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Adipose lipid turnover and long-term changes in body weight

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The worldwide obesity epidemic[1] makes it important to understand how lipid turnover (the capacity to store and remove lipids) regulates adipose tissue mass. Cross-sectional studies have shown that excess body fat is associated with decreased adipose lipid removal rates[2,3]. Whether lipid turnover is constant over the life span or changes during long-term weight increase or loss is unknown. We determined the turnover of fat cell lipids in adults followed for up to 16 years, by measuring the incorporation of nuclear bomb test-derived ^{14}C in adipose tissue triglycerides. Lipid removal rate decreases during aging, with a failure to reciprocally adjust the rate of lipid uptake resulting in weight gain. Substantial weight loss is not driven by changes in lipid removal but by the rate of lipid uptake in adipose tissue. Furthermore, individuals with a low baseline lipid removal rate are more likely to remain weight-stable after weight loss. Therefore, lipid turnover adaptation might be important for maintaining pronounced weight loss. Together these findings identify adipose lipid turnover as an important factor for the long-term development of overweight/obesity and weight loss maintenance in humans.

Adipocytes are large triglyceride-filled cells that form >90% of the white adipose tissue (WAT) mass[4]. Because the triglycerides in adipocytes constitute >95% of the adipocyte volume, the balance between triglyceride storage and removal (lipid turnover) determines the WAT size. Long-term lipid turnover studies in humans have not been performed due to methodological limitations. Previous studies relied on short-term isotope labeling experiments[5–9] or determining enzymatic hydrolysis (lipolysis) of lipids in vitro[10]. Our previous studies revealed altered adipose lipid turnover in the overweight and obese states[2,3]. However, the cross-sectional design of these studies precluded us from determining the change in lipid turnover with either aging or altered body weight within an individual. Only longitudinal investigations can elucidate how growth, reduction or maintenance of WAT mass are regulated by lipid turnover.

To assess lipid turnover across the adult life span or following long-term substantial body weight change, we performed longitudinal analyses (up to 16 years) of lipid age in human WAT (Fig. 1a). Subcutaneous WAT is easily accessible and more sensitive to lipid turnover changes than visceral depots[11]. Lipid age was assessed by measuring the $^{14}C/^{12}C$ ratio in the lipids of subcutaneous adipocytes. Atmospheric ^{14}C levels have remained relatively stable (with respect to total carbon) for the last several thousand years. However, aboveground nuclear bomb tests during the Cold War (1955–1963) doubled the $^{14}C/^{12}C$ concentration ratio in the atmosphere[12]. After the Partial Test Ban Treaty in 1963, ^{14}C levels have dropped exponentially due to mixing with large oceanic and terrestrial carbon reservoirs[13–15]. By assessing the incorporation of ^{14}C into fat cell lipids, it is possible to retrospectively determine the age and thus the turnover of lipids during the lifetime of an individual[2,11].

Two cohorts were investigated (Fig. 1a and Extended Data Fig. 1). Cohort 1 consisted of 54 individuals (10 males). During a mean follow-up time of 13 years (range 7–16 years), individuals increased, remained stable or decreased in body weight (Extended Data Fig. 1 and Methods). Cohort 2 included 41 morbidly obese women followed for 4–7 years (average 5 years) after bariatric surgery, which resulted in pronounced weight loss. In both cohorts, subcutaneous abdominal WAT biopsies were taken at baseline and follow-up (Methods).

WAT mass is set by a balance between lipid uptake and removal due to lipolysis, lipid oxidation and/or ectopic deposition into nonadipose depots[16]. The mean lipid age (in years) is the average time lipids spend in WAT and is determined by measuring the incorporation of radioactive carbon into triglycerides, as shown in Fig. 1b. The lipid removal rate (K_{out}) represents the approximate fraction of lipid replaced each year and is the inverse of lipid age ($K_{out} = 1/\text{lipid age}$), such that a high lipid age reflects a low lipid removal rate (Methods). If lipid removal and total fat mass data are known, the net lipid uptake (K_{in}) can be determined by their product ($K_{in} = \text{kg fat mass} \times \text{year}^{-1}$)[2], reflecting the storage capacity of fatty acids into triglycerides. ^{14}C levels in triglycerides followed atmospheric levels, although they were positioned above the bomb spike, indicating that lipids were stored well before the collection date (Fig. 1c). Modeling based on different sizes of long-lived lipid pools (Fig. 1d and Methods) showed that the ^{14}C data suggested that there is no long-lived pool of lipids in subcutaneous WAT.

In cohort 1, lipid age rose significantly by 0.6 ± 0.8 years over approximately 13 years (Fig. 2a) despite large interindividual variations. Participant and lipid age did not correlate at either time point ($r = 0.02–0.07$; $P = 0.60–0.89$), which is further illustrated in Extended Data Fig. 2. Furthermore, the increase over time in lipid age (Δlipid age), which reflects a decrease in lipid removal rate from WAT, was not dependent on the participant's starting age (Fig. 2b). Body weight change over time and Δlipid age did not correlate (Fig. 2c, slope not significantly different from zero). Instead, irrespective of weight change, mean lipid age increased in 42 out of 54 participants (Fig. 2c, positive y intercept). Even after separating

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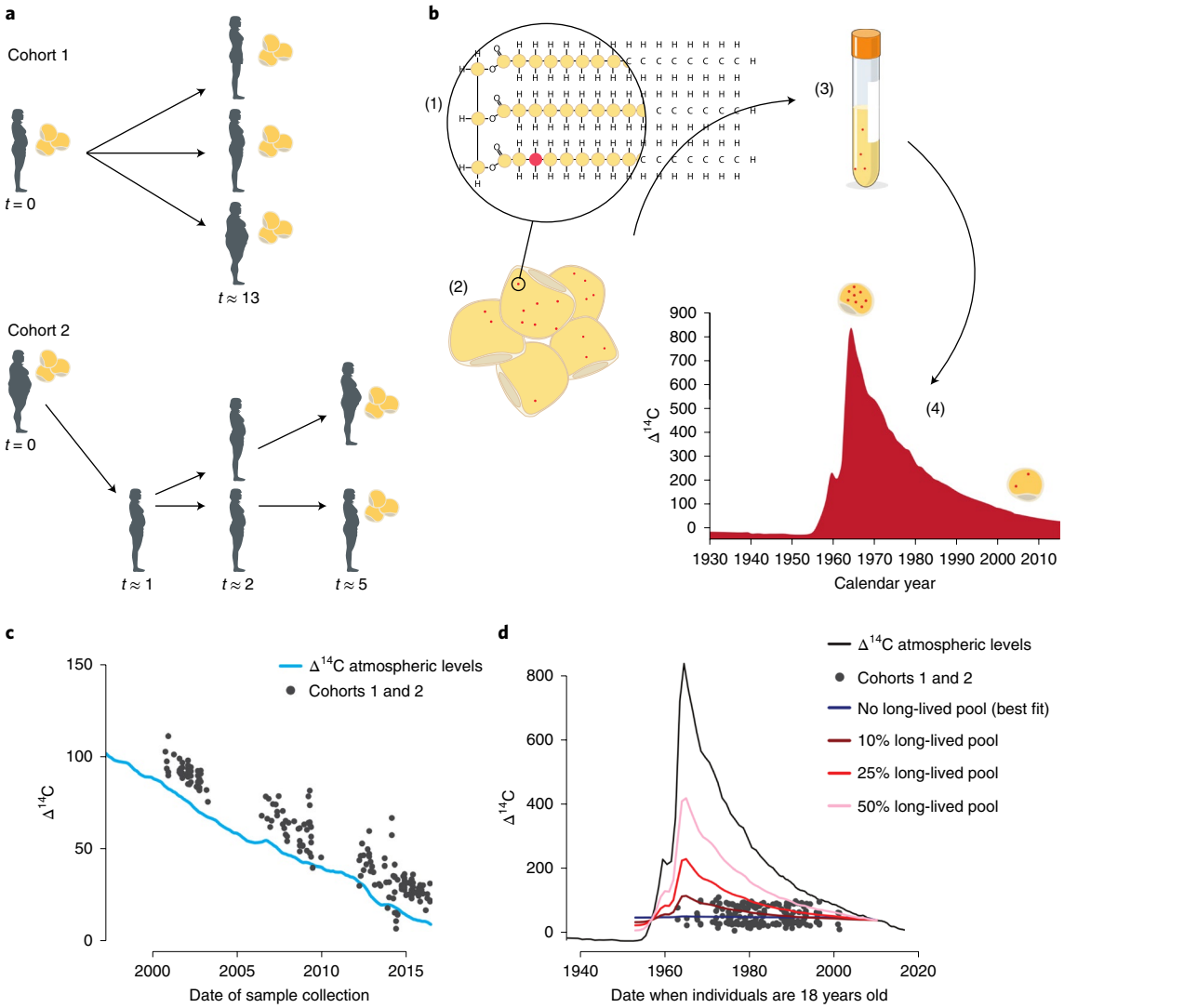

Fig. 1 | Experimental setup and carbon dating of adipose lipids. a, Experimental design of the two patient cohorts. **b**, (1) The ratio of radioactive carbon (^{14}C, shown in red) to stable carbon (^{12}C, shown in yellow) in triglycerides reflects the atmospheric levels of radioactive carbon at the time the triglycerides were formed; (2) triglycerides containing ^{14}C (red dots) display variable concentrations due to continuous lipid turnover. The peak of the curve corresponds to a $^{14}C/^{12}C$ ratio of approximately 2×10^{-12}; (3) triglycerides from adipose biopsies are pooled and the average ^{14}C:^{12}C is determined[13,14]; (4) triglycerides with more ^{14}C content will be older due to decreasing atmospheric ^{14}C levels over time. **c**, Measured ^{14}C levels ($\Delta^{14}C$) in cohorts 1 and 2 (black dots) versus sample collection date. $\Delta^{14}C$ levels follow the atmospheric ^{14}C levels in Western Europe (blue line)[2,3]. **d**, Theoretical impact of long-lived lipid pools (0, 10, 25 and 50% of the total lipid mass). Long-lived lipids were assumed to be formed around the age of 18 and to have persisted since (see Methods). Each curve simulates an individual turning 18 at the year given on the *x* axis. Atmospheric ^{14}C levels peaked in 1960–1970; formation of long-lived lipids during these years should result in elevated $\Delta^{14}C$ levels, as indicated.

participants into weight losers (≥7% weight loss) and gainers (≥7% weight gain) as defined in Arner et al.[10], Δlipid age did not differ between groups (Fig. 2d). Altogether, these results suggest that lipid removal in WAT slows down with aging during adulthood. Unless compensated by changes in lipid uptake, the reduced lipid removal rate will result in fat accumulation over time. Participants who displayed no change in lipid uptake during the observation period displayed a substantial (approximately 20%) increase in body weight (Fig. 2e, where the regression line crosses zero). Conversely, weight losers decreased K_{in} compared to weight gainers (Fig. 2f). Percentage body weight change was used in Fig. 2c,e; similar results were obtained using other weight-related parameters (Supplementary Tables 1 and 2). Lipid storage (K_{in}) mainly mirrors food intake while lipid removal (K_{out}) mostly reflects lipolysis/lipid oxidation. However, several other factors, such as physical activity or dietary composition, may also influence lipid deposition into WAT, as evidenced from human adipocyte lipolysis studies[17,18]. Participants in cohort 1 reported increased physical activity over time (Extended Data Fig. 1), which was unrelated to changes in lipid age ($r=0.02$, $P=0.88$). According to self-reported dietary habits, there were no marked qualitative changes in food composition over time. Previous population-based studies have shown that the main driver for obesity is calorie intake, not energy expenditure[19]. Our results add a dynamic dimension to this, suggesting that a failure to reduce calorie intake in aging leads to weight increase.

In cohort 1, participants showed modest changes in body weight over time. We investigated severely obese patients undergoing gastric bypass surgery (cohort 2) who demonstrated a marked and sustained decrease in body mass index (BMI) over 5 years (Fig. 3a). There was no significant change in lipid age (Fig. 3b), suggesting that alterations in lipid removal are not central for pronounced weight loss. Admittedly, we cannot exclude that an aging-related

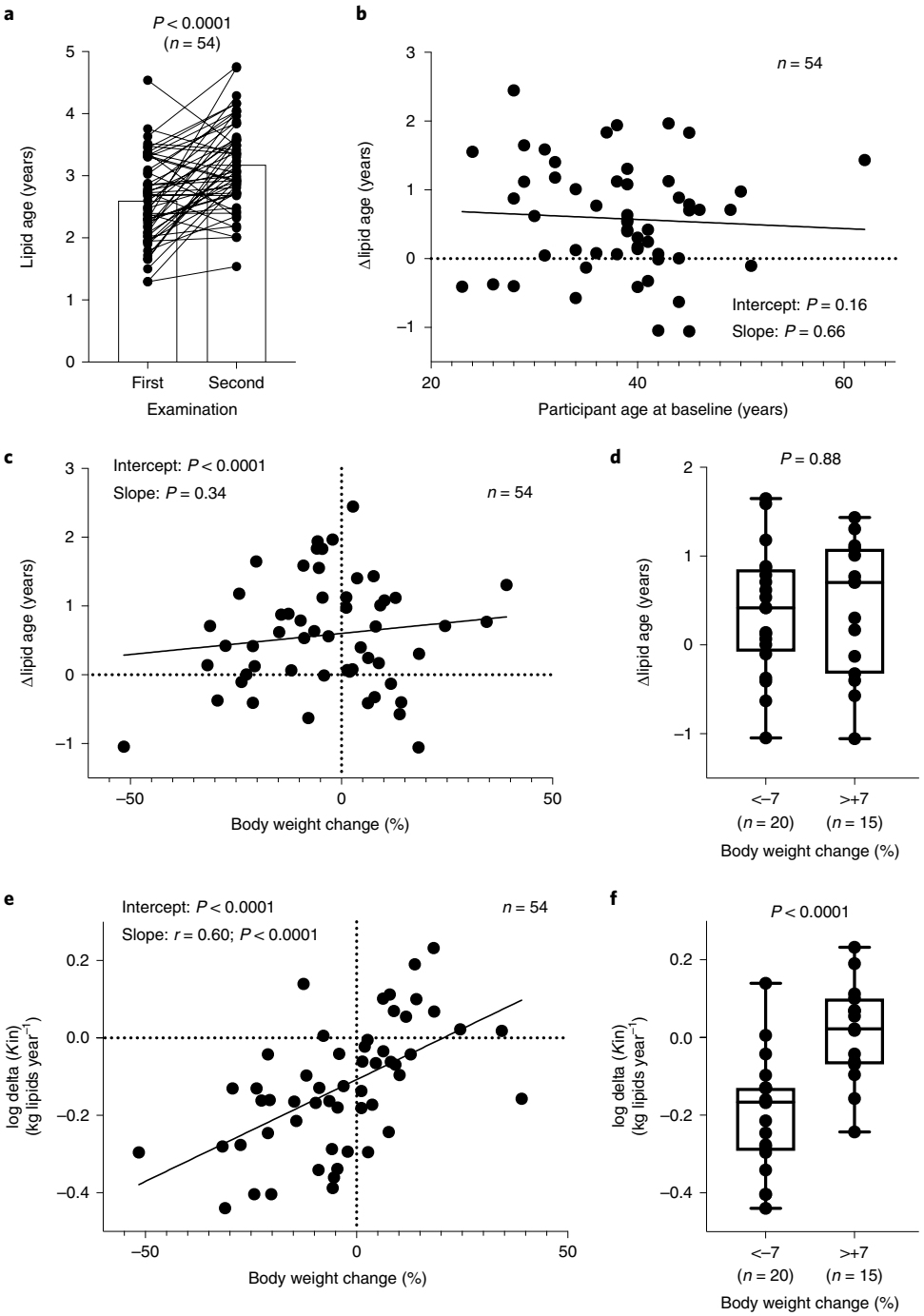

Fig. 2 | Adipose lipid turnover with age (cohort 1). a, Lipid age increased significantly between the first (2.6 ± 0.7 years) and second (3.2 ± 0.7 years) examination. **b**, Participant age at first examination versus Δlipid age (change between first and second examination). **c**, Percentage of change in body weight versus Δlipid age. **d**, Δlipid age between participants who had at least 7% body weight change over time. **e**, Percentage of change in body weight versus change in lipid uptake ($\log_{10}(K_{in})$) over time. **f**, Change in lipid uptake in participants who had at least a 7% body weight change. Results are individual values or group values represented by the box plots. Comparisons were made by paired two-sided *t*-test (**a**), unpaired two-sided *t*-test (**d**,**f**) or linear regression (**b**,**c**,**e**). Values are individual (**a**–**c**,**e**) or box plots (minimum–maximum) with individual data. The bars in **a** indicate the mean value. The number of participants (*n*) are given in the panels.

slowdown in lipid removal is masked by depletion of lipid stores during body weight reduction (Extended Data Fig. 3). Nevertheless, there was a significant decrease in K_{in} between years 0 and 5 (Fig. 3c) demonstrating that lipid uptake is the main determinant driving weight loss following bariatric surgery.

Weight loss, expressed as ΔBMI between years 0 and 5, correlated significantly with lipid age at year 0 (Fig. 3d), with the largest ΔBMI observed in individuals with the oldest lipids. Similar data were obtained if other measures were used instead of ΔBMI (Extended Data Fig. 4). When controlling for initial BMI by analysis of variance (ANOVA), the effect of lipid age on ΔBMI remained significant (values not shown). Similar results were obtained when participants were grouped into tertiles according to ΔBMI, defined as weight-stable (tertiles 2 and 3) or weight rebounders (tertile 1) at year 5 (Fig. 3e). While there was no difference in BMI at year 0 between tertile 1 and tertiles 2 and 3 (Fig. 3e), lipid age differed significantly

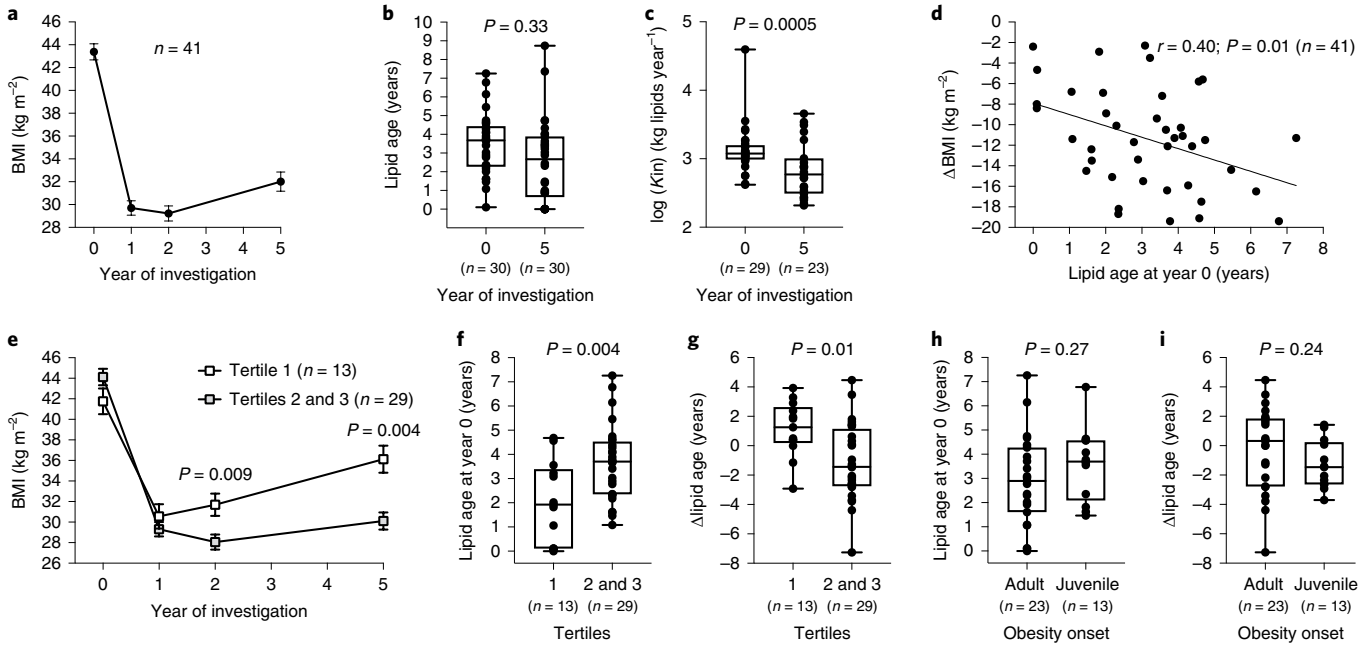

Fig. 3 | Adipose lipid turnover following substantial weight loss (cohort 2). a, BMI at baseline (0 year) and 1, 2 and 5 years following bariatric surgery for obesity (mean, s.e.m.). **b**,**c**, Comparisons of lipid age (**b**) and lipid uptake (**c**) between 0 and 5 years. **d**, Lipid age at baseline versus ΔBMI. **e**, BMI at years 0, 1, 2 and 5, with participants divided into tertiles based on the change in BMI from years 2 to 5 (mean, s.e.m.). Tertile 1 is participants whose weight rebounded from years 2 to 5, while participants in tertiles 2 and 3 remained weight-stable. **f**, Lipid age at baseline in tertile 1 and tertiles 2 and 3. **g**, Δlipid age in tertile 1 and tertiles 2 and 3. **h**,**i**, Lipid age at baseline (**h**) and changes in lipid age over time (**i**) in adult-onset (≥18 years old) or juvenile-onset (<18 years old) obesity. A paired two-sided *t*-test was used in **b**,**c** and an unpaired two-sided *t*-test in **e**–**i**. Linear regression was used in **d**. Δ is a change in parameter between second and first examination. Values in **a** and **e** are the mean ± s.e.m. Values in **b**,**c** and **f**–**i** are box plots (minimum–maximum) with individual values. Values in **d** are individual values; the regression line using simple regression is shown together with *r* and *P* values. The number of participants (*n*) are given in the panels.

(Fig. 3f). Individuals at year 0 having the oldest lipid age (slowest lipid removal rate), showed the greatest maintenance of weight loss between years 1 and 5, whereas those with a younger lipid age regained weight over this period (Fig. 3e). Δlipid age (from years 0 to 5) also differed between the two groups (Fig. 3g): lipid age decreased in weight-stable individuals ($P=0.03$) and increased in weight rebounders ($P=0.05$). This suggests that increased lipid removal following substantial weight reduction contributes to successful weight loss maintenance; such an effect may also explain the findings in Fig. 3b. Individuals with a lower lipid removal rate before weight loss may have a larger window for improving their lipid removal than those with a higher initial removal rate. Therefore, lipid removal rates may adjust following weight loss and thus be an important determinant for long-term weight loss. Similar results were obtained in individuals with juvenile- or adult-onset obesity (Fig. 3h,i).

Age- and weight-related changes in lipid turnover might represent compensatory mechanisms of ectopic fat deposition in the liver or visceral adipose tissue, which was assessed in cohort 2 (see Methods). No association between changes over time in lipid age and fatty liver index (FLI) or visceral fat mass were found (Extended Data Fig. 5). Increased adipose inflammation over time might also influence lipid turnover. However, in both cohorts, fat cell volume decreased while fat cell number increased (Supplementary Table 3). The ensuing hyperplasic adipose tissue (many small fat cells) is associated with less inflammation[20]. Finally, no influence of eating behavior (Supplementary Table 3) on lipid age was seen (Extended Data Fig. 6). It is less probable that other psychological behaviors influenced the results.

Additional clinical parameters for both cohorts, reported in Supplementary Table 3, had no apparent influence on the presented ^{14}C data. We analyzed (1) participants who were overweight/obese or not at 18 years of age or having stable/unstable body weight before examination (cohort 1), (2) excluding bariatric surgery patients in cohort 1 and (3) participants having unchanged medication or menstruation dynamics (both cohorts). None of these factors impacted lipid age (Supplementary Table 4). This study primarily investigated women and as such we cannot rule out a sex effect on lipid turnover[21]. However, previous cross-sectional studies have not shown any sex differences in adipose lipid age or turnover[2,11]. Lipid turnover parameters in other various fat depots may differ[11]. Unfortunately, depots other than subcutaneous fat cannot be investigated in long-term prospective studies for practical/ethical reasons. As discussed by Spalding et al.[11], variations in food composition are probably not important in influencing the ^{14}C results.

Changes in total energy expenditure and lipid oxidation have an impact on fat mass. Our study suggests that the turnover of adipose lipids is another important and independent regulator. To determine whether there was a correlation between these measures, we compared lipid age data with results from indirect calorimetry for both cohorts at baseline ($n=92$). Neither resting energy expenditure nor respiratory quotient correlated with adipose lipid age (Extended Data Fig. 7). Thus, the adipose lipid removal rate does not merely reflect energy expenditure or lipid oxidation.

Is adipose lipid turnover a treatment target? At present there are no specific pharmacological agents targeting adipose lipid uptake and removal. However, it is possible to improve catecholamine-induced fat cell lipolysis, an important regulator of adipose lipid age[2,11]. For example, endurance training enhances the lipolytic activity of fat cells[17,22,23] and could therefore be efficient in individuals with young lipid age. The sympathoadrenal system activity decreases during aging[24] and may diminish the adipose lipid turnover rate. Along this line, catecholamine activation of lipolysis in vivo is impaired in aged individuals[25].

This study sheds a new perspective on changes in fat mass over time in adult humans. Irrespective of long-term (≅13 years) body weight development, lipid turnover in subcutaneous WAT decreases. Thus, maintenance of body weight during aging must be accompanied by a decrease in lipid storage rate, otherwise body weight will increase. Herein, this imbalance corresponded to an average weight gain of +20% over 13 years. We also show that therapeutic weight loss over a 5-year period is primarily explained by decreased lipid storage. Even though not assessed, lipid oxidation probably increases during weight loss, since the average loss of fat mass exceeds what could be expected from normal removal rates only (Methods). Participants with a low baseline lipid removal rate displayed an increase in this measure. This was linked to a more stable weight reduction and thus to better energy balance. Therefore, interindividual variations in the adaptability of lipid turnover to caloric restriction may at least partly explain the difficulties many individuals encounter in maintaining weight loss following dietary interventions[26–29]. These results encourage the development of therapeutic and lifestyle strategies to counteract age-related decreases in lipid turnover rates and recognize the importance to adapt adipose lipid turnover for the maintenance of normal weight or weight loss. Therefore, identifying factors affecting lipid turnover could be of clinical relevance.

Online content

Any methods, additional references, Nature Research reporting summaries, source data, statements of code and data availability and associated accession codes are available at https://doi.org/10.1038/s41591-019-0565-5.

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Author contributions

K.L.S. and P.A. designed the study. A.T. recruited the patients. P.A., D.P.A. and M.R. examined the patients. L.A. and K.-Y.F. prepared the adipose samples. M.S. performed the ^{14}C AMS measurements. S.B. performed the mathematical modeling. K.L.S., P.A., S.B. and M.R. analyzed the data. K.L.S., P.A., M.R. and S.B. wrote the first version of the paper. All authors contributed and approved the final version of the paper.

Competing interests

The authors declare no competing interests.

Additional information

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Methods

Participants. We identified 84 individuals examined at our unit between 2001 and 2003, where abdominal subcutaneous WAT (sWAT) was available (cohort 1: study identifier SOWOT; clinicaltrials.gov trial registration number NCT02227043). Forty-five women and ten men accepted follow-up and attended the unit for clinical examinations and sWAT biopsies. In one woman, it was not possible to make a reliable calculation of lipid turnover parameters and she was excluded from the study. The mean ± s.d. follow-up time was 13 ± 1 years (range 7–16 years). None of the individuals had diabetes. We used our recently published procedure to categorize participants according to body weight changes over time[10]. A weight change cutoff between baseline and follow-up of 7% was used to subdivide participants into weight gainer (≥7%, $n = 15$), weight-stable (<±7%, $n = 19$) and weight loser (≤7%, $n = 20$) categories. This value was based on a separate analysis of the Stockholm Pregnancy and Women's Nutrition study[30] where we followed the body weight development of 563 women 15 years after childbirth. In that study, the 25th percentile in body weight increase corresponded to 7% (data not shown). Regarding the weight loser group, 10 participants had undergone bariatric surgery due to obesity (Roux-en-Y gastric bypass) 3–11 years before the second examination. Body weight had been stable for at least 6 months according to self-report at the follow-up examination. Individuals were asked in detail for their dietary habits at the second examination. None ate predominantly marine food products. One individual became a strict vegetarian after the first examination. There were no other important qualitative changes between the two examinations in terms of food composition. A second group of women participated in a longitudinal trial of Roux-en-Y because of obesity (cohort 2: study identifier DEOSH; clinicaltrials.gov trial registration number NCT01785134). This trial was conducted on women only. Initially, 80 women were enrolled with their baseline characteristics published[31]. None of the women had followed any hypocaloric diet before the first examination and all were weight-stable (weight change <2 kg) for at least 1 year before their first preoperative visit. Postsurgery, women reported their actual body weight after 1 and 2 years and were reexamined after 4–7 years when 49 women returned. Some clinical and adipose data (the latter not included in this study) have been reported[32]. Of these women, 41 had sWAT tissue saved from their first examination and were thus included in the present study. The follow-up time was 5 ± 0.5 (mean ± s.d.) years. At the second examination, body weight had been stable for at least 3 months according to self-report. Postoperative dietary recommendations included intake of fluids and semisolid food for 2 weeks and solid food thereafter. Patients were instructed to adhere to a diet rich in protein and with a calorie content of approximately 800–1,000 kcal for the first 6 postoperative weeks. After this period, more general instructions were given, including frequent and small servings with low calorie content and preferably high content of proteins and whole-grain products. Patients were encouraged to adhere to three main meals daily with up to a total of four snacks in between these meals. To avoid dumping, patients were instructed to avoid nutrients with high content of, in particular, simple carbohydrates and fat and to restrain from intake of fluids until after the meal. Except for this, no specific recommendations were given regarding the relative energy content of fat, carbohydrates and proteins. According to self-report, the patients had not made any important changes in the proportional intake of these components after the initial postoperative period.

Participants came to the laboratory in the morning after an overnight fast. Height, weight, circumferences of hip and waist and total body fat mass by dual-energy X-ray absorptiometry (DEXA; GE Health Care) in cohort 2 and by bioimpedance (Quadscan 4000; Bodystat) in cohort 1 were measured. Participants filled in a questionnaire about physical activity, which was graded in three levels as described in detail in Arner et al.[10] and Hoffstedt et al.[32]. A venous blood sample was obtained for routine clinical chemistry measures as described previously[32]. Finally, an sWAT biopsy was obtained from the periumbilical area as described previously[4]. The subcutaneous abdominal fat mass corresponding to the area for the estimated subcutaneous adipose tissue (ESAT) biopsy was calculated from the DEXA measures as described in detail in cohort 2[33] and, in cohort 1, from an algorithm based on age, sex, total fat mass, waist-to-hip ratio and waist circumference, as described in detail by Andersson et al.[33]. Using DEXA, it was also possible to determine the estimated visceral adipose tissue (EVAT) mass in cohort 2 as described in detail by Andersson et al.[33]. In cohort 2, we also indirectly determined liver fat using a score based on clinical chemistry and anthropometric measures[34]. In both cohorts, mean fat cell size and number were measured in the area of the adipose biopsy (that is, ESAT) as described previously[33]. In both cohorts, eating behavior was also assessed using a questionnaire focused on so-called binge eating[35]. Thirty-three self-report measures were used and subdivided into two categories: BITE-A measured symptom magnitude and BITE-B symptom severity.

Additional clinical details of cohorts 1 and 2 can be found in Supplementary Table 1. Ethical permission for all studies was given by the ethics committee at Karolinska Institutet and written informed consent was obtained from all participants.

Extraction of lipids from adipose tissue preparations. Subcutaneous adipose tissue samples were stored at −70 °C. Lipids were extracted from 50–100 mg subcutaneous adipose tissue. A 7 ml Dounce homogenizer was used for homogenization with two different pestle sizes. Tissue was homogenized with 2 ml isopropanol–heptane–H_2SO_4 (300 ml isopropanol/75 ml heptane/7.5 ml of 0.5 M H_2SO_4) until the solution was homogenous. The homogenous solution was transferred to a glass tube filled with 4 ml water. The homogenizer tube was rinsed with 3 ml isopropanol–heptane–H_2SO_4 and the rinse was pooled with the homogenous solution. An additional 3 ml aliquot of heptane was added to the mixed solution and the tube was sealed with a cork and shaken. After settling, the upper layer containing the heptane and lipids was transferred to clean glass vials using a Pasteur glass pipette. The lipids were extracted two more times by adding 3 ml heptane, shaking and transferring the supernatant. The sample in the glass vial was concentrated in a sample concentrator with nitrogen gas and finally transferred to a 4 ml glass vial with assembled cap (Scantec Nordic). The weight of tissue and lipids were measured before and after extraction; the outcome should be around 70–80% of the starting weight. The resulting lipid sample was processed for ^{14}C measurement as described later on. As discussed previously[2], the extracted lipids mainly represent triglycerides in fat cells. Because the half-life of ^{14}C is approximately 5,700 years, sample storage time does not affect triglyceride age measurement.

Accelerator mass spectrometry (AMS) measurements. The age of the extracted lipid samples was determined using the carbon isotope ratio ($^{14}C/^{12}C$) measurement, employing the AMS technique described previously[2]. Briefly, a dedicated sample preparation method was developed, which facilitates the analysis of samples with total carbon masses from mg to µg[36]. An important feature of the method is the low amount of stray carbon introduced into the samples, which reduces sample-to-sample fluctuations and improves measurement accuracy. For these studies, we have an ample supply of many milligrams of triglycerides, which allow the use of optimal and standard-sized samples. All samples were prepared by initial conversion into CO_2 gas and subsequent reduction into graphite, as described later on. Copper oxide was used as the oxidizing agent, which was added to the samples in quartz tubes. The tubes were subsequently evacuated and sealed with a high temperature torch. The quartz tubes were placed in a furnace set at 900 °C for 3.5 h to combust all carbon to CO_2. The formed gas was cryogenically purified and trapped. The collected CO_2 gas was then reduced into graphite in individual submilliliter reactors at 550 °C for 6 h in the presence of zinc powder as the reducing agent and iron powder as the catalyst. The graphite targets, all containing approximately 500 µg of carbon, were pressed into target holders and measured at the Department of Physics and Astronomy, Ion Physics, Uppsala University, using a 175 kV Mini radioCarbon Dating System (MICADAS) compact accelerator mass spectrometer, which uses permanent low- and high-energy mass-analyzing magnets as well as online $\delta^{13}C$ measurement and isotope fractionation correction[37]. Stringent and thorough laboratory practice was implemented to minimize the introduction of contaminant carbon into the samples, such as preheating all glassware and chemicals under oxygen flow before sample preparation to eliminate surface absorbed CO_2. In addition, the CO_2 from the samples was split and a small fraction (50 µg C) was used to measure the isotopic ratio ($\delta^{13}C$) of the triglycerides in the gas phase by a separate stable isotope ratio mass spectrometer. The results are presented as per mille deviation from a reference sample. All ^{14}C data are $^{14}C/^{12}C$ ratios reported as decay-corrected $\Delta^{14}C$ in per mille deviation from a standard[38] or Fraction Modern[39]. The measurement error was determined for each sample and was typically in the range of ±8–12‰ (2 s.d.) $\Delta^{14}C$. All AMS analyses were performed blind to age and origin of the sample. Triglyceride age in vivo was estimated using measures of $^{14}C/^{12}C$ in lipid set in relation to the corresponding atmospheric levels at the time of sample collection.

Statistics. Values are given as the mean ± s.d. in the text and tables. The box plots indicate the median (central line), 25th and 75th percentiles (box) and minimum–maximum values (whiskers). Paired data were tested for differences in means with paired, two-sided *t*-tests after an *F*-test for comparison of variance. (Only the *t*-tests are reported; variance did not differ significantly in any sample pair.) Correlations were tested using Pearson's linear correlation. Data for separate groups were tested for differences using unpaired, two-sided *t*-test. Before the analysis of adipose samples, a statistical analysis of paired differences was made assuming an s.d. of difference in lipid age of 0.9 years. In cohort 1, we detected a 0.4 year change in lipid age at $P = 0.05$ with almost 90% statistical power using 50 paired samples. In cohort 2, we detected the same difference in 40 paired samples with 80% statistical power.

Transparency and reproducibility information is available in the Nature Research Reporting Summary.

Mathematical modeling methods. *Atmospheric ^{14}C integration in adipose lipids.* We followed a ^{14}C dating method previously developed for adipose tissue samples[2,11,40]. Aboveground nuclear testing-derived atmospheric ^{14}C is continuously integrated into the food in the food chain and consequently in the lipids stored in adipose tissue. To relate changes in ^{14}C content over time with lipid aging and renewal, we used a linear partial differential equation structured in age:

$$\mathrm{d}f(t,a)/\mathrm{d}a + \mathrm{d}f(t,a)/\mathrm{d}t = -K_{\mathrm{out}}(a,t)f(t,a) \quad (1)$$

$$f(t, a = 0) = K_{\mathrm{in}}(t) \quad (2)$$

$$f(t = t0, a) = f0(a) \quad (3)$$

The time t refers to the age of an individual and the age a refers to the age or residence time of lipids within the adipose tissue. The lipid age density $f(t,a)$ is the density of lipids of age a at time t (unit: cells year^{-1}). In the partial differential equation (equation (1)), the terms on the left are conservation terms stating that lipids advance in age at the same speed as time. The lipid removal rate $K_{out}(a,t)$ controls the rate at which lipids are removed from the adipose tissue (unit: year^{-1}) and can depend on a and t. The boundary condition (equation (2)), prescribes that new lipids (lipid with age $a=0$) enter the adipose tissue at the lipid uptake rate $K_{in}(t)$ (unit: kg year^{-1}), which depends only on t. To specify the model completely, an initial lipid age density $f0(a)$ at participant age $t=t0$ must be provided. The initial condition (equation (3)) states that the initial lipid density is negligible. The model defined by equations (1–3) is a one-compartment model where lipids enter adipose tissue at age 0 and enter only once; when they leave the adipose tissue, lipids are not recycled.

The lipid age density f is related to the total body fat mass F in the following way:

$$F(t) = \int f(t,a)\mathrm{d}a \quad (4)$$

If the removal rate K_{out} and the lipid uptake K_{in} are approximately constant, that is, they do not depend on time or lipid age, integrating equation (1) with respect to age, and using equations (2–4) to replace the integral and boundary terms, lead to a linear ordinary differential equation:

$$\mathrm{d}F/\mathrm{d}t = K_{in} - K_{out}\ F \quad (5)$$

$$F(t0) = F0 = \int f0(a)\mathrm{d}a \quad (6)$$

The ordinary differential equation (equation (5)) and the initial condition (equation (6)) describe the time evolution of the fat mass, starting at mass 0 at time 0. The ordinary differential equation can be solved exactly as:

$$F(t) = F0\ \exp(-K_{out}[t - t0]) + K_{in}/K_{out}[1 - \exp(-K_{out}[t - t0])] \quad (7)$$

With time, the exponential terms vanish, and $F(t)$ approaches the fat mass equilibrium:

$$F^* = K_{in}/K_{out} \quad (8)$$

Fat mass equilibrium is reached at an exponential rate with the rate constant K_{out}. When the fat mass is at equilibrium, the total amount of lipids (in kg) replaced each year is $K_{out}\ F^*$ (F^* is given in equation (8)), and the relative amount of lipids replaced each year, K_{out}, is termed the turnover or removal rate. The turnover represents roughly the fraction, which may be greater than 1, of lipids that are replaced each year. At equilibrium, the mean lipid residence time in the adipose tissue is:

$$\langle a \rangle = 1/K_{out} \quad (9)$$

Estimating lipid removal rate and mean age. For each lipid sample s, two independent measurements are available: the total fat mass Fs of the donor and the ^{14}C abundance in the lipid sample Cs. In the following, we assume that the total fat mass F of each donor was at equilibrium F^*. This assumption implies that the removal rate K_{out} and the uptake rate K_{in} are constants, and that K_{out} is a turnover rate. The ^{14}C dating method provides a way to estimate the parameters K_{in} and K_{out} in equations (1) and (2). The equilibrium assumption does not need to hold for estimating K_{in} and K_{out}, but it simplifies the analysis. In the equilibrium assumption, it is possible to estimate K_{out} independently from K_{in}, for each participant. K_{in} can then be estimated directly from the known K_{out} and F^*, with the equality $K_{in} = K_{out}\ F^*$. Out of equilibrium, additional factors such as nonconstant K_{in} and K_{out}, or the state of the adipose tissue in the past, need to be taken into account; it is generally impossible to do that for each participant. A few specific issues arise with the analysis of cohorts 1 and 2.

First, the datasets are longitudinal and the removal rate may change over time. Based on previous results[8,13], mean lipid ages range between 2.5 and 3.5 years. For cohort 1, this is considerably shorter than the mean follow-up time; a change in K_{out} or K_{in} over that timescale would be sufficiently slow for the fat mass to adapt and stay at equilibrium F^*(slowly changing t) $= K_{in}(t)/K_{out}(t)$. For cohort 2, mean lipid ages are in the same range as the follow-up time, so it is not clear if the fat mass would have time to reach a new equilibrium. Moreover, cohort 2 lost a substantial amount of fat mass during year 1, so the equilibrium assumption is clearly not respected.

Second, the lipid removal rate could also depend on the age of the lipids. For instance, lipid could follow a first in, first out rule whereby newly stored lipids are the first to be mobilized, while older lipids more probably stay in the adipose tissue. This would suggest decreasing K_{out} as a function of lipid age. In the more extreme scenario, there could be a pool of long-lived lipids with the removal rate $K_{out} = 0$. The first in, first out hypothesis has been explored before[2]; we found no evidence to support this hypothesis over timescales of years.

We address two remaining issues: the nonequilibrium of cohort 2 and the possibility of having a pool of long-lived lipids. Before that, we present the ^{14}C dating method for the equilibrium assumption with a given fraction of long-lived lipids, and provide a first estimate for K_{out} and K_{in} that will be used to check the consistency of the method.

We assume that at time $t=t0$, the adipose tissue is formed of lipids of age $a=0$. We consider two situations: (1) there is no lipid at birth, $t0=0$ year, $f0(a)=0$ and $K_{in} = K_{out}\ F^*$ for $t > t0$; or (2) the adult adipose tissue is formed at $t0=18$ years, $F0(t0) = F^*$ and $K_{in} = K_{out}\ (1-r)\ F^*$, and only a fraction $(1-r)$ of lipids are subject to removal. The other fraction r is never removed and is aged $t-18$ years, for $t > 18$ years. The fraction r is the size of the long-lived lipid pool.

We illustrate situation 1. The ^{14}C abundance predicted by equations (1–3) for a lipid sample collected at year y is:

$$\hat{C} = \int K(y - a) f(t,a)\mathrm{d}a/F^*$$

where the integral runs from $a=0$ to $a=t$, the age of the participant at sample collection. The function $K(y)$ is the atmospheric ^{14}C at calendar year y. Under the equilibrium assumption, the lipid age density $f(t,a)$ does not depend on K_{in}, so there is only K_{out} left to be estimated.

The removal of K_{out} was calculated based on $\hat{C}$ with MATLAB (MathWorks) using the fzero routine.

For each ^{14}C sample C, we solved the equation for K_{out}, the removal rate for sample C:

$$\hat{C}(K_{out}) - C = 0 \quad (10)$$

Equation (10) usually has one or two solutions because of the unimodal nature of the atmospheric curve. When two solutions were found, the highest removal rate was selected as the correct solution. Low removal solutions were considered as having no physiological meaning, since they can be found only in participants born before the rise of the ^{14}C levels around 1955. When C was below contemporary levels, no removal or only a low removal corresponding to the rising part of the atmospheric curve could be found. In that case, we assumed that the sample was contemporary and assigned an infinite removal rate to it (an age $\langle a \rangle = 0$).

Based on the removal rates found, the average age of lipids with the removal rate K_{out} in a donor aged t was calculated as:

$$a(t) = [-t\ \exp(-K_{out}\ t) + (1 - \exp(-K_{out}\ t))/K_{out}]/[1 - \exp(-K_{out}\ t)] \quad (11)$$

which in practice is very similar to the mean lipid residence time $<a>$ given in equation (9) The only difference is that $a(0)=0$ and increases until it reaches $a(t) = <a>$ for large t. For samples with infinite removal rates, $a(t)=0$ year for all t.

Removal rates and lipid ages were estimated for all samples in cohort 1 ($n=55$, at year 0 and at the end of the follow-up period) and in cohort 2 ($n=41$, at year 0 and at the end of the follow-up period). In cohort 1, one sample had an estimated lipid age of 26.5 years, ten times older than the average lipid age seen in other samples. This was explained by a very high level of ^{14}C that was not seen in other samples. For that reason, this sample was excluded from the study and treated as a missing value. The probability of a mistake in reporting ^{14}C or the risk of contamination far outweighs the probability that the lipid age was really around 26 years.

Long-lived lipid pool. We tested the presence of a long-lived lipid pool. Long-lived lipids were assumed to be formed in participants around the age of 18 and to have persisted since. Atmospheric ^{14}C levels peaked during the years from 1960 to 1970 and formation of long-lived lipid pools during these years should leave measured ^{14}C levels also elevated. We tested 10, 25 and 50% long-lived pool sizes.

Lipid age dynamics and energy balance. *Out-of-equilibrium lipid age dynamics.* To see how lipid age is affected by changes in fat mass, K_{in} or K_{out}, we can write an equation for the mean lipid age, obtained by calculating the expectation of the lipid age density:

$$A = E[a] = \int a\, f(a,t)\mathrm{d}a/F(t) \quad (12)$$

This results in an ordinary differential equation for A:

$$\mathrm{d}A/\mathrm{d}t = 1 - K_{out}\ A - (\mathrm{d}F/\mathrm{d}t)/F\ A = 1 - K_{in}/F\ A \quad (13)$$

$$A(0) = 0$$

where $\mathrm{d}F/\mathrm{d}t$ is the time derivative of F as given by equation (5). Equation (13) is valid even under time changes in K_{in} or K_{out} (but not if K_{out} depends on a). During weight loss, $\mathrm{d}F/\mathrm{d}t$ is negative, either because K_{in} decreases or because K_{out} increases. If K_{in} decreases, new lipid comes at a slower rate and A will go up. This increase will be transient, since K_{in} has no effect on lipid age at equilibrium. If K_{out} increases, F will decrease and A will go down permanently. A combination of a decrease in K_{in}

and an increase in K_{out} would leave A unaffected and would then drop once the new equilibrium is reached.

What does it mean in terms of removal estimates? We first take the situation where K_{in} decreases, but the true K_{out} stays constant. Then we expect the lipid age to go up transiently; this should be reflected in the ^{14}C signature and be interpreted as a decrease in removal rate. We do not see any change in the estimated removal rates between year 0 and year 5, which means that the transient increase in age is not strong enough to affect the estimates. If we now take the situation where K_{out} increases, lipid age would go down and this should again be reflected in the ^{14}C signature and be correctly interpreted as an increase in lipid removal. Again, we do not see a significant reduction in the estimated removal rates between years 0 and 5. The final scenario is the situation where K_{in} decreases, but K_{out} increases at the same time. We would then expect the lipid age to remain relatively stable for a few years and then decrease. The estimated removal rates should then be similar between years 0 and 5, although the true K_{out} has increased. This situation is in agreement with the ^{14}C data, which shows no change between years 0 and 5. However, it is not possible to validate this scenario because we do not have an independent estimate for K_{in}.

We ran consistency check simulations for the system of equations (5) and (13), where K_{in} and K_{out} are estimated by the ^{14}C dating method described earlier, for the 41 individuals in cohort 2. For each individual, we assumed that K_{in} and K_{out} changed immediately at year 0 and remained constant after that. K_{in} could not be estimated for all samples because of missing data on fat mass or because the estimated removal rate was infinite. Lipid dynamics were consistent if the lipid age calculated with equation (13) was similar to the estimated K_{in} and K_{out}. Of the 32 individuals for which K_{in} and K_{out} were valid, 23 had consistent K_{in}/K_{out} dynamics, and 9 had lipid age overshoots after year 0 (see graphical representation later on). For these nine individuals, it could be argued that the estimated removal is too low. For instance, individual 4662 had estimated removal rates at years 0 and 5 that were almost identical (0.26 and 0.25 per year). Since we may expect an increase in lipid age due to the drop in K_{in}, the second removal might be too small.

We conclude that for most individuals, the equilibrium assumption works well. Underestimation of the true removal rate at year 5 might occur for certain samples, although it is difficult to assess by how much. Given that we could see no increase in removal rates in general, we took the conservative approach of reporting the equilibrium removal rates.

Link between lipid removal and energy balance. The success of clinical interventions for weight management ultimately relies on modifying the energy balance. Energy balance can be expressed in terms of energy input and energy expenditure or in terms of energy stored (ES) and energy metabolized (EM).

$$\Delta E = \text{energy intake} - \text{energy expenditure}$$

$$\Delta S = \text{ES} - \text{EM}$$

From the law of conservation of energy, the two expressions are equivalent:

$$\Delta E = \Delta S$$

ΔS is the net amount of ES as lipids in the body per unit time. Most of the extra energy is stored as lipids in the WAT (fat mass), but some is stored in the liver and the muscles as glycogen and proteins (lean mass):

$$\text{ES} = \text{ES}_{\text{fat}} + \text{ES}_{\text{lean}}$$

Likewise, energy can be metabolized from fat and lean mass:

$$\text{EM} = \text{EM}_{\text{fat}} + \text{EM}_{\text{lean}}$$

At energy balance $\Delta E = 0$, the net energy storage is also null, but ES and EM are not: part of the energy intake is stored while some of the stored energy is used. The lipid removal rate measured from ^{14}C data relates directly to EM_{fat}: we have measured the fraction of the total fat mass exchanged daily under the assumption that $\text{ES}_{\text{fat}} = \text{EM}_{\text{fat}}$. Thus, the lipid removal rate K_{out} is:

$$K_{out} = F_{mob}/F_{mass}$$

where F_{mob} is the mass (in kg) and F_{mass} is the total fat mass (in kg). The energy density of fat is $r_{fat} = 39.5\,\text{MJ kg}^{-1}$, or $9.4\,\text{kcal g}^{-1}$ (the energy produced per unit mass of oxidized lipids from adipose tissue)[29]. The amount of lipids metabolized per unit time can be expressed in terms of EM_{fat}:

$$F_{mob} = \text{EM}_{\text{fat}}/\text{r}_{\text{fat}}$$

Lipid removal is then:

$$K_{out} = \text{EM}_{\text{fat}}/r_{\text{fat}}/F_{mass}$$

or

$$\text{EM}_{\text{fat}} = K_{out} \times F_{mass} \times r_{\text{fat}}$$

The daily amount of energy coming from lipid storage is the lipid removal rate × fat mass × energy per unit mass of lipids. Likewise, lipid uptake K_{in} is determined by the amount of ES:

$$K_{in} = \text{ES}_{\text{fat}}/r_{\text{fat}}$$

A full picture of energy balance would be provided by EM_{lean}. Around 88% of the weight loss in cohort 2 was from fat mass, so even though lean energy storage is important for daily energy balance, the main component driving weight loss is $\text{ES}_{\text{fat}} - \text{EM}_{\text{fat}}$.

Weight loss is determined by energy balance (ΔE)[29], but loss of fat mass is determined by the balance between energy storage and removal (ΔS). In terms of body weight change, only the change in energy storage ΔS is relevant, not ES and EM themselves. ES and EM probably depend on ΔE, but they have an independent component: even at energy balance $\Delta E = \Delta S = 0$, lipids are constantly stored and metabolized, and no long-term lipid pools seem to exist. Thus, ES and EM are positive even at energy balance.

Two independent lines of evidence show that adipose tissue lipids contribute to daily energy needs ($\text{EM} > 0$); therefore, they are subject to continuous removal. ^{13}C tracing of dietary lipids shows that around 50% of them are stored instead of being burned for daily expenditure[41]. These numbers are in line with present and previous ^{14}C dating studies of adipose lipids, which showed that stored lipids have an average age of 1.3 years in lean individuals and >2.0 years in obese individuals[2,11]. Indeed, dietary guidelines recommend around 20–35% of daily energy intake to be in lipids[42]. At 25% energy from fat, assuming $9\,\text{kcal g}^{-1}$ food fat, $0.25 \times 2{,}000\,\text{kcal d}^{-1} = 500\,\text{kcal d}^{-1}$, or 56 g food lipids d^{-1}. Assuming that adipose tissue lipids with a 1.3 years average age have a daily removal rate of 1/1.3 year/365 d/year = $0.0021\,\text{d}^{-1}$, for a 60 kg woman at 20% fat mass (12 kg fat mass), this means that $12\,\text{kg} \times 0.0021\,\text{d}^{-1} = 25\,\text{g}$ will be replaced daily in adipose tissue. This corresponds to 25 g/56 g = 45% of the daily lipid intake. Because energy is stored at a higher density in adipose tissue ($9.4\,\text{kcal g}^{-1}$ versus roughly 9.0 in conventional diets), the energy release by stored lipids may account for a slighter larger fraction than 45%. Therefore, lipid removal is not only determined by energy balance.

From a physical point of view, energy deficit ($\Delta E < 0$) has to be compensated for by an increase in lipid removal (K_{out} up), by a decrease in lipid storage rate (K_{in} down) or by depletion of other energy storage (such as glycogen in muscles and liver). The rates of fat removal are potentially limited by several factors, such as mobilization from adipose tissue and transport[43]. According to the cohort 2 clinical data, individuals lost on average $100\,\text{g d}^{-1}$ during the first year following gastric bypass, of which 83 g was fat (cohort 2: average total body weight change between year 0 (118.5 kg) and year 1 (81.8 kg); total body weight loss of 36.7 kg; average total body fat mass (DEXA) change between year 0 (61.5) and year 2 (30.9); total fat mass loss of 30.6 kg; ratio of fat mass loss to total body mass loss (0.83)). Total fat mass was not available at year 1, but there was little change in total body mass between year 1 and year 2. Given a baseline average fat mass of 61 kg (cohort 2: average total body fat (DEXA), year 0) and average lipid removal rate of 0.31 per year (cohort 2: calculated as 1/average lipid age, with average lipid age = 3.1 years), we would predict that only 52 g of fat is oxidized every day. This suggests that in fact lipid removal is increased during weight loss (to match the $83\,\text{g d}^{-1}$), but also that lipid removal might be a factor limiting the rate of weight loss. During the maintenance phase (years 1–5), fat mass is more or less maintained by the balance in lipid storage and removal rates. The lipid removal rate is affected by several physiological and lifestyle factors, but not by energy balance because fat mass is relatively stable.

We found that weight rebounders initially had higher lipid removal rates (Fig. 3f), which tended to decrease at year 5 (Fig. 3g). In contrast, weight-stable individuals initially had lower removal rates (Fig. 3f), which tended to increase at year 5 (Fig. 3g). Weight loss is generally accompanied by a decrease in daily energy expenditure, so that maintenance of weight loss can only be achieved by keeping a low energy intake to match energy expenditure at the new weight.

Reduced lipid removal rates in weight rebounders suggests that energy expenditure decreased more than expected, explaining weight rebound. In the weight-stable, the increase in lipid removal rates suggests that weight maintenance is achieved by a relatively higher energy expenditure. Although lipid uptake K_{in} was reduced in all individuals, K_{in} was not correlated with weight maintenance. Together, these results indicate that the success of long-term weight loss after bariatric surgery is predicted by lipid removal rate status and that individuals with a lower baseline removal rate may have more 'room' to attain energy balance.

Reporting Summary. Further information on research design is available in the Nature Research Reporting Summary linked to this article.

Data availability

The datasets generated and/or analyzed during the current study are available from the corresponding author upon reasonable request.

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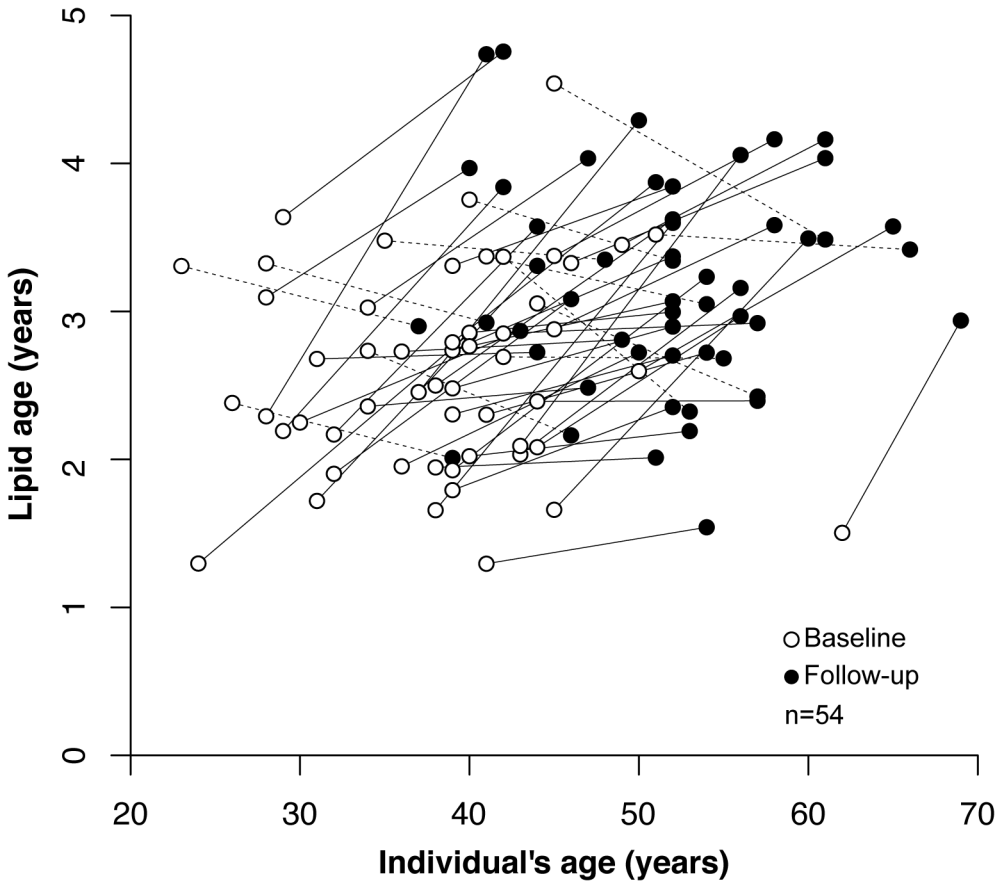
Table 1. Clinical data on examined cohorts

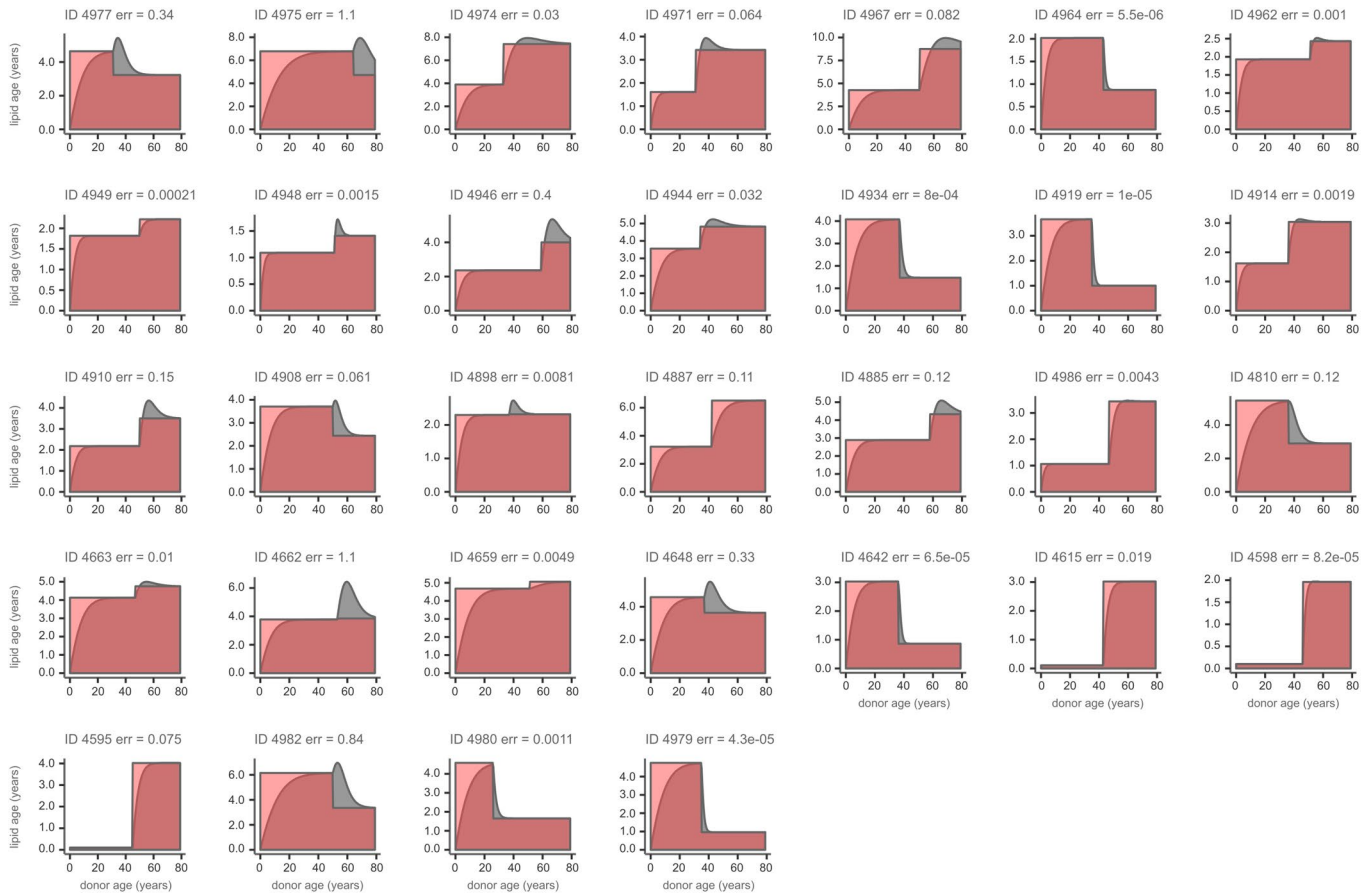
	Cohort 1												*Cohort 2*		
	All subjects			*Weight stable*			*Weight gain*			*Weight loss*					
Phenotype	***First exam***	***Second exam***	***P-value***	***First exam***	***Second Exam***	***p-value***	***First exam***	***Second exam***	***p-value***	***First exam***	***Second exam***	***p-value***	***First exam***	***Second exam***	***p-value***
Age, years	38 ± 7 (23-62)	51 ± 7 (37-69)	-	38 ±6 (24-50)	52 ± 6 (40-65)	-	39 ± 8 (28-62)	52 ± 7 (41-69)	-	38 ±7 (23-51)	51 ±7 (37-66)	-	43 ±9 (26-64)	48 ±9 (31-69)	-
Body mass index, kg/m^2	34 ± 6 (22-50)	32 ± 7 (21-59)	0.10	32 ± 7 (22-50)	32 ±6 (21-46)	0.75	32 ± 5 (23-42)	35 ± 8 (27-58)	<0.001	37 ± 6 (31-49)	31 ±5 (23-37)	<0.0001	43 ±5 (35-55)	32 ±5 (22-45)	<0.0001
Total body fat, kg	50 ± 18 (12-106)	43 ± 16 (15-90)	0.03	42 ±16 (16-76)	40 ±13 (15-60)	0.22	42 ± 13 (12-76)	50 ± 17 (22-90)	<0.000 1	57 ± 20 (38-106)	37 ± 12 (18-62)	<0.0001	73 ±15 (44-115)	43 ±19 (17-103)	<0.0001
Physical activity, score	1.7 ±0.7 (1-3)	2.0 ±0.7 (1-3)	0.007	1.8 ± 0.7 (1-3)	2.2 ± 0.6 (1-3)	0.03	1.7 ±0.7 (1-3)	1.9 ± 0.6 (1-3)	0.17	1.8 ±0.7 (1-3)	2.1 ± 0.7 (1-3)	0.02	1.6 ±0.6 (1-3)	2.0 ±0.5 (1-3)	0.001

Values are mean ± SD and (range). Conditions are compared using a paired t-test. Gender distribution between weight groups had a p-value of 0.74 in Cohort 1 by Fisher´s exact test. Cohort 2 was composed of women only. Body fat was measured with bio-impedance. See online methods for a definition of weight groups in cohort 1.

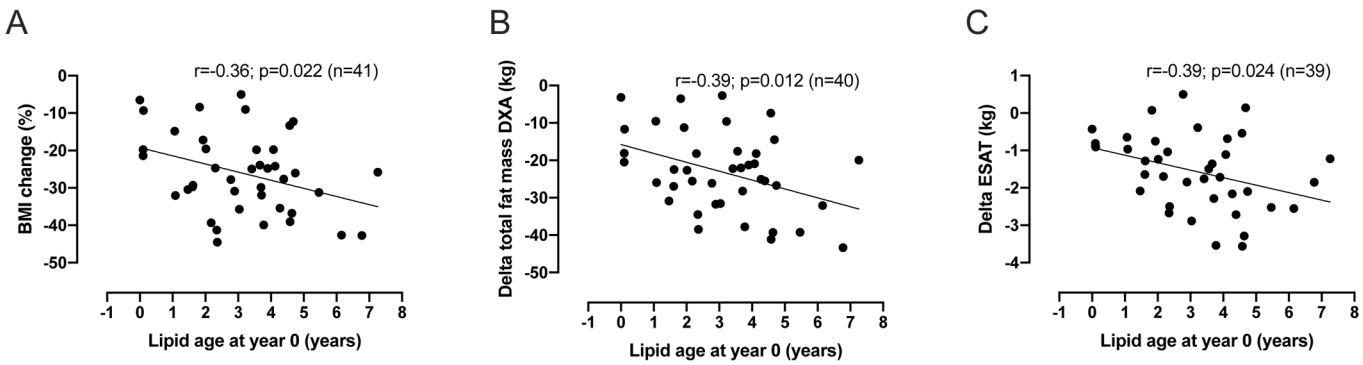
Extended Data Fig. 1 | Clinical data on the examined cohorts. Values are the mean ± s.d. and (range). Conditions are compared using a paired *t*-test. Sex distribution between weight groups had a *P* value of 0.74 in cohort 1 by Fisher's exact test. Cohort 2 was composed of women only. Body fat was measured with bioimpedance. See Methods for a definition of weight groups in cohort 1.



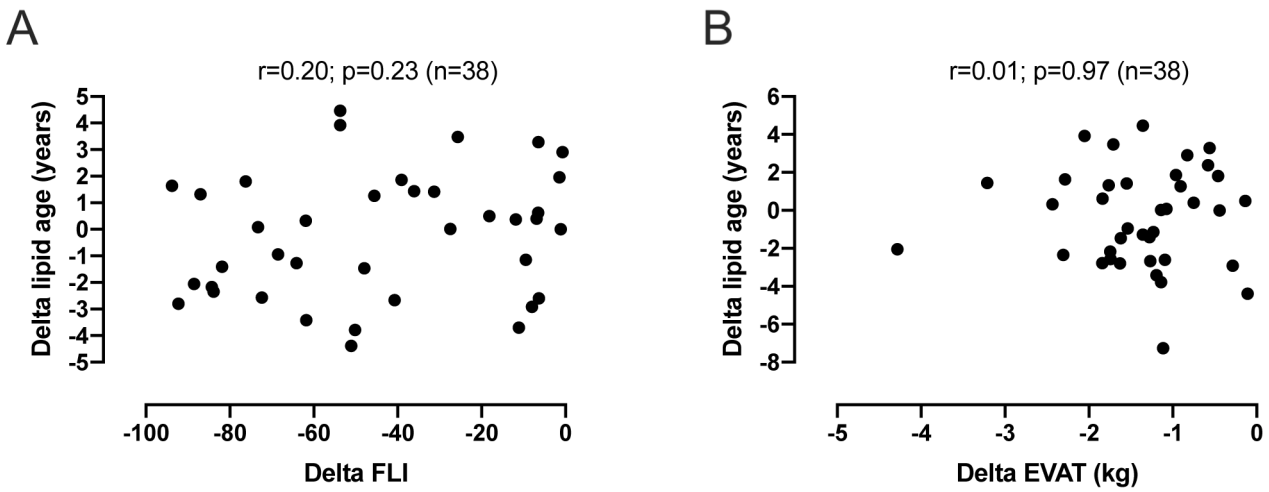
Extended Data Fig. 2 | Relationship between changes in lipid and participant age over time. Cohort 1 was investigated twice with approximately a 13-year interval. The open circles are the first (baseline) and the closed circles the second (follow-up) examination. A large interindividual variation was observed. Despite this, lipid age increased in 42 out of 54 participants examined ($P < 0.0001$ by two-sample paired sign test).



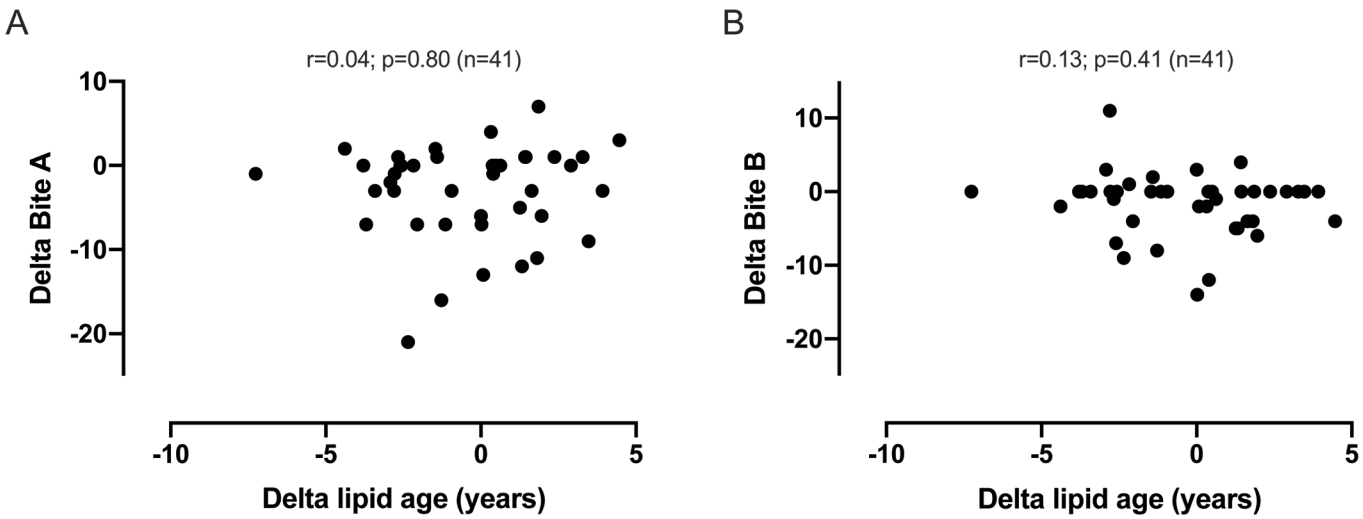
Extended Data Fig. 3 | Simulation of lipid dynamics. Simulation of lipid dynamics (see and equation (13)) with estimated K_{in} and K_{out} for 33 individuals in cohort 2 for whom all data were available. Simulations (gray) closely follow the estimated lipid age (red) if estimates are consistent with the equilibrium assumption. For nine individuals, the simulation deviated significantly, indicating that the equilibrium assumption might not hold, leading to underestimating the true removal rate (and overestimating the true *K*in).



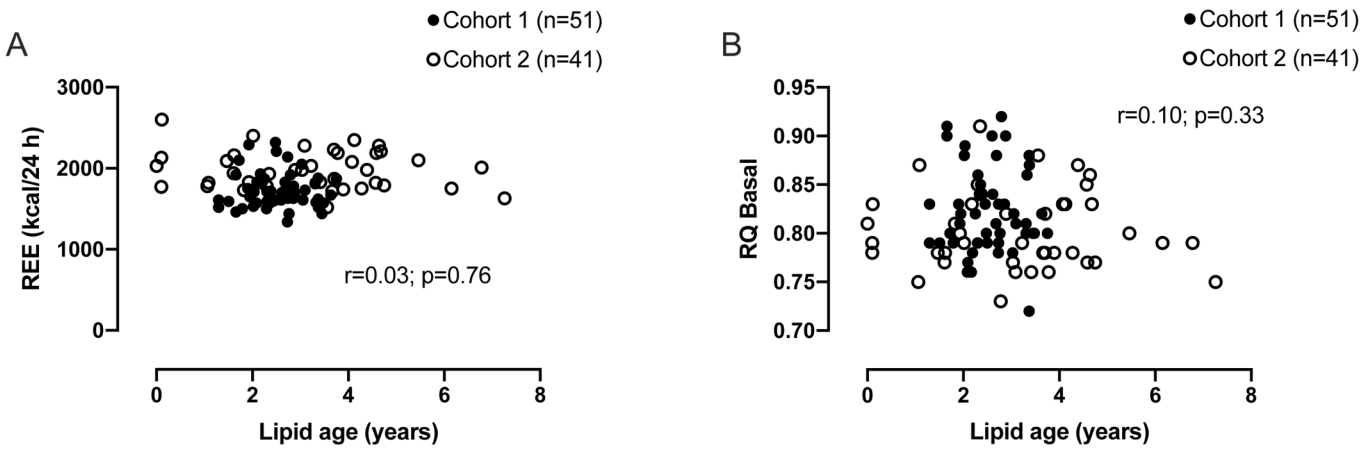
Extended Data Fig. 4 | Relationship between lipid age at first examination and changes in body composition over time (cohort 2). Relationship between lipid age at first examination and changes (second minus first examination) in body composition over time (cohort 2). **a**, Percentage change in BMI. **b**, Changes (Δ) in total fat mass determined by DEXA. **c**, Changes (Δ) in abdominal subcutaneous fat mass corresponding to the site of adipose biopsy (ESAT). Cohort 2 was examined by linear regression. The number of individuals (*n*) are indicated. See Methods for further details.



Extended Data Fig. 5 | Relationship between changes in lipid age and FLI or EVAT mass in cohort 2. a,**b**, Relationship between changes (Δ, second minus first examination) in lipid age and FLI (**a**) or EVAT (**b**) in cohort 2. Cohort 2 was examined using linear regression. The number of individuals (*n*) are indicated. See Methods for further details.



Extended Data Fig. 6 | Relationship between changes in eating behavior and lipid age in cohort 2. Relationship between changes (Δ, second minus first examination) in eating behavior and lipid age in cohort 2. **a**,**b**, The questionnaire on eating behavior (BITE) was used and is detailed in the Methods. BITE-A, magnitude of symptoms; BITE-B, severity of symptoms. Data were examined using linear regression; *r* and *P* values are shown. The number of individuals (*n*) are indicated.



Extended Data Fig. 7 | Relationship between lipid age and measures of indirect calorimetry at first examination. a, Data for resting energy expenditure. **b**, Data for respiratory quotient. Values for both cohorts combined were subjected to linear regression analysis. $n = 51$ and 41 for cohorts 1 and 2, respectively. r and P values are shown. When the cohorts were analyzed separately, the correlation parameters were $r = 0.02$–0.12 (**a**) and $P = 0.45$–0.89 (**b**). Data were examined by linear regression; r and P values are shown. The number of individuals (n) are indicated.

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n/a	Confirmed	
☐	☒	The exact sample size (*n*) for each experimental group/condition, given as a discrete number and unit of measurement
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☐	☒	For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
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Our web collection on statistics for biologists contains articles on many of the points above.

Software and code

Policy information about availability of computer code

Data collection	No commercial, custom use or open source code/software was used in this manuscript
Data analysis	14C modelling done with Matlab R2017a. Statistical analysis

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors/reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research guidelines for submitting code & software for further information.

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All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:

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The datasets generated during and/or analysed during the current study are available from the corresponding author on reasonable request.

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For a reference copy of the document with all sections, see nature.com/documents/nr-reporting-summary-flat.pdf

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size	Prior to the analysis of adipose samples, a statistical analysis of paired differences was made assuming a SD of difference in lipid age of 0.9 years. In Cohort 1 we could detect a 0.4 years change in lipid age at p=0.05 with almost 90% statistical power using 50 paired samples. In Cohort 2 we could detect the same difference in 40 paired samples with 80% statistical power.
Data exclusions	One subject was excluded due to default analysis. This is described on page 8 of the Online Methods "In Cohort 1, one sample had an estimated lipid age of 26.5 years, ten times older than the average lipid age seen in other samples. This was explained by a very high level of 14C that was not seen in other samples. For that reason, this sample was excluded from the study and treated as a missing value. The probability of a mistake in reporting the 14C or that there was contamination far outweighs the probability that the lipid age was really around 26 years."
Replication	Experimental replication was not performed since each subject did not undergo multiple biopsies at each time of investigation.
Randomization	Randomization was not applicable to this study since subject grouping was based on non-randomixable variables, such as BMI, sex, weight gain/loss etc.
Blinding	All samples are processed for AMS blind, with operators having no prior knowledge of which group each sample belongs to.

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

n/a	Involved in the study
☒	☐ Antibodies
☒	☐ Eukaryotic cell lines
☒	☐ Palaeontology
☒	☐ Animals and other organisms
☐	☒ Human research participants
☐	☒ Clinical data

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☒	☐ Flow cytometry
☒	☐ MRI-based neuroimaging

Human research participants

Policy information about studies involving human research participants

Population characteristics	Clinical characteristics of Cohort 1 and 2 are detailed in Table 1.
Recruitment	Subjects in cohort 1 were recruited from a diet intervention study (NUGENOB) or through local advertisement. Subjects in cohort 2 were recruited at Ersta and Danderyd hospital among individuals undergoing bariatric surgery due to obesity. Recruitments in both cohorts were perfromed as detailed in the granted ethical permits.
Ethics oversight	Regional ethics board of Stockholm

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Clinical data

Policy information about clinical studies

All manuscripts should comply with the ICMJE guidelines for publication of clinical research and a completed CONSORT checklist must be included with all submissions.

Clinical trial registration	Cohort 1:NCT02227043, Cohort 2:NCT01785134
Study protocol	Explained in detail in Supplement 1 and at clinicaltrials.gov
Data collection	Baseline assessments in both cohorts were performed over a 2 year period.
Outcomes	The main outcome herein was changes in body weight determined by BMI.