

Dynamics of fat cell turnover in humans

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Obesity is increasing in an epidemic manner in most countries and constitutes a public health problem by enhancing the risk for cardiovascular disease and metabolic disorders such as type 2 diabetes^{1,2}. Owing to the increase in obesity, life expectancy may start to decrease in developed countries for the first time in recent history³. The factors determining fat mass in adult humans are not fully understood, but increased lipid storage in already developed fat cells (adipocytes) is thought to be most important^{4,5}. Here we show that adipocyte number is a major determinant for the fat mass in adults. However, the number of fat cells stays constant in adulthood in lean and obese individuals, even after marked weight loss, indicating that the number of adipocytes is set during childhood and adolescence. To establish the dynamics within the stable population of adipocytes in adults, we have measured adipocyte turnover by analysing the integration of ¹⁴C derived from nuclear bomb tests in genomic DNA⁶. Approximately 10% of fat cells are renewed annually at all adult ages and levels of body mass index. Neither adipocyte death nor generation rate is altered in early onset obesity, suggesting a tight regulation of fat cell number in this condition during adulthood. The high turnover of adipocytes establishes a new therapeutic target for pharmacological intervention in obesity.

The fat mass can expand by increasing the average fat cell volume and/or the number of adipocytes. Increased fat storage in fully differentiated adipocytes, resulting in enlarged fat cells, is well documented and is thought to be the most important mechanism whereby fat depots increase in adults^{4,5}. To analyse the contribution of the fat cell volume in adipocytes to the size of the fat mass, we first analysed the relationship between fat cell volume and total body fat mass (directly measured with bioimpedance or estimated from body mass index (BMI), sex and age in a large cohort of adults). As expected, there was a positive correlation between the measures of fat mass and fat cell volume both in subcutaneous fat (Fig. 1a–c), which represents about 80% of all fat, and in visceral fat (Fig. 1d), which has a strong link to metabolic complications of obesity. However, the relationship between fat cell volume and fat mass markedly differed from a linear relationship (likelihood ratio test $P < 0.001$, and Akaike information criterion, described in Supplementary Information 1) in both subcutaneous and visceral adipose regions and both sexes, indicating that fat mass is determined by both adipocyte number and size. In the nonlinear case, both fat cell number and fat cell size determine fat mass. If the relationship had been linear, fat cell volume would be the only important determinant of fat mass.

The generation of adipocytes is a major factor behind the growth of adipose tissue during childhood⁷, but it is unknown whether the number of adipocytes changes during adulthood. We assessed the

total adipocyte number in 687 adult individuals and combined this data with previously reported results for children and adolescents⁸. Although the total adipocyte number increased in childhood and adolescence, this number levelled off and remained constant in adulthood in both lean and obese individuals (adults over 20 yr, grouped in 5-yr bins; ANOVA, lean $P = 0.68$, obese $P = 0.21$; Fig. 2a and Supplementary Information 3). Thus, the difference in adipocyte number between lean and obese individuals is established during childhood^{7,8} and the total number of adipocytes for each weight category stays constant during adulthood (Fig. 2b). The small variation in adipocyte number for each BMI category demonstrates that this is a stable cell population during adulthood.

To analyse whether alterations in adipocyte number may contribute to changed fat mass under extreme conditions, we next asked whether fat cell number is reduced during major weight loss (mean body weight loss, $18 \pm 11\%$, mean \pm s.d.) by radical reduction in calorie intake by bariatric surgery (reduction of the stomach with the purpose of facilitating weight loss). The surgical treatment resulted in a significant decrease in BMI and fat cell volume; however, this failed to reduce adipocyte cell number two years post surgery (Fig. 2b, c and Supplementary Information 4), in line with previous studies using different methodology^{9–12}. Similar results were found in a complementary longitudinal study¹³. Ref. 13 found that significant weight gain (15–25%) over several months in non-obese adult men resulted in a significant increase in body fat, which was accompanied by an increase in adipocyte volume, but no change in adipocyte number. Similar to our findings, subsequent weight loss back to baseline resulted in a decrease of adipocyte volume, but, again, no change in adipocyte number. Although we cannot rule out that a more prolonged period of weight gain in adulthood could result in an increase in adipocyte number, these results and ours indicate that fat cell number is largely set by early adulthood and that changes in fat mass in adulthood can mainly be attributed to changes in fat cell volume. This may indicate that the number of adipocytes is set by early adulthood with no subsequent cell turnover. Alternatively, the generation of adipocytes may be balanced by adipocyte death, with the total number being tightly regulated and constant.

We next set out to establish whether adipocytes are replaced during adulthood, and, if so, at what rate. Adipocytes can be generated from adult human mesenchymal stem cells and pre-adipocytes *in vitro*¹⁴ and may undergo apoptosis or necrosis^{15–17}, but it is unclear whether adipocytes are generated *in vivo*¹⁴. Cell turnover has been difficult to study in humans. Methods used in experimental animals, such as the incorporation of labelled nucleotides, cannot readily be adapted for use in humans owing to potential toxicity. The detection of cells expressing molecular markers of proliferation can give

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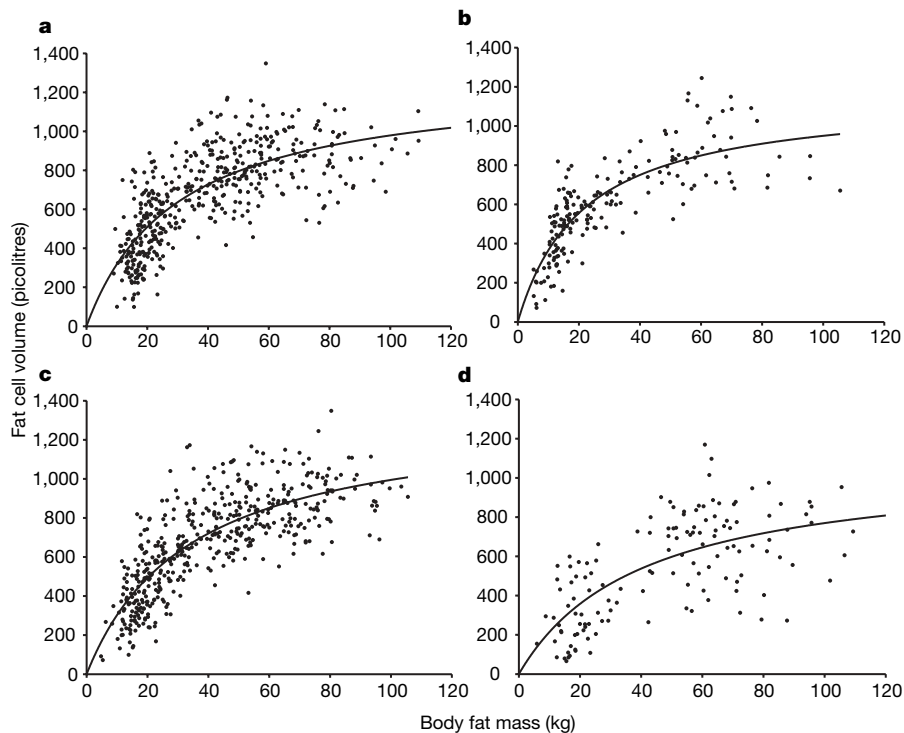


Figure 1 | Fat mass is determined by both adipocyte number and size. **a–d**, The relationship between fat mass and fat cell volume was curvilinear across the range of body fat mass in female (**a**) and male (**b**) subcutaneous fat (n female = 480; n male = 190), in combined female and male subcutaneous fat (**c**; n female = 357, n male = 117), and in male and female visceral fat (**d**; n female = 84, n male = 51). This demonstrates that both adipocyte number

and adipocyte size are determinates of body fat mass. In **a**, **b** and **d**, body fat mass was estimated from BMI using a previously described formula (conversion formula is described in Supplementary Information 1 and 4); in **c**, fat mass was determined using bioimpedance. Fat cell volume is given in picolitres, where 10^{-12} litres = 10^{-9} cm^3 .

insights about mitotic activity, but fail to provide information regarding the fate of the progeny of the dividing cells. This is a limitation when studying postmitotic cell types, which do not divide or express mitotic markers themselves (for example, neurons or adipocytes) but may be replenished from proliferating stem or progenitor cells, such as preadipocytes.

In this study, we used a recently developed method that is based on the incorporation of ^{14}C from nuclear bomb tests into genomic DNA and allows the analysis of cell turnover in humans^{6,18}. Levels of ^{14}C in the atmosphere were relatively stable until the Cold War, when above-ground nuclear bomb tests (1955–1963) caused a notable increase^{19,20} (Fig. 3a, b). Even though the detonations were conducted at a limited number of locations, increased ^{14}C levels in the atmosphere rapidly equalized around the globe. Since the Test-Ban Treaty in 1963, the ^{14}C levels have dropped exponentially, not because of radioactive decay (half-life 5,730 yr), but by diffusion from the atmosphere²¹. Atmospheric ^{14}C reacts with oxygen to form CO_2 , which is

incorporated into plants by photosynthesis. By eating plants, and animals that live off plants, the ^{14}C concentration in the human body closely parallels that in the atmosphere at any given point in time^{22–24}. Because DNA is stable after a cell has gone through its last cell division, the ^{14}C level in DNA serves as a date mark for when a cell was born; this can be used to retrospectively birth-date cells in humans^{6,18}.

To address whether adipocytes are generated from newborn cells in adulthood, we isolated fat cells ($\geq 98\%$ purity, Supplementary Information 4) from adipose tissue collected during liposuction or abdominal wall reconstruction from 35 adult lean or obese individuals. The pure isolation of adipocytes is important because non-adipose cells are present in adipose tissue and these cell types may have a different turnover rate (see Supplementary Information 4 for a full discussion). Genomic DNA was extracted from the purified adipocytes, and ^{14}C levels were measured by accelerator mass spectrometry and related to atmospheric ^{14}C data (Fig. 3c, d and Supplementary Information 4). We first analysed individuals born

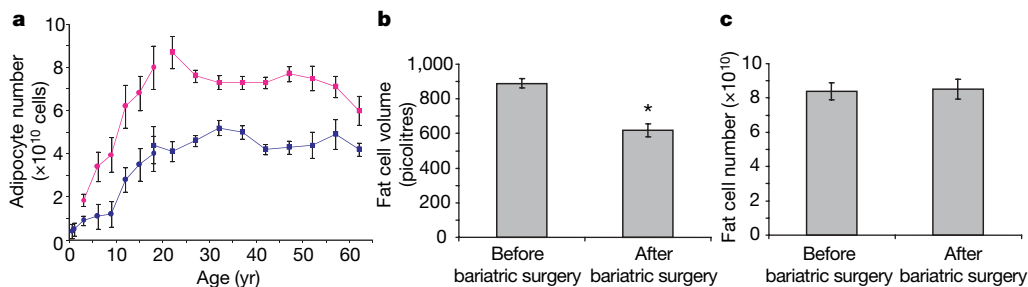


Figure 2 | Adipocyte number remains stable in adulthood, although significant weight loss can result in a decrease in adipocyte volume. Total adipocyte number from 595 (n lean = 253; n obese = 342) adult individuals (squares) was combined with previous results for children and adolescents⁸ (circles; n lean = 178; n obese = 120). **a**, The adipocyte number increases in

childhood and adolescence, with the number levelling off and remaining constant in adulthood in both lean (blue) and obese (pink) individuals. **b**, **c**, Major weight loss by bariatric surgery results in a significant decrease in cell volume (**b**), however fails to reduce adipocyte cell number (**c**), 1–2 yr post surgery ($n = 20$). All error bars represent s.e.m.; asterisk, $P < 0.0001$.

well before the period of nuclear bomb tests. This provides a high sensitivity to detect the generation of cells born after the time of onset of the nuclear bomb tests (1955), because ^{14}C levels above those present before the Cold War can be detected even if only a small (1%) proportion of cells in a population are renewed⁶. In all analysed individuals born before 1955 ($n = 10$), the ^{14}C levels were substantially higher than the atmospheric levels before the nuclear bomb tests, indicating that generation of adipocytes had taken place after 1955 (Fig. 3c, see Supplementary Information 2 for all ^{14}C measurements and associated data). The individuals were 0–22 years old at the onset of nuclear bomb tests, establishing that adipocytes are generated during adolescence and in early adulthood. New adipocytes may also be formed by differentiation of existing post-mitotic pre-adipocytes; hence, DNA integration of ^{14}C provides a lower bound to the generation of adipocytes.

Analysis of individuals born before the onset of the nuclear bomb tests provides a high sensitivity to detect cell turnover, but alone does not allow the establishment of the turnover rate because a certain ^{14}C level can correspond to the rising or the falling part of the atmospheric ^{14}C curve. However, the integration of data from individuals born before and after the period of nuclear bomb tests allows determination of cell turnover as well as the relative contribution of cell death and cell renewal to this process (see Supplementary Information 3). We therefore also analysed ^{14}C levels in adipocyte genomic DNA from individuals born after the period of nuclear bomb tests ($n = 25$). In all of these individuals, the ^{14}C levels corresponded to surprisingly contemporary time points (Fig. 3d and Supplementary Information 2), providing a first indication that there is continuous and substantial turnover of adipocytes in adult humans.

We next calculated the dynamics of fat cell turnover using a simple birth and death model (detailed in Supplementary Information 3). The model's assumptions allow the calculation of kinetic rates for individual subjects. The death rate of adipocytes is approximately $8.4 \pm 6.2\%$ per yr (median \pm average deviation) in the total fat pool of the body. The distribution of death rates is skewed towards lower values and is not the normal gaussian (Jarque–Bera test for normality, $P < 0.05$); therefore, the median \pm average deviation is more informative than the mean \pm s.d.²⁵. To test the reliability of the death-rate estimates, we used three different scenarios concerning the generation of adipocytes early in life, and confirmed that different estimates of the death rates do not differ from the median (sign test, $P > 0.3$; see Supplementary Information 3 for description of the scenarios). We divided the data set into lean ($\text{BMI} < 25 \text{ kg per m}^2$) and obese ($\text{BMI} \geq 30 \text{ kg per m}^2$, all of which had early onset obesity, see Supplementary Information 4) for analyses of the influence of obesity on adipocyte death rate. No significant difference in adipocyte death rate was seen across the different BMIs, with obese individuals having a median adipocyte death rate of $9.5 \pm 5.1\%$ (median \pm average deviation) per yr, versus $8.2 \pm 5.3\%$ (median \pm average deviation) per yr for lean individuals ($P = 0.6$ using the Kruskal–Wallis test, which tests for equality of medians; Fig. 4a). We found no trend for an increase in average cell number in subjects aged 20–70 yr using data presented in Fig. 2b ($n = 650$ and $P = 0.19$ by linear regression analysis), arguing that the adipocyte death rate per yr must be matched with a similar birth rate. This translates into an adipocyte turnover rate similar for all weight categories. We calculate a median turnover rate of $8.4 \pm 6.2\%$ (median \pm average deviation) per yr, with half of the adipocytes replaced every 8.3 yr.

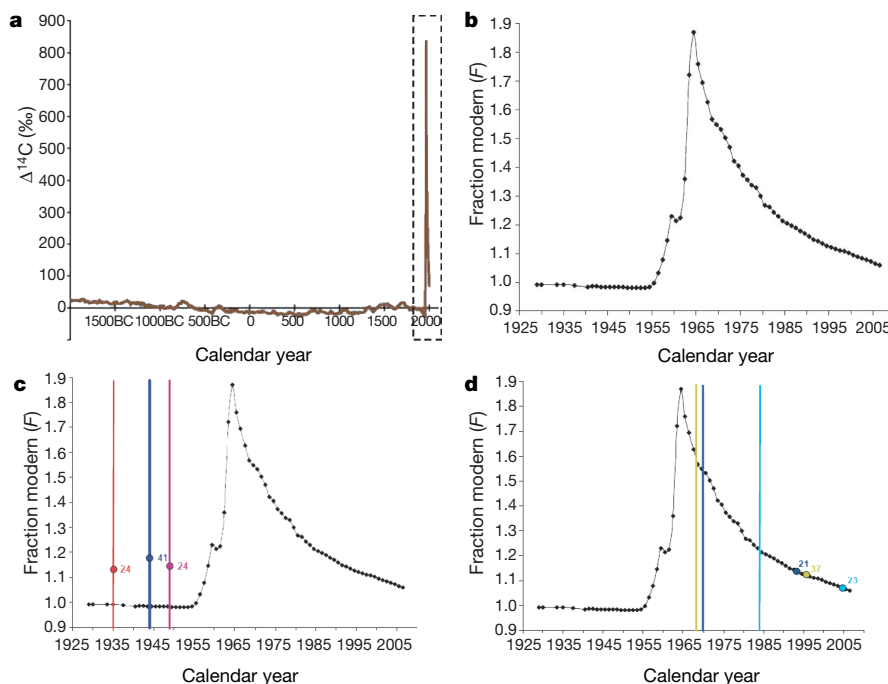


Figure 3 | Turnover of adipocytes in adulthood. **a, b**, The levels of ^{14}C in the atmosphere have been relatively stable over long time periods, with the exception of a large addition of ^{14}C in 1955–1963 as a result of nuclear bomb tests²¹. The boxed region in **a** is shown in more detail in **b**. ^{14}C levels from modern samples are by convention given in relation to a universal standard and corrected for radioactive decay, giving the $\Delta^{14}\text{C}$ value³⁰. **c, d**, Adipocyte age in adult human subjects born before (**c**) and after (**d**) nuclear bomb tests were analysed by determining the ^{14}C concentration in adipocyte genomic DNA using accelerator mass spectrometry. The measured ^{14}C value is related to the recorded atmospheric levels to establish at what time point

they corresponded. The year is plotted on the x axis, giving the birth date of the cell population. Three representative individuals born at different times before the onset of the bomb tests reveals the generation of adipocytes after birth (**c**). Analysis of the oldest individuals established that adipocytes are born in adolescence and in adulthood (**c**). ^{14}C levels analysed in people born after the period of nuclear bomb tests showed continuous and substantial turnover of adipocytes in adult humans (**d**). The time of birth of the person is indicated by a vertical line in each graph and the BMI is shown numerically (**c, d**). Error bars for the accelerator mass spectrometry readings are too small to be visualized in this graph. Each dot represents one individual.

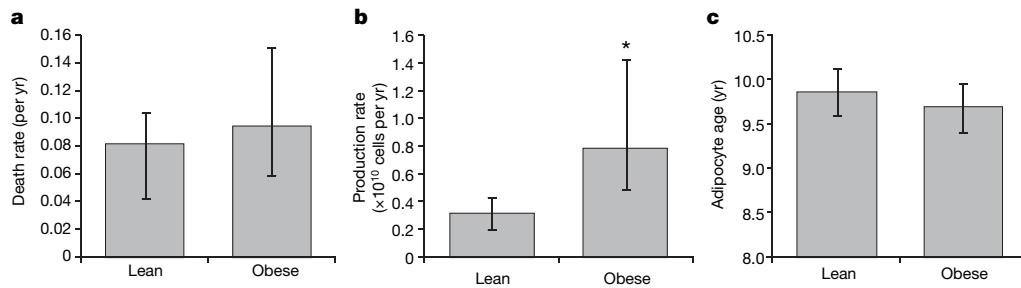


Figure 4 | Effect of obesity on adipocyte generation and death. **a**, No significant difference in adipocyte death rate per year was seen across the different BMIs. **b**, Obese individuals had a significantly greater number of adipocytes added per year than lean individuals. **c**, No significant difference in

Using the death-rate estimates and the fat cell numbers calculated for individual subjects, absolute fat cell production was calculated. Obese individuals were found to have a significantly greater number of adipocytes added per year than lean individuals: $(0.8 \pm 0.5) \times 10^{10}$ cells per yr versus $(0.3 \pm 0.2) \times 10^{10}$ cells per yr (median \pm average deviation; $P < 0.01$, ANOVA; Fig. 4b). Loss of fat cells is therefore compensated by the production of new fat cells, which is twice as high in obese subjects compared with lean subjects. The fact that the total number of new adipocytes added each year is greater in obese compared with lean individuals, yet the proportion of newborn adipocytes added each year (the turnover) is the same for both groups, argues that the difference in cell number between the lean and obese adults occurs before adulthood. In support of this, we found no significant difference in the average age of adipocytes in lean 9.9 ± 3.5 yr (mean \pm s.d.) versus obese 9.7 ± 4.0 yr (mean \pm s.d.) individuals (Fig. 4c). No significant correlation between the age of subjects and cell death or between the age of patients and adipocyte generation was found (Supplementary Information 3), suggesting a constant turnover rate throughout adult life.

If the number of adipocytes is set to a higher level in obese people before adulthood, this could be because cell-number expansion begins earlier (age of onset), because expansion is faster (growth relative to the initial cell number (IC) at age of onset), or because expansion ends later (age at 90% of adult cell number). We used combined adipocyte number data (Fig. 2a) to see whether one or more of these factors determine adipocyte number. Using our birth and death model, we determined that age at onset of adipocyte number expansion is significantly earlier in obese (2.1 ± 0.9 yr) than in lean (5.7 ± 0.8 yr) subjects; the relative increase in adipocyte number is higher in obese (2.4 ± 0.6 IC yr $^{-1}$) than in lean (1.3 ± 0.3 IC yr $^{-1}$) subjects, but end of expansion of adipocyte number is earlier in obese (16.5 ± 1.3 yr) than in lean (18.5 ± 0.7 yr) subjects (all values are predicted values \pm 95% confidence interval, Supplementary Information 3). Therefore, adult cell number is set earlier in obese subjects and is not caused by a prolonged expansion period in adulthood.

We find that the number of adipocytes for lean and obese individuals is set during childhood and adolescence, and that adipocyte numbers for these categories are subject to little variation during adulthood. Even after significant weight loss in adulthood and reduced adipocyte volume, the adipocyte number remains the same. Although we show that the adipocyte number is static in adults, we also demonstrate that there is remarkable turnover within this population, indicating that adipocyte number is tightly controlled and not influenced by the energy balance. Studies of previously obese individuals after weight loss show that their adipose tissue hypercellularity is associated with leptin deficiency, which is likely to increase appetite and to lower energy expenditure²⁶. These factors promote lipid accumulation in fat cells and weight gain towards the status before weight loss. Thus, a tight regulation of adipocyte number, together with mechanisms maintaining their energy balance, may contribute to why obese individuals have difficulties maintaining weight loss.

the average age of adipocytes in lean versus obese individuals was found. In **a** and **b**, values are the medians and error bars indicate the location of the first and third quartiles; in **c**, data are shown as mean \pm s.e.m. Asterisk, $P < 0.01$ for lean ($n = 13$) versus obese ($n = 14$) individuals, Kruskal–Wallis test.

It should be stressed that our conclusions on the rates of adipocyte turnover (^{14}C data) were obtained from studies on subjects with early onset of obesity. We cannot rule out that those who gradually gain significant weight over years in adulthood may initially increase their adipocyte size until a threshold is reached and thereafter recruit new fat cells from committed precursor cells or mesenchymal stem cells. Most obese adults have been obese since childhood, with less than 10% of children with normal weight going on to develop adult obesity²⁷. By contrast, over three-quarters of obese children go on to become obese adults²⁷. Thus, understanding the dynamics of adipocyte turnover in adults who have been obese since childhood is of great importance, especially given the current trend for an increase in childhood obesity.

The size of organs can be regulated by different mechanisms, and the number of cells in some tissues is controlled by a systemic feedback mechanism²⁸. This is best understood for skeletal muscle, in which growth and differentiation factor 8 (GDF8), also known as myostatin, is secreted from myocytes and negatively regulates the generation of new muscle cells and thereby sets the number of cells²⁹. Loss-of-function mutations in *GDF8* result in a large increase in the number (and size) of myocytes in animals and humans²⁹. The steady production of adipocytes in adults results in a stable size of the constantly turning over adipocyte population. Feedback mechanisms that control adipocyte turnover will be important to identify at a molecular level because this may offer a novel target for pharmacological therapy when obesity is established and for other types of intervention during childhood and adolescence when the final number of fat cells in the body is being set.

METHODS SUMMARY

Subjects. The relationship between subcutaneous or visceral fat cell volume, BMI and fat mass was studied in two separate cohorts, and fat cell turnover was studied in a third cohort, all of which are described in Supplementary Information 4.

Isolated fat cells. Fat cells were isolated from the adipose tissue as described in Supplementary Information 4. Details on how to measure weight, volume and the number of fat cells as well as determination of the purity of the adipocytes are given in Supplementary Information 4.

^{14}C analysis. Genomic DNA was prepared from isolated fat cells, and was purified and subjected to accelerator mass spectrometry analyses, as described in Supplementary Information 4 and tabled in Supplementary Information 2.

Data analysis. The calculations of relationship between fat cell volume and BMI or fat mass are described in detail in Supplementary Information 1. The calculations of fat cell death and generation are described in detail in Supplementary Information 3.

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Supplementary Information is linked to the online version of the paper at www.nature.com/nature.

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