

# Regulation of Dietary Fatty Acid Entrapment in Subcutaneous Adipose Tissue and Skeletal Muscle

Kevin Evans,<sup>1</sup> Graham C. Burdge,<sup>2</sup> Stephen A. Wootton,<sup>2</sup> Mo L. Clark,<sup>3</sup> and Keith N. Frayn<sup>3</sup>

**Using stable isotopic labeling of dietary fatty acids in conjunction with arteriovenous difference measurements, we have assessed the regulation of lipoprotein lipase-derived fatty acid entrapment in subcutaneous adipose tissue and forearm muscle in healthy subjects in the postprandial state. Eight volunteers fasted overnight and were then given a mixed meal containing [1-<sup>13</sup>C]palmitic acid and [1-<sup>13</sup>C]oleic acid. At baseline and for 6 h after the meal, blood samples were obtained from an arterialized hand vein and veins draining subcutaneous abdominal adipose tissue and forearm muscle, and arteriovenous differences were calculated. Entrapment of labeled fatty acids released by circulating triacylglycerol hydrolysis was close to 100% at 60 min, decreasing to 10–30% by 360 min. Entrapment of labeled fatty acids in forearm muscle was >100% and did not change with time. This study shows that entrapment of dietary fatty acids in adipose tissue in the postprandial period is a highly regulated process (varying with time) and that this can be studied in humans using stable isotope-labeled fatty acids in combination with measurement of appropriate arteriovenous differences. Also, fatty acid trapping in skeletal muscle is fundamentally different from that in adipose tissue, in that all the fatty acids released by lipoprotein lipase in skeletal muscle are taken up by the tissue. *Diabetes* 51: 2684–2690, 2002**

**O**besity, a major risk factor for development of type 2 diabetes, reflects an excess of fatty acid deposition in adipose tissue compared with oxidation in other tissues. The regulation of fat storage is tightly coupled with energy balance, although the means by which the pathways of fat deposition and fat mobilization interact to bring this about are not clear. It could be that the adipocyte acts as a “sink” to absorb excess circulating fatty acids. However, the process of fatty acid uptake by adipocytes occurs in two stages (1): hydrolysis of circulating triacylglycerol (TAG) by lipoprotein lipase (LPL) bound to the capillary endothelium,

followed by tissue uptake of the resultant fatty acids. It has long been known that the latter process is not fully effective, and that a proportion of fatty acids generated by intravascular TAG hydrolysis will be liberated into the plasma as nonesterified fatty acids (NEFAs) bound to albumin. This is shown by the rapid appearance in plasma of labeled NEFAs after ingestion of labeled fat (2) and by the change in NEFA composition that occurs in the postprandial period, to mirror the dietary fatty acids (3–5). If the rate of intravascular TAG hydrolysis by LPL exceeds the rate of tissue uptake, then the possibility exists that tissue uptake of fatty acids derived from circulating TAG is a pathway that is regulated according to the need of the tissue for fatty acids.

Tissue-specific studies, based mostly on arteriovenous difference measurements, suggest that release of plasma TAG-fatty acids as NEFAs occurs in adipose tissue, but perhaps not in other tissues such as skeletal muscle (6,7). That would imply that the adipocyte is not simply a “sink” to take up excess fatty acids, but that fatty acid uptake is regulated by some means, and that the adipocyte’s handling of fatty acids derived from circulating TAG might be fundamentally different from that of other tissues.

Understanding the mechanisms by which dietary fatty acids are entrapped by tissues is clearly important in understanding the regulation of fat mass and hence the development of obesity. It is also possible that impaired regulation of fatty acid entrapment in adipose tissue might underlie dyslipidemia and insulin resistance (8). Accelerated release of NEFAs and of partially hydrolyzed chylomicron remnants from adipose tissue in the postprandial period could have adverse implications for insulin-stimulated glucose metabolism in other tissues and for hepatic lipid metabolism. There is direct evidence in people with type 2 diabetes for impaired entrapment in response to insulin (9), which contributes to the impaired ability of insulin to suppress plasma NEFA concentrations. Furthermore, it is possible that enhanced fatty acid entrapment in adipose tissue may be an important aspect of the action of the thiazolidinedione insulin-sensitizing agents (10).

In this study, we have therefore used stable isotope labeling of dietary fatty acids in conjunction with arteriovenous difference (A-V) measurements to assess the regulation of LPL-derived fatty acid entrapment in subcutaneous adipose tissue and forearm muscle in normal, healthy subjects in the postprandial state.

## RESEARCH DESIGN AND METHODS

**Subjects and protocol.** Eight healthy volunteers (four men and four women) were studied after an overnight fast

From the <sup>1</sup>Department of Clinical Chemistry, Staffordshire General Hospital, Stafford, U.K.; the <sup>2</sup>Institute of Human Nutrition, University of Southampton, Southampton, U.K.; and the <sup>3</sup>Oxford Centre for Diabetes, Endocrinology, and Metabolism, Oxford, U.K.

Address correspondence and reprint requests to Dr. Kevin Evans, Department of Clinical Chemistry, Staffordshire General Hospital, Weston Road, Stafford, ST16 3SA UK. E-mail: kevin.evans@msg-h-tr.wnids.nhs.uk.

Received for publication 7 January 2002 and accepted in revised form 4 June 2002.

ATBF, adipose tissue blood flow; A-V, arteriovenous difference; FAME, fatty acid methyl ester; GC-IRMS, gas chromatography–isotope ratio mass spectrometry; HSL, hormone-sensitive lipase; LPL, lipoprotein lipase; NEFA, nonesterified fatty acid; TAG, triacylglycerol; V-A, veno-arterial difference.

TABLE 1  
Subject characteristics

	Mean	Range
Age (years)	43	36–54
Body mass (kg)	71.6	53.5–85.8
Height (m)	1.69	1.57–1.83
BMI (kg/m <sup>2</sup> )	25.1	23.2–28.2
Fasting plasma concentrations		
Glucose (mmol/l)	5.2	5.0–5.6
Total cholesterol (mmol/l)	5.1	4.3–6.0
HDL cholesterol (mmol/l)	1.4	0.7–2.0
Triacylglycerol (mmol/l)	1.0	0.7–1.8

(Table 1). Subjects refrained from strenuous exercise and alcohol for 24 h before the study and were given instructions to consume a low-fat meal on the evening before the study and then to fast beginning at 8:00 P.M. None of the subjects were smokers. The study was approved by the Central Oxford Research Ethics Committee, and all subjects gave written informed consent.

**Experimental methods.** A 10-cm, 22-gauge Hydrocath catheter (Becton Dickinson) was introduced over a guide wire into a superficial vein on the anterior abdominal wall and threaded toward the groin so that its tip lay just superior to the inguinal ligament. As described previously (6), this provided access to the venous drainage from the subcutaneous abdominal adipose tissue, uncontaminated by muscle drainage and with a relatively minor contribution from skin. This adipose tissue depot has been shown to be representative of whole-body adipose tissue (11). A retrograde cannula was placed in a vein draining the hand, which was warmed in a hot-air box maintained at 60°C to obtain arterialized blood. A cannula was placed retrogradely in an antecubital vein draining the forearm muscle. The cannulas were kept patent by a slow infusion of 0.9% (wt/vol) saline.

Adipose tissue blood flow (ATBF) was measured immediately after each blood sample using the <sup>133</sup>Xe washout method (12) after injecting 2 MBq <sup>133</sup>Xe into the region drained by the subcutaneous abdominal catheter. The first ATBF measurement was not taken until at least 30 min later to allow recovery from the hyperemic phase caused by the injection. Forearm blood flow was measured by strain-gauge plethysmography (13) on the arm with the deep antecubital cannula, just after each set of blood samples.

Two sets of basal blood samples, 20 min apart, were taken simultaneously from the arterialized vein, the abdominal vein, and the forearm vein. To occlude superficial flow from the hand, a blood pressure cuff was inflated to a pressure of 200 mmHg around the wrist for 2 min before taking the deep venous samples. After basal blood samples were taken, subjects consumed a meal containing 37 g fat and 100 g carbohydrate, including 800 mg each of [1-<sup>13</sup>C]palmitic acid (isotope purity 99.0%; Tracer Technologies, Cleveland, OH) and [1-<sup>13</sup>C]oleic acid (isotope purity 99.3%; Masstrace, Woburn, MA) in emulsified form (Table 2). Blood samples were taken for 6 h after the meal.

**Analytical methods.** Blood samples were collected into heparinized syringes. A portion of each blood sample was rapidly deproteinized with 7% (wt/vol) perchloric acid. Plasma was separated rapidly from the remaining blood by

TABLE 2  
Nutrient composition of test meal

	Carbohydrate (g)	Fat (g)	Protein (g)
Cornflakes (30 g)	24.9	0.2	2.4
Whole milk (130 g)	6.5	5.1	4.2
Plain chocolate (70 g)	45.4	20.4	3.3
Banana (50 g)	11.6	0.2	0.6
Chocolate milkshake	11.3	11.2	5.7
Total	99.7	37.1	16.2

Determined from manufacturers' data and food tables (16).

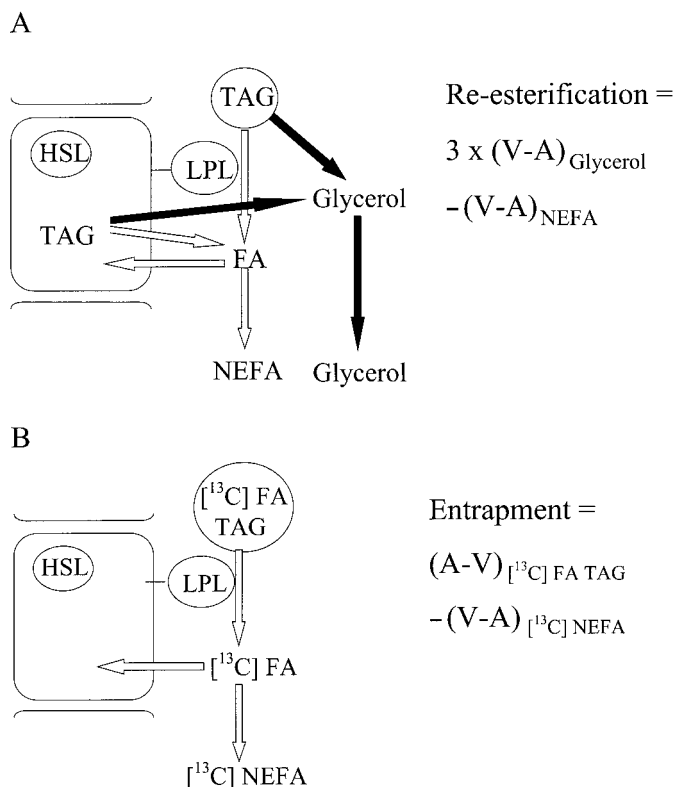
centrifugation at 4°C. Plasma TAG, NEFA, and glucose concentrations and blood glycerol concentrations were measured using enzymatic methods on an IL Monarch centrifugal analyzer (Instrumentation Laboratory, Warrington, U.K.). Plasma insulin was measured using a double-antibody radioimmunoassay (Pharmacia & Upjohn, Milton Keynes, U.K.).

For analysis of enrichment, lipids were extracted from plasma using chloroform-methanol (2:1 vol:vol) (14). NEFA and TAG fractions were separated by one of two methods. In two subjects, thin-layer chromatography was used as described previously (4); in three subjects, solid-phase extraction was used (15); and in three subjects, a combination of methods was used. The two methods give identical results (15), although recovery was greater, and the samples were found to be much cleaner with the solid-phase extraction. After methylation of fatty acids with methanolic sulfuric acid, gas chromatography–isotope ratio mass spectrometry (GC-IRMS) was used to measure isotope enrichment. Fatty acid methyl esters (FAMES) were resolved on a 50 m × 0.25 μm × 0.32 mm BPX-70 fused silica capillary column (SGE Europe, Milton Keynes, U.K.) using an HP6890 GC (Hewlett Packard, Wokingham, Berkshire, U.K.) with an Orchid IRMS interface (PDZ-Europa, Crewe, Cheshire, U.K.). FAMES were converted to CO<sub>2</sub> by heating at 860°C in the presence of PtCuO, and the <sup>13</sup>CO<sub>2</sub>/<sup>12</sup>CO<sub>2</sub> ratio was determined using a 20/20 Stable Isotope Analyser (PDZ-Europa). Tricosanoic acid methyl ester was used as isotope enrichment standard (1.134 atom percent). Fatty acids were identified routinely by their retention times relative to authentic standards. Individual fatty acid concentrations were calculated from their proportion of total fatty acids (determined by capillary GC [Chrompak, Milharbour, London, U.K.] as described previously [4]) and from total lipid concentrations in plasma. Fractional <sup>13</sup>C enrichments of plasma fatty acids were derived from enrichment calibration curves for [1-<sup>13</sup>C]palmitate and [1-<sup>13</sup>C]oleate. <sup>13</sup>C fatty acid concentrations were calculated by expressing fractional <sup>13</sup>C enrichment as a proportion of the total concentration of each fatty acid.

A test meal was also homogenized and analyzed as above to determine the specific fatty acid composition. The major fatty acids in the meal were palmitic acid (27.4%), stearic acid (24.7%), and oleic acid (34.5%). This agrees closely with the fatty acid composition determined from food tables (26.6% palmitic acid, 24.9% stearic acid, and 34.4% oleic acid [16]).

#### Calculations

**Calculations from mass balances.** TAG is hydrolyzed



**FIG. 1.** Calculation of fatty acid entrapment using mass balance (A) and stable isotope (B) methods. FA, fatty acids.

to fatty acids and glycerol by both LPL and hormone-sensitive lipase (HSL) (Fig. 1A).

If the glycerol appears in the adipose venous circulation, then three times the veno-arterial difference (V-A) for glycerol represents total fatty acid release by TAG hydrolysis. The fatty acids may be released into the circulation or taken up into adipose tissue. For re-esterification calculations, plasma NEFA concentrations (P) were converted to those in whole blood (B) using the hematocrit (H):

$$B = P \times (1 - H)$$

These values were then multiplied by blood flow to obtain true fluxes of fatty acids. Fatty acid re-esterification is therefore calculated as

$$\text{Overall fatty acid re-esterification} \\ = [3 \times (V-A)_{\text{glycerol}} - (V-A)_{\text{NEFA}}] \times \text{blood flow}$$

Percentage fatty acid re-esterification is therefore defined as

$$\% \text{ fatty acid re-esterification} \\ = 100 \times [3 \times (V-A)_{\text{glycerol}} - (V-A)_{\text{NEFA}}] / [3 \times (V-A)_{\text{glycerol}}]$$

Fatty acid re-esterification calculated on this basis involves a number of assumptions: 1) complete hydrolysis of TAG, with no release into the circulation of mono- and diacylglycerols, and 2) no reutilization of glycerol in adipose tissue. Also, this calculation gives overall re-esterification for fatty acids derived from both LPL and HSL action. Whereas these assumptions may be true for adipose tissue (discussed below), avoiding these assump-

tions will give more robust and unambiguous data. This calculation is not valid for forearm tissue, as there is significant glycerol reutilization in skeletal muscle (17,18).

Glucose uptake was calculated by converting to whole-blood values and multiplying the arteriovenous concentration difference by blood flow to obtain values for tissue glucose uptake.

**Calculations using isotope-labeled fatty acids.** Using stable isotopes allows for the first time direct measurement of entrapment of LPL-derived dietary fatty acids (Fig. 1B). Fatty acids released by LPL hydrolysis of TAG are measured as the A-V of labeled (dietary) fatty acids in the TAG fraction ( $[^{13}\text{C}]\text{FA TAG}$ ). Labeled fatty acids released into the circulation are measured as the V-A of labeled fatty acids in the NEFA fraction ( $[^{13}\text{C}]\text{NEFA}$ ). The difference represents fatty acids that have been trapped in adipose tissue.

Dietary fatty acid entrapment

$$= [(A-V)_{[^{13}\text{C}]\text{FA TAG}} - (V-A)_{[^{13}\text{C}]\text{NEFA}}] \times \text{blood flow}$$

Percentage fatty acid trapping is then defined as

Percentage entrapment

$$= 100 \times [(A-V)_{[^{13}\text{C}]\text{FA TAG}} - (V-A)_{[^{13}\text{C}]\text{NEFA}}] / (A-V)_{[^{13}\text{C}]\text{FA TAG}}$$

This calculation avoids the assumptions made in calculating entrapment by mass balance. Thus estimates of fatty acid partitioning can be made more precisely and unambiguously by the use of isotope tracers.

**Statistical methods.** Repeated-measures ANOVA with SPSS (SPSS UK, Chertsey, U.K.) were used to test for the significance of changes in plasma concentrations with time and for differences between arterial, forearm venous, and adipose venous samples as well as differences between palmitic and oleic acids. The results for TAG were log-transformed before statistical analysis. The significance of arteriovenous differences for labeled TAG were determined by paired *t* tests on areas under the curve.

## RESULTS

**Blood flow.** Adipose tissue blood flow appeared to increase after the meal (Fig. 2A), but this was not significant ( $P = 0.46$ ). There was no significant change in forearm blood flow after the meal ( $P = 0.24$ ) (Fig. 2A).

**Glucose and insulin.** After the meal, arterial glucose concentrations increased to peak at 30 min ( $P < 0.001$ ) (Fig. 2B), and insulin concentrations peaked at 30–45 min ( $P < 0.001$ ) (Fig. 2C). Adipose venous glucose concentrations also increased ( $P < 0.001$ ) (Fig. 2B), whereas there were no significant changes in forearm venous glucose concentrations ( $P = 0.25$ ) (Fig. 2B). There were significant increases in glucose uptake across both adipose tissue and forearm muscle ( $P < 0.001$ ), with a much greater uptake across forearm muscle ( $P = 0.01$  for time effect,  $P < 0.001$  for time by tissue interaction).

**TAG.** Arterial, forearm venous, and adipose venous TAG concentrations all increased after the meal, as expected (all  $P < 0.001$ ) (Fig. 3A).

Plasma TAG concentrations were lower in adipose venous than in arterialized samples ( $P = 0.003$ ), and TAG A-V concentrations across adipose tissue increased following the meal ( $P = 0.031$ ). In forearm venous plasma, TAG concentrations were lower than in arterial samples ( $P =$



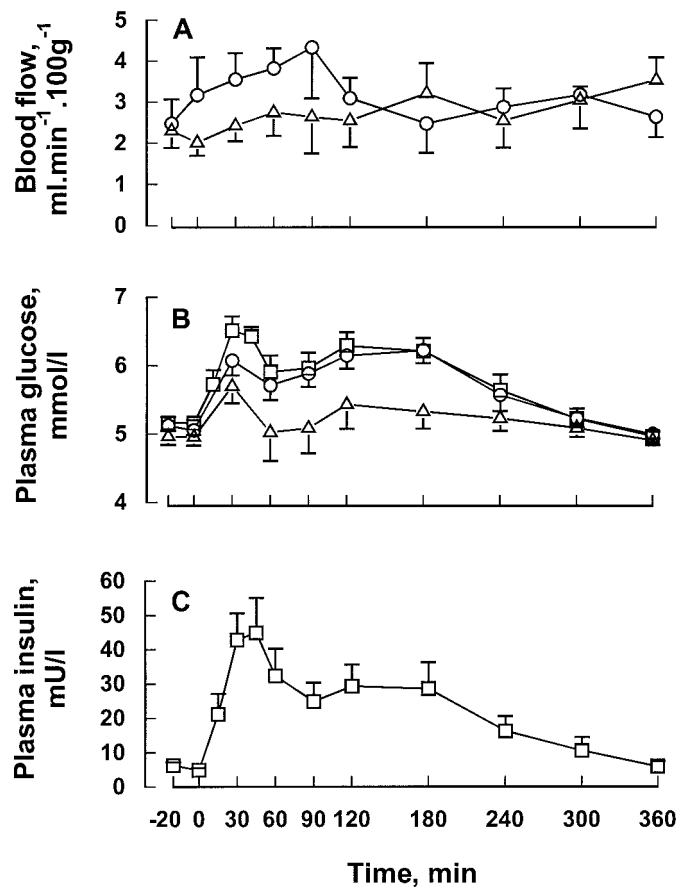


FIG. 2. Changes following a mixed meal. A: Blood flow: adipose (○) and forearm (△) tissue. B: Plasma glucose: arterialized (□), forearm venous (△), and adipose venous (○). C: Plasma insulin concentrations.

0.015), but the A-V did not change following the meal ( $P = 0.33$ ). Labeled TAG appeared in the circulation by 60 min and peaked at 240 min ( $P < 0.001$ ) (Figs. 3B and C). There was clear extraction of labeled TAG across adipose tissue from 60 min ( $P = 0.001$  for palmitic acid,  $P = 0.013$  for oleic acid) and across forearm muscle ( $P = 0.005$  for palmitic acid,  $P = 0.025$  for oleic acid). The plasma concentrations of labeled palmitic and oleic acids in the arterial TAG fraction did not differ from each other ( $P = 0.59$ ), nor did the concentrations of labeled palmitic and oleic acids in TAG in adipose venous ( $P = 0.10$ ) or forearm venous ( $P = 0.16$ ) samples.

**NEFAs.** Arterial, forearm venous, and adipose venous NEFA concentrations all decreased following the meal, before increasing in the late postprandial period (all  $P < 0.001$ ) (Fig. 4A).

The veno-arterial concentration difference across adipose tissue also decreased after the meal, before increasing in the late postprandial period ( $P < 0.001$ ). There were no changes in veno-arterial concentration difference across forearm muscle ( $P = 0.89$ ).

Labeled palmitic and oleic acids appeared in the circulation soon after the meal and increased in concentration throughout the 6 h of the study ( $P < 0.001$  for arterial, forearm venous, and adipose venous for both palmitic and oleic acids) (Figs. 4B and C). There was a steady increase in V-A of labeled palmitic and oleic acids across adipose tissue ( $P < 0.001$  for both palmitic and oleic acids). The

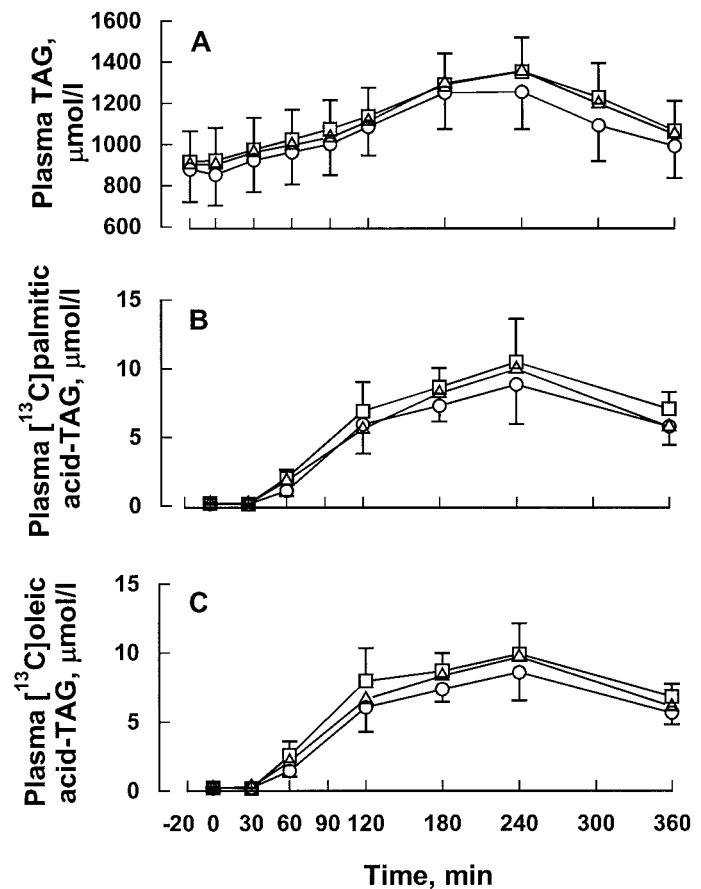


FIG. 3. Changes following a mixed meal in plasma TAG (A), [<sup>13</sup>C]palmitic acid in the TAG fraction (B), and [<sup>13</sup>C]oleic acid in the TAG fraction (C). □, arterialized plasma; △, forearm venous plasma; ○, adipose venous plasma. Units refer to TAG concentrations.

rates of increase in concentration of labeled palmitic and oleic acids in the arterial circulation were the same ( $P = 0.90$ ), as were their rates of increase in concentration in adipose venous ( $P = 0.67$ ) and forearm venous ( $P = 0.71$ ) samples. There was no significant release of labeled fatty acids across forearm muscle ( $P = 0.16$  for palmitic acid,  $P = 0.25$  for oleic acid).

**Fatty acid entrapment.** For clarity, data on fatty acid entrapment are presented only for labeled palmitic acid. The results for labeled oleic acid were very similar.

In the early postprandial period (60–120 min), most of the labeled fatty acids released by circulating TAG hydrolysis in adipose tissue were trapped within the tissue. In the late postprandial period, more of the fatty acids escaped into the circulation ( $P = 0.014$  for difference between release and entrapment) (Fig. 5A).

In forearm tissue, all the labeled fatty acids released by circulating TAG hydrolysis were trapped in the tissue, with no release into the circulation ( $P = 0.25$ ) (Fig. 5B).

Total fatty acid re-esterification in adipose tissue (from mass balance) increased after the meal to peak at 83% at 120 min, before decreasing to 21% at 360 min (Fig. 5C). There was too little fatty acid release to calculate percentage entrapment of labeled fatty acids at 30 min. At 60 min, entrapment was 92% for labeled palmitic acid (Fig. 5C) and 101% for labeled oleic acid (data not shown). Percentage entrapment then decreased until 360 min. There were no

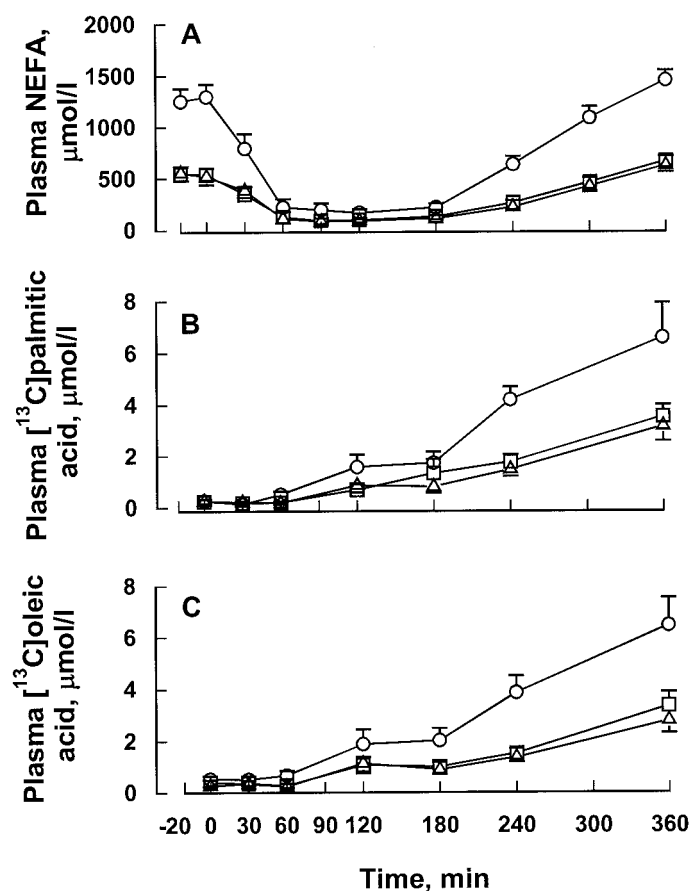


FIG. 4. Changes following a mixed meal in plasma NEFAs (A), [ $^{13}\text{C}$ ]palmitic acid in the NEFA fraction (B), and [ $^{13}\text{C}$ ]oleic acid in the NEFA fraction (C).  $\square$ , arterialized plasma;  $\Delta$ , forearm venous plasma;  $\circ$ , adipose venous plasma.

significant differences between total and labeled fatty acid entrapment ( $P = 0.27$ ). Percentage labeled fatty acid entrapment in forearm muscle was significantly greater than in adipose tissue ( $P = 0.001$ ), did not change with time, and was significantly greater than 100% ( $P = 0.019$ ).

## DISCUSSION

The most striking feature of this study is that the entrapment of dietary fatty acids in adipose tissue in the postprandial period varies markedly with time, suggesting a highly regulated process. We have also shown that this process can be studied in humans using stable isotope-labeled fatty acids in combination with measurement of appropriate arteriovenous differences. Also, we showed that fatty acid trapping in skeletal muscle is fundamentally different from that in adipose tissue, in that all the fatty acids released by LPL in skeletal muscle are taken up by the tissue.

We used two tracers, [ $^{13}\text{C}$ ]palmitic acid and [ $^{13}\text{C}$ ]oleic acid. We found no significant differences in the handling of the two fatty acids. Because these two fatty acids are the major fatty acids found in plasma, their metabolism can be taken to be representative of the bulk of plasma fatty acids. The plasma concentrations of labeled palmitic and oleic acids in the TAG fraction were very similar. Because equal amounts of each labeled fatty acid (800 mg) were added to the meal, it appears that absorption and

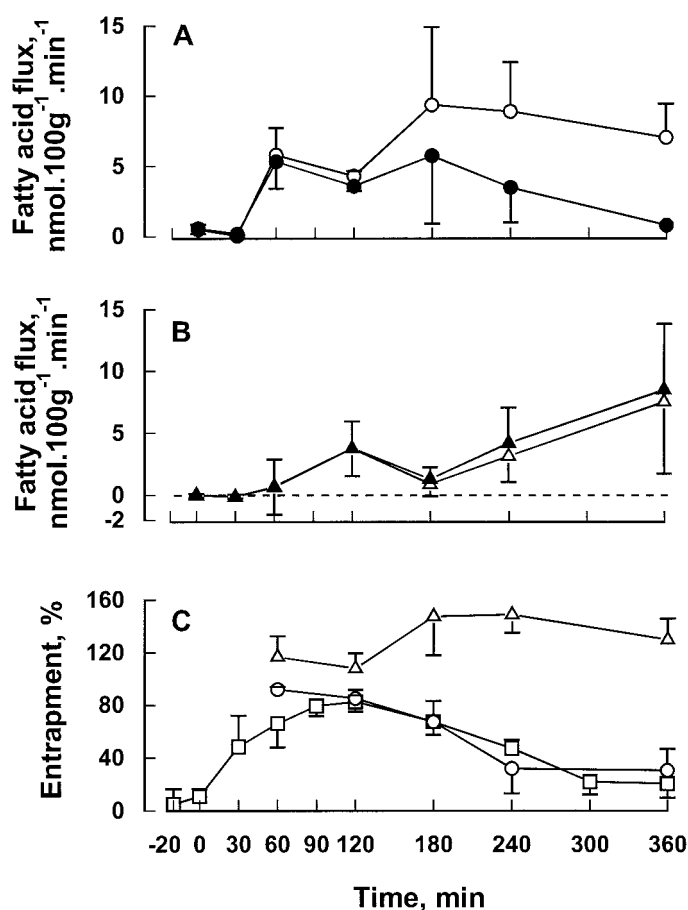


FIG. 5. Changes following a mixed meal in adipose tissue (A) and forearm tissue (B).  $\circ$  and  $\Delta$ , [ $^{13}\text{C}$ ]palmitic acid released from circulating TAG;  $\bullet$  and  $\blacktriangle$ , entrapment. C: Percentage entrapment of fatty acids in adipose tissue from mass-balance ( $\square$ ), percentage entrapment of [ $^{13}\text{C}$ ]palmitic acid in adipose tissue ( $\circ$ ), and percentage entrapment of [ $^{13}\text{C}$ ]palmitic acid in forearm tissue ( $\Delta$ ).

incorporation into chylomicrons is very similar for palmitic and oleic acid. The rates of increase in concentration of labeled palmitic and oleic acids in the circulation were also identical, suggesting that hydrolysis and release into the circulation of these fatty acids is the same. The lack of differences is in agreement with previous studies that have shown both palmitic and oleic acids to be reasonably representative of all fatty acids (19,20), although Summers et al. (21) showed some preferential uptake of monounsaturated fatty acids into adipose tissue in the postprandial period. In discussing the results below we have not distinguished the two tracers.

Adipose tissue TAG extraction increased after the meal, reflecting an increased rate of action of LPL, as we have shown previously (7,22,23). The extraction of labeled fatty acids from the TAG fraction was particularly clear, since they start from a zero baseline, and the tracers are contained mainly in the chylomicron fraction, which is the preferred substrate for LPL action (24–26). Plasma NEFA concentrations, and the release of NEFA from adipose tissue, were suppressed after the meal as expected (22,27), indicating decreased HSL action and increased trapping of LPL-derived fatty acids.

It is well known that dietary fatty acids will appear in the NEFA fraction soon after a meal (2,3,5). We found that

stable isotope-labeled NEFAs were released into the adipose venous circulation soon after the meal was consumed. This demonstrates clearly that trapping of LPL-derived fatty acids in adipose tissue is not 100% efficient. Labeled NEFA release into the circulation increased in the later postprandial period. Previous arteriovenous studies of NEFA balance have measured only net release of NEFAs, which may be a result of both intracellular hydrolysis and NEFA release into the circulation from LPL action. Using stable isotope tracers given with the meal therefore permits unambiguous demonstration of the release into the circulation of LPL-derived NEFAs from adipose tissue in the postprandial period. This would not have been possible had the tracers been given by intravenous infusion. In contrast, the lack of release of labeled fatty acids into the circulation from skeletal muscle implies that all the fatty acids released by LPL in muscle are taken up into the muscle; i.e., fatty acid trapping in skeletal muscle is fundamentally different from that in adipose tissue. Entrapment of labeled fatty acids in forearm muscle of >100%, as we found, implies some uptake of circulating NEFAs as well as entrapment of all fatty acids released by LPL action. Thus, there is a pathway for delivery of dietary fatty acids to skeletal muscle that involves adipose tissue hydrolysis of circulating TAG with release of NEFAs, which are then delivered to the muscle.

The difference between adipose tissue and skeletal muscle in entrapment of LPL-derived fatty acids may be explained by a fatty acid concentration gradient between the circulation and skeletal muscle that is always strongly in favor of fatty acid uptake (28). In contrast, the concentration gradient for fatty acids across the adipose tissue capillary, or adipocyte cell membrane, must vary in direction with time. In that respect, the handling of fatty acids by adipose tissue is analogous to glucose metabolism in the liver, with release from intracellular stores during fasting but net uptake in the fed state. We have speculated previously that coordinated regulation of HSL, LPL, and the processes of fatty acid uptake and esterification account for this changing concentration gradient (29). The present results highlight very clearly the regulation of fatty acid entrapment in the tissue.

Previous measurements of fatty acid entrapment have relied on mass balance calculations, with its necessary assumptions (see RESEARCH DESIGN AND METHODS). Some of these assumptions are more robust than others. There is strong evidence for complete hydrolysis of circulating TAG with no release of mono- and diacylglycerols into the circulation (30,31). Although it has often been stated that glycerol is not reutilized in adipose tissue (32), some studies (33,34), although not all (35), have now shown uptake into adipose tissue of labeled glycerol from the circulation. In addition, there is no direct evidence that fatty acids derived from HSL hydrolysis are handled by adipose tissue in the same way as LPL-derived fatty acids, and so it is possible to calculate only overall fatty acid re-esterification using mass balance techniques, and not specifically trapping of dietary (i.e., LPL-derived) fatty acids. These assumptions are clearly not valid for skeletal muscle, in which there is considerable evidence of glycerol uptake (in some studies even net uptake) (17,18,35),

and so it is not possible to calculate fatty acid trapping in skeletal muscle using mass balance techniques.

Entrapment of fatty acids in adipose tissue for both tracers was ~100% at 60 min and fell during the 6-h postprandial period to ~10–30% at 360 min. The upregulation of fatty acid entrapment in adipose tissue after the meal follows a time course similar to that of the increase in insulin concentrations. This upregulation of fatty acid entrapment has been shown previously at a whole-body level in experiments involving both glucose infusion (36) and insulin infusion (37). In tissue-specific experiments, it has been shown previously to occur in adipose tissue after consumption of a normal meal (22,23), but these results were based on mass balance calculations. In the present study, we found good agreement between fatty acid entrapment calculated using mass balance and stable isotope techniques. Unfortunately, it was not possible to calculate entrapment using stable isotopes at 30 min, as there were insufficient labeled fatty acids in the circulation for accurate calculation.

In conclusion, this study illustrates the feasibility of using stable isotope methodology for determining fatty acid entrapment, avoiding the untested assumptions used in calculations based on mass balance. It shows clearly that there are fundamental differences in the behavior of adipose tissue and skeletal muscle, and the data imply that fatty acid entrapment in the former is a highly regulated process. It will be interesting in future work to explore disturbances in this system and their consequences for insulin resistance and dyslipidemia.

#### ACKNOWLEDGMENTS

We thank the Wellcome Trust for supporting this project and Barbara Fielding, Sandy Humphreys, Fredrik Karpe, Michael Stolinski, and Arshad Humayan for their support and assistance.

#### REFERENCES

- Sniderman AD: Postprandial hypertriglyceridaemia(s): time to enlarge our pathophysiologic perspective. *Eur J Clin Invest* 30:935–937, 2000
- Binnert C, Pachiaudi C, Beylot M, Croset M, Crohen R, Riou J, Laville M: Metabolic fate of an oral long-chain triglyceride load in humans. *Am J Physiol* 270:E445–E450, 1996
- Heimberg M, Dunn GD, Wilcox HG: The derivation of plasma-free fatty acids from dietary neutral fat in man. *J Lab Clin Med* 83:393–402, 1974
- Griffiths JA, Humphreys SM, Clark ML, Fielding BA, Frayn KN: Immediate metabolic availability of dietary fat in combination with carbohydrate. *Am J Clin Nutr* 59:53–59, 1994
- Fielding BA, Callow J, Owen RM, Samra JS, Matthews DR, Frayn KN: Postprandial lipemia: the origin of an early peak studied by specific dietary fatty acid intake during sequential meals. *Am J Clin Nutr* 63:36–41, 1996
- Coppack SW, Fisher RM, Gibbons GF, Humphreys SM, McDonough MJ, Potts JL, Frayn KN: Postprandial substrate deposition in human forearm and adipose tissues in vivo. *Clin Sci* 79:339–348, 1990
- Evans K, Clark ML, Frayn KN: Effects of an oral and intravenous fat load on adipose tissue and forearm lipid metabolism. *Am J Physiol* 276:E241–E248, 1999
- Sniderman AD, Cianflone K, Arner P, Summers LKM, Frayn KN: The adipocyte, fatty acid trapping and atherogenesis. *Arterioscler Thromb Vasc Biol* 18:147–151, 1998
- Riemens SC, Sluiter WJ, Dullaart RPF: Enhanced escape of non-esterified fatty acids from tissue uptake: its role in impaired insulin-induced lowering of total rate of appearance in obesity and type II diabetes mellitus. *Diabetologia* 43:416–426, 2000
- Oakes ND, Thalén PG, Jacinto SM, Ljung B: Thiazolidinediones increase plasma-adipose tissue FFA exchange capacity and enhance insulin-mediated control of systemic FFA availability. *Diabetes* 50:1158–1165, 2001
- Frayn KN, Coppack SW, Humphreys SM: Subcutaneous adipose tissue

- metabolism studied by local catheterization. *Int J Obesity* 17 (Suppl. 3):S18–S21, 1993
12. Larsen OA, Lassen NA, Quaade F: Blood flow through human adipose tissue determined with radioactive xenon. *Acta Physiol Scand* 66:337–345, 1966
  13. Greenfield ADM, Whitney RJ, Mowbray JF: Methods for the investigation of peripheral blood flow. *Br Med Bull* 19:101–109, 1963
  14. Folch J, Lees M, Sloane Stanley G: A simple method for the isolation and purification of total lipids from animal tissues. *J Biol Chem* 226:497–509, 1957
  15. Burdge GC, Wright P, Jones AE, Wootton SA: A method for separation of phosphatidylcholine, triacylglycerol, non-esterified fatty acids and cholesterol esters from plasma by solid-phase extraction. *Br J Nutr* 84:781–787, 2000
  16. Holland BA, Welch AA, Unwin ID, Buss DH, Paul AA, Southgate DAT: *McCance and Widdowson's The Composition of Foods*. 5th ed. Cambridge, U.K., Royal Society of Chemistry and Ministry of Agriculture, Fisheries and Food, 1991
  17. Elia M, Khan K, Calder G, Kurpad A: Glycerol exchange across the forearm assessed by a combination of tracer and arteriovenous exchange techniques. *Clin Sci* 84:99–104, 1993
  18. Frayn KN, Coppack SW, Humphreys SM: Glycerol and lactate uptake in human forearm. *Metabolism* 40:1317–1319, 1991
  19. Hagenfeldt L: Turnover of individual free fatty acids in man. *Federation Proc* 34:2246–2249, 1975
  20. Halliwell KJ, Fielding BA, Samra JS, Humphreys SM, Frayn KN: Release of individual fatty acids from human adipose tissue in vivo after an overnight fast. *J Lipid Res* 37:1842–1848, 1996
  21. Summers LKM, Barnes SC, Fielding BA, Beysen C, Ilic V, Humphreys SM, Frayn KN: Uptake of individual fatty acids into adipose tissue in relation to their presence in the diet. *Am J Clin Nutr* 71:1470–1477, 2000
  22. Coppack SW, Evans RD, Fisher RM, Frayn KN, Gibbons GF, Humphreys SM, Kirk MJ, Potts JL, Hockaday TDR: Adipose tissue metabolism in obesity: lipase action in vivo before and after a mixed meal. *Metabolism* 41:264–272, 1992
  23. Kalant D, Phélis S, Fielding BA, Frayn KN, Cianflone K, Sniderman AD: Increased postprandial fatty acid trapping in subcutaneous adipose tissue in obese women. *J Lipid Res* 41:1963–1968, 2000
  24. Potts JL, Fisher RM, Humphreys SM, Coppack SW, Gibbons GF, Frayn KN: Peripheral triacylglycerol extraction in the fasting and post-prandial states. *Clin Sci* 81:621–626, 1991
  25. Karpe F, Hultin M: Endogenous triglyceride-rich lipoproteins accumulate in rat plasma when competing with a chylomicron-like triglyceride emulsion for a common lipolytic pathway. *J Lipid Res* 36:1557–1566, 1995
  26. van Beek AP, van Barlingen HHJJ, de Ruijter-Heijstek FC, Jansen H, Erkelens DW, Dallinga-Thie GM, de Bruin TWA: Preferential clearance of apoB-48-containing lipoproteins after heparin-induced lipolysis is modulated by lipoprotein lipase activity. *J Lipid Res* 39:322–332, 1998
  27. Frayn KN, Shadid S, Hamlani R, Humphreys SM, Clark ML, Fielding BA, Boland O, Coppack SW: Regulation of fatty acid movement in human adipose tissue in the postabsorptive-to-postprandial transition. *Am J Physiol* 266:E308–E317, 1994
  28. van der Vusse GJ, Reneman RS: Lipid metabolism in muscle. In: *Handbook of Physiology*. Section 12. Rowell LB, Shepherd JT, Eds. New York, American Physiological Society, 1996, p. 952–994
  29. Frayn KN, Coppack SW, Fielding BA, Humphreys SM: Coordinated regulation of hormone-sensitive lipase and lipoprotein lipase in human adipose tissue *in vivo*: implications for the control of fat storage and fat mobilization. *Adv Enz Reg* 35:163–178, 1995
  30. Fielding BA, Humphreys SM, Shadid S, Frayn KN: Arterio-venous differences across human adipose tissue for mono-, di- and tri-acylglycerols before and after a high-fat meal. *Endocrinol Metab* 2:13–17, 1995
  31. Summers LKM, Fielding BA, Herd SL, Ilic V, Clark ML, Quinlan PT, Frayn KN: Use of structured triacylglycerols containing predominantly stearic and oleic acids to probe early events in metabolic processing of dietary fat. *J Lipid Res* 40:1890–1898, 1999
  32. Lin ECC: Glycerol utilisation and its regulation in mammals. *Annu Rev Biochem* 46:765–795, 1977
  33. Kurpad A, Khan K, Calder AG, Coppack S, Frayn K, Macdonald I, Elia M: Effect of noradrenaline on glycerol turnover and lipolysis in the whole body and subcutaneous adipose tissue in humans *in vivo*. *Clin Sci* 86:177–184, 1994
  34. Samra JS, Clark ML, Humphreys SM, Bannister PA, Summers LKM, Macdonald IA, Frayn KN: Evidence for glycerol uptake by subcutaneous adipose tissue (Abstract). *Proc Nutr Soc* 58:164A, 1999
  35. Coppack SW, Persson M, Judd RL, Miles JM: Glycerol and nonesterified fatty acid metabolism in human muscle and adipose tissue in vivo. *Am J Physiol* 276:E233–E240, 1999
  36. Wolfe RR, Peters EJ: Lipolytic response to glucose infusion in human subjects. *Am J Physiol* 252:E218–E223, 1987
  37. Campbell PJ, Carlson MG, Hill JO, Nurjhan N: Regulation of free fatty acid metabolism by insulin in humans: role of lipolysis and reesterification. *Am J Physiol* 263:E1063–E1069, 1992