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The cholesterol-lowering effect of oat bran cereals in rats: Influence of processing

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In order to study the cholesterol-lowering properties of oat bran-based ready-to-eat cereal (RTE), rats (12/group) were fed the following diets: basal semipurified (B), B plus 1% cholesterol (BC), BC plus 10% cellulose (fiber control), BC + 67% of an extruded RTE cereal; BC + 67% of a conventionally cooked RTE cereal; and BC + 67% of the raw ingredients of the latter. All the RTE cereal products were oat bran-based, and 1% cholesterol was included in the dietary formulations. At the end of a 3-week feeding period, serum total cholesterol was significantly increased from 2.02 ± 0.13 mmol/L to 3.57 ± 0.18 mmol/L due to cholesterol feeding alone. The presence of either cellulose or oat bran products in the diet negated the hypercholesterolemia. Inclusion of 1% cholesterol in the diet raised liver cholesterol almost 10 fold. All three of the oat bran-based products significantly inhibited the increase but cellulose was ineffective. Liver cholesterol content was due primarily to the accumulation of cholesteryl esters. Liver triglyceride was also increased by dietary cholesterol and the presence of cellulose or oat bran in the diet appeared to enhance this effect. The method of processing the RTE appears to affect the efficacy of oat bran. (J. Nutr. Biochem. 6:246–249, 1995.)

Keywords: cellulose; cereal processing; cholesterolemia; oat bran; Triglycerides

Introduction

There is no single animal model that fulfills all the requirements of an ideal model for human atherosclerosis but many different models are useful for studying different aspects of the disease.^{1,2} Though Russell and Amy have reported on early atherosclerotic lesions in a susceptible rat model³ it is generally accepted that rodents do not develop arterial lesions resembling those of human atherosclerosis. However, rats can be manipulated to exhibit changes in lipid profiles in response to dietary stimuli. This feature has been used

heavily^{4–14} and can be considered helpful in screening test materials for efficacy before larger animal or expensive human studies are undertaken.

We have examined the serum and liver lipid altering effects of an extruded oat bran cereal product, a rotary cooked (conventional dried), and flaked oat product, and the raw uncooked mixture of the flaked product in the presence of dietary cholesterol. The results of our studies are detailed here.

Methods and materials

Male Wistar rats weighing 100 to 120 g were fed a commercial laboratory regimen for 10 days and were then randomized into six groups of 12 rats each. The rats were housed three to a double cage in a temperature- and light-controlled environment of 21°C on a 12-hr light–dark cycle. They were fed semipurified diets (Tables 1 and 2) for 3 weeks. Food and water were supplied ad libitum. All the diets were formulated to have equal calculated nutrient/energy

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Table 1 Composition of diets (parts/total)

| Diet | Group | | | | | |
|-------------------|-------|-------------------------|-----------------------|----------------------------|-------|------------|
| | 1 | 2 | 3 | 4 | 5 | 6 |
| | Basal | Basal + cholesterol (C) | Basal + C + cellulose | Oat bran products | | Flakes + C |
| | | | Extruded product + C | Uncooked flake mixture + C | | |
| Corn starch | 30.5 | 30.5 | 30.5 | 0.0 | 0.0 | 0.0 |
| Sucrose | 15.5 | 15.5 | 15.5 | 5.2 | 5.2 | 5.2 |
| Casein | 7.8 | 7.8 | 7.8 | 7.8 | 7.8 | 7.8 |
| Soy protein | 10.2 | 10.2 | 10.2 | 0.0 | 0.0 | 0.0 |
| L-lysine | 0.0 | 0.0 | 0.0 | 0.3 | 0.3 | 0.3 |
| DL-methionine | 0.2 | 0.2 | 0.2 | 0.0 | 0.0 | 0.0 |
| Beef tallow | 15.0 | 15.0 | 15.0 | 15.0 | 15.0 | 15.0 |
| Stripped corn oil | 5.0 | 5.0 | 5.0 | 1.8 | 1.8 | 1.8 |
| Cholesterol | 0.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 |
| AIN vitamin mix | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 | 1.0 |
| AIN salt mix | 4.0 | 4.0 | 4.0 | 4.0 | 4.0 | 4.0 |
| Cellulose | 0.0 | 0.0 | 10.0 | 0.0 | 0.0 | 0.0 |
| Cereal product | 0.0 | 0.0 | 0.0 | 67.0 | 67.0 | 67.0 |
| Total | 89.2 | 90.2 | 100.2 | 103.1 | 103.1 | 103.1 |

ratios. Body weights and food intake per cage were monitored weekly.

After 3 weeks of dietary treatment, each rat was killed (by barbiturate injection) following an overnight fast. Serum and liver were collected and frozen for subsequent lipid analysis. Serum was assayed for total cholesterol,¹⁵ high density lipoprotein (HDL) cholesterol,¹⁶ and triglycerides.¹⁷ Liver aliquots were extracted with chloroform: methanol,¹⁸ and the extracts used for determination of total cholesterol,¹⁵ free cholesterol,¹⁹ and triglycerides¹⁷ were also determined. The cholesteryl ester content of liver was estimated by subtracting free cholesterol from total cholesterol. Data were analyzed by analysis of variance (ANOVA) to determine the main treatment effects and by Studentized-Newman-Keuls multiple means comparisons^{20,21} for specific effects. Tests were considered significant at $P < 0.05$.

Results and discussion

Food consumption among the groups was not significantly different (Table 3). Calculation of feed efficiencies (weight

gain/food intake) in the six groups gave an average of 0.353 ± 0.016 . As shown in Table 3, oat bran-fed rats (Groups 4, 5, and 6) had lower absolute weight gains than the control rats fed cellulose (Group 3). Liver weights tended to reflect body weight, hence both the cellulose- and oat bran-fed groups had similar relative liver weights. The inclusion of cholesterol in the diet led to significantly higher relative liver weights. (Group 1 versus Groups 2–6), reflecting the accumulation of lipid in the liver. Ershoff and his associates^{22,23} reported similar findings in rats. As expected, serum cholesterol almost doubled in response to cholesterol feeding (Group 1 versus Group 2; Table 4) and HDL cholesterol (HDL-C) showed no significant change. The net result of this was that HDL-C expressed as a percentage of total serum cholesterol was significantly lower (21.8 versus 48.6%) in the cholesterol-fed group. The hypercholesterolemia induced by dietary cholesterol was reduced by the inclusion of cellulose and prevented by oat bran in the diet (Table 4). Chen and Anderson⁴ and Jennings et al⁶ found

Table 2 Profile of diets (parts)

| Diet | Group | | | | | |
|---------------------|-------|-------------------------|-----------------------|----------------------------|-------|------------|
| | 1 | 2 | 3 | 4 | 5 | 6 |
| | Basal | Basal + cholesterol (C) | Basal + C + cellulose | Oat bran products | | Flakes + C |
| | | | Extruded + C | Uncooked flake mixture + C | | |
| Fiber type | 0 | 0 | Cellulose | Oat + brown rice | Oat | Oat |
| Total parts | 89.2 | 90.2 | 100.2 | 103.1 | 103.1 | 103.1 |
| Total dietary fiber | 0.0 | 0.0 | 10.0 | 5.7 | 7.0 | 7.0 |
| Sucrose | 15.5 | 15.5 | 15.5 | 18.9 | 14.0 | 14.0 |
| Starch | 30.5 | 30.5 | 30.5 | 37.0 | 35.3 | 35.3 |
| Plant protein | 10.2 | 10.2 | 10.2 | 7.4 | 8.4 | 8.4 |
| Animal protein | 7.8 | 7.8 | 7.8 | 7.8 | 7.8 | 7.8 |
| Plant fat | 5.0 | 5.0 | 5.0 | 2.5 | 4.4 | 4.4 |
| Animal fat | 15.0 | 15.0 | 15.0 | 15.0 | 15.0 | 15.0 |

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Table 3 Food consumption, weight gain, and liver weights of rats fed diets containing different amount or kind of fiber*†

| | Food consumption (g/rat/day) | Net weight gain (g) | Liver weight (g) | Relative liver weight‡ |
|--------------------------------------|---------------------------------|-----------------------|------------------|--------------------------|
| <u>Control groups</u> | | | | |
| Group 1, basal | 21.2 ± 0.6 | 161 ± 6 ^a | 8.8 ± 0.4 | 2.90 ± 0.04 ^b |
| Group 2, basal + cholesterol (C) | 20.6 ± 0.5 | 143 ± 5 ^b | 10.1 ± 0.3 | 3.53 ± 0.05 ^a |
| Group 3, basal + C + cellulose | 18.7 ± 0.5 | 167 ± 6 ^a | 11.7 ± 0.7 | 3.71 ± 0.13 ^a |
| <u>Oat Bran Groups</u> | | | | |
| Group 4, extruded + C | 18.2 ± 0.5 | 123 ± 5 ^c | 9.7 ± 0.3 | 3.56 ± 0.05 ^a |
| Group 5, uncooked flakes mixture + C | 18.5 ± 0.5 | 138 ± 6 ^{bc} | 10.7 ± 0.4 | 3.71 ± 0.10 ^a |
| Group 6, flakes + C | 17.9 ± 0.4 | 122 ± 6 ^c | 9.3 ± 0.4 | 3.47 ± 0.01 ^a |

*Mean ± SEM.

†Values within each column followed by different superscripts are significantly different ($P < 0.05$) by Student–Newman–Keuls test.

‡Liver expressed as percentage of body weight.

cellulose to be significantly more cholesterolemic than oat bran and oat gum, respectively. The diets fed by these two groups of researchers contained 0.2% cholic acid in addition to 1% cholesterol, and that may account for some of the differences in the results. In whole animal experiments, cellulose was shown to increase total body cholesterol in rats.²⁴ Oat gum is presumably more active than oat bran in affecting serum cholesterol levels.⁹ In this study the only oat bran treatment group that showed significantly lower serum cholesterol relative to the control cellulose-fed group was the group consuming the extruded oat bran product.

Cholesterol feeding led to significantly lowered serum triglyceride concentrations (Group 1, 0.087 ± 0.006 mmol/L versus Group 2, 0.064 ± 0.003 mmol/L) while addition of cellulose plus cholesterol to the basal diet raised serum triglyceride concentrations relative to the basal plus cholesterol diet (Group 3 versus Group 2) (Table 4). Lipidemia induced by the dietary treatments in this study is probably indicative of the severity of the dietary stimulus because when diets were supplemented with half as much cholesterol (0.5%) there was little effect on lipidemia even in the presence of different fiber types.⁷ Hepatic total cholesterol was almost 10 fold higher when cholesterol was added to the basal diet (Group 1 versus Group 2) (Table 5). This effect was moderated significantly by the oat bran products but not by cellulose. The oat bran–fed groups still had total liver cholesterol levels that were approximately

five times the levels found in the control no-cholesterol group (Groups 4–6 versus Group 1).

All groups fed cholesterol exhibited only slightly higher levels of free cholesterol in the liver; the elevation in hepatic cholesterol was due mainly to the accumulation of cholesterol esters. Liver triglyceride levels were also increased by dietary cholesterol but unlike the case of liver cholesterol both cellulose and oat bran caused a further significant increase in liver triglyceride accumulation. We computed the serum plus liver pool of cholesterol (Table 5). The addition of cholesterol to the fiber-free diet resulted in an almost 10 fold increase in this value. The serum-liver cholesterol pool (to which the liver makes the major contribution) was significantly reduced by all three cereal preparations; the lowest pool was seen in rats fed the extruded cereal. The differences were not due to differences in body size or liver weight, both of which are included in the liver-serum pool calculation. The rats fed the basal-cholesterol-cellulose diet weighed 12 to 20% more than those fed the cereal diets but their serum-liver pool was 81% higher than that of Group 4, 58% higher than that of Group 5, and 72% higher than that of Group 6. These differences reflect an absolute effect on liver cholesterol accumulation.

This study was the first of many studies on the potential lipid lowering effects of several plant products and as such the study was designed to validate a rat model. To this effect there were three control groups: basal diet alone (1),

Table 4 Serum lipid profile of rats fed cholesterol and cellulose or oat bran*†

| | Cholesterol (mmol/L) | HDL cholesterol (mmol/L) | % HDL cholesterol | Triglyceride (mmol/L) |
|--------------------------------------|---------------------------|-----------------------------|-----------------------|-----------------------------|
| <u>Control Group</u> | | | | |
| Group 1, basal | 2.02 ± 0.13 ^{bc} | 1.00 ± 0.10 | 49 ± 2.2 ^a | 0.087 ± 0.006 ^a |
| Group 2, basal + cholesterol (C) | 3.57 ± 0.18 ^a | 0.79 ± 0.07 | 22 ± 1.4 ^c | 0.064 ± 0.003 ^{bc} |
| Group 3, basal + C + cellulose | 2.40 ± 0.13 ^b | 0.83 ± 0.06 | 36 ± 3.0 ^b | 0.088 ± 0.006 ^a |
| <u>Oat Bran Group</u> | | | | |
| Group 4, extruded + C | 1.73 ± 0.08 ^c | 0.90 ± 0.04 | 54 ± 3.1 ^a | 0.060 ± 0.005 ^c |
| Group 5, uncooked flakes mixture + C | 2.09 ± 0.10 ^{bc} | 0.77 ± 0.05 | 37 ± 2.8 ^b | 0.071 ± 0.005 ^{bc} |
| Group 6, flakes + C | 2.15 ± 0.10 ^{bc} | 0.81 ± 0.06 | 38 ± 2.5 ^b | 0.081 ± 0.007 ^{ab} |

*Mean ± SEM.

†Values within each column followed by different superscripts are different ($P < 0.05$) by Student–Newman–Keuls test.

Table 5 Serum plus liver cholesterol pool and liver lipid profile of rats fed cholesterol and cellulose or oat bran*†

| | Liver | | | | |
|--------------------------------------|---------------------------------|----------------------------------|---------------------------------|-----------------------|---------------------------|
| | Serum + liver pool (mmol) | Total cholesterol (mmol/g) | Free cholesterol (mmol/g) | Ester (%) | Triglyceride (mmol/g) |
| Control Groups | | | | | |
| Group 1, basal | 1.81 ± 0.09 ^a | 0.18 ± 0.008 ^c | 0.065 ± 0.003 ^b | 65 ± 2.6 ^b | 4.09 ± 0.34 ^d |
| Group 2, basal + cholesterol (C) | 17.83 ± 1.13 ^b | 1.73 ± 0.09 ^a | 0.080 ± 0.003 ^a | 95 ± 0.3 ^a | 6.92 ± 0.57 ^c |
| Group 3, basal + C + cellulose | 18.99 ± 1.55 ^b | 1.56 ± 0.10 ^a | 0.070 ± 0.003 ^a | 95 ± 0.3 ^a | 9.76 ± 0.45 ^b |
| Oat Bran Groups | | | | | |
| Group 4, extruded + C | 10.51 ± 0.66 ^c | 1.06 ± 0.06 ^b | 0.060 ± 0.003 ^a | 94 ± 0.3 ^a | 12.49 ± 0.45 ^a |
| Group 5, uncooked flakes mixture + C | 12.04 ± 0.91 ^c | 1.12 ± 0.10 ^b | 0.067 ± 0.005 ^a | 94 ± 0.8 ^a | 11.69 ± 0.34 ^a |
| Group 6, flakes + C | 11.77 ± 1.09 ^c | 1.24 ± 0.12 ^b | 0.067 ± 0.005 ^a | 94 ± 0.6 ^a | 9.99 ± 0.34 ^b |

*Mean ± SEM.

†Values within each column followed by different superscripts are different ($P < 0.05$) by Student–Newman–Keuls test.

basal diet + cholesterol (2), and basal diet + cholesterol + cellulose (3). This design made it possible to compare the effects of the addition of cholesterol independent of fiber status. There were two basic oat bran-based products (extruded products and flaked product). Since there were slight formulation differences between the two types of products (Table 2), the raw uncooked mixture of the flaked product was added as a separate group to determine whether cooking made any difference in efficacy. In examining both liver and serum data, there was no indication that cooking per se produced any significant hypolipidemic effects (Group 5 versus Group 6). Processing method (extrusion versus conventional) produced a significantly better profile of serum cholesterol.

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