Uptake of individual fatty acids into adipose tissue in relation to their presence in the diet\textsuperscript{1–4}

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ABSTRACT

Background: The fatty acid composition of adipose tissue triacylglycerol reflects, but is not identical to, the fatty acid composition of the habitual diet.

Objective: We investigated whether the fatty acid composition of adipose tissue is explained by differences between fatty acids in early storage in adipose tissue after a meal.

Design: Nine healthy men ate a meal containing several fatty acids. Blood samples were taken for 6 h after the meal from an arterialized hand vein and a vein draining the anterior abdominal subcutaneous adipose tissue.

Results: Net storage of fatty acids in adipose tissue occurred between 1 and 4 h after the meal. In relation to the amount fed, storage of fatty acids differed \((P < 0.01)\) between classes \((n - 3)\) polyunsaturated < saturated < monounsaturated); oleic acid was stored in the greatest amounts. These differences agreed closely with published data, except for \(n - 3\) polyunsaturated fatty acids. The only individual metabolic step at which significant differences between fatty acids was shown was incorporation of fatty acids into chylomicron triacylglycerol. Differences between fatty acids in rate of extraction from chylomicron triacylglycerol and net uptake into adipose tissue in the postprandial period were significant \((P < 0.01)\), but not when expressed in relation to proportions in chylomicron triacylglycerol.

Conclusions: The characteristic fatty acid pattern of adipose tissue may predominantly reflect the early metabolic handling of different fatty acids. Adipose tissue uptake of \(n - 3\) polyunsaturated fatty acids is slow in relation to that of other fatty acids.


KEY WORDS Adipose tissue, chylomicrons, dietary fatty acids, lipoprotein lipase, postprandial period, triacylglycerol, fatty acid uptake, men

INTRODUCTION

The fatty acid composition of adipose tissue triacylglycerol has been used in epidemiologic studies as a marker of habitual dietary fatty acid intake. The composition of adipose tissue triacylglycerol is not identical to that of the diet, however. As for most biomarkers of habitual fatty acid intake, such as plasma cholesteryl ester fatty acids and red blood cell phospholipids, adipose tissue has a characteristic profile in which the proportion of monounsaturated fatty acids (MUFAs) is greater than that in the diet (1–5).

The mechanism by which this fatty acid profile arises is unclear. Saturated fatty acids (SFAs) and MUFAs can be synthesized de novo and so a close relation with dietary fatty acids is not necessarily to be expected. The characteristic pattern could also arise through differences in the early metabolic processing of individual dietary fatty acids, leading to greater storage for some relative to others. For instance, it has been suggested that long-chain \(n - 3\) polyunsaturated fatty acids (PUFAs) are not stored in the short term in adipose tissue (6, 7). Uptake of dietary fatty acids by adipose tissue involves several metabolic steps, including absorption in the small intestine, incorporation into chylomicron triacylglycerol, hydrolysis of this triacylglycerol by adipose tissue lipoprotein lipase (LPL), and uptake of fatty acids into and esterification within adipocytes. It was shown in rat chylomicrons in vitro that eicosapentaenoic acid (EPA; 20:5\(\text{n-3}\)) esters are relatively resistant to hydrolysis by LPL (8). Therefore, triacylglycerol and diacylglycerol species containing 20:5\(\text{n-3}\) may accumulate at the surface of chylomicron remnants and escape immediate uptake.

Alternatively, the characteristic fatty acid pattern of adipose tissue may be the result of interprandial remodeling. There are well-defined preferences for particular fatty acids in the process of mobilization from white adipose tissue in rats (9, 10) and rabbits (11). In vitro studies with rat adipocytes under conditions of stimulated lipolysis show that for a given fatty acid chain length, relative mobilization \([\text{calculated as the percentage of a particular fatty acid in total nonesterified fatty acid (NEFA) released from the cells divided by the percentage of that fatty acid in adipocyte triacylglycerol}]\) increases exponentially with increases in unsaturation (9). However, for a given degree of unsaturation, relative mobilization decreases as chain length increases (9). These differences (12) reflect characteristic preferences for particular fatty acids.

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differences in relative mobilization were also shown in humans after an overnight fast (12). Again, though, it is not clear how a preferential loss of unsaturated fatty acids would lead to an enrichment of MUFA's in adipose tissue triacylglycerol.

We reasoned that differences in the acute, postprandial uptake of fatty acids into adipose tissue could occur at many stages. There might be differential incorporation into chylomicron triacylglycerol or selectivity of the hydrolysis of particular fatty acids from chylomicron triacylglycerol by LPL. Furthermore, there might be preferential partitioning of those fatty acids released by LPL between tissue uptake or release into plasma. Because differences between fatty acids might be small, the best way to investigate these steps is to feed subjects a meal containing a range of fatty acids and to compare their representation at various stages of the pathway outlined above within individuals.

In the present study we report such studies. We compare the differential uptake of dietary fatty acids that we observed with published data on fatty acid concentrations in adipose tissue triacylglycerol and in the habitual diet. Some of the results were published previously in abstract form (13).

### SUBJECTS AND METHODS

#### Subjects

The subjects were 9 healthy men with a median age of 40 y (range: 21–53 y) and a median body mass index (in kg/m²) of 23.5 (range: 19.8–28.6). Subjects had a median fasting total cholesterol concentration of 4.5 mmol/L (range: 3.3–6.5 mmol/L), a median HDL-cholesterol concentration of 1.1 mmol/L (range: 0.7–1.7 mmol/L), and a median triacylglycerol concentration of 0.9 mmol/L (range: 0.5–2.7 mmol/L). The studies were approved by the Central Oxford Research Ethics Committee and all subjects gave informed consent.

#### Methods

A catheter (22 gauge × 10 cm, Secalon Hydrocat; Ohmeda, Swindon, United Kingdom) was introduced over a guide wire into a small vein on the abdominal wall and advanced until its tip lay near, but superior to, the inguinal ligament (14). As described previously (15), blood obtained from such a vein has all the characteristics expected of the venous drainage from adipose tissue. A cannula was inserted retrogradely into a vein draining a hand that was warmed in a box with an air temperature of 65°C to provide arterialized blood samples (16). Cannulas were kept patent with a slow infusion of saline; no heparin was used.

Simultaneous arterial and venous adipose tissue venous blood samples were collected at −20, 0, 30, 60, 90, 120, 180, 240, 300, and 360 min. A meal was given at 0 min. For consistency, the meal was prepared from standard ingredients and consisted of 100 g toasted white bread, 210 g peeled banana, and 150 g canned tuna fish with the brine drained off. To the tuna fish were added 25 g MaxEPA (Seven Seas, Hull, United Kingdom), 10 g shea stearine (Unilever Research, Sharnbrook, United Kingdom), and 6 g safflower oil. The meal contained 99 g carbohydrate and 44 g fat; the fatty acid composition is given in Table 1. Subcutaneous abdominal adipose tissue blood flow (ATBF) was measured with the 133Xe washout method immediately after each blood sample was taken (17, 18).

Plasma total NEFA and triacylglycerol and whole-blood glycerol concentrations were measured in both arterial and venous adipose tissue samples at all time points. Glucose and insulin concentrations were measured at all time points in arterial samples only. At 120, 180, 240, 300, and 360 min—when circulating chylomicron-triacylglycerol concentrations were expected to be readily measurable—the concentration and specific fatty acid composition of the chylomicron-triacylglycerol fraction in samples from both sites were analyzed. The specific fatty acid compositions of the arterial and venous NEFA fractions were analyzed at all time points and the composition of total plasma triacylglycerol was analyzed at all time points except −20 min. Plasma glucose was not measured in 3 subjects and the specific fatty acid composition of the total triacylglycerol fraction was not analyzed in 1 subject because of an insufficient sample volume.

#### Analyses

Heparin was added to a small portion of each arterial blood sample that was used for blood gas analysis and hematocrit estimation. Arterial oxygen saturation was always >97%. A portion of both arterial and venous samples was rapidly deproteinized with 7% (wt:vol) perchloric acid and the remainder was used to prepare plasma. Whole-blood glycerol and plasma NEFA and glucose concentrations were measured by using enzymatic methods with a Monarch centrifugal analyzer (Instrumentation Laboratory Ltd, Warrington, United Kingdom). Plasma triacylglycerol concentrations were also measured enzymatically (with correction for free glycerol) (19). Plasma insulin concentrations were measured by using a double-antibody radioimmunoassay method (Kabi Pharmacia Ltd, Milton Keynes, United Kingdom).

A chylomicron-rich fraction was prepared from plasma by layering 0.75-mL portions of plasma underneath a solution with a density of 1006 g/L in centrifuge tubes (11 × 34 mm) and centrifuging at 59900 × g at 4°C in a rotor type TLS 55 for 20 min in an Optima TLX ultracentrifuge (Beckman Instruments Ltd, High Wycombe, United Kingdom). The chylomicron-rich fraction was separated by slicing. For analysis of specific fatty acids, lipids were extracted from plasma or from the chylomicron-rich fraction by using chloroform:methanol (2:1, by vol). After separation of the lipid classes by thin-layer chromatography and methylation of fatty acids with methanolic sulfuric acid, gas chromatography was used to analyze the fatty acid composition.
The net transcapillary flux of individual and total fatty acids was calculated on the assumption that fatty acids are neither produced nor interconverted within the capillaries. Thus, net transcapillary flux represents the difference between fatty acids in arterial plasma (triacylglycerol and NEFA) and venous plasma (triacylglycerol and NEFA). It was calculated as follows:

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\text{Net transcapillary flux} = \frac{\text{(NEFA + 3 × TG)}_{\text{art}} - (\text{NEFA + 3 × TG})_{\text{cel}}}{\text{ATBF}}
\]

where concentrations are in whole blood. Positive values represent net uptake of fatty acid into adipose tissue; negative values represent net release. The calculation of net transcapillary flux involves 2 differences between quantities that may be relatively small. Some individual fatty acid values were therefore imprecise and were averaged over consecutive postprandial periods to improve precision: usually 60–120 min (shown as 90 min), 180–240 min (shown as 210 min), and 300–360 min (shown as 330 min). In these cases, derived values were calculated at each time point and then averaged. Because chylomicron triacylglycerol was not analyzed until 120 min, comparisons between net transcapillary flux and chylomicron-triacylglycerol composition were made by averaging over the period from 120 min.

Data were analyzed by using SPSS for WINDOWS (release 8.0; SPSS Inc, Chicago). Repeated-measures analysis of variance (ANOVA) was used to compare metabolite concentrations in each subject, analyzing the effects of time and the site from which the sample was taken, and where appropriate, to compare specific fatty acids. Analysis of covariance with fatty acid as a factor was used to look at relations between metabolic indexes (eg, net transcapillary flux of fatty acids versus the number of moles of that fatty acid in relation to the number of moles of all fatty acids in chylomicron triacylglycerol). When an overall effect of fatty acid type was found, post hoc tests with Bonferroni correction were conducted. For the 8 major fatty acids analyzed there were 28 possible comparisons, so post hoc P values were multiplied by 28 (21).

RESULTS

Adipose tissue blood flow, insulin, and metabolites

There was a significant postprandial rise in ATBF (P < 0.005), with a peak at 60 min, as found previously (18, 22). Arterial glucose and insulin concentrations (not shown) rose significantly after the meal (main effect of time, P < 0.005; repeated-measures ANOVA), both peaking at 60 min. Glycerol concentrations (not shown) were consistently higher in the adipose tissue venous effluent than in arterial blood (P < 0.001). Arterial and venous glycerol concentrations decreased after the meal (P < 0.001) and were lowest at 120 and 90 min, respectively.

Total plasma triacylglycerol and chylomicron triacylglycerol

Arterial plasma triacylglycerol concentrations peaked at 300 min (Figure 1). The A-V difference for triacylglycerol across adipose tissue also increased in the postprandial period, peaking at 180 min. The fractional extraction of triacylglycerol by adipose tissue was greatest at 180 min and triacylglycerol clearance was greatest at 240 min. Arterial and venous chylomicron-triacylglycerol concentrations, measured from 120 min onward, did not change significantly with time. The fractional extraction and clearance of chylomicron-triacylglycerol did not change significantly with time.
The fatty acid composition of arterial chylomicron triacylglycerol, expressed as a percentage of total chylomicron-triacylglycerol fatty acids, resembled, but was not identical to, the meal composition (Figure 2). The absolute concentration of individual fatty acids did not change significantly with time in either the arterial or the venous chylomicron-triacylglycerol fractions. When these concentrations were expressed as percentages of total chylomicron-triacylglycerol fatty acid concentrations, only the percentage of 20:5n−3 changed significantly ($P < 0.05$), increasing to a maximum at 300 min in arterial samples. There were significant differences ($P < 0.001$) between the molar percentages of fatty acids in chylomicron triacylglycerol and those in the meal, with palmitic acid (16:0) and oleic acid (18:1n−9) being especially overrepresented compared with 20:5n−3.

The fractional extraction and clearance of specific fatty acids within the chylomicron-triacylglycerol fraction did not change significantly with time (Figure 3). The A-V differences varied between individual fatty acids in the chylomicron-triacylglycerol fraction ($P < 0.001$; repeated-measures ANOVA), but when expressed as fractional extraction or clearance these differences between fatty acids were not significant. Additionally, the fractional extraction and clearance of the different fatty acids did not differ significantly from each other.

**Nonesterified fatty acids**

Total plasma NEFA concentrations were consistently higher in venous than in arterial plasma (Figure 1), indicating net release of fatty acids from adipose tissue. Concentrations and the V-A difference changed significantly with time; the lowest values were at 90 min. The composition of both the arterial and venous plasma NEFA fractions changed with time ($P < 0.001$) when the specific fatty acids were expressed as absolute concentrations or as percentages of the total NEFA concentration. In both the fasting state and after the meal, 16:0 and 18:1n−9 were the predominant fatty acids; 20:5n−3 and docosahexaenoic acid (22:6n−3) were initially present in negligible amounts, but after 360 min they represented $<1$% and 2%, respectively, of the arterial plasma NEFA fraction. The V-A differences for all of the individual fatty acids changed with time ($P < 0.001$; Figure 4). In particular, the V-A difference for 20:5n−3 and 22:6n−3 was initially close to zero, but increased significantly after the meal.

The net transcapillary flux of total fatty acids in adipose tissue changed significantly with time (Figure 5). Initially, the flux was negative, indicating net release of fatty acids from the adipocytes into the venous plasma as a result of fat mobilization in the fasting state. As anticipated, the flux then became positive, reaching a maximum between 90 and 180 min, indicating net...
uptake of fatty acids produced by the action of LPL from the capillaries into the adipocytes. It became negative again between 240 and 300 min, reflecting the return of net fat mobilization toward fasting values.

The net transcapillary flux of each specific fatty acid followed a similar pattern (not shown), becoming positive during the middle of the postprandial period (90–210 min). Net transcapillary flux differed significantly between different fatty acids. For the more highly represented fatty acids (eg, 18:1n−9 and 16:0), the flux began at a lower level (more negative) and became more positive during the intermediate postprandial period (fatty acid type × time interaction: \( P = 0.001 \); repeated-measures ANOVA). When the middle (positive) portion of the postprandial period (90–210 min) was examined, there was a significant main effect of fatty acid type (\( P < 0.001 \)).

These differences in transcapillary flux were clearly related to the proportion of each fatty acid in the chylomicron-triacylglycerol fraction. There was an approximately linear relation between representation in the chylomicron-triacylglycerol fraction and net uptake into adipose tissue between 120 and 240 min (Figure 6). When net uptake was expressed as a ratio to the proportion of that fatty acid within each person, it did not differ significantly between fatty acids. However, when net uptake was expressed in the same way in relation to the proportion of that fatty acid in the meal, there were significant differences between fatty acids (Figure 7).

Comparison with other studies

Data were taken from other published studies in which proportions of different classes of fatty acids (SFAs, MUFAs, and PUFAs) in the habitual diet were compared with proportions in adipose tissue triacylglycerol (1–5, 23–25). [One of these studies (25) was of subjects with type 2 diabetes and was included because few data for n−3 PUFAs were available; only the data for n−3 PUFAs from that study were included.] The ratio of the proportion in adipose tissue triacylglycerol to that in the diet was calculated for each of these fatty acid classes. Our data for the ratio of net uptake into adipose tissue over the middle part of the postprandial curve (90–210 min) were summed for the fatty acids in each group (SFAs: 14:0, 16:0, and 18:0; MUFAs: 16:1n−7 and 18:1n−9; n−6 PUFAs: 18:2n−6; and n−3 PUFAs: 20:5n−3 and 22:6n−3) and expressed as a ratio to the molar proportion of that fatty acid group in the meal. These data were then compared with the published data (Figure 8). There was striking agreement between the published data and the ratios in the present study for SFAs, MUFAs, and n−6 PUFAs. For n−3 PUFAs, the present data were lower than any of the 4 published sets of data.

DISCUSSION

We showed that there are differences in the extent to which individual fatty acids in the diet are taken up into subcutaneous adipose tissue in the short term. For SFAs, MUFAs, and n−6 PUFAs, these differences closely reflect the differences noted in other studies between the proportions of various fatty acids in the habitual diet and those in adipose tissue triacylglycerol.

It is surprising that these clear differences emerged from such a short-term study. The processes by which dietary fatty acids are stored in adipose tissue are complex, as shown in studies in which a radiolabeled fatty acid was given orally. The label continued to appear in adipose tissue over several weeks, reaching a maximum at 1 mo (26). Such results imply that dietary fatty acids may enter other body pools and perhaps recycle through VLDL many times before eventual storage in adipose tissue.
However, it may be that the specificity of the different processes involved is similar to the more acute processes studied here.

The main result of these studies is that the major step at which differences in storage of fatty acids occur is an early one, that of intestinal absorption and incorporation into plasma chylomicron triacylglycerol. Beyond that, although we showed marked differences between fatty acids, for instance in extraction from chylomicron triacylglycerol and in net uptake, these differences were largely explained by differential representation of the fatty acids in chylomicron triacylglycerol. The fatty acid composition of chylomicron triacylglycerol was examined before. In humans, Wood et al (27) found the proportion of 18:1n-9 to be greater in chylomicron triacylglycerol than in the meal; Griffiths et al (28) found the proportion of 16:0 and Sakr et al (29) found the proportion of both 16:0 and 18:1n-9 to be greater in chylomicron triacylglycerol than in the meal. Therefore, our data fit broadly with this pattern. The relation between meal and chylomicron-triacylglycerol composition appears to be determined by the fatty acid content of the meal. In studies in rats, the proportion of 16:0 in chylomicron triacylglycerol was lower than that in the test meal when the meal was high in 16:0, but was higher in chylomicron triacylglycerol when the meal was low in 16:0 (30). It could be that the fatty acids that had lower proportions in chylomicron triacylglycerol than in the meal were present in the chylomicron-phospholipid fraction. We analyzed this fraction to test this possibility but did not present the results in detail for simplicity. In fact, very small amounts of each fatty acid were found in the phospholipid fraction relative to the triacylglycerol fraction.

It was also surprising that we found no selectivity of LPL action on chylomicron triacylglycerol because, in some studies in vitro, its action decreases with increasing chain length (8, 31), although other authors did not show fatty acid preferences (32). However, we showed previously that many features of LPL action seen clearly in vitro are not reproduced in vivo, suggesting that LPL acts very efficiently in vivo in a highly structured environment (33). Again, it was surprising that the fate of fatty acids beyond removal by LPL—release as NEFA into the plasma or uptake into adipose tissue—did not differ significantly. There is some circumstantial evidence that SFAs are more likely to be released into the plasma after the action of LPL (28, 34), although this was not confirmed at the level of adipose tissue in vivo (33, 35).

Acute storage of n-3 PUFAs relative to the amount fed was lower than in the published long-term comparisons of diet and adipose tissue (Figure 8). The amounts of these fatty acids were lower in chylomicron triacylglycerol than in the test meal. It was shown

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**Figure 6.** Relation between net uptake of fatty acids into adipose tissue (net transcapillary flux) and representation in arterial chylomicron triacylglycerol (TG) over the middle postprandial period (120–240 min). Saturated fatty acids are indicated by the filled circles. $r^2 = 0.713, P = 0.000$ (analysis of covariance, with fatty acid as a factor).

**Figure 7.** Mean (±SEM) net uptake of individual fatty acids into adipose tissue (transcapillary flux at 90–210 min) expressed as a ratio to the molar percentage of the fatty acid in the meal. There was a significant main effect of fatty acid type, $P = 0.007$ (repeated-measures ANOVA). $n = 9$. 

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in rats that endogenous 20:5n−3 and 22:6n−3 may be added to chylomicrons when their presence in the diet is low (30). However, in that study, chylomicron lipids were not separated and it may be that the n−3 PUFAs are added to the phospholipid component of chylomicrons, whereas we looked specifically at chylomicron triacylglycerol. In the present study as well, the appearance of these fatty acids in chylomicron triacylglycerol occurred relatively slowly; the amount of 20:5n−3 increased with time during the experiment, whereas a steady proportion of the other fatty acids was maintained throughout the time of sampling. Sinclair and Gale (7) reported that consumption of an Eskimo diet led to only a small accumulation of 20:5n−3 in adipose tissue over 100 d, and Wood et al (6) claimed that 20:5n−3 is not stored in adipose tissue (6). More recent studies, however, showed that they are clearly present in adipose tissue triacylglycerol, in amounts that reflect habitual fish intake (4, 24, 36). Although net storage of n−3 PUFAs in adipose tissue in this acute experiment was low relative to that of other fatty acids, it certainly occurred. It seems, therefore, that storage of n−3 PUFAs is relatively slow and that to some extent they cycle through other lipid pools before storage. In the present study it was clear that a proportion of the n−3 PUFAs released by the action of adipose tissue LPL was delivered as plasma NEFA (Figure 4) and the concentrations of nonesterified n−3 PUFAs increased from <1 μmol/L in the fasting state to an average of 18 μmol/L at 360 min after the meal. These fatty acids would be available for hepatic uptake and potential recycling in lipoproteins.

One limitation of our data must be borne in mind. A-V differences are unreliable in non-steady states; the uptake of substrates is overestimated when their plasma concentrations are rising and vice versa (37, 38). Plasma concentrations of total NEFAs, individual fatty acids, and triacylglycerols were certainly changing during the period of study, and the concentration of 20:5n−3 changed in the chylomicron-triacylglycerol fraction. We tried to overcome these limitations by averaging data over periods that would be long in comparison with transit times through adipose tissue. We suggested previously that the problem of non-steady states is most acute for water-soluble substances that need to equilibrate with tissue water (39). Therefore, we believe that our measurements of net fluxes of hydrophobic molecules (fatty acids and triacylglycerol), which were made over relatively longer periods, were not substantially affected. In any case, there is no reason to suppose that non-steady-state conditions would introduce differential effects between individual fatty acids.

We showed that the relation between the composition of adipose tissue triacylglycerol stores and dietary fatty acid intake is largely determined by early metabolic events. This is again surprising in view of the well-documented selective mobilization of specific fatty acids during times of fasting (9, 11, 12, 40), in which the shorter the chain length and the higher the degree of unsaturation, the more highly mobilized is the fatty acid in relation to adipose tissue stores. That process might be expected to lead to significant remodeling of adipose tissue triacylglycerol. It is not immediately clear how these observations may be reconciled. One possible explanation is that selective mobilization of fatty acids from adipocytes was shown mainly in vitro under conditions of stimulated lipolysis (9, 40), and the one human study agreeing with these studies in vivo was carried out after a prolonged fasting period (12). It is therefore possible that, because humans spend most of their day in a postprandial condition, the selective mobilization of fatty acids seen under postabsorptive conditions does not have a major effect on the adipocyte fatty acid composition and therefore does not need to be balanced by the preferential uptake of specific fatty acids.

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