Down-regulation of adipose tissue fatty acid trafficking in obesity: a driver for ectopic fat deposition?

Running title: Adipose tissue fatty acid trafficking in obesity

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**Objective:** Lipotoxicity and ectopic fat deposition reduces insulin signalling. It is not clear whether excess fat deposition in non-adipose tissue arises from excessive fatty acid delivery from adipose tissue, or impaired adipose tissue storage of ingested fat.

**Research Design and Methods:** To investigate this we used a whole-body integrative physiological approach with multiple and simultaneous stable-isotope fatty acid tracers to assess delivery and transport of endogenous and exogenous fatty acid in adipose tissue over a diurnal cycle in lean (n=9) and abdominally-obese men (n=10).

**Results:** Abdominally-obese men had substantially (2.5-fold) greater adipose tissue mass than lean controls but rates of delivery of non-esterified fatty acids (NEFA) were down-regulated, resulting in normal systemic NEFA concentrations over a 24-hour period. However, adipose tissue fat storage after meals was substantially depressed in the obese men. This was especially so for chylomicron-derived fatty acids, representing the direct storage pathway for dietary fat. Adipose tissue from the obese men showed a transcriptional signature consistent with this impaired fat storage function.

**Conclusions:** Enlargement of adipose tissue mass leads to an appropriate down-regulation of systemic NEFA delivery with maintained plasma NEFA concentrations. However, the implicit reduction in adipose tissue fatty acid uptake goes beyond this and shows a maladaptative response with a severely impaired pathway for direct dietary fat storage. This adipose tissue response to obesity may provide the pathophysiological basis for ectopic fat deposition and lipotoxicity.

The release of non-esterified fatty acids (NEFA) from upper body subcutaneous adipose tissue is a major determinant of systemic NEFA plasma concentrations (9) whereas visceral fat may contribute fatty acids (FA) specifically to the liver. Increased fasting concentrations of NEFA have been related to adipose tissue mass (10) and the presence of type 2 diabetes (11). However, complete absence of such relationships has also been described in non-diabetic people (12-14). After a single meal, the postprandial concentrations of NEFA tend to remain somewhat higher in obese compared with lean people (15; 16).

Although adipose tissue masses may vary over a 10-fold range between people, reported fasting NEFA
concentrations typically only vary within a 2-fold range. This clearly implies a restriction in the release of NEFA from obese people’s adipose tissue. Although the detailed mechanism for this restriction is unknown it has been observed that adipocytes isolated from overweight subjects respond subnormally to adrenergic lipolytic stimulation (17). However, it is less clear if uptake mechanisms of FA by adipose tissue are balanced by similar regulatory mechanisms. In the postprandial period, insulin stimulates fat deposition into adipose tissue. This occurs by hydrolysis of chylomicron triglycerides (TG) by lipoprotein lipase (LPL) bound to capillary endothelium, and subsequent uptake of the released FA by adipose tissue and re-esterification (storage). Following secretion of a single mixed meal, impaired postprandial plasma TG clearance by adipose tissue was reported in obese subjects (18). This is partly explained by a lower functional LPL activity per unit fat mass in combination with the absence of postprandial up-regulation of adipose tissue LPL in obesity (19). A pertinent question is therefore if adipose tissue in obese people, despite its enlargement, will induce its fat storing capacity in the postprandial state as efficiently as in lean people (20).

In line with the known down-regulation of lipolysis in obesity (17) and the absence of upregulation of adipose tissue LPL activity (19), we hypothesize that individuals with increased adiposity fail to appropriately up-regulate meal-fat storage. Therefore, we investigated the impact of obesity on systemic NEFA concentrations and NEFA delivery from adipose tissue, and then did detailed tissue-specific investigation of adipose tissue FA trafficking of endogenous and meal-derived FA over a 24-h period using stable isotope tracer methodology, along with transcriptional profiles of the FA trafficking pathways.

MATERIALS AND METHODS

Background population. The Oxford Biobank (21) is a population-based collection of healthy men and women aged between 30-53 years (n=1,900) which was used for the recruitment of participants. We analyzed fasting plasma TG, NEFA, insulin and glucose concentrations in male subjects within two BMI ranges, 19-25 kg/m^2 (lean) and 27-32 kg/m^2 (abdominally-obese), and age range 30-53 years (background cohort for the tissue-specific investigation). We excluded subjects with fasting hyperglycemia (> 6.1 mmol/L) and overt hypertriglyceridemia (>3.0 mmol/L). Fat mass was calculated from bioelectric impedance. Subcutaneous adipose tissue biopsies are available from 160 of the participants in the Oxford Biobank and we selected one lean (n=10, BMI: 23.4±0.3 kg/m^2) and an obese group of men (n=10, BMI: 33.6±1.7 kg/m^2) for studies of quantitative mRNA content of genes involved in FA trafficking.

Participants in the tissue-specific investigation. Nine lean and ten abdominally-obese males, comparable for age, were randomly invited from the Oxford Biobank and we selected one lean (n=10, BMI: 23.4±0.3 kg/m^2) and an obese group of men (n=10, BMI: 33.6±1.7 kg/m^2) for studies of quantitative mRNA content of genes involved in FA trafficking. All volunteers recruited were healthy non-smokers, not on any medication known to affect lipid metabolism and fulfilled the above inclusion criteria. We also required the obese participants to have a waist circumference >99cm. The study was approved by the Oxfordshire Clinical Research Ethics Committee and all subjects gave written informed consent.
**Study protocol:** Subjects arrived to the clinical research unit, having fasted from 10 pm the night before. They were asked to refrain from strenuous exercise and alcohol for 24 h before the study. Subcutaneous abdominal adipose tissue metabolism and blood flow was investigated by arterio-venous blood sampling and as described previously (20). A constant infusion of $[^2\text{H}_2]$palmitate (Cambridge Isotopes, CK gases Cambridge, UK) was given intravenously (0.01 µmol/kg per min) in a total volume of 400 ml human albumin (4.5%) over the 24h. The infusion was started more than ~60 min before the first blood sample.

Meals were given at time points breakfast (0), lunch (5h) and dinner (10h). The quantity of food was adjusted to individual basic metabolic rate (BMR) estimated by bioimpedance (1.25*BMR) and divided by three to give the number of calories in each meal. Food items were weighed to the nearest gram. Breakfast was tagged with 100 mg [U-$^{13}$C]linoleic acid, lunch and dinner were tagged with 100 mg [U-$^{13}$C]oleic and [U-$^{13}$C]palmitic acid, respectively. Over the 24 h period, there were 29 blood sampling time points with simultaneous sampling from arterial and adipose venous sites. Further details on the administration, methodology and calculation using the FA stable isotope tracer as well as biochemistry methods are essentially identical to a recent report (20) and also provided in the online appendix available at http://diabetes.diabetesjournals.org.

**RESULTS**

**Relationship between fat mass and NEFA concentration in the population.** We first studied the relationship between fasting plasma NEFA concentrations and fat mass in the background population of 244 lean (BMI 19-25 kg/m$^2$) and 210 abdominally-obese (27-32 kg/m$^2$) from the Oxford Biobank (21). Despite an almost 2-fold difference in total body fat mass between the groups, there was no difference in systemic NEFA concentrations (Table 1). However, compared with the lean group, the abdominally-obese men showed the expected higher fasting plasma TG, glucose and insulin concentrations, and lower HDL-cholesterol concentration.

**Effect of fat mass on expression of genes involved in adipose tissue fatty acid trafficking.** Of 12 selected candidate mRNA for key points in the regulation of FA trafficking genes 11 were significantly down-regulated in adipose tissue of obese men with the exception of

Adipose tissue mRNA quantification: After local anesthesia with 1% lignocaine, adipose tissue biopsies were taken from subcutaneous abdominal depots using a 12-gauge needle in the fasted state. Tissue samples were immediately extracted using TrizolR reagent (GIBCO-Life Technologies Inc., Grand Island, NY). Between 0.5 and 1 µg of RNA were reverse transcribed to cDNA using random Hexamers Primers and Invitrogen Super Script III First-Strand Synthesis System (Invitrogen, Carlsbad, CA). For the real-time quantitative PCR analyses, 4.5 µl of 1 in 100 or 1 in 20 dilution of cDNA per reaction were used in a final reaction volume of 10 µl. Seventeen genes were analyzed (ANGPTL4, LPL, GPIHBP1, CD36, ACLS1, AGPAT9, GPAM, DGAT1, DGAT2, HSL, PLIN, ATGL, ADBR2, LEP, CD68, CD11b and CD163) using predesigned TaqMan Assays-on-Demand (Applied Biosystems, Foster City, CA) using the ABI PRISM 7900HT Sequence Detection System (Applied Biosystems). All the samples were analyzed in quadruplicate and normalized to PPIA, and RPLP0.
CD36 (Supplementary Table 1). To confirm that the rather uniform down-regulation of genes involved in FA trafficking was not part of a universal effect brought about by obesity, we also analysed leptin (LEP) and CD68 mRNA, CD163 and CD11b as possible positive controls. The expression of the latter three mark macrophage infiltration in adipose tissue and are expected to be increased in obese subjects. All macrophage markers were increased (66-98%) (P<0.01 to P<0.001) whereas LEP was unchanged (+4%, ns). These findings suggest a co-ordinated down-regulation of genes involved FA trafficking in adipose tissue of abdominally-obese men.

Fatty acid trafficking. Subject characteristics: As in the background population, there was no difference in fasting NEFA concentrations between the lean (n=9) and the abdominally-obese (n=10) group with expected differences in fasting lipids and lipoproteins for the degree of obesity. Total fat mass was ~2.5 times higher in the abdominally-obese group (Table 1). There were clear signs of insulin resistance in the abdominally-obese group; fasting insulin was increased despite near-identical fasting blood glucose between the groups (Table 1). There were clear signs of insulin resistance in the abdominally-obese group; fasting insulin was increased despite near-identical fasting blood glucose between the groups (Table 1). Obese men had slightly higher ALT (p<0.05), which could be taken as an indicator of increased liver fat. We have recently reported on liver FA partitioning in these groups of people (22).

Diurnal plasma concentrations of TG, NEFA, glucose and insulin: In order to observe diurnal metabolic patterns using a physiologically relevant situation the participants were given three standardised meals, each separated by 5 hours. The significant differences in fasting TG and insulin between the groups were augmented postprandially (Table 1; Figure 1B, 1D), but the postprandial glucose concentrations were similar between the groups (Fig. 1C). As each meal was labelled with an isotopically-labeled FA, the plasma concentration of [13C]-TG reflected the chylomicron/chylomicron remnant TG concentrations; these were marginally higher in the abdominally-obese compared with the lean males (p=0.08, area under curve (AUC) for the 5-hour period after each meal intake, RM-ANOVA).

There were small differences in the postprandial NEFA concentrations (Fig. 1A) between the groups. Both groups showed distinct suppression of NEFA concentrations immediately after the first meal, but the abdominally-obese group remained ~100 µmol/l higher than the lean group within the first meal period (+40 min to +240 min, p<0.05 to p=0.003 for the 6 consecutive time-points measured). However, after this there were no statistically significant differences between the groups over the entire 24-hour period. Neither of the groups started to return towards the initial high fasting NEFA concentrations until the early morning.

Rate of appearance of NEFA in the systemic circulation: The diurnal NEFA delivery curves were essentially parallel between the groups. Although the fasting rate (790±100 (lean) vs 970±130 µmol/min (abdominally-obese)) was not statistically different between the groups, the time-averaged diurnal effect was very clear (450±35 vs 650±50 µmol/min, p=0.009) (Fig. 2B). Both groups showed an immediate reduction in NEFA delivery in response to the breakfast: the NEFA delivery was reduced by 38±6% (p=0.002) in the lean group with a less distinct response in the abdominally-obese group (-19±10% p=0.046).
Expressed as NEFA disappearance per unit of lean body mass, the difference between the groups disappeared (Fig. 2B). The rationale behind this is based on the assumption that NEFA delivery equals NEFA catabolism and that lean body mass is proportional to the skeletal muscle and liver. However, normalizing the NEFA delivery to fat mass the abdominally-obese group showed markedly lower fasting as well time-averaged diurnal NEFA delivery compared with the lean group (Fig. 2C). This highlights the down-regulation of NEFA delivery from the expanded adipose tissue in the abdominally-obese state.

**Adipose tissue blood flow:** The abdominally-obese men had a profoundly suppressed baseline adipose tissue blood flow (ATBF) compared with lean men (2.9±0.5 v 6.3±1.5 ml.100 g⁻¹.min⁻¹, respectively, p=0.01) and also displayed an unresponsive postprandial ATBF regulation after meal intake (Fig. 3A).

**Adipose tissue fatty acid trafficking:** To more precisely define the FA trafficking in subcutaneous adipose tissue we established arterio-venous blood sampling together with the ATBF measurements. In line with the RaNEFA per unit fat mass, the abdominally-obese males had significantly lower abdominal subcutaneous adipose tissue NEFA release, expressed per 100 g tissue, in the fasting state (p=0.008) and across the second and third postprandial periods (p≤0.015) (Fig. 3B), thus confirming the systemic measurements (RaNEFA normalized for fat mass). The fasting RaNEFA normalized to fat mass was correlated with net abdominal subcutaneous adipose tissue NEFA release (r=0.68, p<0.001, n=19).

Having established the quiescence in lipolytic function in the abdominally-obese adipose tissue, we focussed on FA uptake by the tissue with the hypothesis that this function would be equally suppressed. Fasting and postprandial removal of TG from blood across adipose tissue reflects LPL rate of action. A lower fasting absolute TG extraction across adipose tissue was observed in the abdominally-obese compared with the lean males (64±19 v 138±34 nmol.100 g⁻¹.min⁻¹, respectively, p=0.07) (Fig. 3C). Postprandially, the abdominally-obese males had an accentuated lower TG extraction (p<0.001 for all meal periods) (Figure 3C). While postprandial adipose tissue TG extraction increased with each meal in lean males (AUC: 360±109, 580±173, 633±114 µmol/100 g, first to third meal, respectively, p≤0.03), it remained unchanged in the abdominally-obese males (AUC: 122±30, 164±47, 142±31 µmol/100 g, first to third meal, respectively, p=0.18). The differences between the groups were further augmented when the extraction of TG carried in chylomicrons/chylomicron remnants were studied: the absolute extraction of \[^{13}\text{C}\]-TG across adipose tissue for all the meals was considerably lower per unit tissue in the abdominally-obese compared to the lean males (p<0.01) (Figure 3E, 3F).

As a significant proportion of the plasma TG is carried by VLDL, we explored the effect on VLDL-TG specifically by making use of the \[^{2}\text{H}_{2}\]palmitate accumulation in the whole plasma TG, which represents newly-synthesised VLDL. The \[^{2}\text{H}_{2}\]palmitate-TG in plasma rose to a plateau concentration after 150 minutes with distinctly higher concentrations in the abdominally-obese group compared with the lean (2.1±0.3 vs. 0.9±0.1 µmol/l, p=0.003), see Supplementary Figure 1. The fractional extraction of \[^{2}\text{H}_{2}\]palmitate-TG across
adipose tissue was similar between the groups (10.0±1.5 vs 13.3±1.7%, ns, average of the time period 150-900 min) despite the considerably higher arterial concentration of [²H₂]palmitate-TG. These data demonstrate a down-regulation of the efficiency of the uptake of FA from all triglyceride-rich lipoproteins in obese adipose tissue. It was therefore also pertinent to note that the glucose uptake by adipose tissue was much lower in the abdominally-obese men compared with the lean ones as a sign of generally lower metabolic substrate utilization (Fig 3D.).

The arterio-venous model allowed us to study the net movement of FA across the tissue bed, the transcapillary flux of FA (23). In the fasting state, the net movement of FAs was directed from adipose tissue to the capillaries and was lower in the abdominally-obese compared with the lean men (-700±210 vs -2950±860 nmol. 100 g⁻¹.min⁻¹, respectively, p=0.06). In both groups, the direction soon changed after the first meal and there was net deposition of FAs into adipose tissue (Fig. 4A).

In the lean men there was a three-fold increase in transcapillary flux of FAs in response to sequential meal intake (comparison of first to third meal, Fig. 4A). In the abdominally-obese men, the transcapillary flux of FAs was overall much lower (AUC for 5-hour postprandial periods p=0.026) and less dynamic with no increase in transcapillary flux with meal sequence (Fig. 4A). These findings were corroborated by studying the transcapillary flux of all [²H₂]palmitate-FA (composite measurement of VLDL-TG and direct uptake of NEFA, where the latter is the minor component (24)). This also showed a lower and non-dynamic flux of FAs in the adipose tissue in abdominally-obese compared with lean individuals (p=0.04, RM-ANOVA, Fig. 4B).

A proportion of FA released by LPL escape reesterification and this pathway has been denoted ‘spillover’ (25) which could make a contribution to the systemic NEFA concentrations (26). We therefore explored the quantitative significance of this pathway. In lean males, the chylomicron-TG derived ([U-¹³C]-FA, representing dietary-derived fat) spillover fraction decreased progressively across meal periods, as determined at the end of each postprandial period (65, 48, 37%, first to third meal, p=0.006) (Supplementary Fig 2A). However, in the abdominally-obese males, it remained unchanged across meal periods, and at a lower levels than in lean men (35, 24, 24%, first to third meal, p=0.13) (Supplementary Fig 2B). We then calculated the relative proportion of the systemic NEFA concentration that originated from spillover from the chylomicron-carried dietary fat. This shows that the proportion of NEFA derived from adipose tissue spillover is consistently lower (p<0.01) in the abdominally-obese men than their lean counterparts over the meal-feeding period (Supplementary Fig 2C).

Estimations of whole body FA deposition into adipose tissue. It is of importance to consider the expanded adipose tissue mass at whole body level in the abdominally-obese individuals to understand the adaptation of the system. In the calculation of whole body effects, we assume that all adipose tissues have similar handling of FA. Thus, accounting for total body fat mass, it was clear that the abdominally-obese males had lower total adipose tissue TG extraction compared with the lean men. Over the three consecutive meal periods they had progressively lower total adipose tissue
TG extraction: over the third meal period (meal-period AUC data from Fig. 3C multiplied by fat mass) the abdominally-obese men’s adipose tissue extracted only 53% (p=0.01) of what was seen in the lean men. The difference between the groups was further amplified when the same calculation was applied to the transcapillary flux of FAs (net fat storage) from the abdominal subcutaneous adipose tissue depot. Over the third meal period (meal-period AUC data from Fig. 4A multiplied by fat mass) the abdominally-obese men stored only 43% of the fat in adipose tissue compared with the lean men (p=0.03). As previously mentioned, the adipose tissue FA uptake from chylomicrons was particularly low in abdominally-obese compared with lean whereas the FA uptake from VLDL was less affected (the fractional extraction was similar between the groups, yet the concentration was higher in abdominally-obese men). The total AUC of the transcapillary flux of \( \text{[}^{2}\text{H}_2\text{]}\)-palmitate (dominated by the \( \text{[}^{2}\text{H}_2\text{]}\)-palmitate carried by TG in VLDL) factored up for fat mass, was twice as high in abdominally-obese compared with lean men (178±50 vs 73±14 \( \mu \text{mol/hour} \) p=0.049) (calculated from Fig 4B). This implies that a higher proportion of the FA stored in adipose tissue originates from VLDL-TG in abdominally-obese compared with lean men.

However, for glucose uptake by adipose tissue the differences between groups disappeared when accounting for total fat mass and net glucose disposal through adipose tissue is likely to be similar between lean and obese people.

In order to estimate the relative quantity of the meal fat that was stored in adipose tissue, the fat content of each meal was divided by the calculated whole body net fat storage during the three consecutive 5 h meal periods. In lean males, there was a progressive increase in the deposition of meal fat into adipose tissue with each meal (13%, 35%, 47%, first to third meal, respectively, p<0.001) (Fig. 4C). In contrast, the abdominally-obese males failed to increase fat storage with sequential feeding (6%, 25%, 18%, first to third meal, respectively, p=0.12) (Fig. 4C). This difference was highly statistically significant for the last meal period (p=0.001).

**DISCUSSION**

Upper-body subcutaneous adipose tissue is the main site of storage and release of FA to the systemic circulation (27) and by comparing lean healthy men with moderately abdominally-obese men, we describe a remarkable adaptation of the expanded adipose tissue. In terms of delivery of NEFA from the tissue, this adaptation appears to be entirely appropriate, resulting in normal diurnal regulation of NEFA concentrations in abdominally-obese men which is brought about by significantly diminished NEFA release rates per unit of adipose tissue. However, storage of primarily dietary FA the adipose tissue shows a corresponding reduction in the per unit uptake of FA in abdominally-obese men but also clear signs of maladaptation; the ability to induce fat storage was lost leading to a net deficiency in channelling dietary fat into adipose tissue. We propose that this is the pathophysiological basis for diversion of FA to be stored in organs not dedicated for fat storage, i.e. ectopic fat deposition.

One such organ is the liver, and in the loops of FA trafficking studied in this work this became very clear. In lean men a high proportion of chylomicron-TG was stored immediately in adipose tissue, whereas a lower proportion was seen in
abdominally-obese men. In contrast, FA originating from VLDL TG appeared to play a greater proportional role in adipose tissue fat storage in the abdominally-obese men. These events are linked, as insufficient extraction of chylomicron-TG produce chylomicron remnants, the TG content of which will be deposited in the liver and thereby provide substrate for VLDL-TG production. The liver in abdominally-obese men is therefore burdened by this seemingly ‘unnecessary’ loop of FA trafficking and also showed slightly higher plasma ALT concentrations as an indicator of increased liver fat content.

Obese subjects are often reported to have higher fasting and postprandial NEFA concentrations than lean subjects (10; 28) which is thought to be due to the expansion of fat mass (29). However, the diurnal systemic NEFA concentrations were essentially not different between lean and abdominally-obese males. Although, the suppression of systemic NEFA concentrations after the first meal was less marked in the abdominally-obese group, which is in line with observations made by others (16), over the 24-h period this was a negligible difference. Also, the fasting concentration of NEFA of much larger numbers of randomly recruited lean and abdominally-obese people were not different from each other. As this observation is made in moderate stages of obesity in seemingly healthy individuals, it suggests that NEFA oversupply to the systemic circulation might not be an early or universal feature of the metabolic complications of obesity. Not only does this finding concur with studies of BMI-matched insulin-resistant and insulin-sensitive men (14), but also with a more extreme example of obesity (13). They are also in line with a recent study by Mittendorfer et al., who describe a similar, but not complete, down-regulation of NEFA Ra in a group exhibiting a very wide range of body fat content (12). It is also noted that very large-scale observations in the Paris Prospective Study showed no association between BMI and fasting NEFA concentrations (30).

Given the similar systemic NEFA concentrations between the groups in the present study, despite a 2 to 2.5 fold difference in the in whole body adipose tissue mass, we interrogated in detail the mechanisms of NEFA delivery from the tissue. In line with earlier observations for LPL (31) and HSL and ATGL (32), we found lower expression of in obese/insulin resistant men, but our findings on transcript regulation also indicate a profound lowering of all processes involved in FA trafficking in obesity. Most NEFAs delivered from adipose tissue originate from intracellular TG via ATGL/HSL-mediated lipolysis. However, a smaller proportion is originating from spillover of FA derived from the LPL-mediated lipolysis of chylomicron and VLDL-TG and subsequently failing to undergo re-esterification. The spillover was generally lower and certainly less dynamic in the abdominally-obese group, both observed locally in the adipose tissue after each meal as well as its relative contribution to the systemic NEFA concentration. As the overall quantity of LPL-mediated FA delivery to the tissue was much smaller in the abdominally-obese group we conclude that once FA has been generated through this pathway they appear to be readily re-esterified. Accordingly, the re-esterification pathway does not seem to be subject to the same degree of down-regulation as the LPL step in obese adipose tissue.

A limitation of this study is the extrapolation of whole body fat storage
from the local measurement in the abdominal subcutaneous tissue knowing that fat depots have different characteristics in this respect. However, from the point of view of NEFA delivery from adipose tissue to the systemic circulation, this extrapolation has validity as the significant majority of NEFAs delivered to the systemic circulation originates from this depot (27). However, with the same argument, we find it difficult to raise support for the argument that excessive NEFA delivery from adipose tissue to the systemic circulation is an early determinant of the insulin resistance in obesity. We have only studied men extrapolations to women cannot be made.

In summary, adipose tissue shows appropriate adaptation to obesity in terms of systemic NEFA delivery and this is part of a global response (silencing of metabolic and vascular functions of the tissue), whereas the corresponding down-regulation of fat storage exhibit a maladaptive response with insufficient sequestration of dietary FA that may trigger ectopic fat deposition.

**Author Contributions.** FK, SmQ, and KNF wrote the manuscript, all co-authors researched the data, all co-authors reviewed/edited manuscript.

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Table 1. Age, anthropometric and biochemical characteristics of participants in the study.

<table>
<thead>
<tr>
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<th>Background population identified from the Oxford Biobank</th>
<th>Adipose tissue fatty acid trafficking study</th>
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<tr>
<td></td>
<td>Lean males (n=244)</td>
<td>Abdominally-obese (n=210)</td>
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<td>Lean males (n=9)</td>
<td>Abdominally-obese (n=10)</td>
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<td>Age (years)</td>
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<td>100 ± 6***</td>
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<td>Fat mass (kg)</td>
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<td><strong>Fasting lipids, insulin and glucose</strong></td>
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<td>Total cholesterol (mmol/L)</td>
<td>5.3 ± 0.9</td>
<td>5.7 ± 1.0***</td>
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<td>HDL cholesterol (mmol/L)</td>
<td>1.3 ± 0.3</td>
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<td>Triglyceride (mmol/L)</td>
<td>1.1 ± 0.5</td>
<td>1.6 ± 0.6***</td>
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<td>Non-esterifed fatty acids (µmol/l)</td>
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<td>449 ± 197</td>
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<td>Glucose (mmol/L)</td>
<td>5.2 ± 0.4</td>
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<td>Insulin (pmol/L)</td>
<td>46 ± 20</td>
<td>67 ± 29***</td>
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<td><strong>Time-averaged postprandial plasma concentrations</strong></td>
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<td>Triglyceride (mmol/L)</td>
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<td>Insulin (pmol/L)</td>
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<td>Glucose (mmol/L)</td>
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<td>NEFA (µmol/L)</td>
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<td>309 ± 36</td>
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Values are mean ± SD. *p<0.05, **p<0.01, ***p<0.001 comparing abdominally-obese and lean groups.

Figure legends

Figure 1
Arterial plasma concentrations of NEFA (panel A), TG (panel B), glucose (panel C) and insulin (panel D) in lean (closed circles) and abdominally-obese males (open circles).

Figure 2
\( R_{NEFA} \) in lean and abdominally-obese men shown as total body Ra and adjusted for lean and fat mass, respectively. The rate of appearance (Ra) of NEFA: whole body (panel A), expressed per lean mass (panel B) and per total fat mass (panel C) in lean (closed circles) and abdominally-obese men (open circles). Three meals were given as indicated by dotted vertical lines. The \( R_{NEFA} \) (µmol/min) was significantly higher in the abdominally-obese group compared with the lean group (Panel A, time * group \( p=0.009 \)). When the data was calculated and expressed as rate of disappearance \( (Rd_{NEFA}) \), i.e. normalized per lean body mass \( (\mu\text{mol.min}^{-1}[\text{lean mass (kg)}])^{-1} \), this difference disappeared \( (p=0.14) \). The abdominally-obese men has significantly lower \( R_{NEFA} \) when expressed per total fat mass \( (\mu\text{mol.min}^{-1}[\text{fat mass (kg)}])^{-1} \, p=0.029 \).

Figure 3
Adipose tissue blood flow and abdominal adipose tissue NEFA release, TG extraction and glucose uptake. The abdominally-obese (open circles) men had significantly lower adipose tissue blood flow (time*group, p=0.001) compared with the lean men (filled circles, Panel A). The release of NEFA and the extraction of TG from plasma (nmol. 100 g\(^{-1}\) min\(^{-1}\)) was significantly lower in the tissue of the abdominally-obese (open circles) men compared with lean (closed circles, Panel B and C, both time*group, p=0.001). Glucose uptake by adipose tissue was lower (p<0.001) in the adipose tissue in the abdominally-obese men (open circles) than in lean men (closed circles, panel D). The extraction of \(^{13}\)C-TG from each meal (meal 1 indicated by black circles, meal 2 by black triangles and meal 3 by open squares) in lean (Panel E) and in abdominally-obese men (Panel F) shows a diminution (p<0.01) of \(^{13}\)C-TG extraction in the abdominally-obese men in line with the net TG extraction (Panel C).

**Figure 4**
Total transcapillary flux of fatty acids across adipose tissue (panel A) in lean (filled circles) and abdominally-obese men (open circles) following ingestion of three meals (as indicated by dotted vertical lines). Panel B shows the transcapillary flux of \([^{2}\text{H}_2]\)-palmitate, which is dominated by the label carried in \([^{2}\text{H}_2]\)-TG (VLDL). Panel C shows The percentage of meal fat content deposited in adipose tissue after three sequential meals (Meal 1, Meal 2, Meal 3) in lean (black bars) and abdominally-obese (white bars) males. This was calculated from the transcapillary flux (Panel A) where each 5-h time period (AUC) multiplied for body fat mass to arrive at total body fat being stored. The denominator for each time period is the fat content in the meal. Lean men show a significant meal*fractional uptake effect (p=0.009), whereas the effect is statistically non-significant in the abdominally-obese men.
Figure 1
Figure 2

A

B

C

Ra NEFA μmol min⁻¹

Rd NEFA μmol min⁻¹ [lean mass (kg)]⁻¹

Ra NEFA μmol min⁻¹ [fat mass (kg)]⁻¹

Time (h)
Figure 3

A

Adipose tissue blood flow (ml/100g·min⁻¹)

0 5 10 15 20 25

B

AT NEFA release (nmol/100g·min⁻¹)

0 5 10 15 20 25

C

AT absolute extraction of TG (nmol/100g·min⁻¹)

0 5 10 15 20 25

D

AT glucose uptake (μmol/100g·min⁻¹)

0 5 10 15 20 25

E

13C-TG absolute extraction (nmol/100g·min⁻¹)

0 5 10 15 20 25

Lean

F

13C-TG absolute extraction (nmol/100g·min⁻¹)

0 5 10 15 20 25

Abdominally-obese
Figure 4