Hepatic de novo lipogenesis in normoinsulinemic and hyperinsulinemic subjects consuming high-fat, low-carbohydrate and low-fat, high-carbohydrate isoenergetic diets[1–3]

Jean-Marc Schwarz, Peter Linfoot, Doris Dare, and Karmen Aghajanian

ABSTRACT

Background: Hypertriglyceridemia is associated with increased risk of cardiovascular disease. Until recently, the importance of hepatic de novo lipogenesis (DNL) in contributing to hypertriglyceridemia was difficult to assess because of methodologic limitations.

Objective: We evaluated the extent of the contribution by DNL to different conditions associated with hypertriglyceridemia.

Design: After 5 d of an isoenergetic high-fat, low-carbohydrate diet, fasting DNL was measured in normoinsulinemic (≤ 85 pmol/L) lean ($n = 9$) and obese ($n = 6$) and hyperinsulinemic (≥ 115 pmol/L) obese ($n = 8$) subjects. Fasting DNL was measured after a low-fat, high-carbohydrate diet in normoinsulinemic lean ($n = 5$) and hyperinsulinemic obese ($n = 5$) subjects. Mass isotopomer distribution analysis was used to measure the fraction of newly synthesized fatty acids in VLDL-triacylglycerol.

Results: With the high-fat, low-carbohydrate diet, hyperinsulinemic obese subjects had a 3.7–5.3-fold higher fractional DNL ($8.5 \pm 0.7\%$) than did normoinsulinemic lean ($1.6 \pm 0.5\%$) or obese ($2.3 \pm 0.3\%$) subjects. With the low-fat, high-carbohydrate diet, normoinsulinemic lean and hyperinsulinemic obese subjects had similarly high fractional DNL ($13 \pm 5.1\%$ and $12.8 \pm 1.4\%$, respectively). Compared with baseline, consumption of the high-fat, low-carbohydrate diet did not affect triacylglycerol concentrations. However, after the low-fat, high-carbohydrate diet, triacylglycerols increased significantly and DNL was 5–6-fold higher than in normoinsulinemic subjects consuming a high-fat diet. The increase in triacylglycerol after the low-fat, high-carbohydrate diet was correlated with fractional DNL ($P < 0.01$), indicating that subjects with high DNL had the greatest increase in triacylglycerols.

Conclusions: These results support the concept that both hyperinsulinemia and a low-fat diet increase DNL, and that DNL contributes to hypertriglyceridemia. *Am J Clin Nutr* 2003;77:43–50.

KEY WORDS Obesity, hyperinsulinemia, hepatic de novo lipogenesis, triacylglycerols, triglycerides, hypertriglyceridemia, hyperlipidemia, cardiovascular disease, heart disease

INTRODUCTION

Humans have a limited capacity to store energy as carbohydrate, and when carbohydrate intake exceeds storage and oxidation capacities, extra carbohydrate energy is converted to fat by de novo lipogenesis (DNL). In the liver, DNL can play a crucial role in glucose homeostasis, even at amounts that may not be important for whole-body fat balance. Indeed, by converting excess carbohydrate and excess gluconeogenic substrates to triacylglycerol, DNL in the liver may be an important pathway for controlling glucose production and blood glucose concentration. Yet, although this process may maintain glucose homeostasis and prevent diabetes, it may also have the detrimental effect of increasing triacylglycerol concentrations, which in turn may increase the risk of cardiovascular diseases (1, 2).

The activity of lipogenic enzymes seems to be very limited in humans on the basis of in vitro measurements (3), but the significance of hepatic DNL in vivo was, until recently, difficult to assess because of methodologic limitations (4). The traditional in vivo method, indirect calorimetry, can only quantify net DNL, which is the difference between overall body fat oxidation and overall DNL. With this method, if an organ such as the liver is converting carbohydrate into fat at a slower rate than the simultaneous overall fat oxidation taking place in all other tissues of the body, there is no net DNL (4). Because of these limitations, the importance of DNL in humans has been overlooked until recently.

Since the early 1990s, we and other investigators have measured DNL by using 2 novel approaches: *1*) a stable-isotope tracer technique, mass isotopomer distribution analysis (5, 6), and *2*) a technique developed on the basis of the endogenous dilution of linoleate, an essential fatty acid that cannot be synthesized de novo (7, 8). Using these new approaches, investigators have established that DNL varies considerably depending on the dietary conditions and health status of the subjects.

Only limited amounts of carbohydrate are available for DNL with the typical Western diet, because of its high fat content (35–40% of energy as fat). When this type of diet was fed to healthy subjects, hepatic DNL was absent or very low (5, 7, 9). However, when healthy subjects were overfed with carbohydrate, both hepatic DNL and glucose production were increased and the increases were proportional to the excess intake of carbohydrate (9). The research on these overfeeding conditions shows the close interaction between glucose production and hepatic DNL. Another dietary condition that leads to increased hepatic DNL, even when subjects are in energy balance, is when healthy subjects are fed a diet that is very low in fat (10% of energy) and very high in carbohydrate (75% of energy) (7, 10). The carbohydrate-induced rise in hepatic DNL was found to be more pronounced when more than half of the carbohydrate was consumed as simple sugars (8). These studies showed the critical role of carbohydrate quality, with simple sugars being more effective than were complex carbohydrates in stimulating hepatic DNL. Another study used a slightly lower proportion of total carbohydrate (68%) but much more complex carbohydrate, and the results showed that hepatic DNL was minimal (11).

In the present study, we measured hepatic DNL in weight-stable subjects after they consumed a balanced, low-fat, high-carbohydrate diet for 5 d. The diet had 68% of energy from carbohydrate, with 54% of total carbohydrates as simple sugars and 46% as complex carbohydrates. This diet was representative of typical low-fat diets. The effect of this diet on hepatic DNL was compared with that of a standard high-fat Western diet.

We and other investigators have also explored how health status affects hepatic DNL. The most intriguing finding is that critically ill patients make fat from carbohydrate even when they consume a high-fat diet. Concurrently, they have high concentrations of triacylglycerols, and carbohydrate intake fails to suppress their endogenous glucose production, a characteristic feature of hepatic insulin resistance and syndrome X (12, 13). Like critically ill patients, hyperinsulinemic obese subjects also exhibit increased triacylglycerol concentrations and increased hepatic glucose production (14). Therefore, we determined whether obese subjects would also have increased DNL. Because hyperinsulinemia does not always occur in obesity, we measured hepatic DNL in obese subjects with and without fasting hyperinsulinemia to investigate whether hyperinsulinemia was a determinant of increased hepatic DNL.

SUBJECTS AND METHODS

Subjects

Volunteers were recruited by advertisement. The study protocol was approved by the University of California San Francisco and University of California Berkeley Committees on Human Research. The protocol was explained in detail to the subjects, who then gave their written informed consent to participate before any procedures were undertaken. The 33 subjects were nonsmoking male volunteers (14 normal weight and 19 obese subjects). Women were excluded from the study because of the known effect of sex on DNL. The subjects had no history of medical illnesses and showed no abnormalities when screened by physical examination or laboratory testing. All subjects had a stable body weight during the 6 mo preceding the study. HIV-positive subjects were excluded because metabolic abnormalities have been documented in asymptomatic seropositive persons (15). Subjects taking medications with potential metabolic effects (eg, β-blockers, theophylline, diuretics, glucocorticoids, β-agonists, and phenytoin) were also excluded. The subjects who met all the enrollment criteria were admitted to the General Clinical Research Center (GCRC) for the study procedures within 2–6 wk after the screening day, depending on the availability of both the subject and the GCRC.

Body mass index (BMI) was calculated as weight (in kg) divided by height squared (in m). Total body composition was measured with dual-energy X-ray absorptiometry (software version 3.65; Lunar model DPX, Madison, WI) (16).

Experimental design

Two different protocols were performed at the GCRC of San Francisco General Hospital. To ensure strict control of the diet composition, energy intake, and physical activity, the subjects were inpatients confined to the GCRC metabolic ward for the duration of the 5-d study. The subjects received the same diet every day, given as 3 meals and 2–3 snacks (depending on subject preference). The overall macronutrient composition was consistent among all subjects, but the food items used to compose the diet were chosen on the basis of each subject's usual diet and preferences. The ratio of complex to simple carbohydrates in the high-fat, low-carbohydrate diet was the same as for the low-fat, high-carbohydrate diet (54 ± 2% simple and 46 ± 2% complex carbohydrates). Fructose was not excluded from the diets, but it represented only a small portion of the simple carbohydrate (mainly in soft drinks and in the form of sucrose). The amounts of n−3 polyunsaturated fatty acids were very low in all the subjects' diets. Initial energy intake was calculated from the basal metabolic rate (determined with the Harris-Benedict equation) multiplied by a factor of 1.3 for activity. Total energy intake was adjusted to maintain a stable body weight throughout the 5-d study. Previous research showed that when subjects stay on a metabolic ward and consume a study diet for 5 d, this is enough time to allow DNL to reach a new equilibrium (9).

The first protocol consisted of 5 d of an energy-balanced, high-fat, low-carbohydrate diet (40.0 ± 0.4% of energy from fat, 45.8 ± 0.5% from carbohydrate, and 14.3 ± 0.3% from protein), followed by an infusion study on the sixth day. This protocol involved 3 different groups of subjects: 9 normoinsulinemic lean subjects (fasting insulin ≤ 100 pmol/L and BMI ≤ 26 at screening), 6 normoinsulinemic obese subjects (fasting insulin ≤ 100 pmol/L and BMI ≥ 27 at screening), and 8 hyperinsulinemic obese subjects (fasting insulin ≥ 135 pmol/L and BMI ≥ 27 at screening); the 2 groups of normoinsulinemic subjects were normotriglyceridemic (fasting triacylglycerols ≤ 1.7 mmol/L).

The second protocol involved 2 different groups of subjects: 5 normoinsulinemic lean subjects and 5 hyperinsulinemic obese subjects. The protocol consisted of 5 d of an energy-balanced, low-fat, high-carbohydrate diet (16.9 ± 1.0% of energy from fat, 67.9 ± 0.7% from carbohydrate, and 15.2 ± 0.7% from protein) followed by the same infusion study that was used in the first protocol.

Infusion study

The infusion study consisted of an overnight intravenous infusion of sodium [1-^{13}C]acetate (0.120 mmol · kg^{-1} · h^{-1}) purchased

TABLE 1
Subject characteristics[1]

| Characteristic | High-fat, low-carbohydrate diet | | | Low-fat, high-carbohydrate diet | |
	Lean normoinsulinemic ($n = 9$)	Obese normoinsulinemic ($n = 6$)	Obese hyperinsulinemic ($n = 8$)	Lean normoinsulinemic ($n = 5$)	Obese hyperinsulinemic ($n = 5$)
Age (y)	36.4 ± 3.0	32.7 ± 1.9	39.5 ± 4.1	37.2 ± 3.8	48.0 ± 5.8
Prediet weight (kg)	75.9 ± 3.4^a	117.2 ± 10.0^b	105.8 ± 6.7^b	71.7 ± 3.2^a	103.1 ± 10.8^b
Postdiet weight (kg)	75.8 ± 3.4^a	116.7 ± 9.8^b	105.7 ± 6.5^b	71.2 ± 3.1^a	102.9 ± 10.6^b
Height (m)	179.3 ± 2.1	180.6 ± 4.1	175.6 ± 1.3	175.5 ± 4.5	172.0 ± 3.3
BMI (kg/m^2)	23.5 ± 0.7^a	34.9 ± 1.9^b	34.3 ± 1.9^b	23.1 ± 0.4^a	34.7 ± 3.1^b
Body fat (%)	17.3 ± 1.2^a	31.7 ± 1.3^b	32.3 ± 1.6^b	16.6 ± 1.4^a	33.8 ± 2.1^b

[1] $\bar{x} \pm$ SEM. Values in the same row with different superscript letters are significantly different, $P < 0.05$.

from Isotec (Miamisburg, OH) or Cambridge Isotope Laboratory (Andover, MA). Isotopic purity was > 98%. At 2130 on day 5 of the dietary protocols, an intravenous line was placed in the antecubital vein of each arm, one intravenous line for administration of the isotope and the other for drawing blood. At 2300, the infusion was started. A blood sample was drawn 10 h later into iced evacuated tubes containing 1 mg EDTA/mL. The samples were mixed thoroughly before centrifugation at 1500 × g for 20 min at 4 °C.

Biochemical and mass spectrometry analysis

Triacylglycerol concentrations were measured with a colorimetric assay (337-B; Sigma Inc, St Louis). Insulin was analyzed with a commercial radioimmunoassay (Coated-tube RIA for Insulin; Diagnostic Products Corp, Los Angeles), and cortisol was measured with the in-house RIA described previously (17). Gas chromatography–mass spectrometry (GC model 6890 and MS model 5973; Hewlett-Packard, Palo Alto, CA) was used for isotopic enrichments of palmitate-methyl esters from VLDL. A 20-m fused DB-1 silica column was used, with electron impact ionization with single ion monitoring. Methyl palmitate ions were monitored at a mass-to-charge ratio of 270–272, representing the parent M_0 through the M_2 isotopomers. Fractional DNL was calculated by mass isotopomer distribution analysis, as described in detail elsewhere (6). The ratio of double-labeled to single-labeled excesses in VLDL-palmitate reveals the isotopic enrichments of the true precursor for lipogenesis (hepatic cytosolic acetyl-CoA). By application of probability logic and on the basis of multinomial expansion, the fractional contribution of DNL to VLDL fatty acids can be calculated by using the precursor-product relation (5, 6). To determine whether fatty acids synthesized de novo in adipose tissue could potentially contribute to hepatic DNL, plasma fatty acids were extracted and then isolated by thin-layer chromatography, followed by measurement of DNL in fatty acids by gas chromatography–mass spectrometry and mass isotopomer distribution analysis.

Statistical analyses

Data are presented as means ± SEMs. Statistical analyses were performed by using STATVIEW, version 4.1 and SUPERANOVA, version 1.11 (both from Abacus Concepts, Berkeley, CA), with $P < 0.05$ considered statistically significant. The significance of mean differences among groups was determined with analysis of variance (ANOVA). Post hoc analyses for between-group comparisons of subject characteristics, glucose and cortisol concentrations, and fractional DNL were performed with the Tukey Compromise test, with a 5% procedure-wise error rate. The relation

between triacylglycerol and DNL was analyzed by using simple linear regression. For insulin and triacylglycerol, comparisons of prediet (screening) and postdiet concentrations were performed with a two-factor repeated measures ANOVA with one group factor (5 groups) and one time factor (prediet and postdiet). If a significant group-by-time interaction was found, the preceding analysis was repeated separately for each diet. Both fractional DNL and the change in triacylglycerol concentrations are expressed as percentages, and the statistical analyses were performed on the log-transformed data.

RESULTS

Subject characteristics and composition of experimental diets

A total of 23 subjects participated in the high-fat, low-carbohydrate diet protocol. These subjects were either lean normoinsulinemic, obese normoinsulinemic, or obese hyperinsulinemic (**Table 1**). Ten subjects, who were either lean normoinsulinemic or obese hyperinsulinemic, participated in the low-fat, high-carbohydrate diet protocol. The lean and obese subjects in the different groups were not significantly different with respect to age and height, but had significantly different values for body weight, BMI, and body fat (Table 1).

In both diet protocols, the subjects were in energy balance (ie, at stable body weight) during the 5 d preceding the infusion protocol. On average, those fed the high-fat, low-carbohydrate diet received 40% of energy from fat, 46% from carbohydrate, and 14% from protein. The low-fat, high-carbohydrate diet supplied 17% of energy from fat, 68% from carbohydrate, and 15% from protein (**Table 2**). Body weight and body fat remained stable throughout the 5 d on the isoenergetic diet protocol.

Plasma glucose, insulin, triacylglycerol, and cortisol

Fasting plasma glucose concentrations measured at the screening visit did not differ significantly between groups (**Table 3**). Because cortisol stimulates DNL, we measured fasting cortisol concentrations after the 5-d diet protocols; cortisol concentrations did not differ significantly between groups.

Fasting plasma insulin and triacylglycerol concentrations in the 5 groups of subjects prediet and postdiet are shown in Table 3. A two-factor repeated-measures ANOVA was performed to evaluate the effects of group (5 subject groups) and time (prediet and postdiet) on insulin and triacylglycerol. For insulin concentration, there was no significant group-by-time interaction ($P = 0.208$). Therefore, the conclusions about time and group effects were obtained

TABLE 2
Actual composition of diets consumed by the subjects[1]

Diet composition	High-fat, low-carbohydrate diet			Low-fat, high-carbohydrate diet	
	Lean normoinsulinemic ($n = 9$)	Obese normoinsulinemic ($n = 6$)	Obese hyperinsulinemic ($n = 8$)	Lean normoinsulinemic ($n = 5$)	Obese hyperinsulinemic ($n = 5$)
Energy (kJ)	12 033 ± 523	14 251 ± 820	12 686 ± 423	11 163 ± 874	13 071 ± 841
Carbohydrate (% of energy)	46 ± 1	45 ± 1	47 ± 1	67 ± 1	69 ± 1
Fat (% of energy)	40 ± 1	41 ± 1	39 ± 1	17 ± 2	16 ± 1
Protein (% of energy)	14 ± 1	15 ± 1	14 ± 1	16 ± 1	15 ± 1

[1] $\overline{x}$ ± SEM for the 5 d of each diet protocol. For both diets, 54 ± 2% of total carbohydrates was simple sugars and 46 ± 2% was complex carbohydrates.

from the main effects. After 5 d of the controlled isoenergetic diet, there was a significant drop in fasting insulin concentration ($P < 0.05$) when compared with fasting insulin at screening. However, screening values were obtained after an overnight fast of 8–10 h, whereas the overnight fast was 12 h in duration at the postdiet measurement. Furthermore, the mean insulin concentration was significantly higher in the hyperinsulinemic obese subjects than in the normoinsulinemic lean and obese subjects ($P < 0.05$).

For triacylglycerol concentrations, there was a significant group-by-time interaction ($P = 0.003$). Therefore, we repeated the analysis for each diet separately and found that there was no significant group-by-time interaction for either diet. Consumption of a high-fat, low-carbohydrate diet for 5 d did not affect plasma triacylglycerol concentrations compared with screening values, so there was no time effect. In contrast, after 5 d of the low-fat, high-carbohydrate diet, there was a significant, 1.5-fold increase in plasma triacylglycerol concentrations ($P < 0.01$).

Fractional DNL

With the high-fat, low-carbohydrate diet, DNL was minimal and did not differ significantly between the lean and obese subjects with normal fasting insulin concentrations. In contrast, hyperinsulinemic obese subjects had a significantly higher DNL compared with both lean and obese normoinsulinemic subjects (**Figure 1**; $P < 0.05$). Interestingly, although DNL did

not differ significantly between the lean normoinsulinemic and the obese hyperinsulinemic subjects who consumed the low-fat, high-carbohydrate diet (Figure 1), DNL was significantly higher ($P < 0.05$) with the low-fat, high-carbohydrate diet for both groups when compared with the normoinsulinemic subjects who consumed a high-fat, low-carbohydrate diet for 5 d (Figure 1; $P < 0.05$).

Because of the significant interaction among groups for fasting triacylglycerol concentrations, the correlation between DNL and fasting triacylglycerol was analyzed separately for the normoinsulinemic and hyperinsulinemic groups after the high-fat, low-carbohydrate diet; no significant correlations were found. Although the plasma triacylglycerol concentration did not correlate with DNL in the subjects who consumed the low-fat, high-carbohydrate diet, the increase in triacylglycerol concentrations induced by this diet was positively correlated with DNL (**Figure 2**; $P < 0.05$, $R = 0.87$). These results suggest that the individuals with the highest DNL were those who experienced the greatest increase in plasma triacylglycerol after a low-fat, high-carbohydrate diet.

When fractional DNL was measured in the plasma fatty acids of the lean and obese subjects with the highest hepatic DNL, we found that the contribution of plasma fatty acids to hepatic DNL was negligible. Although it can be argued that theoretically, fatty acids synthesized de novo in adipose tissue can be released into

TABLE 3
Fasting concentrations of glucose, hormones, and triacylglycerol[1]

Biochemical index	High-fat, low-carbohydrate diet			Low-fat, high-carbohydrate diet	
	Lean normoinsulinemic ($n = 9$)	Obese normoinsulinemic ($n = 6$)	Obese hyperinsulinemic ($n = 8$)	Lean normoinsulinemic ($n = 5$)	Obese hyperinsulinemic ($n = 5$)
Prediet glucose (mmol/L)	4.4 ± 0.1	4.5 ± 0.2	5.0 ± 0.4	4.1 ± 0.4	5.0 ± 0.5
Postdiet cortisol (nmol/L)	221 ± 25	168 ± 41	262 ± 14	281 ± 41	168 ± 22
Prediet insulin (pmol/L)[2]	47 ± 9	79 ± 10	187 ± 17	66 ± 12	240 ± 28
Postdiet insulin (pmol/L)	37 ± 5	70 ± 12	157 ± 7	51 ± 7	139 ± 13
Insulin ratio (postdiet:prediet)	0.84 ± 0.11	0.86 ± 0.08	0.85 ± 0.06	0.82 ± 0.08	0.62 ± 0.10
Prediet triacylglycerol (mmol/L)[3]	0.82 ± 0.07	1.64 ± 0.52	2.92 ± 0.44	1.05 ± 0.31	2.72 ± 0.76
Postdiet triacylglycerol (mmol/L)	0.78 ± 0.07	1.73 ± 0.59	2.51 ± 0.46	1.51 ± 6.30	4.18 ± 0.76
Triacylglycerol ratio, postdiet:prediet	0.96 ± 0.06	1.04 ± 0.14	0.88 ± 0.10	1.44 ± 0.16	1.52 ± 0.13

[1] $\overline{x}$ ± SEM.

[2] There was no significant interaction between group and time. The main effect of time was significant ($P < 0.05$). The main effect of group was also significant: 3 groups with normal fasting insulin concentrations had significantly lower fasting insulin values than did the 2 groups with high fasting insulin values ($P < 0.05$).

[3] There was a significant interaction between time and group for triacylglycerol concentration ($P < 0.003$), but there was no significant group-by-time interaction for either diet when each diet was analyzed separately. Within the low-fat, high-carbohydrate diet groups, there was a significant main effect of time ($P < 0.01$).
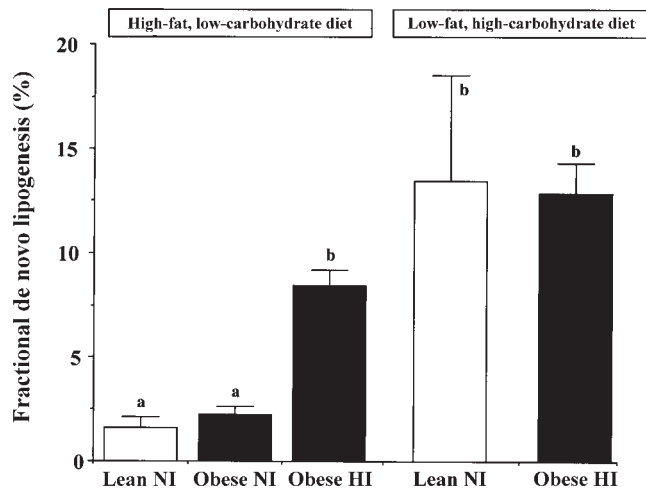
FIGURE 1. Mean (± SEM) fractional de novo lipogenesis in lean normoinsulinemic (NI), obese NI, and obese hyperinsulinemic (HI) subjects after 5 d of consuming a high-fat, low-carbohydrate diet and in different lean NI and obese HI subjects after 5 d of consuming a low-fat, high-carbohydrate diet. Values with different superscript letters are significantly different, $P < 0.05$.
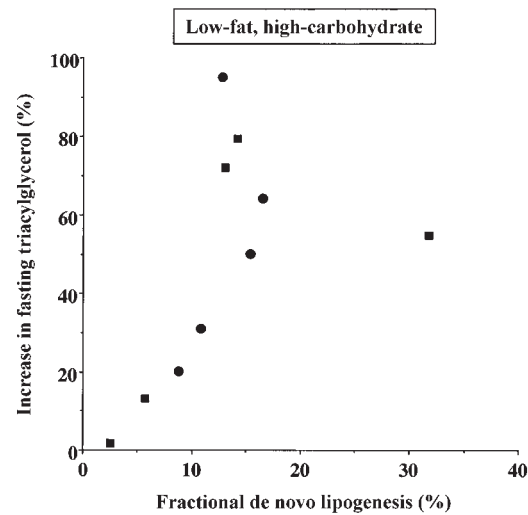

FIGURE 2. Relation between fractional de novo lipogenesis and the percentage increase in fasting triacylglycerol concentration in lean normoinsulinemic (■) and obese hyperinsulinemic (●) subjects after 5 d in energy balance while consuming a low-fat, high-carbohydrate diet ($P < 0.05$, $R^2 = 0.87$).

the bloodstream, reesterified in the liver, and re-secreted as VLDL-triacylglycerol, our data support the concept that, in the time frame of our labeling experiment (10 h), DNL measured from VLDL-triacylglycerols is essentially hepatic. When we measured DNL directly from fatty acids in the 4 subjects in our study who had the highest DNL, we found that 3 of the 4 subjects had fractional DNL measured from fatty acids (**Table 4**). However, the precursor pool enrichment values calculated from fatty acids and from VLDL-triacylglycerol were virtually identical. This suggests that these fatty acids were synthesized in the liver. However, we cannot exclude the possibility that adipose tissue made a small contribution to the otherwise hepatic DNL.

DISCUSSION

Until recently, DNL was believed to be an insignificant pathway in humans who eat a Western, high-fat diet. In addition, very little was known about the effect of the often recommended alternative diet, which is a low-fat, high-carbohydrate diet, on hepatic metabolism. However, with the development of new methodologies for measuring hepatic DNL, it was shown that hepatic DNL varies greatly depending on the health of the subjects (12, 13, 15)

and the types of diets they consume (4, 5, 7, 9–11, 18, 19). The present study sought to answer 3 questions. First, do hyperinsulinemic obese subjects fed a Western, high-fat diet have higher fractional hepatic DNL than do normoinsulinemic lean or obese subjects fed the same diet? Second, is hepatic DNL increased when simple carbohydrate represents 54% of a low-fat, high-carbohydrate diet? And third, what is the relation of increased hepatic DNL to VLDL-triacylglycerol concentrations?

Effect of obesity, hyperinsulinemia, and a high-fat Western diet on fractional hepatic DNL

Our results show for the first time that, with a high-fat, Western diet, hyperinsulinemic obese subjects have significantly higher DNL than do normoinsulinemic lean subjects consuming the same diet (Figure 1). More importantly, we showed that the increase occurred only in obese subjects who were hyperinsulinemic, because obese subjects without hyperinsulinemia did not have elevated DNL. To ensure strict control of dietary energy intake and to achieve energy balance, the subjects stayed in a metabolic ward and received a high-fat, low-carbohydrate Western diet for 5 d before fasting DNL was measured. Weight was measured daily and, if necessary, food intake was adjusted slightly to maintain a

TABLE 4
Fractional de novo lipogenesis (DNL) and precursor pool enrichment from VLDL-triacylglycerol and from plasma fatty acids

Subjects[1]	Fractional DNL measured from VLDL-triacylglycerol	Precursor pool enrichment calculated from VLDL-triacylglycerol	Fractional DNL measured from fatty acids	Precursor pool enrichment calculated from fatty acids
	%		%	
Lean 1	14.2	0.048	0.0	0.00
Lean 2	31.8	0.068	5.1	0.067
Obese 1	15.4	0.036	5.3	0.035
Obese 2	16.5	0.038	7.3	0.039

[1] The subjects were the 4 subjects in the study who had the highest DNL.

constant body weight. Furthermore, to enroll in the study, subjects had to be weight-stable during the 6 mo preceding the study. Therefore, in this study, overeating could not be responsible for the observed changes in DNL. Previous studies showed that DNL is absent or accounts for < 2–3% of VLDL-triacylglycerol in lean subjects in energy balance on a high-fat, Western diet (4, 5, 7, 9). In addition to confirming previous results in lean subjects, the present study shows that obese subjects fed a high-fat diet do not have significantly increased DNL when they are normoinsulinemic (Figure 1; $P < 0.05$). Remarkably, however, hyperinsulinemic obese subjects eating the same nonlipogenic diet have significantly higher DNL, at ≈3–4 times the DNL measured in normoinsulinemic subjects (Figure 1). Our data suggest that in obese subjects, hyperinsulinemia or another unknown unmatched variable, but not body composition, is a determining factor for stimulating hepatic DNL.

These results are intriguing because these hyperinsulinemic subjects did not receive excess energy from carbohydrate but rather consumed a high-fat diet (40% of energy from fat) with a low proportion of carbohydrate (47% of energy). Why would their livers continue to convert carbohydrate into fat when there was a relatively small amount of carbohydrate available? Our study was not designed to answer this question, but 2 hypotheses can be considered. The first one is that DNL in these subjects is the result of reduced glucose uptake by extrahepatic tissues, leading to an increased supply and availability of carbohydrate for hepatic metabolism. Furthermore, gluconeogenic precursors, which are increased with insulin resistance (20–22), may provide an additional source of carbon for hepatic fat synthesis. The second hypothesis is that hyperinsulinemia itself leads to increased DNL in obese subjects.

Interestingly, in insulin-resistant, lipoatrophic mouse models, Shimomura et al (23) showed that there are 2 independent hepatic insulin-signaling pathways. One pathway, controlled by insulin receptor substrate-2, is responsible for gluconeogenesis and is insulin-resistant (lacking proper inhibition of gluconeogenesis by insulin, thus causing hyperglycemia), whereas the other insulin-signaling pathway, controlled by the transcription factor SREBP-1C, remains insulin-sensitive and leads to increased amounts of mRNA for the key enzymes of lipogenesis [glucokinase (EC 2.7.1.1–2), malic enzyme (EC 1.1.1.38), acetyl-CoA carboxylase (EC 6.4.1.2), and fatty acid synthase (EC 2.3.1.85)]. With the increased quantity of insulin present in insulin resistance, the insulin-sensitive signaling pathway (SREBP-1C) is indeed stimulated and leads to increased lipogenesis. This mixed pattern of hepatic insulin resistance and sensitivity may be the molecular foundation for the association between dyslipidemia and type 2 diabetes. Our results in hyperinsulinemic obese subjects are compatible with these recent findings in insulin-resistant mouse models (23). The 2 hypotheses discussed above are not mutually exclusive, and the effects of both in parallel may promote hepatic DNL. In one condition, the extrahepatic insulin resistance redirects substrates and provides more carbohydrate and lipogenic precursors for the liver; in the other, the liver, itself in lipogenic mode because of hepatic insulin resistance, would take up more glucose (stimulated by glucokinase) and convert more carbohydrate into fat (stimulated by lipogenic enzymes).

Our measurements assess overall hepatic DNL and show that the pathway is indeed stimulated even in hyperinsulinemic obese subjects consuming a low-carbohydrate diet. Lipogenesis in these dietary conditions suggests that in the presence of hyperinsulinemia,

the liver converts excess carbohydrate to fat to control blood glucose and prevent hyperglycemia. However, this homeostasis that preserves normal glucose concentrations disrupts fat metabolism by increasing triacylglycerol concentrations, a factor that itself might exacerbate insulin resistance and set up a vicious cycle (24).

Effect of a low-fat, high-carbohydrate diet on DNL in lean normoinsulinemic and obese hyperinsulinemic subjects

Our results showed that after 5 d in energy balance on a low-fat, high-carbohydrate diet, normoinsulinemic lean and hyperinsulinemic obese subjects had significantly higher fractional DNL than did normoinsulinemic lean and obese subjects fed a high-fat diet (Figure 1). Our low-fat, high-carbohydrate diet matched the proportions of fat and carbohydrate used previously by Parks et al (11); however, the ratio of simple to complex carbohydrates in the present study was 54:46, compared with 40:60 in the study by Parks et al. In the present study, this higher proportion of simple sugars (54%) led to elevated fractional hepatic DNL, whereas when only 40% of the carbohydrate consisted of simple sugars, hepatic DNL was minimal (11). Overall, these findings confirm and extend previous results from Hudgins et al (8), who found that the proportion of simple sugars in very-low-fat, high-carbohydrate diets was the determining factor for hepatic DNL in healthy subjects.

In view of this striking difference in fractional hepatic DNL between a high-fat, low-carbohydrate diet (DNL < 3%) and a low-fat, high-carbohydrate diet (DNL > 12%) in lean subjects (Figure 1), it was particularly interesting to test the effect of the low-fat, high-carbohydrate diet in a group of hyperinsulinemic obese subjects whose DNL was already elevated (DNL > 8%) when consuming a high-fat diet. Although fractional DNL appeared to be slightly higher in hyperinsulinemic obese subjects consuming the low-fat, high-carbohydrate diet (DNL > 12%) than in those consuming a high-fat diet, this difference was not statistically significant; a crossover design would have tested this question more adequately. Yet, it appears that these preliminary results comparing 2 small groups of hyperinsulinemic subjects reveal a trend of higher fractional DNL after a low-fat, high-carbohydrate diet than after a high-fat, low-carbohydrate diet. Furthermore, our data clearly show that a low-fat, high-carbohydrate diet with a large proportion of simple sugars stimulates a lipogenic mode in the livers of insulin-sensitive subjects; this mode is comparable with the one observed in hyperinsulinemic subjects consuming a high-fat diet.

Relation between hepatic DNL and triacylglycerol concentrations

A high blood triacylglycerol concentration is an independent risk factor for coronary heart disease. This new understanding was mainly derived from the strength of evidence found in a meta-analysis of 17 prospective population-based studies (1, 2, 25). Moderately increased VLDL-triacylglycerol concentrations are associated with a constellation of abnormalities in serum lipoproteins, such as reduced HDL (26, 27) and a predominance of small, dense LDL (28, 29), both of which are predisposing factors for cardiovascular disease.

Overall, high triacylglycerol concentrations result from an imbalance between the rates of VLDL-triacylglycerol production and VLDL-triacylglycerol clearance. The main factor responsible for hepatic triacylglycerol secretion is fatty acid availability (30).

In the liver, fatty acids may originate from either DNL, breakdown of stored triacylglycerol, or circulating fatty acids (30).

Hepatic DNL may increase VLDL-triacylglycerol secretion by way of 2 processes. The first is a direct effect of the synthesis of new fatty acids made available for triacylglycerol production. The second is an indirect effect of the lipogenic mode of the liver, causing inhibition of fatty acid oxidation and consequently increasing reesterification of circulating fatty acids. The biochemical basis for the second effect was discovered by McGarry et al (31, 32), who showed that the increased concentration of malonyl-CoA (a substrate in the DNL pathway) that is present when DNL is increased is a potent inhibitor of the transport of fatty acids into the mitochondria, where they are oxidized. Therefore, by both indirect and direct mechanisms, high hepatic DNL leads to an increased rate of production of VLDL-triacylglycerol that may in turn lead to increased triacylglycerol concentrations.

Although the triacylglycerol concentrations reported in this study are 2 single measurements obtained prediet and postdiet, and this may have resulted in more variable data, we nonetheless found that hyperinsulinemic obese subjects consuming a high-fat, Western diet had significantly higher triacylglycerol concentrations, as previously shown by others. The new finding in the current study, however, is that these hyperinsulinemic obese subjects also had higher fractional hepatic DNL when compared with normoinsulinemic lean and obese subjects. More importantly, the low-fat, high-carbohydrate diet induced a change in triacylglycerol concentrations that was correlated with fractional hepatic DNL in normoinsulinemic lean and hyperinsulinemic obese subjects (Figure 2; $P < 0.05$). Because we measured the change in triacylglycerol concentrations in the same subjects (Table 3), the association between fractional hepatic DNL and the increase in triacylglycerol concentrations supports a role for hepatic DNL as a factor in this increase (Figure 2).

The change in triacylglycerol concentrations in our study was calculated from 2 single measurements (prediet and postdiet). One cannot rule out that, with our experimental design, a high day-to-day variability and a long interval between the 2 measurements may explain the lack of consistency in triacylglycerol response on the high-fat, low-carbohydrate diet. Many studies have established previously that high-carbohydrate diets increase triacylglycerol concentrations (33, 34). In addition to confirming these findings, our data, as well as those of others (10), support the hypothesis that hepatic DNL contributes to this process (Figure 2). In this study, we showed that hyperinsulinemic obese subjects have high hepatic DNL under a dietary condition (high-fat diet) in which normoinsulinemic subjects have no DNL. We also found that when normoinsulinemic and hyperinsulinemic subjects consume a low-fat, high-carbohydrate diet with more than half of the carbohydrate in the form of simple sugars, hepatic DNL is high and triacylglycerol concentrations are increased.

Animal studies have shown that in rodents and dogs, chronic lipogenic diets are associated with hypertriglyceridemia and the development of insulin resistance (35–37). Two of our previous acute human studies also support a link between hepatic DNL and insulin resistance. Our acute carbohydrate overfeeding study showed a significant increase in fasting glucose production (a sign that hepatic insulin sensitivity was reduced) with increased DNL in healthy subjects (9). In a second study, we found that trauma patients who become critically ill develop a lack of proper inhibition of glucose production by carbohydrate feeding (hepatic insulin resistance). They also have higher fractional DNL than do healthy subjects (12). Although it would be premature at this stage to propose a relation between increased hepatic DNL and hyperinsulinemia, more data that support this idea are accumulating. The primary role of the liver in the etiology of insulin resistance is supported by elegant animal studies showing that loss of insulin signaling in hepatocytes leads to severe insulin resistance (38). The low-fat, high-carbohydrate diet often recommended as a substitution for high-fat, low-carbohydrate diets may not be the best possible choice if it induces hepatic DNL and insulin resistance, especially when most of the carbohydrate is in the form of simple sugars.

This study supports the hypothesis that hepatic DNL is one of the mechanisms by which low-fat, high-carbohydrate diets induce hypertriglyceridemia in human subjects. Furthermore, hepatic DNL may also be a determining factor in inducing hypertriglyceridemia in insulin-resistant subjects, irrespective of their diets. Finally, chronic consumption of a low-fat, high-carbohydrate diet with a high simple sugar content may trigger undesirable changes in hepatic metabolism, leading not only to hypertriglyceridemia, but perhaps also to insulin resistance. Indeed, the new dietary guidelines from the American Heart Association do not recommend very-low-fat diets because they may amplify metabolic abnormalities such as hypertriglyceridemia, insulin resistance, and low HDL concentrations (39).

Further longitudinal human studies are needed to confirm the critical role of hepatic DNL in increasing triacylglycerol concentrations and to determine whether chronic hypertriglyceridemia induced by DNL leads to insulin resistance. Such studies should help to clarify how diet composition is related to hyperinsulinemia and diabetes. ❦

REFERENCES

1. Austin MA. Epidemiology of hypertriglyceridemia and cardiovascular disease. Am J Cardiol 1999;83:13F–6F.
2. Austin MA, Hokanson JE, Edwards KL. Hypertriglyceridemia as a cardiovascular risk factor. Am J Cardiol 1998;81:7B–12B.
3. Weiss L, Hoffmann GE, Schreiber R, et al. Fatty-acid biosynthesis in man, a pathway of minor importance. Purification, optimal assay conditions, and organ distribution of fatty-acid synthase. Biol Chem Hoppe Seyler 1986;367:905–12.
4. Hellerstein MK, Schwarz JM, Neese RA. Regulation of hepatic de novo lipogenesis in humans. Annu Rev Nutr 1996;16:523–57.
5. Hellerstein MK, Christiansen M, Kaempfer S, et al. Measurement of de novo hepatic lipogenesis in humans using stable isotopes. J Clin Invest 1991;87:1841–52.
6. Hellerstein MK, Neese RA. Mass isotopomer distribution analysis at 8 y: theoretical, analytic, and experimental considerations. Am J Physiol 1999;276:E1146–70.
7. Hudgins LC, Hellerstein M, Seidman C, Neese R, Diakun J, Hirsch J. Human fatty acid synthesis is stimulated by a eucaloric low fat, high carbohydrate diet. J Clin Invest 1996;97:2081–91.
8. Hudgins LC, Seidman CE, Diakun J, Hirsch J. Human fatty acid synthesis is reduced after the substitution of dietary starch for sugar. Am J Clin Nutr 1998;67:631–9.
9. Schwarz JM, Neese RA, Turner S, Dare D, Hellerstein MK. Short-term alterations in carbohydrate energy intake in humans. Striking

effects on hepatic glucose production, de novo lipogenesis, lipolysis, and whole-body fuel selection. J Clin Invest 1995;96:2735–43.

10. Hudgins LC, Hellerstein MK, Seidman CE, Neese RA, Tremaroli JD, Hirsch J. Relationship between carbohydrate-induced hypertriglyceridemia and fatty acid synthesis in lean and obese subjects. J Lipid Res 2000;41:595–604.

11. Parks EJ, Krauss RM, Christiansen MP, Neese RA, Hellerstein MK. Effects of a low-fat, high-carbohydrate diet on VLDL-triacylglycerol assembly, production, and clearance. J Clin Invest 1999;104:1087–96.

12. Schwarz JM, Chiolero R, Revelly JP, et al. Effects of enteral carbohydrates on de novo lipogenesis in critically ill patients. Am J Clin Nutr 2000;72:940–5.

13. Tappy L, Schwarz JM, Schneiter P, et al. Effects of isoenergetic glucose-based or lipid-based parenteral nutrition on glucose metabolism, de novo lipogenesis, and respiratory gas exchanges in critically ill patients. Crit Care Med 1998;26:860–7.

14. Reaven GM. Pathophysiology of insulin resistance in human disease. Physiol Rev 1995;75:473–86.

15. Hellerstein MK, Grunfeld C, Wu K, et al. Increased de novo hepatic lipogenesis in human immunodeficiency virus infection. J Clin Endocrinol Metab 1993;76:559–65.

16. Mazess RB, Barden HS, Bisek JP, Hanson J. Dual-energy X-ray absorptiometry for total-body and regional bone-mineral and soft-tissue composition. Am J Clin Nutr 1990;51:1106–12.

17. Murphy BE. Some studies of the protein-binding of steroids and their application to the routine micro and ultramicro measurement of various steroids in body fluids by competitive protein-binding radioassay. J Clin Endocrinol Metab 1967;27:973–90.

18. Hudgins LC. Effect of high-carbohydrate feeding on triacylglycerol and saturated fatty acid synthesis. Proc Soc Exp Biol Med 2000;225:178–83.

19. Aarsland A, Wolfe RR. Hepatic secretion of VLDL fatty acids during stimulated lipogenesis in men. J Lipid Res 1998;39:1280–6.

20. Zawadzki JK, Wolfe RR, Mott DM, Lillioja S, Howard BV, Bogardus C. Increased rate of Cori cycle in obese subjects with type 2 diabetes and effect of weight reduction. Diabetes 1988;37:154–9.

21. Felig P, Wahren J, Hendler R. Influence of maturity-onset diabetes on splanchnic glucose balance after oral glucose ingestion. Diabetes 1978;27:121–6.

22. Reaven GM, Hollenbeck C, Jeng CY, Wu MS, Chen YD. Measurement of plasma glucose, free fatty acid, lactate, and insulin for 24 h in patients with type 2 diabetes. Diabetes 1988;37:1020–4.

23. Shimomura I, Matsuda M, Hammer RE, Bashmakov Y, Brown MS, Goldstein JL. Decreased IRS-2 and increased SREBP-1c lead to mixed insulin resistance and sensitivity in livers of lipodystrophic and ob/ob mice. Mol Cell 2000;6:77–86.

24. McGarry JD. What if Minkowski had been ageusic? An alternative angle on diabetes. Science 1992;258:766–70.

25. Hokanson JE, Austin MA. Plasma triacylglycerol level is a risk factor for cardiovascular disease independent of high-density lipoprotein cholesterol level: a meta-analysis of population-based prospective studies. J Cardiovasc Risk 1996;3:213–9.

26. Grundy SM, Vega GL. Two different views of the relationship of hypertriglyceridemia to coronary heart disease. Implications for treatment. Arch Intern Med 1992;152:28–34.

27. Jeppesen J, Hein HO, Suadicani P, Gyntelberg F. Triacylglycerol concentration and ischemic heart disease: an 8-y follow-up in the Copenhagen Male Study. Circulation 1998;97:1029–36.

28. Dreon DM, Fernstrom HA, Williams PT, Krauss RM. Reduced LDL particle size in children consuming a very-low-fat diet is related to parental LDL-subclass patterns. Am J Clin Nutr 2000;71:1611–6.

29. Stampfer MJ, Krauss RM, Ma J, et al. A prospective study of triacylglycerol level, low-density lipoprotein particle diameter, and risk of myocardial infarction. JAMA 1996;276:882–8.

30. Lewis GF. Fatty acid regulation of very low density lipoprotein production. Curr Opin Lipidol 1997;8:146–53.

31. McGarry JD, Leatherman GF, Foster DW. Carnitine palmitoyltransferase I. The site of inhibition of hepatic fatty acid oxidation by malonyl-CoA. J Biol Chem 1978;253:4128–36.

32. McGarry JD, Mannaerts GP, Foster DW. A possible role for malonyl-CoA in the regulation of hepatic fatty acid oxidation and ketogenesis. J Clin Invest 1977;60:265–70.

33. Katan MB, Grundy SM, Willett WC. Should a low-fat, high-carbohydrate diet be recommended for everyone? Beyond low-fat diets. N Engl J Med 1997;337:563–6.

34. Jeppesen J, Schaaf P, Jones C, Zhou MY, Chen YD, Reaven GM. Effects of low-fat, high-carbohydrate diets on risk factors for ischemic heart disease in postmenopausal women. Am J Clin Nutr 1997;65:1027–33.

35. Pagliassotti MJ, Prach PA, Koppenhafer TA, Pan DA. Changes in insulin action, triacylglycerol, and lipid composition during sucrose feeding in rats. Am J Physiol 1996;271:R1319–26.

36. Thorburn AW, Storlien LH, Jenkins AB, Khouri S, Kraegen EW. Fructose-induced in vivo insulin resistance and elevated plasma triacylglycerol levels in rats. Am J Clin Nutr 1989;49:1155–63.

37. Martinez FJ, Rizza RA, Romero JC. High-fructose feeding elicits insulin resistance, hyperinsulinism, and hypertension in normal mongrel dogs. Hypertension 1994;23:456–63.

38. Michael MD, Kulkarni RN, Postic C, et al. Loss of insulin signaling in hepatocytes leads to severe insulin resistance and progressive hepatic dysfunction. Mol Cell 2000;6:87–97.

39. Krauss RM, Eckel RH, Howard B, et al. AHA Dietary Guidelines: revision 2000: a statement for healthcare professionals from the Nutrition Committee of the American Heart Association. Circulation 2000;102:2284–99.