Effect of High-Carbohydrate Feeding on Triglyceride and Saturated Fatty Acid Synthesis (44564B)

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Abstract. **It has been known for decades that low-fat, high-carbohydrate diets can increase plasma triglyceride levels, but the mechanism for this effect has been uncertain. Recently, new isotopic and nonisotopic methods have been used to determine *in vivo* whether low-fat, high-carbohydrate diets increase triglyceride levels by stimulating fatty acid synthesis. The results of a series of studies in lean and obese weight-stable volunteers showed that very-low-fat (10%), high-carbohydrate diets enriched in simple sugars increased the fraction of newly synthesized fatty acids, along with a proportionate increase in the concentration of plasma triglyceride. Furthermore, the concentration of the saturated fatty acid, palmitate, increased and the concentration of the essential polyunsaturated fatty acid, linoleate, decreased in triglyceride and VLDL triglyceride. The magnitude of the increase in triglyceride varied considerably among subjects, was unrelated to sex, body mass index, or insulin levels, and was higher when fatty acid synthesis was constantly elevated rather than having a diurnal variation. It was notable that minimal stimulation of fatty acid synthesis occurred with higher fat diets (>30%) or with 10% fat diets enriched in complex carbohydrate. Public health recommendations to reduce dietary fat must take into account the distinct effects of different types of carbohydrate that may increase plasma triglycerides and fatty acid synthesis in a highly variable manner. The mediators and health consequences of this dietary effect deserve further study.** [P.S.E.B.M. 2000, Vol 225:178–183]

Many recommendations have been made to the public to reduce dietary fat (1), with some claims that maximum health benefit will be achieved when fat is as low as 10% of total calories (2). However, when calorie balance is maintained, the reduction in dietary fat is mostly accompanied by an increase in carbohydrate, which, in turn, may be converted to fat. I will describe the results of recent studies that answer the question: Does a decrease in dietary fat and an equicaloric increase in dietary carbohydrate stimulate fatty acid synthesis in humans? The answer is important, since increased fatty acid synthesis may not only increase hepatic triglyceride synthesis and plasma triglyceride concentrations, but also enrich the triglyceride with palmitate, 16:0, the saturated fatty acid preferentially formed by mammalian fatty acid synthase. The plasma fatty acid pattern would be similar to that produced by a high-saturated-fat diet believed to promote atherosclerosis.

The substitution of dietary carbohydrate for fat has been known to increase plasma triglyceride since the 1950s, but studies of the mechanisms for this increase have produced conflicting results (3). An increase in the concentration of triglyceride in plasma may occur by decreased clearance of the triglyceride-rich lipoproteins (VLDL and/or chylomicrons) and/or increased triglyceride synthesis from either preformed or newly synthesized fatty acids and secretion from the liver into the plasma. When triglyceride synthesis is increased, the synthesis of apolipoprotein B carried in VLDL and the number of lipoprotein particles may also increase. Kinetic studies with labeled glycerol and fatty acid in small numbers of subjects fed low-fat or fat-free liquid formula diets supported decreased triglyceride fractional clearance (4), increased triglyceride synthesis (5, 6), or both (7) as important mechanisms. All studies showed large differences in response among individuals.

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Until recently, it was not possible to measure *in vivo* the relative contributions of preformed and newly synthesized fatty acids in humans. Using techniques of indirect calorimetry, it was concluded that fatty acid synthesis was a minor biochemical pathway in weight-stable humans (8). New stable isotopic methods are now available using ^{13}C-acetate or deuterated water as tracers (9–12). For our studies, we developed a nonisotopic method based on the dilution of the essential fatty acid, linoleate, by newly synthesized fatty acids in VLDL triglyceride and compared the method with stable isotopic techniques.

Measurement of Fatty Acid Synthesis *in Vivo*

The linoleate dilution method is based on the model that there are three major sources of fatty acids in VLDL triglyceride: preformed fatty acids from the diet and the adipose tissue, and newly synthesized, or *de novo*, fatty acids formed from carbohydrate (Fig. 1). Only two sources provide the essential fatty acid linoleate (18:2), a polyunsaturated fatty acid that we cannot synthesize. When the fatty acid composition of the diet is matched to the composition of each subject's adipose tissue, and there is no fatty acid synthesis, the proportion of 18:2 in triglyceride will be equal to the proportion in the diet and adipose tissue. But, with fatty acid synthesis, the concentration of 18:2 in triglyceride will be lower than the concentration in the diet and adipose tissue due to dilution by *de novo* fatty acids, mainly 16:0. The fraction of newly synthesized fatty acids in VLDL triglyceride is calculated from the percentage decrease in 18:2 below levels in the diet and adipose tissue, as previously described in detail (13). When weight is stable, the half-life of adipose tissue fatty acids is at least 1 year, and thus will minimally change during studies of short duration.

This method was validated by the simultaneous measurement of fatty acid synthesis after the intravenous infusion of ^{13}C-acetate. Using this procedure, the fraction of newly formed 16:0 in VLDL triglyceride was calculated by the MIDA (mass isotopomer distribution analysis) method (14). At the end of each diet period, ^{13}C-acetate was infused intravenously for 15 hr before the first meal and continued for 24–48 hr, with meals consumed at the usual intervals. After the isolation and fatty acid analysis of VLDL triglyceride, the enrichment of ^{13}C-labeled 16:0 was measured using electron-impact ionization and selective ion monitoring by gas chromatography mass spectrometry. The proportions of single- and double-labeled 16:0 were used to calculate the ^{13}C enrichment of the acetate precursor pool and the fraction of newly formed 16:0 in VLDL triglyceride.

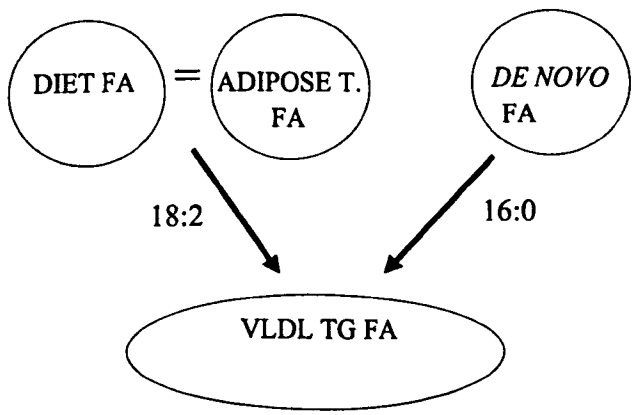

Figure 1. Model of the linoleate dilution method to measure fatty acid synthesis. The fatty acid compositions of the diet and adipose tissue are matched, and the fractional input of *de novo* fatty acids is calculated from the decrease, or dilution, of 18:2 in VLDL triglyceride below the concentrations of the diet and adipose tissue. For details, see Ref. 13.

Effects of a Low-Fat, High-Simple-Carbohydrate Formula Diet

Using these two *in vivo* methods, we measured fatty acid synthesis in 10 healthy male and female volunteers who consumed liquid formula diets for 25 days at The Rockefeller University General Clinical Research Center (13). The diets had either 10% calories as fat, 75% carbohydrate (seven subjects) or 40% fat, 45% carbohydrate (three subjects), given as five equicaloric meals per day and sufficient calories to maintain constant weight. In both diets, the carbohydrate was short-chained glucose polymers (Polycose). As required by the linoleate dilution method, the fatty acid composition of the dietary fat was matched to the adipose tissue composition of each subject by mixing olive oil, corn oil, and lard. The cholesterol content of all diets was 200 mg/day.

Figure 2 shows the marked alteration in the fatty acid composition of VLDL triglyceride on the 10% fat diet (squares). The top panel shows the 44% decrease in the fasting percentage 18:2 in VLDL triglyceride from baseline levels, with a plateau reached slowly by 10 days. This indicated that 44% of the fatty acid in VLDL triglyceride was newly synthesized on the 10% fat diet. The lower panel shows the corresponding increase in 16:0 to concentrations 54% above the concentrations in the diet and adipose tissue. The other major fatty acid, oleate (18:1) showed minor changes. At plateau, levels of VLDL triglyceride and total triglyceride were twice baseline.

In contrast to the marked change in the fatty acid composition on the 10% fat diet, the percentage 18:2 and 16:0 in VLDL triglyceride, diet, and adipose tissue were similar on the 40% fat diet (triangles). This indicated that the high-fat diet minimally stimulated fatty acid synthesis and that only preformed fatty acids from the diet and adipose tissue were incorporated into VLDL triglyceride. On both diets, the fatty acid composition of VLDL triglyceride was constant over 24 hr and did not fluctuate with meals. A similar constant 24-hr pattern of increased newly synthesized 16:0 in VLDL triglyceride on the 10% fat diet compared with the 40% fat diet was found after the infusion of ^{13}C-acetate at the end of each diet. The results confirmed previous reports of low levels of fatty acid synthesis when dietary fat was 30% of calories or higher (10,15).

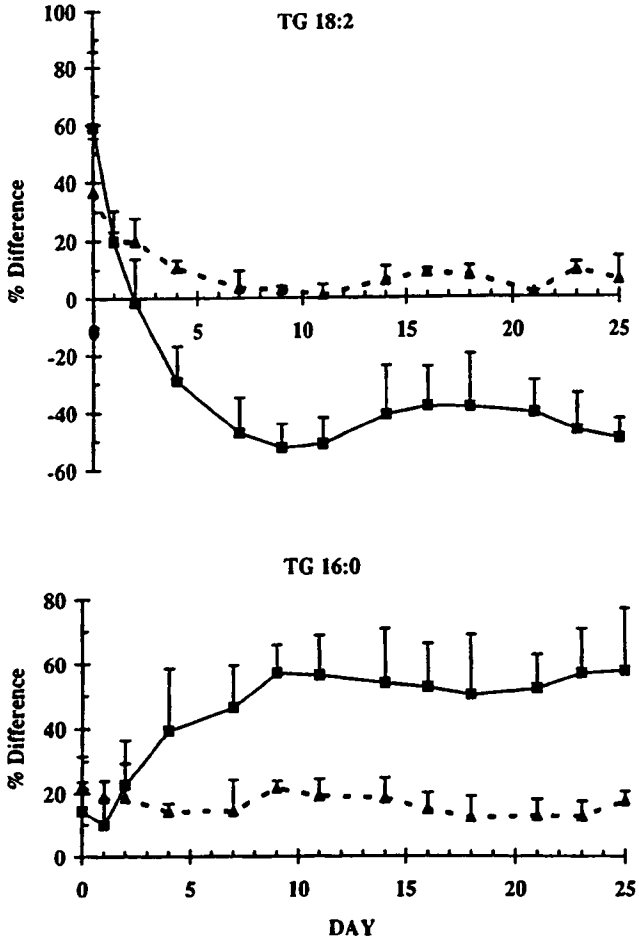

Figure 2. Percentage difference (mean ± SD) between the fatty acid compositions of VLDL triglyceride and the diet/adipose tissue for a 10% fat diet (squares, $n = 7$) and 40% fat diet (triangles, $n = 3$). Top, triglyceride 18:2; bottom, triglyceride 16:0. Reproduced from Ref. 13 by copyright permission from The American Society for Clinical Investigation.

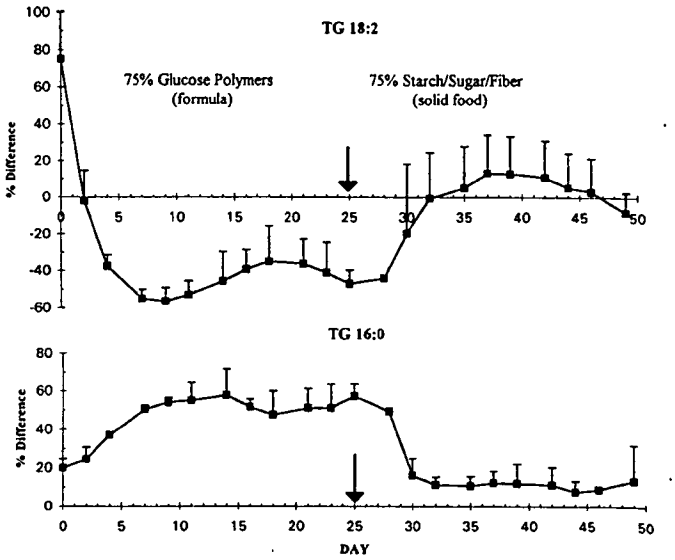

Figure 3. Percentage difference (mean ± SD) between the fatty acid compositions of VLDL triglyceride and the diet/adipose tissue. The arrow marks the change from the 10% fat, 75% glucose polymer formula diet to the 10% fat, 75% complex carbohydrate solid food diet. Top, triglyceride 18:2; bottom, triglyceride 16:0. Reproduced from Ref. 16 by copyright permission from The American Journal of Clinical Nutrition.

Effects of a Low-Fat, High-Complex-Carbohydrate, Solid-Food Diet

To determine whether a similar *de novo* lipogenic response would occur after a low-fat, solid-food diet, we studied four of the seven subjects who consumed the low-fat formula diet for an additional 25 days (16). They consumed single-menu isocaloric diets with 10% fat, 75% carbohydrate, and made with foods consumed in the general population. Unlike the formula diet made with simple carbohydrate, this diet was high in complex carbohydrates (starch/sugar 60/40; 12 g fiber/1000 cal). The fatty acid composition of each diet was matched to each subject's adipose tissue by adding a mixture of oils to each of the three meals.

Unexpectedly, we found a rapid inhibition of fatty acid synthesis as manifested by an increase in fasting percentage 18:2 in VLDL triglyceride on the low-fat, high-complex-carbohydrate diet (Fig. 3, at arrow). Accompanying the increase in 18:2 was a decrease in 16:0 in VLDL triglyceride (lower panel). Thus, the composition of the carbohydrate appeared to be an important variable affecting the lipogenic response to a low-fat, high-carbohydrate diet.

To distinguish the effects of starch, fiber, and sugar, we recruited nine more healthy volunteers who first consumed the same low-fat formula diet with 10% fat, 75% glucose polymers for 10 days. They then consumed one of three 10% fat formula diets made to resemble the simple sugar, starch, or fiber content in the solid food diet (16). This was achieved by using a mixture of simple sugars (glucose, fructose, sucrose, lactose), cornstarch/sugar (50/50), or glucose polymers plus sugarbeet fiber (12 g/1000 cal).

Figure 4 shows at the arrow the rapid increase in fasting concentration of 18:2 in fasting VLDL triglyceride after switching from the 10% fat diet made with glucose polymers to the 10% fat diet with starch. The concentration of 16:0 also declined. The addition of fiber had no inhibitory effect, and the mixture of sugars had a similar stimulatory effect as the glucose polymers (not shown). Thus, the low-fat, high-starch formula diet replicated the suppression in fatty acid synthesis observed with the low-fat solid food diet. These results suggested that the relative amounts of dietary starch and simple carbohydrate determined the *de novo* lipogenic response to a low-fat, high-carbohydrate diet.

That very-low-fat, high-complex-carbohydrate, solid-food diets minimally stimulate fatty acid synthesis was supported by data from a recent study of 10 normal volunteers with triglyceride levels <200 mg/dl (17). Volunteers first consumed a solid food diet with 35% fat, 50% carbohydrate for 1 week, and then an isocaloric diet with 15% fat, 70% carbohydrate provided as packed meals or consumed on site for 5 weeks. Both diets had a high ratio of complex carbo-

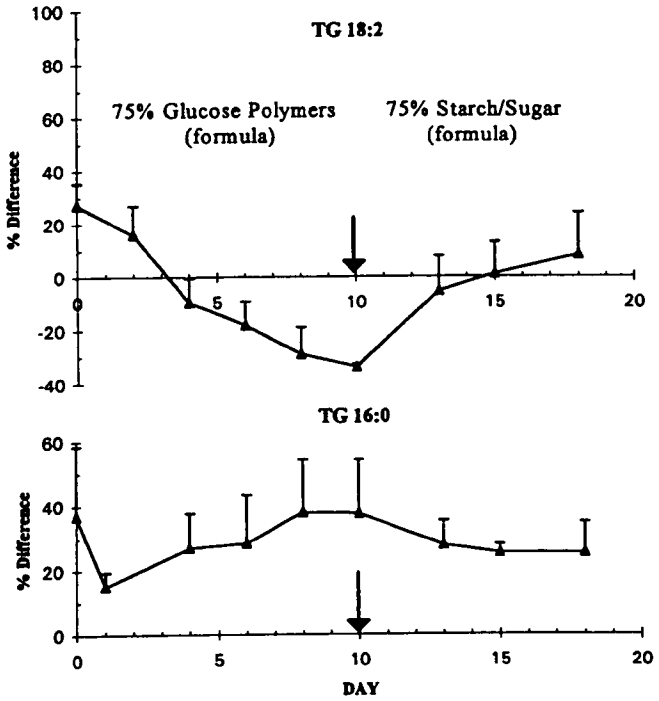

Figure 4. Percentage difference (mean ± SD) between the fatty acid compositions of VLDL triglyceride and the diet/adipose tissue. The arrow marks the change from the 10% fat, 75% glucose polymer formula diet to a 10% fat, 75% starch and sugar formula diet. Reproduced from Ref. 16 by copyright permission from *The American Journal of Clinical Nutrition.*

hydrates to simple sugar. On the 15% fat diet, although triglycerides increased by 60%, there was minimal fractional synthesis of palmitate, measured by MIDA after the intravenous infusion of ^{13}C-acetate. There was also little effect on triglyceride synthesis and hepatic secretion into the plasma, using labeled palmitate and glycerol. Instead, the major mechanism for the increase in triglyceride was attributed to decreased triglyceride clearance. Therefore, the mechanism for increase in triglyceride after a low-fat diet may differ according to the type of carbohydrate.

Effects of a Low-Fat, High-Simple-Carbohydrate, Solid-Food Diet

We had shown that low-fat formula diets made with simple carbohydrate increased fatty acid synthesis and markedly altered the composition of VLDL triglyceride, but had yet to show that fatty acid synthesis was stimulated by low-fat, solid-food diets composed of a mixture of simple and complex carbohydrates typically consumed in the general population. Since fatty acid synthesis is elevated in several rodent models of obesity (18, 19), possibly due to the higher serum insulin and lower glucagon levels, we also wanted to know whether obese individuals were more sensitive to carbohydrate-induced fatty acid synthesis than lean individuals. Using the MIDA method in a small number of lean and severely obese, hyperinsulinemic subjects after an uncontrolled, *ad libitum* diet, fractional palmitate synthesis was positively related to body mass index (20).

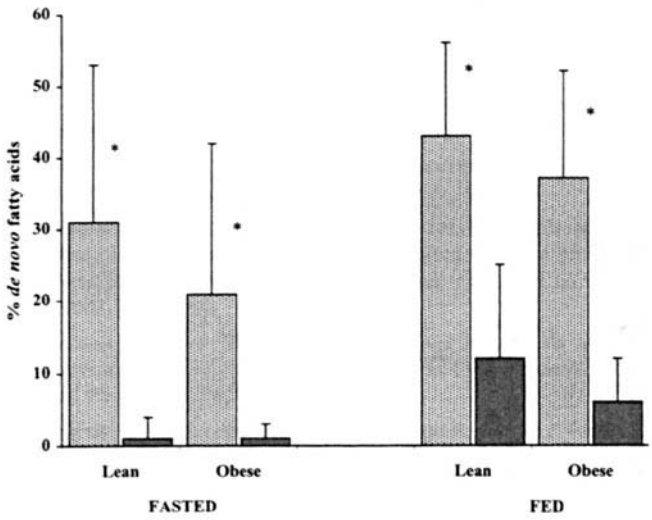

Figure 5. Percentage of newly synthesized fatty acids in VLDL triglyceride (mean ± SD) calculated by the linoleate dilution method in 19 lean and obese subjects on two solid food diets high in simple sugars. Light bar, 10% fat diet; dark bar, 30% fat diet. *$P < 0.01$, 10% vs. 30% fat diet, fasted and fed. For details, see Ref. 21.

Twelve lean and seven obese male and female volunteers with normal lipid levels and 3-hr oral glucose tolerance tests were studied for 1 month at the Rockefeller GCRC. We compared two isocaloric solid food diets that differed in the amount of carbohydrate and fat. A single menu of three meals was given each day for 2 weeks in random order without a washout period. The total calories required minimal adjustment to maintain weight within 0.5 kg and were the same for both diets. One diet had 10% of calories as fat and 75% as carbohydrate; the other 30% of calories as fat and 55% as carbohydrate. Both diets had a high ratio of sugar to starch (60/40). Fiber, about 1/3 soluble, was 12 g/1000 cal on the 10% fat diet and propor-

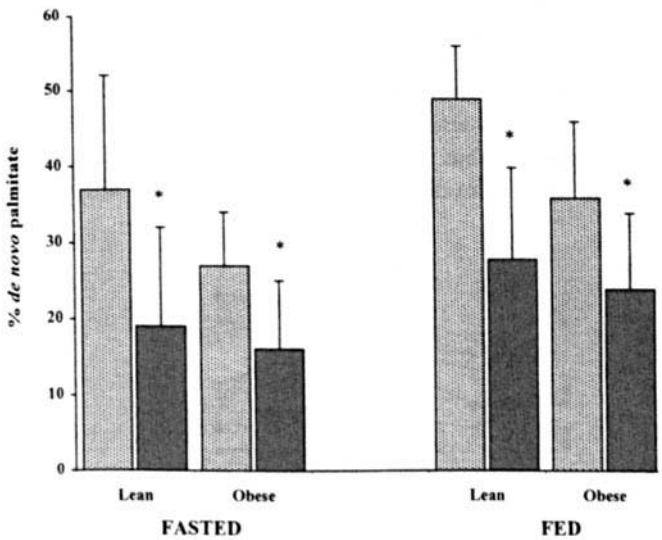

Figure 6. Percentage of newly synthesized palmitate in VLDL triglyceride (mean ± SD) calculated by mass isotopomer distribution analysis (MIDA) on a 10% and 30% solid food diet high in simple sugars. Legend as in Figure 5. *$P < 0.01$, 10% vs. 30% fat diet, fasted and fed. For details, see Ref. 21.

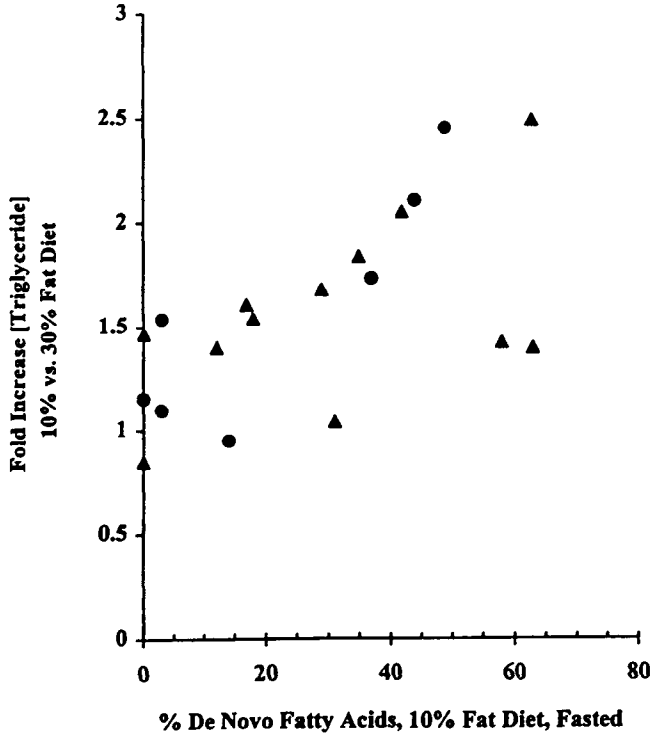

Figure 7. Plot of the relationship between the fasting percentage of synthesized fatty acids in VLDL triglyceride calculated by the linoleate dilution method on the 10% fat solid food diet high in simple sugars and the fold increase in the fasting concentration of triglyceride on the 10% vs. 30% fat diets. Circles = obese; triangles = lean. Regression analysis showed a significant positive relationship, $P = 0.004$; $R^2 = 0.40$. Reproduced from Ref. 21 by copyright permission from *The Journal of Lipid Research.*

tionately reduced to 9 g/1000 cal as the total carbohydrate was reduced on the 30% fat diet. For each subject, the fatty acid compositions of the two diets were identical and matched to the adipose tissue.

The effects of these solid food diets on fatty acid synthesis are shown in Figure 5 (21). On the left is shown the higher fraction of newly formed fatty acids in fasting VLDL triglyceride at the end of the low-fat diet compared with the higher-fat diet (mean of 31 vs 1% in lean subjects and 21 vs 1% in obese subjects). Contrary to our expectation, there was no significant difference between the responses of lean and obese subjects, despite a doubling of fasting and 24-hr insulin levels and lower glucagon levels in the obese. On the right is the maximum fractional synthesis reached during the day, which occurred between 2100 hr and 0300 hr, 4–10 hr after dinner. Again, the fraction of newly formed fatty acids was higher on the 10% fat diet compared with the 30% fat diet: 43 vs 12% for the lean and 37 vs 6% for the obese, with no difference in response between lean and obese subjects. As in previous studies with formula diets, VLDL triglyceride fatty acid composition was altered primarily by an increase in 16:0 and decrease in 18:2.

Figure 6 shows that qualitatively similar results were obtained for the fraction of newly formed 16:0 measured after the intravenous infusion of ^{13}C-acetate at the end of each diet period. These values averaged as high as 49% for the lean and 36% for the obese in the late evening on the 10% fat diet.

Relation Between Carbohydrate-Induced Hypertriglyceridemia and Fatty Acid Synthesis

Average triglyceride levels were also 50%–60% higher on the 10% fat diet, in both lean and obese, after fasting and over 24 hr. VLDL triglyceride levels followed levels of total triglyceride. Half of the subjects had abnormal fasting triglycerides (>150 mg/dl) on the 10% fat diet. HDL cholesterol levels were slightly but significantly lower and inversely related to triglyceride levels.

There was a positive relationship between the increase in triglyceride on the 10% vs 30% fat diets and fraction of newly synthesized fatty acids in VLDL triglyceride (Fig. 7). The strongest relationship was with values obtained after an overnight fast, when there was the greatest intersubject variablity. Thus, those subjects who had the greatest fractional fatty acid synthesis had the greatest diet-induced change in triglyceride. A close look revealed two patterns over 24 hr: "constant," in subjects who had fasting fractional synthesis of >25% and "diurnal," in those who had fasting values <25%, a peak in the evening, then a return to low values (Fig. 8). Note that the mean fasting triglyceride level was twice as high (197 mg/dl vs 108 mg/dl) in subjects with the "constant" versus the "diurnal" pattern. There were no dis-

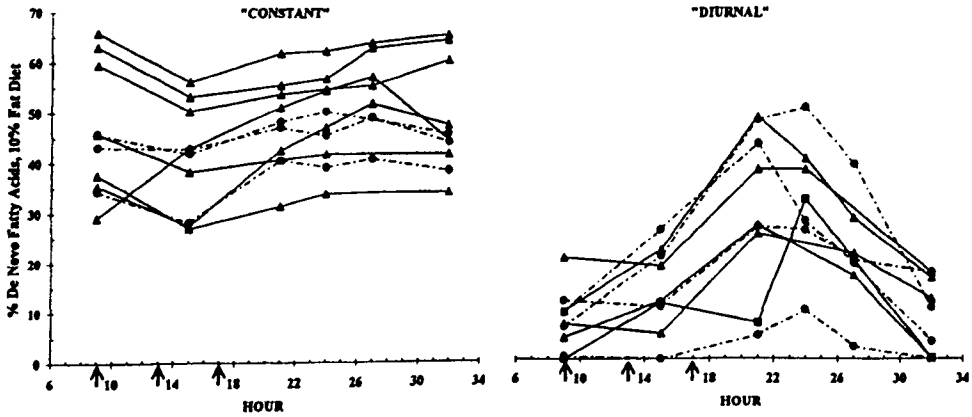

Figure 8. Percentage synthesized fatty acids in VLDL triglyceride calculated by the linoleate dilution method over 24 hr at the end of the 10% fat solid food diet high in simple sugars. Symbols as in Figure 7. Lean and obese subjects were divided into those with high fasting values (>25%, left panel, $n = 10$) and low fasting values (<25%, right panel, $n = 9$). The arrow shows the times of the meals. Reproduced from Ref. 21 by copyright permission from *The Journal of Lipid Research.*

tinguishing clinical characteristics, including BMI and insulin levels. These results suggested that the individual variability in the triglyceride response to low-fat diets may relate to insulin-independent differences in fatty acid synthesis, such as differences in carbohydrate digestion.

Conclusions

Very-low-fat formula and solid-food isocaloric diets with a high ratio of sugar to starch (> 60/40) increased the synthesis of the saturated fatty acid, palmitate. This occurred to a similar extent in lean and obese subjects. Although there were no detected effects on energy balance, there were large alterations in the concentration and fatty acid composition of plasma triglyceride with substantial interindividual variation that was unrelated to body mass index or insulin levels. Recommendations to the public to reduce dietary fat must take into consideration the stimulatory effect of increased dietary sugar on fatty acid synthesis and plasma triglyceride levels. The reasons for the variability in response among individuals and the consequences of these effects on the cardiovascular system need further study.

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