Box 1 | Brown and beige adipogenesis**

In contrast to the energy-storing white adipocyte, brown and beige adipocytes are characterized by the capability for energy expenditure through thermogenesis. Characteristically, these adipocytes have an increased number of mitochondria and many small lipid droplets, and they express high levels of uncoupling protein 1 (UCP1), which is considered the key driver of the thermogenesis programme. In mice, brown and beige adipocytes can be distinguished with high confidence. Brown adipocytes develop from an MYF5 lineage, whereas beige adipocytes do not. Brown adipocytes exist in anatomically distinct depots (most notably, the interscapular depot), whereas beige adipocytes are found in white depots (most notably in subcutaneous depots). Human infants have an interscapular brown adipose depot, whereas adult humans lose this depot and instead can develop thermogenic adipose tissue in the paraspinal depot and supraclavicular depot with adipocytes molecularly similar to both brown and beige adipocytes.

As discussed, there exists a degree of lineage plasticity between white and beige preadipocytes, although there are several hallmarks that distinguish them. Lineage tracing in vivo reveals that the beige preadipocytes, like white preadipocytes, are perivascular cells. The brown and beige specific transcription factor EB2 (REFS [353,354]) recruits peroxisome proliferator-activated receptor-γ (PPARγ) to thermogenic gene targets and is necessary for the adipogenesis of beige adipocytes after β3 adrenergic stimulation (an experimental surrogate of cold exposure). It is also sufficient to drive adipocyte precursors isolated from white adipose tissue to differentiate into beige adipocytes. The white adipocyte commitment factor ZFP423 directly represses EB2 transcriptional activity to allow preadipocytes to form classically white adipocytes [355]. The gonadal depot of mouse adipose tissue is relatively resistant to beige adipogenesis, and the expansion of the beige adipose tissue preferentially occurs subcutaneously [356,357]. However, deletion of ZFP423 in preadipocytes ‘unlocks’ beige adipogenesis in the gonadal depot [358]. Little is known about the molecular and cellular regulation determining whether a precursor forms a white or beige adipocyte, but some data suggest that bone morphogenetic protein (BMP) signalling may disrupt the ZFP423–EB2 interaction, thereby promoting the beige adipocyte fate [359].

Many molecular inputs are described to influence thermogenic adipocyte differentiation and activation (reviewed extensively elsewhere [360,361]), but we briefly draw attention to the role of ageing. Notably, diabetes and metabolic disease show high incidence in the aged population and are accompanied by a considerable decrease in basal and cold-recruited brown adipocyte activity in vivo. In addition, human preadipocytes from elderly individuals show decreased beige adipogenesis in vitro [362]. This decline in beige adipogenesis has been linked to cellular senescence and could be counteracted by overexpression of the NAD-dependent histone deactylase SIRT1, which counteracts cellular senescence [363]. Ageing-induced cellular senescence also appears to diminish beige adipocyte differentiation in vivo, as inhibition of cellular senescence in mouse preadipocytes restored beige adipocyte differentiation [364].

**Early adipocyte commitment factors.** Adipogenic commitment is the process whereby a multipotent precursor becomes restricted to the adipocyte lineage and incapable of forming other mesenchymal cell types, such as myoblasts, chondroblasts or osteoblasts (reviewed elsewhere [365–367]).

Early studies identified that bone morphogenetic protein 2 (BMP2) and BMP4 are sufficient to drive adipocyte commitment in cultured fibroblasts and are required for in vitro adipogenic differentiation [368–370]. BMPs bind and signal through BMP receptors to activate the SMAD4 transcription factor by activating its
Gluconeogenesis
The generation of glucose from non-carbohydrate carbon sources (glycerol, odd-chain fatty acids, lactate or certain amino acids). In mammals, the liver, kidney, intestine and skeletal muscle are the only organs capable of gluconeogenesis.

Box 2 | Identity of the white adipocyte precursor

Until recently, the predominant source of our knowledge regarding adipocyte differentiation derived from various in vitro mixed-cell-type populations including mouse embryonic fibroblasts, the stromal vascular fraction of adipose tissue and the 3T3-L1 fibroblast-like cell line (see Supplementary Box 1). However, it has remained unclear whether these in vitro models mimic relevant in vivo precursors and what the in vivo characteristics of adipose precursors are.

One of the broadest markers of the adipocyte lineage is platelet-derived growth factor α (PDGFRα), and the overwhelming majority of adult adipocytes derive from this lineage). However, PDGFRα is not expressed in mature adipocytes, reflecting that its expression is restricted to precursors. Within adipose depots, PDGFRα+ cells reside near the vasculature. Alternative approaches attempted to characterize the resident adipocyte precursor by identifying characteristics of fibroblasts expressing peroxisome proliferator-activated receptor-γ (PPARγ). Nearly all proliferating PPARγ+ cells were localized to the blood vessel walls of adipose depots but not in other tissues. These cells resemble mural cells (also known as pericytes or vascular smooth muscle cells) and express common mural markers, such as α-smooth muscle actin (αSMA) and PDGFRβ. Independently, screening identified the zinc-finger protease ZFP423 as a key commitment factor of the white adipocyte lineage, and in vivo tracing localized these highly adipogenic cells to the perivascular mural compartment. Several in vivo, lineage-tracing studies confirm that perivascular αSMA+ cells and PDGFRβ+ cells contribute to adipogenesis in mice during high-fat-diet feeding.

While these perivascular lineage markers, including αSMA, PDGFRβ and PDGFRα, all mark the adipocyte lineage, they also mark many other cell types throughout the body, and thus their utility for identifying preadipocytes is limited. Various combinations of cell surface markers can enrich for preadipocytes (reviewed elsewhere), but we still lack a reliable means of identifying adipogenic precursors at the exclusion of other cell types. However, it has recently been shown that a single-cell sequencing approach is able to identify adipogenic populations of stem cells. Despite the heterogeneous development and function of different adipose tissue depots, it appears that the molecular characteristics of adipogenic cells differ between subcutaneous and visceral depots, highlighting the need for increasingly selective markers of preadipocytes. Future work will address the unresolved questions of the in vivo functional importance and lineage restriction of these populations of stromal cells.

Finally, adipocyte differentiation is not a unidirectional process, and certain stimuli can prompt adipocytes to return to a phenotypically fibroblast-like precursor state. Lineage tracing supports this de-differentiation in physiological processes such as wound healing, tumour growth and lactation. In the case of wound healing and lactation, it appears that some adipocytes may pass reversibly through a precursor stage many times while always retaining the capacity to form a mature adipocyte. These fascinating demonstrations of adipocyte plasticity call into question the notion of the adipocyte as a terminally differentiated cell type. It would also be interesting to study whether these fibroblast-like cells derived from an adipocyte lineage are restricted to form only adipocytes or gain pluripotency as a result of de-differentiation.
and it has been proposed that they are able to return to a fibroblast-like precursor state. Lineage-tracing studies in animal models suggest that fibroblasts within healing cutaneous lesions, fibrotic dermis, tumour stroma and and lactating mammary glands derive from an adipogenic lineage. In the case of wound healing and lactation, the adipocyte de-differentiation is reversible, and the fibroblastic cells resulting from the de-differentiation of adipocytes are capable of reforming adipocytes. It is unclear to what degree these fibroblastic adipocyte precursors are restricted to the adipocyte lineage and whether they are capable of forming other cell types in vivo. We also know very little about what (if any) functional purpose this de-differentiation event serves. For example, multiple studies have shown that adipose tissue shrinking during weight loss does not decrease adipocyte number — at least not within the accuracy of the methodologies used, which are not accurate enough to detect alterations within a 10–20% change in cell numbers — and therefore, de-differentiation of adipocytes is not driving cell number control in the adipose tissue, at least not in response to changes in calorie balance. Nevertheless, understanding of the mechanisms controlling the reversibility of adipose differentiation may provide key insights into certain malignancies. Liposarcomas are malignant tumours resembling adipocytes and adipose precursors, with malignancy inversely correlated with the prevalence of well-differentiated adipocytes in the tumour tissue. Other types of cancer, such as haematomas and haemangiomas, also contain adipocytes at various stages of differentiation. Thus, a greater understanding of what molecular signals promote or sustain adipose de-differentiation can provide novel avenues for anticancer therapies and potentially also for controlling the number of adipocytes to promote weight loss.

Sites of adipogenesis

It is helpful to consider adipose tissue as divided into subcutaneous (localized beneath the skin) and visceral (distributed in the abdominal cavity) depots or fat pads. In humans, the predominant subcutaneous depot is the gluteofemoral depot, localized around the hips and thighs. The predominant human visceral depot is the omental depot, which is a large peritoneal fold connecting the stomach and other visceral organs. In rodent models, the inguinal adipose depot (around the groin) and the epididymal (or gonadal) adipose depot are the predominant models of subcutaneous and visceral adipose tissue, respectively. In this Review, we continue to use subcutaneous and visceral as shorthand to refer to the broad categories of adipose tissue but emphasize that important differences in depot function exist within these categorizations (reviewed elsewhere). Adipocytes are also physiologically or pathologically associated with nearly every non-neuronal organ in the body, including the skin, liver, bone marrow, skeletal muscle, vasculature, intestine, adrenal glands, pericardium and ovaries.

Most adipocytes emerge during development, and their emergence is tightly associated with blood vessels. Increases in adipose tissue in adults are mostly driven by adipocyte hypertrophy, but hyperplasia can be induced in some circumstances. Notably, the propensity to generate new adipocytes varies between the different adipogenic sites, and these processes are differentially regulated and have different consequences.

Fig. 2 | Overview of molecular mechanisms of adipogenesis. Multipotent, fibroblast-like mesenchymal precursors (marked by α-smooth muscle actin (αSMa) and platelet-derived growth factor receptors (PDGFRa and/or PDGFRb)) serve as adipocyte precursors but are also capable of forming myoblasts, osteoblasts and chondroblasts. Bone morphogenetic protein (BMP) signalling restricts these mesenchymal precursors to the adipocyte lineage as part of a process known as ‘commitment’. At the time of commitment, the precursors are still morphologically fibroblast-like and importantly express the transcription factor ZFP423. When the committed preadipocyte arrests its growth, it activates the master regulator of adipogenesis peroxisome proliferator-activated receptor-γ (PPARγ) and transcription co-activators CCAAT/enhancer-binding protein α and β (C/EBPα and C/EBPβ). Lipid accumulation drives the expression of the adipocyte fatty-acid binding protein (AP2) and the insulin-sensitive transporter GLUT4, marking adipocytes in early stages of differentiation. At the completion of differentiation, mature adipocytes express all the markers of early adipocyte differentiation as well as the peptide hormones adiponectin and leptin; the lipases adipose triglyceride lipase (ATGL) and lipoprotein lipase (LPL); and high levels of the lipid-droplet-associated protein perilipin 1. There is now evidence that adipocytes can undergo de-differentiation back to fibroblast-like preadipocytes. Whether these preadipocytes are committed to the adipocyte lineage or whether they can generate other mesenchymal cell types is unknown.
**Fetal adipogenesis.** In humans, adipose tissue appears early in the second trimester of pregnancy in a cranial to caudal and then medial to lateral fashion\(^\text{73}\). In mice, subcutaneous adipose is formed embryonically, whereas the visceral depots develop during the first 2 postnatal weeks\(^\text{1}\). Evidence is mounting that fetal and adult adipogenesis involves different precursor populations and distinct regulatory processes. Early lineage-tracing studies provide data to suggest that adult and fetal precursors are distinctly defined populations\(^\text{81}\). Unlike in adults, fetal adipogenesis appears to be surprisingly C/EBPα independent\(^\text{83}\) but requires ZFP423 (REF \(^\text{8}\)). Evidence also suggests that the perivascular phenotype of adipose precursors is adopted postnatally, whereas fetal precursors are found throughout the developing stroma of adipose depots\(^\text{84}\). Additionally, fetal precursors might be at a slightly more differentiated state than their adult counterparts, evidenced by their expression of the lipid droplet protein perilipin and the adipokine adiponectin, the expression of which is conventionally restricted to mature adipocytes in adult animals\(^\text{85}\).

The difference in the molecular identity of fetal and adult precursors has implications for research and physiology. First, expression of adiponectin in fetal precursors means that targeting adipocytes using the commonly employed adiponectin promoter\(^\text{86}\) also targets fetal adipocyte precursors, while this targeting is limited to fully differentiated adipocytes in adults\(^\text{87}\). Careful interpretation of mouse models developed with this strategy is then required, as adipogenesis and development of the adipose depots can be altered in these mice.

Physiologically, the advanced state of differentiation of fetal precursors suggests that these cells are primed to respond to external and internal cues of adipogenesis differently. It is easy to imagine factors such as maternal nutrition state, that could influence the developmental patterning of adipose tissue, with lifelong implications for the organism.

**Visceral and subcutaneous white adipose tissue depots in adults.** The bulk of adipose mass in adults is present within the broadly defined subcutaneous and visceral depots demarcated by the peritoneum. These two classes of adipose depots have intrinsically different metabolic functions, including secretion of adipokines and inflammatory cytokines, lipolysis rates and thermogenic potential. Numerous human and rodent studies correlate increased subcutaneous adiposity relative to visceral adiposity with preserved metabolic health for subjects of similar weights (reviewed elsewhere\(^\text{89}\)).

Subcutaneous and visceral adipose depots also diverge in their developmental formation and remodeling potential upon overnutrition. Monitoring de novo adipocyte formation in mice revealed that subcutaneous adipose commitment and differentiation are established by embryonic days 14–18, and in adults, this depot...
expands primarily through hypertrophy in response to overfeeding. By contrast, the development of the visceral adipose depots occurs primarily postnataally, but this tissue is capable of expanding through hyperplasia as well as hypertrophy. Results utilizing alternative lineage-tracing systems and stable isotope labelling have led to similar conclusions.

How can we reconcile the metabolically protective expansion of subcutaneous fat in mice and humans with the lack of observable preadipocyte hyperplasia within this depot in mouse models? We suggest two possible explanations. First, it is important to consider that the vast majority of studies of adipogenesis in rodents use the C57/B6 mouse strain, partially because of the increased propensity of this strain to develop insulin resistance to model metabolic decline associated with obesity. We cannot rule out that a genetically diminished capacity of subcutaneous hyperplastic expansion is both a contributor and a consequence of this genetic susceptibility to insulin resistance, highlighting the need to expand the lineage-tracking approaches to other mouse strains. Notably, however, when isolated and cultured in vitro, cells from the subcutaneous depot of C57/B6 mice readily differentiate towards adipocytes. In addition, certain metabolically healthy obese states (promoted by TZD treatment or transgenic overexpression of adiponectin) display an increase in subcutaneous adipogenesis. This observation suggests that subcutaneous preadipocytes retain adipogenic capacity but are constrained in vivo under ‘normal’ experimental conditions of overnutrition. Stable isotope tracing of adipocyte turnover in mice also demonstrates that subcutaneous adipose tissue is capable of hyperplasia in juvenile mice but not in adults. This pattern of subcutaneous adipogenic potential followed by decline with ageing and obesity is recapitulated in humans, where subcutaneous adipose depots of young, lean humans expand through adipogenesis during overfeeding, but this capacity is diminished in individuals of advanced age and/or with obesity.

Adipogenesis outside of the main adipose depots.
Apart from those in the main subcutaneous and visceral fat depots, adipogenic events have also been described in other locations, including skin, skeletal muscle and bone marrow. Whereas adipogenesis appears to be beneficial in the skin, adipogenesis in the skeletal muscle and bone marrow is associated with pathology.

Skin is the largest organ of the body and has complex roles in immunity, thermoregulation and maintenance of the mechanical integrity of the organism. Skin contains many adipocytes that are anatomically localized directly below the dermis. These dermal adipocytes are clearly distinct from those in the subcutaneous depot. In rodents, the panniculus carnosus anatomically divides dermal from subcutaneous white adipose tissue. Humans (and various other mammals such as pigs) lack a panniculus carnosus, but dermal adipocytes are anatomically organized into ‘dermal cones’ superficial to the classical subcutaneous adipose tissue. Adipocytes in the skin are most highly appreciated for their mechanical cushioning, but recent studies have revealed new roles of dermal adipocytes in thermoregulation, hair follicle cycling, and fibroblast recruitment and extracellular matrix deposition during wound healing.

Adipogenesis in the dermal adipose depot is most strongly established during wound healing. During wound closure, adipocytes morphologically revert to a fibroblast-like state through a process termed adipocyte-myofibroblast transition (AMT) to aid in wound closure. A subset of myofibroblasts at a cutaneous wound site derive from an adiponectin-positive lineage, implying that they arise through an adipocyte de-differentiation event. Furthermore, there is increasing evidence that this transition is likely reversible, as complementary lineage tracing has demonstrated that dermal adipocytes arise from the myofibroblast lineage following wound closure. While AMT may represent de-differentiation of adipocytes to a less lineage-restricted myofibroblast, several studies also implicate the role of de novo adipogenesis in wound healing. Genetically lipoatrophic mice (‘fatless’ mice, which are genetically incapable of forming adipocytes) and mice treated with PPARγ antagonists to inhibit adipogenesis have profound wound-healing deficiencies. This finding supports the notion that adipocyte precursor proliferation may be involved in the wound-healing process. Clinically, preadipocytes (in clinical approaches referred to as adipose stem cells) are gaining much attention for their potential to improve healing in acute burns and chronic non-healing ulcers, and they are the subject of a number of relevant clinical trials for scars and other non-healing skin conditions (NCT02590042, NCT03113747, NCT02923219, NCT03427905 and NCT03264573). It is also of note that one of the most common sequelae of clinical diabetes is impaired wound healing, and several studies note improved wound healing in patients with diabetes who were treated with adipose stem cell therapy. Classically, wound healing in people with diabetes is attributed to increased inflammation or vascular insufficiency, but these studies raise the possibility of increasing dermal adipocyte number and quality as a novel therapeutic avenue to improve the wound-healing process in patients with diabetes.

Adipocyte deposition in skeletal muscle is associated with insulin resistance, sarcopenia, muscular dystrophy and muscle injury. In this case, the development of adipocytes is associated with a decline in muscle function, which is in contrast to the situation in adipose depots of the skin. In early 2010, two independent studies identified that these infiltrating adipocytes in skeletal muscle arise from precursors different from muscle fibre precursors, myoblasts. Uniquely, they further described the resident fibro-adipogenic progenitors (FAPs), which expressed low levels of PPARγ at baseline and could be prompted to undergo differentiation by niche factors intrinsic to the injured muscle. New data suggest that adipogenesis during muscle repair exacerbates muscle injury. Reducing adipogenic differentiation of FAPs (by blocking Hedgehog signalling; see below for a discussion of signalling modulators of adipogenesis) enhances muscle regeneration during acute muscular injury and in a model of muscular dystrophy.

The characteristically yellow appearance of bone marrow derives from a high quantity of adipocytes.
Young mice and humans have a small quantity of mature adipocytes within the bone marrow, but a great majority of the haematopoietic tissue of long bones is replaced with adipocytes by early adulthood, which were long thought of as simple filler for other bone marrow cells\(^a\). It is now appreciated that a variety of physiological factors, including obesity, anorexia, TZDs, glucocorticoids, irradiation and ageing, can induce further differentiation of bone marrow adipocytes\(^a\). The apposition of adipocytes and haematopoietic stem cells in bone marrow underlies extensive crosstalk between the two tissues. Indeed, bone marrow adipocyte-derived adipokines regulate haematopoietic regeneration\(^b\), as well as macrophage\(^c\), B cell\(^d\) and osteoclast development\(^e\). As in other adipose depots, bone marrow adipocytes arise from a mesenchymal precursor shared with osteoblasts and chondroblasts\(^f\). Uniquely, however, the preadipocytes of bone marrow are leptin receptor-positive (LepR\(^+\))\(^g\). Leptin has been shown to directly promote adipogenesis of bone marrow mesenchymal precursors and to inhibit osteogenic differentiation\(^h\). Impaired leptin receptor signalling in bone marrow mesenchymal precursors reduces differentiation to adipocytes following fracture and improves bone regeneration in mouse models\(^i\). Similarly, impairment of adipogenesis (in lipoatrophic mice or induced by treatment with a PPAR\(\gamma\) inhibitor) after irradiation improves bone marrow engraftment and supports haematopoietic recovery\(^j\). Interestingly, leptin does not appear to be required for adipogenesis following bone marrow irradiation\(^k\), implying that different signals may influence adipogenesis after different injuries. More recently, a separate study identified the adipogenic fraction of mesenchymal precursors that proliferate after bone fracture as ZFP423\(^+\) cells and demonstrated that increased adipogenesis is sufficient to inhibit fracture healing\(^l\). Thus, in both muscle and bone marrow, selective repression of local adipogenesis following a traumatic event is a new and promising therapeutic strategy to improve tissue regeneration.

**Importance of the niche in adipogenesis.** Given the heterogeneity and differences in the function of adipogenesis in different niches, a natural question arises: what defines these differences?

A number of observations indicate a high degree of plasticity of preadipocytes and implicate local niche factors in regulating adipogenesis. For example, donor preadipocytes isolated from subcutaneous or visceral depots behave in a manner consistent with the site of injection in the recipient rather than their depot of origin during high-fat feeding (undergoing differentiation in the visceral but not subcutaneous depot)\(^m\). This plasticity of preadipocytes is further implied by recent observations that PDGFR\(\alpha\) cells are able to form either beige adipocytes in response to \(\beta_3\)-adrenergic stimulation (BOX 1) or phenotypically white adipocytes during high-fat feeding\(^n\). Perhaps most striking is the new report that PPAR\(\gamma\) expression, though sufficient to drive adipogenesis in cultured fibroblasts\(^o\), is insufficient to stimulate adipogenesis in vivo in contexts when adipogenesis is physiologically limited (such as in subcutaneous adipose depots or under chow diet in mice)\(^p\).

These results raise the critical and thus far unaddressed questions — what signals constrain or permit adipogenesis in vivo, what are the cells of origin for these signals and how are they differentially regulated among adipose depots? These questions are especially difficult to address because visceral and subcutaneous adipose depots have functionally different adipocytes and feature considerable differences of tissue organization, including different densities of innervation and vascularization. In addition, recent single-cell sequencing data have demonstrated that the stromal populations of inguinal and visceral adipose tissues contain different cell subpopulations, some of which appear anti-adipogenic\(^q\),\(^r\), indicating differences in stromal cell populations between the different depots. As the tools for studying these subpopulations of stromal cells improve, it seems likely that they will provide insight into the niche-specific regulation of adipogenesis.

Single-cell sequencing reveals heterogeneity among preadipocytes in various depots (BOX 2), although it remains to be seen whether these preadipocytes represent different stages of differentiation or whether their molecular signatures are stable and characteristic of certain depots\(^s\). Some data also suggest an intrinsic limitation to the plasticity of adipose precursors. For example, brown adipocytes but not white or beige adipocytes derive from a myogenic factor 5-positive (MYF5\(^+\)) lineage (BOX 1), and suppression of brown adipogenesis through deletion of the thermogenic transcription co-regulator PRDM16 promotes acquisition of muscle morphology but not white adipocyte morphology\(^t\). By contrast, deletion of PRDM16 during beige adipogenesis promotes white adipocyte differentiation\(^u\). This suggests the existence of lineage plasticity between white and beige adipocytes that is not conserved in brown adipocytes. Notably, the point of divergence of white and brown adipocyte lineages is controversial, because some studies find some contribution of MYF5\(^+\) progenitors to the white adipose lineage\(^v\), suggesting greater potential for lineage plasticity in favourable conditions.

**Signalling modulators of adipogenesis**

Many important signalling hormones and ligands modulate the process of preadipocyte differentiation into a mature adipocyte (FIG. 4).

**Insulin signalling.** Insulin is an anabolic pancreatic peptide hormone secreted in response to increased plasma glucose to promote plasma glucose uptake into peripheral tissues, such as skeletal muscle and adipocytes, for utilization or storage. Insulin signals through high-affinity binding to the insulin receptor in specialized tissues or with lower-affinity binding through the related insulin-like growth factor 1 (IGF1) receptor found on all cells. Intracellularly, the insulin-signalling cascade then activates insulin receptor substrates (primarily IRS1) and then PI3K and AKT1 or AKT2 kinase. This cascade leads to the activation of CREB, mTOR and the family of forkhead proteins (FOXOs)\(^w\).
Insulin is an essential component of in vitro adipocyte differentiation medium, and highly adipogenic cells will differentiate spontaneously in vitro in the presence of insulin without other hormonal cocktails. Disruption of the components of the insulin signalling cascade, including the insulin receptor, IRS family members, PI3K, Akt1 or Akt2, mTOR, and the FOXO family, leads to failure of adipogenesis in vitro. In vivo, elimination of the insulin receptor from late-stage differentiating adipocytes using an AP2-promoter-driven Cre recombinase curiously does not lead to a failure of adipogenesis, suggesting that the IGF1 receptor is capable of compensating for the loss of the insulin receptor in vivo (at least during the late stages) provided the downstream signalling is intact.

Considering the physiological role of insulin in glucose metabolism, an interesting implication is that insulin serves as a permissive differentiation signal to preadipocytes. Persistent elevation of plasma insulin is a hallmark of overnutrition, and a logical adaptive response to this overnutrition would be to increase the adipocyte number to create additional safe storage sites for macronutrients. Considering that IGF1 receptors are expressed at much higher levels in preadipocytes than insulin receptors are, higher levels of insulin in vivo would be required to activate intracellular insulin signalling in undifferentiated preadipocytes than needed for mature adipocytes. However, many unanswered questions relating to the role of insulin in adipogenesis remain. Importantly, understanding of how preadipocytes discriminate between normal postprandial insulin elevations and insulin elevations associated with over-nutrition is elusive. Along similar lines, it is unclear how the response to insulin is regulated in preadipocytes, and signals that facilitate or oppose insulin signalling need to be refined.

**Glucocorticoid signalling.** Glucocorticoids are steroid hormones that signal through the nearly ubiquitously expressed glucocorticoid receptor to suppress inflammation and promote mobilization of nutrients from metabolic tissues. In healthy individuals, plasma glucocorticoids peak shortly after waking from sleep and fall throughout the rest of the day, whereas in individuals with obesity, elevated levels of plasma glucocorticoids persist. Glucocorticoids are one of the three critical components of in vitro adipocyte differentiation medium and are thus considered crucial pro-adipogenic signals. In preadipocytes, glucocorticoids facilitate growth arrest and are required for terminal differentiation. They also upregulate several transcription factors required for differentiation, including C/EBPs. Moreover, glucocorticoid signalling sensitizes preadipocytes to insulin signalling, enhancing the adipogenic actions of the insulin pathway.

**BMP signalling.** BMPs are a class of conserved signalling molecules in the transforming growth factor-β (TGFβ) family of proteins, originally identified for their ability to induce bone and cartilage formation but now known to act as growth factors throughout the body. BMP signalling through BMP receptors results in the intracellular phosphorylation and activation of SMAD proteins. As previously discussed, BMP signalling is required for adipogenesis in vitro, and active SMAD4 results in transcription of PPARγ and facilitation of adipogenesis. Different members of the BMP family have been shown to selectively promote brown or white adipocyte differentiation. Consistent with a role of BMP4 as a pro-adipogenic factor for white adipose tissue, individuals identified as obese but without diabetes have elevated circulating BMP4, suggesting that the elevation of BMP signalling allows for adequate adipogenesis to avoid metabolic decline in obesity.

**WNT signalling.** WNTs are a highly conserved family of autocrine and paracrine ligands known for their roles in embryonic development and carcinogenesis. Despite their generally pro-growth roles in these processes, WNTs are perhaps the most established suppressors of adipogenesis. Canonical WNT signalling results in the stabilization of β-catenin. In preadipocytes, this stabilization results in a failure of PPARγ and C/EBPα induction and, in some cases, a shift towards an osteoblastic or immune cell phenotype. In vivo, hypoxia
and the vascular endothelium were shown to increase WNT levels, providing an interesting means of limiting adipogenesis in regions where the vasculature is unable to support additional adipocytes.

**Hedgehog signalling.** The Hedgehog family of ligands is conserved from flies to humans and is critically involved in body patterning during embryogenesis. Hedgehog signalling decreases adipogenesis in rodent fibroblasts by interfering with BMP signalling and insulin signalling. Interestingly, Hedgehog signalling appears to redirect adipose precursors towards an osteogenic fate in vivo. It also restricts adipogenesis in the muscle after an injury to promote healing.

**Systemic regulation of adipogenesis**

Several key physiological states and cues can influence adipogenesis. These are more integrative than simple signalling inputs and could modulate adipogenesis more globally.

**Inflammation.** Chronic, low-grade inflammation is one of the hallmarks of diet-induced metabolic disease. The low-level inflammation during obesity is clearly different from the inflammation evoked during an acute infection but involves many of the same players, including macrophages.

Early studies utilizing in vitro differentiation of 3T3-L1 fibroblasts demonstrated that the addition of pro-inflammatory factors, such as TNF and IL-6 cytokines, to the culture medium impairs adipocyte differentiation. Macrophages are the main inflammatory cell type infiltrating adipose tissue and contribute considerably to the local levels of these pro-inflammatory agents during high-fat feeding. Interestingly, it has been demonstrated that a strong positive correlation exists between adipocyte size and the extent of macrophage infiltration in white adipose tissue. This finding raises the question — does in vivo inflammation suppress adipogenesis or is inflammation a secondary consequence of unhealthy adipose expansion?

The most established example for an anti-adipogenic inflammatory molecule is TGFβ. TGFβ is secreted from hypertrophic, dysfunctional adipocytes and from immune cells recruited to the adipose depot during obesity in mice and humans. Since the 1980s, elevated TGFβ has been shown to inhibit adipocyte differentiation in vitro, and mice overexpressing TGFβ have a lipodystrophic phenotype with severely impaired adipocyte differentiation. Molecularly, TGFβ signals through SMAD3 and directly inhibits PPARγ–C/EBPα complex formation and, in consequence, adipogenesis. Other inflammatory factors, such as TNF, are also known to directly impair adipogenesis but also promote the elevation of TGFβ as part of their downstream signalling, creating a synergistic effect.

Preadipocytes can adopt a macrophage-like inflammatory phenotype with increased expression of pro-inflammatory cytokines and decreased adipogenic capacity in response to inflammatory stimuli, which can eventually lead to adipose tissue fibrosis. A recent study suggested that adipogenic precursors do not necessarily need to adopt a macrophage phenotype to be inflammatory. A subset of fibroblast-like PDGFRα− precursors marked by high expression of the cell surface marker CD9, which are increased in individuals with diabetes, were found to contribute to tissue inflammation and fibrosis, whereas precursors with low CD9 expression, which are capable of adipogenesis, were depleted in individuals with diabetes. Such fibro-inflammatory progenitors (FIPs) were independently described in another study as a distinct cell population of PDGFRβ− perivascular cells within visceral adipose depots of mice. These FIPs were shown to express higher levels of inflammatory cytokines than regular adipocyte precursors and responded more strongly to inflammatory stimuli. Most surprisingly, FIPs could directly repress the differentiation of adipogenic precursors. This work raises the interesting possibility of heterogeneity within the adipogenic lineage in vivo and provides new insights into the mechanisms underlying the detrimental role of inflammation on adipogenesis.

Importantly, we draw a distinction between acute and chronic inflammation. Chronic inflammation contributes to the pathophysiology of most adipose tissue dysfunction, including the impairment of adipogenesis that contributes to insulin resistance and metabolic disease. Notably, however, transient acute inflammation of adipose depots seems to have a beneficial role in adipogenesis and metabolic homeostasis. For example, TGFβ signalling, when induced acutely, was shown to promote beige adipogenesis in white adipose depots, which is metabolically beneficial. By contrast, impairing adipose tissue inflammation during high-fat feeding is associated with decreased adipogenesis in vivo (although no defects are observed in vitro). These results suggest that transient, localized inflammation may be a potent stimulant of preadipocyte differentiation.

In summary, we find that transient inflammation is likely an important part of the tissue remodelling process required for adipogenesis. However, sustained chronic inflammation likely derives from insufficient adipogenesis within adipose tissue and forms a vicious cycle where inflammation further inhibits adipogenic precursor differentiation.

**Circadian rhythms.** Peripheral and central circadian oscillators are important to coordinate day and night cycles, behaviour (including feeding) and the overall physiological state. Disruption of normal circadian patterns in humans (for example as a result of shift work) increases the risk of obesity, including abdominal obesity associated with the metabolic syndrome. Emerging evidence from mouse models suggests that the increased adiposity in individuals with disrupted circadian rhythm is regulated at the level of the preadipocyte. The expression of the master adipogenic factor, PPARγ, is cyclic in white adipose tissue and liver but not in other peripheral metabolic tissues such as brown adipose tissue or skeletal muscle. Preadipocytes also show specific oscillations in the expression of core circadian clock components, including PER2 and PER3. Moleculately, both PER2 and PER3 restrain adipogenesis — PER2 through direct repression of PPARγ and PER3...
through the repression of KLF15, which was shown to promote adipogenesis of 3T3-L1 cells\(^{170}\), or potentially also through direct repression of PPAR\(_{\gamma}\)\(^{170}\). Additionally, the circadian oscillator BMAL1 and its downstream target, nocturnin, both oscillate in an antiphase manner to the PER paralogues and promote adipogenesis\(^{170}\) by enhancing PPAR\(_{\gamma}\) nuclear localization\(^{170}\).

Crucially, these studies implicate circadian cycling as a regulator of adipogenesis. However, a true understanding of the physiological impact of these factors is still missing. Most mouse models used to study circadian core components use constitutively null alleles, which are often deleted in multiple tissues other than preadipocytes (that is systemic deletions, adipocyte deletions and others). Such manipulations pose a risk of alterations in adipose tissue development, which would confound interpretation of adipogenic phenotypes in adults. Nonetheless, we can envision a scenario where the correct temporal combination of a circadian signal and a systemic or niche differentiation signal must align to induce an adipogenic event. A proof of concept for this systemic cue–circadian coordination of adipogenesis is observed with the temporal requirements for glucocorticoid signalling in differentiating preadipocytes. In healthy animals, plasma glucocorticoids oscillate, with elevations during the active (awake) period. These oscillations flatten in obese animals, leading to persistently high levels of circulating glucocorticoids. Similar to the persistent glucocorticoid signal in individuals with obesity that facilitates adipogenesis, it is standard to induce adipogenic differentiation in vitro with approximately 24 hours of treatment with the corticosteroid dexamethasone, which is an agonist of the glucocorticoid receptor. However, if this same total dose of dexamethasone is fractionated into 12-hour-on and 12-hour-off increments, then preadipocytes fail to differentiate\(^{172}\). Importantly, if the temporal exposure to dexamethasone is lengthened to 18 hours or longer, preadipocytes undergo adipogenic differentiation, suggesting the length of exposure to glucocorticoid signalling regulates differentiation independently of the total dose\(^{172}\).

This suggests that the controlled elevation of hormone levels allows for temporal coordination of PPAR\(_{\gamma}\) activity with the preadipocyte clock programme. Again, these results are limited to in vitro studies but support a model for systemic and circadian coordination of adipogenesis.

**Reactive oxygen species.** Reactive oxygen species (ROS) are molecules including free oxygen and superoxide with an unpaired electron. Normal mitochondrial oxidative metabolism generates a low amount of ROS, usually quenched by intracellular antioxidant enzymes. However, if the amount of ROS generated exceeds the buffering capacity of the cell, these electrophilic ROS damage DNA and RNA and oxidize lipids and amino acids in a process known as oxidative damage\(^{173}\). In liver, adipocytes and other metabolically active tissues, oxidative damage, especially to mitochondrial electron-transport-chain components, leads to inefficient ATP production and metabolic dysfunction\(^{173}\). In preadipocytes, early studies demonstrate that increased generation of intracellular ROS by interfering with the mitochondrial respiratory chain decreases preadipocyte differentiation in vitro, which was reversible with antioxidant treatment\(^{173}\).

However, compelling and seemingly contradictory data demonstrate that ROS may facilitate adipocyte differentiation. The addition of 100 µM hydrogen peroxide to a standard hormonal differentiation cocktail markedly improved adipogenic differentiation in the 3T3-L1 preadipocyte cell line. Interestingly, this effect was stronger in the absence of insulin\(^{173}\). Additional work showed that in human mesenchymal stem cells differentiating into adipocytes, generation of ROS is promoted by mTORC1 through activation of mitochondrial electron transport complex III. In addition, antioxidants targeted to the mitochondria are capable of substantially reducing adipocyte differentiation in vitro\(^{176}\). Since the publication of these initial results, several independent groups have further confirmed that decreasing cellular ROS or providing an extracellular antioxidant impairs adipocyte differentiation\(^{177–179}\).

At a molecular level, there appears to be a high level of crosstalk between insulin signalling, ROS and adipogenesis. Insulin signalling generates ROS through activation of the NADPH oxidase NOX4, which in turn inhibits PTP1B, an intracellular protein phosphatase and inhibitor of the insulin signalling pathway\(^{180,181}\). This property of ROS is associated with the enhancement of insulin signalling and increased adipogenesis. However, sustained high levels of ROS produced from NADPH oxidases during unhealthy obesity\(^{182}\) inhibit insulin signalling and thus inhibit adipogenesis\(^{183,184}\). How do we reconcile these seemingly paradoxical results? One interesting possibility is that there is a certain basal level of ROS required for preadipocyte differentiation. It is possible that a mild exogenous ROS signal sent from adipocytes to precursors serves as a developmental cue, promoting adipogenesis, whereas high levels of ROS in the tissue cause cell dysfunction and damage. Understanding the physiological sources of ROS during adipogenesis and the thresholds at which pro-adipogenic and anti-adipogenic responses occur is critical to reconciling this seeming paradox.

**Adipogenesis in health and disease.** Lack of developmental adipogenesis manifests as lipo-dystrophies (characterized by a complete or partial lack of adipose tissue), which has considerable detrimental effects on systemic glucose and lipid metabolism. Adipose tissue mass also declines with ageing, which could contribute to metabolic decline in old age. Finally, because adipose tissues support various aspects of metabolism, insufficient adipogenesis during obesity exacerbates the metabolic syndrome.

**Perturbation of developmental adipogenesis in lipodystrophies.** Lipodystrophies are a class of metabolic diseases caused by a decreased amount (or near complete absence) of adipose tissue. Individuals with lipodystrophies have very limited fat tissues but have profound insulin resistance and dyslipidaemia, because the lipid ordinarily stored in adipose tissue prematurely accumulates in secondary peripheral tissues.
Caveolae
Small, flask-like invaginations of plasma membrane that are abundant in many mammalian cell types including adipocytes. They have been implicated in various processes, including endocytosis, signalling, lipid regulation and mechanosensing.

Congenital generalized lipodystrophy (CGL) is the most severe form of lipodystrophy and presents as almost no adipose tissue in all locations from infancy. Several mutations have been identified to cause CGL, including mutations in the lysophosphatidic acid acyl-transferase, AGPAT2 (REF.187), the lipid droplet associated protein BSCL2 (REF.188), the plasma membrane scaffold- ing proteins associated with caveole CAV1 (REF.187) and PTFR188. AGPAT2 and BSCL2 are crucial for lipid biosynthesis and storage, a process required in adipocyte formation189,190. Specifically, AGPAT2-deficient preadipocytes fail to induce phospholipases required for terminal adipocyte differentiation, whereas BSCL2-deficient preadipocytes fail to sustain an upregulation of PPARγ, C/EBPα and other terminal adipocyte differentiation drivers in late-stage adipocyte differentiation191,192. Caveolae are induced nearly tenfold during adipogenesis193, and mice with deficiencies of CAV1 partially mimic the human lipodystrophic phenotype with severe adipose tissue dysfunction194. Although not tested directly, CAV1 may enhance adipogenesis through crosstalk with the insulin signalling pathway, as it has been shown to enhance intracellular insulin signalling195. Similarly, PTFR is required for functional caveole formation, and lack of PTFR prevents proper formation and release of caveole from cells196. CAV1-containing extracellular vesicles are essential for crosstalk between cells in adipose tissue in vivo197, and loss of this communication may reduce crucial cell–cell adipogenic signalling. Interestingly, extracellular vesicles are gaining attention for their potential therapeutic use198, and restoration of extracellular-vesicle-mediated communication in adipose tissue is an exciting new target for some of these most severe lipodystrophies.

Interestingly, both humans199 and mice200 with complete loss of adipose tissue show robust metabolic improvement with leptin administration. However, leptin does not restore fat mass; rather, it only attenuates insulin resistance and dyslipidaemia. Using the inductive fatless mouse (in which the loss of adipose tissue can be induced specifically in the adult), it was established that the loss of adipose tissue together with the loss of leptin presents with a more severe metabolic dysfunction than in the absence of leptin alone200. This finding suggests that the adipose tissue has profound regulatory inputs into systemic metabolism, beyond secretion of adipokines, and that restoration of adipose mass, in addition to the mere restoration of adipokine levels, is a beneficial therapeutic avenue for lipodystrophy patients in the future.

Familial partial lipodystrophy (FPLD) has a similar presentation to CGL, except the loss of adipose tissue is mainly from the subcutaneous depot. Mutations in lamin A and PPARy are most common, although mutations in lipid droplet biogenesis and metabolism factors (perilipin 1, CIDEC and lipase E) and in the adenosine 2A receptor, which is implicated in thermogenesis in brown and beige adipocytes, have been reported202. Deficiencies in adipogenesis resulting from mutations in PPARy are not surprising, but it is interesting to note that mutations in lamin A, which is a ubiquitous nuclear envelope protein, also cause primary defects in adipocyte differentiation203. The clinical spectrum of symptoms is much more varied in FPLD than in CGL, and leptin supplementation remains an efficacious treatment for patients, especially in those with marked hypoleptinaemia. However, unlike CGL, there is some evidence that TZDs may be efficacious at reversing the adipose tissue loss associated with FPLD and consequently alleviating metabolic dysfunction204–206.

Blunted adipogenesis during ageing. Ageing is generally associated with organ and tissue functional decline, and adipose tissue is no exception. Loss of subcutaneous adipose tissue mass along with the progressive ectopic lipid deposition in muscle and liver is a hallmark of age-related decline and frailty (reviewed elsewhere207). Ageing is also associated with the decline in adipogenic potential, which is caused by the acquisition of a senescent phenotype by preadipocytes, which restricts preadipocyte proliferation and differentiation capacity and contributes to adipose tissue deterioration during ageing208,209. In addition, these senescent preadipocytes actively decrease adipose tissue insulin sensitivity through secretion of pro-inflammatory cytokines, such as TNF210 and IL-6 (REF.211), both of which also inhibit adipogenesis156–158. Senescent preadipocytes were also shown to secrete activin A, a protein belonging to the TGFβ superfamily, which similar to TGFβ inhibits adipogenesis210. Pharmacological depletion of senescent cells is sufficient to maintain preadipocyte adipogenic capacity and preserves adipose tissue function in old age112,211. Targeting and reversing cellular ageing of preadipocytes are interesting therapeutic concepts to maintain adipose tissue homeostasis and metabolic health in aged individuals.

Furthermore, hyperplasia of adipose tissue can have indirect benefits on lifespan. Decreased insulin levels and insulin signalling are among the strongest factors associated with extended lifespan212–214. As illustrated in this Review, adipogenesis during overnutrition preserves metabolic health, leading to systemically decreased insulin levels, and dampens insulin signalling, thus potentially indirectly contributing to extended lifespan. Additionally, several adipokines including adiponectin21 and FGF21 (REF.215) correlate with extended lifespan. FGF21 can directly activate PPARγ to promote adipogenesis216, and there is evidence that smaller cells formed through adipogenesis express higher levels of adiponectin17. Taken together, these studies suggest the presence of a healthy, self-reinforcing cycle whereby healthy hyperplastic expansion of adipose tissue leads to a longevity-inducing adipokine profile, which in turn maintains systemic insulin sensitivity and metabolic health and could feed back to further promote adipogenesis.

Promoting adipogenesis for metabolic health. Many years of research using humans and animal models have established a strong correlation of adipogenesis and preserved metabolic health.

More recently, genome-wide association studies have identified several loci involved in adipocyte differentiation as susceptibility loci for the development of insulin resistance and ectopic fat deposition17,218. An interesting question then arises: can simply increasing adipose tissue mass enhance metabolic health?
Emerging work in mouse models suggests that improved metabolic health in obese animals can be induced upon further, healthy expansion of fat mass. In these mice, adipogenesis allows adipose tissue to expand while limiting hypoxia, chronic inflammation and fibrosis. In the absence of these pathologies, adipose tissue metabolic function is fully preserved. These findings raise the interesting possibility that obesity-associated metabolic decline is not due to adiposity, per se, but rather is a result of an insufficient capacity of adipose tissue to further expand. This leads to adipose tissue dysfunction and persistently elevated levels of plasma glucose and lipids, which accumulate in other tissues and contribute to insulin resistance.

Several transgenic mouse models of metabolically healthy obesity from our group and others demonstrate considerable adipose tissue expansion in comparison with that of the wild type but with the presence of smaller adipocytes. Despite their increased body mass compared with controls, these healthy obese mice all display extraordinarily preserved systemic metabolic health. It is important to note, however, that these mouse models certainly represent genetic manipulations (overexpression of factors promoting adipogenesis) and almost certainly also have developmental differences in adipose tissue formation. However, these models demonstrate that excess calories stored in healthy, hyperplastic adipose tissue do not contribute to metabolic decline. Consistent with the notion of healthy hyperplastic expansion in obesity demonstrated in these mouse models, treatment with TZDs to activate PPARγ has been reported to increase adipose tissue mass, decrease adipocyte size and improve systemic metabolism in rodents and humans. The expansion of the subcutaneous fat tissue is an integral component of these improvements.

More recently, studies in several new mouse models have highlighted the important role for hyperplastic expansion of the visceral depot in metabolic health maintenance. Tenomodulin is a transmembrane protein preferentially enriched in the adipocytes of obese individuals. Notably, tenomodulin has been recently shown as a factor required for adipogenesis, whereby constitutive overexpression of tenomodulin in adipocytes leads to visceral adipose tissue expansion through adipogenesis. Despite the fact that high visceral adiposity usually correlates with metabolic decline, the adipogenic nature of the expansion in this particular mouse model allows for improved metabolic health compared with control animals. In another study, inhibiting adult adipogenesis of PDGFRβ+ perivascular cells through deletion of PPARγ led to a pathological, hypertrophic white adipose tissue expansion upon high-fat-diet feeding. Conversely, stimulating adult visceral adipogenesis during overnutrition by overexpression of PPARγ had beneficial effects, including reduced levels of local inflammation, preserved serum adiponectin levels and improved metabolic health, without impacting body weight or adiposity. These data provide proof of concept that expanding the number of visceral adipocytes can occur without increasing overall adiposity and body weight, per se, even under increased caloric intake.

Right now, the question of whether adipogenesis confers a benefit to metabolically healthy individuals needs further exploration. It is challenging to manipulate adipogenesis in animal models without also influencing confounding factors, such as total adiposity, especially in light of the fact that physiological rates of adipogenesis in lean animals are very low. Additionally, multiple physiological brakes on adipogenesis exist in vivo (for example, overexpression of PPARγ in PDGFRβ+ perivascular cells does not drive differentiation under standard chow feeding conditions), and multiple cues are required to induce adipocyte hyperplasia. It is also important to note that, before the adoption of in vivo lineage-tracing models, adipogenesis was quantified in vivo by examining adipose cellularity, which would not detect changes in adipocyte turnover (balanced adipogenesis and apoptosis of adipocytes), which may confer an independent metabolic benefit by maintaining a constant adipose tissue mass. The difficulty of generating a lean mouse with increased adipogenesis is an endeavour, but such models are required to determine whether adipogenesis, per se, confers metabolic benefits.

Correlative studies in the clinic provide clues that adipogenesis in lean individuals may indeed be beneficial. For example, humans predisposed to hypertrophic obesity have reduced insulin sensitivity, even in the lean state, and this is a common feature seen for individuals without obesity but with clinical insulin resistance. Furthermore, follow-up studies of babies born during food shortages in Europe indicated that a decreased birthweight predisposes the child to obesity and insulin resistance later in life. While these early studies predominantly used birthweight as a surrogate for adiposity, several hints suggest that adipogenesis is specifically impaired. Maternal food restriction during the second trimester of pregnancy, when adipose tissue is actively being formed, results in severe metabolic impairments later in life, while restriction in the third trimester, after the tissue is formed, does not produce such lifelong metabolic consequences.

There is also evidence that too much adiposity at birth also predisposes individuals to metabolic disease. Cross-sectional studies comparing babies of European descent with Indian babies demonstrate that Indian babies of the same birthweight have relatively higher adiposity than their European-descent counterparts. This increased abdominal adiposity at birth carried an increased risk of obesity and diabetes in adulthood, suggesting that fetal expansion of abdominal adipose tissue may not be desirable.

There is clearly a high degree of crosstalk between maternal nutrition state, fetal adipose tissue development and lifelong risk of developing metabolic disease. Greater clarity about which factors influence adipogenesis during which stages of fetal development has enormous public health potential and is a very promising area for future study.

Conclusions and perspective
We are beginning to understand the complex web of molecular components and cues governing adipocyte differentiation. Several key themes emerge. First, adipogenesis
is a physiological process that enhances the ability of the tissue to safely sequester lipids and prevent lipotoxicity in peripheral organs. This is true during fetal development, where defective adipogenesis leads to lipodystrophies, as well as in adulthood, where preserved metabolic health during obesity correlates with and is facilitated through adipogenesis. Second, preadipocytes show remarkable heterogeneity and lineages plasticity and likely are perivascular. White adipose tissue contains a set of preadipocytes capable of producing both white and beige adipocytes in vivo. Adipocyte precursors are also found outside of adipose depots and have important roles (beneficial or detrimental) in skin, muscle and bone homeostasis. It has also been recently appreciated that adipocytes may return to a fibroblast ‘preadipocyte-like’ state, suggesting that adipocyte differentiation may not be terminal as previously thought. Third, local signalling and systemic cues, such as inflammation, ROS and other factors, influence adipogenesis. There is also evidence that a niche can modulate preadipocyte fate, fine tuning adipogenesis.

In addition, adipogenic precursors themselves — by modulating tissue inflammation — can impact adipogenesis. Finally, it is now evident that adipose depot health is more important than its size alone and that increased adipogenesis during weight gain can offset the negative metabolic consequences of high fat exposure.

Overall, adipogenesis now emerges as a viable therapeutic target. However, PPARγ agonists (TZDs) used as pro-adipogenic compounds were met with mixed enthusiasm and caused controversy upon their initial introduction to the market owing to the associated weight gain frequently observed and certain cardiac side effects (which later proved to be of no major concern). We believe that an increased understanding of adipogenesis holds great promise as a new avenue to treat various diseases, including metabolic disorders, lipodystrophies and healing and regeneration of tissues, such as skin, muscle and bone marrow, which should be made possible with locally acting modulators of adipogenesis.

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