Hepatic and whole-body fat synthesis in humans during carbohydrate overfeeding[1–3]

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ABSTRACT The magnitude of the capacity to convert carbohydrate to fat in the human body is still controversial, as is the extent to which it takes place in the liver as opposed to the adipose tissue. We calculated whole-body net fat synthesis from indirect calorimetry and substrate balance data from five healthy men in the basal state and after 1 and 4 d on a hyperenergetic carbohydrate diet (≈2.5 times energy expenditure). At the same time, the secretion of fatty acids synthesized in the liver was measured to determine the extent to which fat synthesis occurs in the liver in a lipogenic state. The respiratory exchange ratio (RER) was 0.81 ± 0.01 in the basal state and 0.99 ± 0.025 and 1.15 ± 0.022 on days 1 and 4, respectively. Although there was net fat oxidation in the basal state (955 ± 139 $mg \cdot kg^{-1} \cdot min^{-1}$), there was net fat synthesis at the whole-body level both during early (day 1; 481 ± 205 $mg \cdot kg^{-1} \cdot min^{-1}$) and late (day 4; 2243 ± 253 $mg \cdot kg^{-1} \cdot min^{-1}$) carbohydrate overfeeding. Although hepatic secretion of fat synthesized de novo increased ≈35-fold during the study (basal state, 1.0 ± 0.3; day 1, 13.8 ± 6.8; and day 4, 43.3 ± 16.3 $mg \cdot kg^{-1} \cdot min^{-1}$) this could only account for a small portion of total fat synthesis. We conclude that the liver plays a quantitatively minor role when surplus carbohydrate energy is converted into fat in the human body. The main site for fat synthesis is likely to be the adipose tissue. *Am J Clin Nutr* 1997;65:1774–82.

KEY WORDS Fat synthesis, lipogenesis, hyperenergetic diet, carbohydrate, liver, adipose tissue, indirect calorimetry, stable isotopes, de novo lipid biosynthesis, fatty acids, very-low-density lipoprotein triacylglycerol

INTRODUCTION

Obesity is caused by ingestion of food energy in excess of expenditure. The excess energy is stored as triacylglycerols in adipose tissue. Excessive intake of carbohydrates in a mixed diet can potentially lead to fat accumulation indirectly by reducing the need for fat as fuel so that consumed fat will be retained, or directly by conversion of excess carbohydrate into fat through lipogenesis. Carbohydrates have usually been assigned a fat-sparing effect (1–3) in the development of obesity in a population consuming a mixed hyperenergetic diet, although the true contribution to de novo lipid biosynthesis and its significance for obesity has not been quantified. In clinical nutrition, formulas rich in carbohydrate are commonly used because of their ease of administration and broad availability. This practice has been questioned because of the lipogenic potential of carbohydrate-rich diets, particularly in critically ill patients at risk of hepatic fat accumulation (4, 5).

De novo lipid biosynthesis has been measured by indirect calorimetry in humans during parenteral (6–9) and enteral (10–12) nutrition. However, indirect calorimetry can determine only net lipogenesis, and cannot measure the true rate of fatty acid synthesis occurring at the same time as fatty acid oxidation. Thus, neither the capability of humans to convert carbohydrates into fat in this circumstance has been quantified, nor has the site of fatty acid synthesis been determined. Although studies with human tissue have shown the presence of the key enzymes for fatty acid synthesis to be both in the liver and adipose tissue (13–17), the actual contribution of the individual tissues to fat synthesis in vivo has not been measured. However, unlike rats, in which de novo synthesis of fatty acids is known to occur in both the liver and adipose tissue (14), humans are believed to synthesize fat primarily in the liver (1, 18). If this is the case, excess carbohydrates converted into fatty acids would be transferred from the human liver as very-low-density-lipoprotein (VLDL) triacylglycerol–bound fatty acids (TGFAs) for final storage in adipose tissue. Because it has been shown that after a single carbohydrate-rich meal only 1–2% of the palmitate of the VLDL TGFAs is derived from de novo synthesis (19), it could be assumed that this process is of minimal physiologic significance. On the other hand, during prolonged carbohydrate intake the limited capacity of the body to store glycogen could lead to saturation of that pathway, thereby resulting in the induction of the lipogenic pathway and, thus, far greater hepatic lipogenesis than after a single meal.

We took advantage of the fact that de novo lipid biosynthesis is principally a polymerization of acetate. By infusing labeled acetate and then measuring the incorporation of label into individual fatty acids isolated from plasma VLDL TGFAs, we quantified the effect of a continuous feeding of a high-carbohydrate diet on hepatic lipogenic activity. At the same time, whole-body fat synthesis was calculated from indirect calorim-

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etry data. We studied the subjects before and 1 and 4 d after the start of a high-carbohydrate diet to determine the extent to which fat synthesis occurs in the liver in a lipogenic state.

SUBJECTS AND METHODS

Subjects

Five normal male volunteers (aged 31 ± 4 y; three black and two white) participated in the study. They were healthy (as indicated by medical history and physical examination) were of normal weight [body mass index (BMI; kg/m^2) 23.22 ± 0.36], were taking no medications, and had normal blood chemistry values (liver function, thyroid function, plasma glucose, plasma insulin, and serum lipids) and hematologic indexes. The subjects were admitted on two occasions to the Clinical Research Center, University of Texas, Medical Branch at Galveston for the performance of the experiments. The study was approved by the institutional review board of the University of Texas, Medical Branch at Galveston. Informed consent from the subjects was obtained for all procedures.

Experimental protocol

Three tracer-infusion studies were performed on each subject: one after an overnight fast (basal state) and one after 1 d and one after 4 d of carbohydrate feeding (days 1 and 4). The studies were performed ≥ 3 d apart to allow for isotope washout. The basal state value was determined in the first session. The values after 1 and 4 d of carbohydrate feeding were determined in the second session.

The subjects were admitted to the Clinical Research Center the night before the first infusion study. They were given a standard meal and then no more meals throughout the study. The next day at 0700 a constant infusion of glucose at 2 $mg \cdot kg^{-1} \cdot min^{-1}$ was started through a catheter placed in an antecubital vein. Glucose was infused to promote a measurable rate of hepatic fatty acid synthesis, which was required by the model with which we calculated VLDL secretion. This infusion rate of glucose was chosen because it is well below the maximal capacity for glucose oxidation in humans (6, 20) and allowed us to provide the subjects with ≈50% of their estimated energy demands during the study, ie, 48 $kJ \cdot kg^{-1} \cdot d^{-1}$. The isotope infusion was started at 0800. After completion of this basal state study, the subjects were discharged.

For the second infusion study the subjects were admitted at 1200 on the day before the infusion study. They had been instructed to not eat on the day of admission. At 1400 an 8-gauge French nasogastric feeding tube was placed into the upper gastrointestinal tract. Proper positioning was confirmed by auscultation and aspiration of gastric fluid. At 1500 a constant intravenous infusion of glucose at 2 $mg \cdot kg^{-1} \cdot min^{-1}$ was started through a catheter placed in the antecubital vein. At the same time, nasogastric feeding with a liquid meal (Vivonex; Sandoz Nutrition Corporation, Minneapolis) was started. Vivonex had been diluted to an energy density of 4184 kJ/L (80.4 g in 250 mL water; total volume 300 mL) with an energy distribution of 83% carbohydrate, 15% amino acids, and 2% fat (linoleic acid). The liquid meal also contained vitamins, minerals, and trace elements to meet the recommended requirements of the subjects. The carbohydrates were derived from partially hydrolyzed corn starch. A comparable diet was shown to eliminate the influx of chylomicrons into circulation during feeding (21).

The feeding was started at an initial rate of 50 mL/h and gradually increased over the next 6 h to a final rate of 2 $mL \cdot kg^{-1} \cdot h^{-1}$, ie, 201 $kJ \cdot kg^{-1} \cdot d^{-1}$. The nasogastric feeding and intravenous glucose infusion were maintained continuously throughout the study. Thus, the background ^{13}C enrichment was constant during the tracer infusion. At the final rate, a total of 8.9 mg carbohydrate $\cdot kg^{-1} \cdot min^{-1}$ was provided; 6.9 $mg \cdot kg^{-1} \cdot min^{-1}$ was delivered through the nasogastric tube and 2 $mg \cdot kg^{-1} \cdot min^{-1}$ via the intravenous infusion. The total amount of energy provided was ≈250 $kJ \cdot kg^{-1} \cdot d^{-1}$ with ≈88% from carbohydrate (215 $kJ \cdot kg^{-1} \cdot d^{-1}$), 10% from amino acids (31 $kJ \cdot kg^{-1} \cdot d^{-1}$), and 1–2% from fat (4 $kJ \cdot kg^{-1} \cdot d^{-1}$). In addition, the subjects had free access to carbohydrate-containing liquids without caffeine. This fluid intake was not recorded because it did not exceed 1–4% of the daily energy intake. To calculate energy intake the carbohydrate and amino acids were assumed to have an energy value of 16.75 kJ/g, and fat an energy value of 37.66 kJ/g.

Tracer infusion

Tetrahydrofluoranamide catheters were placed percutaneously into an antecubital vein, and a sampling catheter was inserted in a dorsal hand vein of the contralateral arm. The heated-hand technique was used to obtain arterialized blood samples (22). After a blood sample was drawn to determine background enrichment, a constant infusion of [1,2-^{13}C]acetate (99% enriched; Isotec Inc, Miamisburg, OH; 2 $\mu mol \cdot kg^{-1} \cdot min^{-1}$, no prime) was started at 0800.

Blood sampling

Blood to measure VLDL-TGFA kinetics was taken 0, 180, 240, 300, 360, 420, 480, 540, and 600 min after the beginning of the infusion study and placed into 10-mL evacuated containers with disodium EDTA. All samples were placed on ice. Plasma was separated by centrifugation (for 10 min at 4 °C and 1000 × *g* average) shortly after sampling. Samples for VLDL isolation were stored at 4 °C until they were processed the next morning. All other samples were stored at −20 °C until they were processed.

Sample analysis

VLDL was isolated from 3 mL plasma by overlaying the plasma with a solution with density 1.006 kg/L (0.9% NaCl) and spinning it in a 70.1 Ti rotor (Beckman Instruments, Palo Alto, CA) at 50 000 rpm (171 500 × *g* average) for 20 h at 15 °C in an ultracentrifuge (model L7–55; Beckman Ultracentrifuge, Beckman Instruments). After ultracentrifugation, the VLDL triacylglycerol was carefully removed along with the density-gradient solution found on top of the tube by slicing the tube. The total volume of the resulting VLDL suspension was 3 mL.

The triacylglycerol concentration in the VLDL suspension and in plasma was determined enzymatically (RA-500; Technicon Instruments Corp, Tarrytown, NY) (23). The total concentration of fatty acids in plasma was measured by using an enzymatic colorimetric assay (Wako Chemicals USA, Richmond, VA). Relative concentrations of individual fatty acids in plasma were determined by gas chromatography (model 5890;

Hewlett-Packard Co, Palo Alto, CA) after isolation by thin-layer chromatography and subsequent derivatization to fatty acid methyl esters.

Triacylglycerols in the VLDL suspension were isolated by thin-layer chromatography, hydrolyzed to fatty acids, and derivatized to fatty acid methyl esters. Relative concentrations of individual fatty acids in the VLDL fraction were determined by gas chromatography (model 5890; Hewlett-Packard Co, Palo Alto, CA). Isotopic enrichment of selected fatty acids was determined by gas chromatography–mass spectrometry (GCMS) (model 5992; Hewlett-Packard Co, Palo Alto, CA,) in the electron impact ionization mode for the ultimate computation of the tracer-tracee ratio. For the palmitate methyl ester the ions with a mass-to-charge ratio (*m/e*) of 270, 271, 272, 273, and 274 were selectively monitored; for the methyl ester of oleate the ions with a *m/e* of 296, 297, 298, 299, and 300 were monitored; and for the methyl ester of stearate the ions with a *m/e* of 298, 299, 300, 301, and 302 were monitored. For the methyl ester of linoleic acid ions with *m/e* 294, 295, and 296 were monitored.

Calculations

Indirect calorimetry

Total energy expenditure (TEE) and fat and carbohydrate oxidation rates were calculated by using stoichiometric equations (24, 25). TEE was calculated by using the following formula:

$$\text{TEE}(\text{J}\cdot\text{kg}\cdot\text{min}^{-1}) = [(3.9 \times \dot{V}\text{CO}_2/\text{RER}) + (1.11 \times \dot{V}\text{CO}_2) - 2.17n] \times 4.184 \quad (1)$$

and fat oxidation, f, by using the following formula:

$$f(\text{g/min}) = 1.67\dot{V}\text{O}_2 - 1.67\dot{V}\text{CO}_2 - 1.92N \quad (2)$$

where $\dot{V}\text{O}_2$ and $\dot{V}\text{CO}_2$ are total oxygen consumption and carbon dioxide production (L/min), respectively, and respiratory exchange ratio (RER) is the ratio of $\dot{V}\text{CO}_2$ to $\dot{V}\text{O}_2$. When the RER was < 1, carbohydrate oxidation rate (C) was calculated by using the following formula:

$$c(\text{g/min}) = 4.55\dot{V}\text{O}_2 - 3.21\dot{V}\text{CO}_2 - 2.87N \quad (3)$$

and when the RER was > 1, the following formula was used (25):

$$c(\text{g/min}) = 1.34(\dot{V}\text{CO}_2 - 4.88N) \quad (4)$$

Urinary nitrogen excretion, N, was assumed to be 190 $\text{mg}\cdot\text{kg}^{-1}\cdot\text{d}^{-1}$. This value equals the nitrogen intake from the liquid meal. We showed previously that resting subjects provided with a protein intake within this range were in protein balance, ie, nitrogen intake equals nitrogen excretion (26). A 30% error in this assumed value [which exceeds the total range of values in a previous study (26)] would have had no effect on the calculated values of fat synthesis during this study.

It has been shown that when indirect calorimetry is used during net fat synthesis, as indicated by a nonprotein RER > 1, the apparent rate of fat oxidation, f, is the difference between the rate of fat oxidation (f_o) and fat synthesis (f_s) ($f = f_o - f_s$) (24, 25, 27). With nonprotein RER > 1 the equation for fat oxidation yields a negative number. Thus, calculated rates of negative fat oxidation quantitatively equal the net rate of fat synthesis.

Measurement of the rate at which palmitate synthesized de novo is secreted as VLDL triacylglycerol

The mass isotopomer method is similar to the traditional approach of calculating the fractional synthetic rate (FSR) by using the relation of the precursor and product enrichments, except that with the mass isotopomer approach, the precursor enrichment is deduced from the product enrichment rather than measured directly or estimated. It is this novelty that allows the de novo synthesis of palmitate to be quantified because it is not feasible to directly measure or even estimate the in vivo precursor enrichment (hepatic acetyl-CoA).

To estimate the in vivo precursor enrichment for de novo fatty acid synthesis (hepatic acetyl-CoA), we used essentially the same method as that described by Hellerstein et al (28, 29). Briefly, we infused [1,2-^{13}C]acetate for 6 h and measured the $M+0$, $M+2$, and $M+4$ enrichments of VLDL-bound palmitate over time. We chose to use doubly labeled acetate as opposed to [1-^{13}C]acetate, as used by Hellerstein et al, because the precision of analysis was greatly improved. This is because singly labeled palmitate contributes significantly to the $M+2$ peak, thereby requiring correction of the apparent enrichment at $M+2$. The $M+2$ peak, on the other hand, makes minimal contribution to the $M+4$ peak. Also, the background abundances of the $M+2$ and $M+4$ isotopomers are low.

The general principle behind the calculation of the precursor enrichment relies on the fact that eight acetyl-CoA molecules are necessary to form one palmitate molecule. Thus, if labeled acetate is infused, palmitate will be produced that has one, two, three, or more labeled acetate molecules. The relative abundance of palmitate labeled with one, as opposed to two, labeled acetate molecules will depend on the precursor enrichment, so that one can work backwards from the observed palmitate enrichment and deduce the precursor enrichment from the relative abundance of palmitate labeled with one or two labeled acetate molecules. For example, if most of the palmitate molecules in a sample contain four labeled acetate molecules, one would conclude that 50% of the acetate is labeled. The exact formula we used to determine precursor enrichment [p] is as follows:

$$p = [2 \times \text{TTR}(M+4)/\text{TTR}(M+2)]/[7 + 2 \times \text{TTR}(M+4)/\text{TTR}(M+2)] \quad (5)$$

where TTR($M+4$) and TTR($M+2$) are the tracer-tracee ratios of VLDL-bound palmitate that contain one or two labeled acetate molecules, respectively. We derived this equation in reference 30.

To calculate the FSR of VLDL-bound palmitate (FSR), we used the following formula:

$$\text{FSR} = [(E_{t2} - E_{t1})/(t_2 - t_1)]/[8p(1-p)^7] \quad (6)$$

where t_1 and t_2 are the times when samples are taken and E_t is the singly labeled enrichment at time t.

The differences between this formula and the traditional formula (31) for calculation of FSR are the factor of 8 in the denominator and the factor $(1 - p)^7$. The factor of 8 accounts

for the fact that it requires eight acetate molecules to form one palmitate molecule. The factor $(1 - p)^7$ accounts for the probability that seven acetate molecules that are not labeled will be incorporated into a palmitate molecule (30).

To obtain absolute synthesis rates, the FSR was multiplied by the pool size of VLDL-bound palmitate. The pool size was estimated by multiplying the measured VLDL-triacylglycerol concentration by the relative concentration of palmitate in the VLDL-triacylglycerol fraction times the assumed plasma volume of 37 mL/kg (32).

Measurement of total VLDL-bound fatty acid kinetics

Quantitatively, palmitate (16:0), oleate (18:1), stearate (18:0), linoleate (18:2), and palmitoleate (16:1) make up > 97% of the fatty acids of VLDL-bound triacylglycerols. To measure the total VLDL-bound triacylglycerol secretion rate, the secretion rates of VLDL-bound stearate, oleate, and linoleate were measured principally as described for VLDL-bound palmitate 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 syntheses. Furthermore, during the study, the concentration of the two fatty acids relative to each other remained constant in the fatty acid pool and the VLDL-triacylglycerol pool. This suggests that the relative flux of the two fatty acids was the same throughout the study.

To differentiate between the secretion rate of de novo synthesized, elongated, or reesterified 18-carbon fatty acids, certain assumptions were made. It is known that palmitate is the primary product of de novo fatty acid synthesis in the liver and that the 18-carbon fatty acids such as stearate and oleate are the products of elongation and desaturation of palmitate (33, 34). As such, VLDL-bound stearate and oleate can come principally from three sources: *1*) palmitate synthesized de novo 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; *2*) 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; and *3*) from uptake from plasma and subsequent reesterification into VLDL triacylglycerol. In this study the term "synthesized de novo" is preserved for the first alternative, in which all the acetate units of the fatty acid are derived from the hepatic pool. Alternatives 2 and 3 are collectively termed reesterification. As such, reesterification encompasses both the process by which palmitate is taken up from plasma and elongated into an 18-carbon fatty acid for subsequent secretion and the process by which stearate and oleate themselves are taken up from plasma and reesterified into VLDL triacylglycerol. Labeling of stearate and oleate with one labeled acetate molecule can be the consequence of elongation of any palmitate, whether or not it was synthesized de novo. Eighteen-carbon fatty acids containing multiple labeled acetate molecules can only be the elongated product of palmitate synthesized de novo. To obtain more than one labeled acetate molecule, the 18-carbon fatty acids must have had labeled palmitate as the immediate precursor. [Palmitate with a minimum of one labeled acetate unit must be synthesized de novo because elongation of shorter-chain fatty acids is not a major pathway (33).] For this reason, we calculated the FSR for the 18-carbon fatty acids using their doubly labeled isotopomers (two labeled acetate molecules) rather than singly labeled stearate and oleate, with the following formula:

$$\text{FSR} = [(E_{t2} - E_{t1})/(t_2 - t_1)]/[36p^2(1 - p)^7] \qquad (7)$$

The denominator $36p^2\ (1 - \text{p})^7$ represents the probability that two labeled acetate molecules will be incorporated into stearate or oleate ($M+4$ fatty acids), given that the enrichment of the acetyl-CoA pool is p. We used the value of p obtained from VLDL palmitate because palmitate, stearate, and oleate are all derived from the same acetyl-CoA pool. For one subject on day 1 the p value was obtained from VLDL stearate, not from VLDL palmitate. For no known reason, the palmitate had no detectable enrichment at this time point. 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 percentage linoleate abundance to percentage palmitate abundance in VLDL as determined by gas chromatography.

Statistical analysis

All results are expressed as means ± SEMs, except for the individually tabulated results. Comparisons of groups were performed by repeated-measures analysis of of variance (ANOVA). When ANOVA indicated statistical significance, Fisher's least-significant-difference test was used to compare differences between groups (35). Significance was accepted at $P \leq 0.05$.

RESULTS

The study was completed by all subjects. The intravenous infusion and nasogastric feeding (combined enteral and parenteral feeding) were maintained uninterrupted during the 4 d of carbohydrate overfeeding except for an interruption of ≈15 min every morning to change infusion lines.

The ratio between erythrocyte volume and plasma volume, ie, hematocrit, was unchanged during the study. This was expected because the amount of blood withdrawn during each infusion study was < 2% of estimated total blood volume and this amount of blood loss should not have affected erythrocyte volume. Thus, plasma volume during the same period was assumed to be unchanged.

Body weight, plasma glucose, and plasma lipid concentrations

The average body weight tended to increase throughout the study, from 73 ± 3 kg in the basal state to 74 ± 4 and 75 ± 4 kg after 1 and 4 d of overfeeding, respectively; this increase was not significant. Plasma glucose concentration was 6.2 ± 0.4 mmol/L in the basal state and 7.1 ± 0.3 and 6.9 ± 0.5 mmol/L on days 1 and 4 of the carbohydrate feeding, respectively, but these increases were not significant. The plasma triacylglycerol concentration increased significantly during the study from an initial value of 0.92 ± 0.22 mmol/L in the basal state to 1.21 ± 0.33 on day 1 and 4.34 ± 1.61 mmol/L on day 4.

Oxygen consumption, carbon dioxide production, respiratory exchange ratio, and resting energy expenditure

$\dot{V}O_2$, $\dot{V}CO_2$, and RER during the study are shown in **Table 1**. RER was 0.81 ± 0.01 after an overnight fast with ongoing glucose infusion (baseline state). After 1 d of carbohydrate feeding the average value was 0.99 ± 0.024 and, finally, an average value of 1.15 ± 0.022 was reached after 4 d of overfeeding. As the carbohydrate feeding proceeded, the resting energy expenditure of the subjects increased from a mean value of 62.3 ± 3.7 $J \cdot kg^{-1} \cdot min^{-1}$ (day 0) to a mean of 97.9 ± 3.7 $J \cdot kg^{-1} \cdot min^{-1}$ on day 4 (Table 1). All respiratory gas measurements were made during ongoing feeding, either during intravenous infusion alone in the basal state or the combined enteral and parenteral feedings (days 1 and 4).

Substrate oxidation rates

According to the respiratory gas measurements, the oxidation rate of fat was 955 ± 139 $mg \cdot kg^{-1} \cdot d^{-1}$ in the basal state (**Table 2**). During the high carbohydrate intake, no fat oxidation was measured by indirect calorimetry. The carbohydrate oxidation rate was 2127 ± 154 $mg \cdot kg^{-1} \cdot d^{-1}$ in the basal state, and after 1 and 4 d of high carbohydrate intake the oxidation rate was 6934 ± 981 and 10 266 ± 863 $mg \cdot kg^{-1} \cdot d^{-1}$, respectively.

Net fat synthesis at the whole-body level and hepatic lipogenesis

Rate of synthesis of fat at the whole-body level was calculated based on respiratory gas measurements (**Table 3**). All subjects except one had net synthesis of fat at the whole-body level after only 1 d of hyperenergetic carbohydrate feeding (day 1) (individual result not shown). The average value for the whole group was 481 ± 205 $mg \cdot kg^{-1} \cdot d^{-1}$ after 1 d of carbohydrate feeding and 2243 ± 253 $mg \cdot kg^{-1} \cdot d^{-1}$ after 4 d of carbohydrate feeding.

The secretion rate of VLDL TGFA synthesized de novo in the basal state was 1.0 ± 0.3 $mg \cdot kg^{-1} \cdot d^{-1}$. After 1 d of carbohydrate overfeeding the value was 13.8 ± 6.8 $mg \cdot kg^{-1} \cdot d^{-1}$ (day 1) and after 4 d the value was 43.3 ± 16.3 $mg \cdot kg^{-1} \cdot d^{-1}$ (Table 3).

DISCUSSION

Accumulation of body fat mass is due principally to storage of alimentary-derived fat or fat produced endogenously. The relative importance of excess carbohydrate in the development of obesity has been ascribed to the fat-sparing effect of carbohydrates (1–3, 36) rather than to the lipogenic source, which has been presumed to be limited (19, 37). On the other hand, carbohydrate overfeeding in either clinical nutrition (7–9) or in experimental settings (6, 10–12) is known to cause net de novo lipid biosynthesis at the whole-body level as measured by indirect calorimetry, suggesting that the human body can adapt and increase the de novo pathway substantially. The capacity for humans to convert carbohydrate to fat is still controversial, as is the extent to which it occurs in the liver, as opposed to the adipose tissue. Although the acetyl-CoA synthetase and fatty acid synthetase complexes (the enzyme system for de novo synthesis of fat) are found both in the liver and adipose tissue, the liver has been assumed to be the principal site for endogenous fat production in humans eating a balanced diet (1, 18). Hepatic de novo lipid biosynthesis has not been measured during overfeeding in humans, but the lipogenic capacity is assumed to be low compared with the rate based on indirect estimates (14).

Indirect calorimetry indicated that our subjects were accumulating fat after only 1 d of hyperenergetic carbohydrate feeding and lipogenesis increased with time after that. After 4 d of high-carbohydrate feeding, net fat synthesis at the whole-body level was ≈2250 $mg \cdot kg^{-1} \cdot d^{-1}$ (ie, ≈170 g/d). Thus, adaptation to a hyperenergetic carbohydrate diet involved a substantial increase in de novo lipid biosynthetic activity. However, at this time the liver produced ≈40 mg $fat \cdot kg^{-1} \cdot d^{-1}$ (ie, ≈3 g/d). Although this value was ≈50-fold greater than the basal rate, hepatic de novo synthesis of fat only accounted for 2% of whole-body fat synthesis after 4 d of hyperenergetic carbohydrate feeding. Most likely, the adipose tissue had adapted to the high carbohydrate load by synthesizing 167 g fat/d.

TABLE 1
Respiratory gas measurements[1]

	Basal state	Day 1	Day 4
Oxygen consumption ($mL \cdot kg^{-1} \cdot min^{-1}$)	3.19 ± 0.19	3.65 ± 0.32	4.61 ± 0.18[2,3]
Carbon dioxide production ($mL \cdot kg^{-1} \cdot min^{-1}$)	2.58 ± 0.14	3.63 ± 0.36[2]	5.32 ± 0.23[2,3]
Respiratory exchange ratio	0.81 ± 0.01	0.99 ± 0.02[2]	1.15 ± 0.02[2,3]
Resting energy expenditure ($J \cdot kg^{-1} \cdot min^{-1}$)	62.3 ± 3.7	74.9 ± 6.7	97.9 ± 3.7[2,3]

[1] $\bar{x}$ ± SEM; n = 5.
[2] Significantly different from basal state, $P \leq 0.05$.
[3] Significantly different from day 1, $P \leq 0.05$.

TABLE 2
Substrate oxidation rate[1]

Substrate	Basal state	Day 1	Day 4
		$mg \cdot kg^{-1} \cdot d^{-1}$	
Fat	955 ± 139	0[2]	0[2]
Carbohydrate	2127 ± 154	7177 ± 708[2]	10 266 ± 450[2,3]

[1] $\bar{x}$ ± SEM; n = 5. All respiratory gas measurements were made during ongoing feeding.
[2] Significantly different from basal state, $P \leq 0.05$.
[3] Significantly different from day 1, $P \leq 0.05$.

TABLE 3
Lipogenesis[1]

	Basal state	Day 1	Day 4
		$mg \cdot kg^{-1} \cdot d^{-1}$	
Hepatic lipogenesis	1.0 ± 0.3	13.8 ± 6.8[2]	43.3 ± 16.3[2,3]
Whole-Body net lipogenesis	0	481 ± 205[2]	2243 ± 253[2,3]

[1] $\bar{x}$ ± SEM; n = 5.
[2] Significantly different from basal state, $P \leq 0.05$.
[3] Significantly different from day 1, $P \leq 0.05$.

Indirect calorimetry and whole-body fat synthesis

The validity and the underlying assumptions of indirect calorimetry in measuring net fat synthesis have been reviewed extensively (24, 25, 27). We used the approach as currently reviewed and obtained values within the range of what others have confirmed previously in comparable experimental settings (10) and during clinical nutrition (6, 8). Note that in the current experiment, indirect calorimetry measured net synthesis of fat at the whole-body level and did not detect simultaneous fat synthesis and oxidation. Thus, in the current experiment fat synthesis measured by indirect calorimetry represented a minimal estimate of whole-body fat synthesis.

The rapid rate of conversion of glucose to fat, as calculated from respiratory gas measurements in our experiment, is supported by the substrate balances calculated from substrates entering the body and substrate expenditure as calculated from the respiratory gas measurements (**Figure 1**). During overfeeding, 10% and 1–2% of the provided energy substrates were amino acids and fat, respectively. For simplicity, the total substrate intake has been considered to be carbohydrate. The rather small difference in energy yield and theoretical lipogenic potential between carbohydrates and amino acids justifies this simplification (25). In the basal state total energy intake was 48 $kJ \cdot kg^{-1} \cdot d^{-1}$ ($\approx 3\ g \cdot kg^{-1} \cdot d^{-1}$), and during early and late carbohydrate overfeeding the total energy provision was 250 $kJ \cdot kg^{-1} \cdot d^{-1}$ (≈ 15 g carbohydrate $\cdot kg^{-1} \cdot d^{-1}$). Carbohydrates fed at rates comparable with those in this study were shown previously to be absorbed almost completely (10) and none of our subjects had any signs of acute malabsorption. The plasma concentration of glucose in the healthy subjects never exceeded the renal threshold, thus, there should have been no significant loss of glucose through the urine. We can then assume that the surplus of carbohydrate that was provided in excess of energy needs would either be stored quantitatively as glycogen or fat in the body. During early carbohydrate overfeeding (day 1) approximately one-half of the carbohydrate entering the body was enough to cover the measured energy needs ($\approx 6.5\ g \cdot kg^{-1} \cdot d^{-1}$). Assuming that 1 g glucose yields 0.35 g fat (25), ≈ 1.5 g carbohydrate $\cdot kg^{-1} \cdot d^{-1}$ is accounted for by net whole-body lipogenesis as measured by indirect calorimetry. The fate of the rest of the carbohydrate load on day 1 was probably mainly glycogen storage (≈ 7.0 g glucose $\cdot kg^{-1} \cdot d^{-1}$, ≈ 500 g/d). At such a high rate of glycogen deposition, nondepleted glycogen stores would be saturated within 1–2 d because they accommodate ≈ 1000 g maximally (10, 38). The daily respiratory gas measurements showed that RER reached a plateau after 2–3 d of overfeeding (data not shown), indicating that a new steady state with respect to carbohydrate oxidation and lipogenesis had been reached. By the forth day of overfeeding when the glycogen stores were saturated, excess glucose was probably all converted to fat. Again, assuming that 1 g glucose yields 0.35 g fat (25), the excess glucose intake on the fourth day of overfeeding (6.4 g glucose $\cdot kg^{-1} \cdot d^{-1}$) would be converted to ≈ 2.24 g fat $\cdot kg^{-1} \cdot d^{-1}$. The corresponding calculated value from the respiratory gas measurements was 2.24 g fat $\cdot kg^{-1} \cdot d^{-1}$.

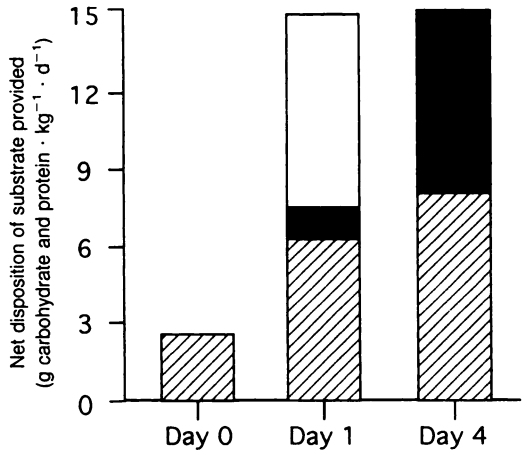

FIGURE 1. Utilization of energy substrates provided. The rate of provision of nutrients in the basal state and during early (day 1) and late (day 4) carbohydrate hyperalimentation (solid bar). The average utilization of substrates for oxidation (lhbox) and fat synthesis ■ was calculated based on indirect calorimetry (*see* Methods). The average rate of glycogen deposition □ was estimated as total rate of substrate provided − (rate of substrate oxidation + substrate utilization for fat synthesis). The carbohydrate and amino acids were assigned an energy value of 16.75 kJ/g. During lipogenesis, 1 g carbohydrate or protein is assumed to yield ≈0.35 g fat (25). The fat provided in the liquid meal (linolenic acid) accounted for 1–2% of the total energy substrate provided. For simplicity, this minor component of the diet was not included in the calculations.

The considerations made above point to the importance of the glycogen stores in buffering normal fluctuations in carbohydrate intake that potentially could lead to stimulation of fat synthesis and subsequent obesity. Usually, large occasional carbohydrate loads are handled primarily by conversion into glycogen. To induce substantial rates of carbohydrate conversion into fat, our results indicate that the glycogen stores must first be enlarged. Even though the daily carbohydrate intake was constant during the overfeeding period in our experiment, there was a 4.6-fold increase in whole-body net lipogenesis between early (day 1) and late (day 4) carbohydrate feeding. Thus, the observed weight gain during the overfeeding period was caused both by glycogen (and water) deposition and fat synthesis.

Hepatic lipogenesis

To our knowledge, this is the first time that the hepatic secretion of fatty acids synthesized de novo has been quantified in human subjects during a prolonged period of net fat synthesis at the whole-body level. We calculated total hepatic secretion of newly synthesized fatty acids by summing the secretion rates of the individual fatty acids, which by mass accounted for $> 97\%$ of the VLDL TGFAs. It is beyond the scope of this report to discuss the kinetics of the individual fatty acids.

The starting point is the estimation of the in vivo precursor enrichment (hepatic acetyl-CoA) for de novo fatty acid synthesis according to the mass isotopomer distribution analysis (28, 29). We discussed recently the details of the calculations and assumptions involved in this approach (30). The conceptual breakthrough of this methodology is that the true precursor enrichment is determined by analysis of the product, thereby eliminating any assumptions regarding the unknown extent of intracellular dilution of the precursor enrichment as measured in plasma. To calculate the absolute rate of secretion we extended the approach used by Hellerstein et al (28, 29) and calculated the absolute secretion rate of fatty acids synthesized de novo by combining the fractional secretion of the individual

VLDL fatty acids with their respective VLDL TGFA pool sizes. Further, because we measured the isotopic enrichment in the individual fatty acids, it was not necessary to make assumptions regarding the extrapolation of the kinetics of one fatty acid (eg, palmitate) to all the fatty acids.

The FSR of the individual fatty acids was calculated based on the precursor enrichment that was determined from the isotopomer distribution of palmitate. 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 precursor enrichment. For the other fatty acids, particularly in the basal state, the isotope enrichment that would frequently allow us to determine the precursor enrichment independently could not be measured. Whenever possible, comparisons were made between precursor enrichments deducted from different fatty acids. A variation in isotope enrichment of the acetyl-CoA pool no more than 10% was found, irrespective of the feeding regimen. This strongly suggests that all the newly synthesized fatty acids in the liver were derived from one precursor pool.

The hepatic lipogenic activity, as quantified by the isotope method, measures the amount of fat synthesized de novo that is secreted from the liver. This would not include simultaneous synthesis and oxidation of fatty acid within the liver, but these processes are not likely to occur at a level of quantitative significance at the same time. In the basal state, de novo synthesis of fatty acids is closely linked to the secretion of VLDL triacylglycerol (39). Faced with a surplus of carbohydrate energy, one would believe this to be even more the case as the dependence on fatty acid–derived energy diminishes.

It is difficult to know whether all fatty acids newly synthesized in the liver were secreted during the time frame of our experiment. During a steady state, one would not expect any net fat accumulation in the liver. At some point during the adaptation to the hyperenergetic carbohydrate diet our subjects were not in a steady state, as indicated by the expansion of the VLDL TGFA pool from day 1 to day 4. However, the time course of this increase was slow in relation to the time frame of the individual infusion studies. During each infusion protocol the VLDL TGFA pool size was relatively constant, suggesting that the hepatic secretion rate also was relatively constant. The time lag between fatty acid synthesis and secretion of VLDL TGFA is in the range of 30 min (40). This delay does not affect our calculation of secretion rate, because we are relying on a steady state situation. Thus, whereas there is a time lag between production and secretion, the secretion rate at any particular time should directly reflect the production rate 30 min earlier.

Hepatic lipogenesis could be underestimated by our method if significant amounts of newly synthesized fatty acids entered the circulation in forms other than VLDL TGFA. Consequently, we also determined the FSR of newly synthesized palmitate in VLDL phospholipids and cholesteryl esters. Secretion of newly synthesized palmitate in VLDL phospholipids or cholesterol esters accounted for only 2–3% of the total secretion of palmitate synthesized de novo bound to VLDL. There are indications that a minor proportion of the high-density lipoprotein (HDL) in circulation is secreted directly from the liver (41). The HDL particle is low in triacylglycerol content but rich in phospholipids. Thus, we believe that HDL secretion from the liver could only be a minor secretory pathway of newly synthesized fatty acids. There is no evidence of hepatic secretion of fatty acids. Consequently, we can be confident that almost all newly synthesized hepatic fatty acids are secreted in VLDL triacylglycerol.

Hepatic compared with whole-body lipogenesis

Previous studies have compared lipogenic activity in adipose and liver tissue samples from humans (13–15). Quantitative comparisons cannot be made between the individual studies because they have used different in vitro assay systems. This is not surprising considering the fact that fat synthesis is a complex process in which several enzymatic steps are integrated and different subcellular compartments can provide substrates needed for the process. Furthermore, the process is under endocrine control, insulin being the strongest promoting hormone and glucagon and epinephrine the principal inhibiting hormones. Thus, depending on the in vitro approach that has been used, either the liver (14) or the adipose tissue (13) has been assigned the most quantitatively active lipogenic role. It should be emphasized that none of these studies were designed to examine the potential effect of either short or prolonged excess substrate provision, ie, excess carbohydrate intake, which may have a tissue-specific inductive effect on lipogenesis. To our knowledge, the only study that has examined the potential tissue-specific effect of hyperenergetic feeding did not show any effect either on lipogenesis in liver or adipose tissue samples (15).

The effect of prolonged excess carbohydrate intake has been studied most extensively in adipose tissue samples studied in vitro. Sjöström (18) measured lipogenic activity in adipose tissue slices obtained from obese healthy volunteers fed a high-carbohydrate diet for 5 d (18). From in vitro data they estimated that in obese subjects 2.7 g palmitic acid was synthesized by the total adipose tissue mass per 24 h. Their conclusion was that "synthesis of fatty acids de novo by adipose tissue is of little quantitative importance for fatty acid storage or consumption of glucose carbon in human adipose tissue" (17). No measurement of whole-body lipogenesis was made in this study. In a later study by Chascione et al (7), de novo lipid biosynthesis was measured in biopsies of adipose tissue from undernourished patients before and after feeding a hyperenergetic high-carbohydrate diet for 6–10 d. They found a much higher rate of lipogenesis during high carbohydrate intake and concluded by extrapolation that adipose tissue could account for $\leq$ 40% of whole-body lipogenesis as measured by indirect calorimetry. Both studies found a marked inductive effect of hyperenergetic high-carbohydrate feeding on lipogenesis in adipose tissue. Increased lipogenesis of 8–79-fold was found depending on the assay system used. Although the two studies assigned the adipose tissue a different lipogenic potential, they have been cited in subsequent studies in which high rates of whole-body net fat synthesis were assumed to occur in the liver (10).

Surplus fat generated in the liver could be retained temporarily in the liver, but eventually it would be transported in the form of VLDL triacylglycerol to the adipose tissue for storage. Assuming that 50% of the net whole-body lipogenesis during carbohydrate overfeeding as measured in this study was to be transferred from the liver to the adipose tissue this would require a transfer of $\approx$1000 mg VLDL triacylglycerol $\cdot$ $kg^{-1} \cdot d^{-1}$ through plasma on

the fourth day. The hepatic production rate of VLDL triacylglycerol has been found to be in the range of 100–350 $mg \cdot kg^{-1} \cdot d^{-1}$ (41–43). Thus, even if the total VLDL triacylglycerol output was derived from fatty acids synthesized de novo, hepatic triacylglycerol secretion and subsequent transport through plasma, as currently understood, would not account for more than ≈10–15% of whole-body fat synthesis. The discrepancy becomes even more obvious when taking into consideration the fact that fatty acids synthesized de novo only make up a fraction of the VLDL TGFAs (19). During the fourth day of carbohydrate feeding, we found that the VLDL-triacylglycerol production rate was ≈320 $mg \cdot kg^{-1} \cdot d^{-1}$ and that newly synthesized fatty acids did not account for more than ≈15% of the VLDL triacylglycerol secreted. Thus, as the transfer process of surplus fat from the liver to adipose tissue is currently understood, it can only accommodate a fraction of the amount of fat that is synthesized according to the respiratory gas measurements in this and previous studies (10).

On the basis of both substrate balance data and respiratory gas measurements, we found that after 4 d of carbohydrate overfeeding our subjects were synthesizing a minimum of ≈170 g fat/d. Even though hepatic lipogenic activity at this time was increased ≈35-fold, it only accounted for 2% of whole-body fat synthesis (≈3 g fat/d). We found about the same proportion between whole-body and hepatic fat synthesis during early (day 1) and late (day 4) carbohydrate overfeeding. Even if hepatic lipogenesis was underestimated by 100% (ie, the liver was synthesizing ≈200 $mg \cdot kg^{-1} \cdot d^{-1}$) and we had overestimated net whole-body lipogenesis by 100% (ie, the whole-body synthesis was ≈1000 $mg \cdot kg^{-1} \cdot d^{-1}$), the conclusion would be the same, ie, the hepatic contribution to whole-body carbohydrate–induced lipogenesis is minor. Other tissues, most likely adipose tissue, play the major role quantitatively when surplus carbohydrate energy is converted into fat in the human body.

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