

Release of individual fatty acids from human adipose tissue in vivo after an overnight fast

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Abstract The objective of this study was to investigate the mobilization of individual fatty acids from human subcutaneous adipose tissue in vivo. Concentrations of individual non-esterified fatty acids were measured in arterialized plasma and in the venous drainage from subcutaneous abdominal adipose tissue in eight normal subjects, after an overnight fast, and for the subsequent 6 h. Whilst the veno-arterial concentration difference for non-esterified fatty acids increased over this period, the relative proportions of different fatty acids remained constant. There was a close relationship between veno-arterial difference and arterialized concentration for the different fatty acids. The arterialized concentration of stearic acid consistently lay above the regression line drawn for unsaturated fatty acids ($P = 0.001$), probably reflecting lower clearance of stearic acid. The release of individual fatty acids was compared with their prevalence in adipose tissue triacylglycerol in biopsies taken from six subjects. Relative release decreased with increasing chain length, and for a given chain length increased with increasing unsaturation, in agreement with animal studies ($P < 0.001$ for differences in relative mobilization according to these two factors). ■ The results suggest that the systemic plasma concentration of individual non-esterified fatty acids is determined by their release from adipose tissue, but that the relationship between release and systemic concentration for stearic acid is different from that for other fatty acids. The results confirm, in humans, differences in relative mobilization found previously in animal studies.—Halliwell, K. J., B. A. Fielding, J. S. Samra, S. M. Humphreys, and K. N. Frayn. Release of individual fatty acids from human adipose tissue in vivo after an overnight fast. *J. Lipid Res.* 1996. **37**: 1842–1848.

Supplementary key words adipose tissue triacylglycerol • fat mobilization • fatty acid clearance • stearic acid

Studies in rats and rabbits have suggested that the mobilization of non-esterified fatty acids (NEFA) from adipose tissue may be selective, with preferential release of shorter-chain rather than longer-chain, and of polyunsaturated rather than saturated, fatty acids (1–4). In these studies the animals have usually been fed on special diets in order to enrich the adipose depots with particular fatty acids (1–4). It is not known whether the

phenomenon of selective fatty acid mobilization occurs to any significant extent in humans in their normal dietary state. There have been some studies of the turnover of individual plasma NEFA in humans (5, 6), but the range of fatty acids studied has not been large, because of the limited availability of tracers. The plasma NEFA profile changes during exercise, a state of rapid fat mobilization, to a more unsaturated pattern, and it has been suggested that this may reflect selective mobilization of different fatty acids (7), but there is little other evidence on this point in humans.

In this study we have assessed the relative mobilization of different fatty acids from human adipose tissue in vivo by measurement of arteriovenous differences across a subcutaneous adipose depot. In the postprandial period NEFA release from adipose tissue reflects the action of lipoprotein lipase (LPL) as well as that of the intracellular hormone-sensitive lipase (HSL) (8). We have therefore studied the period from 14 to 20 h of starvation when fatty acid release is dominated by the release of intracellular fatty acids (9). Because we wished to study humans in their normal dietary state, plasma concentrations of some of the fatty acids of interest were very low, and the measurement of veno-arterial differences was, therefore, potentially subject to considerable analytical imprecision. By taking sequential samples over a 6-h period we have increased the precision of measurement of these concentrations.

Some of the results have been reported previously in abstract form (10).

Abbreviations: BHT, butylated hydroxytoluene; HSL, hormone-sensitive lipase; LPL, lipoprotein lipase; NEFA, non-esterified fatty acids.

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METHODS

Subjects and experimental protocol

Eight normal subjects (2 female) were studied. Their ages were 19–43 yr (median 32), their body mass indices 20.0–25.3 kg · m⁻² (median 21.9). They were a sub-group of the subjects described elsewhere (9): all were normolipidemic and the females were not taking oral contraceptive agents. They gave informed consent to participate in the study, which had been approved by the Central Oxford Research Ethics Committee. They arrived at the laboratory after a normal day, but had refrained from smoking or unaccustomed exercise. They were given a low-fat meal (2.52 MJ, 53 g carbohydrate, 21 g fat) at 18:00 h. A catheter (Secalon Hydrocath 22-gauge, 10 cm; Ohmeda, Swindon, UK) was then introduced into a superficial vein draining the subcutaneous abdominal adipose tissue and threaded antero-gradely over a guide wire until its tip lay near to the inguinal ligament, as described previously (11). We have shown that blood drawn from such a catheter represents almost pure drainage from adipose tissue with at most a minor contribution from the overlying skin and no detectable contribution from muscle (12). The catheter was filled with saline, taped to the skin, and the subject slept in the hospital overnight. At 07:00 the following morning a cannula was introduced into a vein draining a hand, which was then warmed in a box maintained at 65°C to provide arterialized blood (13). From 08:00 pairs of arterialized and adipose venous blood samples were drawn hourly until 14:00. During this time the subject lay quietly on a bed.

Analytical methods

Blood samples were taken into heparinized syringes (Monovette, Sarstedt, Leicester, UK). Plasma was rapidly separated at 4°C and stored at -20°C before analysis. Plasma samples (0.5 or 0.75 ml) were extracted by the method of Folch, Lees, and Sloane Stanley (14) in tubes containing heptadecanoic acid as an internal standard, and butylated hydroxytoluene (BHT) at 50 mg/l. The NEFA fraction was isolated on thin-layer chromatography with BHT at 500 mg/l, and fatty acids were methylated using methanolic sulfuric acid as described previously (15). Fatty acids were separated and quantitated by gas chromatography (Chrompack 9000, with FFAP-CB 25 m × 0.32 mm fused silica capillary column; Chrompack UK, Milharbour, London, UK).

The following fatty acids were resolved and measured on most samples: C12:0, C14:0, C16:0, C16:1, C18:0, C18:1, C18:2, C18:3, C20:0, C20:1, C20:3, C20:4, C20:5, C22:0, C22:5, and C22:6. Different isomers of C18:3 were usually resolved, and of C18:1 sometimes resolved, but they have been combined for the purposes of this

analysis. The plasma NEFA concentration was estimated from the sum of the individual fatty acids. It was also compared with an enzymatic measurement of plasma NEFA concentration (Wako NEFA-C, from Alpha Laboratories, Eastleigh, UK, adapted to an IL Monarch centrifugal analyzer, Instrumentation Laboratory, Warrington, UK). The mean recovery (gas chromatography versus enzymatic) was 104 ± 30 (SD) % on 102 samples.

Adipose tissue biopsies

Adipose tissue needle-biopsies were obtained by aspiration (Vacutainer, Becton-Dickinson, Cowley, UK) (16) from the subcutaneous abdominal adipose tissue in the region drained by the catheter, either at the end of the study or on another occasion as near as possible to the study day. Triacylglycerol-fatty acids were analyzed by the procedure described above but without an internal standard. Proportions of fatty acids were expressed as molar percentages.

Completeness of data and statistical analysis

For one male subject (not biopsied) plasma samples were not available at 08:00, nor at 13:00 and 14:00. From another male subject the 08:00 samples were not available. From one female subject the 12:00 samples were not available. Biopsies were only obtained from six subjects; their composition has been analyzed in comparison only with the plasma concentrations from the same six subjects.

Changes with time were assessed by repeated measures ANOVA. Other statistical methods used are described in the text. All were performed using SPSS for Windows Release 6.1 or 7.1 (SPSS Inc., Chicago, IL).

RESULTS

Arterialized and adipose venous plasma concentrations

The adipose venous NEFA concentration was consistently around twice the arterialized concentration (Fig. 1A), as we have observed before using enzymatic methods of analysis after an overnight fast (11). Whilst the arterialized NEFA concentration was steady over the subsequent 6 h, the adipose venous concentration rose steadily with continued fasting. The veno-arterial differences for individual plasma NEFA followed the same pattern (Fig. 1B). For further analysis, the plasma concentrations and veno-arterial differences were averaged over the seven time-points within each subject.

The mean plasma concentrations of the individual fatty acids in the eight subjects are shown in Table 1. The table emphasizes the low concentrations of some of the individual fatty acids.

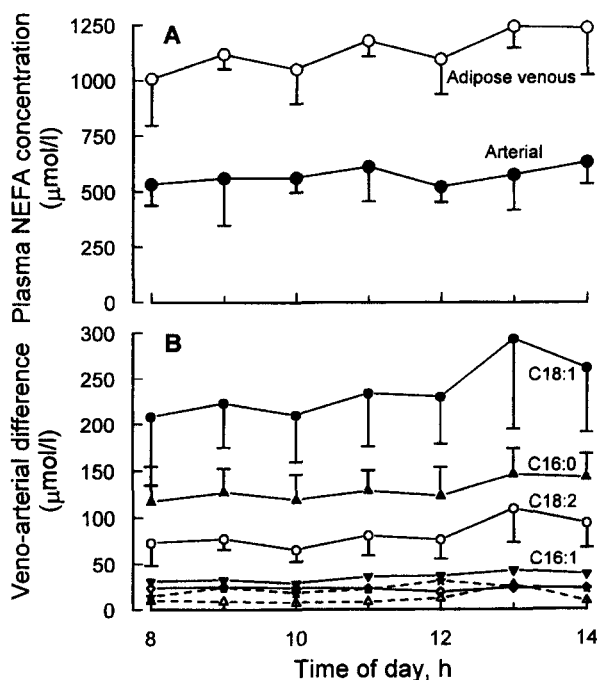


Fig. 1. Plasma concentrations and veno-arterial differences for plasma non-esterified fatty acids (NEFA). A: total plasma NEFA concentrations in arterialized (●) and adipose venous (○) plasma. Repeated measures ANOVA shows site difference (arterial versus venous) significant at $P < 0.01$, effect of time $P = 0.06$. Regression of mean values on time: arterialized, nonsignificant; adipose venous, $P < 0.02$; veno-arterial difference, $P < 0.05$. B: veno-arterial differences for individual fatty acids: ●, C18:1; ▲, C16:0; ○, C18:2; ▼, C16:1; ★, dashed line, C14:0; ◇, C18:0; Δ dashed line, C18:3. Values are shown as mean \pm SEM, but error bars are omitted from minor fatty acids for clarity. Only individual fatty acids whose average veno-arterial difference exceeded 10 $\mu\text{mol/l}$ are shown.

There were differences in absolute NEFA concentrations amongst the eight subjects. Those subjects with higher total NEFA concentrations tended to have larger veno-arterial differences, and this pattern extended to the individual fatty acids (Fig. 2). This was also true when comparing one fatty acid with another within subjects. Thus, within each of the eight subjects there was a relationship between veno-arterial difference and arterialized concentration over the different fatty acids ($r =$ from 0.85 to 0.99, each $P < 0.001$).

Concentrations of each fatty acid (already averaged over time-points within subjects) were averaged over all subjects. Figure 3 shows that there was a close relationship between the mean arterial concentration for each fatty acid, and its mean veno-arterial difference. It was notable, however, that both palmitic acid and stearic acid lay away from the regression line of arterialized concentration against veno-arterial difference. This was tested by calculation of the regression line omitting saturated fatty acids in individual subjects, and then predicting the arterialized concentrations of palmitic

and stearic acids from their veno-arterial differences. For every subject the measured concentration of stearic acid was greater than predicted from the relationship amongst unsaturated fatty acids (predicted mean 23.1 $\mu\text{mol/l}$, actual mean 50.5 $\mu\text{mol/l}$; $P = 0.001$ by paired t -test). For palmitic acid the discrepancy was neither so large nor so consistent (predicted mean 108 $\mu\text{mol/l}$, actual mean 127 $\mu\text{mol/l}$; not significant by paired t -test). Repeating this procedure on the group-average data (shown in Fig. 3) for stearic acid, the predicted arterialized concentration is 22.1 $\mu\text{mol/l}$, compared with actual mean concentration of 50.5 $\mu\text{mol/l}$.

Mobilization of fatty acids in relation to adipose tissue content

The composition of adipose tissue triacylglycerol fatty acids in the six subjects from whom biopsies were taken is shown in Table 1.

Results for the longer-chain polyunsaturated fatty acids, whose concentrations were very low, were variable from subject to subject, and the data on Fig. 4 are based on group mean values for the six subjects from whom biopsies were obtained. Figure 4 shows average mobilization (mean veno-arterial difference over 6 h) expressed in relation to adipose tissue content, all normalized arbitrarily to palmitic acid, for all fatty acids whose veno-arterial concentration difference expressed in this way exceeded 1 unit (i.e., was greater than 1% of the value for palmitic acid). The data are shown in two ways. In Figure 4A, both tissue content and veno-arterial

TABLE 1. Plasma concentrations and adipose tissue contents of the fatty acids measured

	Plasma Concentration		Adipose Tissue Biopsy
	Arterialized	Adipose Venous	
	$\mu\text{mol/l}$		molar percentage
C12:0	18.2 \pm 3.4	29.3 \pm 6.1	1.32 \pm 0.33
C14:0	25.9 \pm 2.2	48.8 \pm 6.7	4.73 \pm 0.22
C16:0	126.6 \pm 14.4	258.9 \pm 33.7	26.25 \pm 0.52
C16:1	24.8 \pm 4.4	61.5 \pm 12.4	3.97 \pm 0.44
C18:0	50.5 \pm 5.3	73.4 \pm 8.1	5.44 \pm 0.39
C18:1	192.9 \pm 32.1	442.3 \pm 82.5	39.65 \pm 0.77
C18:2	71.0 \pm 8.6	154.5 \pm 23.5	15.12 \pm 0.97
C18:3	8.6 \pm 1.3	19.8 \pm 4.0	0.78 \pm 0.08
C20:0	3.1 \pm 0.4	4.6 \pm 0.9	0.31 \pm 0.03
C20:1	3.7 \pm 0.8	4.1 \pm 0.8	0.67 \pm 0.09
C20:3	7.6 \pm 0.9	8.9 \pm 1.2	0.37 \pm 0.07
C20:4	9.6 \pm 1.4	12.3 \pm 2.0	0.28 \pm 0.10
C20:5	10.4 \pm 2.4	12.0 \pm 2.4	0.23 \pm 0.09
C22:0	9.9 \pm 1.2	9.7 \pm 1.5	0.28 \pm 0.09
C22:4	8.9 \pm 3.7	14.5 \pm 4.0	0.25 \pm 0.13
C22:5	8.0 \pm 1.9	8.3 \pm 1.9	0.20 \pm 0.07
C22:6	5.7 \pm 1.2	7.2 \pm 1.3	0.13 \pm 0.03

For plasma, data were averaged within subjects over the 16-h period of study. Results are shown as mean \pm SEM for eight subjects (plasma data) and for six subjects (biopsy data).

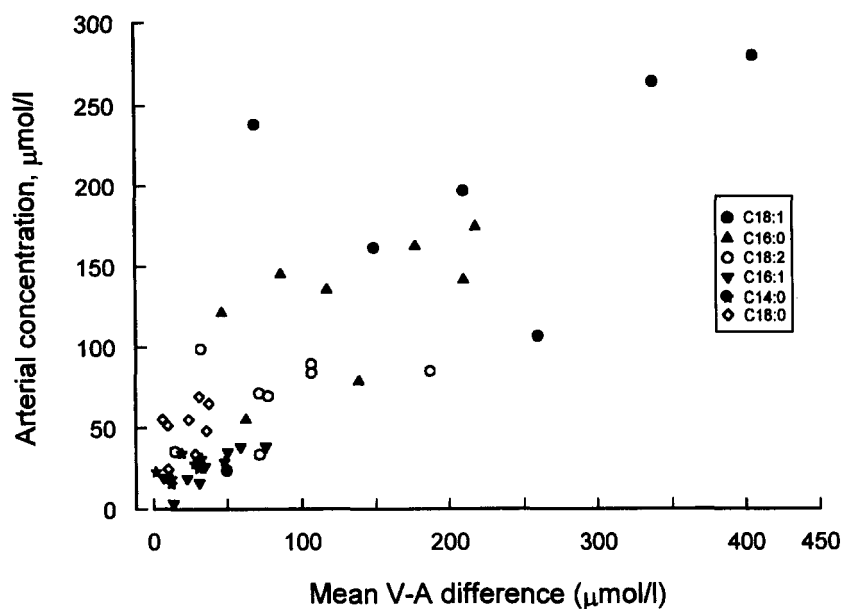


Fig. 2. Relationship between arterial concentration and veno-arterial concentration difference across adipose tissue for individual fatty acids. Each point represents the concentrations averaged over the 6-h study period in one individual. Only individual fatty acids whose average arterial concentration exceeded 10 $\mu\text{mol/l}$ are shown.

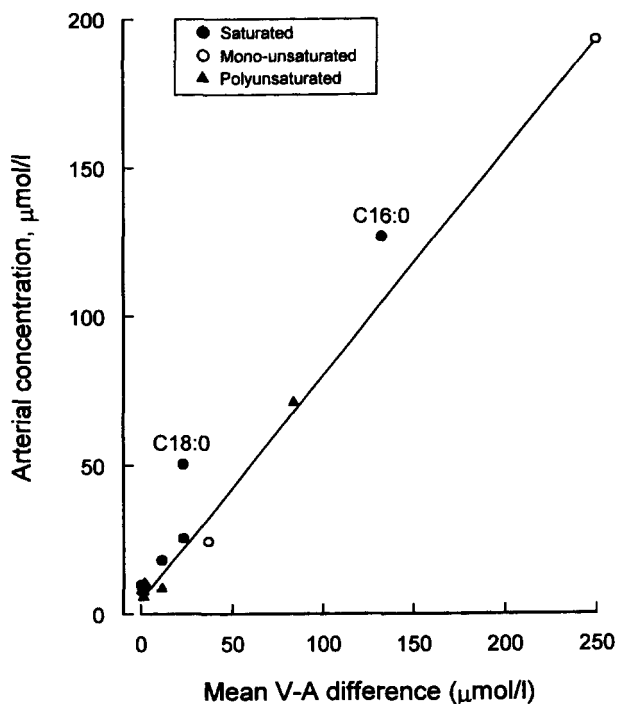


Fig. 3. Relationship between average arterial concentration and average veno-arterial concentration difference across adipose tissue for individual fatty acids. Each point represents the mean concentrations for the six subjects. Only individual fatty acids whose average arterial concentration exceeded 5 $\mu\text{mol/l}$ are shown. The regression line is drawn for all unsaturated fatty acids: $r = 0.98$, $P < 0.001$.

difference are expressed relative to palmitic acid as 100; this shows the low prevalence of the longer-chain fatty acids. In Figure 4B, mobilization of each fatty acid is expressed relative to its tissue content (set at 100%). For a given degree of unsaturation, the relative mobilization decreased with increasing chain length. For instance, for the series C12:0 to C20:0 the correlation coefficient for relative mobilization versus number of carbon atoms, as quoted by Connor, Lin, and Colvis (3) for their data in rabbits, was -0.88; for the series C16:1 to C20:1 it was -0.99. In contrast, for a given chain length, the relative mobilization increased with increasing unsaturation. For the series C18:0 to C18:3 the correlation coefficient for relative mobilization versus number of double bonds was +0.87, and for the series C20:0, C20:4, C20:5, C22:6 (shown on Fig. 4B) it was +0.96.

The significance of these differences in relative mobilization was tested as follows. We used a generalized linear model with relative mobilization (calculated as in Fig. 4B) as the dependent variable, subject as a fixed factor and chain length (12 to 22) and number of double bonds (0 to 6) as co-variates. The overall differences in relative mobilization assessed in this way were highly significant ($P < 0.001$), as was the effect of number of double bonds ($P < 0.002$).

We compared our results for relative mobilization of fatty acids in humans with those of Connor et al. (3) in rabbits. For the two series of fatty acids for which we have comparable data, there was remarkable agreement (Fig. 5).

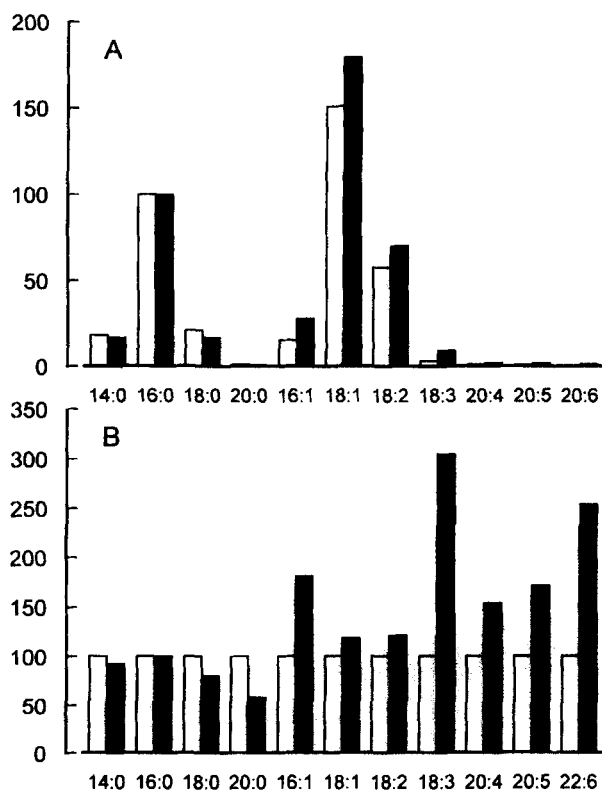


Fig. 4. Relationship between tissue content (open bars) and adipose tissue release (solid bars) of individual fatty acids in six normal subjects. A: the mean veno-arterial concentration difference for release from subcutaneous adipose tissue, and the mean adipose tissue content, each expressed in molar terms, are shown as percentages of the value for palmitic acid. The figure illustrates the very low relative concentrations of some of the fatty acids studied. B: the tissue content for each fatty acid has been set to 100, and the veno-arterial concentration difference is expressed relative to this: again, the value for palmitic acid was arbitrarily set to 100. Only fatty acids whose veno-arterial difference exceeded 1 unit (relative to palmitic acid) are shown. Relative mobilization shown by the solid bars on panel (B) has the same meaning as used by Raclot et al. (2) and Connor et al. (3).

DISCUSSION

These are the first direct observations of the relative mobilization of individual fatty acids from human adipose tissue *in vivo*. Such studies pose great technical problems, both because of the difficulty of measuring release of individual fatty acids *in vivo*, and because of the very low concentrations of some of the fatty acids of interest in normal humans. Our use of a design in which multiple sequential samples were taken from each subject enabled us to overcome the latter difficulty to some extent.

Broadly, there are qualitative similarities in the behavior of all fatty acids: the release of each fatty acid makes up a relatively constant proportion of the total NEFA

released from adipose tissue during this period of early starvation, and for each fatty acid there is a relation between the rate of release from adipose tissue (as reflected in the veno-arterial concentration difference) and the arterial concentration. These observations confirm the findings from animal studies (1-4, 17) that there are differences in the mobilization of each fatty acid relative to its adipose tissue content: for a given chain length, the relative mobilization increases with increasing unsaturation, and for a given degree of unsaturation the relative mobilization decreases with increasing chain length.

The close relationship found overall between the arterial concentration and the veno-arterial difference (Figs. 2, 3) is in accord with the common view that the plasma NEFA concentration is determined by the rate of release of fatty acids from adipose tissue (18, 19). These findings also suggest that the particular adipose depot studied in the present experiments behaves in a manner which is at least qualitatively typical of adipose tissue throughout the body. Although adipose tissue blood flow was measured in these studies it has not been

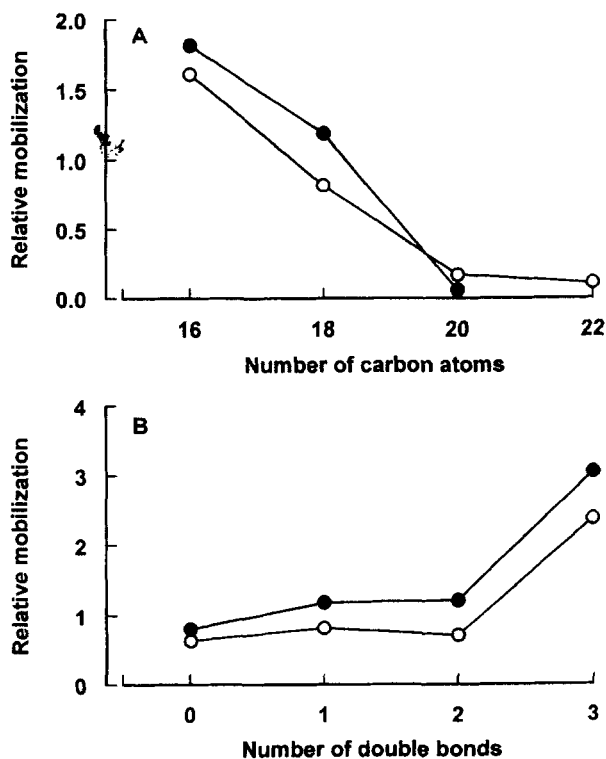


Fig. 5. Comparison of present results in humans with the results of Connor et al. (3) in rabbits. Solid points: present study. Open points: data from Connor et al. A: mono-unsaturated fatty acids; relative mobilization plotted against number of carbon atoms. B: relative mobilization for the series C18:0 to C18:3, plotted against number of double bonds. Relative mobilization for present data is as defined in Fig. 4 but divided by 100 for comparability with Connor et al. (3).

reported here. The relationship shown in Fig. 3 will be unaffected by differences in blood flow among different subjects, as mean values over all subjects are shown; variation in blood flow between subjects may account for some of the additional variability shown in Fig. 2.

One striking observation was the anomalous behavior of stearic acid, such that its arterial concentration was about twice that predicted from its veno-arterial difference. This cannot reflect any abnormality in the release from adipose tissue (which is indicated simply by the veno-arterial difference). It can only be explained by postulating either that the adipose depot we have studied is atypical in its stearic acid content, or that the clearance of stearic acid in other tissues is about half that of other fatty acids. There is no evidence for the first of these suggestions: human subcutaneous abdominal adipose tissue contains more stearic acid than gluteal (20, 21), and a typical amount when compared to other depots throughout the body (22); it would have to contain markedly less stearic acid than other depots contributing to the plasma NEFA pool for this to be the explanation. It therefore seems probable that the clearance of stearic acid is considerably less than that of other plasma NEFA. There is some evidence for this from other studies. Hagenfeldt et al. (23) showed that the splanchnic fractional uptake of stearic acid was 0.11, compared with 0.25 for palmitic and oleic acids, although the fractional uptake of stearic acid by forearm muscle was similar to that of other fatty acids at rest and during exercise (24). Although in our results there was a tendency towards a similar discrepancy for palmitic acid, this was less marked. The common usage of labeled palmitic acid as a tracer for total NEFA turnover is therefore warranted from these observations, although oleic acid, as used in several early studies on NEFA turnover (25), might be even more representative.

Although we have observed differences in the relative mobilization of different fatty acids in humans, as others have in animals (1–4), the composition of adipose tissue triacylglycerol reflects that of the dietary fat, and changes to mirror changes in the dietary fat composition (26, 27). This must imply that the uptake of individual fatty acids varies in the same way, with greater relative uptake of those showing greater relative mobilization. There is indirect evidence for this. After a single meal or during an infusion of triacylglycerol emulsion the plasma NEFA pool changes as triacylglycerol-fatty acids, released by lipoprotein lipase, enter the plasma. However, the plasma NEFA pool becomes enriched more with saturated fatty acids, implying lower tissue uptake of these compared with the unsaturated (28, 29). The need for a match between deposition and mobilization may suggest that selectivity occurs through physico-chemical characteristics such as lipid solubility, rather

than through specificity of any particular enzyme. It has been noted previously that relative mobilization reflects relative retention time on a nonpolar gas chromatography column (1). The striking similarity between our results in humans and the results of Connor et al. (3) in rabbits again suggests that differences in relative mobilization reflect physico-chemical characteristics of individual fatty acids rather than some specialized physiological mechanism.

We conclude that the release of individual plasma NEFA from human adipose tissue broadly determines the composition of plasma NEFA although the stearic acid concentration in plasma is consistently about twice that predicted from its release. The release of individual fatty acids in relation to their concentration in adipose tissue is as expected from work in animals, varying considerably with chain length and degree of unsaturation. ■■

We thank the Wellcome Trust for support of these studies. The Oxford Lipid Metabolism Group is supported by the Oxford Diabetes Trust. Mo Clark provided excellent technical help.

Manuscript received 29 January 1996, in revised form 20 May 1996, and in re-revised form 10 June 1996.

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