Relationship between carbohydrate-induced hypertriglyceridemia and fatty acid synthesis in lean and obese subjects

Lisa C. Hudgins,[1,*] Marc K. Hellerstein,[†] Cynthia E. Seidman,[*] Richard A. Neese,[†] Jolanta D. Tremaroli,[*] and Jules Hirsch[*]

Rockefeller University, 1230 York Avenue, New York, NY 10021-6399 and Department of Nutritional Sciences, University of California-Berkeley, Berkeley, CA 94720

Abstract **We previously reported that a eucaloric, low fat, liquid formula diet enriched in simple carbohydrate markedly increased the synthesis of fatty acids in lean volunteers. To examine the diet sensitivity of obese subjects, 7 obese and 12 lean volunteers were given two eucaloric low fat solid food diets enriched in simple sugars for 2 weeks each in a random-order, cross-over design (10% fat, 75% carbohydrate vs. 30% fat, 55% carbohydrate, ratio of sugar to starch 60:40). The fatty acid compositions of both diets were matched to the composition of each subject's adipose tissue and fatty acid synthesis measured by the method of linoleate dilution in plasma VLDL triglyceride. In all subjects, the maximum % de novo synthesized fatty acids in VLDL triglyceride 3–9 h after the last meal was higher on the 10% versus the 30% fat diet. There was no significant difference between the dietary effects on lean (43 ± 13 vs. 12 ± 13%) and obese (37 ± 15 vs. 6 ± 6%) subjects, despite 2-fold elevated levels of insulin and reduced glucagon levels in the obese. Similar results were obtained for de novo palmitate synthesis in VLDL triglyceride measured by mass isotopomer distribution analysis after infusion of [^{13}C]acetate. On the 10% fat diet, plasma triglycerides (fasting and 24 h) were increased and correlated with fatty acid synthesis. Triglycerides were higher when fatty acid synthesis was constantly elevated rather than having diurnal variation. Thus, eucaloric, solid food diets which are very low in fat and high in simple sugars markedly stimulate fatty acid synthesis from carbohydrate, and plasma triglycerides increase in proportion to the amount of fatty acid synthesis. However, this dietary effect is not related to body mass index, insulin, or glucagon levels.**—Hudgins, L. C., M. K. Hellerstein, C. E. Seidman, R. A. Neese, J. D. Tremaroli, and J. Hirsch. **Relationship between carbohydrate-induced hypertriglyceridemia and fatty acid synthesis in lean and obese subjects.** ***J. Lipid Res.*** **2000.** 41: **595–604.**

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The stimulation of fatty acid synthesis by dietary carbohydrate has been studied for decades in animal and in vitro models (1–3) but only recently evaluated in vivo in weight-stable humans (4–7). We reported a marked increase in newly synthesized palmitate (16:0) and decrease in the essential fatty acid, linoleate (18:2), in plasma triglyceride (TG) and VLDL TG after the equicaloric substitution of dietary simple carbohydrate for fat in lean volunteers fed liquid formula diets (4). A subsequent study showed a similar increase in fatty acid synthesis and alteration in the fatty acid composition of VLDL TG by the same very low fat formula diet after the equicaloric substitution of simple carbohydrate for complex carbohydrate (5). In contrast, very low fat formula or solid food diets with a low ratio of simple to complex carbohydrate had much lesser effects on fatty acid synthesis. Thus, in humans, as had previously been shown in other mammals, the amount of fatty acid synthesis is determined by both the amount and type of dietary carbohydrate.

It has been generally accepted that the fatty acid biosynthetic pathway is quantitatively minor in humans except when a large excess of carbohydrate energy is consumed (6, 8). From our studies, it was evident that although the absolute quantity of carbohydrate converted to fat on very low fat eucaloric diets may be small, over time, the marked shift in the fatty acid composition of lipoprotein TG could change the composition of storage TG, cell membrane phospholipid, and other cellular fatty acid pools important for cell function (2, 9–11). Furthermore, an increase in de novo synthesized fatty acids may contribute to the increase in hepatic TG synthesis, VLDL secretion, and concentration of plasma TG after very low fat, high carbohydrate diets (12–14). An increase in TG-rich lipoproteins is associated with and may cause an increase in small dense

Abbreviations: TG, triglyceride; MIDA, mass isotopomer distribution analysis; HDL-C, high density lipoprotein-cholesterol; REE, resting energy expenditure; RQ, respiratory quotient; VLDL, very low density lipoprotein.

[1] To whom correspondence should be addressed at the Rogosin Institute, Box 72, Rockefeller University.

LDL (15) and a decrease in HDL cholesterol (HDL-C). This lipid profile may accelerate atherosclerosis (16, 17).

The same lipid profile is often associated with obesity and, in particular, an excess of visceral fat in the abdomen, as well as increased fasting and postprandial blood insulin levels and decreased tissue sensitivity to insulin (16, 18). It is unknown whether obese subjects who commonly have insulin resistance, high insulin levels, and low glucagon levels are more sensitive to the de novo lipogenic effects of low fat, high carbohydrate diets which may also raise insulin levels (19). Fatty acid synthesis is increased in most rodent models of obesity that also exhibit hyperinsulinemia and insulin resistance (20–24). In vitro, insulin stimulates and glucagon inhibits the expression and activity of many lipogenic enzymes by transcriptional and postranscriptional mechanisms (1). Furthermore, using mass isotopomer distribution analysis (MIDA) to measure fatty acid synthesis in vivo, fatty acid synthesis was positively related to body mass index in a small number of lean and severely obese, hyperinsulinemic subjects after an uncontrolled ad libitum diet (25).

To explore the effects of diet and obesity on fatty acid synthesis, we hypothesized that fatty acid synthesis is increased in obese subjects and that the increase is most pronounced on very low fat diets. As in our previous study (4), we simultaneously used isotopic and nonisotopic methods to measure fatty acid synthesis and compared the response of lean and obese volunteers to two isocaloric solid food diets with the same high ratio of simple to complex carbohydrate but with different amounts of fat and carbohydrate.

METHODS

Subjects

Twelve lean and 7 obese volunteers were studied as inpatients at the Rockefeller University Clinical Research Center (**Table 1**). Subjects were eligible for the study if weight was between 80 and 150% of desirable weight based on the 1959 Metropolitan Life Insurance tables, no less than 10% of their maximum weight and stable within 10% for the previous 6 months. "Lean" subjects were defined as having <120% desirable weight; "obese" as >120% desirable weight. Participants with systemic illness, on oral prescription medicines, who smoked cigarettes, or who had abnormal lipid levels (LDL-cholesterol >160 mg/dl, TG >200 mg/dl, HDL-cholesterol <35 mg/dl), blood pressure (>140/90), or 3 h oral glucose tolerance tests were excluded. Lipid levels were similar in lean and obese subjects. One of the female subjects was post-menopausal. One lean male subject admitted to smoking 2–3 cigarettes/day during the course of the study.

TABLE 1. Subject characteristics

Variable	Lean	Obese
Sex	6 F, 6 M	4 F, 3 M
Age (yr)	31 (18–62)	42 (22–61)
BMI (kg/m^2)	23 (20–27)	34 (31–38)[a]
W/H	0.87 (0.76–1.02)	0.99 (0.80–1.10)[a]
Triglyceride (mg/dl)	78 (36–128)	111 (67–177)
Cholesterol (mg/dl)	172 (99–228)	191 (162–234)
HDL cholesterol (mg/dl)	53 (29–99)	56 (46–76)

Values are given as mean (range); BMI, body mass index; W/H, waist:hip circumference ratio.

[a] $P < 0.01$, lean versus obese.

The waist circumference at the umbilicus and the widest hip circumference were measured with a fiberglass tape to the nearest millimeter. The mean waist-to-hip circumference ratios were significantly higher in both male and female obese subjects; compared to lean subjects; 3/12 lean and 6/7 obese had high waist:hip circumference ratios (>0.85 for females and >0.95 for males (26)).

The studies were approved by the Rockefeller University Institutional Review Board, and informed consent was obtained from all participating subjects.

Diets

Two solid food diets were given for 2 weeks each in a random-order, cross-over design. One diet was 10% fat, 75% carbohydrate; the other was 30% fat, 55% carbohydrate. The initial total calories were estimated as 1360 kcal/m^2 and required minimal adjustment across diets to keep weight constant. Each diet had a single-day menu (same food items each day) with three meals of similar energy and macronutrient composition.

The fatty acid compositions of the two diets were identical and matched to each subject's adipose tissue composition by adding an appropriate mixture of lard, olive oil, and corn oil to each meal, as required by the linoleate dilution method for measuring fatty acid synthesis (4). Cholesterol was added to the oil in both diets as needed to total 200 mg/d. Gas chromatographic analysis of the total fat and fatty acid compositions of the lipid (>95% TG) from homogenates of the diets confirmed a close match to the desired values.

Both diets had identical carbohydrate compositions with ratios of sugar/starch (60:40) chosen to maximize fatty acid synthesis (5) but not to exceed the upper 10% of the dietary ratio of sugar to starch in the US population (27, 28). The sugar was 50% sucrose, 15% fructose, 8% glucose, and 22% lactose (University of Minnesota Nutrition Data System, version 2.8/10/25, Nutrition Coordinating Center, University of Minnesota School of Public Health, Minneapolis). The total fructose intake (including fructose in sucrose) was 18% energy, or 113 g/2500 kcal on the 10% fat diet and 13% energy, or 81 g/2500 kcal, on the 30% fat diet. The total fiber was 6.5 g/100 g carbohydrate and the soluble/insoluble fiber was 0.25 on both diets (29). The food items of both diets were the same as those described in our previous publication (5): cereal, rice, beans, pasta, bread, carrots, lettuce, tomatoes, pears, boiled egg white, yogurt, skim milk, and grape juice.

Measurements of fatty acid synthesis

Two independent and complimentary methods were simultaneously used to measure fatty acid synthesis in vivo: the linoleate dilution method and MIDA, as previously described (4, 6, 30). Using the linoleate dilution method, the fraction of total de novo synthesized fatty acids in VLDL TG was calculated from the decrease, or dilution, of 18:2 relative to the concentration in the diet and adipose tissue (made the same) by de novo synthesized fatty acids. This essential fatty acid, which cannot be synthesized, serves as a nonisotopic marker for preformed, as opposed to de novo synthesized, fatty acids. The method offers the advantage of repeated measurements over time of total de novo fatty acids (16:0 and its elongase/desaturase products, 18:0, 9c-16:1, 9c-18:1, and 11c-18:1), but depends on the assumption of minimal selectivity in the metabolism of the major fatty acids. Using the MIDA method, the fraction of de novo synthesized 16:0 in VLDL TG was measured after the intravenous infusion of [^{13}]C-acetate and calculation of the ^{13}C enrichment of the acetate precursor pool from the ratio of double to single labeled [^{13}C]16:0 (6). Although total de novo fatty acids are not assessed, this

method detects smaller increases in fatty acid synthesis than the linoleate dilution method which depends on a sufficient increase in 16:0 and other de novo fatty acids to dilute (decrease) 18:2. We assumed with both methods that the fraction of de novo fatty acids reflects the absolute rate of fatty acid synthesis.

In this study, as in our previous report (4), the intravenous infusion of sodium [1-^{13}C]acetate (Cambridge Isotope Laboratories, Andover, MA) at 370 mg/h began 15 h before breakfast and continued in most subjects for at least an additional 24 h until the following morning, for a total of 39 h. Four of the earliest subjects had the infusion continued for 30 h until midnight. Meals were provided at 9 am, 1 pm, and 5 pm. As the second morning value was higher than the first in 8 lean and obese subjects on one or both diets, the second (39 h) fasted value was used in data analysis. The total infusion time was increased from 39 to 63 h in 4 subjects (3 lean, 1 obese) to confirm that a maximum fasting 16:0 enrichment had been nearly reached by 39 h. In all subjects on both diets, the calculated ^{13}C enrichment in the acetyl CoA precursor pool (p) was stable at ~4% for the duration of each study.

Isolation of VLDL, lipid extraction, and fatty acid analysis

Every 2–3 days, at least 12 h after the last meal, 15 ml of blood was drawn in EDTA on ice, and the plasma was separated by low-speed centrifugation at 4°C for 20 min. At the end of each diet period during the [^{13}C]acetate infusion, blood was sampled every 3–6 h for 24–48 h. Chylomicrons were removed and VLDL was isolated by density gradient ultracentrifugation, as previously described (4). The fatty acid composition of VLDL TG was analyzed by capillary gas chromatography after chloroform–methanol extraction and separation by thin-layer chromatography (4). The fatty acid compositions of the diets and subcutaneous adipose tissue sampled from abdominal and gluteal sites before admission and at the end of each diet period were similarly analyzed.

Analysis of cholesterol, triglyceride, and HDL cholesterol

In EDTA-treated plasma obtained every 2–3 days after an overnight fast and every 3 h over 24 h at the end of each diet period, the concentrations of cholesterol and TG were measured enzymatically (Boehringer Mannheim reagents, Indianapolis) and the HDL-C was measured after precipitation of apolipoprotein B-containing lipoproteins by dextran sulfate (31).

Analysis of glucose, insulin, and glucagon

At the end of each diet period, glucose, insulin, and glucagon were measured after an overnight fast. Meals were provided at 9 am, 1 pm, and 5 pm. Samples were obtained at 0, 0.5, 1, 1.5, 2, 3 h after breakfast, then every 2 h (2 h after lunch, just before dinner, and 2 and 4 h after dinner), then every 3 h until the following morning. Glucose was measured by a glucose oxidase assay and insulin was analyzed by ELISA (Abbott Laboratories). Glucagon was measured in duplicate by double antibody radioimmunoassay of EDTA/aprotinin-treated plasma (Diagnostic Products).

Indirect calorimetry

The resting energy expediture (REE) and respiratory quotient (RQ) were measured with a DeltaTrac Metabolic Cart (Sensormedic, Yorba Linda, CA) at the end of each diet period in the morning, 12 h after the last meal and after 20 min rest. Breath samples were analyzed from a ventilated hood for carbon dioxide production and oxygen consumption every min for 30 min. The mean of values obtained during the second 15 min was used for analysis. The RQs were corrected for protein oxidation, which was estimated from nitrogen excretion (as urea, creatinine, and uric acid) in 24 h urine collections from the same day.

Statistical methods

Differences in fatty acid synthesis and other variables between lean and obese subjects were statistically evaluated as a function of diet using 2-way repeated analysis of variance (ANOVA) and a significance value of $P < 0.01$. Simple linear regression was used to assess relationships among continuous variables. Scatter plots were drawn to check for nonlinear relationships and outliers. The meal-stimulated and 24-h areas under the curves for insulin, glucose, and glucagon were computed using the trapezoid rule. A lack of period and cross-over effects was confirmed using unpaired *t* testing of the results obtained from each diet sequence. Data analysis was performed using Excel and Statistica statistical software.

RESULTS

Fatty acid synthesis

A marked increase in the % de novo synthesized fatty acids in VLDL TG on the 10% fat diet compared to the 30% fat diet by the linoleate dilution method is shown in **Fig. 1**. On the 10% fat diet after an overnight fast (FASTED), newly synthesized fatty acids in VLDL TG comprised 27 ± 22% of total fatty acids compared to 1 ± 3% on the 30% fat diet ($P < 0.001$). Contrary to our expectations, lean (solid line) and obese (dotted line) subjects showed no significant difference in response to the two diets (31 ± 22% vs. 1 ± 3% for lean compared to 21 ± 21 vs. 1 ± 2% for obese). The fasting fatty acid compositions in VLDL TG were constant the last 4 days of the 14-day diets, indicating that maximal stimulation or suppression of fasting fatty acid synthesis had occurred by day 10, as was also observed in our previous study of 25 days (4). As shown on the right (FED), the maximum values in the evening after meals 3–9 h after the last meal were also higher on the 10% fat diet compared to the 30% fat diet (41 ± 13 vs. 10 ± 11%, $P < 0.001$). Again, there was no significant difference in response between lean and obese subjects (43 ± 13 vs. 12 ± 13% for lean compared to 37 ± 15 vs. 6 ± 6% for obese).

The synthesis of 16:0 calculated by the MIDA method is shown in **Fig. 2**. On the left, the results after an overnight fast on the 10% fat versus 30% fat diets were qualitatively the same as those obtained with the linoleate dilution method for the overall group (33 ± 13 vs. 18 ± 11%, $P = 0.002$), for the lean (37 ± 15 vs. 19 ± 13) and the obese (27 ± 7 vs. 16 ± 9). On the right, the maximum fed values for the overall group were 44 ± 10 versus 27 ± 11 ($P < 0.001$), for the lean, 49 ± 7 versus 28 ± 12%, and the obese, 36 ± 10 versus 24 ± 10%. Again, for both diets, whether fasted or fed, there was no significant difference in response between lean and obese subjects.

There was large intersubject variability with both methods on the 10% fat diet in both lean and obese subjects. A further inspection of this finding revealed two different patterns in fatty acid synthesis over 24-h. In **Fig. 3**, the individual plots of the 24-h time courses of % de novo fatty acids in VLDL TG by the linoleate dilution method at the end of the 10% fat diet are divided into two groups. On the left are lean and obese subjects with fasting values >25% (mean of 3 days, 45 ± 13%, n = 10); on the right

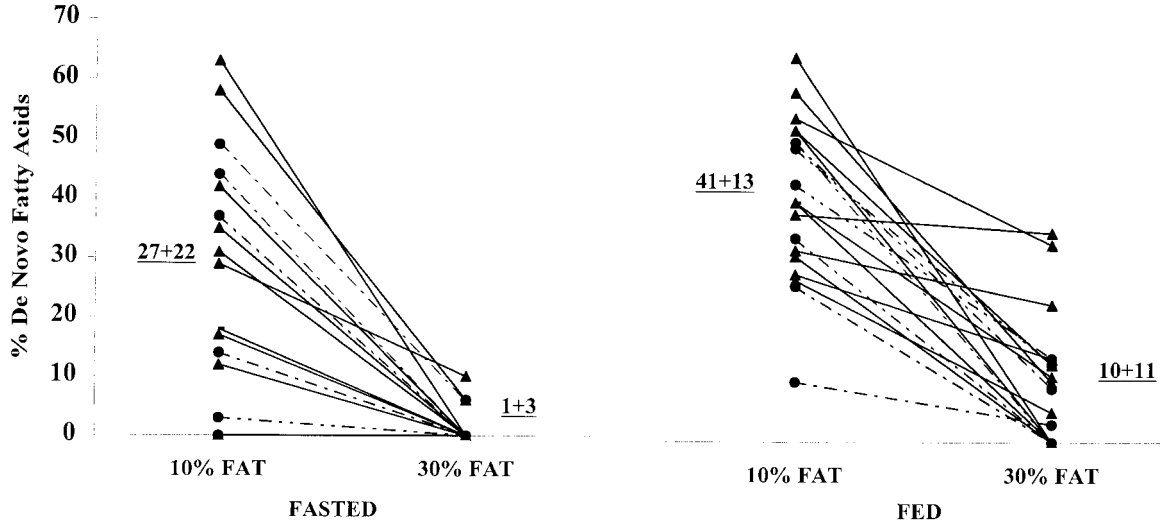

Fig. 1. Percent de novo synthesized fatty acids in VLDL TG calculated by the linoleate dilution method on the 10% and 30% fat diets. Triangles, lean; Circles, obese. Fasted, mean of 3 values/subject after an overnight fast in the last week of each diet; Fed, value at time of maximum fractional synthesis (9 pm–3 am) in samples taken over 24 h at the end of each diet. Mean ± SD for all subjects (n = 19) on each diet shown to the left and right of symbols ($P < 0.01$, 10% vs. 30% fat diet, fasted and fed).

are subjects with fasting values <25% (mean of 3 days, 7 ± 8%, n = 9). The subjects on the left with high fasting fatty acid synthesis showed a sustained "constant" elevation over 24 h. This pattern closely resembled the 24-h pattern obtained in our previous study with formula diets made with 75% glucose polymers (mean fasting value, 44 ± 10%) (4). In contrast, most of the subjects on the right with low fasting fatty acid synthesis showed a "diurnal" pattern, with a large increase after meals, peaking between 9 pm and 3 am (peak maximum 33 ± 12%) and then returning to baseline by 9 am the next morning.

These two 24-h patterns for total de novo fatty acids were mirrored in our measurements of de novo 16:0 synthesis by the MIDA method (mean fasting and maximum fed values for "constant" group: 43 ± 11% and 45 ± 9%, $P = 0.71$; for "diurnal" group: 23 ± 6% and 43 ± 11%, $P < 0.001$). In addition, there was general congruence between the 24-h patterns on the 10% fat and 30% fat diets in individual subjects.

The "constant" and "diurnal" groups did not differ by age, sex, body mass index, waist-to-hip circumference ratio, or other clinical characteristic. For all subjects, there was no statistically significant difference in fatty acid synthesis between males and females by either method. However, only 2/9 premenopausal women received the 10% fat diet during the follicular phase of the menstrual cycle when fatty acid synthesis has been reported to be higher than in males (25).

Fatty acid composition of VLDL TG

Table 2 shows the effects of the diets on the concentrations of each of the major fatty acids (18:2, 16:0, and 9*c*-18:1) in VLDL TG. The first column lists the mean con-

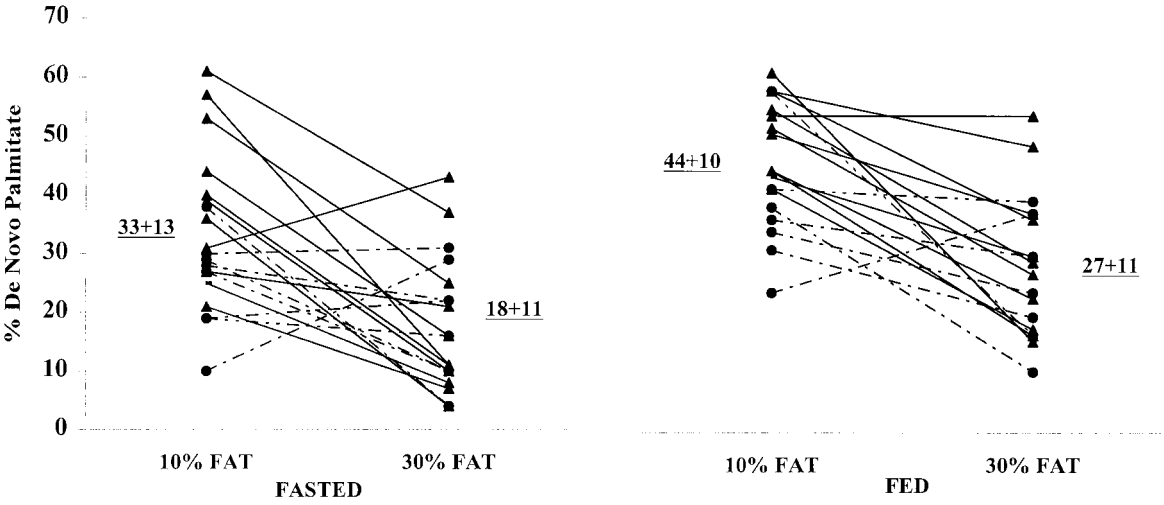

Fig. 2. Percent de novo synthesized 16:0 in VLDL TG calculated by mass isotopomer distribution analysis (MIDA) on the 10% and 30% fat diets ($P < 0.01$, 10% vs. 30% fat diet, fasted and fed). Symbols as in Fig. 1.

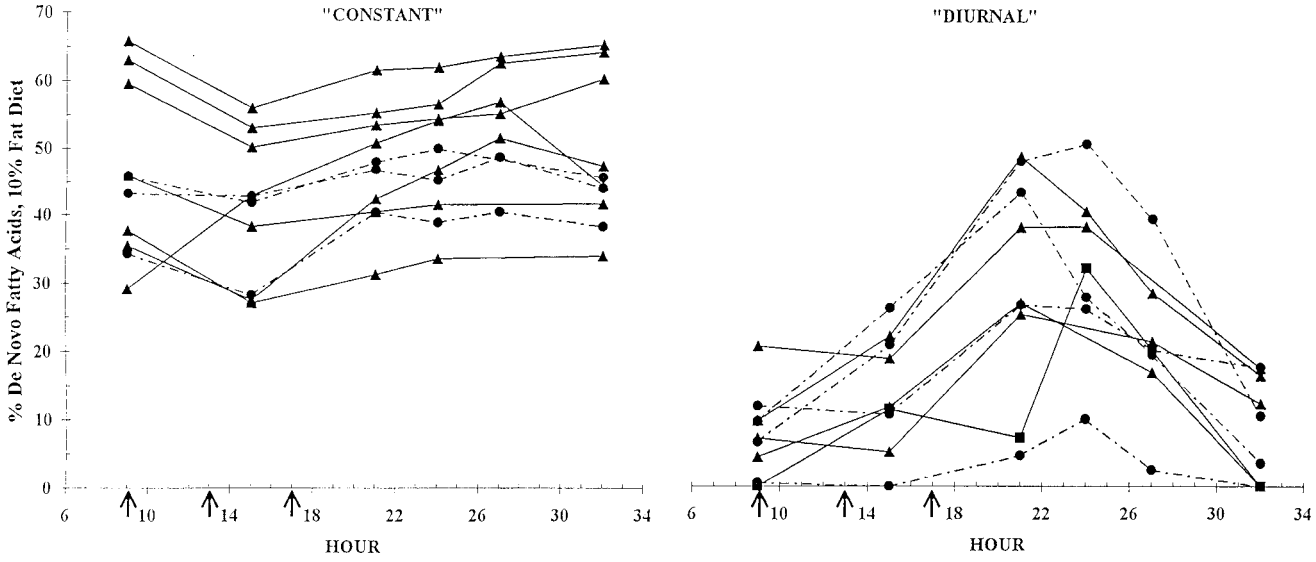

Fig. 3. Percent de novo synthesized fatty acids in VLDL TG calculated by the linoleate dilution method over 24 h at the end of the 10% fat diet. Symbols as in Fig. 1. Subjects were divided into those with high fasting values (>25%, left panel, n = 10) and low fasting values (<25%, right panel, n = 9). Most subjects with high fasting values sustained these values in a "constant" pattern over 24 h; most with low values had a "diurnal" pattern, with a peak between 9 pm and 3 am, and then return to baseline. The arrows show the times of the meals.

centrations of the major fatty acids in the adipose tissue and diet in lean and obese subjects on both diets. As expected, the adipose tissue fatty acid compositions to which the diet compositions were matched were stable for the duration of the study (range of %18:2 for all subjects was 14–25%). There were no differences in the average fatty acid composition of the diet or adipose tissue between lean and obese subjects or subjects with the "constant" and "diurnal" pattern of fatty acid synthesis.

TABLE 2. 10% versus 30% fat diet in lean and obese subjects; mean fatty acid compositions of adipose tissue, diet, and VLDL TG

Diet	Diet and AT	VLDL TG		Difference[a]	
		Fasted[b]	Fed[c]	Fasted[b]	Fed[c]
	% weight of total fatty acids			*%*	
10% fat					
18:2					
Lean	17.4	12.4	9.9	−29 ± 25[d]	−43 ± 12[d]
Obese	17.6	14.1	11.0	−20 ± 24[d]	−37 ± 15[d]
16:0					
Lean	19.8	27.7	32.7	40 ± 23[d]	65 ± 13[d]
Obese	20.2	29.6	35.3	47 ± 26[d]	76 ± 22[d]
9*c*-18:1					
Lean	37.8	33.2	32.1	−12 ± 6[e]	−15 ± 9[e]
Obese	38.9	30.5	29.6	−21 ± 5	−24 ± 7[d]
30% fat					
18:2					
Lean	17.1	19.5	15.6	14 ± 10	−9 ± 16
Obese	17.1	18.8	16.9	10 ± 10	−2 ± 12
16:0					
Lean	19.6	22.5	25.7	15 ± 10	32 ± 21
Obese	19.9	24.9	26.7	25 ± 11	34 ± 12
9*c*-18:1					
Lean	38.1	35.0	34.9	−8 ± 7	−8 ± 12
Obese	39.5	33.6	35.2	−15 ± 5	−11 ± 5

[a] Percent (%) difference for each fatty acid: (VLDL TG minus mean of diet and adipose tissue) × 100/mean of diet and adipose tissue (AT); % difference for 18:2 = % de novo fatty acids in VLDL TG (when < 0) by the linoleate dilution method.

[b] Mean of three values per subject (±SD) after an overnight fast in the last week of each diet.

[c] Value at time of maximum fractional synthesis (9 pm–3 am) in samples taken over 24 h at the end of each diet (±SD).

[d] $P < 0.01$, 10% vs. 30% fat diet.

[e] $P < 0.01$, lean vs. obese.

In the next two columns, as in our previous studies, on the 10% fat diet, there was a large, decrease in %18:2 and increase in %16:0 in VLDL TG compared to the percentages in the diet and adipose tissue. The decrease in 18:2 in VLDL TG relative to the diet/adipose tissue (% difference) was equivalent to the % de novo fatty acids and, as previously described, was significantly greater at both Fasted and Fed time points on the 10% versus 30% diet but was similar in lean and obese subjects. The % difference for 16:0 also was significantly higher on the 10% versus 30% fat diet at both Fasted and Fed time points ($P <$ 0.01) and was similar in lean and obese subjects.

The % difference for 9*c*-18:1 was significantly negative on the 10% versus 30% fat diet (fed only) and in obese subjects compared to lean subjects (fasted and fed, $P <$ 0.01). The decrease in 9*c*-18:1 did not appear to be due to a difference in stearyl Δ 9 desaturase activity, as the ratio of saturates to monounsaturates (16:0 + 18:0/9*c* − 16:1 + 9*c* − 18:1) did not differ between diets or subject groups. As expected, large decreases were also observed in the minor fatty acids which are not synthesized de novo (*trans* and n–3 linolenate) in VLDL TG relative to levels in the diet and adipose tissue (data not shown). There were no discernible differences in the fatty acid compositions of VLDL TG in subjects with the "constant" versus "diurnal" pattern of fatty acid synthesis, other than the previously described different shifts in 18:2/16:0 in parallel with the 24 h pattern of % de novo fatty acids.

It should be noted in Table 2 that on the 30% fat diet in both lean and obese subjects, the % increase for 16:0 was

high (15–34%) despite the small % decrease for 18:2 (0–9%). This probably represents an accumulation of newly synthesized 16:0 but low conversion to 18:0, 9*c*-18:1, and 9*c*-16:1 and an insufficient accumulation of total de novo fatty acids to "dilute" 18:2. In support of this, the % difference for 16:0 (Table 2) closely agreed with values for % de novo 16:0 by MIDA (Figure 2), which were also higher than the % total de novo fatty acids calculated by the linoleate dilution method (Figure 1). Despite the differences in the ability to detect low levels of synthesis and metabolites of 16:0, there was good correlation between measurements of the % de novo 16:0 by MIDA and the % total de novo fatty acids by the linoleate dilution method for fasted and fed time points on both diets ($R^2 = 0.63$, $P < 0.001$).

Total triglyceride, cholesterol, and HDL cholesterol

Table 3 shows the much higher concentrations of plasma TG (50–60%), small but significantly lower HDL-C (10–18%), and lack of difference in total cholesterol on the 10% compared to the 30% fat diets, after an overnight fast and over 24 h, in both lean and obese subjects. The higher levels of TG were paralleled by higher levels of VLDL TG (not shown). TG levels tended to be higher in obese than lean subjects on both diets, but the difference was not statistically significant. On both diets, in lean, obese, "constant" and "diurnal" groups, the TG increased after breakfast, peaked after dinner and then dipped below baseline during the early morning hours, with a return to baseline by 9 am. HDL-C levels were inversely related to TG on both diets at borderline statistical significance.

As with fatty acid synthesis, in both lean and obese subjects, there was large intersubject variability in the TG level on each diet and in the change in TG level on the 10% versus 30% fat diets (−15% to +249%). Of note, on the 10% fat diet, 9/19 subjects had fasting TG values of >150 mg/dl, and 8/9 of these subjects had high fasting fatty acid synthesis ("constant" pattern). Furthermore, the fasting and 24-h TG concentrations in subjects with the "constant" pattern were double those in subjects with the "diurnal" pattern (fasting: 197 vs. 108 mg/dl; 24 h average: 203 vs. 107 mg/dl, $P < 0.001$). Thus, on the 10% fat diet, the "constant" pattern of fatty acid synthesis was associated with much higher TG levels than the "diurnal" pattern. Fasting and 24-h HDL-C levels were 20% lower in the "constant" versus "diurnal" group, at a borderline level of statistical significance. There were no differences in fasting or 24 h TG and HDL-C between the two groups on the 30% fat diet.

Figure 4 shows on the left the positive relation between the fold increase in the fasted concentration of TG on the 10% fat versus 30% fat diet and the fasted % de novo fatty acids in VLDL TG on the 10% fat diet. A similar relation was found for the % de novo 16:0 by MIDA, shown on the right. An equally strong positive relationship existed for the ratio of the 24 h average TG on the two diets (data not shown). Similar, though less strong, positive relations were found with the linoleate dilution method between the maximum fed % de novo fatty acids on the 10% fat diet and the TG response ratios ($P = 0.01$) and between the fasting % de novo fatty acids and the TG level on the 10% fat diet ($P = 0.03$). The ratio of HDL-C on the two diets was not related to fatty acid synthesis calculated by either method.

Insulin, glucose, and glucagon

To explore the mechanism for the increase in fatty acid synthesis and the associated increase in TG on the 10% fat diet, levels of blood insulin, glucose, and glucagon were measured over 24 h at the end of each diet period. The results, summarized in **Table 4**, show that, as expected, fasted, meal-stimulated, and 24-h insulin levels were about double in obese compared to lean subjects. Also as expected, meal-stimulated and 24-h, but not fasting, insulin levels were higher on the 10% fat diet compared to the 30% fat diet in both lean and obese subjects. Glucose levels were higher and glucagon levels were lower in the obese compared to the lean at borderline significance levels ($P = 0.03$), but were not different between diets. There were no significant differences in insulin, glucose or glucagon levels between "constant" and "diurnal" groups.

Regression analysis failed to show a relationship between fasted, meal-stimulated, or 24-h insulin levels and fasted or fed % de novo fatty acids on either diet calculated by linoleate dilution (**Fig. 5**) or MIDA. The same held true for glucose and glucagon values or ratios of insulin to glucose or insulin to glucagon. Thus, the increase in de novo synthesized fatty acids on the 10% fat diet was

TABLE 3. 10% versus 30% fat diet in lean and obese subjects: triglyceride, cholesterol, and HDL cholesterol

	Triglyceride		HDL Cholesterol		Cholesterol	
Diet	Fasted	24 H	Fasted	24 H	Fasted	24 H
	mg/dl		*mg/dl*		*mg/dl*	
10% fat						
Lean	139 ± 52[a]	138 ± 61[a]	37 ± 10[a]	37 ± 10[a]	159 ± 15	156 ± 13
Obese	183 ± 101[a]	190 ± 101[a]	37 ± 10[a]	35 ± 7[a]	177 ± 25	171 ± 24
30% fat						
Lean	90 ± 33	108 ± 46	45 ± 15	44 ± 14	165 ± 19	163 ± 14
Obese	113 ± 38	129 ± 49	41 ± 11	38 ± 10	177 ± 29	168 ± 29

Values given as mean ± SD; fasted, mean of 3 values after an overnight fast in the last week of each diet; 24 H, average of 8 samples obtained every 3 h over 24 h at the end of each diet.

[a] $P < 0.01$, 10% vs. 30% fat diet; no significant differences for cholesterol or lean vs. obese.

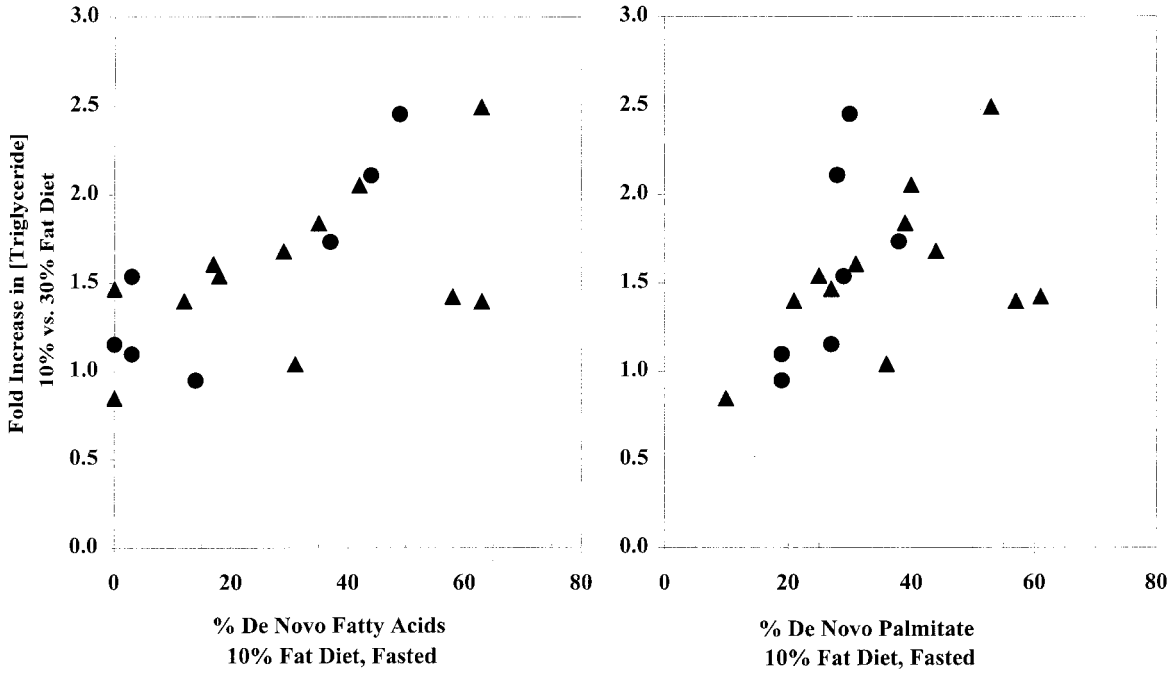

Fig. 4. Left plot: the relationship between the fasting percent de novo synthesized fatty acids in VLDL TG calculated by the linoleate dilution method on the 10% diet and the fold increase in the fasting concentration of triglyceride on the 10% vs. the 30% fat diets. Symbols as in Fig. 1. Regression analysis showed a significant positive relationship, $P = 0.004$; $R^2 = 0.40$. Right plot: values calculated by MIDA for the fasting percent de novo synthesized 16:0 in VLDL TG on the 10% fat diet, $P = 0.07$, $R^2 = 0.18$.

mediated by a mechanism not reflected in blood insulin, glucose, or glucagon concentrations.

In contrast, meal-stimulated and 24-h insulin levels were related to TG levels on each diet as reported by others (19, 32, 33). The strongest relationship was between the fasted TG versus 24-h insulin levels on the 30% fat diet ($P = 0.009$). Insulin levels were also positively related to the waist:hip circumference ratio ($P < 0.01$). Because fatty acid synthesis was low on the 30% fat diet, the TG increase associated with insulin levels is most likely related to other mechanisms, such as delayed TG clearance (19).

Energy expenditure

As in our previous studies (4, 5), there were no detectable differences in energy homeostasis between the 10% and 30% fat diets, despite differences in fatty acid synthesis. REE on both diets was identical for the lean (1445 ± 215 vs. 1412 ± 227 kcal) and obese (1568 ± 276 vs. 1564 ± 242 kcal); the values were higher in obese than lean subjects, as was expected. The total calories to maintain constant body weight were also the same on the two diets (lean = 2431 ± 264 vs. 2454 ± 311 kcal; obese = 2793 ± 323 vs. 2794 ± 324 kcal) and were similar per surface area in lean and obese subjects (1360 kcal/m^2). The fasting nonprotein RQ was less than 1.0 in all subjects after both diets, consistent with a lack of net fat synthesis and storage. It was slightly higher after the 10% than 30% fat diet, as expected due to the greater carbohydrate content of the 10% fat diet (lean = 0.89 ± 0.07 vs. 0.87 ± 0.04; obese = 0.88 ± 0.06 vs. 0.84 ± 0.05). Finally, subjects with high and low fasting fatty acid

TABLE 4. 10% versus 30% fat diets in lean and obese subjects: insulin, glucose, and glucagon

	Insulin			Glucose			Glucagon		
Diet	Fasted	Meal	24 H	Fasted	Meal	24 H	Fasted	Meal	24 H
	microunits/ml			*mg/dl*			*pmol/ml*		
10% fat									
Lean	6 ± 2[a]	126 ± 56[ab]	485 ± 155[ab]	84 ± 5	278 ± 28	2254 ± 28	71 ± 18	226 ± 61	1654 ± 472
Obese	13 ± 6	241 ± 130[b]	924 ± 340[b]	85 ± 7	325 ± 35	2411 ± 84	56 ± 8	152 ± 46	1194 ± 333
30% fat									
Lean	6 ± 1[a]	93 ± 29[a]	361 ± 66[a]	83 ± 6	282 ± 26	2237 ± 112	69 ± 20	224 ± 59	1654 ± 395
Obese	10 ± 2	169 ± 51	635 ± 192	86 ± 4	328 ± 34	2410 ± 124	56 ± 10	180 ± 33	1265 ± 340

Values given as mean ± SD; fasted, 12 h overnight fast at the end of each diet; meal, area under the curve over 3 h after the breakfast meal at the end of each diet; 24 h, area under the curve over 24 h at the end of each diet.

[a] $P < 0.01$, lean vs. obese; insulin levels were significantly higher in obese vs. lean subjects. Insulin results from one lean female excluded because of partial missing data.

[b] $P < 0.01$. 10% vs. 30% fat diet; insulin levels were significantly higher on the 10% vs. 30% fat diet (meal and 24 h).

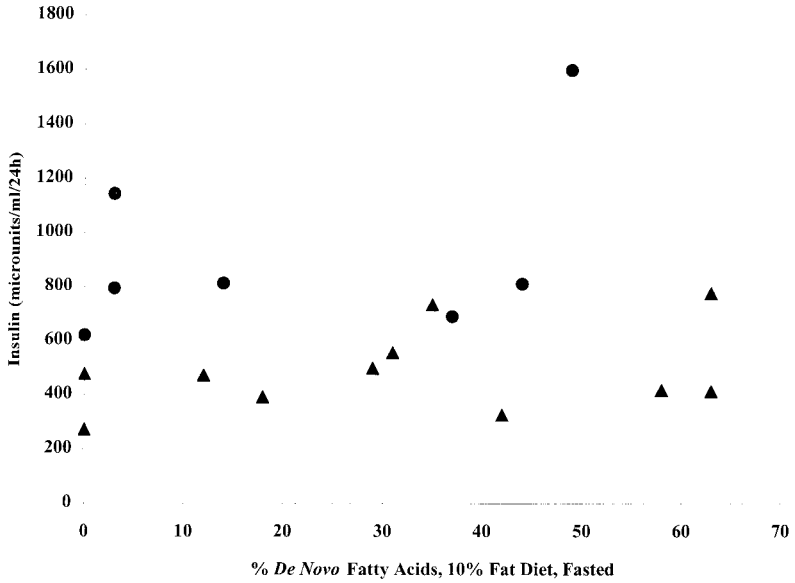

Fig. 5. Plot of the relationship between the fasting percent de novo synthesized fatty acids in VLDL TG calculated by the linoleate dilution method and the concentration of insulin (24 h area under the curve) at the end of the 10% fat diet. Symbols as in Fig. 1. Regression analysis showed no significant relationship, $P = 0.724$. Data from one lean female excluded because of partial missing insulin data.

synthesis on the 10% fat diet ("constant" and "diurnal" patterns) had similar fasting nonprotein RQs.

DISCUSSION

Until recently, it was generally believed that the synthesis of fatty acids was negligible in weight-stable humans. We previously demonstrated that human fatty acid synthesis was markedly stimulated by eucaloric, very low fat, formula diets made entirely with simple carbohydrate (4), and was low when the carbohydrate had a low ratio of simple to complex carbohydrate (40:60) (5). The present study utilized eucaloric, very low fat diets with a high ratio of simple to complex carbohydrate (60:40), composed of solid foods that are commonly consumed by the general population. Similar to our studies with formula diets, fatty acid synthesis was increased and associated with a large increase in 16:0, the fatty acid preferentially synthesized by mammalian fatty acid synthase, and a decrease in the essential fatty acid, 18:2, in VLDL TG.

We had expected that obese subjects would be more sensitive to the effects of dietary carbohydrate due to the lipogenic effects of their high insulin levels. However, despite almost 2-fold higher insulin levels in the obese, fatty acid synthesis was similar in the lean and obese when measured by either the linoleate dilution or MIDA method. Furthermore, no significant relationship was found between insulin levels and fatty acid synthesis in either lean or obese subjects. Thus, the stimulation of human fatty acid synthesis by dietary carbohydrate is not directly related to body mass index or insulin levels.

We observed a significant positive association between fatty acid synthesis and the increase in TG levels produced by the low fat diet. As has been reported in previous studies (13), the magnitude of the increase in TG levels on the low fat diet was highly variable. A similar high intersubject variability was found for fatty acid synthesis when measured after an overnight fast. Evaluation of the data revealed that the TG increases on the low fat diet were related to two distinct 24-h patterns of fatty acid synthesis. Subjects with a "constant" pattern characterized by high fasting fatty acid synthesis (>25% de novo fatty acids in VLDL TG), with a sustained elevation over 24 h, had the highest fasting TG levels. In contrast, subjects with a "diurnal" pattern characterized by a low fasting fractional fatty acid synthesis (<25% de novo fatty acids in VLDL TG) followed by a peak in the late evening and return to low levels the next morning had much lower TG levels. Only the "constant" pattern was observed in 16 subjects in our previous studies with 10% fat, liquid formula diets made entirely with simple carbohydrate (short chain glucose polymers or a mixture of glucose, sucrose, fructose, and lactose). Thus, when very low fat diets are composed of both simple and complex carbohydrate, interindividual differences in the 24-h pattern of fatty acid synthesis are associated with interindividual differences in TG levels.

Although the metabolic basis for the "constant" and "diurnal" patterns of fatty acid synthesis remains uncertain, the two patterns may relate to *1*) an immediate increase in lipogenesis by fructose (14, 34) and *2*) a more gradual, sustained up-regulation of de novo lipogenic enzymes by simple carbohydrate (glucose, fructose, glucose polymers). The first mechanism may be operative in the "diurnal" pattern with low fasting fatty acid synthesis, whereas the second may be dominant in the "constant" pattern with high fasting fatty acid synthesis. Alternatively, the two patterns may result from differential inhibition by com-

plex carbohydrates (resistant starch, soluble fiber) which, upon fermentation in the large intestine, produce short chain fatty acids taken up by the liver with effects on lipid metabolism (35–37). This may be a less likely mechanism, as we previously showed that, in contrast to the marked inhibitory effects of starch, the addition of soluble fiber to a very low fat formula diet made with simple carbohydrate did not inhibit fatty acid synthesis (5).

In vitro, insulin, glucagon, glucose/fructose, and fatty acids act as direct mediators of the transcription of fatty acid synthase and other enzymes in the biosynthetic pathway (1, 38). These hormones and nutrients signal response elements in many of the genes coding for these enzymes. Furthermore, several diet-responsive nuclear transcription factors (e.g., SREBP1, GRBP, USF) regulating the synthesis of lipogenic enzymes have recently been identified (2, 39–42). These studies suggest that the differences in insulin levels, glucose, and fatty acid flux between the lean and obese should lead to differences in the transcription and activity of the enzymes controlling fatty acid synthesis. Yet, the current study suggests that other regulators common to both lean and obese subjects, such as gastrointestinal hormones or factors influencing the digestion of carbohydrates, may be more relevant to the dietary regulation of fatty acid synthesis in vivo in humans.

In contrast to the current study, we previously showed that 10% fat solid food or formula diets with a lower sugar/starch (40:60 vs. 60:40) produced near zero fasting % de novo fatty acids in VLDL TG followed by a small increase in the late evening (4, 5). Another recent study also found low fasting synthesis of 16:0 by the MIDA method (~3%) in normal and mildly hypertriglyceridemic subjects after 5 weeks of a 15% fat eucaloric solid food diet with a similar low ratio of sugar to starch (7). Kinetic studies showed that the higher TG level on the 15% versus the 35% fat control diet was mainly due to delayed clearance of VLDL TG synthesized from preformed plasma nonesterified fatty acids. Thus, depending on the source of carbohydrate, very low fat diets can increase TG levels by mechanisms other than increased fatty acid synthesis.

It should be noted that higher fatty acid synthesis in adipose tissue of obese compared to lean subjects would be detected by the methods in this study only if there is immediate release into the plasma of newly synthesized fatty acids which are then incorporated into VLDL TG after uptake by the liver. Other in vitro studies, however, have not found a difference in fatty acid synthesis between obese and lean human adipose tissue when expressed per gram of fat (43, 44). Furthermore, when obese subjects were overfed a high carbohydrate diet, only a minor increase in fatty acid synthesis was estimated for the total adipose tissue mass (45). Additional studies are needed for a thorough analysis of the impact of dietary carbohydrate on adipocyte fatty acid synthesis and function.

Another matter of interest in this study was the effect of de novo lipogenesis on energy balance. There were no detected differences in energy expenditure between the 10% and 30% fat diets, thus it is likely that the calories expended for the conversion of glucose or fructose into fat were small. This indicates that any advantages of low fat diets for the treatment of obesity are not related to the increased energy expenditure needed for the conversion of carbohydrate to fat.

In conclusion, lean and obese subjects had the same increase in fatty acid synthesis on eucaloric, very low fat solid food diets enriched in simple sugars. The increase in synthesis was not related to insulin, glucagon, or glucose levels. Both the linoleate dilution and MIDA methods showed that the increase in fatty acid synthesis followed two distinct 24-h patterns and was correlated with the highly variable increase in TG. As yet undefined mediators of the carbohydrate-induced stimulation of human fatty acid synthesis need to be identified. The effect of high carbohydrate diets on energy balance is small, yet the impact of de novo synthesized saturated fatty acids on the fatty acid composition and production of VLDL TG is large due to the relatively small pool of fatty acids in the plasma. The physiological consequences and potential atherogenicity of such changes must be elucidated, given the increasing public consumption of simple sugars (27). Future studies of these effects of low fat, high carbohydrate diets should lead to better dietary therapy for hypertriglyceridemia and cardiovascular disease.

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