Contribution of de novo fatty acid synthesis to very low density lipoprotein triacylglycerols: evidence from mass isotopomer distribution analysis of fatty acids synthesized from [$^2H_6$]ethanol

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Abstract A detailed comparison of the structures of plasma very low density lipoprotein (VLDL) and liver triacylglycerols (TG) (Yang et al. 1995. *J. Lipid Res.* **36:** 125–136) has demonstrated that a minimum of 60% of the secreted TG could have been derived from partial lipolysis and reesterification of stored TG and a maximum of 40% could have been derived from direct secretion of newly made TG. To investigate the processes involved in the transfer of TG to VLDL in vivo, we have determined the distribution of deuterium among the molecular species of the liver-TG and VLDL-TG during the infusion of perdeuterated ethanol along with fructose or glucose and during the provision of either glucose or fructose in the drinking water for 2 weeks. The deuterium labeling (percent excess and percent replacement) of the total fatty acids was determined by GC/MS of the methyl esters while the labeling of the glycerol and the glycerol plus fatty acids of the enantiomeric diacylglycerol moieties of TG was determined by LC/MS with on-line mass spectrometry. Supplementation of the diet for 2 weeks with either glucose and fructose stimulated the synthesis of TG containing new fatty acids and glycerol. The proportion of the newly made to preexisting TG differed in VLDL from that in the liver. The ^2H % replacement in glycerol and in total fatty acids was greater in VLDL-TG than in the liver-TG. On the basis of the mass isotopomer distribution analysis it was estimated that a maximum of 30% of the VLDL-TG could have been derived directly from TG that was made de novo and did not equilibrate with the liver-TG stores. The transfer of the stored TG to VLDL was best accounted for by a degradation to 2-monoacylglycerols and resynthesis via the 2-monoacylglycerol pathway with addition of an excess of newly synthesized fatty acids to the resynthesized TG.–**Yang, L-Y., A. Kuksis, J. J. Myher, and G. Steiner.** Contribution of de novo fatty acid synthesis to very low density lipoprotein triacylglycerols: evidence from mass isotopomer distribution analysis of fatty acids synthesized from [$^2H_6$]ethanol. *J. Lipid Res.* 1996. **37:** 262–274.

Supplementary key words VLDL • liver • triacylglycerol • GC–MS • LC–MS • [$^2H_6$]ethanol • isotopomer • mass isotopomer distribution analysis • lipogenesis

The origin of the triacylglycerol (TG) moiety of plasma very low density lipoprotein (VLDL) has received much recent attention as it has become clear that the bulk of the secreted TG is not derived directly from new synthesis (1–3). On the basis of detailed analysis of the stereospecific positional distribution and molecular association of the fatty acids of TG in the plasma VLDL and liver stores, we have concluded (3) that a minimum of 60% of the VLDL-TG could have been derived via lipolysis hepatic TG stores to diacylglycerol (DG) and/or monoacylglycerol (MG) and reesterification, while a maximum of 40% could have originated via direct secretion of newly formed TG. These estimates are comparable to those derived from studies with radioactive fatty acids (2). The mechanism of the remodeling and secretion of the stored TG has not been determined. In order to obtain further information about the lipolysis and esterification pathways, we have now studied the stereospecific distribution of the deuterium-labeled fatty acids and glycerol between VLDL-TG and liver-TG during an infusion of perdeuterated ethanol. We examined the contributions of the phosphatidic acid (PA) and the MG pathways both under basal conditions and when lipogenesis and VLDL pro-

Abbreviations: TG, triacylglycerol; VLDL, very low density lipoprotein; DG, diacylglycerol; MG, monoacylglycerol; PA, phosphatidic acid; TLC, thin-layer chromatography; CE, cholesteryl ester; FFA, free fatty acids; FC, free cholesterol; PL, phospholipid; PC, phosphatidylcholine; GLC, gas–liquid chromatography; MS, mass spectrometry; DNPU, 3,5-dinitrophenylurethane; HPLC, high performance liquid chromatography; NCI, negative chemical ionization; MGAT, 2-monoacylglycerol acyltransferase; MIDA, mass isotopomer distribution analysis.

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duction were increased by subjecting the animals to carbohydrate supplementation. The mass isotopomer distribution in these studies shows that the PA pathway could not have contributed more than 30% of the VLDL-TG during carbohydrate feeding, a value similar to that previously estimated (3).

MATERIALS AND METHODS

Materials

[$^2H_6$]ethyl alcohol (anhydrous) was purchased from MSD isotopes (Division of Merck Frosst Canada Inc., Montreal, Canada). Phospholipase C (type I and type V) was obtained from Sigma Chemical Co. (St. Louis, MO), while 3,5-dinitrophenyl isocyanate was from Sumka Chemical Analysis Service Ltd (Osaka, Japan). Silica gel G soft layer thin-layer chromatography (TLC) plates were purchased from Fisher Scientific Chemical Co. (Rockford, IL). Ethyl magnesium bromide was from Aldrich Chemical Co. (Milwaukee, WI). Somnotal (sodium pentobarbital) was from M.T.C. Pharmaceutical (Canada Packers Inc., Cambridge, Canada).

Animals

Male Wistar rats from Charles Rivers Canada (Division of Bausch and Lomb Canada Inc. St-Constant, Que.) were used. There were three groups of rats: *1)* control (n = 6); *2)* fructose-supplemented (n = 5); and *3)* glucose-supplemented (n = 5). All rats weighed 100 gm at the beginning of their 2-week pre-study feeding period. They were fed standard lab chow (Ralston Purina Co., St. Louis, MO) and housed under normal light. The control rats had ad libitum access to water. Group 2 was given a 10% solution of fructose to drink ad libitum in place of water. Group 3 was given a 10% of glucose to drink ad libitum in place of water. All the rats weighed 240–260 gm at the end of 2 weeks. There were no significant differences in weight among the groups.

At 2:00 PM on the day of the experiment the rats were anesthetized with Somnotal (50 mg/kg) and an intraperitoneal cannula was inserted. Thereafter the rats were allowed access to their previous drinking solutions, but were denied access to solid food. After the rats woke, at 10:00 PM, a 10% solution of perdeuterated ethanol in saline was infused at a rate of 1.1 ml/h for 10 h through the intraperitoneal cannula (4, 5). Groups 2 and 3 also had fructose (20% wt:vol) or glucose (20% wt:vol) in the infusate.

Two other normal rats weighing 350 gm, that were a part of another study, were examined (Tables 1–4). They had not received carbohydrate supplementation. These two rats were infused with a 10% solution of perdeuterated ethanol in saline that contained fructose (20% wt/vol) for 16 h. The blood and liver of the two rats were taken 3 h after the infusion was stopped.

Sample preparation

After the infusion period each rat was anesthetized as previously noted and then killed by exsanguination from the abdominal aorta. The blood was immediately chilled to 4°C and the plasma was separated within 20 min. It was then ultracentrifuged in a Ti 70 rotor (Beckman Instruments Canada, Toronto, Canada) at 35,000 rpm at 10°C for 18 h in order to obtain VLDL. The liver was removed simultaneously and frozen at -20°C. Its protein was measured according to previously described methods (6). The total lipids were extracted with chloroform–methanol 2:1 and stored at -20°C under N_2.

Separation of lipid classes

The total lipid extracts were resolved into cholesteryl esters (CE), TG, free fatty acids (FFA), free cholesterol (FC), and total phospholipids (PL) fractions by TLC on silica gel H plates developed in heptane–isopropyl ether–acetic acid 60:40:4 (by vol) (5). The PL were extracted from the origin with chloroform–methanol–water–acetic acid 50:39:10:1 (by vol) (7). Phosphatidylcholine (PC) was isolated from PL by TLC on silica gel H using chloroform–methanol–acetic acid–water 25:15:4:2 (by vol) as the developing solvent (5).

Total lipid profiles

The plasma and liver total lipid profiles were determined by gas–liquid chromatography (GLC) after digestion with phospholipase C type I and type V, respectively. The digestion converted the PL to the corresponding DG and ceramides, which were trimethylsilylated along with other neutral lipids prior to the GLC analysis (8).

Analysis of the composition of fatty acids

Gel scrapings of the TLC-separated individual lipid class were subjected to acid methanolysis using 6% H_2SO_4 in methanol at 80°C for 2 h and the fatty acid methyl esters were analyzed by GLC (8).

GLC/MS of fatty acid methyl esters

^2H incorporation into fatty acids was determined by gas–liquid chromatography–mass spectrometry (GLC/MS) with electron impact ionization. The analysis was done on a Hewlett-Packard (Palo Alto, CA) model 5890 series II capillary gas chromatograph interfaced with a model 5989 quadruple mass spectrometer, a model 98785A computer terminal, and model 6000/330S data unit. The GLC column (12 m × 0.2 mm ID) was coated with HP1 (Hewlett-Packard) cross-linked methyl silicone gum (0.33 μm film thickness). The elec-

tron energy was 70 eV. The fatty acid methyl esters were eluted by temperature programming from 100–190°C at 20°C/min and then up to 255°C at 5°C/min. The ^2H incorporation was quantitated as % replacement of old molecules by new molecules (9). Correction for the natural abundance of ^2H and ^{13}C was done by subtraction of the parent ion plus 1 to 3 in the corresponding lipids from rats that had not received of perdeuterated ethanol. The approaches used to calculate deuterium % replacement and % excess are explained in a later subsection.

Preparation of DG and DNPU derivatives

The *sn*-1,2(2,3)-DG of TG were prepared by random degradation with Grignard reagent and were resolved by borate TLC using chloroform–acetone 97:3 (by vol) as developing solvent (7). The DG were converted into the 3,5-dinitrophenylurethane (DNPU) derivatives by reacting with dinitrophenylisocyanate and the DNPU derivatives were purified by TLC on silica gel G using hexane–dichloromethane–ethanol 40:10:3 (by vol) prior to chiral phase high performance liquid chromatography (HPLC) (10).

Chiral and reverse phase HPLC/MS

The enantiomeric DG were resolved as DNPU derivatives on a chiral phase HPLC column (A-KO3) containing (R)-(+)-1-(naphthyl) ethylamine polymer using hexane–dichloromethane–ethanol 40:10:1 as a mobile phase (10). The molecular species of the DG were separated as the DNPU derivatives by LC/MS (3) on reversed phase Supelcosil C-18 column (25 cm × 0.4 cm ID) using a linear gradient of 20–50% isopropanol in methanol over 30 min as the solvent at a flow rate of 1 ml/min. The HPLC was done on Hewlett-Packard Model 1090 Liquid Chromatograph, Series II. The HPLC elution profile was obtained by recording the UV absorption at 254 nm. The HPLC was interfaced with Hewlett-Packard Model 5989A quadruple mass spectrometer using the Hewlett-Packard thermospray interface. Negative chemical ionization (NCI) was obtained by post column addition of methanol–0.2 M ammonium acetate 50:50 at 0.2 ml/min. The NCI profiles were recorded by using the filament on mode with a fragmenter setting of 100. The deuterium content of the DG determined by reverse phase LC/MS was corrected for the natural abundance of the stable isotopes as described above.

Calculation of deuterium % replacement and % excess

To quantitate the deuterium in methyl esters of fatty acids, the *m/z* range 100–350 is scanned repeatedly for the entire GLC peak. Every newly made molecule of fatty acid is labeled. For palmitate, the labeled methyl ester molecular ions are found in the region *m/z* 271–280. The methyl esters of the preexisting, unlabeled, palmitate have *m/z* 270, 271 and 272. The last two masses are due to the natural occurrence of ^{13}C, ^2H and ^{17}O and ^{18}O. The GLC/MS will provide mass chromatograms giving areas of ion intensities from *m/z* 270–280 for methyl palmitate, *m/z* 298–305 for methyl stearate, and 296–303 for methyl oleate. To determine the percentage of the relative replacement of a compound, the ion areas must be corrected for the natural occurrence of heavy isotope. This will give the amount of newly made labeled molecules. The methyl palmitate, *m/z* 270, is unlabeled and therefore 100% of it is made up of preexisting molecules. By examining the palmitate from rats that were not given perdeuterated ethanol it was found that the area of *m/z* 271 in the mass spectra was 19% of *m/z* 270, and *m/z* 272 was 2.1% of *m/z* 270. For methyl stearate, the corresponding natural abundances of stable isotope for *m/z* 298, 299, and 300 were 100%, 21.3%, and 2.6%, respectively. These values for the naturally occurring heavy isotope contributions were subtracted from the total amounts of the *m/z* 270–280 and *m/z* 298–305 species to give the amounts of palmitate and stearate, respectively, that were made from perdeuterated ethanol.

The percentage replacement of palmitate is defined as:

$$R_{16:0} = \frac{\text{newly made palmitate}}{\text{newly made palmitate + preexisting palmitate}} \times 100$$

$$R_{16:0} = \frac{\sum_{n=271}^{n=280} Ac}{\sum_{n=270}^{n=280} At} \times 100$$

Where Ac is the ion area from *m/z* = n after correction for the natural heavy isotope abundance, and At is the total ion area for *m/z* = n.

The ^2H % excess is the total number of ^2H atoms in 100 molecules of palmitate. This is obtained as follows. After correcting for natural abundance, the number of ^2H atoms in a given molecular ion (e.g., *m/z* 271, *m/z* 272, etc. to *m/z* 280) is multiplied by the proportion, expressed as a percent, of this molecular ion of all molecular ions of methyl palmitate (e.g., *m/z* 270 to *m/z* 280). These are then all added together.

$$^2H\ \%\ \text{excess} = \sum_{n=271}^{n=280} \left[\frac{[\text{Ac for m/z n}] \times [\text{Number of } ^2H \text{ in } m/z\ n]}{\sum_{n=270}^{n=280} At} \times 100 \right]$$

The ^2H incorporation into the glycerol of TG was estimated from the analysis of DG labeling. It is not known how the labeled fatty acids and glycerol are combined together when ^2H-labeled DG are expressed as % replacement that reflects the number of the ^2H-labeled DG in per 100 DG molecules. In order to know the ^2H labeling in the glycerol, one has to use the concept of ^2H excess, which represents the number of the excess ^2H over the natural abundance of heavy isotope in 100 molecules. The total ^2H excess in the DG molecule is the sum of the ^2H excess in glycerol and that in the fatty acids. Thus the deuterium labeling in the glycerol portion of a DG species was determined by subtracting the label residing in its two fatty acids:

$$\%\ ^2H \text{ excess (glycerol)} = \%\ ^2H \text{ excess (diacylglycerol)} - \Sigma\ \%\ ^2H \text{ excess (2 fatty acids)}$$

RESULTS

Stereochemical structure of TG

Table 1 summarizes the enantiomer composition of the molecular species of rat liver-TG and VLDL-TG, as obtained by stereospecific analyses of samples obtained from the two rats described earlier (3). These animals were infused with fructose and perdeuterated ethanol without prefeeding the sugar water for 2 weeks. Only the major species are shown. The liver-TG species containing both saturated and unsaturated fatty acids were highly asymmetric with the enantiomer containing the saturated fatty acid in the *sn*-1-position, in many instances, being present in nearly 100-fold excess. For example, in rat 1, 2.14% of the liver-TG was 16:0–16:0–18:1, while only 0.02% of the liver-TG was 18:1–16:0–16:0. In contrast the VLDL-TG were considerably more racemic and the enantiomers with saturated fatty acids in the *sn*-1-position were frequently present in only 10-fold excess. In contrast, the TG containing unsaturated fatty acids in both primary positions were present in nearly racemic proportions in both liver and VLDL. Although the differences between VLDL and liver-TG were much smaller, exactly the same direction of change was seen in rat 2. This, suggests the interesting possibility that TG became partly racemized during transfer to VLDL and racemization may occur during the partial hydrolysis of the stored hepatic TG and reesterification (11).

TABLE 1. Calculated major enantiomer composition of the molecular species of normal rat liver-TG and VLDL-TG

	Liver TG		VLDL TG	
	Rat 1	Rat 2	Rat 1	Rat 2
	%			
16:0–16:0–18:2	2.33	1.53	3.54	4.15
18:2–16:0–16:0	0.02	0.09	0.11	0.29
16:0–18:2–16:0	0.17	1.94	0.66	2.34
16:0–16:0–18:1	2.14	1.43	2.16	2.30
18:1–16:0–16:0	0.02	0.12	0.06	0.29
16:0–18:1–16:0	0.15	1.57	0.61	1.89
16:0–18:2–18:1	3.82	5.52	4.17	4.61
18:1–18:2–16:0	0.03	0.47	0.12	0.58
16:0–18:1–18:2	3.82	4.77	6.36	6.73
18:2–18:1–16:0	0.03	0.27	0.20	0.48
16:0–18:2–18:2	4.16	5.90	6.84	8.32
18:2–18:2–16:0	0.04	0.33	0.22	0.59
18:0–16:0–18:2	0.51	0.26	1.17	1.05
18:1–18:2–18:2	0.75	1.43	1.25	2.06
18:2–18:2–18:1	0.84	0.93	1.38	1.16
18:2–18:1–18:2	0.84	0.81	2.11	1.70
18:1–18:1–18:2	0.69	1.16	1.17	1.67
18:2–18:1–18:1	0.77	0.76	1.28	0.94
18:1–18:2–18:1	0.69	1.33	0.76	1.14
18:1–16:0–18:2	0.42	0.37	0.65	1.03
18:2–16:0–18:1	0.47	0.24	0.71	0.58

The calculation was based on the known fatty acid composition in each position of TG (3) and the knowledge of random distribution of the fatty acids in *sn*-1-, *sn*-2-, and *sn*-3-position.

Stereochemical distribution of ^2H in TG

In order to investigate the mechanism involved in VLDL-TG synthesis and secretion, we have compared the relative distribution of deuterium-labeled glycerol and fatty acids among the molecular species of TG of the liver and VLDL after an infusion of perdeuterated ethanol. Perdeuterated ethanol contributes deuterium mainly to the newly synthesized glycerol and fatty acids, although some deuterium may also be introduced into existing fatty acids during chain elongation. The biochemical steps involved are summarized in **Fig. 1.** The glycerol becomes labeled from NAD^{2}H during reduction of the glyceraldehyde and dihydroxyacetone phosphate. The incorporation of the deuterium into the fatty acids takes place during de novo lipogenesis. The deuterium present in the malonyl CoA is largely lost during the biosynthetic process. The deuterium label in the newly synthesized fatty acids is mainly derived from NADPH (12, 13) and also from labeled acetate. The extent of fatty acid labeling depends on the labeling of the NADPH, as this in turn depends on the amount of perdeuterated ethanol infused and oxidized. The contribution of labeled glycerol and fatty acids to the deuterated TG can be estimated using the approach described in the methods section.

Figure 2 shows the GLC/MS profiles of fatty acids of VLDL-TG from a rat infused with perdeuterated ethanol

ETHANOL OXIDATION

1. $C^2H_3\text{-}C^2H_2\text{-}O^2H + NAD^+ \longrightarrow C^2H_3\text{-}C^2HO + NAD^2H + {}^2H^+$
(Alcohol Dehydrogenase)

2. $C^2H_3\text{-}C^2HO + NAD^+ \longrightarrow C^2H_3\text{-}COO^- + NAD^2H + {}^2H^+$
(Alcohol Dehydrogenase)

$NAD^2H + NADP^+ \longleftrightarrow NADP^2H + NAD^+$
(Pyridine Nucleotide Transhydrogenase)

FATTY ACID SYNTHESIS

1. $^2H_3C\text{-}C(O)\text{-}C^2H_2\text{-}C(O)\text{-S-ACP} + NADP^2H + {}^2H^+ \longrightarrow$
$\longrightarrow {}^2H_3C\text{-}C^2H(O^2H)\text{-}C^2H_2\text{-}C(O)\text{-S-ACP} + NADP^+$
(Beta-Ketoacyl-ACP-Reductase)

2. $^2H_3C\text{-}C^2H{=}C^2H\text{-}C(O)\text{-S-ACP} + NADP^2H + {}^2H^+ \longrightarrow$
$\longrightarrow {}^2H_3C\text{-}C^2H_2\text{-}C^2H_2\text{-}C(O)\text{-S-ACP} + NADP^+$
(Enoyl-ACP Reductase)

GLYCEROL-3-PHOSPHATE SYNTHESIS

1. $(O^-)_2(O)P\text{-}O\text{-}C(O)\text{-}CH(OH)\text{-}CH_2\text{-}O\text{-}P(O)(O^-)_2 + NAD^2H + H^+ \longleftrightarrow$
$\longleftrightarrow O^2HC\text{-}CH(OH)\text{-}CH_2\text{-}O\text{-}P(O)(O^-)_2 + NAD^+ + P_i$
(Glyceraldehyde 3-Phosphate Dehydrogenase)

2. $O^2HC\text{-}CH(OH)\text{-}CH_2\text{-}O\text{-}P(O)(O^-)_2 \longleftrightarrow C^2HH(OH)\text{-}C(O)\text{-}CH_2\text{-}O\text{-}P(O)(O^-)_2$
(Triose Phosphate Isomerase)

3. $C^2HH(OH)\text{-}C(O)\text{-}CH_2\text{-}O\text{-}P(O)(O^-)_2 + NAD^2H + {}^2H^+ \longleftrightarrow$
$\longleftrightarrow C^2HH(OH)\text{-}C^2H(OH)\text{-}CH_2\text{-}O\text{-}P(O)(O^-)_2$
(Dihydroxyacetone Reductase)

Fig. 1. The incorporation of deuterium into fatty acids and glycerol of TG from perdeuterated ethanol. Abbreviations: ^2H, deuterium; ACP, acyl carrier protein.

and fructose. The molecular ions of palmitate (16:0) and oleate (18:1) are 270 and 296, respectively. These are seen in the middle and lower panels of Fig. 2. The excess ^2H incorporated in palmitate is seen in the seven peaks following the parent ion 270. In the lower panel it may be noted that the labeling of oleate is very low.

Table 2 compares the deuterium % replacement (upper panel) and % excess (lower panel) of fatty acids in the enantiomeric DGs derived from liver-TG and VLDL-TG. The ^2H replacement represents the number of the ^2H-labeled fatty acid molecules per 100 molecules. The ^2H excess represents the excess of deuterium over the natural abundance of the heavy isotope per 100 fatty acid molecules. Table 2 shows that the labeling in 14:0 and 16:0 is much higher than that of 18:0 and 18:1. As 18:2 is not synthesized it is not labeled. The deuterium excess and replacement data taken together indicate that the measured fatty acids contain, on average, four deuteriums per molecule. The ^2H labeling is higher in *sn*-1,2-DG moieties than that in *sn*-2,3-DG moieties of TG. This is because the major enantiomeric DG moieties share the same fatty acids in *sn*-2-position, and the ^2H-labeled fatty acid distribution between *sn*-1 and -3 positions is asymmetrical.

Figure 3 shows the positional distribution of ^2H in the fatty acids of liver-TG and VLDL-TG. The ^2H distribution differs among the three positions. The greatest labeling is in the *sn*-1-position.

Table 3 shows the determined deuterium incorporation of the sn-1,2- and -2,3-DG moieties of liver- and VLDL-TG. The upper panel shows ^2H replacement. For most of the DG species with saturated fatty acids, the ^2H labeling in *sn*-1,2-DG moieties is higher than that in the *sn*-2,3-DG, this is consistent with the positional distribution of labeled fatty acids. The DG species containing only unsaturated species are much less labeled. This is because the unsaturated fatty acids are not readily made from 16:0 and 18:0. Hence, in the DG species containing

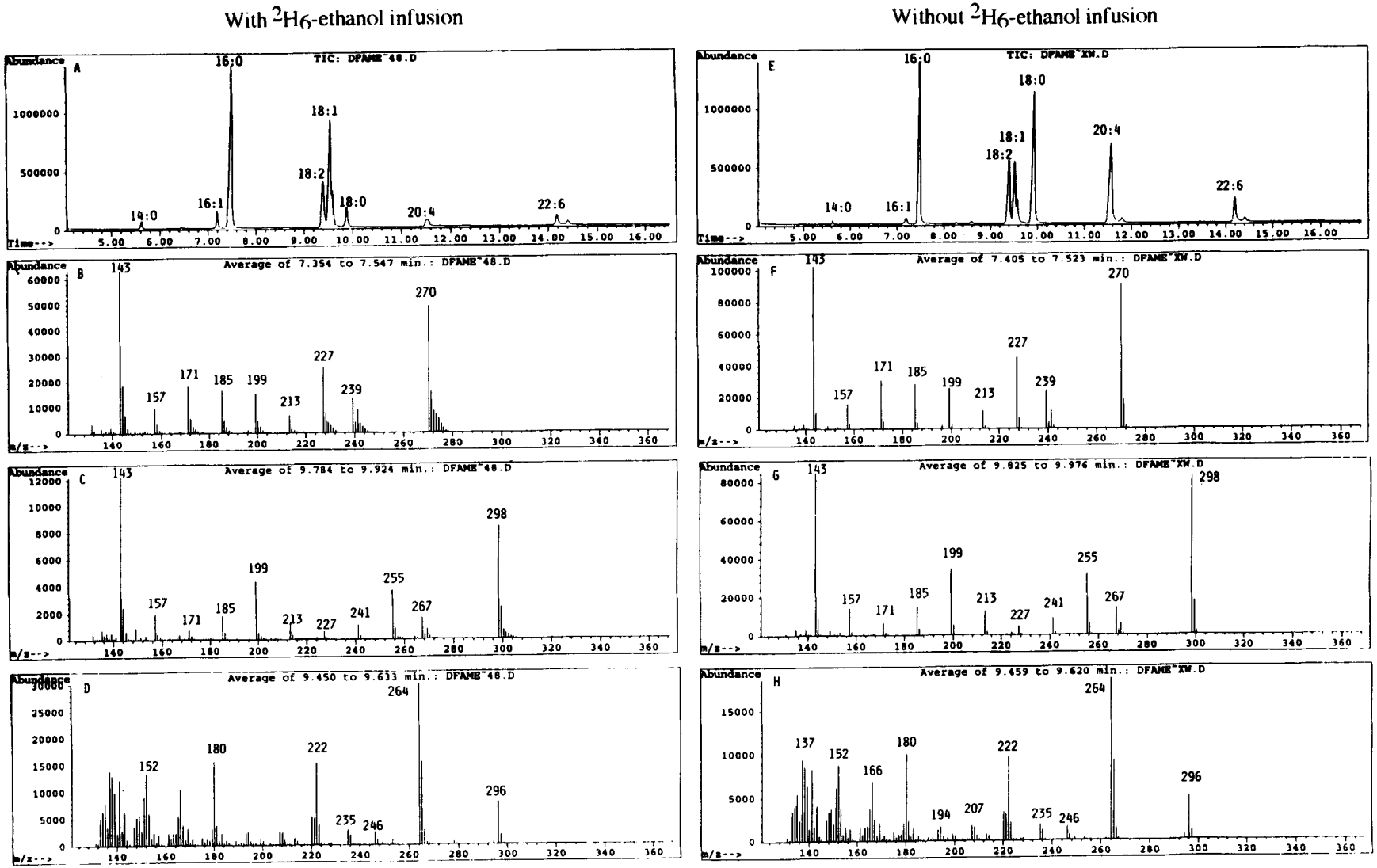

Fig. 2. GLC/MS profile of fatty acid methyl esters from VLDL-TG of a rat infused with fructose and perdeuterated ethanol (left side panels: A,B,C,D) and from liver total lipids of a rat without infusion of deuterated ethanol as control for the natural abundance of stable isotope (right side panels: E,F,G,H). Panels A and E are the total ion current of fatty acid methyl esters, each acid is identified in figure. Panels B and F are the mass/charge ratio of palmitate. Its molecular ion is 270. The series of peaks following 270 (panel B) show that palmitate is extensively labeled with ^{2}H in addition to the natural abundance of the stable isotope. The natural abundance of stable isotope in 270 is shown in panel F. The rest of the peaks are the fragments of palmitate. Panels C and G are stearate. The molecular ion is 298. In panel C the series of peaks following 298 show that the stearate is labeled with ^{2}H in addition to the natural abundance of the stable isotope. The natural abundance of the stable isotope in stearate is shown in panel G. The remaining peaks are fragments of stearate. Panels D and H are oleate. The molecular ion of oleate is 296, its major fragment is 264. After [^{2}H]ethanol infusion, oleate contains only very small amount of ^{2}H labeling (panel D) besides the natural abundance of stable isotope (panel H). The instrument and condition of GLC/MS is as described in methods.

TABLE 2. Deuterium incorporation into the fatty acids of the *sn*-1,2-DG and *sn*-2,3-DG moieties of liver-TG and VLDL-TG of normal rats infused with [$^2H_6$]ethanol and fructose

	Fatty Acids	Liver-TG			VLDL-TG		
		*sn*1,2	*sn*2,3	TG	*sn*1,2	*sn*2,3	TG
				deuterium % replacement			
Rat 1	14:0	37.8	18.0	26.3	24.4	16.4	20.0
	16:0	31.1	27.5	30.1	22.5	18.9	22.9
	16:1	25.7	29.5	33.1	22.0	27.1	23.8
	18:0	5.1	4.1	6.3	5.7	3.9	7.3
	18:1	4.9	5.2	5.0	4.4	2.7	1.0
	18:2	ND	ND	ND	ND	ND	ND
Rat 2	14:0	17.0	9.7	13.7	5.9	6.4	7.7
	16:0	15.4	11.8	15.0	9.9	5.3	9.9
	16:1	5.6	9.1	11.4	7.9	6.0	8.1
	18:0	2.6	2.3	3.3	3.7	3.5	5.3
	18:1	1.7	1.3	1.0	1.2	2.0	2.0
	18:2	ND	ND	ND	ND	ND	ND
				deuterium % excess			
Rat 1	14:0	155.6	65.0	107.1	91.6	63.9	68.0
	16:0	125.2	110.2	113.1	89.1	76.8	91.3
	16:1	120.5	123.4	141.2	95.4	117.1	100.5
	18:0	20.2	15.3	20.7	18.4	11.7	29.9
	18:1	14.2	14.2	14.3	11.4	9.4	8.0
	18:2	ND	ND	ND	ND	ND	ND
Rat 2	14:0	61.8	35.4	52.0	19.9	22.9	25.0
	16:0	62.5	45.7	61.2	37.4	25.8	39.0
	16:1	27.8	35.9	50.1	36.2	28.7	35.6
	18:0	9.6	7.6	12.0	10.4	15.2	20.1
	18:1	2.4	2.7	0.2	1.3	2.0	4.5
	18:2	ND	ND	ND	ND	ND	ND

ND, not detected.

only unsaturated fatty acids, the labeling is mainly in glycerol. In the unsaturated species, the deuterium labeling is similar among the different DG moieties of TG, and the deuterium labeling is also similar between the unsaturated *sn*-1,2- and *sn*-2,3-DG of TG. This is because the major DG species share the same glycerol backbone. The lower panel of Table 3 gives the % excess deuterium in the molecular species of the *sn*-1,2- and *sn*-2,3-DG. The relative labeling of the different 16:0 fatty acid containing species is higher in *sn*-1,2-DG than that in *sn*-2,3-DGs. The large differences in 16:0-containing species between *sn*-1,2- and *sn*-2,3-DG may reflect the relatively small proportion of TG with 16:0 in the *sn*-3-position.

Table 4 gives the % deuterium excess in the glycerol moiety of the liver- and VLDL-TG as estimated from the unsaturated *sn*-1,2-DG and *sn*-2,3-DG moieties of TG and the C_{16} saturated *sn*-1,2-DG and *sn*-2,3-DG moieties after the contributions due to the deuterated fatty acids have been subtracted. As both the *sn*-1,2-DG and -2,3-DG moieties were prepared from the same TG, the labeling of their glycerol should have been the same and this was found to be the case. The labeling of glycerol in the unsaturated and saturated species was similar. The present data show that there is no need to resolve the enantiomeric *sn*-1,2(2,3)-DGs in order to determine the glycerol labeling.

^2H incorporation into liver- and VLDL-TG in fructose- and glucose-treated rats

The deuterium labeling of the VLDL-TG and liver-TG was further examined in rats in which lipogenesis was increased by prefeeding with either glucose or fructose. The sugar treatment increased the liver weighs 10–30% ($P < 0.05$) over those of the control animals, but the liver weights did not significantly differ between the two sugar groups. There was no significant difference in liver-TG content per gm protein among the three groups (range 40–60 mg/g liver protein). Both sugar-treated groups had significantly increased plasma-TG (fructose group 30.2 ± 3.8 mg/dl, glucose group 31.8 ± 2.4 mg/dl, control group 21.1 ± 2.1 mg/dl) and VLDL-TG levels (fructose group 17.2 ± 3.6 mg/dl, glucose group 20.8 ± 5.2 mg/dl, control group 8.0 ± 1.0 mg/dl).

Figure 4 shows the ^2H % replacement and excess in total fatty acids of liver-TG and VLDL-TG. The pattern was similar for both replacement and excess. The order of fatty acid labeling was 14:0 > 16:0 > 16:1 = 18:0. In control rats, the ^2H % replacement in 14:0 was equal to

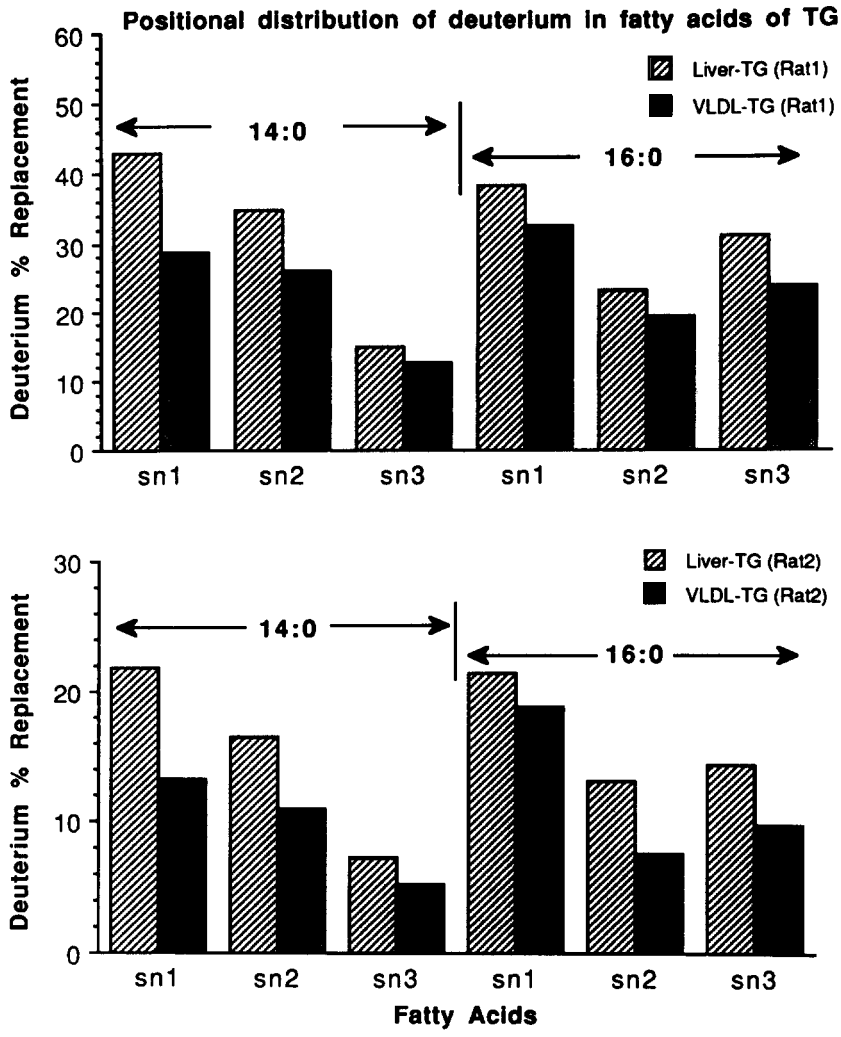

Fig. 3. Positional distribution of ^2H in 14:0 and 16:0 of VLDL-TG and liver-TG obtained from rats infused with fructose and perdeuterated ethanol.

that in 16:0, whereas in both sugar groups the % replacement of 14:0 was higher than that of 16:0. The labeling in 18:1 was minimal (0.5–1.0% replacement); 18:2 was not labeled. Fructose and glucose significantly increased the ^2H % replacement both in VLDL-TG and liver-TG. The 14:0 and 16:0 in VLDL-TG was 1.1–1.8 (average 1.5) times more labeled than the liver-TG. Figure 4 also compares the ^2H % replacement in 16:1 and 18:0 of TG in VLDL and liver. Both 16:1 and 18:0 of TG are less labeled than that of 14:0 and 16:0. This probably reflects a the low activity of the delta-9 desaturase and a slow rate of elongation of 16:0 to 18:0.

Table 5 compares the ^2H % replacement of *sn*-1,2(2,3)-DG moieties of VLDL- and liver-TG. The species containing saturated acids were more labeled than those containing only unsaturated species. Because of the lack or virtual lack of labeling in 18:1 and 18:2 described above, all the ^2H in the DG moieties containing only 18:1 and 18:2 came from glycerol. In contrast, ^2H labeled both the fatty acids and glycerol in those DG moieties that contained saturated acids. In the sugar groups, the ^2H labeling in DG of VLDL-TG was higher than that of liver-TG and ranged from 1.0 to 1.6 (average 1.4) for glucose and from 1.4 to 2.2 (average 1.7) for the fructose group. A similar tendency was seen for several other species in the control rats. However, because the labeling in these rats was much lower than in the sugar-supplemented rats (Figs. 4 and 5), we could not draw any conclusions from the control rat liver-TG versus VLDL-TG. As in the case of fatty acids (Fig. 4), in the sugar-supplemented rats, the glyceride-glycerol in VLDL was more labeled than that in the liver (see the species containing only 18:1 and 18:2, Table 5).

Figure 5 shows the calculated ^2H excess in glycerol in the *sn*-1,2(2,3)-DG moieties of VLDL-TG and liver-TG. The calculation was based on the determined values of

TABLE 3. Deuterium incorporation into the *sn*-1,2-DG and *sn*-2,3-DG moieties of liver-TG and VLDL-TG of normal rats infused with [$^2H_6$]ethanol and fructose

	Rat 1				Rat 2			
	Liver-TG		VLDL-TG		Liver-TG		VLDL-TG	
DG Species	*sn*1,2	*sn*2,3	*sn*1,2	*sn*2,3	*sn*1,2	*sn*2,3	*sn*1,2	*sn*2,3
	deuterium % replacement							
16:0–16:1	59.3	44.9	54.4	40.8	36.1	23.4	24.7	24.0
16:0–16:0	53.2	41.3	51.4	36.0	39.5	25.1	31.0	28.5
16:0–18:2	37.2	37.9	25.4	29.0	23.4	20.4	15.8	17.3
16:0–18:1	45.6	39.7	34.7	35.0	25.0	25.0	15.3	15.2
18:2–18:2	15.1	16.0	12.8	8.9	13.2	15.6	14.6	9.0
18:1–18:2	21.4	18.7	11.5	13.3	13.3	13.9	11.1	6.9
18:1–18:1	18.1	19.2	14.3	16.3	17.2	15.9	13.1	8.4
	deuterium % excess							
16:0–16:1	285.7	147.3	245.0	165.7	148.3	636.	92.3	105.5
16:0–16:0	247.2	167.9	194.0	145.1	149.2	71.7	128.2	116.8
16:0–18:2	132.0	145.8	90.4	116.4	85.4	61.1	46.6	55.8
16:0–18:1	167.3	136.0	135.9	116.1	92.7	89.3	48.3	55.0
18:2–18:2	34.0	30.2	25.3	20.8	24.8	26.9	20.3	17.4
18:1–18:2	57.8	36.8	34.2	39.4	37.5	30.8	23.9	23.8
18:1–18:1	50.7	50.3	32.4	36.5	28.7	26.2	27.4	12.9

^2H excess in the fatty acids (Fig. 4) and in the *sn*-1,2(2,3)-DG of VLDL-TG and liver-TG (data not shown). The ^2H excess of glycerol in TG was obtained by subtracting the ^2H excess of fatty acids of TG from the total ^2H excess of DG molecules. This calculation assumed that the same fatty acids were equally labeled in the different TG species. The glycerol labeling in the different DG moieties of liver-TG was very similar. The glyceride–glycerol labeling of VLDL-TG was about 1.5 times higher than that of liver-TG. Although the fatty acid labeling in the TG of control group of rats was much lower than the sugar-supplemented rats (Fig. 4), the glyceride–glycerol labeling was similar in all three groups of rats.

DISCUSSION

The present studies demonstrate the utility of perdeuterated ethanol to study hepatic lipid synthesis and secretion in vivo. Early experiments showed that ^2H from deuterated water was readily incorporated into liver fatty acids thereby providing a measurement of fatty acid synthesis (5, 14). However, in vivo studies would need large volumes of ^2H_2O to achieve detectable ^2H incorporation. Cursted and Sjövall (4), Cronholm, Cursted, and Sjövall (12), and Cursted (13) showed that ^2H can be supplied more efficiently as deuterated ethanol. In the present experiment, we used perdeuterated ethanol. This form of ethanol ([$^2H_6$]ethanol) has 2.5 times more deuterium per mole of ethanol than does [$^2H_2$]ethanol, which was used by the previous investigators (4, 13). The ^2H on carbon 1 of ethanol is transferred to reducing nucleotides and can go on to label the fatty acids and glycerol. The ^2H on carbon 2 of ethanol can be incorporated via acetate into the terminal methyl group of fatty acid. The intraperitoneal route of infusion

TABLE 4. Deuterium % excess in glycerol of the *sn*-1,2-DG and *sn*-2,3-DG moieties of lfiver-TG and VLDL-TG of normal rats infused with [$^2H_6$]ethanol and fructose

	Rat 1				Rat 2			
	Liver-TG		VLDL-TG		Liver-TG		VLDL-TG	
DC Species	*sn*1,2	*sn*2,3	*sn*1,2	*sn*2,3	*sn*1,2	*sn*2,3	*sn*1,2	*sn*2,3
	%							
16:0–16:1	45.6	40.5	30.5	28.7	31.5	28.2	18.7	13.8
16:0–16:0	33.9	26.5	26.2	21.3	24.1	26.0	23.4	20.4
16:0–18:2	36.3	35.6	33.2	25.6	22.9	15.4	19.1	22.1
16:0–18:1	37.2	25.8	27.2	20.2	20.5	23.2	21.4	20.5
18:2–18:2	34.0	30.2	25.3	20.8	24.8	26.9	20.3	17.4
18:1–18:2	43.8	22.6	22.8	30.0	35.1	28.1	22.6	21.8
18:1–18:1	22.3	21.9	9.6	17.7	23.9	20.8	24.8	8.9

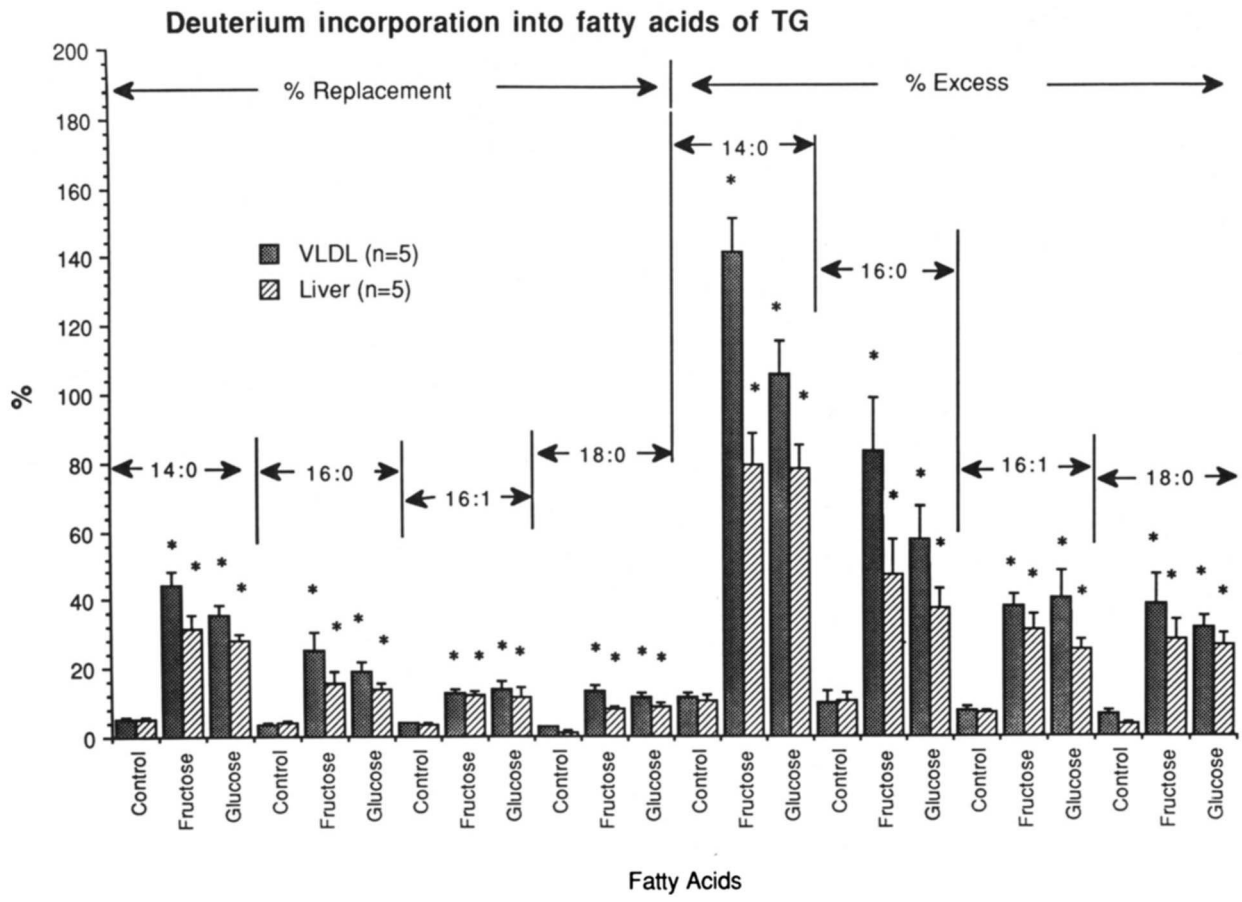

Fig. 4. ^2H % replacement and excess in the fatty acids of VLDL- and liver-TG. Each acid is identified in figure. As there is only 0.5–1.0% ^2H replacement in 18:1 and 18:2 is not labeled, they are not shown. *$P < 0.01$ compared to control group.

was chosen to provide a route that might supply more [$^2H_6$]ethanol to the liver than to the periphery. Furthermore, as alcohol dehydrogenase is primarily a hepatic enzyme, one would anticipate that this would generate a maximal amount of labeled precursors at the site of lipogenesis for VLDL.

Stereochemical formation of liver- and VLDL-TG

A comparison of the distribution of saturated and unsaturated fatty acids between the two outer positions of TG enantiomers (Table 1) shows that the TG of VLDL is more racemic than the TG of liver. This suggests that the *sn*-1-position fatty acid of VLDL-TG is acylated via a different pathway from the liver-TG. It is known that the phosphatidic acid pathway preferentially incorporates saturated fatty acids in *sn*-1-position and unsaturated fatty acids in *sn*-2-position of phosphatidic acid (11, 15). This distribution is retained in the liver-TG. The *sn*-3-position may contain either saturated or unsaturated fatty acids in variable proportions. In contrast, the VLDL-TG contains higher proportions of unsaturated fatty acids in the *sn*-1-position. We have previously (3, 11) proposed that such a discrepancy could arise as a result of two independent pathways that contribute to VLDL-TG formation: the phosphatidic acid and monoacylglycerol pathways. The present studies support this proposal.

Several investigators (1–3) suggested that liver-TG are hydrolyzed and reacylated before secretion as VLDL-TG. However, the processes and pathways remain unclear. If the hydrolysis of liver-TG were only as far as *sn*-1,2-DG, one would expect that the *sn*-1,2-DG in VLDL-TG would be the same as that in liver-TG. The relative racemic change in *sn*-1- and *sn*-3-fatty acids that we observed in VLDL-TG (Table 1) indicates that the liver-TG hydrolysis proceeded beyond that point. It indicates that some of the liver-TG is hydrolyzed into 2-monoacylglycerols and reacylated at *sn*-1- and *sn*-3-positions. This, in turn, suggests that a 2-monoacylglycerol acyltransferase (MGAT) pathway exists in the liver. The ability of MGAT to put unsaturated fatty acids in *sn*-1-position has been demonstrated in intestine (11). Although 2-MG could not be recovered from rat liver in this experiment, the presence of a quantitatively small, rapidly turning over pool could not be excluded. Liver MGAT has been shown to have a very high activity in neonatal rats and to persist with a low activity in adult rats (16).

TABLE 5. Deuterium % replacement in the *sn*-1,2(2,3)-DG moieties of VLDL-TG and liver-TG

DG moieties of TG		Control VLDL (n = 2) Liver (n = 4)	Fructose VLDL (n = 2) Liver (n = 3)	Glucose VLDL (n = 3) Liver (n = 3)
14:0–18:2	VLDL-TG	40.7 ± 2.5	50.3 ± 6.9	45.9 ± 5.0
	Liver-TG	34.3 ± 3.2	33.7 ± 10.3	31.8 ± 1.9
16:0–16:1	VLDL-TG	46.0 ± 3.5	49.1 ± 6.6	54.6 ± 4.1
	Liver-TG	43.1 ± 2.8	38.0 ± 7.0	36.9 ± 3.0
16:0–16:0	VLDL-TG	30.4 ± 1.2	54.0 ± 11.6	51.9 ± 5.2
	Liver-TG	41.2 ± 3.7	40.4 ± 2.7	52.5 ± 6.8
16:0–18:2	VLDL-TG	43.9 ± 4.3 (n = 3)	39.2 ± 4.4	42.0 ± 1.6
	Liver-TG	37.2 ± 0.9	28.4 ± 2.0[a]	31.5 ± 0.6[a]
16:0–18:1	VLDL-TG	32.0 ± 0.9	42.1 ± 3.7	46.0 ± 3.9
	Liver-TG	33.8 ± 0.8	26.5 ± 5.7	31.4 ± 0.8
18:2–18:2	VLDL-TG	28.6 ± 5.7	37.9 ± 1.4	31.0 ± 3.8
	Liver-TG	29.6 ± 2.6	22.7 ± 1.9	25.4 ± 2.1
18:1–18:2	VLDL-TG	29.9 ± 1.1	36.5 ± 1.3	32.5 ± 1.4
	Liver-TG	33.7 ± 1.7	17.6 ± 5.4[a]	22.6 ± 3.3[a]
18:1–18:1	VLDL-TG	30.7 ± 4.8 (n = 3)	32.5 ± 6.2	33.7 ± 2.0
	Liver-TG	27.3 ± 0.7	19.5 ± 0.6[a]	21.5 ± 2.3[a]

Values are mean ± SEM, unless n = 2 in which case the data are given as mean ± range/2.
[a]$P < 0.05$ compared to control group.

VLDL-TG secretion from liver

The ^2H labeling in 14:0, 16:0, and 18:0 of TG of VLDL in sugar-supplemented rats was approximately 1.5-times higher than that of their liver-TG. This indicated that the 14:0, 16:0, and 18:0 of VLDL-TG was not derived only from the liver-TG storage pool, but that a significant amount (~30–40%) was made de novo and directly incorporated into VLDL-TG. This was similar to the conclusion reached in our earlier work on the structure of the VLDL-TG (3). The ^2H labeling of glyceride-glycerol was similar in all liver-TG species. This was not affected by sugar administrated. As in the case of the fatty acids, the VLDL-TG glycerol was approximately 1.5-times more labeled than the liver-TG glycerol. This suggested that 30–40% of VLDL-TG glycerol was also derived from newly synthesized glycerol. The balance of the fatty acids and the glycerol in VLDL-TG was probably derived from hydrolysis and reesterification of the TG stored in the liver. This is consistent with the results obtained by Wiggins and Gibbons using radioisotopes (2). The similarity of the increased labeling of both glycerol and fatty acids in VLDL-TG compared to those in liver-TG suggests that the TG stored in the liver is not hydrolyzed completely (i.e., to glycerol and fatty acids). Rather, liver-TG is probably hydrolyzed to partial glycerides. Our study also indicates that hydrolysis to 2-monoacylglycerols (previously reported in the intestine) (11) may be more important in the liver than previously thought.

Recently, Swift (17) reported that rat livers produce apoB-100- and apoB-48-containing lipoproteins. This observation raises another possible explanation for our findings. Perhaps each of these two lipoprotein fractions has TG of different metabolic origins.

In control rats, VLDL-TG glycerol was labeled to the same extent as it was in sugar-supplemented rats (Fig. 5). However, the VLDL-TG fatty acids were much less labeled in the control rats than in the sugar-supplemented ones (Fig. 4). This implies that sugar supplementation does not alter glycerol synthesis. On the other hand, as previously suggested (22), sugar supplementation does increase de novo fatty acid synthesis.

Fructose and glucose effect on ^2H incorporation into lipids

In the present study, the control group, that had no sugar supplement and was infused i.p. only with normal saline and perdeuterated ethanol, had the very little ^2H labeling in fatty acids. The highest ^2H labeling of TGFA was greatest in the fructose-fed rats, the next was in the glucose-fed rats, and the least was in the control rats. This was consistent with the observation of other investigators using other approaches (18–21). The lipogenic effect of fructose has been reported previously (4, 20–22). This is partly due to fructose-induced increase in the activity of several lipogenic enzymes, glucose-6-phosphate dehydrogenase, malic enzyme, acetyl-CoA carboxylase, fatty acid synthetase, pyruvate dehydro-

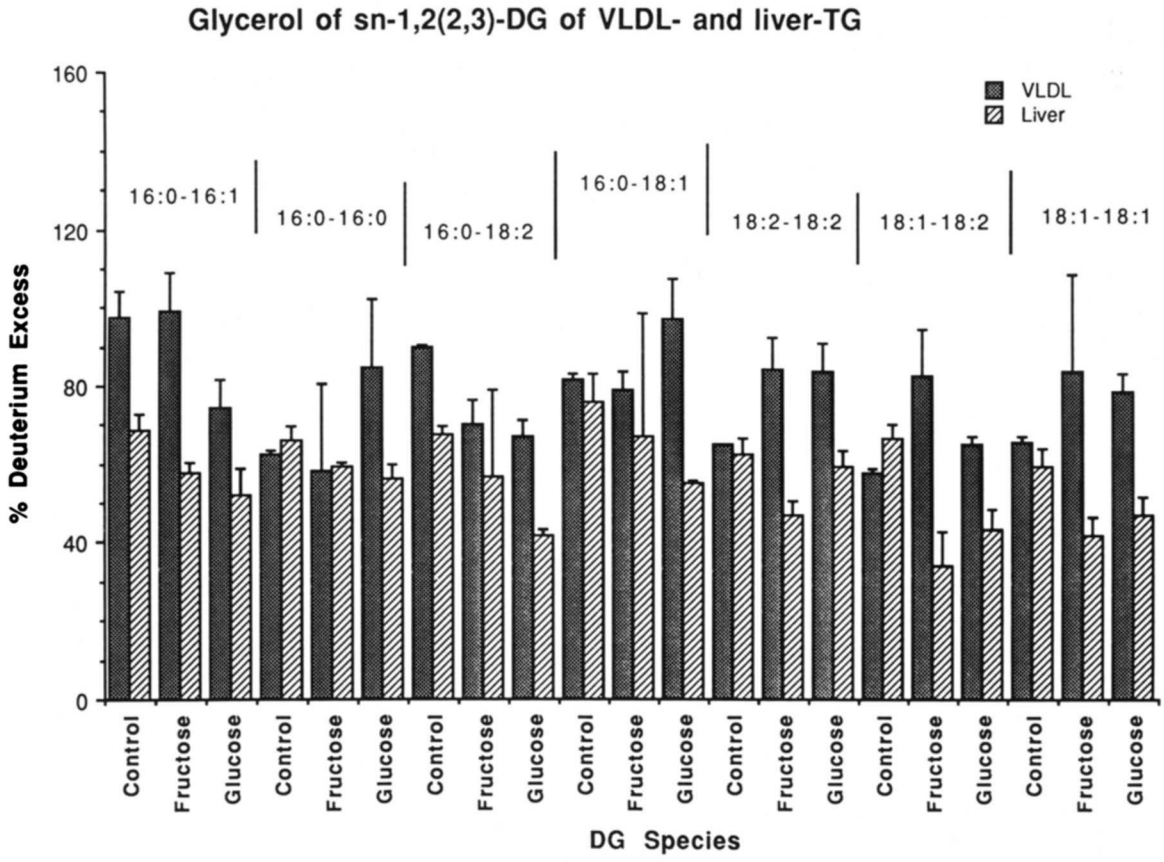

Fig. 5. ^{2}H excess in glycerol of *sn*-1,2(2,3)-DG of VLDL- and liver-TG. Each DG species is identified in figure. There are three rats for each group.

genase, and stearoyl-CoA desaturase (22–24). Also, glucose is used mainly by peripheral tissues (19), whereas fructose, perhaps because its transporters are located mainly in the liver and intestine, is metabolized predominantly in the liver (25).

Conclusions

This stable isotope study demonstrated that [$^{2}H_6$]ethanol is an effective way to label TG glycerol and TGFA and thus to study lipogenesis and lipoprotein secretion in vivo. The fatty acids in VLDL-TG are more symmetrically distributed than those of liver-TG. Both in the case of TG-glycerol and TGFA, VLDL-TG labeling is greater than that of liver-TG, indicating that VLDL-TG do not have a simple product-precursor relationship to liver-TG. These data suggest that 30–40% of the glycerol and fatty acids in VLDL-TG are not the direct product of TG stored in the liver. The data also suggest that liver TG is hydrolyzed to partial glycerides and reesterified before being incorporated into VLDL and that the monoacylglycerol pathway may be more important in this than previously thought.

Finally, these studies may have implications for the use of mass isotopomer distribution analysis (MIDA) in the calculation of VLDL-TGFA synthesis. That approach is based on the assumption that [^{13}C]acetate infused for a sufficient time will label the immediate precursor pool from which fatty acid is made and that one can estimate the labeling of the precursor from the labeling of the fatty acid (26). If VLDL-TGFA is derived both from de novo fatty acid synthesis and from mobilized hepatic-TGFA and if the hepatic-TGFA and VLDL-TGFA have not reached equilibrium in terms of labeling, one may have to account for the different contributions of label from each source to the TGFA in VLDL when MIDA is used to calculate VLDL-TGFA synthesis.

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REFERENCES

1. Francone, O. L., A-D. Kalopissis, and G. Griffaton. 1989. Contribution of cytoplasmic storage triacylglycerol to VLDL-triacylglycerol in isolated rat hepatocytes. *Biochim. Biophys. Acta.* **1002:** 28–36.
2. Wiggins, D., and J. F. Gibbons. 1992. The lipolysis/esterification cycle of hepatic triacylglycerol. *Biochem. J.* **284:** 457–462.

3. Yang, L-Y., A. Kuksis, J. J. Myher, and G. Steiner. 1995. Origin of the triacylgycerol moiety of plasma very low density lipoproteins in the rat: structural studies. *J. Lipid Res.* **36:** 125–136.
4. Cursted, T., and J. Sjövall. 1974. Biosynthetic pathways and turnover of individual biliary phosphatidylcholine during metabolism of [1,1-$^2H_2$]ethanol in the rat. *Biochim. Biophys. Acta.* **369:** 173–195.
5. Pind, S., A. Kuksis, J. J. Myher, and L. Marai. 1985. Deacylation of endogenously deuterated rat liver microsomal phospholipids by endogenous phospholipase. *Can. J. Biochem. Cell Biol.* **63:** 137–144.
6. Smith, P. K., R. I. Krohn, G. T. Hermanson, A. K. Mallia, F. H. Garther, M. D. Provenzano, E. K. Fujimoto, N. M. Geoke, B. J. Oslon, and D. G. Klenk. 1985. Measurement of protein using bicinchonic acid. *Anal. Biochem.* **150:** 76–85.
7. Myher, J. J., A. Kuksis, L-Y. Yang, and L. Marai. 1987. Stereochemical course of intestinal absorption and transport of mustard seed oil triacylglycerol in the rat. *Biochem. Cell Biol.* **65:** 811–821.
8. Yang, L-Y., A. Kuksis, and J. J. Myher. 1990. Intestinal absorption of menhaden and rapeseed oil and their fatty acid methyl and ethyl esters in the rat. *Biochem. Cell Biol.* **68:** 480–491.
9. Kuksis, A., J. J. Myher, L. Marai, S. K. F. Yeung, I. Steiman, and S. Mookerjea. 1975. Quantitation of newly formed fatty acids among glycerolipids of isolated perfused rat liver. *Can. J. Biochem.* **53:** 509–518.
10. Itabashi, Y., A. Kuksis, L. Marai, and T. Takagi. 1990. HPLC resolution of diglycerol moieties of natural triacylglycerols on a chiral phase consisting of bonded (R)-(+)-(1-naphthyl)ethylamine. *J. Lipid Res.* **31:** 1711–1717.
11. Yang, L-Y., and A. Kuksis. 1991. Apparent convergence (at 2-monoacylglycerol level) of phosphatidic acid and 2-monoacylglycerol pathways of synthesis of chylomicron triacylglycerols. *J. Lipid Res.* **32:** 1173–1186.
12. Cronholm, T., T. Cursted, and J. Sjövall. 1982. Formation of bile acids and glycerophosphatides in liver. *In* Metabolic Compartmentation. H. Sies, editor. Academic Press, London. 331–359.
13. Cursted, T. 1974. Biosynthesis of molecular species of phosphatidylcholine in bile, liver and plasma of rats given [1,1-$^2H_2$]ethanol. *Biochim. Biophys. Acta.* **369:** 196–208.
14. Wadke, M., H. Brunengraber, J. M. Lowenstein, J. J. Dolhun, and G. P. Arsenault. 1973. Fatty acid synthesis by liver perfused with deuterated and tritiated water. *Biochemistry.* **12:** 2619–2624.
15. Possmayer, F., G. L. Scherphof, R. M. A. R. Dubbelman, L. M. G. Van Glode, and L. L. M. Van Deenen. 1969. Positional specificity of saturated and unsaturated fatty acids in phosphatidic acid from rat liver. *Biochim. Biophys. Acta.* **176:** 95–100.
16. Coleman, R. A., and E. B. Haynes. 1984. Hepatic monoacylglycerol acyltransferase. Characterization of an activity associated with the suckling rats. *J. Biol. Chem.* **259:** 8934–8938.
17. Swift, L. L. 1995. Assembly of very low density lipoproteins in rat liver: a study of nascent particles recovered from rough endoplasmic reticulum. *J. Lipid Res.* **36:** 395–406.
18. Bar-On, H., and Y. Stein. 1968. Effect of glucose and fructose administration on lipid metabolism in the rat. *J. Nutr.* **94:** 95–105.
19. Nikkila, E. A. 1969. Control of plasma and liver triglyceride kinetics by carbohydrate metabolism and insulin. *Adv. Lipid Res.* **7:** 63–104.
20. Carmona, A., and R. A. Freedland. 1989. Comparison among the lipogenic potential of various substrates in rat hepatocytes: the differential effects of fructose-containing diets on hepatic lipogenesis. *J. Nutr.* **119:** 1304–1310.
21. Reiser, S., and J. Hallfrich. 1987. Lipogenesis and blood lipids. *In* Metabolic Effects of Dietary Fructose. S. Reiser and J. Hallfrich, editors. CRC Press Inc. Boca Raton, FL. 83–111.
22. Mayes, P. A. 1993. Intermediary metabolism of fructose (review). *Am. J. Clin. Nutr.* **58:** 754S–765S.
23. Spence, J., and H. C. Pitot. 1982. Induction of lipogenic enzyme in primary cultures of rat hepatocytes. Relationship between lipogenesis and carbohydrate metabolism. *Eur. J. Biochem.* **128:** 15–20.
24. Park, O. J., D. Cesar, D. Faix, K. Wu, C. H. L. Shackleton, and M. K. Hellerstein. 1992. Mechanisms of fructose-induced hypertriglyceridemia in the rat. Activation of hepatic pyruvate dehydrogenase through inhibition of pyruvate dehydrogenase kinase. *Biochem. J.* **282:** 753–757.
25. Gould, G. W., and G. D. Holman. 1993. The glucose transporter family: structure, function and tissue-specific expression. *Biochem. J.* **295:** 329–341.
26. Hellerstein, M. K. 1995. Method for measurement of fatty acid and cholesterol metabolism. *Curr. Opin. Lipidol.* **6:** 172–181.