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# Adzuki resistant starch lowered serum cholesterol and hepatic 3-hydroxy-3-methylglutaryl-CoA mRNA levels and increased hepatic LDL-receptor and cholesterol 7α-hydroxylase mRNA levels in rats fed a cholesterol diet

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We examined the effects of adzuki bean resistant starch on serum cholesterol and hepatic mRNA in rats fed a cholesterol diet. The mRNA coded for key regulatory proteins of cholesterol metabolism. The control rats were fed 15 % cornstarch (basal diet, BD). The experimental rats were fed BD plus a 0·5 % cholesterol diet (CD), or a 15 % adzuki resistant starch plus 0·5 % cholesterol diet (ACD) for 4 weeks. The serum total cholesterol and VLDL + intermediate density lipoprotein + LDL-cholesterol levels in the ACD group were significantly lower than those in the CD group throughout the feeding period. The total hepatic cholesterol concentrations in the CD and ACD groups were not significantly different. The faecal total bile acid concentration in the ACD group was significantly higher than that in the BD and CD groups. Total SCFA and acetic acid concentrations in the ACD group were significantly higher than those in the CD group but there were no significant differences in the concentrations between the ACD and BD groups. The hepatic LDL-receptor mRNA and cholesterol 7α-hydroxylase mRNA levels in the ACD group were significantly higher than those in the CD group and the hepatic 3-hydroxy-3-methylglutaryl (HMG)-CoA reductase mRNA level in the ACD group was significantly lower than in the CD group. The results suggest that adzuki resistant starch has a serum cholesterol-lowering function via enhancement of the hepatic LDL-receptor mRNA and cholesterol 7α-hydroxylase mRNA levels and faecal bile acid excretion, and a decrease in the hepatic HMG-CoA reductase mRNA level, when it is added to a cholesterol diet.

**Adzuki resistant starch: Serum cholesterol: Short-chain fatty acid: Bile acid: Hepatic mRNA**

Arteriosclerosis is the basis of coronary artery disease, one of the most serious diseases in man. Increased blood lipid and serum cholesterol concentrations contribute to the aetiology of CVD (Castelli *et al.* 1986). The possibility of lowering the plasma cholesterol concentration by interfering with the absorption of cholesterol and bile acids has been extensively investigated using both compounds of natural origin (e.g. gel-forming fibre, resistant starch, phytosterols and saponins) and synthetic sequestrants such as cholestyramine (Stedronsky, 1994). Fibre is known to be a non-nutritional substrate. On the other hand, starch is generally the major constituent of the human diet and has been regarded to be almost entirely digested in the upper part of the digestive tract. Soluble fibres are generally broken down by large-intestine microflora, and the resulting production of SCFA may be involved in the metabolic effects of fibres (Rémésy *et al.* 1995). The main fermentative substrates are undigested dietary carbohydrates, including NSP and resistant starch. Of these, resistant starch seems to be the more important substrate, quantitatively (Cummings & Macfarlane, 1991; Topping & Clifton, 2001). Rats fed resistant starch have significantly lower serum cholesterol and triacylglycerol concentrations than control rats fed a cornstarch diet (Fukushima *et al.* 2001; Han *et al.* 2003*a*). SCFA may be involved in lowering the serum cholesterol concentration (Chen & Anderson, 1984).

Beans are unique foods, rich in complex carbohydrates, proteins, dietary fibres and starch. Relatively few studies have investigated the digestibility of resistant starch from beans in the small intestine of human subjects (Schweizer *et al.* 1990) and the content of SCFA in the hindgut of rats fed processed bean flours (Henningsson *et al.* 2001). We have previously reported that enzyme-resistant adzuki (*Vigna angularis*) or tebou (*Phaseolus vulgaris*) starches are hypocholesterolaemic compared with cornstarch (Han *et al.* 2003*a*). On the other hand, it has been reported that rye reduces the total plasma cholesterol concentration only in rats maintained on a high cholesterol diet (Lund *et al.* 1993). It has also been reported that addition of β-cyclodextrin to a cholesterol-rich diet results in a triacylglycerol-lowering action, enhancement of bile acid synthesis and excretion (Garcia-Mediavilla *et al.* 2003). However, it has not previously

**Abbreviations:** ACD, adzuki resistant starch + cholesterol diet; BD, basal diet; CD, basal + cholesterol diet; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; HMG, 3-hydroxy-3-methylglutaryl; IDL, intermediate density lipoprotein; SR-BI, scavenger receptor type BI.

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been reported that SCFA mediate the hypocholesterolaemic effects of retrograded bean starches in cholesterol-fed rats. Lund *et al.* (1993) have reported that rye causes a reduction in circulating cholesterol in rats on a high cholesterol diet, which they interpret to result from a malabsorption of bile acids and cholesterol possibly due to an increase in unstirred layer resulting from the presence of viscous fibre.

In the present study, we investigated the effects of adzuki resistant starch on serum lipids, liver lipids, faecal lipids and hepatic mRNA in hypercholesterolaemic rats. The mRNA coded for key regulatory proteins of cholesterol metabolism.

## Material and methods

### *Animals and diets*

Male F344/DuCrj rats (7 weeks old) were purchased from Charles River Japan Inc. (Yokohama, Japan). The rats were housed individually in cages with free access to food and water. The animal facility was maintained on a 12 h light/dark cycle at a temperature of 23 ± 1°C and relative humidity of 60 ± 5 %. Animals were randomly assigned into three groups ($n$ 5). The composition of each diet is shown in Table 1. Adzuki bean starch was retrograded by slow refrigeration after boiling. The control rats were fed 15 % cornstarch (basal diet, BD). The experimental rats were fed a BD plus 0·5 % cholesterol diet (CD), or a 15 % adzuki resistant starch plus 0·5 % cholesterol diet (ACD) for 4 weeks. The approximate composition of adzuki starch is as follows (as %): moisture, 2·7; total dietary fibre, 7·7 (insoluble fibre, 7·6; water-soluble fibre 0·1); protein (N < 6·25), 4·9; lipids, 0·1; ash, 3·1; carbohydrate, 81·5. Total dietary fibre, insoluble fibre, water-soluble fibre, protein, lipids, moisture, ash and carbohydrate were determined by the procedure of the Association of Official Analytical Chemists (1990). The adzuki resistant starch was a gift from the Hokkaido Tokachi Area Regional Food Processing Technology Center (Obihiro, Hokkaido, Japan). Body weight and food consumption were recorded weekly and daily, respectively. This experimental design was approved by the Animal Experiment Committee of Obihiro University of Agriculture and Veterinary Medicine. All animal procedures conformed to standard principles described in *Guide for the Care and Use of Laboratory Animals* (National Research Council, 1985).

**Table 1.** Composition of experimental diets (g/kg diet)

| | Dietary group | | |
|---|---|---|---|
| Component | BD | CD | ACD |
| Casein | 250 | 250 | 250 |
| Maize oil | 100 | 100 | 100 |
| Mineral mixture* | 35 | 35 | 35 |
| Vitamin mixture* | 10 | 10 | 10 |
| Cellulose powder | 50 | 50 | 50 |
| Cornstarch | 150 | 150 | – |
| Adzuki starch | – | – | 150 |
| Cholesterol | – | 5 | 5 |
| Choline chloride | 2 | 2 | 2 |
| Sodium cholate | – | 1·25 | 1·25 |
| Sucrose | 1000 | 1000 | 1000 |

ACD, adzuki resistant starch + cholesterol diet; BD, basal diet; CD, basal + cholesterol diet.
* AIN-76 mineral mixture and vitamin mixture (American Institute of Nutrition, 1977).
For details of diets and procedures, see this page.

### *Analytical procedures*

Blood samples (1 ml) were collected between 08.00 and 10.00 hours from the jugular veins of fasting rats anaesthetized by sodium pentobarbital. The samples were taken into tubes without an anticoagulant. After the samples were allowed to stand at room temperature for 2 h, the sera were separated by centrifugation at 1500 ***g*** for 20 min. All faecal excretions were collected during the last 2 d of the experimental period (4 weeks). At the end of the experimental period of 4 weeks, the rats were killed by exsanguinations while anaesthetized by diethyl ether. Subsequently, the liver and caecum were quickly removed, washed with cold saline, dehydrated on filter paper and weighed before freezing for storage.

### *Chemical analysis*

Total cholesterol and HDL-cholesterol concentrations in the serum were determined enzymatically using commercially available reagent kits (assay kits for the TDX system; Abbott Laboratory Co., Irving, TX, USA). The VLDL + intermediate density lipoprotein (IDL) + LDL-cholesterol concentration was calculated as follows: [VLDL + IDL + LDL-cholesterol] = [total cholesterol] − [HDL-cholesterol].

Total lipids were extracted from liver and faeces by a mixture of chloroform–methanol (2:1, v/v) (Folch *et al.* 1957). The neutral steroids in each lipid sample obtained by saponification were acetylated (Matsubara *et al.* 1990) and analysed by GLC using a Shimadzu 14A apparatus (Kyoto, Japan) and a DB17 capillary column (0·25 mm × 30 m; J & W Scientific, Folsom, CA, USA) with nitrogen as the carrier gas. Acidic steroids in faeces were measured by GLC according to the method of Grundy *et al.* (1965). The caecum was anaerobically infused out into deionized water, and carefully suspended without exposure to air. The suspension of caecal contents was deproteinized with ice-cooled perchloric acid (final concentration 50 g/l), and the resulting supernatant was neutralized with NaOH so as to precipitate perchloric acid and change SCFA into sodium salts. Individual SCFA were measured by GLC using a glass column (2000 × 3 mm) packed with 80–100 mesh Chromosorb W-AW DMCS with $H_3PO_4$ (100 ml/l) as the liquid phase according to the procedure of Hara *et al.* (1994).

### *RNA isolation, reverse transcription-PCR and Southern blot analysis*

Total RNA was isolated from the liver by the acid guanidium–phenol–chloroform method, using Isogen (Nippon Gene, Tokyo, Japan; Chomczynski & Sacchi, 1987). mRNA encoding the LDL-receptor, cholesterol 7α-hydroxylase, 3-hydroxy-3-methylglutaryl (HMG)-CoA reductase, scavenger receptor type BI (SR-BI) and glyceraldehyde-3-phosphate dehydrogenase (GAPDH, used as an invariant control) were analysed by semi-quantitative reverse transcription-PCR and subsequent Southern hybridization of PCR products with each inner oligonucleotide probe. Total RNA samples were treated with DNase RQ1 (Promega, Madison, WI, USA) to remove genomic DNA and then subjected to reverse transcription-PCR using Moloney murine leukaemia virus RT (Gibco-BRL,

Gaithersburg, MD, USA) and EX-Taq polymerase (Takara, Tokyo, Japan) with LDL-receptor primers of oligonucleotides (upstream primer, 5′-ATTTTGGAGGATGAGAAGCAG-3′; downstream primer, 5′-CAGGGCGGGGAGTGTGAGAA-3′), cholesterol 7α-hydroxylase primers of oligonucleotides (upstream primer, 5′-GCCGTCCAAGAAATCAAGCAGT-3′; downstream primer, 5′-TGTGGGCAGCGAGAACAAAGT-3′), HMG-CoA reductase primers of oligonucleotides (upstream primer, 5′-GCGTGCAAAGACAATCCTGGAG-3′; downstream primer, 5′-GTTAGACCTTGAGAACCCAATG-3′), SR-BI primers of oligonucleotides (upstream primer, 5′-GTAGGGCCCAGAAGACACCAC-3′; downstream primer, 5′-CCTGCCACCGCTGCCACTTAC-3′) and GAPDH primers of oligonucleotides (upstream primer, 5′-GCCATCAACGACCCCTTCATT-3′; downstream primer, 5′-CGCCTGCTTCACCACCTTCTT-3′). The reaction mixtures for the PCR contained 25 pmol of each primer, 1·25 U EX-Taq polymerase, 1 × PCR buffer (Takara) and 200 μM-dNTP in a 50 μl reaction volume. The expected sizes of DNA fragments amplified with these primers were 931 bp for the LDL-receptor, 306 bp for cholesterol 7α-hydroxylase, 245 bp for HMG-CoA reductase, 539 bp for SR-BI and 702 bp for GAPDH. Temperature cycling was as follows: first cycle, denaturation at 94°C for 3 min, annealing at 60°C for 1 min and extension at 72°C for 2 min; subsequent cycles, denaturation at 94°C for 1 min, annealing at 60°C for 1 min and extension at 72°C for 2 min. The thermal cycling was completed by terminal extension at 72°C for 10 min. In total, there were twenty cycles for the GAPDH amplification, twenty-five cycles for the LDL-receptor, cholesterol 7α-hydroxylase and SR-BI, and thirty cycles for HMG-CoA reductase. Amplification products were separated by electrophoresis using 2 % agarose gel, and transferred to a nylon membrane (Biodyne B; Pall Bio-Support, East Hills, NY, USA). Blots were hybridized with an LDL-receptor probe of a fifty-four-base oligonucleotide (5′-GTGAACTTGGGTGAGTGGGCACTGATCTGAGGGGCAGGCAGGCACATGTACTGG-3′), cholesterol 7α-hydroxylase probe of a fifty-four-base oligonucleotide (5′-CCCGAAGGCCTGTTTAAGTGATGACTCTCAGCCGCCAAGTGACATCATCCAGTG-3′), HMG-CoA reductase probe of a fifty-four-base oligonucleotide (5′-GATCTGTTGTGAACCATGTGACTTCTGACAAGATGTCCTGCTGCCAATGCTGCC-3′), SR-BI probe of a fifty-four-base oligonucleotide (5′-TGCCGTGTGGACAGTGTGACATCTGGGGGCTCAGGACGTGGCACTGGCGGGGTTG-3′) and GAPDH probe of a fifty-four-base oligonucleotide (5′-TGATGACCAGCTTCCCATTCTCAGCCTTGACTGTGCCGTTGAACTTGCCGTGGG-3′). The probe was 3′-tailing labelled with digoxigenin, using a digoxigenin oligonucleotide tailing kit (Boehringer Mannheim, Mannheim, Germany). Prehybridization, hybridization and detection were carried out with a digoxigenin luminescent detection kit (Boehringer Mannheim) as recommended by the manufacturer. The relative quantity of mRNA was estimated by densitometry scanning with X-ray film.

### *Statistical analysis*

Data are presented as means and standard deviations for serum total cholesterol, HDL-cholesterol and VLDL + IDL + LDL-cholesterol at prescribed times. Differences among experimental groups were evaluated by Duncan's multiple range test (SAS Institute, Cary, NC, USA) following ANOVA and considered significant at $P<0·05$.

## Results

There was no difference in food intake among the groups (Table 2). The body weight gains and liver weights in the CD and ACD groups were significantly ($P<0·05$) higher than those in the BD group. The liver weight in the ACD group was significantly ($P<0·05$) lower than that in the CD group. There was no significant difference in the caecal contents among the groups and caecum pH levels in the CD and ACD groups were significantly ($P<0·05$) lower than in the BD group.

Fig. 1 shows the serum total cholesterol, VLDL + IDL + LDL-cholesterol and HDL-cholesterol concentrations in rats fed cholesterol or cholesterol plus adzuki resistant starch. The serum total cholesterol and VLDL + IDL + LDL-cholesterol levels in the CD and ACD groups were significantly ($P<0·05$) higher than those in the BD group, and those in the ACD group

**Table 2.** Body weight, food intake, relative liver weight and caecal content, caecal pH and caecal SCFA concentration in rats fed experimental diets for 4 weeks*

(Mean values and standard deviations for five rats per group)

| | Dietary group | | | | | |
|---|---|---|---|---|---|---|
| | BD | | CD | | ACD | |
| Component | Mean | SD | Mean | SD | Mean | SD |
| Initial body weight (g) | 180[a] | 6 | 179[a] | 3 | 176[a] | 5 |
| Body weight gain (g/4 weeks) | 52[a] | 8 | 64[a] | 3 | 62[a] | 3 |
| Food intake (g/4 weeks) | 414[a] | 39 | 406[a] | 13 | 398[a] | 16 |
| Liver weight (g/100 g body) | 4·06[a] | 0·23 | 4·92[a] | 0·22 | 4·57[a] | 0·31 |
| Caecal content (g/100 g body) | 1·18[a] | 0·07 | 1·35[a] | 0·19 | 1·27[a] | 0·19 |
| Caecal pH | 6·87[a] | 0·20 | 6·44[b] | 0·06 | 6·43[b] | 0·10 |
| Caecal SCFA (μmol/g caecal content) | | | | | | |
| Total | 62·4[a] | 9·4 | 49·3[b] | 6·6 | 61·1[a] | 5·4 |
| Acetic | 51·2[a] | 8·0 | 41·3[b] | 5·9 | 50·6[a] | 4·2 |
| Propionic | 7·3[a] | 1·2 | 5·3[b] | 0·9 | 6·0[ab] | 1·2 |
| Butyric | 3·8[ab] | 1·4 | 2·6[b] | 0·5 | 4·4[a] | 0·7 |

ACD, adzuki resistant starch + cholesterol diet; BD, basal diet; CD, basal + cholesterol diet.
[a,b]Mean values within a row with unlike superscript letters were significantly different ($P<0·05$).
* For details of procedures and diets, see p. 903 and Table 1.

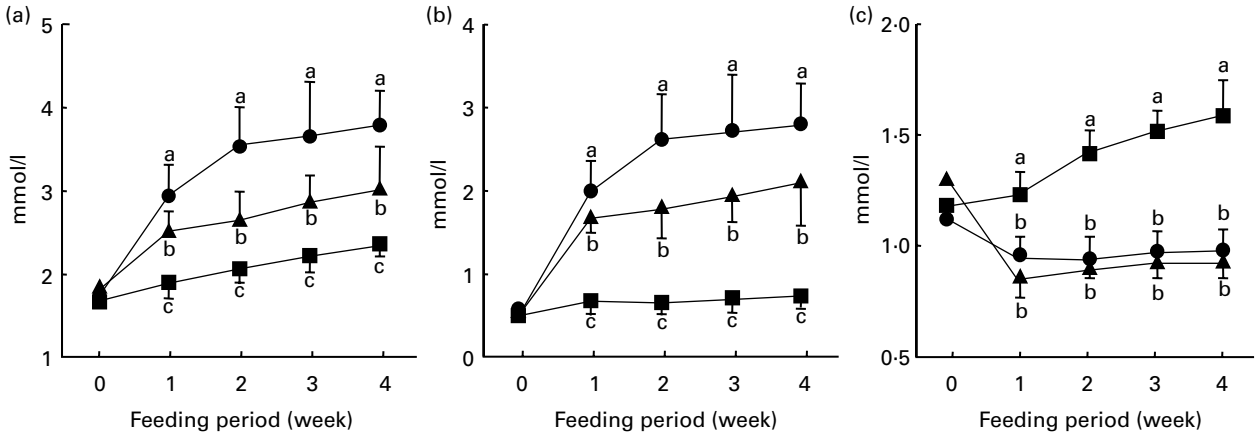


**Fig. 1.** Serum total cholesterol (a), VLDL + intermediate density lipoprotein + LDL-cholesterol (b) and HDL-cholesterol (c) concentrations in rats fed basal diet (■), basal + cholesterol diet (●) or adzuki resistant starch + cholesterol diet (▲) for 4 weeks. For details of procedures and diets, see p. 903 and Table 1. Values are means and standard deviations depicted by vertical bars (data obtained from five animals). [a,b,c]Mean values were significantly different ($P<0·05$), as determined by ANOVA with Duncan's multiple range test.

were significantly ($P<0·05$) lower than those in the CD group throughout the feeding period. The HDL-cholesterol concentrations in the CD and ACD groups were significantly ($P<0·05$) lower than that in the BD group throughout the feeding period.

The liver cholesterol concentrations in rats fed cholesterol or cholesterol plus adzuki resistant starch at the end of the experimental period are shown Fig. 2. The liver cholesterol concentrations in the CD and ACD groups were significantly ($P<0·05$) higher than that in the BD group, but there was no significant difference between the CD and ACD groups.

The relative quantities of mRNA were determined by Southern hybridization of PCR-amplified LDL-receptor cDNA, cholesterol 7α-hydroxylase cDNA, HMG-CoA reductase and SR-BI cDNA in the rat liver. The values of LDL-receptor, cholesterol 7α-hydroxylase, HMG-CoA reductase and SR-BI mRNA were expressed relative to the value of GAPDH mRNA in all groups. The relative quantities of hepatic LDL-receptor and cholesterol 7α-hydroxylase (Fig. 3) in the ACD group were significantly ($P<0·05$) higher than those in the CD group. There was no significant difference in the cholesterol 7α-hydroxylase level (Fig. 3) between the BD and CD groups. The hepatic HMG-CoA reductase mRNA level (Fig. 3) in the ACD group was significantly ($P<0·05$) lower than in the BD and CD groups. There was no difference in SR-BI mRNA level among the groups.

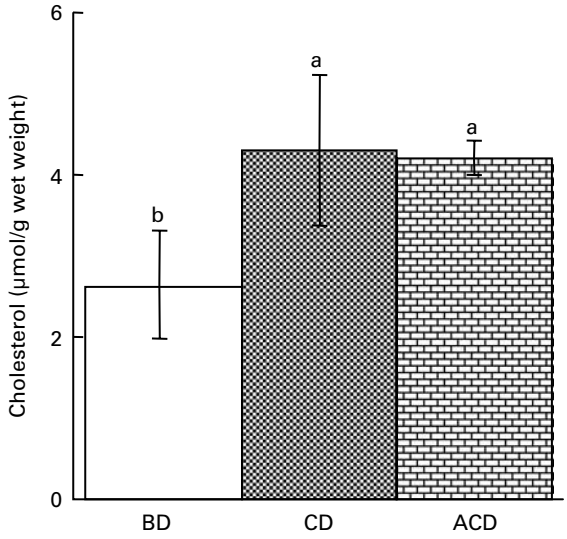


**Fig. 2.** Liver cholesterol concentration in rats fed basal diet (BD), basal + cholesterol diet (CD) or adzuki resistant starch + cholesterol diet (ACD) for 4 weeks. For details of procedures and diets, see p. 903 and Table 1. Values are means and standard deviations depicted by vertical bars (data obtained from five animals). [a,b]Mean values were significantly different ($P<0·05$), as determined by ANOVA with Duncan's multiple range test.

Table 2 also shows the SCFA concentrations in the rat caecum. The caecal acetate, propionate and total SCFA concentrations in the CD group were significantly ($P<0·05$) lower than those in the BD group and the caecal acetate, *n*-butyrate and total SCFA concentrations in the ACD group were also significantly ($P<0·05$) higher than in the CD group. However, there were no significant differences for any SCFA concentration between the BD and ACD groups. Table 3 shows the cholesterol and bile acid concentrations in the faeces of rats. The faecal cholesterol excretion in the CD and ACD groups was significantly ($P<0·05$) greater than in the BD group. However, there was no significant difference in the faecal cholesterol concentration between CD and ACD groups. The faecal cholic acid, deoxycholic acid, chenodeoxycholic acid, lithocholic acid and total bile acid concentrations in the ACD group were significantly ($P<0·05$) higher than those in the BD group, and the cholic acid and lithocholic acid concentrations in the ACD group were significantly ($P<0·05$) higher than in the CD group.

## Discussion

In the present study, we examined the effects of adzuki resistant starch on serum cholesterol and hepatic mRNA levels in rats fed a cholesterol diet. The serum total cholesterol level in the ACD group was significantly lower than that in the CD group. Most of the serum cholesterol in animals fed cholesterol and/or high fat diets is associated with LDL-cholesterol (Brown & Goldstein, 1986; Fukushima & Nakano, 1995). Therefore, lowering the LDL-cholesterol level may be an important factor in lowering the serum total cholesterol level in rats fed a cholesterol diet. In fact, a high correlation was found between the serum VLDL + IDL + LDL-cholesterol concentration ($r$ 0·967, $P<0·001$) and

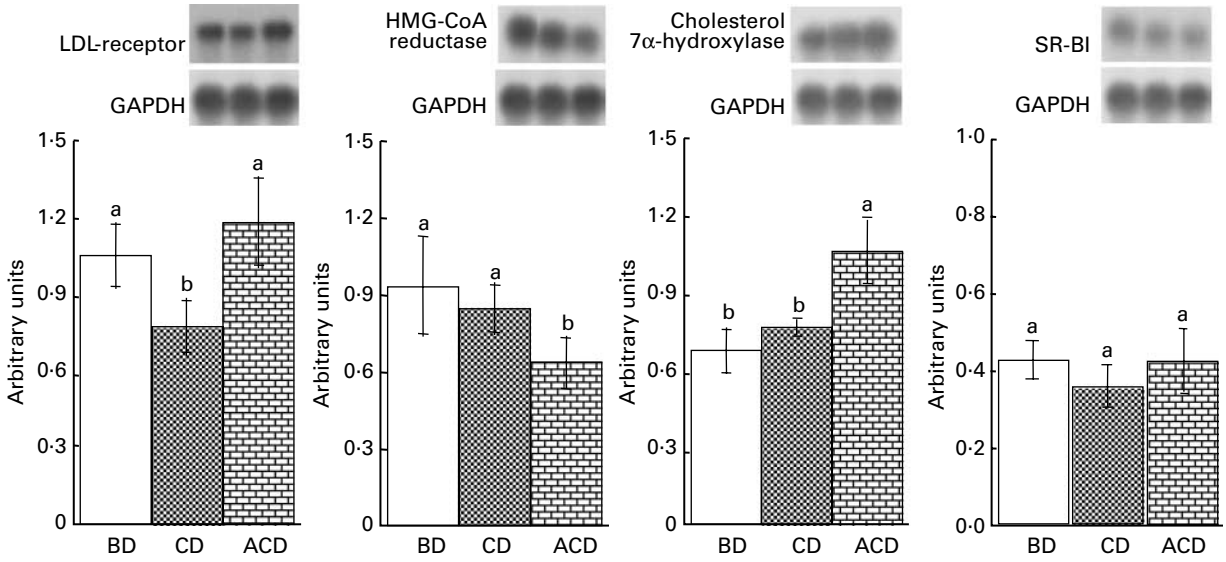


**Fig. 3.** Hepatic cholesterol LDL-receptor mRNA, 3-hydroxy-3-methylglutaryl (HMG)-CoA reductase mRNA, 7α-hydroxylase mRNA and scavenger receptor type BI (SR-BI) mRNA expressions in rats fed basal diet (BD), basal + cholesterol diet (CD) or adzuki resistant starch + cholesterol diet (ACD) for 4 weeks. For details of procedures and diets, see p. 903 and Table 1. Values are means and standard deviations depicted by vertical bars (data obtained from five animals). [a,b]Mean values were significantly different ($P<0·05$), as determined by ANOVA with Duncan's multiple range test. The values for LDL-receptor, HMG-CoA reductase mRNA, 7α-hydroxylase mRNA and SR-BI mRNA are expressed relative to glyceraldehyde-3-phosphate dehydrogenase (GAPDH) mRNA in all groups. Insets illustrate representative Southern hybridization of PCR-amplified LDL-receptor, HMG-CoA reductase, 7α-hydroxylase and SR-BI cDNA of hepatic RNA.

the serum total cholesterol concentration, and the serum LDL-cholesterol concentration in the ACD group was significantly lower than that in the CD group in the present experiment.

Dietary cholesterol has been shown to suppress hepatic LDL-receptor activity (Ma *et al.* 1986; Goldstein & Brown, 1990). The changes in hepatic LDL-receptors contribute to the elevation in blood cholesterol levels induced by high-cholesterol diets and to the reduction that follows hepatic cholesterol depletion (Brown & Goldstein, 1986). In the present study, it was also observed that 5 % cholesterol with 0·125 % sodium cholate in the diet suppressed the hepatic LDL-receptor mRNA level. In the case of animals fed a cholesterol-free diet, the mechanism by which the hypocholesterolaemic effect of adzuki bean starch is caused seems to have already been identified as the enhancement of the excretion of bile acid (Han *et al.* 2003*a*,*b*). Fukushima *et al.* (2001) also reported that diets containing 15 % (w/w) adzuki resistant starch reduced the serum LDL-cholesterol concentration and induced hepatic LDL-receptor mRNA in rats fed a cholesterol-free diet. However, in the case of those fed a diet supplemented with cholesterol, the mechanism has not yet been clarified. In the present study, a cholesterol diet was used to clearly understand the cholesterol-lowering mechanism of the adzuki resistant starch diet in rats. The reduction in LDL-receptor found in response to a high-cholesterol diet in the present study is consistent with data from previous studies (Ma *et al.* 1986; Goldstein & Brown, 1990). However, there was no significant difference in the hepatic LDL-receptor mRNA level between the ACD and BD groups. The present result seems to be interesting because adzuki starch may inhibit and suppress hepatic LDL-receptor activity by dietary cholesterol.

**Table 3.** Faecal steroid concentrations (μmol/100 g body weight per d) in rats fed experimental diets for 4 weeks*

(Mean values and standard deviations for five rats per group)

| | Dietary group | | | | | |
|---|---|---|---|---|---|---|
| | BD | | CD | | ACD | |
| Component | Mean | SD | Mean | SD | Mean | SD |
| Cholesterol | 2·56[b] | 0·87 | 15·95[a] | 2·76 | 15·61[a] | 8·62 |
| Coprostanol | 0·79[a] | 0·52 | 0·77[a] | 0·17 | 1·50[a] | 1·03 |
| Cholic | <0·01 | | 0·06[b] | 0·05 | 0·22[a] | 0·15 |
| Deoxycholic | 0·06[b] | 0·04 | 0·38[a] | 0·16 | 0·55[a] | 0·28 |
| Chenodeoxycholic acid | 0·03[b] | 0·02 | 0·06[ab] | 0·03 | 0·07[a] | 0·03 |
| Lithocholic | 0·14[c] | 0·06 | 0·40[b] | 0·16 | 0·66[a] | 0·28 |
| Total bile acid | 0·24[b] | 0·10 | 0·89[ab] | 0·36 | 1·49[a] | 0·67 |

ACD, adzuki resistant starch + cholesterol diet; BD, basal diet; CD, basal + cholesterol diet.
[a,b,c]Mean values within a row with unlike superscript letters were significantly different ($P<0·05$).
* For details of procedures and diets, see p. 903 and Table 1.

In our previous paper, we reported that leguminous resistant starch reduces the serum HDL-cholesterol concentration and increases the hepatic SR-BI mRNA level in rats fed a cholesterol-free diet (Han *et al.* 2003*a*). In the present study, however, the serum HDL-cholesterol concentration in the CD and ACD groups, and the hepatic SR-BI mRNA level among all groups, were not significantly different. The present findings suggest that the hepatic SR-BI receptor levels might not be involved in changing serum HDL-cholesterol concentration in rats fed a cholesterol diet. Furthermore, the difference between previous (Han *et al.* 2003*a*) and present results concerning serum HDL-cholesterol concentrations may be due to the difference in the ratio of indigestible fraction in leguminous starch, because the retrograded starch was further treated enzymatically with pancreatin and pepsin in the previous study (Han *et al.* 2003*a*).

The activity of HMG-CoA reductase is also regulated by changes in the exogenous cholesterol concentration (Goldstein & Brown, 1990). However, there was no significant difference in the hepatic HMG-CoA reductase mRNA level between the CD and BD groups, but that in the ACD group was significantly lower than in the CD group. The decrease in HMG-CoA reductase mRNA is surprising and conflicts with previous reports. Lund *et al.* (1993) and Moundras *et al.* (1997), using viscous fibres, have reported that dietary fibre increases hepatic HMG-CoA reductase activity. However, their results might not be sufficient to account for serum cholesterol reductions. Our present findings suggest that dietary resistant starch suppresses the rate-limiting enzyme in endogenous sterol biosynthesis by an additive effect with a cholesterol diet. In fact, we have reported that the hepatic HMG-CoA reductase mRNA level is unaffected by leguminous resistant starch in rats fed a cholesterol-free diet (Han *et al.* 2003*a*). Furthermore, the mechanism of reduction of the rate-limiting enzyme of cholesterolgenesis by adzuki resistant starch is probably related to an increase in the liver cholesterol concentration, which may inhibit the hepatic HMG-CoA reductase mRNA level. There was no significant difference between liver cholesterol concentrations in the CD and ACD groups, although this result may have been due to the increasing of LDL-receptor and cholesterol 7α-hydroxylase mRNA levels in the ACD group as compared with the CD group. Therefore, the exact reason for this result remains unclear.

The hypocholesterolaemic effect of dietary fibre has been attributed to its ability to inhibit intestinal absorption of bile acids and neutral steroids, resulting in greater faecal bile acid and total steroid excretions. Buhman *et al.* (1998) demonstrated that feeding psyllium to rats enhanced the hepatic cholesterol 7α-hydroxylase mRNA level, and the faecal bile acid and total steroid excretions. In the present study, feeding adzuki resistant starch significantly increased faecal bile acid compared with the group fed a cholesterol diet, supporting this hypothesis. We also observed that the cholesterol 7α-hydroxylase mRNA level in the ACD group was significantly higher than that in the CD group. Thus, our present results agreed with reports mentioned earlier.

The type of indigestible carbohydrates consumed can influence the distribution of SCFA in the hindgut. For example, guar gum consumption by rats results in a high proportion of propionic acid and pectin in a high proportion of acetic acid upon fermentation (Brighenti *et al.* 1989; Berggren *et al.* 1993). On the other hand, starch appears to be a food source of butyric acid in the rat hindgut (Morita *et al.* 1999) and in the human faecal inocula (Scheppach *et al.* 1988; Cummings & Macfarlane, 1991). In the present study, the caecal butyric acid concentration in the ACD group was higher than that in the CD group. One factor that may have an effect on butyric acid production is the transit time through the gastrointestinal tract. Mathers & Dawson (1991) found a relationship between the molar proportion of butyric acid in caecal contents and the caecal transit time in rats fed various diets. In the present study, excreted faecal contents in the ACD group were greater than those in the CD group. It may be assumed that the increased butyric acid concentration in the ACD group elevated the excretion of steroid. The present findings might suggest that the greater reduction in serum cholesterol level in the ACD group is due to a greater fermentable carbohydrate load. However, we do not know whether the resistant starch really can reduce the serum cholesterol level in man. Although several studies have shown that resistant starch can lower blood lipid levels in rats (Younes *et al.* 1995; Levrat *et al.* 1996; Fukushima *et al.* 2001), similar findings have not been reported in man (Noakes *et al.* 1996; Jenkins *et al.* 1998).

In conclusion, the effects of adzuki resistant starch in rats were clearly observed when compared with rats fed a cholesterol diet. The adzuki resistant starch elevated hepatic LDL-receptor and cholesterol 7α-hydroxylase mRNA levels, reduced the hepatic HMG-CoA reductase mRNA level, and lowered the serum VLDL + IDL + LDL-cholesterol and total cholesterol concentrations.

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