

Preferential Uptake of Dietary Fatty Acids in Adipose Tissue and Muscle in the Postprandial Period

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Despite consistent evidence that abnormalities of fatty acid delivery and storage underlie the metabolic defects of insulin resistance, physiological pathways by which fat is stored in adipose tissue and skeletal muscle are not clear. We used a combination of stable isotope labeling and arteriovenous difference measurements to elucidate pathways of postprandial fat deposition in adipose tissue and skeletal muscle in healthy humans. A test meal containing [¹³C]palmitate was combined with intravenous infusion of [²H₂]palmitate to label plasma fatty acids and VLDL-triglyceride. Both dietary (chylomicron) and VLDL-triglyceride were cleared across adipose tissue and muscle, though with greater fractional extraction of the chylomicron-triglyceride. In adipose tissue there was significant uptake of plasma nonesterified fatty acids (NEFAs) in the postprandial but not the fasting state. However, this was minor in comparison with chylomicron-triglyceride fatty acids. We modeled the fate of fatty acids released by lipoprotein lipase (LPL). There was clear preferential uptake of these fatty acids compared with plasma NEFAs. In muscle, there was unexpected evidence for release of LPL-derived fatty acids into the plasma. With this integrative physiological approach, we have revealed hidden complexities in pathways of fatty acid uptake in adipose tissue and skeletal muscle. *Diabetes* 56:168–176, 2007

Disturbances in fat metabolism, most notably raised plasma concentrations of nonesterified fatty acids (NEFAs) and triglycerides, have been implicated in the pathophysiology of type 2 diabetes and consequent cardiovascular disease (1–3). Increased deposition of triglycerides in tissues outside adipose tissue is intimately associated with the development of insulin resistance, the so-called “fat overflow” hypothesis.

The pathways by which fatty acids are delivered from

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LPL, lipoprotein lipase; NEFA, nonesterified fatty acid; TTR, tracer-to-tracee ratio.

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and taken up by tissues are not well understood, even in healthy subjects. In the fasting state, lipolysis of stored triglycerides in adipocytes delivers NEFAs to the plasma, from where they are taken up by the consuming tissues, mainly skeletal muscle, heart, and liver. However, even in the fasting state, lipoprotein lipase (LPL)-mediated intravascular lipolysis of circulating triglycerides may contribute significantly to NEFA release from adipose tissue (4). In skeletal muscle, the relative importance of fatty acid uptake from the action of LPL on circulating triglycerides and of direct NEFA uptake is not well understood. Some studies suggest a major contribution of triglyceride-derived fatty acids (5), whereas others have suggested that this contribution is unmeasurably small (6). In the postprandial state, the situation is further complicated by the delivery of dietary fat as chylomicron-triglyceride and the suppression of intracellular lipolysis in adipose tissue by insulin. Many studies have suggested that chylomicron-triglyceride is the preferred substrate for LPL and competes with VLDL-triglyceride for lipolysis. This has not been shown conclusively in vivo because of the difficulties of complete separation of chylomicron and VLDL particles. Again, the handling of these sources of triglycerides in muscle is unclear. Direct uptake of NEFAs by adipose tissue has been shown during glucose infusion (7), but it is not known whether this is a physiologically important pathway for fat deposition in adipose tissue.

We have used a combination of differential stable isotope labeling of endogenous and meal-derived fatty acids, with arteriovenous tracer and tracee concentration difference measurements across adipose tissue and muscle, to probe the components of fat metabolism in the fasted and fed states. We believe this particular combination of techniques is novel in its application to the fasting and postprandial states, but they build upon earlier seminal studies of fatty acid metabolism in tissues using tracer techniques (7–11). The results show that all of these pathways interact to determine fat uptake by adipose tissue and muscle in the postprandial state. This study provides novel fundamental knowledge, which can lead to a better understanding of the abnormalities of fat metabolism in insulin resistance and in type 2 diabetes.

RESEARCH DESIGN AND METHODS

Fourteen healthy male volunteers were studied after an overnight fast from 8:00 P.M. the previous evening. Subjects were asked to refrain from strenuous exercise and alcohol for 24 h before the study and to avoid foodstuffs naturally enriched in ¹³C for 48 h before the study. The study was approved by the Oxfordshire Clinical Research Ethics Committee, and all subjects gave written informed consent.

We aimed to quantify the uptake into adipose tissue and skeletal muscle of fatty acids derived from the following three sources: chylomicron-triglyceride,

labeled with [$U\text{-}^{13}\text{C}$]palmitic acid, VLDL-triglyceride, labeled endogenously with [$^2\text{H}_2$]palmitic acid, and circulating NEFAs, labeled with [$^2\text{H}_2$]palmitic acid. On the study day, serial blood samples were taken in the fasting state and for 6 h after consumption of a mixed meal (40 g fat and 40 g carbohydrate) containing 100 mg [$U\text{-}^{13}\text{C}$]palmitic acid (isotope purity 98%; CK Gas Products, Hampshire, U.K.) to label chylomicron-triglyceride. The meal comprised a fat emulsion containing 40 g olive oil and Rice Krispies (Kellogg, Manchester, U.K.) with skimmed milk, as described in more detail previously (12). Subjects also received a continuous intravenous infusion of [$^2\text{H}_2$]palmitic acid (isotope purity 97%; CK Gas Products) complexed to human albumin ($0.04 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$) to label the plasma NEFA pool. The infusion commenced at least 60 min before blood sampling.

Blood sampling. The superficial epigastric vein was cannulated (13) to sample the venous effluent of subcutaneous abdominal adipose tissue. Arterial blood was sampled from a catheter in the femoral artery. Venous blood from forearm muscle was obtained from a cannula placed retrogradely in a deep antecubital vein. To avoid contamination of the blood from the forearm muscle with blood from the hand, a wrist cuff was inflated to 200 mmHg for 3 min before sampling. All catheters were kept patent by continuous saline infusion. Blood sampling was performed simultaneously from all three sites.

Tissue blood flow. Subcutaneous abdominal adipose tissue blood flow was measured by ^{133}Xe washout (14). Forearm muscle blood flow was assessed by venous occlusion strain-gauge plethysmography with an inflated wrist cuff (15). Blood flow measurements were made immediately after blood sampling.

Analyses. Whole blood was collected into heparinized syringes (Sarstedt, Leicester, U.K.) for measurement of metabolite and insulin concentrations. From 10 of the subjects, separations of chylomicrons ($S_f > 400$) and "total VLDL" ($S_f 20\text{--}400$) were made by sequential flotation in an ultracentrifuge as described in detail previously (16). This was done with one basal sample and hourly samples from 2 h onward. Plasma triglyceride and NEFA concentrations were determined enzymatically using an ILab 600 Multianalyser (Instrumentation Laboratory, Warrington, U.K.). Insulin levels were determined by radioimmunoassay (Linco Research, St. Charles, MO). To determine fatty acid composition and isotopic enrichment, total lipids were extracted from plasma and lipoprotein fractions, and methyl esters were prepared from NEFA and triglyceride fractions as previously described (5). Fatty acid compositions (micromoles per 100 μmol total fatty acids) were determined by gas chromatography (5), and palmitate concentrations were calculated by multiplying the proportion of palmitate by the corresponding plasma concentrations of NEFAs or triglycerides determined enzymatically. [$U\text{-}^{13}\text{C}$] and [$^2\text{H}_2$]palmitate enrichments were determined simultaneously by gas chromatography-mass spectrometry using a 5890 gas chromatograph coupled to a 5973N mass spectrometer (Agilent Technologies, Stockport, U.K.). The gas chromatograph was equipped with a DB-Wax 30-m capillary column (inside diameter 0.25 mm, film thickness 0.25 μm ; Agilent), and ions with mass-to-charge ratios of 270 ($M + 0$), 272 ($M + 2$), and 286 ($M + 16$) were determined by selected ion monitoring. Dwell time was 100 ms. Tracer-to-tracee ratios (TTRs) for [$U\text{-}^{13}\text{C}$]palmitate ($M + 16$)/($M + 0$) and [$^2\text{H}_2$]palmitate ($M + 2$)/($M + 0$) were multiplied by the corresponding palmitate-NEFA or palmitate-triglyceride concentrations to give plasma tracer concentrations.

Calculations. Plasma concentrations of lipids were converted to whole blood values using the hematocrit. Arteriovenous and venoarterial differences in all metabolite concentrations (labeled and unlabeled) were calculated across adipose tissue and skeletal muscle. Absolute net flux was calculated as the product of arteriovenous or venoarterial difference and tissue blood flow. A positive arteriovenous difference in metabolite concentrations implies net uptake, or extraction, across a tissue whereas a positive venoarterial difference implies net release. Fractional extraction was calculated as the arteriovenous difference divided by the arterial concentration, expressed as a percentage (17).

Fractional uptake of [$U\text{-}^{13}\text{C}$]palmitate derived from triglyceride hydrolysis by LPL. The arteriovenous difference of [$U\text{-}^{13}\text{C}$]palmitate in the triglyceride fraction represents the amount of labeled palmitate that is released from lipolysis of $U\text{-}^{13}\text{C}$ -labeled triglyceride by LPL. In adipose tissue, a proportion of the fatty acids liberated by LPL will "spill over" into the systemic circulation (5). This spillover can be measured as the venoarterial difference of nonesterified [$U\text{-}^{13}\text{C}$]palmitate. The difference between the fatty acids liberated from triglyceride and those passing into the systemic circulation represents chylomicron-fatty acid uptake into adipose tissue. Thus, the fractional uptake, or "percentage entrapment" (5), of [$U\text{-}^{13}\text{C}$]palmitate into adipose tissue can be calculated as

$$\text{Fractional uptake of } [U\text{-}^{13}\text{C}] \text{ palmitate derived from hydrolysis of chylomicron-TG} \\ = 100 \times \frac{A\text{-V}[^{13}\text{C}]\text{TG}_{\text{FA}} - V\text{-A}[U\text{-}^{13}\text{C}]\text{palmitate}}{A\text{-V}[^{13}\text{C}]\text{TG}_{\text{FA}}} \quad (1)$$

where TG is triglyceride, $A\text{-V}[^{13}\text{C}]\text{TG}_{\text{FA}}$ is the arteriovenous difference in concentration of labeled palmitate in the TG fraction, and $V\text{-A}[U\text{-}^{13}\text{C}]\text{palmitate}$ is the venoarterial difference in concentration of labeled nonesterified palmitic acid. This calculation was also performed for muscle, albeit with the expectation that all fatty acid liberated from triglyceride by LPL would be taken into the tissue (5).

Fractional uptake of nonesterified [$^2\text{H}_2$]palmitate. [$^2\text{H}_2$]Palmitate was infused to act as a tracer of both the plasma NEFA pool and of VLDL-triglyceride (produced in the liver after hepatic uptake of labeled plasma NEFAs). In calculating the fractional uptake of [$^2\text{H}_2$]palmitate from the NEFA pool, the contribution of [$^2\text{H}_2$]palmitate derived from hydrolysis of $^2\text{H}_2$ -labeled triglyceride and escaping tissue uptake (spillover) must be taken into account. In muscle, the assumption is made that all fatty acids derived from triglyceride hydrolysis are taken up; however, the same is not true for adipose tissue (5). The fractional uptake of fatty acid derived from VLDL-triglyceride hydrolysis has never been measured in adipose tissue. Thus, three models were used to encompass the range of possibilities. The first model, which was also used for the calculation of fractional uptake across muscle, assumes that all of the [$^2\text{H}_2$]palmitate derived from hydrolysis of $^2\text{H}_2$ -labeled triglyceride is taken up into the tissue (0% spillover). Thus, the arteriovenous difference in [$^2\text{H}_2$]palmitate represents uptake across the tissue and the fractional uptake can be expressed as

$$\text{Fractional uptake of non-esterified } [^2\text{H}_2] \text{ palmitate (model 1)} \\ = 100 \times \frac{A\text{-V}[^2\text{H}_2] \text{ palmitate}}{\text{Art}[^2\text{H}_2] \text{ palmitate}} \quad (2)$$

where $\text{Art}[^2\text{H}_2]\text{palmitate}$ is the arterial concentration of [$^2\text{H}_2$]palmitate. The second model assumes that none of the [$^2\text{H}_2$]palmitate derived from hydrolysis of $^2\text{H}_2$ -labeled triglyceride is taken up into the tissue (100% spillover). The arteriovenous difference in [$^2\text{H}_2$]TG_{FA} is a measure of the [$^2\text{H}_2$]palmitate derived from the hydrolysis of labeled triglyceride. Thus, $A\text{-V}[^2\text{H}_2]\text{TG}_{\text{FA}}$ is subtracted from the venous [$^2\text{H}_2$]palmitate concentration and the fractional uptake can be calculated as

$$\text{Fractional uptake of non-esterified } [^2\text{H}_2] \text{ palmitate (model 2)} \\ = 100 \times \frac{\text{Art}[^2\text{H}_2] \text{ palmitate} - (\text{Ven}[^2\text{H}_2] \text{ palmitate} - A\text{-V}[^2\text{H}_2]\text{TG}_{\text{FA}})}{\text{Art}[^2\text{H}_2] \text{ palmitate}} \quad (3)$$

where $\text{Ven}[^2\text{H}_2]\text{palmitate}$ is the venous concentration of [$^2\text{H}_2$]palmitate.

The final model assumes that the uptake of [$^2\text{H}_2$]palmitate derived from hydrolysis of $^2\text{H}_2$ -labeled triglyceride is identical to the uptake of [^{13}C]palmitate derived from the hydrolysis of chylomicron-triglyceride, calculated above (i.e., VLDL spillover equal to chylomicron spillover). Therefore,

$$\text{Uptake of } [^2\text{H}_2] \text{ palmitate derived from hydrolysis of } [^2\text{H}_2] \text{ labeled triglycerides} \\ = \left(1 - \frac{\text{Eq. 1}}{100}\right) \times A\text{-V}[^2\text{H}_2]\text{TG}_{\text{FA}} \quad (4)$$

Thus, the fractional uptake can be calculated as

$$\text{Fractional uptake of non-esterified } [^2\text{H}_2] \text{ palmitate (model 3)} \\ = 100 \times \frac{\text{Art}[^2\text{H}_2] \text{ palmitate} - (\text{Ven}[^2\text{H}_2] \text{ palmitate} - \text{Eq. 4})}{\text{Art}[^2\text{H}_2] \text{ palmitate}} \quad (5)$$

Total unidirectional fatty acid fluxes. To assess the quantitative significance of unidirectional fatty acid uptake from the circulating NEFA pool across adipose tissue, total NEFA uptake was calculated from the fractional uptake of labeled palmitic acid. Uptake of fatty acids liberated from triglycerides by LPL could be differentiated from uptake of fatty acids from the circulating NEFA pool by using the fractional uptake of the appropriate isotope of palmitic acid (i.e., [$U\text{-}^{13}\text{C}$]palmitate for fatty acids from triglyceride hydrolysis and [$^2\text{H}_2$]palmitate for circulating fatty acids).

For triglyceride-derived fatty acids, the total uptake of palmitate derived from triglyceride hydrolysis was taken as the product of the fractional uptake of LPL-derived [$U\text{-}^{13}\text{C}$]palmitate (as in Eq. 1) and the arteriovenous difference in triglyceride-palmitate concentration (i.e., total palmitate made available by LPL). The uptake of total fatty acids derived from triglyceride hydrolysis was then calculated as the total palmitate uptake divided by the fraction of palmitate in the "pool" of fatty acids derived from triglyceride hydrolysis, expressed as a percentage. The latter fraction was calculated as follows: the arteriovenous difference in total triglyceride concentration determined the total fatty acids derived from triglyceride hydrolysis available for uptake. Similarly, the arteriovenous difference in palmitate in the triglyceride fraction

was the palmitate derived from triglyceride hydrolysis available for uptake. Dividing the latter by the former determined what fraction of total fatty acids derived from triglyceride hydrolysis was made up of palmitate. The calculations can be summarized as

$$\begin{aligned} & \text{Uptake of palmitate derived from triglyceride hydrolysis} \\ & = \text{Eq. 1} \times \text{A-V TG-palmitate} \end{aligned} \quad (6)$$

$$\begin{aligned} & \text{Palmitate fraction of FAs liberated by triglyceride hydrolysis} \\ & = \frac{\text{A-V TG-palmitate}}{\text{A-V TG}} \end{aligned} \quad (7)$$

$$\text{Uptake of total FA from triglyceride hydrolysis} = \frac{\text{Eq. 6}}{\text{Eq. 7}} \quad (8)$$

where A-V TG-palmitate is the arteriovenous difference in triglyceride-palmitate concentration and A-V TG is the arteriovenous difference in triglyceride concentration. This calculation makes two assumptions. First, in terms of uptake, adipose tissue does not discriminate between fatty acids dependent on the triglyceride from which they derive, i.e., fatty acids liberated from chylomicrons are treated analogously to fatty acids derived from VLDL (equal spillover). This is the same assumption made in the final model of [$^2\text{H}_2$]palmitate uptake above. Second, the calculation assumes complete hydrolysis of triglycerides.

For uptake of fatty acids from the circulating NEFA pool, a similar calculation was made. The total palmitate uptake was calculated as a product of the fractional uptake of [$^2\text{H}_2$]palmitate, using the third model described above, and the arterial palmitate concentration. The total NEFA uptake was then calculated as the total palmitate uptake divided by the fraction of palmitate in the arterial NEFA pool, expressed as a percentage. The calculations can be summarized as

$$\text{Total non-esterified palmitate uptake} = \text{Eq. 5} \times \text{Art palmitate} \quad (9)$$

$$\text{Fraction of palmitate in NEFA pool} = \frac{\text{Art palmitate}}{\text{ArtNEFA}} \quad (10)$$

$$\text{Total NEFA uptake} = \frac{\text{Eq.9}}{\text{Eq.10}} \quad (11)$$

where Art NEFA is the arterial concentration of NEFA.

Statistical methods. Data were analyzed using SPSS for Windows v11 (SPSS U.K., Chertsey, U.K.). Statistical significance was set at $P < 0.05$. All data are presented as means \pm SE unless otherwise stated. Repeated measures ANOVA with time and, when appropriate, tissue or isotope as within-subject factors was used to identify time effects, differences between tissues, isotope effects, time and tissue interactions, and time and isotope interactions. The difference between fasting muscle and adipose tissue triglyceride extraction was assessed by paired t test.

RESULTS

The clinical characteristics of the subjects are shown in Table 1. A summary of the principal study results is presented in Table 2. The test meal was given at time 0.

Tissue blood flow and unlabeled metabolites. Adipose tissue blood flow was greater than forearm muscle blood flow, with no significant change in either tissue after the test meal. Arterial triglyceride concentrations rose postprandially (Fig. 1A). Chylomicron-triglyceride concentrations rose to a peak at 3 h in parallel with plasma triglyceride, whereas VLDL-triglyceride concentrations rose to a broader peak ($P = 0.01$ for time) (Fig. 1A).

During the fasting period, absolute extraction of triglyceride (arteriovenous difference multiplied by blood flow) was higher across adipose tissue than across muscle (Table 2) ($P = 0.011$). After the meal, absolute extraction of triglyceride increased in both adipose tissue and muscle (both $P < 0.001$). Similarly, fractional extraction of triglyceride (arteriovenous difference divided by arterial concentration) was higher during fasting in adipose tissue than in muscle; however, there was no statistical difference between the tissues postprandially (Table 2).

TABLE 1
Subject characteristics

	Mean	Range
Age (years)	43	32–54
Weight (kg)	87	73–102
BMI (kg/m^2)	28	23–34
Systolic blood pressure (mmHg)	127	104–143
Diastolic blood pressure (mmHg)	82	73–96
Fasting plasma concentrations		
Glucose (mmol/l)	5.0	4.4–6.0
Insulin (mU/l)	12.2	4.9–22.6
Triglycerides (mmol/l)	1.8	0.7–3.3
HDL (mmol/l)	1.0	0.9–1.7

Arterial NEFA concentrations fell during the early postprandial period before rising again toward the end of the study (Fig. 1B). Net adipose tissue NEFA output (venoarterial difference multiplied by blood flow) fell after the meal and then increased again later in the postprandial period (not shown, but similar to results in previous studies) (5). There was net NEFA uptake across muscle (arteriovenous difference multiplied by blood flow) throughout the study period (Table 2), similar to previous studies (5).

Labeled triglycerides and NEFAs

^{13}C -Labeled triglycerides and NEFA. ^{13}C -Labeled (dietary) triglycerides appeared in plasma at 60 min, and the concentration peaked at 240 min ($P < 0.001$) (Fig. 1C). ^{13}C -Labeled triglyceride concentrations in the chylomicron fraction were similar to those in arterial whole plasma until 180 min and then were somewhat lower (Fig. 1C), reflecting the incorporation of ^{13}C -labeled (dietary) fatty acids into VLDL during the later postprandial phase (18). Extraction of ^{13}C -labeled triglycerides across adipose tissue and muscle was detectable from 60 min. Absolute extraction peaked at 120 min in adipose tissue and at 180 min in muscle (not shown). There was greater absolute extraction of ^{13}C -labeled triglyceride across adipose tissue than across muscle ($P = 0.017$).

[U- ^{13}C]Palmitate also appeared in the plasma NEFA pool from 60 min but reached a peak concentration later at 300 min. The arterial plasma [U- ^{13}C]palmitate concentration rose between 3 and 5 h, when the ^{13}C -labeled triglyceride concentration was decreasing. In muscle, there was a net uptake of diet-derived fatty acids throughout the postprandial period (negative venoarterial difference in [U- ^{13}C] palmitate multiplied by blood flow) (Fig. 2B). In contrast, there was net efflux of [U- ^{13}C]palmitate from adipose tissue (venoarterial difference multiplied by blood flow), which increased during the study ($P < 0.001$). This efflux must reflect release of dietary fatty acids, derived from chylomicron hydrolysis, into the systemic circulation (Fig. 2B). Increasing release of [U- ^{13}C]palmitate from adipose tissue in the later postprandial period would reflect decreasing entrapment of the LPL-derived fatty acids in the tissue, as we have described before (5).

$^2\text{H}_2$ -labeled NEFAs and triglycerides. The arterial concentration of [$^2\text{H}_2$]palmitate (the infused tracer) and the TTR did not vary before the meal (Fig. 3A), implying that steady-state conditions had been reached before any blood sampling. Arterial, forearm venous, and adipose venous [$^2\text{H}_2$]palmitate concentrations fell postprandially but returned to baseline by the end of the study (all $P < 0.001$). We examined the arteriovenous differences for the [$^2\text{H}_2$]palmitate tracer. There was no significant flux (veno-

TABLE 2
Summary of principal study results

	Fasting	Postprandial period			ANOVA	
		Early	Mid	Late	Variable	<i>P</i>
Blood flow						
Adipose (ml · 100 g ⁻¹ · min ⁻¹)	2.8 ± 0.3	3.0 ± 0.4	2.3 ± 0.2	2.9 ± 0.3	Tissue	0.016
Muscle (ml · 100 ml ⁻¹ · min ⁻¹)	1.8 ± 0.1	1.9 ± 0.2	2.0 ± 0.2	2.2 ± 0.3		
Net NEFA flux (nmol · 100 g⁻¹ · min⁻¹)						
Adipose (output)	1080 ± 206	423 ± 71	231 ± 33	858 ± 166	Time	<0.001
Muscle (uptake)	43 ± 31	17 ± 21	69 ± 19	82 ± 32	Time	0.065
NEFA uptake across adipose tissue (nmol · 100 g⁻¹ · min⁻¹)						
LPL-derived fatty acids	NA	371 ± 60	380 ± 82	278 ± 52	Fatty acid source	0.001
Fatty acids from NEFA pool	NA	82 ± 23	183 ± 41	-27 ± 54		
Absolute triglyceride extraction (nmol · 100 g⁻¹ · min⁻¹)						
Adipose	120 ± 27	132 ± 20	158 ± 38	146 ± 31	Tissue (fasting)	0.011
Muscle	32 ± 24	113 ± 21	182 ± 41	41 ± 16	Tissue (postprandial)	0.065
Fractional triglyceride extraction (%)						
Adipose	5 ± 1	5 ± 1	6 ± 1	6 ± 1	Tissue (fasting)	0.048
Muscle	1 ± 1	6 ± 1	6 ± 1	2 ± 1	Tissue (postprandial)	0.243
Fractional extraction of labeled triglycerides across adipose tissue (%)						
¹³ C	NA	22 ± 4	11 ± 3	12 ± 3	Isotope	<0.001
² H ₂	NA	3 ± 2	6 ± 1	4 ± 2		
Fractional extraction of labeled triglycerides across forearm (%)						
¹³ C	NA	23 ± 3	10 ± 2	6 ± 2	Isotope	<0.001
² H ₂	NA	7 ± 1	5 ± 1	2 ± 1		
Fractional extraction of labeled palmitate across adipose tissue (%)						
¹³ C	NA	83 ± 4	72 ± 5	55 ± 8		
² H ₂						
Model 1		16 ± 6	25 ± 5	-7 ± 9	Isotope	<0.001
Model 2		36 ± 8	63 ± 8	16 ± 15	Isotope	<0.001
Model 3		20 ± 6	35 ± 6	6 ± 12	Isotope	<0.001
Fractional uptake of labeled palmitate across forearm (%)						
¹³ C	NA	103 ± 2	104 ± 3	101 ± 6	Isotope	<0.001
² H ₂	39 ± 4	42 ± 4	46 ± 4	30 ± 4		

Data are means ± SE for fasting and postprandial states. The postprandial phase is divided into 2-h periods: early postprandial (0–2 h), mid-postprandial (2–4 h), and late postprandial (4–6 h). Repeated-measures ANOVA has been used to calculate significant differences between datasets. The parameters under comparison are described in the “variable” column. “Tissue” implies a comparison of adipose tissue and muscle, “Time” implies a calculation of time effect, “Fatty acid source” implies a comparison of LPL-derived and circulating fatty acids, and “Isotope” implies a comparison of ¹³C- and ²H₂-labeled triglycerides or fatty acids. ANOVA calculations refer to the whole study period unless otherwise stated. NA, not applicable.

arterial difference multiplied by blood flow) of [²H₂]palmitate across adipose tissue during fasting (mean 0.132 nmol · 100 g⁻¹ · min⁻¹ [95% CI -0.61 to 0.87]) or at 360 min (1.67 nmol · 100 g⁻¹ · min⁻¹ [-0.42 to 3.76]). However, during the postprandial period there was a net uptake of NEFAs by adipose tissue from the circulating NEFA pool from 60 to 300 min (negative venoarterial difference in [²H₂]palmitate multiplied by blood flow) (Fig. 2A). This shows an uptake of plasma NEFAs by adipose tissue in the postprandial period, despite an overall net release of NEFA from adipose tissue. There was consistent uptake of circulating NEFAs by muscle (negative venoarterial difference in [²H₂]palmitate multiplied by blood flow) during the study, which did not vary with prandial state (*P* = 0.19) (Fig. 2A).

²H₂-Labeled triglycerides were detected in the first blood samples, taken 1 h before the meal (mean concentration at -60 min 0.19 μmol/l [95% CI 0.11–0.28]) and increased consistently throughout the study (*P* < 0.001)

(Fig. 1C). This reflects incorporation of the infused [²H₂]palmitate tracer into VLDL in the liver. In confirmation, the incorporation of [²H₂]palmitate into VLDL-triglyceride (separated in the ultracentrifuge) mirrored very closely the appearance in total plasma (Fig. 1C). We examined the arteriovenous differences for the ²H₂-labeled triglycerides as a marker of tissue handling of VLDL-triglyceride. Extraction of ²H₂-labeled triglycerides was detectable across both adipose tissue and muscle from 60 min (Fig. 4A and B). There was no difference in absolute or fractional extraction of ²H₂-labeled triglycerides between the two tissues (*P* = 0.32 and *P* = 0.90, respectively).

Comparison of dietary (¹³C-labeled) and endogenous (²H-labeled) fatty acid handling in adipose tissue and muscle

TTRs. As expected, the venous TTR (isotopic enrichment) of [²H₂]palmitate was significantly lower than the arterial TTR across adipose tissue (*P* < 0.001) (Fig. 3A) because the circulating tracer is diluted by unlabeled fatty

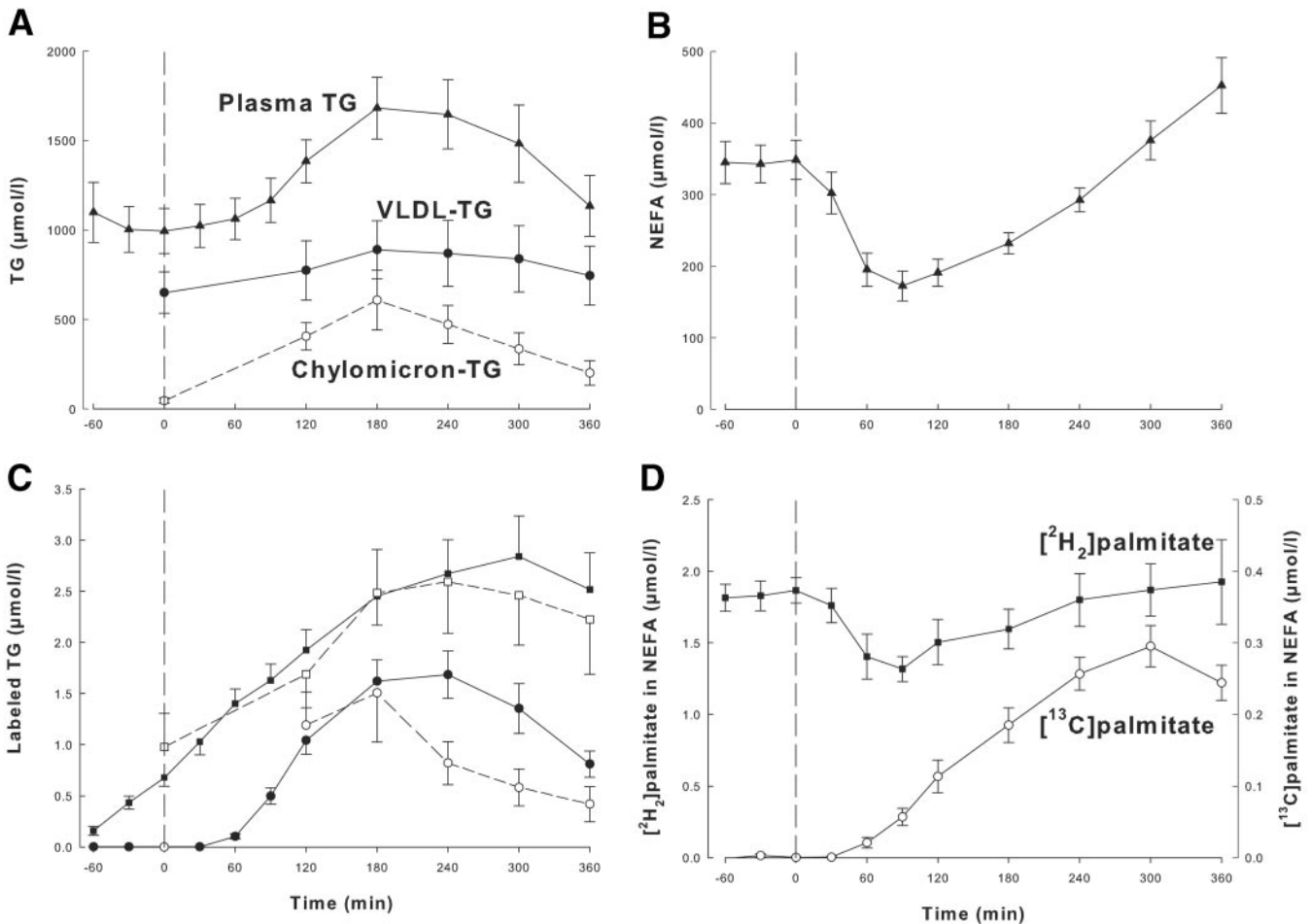


FIG. 1. Whole plasma and lipoprotein fraction arterial concentrations of triglycerides (TG) and fatty acids after a mixed meal: **A:** Unlabeled triglycerides: \blacktriangle , whole plasma triglycerides; \bullet , VLDL (S_r 20–400)-triglyceride; \circ , chylomicron ($S_r >400$)-triglyceride. **B:** Unlabeled NEFAs. **C:** Triglycerides labeled with [²H₂]palmitic acid or [¹³C]palmitic acid: \blacksquare , whole plasma ²H₂-labeled triglycerides; \square , ²H₂-labeled triglycerides in the VLDL (S_r 20–400) fraction; \bullet , whole plasma U-¹³C-labeled triglycerides; \circ , U-¹³C-labeled triglycerides in the chylomicron ($S_r >400$) fraction. **D:** Nonesterified [²H₂]palmitic acid (\blacksquare) and [¹³C]palmitic acid (\circ). For whole plasma data, $n = 14$; for lipoprotein fractions, $n = 9$ or 10 .

acids released from adipose tissue lipolysis. However, an unexpected and striking finding was that the muscle venous TTR of [²H₂]palmitate was also significantly lower than the arterial TTR ($P < 0.001$) (Fig. 3A). This must imply the release of fatty acids not labeled with [²H₂]palmitate across both tissues. In contrast, when we examined the differences in TTRs of [U-¹³C]palmitate across muscle or adipose tissue, there were no consistent differences in TTRs (Fig. 3B).

Labeled triglycerides. By assuming that ¹³C-labeled triglyceride is a marker of chylomicron-triglyceride (at least in the early postprandial period) and that ²H₂-labeled triglyceride is a tracer for VLDL-triglyceride, it is possible to compare the handling of these two lipoproteins in the tissues. The fractional extraction of ¹³C-labeled triglyceride (marking chylomicrons) was significantly higher across both adipose tissue and muscle than that of ²H₂-labeled TG (marking VLDL) ($P < 0.001$ for adipose tissue and muscle) (Fig. 4A and B), implying a preferential hydrolysis of chylomicron-triglyceride over VLDL-triglyceride by LPL in the postprandial period. There was no difference in fractional extraction between the tissues (adipose tissue versus muscle, $P = 0.62$) (Fig. 4A and B and data in Table 2).

Labeled fatty acids. We asked the question: Once fatty acids have been liberated from circulating triglycerides by

LPL, do they effectively mix with circulating NEFAs, or is there preferential uptake by tissues? We calculated the fractional uptake of [¹³C]palmitate derived from the LPL-mediated hydrolysis of ¹³C-labeled triglycerides (Fig. 5A, \blacktriangle). This graph may be interpreted as follows. At 60 min after the meal, $87 \pm 4\%$ (mean \pm SE) of chylomicron-palmitate liberated via LPL was taken up into adipose tissue, decreasing to $48 \pm 12\%$ by 360 min. This fractional uptake of chylomicron-triglyceride palmitate liberated by LPL was consistently greater than that of nonesterified [²H₂]palmitate across adipose tissue, irrespective of which model for fractional uptake of [²H₂]palmitate originating from hydrolysis of ²H₂-labeled triglycerides across adipose tissue was used ($P < 0.001$ for models 1, 2, and 3) (Fig. 5A). (These models set outer boundaries for the spillover of VLDL-derived ²H₂-labeled fatty acids.) Thus, in adipose tissue, fatty acids derived from triglyceride hydrolysis are more likely to be taken up than fatty acids from the circulating NEFA pool. Given the demonstration of NEFA uptake by adipose tissue in the postprandial period (Fig. 2B), we now asked: What is the quantitative importance of this route for adipose tissue fat accumulation in the postprandial period? We therefore calculated the (unidirectional) uptake of total fatty acid derived from triglyceride hydrolysis (Fig. 5B, \circ) and compared this with the unidirectional uptake of fatty acids from the circulating

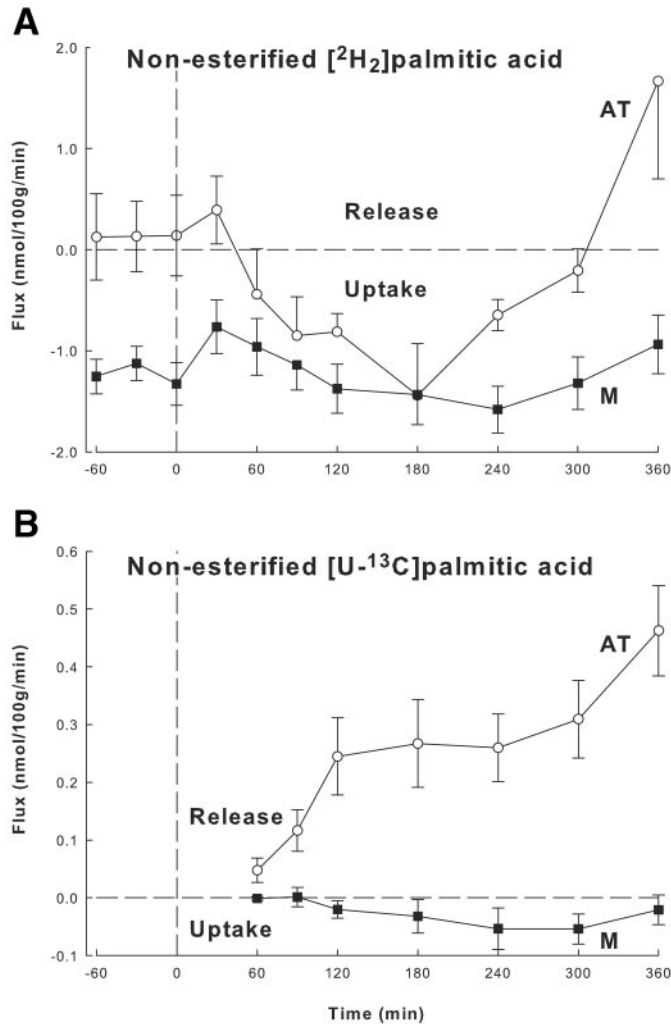


FIG. 2. Nonesterified [²H₂]palmitic acid (A) and [U-¹³C]palmitic acid (B) flux across adipose tissue (AT) (○) and muscle (M) (■) after a mixed meal. Flux is calculated as the venoarterial difference multiplied by the appropriate tissue blood flow. A positive flux implies output, whereas a negative flux implies uptake. For [²H₂]palmitic acid, there was consistent extraction across forearm muscle, as expected, but unexpected uptake during the postprandial period into adipose tissue. [U-¹³C]Palmitic acid was extracted, again as expected, across muscle but released across adipose tissue, reflecting the action of LPL on circulating chylomicron-triglyceride.

NEFA pool (Fig. 5B, ●). Triglyceride-derived fatty acid uptake was significantly greater than NEFA uptake ($P = 0.001$).

DISCUSSION

Disturbances in fat metabolism have been implicated in the pathophysiology of type 2 diabetes and consequent vascular disease (1–3). However, the pathways of normal fatty acid metabolism are not fully understood. In this study, the most striking findings were the postprandial uptake of fatty acids from the circulating NEFA pool by adipose tissue, the direct confirmation that chylomicrons are the preferred substrate of LPL over VLDL, the preferential channeling of fatty acids derived from LPL-mediated chylomicron hydrolysis into adipose tissue, and the postprandial release of fatty acids across the forearm.

We have used a combination of stable isotope tracers to label specifically the chylomicron and VLDL fractions. Because this is a novel methodology in the present con-

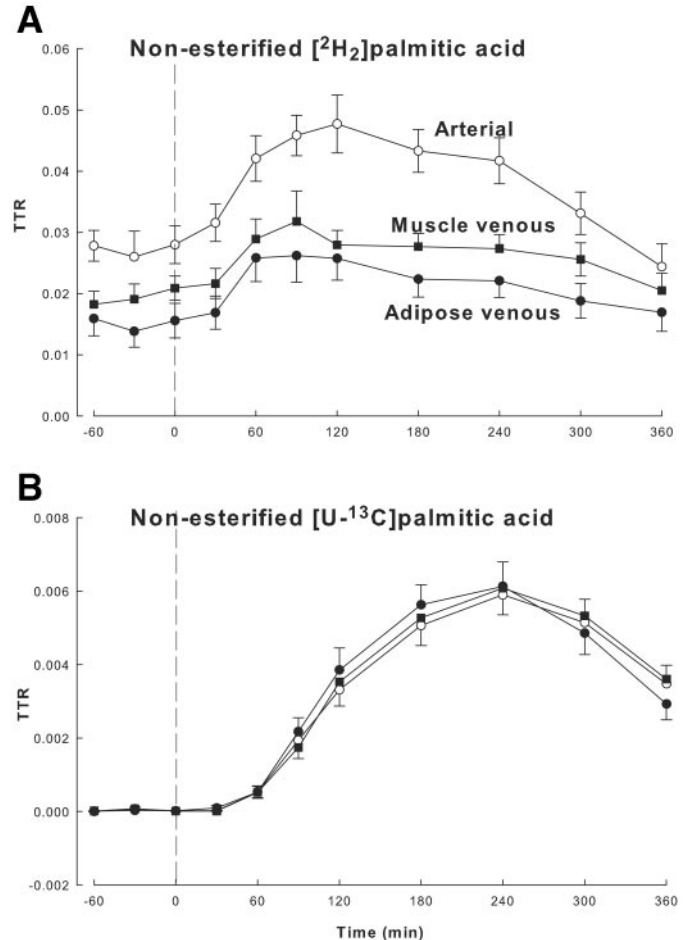


FIG. 3. Changes in nonesterified [²H₂]palmitic acid (A) and [U-¹³C]palmitic acid (B) TTRs (isotopic enrichment) after a mixed meal. Arterial (○), muscle venous (■), and adipose venous (●) TTRs are shown. Although the TTR of [²H₂]palmitic acid is lower in venous samples (from both muscle and adipose tissue) than in arterial plasma, there is no corresponding “dilution” of the [U-¹³C]palmitic acid across the tissues.

text, we sought some validation by direct measurements on separated lipoprotein fractions. The appearance of the ¹³C-labeled (dietary) fatty acids in the chylomicron-triglyceride fraction paralleled closely their appearance in whole plasma triglycerides until 3 h after the meal; beyond that, there was more label in whole plasma triglycerides, as expected, as some dietary fatty acids by that time have moved into VLDL-triglyceride (18). Thus, our conclusions on metabolic handling of chylomicron-triglyceride are strongest in the early postprandial period. The appearance of the infused tracer, [²H₂]palmitic acid, in plasma triglycerides was taken to be a marker of VLDL, and indeed [²H₂]palmitic acid appearance in whole plasma triglycerides paralleled closely its appearance in the VLDL fraction throughout the study. The rapidity of the increase in [²H₂]palmitate enrichment in triglycerides confirms other studies demonstrating high VLDL-triglyceride synthesis rates (18–20). As the calculations of isotope fractional uptake illustrate, it is essential to measure ²H₂-labeled triglycerides when performing isotope infusion studies, because fatty acids derived from hydrolysis of labeled triglycerides have an impact on calculations of labeled fatty acid flux. Although in this study we did not examine arteriovenous differences for isolated lipoprotein fractions, we have done this previously without the use of

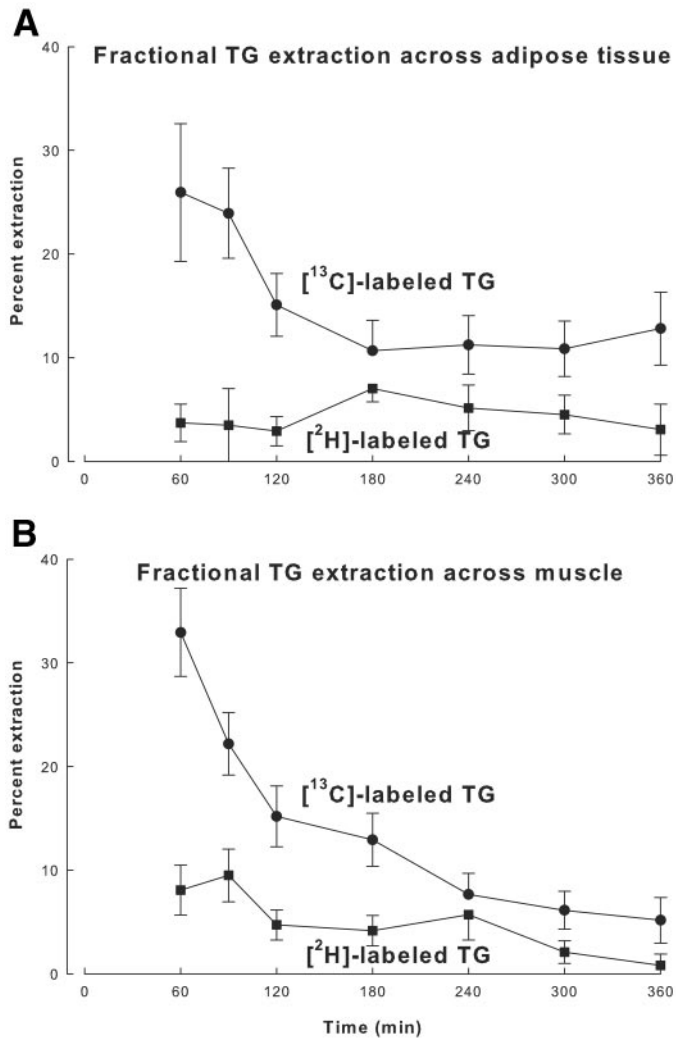


FIG. 4. Fractional extraction of ¹³C-labeled triglycerides (●, marking the chylomicron fraction) and ²H₂-labeled triglycerides (■, marking VLDL-triglyceride) across adipose tissue (A) and muscle (B). Fractional triglyceride extraction was calculated as the arteriovenous difference in labeled triglycerides divided by the arterial concentration of labeled triglycerides and expressed as a percentage. For both tissues, fractional extraction of chylomicron-triglyceride was greater than that of VLDL-triglyceride.

isotopic tracers, and the major result from this study of preferential extraction of chylomicron-compared with VLDL-triglyceride was consistent with those earlier studies (21,22).

Direct uptake by adipose tissue of circulating NEFA has previously only been demonstrated during glucose infusion (7). The calculation of total NEFA uptake across adipose tissue (Fig. 5) illustrates that the postprandial uptake of fatty acids from the circulating NEFA pool is of quantitative significance. Indeed, during the mid-postprandial period, more than one-third of the total fatty acids taken up by adipose tissue came from the circulating NEFA pool (Table 2). The variation in flux with the prevailing metabolic conditions suggests that NEFA uptake by adipose tissue is a regulated process.

The difference between ¹³C- and ²H₂-labeled triglyceride extraction across both adipose tissue and muscle implies a preferential hydrolysis of chylomicron-triglyceride by LPL. This has been suggested previously (16,21,23–25). However, earlier studies relied on ultracentrifugation for sep-

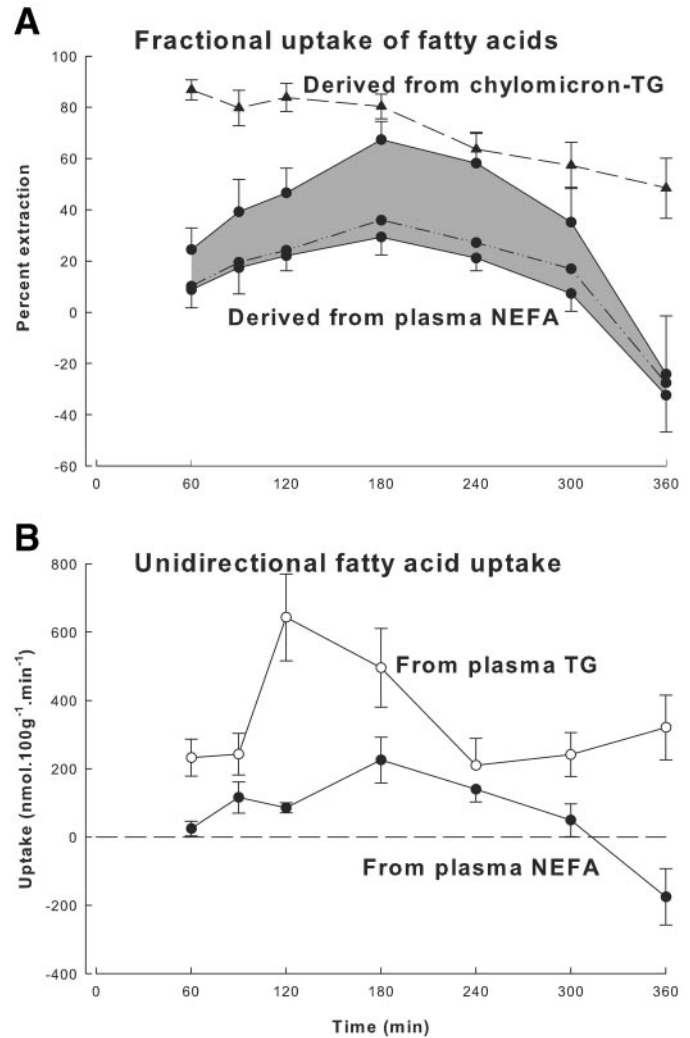


FIG. 5. Adipose entrapment of LPL-derived fatty acids. A: Comparison of the fractional uptake of [¹³C] palmitic acid derived from chylomicron hydrolysis (▲---▲) and of [²H₂]palmitic acid (●—●) from the circulating NEFA pool across adipose tissue after a mixed meal. In calculating [²H₂]palmitic acid fractional uptake, it is necessary to allow for fatty acids derived from VLDL hydrolysis. The shaded area represents the range of possible outcomes for [²H₂]palmitic acid uptake. The upper border assumes 0% uptake of VLDL-derived fatty acids (model 2 in the text), whereas the lower border assumes 100% uptake of VLDL-derived fatty acids (model 1 in the text). The dashed line within the shaded area results if the fractional uptake of fatty acids derived from VLDL hydrolysis is taken to be the same as the fractional uptake of fatty acids derived from chylomicron hydrolysis (Eqs. 1–5). B: Unidirectional adipose tissue uptake of total fatty acids from the circulating NEFA pool (●) and total fatty acids derived from LPL-mediated triglyceride hydrolysis (○) across adipose tissue after a mixed meal (Eqs. 6–11).

aration of chylomicron-triglyceride and VLDL-triglyceride with the limitation that some small chylomicrons and chylomicron remnants contaminate the VLDL-triglyceride fraction. The use of different stable isotope labels for the chylomicron- and VLDL-triglyceride pools allows a more accurate assessment of LPL preference, especially during the first half of the postprandial period. One possible explanation for the preference for chylomicron-triglyceride is that the larger size of chylomicrons allows them to bind to more LPL molecules per particle than VLDL (26). The difference between the fractional extraction of ¹³C- and ²H₂-labeled triglycerides was less marked at the end of the study. Dietary fatty acids may appear in VLDL-triglyc-

eride as early as 3 h postprandially (19). Thus, by 6 h after the meal, it is no longer correct to assume that the [^{13}C]palmitate in the triglyceride fraction is restricted to chylomicrons.

The calculation of fractional isotopic uptake across adipose tissue enables differentiation between the uptake of circulating NEFA, represented by the uptake of [$^2\text{H}_2$]palmitate, and the uptake of fatty acids derived from chylomicron hydrolysis, represented by the uptake of [$\text{U-}^{13}\text{C}$]palmitate. Irrespective of which model of [$^2\text{H}_2$]palmitate uptake was used, there was a significantly lower uptake of fatty acids from the circulating NEFA pool compared with fatty acids derived from chylomicron hydrolysis. This has been suggested before (7); however, the earlier report did not take into account the contribution of fatty acids derived from labeled triglyceride hydrolysis in the calculation of NEFA uptake. It is likely that the physical positioning of LPL on the vascular endothelium results in the generation of fatty acids immediately adjacent to the adipocyte cell membrane. There is evidence of the juxtapositioning of chylomicron particles and the capillary endothelium to support this hypothesis (27,28). In contrast, circulating NEFA flows more centrally within the lumen of the capillary.

The changes in fatty acid isotope TTRs across adipose tissue and muscle are striking. Assuming that there is no discrimination between tracer and tracee for tissue uptake, then a decrease in fatty acid TTRs across the tissue must reflect release of fatty acids not labeled with the isotope of interest. This is expected in adipose tissue and was seen clearly for [$^2\text{H}_2$]palmitate. This dilution in [$^2\text{H}_2$]palmitate enrichment reflects both the release of unlabeled fatty acids from intracellular lipolysis, and (in the postprandial period) the release of unlabeled palmitate and [$\text{U-}^{13}\text{C}$]palmitate from intravascular chylomicron-triglyceride lipolysis. However, it was unexpected to see almost the same degree of dilution in [$^2\text{H}_2$]palmitate enrichment across the forearm. That dilution must, again, reflect the release of non- $^2\text{H}_2$ -labeled fatty acids from the tissue bed.

Information on the source of these "diluting" fatty acids comes from the changes in TTRs of [$\text{U-}^{13}\text{C}$]palmitate across the tissues. In both adipose tissue and muscle, there was no consistent change in [$\text{U-}^{13}\text{C}$]palmitate enrichment across the tissue, implying an equal release of labeled and unlabeled fatty acids. In adipose tissue this is not entirely unexpected. Intracellular lipolysis in adipose tissue is suppressed in the fed state. Consequently, there will be no excess release of unlabeled fatty acids and no change in TTR. The data suggest that the major source of dilution of the [$^2\text{H}_2$]palmitate across adipose tissue in the postprandial state is fatty acid release from intravascular lipolysis of chylomicron-triglyceride. The degree to which this dominates fatty acid release from adipose tissue throughout the postprandial period was unexpected. The dilution of [$^2\text{H}_2$]palmitate across the forearm in the fasting state (-60 to 0 min on Fig. 3A) implies release of unlabeled NEFA from the tissue. This has been observed previously in the leg (29), where it was attributed to fatty acids released from turnover of intramuscular triglyceride. An alternative explanation might be that the forearm also contains some adipose tissue, NEFA release from which dilutes the circulating tracer in the fasting state. After the meal, however, this NEFA release is likely to be suppressed and, when taken together with the lack of dilution of [$\text{U-}^{13}\text{C}$]palmitate, may imply that in forearm muscle, as

in abdominal adipose tissue, there is significant release of fatty acids from intravascular lipolysis of chylomicron-triglyceride. Despite this, there was no net release of [$\text{U-}^{13}\text{C}$]palmitate from intravascular lipolysis of chylomicron-triglyceride in the forearm; rather, there was consistent net uptake (Fig. 2B) as we have observed before (5). These interpretations apply most strongly to the mid-postprandial period. In the early period there is insufficient labeling of chylomicron-triglyceride, and in the later period [$\text{U-}^{13}\text{C}$]palmitate will have recycled as VLDL-triglyceride.

We acknowledge the limitation that our measurements are made in non-steady-state conditions after a meal. It has been argued that arteriovenous difference measurements made in non-steady states are difficult to interpret (30). However, more recent measurements of transit times through the forearm suggest that the problem is not as great as once thought (31). We do not know the transit time through subcutaneous adipose tissue, but given the relatively low blood volume and high blood flow compared with resting muscle, it is not likely to be greater. Our main results here are based on time courses that are relatively stable over a period of hours, not on individual time points, and so we do not feel the problems with non-steady state have affected our conclusions.

In conclusion, the combination of stable isotope methodology and arteriovenous difference measurements has demonstrated new facets of the postprandial deposition of fat in extrahepatic tissues. These results and the techniques used can now be taken forward to examine the effects on adipose tissue and muscle fat metabolism of differing physiological and pathological conditions such as dietary modification and insulin resistance.

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