Effects of moderate increases in dietary polyunsaturated : saturated fat on plasma triglyceride and cholesterol levels in man

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1. The effects of two isoenergetic diets differing only in the values for polyunsaturated: saturated fat (P : S values of 0.2 v. 2.0) were studied in twenty adult human volunteers.
2. A period of 14 d on the high P : S diet failed to produce significant changes in fasting triglyceride levels, though there were individual variations. On the other hand, fasting cholesterol levels dropped by 10% (P < 0.005). High-density-lipoprotein-cholesterol concentrations were not influenced by changes in the P : S value.
3. Investigations into the mechanism by which changes in the P : S value might affect plasma triglyceride values revealed no consistent effects on very-low-density-lipoprotein kinetics, insulin secretion, insulin sensitivity or free fatty acid concentrations.
4. The results of this study suggest that the largest increase in dietary P : S values that is likely to be obtained on a long-term basis may have only a small effect on plasma triglyceride and cholesterol concentrations.

It has been known for some time that diets in which polyunsaturated fats have been substituted for saturated fats will lower serum cholesterol levels (Kinsell et al. 1952; Kinsell et al. 1953; Ahrens et al. 1957). What is not well appreciated is the possibility that plasma triglyceride (TG) levels will also fall in response to the same dietary change (Ahrens et al. 1957; Antonis & Bersohn, 1961; Engelberg, 1966; Grundy, 1975). Since it appears that the risk of developing coronary heart disease is greater in patients with hypercholesterolaemia (Bronte-Stewart et al. 1955; Kannel et al. 1971) or hypertriglyceridaemia (Carlson & Bottiger, 1972; Goldstein et al. 1973) or both, the potential therapeutic benefit of manipulating the kind of dietary fat is self-evident. On the other hand, it is important to note that the effect of an increased polyunsaturated fat intake on plasma TG levels has not been unequivocally established. Thus, several studies have involved weight reduction in addition to substitution of polyunsaturated fats for saturated fats (Ahrens et al. 1957; Antonis & Bersohn, 1961; Wilson et al. 1971; Hall et al. 1972), and the influence that changes in body-weight have on TG metabolism is well known (Olefsky, Reaven et al. 1974). Furthermore, when weight has been maintained at a constant, polyunsaturated : saturated fat (P : S) values of such extreme amounts have generally been employed that the relevance of the results to clinical settings is unclear (Spritz & Mishkel, 1969; Nestel et al. 1970; Conner et al. 1975; Durrington et al. 1977; Shephard et al. 1978; Shephard et al. 1980).

Given all these considerations, the clinical utility of substituting polyunsaturated for saturated fat in patients with hypertriglyceridaemia remains conjectural. Furthermore, there is relatively little information as to the mechanism by which an increased polyunsaturated fat intake lowers plasma TG levels. The present study was undertaken in order to address both questions.
Materials and Methods

Subjects

Twenty adult subjects (nine males and eleven females) were studied while hospitalized on the Stanford University General Clinical Research Center. Their ages ranged from 21 to 69 years, and their relative body-weights (Metropolitan Life Insurance Co., 1959) from 0.77 to 1.10. All subjects had fasting plasma glucose values of less than 6.11 mmol/l. They were all in good general health without evidence of cardiac or hepatic disease. Informed consent was obtained from each subject.

Experimental design

The effects of two isoenergetic liquid-formula diets differing only in their P:S value were investigated. Each diet contained (g/kg): 480 carbohydrate, 400 fat, 120 protein, and 500 mg/d cholesterol. In both diets 94% of the protein was supplied as milk protein, and the carbohydrate content consisted of (g/kg): 458 lactose, 312 maltose and 228 dextrin. One diet (diet S) had a P:S value of 0.2 with fat supplied as soya-bean oil, palm oil and butter; while the other diet (diet P) had a P:S value of 2.0 with corn oil (linoleic 61%) as the main source of fat. Total energy intake of 8.36 kJ/kg per d was divided into portions of one-fifth, two-fifths and two-fifths, and consumed at 08.00, 12.00 and 18.00 hours. Subjects were randomly assigned to the order of their diets and consumed each diet for 14 d. Experimental studies were carried out after 10 d of metabolic stabilization on each diet. Following the first experimental period, subjects were switched to the second diet and studies were again performed after another 10 d of stabilization. No subject’s weight changed by more than ±1 kg during the experimental period.

Fasting concentrations

After 10 d on each diet, plasma was sampled on three occasions for determination of plasma glucose, insulin, TG, cholesterol, high-density-lipoprotein (HDL)-cholesterol and free fatty acids (FFA) concentrations after an overnight fast. Values were expressed as the mean of three determinations for each subject.

Postprandial concentrations

Blood was obtained at hourly intervals from 09.00 to 17.00 hours during a day in which all subjects consumed their formulas at the usual times (08.00 and 12.00 hours). These samples were used to compare the day-long postprandial glucose, insulin, TG and FFA responses to the two diets. These values were expressed as the integrated areas under the response curves.

Very-low-density lipoprotein (VLDL)-TG secretion rate

The technique used to measure VLDL-TG secretion rate has previously been described (Reaven et al. 1965; Farquhar et al. 1965). Briefly, blood is obtained at 1,2,3,4,6,9 and 12 h after the intravenous injection of 300 μCi of [3H]glycerol, and the specific activity time-curve of the endogenously-labelled VLDL-TG is determined. From this time-curve the fractional turnover rate is calculated. The pool size of VLDL-TG is obtained from the product of the plasma VLDL-TG concentration and the plasma volume. The product of the fractional turnover rate and the VLDL-TG pool size gives the total VLDL-TG turnover rate. Since plasma VLDL concentration is in a steady state through the study, VLDL-TG turnover rate, removal rate and secretion rate are all equal.
Measurement of insulin sensitivity

The ability of insulin to stimulate glucose removal from plasma was determined by a previously-validated infusion technique (Shen et al. 1970). Epinephrine (6 μg/min), propranolol (0.08 mg/min), insulin (30 μU/min), and glucose (6 mg/kg per min) were infused at a constant rate for 150 min, starting at 08.00 hours after an overnight fast. Under these conditions, endogenous insulin secretion is inhibited, and steady-state plasma glucose (SSPG) and exogenous insulin (SSPI) levels are reached by 90 min. Blood samples were drawn for determination of plasma glucose and insulin at 5 min intervals during the last 30 min of the 150 min infusion period. Since similar SSPI levels are achieved in all subjects, the height of the SSPG level provides a direct estimate of the ability of insulin to promote disposal of the infused glucose load.

Analytical methods

Blood samples were drawn into test-tubes containing EDTA. Plasma was separated and portions stored at −20° until analysis. Glucose was measured by an automated glucose oxidase method (Beckman Instruments, Fullerton, CA), and insulin by the method of Desbuquois & Aurbach (1971). FFA was measured by a modification of the procedure of Akio et al. (1973), and cholesterol and triglyceride were analyzed on a Technicon Auto Analyzer (Technicon Corp., Tarrytown, N.Y.) (Allain et al. 1975; Kessler & Lederer, 1976). HDL-cholesterol was measured by phosphotungstate precipitation with Auto-Isopol precipitating reagent (Bernstein et al. 1970).

Statistical analysis

Statistical comparisons were performed as Student's paired t tests on an IBM 370/3033 computer in the Stanford Computer Center utilizing the Statistical Package for the Social Sciences.

RESULTS

Table 1 shows some clinical characteristics of the subjects studied, as well as their fasting TG, cholesterol and HDL-cholesterol concentrations on diets S and P. Each value represents the mean of three values for each subject, and patients have been listed in order of increasing fasting TG concentration on diet S. Fasting TG concentrations on diet S ranged from 0.51 to 5.04 mmol/l. Plasma TG levels were lower on diet P in seventeen subjects, but the differences were very small in most instances. Thus, the group mean was slightly, but not significantly, lower on diet P as compared to diet S. If we focus on the seven patients with TG levels of more than 1.69 mmol/l, it can be seen that the mean TG level on diet P was lower in one subject (20), essentially similar in four subjects (14, 16, 17 and 18) and actually higher in two subjects (15 and 19). The values obtained would seem to indicate that the differences in the P:S value of the amount used in the present studies do not modify fasting plasma TG concentrations.

In contrast, plasma cholesterol levels were significantly lower (P < 0.005) on diet P as compared to diet S. However, even in this instance, the magnitude of the change was modest (10%), and was only observed in fourteen subjects. On the other hand, the fasting cholesterol level of the group was only 4.60 mmol/l on diet S, and more dramatic changes might have been seen with higher base-line cholesterol levels.

Finally, it is apparent from the values in Table 1 that the fasting HDL-cholesterol concentrations were not influenced by differences in the P:S value.

The relationship between VLDL-TG secretion rate and TG concentration is shown in Fig. 1. It is obvious that differences in dietary P:S had little effect on either variable, and
Table 1. Subject characteristics and lipid concentrations

<table>
<thead>
<tr>
<th>Subject no.</th>
<th>Sex</th>
<th>Age (years)</th>
<th>Body-wt (kg)</th>
<th>TG (mmol/l)</th>
<th>Cholesterol (mmol/l)</th>
<th>HDL-Cholesterol (mmol/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Diet S</td>
<td>Diet P</td>
<td>Diet S</td>
</tr>
<tr>
<td>1</td>
<td>♂</td>
<td>35</td>
<td>50.6</td>
<td>0.51</td>
<td>0.45</td>
<td>3.80</td>
</tr>
<tr>
<td>2</td>
<td>♂</td>
<td>63</td>
<td>45.5</td>
<td>0.64</td>
<td>0.62</td>
<td>5.92</td>
</tr>
<tr>
<td>3</td>
<td>♂</td>
<td>55</td>
<td>69.6</td>
<td>0.67</td>
<td>0.63</td>
<td>3.82</td>
</tr>
<tr>
<td>4</td>
<td>♂</td>
<td>21</td>
<td>85.0</td>
<td>0.62</td>
<td>0.53</td>
<td>3.18</td>
</tr>
<tr>
<td>5</td>
<td>♂</td>
<td>69</td>
<td>73.1</td>
<td>0.73</td>
<td>0.72</td>
<td>4.42</td>
</tr>
<tr>
<td>6</td>
<td>♂</td>
<td>34</td>
<td>81.2</td>
<td>0.79</td>
<td>0.62</td>
<td>4.83</td>
</tr>
<tr>
<td>7</td>
<td>♂</td>
<td>51</td>
<td>53.8</td>
<td>0.82</td>
<td>0.80</td>
<td>3.13</td>
</tr>
<tr>
<td>8</td>
<td>♂</td>
<td>31</td>
<td>54.9</td>
<td>0.89</td>
<td>0.75</td>
<td>5.40</td>
</tr>
<tr>
<td>9</td>
<td>♂</td>
<td>63</td>
<td>80.0</td>
<td>0.95</td>
<td>0.78</td>
<td>5.30</td>
</tr>
<tr>
<td>10</td>
<td>♂</td>
<td>44</td>
<td>59.6</td>
<td>1.02</td>
<td>0.88</td>
<td>4.75</td>
</tr>
<tr>
<td>11</td>
<td>♂</td>
<td>54</td>
<td>69.9</td>
<td>1.11</td>
<td>0.86</td>
<td>3.72</td>
</tr>
<tr>
<td>12</td>
<td>♂</td>
<td>48</td>
<td>73.2</td>
<td>1.16</td>
<td>0.89</td>
<td>4.78</td>
</tr>
<tr>
<td>13</td>
<td>♂</td>
<td>38</td>
<td>59.1</td>
<td>1.40</td>
<td>1.38</td>
<td>4.75</td>
</tr>
<tr>
<td>14</td>
<td>♂</td>
<td>46</td>
<td>66.3</td>
<td>1.76</td>
<td>1.71</td>
<td>5.09</td>
</tr>
<tr>
<td>15</td>
<td>♂</td>
<td>54</td>
<td>83.0</td>
<td>1.97</td>
<td>2.54</td>
<td>3.90</td>
</tr>
<tr>
<td>16</td>
<td>♂</td>
<td>53</td>
<td>78.2</td>
<td>2.88</td>
<td>2.88</td>
<td>4.34</td>
</tr>
<tr>
<td>17</td>
<td>♂</td>
<td>58</td>
<td>79.0</td>
<td>2.99</td>
<td>2.89</td>
<td>5.48</td>
</tr>
<tr>
<td>18</td>
<td>♂</td>
<td>51</td>
<td>59.4</td>
<td>3.05</td>
<td>2.98</td>
<td>5.87</td>
</tr>
<tr>
<td>19</td>
<td>♂</td>
<td>53</td>
<td>92.2</td>
<td>3.29</td>
<td>3.91</td>
<td>4.75</td>
</tr>
<tr>
<td>20</td>
<td>♂</td>
<td>43</td>
<td>94.5</td>
<td>5.04</td>
<td>4.16</td>
<td>4.63</td>
</tr>
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</table>

Mean ± SE:

<table>
<thead>
<tr>
<th>Statistical significance of difference between diets</th>
</tr>
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<tbody>
<tr>
<td>Mean ± SE</td>
</tr>
<tr>
<td>NS</td>
</tr>
<tr>
<td>P &lt; 0.005</td>
</tr>
<tr>
<td>NS</td>
</tr>
</tbody>
</table>

NS, not significant; HDL, high-density-lipoprotein.

Fig. 1. Relationship between fasting triglyceride (TG) concentration and very-low-density lipoprotein (VLDL)-TG secretion rate (μmol/kg per h) in adult human subjects given diets S (●) and P (→). (○). Mean and 95% confidence interval of ninety-five normal subjects studied with this method in our laboratory. For details of the diets, see p. 260.
Table 2. Effect of diet on fasting and postprandial glucose, insulin and FFA concentration in adult human subjects

<table>
<thead>
<tr>
<th></th>
<th>Fasting</th>
<th>Postprandial*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Diet S</td>
<td>Diet P</td>
</tr>
<tr>
<td>Mean</td>
<td>SE</td>
<td>Mean</td>
</tr>
<tr>
<td>Glucose (mmol/l)</td>
<td>4.83</td>
<td>0.08</td>
</tr>
<tr>
<td>Insulin (μU/ml)</td>
<td>10.5</td>
<td>1.9</td>
</tr>
<tr>
<td>FFA (mmol/l)</td>
<td>0.42</td>
<td>0.03</td>
</tr>
</tbody>
</table>

FFA, free fatty acids.

* The total area above (glucose and insulin) or below (FFA) the 8.00 concentration during the day-long measurement of postprandial glucose, insulin and FFA responses.

† Values expressed on a /h basis.

that the relationship between the two variables was comparable to what we had previously observed (Reaven et al. 1965; Farquhar et al. 1965; Olefsky, Farquhar et al. 1974).

Fasting and postprandial glucose, insulin and FFA levels on the two diets are shown in Table 2. It is apparent that changes in dietary P:S did not modify any of these variables. Parenthetically, the postprandial plasma TG levels were also the same on the two diets.

Finally, the values in Fig. 2 demonstrate that both SSPG and SSPI levels were similar on the two diets. Thus, varying dietary P:S values did not lead to any change in insulin sensitivity.
DISCUSSION

The aims of this study were twofold. First, we wished to document the effect of a realistic increase in dietary P:S on TG metabolism. More specifically, diet S was an attempt to match closely a conventional American diet in both amount and type of fat, and diet P was meant to simulate the increase in polyunsaturated fat intake which could be readily attained and maintained in a free-living state. With this approach, it is clear that we could not produce a significant fall in plasma TG levels of the twenty subjects that we studied. In contrast, a small, but statistically significant, fall in plasma cholesterol levels was achieved.

On one level these results seem to be at odds with the bulk of previously published observations on the effect of increasing the dietary P:S value. However, on more detailed analysis it appears that our results may not be that aberrant. First, many previous studies examining the effects of polyunsaturated fats on plasma triglyceride were long term, and involved dietary manipulations other than simply substituting polyunsaturated for saturated fats (Ahrens et al. 1957; Antonis & Bersohn, 1961; Wilson et al. 1971; Hall et al. 1972). In particular, some extent of weight reduction was also achieved, and the influence that changes in body-weight have on TG metabolism is well known (Olefsky, Reaven et al. 1974). Therefore, it is more reasonable to compare our results to short term controlled observations. When this is done, it is clear that these studies have differed dramatically in the extent to which polyunsaturated fats were substituted for saturated fats. It is not possible to determine the exact P:S value used in some studies (Engelberg, 1966; Grundy, 1975), which makes interpretation of their results difficult. When accurate P:S values are reported (or can easily be calculated), it is apparent that most investigators employed more extreme changes in P:S than we with values 4–7.5 (Spritz & Mishkel, 1969; Nestel et al. 1970; Conner et al. 1975; Durrington et al. 1977; Shephard et al. 1978; Shephard et al. 1980). Thus, our inability to lower plasma TG levels may be a simple reflection of the fact that we only increased the P:S value from 0.2 to 2.0. Indeed, the only two investigators of whom we are aware who studied a reasonable number of patients and used comparable changes in P:S, were Chait et al. (1974) and Vessby et al. (1980). While their results differ somewhat from ours, there are many similarities. Chait et al. (1974) reported a 35% decrease in plasma TG concentration in subjects similar to ours when they raised the P:S value from 0.2 to 2.4; however, if two hypertriglyceridaemic patients who displayed large reductions in TG levels are excluded from their analysis, the reduction in TG concentration for the remaining twenty-one patients is less dramatic. In addition, variation of the results of the studies may be due to subtle differences in the diets employed. Though the sources and P:S values of the dietary fats were similar in the work of Chait et al. (1974) and our study, there are differences in the protein and carbohydrate sources. The protein in our study was almost exclusively casein, while the protein of Chait et al. (1974) was meat and milk. Our carbohydrate was composed of mostly lactose and maltose, while starch, sucrose and fructose were used in the latter study. Differences in dietary components of these types have been shown to influence lipid responses dramatically (Kritchevsky, 1976; Carroll, 1978). In particular, carbohydrate sources such as sucrose and fructose have much greater effects on lipid responses than lactose, and may accentuate lipid responses that are induced by protein or fat. Therefore, interaction involving each of the dietary components may have contributed to any differences in our TG results. Vessby et al. (1980) observed a 13% reduction in plasma TG concentrations in subjects with type IIb and type IV hyperlipoproteinaemia when the P:S value was changed from 0-2 to 2-0, a result not markedly different from the 4-3% reduction seen in our subjects. The subjects studied by Vessby et al. (1980) had extreme elevations of TG levels with mean values of 3-93 and 4-89 mmol/l, respectively,
as compared to our subject mean of 1.62 mmol/l. Since patients with the most severe TG elevations appear to benefit the most from an increase in dietary P:S (Chait et al. 1974), our results and those of Vessby et al. (1980) are very comparable. Furthermore, it is interesting to note that the plasma cholesterol levels of the patients in both studies fell by 16 and 12%, which is similar in magnitude to the fall of 10% that we observed.

The second goal of our experiments was to investigate the mechanism by which increases in dietary P:S value reduce plasma TG concentrations. We wished to see if the changes in TG kinetics resulting from the increased P:S value were associated with differences in either insulin secretion or insulin sensitivity or both. The results presented clearly demonstrated that a rise in P:S from 0-2 to 2-0 had no consistent effect on any of the metabolic variables we measured. Thus, neither plasma TG concentrations nor VLDL-TG demonstrated that a rise in P:S has been increased to higher values (Spritz & Meshkel, 1969; Durrington et al. 1977; Shephard et al. 1978; Shephard et al. 1980). On the other hand, the present results raise the possibility that the increase in dietary P:S that can be attained by conventional diets may have, at best, only a modest effect on plasma lipid levels.

In conclusion, increasing dietary P:S from 0-2 to 2-0 did not lead to any dramatic improvement in a number of metabolic variables. Indeed, the only significant change noted was a 10% reduction in fasting plasma cholesterol. One interpretation of these results is that it takes longer than 14 d of dietary adjustment before significant changes in VLDL-TG metabolism can occur; however, lower TG levels have stabilized within 14 d when the dietary P:S has been increased to higher values (Spritz & Meshkel, 1969; Durrington et al. 1977; Shephard et al. 1978; Shephard et al. 1980). On the other hand, the present results support the possibility that the increase in dietary P:S that can be attained by conventional diets may have, at best, only a modest effect on plasma lipid levels.

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266 F. B. KRAEMER, M. GREENFIELD, T. A. TOBEY AND G. M. REAVEN


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