

Dietary acetic acid reduces serum cholesterol and triacylglycerols in rats fed a cholesterol-rich diet

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To investigate the efficacy of the intake of vinegar for prevention of hyperlipidaemia, we examined the effect of dietary acetic acid, the main component of vinegar, on serum lipid values in rats fed a diet containing 1% (w/w) cholesterol. Animals were allowed free access to a diet containing no cholesterol, a diet containing 1% cholesterol without acetic acid, or a diet containing 1% cholesterol with 0.3% (w/w) acetic acid for 19 d. Then, they were killed after food deprivation for 7 h. Cholesterol feeding increased serum total cholesterol and triacylglycerol levels. Compared with the cholesterol-fed group, the cholesterol and acetic acid-fed group had significantly lower values for serum total cholesterol and triacylglycerols, liver ATP citrate lyase (ATP-CL) activity, and liver 3-hydroxy-3-methylglutaryl-CoA content as well as liver mRNA levels of sterol regulatory element binding protein-1, ATP-CL and fatty acid synthase ($P < 0.05$). Further, the serum secretin level, liver acyl-CoA oxidase expression, and faecal bile acid content were significantly higher in the cholesterol and acetic acid-fed group than in the cholesterol-fed group ($P < 0.05$). However, acetic acid feeding affected neither the mRNA level nor activity of cholesterol 7 α -hydroxylase. In conclusion, dietary acetic acid reduced serum total cholesterol and triacylglycerol: first due to the inhibition of lipogenesis in liver; second due to the increment in faecal bile acid excretion in rats fed a diet containing cholesterol.

Acetic acid: Hyperlipidaemia: Lipogenesis: Rats

Acetic acid is a main component of vinegar at a concentration of 3–9% for consumer use (Ren *et al.* 1997). Vinegar is not only used commonly as a condiment but also traditionally as a folk medicine (Ross & Poluhowich, 1984; Nakazawa & Muraoka, 1989). In animal studies, we showed previously that a diet containing acetic acid or vinegar at concentrations found in traditional diets has such effects as enhancement of glycogen repletion (Fushimi *et al.* 2001, 2002), prevention of hypertension (Kondo *et al.* 2001) and stimulation of Ca absorption (Kishi *et al.* 1999). However, very little is known about the effect of dietary acetic acid on lipid metabolism.

Acetic acid is one of the SCFA, which are products of the caecal and colonic fermentation that is stimulated by the ingestion of dietary fibre. Feeding highly fermentable dietary fibre is reported to reduce the increments of serum cholesterol (Aritsuka *et al.* 1989). Previously, Hara *et al.* (1999) reported that a fibre-free diet containing SCFA decreases serum cholesterol. However, the diet used in that study contained 3.5% sodium acetate as a Na salt, which is excessive in comparison with foods prepared with vinegar, such as sushi containing 0.2–0.6% acetic acid (Mine *et al.* 1982; Fujii *et al.* 1992; Chang *et al.* 1994).

Absorbed acetic acid is taken up and then metabolised via acetyl-CoA in the tricarboxylic acid cycle in the liver and peripheral tissues (Ballard, 1972; Spydevold *et al.* 1976; Crabtree *et al.* 1990). Thus, acetate has been utilised as a substrate of lipogenesis in many cell studies. On the other hand, when acetate is metabolised by acetyl-CoA synthetase, AMP is simultaneously produced: acetate + CoA + ATP \leftrightarrow acetyl-CoA + AMP + pyrophosphate. Based on this point, Kawaguchi *et al.* (2002) reported that in hepatocytes acetate activated AMP-activated protein kinase (AMPK), the inhibitor of fatty acid and sterol synthesis (for reviews, see Hardie *et al.* 1998; Winder & Hardie, 1999; Hardie, 2003). We very recently reported that dietary acetic acid reduced the increment of malonyl-CoA content in liver of rats under postprandial conditions (Fushimi & Sato, 2005). Since in a review article it was pointed out that malonyl-CoA content was down regulated by the activation of AMPK (Saha & Ruderman, 2003), it could be supposed that dietary acetic acid may influence lipid synthesis in rats.

Considering these findings, it remains unclear whether a diet containing acetic acid at a concentration found in traditional diets affects lipid metabolism. In the present study, we explored the efficacy of vinegar for prevention of

Abbreviations: ACC, acetyl-CoA carboxylase; AMPK, AMP-activated protein kinase; AOX, acyl-CoA oxidase; ATP-CL, ATP citrate lyase; CYP7A1, cholesterol 7 α -hydroxylase; FAS, fatty acid synthase; GAPDH, glyceraldehyde phosphate dehydrogenase; HMG-CoA, 3-hydroxy-3-methylglutaryl-CoA; SREBP-1, sterol regulatory element binding protein-1.

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hyperlipidaemia and examined whether a diet containing 0.3% acetic acid, a concentration corresponding to that in sushi, a traditional Japanese food (Mine *et al.* 1982; Fujii *et al.* 1992; Chang *et al.* 1994), would affect lipid metabolism in rats fed a cholesterol-rich diet.

Materials and methods

Animals, feeding protocol and diets

Male rats aged 5 weeks (Sprague–Dawley; Japan SLC, Hamamatsu, Japan) weighing 145 (SEM 1) g were individually housed in a temperature-controlled room (23–25°C) with a 12 h light–dark cycle. The light period began from 07.00 hours. The animals had free access to water and a powdered AIN-93G (Reeves *et al.* 1993) purified diet (Oriental Yeast Co., Ltd, Tokyo, Japan) for 9 d. On the 8th day of the acclimatisation period food was withdrawn at 08.00 hours. After a 7 h period of food deprivation, blood was collected from the retro-orbital plexus of the rats under anaesthesia with diethyl ether. On the next day (defined as day 0 of the experimental period), serum cholesterol, triacylglycerol and NEFA values were measured by a clinical automatic analyser (Automatic analyser 736–10; Hitachi, Tokyo, Japan). On the 1st day of the experimental period, the animals were divided into three groups of eight, each with a similar mean body weight (184 (SEM 2) g) and a similar mean concentration of serum total cholesterol (855 (SEM 30) mg/l), triacylglycerols (551 (SEM 31) mg/l) and NEFA (0.73 (SEM 0.04) mmol/l). The rats were cared for in accordance with the Guidelines for Animal Experimentation at the 34th Annual Meeting of the Japanese Association for Laboratory Animal Science, held 22 May 1987.

From the 1st day of the experimental period, each group was given one of three experimental diets for the 19 d experimental period (Table 1). The experimental diets, based on the powdered AIN-93G diet, contained no cholesterol (no-cholesterol diet), 1% cholesterol without acetic acid (cholesterol diet), or 1% cholesterol and 0.3% acetic acid (cholesterol and acetic acid diet). Food intake on day 7 and day 14 and body weight on day 9 and day 16 were measured. On day 13, of the experimental period, rats were removed to a metabolism cage for 48 h, and faeces were collected during that period. On day 20, food was withdrawn at 07.00 hours. After a 7 h period of food deprivation, whole blood was

drawn from the abdominal aorta under anaesthesia with diethyl ether. Livers were dissected quickly and a portion was treated for the measurement of enzyme activities; the remaining portion was immediately submerged and powdered in liquid N₂. Serum and powdered liver were stored at –80°C until assay.

Measurements of serum metabolites and hormones

Serum total cholesterol and triacylglycerols were measured by an automatic analyser system as described earlier. Serum insulin, glucagon, glucagon-like peptide 1, cholecystokinin and secretin were assayed by ELISA kits (ultra sensitive rat insulin ELISA kit (Morinaga Bioscience, Kanagawa, Japan); rat glucagon ELISA kit Wako (Wako Pure Chemicals, Osaka, Japan); glucagon-like peptide 1 EIA kit (Yanaihara, Fujinomiya, Japan); cholecystokinin 26–33 EIA kit and secretin EIA kit (Phoenix Pharmaceuticals, Inc., Belmont, CA, USA)).

Ribonucleic acid isolation and Northern blotting

Total RNA from rat liver was extracted using TRI reagent (Molecular Research Center, Inc., Cincinnati, OH, USA) according to the supplier's isolation procedures. Northern blot analysis was performed using ³²P-labelled cDNA probes as described previously (Goda *et al.* 1994). The cDNA probes used in the Northern blot analysis were generated by the reverse-transcription PCR method as follows: rat liver X receptor type α , a fragment corresponding to +1 to +1562 (Apfel *et al.* 1994); rat cholesterol 7 α -hydroxylase (CYP7A1), a fragment corresponding to +49 to +1009 (Noshiro *et al.* 1989); rat acyl-CoA oxidase (AOX), a fragment corresponding to +131 to +591 (GenBank accession no. J02752) (Mochizuki *et al.* 2001); mouse sterol regulatory element binding protein-1 (SREBP-1), a fragment corresponding to +169 to +1196 (GenBank accession no. NM011480); rat glyceraldehyde phosphate dehydrogenase (GAPDH), a fragment corresponding to +1087 to +1407 (GenBank accession no. NM017008) (Mochizuki *et al.* 2002). Specific mRNA signals were scanned using STORM (Amersham Pharmacia Biotech, Tokyo, Japan) and signal densities were standardised for the corresponding GAPDH mRNA levels.

Table 1. Dietary composition (g/kg diet)

Diet...	No-cholesterol	Cholesterol	Cholesterol and acetic acid
Maize starch	469	459	459
Casein	200	200	200
Sucrose	100	100	100
Soyabean oil	100	100	100
Cellulose	50	50	50
AIN-93G mineral mix*	35	35	35
AIN-93 vitamin mix*	10	10	10
L-Cystine	3	3	3
Choline bitartrate	2.5	2.5	2.5
<i>tert</i> -Butylhydroquinone	0.014	0.014	0.014
Cholesterol	0	10	10
Distilled water	30	30	–
Acetic acid (10% solution)	–	–	30

* Reeves *et al.* (1993). The mixes were purchased from Oriental Yeast, Tokyo, Japan.

Semi-quantitative reverse transcriptase polymerase chain reaction

The relative abundances of lipogenic mRNA (ATP citrate lyase (ATP-CL), fatty acid synthase (FAS) and acetyl-CoA carboxylase (ACC)) were determined by a semi-quantitative RT-PCR method. Total RNA from rat liver was extracted as described earlier and reverse transcription was performed by incubating 1 µg total RNA, random hexamers (2.5 mmol/l; Takara Bio. Co., Kyoto, Japan), the four deoxyribonucleotide triphosphates (each 0.5 mmol/l), 1X reaction buffer, 10 U RNasin (TOYOBO Co., Osaka, Japan), and 4 U Omniscript RT (Qiagen, Tokyo, Japan) in a final volume of 20 µl at 37°C for 1 h. The PCR were then done in a 12.5 µl reaction volume containing 1 µl cDNA, the four deoxyribonucleotide triphosphates (each 0.2 mmol/l), 1X Ex-Taq reaction buffer, the appropriate oligonucleotide primers (0.5 µmol/l; Table 2) and 0.5 U Takara EX-Taq DNA polymerase (Takara Bio. Co., Kyoto, Japan). The number of cycles was selected to allow linear amplification of the cDNA. All PCR reactions used a hot-start procedure (incubation at 94°C for 2 min), denaturation at 94°C for 30 s, annealing at an appropriate temperature (see Table 2) for 30 s, extension at 72°C for 30 s and final incubation at 72°C for 5 min. PCR products were electrophoresed on 1% agarose-tri(hydroxymethyl)-aminomethane-borate EDTA gels, stained with ethidium bromide, and analysed on a Fluoro Imager (Molecular Dynamics, Grefeld, Germany). mRNA abundance was expressed as band intensity relative to the corresponding 18S rRNA band intensity.

Determinations of liver metabolites and enzyme activity

Lipids in liver were extracted from freeze-stored liver by Folch's method (Folch *et al.* 1957), and extracted cholesterol and triacylglycerols were measured by individual assay kits (cholesterol C-test Wako and triglyceride E-test Wako, respectively; Wako Pure Chemicals, Osaka, Japan). 3-Hydroxy-3-methylglutaryl-CoA (HMG-CoA) was determined by a previously reported HPLC method (DeBuysere & Olson, 1983) with a slight modification. In brief, mobile-phase solvents were A (sodium phosphate (0.2 mol/l), 1% chloroform (v/v), pH 5.0) and B (sodium phosphate (0.2 mol/l), 49% methanol, 1% chloroform (v/v), pH 5.0).

The percentage of solvent B was 12 at time 0, 16 at 8 min, 26 at 14 min, 40 at 20 min, and 90 at 32 min, rising in a linear manner. After the final composition of solvent B reached 90%, it was held for 3 min and the initial condition was re-established for 10 min in a linear manner. This composition was kept for 10 min additionally, and the column was then ready for the next injection. ATP-CL and CYP7A1 activities were measured as previously described (Takeda *et al.* 1969; Yokogoshi *et al.* 1999, respectively).

Measurements of faecal neutral steroids and bile acids

Faecal neutral steroids and acid steroids were extracted from collected faeces as described previously (Moundras *et al.* 1997) and measured by individual assay kits (cholesterol test Wako and total bile acids test Wako; Wako Pure Chemicals, respectively).

Statistical analysis

Data are expressed as mean values with their standard errors. Homogeneity of variance was analysed by Levene's test. Statistical evaluation of the results with homogeneous variances was performed by one-way ANOVA. Fisher's least significant difference test was used to determine whether mean values were significantly different at $P < 0.05$. Results with heterogeneous variance were analysed by the Kruskal-Wallis non-parametric test. SPSS for Windows (version 11.5 J; SPSS, Inc., Chicago, IL, USA) was used for all analyses.

Results

Body weight and food intake

There were no significant differences in either body weight or food intake among the three groups (Table 3).

Serum lipids

In the cholesterol diet group, serum total cholesterol and triacylglycerol concentrations were significantly higher than in the no-cholesterol group (Table 4). The cholesterol and acetic acid diet group had a significantly higher serum total

Table 2. Sequences of polymerase chain reaction primers and polymerase chain reaction conditions for the analysis of specific mRNA

MRNA	GenBank accession no.	Primer sequences (5'-3')	PCR reaction conditions				Size of PCR product (bp)
			Denature	Annealing	Extension	Cycles	
FAS	M76767	(Forward) CCCTGAGCAGAGATCCTGAG (Reverse) GCACACAGGGACCGAGTAAT	94	62	72	28	586
ACC	J03808	(Forward) AGGAGGGAAGGGAATCAGAA (Reverse) ATCACCCCAAGGAGATACCC	94	62	72	28	514
ATP-CL	NM016987	(Forward) GTGGGTGTGGACGAGAACT (Reverse) CTTCCCAACTTCTCCCATCA	94	62	72	28	729
18S rRNA	V01270	(Forward) CCGCAGCTAGGAATAATGGA (Reverse) AACTAAGAACGGCCATGCAC	94	65	72	20	498

FAS, fatty acid synthase; ACC, acetyl-CoA carboxylase; ATP-CL, ATP-citrate lyase.

Table 3. Body weight and food intake of rats fed a no-cholesterol, cholesterol, or cholesterol and acetic acid diet*

(Mean values with their standard errors; eight rats per group)

Diet...	No-cholesterol		Cholesterol		Cholesterol and acetic acid	
	Mean	SEM	Mean	SEM	Mean	SEM
Body weight (g)†						
Day 1	184	3	184	4	183	4
Day 9	242	5	245	8	240	5
Day 16	274	5	283	9	275	5
Food intake (g/d)‡						
Day 7	16.4	0.8	16.2	0.8	16.4	0.3
Day 14	18.2	0.9	19.2	0.9	18.9	0.5

* Rats were fed an experimental diet for 19 d and grouped according to a diet containing either no cholesterol without acetic acid (no-cholesterol group), 1% cholesterol without acetic acid (cholesterol group), or 1% cholesterol with 0.3% acetic acid (cholesterol and acetic acid group).

† Body weight was measured on day 1, day 9 and day 16 of the experimental period (see p. 917).

‡ Food intake was measured on day 7 and day 14 of the experimental period (see p. 917).

cholesterol level than the no-cholesterol diet group, but serum triacylglycerol levels did not differ significantly between the two groups. Further, the cholesterol and acetic acid diet group had significantly lower serum total cholesterol and triacylglycerol concentrations than the cholesterol diet group.

Serum hormones

Serum insulin levels did not differ significantly between the no-cholesterol diet group and the cholesterol diet group. In the cholesterol and acetic acid diet group, the mean serum insulin level was significantly lower ($P < 0.05$) than in the no-cholesterol diet group, and tended to be lower ($P < 0.1$) than in the cholesterol diet group (Table 4). The serum secretin level was significantly higher in the cholesterol and acetic acid diet group than in the other two groups. There were no significant differences in the concentrations of glucagon, glucagon-like peptide 1 or cholecystokinin among the groups (data not shown).

Gene expressions in the liver

To examine whether gene expressions of lipogenic enzymes are affected by dietary acetic acid, transcript levels

of SREBP-1, a transcription factor involved in regulation of lipogenesis, and ATP-CL, FAS and ACC were measured in the liver. As shown in Fig. 1, the cholesterol diet group had significantly greater SREBP-1 mRNA levels in the liver than the no-cholesterol diet group. However, in the cholesterol and acetic acid diet group, SREBP-1 mRNA levels in the liver were significantly lower than in the cholesterol diet group. The SREBP-1 mRNA levels in the cholesterol and acetic acid diet group did not differ significantly from those in the no-cholesterol diet group. Whereas no significant differences were observed in mRNA levels of ATP-CL or FAS between the no-cholesterol and cholesterol diet groups, the cholesterol and acetic acid diet group had significantly lower ATP-CL and FAS mRNA levels in the liver than did the other two groups. The transcript levels of ACC in the liver of the no-cholesterol diet group were significantly higher than in the other two diet groups. In addition, the cholesterol and acetic acid diet group tended to have lower mRNA levels of ACC than the cholesterol diet group ($P < 0.1$) (Fig. 1).

To examine whether β -oxidation of fatty acids and/or cholesterol metabolism in the liver was influenced by dietary acetic acid, we next determined the transcript levels of AOX, CYP7A1, a rate-limiting enzyme producing bile acid, and liver X receptor α , a nuclear receptor known to play a pivotal role in cholesterol homeostasis in hepatic cells. As shown in Fig. 2, the AOX mRNA level in the cholesterol and acetic acid diet group was significantly greater than in the other groups. The CYP7A1 mRNA levels in both the cholesterol diet group and the cholesterol and acetic acid diet group were significantly greater than in the no-cholesterol diet group; these levels, however, did not differ significantly between the cholesterol diet and cholesterol and acetic acid diet groups. There was no significant difference in the expression of liver X receptor α mRNA among the groups.

Metabolites and enzyme activity in liver

The concentrations of cholesterol, triacylglycerols and HMG-CoA and the ATP-CL activity in the liver are summarised in Table 5. Feeding a cholesterol-rich diet caused significantly elevated cholesterol and triacylglycerol concentrations in the liver compared with feeding of the no-cholesterol diet. In the cholesterol and acetic acid diet group, the hepatic concentrations of both cholesterol and triacylglycerols

Table 4. Serum metabolites and hormones in rats fed a no-cholesterol, cholesterol, or cholesterol and acetic acid diet*

(Mean values with their standard errors; eight rats per group)

Diet...	No-cholesterol		Cholesterol		Cholesterol and acetic acid	
	Mean	SEM	Mean	SEM	Mean	SEM
Total cholesterol (mg/l)	573	34	1050†	79	854‡	72
Triacylglycerols (mg/l)	593	91	1125†	137	736†	118
Insulin (μ g/l)	2.93	0.37	2.61	0.33	1.69†	0.24
Secretin (pmol/l)	3.02	0.31	3.36	0.53	5.21†‡	0.83

* For details of diets and procedures, see Tables 1 and 3. Serum was collected from the abdominal aorta after a 7 h period of food deprivation on day 20 of the experimental period (see p. 917).

† Mean value was significantly different from that of the no-cholesterol group ($P < 0.05$).

‡ Mean value was significantly different from that of the cholesterol group ($P < 0.05$).

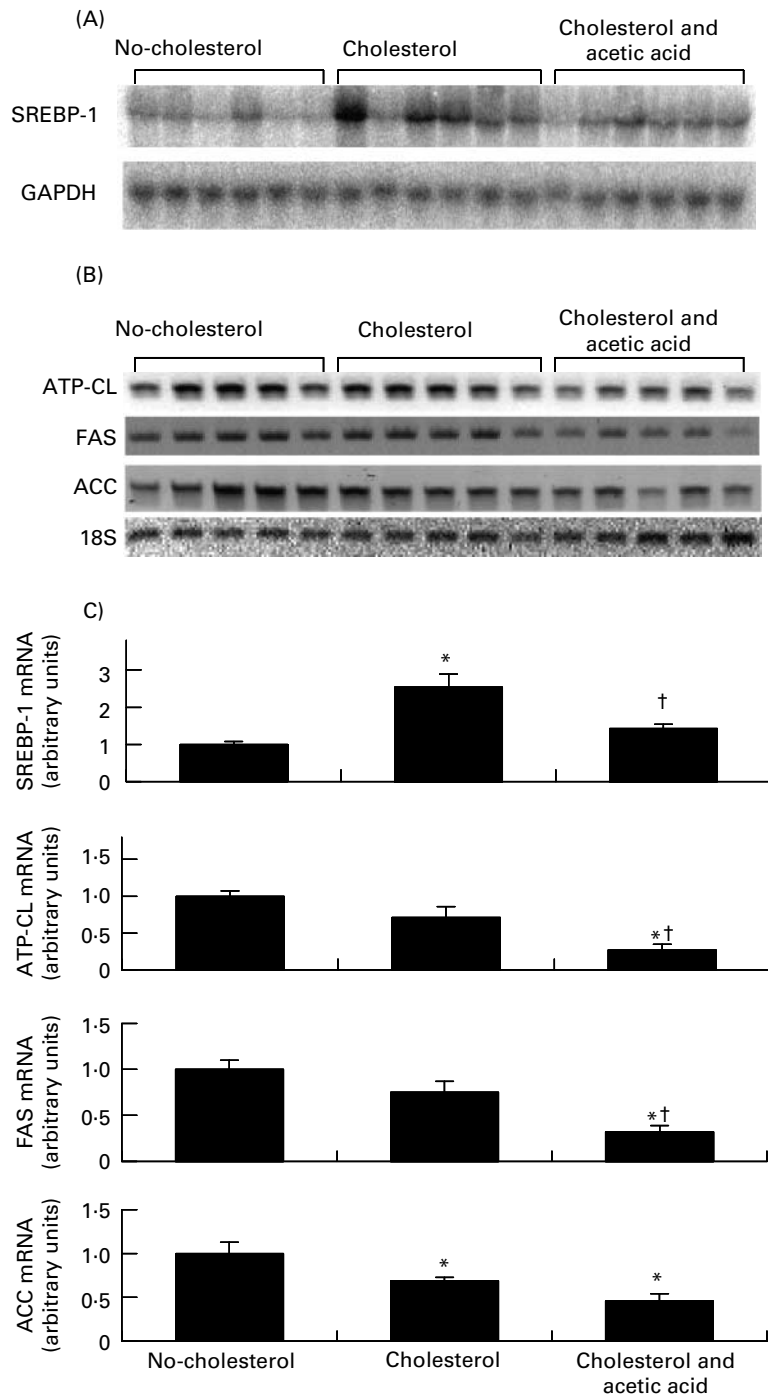


Fig. 1. mRNA levels of sterol regulatory element binding protein-1 (SREBP-1), ATP citrate lyase (ATP-CL), fatty acid synthase (FAS) and acetyl-CoA carboxylase (ACC) in liver of rats fed a no-cholesterol, cholesterol, or cholesterol and acetic acid diet. For details of diets and procedures, see Tables 1 and 3. Liver was dissected after a 7 h period of food deprivation on the 20th day. (A) Representative Northern blots. Liver total RNA (35 µg) was analysed by Northern blot hybridisation of SREBP-1c and glyceraldehyde phosphate dehydrogenase (GAPDH) mRNA. (B) Representative agarose gel electrophoresis of RT-PCR products. Liver total RNA was analysed by semi-quantitative RT-PCR for ATP-CL, FAS and ACC mRNA levels and 18S rRNA levels. (C) mRNA levels for SREBP-1, ATP-CL, FAS and ACC. Results were normalised to the corresponding GAPDH mRNA (Northern blot) or 18S rRNA (RT-PCR) signals and then expressed in arbitrary units. Values are means with their standard errors shown by vertical bars (eight rats per group). * Mean value was significantly different from that of the no-cholesterol group ($P < 0.05$). † Mean value was significantly different from that of the cholesterol group ($P < 0.05$)

tended to be lower than in the cholesterol diet group ($P < 0.1$). Although there was no difference in HMG-CoA concentration in the liver between the no-cholesterol diet and cholesterol diet groups, this value was significantly lower in the cholesterol and acetic acid diet group. Similarly,

there were no significant differences in ATP-CL activity in the liver between the no-cholesterol diet and cholesterol diet groups, but such activity was significantly lower in the cholesterol and acetic acid diet group than in the other groups. With regard to CYP7A1 activities, differences were

not significant among the three groups; neither were mRNA levels.

Faecal steroids

Faecal neutral steroid excretion was similarly elevated in both the cholesterol and cholesterol and acetic acid diet groups compared with the no-cholesterol diet group. However, no significant difference was observed between the cholesterol and cholesterol and acetic acid diet groups (Table 5). Faecal bile acid excretion in the cholesterol diet group was 3.5 times as great as that in the no-cholesterol diet group. Excretion of

bile acid in the faeces was slightly but significantly greater in the cholesterol and acetic acid diet group than the cholesterol diet group (Table 5).

Discussion

In the present study, we demonstrated that dietary acetic acid reduces serum concentrations of total cholesterol and triacylglycerols in rats fed a cholesterol-rich diet, with a tendency for a reduction of cholesterol and triacylglycerol concentrations in the liver.

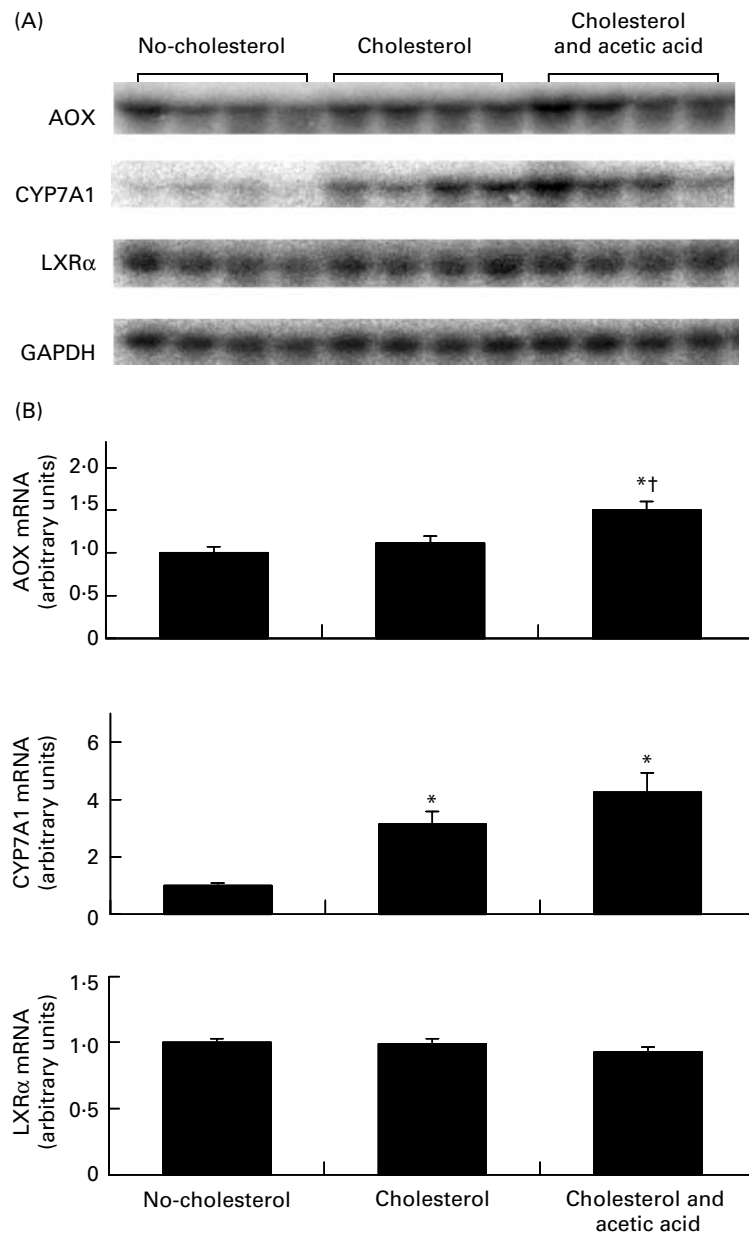


Fig. 2. mRNA levels of acyl-CoA oxidase (AOX), cholesterol 7 α -hydroxylase (CYP7A1) and liver X receptor α (LXR α) in liver of rats fed a no-cholesterol, cholesterol, or cholesterol and acetic acid diet. For details of diets and procedures, see Tables 1 and 3. (A) Representative Northern blots. Liver total RNA (35 μ g) was analysed by Northern blot hybridisation for AOX, CYP7A1, LXR α and glyceraldehyde phosphate dehydrogenase (GAPDH) mRNA. (B) mRNA levels for AOX, CYP7A1 and LXR α . Results were normalised to the corresponding GAPDH mRNA signal and then expressed in arbitrary units. Values are means with their standard errors shown by vertical bars (eight rats per group). * Mean value was significantly different from that of the no-cholesterol group ($P < 0.05$). † Mean value was significantly different from that of the cholesterol group ($P < 0.05$).

Table 5. Metabolites and enzymic activity in the liver and faecal steroids of rats fed a no-cholesterol, cholesterol, or cholesterol and acetic acid diet*

(Mean values with their standard errors; eight rats per group)

Diet...	No-cholesterol		Cholesterol		Cholesterol and acetic acid	
	Mean	sem	Mean	sem	Mean	sem
Liver						
Cholesterol (mg/g tissue)	3.3	0.2	34.7†	4.7	26.5†	3.3
Triacylglycerols (mg/g tissue)	13.4	1.0	28.7†	3.9	21.6†	1.7
HMG-CoA (nmol/g tissue)	5.14	0.46	4.68	0.44	3.46†‡	0.29
ATP-CL (nmol/min per g protein)	41.1	2.8	46.5	4.9	27.7†‡	3.8
CYP7A1 (nmol/min per g protein)	1.28	0.33	9.35	2.59	8.59	3.01
Faecal steroids						
Neutral steroids (mg/d)	8.0	0.3	121†	7	129†	8
Bile acids (mg/d)	7.6	0.4	27.0†	1.3	30.7†‡	1.3

HMG-CoA, 3-hydroxy-3-methylglutaryl-CoA; ATP-CL, ATP citrate lyase; CYP7A1, cholesterol 7 α -hydroxylase.

* For details of diets and procedures, see Tables 1 and 3. Liver was dissected after a 7 h period of food deprivation on day 20, and faeces were collected on day 15 of the experimental period (see p. 917).

† Mean value was significantly different from that of the no-cholesterol group ($P < 0.05$).‡ Mean value was significantly different from that of the cholesterol group ($P < 0.05$).

Dietary cholesterol has been reported to stimulate hepatic triacylglycerol biosynthesis by reduction of fatty acid oxidation, leading to an increase in plasma triacylglycerol concentration (Fungwe *et al.* 1993, 1994). In the present study, we found that AOX gene expression was elevated in the cholesterol and acetic acid diet group, suggesting that dietary acetic acid might attenuate the cholesterol-mediated increase in hepatic triacylglycerol synthesis and eventually suppress the elevation of serum triacylglycerol concentrations.

We also found that the mRNA level of SREBP-1, a nuclear factor known to play a pivotal role in the regulation of fatty acid synthesis in the liver (Foufelle & Ferré, 2002), was significantly reduced by dietary acetic acid in rats fed a cholesterol-rich diet. Indeed, mRNA levels of ATP-CL, FAS and ACC, which are related to fatty acid synthesis and regulated transcriptionally (Sato *et al.* 2000; Bennett *et al.* 1995; Lopez *et al.* 1996, respectively), were reduced in the cholesterol and acetic acid diet group. The cholesterol diet group had a significantly higher SREBP-1 mRNA level than the no-cholesterol diet group. However, mRNA levels of SREBP-1-responsive genes for ATP-CL, FAS and ACC in the cholesterol diet group were generally lower than those in the no-cholesterol diet group. Cholesterol has been reported to reduce mRNA levels of ATP-CL, FAS and ACC in hepatocytes (Sato *et al.* 2000; Bennett *et al.* 1995; Lopez *et al.* 1996, respectively). Thus, it is not unexpected that these mRNA levels in the cholesterol diet group were lower than those in the no-cholesterol diet group. Further, the cholesterol and acetic acid group had lower mRNA levels than the cholesterol group, which had lower mRNA levels than the no-cholesterol group, because the cholesterol and acetic acid diet group had significantly lower SREBP-1 mRNA levels than the cholesterol diet group. Therefore, it is very likely that dietary acetic acid inhibits fatty acid synthesis through a decrease in SREBP-1 mRNA. Indeed, it should be noted that the activity of ATP-CL, which plays an important role in supplying acetyl-CoA to pathways of both cholesterologenesis and lipogenesis (Pearce *et al.* 1998), was markedly reduced in the cholesterol and acetic acid diet group.

Further, the hepatic concentration of HMG-CoA, a main substrate for cholesterol synthesis, was reduced by dietary acetic acid. Since HMG-CoA is a metabolite on the cholesterol synthesis pathway from acetyl-CoA, the lower HMG-CoA content in the cholesterol and acetic acid diet group might result from the reduction of both the mRNA level and activity of ATP-CL by acetic acid feeding. HMG-CoA synthesis is catalysed by HMG-CoA synthase. While the transcription of this enzyme is regulated by SREBP-1a or -2 (Horton *et al.* 1998), it has been reported that cholesterol reduced the mRNA level of HMG-CoA synthase in hepatocytes (Bennett *et al.* 1995; Lopez *et al.* 1996). Therefore, the HMG-CoA content in the cholesterol diet group was about 90% of that in the no-cholesterol diet group, a difference that was without significance, although the cholesterol diet group had a significantly higher SREBP-1 mRNA level than the no-cholesterol diet group.

Taken together, these results suggest that dietary acetic acid may reduce the supply of acetyl-CoA, as a common substrate for cholesterol and fatty acid synthesis, through the reduction of both the mRNA level and activity of ATP-CL caused by the suppression of SREBP-1 gene expression. We also found that dietary acetic acid caused an increase in AOX gene expression, which indicates elevated fatty acid β -oxidation. Thus, it seems likely that dietary acetic acid affects not only fatty acid synthesis and cholesterol synthesis in the liver, but also fatty acid oxidation, leading eventually to reduction of serum cholesterol and triacylglycerol concentrations.

It is well known that through SREBP-1, insulin up regulates the expression of lipogenic genes, including ATP-CL, FAS and ACC (Foufelle & Ferré, 2002). The present results showed that the serum insulin level was reduced in the cholesterol and acetic acid diet group. In a study on human subjects, intake of a starchy meal with vinegar was reported to lead to suppression of postprandial increments in serum glucose and insulin by a delayed gastric emptying rate (Liljeberg & Björck, 1998). Hence, continuous acetic acid feeding may lead to a chronically lowered serum insulin value. On the other hand, it was recently reported that AMPK was activated

in hepatocytes cultured in the presence of acetate (Kawaguchi *et al.* 2002). The activation of AMPK has been shown to repress the hepatic SREBP-1 mRNA level and the amount of the mature form of SREBP-1 protein in nuclei, concomitant with reduced expressions of lipogenic genes including FAS and ACC (Zhou *et al.* 2001), as well as lowered fatty acid synthesis and cholesterol synthesis (for reviews, see Hardie *et al.* 1998; Winder & Hardie, 1999; Hardie, 2003). Although the present study had no data on the amount of the mature form of SREBP-1 protein in nuclei, we found that acetic acid feeding reduced mRNA levels of SREBP-1 responsive genes (ATP-CL, FAS and ACC) as well as that of SREBP-1. In consideration of these factors, we speculate that the suppression of SREBP-1 mRNA expression observed in the liver of rats fed the cholesterol and acetic acid diet is attributable to a lowering of serum insulin levels and/or an acetic acid-mediated activation of AMPK.

In the present study, we showed that faecal bile acid excretion was enhanced by dietary acetic acid in rats fed a cholesterol-rich diet. Although both the level of CYP7A1 mRNA and its activity were unaffected by dietary acetic acid, it is likely that bile acid secretion from the liver may be enhanced in the cholesterol and acetic acid diet group, because this group had a significantly higher serum secretin concentration than the cholesterol diet group. Previous reports showed that secretin enhances the release of bile acid in hepatocytes (Shimizu *et al.* 1987), and that intraduodenal perfusion of acetic acid at a higher concentration than physiological levels was accompanied by secretin release (Sanchez-Vincente *et al.* 1995). Thus, it could be assumed that acetic acid feeding promotes bile acid excretion through the stimulation of secretin release. However, there is no report about the correlation between serum secretin levels and faecal bile acid content *in vivo*. In order to confirm this interpretation, it should be made clear whether secretin directly increases faecal bile acid excretion *in vivo*, and whether dietary acetic acid directly stimulates secretin release at physiological concentrations.

In summary, the present results suggest that dietary acetic acid reduces serum cholesterol and triacylglycerol concentrations in rats fed a cholesterol-rich diet through inhibition of metabolic pathways of cholesterologenesis and lipogenesis in the liver, together with a concomitant enhancement of fatty acid oxidation and a stimulation of faecal bile acid excretion. In future, it is important to confirm the same effect of dietary acetic acid on lipid metabolism in human subjects. Thus, hamsters should be studied at the next stage of this investigation, as their lipid metabolism is closer to that of man than lipid metabolism in the rat.

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