gravitational energy to answer this question, but planets and stars do. With self-gravity in the picture, a concept called the strong equivalence principle comes into play. This principle singles out the general theory of relativity from its competitors. In Einstein's theory, all bodies — hammers, feathers, planets, neutron stars, white dwarfs and even black holes — fall with the same acceleration. But in most alternative theories of gravity, such as scalar–tensor theories[6], the equivalence principle is violated for bodies that have self-gravity.

For almost 50 years, researchers have measured how long it takes for laser pulses to make the round trip from Earth to the Moon and back — a technique known as lunar laser ranging. Analyses of these data[7,8] have verified the strong equivalence principle, by showing that the accelerations of the two bodies towards the Sun differ by no more than a few parts per 10^{13}. Because about 5 parts in 10^{10} of Earth's mass is gravitational energy[9], this result implies that the accelerations of gravitational energy and matter differ by less than a few parts per 10^{4}.

Archibald and colleagues' study breaks new ground because the gravitational energy inside a neutron star can account for as much as 20% of the body's mass[10]. The authors' results therefore imply that the accelerations of gravitational energy and matter differ by no more than a few parts per 10^{5} — a tenfold improvement over the bound from lunar laser ranging.

More importantly, the authors have provided what is known as a strong-field test of general relativity. Unlike the Solar System, for which Einstein's theory predicts only small deviations from Newton's theory of gravity, the motion of a neutron star in a gravitational field invokes full general relativity in all its complex glory. Einstein's theory passes this strong-field test with flying colours.

Because general relativity predicts a null effect, the grading is a simple pass or fail. But for alternative theories, invoking strong-gravity effects substantially complicates the interpretation of the results. Archibald *et al.* demonstrate this complexity using scalar–tensor theories as an example. For these theories, the interpretation of the results depends on the internal structure assumed for the neutron star and on the values chosen for quantities known as coupling constants. The authors show that their results improve on certain pre-existing constraints on the parameters that govern these theories — some arising from Solar System measurements and some from data on binary systems containing a pulsar. Although the theories are not completely quashed, their hopes for validity have been made that much fainter. ■

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DEVELOPMENTAL BIOLOGY

Fat gets a brake

Single-cell transcriptional profiling of stem and progenitor cells in fat tissue identifies distinct cell subpopulations, one of which inhibits fat growth by signalling to neighbouring cells. SEE LETTER P.103

DAVID A. GUERTIN

Fat tissue has a remarkable capacity for growth. It can expand in two ways: by increasing the size of individual fat cells (adipocytes), and by making adipocytes from progenitor cells through the process of adipogenesis. Fat is essential for metabolic fitness, but having too much fat in the wrong places can be harmful. Obesity is a precursor to serious medical conditions such as type 2 diabetes, cardiovascular disease and cancer, which are ravaging health-care systems worldwide. Key to combating obesity is understanding how mature adipocytes develop from precursor cells, but the identity of these precursors has so far been elusive. On page 103, Schwalie *et al.*[1] define three populations of fat-precursor cell, one of which unexpectedly functions to suppress adipocyte production.

The origin of the body's adipocytes has been a mystery, complicated by the fact that fat tissues, called depots, contain many cell types other than adipocytes. In addition, adipocytes at different anatomical locations originate from different early embryonic precursors[2]. There is also metabolic variation between fat depots around the body, and even between adipocytes in the same depot[3–5].

A pool of adipocyte stem and progenitor cells (ASPCs) can be isolated from fat depots using a technique called fluorescence-activated cell sorting (FACS), which separates cells on the basis of specific cell-surface proteins[6,7]. Many of the surface markers currently used to isolate ASPCs through FACS were selected because they distinguish stem-like cells from other tissues. However, the pools isolated when trying to obtain ASPCs using this method contain a mixture of cell types, and the molecular profiles of the true stem and progenitor cells within the mix has remained largely undefined.

Schwalie and colleagues started with a mixture of 208 ASPCs isolated from mouse fat tissue using FACS. They performed single-cell RNA sequencing to determine which genes are expressed in each cell. Using computer algorithms to group the cells according to their gene-expression profiles, the authors discovered that at least three distinct subpopulations exist within the ASPC pool. They then confirmed the existence of these three subpopulations using an alternative cell-isolation strategy combined with a different method of grouping ASPCs by their gene-expression signatures.

Most of the ASPCs fell into two of three groups discovered by the authors. The first group, designated P1, expressed high amounts of stem-cell markers. The second group, designated P2, expressed many genes that regulate the early steps of adipocyte formation. But it was the smallest group, P3, representing less than 10% of the cell population, that drew the authors' attention. Unlike the other groups, P3 cells did not form mature adipocytes when

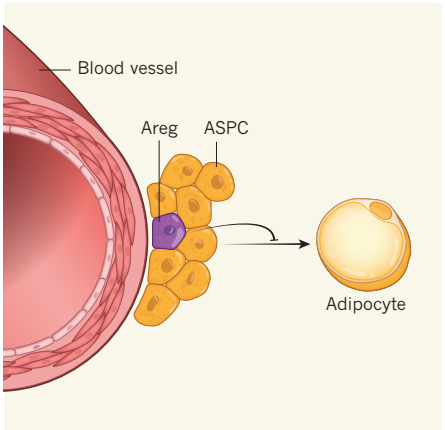

Figure 1 | A cell population that inhibits fat growth. A pool of adipocyte stem and progenitor cells (ASPCs) that is found around blood vessels gives rise to fat cells, which are called adipocytes. Schwalie *et al.*[1] identified a subpopulation of cells within the ASPC pool, dubbed Aregs. These cells release signals to inhibit the formation of adipocytes from ASPCs.

induced to differentiate in a cell-culture dish. In addition, removing P3 cells from the ASPC pool improved the ability of the other cells in the dish to differentiate into adipocytes.

These data suggest that the low-abundance P3 cells inhibit adipogenesis. Schwalie and colleagues named these cells adipogenesis regulators (Aregs), and confirmed the cells' function *in vivo*. First, they transplanted two mixtures of ASPCs — one lacking Aregs, the other containing the entire cell pool — into mice. Each mouse received both mixtures, one on each side of the body. Next, the researchers fed the mice a high-fat diet to induce adipogenesis. Over a few weeks, the implanted cell mixture lacking Aregs grew many more adipocytes than the other mixture did, indicating that Aregs inhibit fat growth. The authors also showed that Aregs exist in human fat, implying that fat-development mechanisms are conserved between mice and humans.

How do Aregs inhibit adipogenesis? Schwalie *et al.* found that the cells reside near the blood vessels of fat tissues in mice, a location that was previously proposed as the site of adipocyte precursor cells[8] (Fig. 1). Next, the authors investigated whether Aregs signal to neighbouring cells through physical contact or by sending chemical (paracrine) signals to nearby cells. Co-culture experiments, in which the authors placed a barrier permeable to small molecules between the Aregs and their target cells, revealed that direct contact is not needed for Aregs to influence fat-cell formation, indicating that the signal is paracrine.

To identify candidate signalling molecules, the researchers inactivated genes that are highly expressed in Aregs. They found that the gene *Rtp3* needed to be turned on to enable Aregs to send their inhibitory signals. Little is known about the Rtp3 protein, and it is not obvious how it works in this context. This is an area ripe for future study, because modulating the signals released by Aregs could have therapeutic potential for controlling fat growth.

Schwalie and colleagues' findings are exciting for several reasons. First, although high variation between ASPC subpopulations had been predicted, this study fills a major gap by adding molecular details to our understanding of that variability. Second, the authors use state-of-the-art technology for single-cell gene-expression profiling, enabling them to identify a regulatory cell type that would have been difficult to predict on the basis of previous studies. It is to be hoped that this study will stimulate other work aimed at elucidating the organization of adipogenesis (the hierarchy of cells that regulate the formation of fat), as has been achieved for blood-cell lineages[9].

The current study adds to the mounting evidence that paracrine signals help to remodel stem- and progenitor-cell function[10], and opens up several avenues for future research. For instance, what is the anti-adipogenic signal, and how does Rtp3 help to stimulate Aregs to produce it? Genetic or age-related differences in Areg number or function might contribute to body-fat patterning or the propensity to become obese, and these possibilities should also be explored.

It will be interesting to determine whether the ASPC pool can be divided into further subpopulations with more-specific functions. Tracing the *in vivo* fates of these different subpopulations would be a powerful strategy for picking apart which cells become adipocytes and which become fat-supporting cells. Finally, perhaps one of the most interesting questions raised by the study is whether there is a true adult adipocyte stem cell, which, by definition, would be capable of producing both committed adipocyte progenitors and more adipocyte stem cells.

As the devastating human costs of obesity-related conditions rise, research and health-care professionals must meet the challenge with breakthroughs in medical management and care. This will require a better understanding of adipogenesis, and Schwalie and colleagues' work has pointed to a new way of advancing knowledge in this important area. ■

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TISSUE ENGINEERING

Living replacement heart valves remodelled

Bioengineered heart valves are a promising treatment for heart-valve disease, but often undergo mechanical failure when implanted. Computational modelling of the initial valve design has now improved their performance in sheep.

CRAIG A. SIMMONS

Heart-valve disease has been described as an emerging epidemic[1], owing to its worldwide prevalence, its potential to kill, and the lack of therapies for its prevention or treatment. Damaged and defective valves can be replaced with prosthetic ones, but the inability of prostheses to grow or adapt to change makes them a poor solution for young patients[2]. An alternative is a living replacement made from bioengineered tissue. But, so far, tissue-engineered heart valves (TEHVs) have failed because detrimental valve-tissue remodelling occurs *in vivo*, impairing normal function. Writing in *Science Translational Medicine*, Emmert *et al.*[3] address this problem using computational modelling to design a TEHV that remodels favourably after implantation.

A promising strategy for heart-valve tissue engineering is to grow tissues in the shape of a valve in the laboratory using cells and a degradable biomaterial, then remove the cells to leave an empty extracellular-matrix scaffold. After implantation in the heart, the tissue scaffold is populated by the recipient's cells, presumably from the blood and the adjacent blood-vessel wall. Some of these cells then transform into contractile cells that degrade the scaffold and replace it with new tissue, while pulling on the tissues to hold everything together, and speed up remodelling. In all animal studies so far, however, this process has led to excessive tissue production, which thickens and stiffens the valve's three leaflets to obstruct blood flow, or to excessive tissue contraction, which causes the leaflets to shorten and retract, preventing proper valve closure and allowing backflow of blood (Fig. 1a).

After valve repair in healthy hearts, the contractile cells typically enter a deactivated state called quiescence, or are cleared from the region by programmed cell death. But certain stimuli, such as biomechanical stresses[4], can cause the cells to persist and remain activated. This can lead to excessive collagen production and tissue contraction — a process known as fibrosis[4]. In the native valve, fibrosis leads to leaflet thickening or retraction, which is similar to the problem seen in TEHVs. Biomechanical activation of fibrosis can be a particular problem in heart valves that are subjected to abnormalities in stretch, compression and pressure changes as they open