

Systemic and Forearm Triglyceride Metabolism

Fate of Lipoprotein Lipase–Generated Glycerol and Free Fatty Acids

John M. Miles,¹ Yongsoon S. Park,² Dorota Walewicz,³ Crystal Russell-Lopez,¹ Sheryl Windsor,³ William L. Isley,¹ Simon W. Coppack,⁴ and William S. Harris³

Little is known about the fate of the lipolytic products produced by the action of lipoprotein lipase (LPL) on circulating triglyceride-rich lipoproteins in humans. We studied eight lean, healthy male subjects after an overnight fast. Subjects received infusions of lipid emulsions containing triolein labeled with ³H on both the glycerol backbone and the fatty acid portion of the molecule; ¹⁴C glycerol and ¹⁴C oleate were coinfused to quantify the systemic and forearm release of ³H glycerol and ³H oleate resulting from LPL action. There was significant forearm uptake of both whole plasma triglyceride (presumed to represent primarily VLDL; extraction fraction $2.6 \pm 0.6\%$, $P < 0.005$ vs. zero) and radiolabeled triglyceride derived from the lipid emulsion (a surrogate for chylomicrons; extraction fraction $31 \pm 4\%$, $P < 0.005$ vs zero). Systemic clearance and forearm fractional extraction of glycerol was greater than that of oleate ($P < 0.001$ and $P < 0.02$, respectively). The systemic and forearm fractional release of LPL-generated glycerol were similar at 51 ± 4 and $59 \pm 1\%$, respectively (NS). In contrast, the forearm fractional release of LPL-generated oleate was less than systemic fractional release (14 ± 2 vs. $36 \pm 4\%$, $P < 0.0001$). These results indicate that there is escape, or spillover, of the lipolytic products of LPL action on triglyceride-rich lipoproteins in humans. They further suggest that LPL-mediated fatty acid uptake is an inefficient process, but may be more efficient in muscle than in adipose tissue. *Diabetes* 53:521–527, 2004

Although it is generally accepted that the action of hormone-sensitive lipase (HSL) in adipocytes is the primary source of plasma free fatty acids (FFAs) in normal postabsorptive individuals (1), the ubiquitous presence of lipoprotein lipase (LPL) (2) represents a potential additional source of

plasma FFAs. Available evidence suggests that VLDL triglyceride is not an important source of plasma FFAs. Wolfe et al. (3) administered biosynthetic labeled VLDL to dogs and found that $>95\%$ of VLDL-triglyceride fatty acids were taken up directly into tissues without traversing the plasma FFA pool; in contrast, glycerol produced by the action of LPL on VLDL triglyceride was quantitatively released into the circulation.

Chylomicrons, on the other hand, appear to be a significant source of FFAs during meal absorption. However, the magnitude of the chylomicron contribution to total FFA flux is controversial. Olivecrona et al. (4) gave chylomicrons labeled with ¹⁴C glycerol and ³H palmitate to rats, and found that $\sim 90\%$ of the administered ³H was recovered in adipose tissue, whereas only trace amounts of ¹⁴C could be found in adipose tissue. Recently, studies in which both chylomicrons and lipid emulsions containing labeled oleate were administered to mice found that 80–90% of the label was recovered in plasma (5). Roust and Jensen (6) gave a meal containing radiolabeled triglyceride to human subjects and found that approximately one-third of dietary fatty acids were released into the systemic circulation before uptake into tissues. Evans et al. (7) administered ¹³C-labeled fatty acids in a mixed meal to healthy subjects and found variable but substantial (20–80%) escape of chylomicron-derived fatty acids into the venous effluent from adipose tissue, but essentially no release of LPL-generated fatty acids from the forearm. The present studies were undertaken in healthy volunteers to determine the extent to which LPL-generated FFAs and glycerol escape into the systemic circulation, using an intravenously administered radiolabeled lipid emulsion as a tracer. We also sought to determine whether significant release of LPL-generated FFAs and glycerol occurs in the forearm, where a substantial portion of total LPL activity resides in muscle tissue.

RESEARCH DESIGN AND METHODS

Eight healthy male subjects, aged 25.6 ± 2.2 years and weighing 73.7 ± 3.1 kg (BMI 24.0 ± 0.6 kg/m²), participated in the study. Individuals on chronic medications or with fasting plasma triglycerides >150 mg/dl were excluded. Subjects refrained from alcohol consumption and vigorous exercise for 48 h before the study. The protocol was approved by the Saint Luke's Hospital Institutional Review Board, and informed, written consent was obtained from the subjects after the nature of the study was explained.

Subjects were admitted to the Clinical Study Unit the afternoon before the experiment and at 1900 h. A mixed meal (50% carbohydrate, 30% fat, and 20% protein) was given containing calories equal to one-third of energy requirements for weight maintenance, calculated as 135% of estimated basal energy

From the ¹Endocrine Research Unit, Mayo Clinic, Rochester, Minnesota; ²Yongsan University, Pusan, South Korea; the ³Mid-America Heart Institute of Saint Luke's Hospital, University of Missouri–Kansas City, Kansas City, Missouri; and ⁴Royal London Hospital, London, U.K.

Address correspondence and reprint requests to John M. Miles, MD, Endocrine Research Unit Mayo Clinic, Rochester, MN 55905. E-mail: miles.john@mayo.edu.

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FE, fractional extraction; FFA, free fatty acid; HPLC, high-performance liquid chromatography; HSL, hormone-sensitive lipase; LPL, lipoprotein lipase; MCR, metabolic clearance rate.

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expenditure (Harris-Benedict equation). The next morning, after an overnight (12–14 h) fast, a 20-gauge catheter was placed in a radial artery under local anesthesia and an additional sampling catheter was placed in an antecubital vein of the ipsilateral forearm. Oxygen saturation was measured in blood from this site to confirm that it was <60%, ensuring that the selected vein was draining primarily deep forearm tissues. An infusion catheter was inserted into a contralateral forearm vein. All three catheters were kept patent by controlled (15 ml/h each) infusions of 0.9% NaCl. After obtaining blood samples for assay blanks at 0800 h, continuous infusions of [^{14}C]oleate (~0.3 $\mu\text{Ci}/\text{min}$), [$1,3\text{-}^{14}\text{C}$]glycerol (~0.45 $\mu\text{Ci}/\text{min}$), [oleyl- $9,10\text{-}^3\text{H}$]triolein (~0.53 $\mu\text{Ci}/\text{min}$), and [glycerol- $1,2,3\text{-}^3\text{H}$]triolein (~1.2 $\mu\text{Ci}/\text{min}$) were started and continued to the end of the study. Forearm blood flow was measured at 0900 h by mercury strain-gauge plethysmography, which produces data expressed in milliliter per unit mass per minute (8). After allowing 2 h to establish isotope equilibrium, seven paired blood samples were taken at 10-min intervals for determination of steady-state concentrations and specific activities of plasma glycerol, free oleate, and triglycerides.

Preparation of tracers. All radioactive materials were purchased from American Radiolabeled Chemicals (St. Louis, MO). [$1,3\text{-}^{14}\text{C}$]glycerol and [^{14}C]oleate were prepared for infusion as previously described (8). The [9,10- ^3H]triolein and the [glycerol- $1,2,3\text{-}^3\text{H}$]triolein were purified by thin-layer chromatography and separately added to aliquots of 10% Liposyn (Abbott Laboratory, Chicago, IL), and labeled triglyceride-rich particles were then isolated by size-exclusion high-performance liquid chromatography (HPLC) (9,10). The material was collected in a sterile vial containing unlabeled lipid emulsion in order to restore the phospholipid excess present in commercial lipid emulsions, thus maintaining particle stability, and autoclaved (10,11). The labeled particles in the emulsion have previously been shown to be a suitable surrogate for the study of chylomicron triglyceride metabolism (10). **Analyses.** Blood samples were taken, immediately transferred to precooled EDTA tubes containing 0.5 mg of paraoxon (an inhibitor of LPL), and kept on ice until centrifugation at 4°C. Plasma triglyceride concentrations were determined by replicate analysis ($n = 15$ on each specimen) using a Cobas Fara analyzer and a lipase/glycerol kinase-based kit (Bayer TO1-1868-02); for each sample, the three highest and three lowest values were dropped, and the remaining nine values were averaged. This approach produces excellent precision, since the intra-assay coefficient of variation on the centrifugal analyzer is already excellent, usually <2%.

The concentration and specific activity of plasma oleate was measured by HPLC (12), using [^3H]₃₁palmitate as an internal standard (13). The concentration and specific activity of plasma glycerol was measured by HPLC (14), using 1,2,6-hexanetriol as an internal standard. In both assays, two standard curves are used: one utilizing the internal standard to determine concentration, and a second “absolute” standard curve containing ^{14}C -labeled oleate or glycerol to determine the mass of material in the unknown sample for the calculation of specific activity. A modified Folch extraction of whole plasma, followed by removal of labeled FFAs with 0.01N Na_2CO_3 , was performed to determine whole plasma ^3H triglyceride concentrations.

Calculations. Steady-state equations were used for all calculations (15).

1) The systemic rates of appearance (R_a) of glycerol and oleate were calculated from the formula:

$$R_a (\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}) = \frac{[^{14}\text{C}] \text{ infusion rate (dpm} \cdot \text{kg}^{-1} \cdot \text{min}^{-1})}{[^{14}\text{C}] \text{ SA}_A (\text{dpm} \cdot \mu\text{mol})}$$

where SA_A is the specific activity in arterial plasma.

2) Systemic metabolic clearance rates (MCRs) of glycerol and oleate were calculated from the formula:

$$\text{MCR (ml} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}) = \frac{R_a (\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1})}{\text{arterial plasma concentration } (\mu\text{mol} \cdot \text{ml}^{-1})}$$

3) Fractional extraction (FE) of glycerol and oleate were calculated from the formula:

$$\text{FE (\%)} = \left(1 - \frac{[^{14}\text{C}] \text{ SA}_V \cdot C_V}{[^{14}\text{C}] \text{ SA}_A \cdot C_A}\right) \cdot 100$$

where SA_V is the specific activity in venous plasma, and C_V and C_A are the concentrations in venous and arterial plasma, respectively.

4) Uptake and release of glycerol and oleate in the forearm tissue beds were calculated from the formulae:

$$\text{Uptake (nmol} \cdot 100 \text{ g}^{-1} \cdot \text{min}^{-1}) = (\text{FE} \cdot C_A) \cdot \text{blood/plasma flow}$$

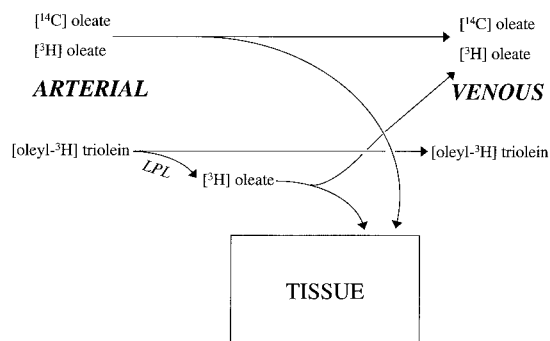


FIG. 1. A schematic representation of the stoichiometric basis for the calculations used for forearm oleate kinetics. The concept applies identically for the calculation of forearm glycerol kinetics.

$$\text{Release (nmol} \cdot 100 \text{ g}^{-1} \cdot \text{min}^{-1}) = \text{uptake} + [(C_V - C_A) \cdot \text{blood/plasma flow}]$$

where plasma flow ($\text{ml} \cdot 100 \text{ g}^{-1} \cdot \text{min}^{-1}$) equals blood flow $\times (1 - \text{hematocrit})$. Calculations are made on the assumption that fatty acids are only carried in plasma, whereas glycerol is distributed in whole blood (16).

5) The systemic rates of appearance of [^3H] were calculated from the formula:

$$[^3\text{H}] R_a (\text{dpm}/\text{min}) = [^{14}\text{C}] \text{ infusion rate} \cdot \frac{[^3\text{H}] \text{ SA}_A}{[^{14}\text{C}] \text{ SA}_A}$$

6) Systemic fractional release (%) was calculated from the formula:

$$\text{Systemic fractional release} = \frac{[^3\text{H}] R_a}{[^3\text{H}] \text{ triolein infusion rate}} \cdot 100$$

assuming that the [^3H] R_a (dpm/min) would equal the [^3H]triolein infusion rate if there were no local uptake of [^3H]glycerol or [^3H]oleate

7) The rate of [^3H]oleate or glycerol release in a tissue bed that would be expected if there were no local uptake of LPL-generated oleate or glycerol was calculated from the uptake of ^3H -triglyceride from arterial plasma according to the formula:

Expected [^3H] release ($\text{dpm} \cdot 100 \text{ g tissue}^{-1} \cdot \text{min}^{-1}$) = ($[^3\text{H}] \text{ TG}_A - [^3\text{H}] \text{ TG}_V$) \cdot blood/plasma flow, where [^3H] TG equals concentration of ^3H triglyceride in dpm/ml.

8) The actual release of triglyceride-derived [^3H] in a tissue was calculated from the formula:

$$\text{Release (dpm} \cdot 100 \text{ g}^{-1} \cdot \text{min}^{-1}) = \{([^3\text{H}] \text{ SA}_V \cdot C_V) - ([^3\text{H}] \text{ SA}_A \cdot C_A) \cdot \text{FE}\} \cdot \text{blood/plasma flow}$$

where the expression ($[^3\text{H}] \text{ SA}_A \cdot C_A$) \cdot FE equals the predicted concentration of ^3H -oleate or ^3H -glycerol in venous plasma if LPL activity in the tissue bed is zero.

9) Forearm fractional release (%) of [^3H]oleate or glycerol was calculated by the formula:

$$\text{Forearm fractional release} = \frac{\text{actual release}}{\text{expected release}} \cdot 100$$

The conceptual basis for formulae 7–9 is illustrated schematically in Fig. 1. After tracer equilibration is achieved, a constant amount of both [^3H] and [^{14}C] is delivered to the tissue bed via arterial blood. Since the [^{14}C] labeled material can only disappear across the tissue bed, the decrement in [^{14}C] concentration represents only uptake. The same is true for the [^3H] triglyceride tracers. It is assumed that the arteriovenous difference in [^3H] triglyceride concentration (see formula 7) is due to complete hydrolysis of labeled triglyceride (17). Only [^3H]oleate and [^3H]glycerol can both be produced and taken up by forearm tissue. If one assumes that LPL activity in the tissue bed is zero, then one can predict (for example) venous [^3H]oleate concentrations from the extraction fraction of [^{14}C]oleate and the arterial concentration of [^3H]oleate (formula 8). If there is LPL activity in the tissue bed, then the release, or escape, of

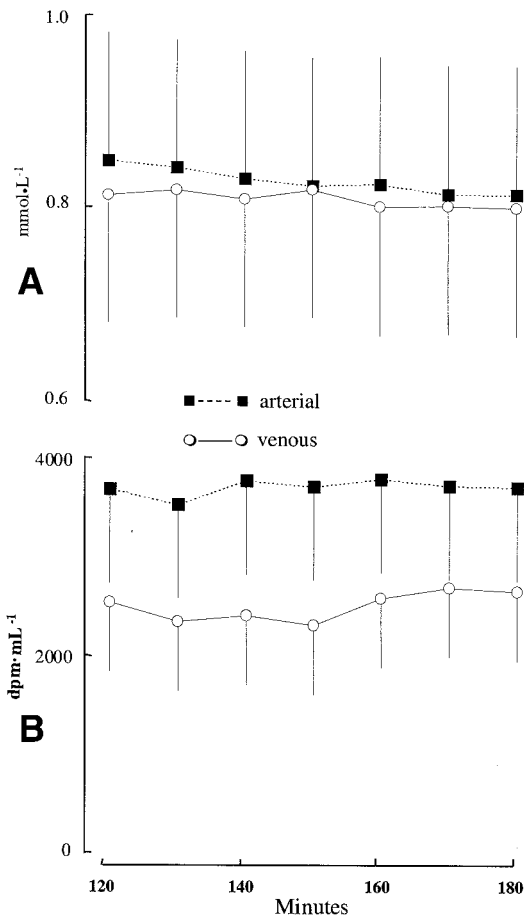


FIG. 2. Plasma triglyceride concentrations (A) and radioactivity (B) in arterial and forearm venous blood in healthy postabsorptive volunteers ($n = 8$).

^3H oleate due to LPL activity can be calculated from the difference between measured and predicted venous ^3H oleate concentration. Fractional release, then, represents the relationship between actual release of ^3H oleate (formula 8) and the disappearance of ^3H triglyceride (formula 7).

Statistical analysis. Means \pm SE for arterial and venous plasma ($n = 7$ each) were calculated for each subject, and a P value of <0.05 was required for statistical significance. The concentrations, radioactivities, and specific activities of oleate, glycerol, and triglyceride, as well as fractional release of oleate and glycerol between venous and arterial plasma, were compared using Student's paired t test, as was the systemic versus forearm fractional release of oleate and glycerol.

RESULTS

Arterial and venous plasma triglyceride concentrations and radioactivity are shown in Fig. 2. Venous plasma

triglyceride concentrations were significantly lower than arterial values (0.811 ± 0.116 vs. 0.830 ± 0.116 mmol/L, $P < 0.005$). Venous plasma triglyceride radioactivity was also lower than arterial ($2,721 \pm 961$ vs. $3,856 \pm 904$ dpm/ml, $P < 0.005$). Forearm FE of whole plasma triglyceride and ^3H -triglyceride was 2.6 ± 0.6 and $31 \pm 4\%$, respectively (Table 1). Forearm total triglyceride uptake was 34 ± 10 nmol \cdot 100 g $^{-1}$ \cdot min $^{-1}$ (Table 1).

Arterial and venous plasma glycerol concentrations were similar, averaging 40 ± 45 $\mu\text{mol/L}$ (Fig. 3). In contrast, venous oleate concentrations were lower than arterial (126 ± 19 vs. 151 ± 22 $\mu\text{mol/L}$, $P < 0.05$, Fig. 3). Plasma oleate specific activities are shown in Table 2. Venous ^{14}C oleate specific activity was lower than arterial (4.2×0.5 vs. 6.6 ± 0.9 dpm/nmol, $P < 0.001$). Venous ^3H oleate specific activity was slightly, but not significantly, lower than arterial (4.3 ± 1.0 vs. 4.9 ± 1.1 dpm/nmol, NS). Plasma glycerol specific activities are depicted in Table 2. Venous ^{14}C glycerol specific activity was lower than arterial (5.0 ± 0.9 vs. 11.4 ± 1.6 dpm/nmol, $P < 0.001$), whereas there was no difference between venous and arterial ^3H glycerol specific activity (7.4 ± 2.9 vs. 7.4 ± 2.2 dpm/nmol, NS).

Forearm blood flow was 2.77 ± 0.27 ml \cdot 100 g $^{-1}$ \cdot min $^{-1}$, and forearm plasma flow was 1.63 ± 0.14 ml \cdot 100 g $^{-1}$ \cdot min $^{-1}$. Systemic R_a and MCR for oleate and glycerol are shown in Table 1. Glycerol MCR was higher than oleate MCR ($P < 0.005$). Forearm uptake, release, and FE of glycerol and oleate are also shown in Table 1. It can be estimated from the specific activity of the infused lipid emulsion (409 ± 55 dpm/nmol, data not shown), the arterial and venous ^3H -triglyceride concentrations ($3,856 \pm 904$ and $2,721 \pm 962$ dpm/ml, respectively) and the forearm triglyceride uptake (18.8 ± 4.5 $\mu\text{mol/L}$) that the contribution of emulsion particles to forearm triglyceride uptake was ~ 0.0028 mmol/L, or $\sim 10\%$ of total uptake. Forearm FE was higher for glycerol than for oleate ($P < 0.02$). If one assumes that 75% of forearm tissue is skeletal muscle and that muscle is 25% of body weight, one can extrapolate from forearm glycerol uptake to a value for whole-body skeletal muscle glycerol uptake [$(66$ nmol \cdot 100 g muscle $^{-1}$ \cdot min $^{-1}$ / 0.75) \times 0.25 \times 73,700 g body wt = ~ 16 $\mu\text{mol/min}$] that is $\sim 14\%$ of whole-body glycerol turnover.

Fractional release of LPL-generated (i.e., ^3H) oleate and glycerol, shown in Fig. 4, were calculated by dividing actual systemic or forearm release by expected systemic or forearm release. Expected release represents the rate of release that would be observed if there were no local

TABLE 1
Systemic and forearm kinetics

	Glycerol	Oleate	Triglycerides	Chylomicrons
Systemic				
R_a ($\mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$)	1.6 ± 0.2	1.4 ± 0.2	—	—
Clearance ($\text{ml} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$)	$42 \pm 4^*$	9.5 ± 0.6	—	—
Forearm				
Uptake ($\text{nmol} \cdot 100\text{g}^{-1} \cdot \text{min}^{-1}$)	66 ± 10	108 ± 18	34 ± 10	—
Release ($\text{nmol} \cdot 100\text{g}^{-1} \cdot \text{min}^{-1}$)	63 ± 5	62 ± 7	—	—
FE (%)	$58 \pm 4^\ddagger$	45 ± 3	$2.6 \pm 0.6^\ddagger$	$31 \pm 4\ \ $

Greater than corresponding value for oleate: $*P < 0.001$, $^\ddagger P < 0.02$. Greater than zero: $^\ddagger P < 0.001$, $^\|\| P < 0.005$. Greater than whole plasma triglycerides: $^\|\| P < 0.0001$.

TABLE 2
Plasma glycerol and oleate specific activities

Time (minutes)	120	130	140	150	160	170	180
Glycerol							
¹⁴ C							
Arterial	11.7 ± 8.0	11.9 ± 2.4	11.8 ± 1.3	11.8 ± 1.9	10.2 ± 1.0	10.5 ± 1.3	11.6 ± 2.5
*Venous	5.2 ± 1.3	4.4 ± 0.7	5.1 ± 1.1	4.8 ± 0.8	5.8 ± 1.2	5.2 ± 0.7	4.3 ± 0.4
³ H							
Arterial	6.9 ± 1.8	8.1 ± 2.3	8.8 ± 2.6	7.9 ± 2.4	6.4 ± 1.7	7.1 ± 2.2	6.9 ± 2.2
Venous	8.2 ± 3.7	6.3 ± 2.3	8.4 ± 3.5	7.3 ± 2.9	8.5 ± 3.7	6.9 ± 2.4	7.4 ± 2.9
Oleate							
¹⁴ C							
Arterial	7.4 ± 1.1	6.9 ± 0.9	6.7 ± 0.9	6.4 ± 0.9	6.2 ± 0.9	6.1 ± 0.8	6.2 ± 1.0
*Venous	4.6 ± 0.7	4.2 ± 0.6	4.2 ± 0.5	4.2 ± 0.6	4.1 ± 0.6	4.1 ± 0.6	3.9 ± 0.5
³ H							
Arterial	4.8 ± 1.0	4.8 ± 1.0	4.8 ± 1.1	5.0 ± 1.2	4.9 ± 1.2	5.0 ± 1.2	5.1 ± 1.3
Venous	4.3 ± 1.0	4.3 ± 1.0	4.2 ± 0.9	4.5 ± 1.0	4.3 ± 0.9	4.4 ± 1.0	4.5 ± 1.0

**P* < 0.001 vs. arterial.

sequestration of LPL-generated [³H]oleate or glycerol and is equal to the amount of radiolabeled triglyceride that is taken up by the forearm (see formulae 5–7 above). Systemic and forearm fractional release of ³H-glycerol were similar (51 ± 4 vs. 59 ± 1%, NS). For oleate, however, forearm fractional release was lower than systemic (14 ± 2 vs. 36 ± 4%, *P* < 0.0001).

DISCUSSION

The present study indicates that a radiolabeled lipid emulsion can be used effectively to investigate the systemic and regional trafficking of LPL-generated glycerol and fatty acids in humans. We have previously demonstrated that the triglyceride-rich particles in our labeled lipid emulsion are metabolized similarly to native chylomicrons (10). A strength of this method is that the labeled lipid emulsions serve as near-massless tracers, which, when used in combination with independent FFA and glycerol tracers, can be used to partition the lipolytic products of LPL action from the lipolytic products of HSL action. Our current results indicate that chylomicrons are a potentially significant source of plasma FFAs in healthy volunteers. We also found that there is significant uptake of triglyceride-derived glycerol in tissues, without prior release into the systemic circulation. The results further suggest that there may be tissue-specific differences in the efficiency of LPL-mediated fatty acid storage.

The chief role of endothelium-bound LPL is the mediation of fat storage (2). In our study, we calculated the fractional release of fatty acids from a radiolabeled triglyceride substrate as a reflection of the relative efficiency of this process. Systemically, ~36% of LPL-generated fatty acids were released into the circulation, suggesting that fat storage is not a completely efficient process. This apparent inefficiency may be important in redistributing FFAs to tissues that lack LPL, such as liver. It also may play a role in maintaining a small supply of FFAs as fuel for tissues that depend on FFAs for energy (liver, myocardium) during the postprandial period, when insulin suppresses FFA availability (18) via its action on HSL in adipose tissue (19). We found markedly lower fractional FFA release by forearm LPL (~14%), indicating that the relative efficiency of LPL-mediated fatty acid storage in forearm tissue is greater than in the whole body. Since adipose tissue is underrepresented (and skeletal muscle overrepresented) in the forearm compared with whole body fat and muscle mass, and since the majority of systemic LPL activity resides in adipose tissue (2), these results suggest that the uptake of LPL-generated fatty acids is less efficient in adipose tissue than in muscle. It should be emphasized that these observations were made under postabsorptive

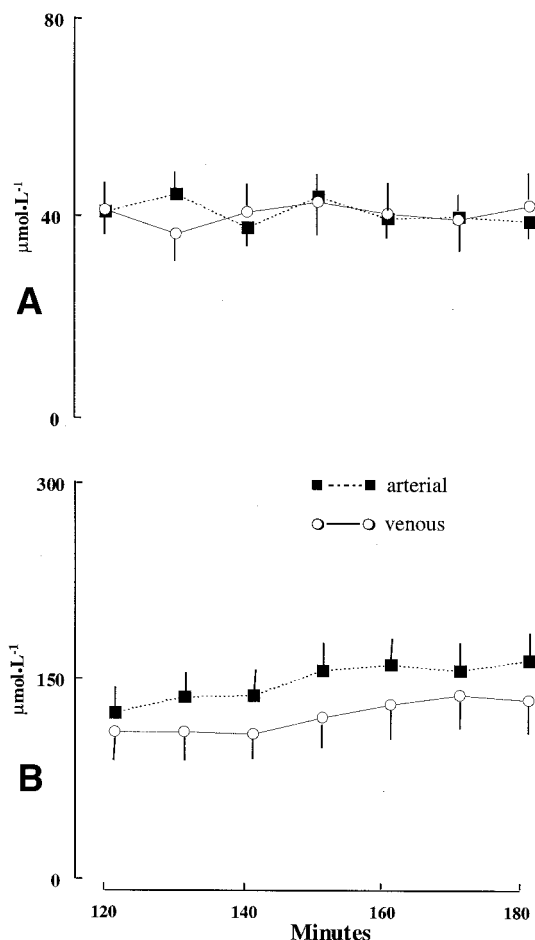


FIG. 3. Plasma glycerol (A) and oleate (B) concentrations in arterial and forearm venous blood of postabsorptive healthy volunteers (*n* = 8)

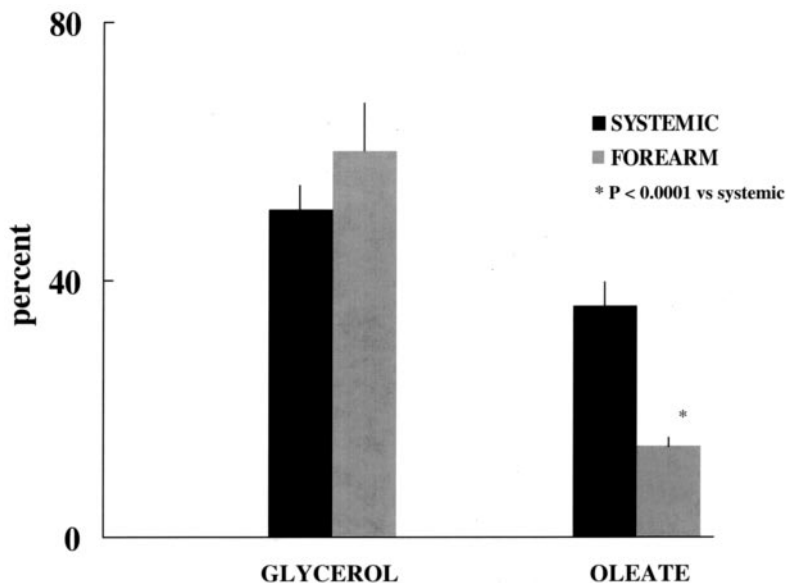


FIG. 4. Systemic and forearm fractional release of triglyceride-derived glycerol and oleate in healthy, postabsorptive volunteers ($n = 8$).

conditions. LPL in adipose tissue and skeletal muscle are regulated reciprocally; specifically, LPL activity increases in adipose tissue and decreases in skeletal muscle after meals (2). These changes are mediated in part by increases in plasma insulin concentrations, and it is possible that insulin could influence the efficiency of fat storage. Thus, since our studies were not done in the postprandial state, they may not be entirely relevant to the storage of fat that occurs after meal ingestion.

The finding that ~36% of LPL-generated fatty acids are released from tissues without initial storage are similar to previous data in humans. Roust and Jensen (6) gave a meal containing ^{14}C -triolein to humans and found that ~33% of the ^{14}C -labeled oleate appeared in the systemic circulation. This suggests that our intravenous radiolabeled lipid emulsion is metabolized by LPL in a fashion similar to native chylomicrons, an interpretation consistent with a previous report from our laboratory (10). Evans et al. (7) recently reported results that were qualitatively similar to our findings, with some quantitative differences. Healthy subjects received a mixed meal containing ^{13}C -labeled oleic acid and palmitic acid. Blood samples were taken from an arterial hand vein and from veins draining abdominal adipose tissue and the forearm. The fractional release of LPL-generated fatty acids from adipose tissue varied from ~20% in the early postprandial period to ~80% at 6 h after the meal. The fractional release from the forearm was essentially zero, although it was not possible to partition uptake of arterial FFAs and release of LPL-generated FFAs because an independent FFA tracer was not administered (7). A strength of the Evans study was the fact that the study was conducted during meal absorption. The general observation that LPL-mediated fatty acid uptake is more efficient in the forearm than in adipose tissue is concordant with our results in postabsorptive subjects showing that the spillover of LPL-generated fatty acids is greater systemically than in the forearm.

These data in humans contrast with results of studies in rodents, however. Olivecrona injected chylomicrons labeled with ^3H in the fatty acid moiety into rats and found

that only ~10% of the triglyceride-fatty acid isotope appeared in plasma FFAs, but a considerable amount of ^3H was recovered in adipose tissue and myocardium (4). Teusink et al. (5) recently reported studies in which both lipid emulsion and chylomicrons labeled with ^3H -triolein were given intravenously to anesthetized mice, and the appearance of ^3H -oleate was traced with ^{14}C -oleate. The results of experiments using the lipid emulsion and chylomicrons were concordant, suggesting that 80–90% of triglyceride fatty acids traverse the plasma FFA pool (5). The explanation for the discrepancy between these results and other studies is not apparent. Olivecrona (4) found an ~10% escape of chylomicron fatty acids into plasma in rats, whereas our results and those of others (6,7) suggest that a higher figure is operative in humans. It is possible that the study of anesthetized animals influenced the results, since general anesthesia has a marked effect on FFA metabolism (20). However, the inconsistencies may relate more to species differences. Human chylomicrons are reported to have particle diameters of 316–352 nm (10,21,22); this correlates well with commercial lipid emulsions, which have particle sizes in the 286- to 340-nm range (10,23). In contrast, rat chylomicrons have been found to have mean diameters of 130–160 nm (24–26). Whether differences in chylomicron particle size could account for differences in the trafficking of LPL-generated fatty acids is not clear.

Although there appears to be general agreement that plasma triglycerides, specifically chylomicrons, contribute to circulating FFAs, recent studies in mice have suggested that the distinction between triglyceride-derived fatty acids and plasma FFAs cannot be made (5). Our data show substantial systemic sequestration of triglyceride fatty acids without appearance in plasma FFAs, indicating that the distinction is both important and measurable in humans. We have previously reported that postabsorptive FFA uptake is substantial in forearm tissue but negligible in subcutaneous adipose tissue in postabsorptive subjects (8). Taken together, these observations suggest that FFAs and triglyceride fatty acids are partitioned in tissues. Such

partitioning could be due to differential transport of FFAs and triglyceride fatty acids through the interstitial space; labeled FFAs are present in fairly high concentrations in lymph during constant tracer infusion in animals (27).

Available evidence suggests that VLDL triglyceride, unlike chylomicron triglyceride, is not a significant source of plasma FFAs. Wolfe et al. (3) administered biosynthetic doubly-labeled VLDLs to dogs (harvested from donor dogs who had been given [^3H]glycerol and [^{14}C]palmitate); they found that >95% of VLDL-triglyceride fatty acids were taken up directly into tissues without traversing the plasma FFA pool. In recent unpublished studies, VLDL labeled with triolein, using both in vivo and ex vivo labeling techniques, was given to normal volunteers and no detectable labeled FFAs were found in plasma (M.D. Jensen, personal communication). Even if 5% of LPL-generated fatty acids from VLDL escaped into the systemic circulation (assuming complete triglyceride hydrolysis by LPL), it would account for only ~0.5% of total FFA appearance, based on VLDL triglyceride turnover rates of $\sim 0.2 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ (28) and FFA flux rates of $\sim 6 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ (8) in normal subjects. The relative contribution of VLDL to total FFA appearance would be expected to increase during the postprandial period because of suppression of HSL (6) if VLDL triglyceride hydrolysis continued at the same rate. However, a 75–90% decrease in VLDL triglyceride hydrolysis occurs during mixed-meal absorption, due to competition for LPL by chylomicrons (29). Although VLDL secretion rates are increased to $\sim 0.4 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ in obese nondiabetic (30) and type 2 diabetic (31,32) individuals, FFA appearance is also increased in the postabsorptive (18,33,34) and postprandial (6,18) states in these individuals. Thus, the relative contribution of VLDL would remain low in normal and insulin-resistant subjects under both postabsorptive and postprandial conditions.

We found that the fractional release of LPL-generated glycerol was 50–60%, with no difference between the systemic and forearm values. The substantial (~40%) uptake of triglyceride-derived glycerol in the forearm is consistent with our previous work (8) and indicates the presence of glycerol kinase in skeletal muscle. However, the observation that glycerol generated by the action of LPL on triglyceride-rich lipoproteins is substantially sequestered in tissues without systemic release contrasts with previous studies in animals showing near-quantitative mixing with the plasma glycerol pool, both in the case of VLDL and with chylomicrons (3,4). Species differences may explain these discrepant findings.

Our results demonstrate significant uptake of triglyceride by the forearm, which in normotriglyceridemic post-absorptive subjects is due almost entirely to LPL's interaction with VLDL (35). The contribution of emulsion particles to forearm triglyceride uptake was ~10% of total uptake. The fractional extraction of radiolabeled triglyceride in the forearm was 31%, which underestimates the avidity of chylomicron triglyceride extraction because the calculation was made on whole plasma triglyceride radioactivity, which includes chylomicron remnants that do not interact with LPL. Nonetheless, it amply demonstrates the preference LPL has for chylomicrons compared with VLDL, as shown previously (16).

The implications of our results concerning the contribution of dietary fat to FFA turnover are considerable and can be estimated. Assuming for a 70 kg adult 1) postabsorptive FFA flux of $\sim 6 \mu\text{mol} \cdot \text{kg}^{-1} \cdot \text{min}^{-1}$ (8); 2) postprandial FFA flux averaging about half of postabsorptive values (18), prevailing for at least half of the 24-h period; 3) dietary fat intake equaling 35% of a daily energy intake of ~2,000 kcal; and 4) fractional systemic release of chylomicron derived fatty acids of 36%, as much as 20–25% of total FFAs may come from LPL. Thus, it is possible that dietary fat content could have a nearly around-the-clock impact on ambient FFA levels. The proportion of FFAs derived directly from triglyceride-rich lipoproteins could be even higher if the relative efficiency of LPL-generated fatty acid storage were impaired in certain disease states. An increased contribution of LPL to the FFA pool could result in FFA concentrations even higher than would occur solely with the increased HSL-generated FFA release that occurs in insulin-resistant states, such as obesity and type 2 diabetes. This could have important pathophysiological consequences, considering that elevated FFAs contribute to systemic insulin resistance (36), impair endothelial function (37), stimulate the sympathetic nervous system (38), and acutely raise blood pressure (39).

In summary, the results of the present study indicate that there is escape, or spillover, of the lipolytic products of LPL action on triglyceride-rich lipoproteins in humans. This suggests that LPL-mediated fat storage is somewhat inefficient. Additional studies will be needed to determine whether the efficiency of fat storage is altered in conditions such as obesity and diabetes.

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REFERENCES

- Coppack SW, Jensen MD, Miles JM: The in vivo regulation of lipolysis in humans. *J Lipid Res* 35:177–193, 1994
- Eckel RH: Lipoprotein lipase: a multifunctional enzyme relevant to common metabolic diseases. *N Engl J Med* 320:1060–1068, 1989
- Wolfe RR, Shaw JHF, Durkot MJ: Effects of sepsis on VLDL kinetics: responses in basal state and during glucose infusion. *Am J Physiol* 248:E732–E740, 1985
- Olivecrona T: Metabolism of chylomicrons labeled with C14-glycerol-H3-palmitic acid in the rat. *J Lipid Res* 3:439–444, 1962
- Teusink B, Voshol PJ, Dahlmans VE, Rensen PC, Pijl H, Romijn JA, Havekes LM: Contribution of fatty acids released from lipolysis of plasma triglycerides to total plasma fatty acid flux and tissue-specific fatty acid uptake. *Diabetes* 52:614–620, 2003
- Roust LR, Jensen MD: Postprandial free fatty acid kinetics are abnormal in upper body obesity. *Diabetes* 42:1567–1573, 1993
- Evans K, Burdge GC, Wootton SA, Clark ML, Frayn KN: Regulation of dietary fatty acid entrapment in subcutaneous adipose tissue and skeletal muscle. *Diabetes* 51:2684–2690, 2002
- 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
- Miles JM, Judd RL, Persson M, Coppack SW: Methods for estimating the

- kinetics of lipid fuels in vivo. In *Research Methodologies in Human Diabetes*. Mogensen CE, Standl W, Eds. New York, Walter de Gruyter, 1995, p. 345–359
10. Park Y, Grellner WJ, Harris WS, Miles JM: A new method for the study of chylomicron kinetics in vivo. *Am J Physiol* 279:E1258–E1263, 2000
 11. Park Y, Damron BD, Miles JM, Harris WS: Measurement of human chylomicron triglyceride clearance with a labeled commercial lipid emulsion. *Lipids* 36:115–120, 2001
 12. Miles JM, Ellman MG, McClean KL, Jensen MD: Validation of a new method for determination of free fatty acid turnover. *Am J Physiol* 252:E431–E438, 1987
 13. Jensen MD, Heiling VJ, Miles JM: Measurement of non-steady-state free fatty acid turnover. *Am J Physiol* 258:E103–E108, 1990
 14. Judd RL, Nelson R, Klein S, Jensen MD, Miles JM: Measurement of plasma glycerol specific activity by high performance liquid chromatography to determine glycerol flux. *J Lipid Res* 39:1106–1110, 1998
 15. Steele R, Wall JS, DeBodo RC, Altszuler N: Measurement of size and turnover rate of body glucose pool by the isotope dilution method. *Am J Physiol* 18:15–24, 1956
 16. 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
 17. Fielding B, Humphreys S, Shadid S, Frayn K: Plasma mono-, di- and triacylglycerol measurements in a study of fat uptake by human adipose tissue in vivo. *Biochem Soc Trans* 23:487S, 1995
 18. Miles JM, Wooldridge D, Grellner WJ, Windsor S, Isley WL, Klein S, Harris WS: Nocturnal and postprandial free fatty acid kinetics in normal and type 2 diabetic subjects: effects of insulin sensitization therapy. *Diabetes* 52:675–681, 2003
 19. Jensen MD, Caruso M, Heiling VJ, Miles JM: Insulin regulation of lipolysis in nondiabetic and IDDM subjects. *Diabetes* 38:1595–1601, 1989
 20. Horber FF, Kraye S, Miles J, Cryer P, Rehder K, Haymond MW: Isoflurane and whole body leucine, glucose, and fatty acid metabolism in dogs. *Anesthesiology* 73:82–92, 1990
 21. Haberbosch W, Poli A, Augustin J: Characterization of human chylomicrons. *Biochim Biophys Acta* 713:398–409, 1982
 22. Ruf H, Gould BJ: Size distributions of chylomicrons from human lymph from dynamic light scattering measurements. *Eur Biophys J* 28:1–11, 1999
 23. Driscoll DF, Etzler F, Barber TA, Nehne J, Niemann W, Bistran BR: Physicochemical assessments of parenteral lipid emulsions: light obscuration versus laser diffraction. *Int J Pharm* 219:21–37, 2001
 24. Martins IJ, Mortimer BC, Miller J, Redgrave TG: Effects of particle size and number on the plasma clearance of chylomicrons and remnants. *J Lipid Res* 37:2696–2705, 1996
 25. Yang LY, Kuksis A: Size and composition of lymph chylomicrons following feeding corn oil or its fatty acid methyl esters. *Biochem Cell Biol* 65:514–524, 1987
 26. Borensztajn J, Kotlar TJ, Meredith SC: Fractionation of chylomicrons by heparin-sepharose chromatography: characterization of two heparin-binding proteins. *J Biol Chem* 260:13047–13052, 1985
 27. Judd RL, Sarr MG, Miles JM: Extravascular transport of free fatty acids and albumin in normal and diabetic dogs (Abstract). *Diabetes* 41 (Suppl. 1):186A, 1992
 28. Beil U, Crouse JR, Einarsson K, Grundy SM: Effects of interruption of the enterohepatic circulation of bile acids on the transport of very low density-lipoprotein triglycerides. *Metabolism* 31:438–444, 1982
 29. Bjorkegren J, Packard CJ, Hamsten A, Bedford D, Caslake M, Foster L, Shepherd J, Stewart P, Karpe F: Accumulation of large very low density lipoprotein in plasma during intravenous infusion of a chylomicron-like triglyceride emulsion reflects competition for a common lipolytic pathway. *J Lipid Res* 37:76–86, 1996
 30. Egusa G, Beltz WF, Grundy SM, Howard BV: Influence of obesity on the metabolism of apolipoprotein B in humans. *J Clin Invest* 76:596–603, 1985
 31. Ginsberg H, Grundy SM: Very low density lipoprotein metabolism in non-ketotic diabetes mellitus: effect of dietary restriction. *Diabetologia* 23:421–425, 1982
 32. Howard BV, Abbott WGH, Beltz WF, Harper IT, Fields RM, Grundy SM, Taskinen M-R: Integrated study of low density lipoprotein metabolism and very low density lipoprotein metabolism in non-insulin-dependent diabetes. *Metabolism* 36:870–877, 1987
 33. Jensen MD, Haymond MW, Rizza RA, Cryer PE, Miles JM: Influence of body fat distribution on free fatty acid metabolism in obesity. *J Clin Invest* 83:1168–1173, 1989
 34. Groop LC, Bonadonna RC, Del Prato S, Ratheiser K, Zyck K, Ferrannini E, DeFronzo RA: Glucose and free fatty acid metabolism in non-insulin dependent diabetes mellitus: evidence for multiple sites of insulin resistance. *J Clin Invest* 84:205–213, 1989
 35. Fisher RM, Miles JM, Kottke BA, Frayn KN, Coppack SW: Very-low-density lipoprotein subfraction composition and metabolism by adipose tissue. *Metabolism* 46:605–610, 1997
 36. Boden G, Jadali F, White J, Liang Y, Mozzoli M, Chen X, Coleman E, Smith C: Effects of fat on insulin stimulated carbohydrate metabolism in normal men. *J Clin Invest*, 1991
 37. Steinberg HO, Tarshoby M, Monestel R, Hook G, Cronin J, Johnson A, Bayazeed B, Baron AD: Elevated circulating free fatty acid levels impair endothelium-dependent vasodilation. *J Clin Invest* 100:1230–1239, 1997
 38. Manzella D, Barbieri M, Rizzo MR, Ragno E, Passariello N, Gambardella A, Marfella R, Giugliano D, Paolisso G: Role of free fatty acids on cardiac autonomic nervous system in noninsulin-dependent diabetic patients: effects of metabolic control. *J Endocrinol Metab* 86:2769–2774, 2001
 39. Stojiljkovic MP, Zhang D, Lopes HF, Lee CG, Goodfriend TL, Egan BM: Hemodynamic effects of lipids in humans. *Am J Physiol* 280:R1674–R1679, 2001