LAWRENCE
LIVERMORE
NATIONAL
LABORATORY

UCRL-JRNL-233117

# Dynamics of Adipocyte Turnover in Humans

K.L. Spalding, E. Arner, P.O. Westermark, S. Bernard, B.A. Buchholz, O. Bergmann, L. Blomqvist, J. Hoffstedt, E. Naslund, T. Britton, H. Concha, M. Hassan, M. Ryden, J. Frisen, P. Arner

July 26, 2007

Nature

# Dynamics of adipocyte turnover in humans

Kirsty L. Spalding[1, *], Erik Arner[1], Pål O. Westermark[2], Samuel Bernard[3], Bruce A. Buchholz[4], Olaf Bergmann[1], Lennart Blomqvist[5], Johan Hoffstedt[5], Erik Näslund[6], Tom Britton[7], Hernan Concha[5], Moustapha Hassan[5], Mikael Rydén[5], Jonas Frisén[1, *] and Peter Arner[5, *].

[1]Department of Cell and Molecular Biology, Karolinska Institute, SE-171 77 Stockholm, Sweden. [2]Institute for Theoretical Biology (ITB), Humboldt University Berlin and Charité, Invalidenstrasse 43, 10115 Berlin, Germany [3]Institute of Applied and Computational Mathematics, Foundation of Research and Technology, 71110 Heraklion Crete, Greece. [4]Center for Accelerator Mass Spectrometry, Lawrence Livermore National Laboratory, 7000 East Ave., L-397, Livermore, CA 94551 USA. [5]Department of Medicine, Karolinska University Hospital, SE-141 86 Stockholm, Sweden. [6]Department of Surgery, Danderyds Hospital, SE-182 88 Stockholm, Sweden Stockholm, Sweden. [7]Department of Mathematics, Stockholm University, 106 91 Stockholm, Sweden.

*Address correspondence to

Kirsty Spalding, PhD, Assistant Professor, Phone +46 70 437 1542, fax +46 8 324927, e-mail kirsty.spalding@ki.se

Jonas Frisén, MD, PhD, Professor, Phone +46 8 52487562, fax +46 8 324927, e-mail jonas.frisen@ki.se

Peter Arner, MD, PhD, Professor, Phone +46 8 58582342, Fax +46 8 58582407, e-mail peter.arner@ki.se

**Obesity is increasing in an epidemic fashion 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 history[3]. The factors determining fat mass in adult humans are not fully understood, but increased lipid storage in already developed fat cells is thought to be most important [4,5].**

**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 and even under extreme conditions, 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 analyzing the integration of $^{14}C$ derived from nuclear bomb tests in genomic DNA[6]. 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 obesity, suggesting a tight regulation of fat cell number that is independent of metabolic profile in 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 thought to be the most important mechanism whereby fat depots increase in adults [4,5]. To analyze the contribution of the fat cell volume in adipocytes to the size of the fat mass, we first analyzed the relationship between fat cell volume and body mass index (BMI, which reflects the fat mass) in a large cohort of adults. As expected, there was a positive correlation between BMI and fat cell volume both in subcutaneous fat, which represents about 80% of all fat (Fig. 1a,b), and in visceral fat (Fig. 1c) which has a strong link to metabolic complications of obesity. If the relationship had been linear fat cell volume would be the only important determinant of BMI. In the non-linear case both fat cell number and fat cell size determine BMI. The relationship was curve-linear indicating that increasing BMI in adulthood is a result of increased number of fat cells plus enlargement of fat cells.

The generation of adipocytes is a major factor behind the growth of adipose tissue during childhood[7], 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 adolescents[8]. Whilst total adipocyte number increased in childhood and adolescence, this number levelled off and remained constant in adulthood in both lean and obese (adults over 20 years, grouped in 5 year bins; ANOVA, lean $p = 0.68$, obese $p = 0.21$; Fig. 2a; Supplement 3). Thus, the difference in adipocyte number between lean and obese individuals is established during childhood[7,8] and the total number of adipocytes stays constant during adulthood, albeit at higher levels with increasing BMI (Fig. 2b). The small variation in adipocyte number during adulthood demonstrates that this is a stable cell population.

To analyze 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% ± 11%) by radical reduction in calorie intake by bariatric surgery and dietary restriction. The calorie reduction resulted in a significant decrease in BMI and fat cell volume, however failed to reduce adipocyte cell number 2 years post surgery (Fig. 2b, c: Supplement 4), in line with previous studies using different methodology[9-12]. Thus, whereas differences in adipocyte generation in lean and obese has a major impact on establishing the fat mass during childhood and adolescence, the number of fat cells stays remarkably stable and fat mass only appears to be regulated by fat cell volume in adults. 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*[13] and may undergo apoptosis or necrosis[14-16], but it is unclear whether adipocytes are generated *in vivo*[13]. 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 due to potential toxicity. The detection of cells expressing molecular markers of proliferation can give 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. We have 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[6,23]. $^{14}C$ levels in the atmosphere have remained relatively stable until the Cold War when above ground nuclear bomb tests caused a dramatic increase[18,19] (Fig. 3a, b). Even though the detonations were

conducted at a limited number of locations, the elevated $^{14}C$ levels in the atmosphere rapidly equalized around the globe. After the test ban treaty in 1963, the $^{14}C$ levels have dropped exponentially, not because of radioactive decay (half-life 5730 years), but by diffusion from the atmosphere[20]. 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[17,21-22]. Since 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 and can be used to retrospectively birth date cells in humans[6,23].

To address whether adipocytes are generated in adulthood, we isolated fat cells (≥98% purity, Supplement 4) from adipose tissue collected at liposuction or abdominal wall reconstruction from 35 adult lean or obese individuals. The pure isolation of adipocytes is important as non-adipose cells are present in adipose tissue, and these cell types may have a different turnover rate (see supplement 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; Supplement 4). We first analyzed individuals born 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), as $^{14}C$ levels above those present prior to the Cold War can be detected even if only a small (1%) proportion of cells in a population are renewed. In all analyzed individuals born before 1955 (n=10), the $^{14}C$ levels were substantially higher than the atmospheric levels prior to the nuclear bomb tests, indicating that generation of adipocytes had taken place after 1955 (Fig 3c, see Supplement 2 for all $^{14}C$ measurements and associated data). The individuals were 0-22 years at the onset of nuclear bomb tests, establishing that adipocytes are generated during adolescence and in early adulthood.

Analysis of individuals born before the onset of the nuclear bomb tests provides high sensitivity to detect cell turnover, but does not alone allow the establishment of the turnover rate, since a certain $^{14}C$ level can correspond to the rising or 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 allow the determination of cell turnover, as well as the relative contribution of cell death and cell renewal to this process (see Supplement 3). We therefore also analyzed $^{14}C$ levels in adipocyte genomic DNA from individuals born after the period of nuclear bomb tests (n=25). In all these individuals the $^{14}C$ levels corresponded to surprisingly contemporary time points (Fig. 3d and Supplement 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 Supplement 3). The model's assumptions allow the calculation of kinetic rates for individual subjects. The death rate of adipocytes is approximately 0.084±0.07 per year (median ± average deviation) in the total fat pool of the body. The distribution of death rates is skewed toward lower values and is not Gaussian (Jarque-Bera test for normality, $p<0.05$); hence the median is more informative than the mean[24]. 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 (Kruskall-Wallis test, $p>0.3$, see Supplement 3 for description of the scenarios). We divided the data set into lean (BMI <25 kg/m$^2$) and obese (BMI ≥30 kg/m$^2$) 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 0.095 ±0.05 per year, versus 0.082 ±0.06 per year for lean individuals (Kruskal-Wallis test for equality of medians, $p=0.6$; Fig. 4a). Since adipocyte number remains constant in adults, the adipocyte death rate will correspond to the rate of adipocyte birth. Thus, we

calculate a median adipocyte turnover rate of 8.35±6.6% per year, with 50% of adipocytes replaced every 8.30 years.

From the death rate estimates and the fat cell numbers calculated for each individual subject, absolute fat cell production was calculated. Obese individuals were found to have a significantly greater number of adipocytes added per year than lean individuals (median values 0.79±0.54 vs. 0.32±0.19x$10^{10}$ cells/y, ($p<0.01$, Kruskal-Wallis test; Fig. 4b). Loss of fat cells is therefore compensated by a production of new fat cells that is twice as high in obese subjects compared to lean subjects. That the total number of new adipocytes added each year is greater in obese individuals compared to lean, yet the proportion of newborn adipocytes added each year is the same for both groups, argues that the difference in cell number between the lean and obese adults occurred prior to adulthood. In support of this, we found no significant difference in the average age of adipocytes in lean (mean 9.86±3.5y) versus obese (mean 9.66±4.0y) individuals (Fig. 4c). No significant correlation between age of subjects and cell death or age of patients and adipocyte generation was found (Supplement 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 is either 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±0.9y) than in lean (5.7±0.8y) subjects; relative increase in adipocyte number is higher in obese (2.4±0.6 IC/y) than in lean (1.3±0.3 IC/y) subjects, but end of expansion of adipocyte number is earlier in obese (16.5±1.3y) than in lean subjects (18.5±0.7y) (predicted values ±95% confidence interval, Supplement 3). Therefore adult cell

number is set earlier in obese subjects and is not due to a prolonged expansion period in adulthood.

We find that the number of adipocytes is set during childhood and adolescence and adipocyte numbers are subject to little variation during adulthood. An increase in fat mass in young is reflected in both increased adipocyte number and volume, but changes in fat mass in adults are the result of altered fat cell volume. Whereas the total fat cell number is static in adults, there is remarkable turnover within this population. That total adipocyte number in adults stays constant, regardless of BMI and even after marked weight loss, and the substantial turnover within this cell population, indicates that the total fat cell number is tightly controlled and not influenced by the energy balance.

The size of organs can be regulated by different mechanisms, and the number of cells in some tissues is controlled by a systemic feedback mechanism[25]. This is best understood for skeletal muscle, where 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 cells[26]. Loss of function mutations in *GDF8* results in a large increase in the number (and size) of myocytes in animals and humans[26]. 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 as it 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.

## Materials and methods

### Subjects

The relationship between subcutaneous or visceral fat cell volume and BMI was studied in two separate cohorts and fat cell turnover was studied in a third cohort, all are described in Supplement 4.

### Isolated fat cells

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

### $^{14}C$ analysis

Genomic DNA was prepared from isolated fat cells, purified and subjected to accelerator mass spectrometry analyses as described in Supplement 4 and tabled in Supplement 2.

### Data analysis

The calculations of relationship between fat cell volume and BMI are described in detail in Supplement 1. The calculations of fat cell death and generation are described in detail in Supplement 3.

## Acknowledgements

We thank M. Stahlberg and T. Bergman for help with HPLC and D. Kurdyla, P. Zermeno and Alan Williams for producing graphite. This study was supported by grants from Knut och Alice Wallenbergs Stiftelse, Human Frontiers Science Program, the Swedish Research Council, the Swedish Cancer Society, the Swedish Heart and Lung foundation, the Novo Nordic Foundation, the Swedish Diabetes Foundation, the Foundation for Strategic Research, the Karolinska Institute, the Tobias Foundation and NIH/NCRR (RR13461). This work was performed in part under the auspices of the U.S. Department of Energy by University of California, Lawrence Livermore National Laboratory under contract W-7405-Eng-48.

## References


1. Van Gaal, L. F., Mertens, I. L. & De Block, C. E. Mechanisms linking obesity with cardiovascular disease. *Nature* **444**, 875-80 (2006).
2. Kahn, S. E., Hull, R. L. & Utzschneider, K. M. Mechanisms linking obesity to insulin resistance and type 2 diabetes. *Nature* **444**, 840-6 (2006).
3. Olshansky, S. J. et al. A potential decline in life expectancy in the United States in the 21st century. *N Engl J Med* **352**, 1138-45 (2005).
4. Bjorntorp, P. Effects of age, sex, and clinical conditions on adipose tissue cellularity in man. *Metabolism* **23**, 1091-102 (1974).
5. Hirsch, J. & Batchelor, B. Adipose tissue cellularity in human obesity. *Clin Endocrinol Metab* **5**, 299-311 (1976).
6. Spalding, K. L., Bhardwaj, R. D., Buchholz, B. A., Druid, H. & Frisen, J. Retrospective birth dating of cells in humans. *Cell* **122**, 133-43 (2005).
7. Prins, J. B. & O'Rahilly, S. Regulation of adipose cell number in man. *Clin Sci (Lond)* **92**, 3-11 (1997).
8. Knittle, J. L., Timmers, K., Ginsberg-Fellner, F., Brown, R. E. & Katz, D. P. The growth of adipose tissue in children and adolescents. Cross-sectional and longitudinal studies of adipose cell number and size. *J Clin Invest* **63**, 239-46 (1979).
9. Miller, M et al., Demonstration of de novo production of adipocytes in adult rats by biochemical and radioautographic techniques. *J Lipid Res* **25**, 336-347 (1984).
10. Kral, J. et al. Body composition and adipose tissue cellularity before and after jejuno-ileostomy in severely obese subjects. *Eur J Clin Inv* **7**, 413-419 (1977).
11. Björntorp, P. et al. Effect of an energy reduced dietry regimen in relation to adipose tissue cellularity in obese women. *Am J Clin Nut* **28**, 445-452 (1975).

12. Häger, A. et al. Adipose tissue cellularity in obese school girls before and after dietary intervention. *Am J Clin Nut* **31**, 68-75 (1978).

13. Rodriguez, A. M., Elabd, C., Amri, E. Z., Ailhaud, G. & Dani, C. The human adipose tissue is a source of multipotent stem cells. *Biochimie* **87**, 125-8 (2005).

14. Petruschke, T. & Hauner, H. Tumor necrosis factor-alpha prevents the differentiation of human adipocyte precursor cells and causes delipidation of newly developed fat cells. *J Clin Endocrinol Metab* **76**, 742-7 (1993).

15. Prins, J. B., Walker, N. I., Winterford, C. M. & Cameron, D. P. Apoptosis of human adipocytes in vitro. *Biochem Biophys Res Commun* **201**, 500-7 (1994).

16. Cinti, S. et al. Adipocyte death defines macrophage localization and function in adipose tissue of obese mice and humans. *J Lipid Res* **46**, 2347-55 (2005).

17. Spalding, K. L., Buchholz, B. A., Bergman, L. E., Druid, H. & Frisen, J. Forensics: age written in teeth by nuclear tests. *Nature* **437**, 333-4 (2005).

18. De Vries, H. Atomic bomb effect: variation of radiocarbon in plants, shells, and snails in the past 4 years. *Science* **128**, 250-1 (1958).

19. Nydal, R. & Lovseth, K. Distribution of radiocarbon from nuclear tests. *Nature* **206**, 1029-31 (1965).

20. Levin, I. & Kromer, B. The tropospheric 14CO2 level in mid latitudes of the northern hemisphere (1959-2003). *Radiocarbon* **46**, 1261-1272 (2004).

21. Libby, W. F., Berger, R., Mead, J. F., Alexander, G. V. & Ross, J. F. Replacement Rates for Human Tissue from Atmospheric Radiocarbon. *Science* **146**, 1170-2 (1964).

22. Harkness, D. D. Further investigations of the transfer of bomb 14C to man. *Nature* **240**, 302-3 (1972).

23. Bhardwaj, R. D. et al. Neocortical neurogenesis in humans is restricted to development. *Proc Natl Acad Sci U S A* **103**, 12564-8 (2006).

24. Altman, D.G., Practical Statistics for Medical Research, Chapman & Hall/CRC, p. 164 (1991).

25. Raff, M.C. Size control: the regulation of cell numbers in animal development. *Cell*. **26**:86(2):173-5 (1996).

26. Joulia-Ekaza, D. & Cabello, G. Myostatin regulation of muscle development: molecular basis, natural mutations, physiopathological aspects. *Exp Cell Res.* **312**(13):2401-14 (2006).

27. Stuiver, M., et al., INTCAL98 radiocarbon age calibration, 24,000–0 cal BP. *Radiocarbon*, **40**(3):1041–1083 (1998).

28. Reimer, P. J., Brown, T. A., and Reimer, R. W. Discussion: reporting and calibration of post-bomb $^{14}C$ data. *Radiocarbon* **46**, 1299-1304 (2004).

## Figure legends

*Figure 1* Fat mass is determined by both adipocyte number and size. The relationship between BMI and fat cell volume was curvilinear across the range of BMI's in female (a) and male (b) subcutaneous fat, as well as in male and female visceral fat (c), demonstrating that both adipocyte number and size are determinates of BMI.

*Figure 2* Adipocyte number remains stable in adulthood, though significant weight loss can result in a decrease in adipocyte volume. Total adipocyte number from 687 adult individuals (squares) was combined with previous results for children and adolescents[7] (circles). 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 (a). Major weight loss by bariatric surgery results in a significant decrease in cell volume (b) however fails to reduce adipocyte cell number, 1-2 years post-surgery (c). Error bars = SEM, Asterisk = $p < 0.0001$.

*Figure 3* Turnover of adipocytes in adulthood. The levels of $^{14}C$ in the atmosphere have been stable over long time periods with the exception of a large addition of $^{14}C$ in 1955–1963 as a result of atmospheric nuclear weapon tests[19-20, 27] (a, b). Historical secular levels of $^{14}C$ (a) are by convention given in relation to a universal standard and corrected for radioactive decay, giving the $\Delta^{14}C$ value[27]. The boxed region in plot (a) is shown in more detail in (b). The $^{14}C$ fraction modern expressed in $F^{14}C$ convention defined for bomb-pulse dating[28] is used in (b)-(d) where $F^{14}C = 1$ is the natural concentration of atmospheric $^{14}C$ in 1890. Adipocyte age in adult human subjects born before (c) and after (d) nuclear bomb tests were analyzed 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 read off 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 adulthood (c). $^{14}C$ levels analyzed 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 and the BMI 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. No significant difference in adipocyte death rate was seen across the different BMI´s (a). Obese individuals had a significantly greater number of adipocytes added per year than lean individuals (b). No significant difference in the average age of adipocytes in lean versus obese individuals was found (c). Values are the median+/-1st and 3rd quartiles (a, b) and mean+/-SD (c). Asterisk = $p < 0.01$ for lean versus obese.

# Supplement 1

## Model and curve fit for fat cell data measuring average fat cell volume against BMI


### Summary

Three data sets relating BMI to the average fat cell volume were analyzed and fitted to a simple quantitative model. Subjects with a wide range of BMI (from leanness to extreme obesity) were investigated. The analysis strongly suggests that increasing BMI is a result of enlargement of fat cells plus increased number of fat cells.


### Theory

Make the following assumptions:

1. The total fat cell volume ($m^3$), denoted $\eta$, is increasing linearly with the BMI with some unknown proportionality constant $k_B$ (unit: $m^5/kg$). Introduce an adjusted BMI denoted as $B$ (unit: $kg/m^2$) and assume $B$ = BMI - $BMI_0$, where $BMI_0$ represents the lower theoretical limit of the BMI corresponding to $\eta = 0$, i.e. a body without fat cells. We have

$$\eta = k_B B$$

2. The number of fat cells, denoted $n$, has a lower limit $n_0$, which could be the number of fat cells from birth, and may also increase linearly with the BMI with a proportionality constant $k_g$ (unit: $m^2/kg$), i.e.

$$n = n_0 + k_g B \qquad (1)$$

Note that a very low $k_g$ corresponds to the notion that fat cells hardly increase in number in adult humans.

We now take interest in the average volume per cell, i.e. $V = \eta / n$ and obtain

$$V = \frac{\eta}{n} = \frac{k_B B}{n_0 + k_g B} = \frac{\frac{k_B}{n_0} B}{1 + \frac{k_g}{n_0} B} \tag{2}$$

This equation describes a saturation curve for the average volume per cell with respect to the BMI. Important to note is that when $k_g$ equals zero, we have:

$$\frac{\eta}{n} = \frac{k_B}{n_0} B \tag{3}$$

i.e. the average volume per cell increases linearly with the BMI if there is no increase in the fat cell number. Also important to note is that the three physical parameters $k_B$, $k_g$, and $n_0$, are not *identifiable*, meaning that we are not able to determine their values from data measuring the average cell volume $V$ against BMI ($B$). Instead, we will estimate the fractions $\frac{k_B}{n_0}$ and $\frac{k_g}{n_0}$, together with $BMI_0$.

A potential caveat that needs to be addressed is the variability of the observable parameters ($\frac{k_B}{n_0}$ and $\frac{k_g}{n_0}$) between individuals. Generally we have, for subject $i$, $y_i = \frac{a_i x_i}{1 + b_i x_i} + e_i$, where $y = V$, $x = B$, $a = \frac{k_B}{n_0}$, $b = \frac{k_g}{n_0}$, and $e$ is the error in measurement, assumed to be independent from $x$. It is immediately obvious that the model is over-parameterized if $a$ and $b$ are allowed to vary freely between individual subjects $i$ – a perfect fit can be obtained by letting $a_i = y_i/x_i$ and $b_i = 0$. In the following, we outline how to assess the individual variability of $a_i$ and $b_i$. Let us introduce the error model $a_i = a + c_i$ and $b_i = b + d_i$. A Taylor expansion gives

$$y_i = \frac{(a + c_i) x_i}{1 + (b + d_i) x_i} + e_i \approx \frac{a x_i}{1 + b x_i} + \frac{c_i x_i}{1 + b x_i} - \frac{d_i a x_i^2}{(1 + b x_i)^2} + e_i, \tag{4}$$

where the first term is the original model and the following three are errors. These errors can be assessed by observing the residuals $\left| y_i - \frac{\hat{a} x_i}{1 + \hat{b} x_i} \right|$, where $\hat{a}$ and $\hat{b}$ are estimated from a

curve fit of equation (2) using a least squares method. If the residuals vary little with respect to $x_i/ (1 + bx_i)$ and $ax_i^2/ (1 + bx_i)^2$, respectively, individual variations in $a$ and $b$ are negligible; if they grow with any of these terms there is significant variation in $a$ and/or $b$, respectively.

Given a low individual variability in the parameters, a likelihood ratio test to compare the model to the null hypothesis, the special case that $k_g$ is 0 (whereby fat cells do not form in the adult human), can be performed accordingly: let $z_1 = \sum_i (y_i - \frac{\hat{a}x_i}{1+\hat{b}x_i})^2$, i.e. the residual sum of squares. Similarly, $z_2 = \sum_i (y_i - \hat{a}x_i)^2$, corresponding to the independent fit of the null hypothesis that $k_g = 0$, and thereby $b = 0$. If $n$ is the number of observations, the null hypothesis is rejected if

$$n \times \log\frac{z_2}{z_1} > \chi 2\ (1). \qquad (5)$$

**Data Fit and Statistical Tests**

We partitioned our data into three data sets: one consisting of samples of visceral fat (men and women), one consisting of samples of subcutaneous fat (women), and one consisting of samples of subcutaneous fat (men). The subjects are described in Supplement 4. From each subject, we have the BMI value and the average cell volume. Curve fits of equation (2) to these three data sets were performed in the least squares sense using Matlab's statistical toolbox. The individual values are shown if Fig.1. The numerical values of the fitted parameters together with corresponding 95% confidence intervals are given in Table 1. The differences between the estimated parameters of different data sets were not significant ($P > 0.05$).

As described in the theory section, we checked whether the fit of equation (2) was significantly better than a fit to the linear equation (3). Regressions were made of the residuals against $x/(1+bx)$ and $ax^2/ (1 - bx)^2$ respectively. As presented in table 2, the coefficient of

determination $r^2$} was always smaller than 0.05, which justified the straightforward use of the likelihood ratio test (equation 5). However, one may note that the variability of the parameters seems to be greater among men than among women.

In all cases (both adipose regions and both sexes), a likelihood ratio test showed that $k_g$ is significantly greater than zero ($P < 0.001$) meaning that the relationship between fat cell volume and BMI differed from a linear relationship.

| Data set | $k_B/n_0$ | $k_g/n_0$ | $BMI_0$ |
|---|---|---|---|
| Visceral fat (men and women) | 78 ± 23 | 0.074 ± 0.032 | 18.6 ± 1.3 |
| Subcutaneous fat (women) | 106 ± 16 | 0.085 ± 0.018 | 16.0 ± 0.8 |
| Subcutaneous fat (men) | 121 ± 20 | 0.10 ± 0.025 | 17.9 ± 0.7 |

Table 1. Estimated parameters. Intervals are 95% confidence intervals.

| Data set | $r^2$ for $R_1$ | $r^2$ for $R_2$ | LR score |
|---|---|---|---|
| Visceral fat (men and women) | 0.042 | 0.041 | 16.8 ($P < 0.001$) |
| Subcutaneous fat (women) | $8.2 \times 10^{-4}$ | $8.9 \times 10^{-4}$ | 66.9 ($P < 0.001$) |
| Subcutaneous fat (men) | 0.039 | 0.041 | 45.3 ($P < 0.001$) |

Table 2. Coefficients of determination $r^2$ for the residual plots against $R_1 = x/(1 + bx)$ and $R_2 = ax^2/(1 - bx)^2$ of the three data sets, and outcome of the likelihood ratio (LR) test (equation 5).

## Supplement 2

Table showing all data from the 35 people whose adipose DNA was carbon dated. DOB = date of birth to the year. For a complete explanation of the terms 'C14' and 'F14C', refer to Supplement 4.

| Table number | Fat sample number | Sex | DOB | Age (yrs) | BMI | Height (cm) | Body weight (kg) | Fat cell volume (pl) | Fat cell weight (mg) | Total number of fat cells in the body x 10^10 | C14 | +/- | pMC | +/- | Yr of analysis |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 16 | Female | 1984 | 21 | 23,1 | 164 | 62 | 497 | 455 | 3,53 | 74 | 29 | 1,081 | 0,029 | 2006,3 |
| 2 | 22 | Man | 1982 | 23 | 21,1 | 171 | 62 | 417 | 382 | 1,89 | 158 | 12,4 | 1,165 | 0,012 | 2005,9 |
| 3 | 41 | Female | 1978 | 27 | 22,8 | 162 | 60 | 597 | 546 | 2,88 | 107 | 7,9 | 1,115 | 0,008 | 2005,9 |
| 4 | 43 | Female | 1976 | 29 | 24,2 | 176 | 75 | 505 | 482 | 4,45 | 122 | 7,5 | 1,129 | 0,007 | 2005,9 |
| 5 | 15 | Female | 1976 | 29 | 29,0 | 164 | 78 | 442 | 405 | 6,89 | 86 | 6,5 | 1,093 | 0,007 | 2005,6 |
| 6 | 53 | Female | 1976 | 29 | 29,1 | 174 | 88 | 249 | 228 | 13,86 | 69 | 6 | 1,076 | 0,006 | 2006,3 |
| 7 | 60 | Man | 1977 | 29 | 27,7 | 170 | 80 | 419 | 383 | 5,50 | 90 | 31,3 | 1,098 | 0,032 | 2007,0 |
| 8 | 34 | Female | 1975 | 30 | 32,8 | 171 | 96 | 452 | 414 | 9,65 | 135 | 13,2 | 1,142 | 0,013 | 2005,9 |
| 9 | 29 | Female | 1975 | 30 | 24,2 | 160 | 62 | 576 | 527 | 3,38 | 144 | 5,4 | 1,152 | 0,005 | 2005,9 |
| 10 | 51 | Female | 1974 | 31 | 35,6 | 165 | 97 | 733 | 671 | 6,64 | 80 | 11,5 | 1,087 | 0,012 | 2006,3 |
| 11 | 18 | Female | 1974 | 31 | 20,5 | 174 | 62 | 465 | 425 | 3,40 | 80 | 11 | 1,087 | 0,011 | 2006,3 |
| 12 | 20 | Female | 1973 | 32 | 24 | 168 | 68 | 492 | 450 | 4,33 | 180 | 5,9 | 1,188 | 0,006 | 2005,6 |
| 13 | 12 | Female | 1973 | 32 | 23,5 | 170 | 68 | 457 | 418 | 4,54 | 100 | 5,3 | 1,107 | 0,005 | 2005,6 |
| 14 | 40 | Female | 1973 | 32 | 25,7 | 165 | 70 | 488 | 446 | 4,90 | 161 | 17,8 | 1,168 | 0,018 | 2005,9 |
| 15 | 38 | Female | 1973 | 32 | 31,2 | 163 | 83 | 668 | 611 | 5,36 | 94 | 30 | 1,101 | 0,030 | 2006,3 |
| 16 | 7 | Female | 1971 | 34 | 38,6 | 168 | 85 | 486 | 445 | 7,27 | 86 | 11,7 | 1,093 | 0,012 | 2005,2 |
| 17 | 8 | Female | 1970 | 35 | 40,2 | 160 | 62 | 190 | 174 | 10,46 | 150 | 4,7 | 1,158 | 0,005 | 2005,2 |
| 18 | 47 | Female | 1970 | 35 | 46,7 | 168 | 98 | 471 | 431 | 14,35 | 91 | 68 | 1,098 | 0,068 | 2006,3 |
| 19 | 4 | Female | 1970 | 35 | 21,2 | 161 | 55 | 462 | 422 | 3,24 | 133 | 9,6 | 1,141 | 0,010 | 2005,7 |
| 20 | 36 | Female | 1969 | 36 | 28,2 | 163 | 75 | 542 | 496 | 5,37 | 150 | 10,8 | 1,158 | 0,011 | 2005,9 |
| 21 | 11 | Female | 1968 | 37 | 37,4 | 173 | 83 | 466 | 408 | 7,09 | 122 | 7 | 1,129 | 0,007 | 2005,2 |
| 22 | 14 | Female | 1968 | 37 | 22,1 | 162 | 58 | 461 | 422 | 3,64 | 124 | 4,8 | 1,132 | 0,005 | 2005,6 |
| 23 | 42 | Female | 1968 | 37 | 22,6 | 176 | 70 | 472 | 432 | 4,41 | 186 | 9,6 | 1,195 | 0,010 | 2005,9 |
| 24 | 21 | Man | 1968 | 37 | 26,6 | 187 | 93 | 707 | 646 | 3,13 | 171 | 10,0 | 1,179 | 0,010 | 2005,7 |
| 25 | 48 | Man | 1966 | 39 | 28,1 | 174 | 85 | 492 | 450 | 4,58 | 199 | 5,9 | 1,207 | 0,006 | 2005,9 |
| 26 | 27 | Female | 1955 | 50 | 59,6 | 169 | 107 | 654 | 599 | 15,08 | 115 | 7,9 | 1,123 | 0,008 | 2005,9 |
| 27 | 5 | Female | 1954 | 50 | 54,1 | 166 | 103 | 414 | 378 | 20,75 | 173 | 15 | 1,181 | 0,015 | 2005,7 |
| 28 | 13 | Female | 1954 | 51 | 35,4 | 163 | 65 | 479 | 438 | 4,73 | 144 | 6,9 | 1,152 | 0,007 | 2005,6 |
| 29 | 50 | Female | 1949 | 56 | 23,9 | 153 | 56 | 540 | 494 | 3,59 | 142 | 6,7 | 1,150 | 0,007 | 2005,9 |
| 30 | 3 | Female | 1948 | 57 | 35,9 | 160 | 72 | 535 | 489 | 5,60 | 150 | 4,7 | 1,157 | 0,005 | 2005,6 |
| 31 | 25 | Female | 1945 | 60 | 26,1 | 167 | 73 | 548 | 502 | 5,16 | 103 | 5,4 | 1,110 | 0,005 | 2005,6 |
| 32 | 46 | Female | 1944 | 61 | 41 | 166 | 80 | 550 | 504 | 6,34 | 179 | 9,4 | 1,187 | 0,009 | 2005,9 |
| 33 | 45 | Man | 1935 | 70 | 24,4 | 173 | 73 | 521 | 477 | 5,23 | 133 | 8,9 | 1,141 | 0,009 | 2007,0 |
| 34 | 19 | Female | 1934 | 71 | 29,4 | 168 | 83 | 718 | 657 | 5,28 | 130 | 18,9 | 1,138 | 0,019 | 2005,9 |
| 35 | 35 | Female | 1933 | 72 | 27,5 | 165 | 75 | 521 | 477 | 6,15 | 75 | 17,1 | 1,082 | 0,017 | 2007,0 |

# Supplement 3

## Modelling of $^{14}C$ data

### Summary

We used a mathematical model for birth and death of adipocytes to estimate adipocyte turnover in 35 adult subjects. Death and birth rates of adipocytes were calculated from DNA $^{14}C$ content in adipocytes, ages of patients, and absolute adipocyte number. We used three scenarios for the evolution of adipocyte formation at early ages and estimated rates in three BMI categories: lean (BMI<25 kg/m$^2$), overweight (25≤BMI<30 kg/m$^2$) and obese (BMI≥30 kg/m$^2$) subjects. We conclude that the adipocyte death rates in the three BMI groups are not different (Kruskal-Wallis test, p=0.6). Absolute production of new adipocytes is significantly higher in obese subjects than in lean subjects (Kruskal-Wallis test, p<0.01). The adipocyte relative turnover number, however, does not differ significantly between BMI groups (p-value>0.1): a median value of 8.35±6.6% adipocytes is replaced each year. Using two other data sets, we determined the age of onset of adipocyte number expansion, and the relative growth in cell number during that expansion.

### Model

We used a linear model with an age-structure for birth and death of adipocytes to estimate kinetic parameters of adipocyte birth and death. Data come from three sources: subjects who underwent cosmetic surgery and subsequent analysis of $^{14}C$ in subcutaneous fat cell DNA (D1), cross-sectional data from a study by Knittle et al (1979) (D2) (ref. 2), and from subjects analysed for the relationship between BMI and subcutaneous fat cell volume (D3). The subjects in D1 and D3 are described in Supplement 4. Available from D1 data are average $^{14}C$ levels in adipocytes samples collected during liposuction, ages of subjects, the absolute body adipocyte count, and their BMI (n=35). From D2 data, we have the average adipocyte

number for different age groups, for normal or obese children (ages range from 0.5 to 18 y). D3 data consist of ages, BMI, and adipocyte numbers (n=687) using lean (BMI<25 kg/m$^2$), and obese (BMI≥30 kg/m$^2$) subjects. We sorted D1 patients in three BMI categories: lean (BMI<25 kg/m$^2$), overweight (25≤BMI<30 kg/m$^2$), and obese (BMI≥30 kg/m$^2$) patients. The ages of subjects ranged from 21 to 72 years (average 39.9±13.8 years, at date of collection) and the BMI values from 20.5 to 59.6 kg/m$^2$ (average 30.4±9.1 kg/m$^2$). Carbon 14 levels varied from 1.0763 to 1.2068 Fraction Modern Carbon (average 1.134±0.036 F$^{14}$C) (Table 1).

Table 1 Summary of subjects' data D1

| n=35 | Birth year | Age (y) | $^{14}$C levels (F14C) | Adipocyte number (x10$^{10}$) | BMI (kg/m$^2$) |
|---|---|---|---|---|---|
| Mean | 1965.09 | 39.91 | 1.13435 | 6.36 | 30.39 |
| STD | 13.86 | 13.81 | 0.03617 | 3.98 | 9.15 |
| Median | 1970.00 | 35.00 | 1.13760 | 5.23 | 27.70 |
| 1st quart | 1954.50 | 30.50 | 1.09990 | 3.98 | 23.95 |
| 3rd quart | 1974.50 | 50.00 | 1.15792 | 6.76 | 35.50 |
| Min Value | 1933.00 | 21.00 | 1.07630 | 1.89 | 20.50 |
| Max Value | 1984.00 | 72.00 | 1.20679 | 20.75 | 59.60 |
| Lean n=13 | 1969.62 | 35.38 | 1.13711 | 3.73 | 22.89 |
| Overweight n=10 | 1961.70 | 43.40 | 1.13099 | 6.08 | 27.74 |
| Obese n=12 | 1963.00 | 41.92 | 1.13416 | 9.44 | 40.71 |

The age-structured model for cell birth and death has four parameters: the cell death rate $\gamma$, the initial age $t_0$ at which the adipocyte distribution is known (we refer to the age of the patient with $t$ and the age of adipocytes with $a$), the initial cell number $n_0$, and the production of new adipocytes from a fat stem cell pool $\beta$. We make the following assumptions.

*Period of expansion of adipocyte number*: Most of the adipocytes in a young adult are born during a specific, critical period (starting at $t_0$) before adulthood. This means that in a

subject aged $t_0$ years, all adipocytes have age 0 years (or perhaps very few older cells from childhood).

*Constant cell death rate*: All adipocytes, regardless of their age, die at a rate $\gamma$ that is constant over the lifetime of the person.

*Constant cell production*: Adipocytes are produced steadily at a rate $\beta$ that is independent of the total cell number and is constant over the lifetime (after age $t_0$). This is because mature adipocytes do not divide and we ignore regulation of new adipocytes by mature cells.

Mathematically, these assumptions may be formulated as a linear partial differential equation with an age-structure:

1

$$\frac{\partial n(t,a)}{\partial t} + \frac{\partial n(t,a)}{\partial a} = -\gamma\, n(t,a).$$

The initial condition at $t = t_0$ is $n(t_0, a) = n_0 \delta_0(a)$, where $\delta$ is the Dirac delta function (i.e. all cells at $t_0$ have age $a = 0$) and $n_0$ is the initial adipocyte number (unit: $10^{10}$ cells). A boundary condition describes the birth of new adipocytes from progenitor cells, $n(t,0) = \beta$ (unit: $10^{10}$ cells/year). This equation may be solved cells with an age smaller than the time elapsed since $t_0$. For $0 \leq a \leq t - t_0$,

2

$$n(t,a) = n_0 \delta_{t-t_0}(a) e^{-\gamma a} + \beta\, e^{-\gamma a},$$

and the average age of a adipocyte in an adult of age $t > t_0$ is

3

$$\langle a \rangle = \frac{\gamma(t-t_0)e_t - b(t-t_0)e_t + b/\gamma(1-e_t)}{\gamma\, e_t + b(1-e_t)},$$

where $e_t = e^{-\gamma(t-t_0)}$. The newly introduce parameter $b$ is the birth rate relative to the initial cell number $n_0$,

$b = \frac{\beta}{n_0}$.

(The parameter b is useful since $\langle a \rangle$ becomes independent of the total cell number.) The total cell number at age $t$, $N(t)$, is found by integrating $n(t,a)$ over the ages of adipocytes $a$,

4

$$N(t) = \int_0^{t-t_0} n(t,a)da = \frac{\beta}{\gamma}\left(1 - e^{-\gamma(t-t_0)}\right) + n_0 e^{-\gamma(t-t_0)}.$$

The equation satisfies the initial condition $N(t_0) = n_0$. If $b > \gamma$, the cell number increases with time, else decreases. The cell number always stabilises to a constant steady state $\bar{N} = \beta/\gamma$. To relate the age of the adipocytes with the $^{14}C$ data, we used a carbon normalization curve $K$ (ref. 3). If the adipocyte age distribution follows Equation 2, the average 14C level $C$ is

5

$$C = \frac{\int_0^{t-t_0} K(a)n(t,a)da}{N(t)}$$

Equation 5 depends on four parameters: $n_0$, $\gamma$, $\beta$, and $t_0$. The parameter $n_0$ can be eliminated by dividing the numerator and denominator by $n_0$, and by replacing the term $\beta/n_0$ with $b$ as in Equation 3. Thus three parameters remain to be estimated: $\gamma$, $b$, and $t_0$. In general, it is not possible from equation 5 alone to estimate all three parameters; different choices of $\gamma$, b, and $t_0$ can yield the same value of $C$. This is why additional assumptions on one or more parameters must be made.

*Default Scenario:* The death rate $\gamma$, and the ages of the patients are sufficiently large to neglect the initial adipocyte population, i.e. $n_0$ is very small. This means that $b = \beta/n_0$ becomes large and $t_0$ can be set to $t_0 = 0$. Parameter $b$ cancels out in Equation 5, and the model has only one parameter left to estimate: the death rate $\gamma$. This allows us to calculate an individual death rate for each patient. The adipocyte production can then be estimated from

the adipocyte count of each subject. Under Default Scenario, $n(t,a) = \beta e^{-\gamma a}$, for $a \leq t$, and 0 otherwise.

By comparing the results with those obtained with different assumptions, we may be able to identify information that is statistically significant. In addition to the Default Scenario, we used the following alternative scenarios:

*Scenario A*: Same as Default Scenario, except that an average death rate is calculated instead of individual values for each subject.

*Scenario B*: The cell turnover as we model it starts in a patient at age $t_0$, and for age $t \geq t_0$ the total cell number is constant, i.e. $b = \beta / n_0 = \gamma$. Thus $\gamma$ and $t_0$ need to be fitted.

*Scenario C*: The cell turnover as we model it starts in a patient at age $t_0$, which has to be guessed. Parameters $\gamma$ and $b$ are fitted.

As shown below, the model is robust with respect to $t_0$ (changing $t_0$ does not affect the estimates of other parameters), as long as this parameter is far from the ages of patients at time of harvest. The consequences of each case are felt on the cell numbers. In the Default Scenario and Scenario A, the population increases to reach a steady state. In Scenario B, the population is constant, by definition, for $t \geq t_0$. In Scenario C, the population can either increase or decrease before reaching the steady state.

**Results**

*Ages of subjects and BMI distribution*

Subjects were divided according to their BMI and age. Data D1 are summarized in Table 1. Overweight patients tend to be older than lean and obese patients, but not significantly (ANOVA, $p>0.05$). Similarly, we found no significant differences in $^{14}C$ levels between young and old patients and between lean and obese subjects (ANOVA, $p=0.8$).

*Estimating birth and death rates*

Table 2 Summary of the adipocyte kinetic rates for three groups of subjects: lean (BMI<25 kg/m$^2$), overweight (25≤BMI<30 kg/m$^2$), and obese (BMI≥30 kg/m$^2$). Units are $\gamma$ $y^{-1}$, $\beta$ $10^{10}$ cells/y, $b$ ICP/y; and $t_0$ and $\langle a \rangle$ y. [CI] 95% confidence interval on the parameters, STD standard deviation, AD absolute deviation, g. guessed. *Kruskal-Wallis test p<0.01.

| | Default Scenario | | Scenario A **Error! Bookmark not defined.** | | Scenario B | | | Scenario C **Error! Bookmark not defined.** | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| BMI category | $\gamma$ mean±STD (median±AD) | $\beta$ mean±STD (median±AD) | $\gamma$ fit [CI] | $\beta$ mean (median) | $\gamma$ fit [CI] | $\beta$ mean (median) | $t_0$ fit | $\gamma$ fit [CI] | $\beta$ mean (median) | $b$ fit | $t_0$ g. |
| lean (n=13) | 0.097±0.08 (0.0823±0.056) | 0.394±0.26* (0.319±0.19) | 0.075 [0.03] | 0.308 (0.290) | 0.080 [0.04] | 0.299* (0.287) | 4.6 | 0.081 [0.05] | 0.292* (0.288) | 0.065 | 9 |
| overweight (n=10) | 0.138±0.1 (0.1195±0.085) | 1.113±1.50 (0.623±0.94) | 0.090 [0.04] | 0.572 (0.489) | 0.091 [0.05] | 0.554 (0.485) | 12.9 | 0.104 [0.06] | 0.543 (0.507) | 0.031 | 9 |
| obese (n=12) | 0.114±0.06 (0.0946±0.051) | 1.057±0.64* (0.793±0.54) | 0.0894 [0.04] | 0.872 (0.671) | 0.078 [0.06] | 0.740* (0.563) | 13.3 | 0.077 [0.04] | 0.804* (0.637) | 729.4 | 9 |
| all (n=35) | 0.114±0.08 (0.0835±0.066) | 0.827±0.93 (0.502±0.58) | 0.085 [0.02] | 0.569 (0.447) | 0.088 [0.02] | 0.562 (0.462) | 16.6 | 0.089 [0.03] | 0.569 (0.468) | 0.088 | 9 |

Table 3 Age of adipocytes, average years ±STD, (median)

| BMI category | Default Scenario | Scenario A | Scenario B | Scenario C |
|---|---|---|---|---|
| lean (n=13) | 9.86±3.5 (10.24) | 10.22±1.4 (10.14) | 8.15±2.1 (8.41) | 9.74±1.1 (9.88) |
| overweight (n=10) | 8.96±4.6 (8.69) | 9.79±0.9 (9.70) | 9.27±1.6 (9.36) | 10.30±0.8 (10.36) |
| obese (n=12) | 9.67±4.0 (9.82) | 9.94±0.7 (9.68) | 9.61±1.2 (9.27) | 10.48±0.6 (10.31) |
| all (n=35) | 9.54±3.9 (10.24) | 9.96±1.0 (9.90) | 8.97±1.8 (9.09) | 10.15±0.9 (10.22) |

Since it is not possible to estimate all three parameters at once from equation for $C$, we used the Default Scenarios and the three scenarios described above. For each of them, the adipocyte death rate, $\gamma$, can be estimated. Moreover, in Default Scenario, $\gamma$ is the only parameter, allowing estimates for individual patients unlike the alternative scenarios. (In Scenario A, we calculated a single average death rate using the same assumptions as in Default Scenario). In Scenario B, $t_0$ is estimated together with $\gamma$, while $b = \gamma$ by definition.

Finally in Scenario C, $\gamma$ and $b$ are estimated together, while $t_0$ is assigned a guessed, fixed value. For each scenario, we calculated the death rates $\gamma$ for the lean, overweight, obese, and all subjects. We estimated the death rates using the nonlinear fitting algorithm *nlinfit* from Matlab (www.mathworks.com).

Once the death rate estimated, we used the estimated number of adipocytes in each patient (Supplement 4 contains the procedure for adipocyte number calculation) and their age to calculate the absolute birth rate $\beta$. This was performed by taking the total number of adipocytes given by the model ($N(t)$ in Equation 4) and solving for $\beta$. This leads to three formulas.

6

Default Scenario and Scenario A, $\beta = \gamma \frac{CELLNUMBER}{1 - e^{-\gamma\, AGE}}$

7

Scenario B, $\beta = \gamma\, CELLNUMBER$

8

Scenario C, $\beta = \gamma\, b \frac{CELLNUMBER}{b - (b - \gamma)e^{-\gamma(AGE - t_0)}}$

The equations are the direct consequence of the assumptions on each scenario. Results are displayed in Table 2. The average age of adipocytes $\langle a \rangle$ was directly calculated from $\gamma, b$, and $t_0$ using Equation 3 (
Table **3**).

**Statistical analysis**

The death rates calculated from various scenarios are very similar, with values just below 0.09 per year (Table 2). This is encouraging, because estimating the absolute birth rates requires robust estimates of the death rates, cf. Equations 6, 7, and 8. No significant difference in death rates could be found between the lean and obese subjects (Kruskal-Wallis test for equality of medians, p=0.6). No significant differences in ages of adipocytes were found

between different BMI categories (average age 9.86±3.5, 8.96±4.6 and 9.67±4.0 years for lean, overweight and obese subjects respectively,
Table **3**), which is a consequence of the $^{14}C$ levels being constant between BMI groups. A constant death rate across BMI categories should correspond to a higher adipocyte production in patients with high BMI. Four estimates were obtained for the birth rate $\beta$. Estimates varied between $0.29x10^{10}$cells/y in lean subjects to $1.05x10^{10}$cells/y in obese patients. For Default Scenario, the difference in $\beta$ between lean and obese subjects is significant (median 0.32 to $0.79x10^{10}$cells/y, Kruskal-Wallis test, p=0.0016, Table 2). When divided into two groups with BMI below and above 27 kg/m$^2$, the difference is also significant (Kruskal-Wallis test, p=0.01). The difference in $\beta$ between lean and overweight, and obese subjects, however, is not significant (p=0.3).

It is possible that the turnover is not uniform among the adipocytes sampled. The $^{14}C$ labelling method measures the ages of cells present in samples and, by extension, their death rates. Strawford et al[2] used heavy water ($^2H_2O$) to label newly synthesised DNA with deuterium and reported extremely high turnover rates, 58%--105% per year, in subcutaneous adipose tissue aspirates in humans. Differences in tissue samples cannot account for the observed difference in rates, thus the difference in turnover rates suggests that the newborn adipocytes, i.e. differentiated post-mitotic pre-adipocytes, have higher turnover than the mature adipocytes. Including fast proliferating cells present in a proportion reported; 10-17% newly divided cells after 9 weeks of labelling[2], would affect our estimates by less than 10%. The actual 7-12 fold difference in turnover rates might represent the excess ratio of pre-adipocyte to adipocyte produced. These turnover rates indicate that most proliferating pre-adipocytes do not permanently join the adipocyte population. Nuclear bomb test $^{14}C$ and heavy water labelling are complementary approaches suitable to detect cell turnover in the older, mature adipocyte fraction and to estimate the faster turnover of the proliferating fraction.

In summary, the $^{14}C$ samples allowed us to estimate reliably two parameters of the model: the annual death rate $\gamma$ and the annual adipocyte production $\beta$. We only obtained poor estimates for the two remaining parameter, $t_0$ and $b$, because of the finite memory of the adipocyte population. These parameters depend on childhood and cannot be estimated in adults. To get an estimate for these two parameters, we used cross-sectional data coming from children and adults.

**Estimating $t_0$ and $b$ from cell number data**

Since $t_0$ and $b$ cannot reliably be estimated from the C14 sample, we used cross-sectional data on cell numbers in subjects with wide range of age (data set D2 and D3, Table 4) to see how the modelled cell number $N(t)$ varies over age. The aggregated data were divided into two groups, normal and obese (normal, BMI<25 kg/m$^2$; obese, BMI>30 kg/m$^2$), corresponding to the division used by Knittle et al[1].

Table 4 shows a summary of the data, divided according to age and BMI. Cell number does not change significantly between age groups in data set D3 (multi-group ANOVA, p=0.68). No data are provided for obese aged less than 3 since no obese were detected at this early age. We thus used data from the normal group for obese below 3 years old. We fitted the four parameters of the cell number $N(t)$ to these data and got estimates of $t_0$ =5.7±0.8y and 2.1±0.9 y and $b$ = 1.3±0.3 and 2.4±0.6 IC/y for normal and obese respectively (

Figure 1). Using the fitted adipocyte number curves, we calculated the age at which 75% or 90% of adult cell number is reached in lean and obese subjects. The adult adipocyte cell number was at 16.5±1.3y (90%) and 10.5±1.3y (75%) in the obese and at 18.5±0.7y, (90%) and 14.5±0.7y, (75%) in the lean. The errors are the 95% confidence intervals on the

predicted ages. The value of $t_0$ is consistent with the conclusions of Knittle et al[1] that cell number expansion starts earlier in obese than in lean children.

Table 4 Adipocyte number for different age groups and BMI categories. Data in italic are D2[1], and in bold D3 (present study)

| | normal | | | obese | | |
|---|---|---|---|---|---|---|
| age | n | cell number | STD | n | cell number | STD |
| *0.5* | *18* | *0.4* | *1.4* | - | - | - |
| *1* | *27* | *0.5* | *1.5* | - | - | - |
| *3* | *31* | *0.9* | *1.2* | *17* | *1.85* | *1.2* |
| *6* | *29* | *1.1* | *3.1* | *23* | *3.4* | *3.3* |
| *9* | *29* | *1.2* | *3.2* | *15* | *3.95* | *3.1* |
| *12* | *22* | *2.8* | *2.6* | *27* | *6.2* | *5.1* |
| *15* | *16* | *3.5* | *3.0* | *29* | *6.8* | *4.3* |
| *18* | *6* | *4.0* | *2.0* | *9* | *8.0* | *3.0* |
| **18** | **6** | **4.4** | **2.1** | - | - | - |
| **22** | **21** | **4.1** | **2.1** | **20** | **8.7** | **3.4** |
| **27** | **42** | **4.6** | **1.7** | **49** | **7.6** | **2.0** |
| **32** | **44** | **5.2** | **2.3** | **55** | **7.3** | **2.4** |
| **37** | **24** | **5.0** | **1.5** | **56** | **7.3** | **2.3** |
| **42** | **56** | **4.2** | **1.8** | **59** | **7.3** | **2.1** |
| **47** | **27** | **4.3** | **1.6** | **47** | **7.7** | **2.4** |
| **52** | **16** | **4.4** | **2.4** | **22** | **7.5** | **2.7** |
| **57** | **9** | **4.9** | **2.1** | **21** | **7.1** | **2.3** |
| **60+** | **8** | **4.2** | **0.9** | **13** | **6.0** | **2.4** |

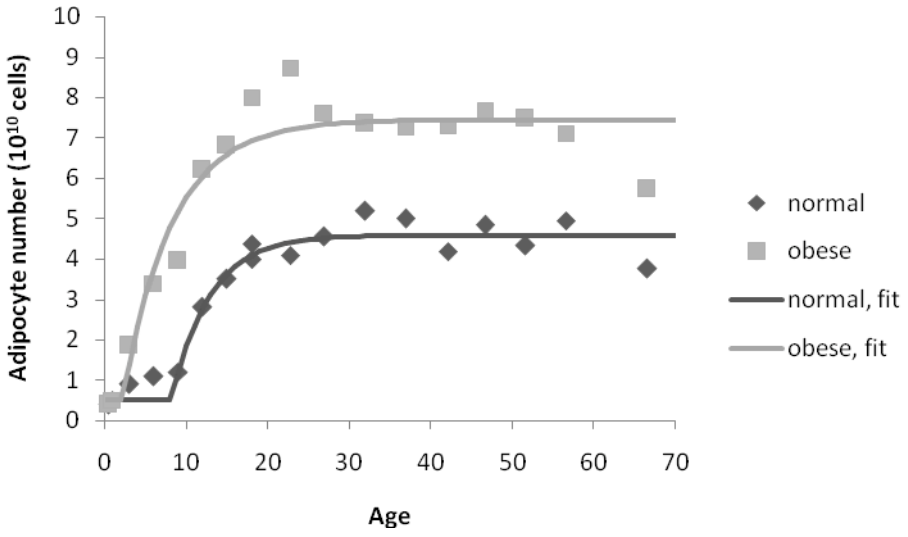



Figure 1 Results of the fitting of the cell number data by N(t)

**References Supplement 3**

1. Knittle, J.L., Timmers, K., Ginsberg-Fellner, F., Brown, R.E., and Katz, D.P. The growth of adipose tissue in children and adolescents. cross-sectional and longitudinal studies of adipose cell number and size. *J Clin Invest*, **63**(2):239–246 (1979).

2. Strawford, A., Antelo, F., Christiansen, M., and Hellerstein, M.K. Adipose tissue triglyceride turnover, de novo lipogenesis, and cell proliferation in humans measured with 2h2o. *Am J Physiol Endocrinol Metab*, **286**(4):E577–E588 (2004).

3. Stuiver, M., et al., INTCAL98 radiocarbon age calibration, 24,000–0 cal BP. *Radiocarbon*, **40**(3):1041–1083 (1998).

**Supplement 4**

**Subjects**

The relationship between BMI and fat cell volume was determined on two study groups. Abdominal subcutaneous adipose was obtained from 204 men and 486 women (age 16-72 years, mean 39 years) by biopsy under local anesthesia. Visceral (omental) adipose tissue was obtained at the beginning of elective abdominal surgery because of a non-malignant disorder from 36 men and 107 women (age 16-32 years, mean 39 years). The effect of weight reduction was studied in 9 men and 11 women (age 26-57 years, mean 43 years) who were operated for obesity using gastric banding. An abdominal subcutaneous fat biopsy was obtained as above before operation when BMI was 42.2 ± 4.5 (mean ± SD) $kg/m^2$ and 2 years thereafter when BMI was 34.6 ± 6.0 $kg/m^2$. For $^{14}C$ analysis, a large piece (about 200 g) of subcutaneous adipose tissue was obtained from 35 separate individuals (age 21 - 72 years). This group included 25 women and 2 men who underwent abdominal subcutaneous liposuction for cosmetic reasons, and 6 women who underwent abdominal wall reconstructive surgery 1-2 years after bariatric surgery for obesity, when weight reduction had caused skin folding of the abdomen. The latter group of subjects was classified as obese regardless of their BMI at the time of reconstructive surgery, since their surgical diagnosis was massive obesity and we can show in this study that weight reduction does not influence fat cell number. The studies were explained to each subject and his/hers consent was obtained. Ethical permission for all studies was approved by the ethics committee at Karolinska Institute.

**Isolation of adipocytes from adipose tissue**

Fat cells were isolated from the remaining components of the tissue according to the collagenase procedure of Rodbell[1]. In brief, tissue was cut into ≈ 20 mg pieces and incubated

(1 g tissue/ml medium) in Krebs-Ringer phosphate (KRP buffer (pH 7,4) containing 4% bovine serum albumin (BSA) and 0.5 mg/ml of collagenase type 1 for 60 min at 37ºC in a shaking water bath. The isolated fat cells were collected on a nylon mesh filter and were washed 4-5 times with 0.1% KRP-BSA buffer. The purity of the isolation procedure was estimated by investigating 200 cells under a light microscope in each subject. The number of isolated cells not resembling fat cells or cell material that was stuck to a fat cell was always 0-2 per 200 counted cells.

In order to exclude possible contamination with even small numbers of non-fat cells in the preparations of mature fat cells, a number of techniques were used. Results are presented in Table 1. As a first step, $10^6$ isolated mature fat cells were centrifuged at 200 g for 10 min. The resulting pellet, predominantly containing contaminating non-fat cells was very small and hardly discernable by visual inspection and was termed the contaminating fraction. The pellet was resuspended in a small amount of minimal essential medium (with no phenol red) and stained with 0.3 mg/ml of MTT (3-[4,5-Dimethylthiazol-2-yl]-2,5-diphenyl-tetrazoliumbromide)) for 1 hr at 37C. Cells were lysed with isopropanol and staining was determined spectrophotometrically at 592 nm as described previously[2]. The amount of living cells in these preparations was compared with known amounts of isolated preadipocytes (see above) isolated from the same specimen and resuspended in different test tubes. With this semiquantitative titration comparison, the total number of surviving cells in the "contaminant" fraction was low and corresponded to 13-16 $x10^4$, i.e. a contamination degree of approximately 1.3-1.6%. In order to determine what type of cells the contaminant fraction was composed of, we performed flow cytometry. For this, contaminant fractions were obtained from approximately $10x10^6$ cells. These cells were washed and re-suspended in phosphate buffered saline (PBS) and incubated on ice for 30 minutes with conjugated, monoclonal antibodies against CD34 (preadipocyte marker), CD45 (common leukocyte

marker), CD31 (endothelial cell marker) (all antibodies from Becton Dickinson, San Jose, CA, USA). Nonspecific fluorescence was determined, using equal aliquots of cell preparation incubated with anti-mouse monoclonal antibodies (Becton Dickinson, San José, CA, USA). Finally, the cells were assayed on a FACS Calibur flow cytometer (Beckton Dickinson, San José, CA, USA) and using Cellquest software (Becton Dickinson, San José, CA, USA). This analysis revealed that the contaminating fraction consisted of approximately 5-6 x $10^4$ cells out of which 67-87% were living cells. The percentage of contaminating cells in total would be less than 0.5% if estimated in this manner. This was in accordance, albeit somewhat less, than the numbers estimated with the MTT assay. When comparing the cell numbers with the total amount of mature fat cell from which the contaminant fractions were obtained, the contamination degree for cells positive for each epitope was the following; CD34-0.041%; CD31-0.11% and for CD45-0.017%. The number of putative leukocytes present in the contaminant fraction was finally assessed by incubation with CD45-Dynabeads (Dynal Biotech, AS, Oslo, Norway). In brief, $10^5$ mature fat cells were resuspended in PBS+0.1% BSA. 1X107 Dynabeads were washed in PBS+0.1% BSA and added to eppendorf vials. After letting the tubes stand in a magnetic rack for 1 minute, the supernatant was removed and the mature fat cells were added to a final volume of 1 ml. Cells were allowed to incubate in the presence of Dynabeads for 20 minutes at +4C after which the tubes were put in magnetic racks. The supernatant was removed and the pellet, containing rosetted leukocytes was resuspended in Dulbecco´s modified Eagles medium (DMEM/F12) and plated on to cell culture plates. After allowing the cells to sink for an hour at 37C the number of rosetted leukocytes was counted. With this method the number of leukocytes in the contaminating fraction was estimated to <0.1%. Finally, the methods employed above indicate that a small proportion of cells, probably 1% or less are present in washed and packed fat cells. However, it is still possible that a small number of cells may attach to large fat cells and thereby still be

present in the mature fat cell fraction. In order to exclude this possibility, washed preparations of mature fat cells were stained with the nuclear stain 4'6-Diamino-2-phenglindole (DAPI) from (SIGMA, St Louise, MO, USA). In brief, mature adipocyte fractions were fixed in cell fix (BD Biosciences) for 20 min, followed by washes in PBS. DAPI (stock dissolved in methanol) was added at 100 ng/ml final concentration for 20 min followed by repeated washes in PBS. Cells were then mounted on microglass slides. Inspection by fluorescent microscopy in 10 random fields revealed clearly discernable nuclei in mature fat cells but no nuclei corresponding to contaminating cells on the surface of any fat cell or in between the adipocytes, clearly demonstrating that the mature fat cell preparations are more or less exclusively composed of mature fat cells with a very low degree of contaminating cells (Fig. 1).

| Method | Contamination degree (mean %) | Range (%) | *N* |
|---|---|---|---|
| MTT | 1.45 | 1.3-1.6 | 2 |
| FACS | | | 3 |
| CD34 | 0.041 | 0.0067-<br>0.07- | |
| CD31 | 0.11 | 0.011-0.2 | |
| CD45 | 0.017 | 0.0061-<br>0.027 | |
| Dynalbeads | <0.1 | | 2 |

Table 1. Assessment of contamination of non-fat cells in the isolated fat cell preparation, using a variety of techniques, as descried in the text. N = number of experiments.

**Measurement of fat cell weight, volume, and number**

Mean fat cell volume and weight was determined on isolated fat cells as follows: Fat cell diameter was determined during direct microscopy and the mean diameter of 100 cells in each individual was determined. The mean fat cell volume and weight were calculated by formulas developed by Hirsch and Gallian[3]. Coefficient of variation for the method is 2-3%. The mean values are essentially the same as those determined from fat cell sizing of intact pieces of

human adipose tissue[4]. Total number of fat cells in the body was calculated in the subjects participating in the 14C study and in the study presented in Fig.2 using a formula based on age, BMI and sex to determine percentage of body fat[5]. In a separate methodological study we evaluated this formula by comparing percentage body fat obtained by the formula and by dual-X-ray absorbmetry in 7 men and 14 women with BMI 17-46 kg/m$^2$ and age 22-79 years. There was an excellent correlation between the two measures (r=0.95 by linear regression) with slope and intercept near 1.0 and zero, respectively. The coefficient of variation for the two measures was 4.1% in non-obese and 3.7% in obese (the group divided at BMI 30 kg/m$^2$). The amount of fat cells in the body was calculated as (% body fat x body weight) /mean fat cell weight. The mean fat cell weight was determined in the abdominal subcutaneous fat sample used for $^{14}$C analysis. It is well known that mean fat cell volume and weight differs between various adipose regions in man. However, the differences are small and only introduce a marginal error when just one depot is used for the calculation of total fat cell number as discussed[6]. In the whole cohort total fat cell number (mean$\pm$SD) was 5.6$\pm$2.6 x $10^{10}$, which is in the same range as in several other studies as discussed[6]. For the obese group investigated before and after gastric banding body fat was determined by bioimpedance and fat cell number as described above.

| Condition | BMI kg/m$^2$ | Fat cell volume picolitres | Fat cell number x$10^{10}$ |
|---|---|---|---|
| Before gastric banding | 42.2±1.0 | 890±28 | 8.4 ±0.5 |
| After gastric banding | 34.6±1.3 | 617±38 | 8.5±0.6 |
| p-value by Student´s paired t-test | <0.0001 | <0.0001 | 0.98 |

Table 2. Effects of gastric banding on BMI, fat cell volume and total fat cell number before and 2 years after surgery on obese subjects. The weight loss was 18±11 %. Values are mean ± SEM.

**DNA extraction**

Purified adipocytes were stored at -80 degrees in 25-35ml aliquots. Frozen adipocytes were placed into plastic bags and mechanically disrupted with a hammer. One ml of digestion buffer (500mM NaCl, 50mM Tris.Cl pH 8.0, 125mM EDTA pH 8.0, 2.5% SDS, 0.5mg/ml proteinase K) per 8ml of adipocytes was added to the plastic bag and the sample incubated at +55 degrees overnight (12hrs). The samples were then extracted with equal amounts of volume of phenol/chloroform/isoamylalcohol. This procedure was repeated until no more protein was seen in the interphase between the two layers. A further extraction was performed using chloroform only. DNA was precipitated by adding half the volume of 7.5M ammonium acetate and two volumes of 95% ethanol to the aqueous phase. DNA was washed thoroughly (4 -6 washes in 70% ethanol for 30-60 mins) before being air dried at +50 degrees. DNA was resuspended in 0.5-1.0 ml DNAse-free pure water and incubated at +60 degrees for several days. DNA purity was confirmed using a spectrophotometer and HPLC analysis.

**AMS analysis of 14C content**

Purified DNA samples suspended in 1.0-1.5 mL water glass vials were lyophilized to dryness, resuspended in 0.25 mL of 18.2 MΩ DDI water and transferred to quartz AMS combustion tubes. Each glass vial was rinsed 3x with 0.25 mL of 18.2 MΩ DDI water and the rinsate was added to resuspended DNA in the quartz combustion tubes. The DNA and rinsate were again lyophilized to dryness. AMS isotopic standards Oxalic Acid II (NIST SRM 4990 C) and ANU sucrose underwent the same drying and transfer as the DNA. Excess copper oxide (CuO) was added to each dry sample, tubes were evacuated and sealed with a $H_2/O_2$ torch. Tubes were placed in a furnace set at 900 C for 3.5 h to combust all carbon to $CO_2$. The evolved $CO_2$ was purified, trapped, and reduced to graphite in the presence of iron catalyst in individual reactors[7]. Magnesium perchlorate water traps were used during graphite

production[8]. Large $CO_2$ samples (> 500 μg) were split and $\delta^{13}C$ was measured by stable isotope ratio mass spectrometry. Graphite targets were measured at the Center for Accelerator Mass Spectrometry at Lawrence-Livermore National Laboratory.

Few of the DNA samples were large enough to get $\delta^{13}C$ splits, but those measured ranged from –21 to –23 per mil, values similar to the whole tissues. We used a $\delta^{13}C$ fractionation correction of –23 +/-2 for all samples. Corrections for background contamination introduced during AMS sample preparation were made following the procedures of Brown and Southon[9]. All $^{14}C$ data are reported in units of fraction modern carbon ($F^{14}C$) with $\delta^{13}C$ fractionation correction. The supplemental information expresses $^{14}C$ data in other nomenclatures, as decay corrected $\Delta^{14}C$ following the dominant convention of Stuiver and Polach[10] and as $F^{14}C$ post-bomb fractionation corrected data[11]. The $\Delta^{14}C$ convention established for reporting radiocarbon data in chronological and geophysical studies was not developed to deal with post-bomb data, but it is the most commonly used. The relatively new $F^{14}C$ is a clearly defined standard nomenclature for post-bomb data[11].

**References Supplement 4**


1. Rodbell, M. Metabolism of Isolated Fat Cells. I. Effects of Hormones on Glucose Metabolism and Lipolysis. *J Biol Chem* **239**:375-380 (1964).
2. Rydén, M., et al., Mapping of early signaling events in tumor necrosis factor-alpha -mediated lipolysis in human fat cells. *J Biol Chem* **277**:1085-1091 (2002).
3. Hirsch, J., and Gallian, E. Methods for the determination of adipose cell size in man and animals. *J Lipid Res* **9**:110-119 (1968).
4. Bjorntorp, P. Size, number and function of adipose tissue cells in human obesity. *Horm Metab Res* Suppl **4**:77-83 (1974).

5. Gallagher, D., et al. How useful is body mass index for comparison of body fatness across age, sex, and ethnic groups? *Am J Epidemiol* **143**:228-239 (1996).

6. Bjorntorp, P. Effects of age, sex, and clinical conditions on adipose tissue cellularity in man. *Metabolism* **23**:1091-1102 (1974).

7. Vogel, J. S., Southon, J. R., and Nelson, D. E. Catalyst and Binder Effects in the Use of Filamentous Graphite for AMS. Nucl. Instrum. *Methods Phys. Res. Sect. B* **29**, 50-56 (1987).

8. Santos, G. M., Southon, J. R., Druffel-Rodriguez, K. C., Griffin, S., and Mazon, M. Magnesium Perchlorate as an Alternative Water Trap in AMS Graphite Sample Preparation: A Report on Sample Preparation at KCCAMS at The University of California, Irvine. *Radiocarbon* **46**, 165-173 (2004).

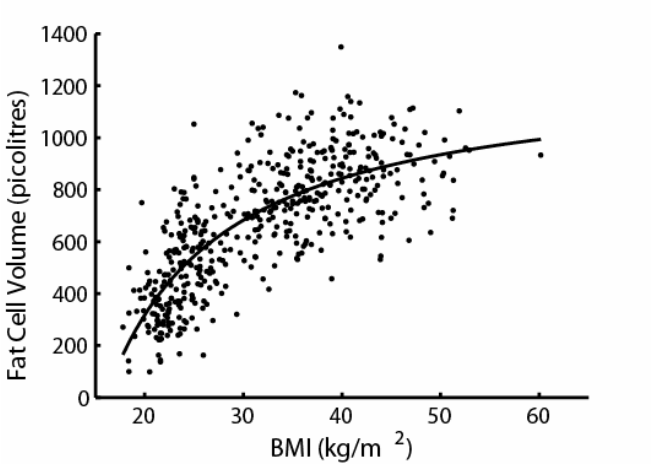
9. Brown, T. A. and Southon, J. R. Corrections for contamination background in AMS $^{14}C$ measurements. Nucl. Instrum. *Methods Phys. Res. Sect. B* **123**, 208-213 (1997).

10. Stuiver, M. and Polach, H. A. Discussion; reporting of C-14 data. *Radiocarbon* **19**, 355-363 (1977).

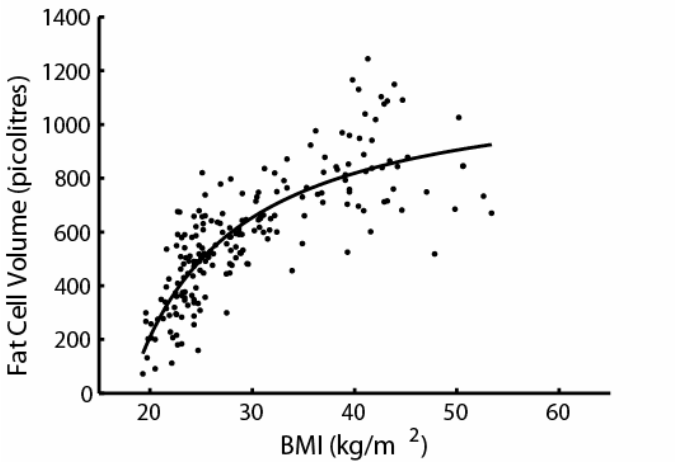
11. Reimer, P. J., Brown, T. A., and Reimer, R. W. Discussion: reporting and calibration of post-bomb $^{14}C$ data. *Radiocarbon* **46**, 1299-1304 (2004).

Figure1

**a**


Fat Cell Volume (picolitres)
1400
1200
1000
800
600
400
200
0
20
30
40
50
60
BMI (kg/m 2)

**b**


Fat Cell Volume (picolitres)
1400
1200
1000
800
600
400
200
0
20
30
40
50
60
BMI (kg/m 2)

**c**

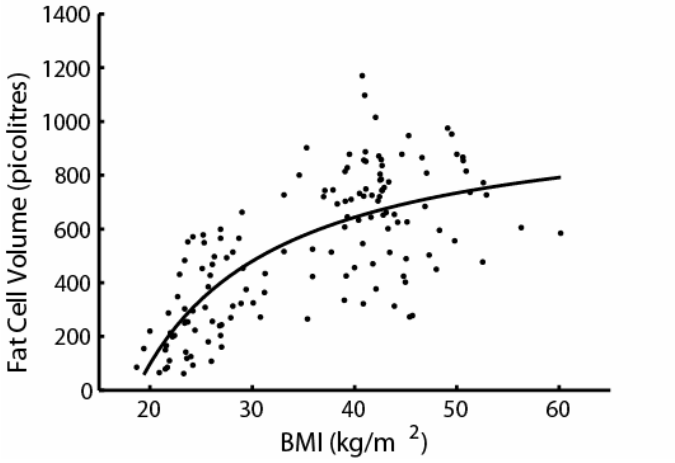
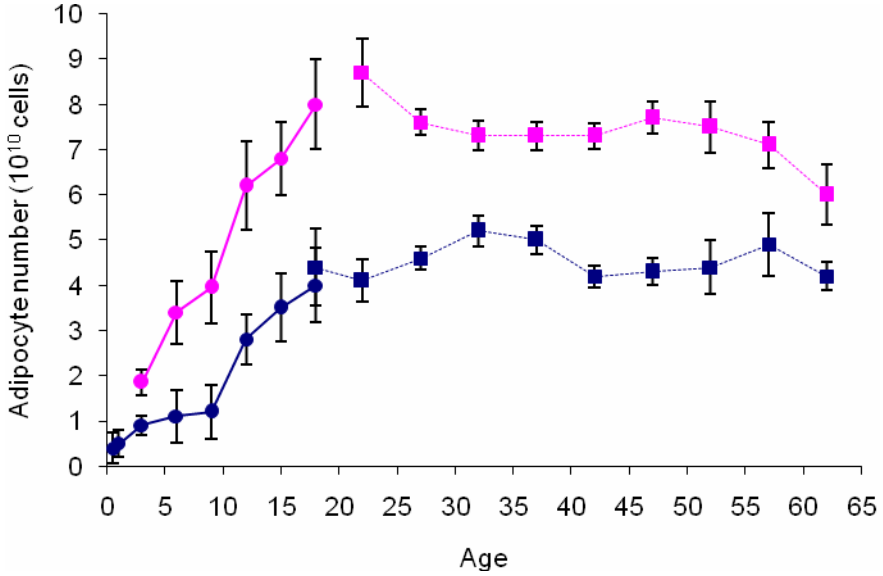
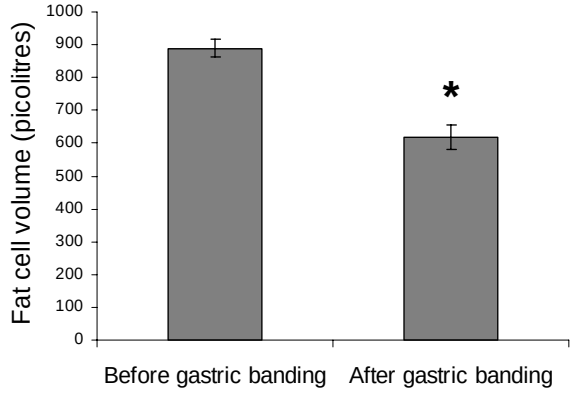

Fat Cell Volume (picolitres)
1400
1200
1000
800
600
400
200
0
20
30
40
50
60
BMI (kg/m 2)

Figure 2

**a**


Adipocyte number ($10^{10}$ cells)
Age
0
1
2
3
4
5
6
7
8
9
10
0
5
10
15
20
25
30
35
40
45
50
55
60
65

**b**


Fat cell volume (picolitres)
1000
900
800
700
600
500
400
300
200
100
0
*
Before gastric banding
After gastric banding

**c**

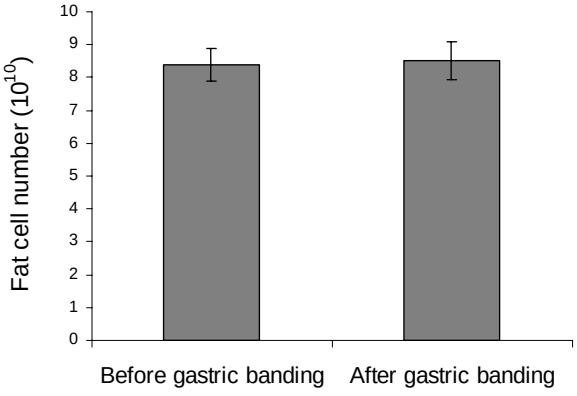

Fat cell number ($10^{10}$)
10
9
8
7
6
5
4
3
2
1
0
Before gastric banding
After gastric banding

Figure 3

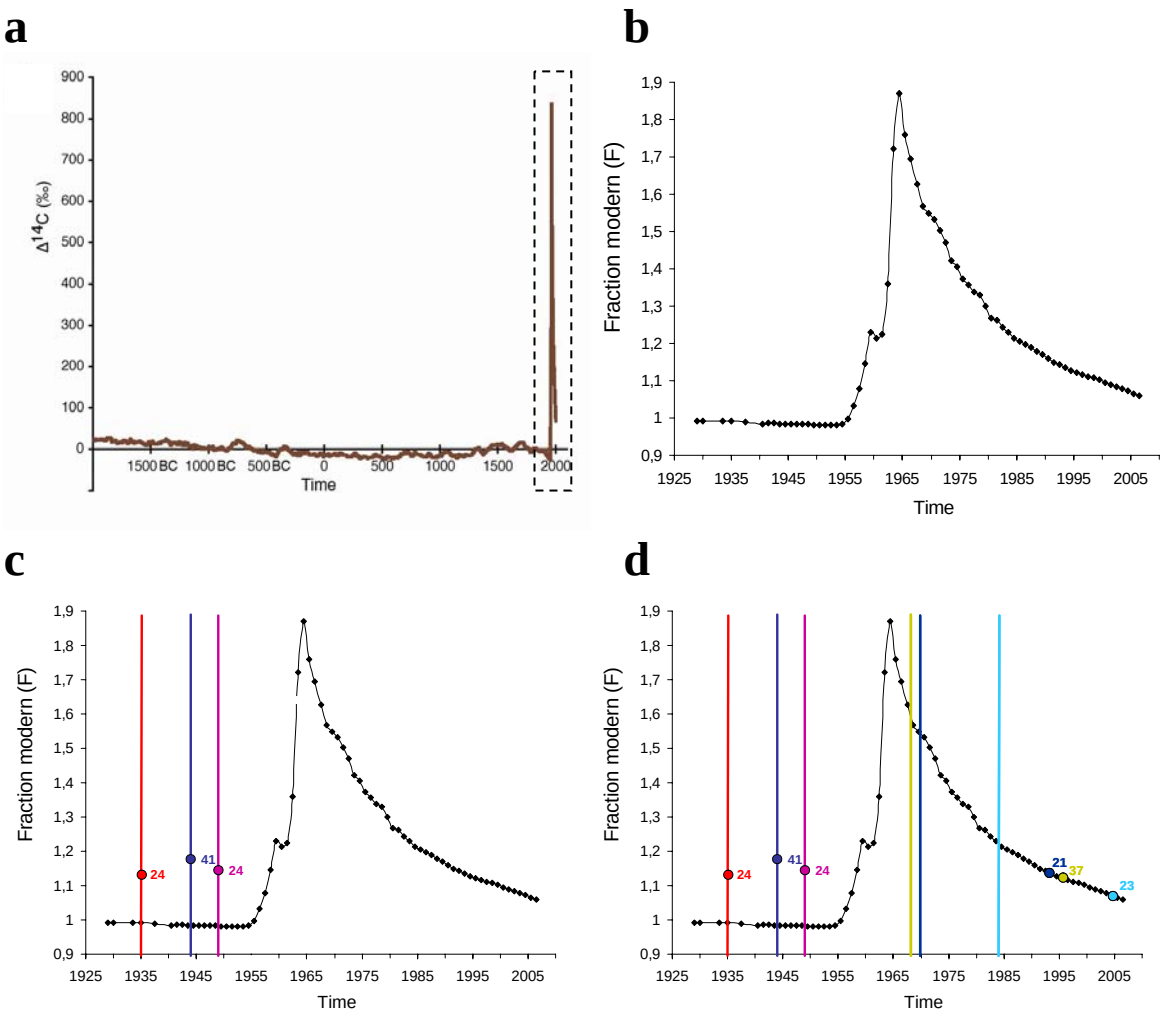
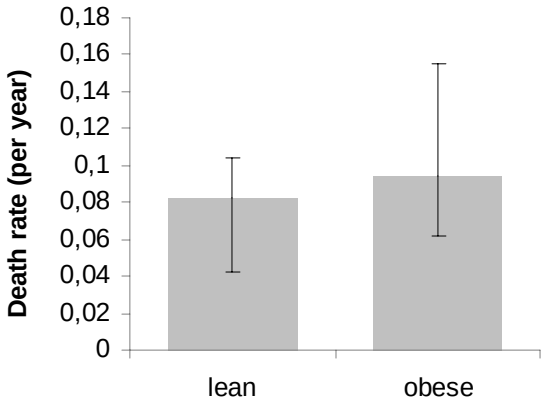
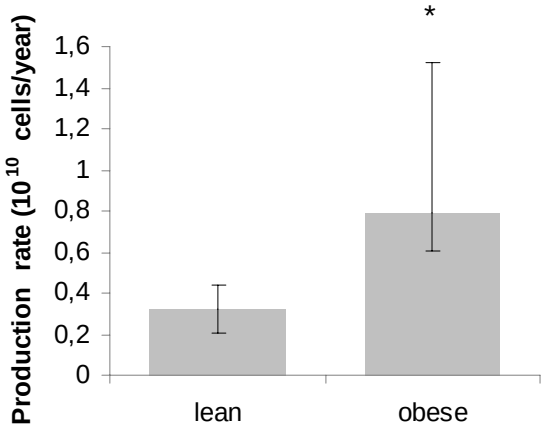


Figure 4

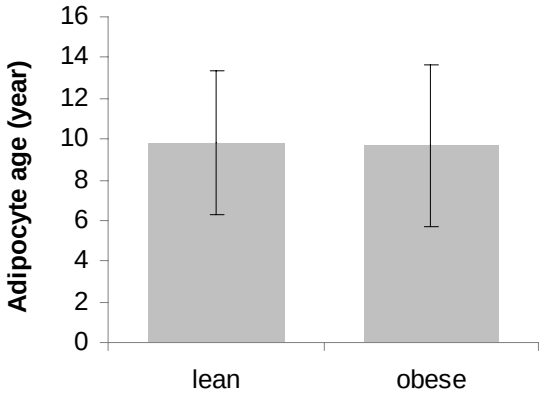
**a**


Death rate (per year)
0,18
0,16
0,14
0,12
0,1
0,08
0,06
0,04
0,02
0
lean
obese

**b**


Production rate ($10^{10}$ cells/year)
*
1,6
1,4
1,2
1
0,8
0,6
0,4
0,2
0
lean
obese

**c**


Adipocyte age (year)
16
14
12
10
8
6
4
2
0
lean
obese