Human fatty acid synthesis is reduced after the substitution of dietary starch for sugar

Lisa C Hudgins, Cynthia E Seidman, Jolanta Diakun, and Jules Hirsch

ABSTRACT Using new nonisotopic and isotopic methods, we showed previously that fatty acid synthesis was markedly stimulated in weight-stable normal volunteers by a very-low-fat formula diet with 10% of energy as fat and 75% as short glucose polymers. In this study, we determined whether fatty acid synthesis was equally stimulated by a very-low-fat solid diet made with foods consumed typically. Four normal volunteers consumed the same very-low-fat formula diet for 25 d and then an isoenergetic solid food diet with 10% of energy as fat and 75% as starch, simple sugars, and fiber for 25 d. To measure fatty acid synthesis, the fatty acid compositions of the diets were matched to the composition of each subject's adipose tissue and compared with the composition of VLDL-triacylglycerol. In all subjects, the large increases in newly formed palmitate and decreases in linoleate in VLDL-triacylglycerol were quickly reversed by the solid food diet, and the fraction of de novo synthesized fatty acids in fasting VLDL-triacylglycerol decreased from 30–54% to 0–1%. In a second group of subjects, the stimulation of fatty acid synthesis by the formula diet with 75% glucose polymers was similarly reduced by a formula diet with amounts of fat, starch, and sugar chosen to mimic those of the solid food diet, but persisted after the addition of fiber or a diet with 75% sugar. In conclusion, an increase in fatty acid synthesis and palmitate-rich, linoleate-poor VLDL-triacylglycerol induced by very-low-fat, high-sugar diets may be reduced by the substitution of dietary starch for sugar with potentially beneficial effects on cardiovascular health. Am J Clin Nutr 1998;67:631–9.

KEY WORDS Triacylglycerol, palmitic acid, linoleic acid, VLDL, carbohydrates, starch, fatty acid synthesis, very-low-fat diet

INTRODUCTION Using two new methods to measure fatty acid synthesis in vivo, we recently reported markedly increased de novo synthesis of the saturated fatty acid palmitate (16:0) in weight-stable normal volunteers who consumed very-low-fat liquid formula diets with 10% of energy as fat and 75% as carbohydrate. In contrast, there was minimal fatty acid synthesis in subjects who consumed formula diets with 40% of energy as fat and 45% as carbohydrate (1). The increase in fatty acid synthesis was associated with increased plasma VLDL and total triacylglycerol, decreased HDL cholesterol, and alteration in the triacylglycerol fatty acid composition that persisted over 24 h. In VLDL and total triacylglycerol there was a large accumulation of palmitate, the fatty acid preferentially formed by mammalian fatty acid synthase, and a decrease in linoleate (18:2), an essential fatty acid that cannot be formed de novo. This fatty acid pattern also occurs after the consumption of diets that are high in saturated fats, which, along with the increase in total blood triacylglycerol and decrease in HDL cholesterol, have been associated with increased risk of cardiovascular disease (2–4).

The carbohydrate in the experimental formula diets was short-chain glucose polymers derived from hydrolyzed cornstarch. This and other similar ingredients are commonly used in experimental and therapeutic diets in place of mono- or disaccharide sugars to improve gastrointestinal tolerance and taste. It is well known from animal studies that fatty acid synthesis may be reduced when a complex carbohydrate such as starch is substituted for sugar (5–7). Therefore, it is important to know in humans whether increases in fatty acid synthesis and alterations in plasma lipid fatty acid composition occur with very-low-fat solid food diets composed of a mixture of typically consumed simple and complex carbohydrates, as is often recommended for the treatment or prevention of hyperlipidemia and cardiovascular disease.

To answer this question, we first compared fatty acid synthesis in normal volunteers who consumed sequentially two very-low-fat diets that differed in carbohydrate composition: a liquid formula diet made with glucose polymers and a solid food diet made with sugars, starches, and fibers. Because the responses differed dramatically, we hypothesized that the nature of the dietary carbohydrate influenced the magnitude of fatty acid synthesis. Therefore, using very-low-fat formula diets made similar in all respects except for the type of carbohydrate, we compared the effects of the diet made with glucose polymers with those of three other diets that separately tested the effects of starch, fiber, and sugar on fatty acid synthesis and composition of VLDL-triacylglycerol.
SUBJECTS AND METHODS

Subjects

The characteristics of 13 normal volunteers who were studied as inpatients at the Rockefeller University Clinical Research Center are shown in Table 1. Subjects 1–4 were participants in our previous study (subjects 4–7 in reference 1) and stayed an additional period to consume a second diet. All were healthy nonsmokers taking no oral prescription medications and from a variety of racial and ethnic backgrounds. Body weights were within 80–120% of ideal body weight and were within 10% of the weight in the previous 6 mo. The waist circumferences at the umbilicus and the widest hip circumferences were measured with a fiberglass tape to the nearest millimeter. Two male subjects (nos. 9 and 13) had high waist-hip ratios (> 0.95). The mean plasma triacylglycerol, cholesterol, and HDL-cholesterol concentrations obtained after 12-h fasts on admission and during outpatient visits within 2 mo of admission were all normal. Blood pressure and fasting blood glucose were also normal. The studies were approved by the Rockefeller University Institutional Review Board, and informed consent was obtained from all participating subjects.

Diets

All subjects received two very-low-fat diets that differed in the type of carbohydrate. Subjects 1–4 consumed diet 1 (formula) and then 2 (solid food) for 25 d each diet period; subjects 5–13 all consumed diet 1 (formula) for 10 d and then diets 3, 4, or 5 (all formula) for 7–10 d. Diet 1 was identical to the very-low-fat formula diet used in our previous report (1). Subjects received the two diets sequentially without an intervening washout period, except for subject 1, who consumed her usual diet for 1 mo between diet periods.

The composition of the tested diets is shown in Table 2. All diets provided 10% of energy as fat and 75% as carbohydrate with the fatty acid compositions matched to the composition of each subject’s adipose tissue by mixing lard, olive oil, and corn oil, as required by the linoleate dilution method used to measure fatty acid synthesis (see below). The fatty acid compositions of the two diets consumed by each volunteer were thus identical. Cholesterol was added to the oil in all diets to total 5.1 mmol (200 mg)/d.

Diet 1 was a liquid formula diet with 75% of energy as short-chain glucose polymers derived from cornstarch (Polycose; Ross Laboratories, Columbus, OH) and 15% as milk protein (Casein; Mead Johnson, Evansville, IN). Diet 2 was a solid food diet with a single-day menu of cereal, rice, beans, pasta, bread, carrots, lettuce, tomatoes, canned pears, yogurt, skim milk, and grape juice. The solid food contributed one-fifth of the total fat, and the remaining fat was spread on toast as a mixture of oils in proportions to make the fatty acid composition of the total diet equal to the composition of each subject’s adipose tissue. The carbohydrate in diet 2 was 57% starch and 43% simple sugars [31% sucrose, 16% glucose, 28% fructose, 23% lactose, and 3% maltose; 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 20% of energy, or 12 g fructose/MJ (125 g/2500 kcal). There was 0.6 g soluble fiber (0.4 g hemicellulose and 0.2 g pectin) and 2.3 g insoluble fiber (0.9 g hemicellulose, 0.2 g pectin, 0.9 g cellulose, and 0.3 g lignin) per MJ (30 g fiber/2500 kcal) (8).

Diet 3, 4, and 5 were formula diets designed to separately test the effects of starch, sugar, and fiber in diet 2. To test the effect of starch, subjects 5–7 received diet 1 followed by diet 3. The carbohydrate in diet 3 was one-half precooked common cornstarch and one-half mixed simple sugars in proportions that were similar to those in diet 2. The cornstarch (The American Maize Company, Hammond, IN) was 70% amylopectin and 30% amylose. To test the effects of fiber, subjects 8–10 received diet 1 followed by diet 4, which was the same as diet 1 except that sugar

<table>
<thead>
<tr>
<th>Diet and subject</th>
<th>Age</th>
<th>BMI</th>
<th>Waist:hip</th>
<th>Triacylglycerol</th>
<th>Cholesterol</th>
<th>HDL cholesterol</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>y</td>
<td>kg/m²</td>
<td></td>
<td>mmol/L</td>
<td>mmol/L</td>
<td>mmol/L</td>
</tr>
<tr>
<td>Diets 1 and 2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1, F</td>
<td>20</td>
<td>19.7</td>
<td>.075</td>
<td>1.49</td>
<td>4.56</td>
<td>1.13</td>
</tr>
<tr>
<td>2, F</td>
<td>24</td>
<td>20.6</td>
<td>.80</td>
<td>0.67</td>
<td>4.03</td>
<td>1.36</td>
</tr>
<tr>
<td>3, M</td>
<td>20</td>
<td>20.0</td>
<td>.82</td>
<td>0.61</td>
<td>4.21</td>
<td>1.46</td>
</tr>
<tr>
<td>4, M</td>
<td>20</td>
<td>21.5</td>
<td>.82</td>
<td>0.93</td>
<td>3.59</td>
<td>0.87</td>
</tr>
<tr>
<td>x ± SD</td>
<td>21 ± 2</td>
<td>20.5 ± 0.8</td>
<td>0.80 ± 0.03</td>
<td>0.93 ± 0.40</td>
<td>4.10 ± 0.41</td>
<td>1.21 ± 0.26</td>
</tr>
<tr>
<td>Diets 1 and 3</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5, F</td>
<td>22</td>
<td>23.7</td>
<td>.82</td>
<td>1.53</td>
<td>5.05</td>
<td>1.18</td>
</tr>
<tr>
<td>6, M</td>
<td>28</td>
<td>22.9</td>
<td>.84</td>
<td>0.60</td>
<td>2.92</td>
<td>1.13</td>
</tr>
<tr>
<td>7, F</td>
<td>20</td>
<td>23.5</td>
<td>.79</td>
<td>0.59</td>
<td>3.38</td>
<td>1.13</td>
</tr>
<tr>
<td>x ± SD</td>
<td>23 ± 4</td>
<td>23.4 ± 0.4</td>
<td>0.82 ± 0.03</td>
<td>0.91 ± 0.54</td>
<td>3.79 ± 1.12</td>
<td>1.15 ± 0.03</td>
</tr>
<tr>
<td>Diets 1 and 4</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8, F</td>
<td>24</td>
<td>20.7</td>
<td>.81</td>
<td>0.93</td>
<td>3.85</td>
<td>1.41</td>
</tr>
<tr>
<td>9, M</td>
<td>57</td>
<td>27.3</td>
<td>1.01</td>
<td>1.30</td>
<td>4.59</td>
<td>1.05</td>
</tr>
<tr>
<td>10, M</td>
<td>38</td>
<td>21.9</td>
<td>.88</td>
<td>0.90</td>
<td>5.10</td>
<td>1.82</td>
</tr>
<tr>
<td>x ± SD</td>
<td>40 ± 17</td>
<td>23.3 ± 3.5</td>
<td>0.90 ± 0.10</td>
<td>1.04 ± 0.22</td>
<td>4.51 ± 0.63</td>
<td>1.43 ± 0.38</td>
</tr>
<tr>
<td>Diets 1 and 5</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>11, F</td>
<td>23</td>
<td>23.3</td>
<td>.80</td>
<td>1.03</td>
<td>3.74</td>
<td>0.82</td>
</tr>
<tr>
<td>12, M</td>
<td>34</td>
<td>26.1</td>
<td>.93</td>
<td>1.09</td>
<td>4.56</td>
<td>1.26</td>
</tr>
<tr>
<td>13, M</td>
<td>59</td>
<td>23.9</td>
<td>.96</td>
<td>1.26</td>
<td>4.85</td>
<td>1.18</td>
</tr>
<tr>
<td>x ± SD</td>
<td>39 ± 18</td>
<td>24.4 ± 1.5</td>
<td>0.90 ± 0.09</td>
<td>1.13 ± 0.12</td>
<td>4.38 ± 0.57</td>
<td>1.09 ± 0.23</td>
</tr>
</tbody>
</table>

*Ratio of waist circumference to hip circumference.
beet fiber (Delta Fiber Foods, Minneapolis) was added to total 1 g soluble fiber and 2 g insoluble fiber/MJ. Finally, to compare the effects of glucose polymers and mono- and disaccharides, subjects 11–13 received diet 1 followed by diet 5, which was 75% mixed simple sugars in proportions that were identical to those in diets 2 and 3.

All diets were well tolerated and subjects had no evidence of gastrointestinal malabsorption. The total energy in each diet was adjusted to maintain weight within 0.5 kg. The initial amount of energy fed was 5.7 MJ (1360 kcal)/m² surface area, a constant found to be close to the needs of subjects studied on the research unit. Some subjects attended work or classes but were advised to avoid strenuous activities and long walks. All diets had the same amounts of sodium chloride (5 g/d) to ensure constant weight across diets. Minimal adjustment of energy was required to stabilize weight with diet 1 (±0.8 MJ, or 200 kcal, in subjects 1 and 4) or across diets (0.4 MJ, or 100 kcal, in subjects 3 and 13). The average weights in the last 3 d of the comparison diets differed by <0.5 kg, except for subjects 5 and 13, who had stable weights that were 1.3 and 1.0 kg lower, respectively, with the second diet for the last 5–6 d.

Diet 2, made with solid food, was given in three meals of equal energy. Formula diets 1, 3, 4, and 5 were given in five equal servings. The last serving was consumed no later than 2000, except on the day at the end of each diet period when blood was sampled. On these days, meal times were standardized to 0900, 1300, and 1700 (solid food), or 0900, 1100, 1300, 1500, and 1700 (formula).

Gas chromatographic analysis of the total fat and fatty acid compositions of the lipid (> 95% triacylglycerol) from samples of the diets closely matched desired values. The total energy per gram formula or solid food homogenate measured by bomb calorimetry came within 5% of expected values.

Measurement of fatty acid synthesis

The linoleate dilution method, as described previously and validated by comparison with mass isotopomer dilution analysis after the intravenous infusion of [13C]acetate (1), was used to measure fatty acid synthesis. In brief, this method is based on the model that the fatty acid composition of VLDL-triaclyglycerol is determined by the relative input of fatty acids from the diet, the adipose tissue, and endogenous synthesis. When the fatty acid composition of the tested diet is matched to the composition of the adipose tissue and there is minimal selectivity in the metabolism of the major fatty acids, then linoleate in VLDL-triaclyglycerol can be used as a marker for the fractional input of preformed fatty acids from the diet and adipose tissue, as opposed to de novo synthesis. The decrease, or dilution, of linoleate in VLDL-triaclyglycerol relative to the concentration in the diet or adipose tissue by de novo synthesized fatty acids can be used to calculate the fraction of de novo synthesized fatty acids in VLDL-triaclyglycerol as follows:

\[
\text{Percentage de novo synthesized VLDL-triaclyglycerol} = \left(\frac{\text{percentage linoleate in diet and adipose tissue}}{\text{percentage linoleate in VLDL-triaclyglycerol}} \right) \times 100
\]

Isolation of VLDL, lipid extraction, and fatty acid analysis

Every 1–3 d, ≥12 h after the last meal, 15 mL blood was sampled and put in EDTA on ice, and the plasma was separated by low-speed centrifugation at 4 °C for 20 min within 1 h of blood sampling. After adding lipase and protease inhibitors (phenylmethylsulfonyl chloride, leupeptin, and pepstatin), VLDL was isolated by density-gradient separation as described previously (1). In samples obtained the last day of each diet period before the meals eaten 1300 and 1700 and every 4 h thereafter over 24 h (subjects 1–4) or 1 h after the last meal (subjects 1–13), chylomicrons were first separated by ultracentrifugation, as described (1). The lipid was extracted from VLDL with chloroform:methanol (2:1), the triacylglycerol fraction was separated by thin-layer chromatography and transmethylated with methanolic hydrochloric acid, and the fatty acid methyl esters analyzed by capillary gas chromatography (1).

The fatty acid compositions of the adipose tissue and diets were analyzed similarly after chloroform:methanol extraction by gas chromatography. Subcutaneous adipose tissue from the abdominal and gluteal region of each subject was aspirated through a needle attached to a syringe after local anesthesia with 1% lidocaine within 3 wk of admission (9) and at the end of each diet period. Because of our previous documentation of stability in the fatty acid composition at abdominal and gluteal sites with short-term dietary changes, only gluteal fat was sampled in subjects 5–13 at the end of diets 1, 3, 4, and 5.

Analysis of cholesterol, triacylglycerol, HDL cholesterol, glucose, and insulin

In EDTA-treated plasma obtained for fatty acid analysis, the concentrations of cholesterol and triacylglycerol were measured enzymatically (Boehringer Mannheim reagents, Indianapolis) and the HDL cholesterol after precipitation of apolipoprotein B–containing lipoproteins by dextran sulfate (10). Glucose and insulin measurements were made on day 0 and every 2–3 d in samples obtained after an overnight fast. Samples were also obtained on the last day of each diet, immediately before the meal eaten at 1300 (subjects 1–4) and 1 h after the last meal (subjects 1–13). Glucose was measured by glucose oxidase or hexokinase assays in serum samples obtained at the same time points. Insulin was analyzed in duplicate by radioimmunoassay (11).

Indirect calorimetry

For subjects 1–4, the resting energy expenditure (REE) and respiratory quotient (RQ) were calculated from the analysis of breath samples by using an MMC Horizon Metabolic Cart (Beckman)
Instruments, Inc, Brea, CA) or a Sensormedic DeltaTrac Metabolic Cart (Sensormedic, Yorba Linda, CA) with a ventilated hood. On day 0 and every 4–5 d, measurements of carbon dioxide production and oxygen consumption were made every 30–60 s for 30 min in the morning, 12 h after the last meal, and after 20 min of rest. For each 30-min measurement, the mean of values obtained during the second 15 min was used for comparative analysis. The RQs were corrected for protein oxidation, which was estimated from the difference between nitrogen intake and nitrogen excretion (in urea, creatinine, and uric acid) in 24-h urine collections from the same day.

**Statistical methods**

For each diet pair group, the difference in percentage de novo synthesized VLDL-triacylglycerol or other variables between diets were analyzed by paired t test. For subjects 1–4, the mean percentage de novo synthesized VLDL-triacylglycerol was calculated from seven to eight fasting samples in the last 2 wk of each 25-d diet period, when a constant fatty acid composition existed. For subjects 5–13, the mean percentage de novo synthesized VLDL-triacylglycerol was calculated from two fasting samples at the end of each 10-d diet period, except for subjects 5, 6, and 8, who received the second diet for 7 d and a single value from the last day was used. Because there was no change over time, the mean values for REE and RQ for subjects 1–4 were calculated from the last three to four measurements in the last 2 wk of study. Data analysis was performed by using EXCEL statistical software (Microsoft, Redmond, WA).

**RESULTS**

**Glucose polymers compared with solid food with sugars, starches, and fibers (diets 1 and 2)**

The results after diet 1 in subjects 1–4 were included in our report of a larger group of subjects consuming this diet (1). As expected, because of very slow turnover, the fatty acid compositions of adipose tissue sampled before and after diet 1 did not change (Table 3). The pre- and posttreatment values for linoleate were, respectively (% by wt of total fatty acids), 15.6±2.2% and 15.2±2.0%, and for palmitate were 19.2±0.9% and 19.6±0.8% (NS). In the third column of the table, note the close resemblance of the diet and the adipose tissue.
adipose tissue compositions should be compared with the mean composition of VLDL-triacylglycerol after an overnight fast in the last 2 wk of study. The percentage difference for each of the three major fatty acids is shown in the next column.

Similar to our results for the larger group who consumed diet 1 (1), the mean concentration of linoleate was 40% lower and that of palmitate was 53% higher than the concentrations in the diet and adipose tissue, whereas that of oleic acid was only slightly decreased. This fatty acid pattern was stable over 24 h and did not fluctuate with meals. Assuming no selective metabolism of linoleate, the 40% decrease for linoleate indicated that 40% of the VLDL-triacylglycerol was formed from de novo synthesized fatty acids, principally palmitate.

In Figure 1, at the arrow, large, rapid changes in linoleate and palmitate in VLDL-triacylglycerol are shown after switching from diet 1 to diet 2. Within 5 d of starting diet 2 in all subjects, linoleate increased and palmitate decreased to concentrations that were similar to those in the diet and adipose tissue (percentage difference between diet 1 and diet 2: —40% compared with 9% and 53% compared with 12%, respectively; \( P = 0.003 \) and 0.004; Table 3). These changes suggest nearly complete suppression of fatty acid synthesis by diet 2. Samples obtained over 24 h at the end of the diet from subjects 1, 2, and 4 showed small increases in percentage de novo synthesized VLDL-triacylglycerol from fasting concentrations that peaked between 1800 and 2200, 1–5 h after dinner (8%, 4%, and 17%, respectively). Subject 3 became ill (pharyngitis) on the day of 24-h blood sampling; his results from that day were excluded because on that day he had an abrupt, large increase in fasting and 24-h blood sampling; his results from that day were excluded.

Percentage differences between the fatty acid compositions of VLDL-triacylglycerol after an overnight fast in the last 2 wk of study. The arrow marks the change from the 10%-fat, 75%-glucose polymer formula diet (diet 1) to the 10%-fat, 75%-mixed carbohydrate solid food diet (no. 2). Results from subject 1 were similar but are omitted because diets 1 and 2 were interrupted by 1 mo of an ad libitum diet. Percentage difference \( = \) [percentage linoleate (18:2) in VLDL-triacylglycerol – percentage linoleate in diet and adipose tissue] \times 100/percentage linoleate in diet and adipose tissue. Negative values for linoleate are equal to the percentage de novo synthesized VLDL-triacylglycerol by the linoleate dilution method described in the text. \( \bar{x} \pm SD; n = 3. \)

Glucose polymers compared with sugars and starch (diets 1 and 3)

The formula diet (diet 3) made to imitate the fat, starch, and sugar composition of the solid food diet (diet 2) replicated the suppressive effect on fatty acid synthesis by this diet. As shown in Figure 2 and Table 4, in all subjects diet 3 quickly reversed the mean 29% decrease in linoleate in VLDL-triacylglycerol below levels in the diet and adipose tissue induced by diet 1. The mean percentage difference for palmitate declined with the increase in linoleate (bottom panel, Figure 2), although the average decline was not as sharp and pronounced as with the solid food diet. This was because of a gradual further increase in palmitate and decrease in palmitoleate (16:1) in subject 5; the other two subjects had increases and decreases in palmitate that were similar to the responses seen with first diet 1 and then diet 2. At the end of diet 3, the mean percentage difference in linoleate was slightly positive (8%) and almost identical to that obtained with diet 2 (9%), indicating no dilution of linoleate by de novo synthesized fatty acids. There were no diurnal changes with diet 1, but similar to diet 2, there were small increases in percentage de novo synthesized VLDL-triacylglycerol in samples obtained 1 h after the last meal in subject 6 (from 5% to 11%) and subject 7 (from 0 to 12%). Thus, these results suggested that the dietary ratio of starch to sugar was an important determinant of the magnitude of de novo fatty acid synthesis induced by very-low-fat diets.

Glucose polymers compared with glucose polymers plus fiber (diets 1 and 4)

To test the effect of dietary fiber, sugar beet fiber was chosen because of its similarity in composition to the fiber in diet 2 and was added in amounts similar to those in diet 1. No subject had a decrease in percentage de novo synthesized VLDL-triacylglycerol after the addition of fiber. In fact, Table 4 shows that the...
mean percentage de novo synthesized VLDL-triacylglycerol tended to be slightly higher after the addition of fiber (41% compared with 28%, \( P = 0.24 \)). Palmitate also increased further (percentage difference: 46% compared with 29%). The fatty acid compositions of VLDL-triacylglycerol sampled before the first meal and after the last meal were similar for each diet. These results suggested that the fiber in diet 2 did not contribute to its suppressive effect on fatty acid synthesis.

Glucose polymers compared with sugars (diets 1 and 5)

The isoenergetic substitution of glucose polymers with a mixture of simple sugars in proportions that were similar to those in diets 2 and 3 resulted in similar stimulation of fatty acid synthesis in all subjects. The mean percentage de novo synthesized VLDL-triacylglycerol after an overnight fast was 58% and 40% with diets 1 and 5, respectively (\( P = 0.17 \)). Palmitate in VLDL-triacylglycerol was 55% and 48% higher than in the diet and adipose tissue. The fatty acid composition of VLDL-triacylglycerol sampled before the first meal and after the last meal was similar with each diet. Thus, there was no evidence for unique stimulation of fatty acid synthesis by glucose polymers in diet 1 or suppression by any of the sugars also present in diets 2 and 3.

**Total triacylglycerol, cholesterol, and HDL-cholesterol concentrations**

The triacylglycerol, cholesterol, and HDL-cholesterol response to diets 1 and 2 in subjects 1–4 is compared in Table 5. As had been reported previously (1), after diet 1, the triacylglycerol concentration rose to four times baseline values by days 4–11 and then decreased and stabilized in the last week at concentrations that were

---

**TABLE 4**

Diet 1 compared with diets 3, 4, and 5: mean fatty acid compositions of adipose tissue, diet, and VLDL-triacylglycerol.

<table>
<thead>
<tr>
<th>Diet and Adipose tissue&lt;sup&gt;1&lt;/sup&gt;</th>
<th>Diet and VLDL-triacylglycerol&lt;sup&gt;1&lt;/sup&gt;</th>
<th>Newly synthesized VLDL-triacylglycerol&lt;sup&gt;1&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>% by wt of total fatty acids</td>
<td>%</td>
<td>%</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Diet and Fatty Acid</th>
<th>Diet 1</th>
<th>Diet 2</th>
<th>Diet 3</th>
<th>Diet 4</th>
<th>Diet 5</th>
<th>Prediet</th>
<th>Postdiet</th>
<th>Diet</th>
<th>VLDL-triacylglycerol</th>
<th>Difference&lt;sup&gt;4&lt;/sup&gt;</th>
<th>Value&lt;sup&gt;5&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>18:2</td>
<td>15.39</td>
<td>15.46</td>
<td>14.52</td>
<td>15.00</td>
<td>15.00</td>
<td>13–19</td>
<td>13–19</td>
<td>10.64</td>
<td>28.41</td>
<td>–29.08 ± 11.19</td>
<td>–15 to –35</td>
</tr>
<tr>
<td>16:0</td>
<td>15.58</td>
<td>15.58</td>
<td>16.01</td>
<td>15.79</td>
<td>15.79</td>
<td>13–20</td>
<td>13–20</td>
<td>17.06</td>
<td>27.85</td>
<td>38.24 ± 14.66</td>
<td>22–49</td>
</tr>
<tr>
<td>9c:18:1</td>
<td>20.98</td>
<td>20.35</td>
<td>20.17</td>
<td>20.15</td>
<td>20.15</td>
<td>18–22</td>
<td>18–22</td>
<td>27.85</td>
<td>35.11</td>
<td>–10.82 ± 3.49</td>
<td>–8 to –15</td>
</tr>
</tbody>
</table>

<sup>1</sup>Mean of each 11 fatty acids in adipose tissue before and after the diets.

<sup>2</sup>Mean of adipose tissue (postdiet) and the corresponding diet; range in parentheses.

<sup>3</sup>Mean fatty acid composition of VLDL triacylglycerol sampled days 7–10 during the diets; range in parentheses.

<sup>4</sup>(VLDL-triacylglycerol–diet and adipose tissue value) × (100/diet and adipose tissue value). \( \bar{x} \pm SD; \) range in parentheses.

<sup>5</sup>Percentage decrease in 18:2 in VLDL-triacylglycerol compared with diet and adipose tissue value.

<sup>6</sup>Significantly different, \( P = 0.04 \).
The fasting total cholesterol concentrations remaining depressed for the duration of diets 1 and 2, with little more quickly, decreasing to 34% below baseline values and suffered from that of triacylglycerol. HDL cholesterol changed over 24 h. The mean triacylglycerol concentrations were not significantly different (1.34 ± 0.38 and 2.00 ± 1.23 mmol/L, or 118 and 176 mg/dL; P = 0.38). The mean total 24-h area under the curve calculated from values obtained every 4–6 h at the end of each diet tended to be lower after diet 2 than after diet 1, but again, the means were not significantly different (37.5 and 45.10 mmol·24 h/L, or 3300 and 3969 mg·24 h/dL; P = 0.51, data not shown). Thus, the higher percentage de novo synthesized triacylglycerol with diet 1 than with diet 2 was not consistently associated with higher concentrations of triacylglycerol.

Note that the time course of changes in HDL cholesterol differed from that of triacylglycerol. HDL cholesterol changed more quickly, decreasing to 34% below baseline values and remaining depressed for the duration of diets 1 and 2, with little change over 24 h. The fasting total cholesterol concentrations decreased to a lesser extent (~15%) and also showed little change between diets or over 24 h. Thus, after both diets 1 and 2, the ratio of total cholesterol to HDL cholesterol was greater in all subjects compared with baseline.

The short duration of studies conducted in subjects 5–13 limits the conclusions that can be drawn regarding the dietary effects of specific carbohydrates on plasma triacylglycerol, cholesterol, and HDL cholesterol. In this group, the changes in plasma triacylglycerol, cholesterol, and HDL-cholesterol response after diet 1 relative to baseline values were similar to the changes in subjects 1–4. Fasting (and fed) triacylglycerol values averaged two times baseline values (range: 1.5–4), whereas mean cholesterol and HDL-cholesterol values decreased by 11% and 30%, respectively. The ratio of total to HDL cholesterol increased in eight of nine subjects from a mean of 3.2 at baseline to 4.1. There were no significant differences in triacylglycerol, cholesterol, HDL cholesterol, or the ratio of total to HDL cholesterol between diets.

Insulin and glucose (diet 1 compared with diets 2–5)

The mean triacylglycerol, insulin, and glucose after all diets after an overnight fast and 1 h after the last meal are compared in Table 6. On day 0, no subject was clearly insulin resistant or glucose intolerant (maximum fasting insulin and glucose of 151 pmol/L and 5.8 mmol/L, respectively). Despite differences in fatty acid synthesis for all diet pairs tested, there were no significant differences between diets in fasting or fed triacylglycerol, insulin, or glucose concentrations.

Energy expenditure (diets 1 and 2)

Despite differences in fatty acid synthesis in the last 2 wk of each diet period, there was no detected difference in the energy required to keep weight constant (9.1 ± 1.2 compared with 9.2 ± 1.0 MJ/d, or 2168 compared with 2193 kcal/d; NS), in the REE measured after an overnight fast (5.6 ± 0.9 compared with 5.4 ± 1.0 MJ/d, or 1336 compared with 1286 kcal/d; NS), or in the nonprotein RQ (0.96 ± 0.10 compared with 0.92 ± 0.06; NS). RQ values < 1.0 indicated that there was no net deposition of newly synthesized fatty acid.

DISCUSSION

These results clearly confirm the findings of our previous study that very-low-fat diets that contain only simple carbohydrate (short-chain glucose polymers) markedly increase fatty acid synthesis in weight-stable normal volunteers. The new finding is that a much lower fraction of plasma VLDL-triacylglycerol was formed from newly synthesized fatty acids after a very-low-fat solid food diet made with a mixture of simple and complex carbohydrates than after the formula diet made with glucose polymers. Further study with formula diets permitted a more exact analysis of the roles of starch, fiber, and sugar in the solid food diet with results that indicated similar effects of formula and solid food diets. As with the solid food diet, the formula diet with a mixture of starch and sugar suppressed the stimulation of fatty acid synthesis by the low-fat diet made with glucose polymers. It also reduced the accumulation of palmitate synthesized de novo and increased linoleate in plasma triacylglycerol, with potential benefits for cardiovascular health.

Many studies in rodents have shown greater stimulation of fatty acid synthesis by high-sugar than by high-starch diets (5–7). The difference is most pronounced with fat-free diets and diminishes with a rise in the percentage of energy from fat. In some strains, the higher fatty acid synthesis is accompanied by higher hepatic triacylglycerol synthesis, secretion, and plasma triacylglycerol concentrations (12). When fatty acid synthesis is increased with fat-free diets—even when triacylglycerol concentrations are not elevated—there are marked increases in de novo synthesized saturated and monounsaturated fatty acids and decreases in linoleate in the plasma and liver triacylglycerol that precede the increase in the Mead acid 20:3n–9, a marker of essential fatty acid deficiency (13, 14).

<table>
<thead>
<tr>
<th>TABLE 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Triacylglycerol, cholesterol, and HDL cholesterol with diet 1 compared with diet 2[^2]</td>
</tr>
<tr>
<td>Subjects</td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>Ad lib</td>
</tr>
<tr>
<td>1</td>
</tr>
<tr>
<td>2</td>
</tr>
<tr>
<td>3</td>
</tr>
<tr>
<td>4</td>
</tr>
</tbody>
</table>

[^2]: Values represent the mean of two to three samples obtained before the study during ad libitum diet (ad lib) or three samples in the last week of diets 1 or 2. There were no significant differences between diets 1 and 2 (P > 0.05).
In humans, there are no previously published comparisons of the effects of diets that differ in the type of carbohydrate on fatty acid synthesis and composition of plasma lipids. However, it is known that the isoenergetic substitution of sugar for starch raises the plasma triacylglycerol concentration to a variable extent, depending on the amount and type of sugar, starch, and fat, as well as duration of the diet and the insulin sensitivity of the subjects (12, 15, 16). Clearly, in addition to increased fatty acid synthesis, increased triacylglycerol synthesis from preformed fatty acids or decreased plasma triacylglycerol concentration to a variable extent, depend-ent on the type of diet and the insulin sensitivity of the subjects (12, 15, 16).

The difference in fatty acid synthesis with diets that vary in the amount of carbohydrate in the diet may be associated with marked increases in fatty acid synthesis and alterations in the plasma lipid composition. The plasma fatty acid changes may in turn influence membrane lipids, receptor function, or insulin sensitivity with slowly developing secondary effects on plasma lipid concentrations (4, 18).

Despite the large differences in percentage de novo synthesized VLDL-triacylglycerol between the liquid and solid food diets (diets 1 and 2), the total energy expenditure, as reflected by the total energy required to keep weight constant, and the REE after an overnight fast were similar. As we noted previously (1), this lack of difference in energy expenditure is consistent with what is predicted given the extra energy cost to convert glucose carbon to fatty acid (19) and an assumed upper limit of triacylglycerol synthesis of ~25 g/d. It suggests that the absolute rate of conversion of glucose carbon to fatty acid during diet 1 was small and below the detection limit of 0.4 MJ (~100 kcal)/d in a 25-d study. Nevertheless, because the plasma triacylglycerol synthesis rate is also relatively small, there was a large fractional increase in newly synthesized fatty acid and a dramatic change in triacylglycerol fatty acid composition in this study.

The difference in fatty acid synthesis with diets that vary in the ratio of starch to sugar may have been due to differences in glucose flux or insulin metabolism. This is supported by the findings that insulin concentrations and hepatic glucose production are higher in rats pair fed sucrose than in those pair fed starch (20) and that acetyl-CoA carboxylase and fatty acid synthase mRNA are expressed at higher levels in rats pair fed sucrose than in those pair fed starch (20) and that acetyl-CoA carboxylase and fatty acid synthase mRNA are expressed at higher levels in rats pair fed sucrose than in those pair fed starch (20) and that acetyl-CoA carboxylase and fatty acid synthase mRNA are expressed at higher levels in rats pair fed sucrose than in those pair fed starch (20) and that acetyl-CoA carboxylase and fatty acid synthase mRNA are expressed at higher levels in rats pair fed sucrose than in those pair fed starch (20) and that acetyl-CoA carboxylase and fatty acid synthase mRNA are expressed at higher levels in rats pair fed sucrose than in those pair fed starch (20). Although the starch molecule is a straight or branched chain of thousands of glucose molecules that enter the bloodstream as glucose after digestion, it may be absorbed more slowly than glucose with lower postprandial rises in insulin and glucose (12). Further- more, it has been recognized recently that the variation in the glycemic indexes of starchy foods (23) may be explained by variations in the ratios of amylose to amylopectin. Amylose, a resistant starch, is incompletely digested in the small intestine and produces smaller increases in postprandial rises in insulin and glucose (12). Furthermore, it has been recognized recently that the variation in the glycemic indexes of starchy foods (23) may be explained by variations in the ratios of amylose to amylopectin. Amylose, a resistant starch, is incompletely digested in the small intestine and produces smaller increases in postprandial rises in insulin and glucose (12). Furthermore, it has been recognized recently that the variation in the glycemic indexes of starchy foods (23) may be explained by variations in the ratios of amylose to amylopectin. Amylose, a resistant starch, is incompletely digested in the small intestine and produces smaller increases in postprandial rises in insulin and glucose (12). Furthermore, it has been recognized recently that the variation in the glycemic indexes of starchy foods (23) may be explained by variations in the ratios of amylose to amylopectin. Amylose, a resistant starch, is incompletely digested in the small intestine and produces smaller increases in postprandial rises in insulin and glucose (12). Furthermore, it has been recognized recently that the variation in the glycemic indexes of starchy foods (23) may be explained by variations in the ratios of amylose to amylopectin. Amylose, a resistant starch, is incompletely digested in the small intestine and produces smaller increases in postprandial rises in insulin and glucose (12). Furthermore, it has been recognized recently that the variation in the glycemic indexes of starchy foods (23) may be explained by variations in the ratios of amylose to amylopectin. Amylose, a resistant starch, is incompletely digested in the small intestine and produces smaller increases in postprandial rises in insulin and glucose (12). Furthermore, it has been recognized recently that the variation in the glycemic indexes of starchy foods (23) may be explained by variations in the ratios of amylose to amylopectin. Amylose, a resistant starch, is incompletely digested in the small intestine and produces smaller increases in postprandial rises in insulin and glucose (12). Furthermore, it has been recognized recently that the variation in the glycemic indexes of starchy foods (23) may be explained by variations in the ratios of amylose to amylopectin. Amylose, a resistant starch, is incompletely digested in the small intestine and produces smaller increases in postprandial rises in insulin and glucose (12). Furthermore, it has been recognized recently that the variation in the glycemic indexes of starchy foods (23) may be explained by variations in the ratios of amylose to amylopectin. Amylose, a resistant starch, is incompletely digested in the small intestine and produces smaller increases in postprandial rises in insulin and glucose (12). Furthermore, it has been recognized recently that the variation in the glycemic indexes of starchy foods (23) may be explained by variations in the ratios of amylose to amylopectin. Amylose, a resistant starch, is incompletely digested in the small intestine and produces smaller increases in postprandial rises in insulin and glucose (12). Furthermore, it has been recognized recently that the variation in the glycemic indexes of starchy foods (23) may be explained by variations in the ratios of amylose to amylopectin. Amylose, a resistant starch, is incompletely digested in the small intestine and produces smaller increases in postprandial rises in insulin and glucose (12). Furthermore, it has been recognized recently that the variation in the glycemic indexes of starchy foods (23) may be explained by variations in the ratios of amylose to amylopectin. Amylose, a resistant starch, is incompletely digested in the small intestine and produces smaller increases in postprandial rises in insulin and glucose (12). Furthermore, it has been recognized recently that the variation in the glycemic indexes of starchy foods (23) may be explained by variations in the ratios of amylose to amylopectin. Amylose, a resistant starch, is incompletely digested in the small intestine and produces smaller increases in postprandial rises in insulin and glucose (12). Furthermore, it has been recognized recently that the variation in the glycemic indexes of starchy foods (23) may be explained by variations in the ratios of amylose to amylopectin. Amylose, a resistant starch, is incompletely digested in the small intestine and produces smaller increases in postprandial rises in insulin and glucose (12). Furthermore, it has been recognized recently that the variation in the glycemic indexes of starchy foods (23) may be explained by variations in the ratios of amylose to amylopectin. Amylose, a resistant starch, is incompletely digested in the small intestine and produces smaller increases in postprandial rises in insulin and glucose (12). Furthermore, it has been recognized recently that the variation in the glycemic indexes of starchy foods (23) may be explained by variations in the ratios of amylose to amylopectin. Amylose, a resistant starch, is incompletely digested in the small intestine and produces smaller increases in postprandial rises in insulin and glucose (12). Furthermore, it has been recognized recently that the variation in the glycemic indexes of starchy foods (23) may be explained by variations in the ratios of amylose to amylopectin. Amylose, a resistant starch, is incompletely digested in the small intestine and produces smaller increases in postprandial rises in insulin and glucose (12). Furthermore, it has been recognized recently that the variation in the glycemic indexes of starchy foods (23) may be explained by variations in the ratios of amylose to amylopectin. Amylose, a resistant starch, is incompletely digested in the small intestine and produces smaller increases in postprandial rises in insulin and glucose (12). Furthermore, it has been recognized recently that the variation in the glycemic indexes of starchy foods (23) may be explained by variations in the ratios of amylose to amylopectin. Amylose, a resistant starch, is incompletely digested in the small intestine and produces smaller increases in postprandial rises in insulin and glucose (12). Furthermore, it has been recognized recently that the variation in the glycemic indexes of starchy foods (23) may be explained by variations in the ratios of amylose to amylopectin. Amylose, a resistant starch, is incompletely digested in the small intestine and produces smaller increases in postprandial rises in insulin and glucose (12). Furthermore, it has been recognized recently that the variation in the glycemic indexes of starchy foods (23) may be explained by variations in the ratios of amylose to amylopectin. Amylose, a resistant starch, is incompletely digested in the small intestine and produces smaller increases in postprandial rises in insulin and glucose (12). Furthermore, it has been recognized recently that the variation in the glycemic indexes of starchy foods (23) may be explained by variations in the ratios of amylose to amylopectin. Amylose, a resistant starch, is incompletely digested in the small intestine and produces smaller increases in postprandial rises in insulin and glucose (12). Furthermore, it has been recognized recently that the variation in the glycemic indexes of starchy foods (23) may be explained by variations in the ratios of amylose to amylopectin. Amylose, a resistant starch, is incompletely digested in the small intestine and produces smaller increases in postprandial rises in insulin and glucose (12). Furthermore, it has been recognized recently that the variation in the glycemic indexes of starchy foods (23) may be explained by variations in the ratios of amylose to amylopectin. Amylose, a resistant starch, is incompletely digested in the small intestine and produces smaller increases in postprandial rises in insulin and glucose (12). Furthermore, it has been recognized recently that the variation in the glycemic indexes of starchy foods (23) may be explained by variations in the ratios of amylose to amylopectin. Amylose, a resistant starch, is incompletely digested in the small intestine and produces smaller increases in postprandial rises in insulin and glucose (12). Furthermore, it has been recognized recently that the variation in the glycemic indexes of starchy foods (23) may be explained by variations in the ratios of amylose to amylopec...
lopectin to amylase (70:30) and thus might be expected to be completely digested in the small intestine and have a high glycemic index, similar to the diet made with glucose polymers. On the other hand, because the absolute amount of dietary starch was high (22 g/MJ, or 231 g/2500 kcal), even the fermentation of a small fraction of the starch might have significant effects.

Because certain fibers may be fermented in a fashion similar to that of short-chain fatty acids and may also slow glucose absorption, the lack of an effect of fiber on fatty acid synthesis in the current study may reflect the choice of sugar beet fiber. Although this fiber (at a 10-g test dose) has been shown to slow glucose absorption in humans in vivo (27) and to be fermented to acetate, butyrate, and propionate by human fecal bacteria in vitro (28), it may not have exactly replicated the effects of the fiber in the solid food diet in our study.

Very-low-fat diets are high in carbohydrates that are chemically diverse with a broad range of physiologic effects. The overall effect of a substitution of dietary starch for sugar on cardiovascular disease is uncertain. A decrease in palmitate and increase in linoleate in plasma VLDL-triacylglycerol after the substitution of dietary starch for sugar is a previously unrecognized effect that resembles the fatty acid pattern produced by the dietary substitution of vegetable fat for animal fat. Whether an increase in the ratio of palmitate to linoleate acid pattern produced by the dietary substitution of vegetable fat for animal fat is unknown, studies that attempt to relate changes in dietary fat intake, or insulin sensitivity, remains to be shown. If so, then moderately low-fat diets that are high in complex carbohydrates rather than simple sugars may be less atherogenic, as suggested by recent large-scale epidemiologic studies (29, 30).

To conclude, because the amount and type of starch that suppresses fatty acid synthesis in various subsets of the population is unknown, studies that attempt to relate changes in dietary fat to disease should take into account both the nature of the dietary carbohydrate and the potential for interindividual variations in carbohydrate-induced fatty acid synthesis. Current studies to better define these variables are in progress (31).

The excellent technical assistance of Orit Gur-Arieh, Stuart Shiff, Ludmila Malkin, Ellen Murphy, and Dave Markell is much appreciated.

REFERENCES


