Exercise Prevents the Accumulation of Triglyceride-Rich Lipoproteins and Their Remnants Seen When Changing to a High-Carbohydrate Diet

Christina Koutsari, Fredrik Karpe, Sandy M. Humphreys, Keith N. Frayn, Adrienne E. Hardman

Abstract—We tested the hypothesis that daily aerobic exercise opposes the fasting hypertriglyceridemia and exaggerated postprandial lipemia observed after substituting dietary fat with carbohydrate. Eight healthy postmenopausal women aged 51 to 66 years consumed the same high-fat mixed meal on 3 occasions: (1) after 3 days on a low-carbohydrate diet (35%, 50%, and 15% energy from carbohydrate, fat, and protein, respectively); (2) after 3 days on an isoenergetic high-carbohydrate diet (corresponding values 70%, 15%, and 15%); and (3) after 3 days on the same high-carbohydrate diet with 60 minutes of brisk walking daily. Plasma triglycerides were higher after the high-carbohydrate diet than after the low-carbohydrate diet: fasting, 1.58 ± 0.19 versus 0.96 ± 0.12 mmol/L, respectively; 6-hour postprandial area under concentration versus time curve, 13.74 ± 1.57 versus 10.12 ± 1.15 (mmol/L) × hour, respectively (both P < 0.01). In the fasted and postprandial states, concentrations of apolipoproteins B-48 and B-100 in the triglyceride-rich lipoprotein fraction were significantly higher after the high-carbohydrate diet, as was the concentration of remnant-like lipoprotein particle cholesterol (a measure of lipoprotein remnants). These carbohydrate-induced increases in the number of circulating triglyceride-rich particles and their remnants were abolished when subjects had exercised daily during the high-carbohydrate diet. (Arterioscler Thromb Vasc Biol. 2001;21:1520-1525.)

Key Words: exercise • dietary carbohydrate • lipoproteins • triglycerides • women

Accumulating evidence links postprandial triglyceride (TG)-rich lipoproteins (TRLs) with coronary heart disease (CHD). These lipoproteins are a heterogeneous group of particles, including chylomicrons (containing apoB-48), VLDLs (containing apoB-100), and their remnants. Remnants are formed from VLDLs and chylomicrons after partial removal of their TGs by the action of lipoprotein lipase (LPL). They have a reduced TG content but are enriched in cholesterol and have been implicated in atherosclerosis. Determination of remnant-like lipoprotein particle (RLP) cholesterol provides a measure of TRL particles with remnant characteristics and can contribute to the assessment of CHD risk.

Current dietary guidelines for reducing the risk of CHD emphasize the replacement of saturated fat with complex carbohydrates so that total fat intake does not exceed 30% of energy intake. Low-fat high-carbohydrate diets effectively reduce LDL cholesterol but, in the absence of weight loss, can also increase fasting and postprandial plasma TG concentrations as well as RLP cholesterol. As explained above, this effect on TRL metabolism may reduce the overall benefit expected from such diets. For these and related reasons, there is currently a debate concerning the effectiveness of high-carbohydrate diets as a means for reducing CHD risk.

However, this debate has largely ignored the potential of exercise to offset these effects. Exercise conditioning is known to decrease fasting TG and postprandial TRL concentrations and to a low-fat high-carbohydrate diet. We used a short-term intervention specifically to examine the influence of exercise on TRL metabolism during the initial period of dietary change, when lipoproteinemic changes are particularly marked.

Methods

Subjects

The study was approved by Loughborough University’s Ethical Advisory Committee, and subjects gave their informed consent. Eight nonsmoking postmenopausal (for at least 2 years) healthy women aged 60 ± 4 years (mean ± SD) with a body mass index of 26.4 ± 2.3 kg/m² participated. Their habitual diets were assessed by the weighed food inventory method over 2 weekdays and 1 weekend day. Inventories were analyzed by using a computerized version of...
**TABLE 1. Composition of Low-CHO and High-CHO Diets**

<table>
<thead>
<tr>
<th></th>
<th>Low-CHO Diet</th>
<th>High-CHO Diet</th>
</tr>
</thead>
<tbody>
<tr>
<td>Energy, MJ</td>
<td>7.45±1.39</td>
<td>7.41±1.39</td>
</tr>
<tr>
<td>Carbohydrate, % energy</td>
<td>35</td>
<td>70</td>
</tr>
<tr>
<td>Simple sugars</td>
<td>18</td>
<td>47</td>
</tr>
<tr>
<td>Starch</td>
<td>17</td>
<td>23</td>
</tr>
<tr>
<td>Fat, % energy</td>
<td>50</td>
<td>15</td>
</tr>
<tr>
<td>Saturated</td>
<td>20</td>
<td>7</td>
</tr>
<tr>
<td>Monounsaturated</td>
<td>16</td>
<td>4</td>
</tr>
<tr>
<td>Polyunsaturated</td>
<td>12</td>
<td>3</td>
</tr>
<tr>
<td>Protein, % energy</td>
<td>15</td>
<td>15</td>
</tr>
<tr>
<td>Cholesterol, mg</td>
<td>331±131</td>
<td>131±25</td>
</tr>
<tr>
<td>Fiber, g</td>
<td>13±3</td>
<td>13±3</td>
</tr>
</tbody>
</table>

Values are mean or mean±SD (n=8 women).

food composition tables (Comp-Eat, version 5.0, Nutrition Systems). The subjects’ habitual diets provided 7.41±1.37 MJ/d, with 46±4% of energy as carbohydrate, 37±5% as fat, and 17±3% as protein. Maximal oxygen uptake (VO₂ max), predicted from the VO₂/heart rate relationship during uphill treadmill walking, was 29±2 mL/kg per minute. Plasma concentrations of TGs and total and HDL cholesterol, measured after an overnight fast on recruitment, were 1.1±0.31, 6.50±0.46, and 1.69±0.29 mmol/L, respectively. Two subjects were taking hormone replacement therapy and maintained this therapy during the study. Other than this, no subject was taking drugs known to influence lipid or carbohydrate metabolism.

**Design**
Subjects consumed a standard high-fat mixed meal after 3 different interventions: (1) 3 days on a low-carbohydrate (low-CHO) diet, (2) 3 days on a high-carbohydrate (high-CHO) diet, and (3) 3 days on the same high-CHO diet with one 60-minute session of brisk walking daily (high-CHO-Ex diet). The order of the interventions was counterbalanced, with 10-day washout periods, during which the subjects resumed their usual physical activity and dietary habits. During the day preceding each intervention, the diet was standardized, and subjects refrained from exercise and from alcohol consumption. No alcohol was consumed during any of the intervention periods.

**Experimental Diets and Exercise Sessions**
The experimental diets were based on normal foods. Their energy and macronutrient contents are shown in Table 1. The energy values matched, on an individual basis, each subject’s habitual energy intake. All food items were provided for the subjects, who prepared 3 meals and 1 snack each day by following a prescribed menu and weighing each item. Both diets were well tolerated, aside from a common complaint that subjects felt excessively full when consuming the high-CHO diet. Compliance, assessed by food inventories and detailed discussions with subjects, was high; ie, subjects followed the diets “to the gram.”

During the diet-only interventions, only activities of daily living were permitted. During the high-CHO-Ex intervention, subjects walked on the treadmill at 1.5±0.1 m/s (mean±SD) up a 3±1% gradient for 60 minutes each afternoon. VO₂ and carbon dioxide production were measured by using Douglas bag techniques, heart rate was determined by short-range telemetry (SPORT-TESTER, Polar Electro), and ratings of perceived exertion were assessed by use of the Borg scale. The average VO₂ was 17.7±1.1 mL/kg per minute, which represented 61±3% of predicted VO₂ max. Heart rate averaged 128±28 bpm, and ratings of perceived exertion were 12±2 (on a scale from 6 to 20). No subject experienced difficulty completing the walk. Gross energy expenditure per session was 1.46±0.10 MJ. On average, 56±11 g of carbohydrate and 13±3 g of fat were oxidized daily during treadmill walking.

**Test Meal Protocol**
Subjects arrived at the laboratory at 8:00 AM, after a 12-hour fast. Blood samples were obtained via a venous cannula in the fasted state and at 15, 30, 45, 60, 90, and 120 minutes after completion of the test meal and then hourly for 6 hours. The meal contained cereal, coconut, nuts, chocolate, fruit, and whipping cream (per kilogram body mass: 1.0 g fat, 0.9 g carbohydrate, and 0.2 g protein). The subjects rested throughout, consumed only water, and were always supine for at least 15 minutes before blood sampling.

**Analytical Methods**
One aliquot of plasma was kept on ice overnight before separation of a TRL fraction. To minimize proteolytic degradation of apoB in this aliquot, to each milliliter of plasma 1.0 μL of phenylmethylsulfonylfluoride (Sigma Diagnostics) (10 mM, dissolved in isopropanol) and 5 μL of aprotinin (Trasylol, Bayer) (1.4 μg/L) were added. The concentrations of TGs in plasma (samples were stored at −20°C) and the TRL fraction (fresh samples) were measured enzymatically, with correction for free glycerol. Plasma samples were also analyzed for total and HDL cholesterol (fasted samples only), and nonesterified fatty acids and glucose by enzymatic colorimetric methods. Aliquots of serum (stored at −70°C) were analyzed for RLP cholesterol (details below), insulin (Coat-a-Count, Diagnostic Products), and 3-hydroxybutyrate (Sigma Diagnostics). Apart from TG analysis in the TRL fraction, all samples from each subject were analyzed in the same batch. The TRL fraction (Sₐ >20) was separated by slicing the tube after preparative ultracentrifugation in an Optima TLX ultracentrifuge (Beckman Instruments Ltd) in a fixed angle rotor (TLA 100 - 4, Beckman Instruments Ltd) for 150 minutes at 543 000 g at 4°C. ApoB-48 and apoB-100 in the TRL fraction were used as specific markers for the concentration of TRL particles of intestinal and hepatic origin, respectively. They were quantified by using analytical SDS-PAGE. ApoE was quantified by using the apoB-100 standard, taking account of the difference in chromogenicity by multiplying by 2.6. RLP cholesterol was determined in the fasted state and 4 hours after a meal by using a novel immunoseparation kit technique (Jmro-II) kindly provided by Japanese Immunoresearch Laboratories Co (Takasaki, Japan).3

**Calculations and Statistics**
Comparisons among interventions were made by using repeated-measures ANOVA, followed by Tukey post hoc tests where applicable. One-way ANOVA was used to compare variables measured in the fasted state and summary measures of postprandial responses (total areas under concentration versus time curves [AUCs]). For RLP cholesterol concentrations, intervention and time effects were determined by using 2-way ANOVA. Whenever data were not normally distributed, statistical analyses were performed after logarithmic transformation. The number of TG molecules per apoB-100–containing TRL particle in the fasted state was calculated as a measure of VLDL particle size. The number of apoE molecules per apoB-100–containing TRL particle in the fasted state was also calculated. For both calculations, fasting TRL concentrations of TGs, apoB-100, and apoE were converted to moles per liter, and the presence of a small number of apoB-48–containing TRL particles was disregarded, because these represent a very small proportion of the TRL pool and are relatively TG poor (Sₐ <400) compared with “normal” chylomicrons. Fasting LDL cholesterol concentration was estimated by using the Friedewald formula. Statistical analyses were performed by using Statistics for Windows, version 5.0, adopting a 5% level of significance.

**Results**
**Plasma, TRL, and Serum Concentrations in the Fasted State**
Plasma TG and TRL-TG concentrations were significantly higher after the high-CHO intervention than after the low-CHO intervention, as were plasma TRL–apoB-48 and TRL–apoB-100 concentrations (Table 2). The high-CHO intervention did not seem to have affected the size of VLDL particles:
the concentrations of TRL-TG and TRL–apoB-100 were increased to the same degree; thus, the number of TG molecules per apoB-100–containing TRL particle was not significantly affected (Table 2). The high-CHO intervention increased the apoE content of VLDL particles (Table 2) as well as the serum RLP cholesterol concentration (Table 3). HDL cholesterol was reduced by the high-CHO intervention (Table 2).

The addition of exercise to the high-CHO diet significantly attenuated the diet-induced increases in plasma TGs, TRL-TGs and concentrations of TRL–apoB-48, TRL–apoB-100, and TRL–apoE (Table 2) and serum RLP cholesterol concentration (Table 3). Exercise did not reverse the reduction in HDL cholesterol concentration observed with the high-CHO intervention (Table 2).

There were no significant differences between the high-CHO-Ex and the low-CHO interventions in any of the above parameters except HDL cholesterol, which was significantly lower after the high-CHO-Ex intervention (Table 2).

Fasting plasma nonesterified fatty acid concentrations were not different among interventions (data not shown), but the 3-hydroxybutyrate concentration was somewhat lower after the high-CHO intervention than after the low-CHO intervention (P<0.07, Table 2). Serum insulin was lower after the high-CHO intervention than after the low-CHO intervention (Table 2).

Postprandial Responses
Plasma and TRL-TG concentrations after the high-fat mixed meal are shown in Figure 1. The AUCs for postprandial plasma TGs and TRL-TGs were greater (36% and 32%, respectively) after the high-CHO intervention than after the low-CHO intervention (Table 4). However, the addition of daily exercise to the high-CHO diet significantly reduced both AUCs, almost to the level observed after the low-CHO intervention (Figure 1, Table 4).

Postprandial plasma concentrations of TRL–apoB-48, TRL–apoB-100, and TRL–apoE are shown in Figure 2. The AUCs for each of these apolipoproteins were significantly

TABLE 2. Plasma, Serum, or TRL Fraction Variables Measured in the Fasted State

<table>
<thead>
<tr>
<th></th>
<th>Low-CHO Diet</th>
<th>High-CHO Diet</th>
<th>High-CHO Diet Ex</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Plasma TG, mmol/L</td>
<td>0.96±0.12</td>
<td>1.58±0.19</td>
<td>1.21±0.10</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>TRL-TG, mmol/L</td>
<td>0.37±0.08</td>
<td>0.85±0.14</td>
<td>0.55±0.09</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>TRL–apoB-48, mg/L</td>
<td>0.08±0.05</td>
<td>0.44±0.12</td>
<td>0.23±0.09</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>TRL–apoB-100, mg/L</td>
<td>9.36±2.27</td>
<td>27.84±5.31</td>
<td>17.63±3.49</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>TRL–apoE, mg/L</td>
<td>3.97±1.33</td>
<td>21.59±6.12</td>
<td>10.30±3.06</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Molecules TG per apoB-100–containing TRL particle</td>
<td>24.400±5300</td>
<td>16.700±1800</td>
<td>22.500±9000</td>
<td>0.42</td>
</tr>
<tr>
<td>Molecules apoE per apoB-100–containing TRL particle</td>
<td>5.43±0.92</td>
<td>12.74±4.11</td>
<td>10.47±3.16</td>
<td>0.02</td>
</tr>
<tr>
<td>Total cholesterol, mmol/L</td>
<td>6.37±0.22</td>
<td>6.37±0.23</td>
<td>6.23±0.18</td>
<td>0.44</td>
</tr>
<tr>
<td>HDL cholesterol, mmol/L</td>
<td>1.39±0.09</td>
<td>1.25±0.08</td>
<td>1.29±0.08</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>LDL cholesterol, mmol/L</td>
<td>4.50±0.16</td>
<td>4.37±0.17</td>
<td>4.36±0.15</td>
<td>0.34</td>
</tr>
<tr>
<td>Serum 3-hydroxybutyrate, mmol/L</td>
<td>0.10±0.02</td>
<td>0.07±0.01</td>
<td>0.10±0.01</td>
<td>0.06</td>
</tr>
<tr>
<td>Serum insulin, μU/mL</td>
<td>10.8±1.3</td>
<td>12.3±1.9</td>
<td>9.8±1.1</td>
<td>0.04</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>Low-CHO Diet</th>
<th>High-CHO Diet</th>
<th>High-CHO Diet Ex</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Values are mean±SEM (n=8 women).</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>*Overall significance of differences among the 3 interventions by 1-way ANOVA for repeated measures.</td>
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<tr>
<td>†P&lt;0.01 vs low-CHO diet, ‡P&lt;0.05 vs low-CHO diet, §P&lt;0.01 vs high-CHO diet, and</td>
<td></td>
<td>P&lt;0.05 vs high-CHO diet by Tukey post hoc test.</td>
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</tr>
</tbody>
</table>

TABLE 3. Serum RLP Cholesterol Concentration in the Fasted State (0 h) and at 4 h After Consumption of High-Fat Mixed Meal

<table>
<thead>
<tr>
<th></th>
<th>Low-CHO Diet</th>
<th>High-CHO Diet</th>
<th>High-CHO Diet Ex</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 hour, mmol/L</td>
<td>0.26±0.02</td>
<td>0.41±0.07*</td>
<td>0.29±0.02†</td>
<td></td>
</tr>
<tr>
<td>4 hour, mmol/L</td>
<td>0.38±0.04</td>
<td>0.55±0.06*</td>
<td>0.41±0.04†</td>
<td></td>
</tr>
</tbody>
</table>

Values are mean±SEM (n=8 women). Main effect of time was assessed as P<0.001; main effect of intervention was assessed as P<0.01.

*P<0.01 vs low-CHO diet, and †P<0.01 vs high-CHO diet by Tukey post hoc test.

Figure 1. Plasma TG and TRL-TG concentrations in the fasted state (0 hours) and for 6 hours after consumption of a high-fat mixed meal after 3 days on a low-CHO diet, after 3 days on a high-CHO diet, and after 3 days on a high-CHO Diet Ex. Values are mean±SEM for 8 women. Statistical analyses based on total AUCs are given in Table 4.
higher after the high-CHO intervention than after the low-CHO intervention (Table 4). After the high-CHO-Ex intervention, the AUCs for all 3 measured apolipoproteins were significantly lower than after the high-CHO intervention (Figure 2, Table 4).

Serum RLP cholesterol concentration increased after meals (main effect of time, \(P_{0.001}\); Table 3). Postprandial concentrations were 40% higher after the high-CHO intervention than after the low-CHO intervention, but exercise prevented this increase.

The serum insulin and 3-hydroxybutyrate responses to the test meal are shown in Figure 3. The 3-hydroxybutyrate AUC was significantly lower after the high-CHO intervention than after the low-CHO intervention (Table 4). The insulin AUC was significantly lower after the high-CHO-Ex intervention than after the low-CHO and high-CHO interventions. No significant differences were observed in the glucose or nonesterified fatty acid responses to the meal (data not shown).

### Discussion

In the present study, quantifications of TRL–apoB-48, TRL–apoB-100, and serum remnant lipoproteins were performed...
after dietary and exercise interventions in postmenopausal women, a group at high risk for CHD.21 This is the first study to show that daily exercise of moderate intensity prevents the increases in fasting and postprandial concentrations of TRL particles and their remnants, which are detected when there is a change from a low-CHO to a high-CHO diet.

In the fasted state, plasma TG and TRL-TG concentrations were higher after the high-CHO intervention than after the low-CHO intervention. It has been suggested that elevated TG synthesis by the liver and inadequate or reduced TG clearance could contribute to carbohydrate-induced hypertriglyceridemia.22 The increased presence of apoB-48–containing TRL particles in the 12-hour fasted state after the high-CHO intervention was a surprising observation, taking into consideration that the subjects had consumed very little fat (~35 g) the day before. These particles may constitute a subpopulation of chylomicron remnants with a slow turnover,23 and increased competition with VLDL particles24 (elevated 3-fold after the high-CHO intervention) could have prolonged their residence time in the circulation.

The carbohydrate-induced enhancement of postprandial TG and TRL responses would arise, in part, from the hypertriglyceridemia observed in the fasted state, because increases in fasting VLDL would increase competition for LPL when newly synthesized chylomicrons appear after a meal.24 It has been suggested that the increase in VLDL-TG secretion rate due to a high-carbohydrate diet results from an adaptive increase in the fractional rate of synthesis of TG in the liver. In line with this thinking, the reduction in the postprandial 3-hydroxybutyrate response by the high-CHO intervention was somewhat attenuated by exercise (Table 4, Figure 3). Another explanation may be that exercise opposed the suppressive effects of a diet high in carbohydrates on LPL activity.33 Exercise conditioning increases LPL activity,34 an effect that would improve the capacity for hydrolysis of VLDL-TGs and chylomicron-TGs. The addition of exercise to the high-CHO diet prevented the exaggerated and protracted postprandial TRL-apoE response observed after the high-CHO intervention. The latter effect probably reflects lower levels of remnant lipoproteins, a suggestion that is supported by the lower postprandial serum RLP cholesterol levels.

In the present study, the subjects’ energy intakes were held constant among interventions, so the effects of exercise may be attributable, in part, to the energy deficit incurred. However, a previous study, also in postmenopausal women, found that the attenuation of postprandial plasma TG concentrations by a single session of exercise was much greater than that attributable to an equivalent energy deficit achieved by reducing energy intake.35 Therefore, other, specifically exercise-related, factors must be involved.

The findings of the present study do not necessarily speak against current population recommendations for the substitution of energy from fat with carbohydrate, not least because our high-CHO diet was extreme and provided a large proportion of energy from sugars. Furthermore, in free-living populations, high-carbohydrate diets may be accompanied by weight loss, which often prevents hypertriglyceridemia.36 However, scientific bodies now recognize that a diet high in carbohydrates may adversely affect the lipid profile, at least in some individuals; thus, they advocate a diet high in unsaturated fat as an alternative.37 Our findings show that the adoption of a physically active lifestyle concurrent with a low-fat high-carbohydrate diet is a further option, although larger controlled randomized studies over a longer time scale are needed to examine this proposition. The present study provides evidence that moderate physical activity could be a means of lessening current anxieties about high-carbohydrate diets.

Acknowledgments
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References
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