Role of very low density lipoproteins in the energy metabolism of the rat

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Abstract The role of very low density lipoproteins (VLDL) in the energy metabolism of conscious, 24-hr fasted rats was studied. VLDL labeled with [2-^{3}H]glycerol and [1-^{14}C]palmitate were infused into the rats, along with [1-^{13}C]palmitate bound to albumin and d-8-glycerol, and various metabolic factors were assessed. The rates of appearance in plasma of fatty acids in VLDL and albumin-bound free fatty acids (FFA) were about equal, on a molar basis, and only a small fraction of the FFA flux was derived from VLDL. The rate of direct oxidation of the fatty acids from VLDL was 4.4 ± 0.9 μmol of FA/kg · min, as compared with the value of 4.0 ± 0.42 μmol of FA/kg · min for plasma FFA. Four percent of the plasma glycerol flux was derived from VLDL. Thus, the direct oxidation of fatty acids in VLDL played an important role in the energy metabolism of the rats, accounting for a percentage of the total CO_2 production that was equal to the amount that arose from the oxidation of plasma FFA. The oxidation of VLDL-fatty acids did not involve prior entry of the fatty acids into the plasma FFA pool to any significant extent. — **Wolfe, R. R., and M. J. Durkot.** Role of very low density lipoproteins in the energy metabolism of the rat. *J. Lipid Res.* 1985. **26:** 210–217.

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The in vivo rates of oxidation of glucose and fat can be calculated by means of indirect calorimetry or with the use of a ^{13}C- or ^{14}C-labeled tracer of glucose or fat. In postabsorptive dogs at rest, the values obtained by the isotopic technique and by indirect calorimetry for glucose oxidation agreed well (1). When a ^{13}C-labeled fatty acid (palmitate) was used to calculate the rate of plasma free fatty acid (FFA) oxidation, however, only about one-third of the total rate of fat oxidation, as determined by indirect calorimetry, could be accounted for (1). The discrepancy between the total rate of fat oxidation and that calculated when a plasma FFA tracer is used has been recognized for several years. The existence of an intramuscular fat pool that provides muscle with substrate for energy metabolism directly has been proposed as an explanation for this finding (2). Another possible source of fat for energy metabolism is the circulating very low density lipoproteins (VLDL).

A wide range has been reported for the rate of VLDL secretion in rats. Although several studies report values in the range of 1 to 2 μmol/kg · min (e.g., ref. 3), values as high as 20 μmol/kg · min also have been found (4). This wide range may reflect the methodological problems of the various techniques that have been used to measure VLDL turnover (5). In any case, if a VLDL secretion rate of 5 μmol/kg · min is assumed, the appearance rate of fatty acids incorporated in VLDL would be 15 μmol/kg · min, approximately the same as the rate of appearance of plasma FFA in rats (6). Consequently, if the fatty acids in VLDL were oxidized as efficiently as plasma FFA, they could contribute equally to the total rate of energy production.

Despite the possible importance of the oxidation of VLDL-fatty acids in overall energy metabolism, studies in which their rate of oxidation has been quantified are limited. Research in rabbits revealed that approximately 15% of triglyceride (TG) released into the plasma was oxidized (7). If this also occurs in rats, it would indicate that the oxidation of TG-fatty acids may be an important source of energy. In the rabbit study, however, as well as in all other studies in which oxidation rates of VLDL-fatty acids have been reported, the oxidation of VLDL-fatty acids was not distinguished from the oxidation of the plasma FFA that were derived from hydrolysis of VLDL. Yet this distinction is crucial in considerations of the physiological importance of VLDL oxidation because, if it is not made, it cannot be determined whether the VLDL oxidation rate is additive to the rate of oxidation plasma FFA, or whether the two values overlap. The distinction is also important for interpretation of the importance of relative changes in lipoprotein lipase (LPL)

Abbreviations: VLDL, very low density lipoproteins; FFA, free fatty acid; LPL, lipoprotein lipase; TG, triglyceride; EGME, ethylene glycol monoethyl ether; TMS, trimethylsilyl; GLC, gas–liquid chromatography; MS, mass spectrometry.

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activity that occur in different tissues under varying physiological conditions. If the main route of VLDL-fatty acid oxidation is through entry into the plasma FFA pool, then changes in LPL activity in individual tissues are of much less importance in determining the nature of substrate oxidation in that tissue than if the VLDL-fatty acids are taken up directly and oxidized by the tissues in which the LPL hydrolyzes the circulating triglyceride.

The direct oxidation of VLDL-fatty acids can only be distinguished reliably from the oxidation of plasma FFA derived from VLDL if VLDL and FFA that are labeled with different tracers of carbon are infused simultaneously. We have, therefore, developed a method in which the use of ^{14}C-labeled VLDL and ^{13}C-labeled palmitate bound to albumin allows assessment of the direct oxidation of VLDL-fatty acids, and also enables the determination of the extent to which VLDL contributes to the appearance and oxidation of plasma FFA.

While studying the interaction between plasma and VLDL-fatty acids, we have concurrently determined the contribution of the glycerol that arises from hydrolysis of VLDL to the total plasma glycerol flux by use of a combination of radioactive (^{3}H) and stable (^{2}H) isotopes. An important question exists about the interpretation of such glycerol turnover data. The rate of appearance of glycerol in plasma is usually considered to result entirely from the rate of lipolysis in peripheral fat tissue (8), but theoretically some plasma glycerol could arise from plasma VLDL.

METHODS

Our method involved the in vivo synthesis of VLDL labeled with [1-^{14}C]palmitate and [2-^{3}H]glycerol, isolation of the labeled VLDL, and infusion of the isotope into conscious rats that were infused concurrently with d-8-glycerol and [1-^{13}C]palmitate bound to albumin.

The experiment was performed in nine Sprague-Dawley rats (350–400 g). One week before the study, the rats were anesthetized with halothane (2% in O_2) and polyethylene catheters (PE 50) were inserted into their jugular veins (for isotope infusion) and carotid arteries (for blood withdrawal) and tunneled subcutaneously so that they exited at the back of the neck. The catheters were flushed with saline and the ends were heat-sealed and taped behind the neck. The rats recovered fully from the surgery within 3 to 4 days, as evidenced by good appetite, weight gain, and general activity. Heparin was not used.

The rats were not fed for 24 hr before the study began. At approximately 9:00 AM on the morning of the study, each rat was placed in an air-tight Plexiglas chamber that was part of a closed system (**Fig. 1**). The air in the chamber was circulated by means of a roller pump (Masterflex) that maintained a high turnover of air. The air passed through a water trap (Drierite) (A, Fig. 1) and then to CO_2 traps. A different system was used to collect CO_2 for enrichment determinations and to quantify total $\dot{V}CO_2$. The air analyzed for the determination of total $\dot{V}CO_2$ passed through Ascarite (asbestos coated with NaOH), which traps all CO_2. The CO_2 excreted over time was then calculated from the change in weight (9). Two Ascarite traps were used in the system (E and F, Fig. 1) to facilitate duplicate assessments of $\dot{V}CO_2$. As the H_2O and CO_2 were trapped, the pressure in the system dropped, thereby activating a pressure-sensitive valve to a calibrated volumeter that allowed oxygen from the cylinder in the volumeter to enter the system until pres-

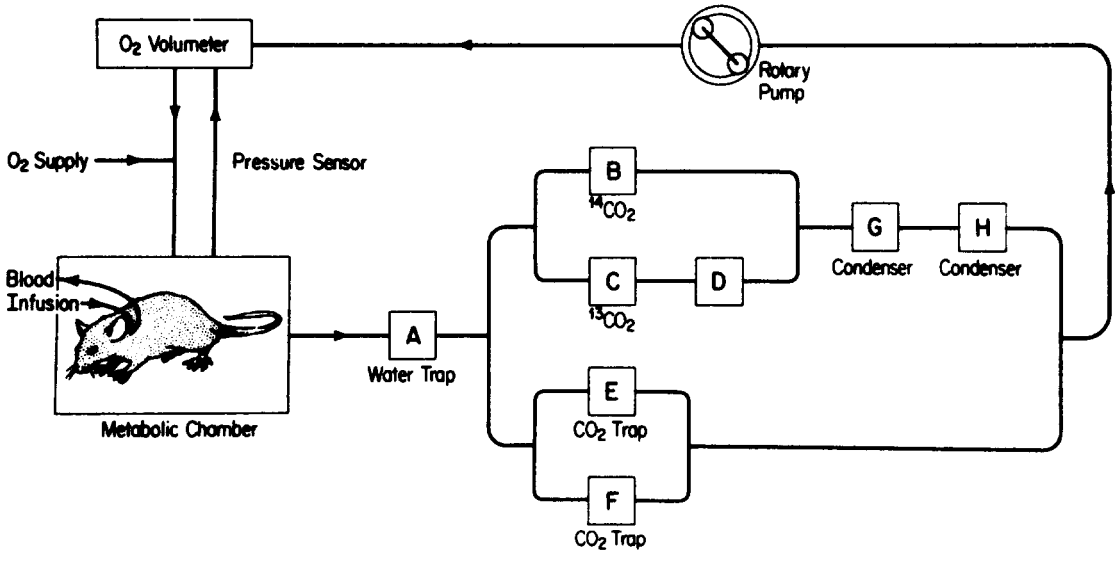

Fig. 1 Schematic diagram of recirculating system for determination of oxygen consumption, carbon dioxide production, and $^{13}CO_2$ and $^{14}CO_2$ enrichment. Rat is in an airtight Plexiglas® box.

sure was equilibrated. The $\dot{V}O_2$ was then calculated from the rate at which the cylinder emptied. For the determination of $^{13}CO_2$ enrichment, the stopcocks were turned for 5 min so that the air was bubbled through 0.1 N NaOH (C, Fig. 1) and then 5.0 N NaOH (D, Fig. 1). The CO_2 trapped in the lower normality NaOH was used for the subsequent $^{13}CO_2$ enrichment determination that was performed by use of a Nuclide 3'-60° isotope ratio mass spectrometer (10). The higher normality solution ensured that all CO_2 was trapped.

For the determination of $^{14}CO_2$ counts, the air was routed through the other trap (B, Fig. 1) for 5 min. The total counts expired were determined over 5-min intervals by trapping all CO_2 in ethanolamine in ethylene glycol monomethyl ether (EGME) at 1:2 v/v. Then, 3 ml of the trapping solution was added to 15 ml of toluene and EGME (2:1, v/v), which contained 5.5 g/l of PPO to determine the radioactivity. The total counts expired were calculated by correcting the observed counts to the extent of the fraction of the original volume of EGME solution that was used for trapping the CO_2. Specific activity (dpm/μmol) was then determined by dividing the total rate of expiration of ^{14}C (dpm/min) by the $\dot{V}CO_2$ (μmol/min). After the air passed through the methanolamine solution, it was passed through two cold-finger condensors in an acetone/dry ice bath (G and H, Fig. 1) to condense any methanol that was formed.

The catheters extended out of airtight holes in the chamber. Before the study, the rats were left in the chamber for about 30 min so that they could become accustomed to the surroundings. In general, they rested quietly throughout the study period. A blood sample (2 ml) and several expired CO_2 samples were taken before the start of the isotope infusion to determine the natural enrichment of glycerol, palmitic acid, and expired CO_2. A constant infusion of ^3H, ^{14}C-labeled VLDL-TG, d-8-glycerol, and albumin-bound [1-^{13}C]palmitic acid, in tracer amounts, was then begun (see below for details). The infusion lasted 150 min, during which time the $\dot{V}O_2$, $\dot{V}CO_2$, $^{13}CO_2$ enrichment and $^{14}CO_2$ specific activity were determined several times. A blood sample (10 ml) was taken as soon as possible after the infusion was stopped.

Isotope infusion

The doubly labeled VLDL-TG was synthesized in vivo because none is available commercially for use as a tracer. The synthesis was performed by infusing [1-^{14}C]palmitate and [2-^{3}H]glycerol into dogs and then isolating the labeled VLDL-TG that was produced. The dog had not eaten since the evening before infusion, but was infused with glucose at a high rate for about 1 hr before the isotope infusion to stimulate VLDL production.

The [1-^{14}C]palmitate (1 mCi; New England Nuclear, Boston, MA) was bound to albumin (Fraction V, Arnel Products Co.) before infusion (10). Then, [2-^{3}H]glycerol (1.5 mCi) was added to the [1-^{14}C]palmitate and the mixture was infused, for 5 min, into anesthetized (Seratol) dogs through a catheter in the portal vein. Twenty-five minutes later, blood was collected through two arterial catheters and transferred to heparinized tubes and stored on ice. The plasma was separated by using a refrigerated centrifuged (Sorrel RC-5), and was pooled before isolation of the labeled VLDL.

Twenty-five milliliters of the dog plasma was pipetted into Beckman No. 326823 polyallomer ultracentrifuge tubes. By use of a syringe, 6 ml of a 0.15 N NaCl solution (d 1.005 g/ml) was layered carefully on top of the plasma. The tubes were balanced and placed in a Beckman 50.2 Ti rotor and spun in a Beckman L2-65 ultracentrifuge at 34,000 rpm for 20 hr at 15°C. After ultracentrifugation, the top layer containing the VLDL was isolated and concentrated by evaporation. An aliquot of the VLDL-TG was taken for subsequent verification of purity. The concentrated VLDL-TG solution was dissolved in approximately 2 ml of a 5% albumin solution and then added to 12 ml of a 5% albumin solution that was prelabeled with [1-^{13}C]palmitic acid (Merck Isotopes, Montreal) (10). Then 43 μmol (4 ng) of d-8-glycerol (Merck Isotopes) was added to the mixture. The d-8-glycerol infusion was primed with 22 μmol of d-8-glycerol, and the bicarbonate pool was primed with 17 μmol/kg of $NaH^{13}CO_3$ and 2×10^6 dpm of $NaH^{14}CO_3$. The isotope mixture was infused into the rats at 0.0382 ml/min by using a Harvard syringe pump; the final isotope infusion rates were ^3H-labeled VLDL, 4×10^4 dpm/min; ^{14}C-labeled VLDL, 5×10^4 dpm/min; d-8-glycerol, 0.15 μmol/min; and [1-^{13}C]palmitate, 0.5 μmol/min. A small stirring bar was put in the syringe so that the infusate stayed well mixed throughout the infusion.

The principal concern with regard to the purity of the labeled VLDL infusion was that no [^{14}C]palmitate be infused that was not in the VLDL fraction. We checked this by placing an aliquot of the infusate on a Sephadex LH-20 column using chloroform–methanol 2:1 and by collecting the eluant in fractions. Either only the labeled VLDL was put on the column or the [1-^{14}C]palmitate was also added. The VLDL eluted as a single peak that was distinct from the [^{14}C]palmitate peak (**Fig. 2**).

Sample analysis

Blood samples were collected in heparinized tubes and stored immediately on ice. Plasma was separated by centrifugation at 3,600 rpm for 15 min and stored until subsequent analysis.

Plasma FFA (^{13}C, ^{14}C)

Plasma (1.0 ml) was processed for quantitative gas-liquid chromatography, as described by McDonald-Gibson and Young (11). The enrichment of the palmitic acid methyl ester was determined by gas chromatogra-

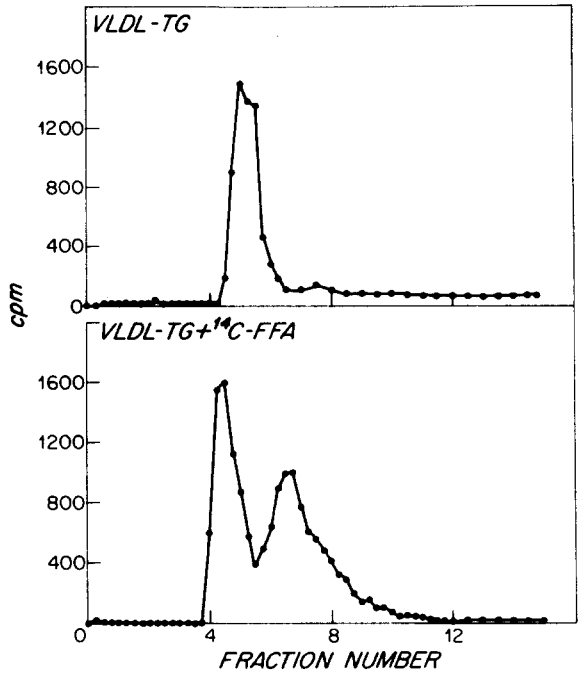

Fig. 2 Results from column chromatography (Sephadex LG-20) showing that labeled VLDL eluted in a single peak that was distinct from the [1-^{14}C]palmitate peak.

phy–mass spectrometry (GCMS), as we have described (10), by using a Hewlett-Packard 5985B GCMS system. Palmitic acid and total FFA concentrations were quantified on a Varian 2100 gas chromatograph and CDS 111 Data System. Specific activity was determined by scintillation counting after isolation of FFA by TLC, and correction for the recovery of a standard of known specific activity.

Glycerol (d-8, ^{3}H)

Plasma (1.0 ml) was processed for the determination of the isotopic enrichment of d-8-glycerol, the specific activity of [2-^{3}H]glycerol, and plasma glycerol concentration by adding an internal standard for glycerol (1, 2, 4-butanetriol) after the plasma was obtained. Plasma proteins were then precipitated by adding $Ba(OH)_2$ and $ZnSO_4$ to the plasma, vortexing immediately, and centrifuging at 15,000 rpm for 15 min. The resulting supernatant was placed on an anion (AG1-X8)/cation (AG-50-W-X8) exchange-resin column. The neutral eluate was collected in screw-top tubes (13 mm × 10 mm) and evaporated to dryness under N_2 gas. The sample was then redissolved in 0.5 ml of distilled H_2O. The dpm/ml plasma was determined by counting a known volume (0.2 ml) of the eluate in a liquid scintillation counter. (Although glucose was also in the eluate that contained the glycerol, the ^{3}H from the 2 position is lost in the synthesis of glucose from glycerol, and therefore, glycerol did not have to be separated from the glucose before scintillation counting.) Plasma glycerol concentration and enrichment were then determined by evaporating the sample to dryness again and making the tri-TMS derivative of glycerol by adding equal volumes (50 μl) of BSFA and pyridine. Plasma glycerol concentration was quantitated on a Varian 2100 gas chromatograph and CDS 111 Data System. The value for glycerol was obtained by measurement of its peak area and relative response in comparison with the internal standard (1, 2, 4-butanetriol). Plasma enrichment was measured by GLCMS. Gas–liquid chromatography separation was performed on a 6-ft glass column packed with 3% OV 17 on 80–100 Supelcoport, with the column temperature going from 80° to 250°C at 10°C/min and a helium carrier gas flow rate of 30 ml/min. We used electron-input ionization and monitored masses at m/e 205.1 and 208.1 selectively throughout the run, with their intensities integrated over the profile of the peak.

VLDL-TG (^{3}H/^{14}C)

The VLDL-TG was isolated from the plasma by placing 1 ml of plasma into a No. 331370 cellulose-nitrate centrifuge tube and topping it off with 10 ml of a 0.15 N NaCl solution (d 1.005 g/ml). The sample was spun in a Beckman L2-65B ultracentrifuge at 29,500 rpm (109,000 *g*) for 20 hr at 15°C by use of a Beckman SW41 rotor. After ultracentrifugation, the top layer of the solution, which contained the VLDL-TG, was removed carefully with a roller aspiring pump. The triglyceride concentration of the fraction that contained the VLDL was measured by using the Harleco Tri-ES kit, and a measured volume of the solution was counted by using Hydrofluor (National Diagnostic, Parsippany, NY) as scintillant and a Searle Analytic Beta Counter with the dual-isotope program.

Calculations

We assumed an isotopic steady state for all calculations. The basis for this assumption was that good plateaus were achieved in expired $^{14}CO_2$ and $^{13}CO_2$ enrichment (see results), and this could only have occurred if the plasma enrichment of the precursors (^{14}C-labeled VLDL and [^{14}C]palmitate) was constant. We have previously described the equations we used to calculate the total rate of appearance of plasma FFA and glycerol and the rate of plasma FFA oxidation (10).

The following calculations were used to calculate VLDL kinetics and oxidation.

(*1*) Rate of appearance of VLDL (RaVLDL) into plasma

$$(\mu\text{mole/kg} \cdot \text{min}) = \frac{^{3}\text{H infusion rate (nCi/kg} \cdot \text{min)}}{\text{VLDL SA } (^{3}\text{H})}$$

where SA is specific activity.

(*2*) Ra of ^{14}C-FFA (nCi/kg · min) = plasma FFA-SA (nCi/μmol) × total Ra of plasma FFA from ([1-^{13}C]palmitate data) (μmol/kg · min).

(3) Ra of FFA into plasma derived from VLDL

$$(\mu\text{mole/kg} \cdot \text{min}) = \frac{\text{Ra of } ^{14}\text{C-FFA (nCi/kg} \cdot \text{min)} \quad (2)}{\text{SA of FFA in infusate (nCi/}\mu\text{mole).}}$$

(4) Fraction of plasma FFA derived from VLDL

$$= \frac{\text{Ra of FFA from VLDL } (\mu\text{mol/kg} \cdot \text{min}) \ (3)}{\text{total RaFFA from } ^{13}\text{C-data.}}$$

(5) Total rate of oxidation rate of fatty acids in VLDL

$$(\mu\text{mole/kg} \cdot \text{min}) = \frac{^{14}CO_2 \text{ expired (nCi/kg} \cdot \text{min)}}{^{14}\text{C infusion rate (nCi/kg} \cdot \text{min)} \times 0.95} \times \text{RaVLDL (1)} \times 3\ \mu\text{mol FA/}\mu\text{mol VLDL-TG.}$$

The 0.95 is included to correct for retention of $^{14}CO_2$ in the bicarbonate pool (9).

(6) Oxidation of plasma FFA derived from VLDL

$$= \frac{\text{RaFFA from VLDL } (1) \ (\mu\text{mol/kg} \cdot \text{min})}{\text{total RaFFA from } ^{13}\text{C-data. } (\mu\text{mole/kg} \cdot \text{min})} \times \text{total plasma FFA oxidation rate } (\mu\text{mol/kg} \cdot \text{min}).$$

Since the total rate of oxidation of the fatty acids in VLDL (5) is equal to the sum of the oxidation of the plasma FFA that have been derived from the VLDL and the fatty acids from VLDL that have been oxidized directly without entering the plasma pool, then:

(7) *Direct oxidation of fatty acids in VLDL (μmol/kg · min)* = (6) − (5).

(8) *Ra glycerol into plasma from VLDL-TG (μmol/kg · min)*

$$= \frac{[^3\text{H}]\text{glycerol SA (nCi/}\mu\text{mol)} \times \text{Ra of glycerol from } d_8 \text{ data } (\mu\text{mol/kg} \cdot \text{min})}{\text{SA of } [^3\text{H}]\text{glycerol in infusate (nCi/}\mu\text{mol).}}$$

(9) *Fraction of glycerol from TG*

$$= \frac{\text{Ra glycerol from VLDL-TG } (\mu\text{mol/kg} \cdot \text{min}) \ (8)}{\text{total Ra glycerol (from } d_8 \text{ data) } (\mu\text{mol/kg} \cdot \text{min}).}$$

Results were expressed as means ± SEM.

RESULTS

Plasma concentration (μmol/ml) of FFA was 0.48 ± 0.06; glucose was 5.83 ± 0.26; glycerol, 0.21 ± 0.08; and triglycerides, 0.67 ± 0.12; all values were in the normal range for this duration of fasting, as was the rate of oxygen consumption, 639 ± 38 μmol/kg · min and carbon dioxide production, 460 ± 24 μmol/kg · min. The respiratory exchange coefficient (R) was 0.72 ± 0.03 μmol/kg · min, which indicated that fat oxidation was the primary source of energy production.

Since proof of the existence of an isotopic equilibrium at the time the blood sample was drawn is crucial for the justification of the calculations we used for plasma kinetics, all the individual values for $^{13}CO_2$ and $^{14}CO_2$ enrichment are presented in **Table 1.** We considered these values to indicate that plateaus in enrichment in expired CO_2 were achieved.

The rate of appearance of VLDL (RaVLDL), as calculated from the ^{3}H data, was 4.8 ± 0.57 μmol/kg · min (**Table 2**). The appearance rate of fatty acids in VLDL (14.4 ± 2.4 μmol/kg · min) was roughly equal to the difference between RaFFA (19.1 ± 1.5 μmol/kg · min) and the rate of oxidation of plasma FFA (4.0 ± 0.38 μmol/kg · min). Hydrolysis of VLDL resulted in a minimal contribution of glycerol or FFA to the respective plasma pools, since only 4.0% (0.9 ± 0.13 μmol/kg · min) of the total plasma glycerol flux of 17.3 ± 2.2 μmol/kg · min and only 3.6% (0.7 ± 0.04 μmol/kg · min) of the total plasma FFA flux of 19.1 ± 1.5 μmol/kg · min was derived from the

TABLE 1. CO_2 enrichment in expired breath in individual rats[a]

Minutes Of Infusion	Rats 1	2	3	4	5	6	7	8	9	$\bar{X}$ ± SEM
$^{13}CO_2$ Enrichment (atom percent excess)										
30–40	.0066	.0076	.0061	.0046	.0070	.0024	.0071	.0045	.0088	.0061 ± .0006
60–70	.0071	.0062	.0080	.0063	.0032	.0022	.0059	.0037	.0054	.0053 ± .0006
90–100	.0075	.0071	.0075	.0069	.0034	.0019	.0060	.0031	.0043	.0053 ± .0007
100–110	.0073	.0074	.0060	.0063	.0040	.0021	.0057	.0048	.0040	.0053 ± .0006
130–140	.0078	.0077	.0068	.0070	.0041	.0022	.061	.0023	.0043	.0054 ± .0007
$^{14}CO_2$ Specific activity (dpm/μmol CO_2)										
30–40	.484	.471	1.144	.865	.308	.242	.721	1.117	.401	.639 ± .113
60–70	.440	.572	1.210	1.113	.290	.273	.635	.767	.431	.636 ± .112
90–100	.277	.528	.871	.548	.286	.238	.447	.468	.461	.458 ± .064
100–110	.218	.596	.793	.565	.288	.247	.431	.348	.440	.436 ± .063
130–140	.218	.618	.843	.532	.288	.240	.466	.308	.455	.440 ± .068

[a] CO_2 was trapped over 5-min intervals.

TABLE 2. Plasma kinetics of VLDL, FFA and glycerol

VLDL appearance (^{3}H-data)	4.8 ± 0.57[a]
Total glycerol appearance (d-8-data)	17.3 ± 2.2
Glycerol appearance from VLDL (^{3}H-data)	0.9 ± 0.13
Total FFA appearance (^{13}C-data)	19.1 ± 1.5
FFA appearance from VLDL (^{14}C-data)	0.3 ± 0.04

[a]Units are μmol/kg · min ± SEM.

hydrolysis of VLDL-TG (Table 2). The direct oxidation of fatty acids derived from VLDL accounted for approximately 15% of the total $\dot{V}CO_2$, which was roughly the same as the % $\dot{V}CO_2$ arising from the direct oxidation of plasma FFA (**Table 3**). Approximately the same fraction of the fatty acids appearing in VLDL was oxidized directly to CO_2 (28%) as that of the fatty acids appearing as plasma FFA (21%). Together, approximately 30% of the total $\dot{V}CO_2$ could be accounted for by the oxidation of the labeled plasma lipids.

DISCUSSION

The values reported for the rate of VLDL release in rats fasted for 24 hr have ranged widely (about tenfold) (3, 4, 12). In most experiments in which isotopes have been used to derive those values, only a single injection of labeled glycerol or palmitate was given and the VLDL kinetics were calculated from the subsequent decay in radioactivity of the VLDL after the labeled precursor had been incorporated. The complexity of the decay process and the problems it creates in the interpretation of results have been discussed previously (13). If reliable data are to be generated, many more samples must be drawn and analyzed than are feasible when a rat is used (5). Although a mathematical model exists that can be used to describe VLDL kinetics if an adequate number of samples are taken (over 48 hr) after a bolus of [^{3}H]glycerol is administered (5), since dietary status (fed versus fasted, length of fast) is important in determining VLDL-kinetics (14), any method that requires several hours of sampling must have interpretive limitations.

We chose to use a continuous infusion technique to quantitate VLDL kinetics because, once an isotopic equilibrium exists in the plasma enrichment, the stochastic approach eliminates many of the assumptions that must apply when single-injection techniques are used. Furthermore, this approach allowed us to measure the rate of oxidation of the VLDL fatty acids, which is problematic when a single-injection technique is used (15). One potential limitation of the constant infusion technique as we have applied it is that we have used dog VLDL as a tracer for rat VLDL kinetics, and it is possible that the dog VLDL may be handled differently. However, the average rate of appearance of VLDL that we calculated (4.8 μmol/kg · min) was similar to that calculated by Palmer, Cooper, and Shipley (12) (4.1 μmol/kg · min) from the incorporation of [^{14}C]palmitate into rat VLDL. This figure is in essence the rate of VLDL production.

We would have preferred to draw at least three blood samples after the isotope infusion began in order to demonstrate the existence of an equilibrium in the enrichments of plasma VLDL, FFA, and glycerol. Unfortunately, the volume of blood required for analysis prohibited multiple sampling. Consequently, we had to rely on the CO_2 enrichment data to substantiate the existence of an isotopic steady state. It is not possible to determine definitively whether a plateau in CO_2 enrichment was achieved in each experiment; however, we feel that our data support the existence of an isotopic plateau. The greater range in values for $^{14}CO_2$ specific activity than in those for $^{13}CO_2$ enrichment resulted because the $^{14}CO_2$ value was calculated by multiplying the total counts expired over 5 min by the $\dot{V}CO_2$ (values that were determined at different times). The $^{13}CO_2$ enrichment, on the other hand, was determined directly by isotope ratio-mass spectrometry. Also, the specific activity of the VLDL infused varied, but only a single source of [1-^{13}C]palmitate bound to albumin was used. Since VLDL are distributed in the plasma, it is not unreasonable to assume that an equilibrium would have been achieved in a length of time comparable to that required for albumin-bound palmitate. We have shown previously that an equilibrium in palmitate enrichment is achieved within 20 min in rats that have been fasted for 24 hr (6).

If, as we believe, we were reasonably close to an isotopic plateau, then our data indicate that, on a molar basis, the rate of appearance of VLDL in plasma in rats is about one-third the rate of appearance of FFA in plasma. Thus, since there are three fatty acids per mole of VLDL-triglyceride, a similar number of fatty acids appear in plasma as FFA as appear in plasma in VLDL. This result is consistent, in a general sense, with the observation that, in animals fasting, the primary determinant of VLDL flux is the rate of plasma FFA flux (16). It is, therefore, not surprising that the oxidation of the VLDL-fatty acids produced a substantial fraction of the total rate of CO_2 production. Furthermore, only about 3% of the VLDL-fatty acids first entered the plasma pool before oxidation.

TABLE 3. VLDL and FFA oxidation

VLDL oxidation, μmol FA/kg · min[a]	4.4 ± 0.9
% of $\dot{V}CO_2$ from VLDL oxidation[a]	15.5 ± 1.8
Plasma FFA oxidation, μmol FA/kg · min[b]	4.0 ± 0.38
% $\dot{V}CO_2$ from FFA[b]	14.6 ± 1.2

[a]Value does not include oxidation of FA that first entered plasma FFA pool.
[b]Contribution from VLDL-derived FA has been subtracted.

Although it has been estimated that about 10% of the plasma FFA turnover in rabbits is derived from the triglyceride fatty acids (7), the well-recognized species difference in VLDL kinetics (17) could account for the lower value we observed. In any case, in postabsorptive rats, the circulating VLDL is an important source of substrate for energy metabolism. Since lipoprotein lipase (LPL) is responsible for the hydrolysis of plasma TG in different tissues, the fact that the VLDL-fatty acids are oxidized in the tissue in which hydrolysis occurs supports the importance of the role of LPL in regional alterations in substrate energy metabolism.

A higher percentage of the plasma glycerol flux originated from VLDL than did the plasma FFA flux. This finding is understandable, since the tissues that probably use the VLDL-fatty acids for energy (for example, muscle) would be expected to take up little glycerol from the plasma because of the low activity of glycerol kinase in those tissues. The important observation in this regard, however, is that the value was only 5% of the total rate of appearance of glycerol in plasma. Thus, the assumption that the rate of appearance of glycerol in plasma is primarily a reflection of the rate of lipolysis in the adipocyte (8) seems to be valid, yet the glycerol data are difficult to interpret in light of the VLDL fatty acid data. The number of fatty acids released as a result of the hydrolysis of VLDL far exceeded the 3:1 ratio that might have been expected on the basis of the observed rate of appearance of [^{3}H]glycerol into the plasma pool. The VLDL oxidation rate alone was more than three times greater than the appearance of glycerol arising from VLDL, which suggests that partial hydrolysis of VLDL-TG occurred to some extent.

The indirect calorimetry data indicated that almost all of the energy derived from the oxidation of nonprotein substrates was derived from the oxidation of fat. These results are consistent with the observations that, in this model, the oxidation of plasma glucose accounts for only 7% of $\dot{V}CO_2$ (6), and the breakdown of muscle glycogen under these conditions is minimal (18). Consequently, since the sum total of rate of oxidation of VLDL and plasma FFA only accounted for 30% of $\dot{V}CO_2$, the origin of more than half of the fat oxidation was unknown. We have, therefore, not only shown VLDL to be important in terms of energy metabolism, but we have also confirmed earlier observations on the significance of nonplasma sources of fat (such as intramuscular pools) (2) as sources of fat in energy metabolism.

As we have discussed previously, such intracellular pools of fat could affect the accuracy of the plasma-tracer technique for determining the rate of VLDL or FFA oxidation (1). Nevertheless, any dilutional effects that these pools may have had on isotope taken up from plasma should have been relatively similar for the ^{13}C-labeled FFA and the ^{14}C-labeled VLDL-fatty acids, and thus should not invalidate the conclusion that VLDL-fatty acid oxidation is as important as plasma FFA oxidation in the energy metabolism of rats fasted for 24 hr.

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