Two paths to fat

Evan D. Rosen

Excess caloric intake leads to both the growth of existing fat cells and the generation of new adipocytes. New findings show that PI3K–Akt2 signalling is involved in the differentiation of adult adipose precursor cells — a pathway not required for adipogenesis in the embryo.

The extent to which most people think about adipose tissue is how they can get rid of it. In the face of a severe obesity epidemic, this seems reasonable. For many biologists, however, it is far more interesting to ponder how fat gets there in the first place, and this has been a far knottier problem than first imagined. Several recent papers have shed light on the process, and another appears in this issue of *Nature Cell Biology*. In a surprising twist, Jeffery *et al.*[1] suggest that the process of forming the mouse fat pad during development is very different from the route by which new adipocytes are recruited in the face of overnutrition.

Many of us were taught that "you are born with all the fat cells you will ever have." Like most old saws, this one turns out to be wrong. Although human adipose tissue is recognizable by around 14 weeks of gestation, and most development is complete by birth, we now appreciate that fat is a dynamic tissue that undergoes a constant process of renewal and elimination; approximately 8–9% of adipocytes are replaced every year[2]. Mice develop fat pads later — the subcutaneous depot appears late in gestation, and visceral fat (that inside the abdominal cavity) does not arise until several days after birth. As with humans, however, mice display continuous adipocyte turnover throughout their lives[3].

Many groups have sought to identify the adipose stem cells that allow for the initial development of the fat pad, with much of the early work in this area done by tissue engineers exploiting the fact that fat is one of the few adult tissues that human subjects do not mind parting with. More recent studies have used flow-cytometric sorting of cells with various surface markers to identify adipose stem cells, and found that $Lin^-Sca1^+CD29^+CD34^+$ cells show excellent adipogenic potential[4]. When these cells were further subdivided into $CD24^+$ and $CD24^-$ fractions, both could be converted to adipocytes *in vitro*, but only the former could reconstitute a functional fat pad when transplanted into a lipodystrophic mouse[5].

But what happens during overnutrition? The adipose depot gets bigger, of course, but is it because more fat cells form (hyperplasia) or because existing adipocytes get larger (hypertrophy)? Or do both processes occur? Histological studies have shown definitively that hypertrophy happens. However, adipocytes have a finite storage capacity (believed to be around 0.7–0.8 μg lipid per cell), and once existing cells have reached that limit, new adipocyte formation is required to prevent lipid deposition in liver, muscle or other inappropriate locations. A cellular fate-mapping study found that the obesity-associated growth of visceral fat is due to a combination of hypertrophy and hyperplasia, while growth of the subcutaneous pad seems to depend almost exclusively on hypertrophy[6]. However, the timing of the obesity effect was not examined in detail, and the assumption was that hypertrophy happens first, with hyperplasia kicking in when the storage capacity of pre-existing adipocytes is reached. A prevalent hypothesis holds that a signal must be released by the 'full' adipocyte, recruiting precursor cells to divide.

Jeffery *et al.* address this issue directly. Using a somewhat different fate-mapping approach, they first confirmed the earlier results by showing that visceral fat in mice responds to overnutrition with a combination of hypertrophy and hyperplasia, whereas subcutaneous adipose tissue grows primarily through hypertrophy (Fig. 1). However, they noted that BrdU (bromodeoxyuridine) incorporation (indicative of

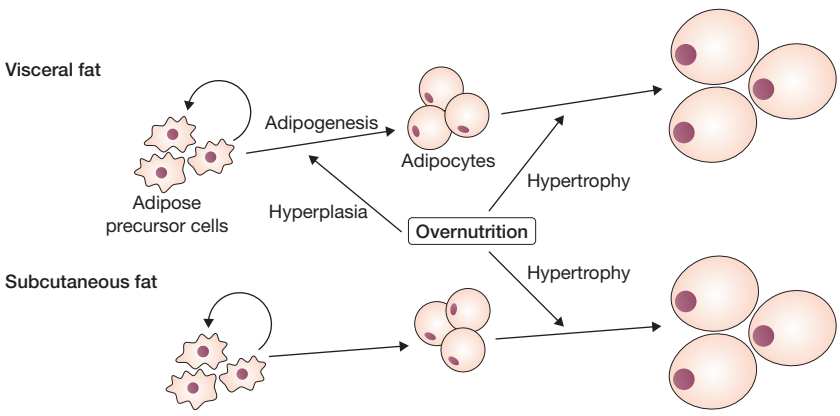

Figure 1 Alternative ways of accommodating additional fat. In the context of overnutrition, both subcutaneous and visceral white adipose tissues expand by hypertrophy (enlargement of existing fat cells) but only visceral fat undergoes a simultaneous hyperplastic response (the formation of new fat cells from adipose precursors).

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cell proliferation) occurred in adipose precursors — defined as $Lin^{-}Sca1^{+}CD29^{+}CD34^{+}$ — only during the first week of high-fat feeding, and only in visceral fat. This was seen in both the $CD24^{+}$ and $CD24^{-}$ subfractions of these cells, although $CD24^{+}$ cell proliferation was noted before $CD24^{-}$ cells became marked with BrdU. This effect was apparent as early as twenty-four hours after the introduction of a high-fat diet, with the highest rate of proliferation seen at three days. This finding has major implications for the previously proposed model, because it shows that adipose precursor cells begin to proliferate almost immediately after exposure to a high-calorie diet, and are thus not 'waiting' for a signal from hypertrophic adipocytes.

The authors found that these newly generated adipose precursor cells do not complete differentiation into mature adipocytes for several weeks, which is much longer than the week or so that it takes for adipogenesis to occur in several *in vitro* models[7]. Presumably, the precursor cells *in vivo* are at an earlier stage of development than the preadipocytes used in these models, and must pass through additional, as yet undetermined, steps before becoming fully differentiated.

So, how might an adipose stem cell detect a state of overnutrition? There are many logical candidates for a nutrient-sensing system, but Jeffery *et al.* focused on insulin signalling, in particular the phosphatidylinositol-3-OH kinase (PI3K)–Akt2 node of the insulin signalling pathway. Insulin levels rise rapidly after feeding, and remain high during a high-fat diet. The authors found that total Akt phosphorylation status was elevated three days after the onset of high-fat diet (coinciding with the peak stage of adipose precursor proliferation), and that this was almost all due to Akt2, and not Akt1, signalling. Functional evidence for the involvement of PI3K–Akt2 was provided by a pharmacological strategy using the PI3K inhibitor wortmannin, which caused reduced precursor-cell proliferation, and by a genetic approach, in which the *Akt2* gene was knocked out in adipose precursor cells, demonstrating a similar effect. Finally, an A*kt2* global knockout mouse showed the same result, but an *Akt1* knockout mouse did not. Thus, PI3K–Akt2 signalling is clearly required for adipose precursor proliferation and the expansion of the visceral fat pad in the setting of increased caloric intake (Fig. 2).

Jeffery *et al.* also observed that young $Akt2^{-/-}$ mice develop apparently normal visceral fat pads, despite the fact that there is a burst of precursor cell proliferation during the first postnatal week of life. This striking result indicates that the mechanism by which fat pads expand in obesity is fundamentally different to how they form in the first place, and it could imply that different stem-cell populations are at play in each scenario. In fact, this is exactly the conclusion drawn by another recent fate-mapping study[8], which identified 'developmental' adipose stem cells as generating the nascent fat pad, and 'adult' stem cells that replenished the adipocyte population during normal turnover (the effect of a high-fat diet was not described). This study also reported that the adult stem cells appear in embryogenesis before the developmental cells. So do those 'adult' stem cells correspond to the Akt2-dependent adipose precursor cells Jeffery *et al.* identify as proliferating in response to a high-fat diet? We cannot yet say for sure, but this is an attractive and parsimonious idea that should be explored.

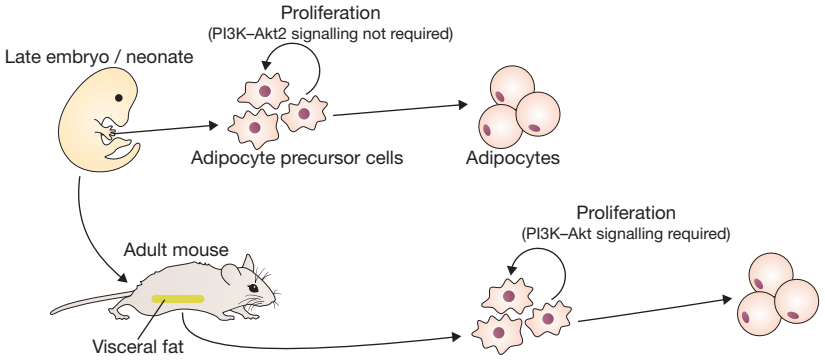

Figure 2 Adipose stem cell signalling in embryos and adults. The stem cells that generate white adipose tissue depots in late embryogenesis and early postnatal life undergo dramatic replication that does not require PI3K–Akt2 signalling. By contrast, adipose precursor cells within the visceral fat of an adult mouse respond to overnutrition with a replicative burst that is dependent on this signalling pathway.

These studies raise several other key issues, including to what extent these findings from murine models can be applied to humans. People with Akt2 loss-of-function mutations have been described, and they have somewhat reduced fat mass in the setting of severe insulin resistance and diabetes, whereas people with gain-of-function mutations in Akt2 have the opposite phenotype[9,10]. There are many possible explanations for this, but the data are at least consistent with an Akt2-dependent increase in proliferative capacity of adipose precursor cells during adult life. Certainly, the fact that patients without Akt2 have fat at all suggests that this pathway is dispensable for nascent fat pad development in humans, as in mice.

Another question concerns the nature of the nutrient-associated signal that induces adipose precursor proliferation in visceral fat. It seems clear that PI3K–Akt2 signalling is required, but that does not imply that the primary signal is insulin, or another insulin-like factor. Glucose, fatty acids or other hormonal signals (such as gut-derived incretins) may also be involved, and this area needs further research.

Finally, the depot-selective nature of the adipose precursor cell proliferative response is striking, and it begs the question of why this effect is limited to visceral fat. Are precursor cells in each depot intrinsically different, perhaps related to different embryological origins, or are there local factors that dictate whether a 'generic' adipose precursor cell is able to proliferate in response to nutrients? Perhaps more importantly, it is worth considering whether adipose precursor proliferation has anything to do with the well-known association between visceral fat expansion and poor metabolic outcomes. Reduced adipose proliferative capacity is associated with insulin resistance[11], so perhaps this capability of visceral fat might be enhanced as a potential therapeutic strategy.

COMPETING FINANCIAL INTERESTS
The authors declare no competing financial interests.

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