# Hepatic secretion of VLDL fatty acids during stimulated lipogenesis in men

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**Abstract** **Fatty acids (FA) that are utilized for triglyceride (TG) synthesis in the liver and principally from two sources: FA synthesized de novo in the liver and preformed FA. We have measured the contribution from the two sources to very low density lipoprotein (VLDL) TG synthesis individually for palmitate, oleate, stearate, and linoleate (≈98% of the total FA of VLDL TG (VLDL TGFA)) by isotopomer analysis. Five healthy men were studied in the basal state, and 1 (day 1) and 4 days (day 4) after the start of a hypercaloric carbohydrate-enriched diet (≈2.5 times energy expenditure). The secretion of de novo palmitate was increased 15- and 43-fold after 1 and 4 days of hyperalimentation (2.6 ± 1.2 (basal state), 40.8 ± 20.0 (day 1), and 113.3 ± 42.0 μmol/kg per d (day 4)). Even though 4 days of hyperalimentation increased the secretion of de novo stearate 43-fold and de novo oleate 70-fold (stearate; 0.2 ± 0.2 (basal), 8.6 ± 3.3 μmol/kg per d (day 4), oleate; 0.4 ± 0.4 (basal), 28.2 ± 12.7 μmol/kg per d (day 4)), palmitate accounted for 75–85% of all the de novo VLDL TGFA. One day of carbohydrate hyperalimentation tended to decrease the secretion while 4 days increased the secretion of all preformed FA in VLDL TG. The rate of secretion of preformed palmitate and oleate were almost identical (palmitate; 80.2 ± 22.2 (basal), 45.1 ± 23.8 (day 1), and 256.2 ± 74.1 μmol/kg per d (day 4), oleate; 95.2 ± 22.8 (basal), 46.2 ± 24.2 (day 1), and 356.8 ± 74.1 μmol/kg per d (day 4)) and collectively these two FA accounted for 80–90% of the secretion from the preformed source. Palmitate is the predominant product of acute and prolonged carbohydrate mediated lipogenesis in the human liver. The pathway of further elongation and subsequent desaturation of de novo synthesized palmitate to generate stearate and oleate is inducible but, quantitatively, of minor significance in hepatic lipogenesis.—**Aarsland, A., and R. R. Wolfe. **Hepatic secretion of VLDL fatty acids during stimulated lipogenesis in men. *J. Lipid Res.* 1998.** 39: **1280–1286.**

**Supplementary key words** de novo synthesis of fatty acids • palmitic • stearic • palmitoleic • oleic • linolenic • elongation • desaturation • stable isotope

Very low density lipoprotein triglyceride (VLDL TG) secreted by the liver provides the rest of the body with long chain fatty acids (FA). FA are highly specialized structural components of various tissues, where they constitute the majority of membrane lipids. The long chain FA also serve as the body's main storage form of energy. To fulfill different functions, FA have different structural characteristics based on their chain length (number of carbon units) and saturation (number of unsaturated bonds). With the exception of the essential FA (linoleic (C18:2) and linolenic FA (C18:3)), the major FA of VLDL TG can alternatively be made within the liver or be derived from the preformed pool. The FA of the preformed pool either come from the diet or from the adipose stores in the peripheral tissues. They are taken up from the plasma and are either temporarily stored in the liver or reesterified directly into VLDL TG. De novo synthesis of FA is principally a polymerization of acetate to form palmitate (C16:0) (consisting of 8 acetate units) as the most common product. Elongation and/or desaturation of de novo synthesized or preexisting FA will yield the longer FA, with stearate (C18:0) and oleate (C18:1) being quantitatively the most significant (pertinent reviews are provided in ref. 1–3).

Several studies have shown that the flux through the pathways that provide FA for VLDL TG synthesis are different for each FA and that the flux can vary depending on the hormonal and the nutritional milieu (4–10). Furthermore, the relative availability of individual FAs can affect the secretion rate and subsequent metabolic faith of the VLDL TG lipoprotein particle (11). Thus, a high carbohydrate intake that favors lipogenesis could potentially cause stimulated total VLDL TG secretion with a relative enrichment in saturated FA. Alternatively, a prolonged carbohydrate intake could spare unsaturated FA from oxidation and a later adaptive up-regulation of the desaturation pathway could channel de novo synthesized FA towards desaturation, thus maintaining the total unsaturation of the VLDL TG.

Because the assembly of the VLDL TG involves several metabolically different FA, an integrated study of this process is complicated. Although the scientific literature pertinent to this field is extensive, our current knowledge is

Abbreviations: VLDL, very low density lipoprotein; TG, triglyceride; FA, fatty acid(s); FSR, fractional secretion rate.

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largely fragmentary, particularly regarding human metabolism. While human in vivo studies have usually been confined to the flux of one single FA, from which the kinetics of all the VLDL TGFA have been extrapolated (12–14), various in vitro preparations have been the most common approach to study the integrated flux of multiple FA (9, 15–21). In view of the need to quantify the flux of the major FA of the VLDL TG in vivo, we have infused labeled acetate and subsequently measured the incorporation of label in individual FA isolated from plasma VLDL TG. With this technique, we have quantified the total VLDL TG bound secretion of palmitate, oleate, stearate and linoleate. Further, we have quantified the contribution from the two major pathways; the secretion of FA de novo synthesized in the liver and the secretion of FA derived from peripheral adipose tissue for each FA. We have studied the subjects before and 1 and 4 days after the start of a hypercaloric carbohydrate-enriched diet in order to document possible adaptive changes in the flux of the FA that may occur over time during stimulated lipogenesis.

## MATERIALS AND METHODS

A detailed description of the subjects, the experimental design, the tracer infusion, the blood sampling, and the analysis of the samples of this study has been provided previously (22). Subjects gave informed written consent after the experimental procedures and risks have been explained in detail. This study was approved by the Institutional Review Board of U.T.M.B. Briefly, three tracer infusion studies were performed on five normal male volunteers. They were studied after an overnight fast (basal state) and after 1 and 4 days of carbohydrate feeding (Day 1 and Day 4). The studies were performed at least 3 days apart to allow for isotope washout. The basal state value was determined in the first session. The values after 1 (Day 1) and 4 days (Day 4) of carbohydrate feeding were determined in the second session.

In the first session the subjects were admitted to the Clinical Research Center the night before the infusion study. They were given a standard meal and then no more meals throughout the study. The basal state value was determined during i.v. infusion of glucose at 2 mg/kg per min. Glucose was infused to promote a measurable rate of hepatic fatty acid synthesis, which was required by the model. This infusion rate was chosen because it is well below the maximal capacity for glucose oxidation in humans (23, 24) and allowed us to provide the subjects with approximately 50% of their estimated energy demands during the study. The constant infusion of [1,2-$^{13}$C] acetate (2 μmol/kg per min, no prime) and the glucose infusion was started at 8 am. After completion of this basal state study, the subjects were discharged.

For the second session the subjects were admitted at noon the day before the infusion study. They had been instructed not to eat on the day of admission. At 3 pm a constant i.v. infusion of glucose at 2 mg/kg per min was started. At the same time nasogastric feeding with a liquid meal (Vivondx, Sandoz Nutrition Corporation, Minneapolis, MN) was started. Vivonex had been diluted to a caloric density of 1 kcal per ml with an energy distribution of 83% carbohydrates, 15% amino acids, and 2% fat (linoleic acid). A comparable diet has been shown to eliminate the influx of chylomicrons into circulation during feeding (25). The feeding was gradually increased over the next 6 h to a final rate of 2 ml/kg per hr, i.e., 50 kcal·kg$^{-1}$ day$^{-1}$. The nasogastric feeding and i.v. glucose infusion were maintained night and day throughout the study. Thus, the carbohydrate-derived background $^{13}$C enrichment was constant during the tracer infusion. At the final rate a total of 8.7 mg/kg per min of carbohydrate was provided; 6.7 mg/kg per min was delivered through the nasogastric tube and 2 mg/kg per min through the i.v. infusion. The total amount of calories provided was Å 60 kcal/kg per day with approximately 90% from carbohydrates.

During the second session a constant i.v. infusion of [1, 2-$^{13}$C] acetate (2 μmol/kg per min, no prime) was started at 8 am the first morning after the start of the high carbohydrate feeding (Å 18 h after the start, Day 1) and at the morning of the fourth day of the carbohydrate feeding (Day 4). During all three isotope infusions, i.e., the one in the first and the two in the second session, [$^{2}H_5$]glycerol was infused i.v. for 90 min to measure lipolysis (22).

### Measurement of VLDL-bound fatty acid kinetics

Quantitatively, palmitate (C16:0), oleate (C18:1), stearate (C18:0), linoleate (C18:2), and palmitoleate (C16:1) make up over 97% of the fatty acids of VLDL-bound triglycerides. To quantify the total secretion of VLDL-bound FA, the secretion of stearate, oleate, and linoleate were measured principally as previously described for VLDL-bound palmitate (20) except with minor modifications. In our calculations the production and secretion of palmitoleate was considered to be the same as palmitate and thus, the two VLDL pools were combined in our calculations. This approach is justified by the fact that the synthesis of palmitoleate proceeds through the initial synthesis of palmitate and that the final desaturation step is the only differentiating step in their synthesis.

VLDL-bound FA secreted by the liver are principally derived from two sources of fatty acid, *1*) fatty acids that are synthesized de novo in the liver and *2*) preformed fatty acids (22). The term preformed fatty acids is used collectively for fatty acids that arise from any other source than de novo synthesis in the liver. Potential sources of preformed fatty acids include the plasma FFA from peripheral lipolysis (adipose tissue), FA from lipolysis of lipoprotein remnants, or FA resulting from hydrolysis of intrahepatic TG. In our experiment, fat was omitted from the diet so that the plasma FFA was entirely derived from lipolysis.

Briefly, our method of measuring the kinetics of VLDL bound FA takes advantage of the fact that acetyl-CoA is the precursor for de novo fatty acid synthesis. Labeled acetate, mixed with the endogenous pool of acetyl-CoA, labels newly formed fatty acids in the liver. A certain fraction of these labeled fatty acids are secreted from the liver as VLDL bound FA. If the enrichment of the precursor pool is known, then the rate of secretion of de novo synthesized FA can be calculated by measuring rate of incorporation of the labeled into the product, i.e., the individual VLDL bound FA. The enrichment of the hepatic precursor pool for FA synthesis cannot be measured directly or even estimated in vivo. Therefore, the novelty of the current method is the examination of precursor enrichment based on the "enrichment pattern" (isotopomer distribution) of the product (VLDL-bound FA). The deduced precursor enrichment enables us to measure not only the rate of secretion of de novo synthesized FA but also the total rate of secretion of the individual VLDL bound FA based on the dilution principle. The rate of secretion of preformed FA is calculated by subtracting the rate of secretion of de novo synthesized FA from the total rate of secretion of the fatty acid.

In the current study, the fractional secretion rate (FSR) of individual fatty acids was calculated as previously described based on the precursor enrichment that was determined from the isotopomer distribution of palmitate (26). The assumption is that all the newly synthesized fatty acids are derived from one or several intrahepatic acetyl-CoA pools with the same precursor enrichment. This concept is supported by recent studies on hepatoma cell lines (20). Generally, palmitate was the fatty acid

with the highest isotope enrichment and thus provided the most reliable determination of isotopomeric distribution. The total rate of VLDL bound FA secretion is obtained by adding the total secretion rate of the individual FA. The specific formulas used to calculate the intrahepatic precursor enrichment for FA synthesis, the rate of secretion of de novo synthesized, and preformed FA bound to VLDL-TG had been provided previously (22).

To differentiate between the secretion rate of de novo synthesized, elongated, or preformed C18 fatty acids, certain assumptions were made (**Fig. 1**). We assume that palmitate is the primary product of de novo fatty acid synthesis in the liver and that the C18 fatty acids such as stearate and oleate are the products of elongation and desaturation of palmitate (3, 19). As such the VLDL bound stearate and oleate can principally come from three sources (Fig. 1): A) from de novo synthesized palmitate with all its acetate molecules coming from the hepatic acetate pool and getting one more acetate unit to become stearate or undergoing further desaturation to become oleate; B) from palmitate that is initially taken up from the plasma and elongated by the addition of one acetate molecule from the hepatic pool to become stearate and oleate; C) and from uptake from plasma and subsequent reesterification into VLDL TG. In this study the term "de novo synthesized" refers to process A where all the acetate units of the FA are derived from the hepatic pool. FA derived from process B and C are collectively termed preformed. As such, preformed encompasses both the FA derived from palmitate that was taken up from plasma and elongated into a C18 FA, and also the stearate and oleate that were taken up from plasma and reesterified into VLDL TG without any modification. The term preformed FA does not assign a specific source of the FA, i.e., plasma FA, membrane lipids, or storage TG, but rather classifies the FA as not de novo synthesized. Labeling of stearate and oleate with one labeled acetate molecule can be the consequence of elongation of any palmitate, de novo synthesized or not. C18 fatty acids containing multiple labeled acetate molecules (2, 3–8) can only be the elongated product of de novo synthesized palmitate. To obtain more than one labeled acetate molecule, the C18 FA must have had labeled palmitate as the immediate precursor (palmitate with a minimum of one labeled acetate unit must be de novo synthesized as elongation of shorter

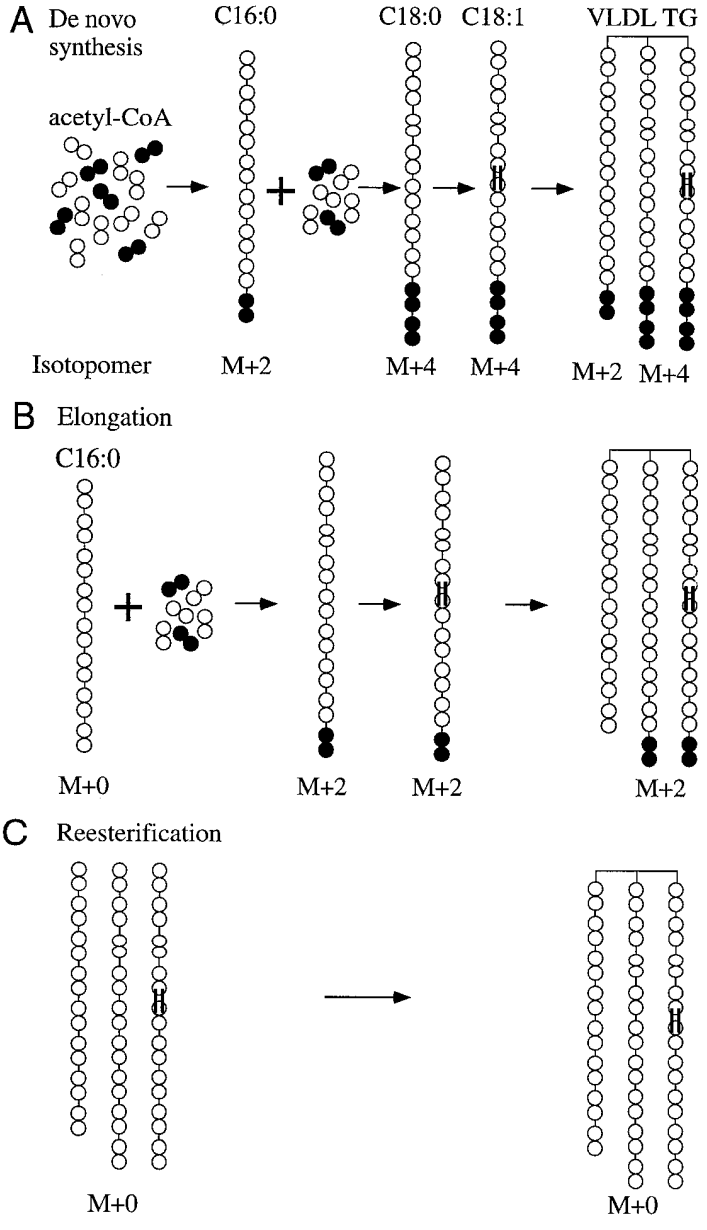


**Fig. 1.** Sources of long chain fatty acids for hepatic VLDL TG synthesis. A) A de novo synthesized FA in our model is defined as having all the carbon atoms coming from the acetyl-CoA pool. During infusion of 1,2$^{-13}$C-labeled acetate, the M+2 isotopomer of palmitate and the M+4 isotopomer of stearate and oleate can only come from de novo synthesis. B) Elongation and subsequent desaturation of unlabeled (M+4) palmitate can generate the M+2 isotopomers of stearate and oleate. C) Reesterification of a FA will not change its isotopomer distribution. FA from B and C and collectively defined as preformed FA. (See text for further details.)

chain fatty acids is not a major pathway (3, 19)). For this reason we calculated the FSR for the C18 fatty acids using their doubly labeled isotopomers(two labeled acetate molecules) rather than singly labeled stearate and oleate.

Linoleate is an essential fatty acid, meaning that it is not synthesized. Thus, the only quantity to be computed regarding linoleate is the secretion rate of VLDL-bound linoleate. This is computed by multiplying the VLDL palmitate secretion rate by the ratio of % linoleate abundance to % palmitate abundance in VLDL as determined by gas chromatography.

### Statistical analysis

All results are expressed as mean and SEM. Comparisons of groups were preformed by means of analysis of variance (ANOVA) (27). When ANOVA demonstrated statistical significance, Fisher's Least Significant Difference Test was used to compare differences between groups. Statistical significance was accepted at $P < 0.05$.

## RESULTS

The results presented below describe the kinetics of the individual VLDL TGFA in the basal state and during 4 days of carbohydrate hyperalimentation. The calculated enrichment of the lipogenic acetyl-CoA pool, the total VLDL TG kinetics, indirect calorimetry values, body weight as well as the plasma glucose and lactate concentration had been reported previously (22).

### Plasma FFA

*Relative concentrations of plasma FFA.* The relative concentrations of the major FFAs in plasma during the carbohydrate hyperalimentation are shown in **Fig 2**. After short term hyperalimentation, there was a significant decrease in oleate abundance from 46.1 ± 4.8% in the basal state to 33.8 ± 1.8% at Day 1. By the fourth day of hyperalimentation, this effect was reversed. The relative abundance of both palmitic and palmitoleic acid in plasma tended to increase during hyperalimentation, from 27.1 ± 3.1% and 3.6 ± 1.0%, respectively, in the basal state to 36.0 ± 0.5% and 10.6 ± 1.3% after prolonged hyperalimentation (Day 4).

*Concentrations of plasma FFA.* The concentrations of the individual FFA in plasma are shown in **Table 1**. After 1 day of carbohydrate feeding, oleate concentration in plasma was reduced 50% from 0.43 ± 0.04 mmol/L in the basal state to 0.20 ± 0.01 mmol/L at Day 1, while prolonged hyperalimentation tended to normalize this trend. Linoleic acid concentration declined steadily throughout the feeding program, from 0.12 ± 0.03 mmol/L in the basal state to 0.04 ± 0.01 mmol/L at Day 4. The concentration of palmitoleic acid increased 100% during the experiment from 0.03 ± 0.01 mmol/L in the basal state to 0.07 ± 0.01 mmol/L after 4 days of hyperalimentation.

TABLE 1. Concentrations of individual plasma FFA

| Fatty Acid | Basal State | Day 1 | Day 4 |
|---|---|---|---|
| | | *mmol/L* | |
| Palmitic | 0.25 ± 0.03 | 0.20 ± 0.03 | 0.26 ± 0.00 |
| Palmitoleic | 0.03 ± 0.01 | 0.04 ± 0.01 | 0.07 ± 0.01[a] |
| Stearic | 0.05 ± 0.01 | 0.06 ± 0.03 | 0.04 ± 0.00 |
| Oleic | 0.43 ± 0.04 | 0.20 ± 0.01[a] | 0.27 ± 0.01[a,b] |
| Linoleic | 0.12 ± 0.03 | 0.07 ± 0.02 | 0.04 ± 0.01[a] |
| Other fatty acids | 0.05 ± 0.02 | 0.03 ± 0.01 | 0.04 ± 0.02 |
| Total fatty acids | 0.93 ± 0.10 | 0.60 ± 0.12[a] | 0.72 ± 0.07[b] |

Results are given as mean ± SEM.
[a] $P < 0.05$, statistically different from Day 0.
[b] $P < 0.05$, different from Day 1.

### Fatty acids bound to VLDL TG

*Relative Concentrations of VLDL TGFA.* Collectively the palmitic, palmitoleic, stearic, oleic, and linoleic acids made up 98–99% of the fatty acids that were esterified into VLDL TG both in the basal state and during the hyperalimentation (Fig. 2). In the basal state, palmitic acid accounted for 28.3 ± 3.0% of the VLDL TGFA, but after 4 days of carbohydrate intake the palmitate contribution was increased to 37.9 ± 0.5%. Plamitoleic acid accounted initially for only 3.8 ± 1.0% of total VLDL TGFA, but by Day 4 this fatty acid made up 10.0 ± 1.3% of total VLDL TGFA. After short term carbohydrate hyperalimentation the relative amount of oleate in VLDL TG decreased from 48.1 ± 4.8% in the basal state to 34.8 ± 1.7% (Day 1), but prolonged hyperalimentation restored the oleate abundance to 40.5 ± 1.9% (Day 4). The relative amount of linoleic acid decreased during the study, from 13.1 ± 3.7% in the basal state to 5.4 ± 1.8 after 4 days of hyperalimentation (Day 4).

*Concentrations and pool sizes of VLDL TGFA.* Throughout the carbohydrate feeding all the identified VLDL TGFA increased in concentration (**Table 2**). The C16 fatty acids palmitate and palmitoleic acid demonstrated the greatest in-

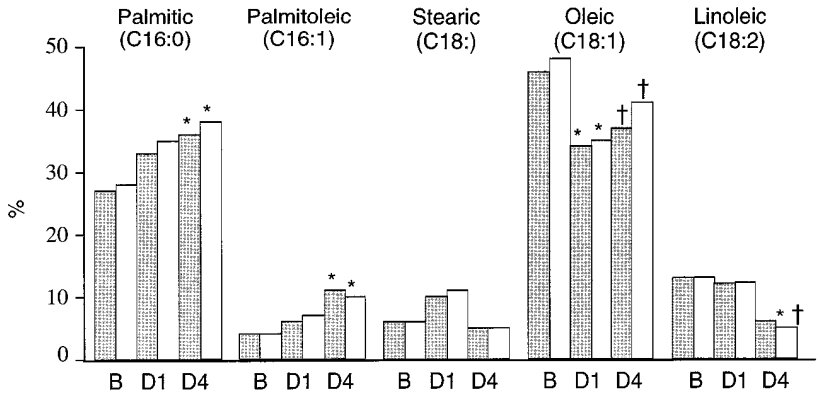


**Fig. 2.** Relative concentrations of plasma FFA and VLDL TGFA. The relative concentrations of plasma free fatty acids (open bars) and VLDL triglyceride-bound fatty acids (filled bars) are shown in the basal state (B), and after 1 day (D1) and 4 days (D4) of carbohydrate hyperalimentation. *$P < 0.05$ statistically different from Day 0; †$P < 0.05$ different from Day 1.

TABLE 2. Concentrations of individual VLDL bound FA

| Fatty Acid | Basal State | Day 1 | Day 4 |
|---|---|---|---|
| | | *mmol/L* | |
| Palmitic | 0.29 ± 0.02 | 0.52 ± 0.01[a] | 3.33 ± 0.04[a,b] |
| Palmitoleic | 0.04 ± 0.00 | 0.14 ± 0.02[a] | 0.87 ± 0.11[a,b] |
| Stearic | 0.05 ± 0.01 | 0.07 ± 0.01 | 0.46 ± 0.05[a,b] |
| Oleic | 0.40 ± 0.02 | 0.56 ± 0.03[a] | 3.55 ± 0.16[a,b] |
| Linoleic | 0.12 ± 0.03 | 0.07 ± 0.05 | 0.47 ± 0.15[a,b] |
| Other fatty acids | 0.01 ± 0.01 | 0.02 ± 0.01 | 0.10 ± 0.02[a,b] |
| Total fatty acids | 0.91 ± 0.23 | 1.38 ± 0.66 | 8.78 ± 1.66[a,b] |

Results are given as mean ± SEM.
[a] $P < 0.05$, statistically different from Day 0.
[b] $P < 0.05$, different from Day 1.

crease in concentrations from the basal state to the fourth day (12- and 20-fold, respectively). Among the C18 fatty acids, the concentration of both stearate and oleate increased approximately 9-fold while the concentration of linoleic acid only increased 4-fold during the carbohydrate feeding.

*Rate of Secretion of VLDL TGFA.* In the basal state and during carbohydrate feeding, secretion of palmitate accounted for 75–85% of the total secretion of de novo synthesized VLDL TGFA (**Table 3**). After only 1 day of carbohydrate hyperalimentation, the secretion rate of de novo synthesized palmitate was increased significantly from 2.6 ± 1.2 to 40.8 ± 20.0 μmol/kg per day and finally reached a level of 113.3 ± 42.0 μmol/kg per day after 4 days of carbohydrate feeding. The rates of secretion of de novo synthesized stearate and oleate were also significantly increased after 4 days of carbohydrate hyperalimentation, going from a basal level of 0.2 ± 0.2 and 0.4 ± 0.4 to 8.6 ± 3.3 and 28.2 ± 12.7 μmol/kg per day, respectively, at day 4. There was no measurable secretion of de novo synthesized linoleic acid.

The total secretion rate of de novo synthesized VLDL TGFA was 3.2 ± 1.1 μmol/kg per min in the basal state. After 1 day of carbohydrate hyperalimentation, the secretion rate increased to 47.6 ± 23.8 μmol/kg per day; after 4 days it reached 150 ± 56.4 μmol/kg per day.

As opposed to the secretion of de novo synthesized fatty acids, the rate of secretion of VLDL TGFA coming from the intrahepatic preformed source tended to decrease after 1 day of carbohydrate hyperalimentation (**Table 4**). This statistically insignificant tendency was evident for all of the measured VLDL TGFA. After 4 days of hyperalimentation the secretion rates of preformed palmitate

TABLE 3. Secretion of de novo synthesized VLDL TGFA

| Fatty Acid | Basal State | Day 1 | Day 4 |
|---|---|---|---|
| | | *μmol/kg/d* | |
| Palmitic | 3 ± 1 | 41 ± 20[a] | 113 ± 42[a,b] |
| Stearic | 0 ± 0 | 2 ± 1 | 9 ± 3[a,b] |
| Oleic | 0 ± 0 | 5 ± 3 | 28 ± 13[a,b] |
| Linoleic | 0 | 0 | 0 |
| Total secretion rate | 3.2 ± 1.1 | 48 ± 24[a] | 150 ± 56[a,b] |

Results are given as mean ± SEM.
[a] $P < 0.05$, statistically different from Day 0.
[b] $P < 0.05$, different from Day 1.

TABLE 4. Secretion of preformed VLDL TGFA

| Fatty Acid | Basal State | Day 1 | Day 4 |
|---|---|---|---|
| | | *μmol/kg/d* | |
| Palmitic | 80 ± 22 | 43 ± 24 | 246 ± 74[a,b] |
| Stearic | 12 ± 3 | 7 ± 2 | 26 ± 5[a,b] |
| Oleic | 95 ± 23 | 48 ± 24 | 256 ± 87[a,b] |
| Linoleic | 25 ± 6 | 14 ± 6 | 27 ± 9 |
| Total secretion rate | 212 ± 52 | 113 ± 52 | 566 ± 157[a,b] |

Results are given as mean ± SEM.
[a] $P < 0.05$, statistically different from Day 0.
[b] $P < 0.05$, different from Day 1.

stearate, and oleate were significantly elevated compared to the basal state and also to the secretion rate after short term carbohydrate feeding (Day 1). The secretion of preformed palmitate was 3.2-fold higher than the basal level after 4 days of carbohydrate feeding. The corresponding values for stearate and oleate were 2.2- and 2.7-fold.

The total secretion rate of preformed VLDL TGFA was 211.9 ± 51.9 μmol/kg per min in the basal state. After 1 day of carbohydrate hyperalimentation, the value was decreased to 112.6 ± 52.4 μmol/kg per day; at day 4 it was significantly increased to 566.2 ± 157.4 μmol/kg per day.

## DISCUSSION

To our knowledge this is the first time that the rate of secretion of the individual VLDL TGFA in humans has been reported in the basal state and during acute (Day 1) and prolonged (Day 4) stimulation of the de novo lipogenic pathway. The study clearly shows that palmitate is quantitatively the major fatty acid produced during hepatic lipogenesis. Irrespective of the feeding regime, hepatic palmitate synthesis accounted for 75–85% of all de novo lipogenesis, while the other major VLDL TGFA, oleate, accounted for 10–20% of the total secretion of de novo synthesized VLDL TGFA. Thus, elongation and subsequent desaturation of newly synthesized palmitate is quantitatively of minor significance in hepatic lipogenesis, even after 4 days of stimulated lipogenesis. At its maximum (Day 1), approximately 45% of all the palmitate secreted came from de novo synthesis, while 10% of the secreted oleate was derived from hepatic de novo synthesis. Thus, there are major differences in the intrahepatic flux of the individual FA bound to VLDL TG, and assumptions regarding the extrapolation of the kinetics of one fatty acid (e.g., palmitate) to all the fatty acids should be interpreted with care.

### Model and assumptions

In this study the isotopic enrichments of 90–97% of the VLDL TGFA were measured and the kinetics of the individual FA were computed independently.

The FSR of individual fatty acids was calculated based on the precursor enrichment that was determined from the isotopomer distribution of palmitate (26). The assumption is that all the newly synthesized fatty acids are derived from one or several intrahepatic acetyl-CoA pools

with the same precursor enrichment. Generally, palmitate was the fatty acid with the highest isotope enrichment and thus provided the most reliable determination of isotopomeric distribution. For the other fatty acids, particularly in the basal state, the isotope enrichment that would allow us to determine the precursor enrichment independently often could not be measured. But, whenever possible, comparisons were made between precursor enrichments deducted from different fatty acids. A variation in isotope enrichment of the acetyl-CoA pool of no more than 10% was found irrespective of the feeding regime. This strongly suggests that all the newly synthesized fatty acids in the liver were derived from one precursor pool.

In our model, it is essential that the synthesis of palmitate proceeds through the polymerization of eight acetate units and not through elongation of shorter, preformed fatty acids by addition of one or more acetate units. In our deduction of the precursor enrichment and subsequent calculations, it is crucial that all of the eight acetate units in newly synthesized palmitate have an equal probability of picking up a labeled acetate unit (26). De novo synthesized palmitate can subsequently be desaturated and/or elongated to produce the other fatty acids (Fig. 1) (3). In our model we have defined a de novo synthesized fatty acid as having all the carbon atoms coming from the acetyl-CoA pool. For newly synthesized C18 fatty acids, this means that they are made out of newly synthesized palmitate by the addition of one more acetate unit. Thus, all the acetate units in the C18 fatty acids also have an equal probability of picking up a labeled acetate unit.

Furthermore, this definition had enabled us to differentiate between the secretion of newly synthesized C18 fatty acids versus the secretion of C18 fatty acids that are derived from the elongation of preformed palmitate (Fig. 1). Any palmitate containing labeled acetate must be de novo synthesized (all or none of the carbons come from the acetate pool). Any C18 fatty acid containing two or more labeled acetate units could only have labeled palmitate (newly synthesized) as an immediate precursor. Thus, the isotopomer of the C18 fatty acids containing two labeled acetate units (m+4) was used to compute the kinetics of oleate and stearate. The isotopomer representing the one labeled acetate unit (m+2) could potentially come from de novo synthesis or from elongation of preformed palmitate.

In selected cases, where the isotopomer distribution allowed for an independent estimation of precursor enrichment, the m+2 isotopomer was used to calculate the rate of secretion of elongated C18 fatty acids. With a known precursor enrichment and a known rate of appearance of the m+4 isotopomer (which is defined by the secretion of newly synthesized fatty acid), one can deduct the expected rate of appearance of the m+2 isotopomer from the secretion of newly synthesized fatty acids. The difference between the deducted and the observed rates of appearance of the m+2 isotopomer represents the secretion of elongated C18 fatty acids. Based on the selected cases in which sufficient information was available, we estimated that the secretion of elongated C18 fatty acids was approximately 10% of the secretion of newly synthesized fatty acids, and no more than 1–2% of the total secretion of C18 fatty acids came from this source of fatty acids.

### Rate of secretion of individual VLDL TGFA

Hepatic lipogenesis predominantly produces palmitate irrespective of feeding regime. Palmitate synthesis accounted for 75–85% of all the de novo lipogenesis. The 43-fold increased synthesis rate of palmitate was mirrored in the relative up-regulation of palmitate concentration in both VLDL TG and plasma FA. This shows that the fatty acid synthase complex in humans is primarily a palmitate-producing complex. After 4 days of carbohydrate overfeeding, the secretion of de novo synthesized stearate and oleate was 43- and 70-fold higher, respectively, than in the basal state. The calculated elongation and/or desaturation activity is a minimal estimate of the flux through this pathway as elongation and/or desaturation of preformed palmitate is not accounted for. Even so, the results clearly show that during stimulated lipogenesis in the liver, the elongation and desaturation pathways of long chain FA are highly inducible. Based on the relatively increased secretion rate of newly synthesized oleate during the fourth day of carbohydrate feeding (Day 4), it is also possible that the up-regulation of oleate production was lagging in time. Palmitoleic acid was the fatty acid that increased its relative concentration the most in VLDL TG. We were not able to measure the isotopic enrichment of this fatty acid due to chromatographic difficulties and thus could not calculate its kinetics. However, the relative increase in concentration suggests that the secretion of de novo synthesized palmitoleic acid may have increased relatively even more than the secretion of newly synthesized palmitate. We did check for isotope enrichment (acetate incorporation) in linoleic acid, but (as expected) this fatty acid did not show any signs of being synthesized in the liver.

The secretion rate of preformed VLDL TGFA was calculated as the difference between the total secretion rate and the secretion rate of de novo synthesized fatty acids. Ultimately, the preformed fatty acids in the liver would come from the plasma FA pool as this is the only source of FA except for newly synthesized fatty acids. But, over the time span of our experiment, the proximate source of these FA might have been plasma FA, TG from remnant particles, hepatic membrane lipids, or storage TG. The shortest transit time for fatty acids from plasma to subsequent VLDL TGFA secretion is probably within 30 min to 2 h. The time it would take to fully equilibrate with a potential intrahepatic storage pool of TG is dependent on the size of the pool and its turnover rate. The later parameters are unknown in our experiment and we would expect them to change during the carbohydrate feeding. Thus, we are unable to quantify the extent of recycling of VLDL TGFA that took place during the experiment. The relative concentration of FA in plasma clearly mirrored the VLDL TGFA secretory pattern (Fig. 2) suggesting that there is a potential for recycling or, alternatively, that FA are synthesized at other locations (adipose tissue). On the

other hand, during our sampling periods, the plasma FFA isotope enrichment did not exceed 10% of the corresponding isotope enrichment in VLDL TGFA, suggesting that the recycling was of minor importance.

The preformed source of fatty acids was the most important contributor to VLDL TG secretion. Collectively, the secretion of preformed fatty acids accounted for 99, 70, and 79% of the total VLDL TGFA secretion during the basal state, early (Day 1), and late (Day 4) carbohydrate feeding, respectively. Preformed oleate was the single fatty acid that contributed the most from this source. It is also this FA that demonstrated most clearly the trend of decreased secretion rate during early (Day 1) versus late (Day 4) carbohydrate overfeeding. This shift in secretion rate was probably partially caused by an early decrease in plasma FA availability which later was counteracted by increased plasma FA availability and decreased intrahepatic FA utilization. The secretion of linoleic acid was maintained and the concentration in VLDL TG tended to increase during the carbohydrate feeding. This trend probably reflected the ongoing supply of linoleic acid in the enteral formula.

Based on these observations, we conclude that the adaptation of the human liver to a hypercaloric high carbohydrate diet involved the induction of the pathways for long chain fatty acid synthesis, elongation, and desaturation. Although the synthesis of stearate an oleate was upregulated, palmitate was quantitatively the predominant product of de novo lipogenesis. Even during maximal hepatic lipogenesis, preformed fatty acids accounted for 80% of all the VLDL TGFA secreted from the liver, almost half as oleate. This study also demonstrates that the flux measurement of individual FA is crucial in understanding the process of VLDL TG assembly.

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## REFERENCES

1. Glickman, R. M., and S. M. Sabesin. 1994. Lipoprotein metabolism. *In* The Liver: Biology and Pathobiology. I. M. Arias, J. L. Boyer, N. Fausto, W. B. Jakoby, D. Schachter, and D. A. Shafritz, editors. Raven Press, New York. 391–414.
2. Goodridge, A. G. 1991. Fatty acid synthesis in eucaryotes. *In* Biochemistry of Lipids, Lipoproteins and Membranes. D. E. Vance and J. Vance, editors. Elsevier Science Publishing, Amsterdam. 111–140.
3. Cook, H. W. 1991. Fatty acid desaturation and chain elongation in eucaryotes. *In* biochemistry of Lipids, Lipoproteins and Membranes. D. E. Vance and J. Vance, editors. Elsevier Science Publishing, Amsterdam. 141–169.
4. Lewis, G. F., K. D. Uffelman, L. Szeto, B. Weller, and G. Steiner. 1995. Interaction between free fatty acids and insulin in the acute control of very low density lipoprotein production in humans. *J. Clin. Invest.* **95:** 158–166.
5. Hughes, T. A., M. Heimberg, X. Wang, H. Wilcox, S. M. Hughes, E. A. Tolley, D. M. Desiderio, and J. T. Dalton. 1996. Comparative lipoprotein metabolism of myristate, palmitate, and stearate in normolipidemic men. *Metabolism.* **45:** 1108–1118.
6. Tijburg, L. B. M., M. J. H. Geelen, and L. M. G. Van Golde. 1989. Regulation of the biosynthesis of triacylglycerol, phosphatidylcholine and phosphatidylethanolamine in the liver. *Biochim. Biophys. Acta.* **1004:** 1–19.
7. Gibbons, G. F. 1990. Assembly and secretion of hepatic very-low-density lipoprotein. *Biochem. J.* **268:** 1–13.
8. Howard, B. V. 1987. Lipoprotein metabolism in diabetes mellitus. *J. Lipid Res.* **28:** 613–628.
9. Gibbons, G. F., and F. J. Burnham. 1991. Effect of nutritional state on the utilization of fatty acids for hepatic triacylglycerol synthesis and secretion as very-low-density lipoprotein. *Biochem. J.* **275:** 87–92.
10. Sparks, J. D., and C. E. Sparks. 1994. Insulin regulation of triacylglycerol rich lipoprotein synthesis and secretion. [Review]. *Biochim. Biophys. Acta.* **1215:** 9–32.
11. Mattson, F. H., and S. M. Grundy. 1985. Comparison of effects of dietary saturated, monounsaturated, and polyunsaturated fatty acids on plasma lipids and lipoproteins in man. *J. Lipid. Res.* **26:** 194–202.
12. Havel, R. J., J. P. Kane, E. O. Balasse, N. Segel, and L. V. Basso. 1970. Splanchnic metabolism of free fatty acids and production of triglycerides of very low density lipoproteins in normotriglyceridemic and hypertriglyceridemic humans. *J. Clin. Invest.* **49:** 2017–2035.
13. Boberg, J., L. A. Carlson, U. Freyschuss, B. W. Lassers, and M. L. Wahlqvist. 1972. Splanchnic secretion rate of plasma triglycerides and of total and splanchnic turnover of plasma free fatty acids in man with normo- and hypertriglyceridaemia. *Eur. J. Clin. Invest.* **2:** 454–466.
14. Binnert, C., C. Pachiaudi, M. Beylot, M. Croset, R. Cohen, J. P. Riou, and M. Laville. 1996. Metabolic fate of an oral long-chain triglyceride load in humans. *Am. J. Physiol.* **270:** E445–E450.
15. Lee, P. W. N., S. Bassilian, Z. Guo, D. Schoeller, J. Edmond, A. E. Bergner, and L. O. Byerley. 1994. Measurement of fractional lipid synthesis using deuterated water ($^2H_2O$) and mass isotopomer analysis. *Am. J. Physiol.* **266:** E372–E383.
16. Fukuda, N., and J. A. Ontko. 1984. Interactions between fatty acid synthesis, oxidation, and esterification in the production of triglyceride-rich lipoproteins by the liver. *J. Lipid Res.* **25:** 831–842.
17. Angeletti, C., and M. J. de Alaniz. 1995. Fatty acid uptake and metabolism in HepG2 human-hepatoma cells. *Mol. Cell. Biochem.* **143:** 99–105.
18. Kvilekval, K., J. Lin, W. Cheng, and N. Abumrad. 1994. Fatty acids as determinants of triglyceride and cholesteryl ester synthesis by isolated hepatocytes: kinetics as a function of various fatty acids. *J. Lipid Res.* **35:** 1786–1794.
19. Ajie, H. O., M. J. Connor, W. N. P. Lee, S. Bassilian, E. A. Bergner, and L. O. Byerley. 1995. In vivo study of the biosynthesis of long-chain fatty acids using deuterated water. *Am. J. Physiol.* **269:** E247–E252.
20. Lee, W. N., L. O. Byerley, S. Bassilian, H. O. Ajie, I. Clark, J. Edmond, and E. A. Bergner. 1995. Isotopomer study of lipogenesis in human hepatoma cells in culture: contribution of carbon and hydrogen atoms from glucose. *Anal. Biochem.* **226:** 100–112.
21. Bjornsson, O. G., J. D. Sparks, C. E. Sparks, and G. F. Gibbons. 1994. Regulation of VLDL secretion in primary culture of rat hepatocytes: involvement of cAMP and cAMP-dependent protein kinases. *Eur. J. Clin. Invest.* **24:** 137–148.
22. Aarsland, A., D. Chinkes, and R. R. Wolfe. 1996. Contributions of de novo synthesis of fatty acids to total VLDL-triglyceride secretion during prolonged hyperglycemia/hyperinsulinemia in normal man. *J. Clin. Invest.* **98:** 2008–2017.
23. Wolfe, R. R., T. F. O'Donnel, M. D. Stone, D. A. Richmand, and J. F. Burke. 1980. Investigation of factors determining the optimal glucose infusion rate in total parenteral nutrition. *Metabolism.* **29:** 892–900.
24. Wolfe, R. R., J. R. Allsop, and J. F. Burke. 1979. Glucose metabolism in man: responses to intravenous glucose infusion. *Metabolism.* **28:** 210–220.
25. Grundy, S. M., H. Y. I. Mok, L. Zeck, and D. Steinberg. 1979. Transport of very low density lipoprotein triglycerides in varying degrees of obesity and hypertriglyceridemia. *J. Clin. Invest.* **63:** 1274–1283.
26. Chinkes, D., A. Aarsland, J. Rosenblatt, and R. R. Wolfe. 1996. A comparison of mass isotopomer dilution methods used to compute production of VLDL fatty acids in vivo in human subjects. *Am. J. Physiol.* **271:** E373–E383.
27. Armitage, P., and G. Berry. 1994. Statistical Methods in Medical Research. Blackwell Scientific Publications, Oxford.