LETTER

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Dynamics of human adipose lipid turnover in health and metabolic disease

Peter Arner[1], Samuel Bernard[2], Mehran Salehpour[3], Göran Possnert[3], Jakob Liebl[4], Peter Steier[4], Bruce A. Buchholz[5], Mats Eriksson[1], Erik Arner[1], Hans Hauner[6], Thomas Skurk[6], Mikael Rydén[1], Keith N. Frayn[7] & Kirsty L. Spalding[8]

Adipose tissue mass is determined by the storage and removal of triglycerides in adipocytes[1]. Little is known, however, about adipose lipid turnover in humans in health and pathology. To study this *in vivo*, here we determined lipid age by measuring ^{14}C derived from above ground nuclear bomb tests in adipocyte lipids. We report that during the average ten-year lifespan of human adipocytes, triglycerides are renewed six times. Lipid age is independent of adipocyte size, is very stable across a wide range of adult ages and does not differ between genders. Adipocyte lipid turnover, however, is strongly related to conditions with disturbed lipid metabolism. In obesity, triglyceride removal rate (lipolysis followed by oxidation) is decreased and the amount of triglycerides stored each year is increased. In contrast, both lipid removal and storage rates are decreased in non-obese patients diagnosed with the most common hereditary form of dyslipidaemia, familial combined hyperlipidaemia. Lipid removal rate is positively correlated with the capacity of adipocytes to break down triglycerides, as assessed through lipolysis, and is inversely related to insulin resistance. Our data support a mechanism in which adipocyte lipid storage and removal have different roles in health and pathology. High storage but low triglyceride removal promotes fat tissue accumulation and obesity. Reduction of both triglyceride storage and removal decreases lipid shunting through adipose tissue and thus promotes dyslipidaemia. We identify adipocyte lipid turnover as a novel target for prevention and treatment of metabolic disease.

A major function of adipose tissue is to store and release fatty acids, which are incorporated into adipocyte triglycerides according to whole-body energy demands. Body fat mass is determined by the balance between triglyceride storage and removal in adipocytes, by either enzymatic hydrolysis (lipolysis) and subsequent fatty acid oxidation and/or ectopic deposition in non-adipose tissues. Little is known about the dynamics of these processes in humans. Although isotope tracer methods have been used to estimate lipid turnover in human adipose tissue, these studies have been limited to short-term experimental conditions[1–4]. To study long-term adipose tissue lipid turnover *in vivo* and across the adult lifespan, we developed a method to retrospectively determine the age of adipocyte triglycerides in humans. Triglycerides are the major component of the adipocyte lipid droplet. Lipid age was assessed by measuring the ^{14}C content in the lipid compartment of adipocytes from human subcutaneous adipose tissue, the major fat depot in humans. ^{14}C levels in the atmosphere remained remarkably stable until above ground nuclear bomb tests between approximately 1955 and 1963 caused a significant increase in ^{14}C relative to stable carbon isotope levels[5] (Fig. 1a). After the Limited Nuclear Test Ban Treaty was signed in 1963, ^{14}C levels in the atmosphere decreased exponentially. This is not due to radioactive decay (half-life ($T_{1/2}$) for ^{14}C is 5,730 years), but to diffusion of $^{14}CO_2$ out of the atmosphere[6]. ^{14}C in the atmosphere oxidises to form CO_2, which is taken up in the biotope by photosynthesis. Because we eat plants, or animals that live off plants, the ^{14}C content in the atmosphere is directly mirrored in the human body.

Radiocarbon dating has been used to study the incorporation of atmospheric ^{14}C into DNA to determine the age of different human cell types, including adipocytes[7–11]. Here, we compared the incorporation of ^{14}C into adipocyte triglycerides with the dynamic changes in atmospheric ^{14}C described earlier. Triglyceride age was determined by using a linear lipid replacement model in which the age distribution of lipids within an individual was exponentially distributed corresponding to a constant turnover rate (per year)[12]. The associated mean age, termed lipid age, is the inverse of the turnover rate and reflects the irreversible removal of lipids from adipose stores (Supplementary Information 1 and Fig. 1 of Supplementary Information 1).

Earlier studies indicate that triglycerides in adipose tissue form two distinct pools with high or low turnover rates, respectively[13,14]. Our data, obtained from individuals born before, during and after bomb testing, do not support the hypothesis of dual large lipid pools with different half-lives (Fig. 1b). ^{14}C data were modelled according to one or more pools of lipids with different lipid removal rates (Supplementary Information 1). The existence of a very small pool of younger lipids cannot be excluded based on data modelling (Supplementary Information 1 and Fig. 2 of Supplementary Information 1). According to a two-pool model the influence on the turnover rate is proportional to the fraction of lipid in the small pool. Triglyceride exchange between adipocytes and other small storage pools can affect turnover estimates. The two-pools model shows, however, that the non-adipose pool can be neglected when it makes up less than 20% of the lipids (Supplementary Information 1, Fig. 3). Small pools with high turnover are more important for short-term (days or weeks) than long-term (years) triglyceride turnover.

Mean lipid age was 1.6 years (Fig. 1c), which is in the same range as in short-term turnover studies[4]. The distribution of lipid age was compared with that of adipocyte age reported previously in a comparable cohort[9]. The mean age of adipocytes was 9.5 years (Fig. 1d). This implies that triglycerides, on average, are replaced six times during the lifespan of the adipocyte, enabling a dynamic regulation of lipid storage and mobilization over time.

There is a large variation in adipocyte size within and between individuals (Supplementary Information 2, Supplementary Table 1)[15]. However, it is unlikely that the rate of triglyceride removal from adipocytes is important for these variations, as lipid age was not related to adipocyte size when set in relation to the body fat mass (Fig. 2a, b), nor was there a difference in lipid age between large and small adipocytes of the same adipose tissue sample (Fig. 2c, d). These data indicate that there is a continuous exchange of lipids between adipocytes within the adipose tissue that is not dependent on adipocyte size. Fatty acids produced by lipolysis in one adipocyte could, for example, be taken up

[1]Department of Medicine, Karolinska University Hospital, SE-141 86 Stockholm, Sweden. [2]Institut Camille Jordan, CNRS UMR 5208, University of Lyon, F-69622 Villeurbanne, France. [3]Department of Physics and Astronomy, Ion Physics, Uppsala University, SE-751 20, Sweden. [4]Faculty of Physics – Isotope Research, University of Vienna, Vienna, A-1090, Austria. [5]Center for Accelerator Mass Spectrometry, Lawrence Livermore National Laboratory, 7000 East Ave., L-397, Livermore, California 94551 USA. [6]Else Kröner-Fresenius Centre for Nutritional Medicine, Technische Universität Münich, D-85350 Weihenstephan, Germany. [7]Oxford Centre for Diabetes, Endocrinology & Metabolism, Churchill Hospital, Oxford OX3 7LJ, UK. [8]Department of Cell and Molecular Biology, Karolinska Institute, SE-171 77, Stockholm, Sweden.

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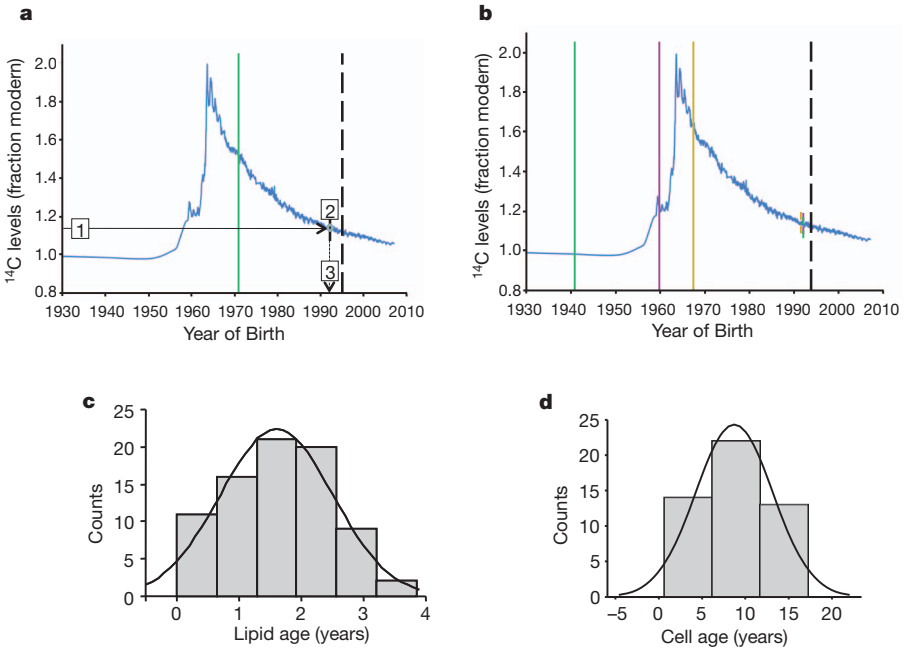

Figure 1 | Atmospheric ^{14}C over time and its use to determine lipid age and adipocyte age. **a**, Above ground nuclear bomb testing during the period of the cold war caused an increase in atmospheric levels of ^{14}C. These values decreased exponentially following implementation of a limited world-wide test ban treaty in 1963 (blue curve). Lipid age is determined by measuring ^{14}C levels in lipids (1) and plotting this value against the bomb curve (2) to determine the difference between the year corresponding to the atmospheric ^{14}C concentration (3) and the biopsy collection date (dashed line). Atmospheric ^{14}C levels are presented as ^{14}C/^{12}C ratios in units of fraction modern (for a definition of 'modern' see Supplementary Information 2). **b**, Lipid age and turnover do not change as a function of person age. Lipid age is shown for three individuals born in 1940.2, 1959.9 and 1967.9. Lipid age was shown to be the same for all individuals, despite markedly different subject ages. Fat biopsies were collected from all individuals on the same date (dashed vertical line). The solid vertical lines indicate the date of birth. The small dashed lines show the ^{14}C lipid value for each individual. **c**, Distribution of values for lipid age in healthy non-obese or obese individuals from cohort 1 ($n = 78$). **d**, The distribution of values for human adipocyte age ($n = 27$). Adipocyte age data are obtained from a previous publication (see main text).

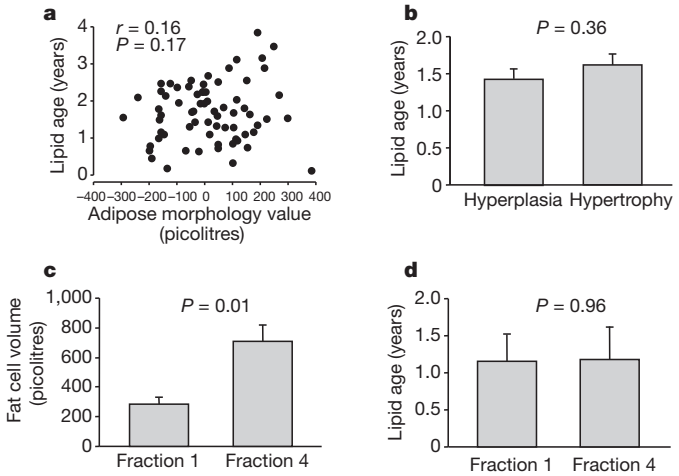

Figure 2 | Relationship between adipocyte size and lipid age. **a**, **b**, Influence of adipocyte cellularity on lipid age. Individuals were assigned a morphometric value, which is the difference between the measured adipocyte volume for the individual minus the average adipocyte volume for all subjects (see Supplementary Information 2). This analysis was carried out across the full range of body masses. Positive values indicate larger adipocytes than expected (fewer but larger adipocytes = hypertrophy). Negative values indicate smaller adipocytes than expected (many but smaller adipocytes = hyperplasia). **a**, Individual values compared by linear regression analysis ($n = 74$). **b**, Data (mean ± standard error) with morphology as a dichotomous variable ($n = 36$ for hyperplasia and $n = 38$ for hypertrophy). An unpaired t-test was used. **c**, **d**, Isolated subcutaneous adipocytes were fractioned into small (fraction 1) or very large (fraction 4) samples ($n = 7$). Adipocyte volume (**c**) and adipose lipid age (**d**) were compared. Values are mean ± standard error. A paired t-test was used. Data in **a** and **b** are from non-obese plus obese individuals in cohort 1 and data in **c** and **d** are from cohort 2.

by adjacent adipocytes and incorporated into their triglycerides. These processes would not be part of lipid removal as measured here.

Lipid age and total fat mass data were used to determine the net triglyceride storage in adipose tissue (kg year^{-1}) (see Supplementary Information 1). The net amount of lipid stored in adipose tissue each year is the sum of exogenous fat incorporation and endogenous synthesis, minus lipid removal. The removal rate represents the hydrolysis of triglycerides (lipolysis) followed by the irreversible removal of lipids by oxidation. A high lipid age therefore mirrors low removal rates. No relationship between lipid storage or removal and person age or gender was seen (Supplementary Information 2 and Fig. 1a–d of Supplementary Information 2).

Two clinical conditions where altered lipid metabolism is observed were investigated—obesity and familial combined hyperlipidaemia (FCHL); the latter is the most common hereditary lipid disorder (reviewed in ref. 16). It has an unknown aetiology and is a common hereditary cause of premature coronary heart disease. Adipocyte lipolysis is impaired in both conditions due to decreased cyclic AMP-dependent signalling, the major lipolytic pathway in adipocytes[17–19]. Both conditions show a similar metabolic phenotype (mixed dyslipidaemia, elevated apolipoprotein B and insulin resistance)[20]. These clinical characteristics are confirmed in our study cohort (Supplementary Information 2, Supplementary Table 1). FCHL individuals may present with a range of body fat levels; however, for our analyses only non-obese FCHL patients were selected so as to remove the confounding factor of obesity from the study.

In obese subjects, the rate of triglyceride storage (Fig. 3a) and mean lipid age (Fig. 3b) were markedly increased compared to non-obese individuals. Both lipid age ($r = 0.38$, $P = 0.0005$) and triglyceride storage ($r = 0.60$, $P < 0.0001$) correlated with body mass index (BMI) when non-obese and obese individual were pooled together. Similarly, in non-obese FCHL individuals lipid age was increased to values

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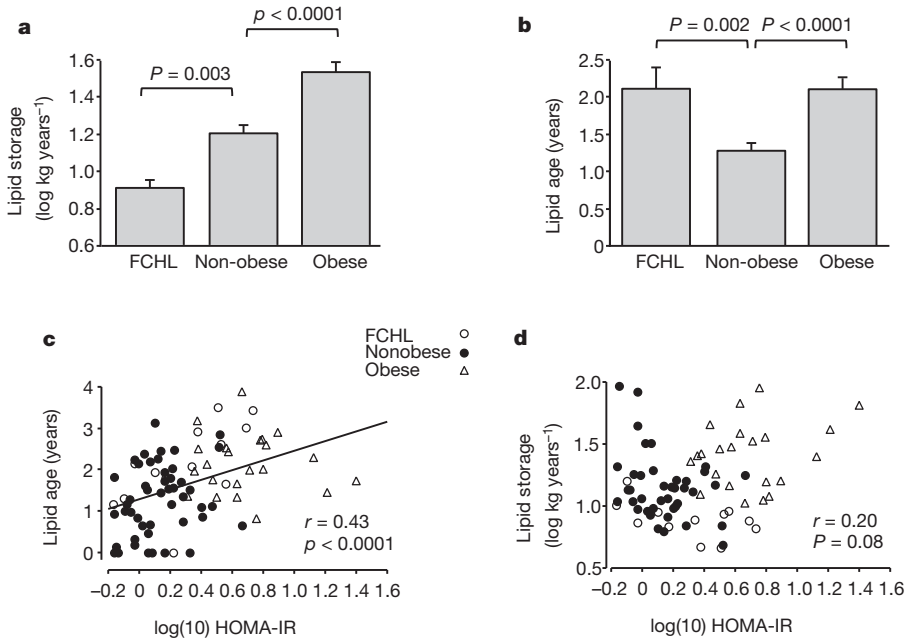

Figure 3 | Lipid turnover in subcutaneous fat. **a**, **b**, Lipid storage (**a**) and lipid age (**b**) were determined in 48 non-obese, 30 obese and 13 non-obese FCHL subjects. Error bars indicate standard error. Overall effect is $P < 0.0001$ by analysis of variance (ANOVA) in **a** and **b**. Results in graphs are from post-hoc test. Data are from cohort 1 (see Supplementary Information 2). A linear regression analysis was performed on all individuals from cohort 1 having insulin resistance measures ($n = 82$). **c**, **d**, HOMA-IR was correlated with lipid age (**c**) and lipid storage (**d**). The relationship between lipid age and HOMA-IR remained significant when BMI, gender or group (non-obese, obese, FCHL) were included in the analysis (partial $r = 0.41$, $P = 0.006$ with BMI using multiple regression analysis and $F = 16.6$, $P = 0.0001$ and $F = 4.8$, $P = 0.03$ for gender or group, respectively, using analysis of covariance (ANCOVA)).

observed in obesity (Fig. 3b). In contrast to obesity, however, the rate of triglyceride storage was markedly decreased compared to non-obese individuals (Fig. 3a). Thus, adipocyte triglyceride turnover is not just a mere reflection of the fat mass. Our data indicate a model where a combination of high storage and low lipid removal rates, as in obesity, facilitates triglyceride accumulation within adipose tissue, thereby promoting the development and/or maintenance of excess body fat mass. Conversely, a low rate of both triglyceride storage and removal, as in FCHL, leads to reduced triglyceride turnover and thereby a decreased ability of adipocytes to store and release fatty acids, despite a normal body fat mass. As discussed in detail elsewhere[21,22], low lipid turnover in adipose tissue may result in fatty acids being shunted to the liver, which drives the synthesis of apolipoprotein B and increases the circulating levels of triglycerides. Adipocyte triglyceride turnover may also be

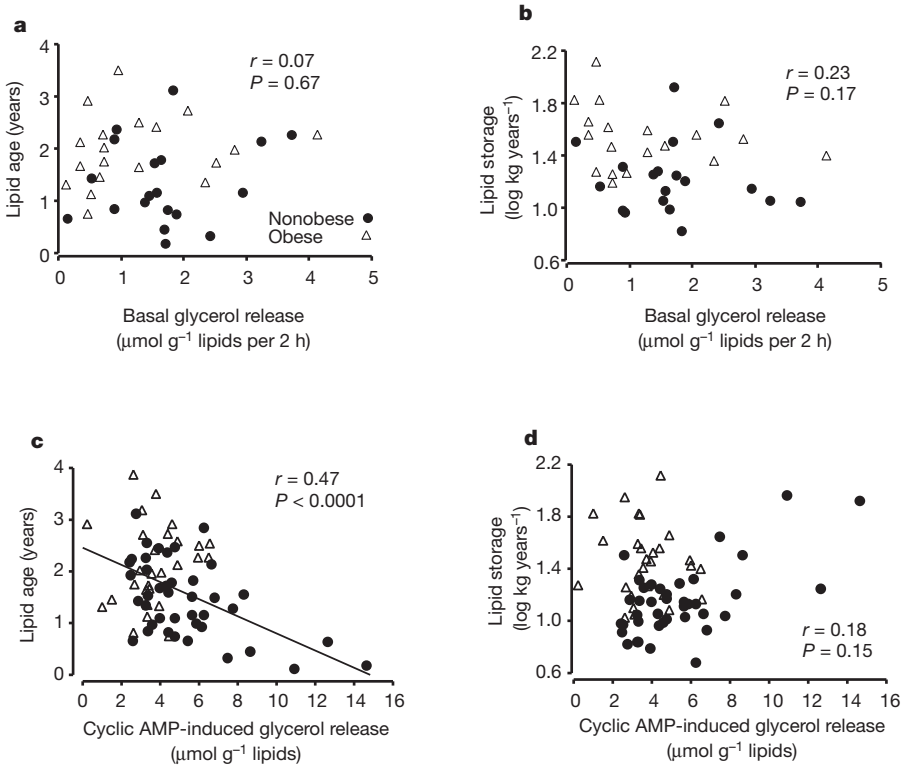

Figure 4 | Correlation between lipid turnover and adipocyte lipolysis. **a**–**d**, Lipid age and lipid removal rates were compared with basal rate of glycerol release (**a**, **b**) and with the rate of glycerol release induced with dibutyryl cyclic AMP (**c**, **d**), which is a phosphodiesterase-resistant and stabile cyclic AMP analogue stimulating the protein kinase A complex. Linear regression analysis was used. Data are from non-obese ($n = 48$) and obese ($n = 28$) individuals from cohort 1. Data with dibutyryl cyclic AMP-induced lipolysis versus lipid age were also significant when analysed using BMI as a covariate in multiple regression analysis (partial $r = -0.40$; $P = 0.0006$).

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involved in determining overall insulin effects. Insulin resistance (indirectly measured by the HOMA-IR index, see Supplementary Information 2) and lipid turnover were assessed in 82 individuals. Triglyceride age was strongly related to levels of insulin resistance (Fig. 3c), although there was no relationship between triglyceride storage and insulin resistance (Fig. 3d). There was no significant interaction between groups (lean, obese and non-obese FCHL) as determined by analysis of co-variance, indicating that the rate of triglyceride removal from adipocytes has an impact on whole-body insulin sensitivity independent of any underlying disorder. Multiple regression analysis showed that the relationship between HOMA-IR and lipid removal was not influenced by plasma triglycerides (partial $r = 0.35$; $P = 0.007$).

We also examined non-obese and obese individuals separately (Supplementary Information 2 and Figure 2a–d of Supplementary Information 2). Variations in BMI were significantly related to lipid age only among non-obese and to lipid storage only among obese individuals. HOMA-IR variations were significantly related to lipid storage when obese subjects were removed from the analysis (no relationship was found among obese subjects themselves). Thus, variations in triglyceride turnover may have a different impact on metabolic status in obese versus non-obese populations. Clearly, this assumption must be confirmed by investigations in much larger samples.

Because adipose tissue lipolysis is the first step in lipid removal, we investigated the ability of the cyclic AMP system to activate lipolysis *in vitro* in adipocytes isolated from lean and obese individuals and compared this with *in vivo* measurements of lipid storage and removal. Spontaneous (basal) lipolysis was not related to lipid turnover (Fig. 4a, b). However, the stimulated rate of lipolysis was positively correlated with triglyceride removal (inversely correlated with lipid age) but was not related to the rate of triglyceride uptake (lipid storage). This was irrespective of whether lipolysis was induced using a cyclic AMP analogue (Fig. 4c, d), by activating endogenous adenylate cyclase (using forskolin; Supplementary Information 2 and Fig. 3a, b of Supplementary Information 2) or by administration of a synthetic β-adrenoceptor-selective catecholamine (isoprenaline; Fig. 3c, d of Supplementary Information 2). These data indicate that lipolysis determines lipid turnover in adipocytes by regulating the rate of triglyceride removal. The impact of subsequent fatty acid oxidation could not be examined in this study; however, decreased lipid oxidation is frequently observed in obesity[23,24]. As there are regional variations in lipolysis and all our studies were performed on one fat depot no attempts were made to extrapolate findings to the whole-body level.

We are in the midst of a global epidemic of obesity with negative health and socio-economic consequences. We propose adipose triglyceride turnover as a novel target for the prevention and treatment of excess body fat and possibly its consequences for insulin resistance. New insights into abnormal triglyceride turnover in FCHL patients may also suggest novel treatment strategies for this complex disease that targets adipocytes.

METHODS SUMMARY

Subjects. Subcutaneous adipose tissue was obtained from two patient cohorts. Patient selection and collection of clinical data are described in Supplementary Information 2.

Preparation of lipids. Triglycerides were extracted from pieces of adipose tissue or isolated adipocytes. Details of lipid extraction and adipocyte isolation are given in Supplementary Information 2. Extracted lipids were subjected to accelerator mass spectrometry analysis, as described in Supplementary Information 2.

Data analysis. Calculations between lipid turnover and clinical or adipocyte phenotypes are described in Supplementary Information 2. Calculations of lipid age and net lipid uptake by adipose tissue are described in Supplementary Information 1. Conventional statistical methods were used to summarize and compare data.

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

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Author Contributions K.L.S. and P.A. designed the study and wrote the manuscript together with K.N.F. and S.B. M.R. co-ordinated writing and data assembly. S.B. and E.A. were responsible for the modelling. K.L.S. performed sample preparation. M.S., G.P., B.A.B., P.S. and J.L. performed ^{14}C accelerator mass spectrometry measurements. P.A., M.E., T.S. and H.H. collected clinical material.

Author Information Reprints and permissions information is available at www.nature.com/reprints. The authors declare no competing financial interests. Readers are welcome to comment on the online version of this article at www.nature.com/nature. Correspondence and requests for materials should be addressed to P.A. (peter.arner@ki.se) or K.L.S. (kirsty.spalding@ki.se).

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Supplement 1: Modelling Lipid Turnover

Summary of the model for lipid turnover

Fat is mainly stored as lipids in adipocytes (fat cells). Change in the size of the fat mass over time is determined by the balance between the amount of lipid storage by adipocytes and the rate of lipid removal from the body. This is expressed by the differential equation

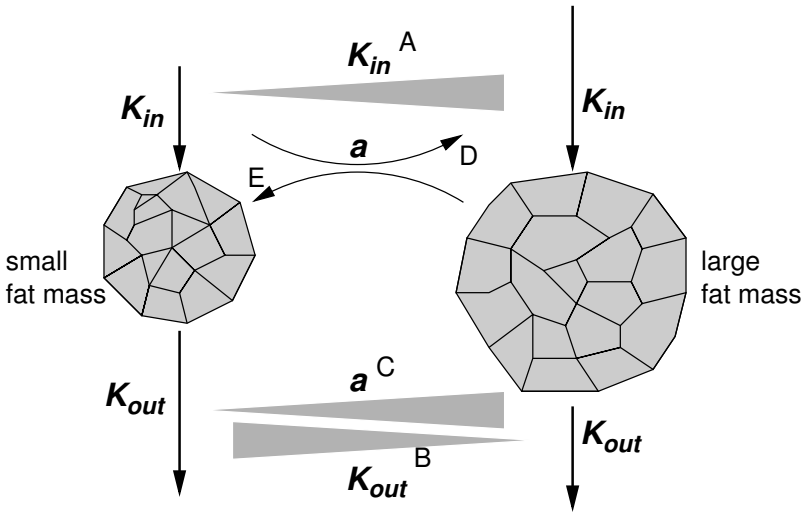
$$\frac{dF(t)}{dt} = K_{in}(t) - R(t, F(t)), \tag{1}$$

where $F(t)$ is the total fat mass (kg), $K_{in}(t)$ is the amount of lipid uptake by the adipose tissue every year (kg/year) and $R(t, F(t))$ is the amount of lipids removed every year (kg/year). The time t is the age of the subject in years. The amount of lipids removed each year clearly depends on the total fat mass, so it is useful to measure the relative removal rate per unit fat mass by dividing R with the total fat mass. The resulting value, $K_{out}(t) = R(t, F(t))/F(t)$, defines the instantaneous turnover rate (per year). $K_{out}(t)$ might change over time, but for $K_{in}(t) = K_{in}$ and $K_{out}(t) = K_{out}$ fixed, the total fat mass always reaches a constant steady state F. This happens when $K_{in} = K_{out}F$. Thus, a larger value of K_{in} sets the mass at a larger value (Supp. 1, Fig. 1A) and a larger value of K_{out} sets the mass at a smaller value (Supp. 1, Fig. 1B). Once the total fat mass is at steady state, the turnover rate K_{out} defines how quickly lipids are replaced in adipocytes. The lipid age (the average time lipids have spent in adipocytes) is the inverse of the turnover rate: $a = 1/K_{out}$ (in years, Supp. 1, Fig. 1C).

This interpretation of the turnover has a meaning only at steady state, and a change in fat mass can affect lipid age. A sudden increase in lipid storage will reduce the average age while the total fat mass is increasing, but only transiently (Supp. 1, Fig. 1D). Once the fat mass has reached a new steady state, the age goes back to its original value, as it does not depend on lipid storage. Similarly, a sudden decrease in lipid storage will increase transiently the average age (Supp. 1, Fig. 1E). Any permanent change in lipid removal rate, however, would affect the average age and its steady state value (Supp. 1, Fig. 1C). Below, we describe in details the mathematical model used for estimating lipid turnover from ^{14}C data.

Mathematical model of lipid turnover

Lipid age was estimated in two different ways. First, the ^{14}C level of each sample was reported on the atmospheric ^{14}C level curve (bomb curve[6]) and the difference between the collection date and the corresponding date was taken. This was called bomb curve age a_{BC}[7] . For the second method, a linear lipid replacement model was used, in which the age distribution of lipid within one subject is exponentially distributed. This corresponds to a constant turnover rate, denoted K_{out}. The associated mean age is $a_{to} = 1/K_{out}$.



Supp. 1, Fig. 1. Lipid turnover in adipocytes.

This age corresponds to the average age of lipids in one individual. Alternative models, where lipids follow a Last In, First Out scheme, or when lipids are exchanged between the adipose and non-adipose tissues, have been tested but do not appear essential to describe the data. A very strong similarity was found between a_{bc} and a_{to}, suggesting that the modelling captures the essential of the lipid dynamics in the adipose tissue.

We assumed that the total body fat mass F evolves according to a balance equation,

$$\frac{dF}{dt} = K_{in} - R, \tag{2}$$

where K_{in} is the net lipid uptake by the adipose tissue (units: kg/year), and R is the net lipid removal rate (units: kg/year). The relative removal rate normalized by the total fat mass is $K_{out} = R/F$. The parameter K_{out} controls the rate at which lipids are replaced, and is termed turnover rate. If the total fat mass does not change too fast, the fat mass is said to be at steady state. The steady state assumption means that the left-hand side of equation (2) is zero. This implies that the body fat mass is determined by the balance between lipid storage and lipid turnover:

$$F = \frac{K_{in}}{K_{out}} \tag{3}$$

Under the steady-state assumption, the average age of lipids is $a_{to} = 1/K_{out}$ (see Section "Estimating the Turnover from ^{14}C Data" below for details). Moreover, the total fat mass satisfies the relationship

$$F = K_{in} a_{to}. \tag{4}$$

This relation defines the relation between the average age of lipids, the total fat mass and the net amount of lipid uptake.

Estimating the Turnover from ^{14}C Data

The method used here has been adapted from a general model for estimating turnover rates in biological samples[9,12] . Atmospheric ^{14}C is continuously integrated into lipids that enter adipocytes. In order to track the change in ^{14}C content over time, it is helpful to recast equation (2) into a linear structured differential equation

$$\frac{\partial f(t,a)}{dt} + \frac{\partial f(t,a)}{da} = - K_{out} f(t,a). \tag{5}$$

$$f(t,0) = K_{in}. \tag{6}$$

The function $f(t,a)$ is the density of lipids with age a at time t and is related to the fat mass in the following way:

$$F(t) = \int f(t,a) da. \tag{7}$$

This is a one-compartment model in which lipids enter adipose tissue at age 0 and and enter only once; lipids are not recycled. In Section "Two-compartment models", we look at the effect of a second compartment on lipid turnover analysis.

The left-hand-side terms are conservation terms saying that lipids advance in age at the same rate as time. The right-hand-side term accounts for the removal of lipids. A boundary condition describes the constant uptake of new lipids ($f(t,0) = K_{in}$). This model has two parameters, K_{in} and K_{out}, which are possibly nonlinear. To specify the system completely, we need initial data, i.e. the age distribution of lipids at subject age $t = 0$. We assume that the total fat mass at birth is negligible $f(0,a) = 0$.

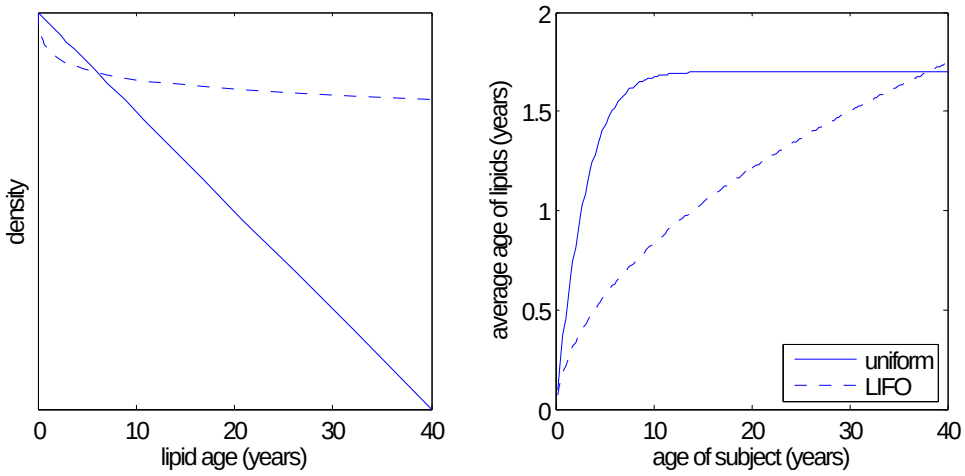
For each subject, two independent measurements are available: the total fat mass (F) and the ^{14}C abundance in lipids. The ^{14}C abundance allows the calculation of the parameter K_{out}. The associated ^{14}C abundance associated to a lipid sample collected at time t_d is

$$C = \frac{\int_0^{t_d - t_b} K(t_d - a) f(t,a) da}{F(t)} \tag{8}$$

The value t_b is the time of birth of the subject. For lipid turnover, it is assumed no lipid is left from birth, due to the relatively rapid turnover. The parameter K_{in} cancels out so the only parameter left is K_{out}. This simple model thus allows individual estimates of K_{out}, the turnover rate. The function K is the atmospheric ^{14}C abundance curve[6] , or the "bomb curve".

Based on that system, the average age of lipids with turnover rate K_{out} in a subject of age t is

$$\langle a \rangle_t = \frac{-t \exp(-K_{out} t) + (1 - \exp(-K_{out} t))/K_{out}}{1 - \exp(-K_{out} t)} \tag{9}$$



Supp. 1, Fig. 2. "Last In, First Out" scenario. (*Left*) Lipid age distribution (in normalized log-scale amount per year), for the uniform turnover (solid) and LIFO (dashed) with $t = 40$ years. Each line represents the relative amount of lipid at a given age. (*Right*) Evolution with subject age of the average lipid age. $K_{out} = 0.59$/year, $k_1 = 1/12$ year, and $k_0 = 18$ years in both panels.

The average lipid age depends strongly on t in young subjects, but as t gets larger, $\exp(-K_{out}t)$ gets very small and the average lipid age approaches $a_{to} = 1/K_{out}$. For the turnover values measured here, this approximation is valid for ages $t > 10$ years (see right panel of Supp. 1, Figure 2). All subjects were adults, so we used this approximation to define the average lipid age.

We compared the lipid age obtained with this turnover method with the lipid age obtained by reading off the bomb curve. The two methods yielded very similar estimates (correlation $\rho = 0.99$), but the bomb curve age gave slightly larger estimates (difference 0.08 years, p-value$<$0.001). Although the estimates were slightly different, these results show that the turnover method did not introduce unwanted artifacts.

Last In, First Out Scenario

In the previous section, we assumed that the turnover rate K_{out} was constant. However, it has been proposed that lipids are not distributed uniformly in the adipocytes, but that last entered lipids are more likely to be removed from the cell than older lipids. This scenario is called "Last In, First Out", or LIFO[13,14] . In terms of the model here, this is expressed as an age-dependent K_{out}. Last entered (young) lipids have a higher turnover than old lipids, thus the turnover must be a decreasing function of lipid age. One possible

such function is the following,

$$k_{out}(a) = \frac{k_0 k_1}{k_1 + a}. \tag{10}$$

(Small letters are used to distinguish from the default one-compartment model.) When the turnover is uniform with age a, the average lipid age is directly related to the turnover $a_{to} = 1/K_{out}$, as above. In the "Last In, First Out", or LIFO scenario, the average lipid age in a subject aged t years is

$$a_{LIFO} = \frac{[-k_0 k_1 + 1]\left[(k_1 + t)^{-k_0 k_1 + 2} - k_1^{-k_0 k_1 + 2}\right]}{[-k_0 k_1 + 2]\left[(k_1 + t)^{-k_0 k_1 + 1} - k_1^{-k_0 k_1 + 1}\right]} - k_1. \tag{11}$$

The parameter k_1 control at which age the turnover is half what it was at age 0, denoted by parameter k_0. From equation (11), the larger the value of k_1, the weaker the LIFO effect. It is therefore interesting to see what happens when k_1 is small. For example, we have chosen $k_1 = 1/12$ year (one month), and $k_0 = 18$ per year and $t = 40$ years. For these values, the average lipid age 1.74 years, close to the average value calculated from ^{14}C data, but the distribution of ages is much wider (Supp. 1, Figure 2, left panel). With these values, there is a strong subject age-depedence due to the slow aging of old lipids that never get replaced (Supp. 1, Figure 2, right panel). Therefore, if the LIFO effect was important, there should be a slow lipid aging amongst older subjects. The absence of such a correlation speaks against the importance of LIFO in determining lipid age. With a uniform turnover rate, the lipid age quickly reaches a steady state in adults, in agreement with the measured lipid ages.

We have fitted the LIFO scenario to the ^{14}C samples. The best fit for the whole population (where the error, denoted SSE, is minimized in the least-square sense) yielded $k_0 = 0.63$ per year and $k_1 = 36$ years (SSE = 0.014). This large value for k_1 means that the turnover rate is almost uniform. With an initial rate of 0.63 per year, essentially all of the lipids would be replaced well before their turnover has decreased significantly. It is still possible that the rapid compartment is fast but small with respect to the whole tissue. To see if this could be compatible with our data, we constrained parameter k_1 to be short ($k_1 = 1$ year), and fitted the parameter . The result, $k_0 = 2.9$ per year (SSE = 0.022) was not as good as the optimal fit, but is still acceptable. Lipid age would be of average 1.05 years, somewhat younger than in a homogeneous pool. Therefore, although the best model seems to be a homogeneous pool of lipid, the data are compatible with the existence of a compartment with a rapid turnover. Assuming the existence of such a compartment does not change the results and conclusion obtained with the constant turnover scenario, but would have a important effect on measurements obtained by short-term labelling studies.

In summary, there is no indication that the LIFO is better than the uniform turnover. However, we cannot rule out that a small proportion of the lipids is turning over rapidly.

Two-compartment models

In Section "Estimating the Turnover from ^{14}C Data", the model used to estimate turnover rate is a one-compartment model. We look here at the effect of having two different pools of lipids, with different turnover rates and possibly exchanges between to two pools. At steady states, the ages distributions for the two pools are given by two coupled linear differential equations

$$\begin{aligned}\frac{\partial p_1(a)}{da} &= -(k_o^1 + k_{12})p_1(a) + k_{12}p_2(a),\\ \frac{\partial p_2(a)}{da} &= -(k_o^2 + k_{21})p_2(a) + k_{21}p_1(a),\\ p_1(0) &= k_{in}^1,\\ p_2(0) &= k_{in}^2.\end{aligned}$$

The solution to this system is

$$p_1(a) = q_1 e^{-\lambda_1 a} + q_2 e^{-\lambda_2 a},$$

where

$$\begin{aligned}q_1 &= -\frac{1}{\sqrt{d}}\left[\frac{k_{in}^1}{2}\left(k_o^2 + k_{21} - k_o^1 - k_{12} - \sqrt{d}\right) + k_{in}^2 k_{21}\right],\\ q_2 &= \frac{1}{\sqrt{d}}\left[\frac{k_{in}^1}{2}\left(k_o^2 + k_{21} - k_o^1 - k_{12} + \sqrt{d}\right) + k_{in}^2 k_{21}\right],\\ \lambda_1 &= \frac{1}{2}\left(k_o^2 + k_{21} + k_o^1 + k_{12} + \sqrt{d}\right),\\ \lambda_2 &= \frac{1}{2}\left(k_o^2 + k_{21} + k_o^1 + k_{12} - \sqrt{d}\right),\\ d &= (k_o^2 + k_{21} - k_o^1 - k_{12})^2 + 4k_{12}k_{21}.\end{aligned}$$

Therefore, adipocyte lipids are removed at two different rates, λ_1 and λ_2, and the relative importance (weight) of each removal rates are given by the coefficients of each of the exponentials in the equation for p_1 above. The average age in pool 1 is therefore

$$\langle a \rangle_1 = \frac{\int_0^\infty a p_1(t,a) da}{\int_0^\infty p_1(t,a) da} = \frac{q_1/\lambda_1^2 + q_2/\lambda_2^2}{q_1/\lambda_1 + q_2/\lambda_2}.$$

A similar relation exists for pool 2, with indices 1 and 2 switched.

Effect of a small pool of high turnover lipids

In the LIFO model, there was a continuum of decreasing turnover rates with lipid ages. To quantify the effect of a small pool of higher turnover lipids, we looked at the linear two-compartment model, in which young lipids (pool 2) are either rapidly removed or enter

the low turnover pool (pool 1). According to these assumptions, no old lipid reenter the young lipid pool ($k_{12} = 0$) and all old lipids transited through the young pool ($k^1_{in} = 0$). Moreover, we assumed that the ^{14}C abundance measured gives us an estimate of the mean age of the total lipid content, in pools 1 and 2. This is slightly different from the LIFO model, in that we do not fit the model to the data, because there are too many parameters (k^1_o, k^2_o, k_{21}) to get a robust fit. From the equation above and the assumptions on parameters, the average age of lipids in pool 1 is

$$\langle a \rangle_1 = \frac{(k^2_o + k_{21} + k^1_o)}{(k^1_o)\,(k^2_o + k_{21})}.$$

Similarly, the average age in pool 2 is

$$\langle a \rangle_2 = \frac{1}{k^2_o + k_{21}}.$$

The fractions of lipids in pool 1 and 2 are respectively

$$s_1 = \frac{k_{21}}{k_{21} + k^1_o},$$
$$s_2 = \frac{k^1_o}{k_{21} + k^1_o}.$$

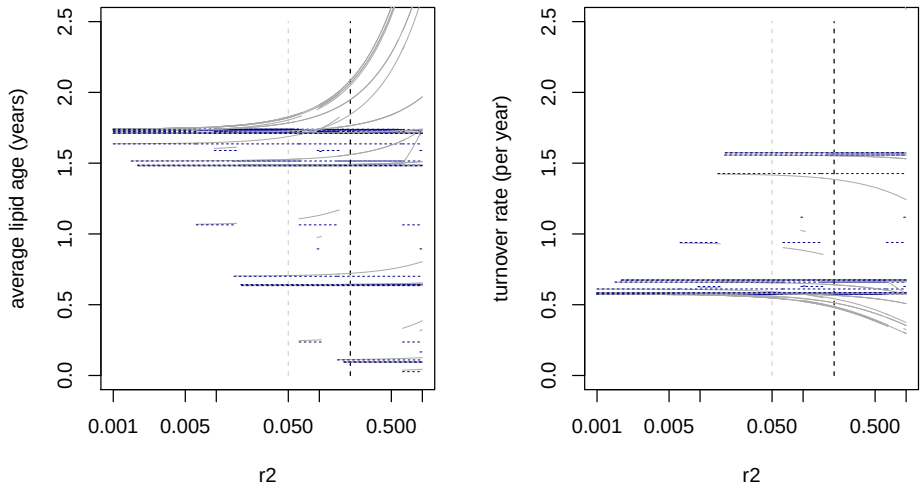
The average lipid age in the two pools is

$$\begin{aligned}\langle a \rangle_{two} =& s_1 \langle a \rangle_1 + s_2 \langle a \rangle_2 \\ =& \frac{k_{21}}{k_{21} + k^1_o} \frac{(k^2_o + k_{21} + k^1_o)}{(k^1_o)\,(k^2_o + k_{21})} + \frac{k^1_o}{k_{21} + k^1_o} \frac{1}{k^2_o + k_{21}}\end{aligned}$$

To see if we can neglect pool 2, we compare the turnover rate obtained in the one-compartment model K_{out} and the slow turnover obtained with the two-compartment model k^1_o, for a given value of the total average lipid age. Equating the two average values: $1/K_{out} \equiv \langle a \rangle_{two}$, we look at the ratio between k^1_o and K_{out}. To do that, we rewrite the high turnover parameter as $k^2_o = z k^1_o$ (with factor $z > 1$) and the transfer rate as $k_{21} = (1 - s_2) k^1_o / s_2$. Then we obtain,

$$\begin{aligned}\frac{k^1_o}{K_{out}} =& (1 - s_2) \frac{z + 1/s_2}{z + (1 - s_2)/s_2} + \frac{s_2}{z + (1 - s_2)/s_2} \\ =& \frac{(1 - s_2) z + 1/s_2 - 1 + s_2}{z + (1 - s_2)/s_2}.\end{aligned}$$

From the assumptions, we know that s_2 is small (small pool) and $r \gg 1$ (high turnover). For large values of z, the ratio $k^1_o/K_{out} \approx 1 - s_2$, and for $z = 1$, the ratio $k^1_o/K_{out} = 1$.



Supp. 1, Fig. 3. Non-adipocyte pool. (*Left panel*) Average lipid age as a function of the ratio between of pool 2 and pool 1 sizes (r_2). The average ages given by the two-compartment model (*grey solid lines*) are well approximated by equation (14) (*blue dashed lines*), for small values of r_2. (*Right panel*) Turnover rates calculated with two-compartment model (*grey solid lines*) and with the approximation (*blue dashed lines*, turnover defined as the inverse of $\langle a \rangle_1$ given by equation (14)). In both panels, pairs of curves were drawn using one set of parameters amongst $k_o^1 = 0.575$, $k_o^2 = k_o^1 f_i$, $k_{12} = f_j$, $k_{21} = f_k$, where $f = (0.01, 0.1, 1, 10, 100)$, and (i, j, k) run from 1 to 5 (n=125 pairs of curves). For most of the parameter values, the average age is close to $1/k_o^1$ or $1/(k_o^1 + k_{12})$.

This means that for small values of z, the ratio between the turnover rates is close to one (s_2 being very small) and for large values of z, the error on turnover rates is of the order of s_2. In fact, the removal rate k_o^1 underestimates the true turnover rate K_{out} because the lipids enter the slow turnover pool with a positive age.

We conclude that the error made by using the one-compartment model, if there is a small lipid pool with a high turnover rate, is at worst of the same order as the size of the high turnover lipid pool.

Non-adipocyte compartment for lipids

A small quantity of lipids is stored outside adipocytes, such as in blood and liver. Exchange between two physiological compartments can affect the measured turnover rate. Only the adipocyte lipids (pool 1) was measured here. We are therefore interested in the

average age of compartment 1 only. The average lipid age in pool 1 is

$$\langle a\rangle_1 = \frac{[k_o^2+k_o^1+k_{12}+k_{21}]k_{21}k_{in}^2+[2k_o^2k_{21}+k_{12}k_{21}+(k_o^2)^2+(k_{21})^2]k_{in}^1}{[k_o^2k_{12}+k_o^2k_o^1+k_{21}k_o^1]k_{21}k_{in}^2+[k_o^2k_{12}k_{21}+2k_{21}k_o^2k_o^1+(k_o^2)^2(k_{12}+k_o^1)+(k_{21})^2k_o^1]k_{in}^1}. \quad (12)$$

The expression above cannot be related easily to the lipid age $1/K_{out}$. Because lipids can cycle between the two pools, the lipid removal rate from one pool is not well defined. Expressing the production rate ratio $w = k_{in}^2/k_{in}^1$, the expression (12) can be simpified to:

$$\langle a\rangle_1 = \frac{[k_o^2+k_o^1+k_{12}+k_{21}]k_{21}w+[2k_o^2k_{21}+k_{12}k_{21}+(k_o^2)^2+(k_{21})^2]}{[k_o^2k_{12}+k_o^2k_o^1+k_{21}k_o^1]k_{21}w+[k_o^2k_{12}k_{21}+2k_{21}k_o^2k_o^1+(k_o^2)^2(k_{12}+k_o^1)+(k_{21})^2k_o^1]}, \quad (13)$$

with one parameter less. The size ratio between pool 2 and pool 1 can be calculated:

$$r_2 = \frac{k_o^1w+k_{12}w+k_{12}}{k_{21}w+k_o^2+k_{21}}.$$

It is biologically relevant to look at the behavior of $\langle a\rangle_1$ when r_2 is small (Supp. 1, Figure 3, left panel). For small values of r_2 (when $r_2 \to 0$), the average age simplifies to

$$\langle a\rangle_1 \approx \frac{k_o^2+k_{21}}{k_o^2k_{12}+k_o^1k_o^2+k_o^1k_{21}}. \quad (14)$$

This last expression is much more tractable, and does not depend on the production rates anymore. However, one must make sure that the production rate ratio is positive, so this expression is valid for r_2 between the two values

$$r_2(w=0) = \frac{k_{12}}{k_o^2+k_{21}}, \quad (15)$$

and

$$r_2(w=\infty) = \frac{k_o^1+k_{12}}{k_{21}}. \quad (16)$$

It is still difficult to relate $\langle a\rangle_1$ with the parameters but we can distinguish three limiting cases. These cases are only valid for small values of r_2, but in pratice, we have found that $r_2 < 0.20$ is small enough.

1) $k_{12} \to 0$ or $k_o^2 \to 0$ or $k_{21} \to \infty$. Then $\langle a\rangle_1 = 1/k_o^1$. All lipids go quickly to the adipocyte pool and the lipid age is only controled by the removal rate in the adipocyte

pool. Therefore the two-compartment model reduces to the one-compartment model by setting $K_{out} = k_o^1$.

2) $k_{21} \to 0$ or $k_o^2 \to \infty$. Then $\langle a \rangle_1 = 1/(k_o^1 + k_{12})$. In this case, the non-adipocyte pool quickly removes all lipids, which are not recycled, so the turnover in the adipocyte pool is the sum of the removal rate k_o^1 and the exchange rate k_{12}. Therefore the two-compartment model reduces to the one-compartment model by setting $K_{out} = k_o^1 + k_{12}$.

3) Other cases. Then

$$\frac{1}{k_o^1 + k_{12}} \leq \langle a \rangle_1 \leq \frac{1}{k_o^1}. \tag{17}$$

This interval gets large when k_{12} is much larger than k_o^1, i.e. when adipocyte lipids are quickly transfered to the non-adipocyte pool rather than permanently removed. Then the average age is best expressed by equation (14).

As noted in the main text, the estimate of lipid age using the bomb curve profile (a_{BC}) and the one-compartment model (a_{to}) are similar, indicating that the estimated lipid age probably reflect well the real lipid age. The discussion above therefore only has an impact on the estimated turnover rate. From the ^{14}C data, ot is not possible to know if lipids going out of the adipocyte pool are stored in a second pool before being removed from the body, so the estimate for K_{out} can only be interpreted as a general removal rate, which corresponds either to k_o^1 or to $k_o^1 + k_{12}$. In practice, it does not matter where lipids go, as long as they are not recycled to the adipose tissue. A case where lipid recycling is important is when k_{21}, k_o^2 and k_{12} are large. Then the ^{14}C-derived turnover rate K_{out} will be in the interval $(k_o^1, k_o^1 + k_{12})$. In such a case, the turnover rate in pool 1 is not well defined. One may tentatively define the turnover rate as the inverse of the average lipid age, as in the one-compartment model,

$$K_{out} \equiv \frac{k_o^2 k_{12} + k_o^1 k_o^2 + k_o^1 k_{21}}{k_o^2 + k_{21}}.$$

Because of lipid exchange, the turnover cannot be expressed in term of pool 1 parameters only. Nevertheless, for small values of r_2, numerical simulation show that the this approximation of the turnover rate is very close to the turnover rate computed from the two-compartment model. We ran the two-comparment model with 125 different parameter sets over 4 orders of magnitude and found that for $r_2 < 0.20$ the turnover rates were well approximated (Supp. 1, Figure 3, right panel).

The numerical simulations indicate that the one-compartment model is able to capture the lipid dynamics of a two-compartment model as long as the size of the adipocyte lipid pool is at least five times larger than the non-adipocyte lipid pool. Since the non-adipocyte lipid pool is estimated to be less than 4% of the entire lipid pool, we conclude that we can safely ignore the non-adipocyte lipid pool in studying the adipocyte lipid turnover dynamics.

Data Analysis

Equation (4) establishes the relation between fat mass, lipid uptake and lipid age. Fat mass and lipid age have been estimated independently, so the annual uptake rate can be estimated as well. The turnover K_{out} was calculated using a direct search method for each ^{14}C sample with Matlab (Naticks, MA).

Analysis was based on 157 samples, of which clinical data was available for 92 individuals. One sample per subject was used for lipid turnover analysis. Total lipid storage K_{in} was calculated as $K_{in} = K_{out}F$, where the total fat mass F was obtained independently from K_{out}. One of the samples had a calculated age of 8.5±0.6 years. This sample was an extreme outlier and was not included in the statistical analysis presented in the main text.

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Supplement 2: Patient Information and Tissue Processing

Subjects. Adipose tissue biopsy samples from Swedish (Cohort 1) and German subjects (Cohort 2) were used in this study. The influence of clinical parameters, adipocyte lipolysis and the morphology of adipose tissue were determined in Cohort 1. Abdominal subcutaneous adipose tissue was obtained in the morning after an overnight fast from healthy subjects non-obese or obese (BMI> kg/m^2) and non-obese patients with familial combined hyperlipidemia (FCHL) by biopsy under local anaesthesia (clinical data in Supplementary Table 1). FCHL was diagnosed as we described previously (1) and diagnosis was confirmed by the author ME who is a clinical lipidologist. Two patients were on a fibrate or a statin. The remaining FCHL subjects received no treatment at the time of investigation. All subjects in Cohort 1 were weight stable for at least 6 months prior to examination according to self report. The non-obese healthy subjects and the FCHL patients had never been obese. In this group adipose samples were collected from 1991-2001. BMI and venous plasma levels of insulin, glucose, triglcyerides total and HDL cholesterol and apolipoprotein B (apoB) were determined as described (2). Insulin sensitivity was determined indirectly by calculating a so called HOMA index from insulin and glucose values as described (2). Fat mass was directly measured with bioimpedance using GuadScand 4000 (BodyStat Ltd, Isle of Man, British Isles). In Cohort 2 (clinical data in Supplementary Table 2) lipid turnover in large and small adipocytes from the same sample were examined. Subcutaneous abdominal adipose tissue was obtained from cosmetic liposuction on healthy subjects (collected in 2009). ^{14}C analysis was performed on tissue samples from both cohorts.

Ethical permission for all studies was approved by the ethics committee at Karolinska Institutet, Stockholm, Sweden (Cohort 1) and at Technische Universität Münich, Germany (Cohort 2) and informed consent was obtained from all subjects.

Separation of adipocytes from the same tissue sample according to size

Details of the method are reported (3). In brief, subcutaneous adipose tissue samples (50-100 g wet weight) were obtained at the Munich site and immediately transported to the laboratory. Isolated fat cells were prepared by the Rodbell method (4). Adipose tissue was minced and digested in Krebs-Ringer-phosphate buffer (KRP) containing 100 U/ml collagenase and 4% albumin for 60 min at 37^{o}C in a shaking water bath. The undigested tissue was removed by filtration. The floating adipocytes were washed three times with KRP containing 0.1% albumin and separated into four fractions exactly as described (3). In brief, ten millilitres of the isolated adipocytes were separated into four fractions (small, medium, large, extra large) adipocyte diameters. The cell volume was calculated from the diameter using a described formula (3). Fraction I (smallest) and IV (largest) were stored at -70^{o}C and subsequently sent to Stockholm for lipid extraction and ^{14}C analysis of lipids.

Determination of adipose tissue morphology

At any level of total body fat adipose tissue may contain many small fat cells (hyperplasia) or few but large fat cells (hypertrophy) as discussed (5). The relationship between mean adipocyte volume and total body fat determines adipocytes morphology and can be used to classify the adipose morphology into hyperplastic or hypertrophic (5). Briefly, the relationship is described by the following equation: $(V = a \times m)/(1+b \times m)$ where V is the average adipocyte volume (pl), m is the amount of body fat (kg), and a and b are variables that can be estimated by fitting the formula to subject data. We used data regarding subcutaneous adipocyte volume and body fat to estimate a to 42.6 pl/kg and b to 0.033 /kg for the total adipose depot. The differences between the observed adipocyte volume and the expected volume (as obtained from the curve fit) at the corresponding level of total body fat

mass was calculated for each subject, who was then classified as being either hyperplastic (negative deviation) or hypertropic (positive deviation) relative to the estimated average value for body fat. The validity of using total body fat and fat cell volume from a single adipose region for the determination of the morphology values has been discussed in detail (5). The morphology values are normally distributed (5). When positive they indicate hypertrophy and when negative they are indicative of hyperplasia (5). Small values represent a minor degree of either form of morphology and suggestive of mixed phenotype. Large values are indicative of an extreme phenotype. We presently utilized both continuous values and a subdivision into a hyperplasia or a hypertrophy phenotype.

Extraction of lipids from adipose tissue preparations

Samples were stored at -70^{o}C. Some samples consisted of pieces of intact adipose tissue, others were purified adipocytes isolated from fat tissue according to the collagenase procedure of Rodbell (4). Essentially all lipids of adipose tissue are contained in adipocytes and initial methodological experiments revealed that ^{14}C data from fat pieces and isolated adipocytes gave identical results. As such, both sample types were used in this study and total lipids were extracted exactly as described (6). In brief, adipose tissue (30-40 mg) or packed isolated adipocytes (200 µl) were homogenized with the extraction mixture of Dole. Thereafter heptane and distilled water were added and the heptane phase removed. This was repeated four times and the resultant lipid sample was processed for ^{14}C measurements. The Dole procedure does not extract phospholipids and cholesterol but predominantly acylglycerols. Since human adipose tissue contains minute amounts of diacyl- and monoacylglycerols, we considered the extracted lipids as triglycerides in adipose tissue and fat cells.

Studies of adiopcytes lipolysis

These experiments were concluded on samples from non-obese and obese in Cohort 1 exactly as described previously (7). Isolated human fat cells were incubated under dilute conditions (2% vol/vol) in an albumin/glucose containing buffer (pH 7.4) for 2 hours at 37^{o}C. At the end of incubation release of glycerol (lipolysis index) was determined. Different agents were added to the medium at the start of incubation. Adenosine deaminase (1 U/ml) was added in order to remove tracers of adenosine which artificially leaks out of isolated fat cells and inhibit the spontaneous (basal) rate of lipolysis (8). Isoprenaline is a synthetic beta-adrenergic receptor selective catecholamine and was added at 10^{-9} – 10^{-5} mol/. Forskolin (10^{-7} – 10^{-4} mol/l) was added in order to stimulate the endogenous production of cyclic AMP (the key mediator of lipolysis) by acting selectively on adenylate cyclase. Dibutyryl cyclic AMP (10^{-5} – 10^{-3} mol/l) is a cyclic AMP analogue that is resistant to phosphodiesterase which is the major enzyme responsible for intracellular degradation of the nucleotide. It was added to activate protein kinase A so that the lipases responsible for hydrolysis of triglycerides to the end products fatty acid and glycerol are activated. The glycerol values at maximum effective concentration of the lipolytic agents were used in each individual experiment to calculate the maximum stimulated rate of lipolysis. In the present experiment the ratio of forskolin/adenosine deaminase induced lipolysis was 4.3 $\pm$ 3.1 (mean $\pm$ SD). Corresponding ratios for dibutyryl cyclic AMP and isoprenaline were 4.2 $\pm$ 3.5 and 4.8 $\pm$ 3.6, respectively. There is no consensus how to express lipolysis rates (per amount of lipids or per number of fat cells). As discussed extensively and shown in independent cohorts entirely different results are obtained when subjects with different BMIs are investigated, presumably due to a strong influence on lipolysis values by fat cell size (7,9). For several reasons we choose to express glycerol release per lipid weight. First, this expression correlates best with lipase expression

and body fat mass (7,9). Second, the adipocyte parameters measured by the present ^{14}C method were related to amount of fat and not number of fat cells.

Accelerator mass spectrometry (AMS) measurements

Carbon isotope ratio measurements using AMS require production of negative ions. The sputter ion source injecting the accelerator necessitates the samples to be in graphite form which provides high and stable ion currents. The samples consequently undergo a preparation procedure prior to the AMS analysis, including two chemical processes: 1) Combustion, where the sample is oxidized into CO_2 gas and 2) Graphitization, where the CO_2 gas is reduced into graphite. All laboratory routines comply with the aim of minimizing the amount of background carbon. This includes pre-heating all glassware including borosilicate and quartz vials, as well as the oxidizing agent, the reducing agent and the catalyst for about 3 hours. The temperature used depends on the material but is in the range of 300-900 ºC. For a general description of the setup see reference (10).

The samples are placed in a quartz tube with 80 mg of CuO (≤0.002% carbon) and vacuum pumped for about an hour to a vacuum of $<5.10^{-5}$ mbar. The tube is sealed under vacuum with a high temperature torch using liquefied petroleum gas and oxygen. The sealed sample is placed in an oven and baked at 950 ºC for 3 hours where the sample is oxidized. The tube containing the CO_2 gas is then moved to a gas transfer setup with a base pressure in the 2.10^{-5} mbar range. The tube is punctured and the CO_2 gas is cryogenically transferred and trapped, using liquid nitrogen, into a borosilicate tube containing 100 mg zinc (<150µm powder, 99.995% pure) as the reducing agent and 2 mg iron powder (325 mesh, 99.9% pure) as the catalyst. Prior to this, any water in the sample is removed using a mixture of ethanol and liquid nitrogen (-80 ºC) and any residual gases are pumped away. A small fraction of the sample is used for off-line $^{13}C/^{12}C$ ($\delta^{13}C$) measurements in a Stable Isotope Ratio Mass

Spectrometer where fractionation of carbon 13 in the samples is measured relative to a standard sample to allow a fractionation correction of the AMS results (11). The $\delta^{13}C$-variation for the samples in this study was typically in the 1 per mil range (variance 0.5‰) that results in a smaller contribution to the ^{14}C measurement uncertainty than the error from the AMS measurements itself. The sample amount is measured by the pressure of the CO_2 gas in a calibrated volume using a capacitance manometer. The samples were typically in the range corresponding to 200-900 µg carbon. The vial containing the CO_2 sample gas is subsequently heated to 550 ºC for 6 hours. The produced graphite is pressed into an aluminium cathode which is then loaded into the ion source of the accelerator at the Uppsala University, Tandem Accelerator Laboratory. The AMS analysis uses 12 MeV carbon ions, where the ^{12}C current is measured using a faraday cup and the ^{14}C ions are detected individually, using a solid state detector where the energy spectrum of the incoming ion is concurrently analyzed for unambiguous identification and detection. The samples are analyzed four times, each acquisition period lasting 300 seconds. This is complemented with data acquired from 3 separate NIST standards (SRM 4990C, Oxalic Acid II) to ensure correct normalization. Data is presented as Fraction Modern, $F^{14}C$, which is the preferred unit for representing isotopic ratio in this application. It is defined as the ratio of the ^{14}C to ^{12}C carbon for a sample divided by that of a well defined standard sample, measured in the same year and incorporating ^{13}C fractionation correction (See reference 12). Fractionation variations in the graphitization process were measured to be below 0.1%. This was done by re-combusting the graphitized samples and analysing them using Stable Isotope Ratio Mass Spectrometry.

Statistical Methods

Values are, unless otherwise stated, mean ± standard error of mean in figures and mean ± standard deviation in tables. They were compared using paired or unpaired t-test, analysis of

variance (ANOVA), analysis of covariance (ANCOVA) and linear or multiple regression analysis. Values for HOM index, plasma triglycerides and lipid storage (K_{in}) were 10 log transformed in order to normalize distribution. Since no previous information existed in adipose lipid age from atmospheric ^{14}C data we used published short-term experimental data on triglyceride turnover in human adipose tissue obtained from 18 men (13). Standard deviation divided by mean value was 0.5. We tested between group differences in lipid age which could be detected with an unpaired or paired t-test at $p < 0.05$ with 80% power. In Cohort 1 (2-3 groups) we could detect a difference of 15-25 % between any group combination with unpaired t-test. In Cohort 2 (2 groups) we could detect a difference of 20-30% with paired t-test.

References Supplement 2

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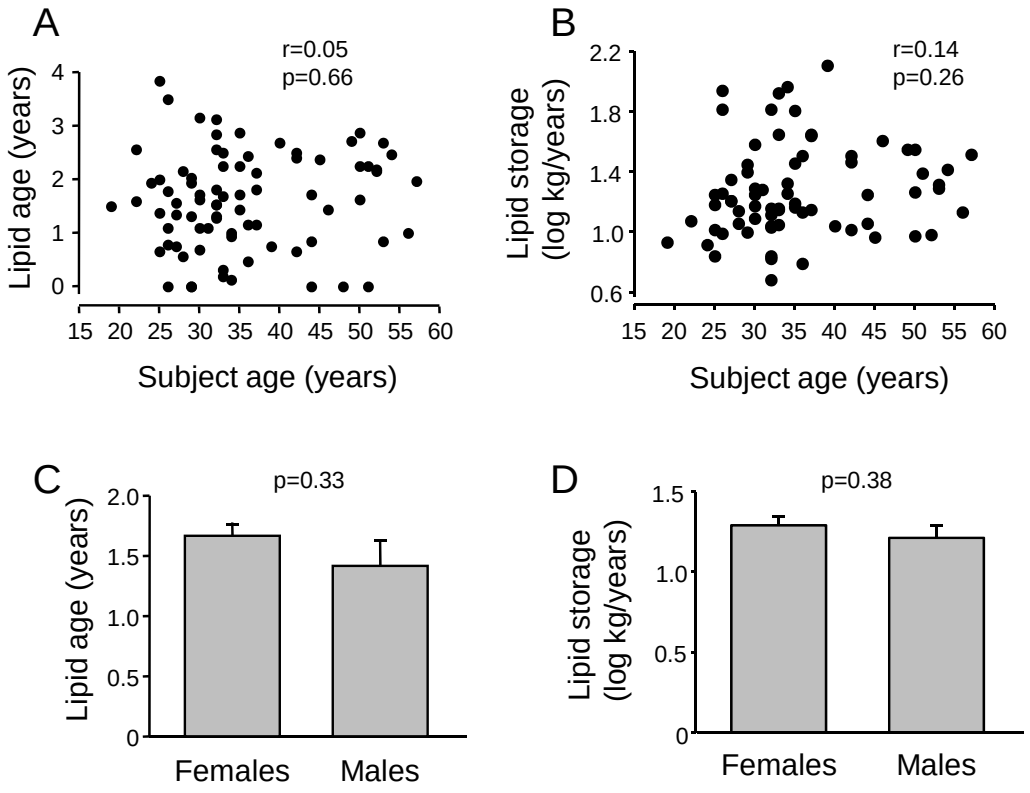
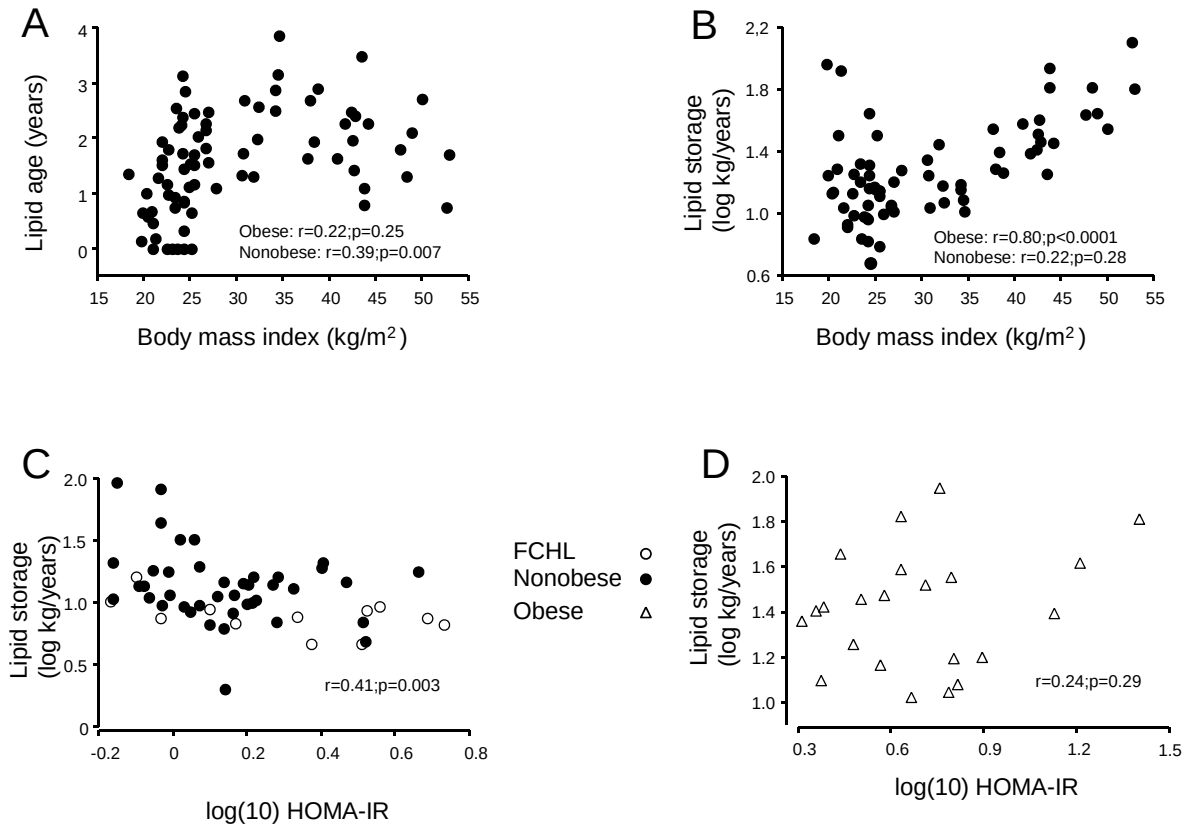
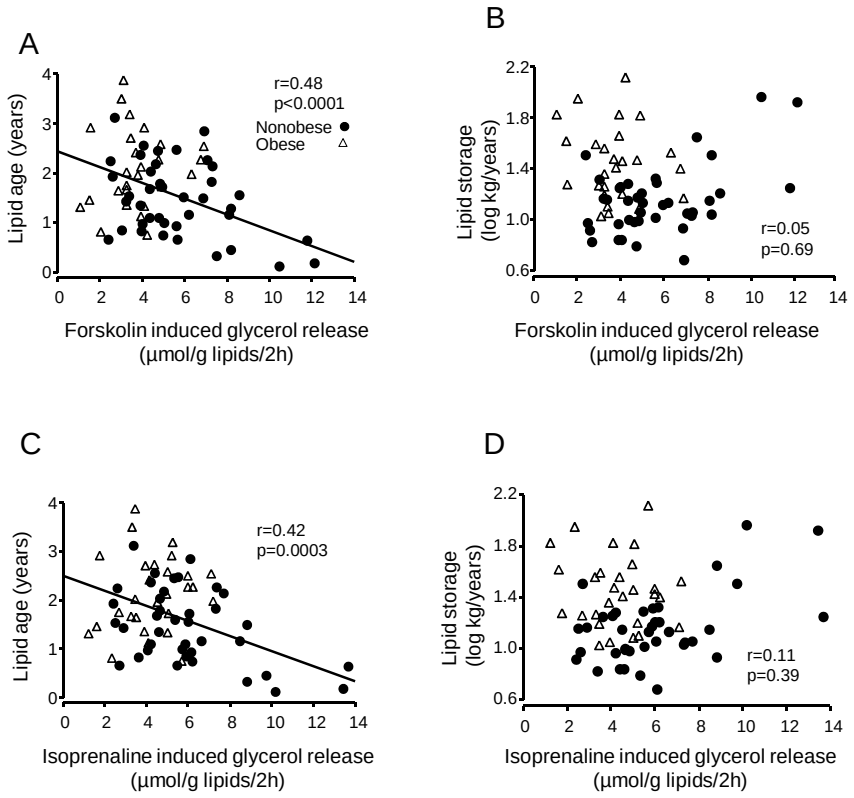


Suppl 2 Fig. 1 *Influence of person age and gender on subcutaneous adipose lipid turnover.* No relationship between person age and lipid age (A) or lipid storage (B) was seen (linear regression analysis). Gender had no influence on lipid age (C) or lipid storage (D) (unpaired t-test). The same was true if percentage body fat was taken into account in the analysis (p-value for gender > 0.2 by ANOVA). Values are mean ± standard error. Data are from Cohort 1.



Suppl 2 Fig. 2 *Lipid turnover in subcutaneous fat among subgroups.* The relationship between BMI and lipid age (A) or lipid storage (B) was determined in 48 non-obese and 30 obese subjects; the subgroups were analyzed separately. The relationship between lipid storage and HOMA-IR was determined in 48 non-obese healthy and 13 non-obese FCHL subjects put together (C) and separately in 30 obese subjects (D). Simple regression analysis was used. Data are from Cohort 1.



Suppl 2 Fig. 3 *Correlation between lipid turnover and adipocyte lipolysis stimulated by lipolytic agents.* Lipid age and lipid removal rates were related to the rate of glycerol release induced with different lipolytic compounds. A and B: Results with forskolin (C,D), a stimulator of adenylate cyclase which stimulates cyclic AMP production and isoprenaline. C and D: Results with a synthetic beta adrenergic receptor-selective catecholamine. Linear regression analysis was used. Data are from non-obese and obese individuals from cohort 1. Data with lipolytic agents versus lipid age were significant when analysed using body mass index as covariate in multiple regression analysis (partial r = -0.35; $p \leq 0.004$).

Supplement. 2. Table 1. Clinical data of individuals subdivided according to their diagnosis (Cohort 1). BMI cut-off point for obesity is >30 kg/m^2. Values are mean ± SE.

Group	Males/ females	Age, years	BMI, kg/m^2	Body fat, %	HOMA index*	Total plasma cholesterol, mmol/l	Plasma HDL cholesterol, mmol/l	Plasma triglyceride, mmol/l*	ApoB, g/l	Fat cell volume ,pico-litres	Fat cell morphology values, picolitres
Non-obese n=48	12/36	35 ± 9	23.6 ±2.1	28 ±7	1.5 ±0.8	5.0 ±1.0	1.5 ±0.4	1.0 ± 0.4	0.9±0.2	495 ±150	-2±152
Obese N=30	7/23	37±10	40.2±6.7	53 ± 15	6.3±5.5	5.5 ±1.1	1.1±0.3	2.5±1.8	1.2±0.2	865± 165	49 ± 197
FCHL n=13	10/3	44±10	25.5±2.3	28±9	2.4±1.5	7.2±1.2	1.1±0.3	4.6±4.9	1.5±0.4	495±140	-25± 132
p-value ANOVA		0.009	<0.0001	<0.001	<0.001	<0.0001	0.0004	<0.0001	<0.0001	<0.0001	0.31
Post-hoc p-value											
Non-obese obese		0.36	<0.0001	<0.0001	<0.0001	0.07	0.0005	0.009	<0.0001	<0.0001	-
Non-obese FCHL		0.002	0.13	0.67	0.02	<0.0001	0.002	<0.0001	<0.0001	0.99	-
Obese-FCHL		0.02	<0.0001	<0.0001	<0.0001	<0.0001	0.93	0.01	0.001	<0.0001	-

*10 log transformed values were used in the statistical tests. Results were compared by ANOVA and Fisher's PLSD post-hoc test.

**Similar results were obtained with a non parametric test.

Supplement 2. Table 2. Clinical data of subjects undergoing investigations of their subcutaneous adipocytes when fractionated according to fat cell size (Cohort 2).

Gender (F/M)	5/2
BMI (kg/m^2) (median and range)	30 (25-36)*
Age (years) mean ± standard error	34 ± 3

*3 obese and 4 non-obese were investigated.