LETTER

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Dynamics of human adipose lipid turnover in health and metabolic disease

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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

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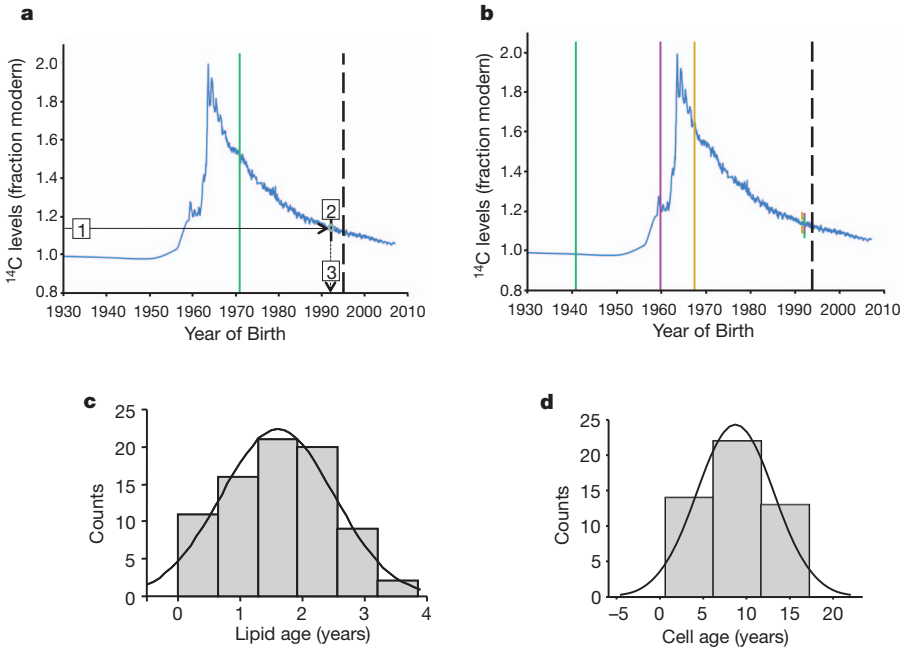

Figure 1 | Atmospheric ^{14}C over time and its use to determine lipid age and adipocyte age. **a**, Above ground nuclear bomb testing during the period of the cold war caused an increase in atmospheric levels of ^{14}C. These values decreased exponentially following implementation of a limited world-wide 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).

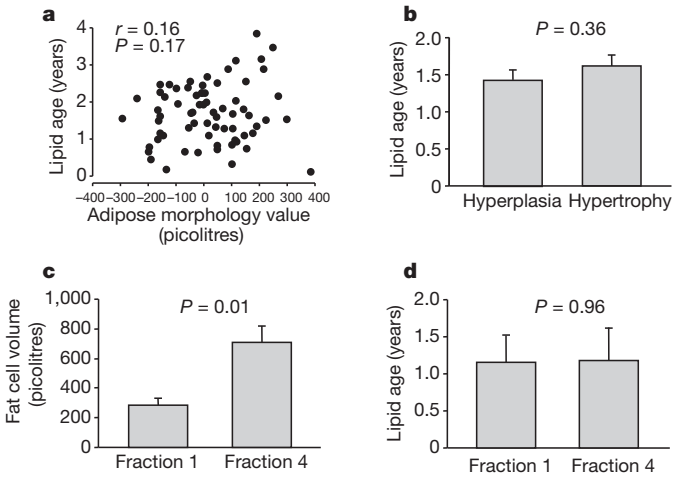

Figure 2 | Relationship between adipocyte size and lipid age. **a**, **b**, Influence of adipocyte cellularity on lipid age. Individuals were assigned a morphometric value, which is the difference between the measured adipocyte volume for the individual minus the average adipocyte volume for all subjects (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

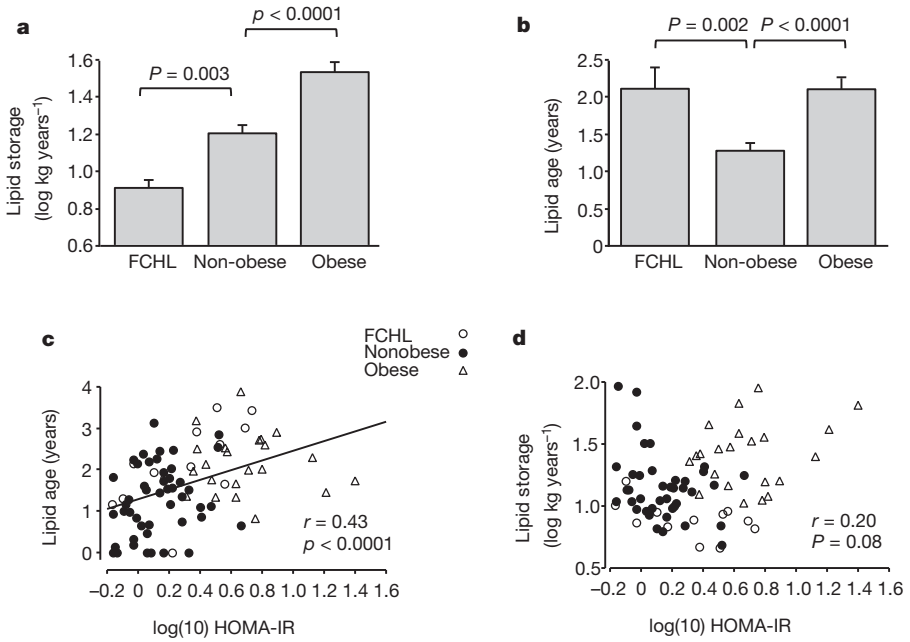

Figure 3 | Lipid turnover in subcutaneous fat. **a**, **b**, Lipid storage (**a**) and lipid age (**b**) were determined in 48 non-obese, 30 obese and 13 non-obese FCHL subjects. Error bars indicate standard error. Overall effect is $P < 0.0001$ by analysis of variance (ANOVA) in **a** and **b**. Results 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

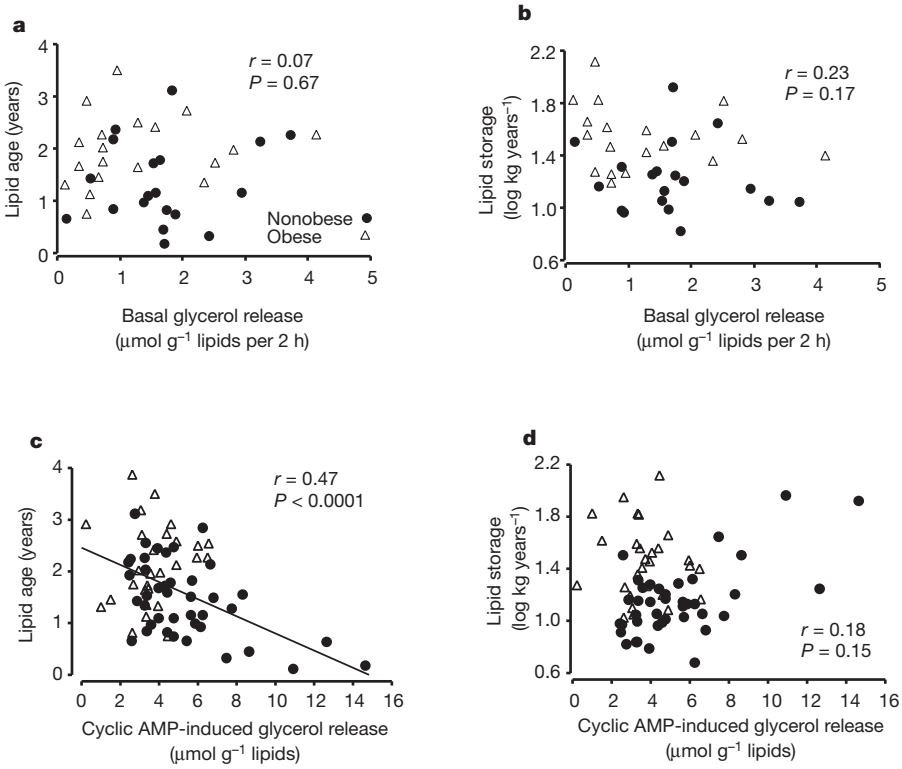

Figure 4 | Correlation between lipid turnover and adipocyte lipolysis. **a**–**d**, Lipid age and lipid removal rates were compared with basal rate of glycerol release (**a**, **b**) and with the rate of glycerol release induced with dibutyryl cyclic AMP (**c**, **d**), which is a phosphodiesterase-resistant 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 $r = -0.40$; $P = 0.0006$).

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.

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