Differences in glucose-dependent insulinotrophic polypeptide hormone and hepatic lipase in subjects of southern and northern Europe: implications for postprandial lipemia1–3

Kim G Jackson, Antonios Zampelas, Jacqueline ME Knapper, Helen M Roche, Michael J Gibney, Anthony Kafatos, Barry J Gould, John W Wright, and Christine M Williams

ABSTRACT

Background: This study was an extension of a previous study that showed different lipemic responses to standard test meals in subjects from southern and northern Europe.

Objective: The aim was to determine in 32 healthy young men from northern and southern Europe whether differences in the secretion of insulin and glucose-dependent insulinotrophic polypeptide (GIP) might explain these findings through the actions of these hormones on lipoprotein lipase.

Design: We investigated in a randomized, single-blind, crossover study the effects of 2 test meals of identical macronutrient composition but different saturated fatty acid (SFA) and monounsaturated fatty acid (MUFA) contents on postprandial GIP, insulin, the ratio of incremental triacylglycerol to apolipoprotein B-48 (a marker of chylomicron size), and the activity of postheparin lipases.

Results: Fasting and postprandial GIP concentrations and postheparin hepatic lipase activities were significantly higher in the southern Europeans (P < 0.001 and P < 0.02, respectively). Lipoprotein lipase activity after the SFA-rich meal was significantly higher in the northern Europeans (P < 0.01). HL activity 9 h after the SFA-rich meal and the area under the curve (AUC) for the postprandial insulin response correlated with the AUC for the postprandial GIP response [r = 0.44 (P < 0.04) and r = 0.46 (P < 0.05), respectively]. There were no significant differences in chylomicron size between the 2 groups for either meal, but when the groups were combined there was a significant difference in chylomicron size between the SFA- and MUFA-rich meals (P < 0.05), which could be due to the formation of larger chylomicrons after the MUFA-rich meal.

Conclusion: The significantly higher GIP and insulin responses and HL activities in southern Europeans may provide an explanation for our previous report of attenuated postprandial lipemia (2–4). Most evidence suggests that the larger size of the PUFA-containing chylomicron particles is responsible for their faster rate of clearance; however, recent evidence showed that the percentage increase in apolipoprotein (apo) B-48 and triacylglycerol in a triacylglycerol-rich lipoprotein fraction was greater after a PUFA-rich test meal than after an SFA-rich test meal (5).

INTRODUCTION

The magnitude and duration of postprandial lipemia has been correlated with the risk of development of coronary heart disease (CHD) (1) and there is interest in dietary factors that can moderate postprandial lipemia. Postprandial studies with meals of various saturated fatty acid (SFA) and polyunsaturated fatty acid (PUFA) contents have confirmed that the fatty acid composition of the chylomicron particle is an important determinant of its synthesis, removal rate, or both, and can influence postprandial lipemia (2–4). Most evidence suggests that the larger size of the PUFA-containing chylomicron particles is responsible for their faster rate of clearance; however, recent evidence showed that the percentage increase in apolipoprotein (apo) B-48 and triacylglycerol in a triacylglycerol-rich lipoprotein fraction was greater after a PUFA-rich test meal than after an SFA-rich test meal (5).

Little was known about the effects of various monounsaturated fatty acid (MUFA) contents in meals on lipemia until recent studies that showed no effect on triacylglycerols (6) or apo B-48 (7) of meals with various MUFA and SFA contents. However, the latter study, which was conducted in men living in southern and northern Europe, showed that although lipemic responses to the different meals were similar, there were notable differences in the patterns of response between the northern and southern European subjects. The patterns of response showed an earlier triacylglycerol peak and a more rapid return to fasting triacylglycerol and apo B-48 concentrations in southern than in northern Europeans. The more rapid decline in apo B-48 in the late postprandial period suggested fewer remnant particles in the

KEY WORDS Glucose-dependent insulinotrophic polypeptide, hepatic lipase, postprandial lipemia, apolipoprotein B-48, Mediterranean diet, saturated fatty acids, monounsaturated fatty acids, Europe, humans

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circulation in the southern Europeans. Although insulin responses to a standard meal were also higher in the southern Europeans, no other differences were evident between the 2 populations.

In the present investigation we wished to explore the possibility that differences in the postprandial secretion of glucose-dependent insulinoergic polypeptide (GIP) and in the activation of lipoprotein lipase might underlie the greater triacylglycerol tolerance of the southern European group. GIP responses to a standard SFA-rich meal and lipoprotein lipase and hepatic lipase activities in response to SFA- and MUFA-rich meals were measured. Previous studies suggest that GIP may play a key role in circulating triacylglycerol homeostasis (4, 8–10). The secretion of this hormone is stimulated by the absorption of fat in the enteroctye and the extent of its secretion is influenced by meal fat content and the type of fat (11). Notably, this gut hormone alone and in combination with insulin promotes the activation of lipoprotein lipase in adipose tissue (12). Therefore, we proposed that greater activation of lipoprotein lipase, through a heightened response of the enteroinusral axis, may underlie the greater triacylglycerol tolerance of the southern Europeans. Postheparin hepatic lipase activities were also determined because this lipolytic enzyme has been implicated in the receptor-mediated removal of chylomicron remnants from the circulation (13), which appeared to be accelerated in our southern European group (7). Because chylomicron particle size has been suggested as one mechanism through which altered meal fat fatty acid composition may influence the clearance of triacylglycerols, and because more recent in vitro studies have suggested that larger chylomicrons are formed in the presence of MUFAs than of PUFAs and SFAs (14), we estimated the incremental triacylglycerol–apo B-48 ratios (as a marker of chylomicron size) in our 2 groups of subjects in response to both the SFA- and MUFA-rich meals.

SUBJECTS AND METHODS

Protocol

A subset of 32 of the 60 subjects reported in reference 7 was investigated in greater detail in the present study. The subjects were healthy men aged 18–30 y, had a body mass index (BMI; in kg/m²) of 20–25, and were recruited mostly from university populations at each center. Sixteen of the subjects were from northern Europe (Ireland and the United Kingdom) and 16 were from southern Europe (Greece). Ethical consent was provided by the relevant ethics committees responsible for each of the 3 investigation centers and subjects gave written consent before the study. Subjects were excluded if they had any metabolic disorders (eg, diabetes mellitus or other endocrine or liver disease), were taking dietary supplements (eg, fish oil or primrose oil), or were smokers, vegetarians, heavy exercisers (ie, more than three 30-min aerobic exercise sessions per week), or heavy drinkers (> 30 units alcohol/wk). A fasting blood sample was taken to ensure that plasma total cholesterol concentrations were < 6.5 mmol/L, triacylglycerols were < 1.5 mmol/L, glucose was < 6.5 mmol/L, and hemoglobin was > 130.0 g/L. Habitual dietary intakes were assessed by dietitians using a combination of food-frequency questionnaires and diet diaries, which detailed the frequency and quantity of foods consumed. Subjects were asked to maintain their usual exercise patterns during the study period and to abstain from alcohol and organized exercise regimens for 24 h before each postprandial study day. Age, BMI, and daily nutritional intake for each group are shown in Table 1.

### Table 1

<table>
<thead>
<tr>
<th>Age (y)</th>
<th>Northern Europeans</th>
<th>Southern Europeans</th>
</tr>
</thead>
<tbody>
<tr>
<td>22.3 ± 3.2</td>
<td>23.3 ± 3.0</td>
<td>23.3 ± 3.0</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>23.0 ± 2.0</td>
<td>23.8 ± 1.5</td>
</tr>
<tr>
<td>Energy (MJ/d)</td>
<td>12.9 ± 3.6</td>
<td>11.1 ± 4.6</td>
</tr>
<tr>
<td>Protein (g/d)</td>
<td>88.5 ± 23.2</td>
<td>94.2 ± 36.4</td>
</tr>
<tr>
<td>Carbohydrate (g/d)</td>
<td>387.0 ± 133.6</td>
<td>282.8 ± 129.0</td>
</tr>
<tr>
<td>Nonstarch polysaccharides (g/d)</td>
<td>15.6 ± 6.5</td>
<td>12.1 ± 3.6</td>
</tr>
<tr>
<td>Fat (g/d)</td>
<td>108.0 ± 32.0</td>
<td>128.8 ± 64.5</td>
</tr>
<tr>
<td>SFA (% of energy)</td>
<td>37.0 ± 6.0</td>
<td>42.9 ± 9.3</td>
</tr>
<tr>
<td>MUFA (% of energy)</td>
<td>14.0 ± 3.0</td>
<td>15.1 ± 3.4</td>
</tr>
<tr>
<td>PUFA (% of energy)</td>
<td>6.0 ± 2.0</td>
<td>4.3 ± 2.3</td>
</tr>
<tr>
<td>MUFA (% of energy)</td>
<td>11.0 ± 2.0</td>
<td>18.3 ± 7.4</td>
</tr>
</tbody>
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Each subject consumed 2 test meals on separate occasions in random order. The macronutrient composition of the test meals was identical and the energy content of the meals was 4.18 MJ, of which 55% was derived from carbohydrate, 8% was from protein, and 35% was from fat. The fatty acid composition of the test meals was modified to provide 12% and 24% of dietary energy as MUFAs. The SFA-rich test meal (SFA meal) had a fatty acid composition typical of a UK diet (12% MUFAs, 17% SFAs, and 5.0% PUFAs) and the MUFA-rich test meal (MUFA meal) had a fatty acid composition typical of the average Cretan diet (24.1% MUFAs, 5.4% SFAs, and 4.6% PUFAs) reported at the time of the Seven Countries Study (15). The test meals were presented in the form of a sandwich with bread (135 g) and strawberry jam (36 g) and a milk shake consisting of skim milk powder (40 g), strawberry milk shake powder (40 g Nesquik; Nestle UK Ltd, Surrey, United Kingdom), 40 g of the relevant test oil, and 200 mL mineral water (Évian, Évian, France). The test oils used (per 40 g meal) in the SFA meal were coconut oil (16 g), palm oil (8 g), olive oil (12 g), and sunflower oil (4 g); olive oil (40 g) was used in the MUFA meal. Coconut oil, which contains significant amounts of medium-chain triacylglycerols (MCTs), was used as a major fat component of the test oil in the SFA meal to represent the typical UK dietary fat profile. This oil was used because it provided the required amounts of stearic acid and MCTs, which are typically present in the UK diet as a result of the high use of dairy products. The ingredients used to prepare the test meals were provided from one source, except for the white sliced bread (low fiber), which was purchased fresh from local food stores at each of the 3 centers according to the agreed criteria for nutrient composition.

The design of the study was a single-blind crossover study in which the subjects attended an investigation unit after a 12-h overnight fast on 2 separate occasions separated by 24 wk. The northern Europeans attended either the Royal Surrey County Hospital (United Kingdom) or Trinity College (Dublin) and the southern Europeans attended the University of Crete (Greece). On arrival, an indwelling cannula was inserted and 2 fasting blood samples were taken. The test meal was presented and consumed within a 20-min period; no other food was allowed during the test period. Blood samples were collected hourly for...
a period of 9 h. Every 2 h, 200-mL decaffeinated, sugar-free drinks were consumed and subjects maintained their normal sedentary activity. After the last blood sample was taken, 7500 IU sodium heparin was injected into the contralateral arm and blood samples were collected at 5 and 15 min for the analysis of hepatic lipase and lipoprotein lipase activities, respectively. Blood samples for the analysis of fasting postheparin lipoprotein lipase and hepatic lipase activities were also collected on a separate occasion after a 12-h overnight fast, ≥7 d after the second test meal.

Blood was collected into lithium heparin-containing tubes for the analysis of triacylglycerol, apo B-48, GIP, insulin, and postheparin lipase activities. Plasma was separated by spinning at 1700 × g for 10 min with a bench centrifuge at room temperature, portioned for each of the analyses, and stored at −20 or −80°C. To protect apo B-48 from proteolytic cleavage, a preservative cocktail was added to the appropriate tubes before addition of the plasma sample to give a final concentration of 5% (by vol) (16). Insulin and GIP concentrations were measured only in samples collected after the SFA meals; other analytes were measured in samples collected after each of the 2 meals.

Analytic methods

To minimize analytic variability, assays for individual lipid and hormonal indexes were carried out at the UK center; frozen samples from the other 2 centers were transported to the UK center on dry ice. Validation studies were carried out for each of the biochemical assays before the multicenter study began, which showed the stability and reproducibility of samples collected and transferred under these conditions.

Plasma triacylglycerol concentrations were measured by using an automated enzymatic-based method with use of a Cobas-Mira analyzer (Roche Diagnostics Ltd, Welwyn Garden City, United Kingdom). Apo B-48 was measured by a specific competitive enzyme-linked immunosorbent assay (ELISA) (17). Briefly, a heptapeptide-thyroglobulin conjugate consisting of the terminal residues of the apo B-48 molecule is used as the coating material in this ELISA format. Samples are incubated with a specific polyclonal anti-apo B-48 antiserum, which recognizes the C-terminal region of the protein on the surface of lipoprotein particles and does not show any cross-reactivity to apo B-100 (18). The intraassay CV was 5.2% for 2.23 mg/L and 3.8% for 4.18 mg/L. The interassay CVs were 9.4% and 7.6%, respectively.

A double-antibody radioimmunoassay technique using specific antisera was used to measure immunoreactive insulin (19) and GIP (20) concentrations after the SFA meal only. Lipoprotein lipase and hepatic lipase activities were measured according to the method described by Nilsson-Ehle and Schotz (21) and modified by Knapper et al (12). Briefly, a triolein emulsion was diluted with 50 mmol tris (containing 0.3% fatty acid–free bovine serum albumin; Sigma Chemical Company, Dorset, United Kingdom), 0.2 or 4.0 mol NaCl/L (containing 0.2 IU heparin/L), and heat-inactivated serum. Plasma was incubated with the emulsion for 30 min at 37°C and skim milk was used as a standard in the assay. Lipoprotein lipase activities (7) were calculated by subtracting the activities from incubation of plasma samples with 4.0 mol NaCl/L (hepatic lipase activity) from that with 0.2 mol NaCl/L (total lipase activity). Enzyme activity is expressed in U/L plasma, which is equivalent to 1 nmol oleate released per minute at 37°C.

Calculation of chylomicron size

Incremental plasma triacylglycerol–apo B-48 ratios were calculated for each of the time points after the SFA and MUFA meals for all individuals in both groups. This was done by dividing individual triacylglycerol concentrations by those for apo B-48 for each time point of the postprandial period and then calculating the incremental triacylglycerol–apo B-48 ratio by subtracting the fasting triacylglycerol–apo B-48 ratio from each of the postprandial triacylglycerol–apo B-48 ratios. The incremental values were used because subtraction of the fasting triacylglycerol–apo B-48 ratio minimizes the contribution to plasma triacylglycerol by VLDLs. It has been estimated that 80–90% of the increase in postprandial triacylglycerol is contributed by chylomicrons rather than VLDLs (22), so that 80–90% of the incremental increase in triacylglycerol is assumed to be chylomicrons. This calculation provides an approximation of the relative amounts of triacylglycerol and apo B-48 in chylomicrons and, therefore, of chylomicron size.

Statistical analysis

Total areas under the curve (AUCs) for time responses were calculated by using the trapezoidal rule on EXCEL spreadsheets (23) and are reported as means ± SEMs. In the tables, data are presented as means ± SDs. The statistical package used was DATA DESK 4.1 (Data Description Inc, New York) and data that were not normally distributed were transformed before statistical analysis. An unpaired Student’s t test was used to compare the dietary intakes and fasting concentrations of lipids and hormones between the northern and southern Europeans. Two-way repeated-measures analysis of variance, with center (northern or southern Europe) and meal as independent variables, was used to investigate changes in postprandial responses for the analytes measured. P values < 0.05 were considered significant.

RESULTS

Subject information

There were no significant differences between the ages, BMIs, or dietary intakes reported earlier for the original group (7) and those described for the subset of 32 subjects in the present study. The subset of 32 subjects fulfilled the inclusion criteria for age, BMI, fasting blood lipids, and other factors. There were no significant differences in age or BMI between the northern and southern Europeans (Table 1). Intakes of carbohydrate and nonstarch polysaccharides (dietary fiber) were significantly lower and the percentage of energy provided as fat was higher in the southern Europeans. The type of fat eaten differed significantly between the 2 groups, with the percentage of energy as MUFAs higher and of PUFAs lower in the southern Europeans.

Fasting plasma lipid, apolipoprotein, and hormone concentrations

The biochemical values for each of the indexes reported here for the subset of 32 subjects were not significantly different from those reported earlier for the original group of 60 subjects (7). Fasting plasma lipid and apo B-48 concentrations were not significantly different on the morning of each of the study days and only data obtained before the SFA meal are shown (Table 2). There were no significant differences in fasting total and LDL
cholesterol or triacylglycerol between the 2 groups. However, apo B-48 and HDL-cholesterol concentrations were significantly lower in the southern Europeans than in the northern Europeans. Both fasting plasma insulin and GIP concentrations were significantly higher in the southern Europeans.

### Postprandial plasma GIP and insulin responses to the SFA meal

After the SFA meal, GIP concentrations rose, peaking at 120 min before returning slowly to baseline concentrations (Figure 1). The AUC for the GIP response in northern Europeans (63.2 ± 3.5 nmol · min/L) was significantly lower than that of the southern Europeans (116.5 ± 7.2 nmol · min/L) (P < 0.0001). Repeated-measures analysis of variance showed a significant difference in the pattern of the GIP responses between the 2 groups over the postprandial time course.

After the test meal, insulin concentrations rose sharply, peaking between 60 and 120 min for the northern Europeans and between 60 and 180 min for the southern Europeans (Figure 2).

Concentrations returned to baseline values at ≈360 min in both groups and were maintained until the end of the 9-h test period. No significant differences in the AUCs were observed between the 2 groups, although there was a trend for a greater response in the southern Europeans (228.6 ± 68.5 nmol · min/L) than in the northern Europeans (104.6 ± 12.3 nmol · min/L). Repeated-measures analysis of variance showed a significant difference in postprandial insulin response patterns over time between the 2 groups. The AUC for insulin was significantly correlated with the AUC of GIP after the SFA meal (r = 0.46, P < 0.05).

### Postprandial plasma triacylglycerol and apolipoprotein B-48 responses

Postprandial triacylglycerol and apo B-48 responses for the subset of 32 subjects (7) are shown in Figures 3 and 4, respectively. As reported previously (7), the summary measures for triacylglycerol and apo B-48 postprandial responses (AUCs and incremental AUCs) did not differ significantly between the 2 European groups. However, a significantly different pattern of response for both triacylglycerol and apo B-48 was observed between the 2 groups. Notably, there was an earlier time to peak triacylglycerol response after the SFA meal in the southern Europeans (104.6 ± 12.3 nmol · min/L), who also showed a more rapid decrease in the declining part of both the triacylglycerol and apo B-48 response curves.

### Incremental plasma triacylglycerol–apolipoprotein B-48 ratios

Calculated incremental triacylglycerol–apo B-48 ratios (chylomicron size) for the 2 groups in response to the 2 meals are shown in Table 3. There were no significant differences between the 2 European groups after either meal, which may in part reflect the considerable heterogeneity in the data and the large SD values. When the data for each meal for the 2 groups were combined (Figure 5), there was a significant difference in the postprandial responses of the incremental ratios to the 2 meals. Using the ratio of incremental triacylglycerol to apo B-48 as a surrogate measure of chylomicron size, it
appears that larger chylomicrons may have been formed in response to the MUFA meal than to the SFA meal, particularly in the early postprandial period. However, there is considerable heterogeneity in these data and the biological significance of the observed differences should be interpreted cautiously.

Postheparin lipoprotein lipase and hepatic lipase activities

Blood samples for the measurement of postheparin lipoprotein lipase activities were collected 15 min after injection of heparin and were obtained under 2 nutritional conditions: 9 h after consumption of the SFA and MUFA meals and on a separate occasion after an overnight fast. There was no significant effect of nutritional status on lipoprotein lipase activities in either group. However, there was a tendency for the 9-h postprandial lipoprotein lipase activities to be higher in the northern European than in the southern European subjects for both meals, and the difference was significant for the SFA meal (Figure 6). Mean fasting lipoprotein lipase activities also tended to be higher in the northern Europeans, but the difference was not significant. There were no correlations between lipoprotein lipase activities, measured under any nutritional conditions, and postprandial insulin or GIP responses.

Blood samples for measurements of postheparin hepatic lipase activities were collected 5 min after the injection of heparin under the same nutritional conditions as for lipoprotein lipase. Postheparin hepatic lipase activities in the southern Europeans were significantly higher than those in the northern Europeans after the SFA and MUFA meals and in the samples collected after an overnight fast (Figure 6). The postheparin hepatic lipase activity measured 9 h after the SFA meal correlated significantly with that of the AUC for GIP ($r = 0.44$, $P < 0.04$).

DISCUSSION

The aim of this study was to determine whether the different patterns of triacylglycerol and apo B-48 responses we reported previously between northern and southern Europeans (7) and in the subset reported in the present study could be explained by differences in gut hormone response, activities of lipoprotein lipase or hepatic lipase, chylomicron particle sizes, or a combination thereof in the 2 study populations. As expected, dietary differences were evident between the 2 populations, with higher intakes of MUFAs and lower intakes of PUFAs in the southern European group. The percentage of energy as total fat was also higher in the southern European group. These data for fat intake were supported by our previous measurements of adipose tissue fatty acid composition in this group (7), which, consistent with the dietary data, showed higher MUFA concentrations in the adipose tissue of southern Europeans.

We speculated that the different lipemic responses to standard test meals observed in the southern Europeans may have resulted from adaptational changes in lipid absorption, chylomicron formation and secretion, or chylomicron remnant clearance, which occur as a direct consequence of the consumption of a high-fat, high-MUFA diet. This possibility is supported by the findings of a recent study in which we observed changes in postprandial triacylglycerol and apo B-48 responses (similar to those evident for the southern Europeans) when subjects were transferred from a typical UK diet to a diet high in MUFA (6). The diets were highly controlled and resulted in changes only in the type of fat consumed (SFA or MUFA) in the 2 diets, with no alteration in total fat, carbohydrate, or fiber. In the present study, subjects from the 2 regions of Europe showed differences in their intakes of carbohydrate, fiber, as well as fat, so that it is not possible to conclude that their different postprandial profiles were solely the result of an adaptational response to a high-MUFA diet. There is limited information available on the effects of background dietary fiber intakes on postprandial lipemic responses and that which is reported provides conflicting information. Whereas one study showed an attenuated postmeal lipemic response when pea fiber was added to the background diet (24), others reported an exaggerated postprandial triacylglycerol response when the amount of soluble fiber in the diet was increased (25, 26) and an earlier study showed no effect on postprandial lipemia with the addition of dietary psyllium fiber (27). In addition to dietary differences, the possibility that genotypic differences between the 2 study populations or differences
Europeans (significant differences over time between the northern and southern European and southern European subjects. There were A)–rich and monounsaturated fatty acid (MUFA; B)–rich meals by 16 after the random consumption of the saturated fatty acid (SFA; response in this group. An increase in postprandial GIP response explain the higher fasting and more pronounced postprandial GIP habituation of subjects. Contrary to the GIP and insulin findings, lipoprotein lipase activities were consistently lower in the southern Europeans, although the difference was only significant after the SFA meal. Clearly, we were unable to measure postheparin lipoprotein lipase activities earlier in the postprandial periods, at time points that might have been more relevant to our hypothesis (when active clearance of chylomicron and VLDL is at its peak), so the data may reflect, in part, the limitation of the late postprandial measurement as an index of insulin- and GIP-stimulated lipoprotein lipase activity. Nevertheless, there was also a trend for fasting concentrations to be lower in the southern European group, which suggests a consistent down-regulation of this enzyme.

The finding of markedly and consistently higher hepatic lipase activities in the southern European group than in the northern European group was surprising and not entirely consistent with current thinking because a favorable lipoprotein phenotype for a lower risk factor of CHD is one in which there is a high lipoprotein lipase activity and a low hepatic lipase activity, resulting in the attenuation of lipemia and a low concentration of small, dense LDL and HDL particles (29). On the other hand, involvement of hepatic lipase with remnant removal suggests that under conditions in which neutral lipid exchange is not enhanced and triacylglycerol enrichment of HDL and LDL is low, an elevated hepatic lipase activity would be advantageous by promoting the rapid uptake and clearance of remnants (13). In the present study, it is possible that the higher hepatic lipase activities were associated with faster clearance of the dietary-derived remnants and could explain the rapid decline in apo B-48 concentration observed in these subjects after each of the test meals (7) and the lower fasting apo B-48 concentrations. The lipoprotein lipase activities reported here were within the range of published values, but hepatic lipase activities were low compared with those reported in other studies (29–32). Thus, it could be argued that our data do not accurately reflect true hepatic lipase activities. Although this is a possibility, the marked differences in the values found in the 2 populations obtained using the same assay and analyzed in a single laboratory suggest that the findings are not simply an artifact of the assay procedure. We observed a significant correlation between postprandial GIP responses and postheparin hepatic lipase activities in the 2 populations. Although GIP has been shown to promote the activation of lipoprotein lipase in vitro (12) and to enhance the clearance of

FIGURE 4. Mean (±SEM) apolipoprotein (apo) B-48 responses after the random consumption of the saturated fatty acid (SFA; A)–rich and monounsaturated fatty acid (MUFA; B)–rich meals by 16 northern European and 16 southern European subjects. There were significant differences over time between the northern and southern Europeans (P < 0.0001, repeated-measures analysis of variance).

in lifestyle factors not accounted for by our inclusion criteria may underlie the differences in postprandial lipemic response that we observed here should be considered.

In the present study we investigated the possible role of hormonal mechanisms or of differences in chylomicron size (which might reflect alterations in chylomicron synthesis and secretion) that might underlie the effects of a chronic high-MUFA diet on postprandial lipemia. In particular, we wished to investigate whether concentrations of the gut hormone GIP, which has been implicated in circulating triacylglycerol homeostasis and which stimulates lipoprotein lipase activity, differed between the 2 populations.

The most important aspect of our findings was that fasting and postprandial GIP and insulin concentrations were significantly higher in the southern than in the northern Europeans. Mean habitual fat intakes were not shown to be significantly higher in the southern Europeans, although a higher fat intake could explain the higher fasting and more pronounced postprandial GIP response in this group. An increase in postprandial GIP response to standard fat-containing meals was shown when fat intake was chronically increased in humans (11). Although no studies have investigated the specific effects of dietary MUFAs on GIP, MUFAs have been shown to stimulate a potent cholecystokinin response (28). This response and the apparently more rapid absorption of dietary triacylglycerol in the southern European group (7) would be expected to enhance GIP secretion because GIP secretion is regulated by chylomicron formation. A biphasic insulin response was observed in the southern Europeans but not in the northern Europeans, which may have been due to the continual high secretion of GIP during the postprandial period, which is known to directly affect the secretion and synthesis of insulin. Therefore, the GIP and insulin data appear to support our original proposal that rapid clearance of the triacylglycerol-rich lipoproteins and return to fasting concentrations previously reported in these subjects (7) may be the result of heightened activity of the enteroinsular axis. However, the latter aspect of our hypothesis is not supported by the values for either late postprandial or fasting lipoprotein lipase activities, which were measured in the 2 groups of subjects. Contrary to the GIP and insulin findings, lipoprotein lipase activities were consistently lower in the southern Europeans, although the difference was only significant after the SFA meal. Clearly, we were unable to measure postheparin lipoprotein lipase activities earlier in the postprandial periods, at time points that might have been more relevant to our hypothesis (when active clearance of chylomicron and VLDL is at its peak), so the data may reflect, in part, the limitation of the late postprandial measurement as an index of insulin- and GIP-stimulated lipoprotein lipase activity. Nevertheless, there was also a trend for fasting concentrations to be lower in the southern European group, which suggests a consistent down-regulation of this enzyme.

The finding of markedly and consistently higher hepatic lipase activities in the southern European group than in the northern European group was surprising and not entirely consistent with current thinking because a favorable lipoprotein phenotype for a lower risk factor of CHD is one in which there is a high lipoprotein lipase activity and a low hepatic lipase activity, resulting in the attenuation of lipemia and a low concentration of small, dense LDL and HDL particles (29). On the other hand, involvement of hepatic lipase with remnant removal suggests that under conditions in which neutral lipid exchange is not enhanced and triacylglycerol enrichment of HDL and LDL is low, an elevated hepatic lipase activity would be advantageous by promoting the rapid uptake and clearance of remnants (13). In the present study, it is possible that the higher hepatic lipase activities were associated with faster clearance of the dietary-derived remnants and could explain the rapid decline in apo B-48 concentration observed in these subjects after each of the test meals (7) and the lower fasting apo B-48 concentrations. The lipoprotein lipase activities reported here were within the range of published values, but hepatic lipase activities were low compared with those reported in other studies (29–32). Thus, it could be argued that our data do not accurately reflect true hepatic lipase activities. Although this is a possibility, the marked differences in the values found in the 2 populations obtained using the same assay and analyzed in a single laboratory suggest that the findings are not simply an artifact of the assay procedure.

We observed a significant correlation between postprandial GIP responses and postheparin hepatic lipase activities in the 2 populations. Although GIP has been shown to promote the activation of lipoprotein lipase in vitro (12) and to enhance the clearance of
dietary triacylglycerol in vivo (33), no studies have investigated the effects of GIP on hepatic lipase; therefore, the possibility that GIP may influence the activity of this enzyme, which is suggested by the present data, is tentative but warrants further investigation.

Calculated chylomicron sizes for each of the postprandial time points after the SFA and MUFA meals were not significantly different between the 2 European groups; therefore, it is unlikely that formation of larger chylomicrons in response to fat-containing meals was the explanation for the observed differences in the postprandial lipemic response between the 2 groups. However, the calculation of chylomicron size is based on the assumption that most of the postprandial triacylglycerol is derived from chylomicrons, which may be incorrect or exaggerated. This assumption, together with the observed heterogeneity in the data, suggests that a more direct measurement of chylomicron size should be undertaken to address this hypothesis effectively. However, the incremental triacylglycerol–apo B-48 ratio was shown to be generally higher after the MUFA meal than after the SFA meal, which suggests that during the postprandial phase, there may be entry of larger chylomicrons into the systemic circulation when an MUFA-rich meal rather than an SFA-rich meal is consumed.

In conclusion, differences in postprandial gut hormone and insulin secretion between southern and northern European subjects in the present study may explain some of the differences in the postprandial lipemic responses we reported previously in these same individuals (7). However, contrary to our original proposal, there was no evidence to suggest greater hormone-induced activation of lipoprotein lipase, the mechanism that could link greater

<table>
<thead>
<tr>
<th>Time postmeal (min)</th>
<th>Northern Europeans</th>
<th>Southern Europeans</th>
<th>Northern Europeans</th>
<th>Southern Europeans</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>SFA meal</td>
<td>MUFA meal</td>
<td></td>
<td></td>
</tr>
<tr>
<td>60</td>
<td>−32.6 ± 43.6</td>
<td>−50.1 ± 135.0</td>
<td>−18.2 ± 58.9</td>
<td>−26.9 ± 98.3</td>
</tr>
<tr>
<td>120</td>
<td>−9.7 ± 69.7</td>
<td>−0.7 ± 120.1</td>
<td>34.8 ± 70.0</td>
<td>64.9 ± 141.6</td>
</tr>
<tr>
<td>180</td>
<td>−45.8 ± 48.3</td>
<td>−37.5 ± 166.2</td>
<td>−12.6 ± 88.2</td>
<td>−30.9 ± 115.4</td>
</tr>
<tr>
<td>240</td>
<td>−59.8 ± 72.0</td>
<td>20.6 ± 163.2</td>
<td>−48.8 ± 90.9</td>
<td>−69.7 ± 133.3</td>
</tr>
<tr>
<td>300</td>
<td>−12.4 ± 79.5</td>
<td>−1.8 ± 165.2</td>
<td>−22.9 ± 86.0</td>
<td>−69.0 ± 99.0</td>
</tr>
<tr>
<td>360</td>
<td>19.2 ± 81.0</td>
<td>−22.4 ± 162.5</td>
<td>72.1 ± 212.4</td>
<td>0.7 ± 120.9</td>
</tr>
<tr>
<td>420</td>
<td>10.1 ± 83.4</td>
<td>14.9 ± 161.1</td>
<td>13.8 ± 99.5</td>
<td>−20.3 ± 157.7</td>
</tr>
<tr>
<td>480</td>
<td>0.30 ± 113.4</td>
<td>31.8 ± 159.1</td>
<td>43.7 ± 104.5</td>
<td>−0.9 ± 120.5</td>
</tr>
<tr>
<td>540</td>
<td>−29.1 ± 79.3</td>
<td>11.4 ± 111.9</td>
<td>0.5 ± 67.3</td>
<td>−41.3 ± 96.4</td>
</tr>
</tbody>
</table>

\[
\text{TABLE 3} \\
\text{Incremental triacylglycerol–apo B-48 ratios in subjects from northern and southern Europe after consuming the SFA-rich and MUFA-rich meals}^{1} \\
\]

\[
\begin{array}{llllll}
\text{Time postmeal (min)} & \text{Northern Europeans} & \text{Southern Europeans} & \text{Northern Europeans} & \text{Southern Europeans} \\
\hline
60 & −32.6 ± 43.6 & −50.1 ± 135.0 & −18.2 ± 58.9 & −26.9 ± 98.3 \\
120 & −9.7 ± 69.7 & −0.7 ± 120.1 & 34.8 ± 70.0 & 64.9 ± 141.6 \\
180 & −45.8 ± 48.3 & −37.5 ± 166.2 & −12.6 ± 88.2 & −30.9 ± 115.4 \\
240 & −59.8 ± 72.0 & 20.6 ± 163.2 & −48.8 ± 90.9 & −69.7 ± 133.3 \\
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360 & 19.2 ± 81.0 & −22.4 ± 162.5 & 72.1 ± 212.4 & 0.7 ± 120.9 \\
420 & 10.1 ± 83.4 & 14.9 ± 161.1 & 13.8 ± 99.5 & −20.3 ± 157.7 \\
480 & 0.30 ± 113.4 & 31.8 ± 159.1 & 43.7 ± 104.5 & −0.9 ± 120.5 \\
540 & −29.1 ± 79.3 & 11.4 ± 111.9 & 0.5 ± 67.3 & −41.3 ± 96.4 \\
\end{array}
\]

\[
^{1} x \pm SD; n = 16. SFA, saturated fatty acids; MUFA, monounsaturated fatty acids.
\]
activation of the enteroinsular axis and improve triacylglycerol homeostasis. In contrast, the activity of hepatic lipase, a lipase whose greater activity has been linked with increased risk of CHD, was higher in the southern Europeans. We speculated that elevated hepatic lipase activity may be advantageous, favoring rapid removal of diet-derived remnants.

We thank Nigel Furlonger and Judith Wilson for their technical assistance.

REFERENCES