Dietary Fiber and Lipoprotein Metabolism in the Genetically Obese Zucker Rat

Joseph N. Wilson, Selma P. Wilson, and R. Philip Eaton

The present investigation was designed to examine the influence of dietary fibers with differing soluble fiber compositions upon the metabolism of lipids in a hyperlipemic animal model, the Zucker fatty rat. The response to fiber was examined using a diet supplemented with cellulose, oat bran, or pectin which have a soluble/insoluble fiber ratio of 0:100, 33:66, and 100:0, respectively. These fibers provided 10% of the total diet weight; the control diet contained no fiber. A rapid increase in plasma triglyceride concentration was observed in all animals given fiber-supplemented diets in correlation with the increased carbohydrate content of the defined diets relative to the prestudy diets. This increase in plasma triglyceride was due to increased production of triglycerides with no change in the rates of clearance. The plasma total cholesterol levels were relatively constant on all diets. However, after 7 weeks on the pectin-supplemented diet, rats showed a 39% elevation in HDL and a 44% reduction in LDL concentration. This diet also resulted in reduced weight gain, in spite of a caloric intake equivalent to the control diets. Our data suggest that the ability of dietary fiber to alter plasma lipoproteins might be predictable from the soluble fiber composition or the pectin content of a given dietary fiber in this model of genetic endogenous hyperlipemia. (Arteriosclerosis 4:147–153, March/April 1984)
Methods

To examine the effects of differing dietary fibers, we designed four synthetic diets containing 70% carbohydrate, 14% fat, and 17% protein. These were a nonfiber diet (no added fiber), a 0% soluble fiber diet (cellulose fiber), a 33% soluble fiber diet (oat bran fiber), and a 100% soluble fiber diet (pectin citrus fiber No. 102587, ICN Pharmaceuticals, Incorporated, Cleveland, Ohio). The composition and caloric distribution of each diet is shown in Table 1. Diets were designed to be equivalent in simple carbohydrate and fat content (cottonseed oil) as prepared by ICN Nutritional Biochemicals (Cleveland, Ohio), although the nonfiber control diet contained more complex carbohydrate (starch) than did the high fiber diets. Before the study, all rats had been fed a standard diet of Wayne Lab-Blox (Continental Grain Company, Chicago, Illinois) with a caloric content of 55% carbohydrate, 12% fat, and 32% protein.

In the first diet study, 7-month-old female Zucker rats with homozygous hyperlipidemia were randomly assigned to the four treatment groups. All diets were fed ad lib simultaneously for 5 weeks, and all rats were weighed weekly. As tabulated in Table 2, the control, nonfiber-fed animals ate 20 ± 2 g of feed each day, providing 83 ± 8 Kcal. Animals eating soluble, fiber-containing diets consumed 19 to 23 g/day (70 to 91 cal/day), while the animals eating the insoluble cellulose ate 26 ± 2 g/day (96 cal/day).

In the second diet study, 7-month-old female Zucker rats were randomly assigned to the four treatment groups for pair-fed investigation over a 7-week period. Daily intake averaged 22 ± 1 g of each diet providing 74 to 83 Kcal/day (See Table 3). At the end of the study, plasma was analyzed for LDL and HDL apoprotein and cholesterol content.

Basal Levels

Triglyceride and cholesterol levels were measured by retro-orbital eye bleeding following a 12-hour fast (water available ad lib) before the diets were given and at weekly intervals. The 5-week samples were obtained after a 24-hour fast (water and 0.45 N NaCl-H2O available ad lib). At the end of the experiment, the animals were decapitated and the blood was collected and analyzed for TG and cholesterol. Triglyceride concentrations were determined in duplicate by the microenzymatic method of Baculo and David.11 Total serum cholesterol concentration was assayed by the direct microenzymatic method of Al-lain et al.12 Lipoproteins were isolated by serial ultracentrifugation in samples from Zucker hyperlipidemic rats as previously reported by Schonfeld et al.13

Triglyceride Secretion Studies

After 21 days of the experimental diet, triglyceride secretion rates (TGSR) were determined in animals which had fasted for 24 hours using the method of Triton hepatic blockade described by Robertson et al.14 and previously reported from this laboratory.15 The Triton blockade of peripheral triglyceride removal results in a rise in plasma TG levels, reflecting TG secretion into the plasma. Retro-orbital blood sam-

Table 1. Diet Composition

<table>
<thead>
<tr>
<th></th>
<th>Control (%)</th>
<th>Cellulose (%)</th>
<th>Oat bran (%)</th>
<th>Pectin (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sucrose</td>
<td>46.5</td>
<td>46.5</td>
<td>46.5</td>
<td>46.5</td>
</tr>
<tr>
<td>Starch</td>
<td>26.0</td>
<td>16.0</td>
<td>16.0</td>
<td>16.2</td>
</tr>
<tr>
<td>Casein</td>
<td>16.2</td>
<td>16.2</td>
<td>14.2</td>
<td>16.2</td>
</tr>
<tr>
<td>dl-Met</td>
<td>0.3</td>
<td>0.3</td>
<td>0.3</td>
<td>0.3</td>
</tr>
<tr>
<td>Vegetal oil</td>
<td>6.0</td>
<td>6.0</td>
<td>6.5</td>
<td>6.0</td>
</tr>
<tr>
<td>Vitamin mix</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>Salt mix</td>
<td>4.0</td>
<td>4.0</td>
<td>4.0</td>
<td>4.0</td>
</tr>
<tr>
<td>Cellulose</td>
<td>—</td>
<td>10.0</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Oat bran fiber</td>
<td>—</td>
<td>—</td>
<td>9.5</td>
<td>—</td>
</tr>
<tr>
<td>Pectin</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>10.0</td>
</tr>
<tr>
<td>(Soluble fiber)</td>
<td>0</td>
<td>0</td>
<td>(33)</td>
<td>(100)</td>
</tr>
</tbody>
</table>

| Caloric content | 3.040 | 4.148 | 3.698 | 3.961 | 3.688 |
| Carbohydrate (%)| 55    | 70    | 68    | 71    | 67    |
| Fat (%)         | 12    | 13    | 14    | 14    | 14    |
| Protein (%)     | 32    | 16    | 18    | 15    | 18    |

*Wayne Lab-Blox, Wayne Feed Division, Continental Grain Company, Chicago, Illinois.
Table 2. Effects of Diet

<table>
<thead>
<tr>
<th>Diet</th>
<th>Body weights</th>
<th>Diet intake†</th>
<th>Plasma concentration</th>
<th>Hepatic concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Initial (g)</td>
<td>Final (g)</td>
<td>(Kcal/day)</td>
<td>(mg/dl) (24-hr fasted)</td>
</tr>
<tr>
<td>Pectin (100% soluble fiber)</td>
<td>6 631 ± 36 660 ± 41</td>
<td>19 ± 2</td>
<td>70 ± 7</td>
<td>401 ± 56†</td>
</tr>
<tr>
<td>Oat bran (33% soluble fiber)</td>
<td>5 620 ± 42 686 ± 53</td>
<td>23 ± 2</td>
<td>91 ± 8</td>
<td>457 ± 95*</td>
</tr>
<tr>
<td>Cellulose (0% soluble fiber)</td>
<td>8 628 ± 47 672 ± 49</td>
<td>26 ± 2</td>
<td>96 ± 7</td>
<td>348 ± 27*</td>
</tr>
<tr>
<td>No fiber</td>
<td>6 647 ± 46 705 ± 60</td>
<td>20 ± 2</td>
<td>83 ± 8</td>
<td>226 ± 67</td>
</tr>
</tbody>
</table>

Values are means ± SEM.
†p < 0.05 compared with no fiber diet.
*tp < 0.15 compared with no fiber diet.
†Determined for two animals per group.

Table 3. Pair-Fed 7-Week Effect on Plasma Lipoproteins

<table>
<thead>
<tr>
<th>Protein content (mg/dl)</th>
<th>Cholesterol content (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HDL (d = 1.063–1.21)</td>
<td>116 ± 11</td>
</tr>
<tr>
<td>LDL (d = 1.019–1.063)</td>
<td>87 ± 5</td>
</tr>
<tr>
<td>HDL (d = 1.063–1.21)</td>
<td>135 ± 24</td>
</tr>
<tr>
<td>LDL (d = 1.019–1.063)</td>
<td>65 ± 25</td>
</tr>
<tr>
<td>HDL (d = 1.063–1.21)</td>
<td>128 ± 11</td>
</tr>
<tr>
<td>LDL (d = 1.019–1.063)</td>
<td>45 ± 11</td>
</tr>
<tr>
<td>HDL (d = 1.063–1.21)</td>
<td>153 ± 20*</td>
</tr>
<tr>
<td>LDL (d = 1.019–1.063)</td>
<td>49 ± 4†</td>
</tr>
</tbody>
</table>

Weight (g)/caloric intake (Kcal)

<table>
<thead>
<tr>
<th>Weight: Initial</th>
<th>533 ± 23</th>
</tr>
</thead>
<tbody>
<tr>
<td>Final</td>
<td>591 ± 22</td>
</tr>
<tr>
<td>Kcal/day</td>
<td>83 ± 8</td>
</tr>
</tbody>
</table>

All animals were pair-fed, averaging 22 ± 1 g of food each day. Values are means ± SEM.
*p < 0.01.
†p < 0.001.
‡p < 0.05.
exogenous lipid load provides insight into the potential capacity of removal mechanisms, but clearance does not directly define the amount of triglyceride being removed in the basal state. Since triglyceride clearance is recognized as a first-order kinetic event, TG clearance under conditions of normal TG concentration must be less than that determined during exposure to an exogenous lipid load.

Liver Triglyceride and Cholesterol

Liver samples were obtained at the end of the treatment period. They were homogenized, extracted with ether, and the total content of TG and cholesterol was determined.

Results

In the first diet study, hyperlipidemic rats with a mean weight of 632 ± 89 g were randomly assigned to the four treatment groups. The rats gained an average of 49 g during the 5 weeks of special diets, accounting for a 0.7% weight gain in these obese animals (weight change: pectin diet = 29 g, p < 0.2; oat bran diet = 66 g; cellulose diet = 44 g; control diet = 58 g). Although the final weights in the different groups were not significantly different, the pectin-fed animals gained the least and had the lowest final weight (Table 2).

In the second study with pair-feeding for 7 weeks, the average weight gain was 69 g, with the pectin-fed animals again showing the least gain (33 ± 4 g, p < 0.01) and the cellulose- and oat bran-fed animals showing the most gain (88 and 98 g) (See Table 3).

Triglyceride Serum Concentration

After the first week of the initial exposure to a high fiber diet, there was a prompt elevation in plasma TG concentration from the 24-hour fasting basal levels of 218 ± 24 mg/dl (Table 2). This fiber-induced hypertriglyceridemia averaged 402 ± 59 mg/dl as compared to the level of 226 ± 67 mg/dl in the control nonfiber-fed rats (p < 0.05) and was sustained throughout the 5 weeks of monitoring. The cellulose-fed animals with the largest caloric ingestion demonstrated the smallest relative degree of triglyceridemia.

Triglyceride Production

Triglyceride production (TGSR) was evaluated in 12 genetic hyperlipidemic rats with exposure to fiber (Figure 1). TGSR increased from a control mean of 1.05 ± 1.0 mg/min to 1.67 ± 0.2 mg/min (p < 0.01) in the oat bran diet (33% soluble) and to 2.05 ± 0.5 mg/min (p < 0.01) on the pectin fiber diet (100% soluble). The pectin-fed animals with the least caloric ingestion and the least weight gain demonstrated the highest TGSR.

Triglyceride Clearance

Plasma intralipid clearance (ILRR) from the plasma averaged 1.15 ± 0.25 mg/min in response to both oat bran and pectin dietary fiber, as compared to that in nonfiber diets of 0.75 ± 0.13 mg/min. However, this difference did not achieve statistical significance.

In Figure 1, a progressive discrepancy between TG production relative to clearance is seen with the

![Figure 1. Effect of fiber intake on triglyceride production and removal in Zucker obese hyperlipemic rats after 3 weeks of diet therapy.](https://example.com/figure1.png)
intake of oat bran and pectin. Thus, the nonfiber-fed animals show a net 0.30 ± 0.12 mg/min balance favoring production over clearance, while the oat bran and pectin fiber-fed animals show a net TG balance of 0.47 ± 0.25 mg/min and 0.95 ± 0.37 mg/min, respectively. Maximal net TG balance thus correlates with the elevation in TG concentration observed with fiber intake.

**Cholesterol Serum Concentrations**

Fasting plasma cholesterol concentrations averaged 236 ± 38 mg/dl in these genetically hyperlipidemic rats, and gradually increased throughout the 5 weeks of monitoring in all dietary groups. Although the lowest mean plasma cholesterol level of 284 ± 45 mg/dl was observed in the rats fed a pectin fiber diet, this observation did not achieve statistical significance when compared with the nonfiber level of 323 ± 45 mg/dl (Table 2).

Examination of the lipoprotein distribution of cholesterol in pair-fed animals after 7 weeks (Table 3) demonstrated a 39% increase in HDL cholesterol (p < 0.05), in association with a 32% increase in HDL apoprotein (p < 0.01) in pectin-fed animals. Parallel to this elevation in plasma HDL, a reduction LDL apoprotein to 56% of control (p < 0.001) and of LDL cholesterol to 71% of control (p < 0.05) in pectin-fed animals was observed.

**Hepatic Triglyceride and Cholesterol Levels**

The weights of the livers were similar in the four groups; 263 ± 13 g, 246 ± 4 g, 264 ± 13 g, and 262 ± 11 g, respectively, in the control, cellulose-fed, oat bran-fed, and pectin-fed animals. Hepatic triglyceride content was reduced from nonfiber-fed levels of 191 ± 42 μg/mg/net weight of liver, to 104 ± 28 μg/mg, 68 ± 13 μg/mg, and 89 ± 21 μg/mg, respectively in cellulose-fed, oat bran-fed, and pectin fiber-fed animals (Table 2). Only the reduction observed in oat bran-fed animals achieved statistical significance (p < 0.05). A similar tendency was seen in the hepatic cholesterol content, with control concentrations of 7.9 ± 0.5 μg/mg reduced to 5.4 ± 0.7 μg/mg with the addition of oat bran or pectin fiber containing soluble components (p < 0.05), but only to 6.4 ± 0.7 μg/mg with insoluble cellulose fiber (p > 0.15).

**Discussion**

Dietary fiber has reportedly reduced plasma cholesterol concentrations in both humans and animals. The present investigation was designed to examine the theory that eating fiber enriched in water-soluble, mucilaginous materials may selectively result in these cholesterol-lowering effects. Our data are consistent with the concept that plasma low density lipoprotein may be reduced by the dietary addition of pectin fiber which contains soluble components. When fiber was given in the form of a totally insoluble cellulose, this effect was lacking. These data are clinically pertinent since this animal model of genetic Type IV hyperlipidemia, the Zucker rat, was sustained on a no cholesterol diet. This finding in the genetically hyperlipidemic rat is similar to the reports that gum guar and oat bran fiber reduced plasma LDL in human hyperlipidemic patients.

We also observed a parallel elevation in plasma HDL as has been reported in cholesterol-fed Sprague-Dawley rats that were fed fiber. The opposite changes in the cholesterol content of plasma LDL and HDL appeared to balance each other, resulting in no significant net change in total plasma cholesterol levels in these hyperlipidemic rats. It is possible that feeding pectin over a longer time might reduce plasma cholesterol, as was reported in normal rats.

Our data confirm previous reports in the literature which examined the addition of various dietary fibers in which specific attention to the soluble or insoluble character of the fiber was not identified as a potential factor in predicting the metabolic response of rats to the diet. Considering the 18 groups of animals fed differing fiber-supplemented diets, it is clear that those fibers that are predominantly soluble (such as pectin, gum guar, carrageenan, or oat bran) are regularly associated with a reduction in both plasma and hepatic cholesterol concentrations, and a rise in plasma HDL. In contrast, those studies using predominantly insoluble fibers (such as alfalfa, wheat bran, or cellulose) showed no reduction in plasma or hepatic cholesterol concentration. The chemical nature of soluble pectin, galacturonic acid units, is distinct from other plant fiber structures, and could be critical to this metabolic response observed with the mucilaginous fibers.

It may be that these changes in plasma cholesterol transport lipoproteins have some influence upon the course of cholesterol deposition within tissues. Indeed, in those studies in which the cholesterol content of the rat aorta has been examined, adding an apparently soluble fiber to the diet of Sprague-Dawley rats resulted in a reduction in cholesterol deposition. Such an effect is consistent with the concept that HDL may transport cholesterol out of tissues, while LDL enhances cholesterol deposition into tissues.

The rise in plasma triglyceride concentration resulting from a fiber-induced increase in TG-production is unexpected. The additional complex carbohydrate (starch) in the diet of the nonfiber-fed control animals may have reduced the plasma TG levels in this control group as has been reported with complex sugars. Consistent with this suggestion, in studies in which fiber has been fed to normal rats, or to cholesterol-fed Sprague-Dawley rats, either no change or a reduction in plasma triglyceride concentration has been reported. We found no relationship between total caloric intake and the degree of triglyceridemia in these studies (See Table 2).
The mechanism(s) for the reduction in plasma LDL in pectin-fed animals is not examined in these studies, but is clearly important in the use of dietary fibers to manage human hypercholesterolemia. Lymphatic absorption of cholesterol in the rat is reduced by dietary fiber, which may explain the observation that the greatest hypocholesterolemic effect of fiber occurs with a cholesterol-supplemented diet. More- over, eating fiber also results in an increased fecal loss of endogenous neutral sterols and of bile acids. Such a fecal loss of endogenous cholesterol would be compatible with increased hepatic conversion of cholesterol to bile acids, leading to a reduction in both plasma LDL cholesterol and hepatic cholesterol availability as seen in our study. The operation of these two mechanisms has been reported during diets including pectin (100% soluble) in the Sprague-Dawley rat. Reduced cholesterol synthesis in everted sacs of ileum as well as in isolated ileum cells after feeding pectin has been reported, suggesting a further mechanism for reduction in LDL with this dietary fiber. However, other events occurring as a result of fiber exposure at other sites of LDL regulation, including the cell surface LDL-receptor, have not been examined. Finally, the relative decrease in weight gain observed in pectin-fed animals may have contributed to the changes in cholesterol transport lipoproteins.

We have no explanation for the reduced weight gain in rats on pectin-supplemented diets, even given a calorically equivalent diet. One likely explanation is that there was a limitation on nutrient absorption, although secondary influences on caloric utilization cannot be excluded. Since intraluminal bile acid concentrations correlate directly with the efficiency of fat absorption, an induced abnormality in bile acid solubilization of lipid to sustain fat digestion could lead to caloric loss in the stool. Heaton has reviewed the physiological obstacles to energy recovery resulting from dietary fiber in his proposal that dietary fiber may play a role in the prevention of obesity. Whatever the mechanism, the resulting relative caloric restriction may be a factor in the lowering of LDL and the elevation in HDL observed in pectin-fed rats, as observed in some investigations on humans.

Our data and those in the literature suggest that the ability of a dietary fiber to cause a reduction in plasma LDL and an elevation in plasma HDL may depend on the solubility characteristics or the pectin content of the fiber.

Acknowledgments

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References


Index Terms. hyperlipemic • LDL • Zucker fatty rat • soluble fiber • cellulose • oat bran • pectin • serum triglyceride • serum cholesterol • HDL
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