Effects of solvent extraction on the hypocholesterolaemic action of oat bran in the rat

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In adult male rats fed on a cholesterol-free synthetic diet, plasma cholesterol concentrations were lowest with oat bran, intermediate with cellulose and highest with wheat bran. Plasma triacylglycerols (TAG) were similar with wheat bran and cellulose but higher with oat bran. The concentrations and pools of caecal volatile fatty acids (VFA) were lowest with cellulose and equally higher with oat bran and wheat bran. Plasma VFA concentrations in the hepatic portal vein reflected those in caecal digesta and were unrelated to plasma cholesterol. Feeding oat bran after extraction with *n*-pentane gave plasma cholesterol concentrations similar to that found with wheat bran. Reconstitution of oat bran with extracted lipids did not restore the cholesterol-lowering effect. Addition of the extracted material to a wheat-bran diet had no effect on plasma cholesterol. Plasma TAG were higher with the oat bran and reconstituted-oat-bran diets than with wheat-bran or cellulose diets. However, extracted oat bran + safflower oil gave similar TAG concentrations to that with wheat bran. These extractions and additions did not change caecal bile acid or neutral sterol concentrations. Effects of these diets on plasma cholesterol were unrelated to their tocotrienol or tocopherol content. Addition of *n*-pentane to oat bran followed by evaporation of solvent gave plasma cholesterol concentrations that were significantly higher than untreated oat bran but lower than similarly treated wheat bran. It is concluded that oat bran affects cholesterol metabolism through a pentane-soluble component as well as non-starch polysaccharides. It appears that the activity of this lipid is not transferable by simple addition of the solvent extract to the whole diet.

Dietary fibre: Hypocholesterolaemia: Oat bran: Rat

Considerable interest has arisen in the potential for lipid lowering of certain plant fibre products. These are generally high in water-soluble, non-starch, non-cellulose polysaccharides (NSP; Schneeman, 1986) and one such preparation is a wholemeal flour prepared from oats, commonly referred to as oat bran. Studies in normal (van Horn *et al.* 1986) and mildly hypercholesterolaemic men (Kirby *et al.* 1981; Anderson *et al.* 1984; Kestin *et al.* 1990) have shown reductions of 3–26% in total plasma cholesterol at intakes of 40–75 g oat bran/d. Possible reasons for this range of responses may lie in the degree of initial hypercholesterolaemia of the volunteers as well as compositional differences between various oat brans. Parallel experiments in rats have confirmed that oat bran lowers plasma cholesterol with diets high (Schinnick *et al.* 1988) or low (Illman & Topping, 1985) in cholesterol. The mechanism for this lipid lowering remains uncertain but appears to be mediated partially through enhanced faecal bile acid excretion (Judd & Truswell 1981; Illman & Topping, 1985). The components responsible for the lowering also remain to be identified although it is generally accepted that the water-soluble NSP are responsible as both oat (Chen *et al.* 1981) and barley β-glucan oppose dietary hypercholesterolaemia in

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the rat (Klopfenstein & Hoseney, 1987). However, some of the cholesterol reduction is believed to be due to the lipid present in oats (Judd & Truswell, 1981) so we have examined the effects of organic solvent treatment of the bran together with addition of the extracted material to oat bran and wheat bran on plasma cholesterol in rats.

METHODS

Materials

The oat bran and wheat bran used in these experiments were obtained from George Weston Foods (Braidwood St, Enfield, NSW 2136). They were analysed for total fat, fatty acid composition and protein as described previously (Cheng *et al.* 1987), and for starch, NSP and lignin by modifications of the methods of Theander & Westerlund (1986) and Englyst & Cummings (1988).

Animals and diets

Adult male rats of the Hooded Wistar strain were used. They were housed in groups of six in stainless-steel cages with wire-mesh bottoms to minimize coprophagy and under conditions of controlled heating and lighting as described previously (Cheng *et al.* 1987). They were given free access to water and to a purified diet based on sucrose and maize starch and containing added vitamins and minerals (Topping *et al.* 1988). There were three basic diets in which wheat bran, oat bran or cellulose were added to provide dietary fibre. The cellulose diet contained 60 g cellulose/kg. In manufacturing the other two experimental diets, wheat or oat bran were added instead of cellulose with appropriate corrections being made in the additions of maize starch, sucrose, casein and maize oil for endogenous starch, simple sugars, protein and fat. On analysis the oat bran was found to contain (g/kg) 496 starch, 108 protein and 107 NSP (+lignin). Corresponding values for the wheat bran were (g/kg) 270 starch, 32 fat, 110 protein and 354 NSP (+lignin). Three separate experiments were performed.

Expt 1

Rats of 190–210 g body-weight were fed on the cellulose, wheat-bran and oat-bran diets for an experimental period of 28 d. Food intake and body-weights were recorded daily from Monday to Friday. At the end of this time animals were lightly anaesthetized with diethyl ether at 09.30–10.00 hours and samples of blood were taken from the hepatic portal vein and systemic aorta and collected into ice-cold tubes. A portion of portal venous plasma was deproteinized with an equal volume of 10% sulphosalicylic acid. The precipitate was removed by centrifugation and the supernatant fraction was removed and stored at $-20°$ for up to 3 weeks before analysis. For this procedure, the volatile fatty acids (VFA) were concentrated by vacuum distillation following addition of heptanoic acid as internal standard. The distillate was dissolved in 50 μ litres 1 M-phosphoric and 1 μ litre chromatographed on a column of Tenax GC (containing 1% phosphoric acid) (Cheng *et al.* 1987). Triacylglycerols (TAG) in arterial plasma were measured using a commercial kit (Boehringer Mannheim, North Ryde, NSW 2113). Cholesterol was measured by gas–liquid chromatography after alkaline hydrolysis (Cheng *et al.* 1987). Whole caeca were excised and the contents extruded, weighed and homogenized with an approximately equal volume of distilled water. A portion of this homogenate was treated in the same way as plasma for the subsequent measurement of VFA.

Expt 2

In this experiment the effects of removal of neutral lipid by solvent extraction of oat bran were examined. Oat bran (3·5 kg) was steeped in 15 litres freshly redistilled analytical reagent grade *n*-pentane (BDH Ltd, Port Fairy, Victoria 3284). The solvent was decanted

and stored in an air-tight container. The steeping procedure was repeated twice and the solvent pooled and filtered to remove fine starch particles. The solvent was evaporated in vacuo at 40° leaving 325 g oil. The delipidated oat bran was air-dried at 20–25° for 12 h. Oat lipid (140 g) was redissolved in 1 litre *n*-pentane and added to 1·5 kg delipidated oat bran. The slurry was stirred thoroughly and the solvent removed by rotary evaporation. Wheat bran (3·5 kg) was similarly extracted with solvent and 140 g oat lipid added to an appropriate quantity of the delipidated bran. There were six experimental groups: oat bran (OB); wheat bran (WB); oat bran extracted and reconstituted with oat oil (OBO); wheat bran extracted and reconstituted with oat oil (WBO); extracted oat bran reconstituted with safflower oil (OBS); and cellulose (C). Samples of the diets were taken and powdered and extracted with *n*-pentane for determination of tocopherols and tocotrienols by high-performance liquid chromatography by a modification of the method of Hatam & Kayden (1979).

Rats (190–210 g body-weight) were fed on the diets for 10 d when the animals were anaesthetized and arterial blood samples taken as described. Whole caeca were excised, and the contents were extruded and lyophilized. Of the resulting powder 100 mg were hydrolysed with 2·5 M-sodium hydroxide following addition of *nor*-deoxycholate and 5-α cholestane as internal standards. Neutral sterols were extracted with light petroleum (b.p. 35–40°) and after acidification, bile acids were extracted with diethyl ether. Neutral sterols were analysed by gas–liquid chromatography on the same fused silica column (BPI; SGE Ltd, Melbourne, Victoria) used for plasma cholesterol determination. Bile acids were converted to the methyl trifluoroacetate derivatives before chromatography on a RSL 400 capillary column (Alltech Ltd, Homebush, NSW 2140).

Expt 3

In order to determine the consequences of *n*-pentane treatment *per se* on the effects of oat bran on plasma cholesterol, 3·5 kg oat bran were immersed in 1 litre solvent and then immediately dried in vacuo by rotary evaporation before diet preparation. There were three experimental groups: oat bran (OB); wheat bran (WB); and solvent-treated oat bran (SBO).

Rats (190–210 g body-weight) were fed on the diets as in Expt 1. After 10 d of dietary modification the animals were anaesthetized and arterial blood samples taken as described previously.

Statistical methods

Results are shown as the means with their standard errors for six observations per group. The statistical significance of differences between groups was established by the analysis of variance using a Genstat (1983) statistical package on a Microvax computer. The pooled estimate of variance was employed to calculate a least significance difference (using a two-tailed *t* test) in comparing individual groups. A value of $P < 0{\cdot}05$ was taken as a criterion of significance.

RESULTS

Expt 1

Food intake was constant over the experimental period with mean intakes (g/rat per d) of 24 (diet C), 22 (diet OB) and 23 (diet WB). Initial body-weight was the same for all three experimental groups and averaged 195 (SE 3, *n* 18) g for all groups, combined. At the end of the experiment mean body-weights did not differ, with means of 327 (SE 8), 335(SE 14) and 325 (SE 10) g for diets C, OB and WB respectively.

Concentrations of plasma total cholesterol and high-density-lipoprotein (HDL)-cholesterol were highest in rats fed on the WB diet, intermediate with diet C and lowest with

Table 1. *Expt 1*. Concentrations (μmol/ml) of plasma total cholesterol and high-density-lipoprotein (HDL)-cholesterol and triacylglycerols (TAG) in rats fed on diets containing oat bran (OB), wheat bran (WB) or cellulose (C)*

(Mean values with their standard errors for six observations per group)

	Cholesterol					
	Total		HDL		TAG	
Diet	Mean	SE	Mean	SE	Mean	SE
OB	2·01[a]	0·06	1·24[a]	0·09	1·71[a]	0·21
WB	2·47[b]	0·04	1·43[b]	0·04	0·91[b]	0·08
C	2·32[b]	0·07	1·51[b]	0·07	0·89[b]	0·13

[a, b] In any column, values with unlike superscript letters were significantly different ($P < 0·05$).
* For details, see p. 436.

Table 2. *Expt 1*. Concentration (μmol/ml) of major individual and total volatile fatty acids (VFA) in caecal digesta of rats fed on diets containing oat bran (OB), wheat bran (WB) or cellulose (C)*

(Mean values with their standard errors for six observations per group)

	Acetate		Propionate		Butyrate		Total†	
Diet	Mean	SE	Mean	SE	Mean	SE	Mean	SE
OB	54[a]	6	32[a]	4	27[a]	3	117[a]	12
WB	68[a]	4	15[b]	2	20[a]	2	106[a]	7
C	45[b]	4	14[b]	1	3[b]	0	65[b]	5

[a, b] In any column, values with unlike superscript letters were significantly different ($P < 0·05$).
* For details, see p. 436.
† The total includes the sum of total major (acetate, propionate and butyrate) and minor (iso-butyrate, valerate, iso-valerate and caproate) VFA.

diet OB (Table 1). The differences between diets OB and WB were statistically significant. Plasma TAG concentrations were the same with diets C and WB and significantly higher with diet OB.

Caecal digesta mass was highest in the OB group with a mean of 2·86 (SE 0·18) g, and significantly ($P < 0·01$) lower in animals fed on diets WB and C with similar means of 2·16 (SE 0·04) and 2·01 (SE 0·28) g respectively. Caecal total VFA concentrations were lowest with cellulose and similar with both bran-based diets (Table 2). Acetate was the major acid present with all treatments and its concentration was the same in the OB and WB groups and significantly higher than with diet C. The oat-bran diet (OB) gave significantly higher propionate concentrations than diets WB and C. Butyrate concentrations were the same with diets OB and WB but this acid was almost absent with the cellulose diet (C). The mass of total VFA (i.e. concentration × caecal fluid volume) was lowest with diet C, averaging 98 (SE 10) μmol. This was significantly ($P < 0·001$) higher with diet WB with a mean of 171 (SE 12) μmol. The highest VFA mass was found with the oat-bran diet with a mean of 219 (SE 24) μmol, although this was not significantly different from diet WB.

Concentrations of total plasma VFA in the hepatic portal vein were the same in the OB and WB groups and significantly higher than with diet C (Table 3). Concentrations of

Table 3. *Expt 1*. Concentration (μmol/ml) of major and total volatile fatty acids (VFA) in hepatic portal venous plasma of rats fed on diets containing oat bran (OB), wheat bran (WB) or cellulose (C)*

(Mean values with their standard errors for six observations per group)

	Acetate		Propionate		Butyrate		Total	
Diet	Mean	SE	Mean	SE	Mean	SE	Mean	SE
OB	1·04[a]	0·14	0·30[a]	0·04	0·20[a]	0·02	1·58[a]	0·20
WB	1·00[a,b]	0·13	0·14[b]	0·03	0·15[a]	0·03	1·33[a]	0·18
C	0·76[b]	0·04	0·11[b]	0·01	0·03[b]	0·00	0·95[b]	0·05

[a, b] In any column, values with unlike superscript letters were significantly different ($P < 0·05$).

* For details, see p. 436.

† The total includes the sum of the major (acetate, propionate and butyrate) and minor (iso-butyrate, valerate, iso-valerate and caproate) VFA.

Table 4. *Expt 2*. Concentrations (μmol/ml) of plasma total cholesterol and high-density-lipoprotein (HDL)-cholesterol and triacylglycerols (TAG) in rats fed on diets containing oat bran (OB), extracted oat bran + oat oil (OBO), extracted oat bran + safflower oil (OBS), wheat bran (WB), wheat bran + oat oil (WBO) or cellulose (C)*

(Mean values with their standard errors for six observations per group)

	Cholesterol					
	Total		HDL		TAG	
Diet	Mean	SE	Mean	SE	Mean	SE
OB	2·21[a]	0·06	1·44[a]	0·07	2·71[a]	0·40
OBO	2·49[b]	0·06	1·63[b]	0·04	2·45[a,b]	0·34
OBS	2·51[b]	0·10	1·61[b]	0·06	1·65[b]	0·30
WB	2·64[b]	0·11	1·69[b]	0·08	1·67[b]	0·21
WBO	2·62[b]	0·10	1·63[b]	0·06	1·68[b]	0·15
C	2·39[a,b]	0·08	1·44[a]	0·07	1·56[b]	0·04

[a, b] In any column, values with unlike superscript letters were significantly different ($P < 0·05$).

* For details, see pp. 436–437.

acetate and butyrate were similar in the OB and WB groups whereas propionate was significantly raised with diet OB. With diet C, plasma propionate was the same as with diet WB but butyrate was almost undetectable.

Expt 2

Initial body-weights were the same in all groups with a combined mean of 285 (SE 2, *n* 36) g. Final body-weights did not differ between groups with mean values of: OB 336 (SE 5), WB 336 (SE 5), OBO 337 (SE 7), WBO 338 (SE 3), OBS 328 (SE 6), C 336 (SE 9) g.

As expected, feeding unextracted oat bran (diet OB) gave significantly lower plasma cholesterol than wheat bran (Table 4). When diet OBO was given the concentrations of plasma cholesterol were the same as with diets WB, WBO and OBS. Feeding diet C resulted in a plasma cholesterol concentration intermediate between diet OB and the other diets, although the differences were not significant. However, HDL-cholesterol was the same with diets C and OB and significantly lower than with all other dietary groups. Plasma TAG

Table 5. *Expt 3*. Concentrations (μmol/ml) of plasma total cholesterol and triacylglycerols (TAG) in rats fed on diets containing oat bran (OB), solvent-treated oat bran (SBO) and solvent-treated wheat bran (SWB)*

(Mean values with their standard errors for six observations per group)

	Cholesterol		TAG	
Diet	Mean	SE	Mean	SE
OB	2·09[a]	0·04	2·61[a]	0·40
SBO	2·32[b]	0·07	2·30[a]	0·16
SWB	2·58[b]	0·10	1·57[b]	0·19

[a, b] In any column, values with unlike superscript letters were significantly different ($P < 0·05$).

* For details, see p. 437.

were the same with diets OBS, WB, WBO, WBS and C but significantly higher with diet OB.

Total caecal bile acids (CBA) and caecal neutral sterols (CNS) in pooled digesta from rats fed on diet OB were 0·65 and 1·64 mg respectively. The corresponding values with the WB diet were 0·66 and 0·87 mg. In rats fed on diets OBO and OBS the findings were similar to those for diet OB with CBA of 0·73 and 0·51 mg respectively, and CNS of 1·74 and 1·46 mg respectively. In animals fed on diet WBO, CBA were unchanged from those of diet WB at 0·71 mg while CNS were much lower at 0·40 mg. CBA content was very low with the cellulose diet being only 0·15 mg, although CNS content was similar to the other diets at 0·72 mg.

Diet OB contained 3 mg tocotrienols/kg and 4·2 mg tocopherols/kg. These levels were essentially unchanged in diet OBO with values of 3 mg tocotrienols/kg and 4·5 mg tocopherols/kg. In diet OBS the tocotrienol content was low at 0·2 mg/kg but the tocopherols were present at 6·3 mg/kg. Diet WB contained no detectable tocotrienols but had 11 mg tocopherols/kg. With diet WBO, tocotrienol and tocopherol levels were comparable to those with diets OB and OBO, i.e. 4·8 and 3·9 mg/kg respectively. Diet C contained traces of tocotrienols (0·8 mg/kg) but similar levels of tocopherols to those with diet WB, i.e. 10 mg/kg.

Expt 3

In order to determine whether solvent treatment modified the plasma lipid response rats were fed on oat bran (OB), solvent-treated oat bran (SOB) or treated wheat bran (SWB). The mean initial body-weight (all groups combined) was 240 (SE 4, *n* 18). Final body-weights were similar in all three groups with means of 300 (SE 7), 293 (SE 7) and 296 (SE 8) for diets OB, SOB and SWB respectively.

Plasma total cholesterol was lowest with diet OB, intermediate with SOB and highest in rats fed on diet WB (Table 5). These differences were statistically significant and the values for diets OB and SWB were similar to those with diets OB and WB in the previous two experiments. Plasma TAG were similar with diets OB and SOB and significantly higher than with diet SWB (Table 5).

DISCUSSION

The findings obtained in the present study confirm previous work by showing that in the rat, plasma cholesterol is lowered with oat bran (Chen *et al.* 1981; Illman & Topping, 1985; Schinnick *et al.* 1988). The present reduction was obtained with a diet low in cholesterol and with an Australian oat bran that contained approximately 50% of the water-soluble NSP

and protein present in the product used in other laboratories (Chen *et al.* 1981; Schinnick *et al.* 1988). This difference in NSP may reflect cultivar variations as well as differences in climate. The first experiment in the present series confirmed that the lowering effect was not restricted to the short term and was observed over 4 weeks (as opposed to 8–12 d in most previous reports). Oat bran gave the lowest plasma cholesterol and wheat bran the highest. These findings with wheat bran are similar to those reported by Asp *et al.* (1981) who also noted an increase in HDL-cholesterol. In the rat more cholesterol transport occurs in HDL than low-density lipoprotein (LDL) (Arbeeny *et al.* 1987) and hepatic uptake of both these lipoproteins occurs through receptor-mediated mechanisms. Activities of the LDL and putative HDL receptor appear to be reciprocally related (Roach *et al.* 1987). In the rat, wheat bran lowers HDL binding activity so that hepatic uptake of cholesterol also may be diminished (Topping *et al.* 1990). This down-regulation may explain the higher cholesterol concentrations with wheat bran but the mechanism whereby it occurs is unknown.

The active agent(s) responsible for these effects of oat bran on lipid metabolism also remain to be identified definitively. It is thought that the water-soluble NSP are substantially responsible as both barley and oats and their respective β-glucans are hypocholesterolaemic in cholesterol-fed rats (Klopfenstein & Hoseney, 1987). However, the mechanism of action of these polysaccharides is still open to question and several have been proposed. For example, NSP may lower cholesterol through inhibition of hepatic cholesterol synthesis by the propionate formed during large bowel NSP fermentation (Chen *et al.* 1981). This mechanism does not seem to be operative in vivo (Illman *et al.* 1988), a conclusion supported by Expt 1 in the present series in which VFA were measured in both the caecum and portal venous plasma. In part, this was done to compare effects of longer-term feeding of different dietary fibres on VFA. Notwithstanding reports that large bowel VFA in the rat were unaffected by dietary fibre (Nyman & Asp, 1985), oat bran raised the concentration of propionate, confirming previous experiments of shorter duration (Illman & Topping, 1985). However, the propionate concentrations recorded in animals fed on diet OB still appear to be insufficient to inhibit hepatic cholesterol synthesis.

Previous studies had shown that in rats fed on a cholesterol-free diet, oat bran increased the faecal excretion of bile acids and (to a lesser extent) of neutral sterols compared with animals fed on cellulose (Illman & Topping, 1985). This finding was supported by the enhanced hepatic cholesterogenesis seen in those animals as well as their raised plasma TAG. Such an increase is typical of bile acid depletion and reflects enhanced hepatic TAG secretion (Nestel & Grundy, 1976). However, the present findings suggest that it is quite possible that oat bran affects plasma lipid metabolism through more than one component and that wheat bran, oat bran and cellulose do not even act through a common mechanism. It seems that, relative to cellulose, oat bran lowers and wheat bran raises plasma cholesterol concentrations. Oat bran raises caecal bile acids (and hence, excretion) relative to cellulose (Illman & Topping, 1985). However, in the present study digesta steroid pools were similar with both oat and wheat bran while delipidation of oat bran abolished its dietary effects on total and HDL-cholesterol without altering caecal steroids. Finally, feeding delipidated oat bran to which safflower oil has been added, gave the same plasma TAG concentrations as did wheat bran.

It should be noted that interest in oats as a dietary aid to lowering cholesterol is of long standing (e.g. de Groot *et al.* 1963) and precedes the relatively recent interest in the hypocholesterolaemic properties of NSP. Indeed, Judd & Truswell (1981) concluded that both the lipid and non-lipid components of oats were major contributors to the lowering of plasma cholesterol by the grain in humans. However, these authors did not discriminate between the major lipids (TAG and phospholipids) and minor components (such as sterols or sterol esters). Our present findings with delipidated oat bran accord with their general

conclusions that oat lipids are important to the lowering of cholesterol. However, it appears there is some relationship between these lipids and other components (e.g. NSP) in native oat bran that enables plasma cholesterol to be lowered in the rat. Thus, with the techniques at our disposal it was not possible to add the *n*-pentane-soluble material back to extracted oat bran and restore the cholesterol-lowering effect. Also this extract when added to wheat bran did not alter the effects of the latter on plasma cholesterol. Moreover, feeding oat bran to which *n*-pentane was added and then evaporated gave similar plasma cholesterol concentrations to diets containing cellulose but plasma TAG concentrations similar to diets containing wheat bran. *Inter alia* these findings suggest that there are at least two components in the *n*-pentane extract that affect plasma lipids. It would appear that these components may be present in relatively small quantities in that the extracted oat lipid did not behave like an oil which conferred its properties on the diet by simple addition. This situation contrasts with recent studies with oils and cereal brans where we found that addition of fish oil to wheat or rice bran produced a significant lowering of plasma TAG and cholesterol (Topping *et al.* 1990). Of course, it could be argued that in the present experiments some of the observed changes could reflect disruption of the structure of the β-glucan or decomposition of a bile component in the lipid fraction due to the solvent treatment. However, these are unlikely in view of the fact that plasma TAG were the same in rats fed on the native and delipidated then relipidated oat bran. It seems more reasonable to conclude that these differences in cholesterol lowering reflect the loss of an interaction between the lipid and the NSP present in the parent bran.

The nature of this lipid remains to be identified but it is reasonably certain that it was not a tocotrienol. Qureshi *et al.* (1986) have shown that in chickens, barley lowered plasma cholesterol by direct inhibition of hepatic cholesterol synthesis. The inhibitor has been identified as being a tocotrienol. However, in the present experiments with extracted oat bran, no relationship could be noted between the dietary content of these compounds or tocopherols and plasma cholesterol.

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