Chinese soft-shelled turtle egg powder lowers serum cholesterol, increases faecal neutral steroids and bile acid excretion, and up-regulates liver cytochrome P450 mRNA level in rats

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The aim of the present study was to investigate the effect of Chinese soft-shelled turtle whole egg powder (TE) on cholesterol metabolism in Sprague–Dawley rats to determine whether it has a cholesterol-lowering effect. Forty male Sprague–Dawley rats were fed a high-fat diet supplemented with TE (0, 0·75, 1·50 or 3·00 g/kg body weight) administrated by gavage for 24 weeks. Serum total cholesterol (TC), HDL-cholesterol (HDL-C), LDL-cholesterol (LDL-C) and faecal total bile acids levels were determined by enzymatic methods. Faecal steroid concentrations were measured by GC. Means and standard deviations were calculated where appropriate for values, and the data were tested by one-way ANOVA. After 24 weeks of feeding a high-fat diet with TE supplementation, serum TC and LDL-C levels, liver cholesterol and liver lipid levels were reduced in rats. TE supplementation did not affect the faecal output, but significantly increased steroid concentrations in faeces, indicating increased steroids excretion. The faecal bile acid excretion was also increased as evidence by elevated mRNA level of liver cytochrome P450, family 7, subfamily A, polypeptide 1. Our results demonstrated that the TE does have a cholesterol-lowering effect by increasing the excretion of total bile acids and neutral steroids.

Lipid-lowering: Faecal bile acid: Steroids: Low cholesterol

One of the basic theories of nutrition in traditional Chinese medicine is food as tonic. Food as tonic refers to using food to make a person stronger and feel better in general. Chinese soft-shelled turtle has always been viewed as this kind of food and has been eaten for thousands of years in China. It has been believed to nourish *yin* (body fluid) and enhance liver function to make a person feel calmer. The eggs of the turtle are also thought to nourish *yin* and supplement deficiency. The medicinal effects of turtle eggs have been depicted in *Materia Medica*.

A number of risk factors are known to contribute to CVD. Elevated levels of plasma total cholesterol (TC) and lower LDL-cholesterol (LDL-C) have long been viewed as primary risk factors for CVD (Consensus Conference, 1995). To avoid the side-effects of lipid-lowering pharmacological drugs, reduction in TC and LDL-C levels can be achieved by dietary means. It has been shown that soya protein, fish protein, PUFA, egg lecithin, dietary fibres and plant sterols can lower cholesterol levels in animal models and human subjects (Fukushima *et al.* 2000; Tammi *et al.* 2001; Blair *et al.* 2002; Garg *et al.* 2003; Noh & Koo, 2003; Wergedahl *et al.* 2004). The mechanisms of reducing plasma cholesterol by soya protein include inducing LDL receptor expression, increasing bile acid synthesis, and decreasing steroid absorption from the intestine (Potter, 1995). Several studies suggest that the amino acids or peptide of soya

protein might be responsible for the changes in blood lipids (Kern *et al.* 2002). Although the cholesterol-lowering effect of soya protein is well known, only a few studies have been performed on the lipid-lowering effects of other proteins, such as fish protein (Zhang & Beynen, 1993; Wergedahl *et al.* 2004), white lupin seed proteins (Sirtori *et al.* 2004) and buckwheat protein (Tomotake *et al.* 2000).

The amino acids profile of turtle egg protein is unique, and its phosphatidylcholine content is rich and cholesterol content is very low. Can it change blood lipids? It has not been studied extensively. Although the search for natural substances capable of lowering blood cholesterol is ongoing in the field of nutrition, research on whole foods containing more than one factor with potential cholesterol-lowering effect is scanty. The objective of the present study was to determine whether Chinese soft-shelled turtle whole egg powder (TE) can lower serum lipids and undertake a preliminary investigation of its mechanism.

Materials and methods

Preparation of Chinese soft-shelled turtle whole egg powder

Turtle egg powder was prepared as follows. Before freeze-drying, the turtle eggs were washed with water and crushed into small pieces, which were filtered through an 80 mesh sieve. The egg

suspension obtained was frozen at $-80°C$ for 72 h and then the samples were transferred to a freeze-dryer and dehydrated. The freeze-dried turtle egg samples were then rapidly powdered, and the resulting powder was stored desiccated at $-20°C$ in sealed plastic bags. The amino acid and fatty acid composition of TE is shown in Table 1.

Animal and diets

Male Sprague–Dawley rats (Experimental Animal Breeding Center, Peking University, Beijing, China) weighing 160–180 g were fed a chow diet (Table 2) for 7 d, then fed a high-fat diet (10 % lard, 10 % yolk powder, 1 % cholesterol, 79 % chow). The rats were maintained at $22 \pm 2°C$ and 60 ± 5 % relative humidity in a room with 12 h light/12 h dark cycles and given free access to food and water at all times. Rats were divided according to serum LDL-C level and weight into four groups of ten rats each and fed the high-fat diet supplemented with TE (0, 0·75, 1·5 or 3·0 g/kg body weight (BW)) administrated by gavage. BW and food intake were recorded weekly during the 24-week study. During the last 7 d of the experimental period, faecal samples were collected from each cage, pooled and then stored at $-20°C$ until analysis. At the end of the experiment, rats were deprived of food for 16 h and blood samples were collected into tubes by femoral puncture and centrifuged at $4°C$ for 15 min at 4000 rpm. Before blood sampling, the rats were anaesthetized by sodium pentobarbital (40 mg/kg BW). After collecting the blood, the livers were removed and weighed and first being washed in ice-cold 0·9 % NaCl and then stored at $-80°C$ until analysis. All animals were handled in accordance with the guidelines established by the Chinese Committee on Experimental Animal Supervision.

Serum total cholesterol, HDL-cholesterol and LDL-cholesterol

Serum TC was determined without extraction by an enzymatic colorimetric method using a kit (#CH3810) provided by Randox Co. (Crumlin, County Antrim, UK). HDL-cholesterol (HDL-C) (kit #CH3811; Randox Co.) and LDL-C (kit #CH3841; Randox Co.) were determined after precipitation with magnesium phosphotungstic acid. All these were accomplished in an automatic machine (7020 Clinical Analyzer; Hitachi, Tokyo, Japan).

Table 1. Amino acid and fatty acid* content of Chinese soft-shelled turtle egg (g/100 g)

Amino acid	Content	Fatty acid	Content
Leucine	2·86	Myristic acid	0·44
Isoleucine	1·77	Palmitic acid	3·14
Lysine	2·99	Stearic acid	0·87
Methionine	0·76	Palmitoleic acid	1·88
Cysteine	0·80	Heptadecenoic acid	0·15
Phenylalanine	1·52	Oleic acid	11·8
Tyrosine	1·63	Eicosenoic acid	0·20
Threonine	1·69	Linoleic acid	0·86
Tryptophan	0·47	Linolenic acid	0·12
Valine	1·86	Arachidic acid	0·42
Histidine	1·01	EPA	1·14
Arginine	2·51	Docosapentaenoic acid	0·44
Alanine	1·93	DHA	1·27
Aspartic acid	2·94	Phospholipid	3·79
Glutamic acid	3·97	Phosphatidylcholine	2·61
Glycine	1·08	Cholesterol	0·85
Proline	1·90		
Serine	3·96		

*Fatty acid content less than 0·1 g/100 g not listed.

Table 2. Composition of the chow diet

Nutrient	Content
Protein (%)	20·26
Fat (%)	4·24
Carbohydrate (%)	25·19
Vitamin A mg/100 g	3·6
Vitamin D mg/100 g	0·25
Vitamin E mg/100 g	44·0
Vitamin K mg/100 g	5·0
Choline chloride (g/kg)	1·10
Mineral mix (g/kg)	25·90

For details of diets and procedures, see p. 314 of proofs.

Liver total lipids, total cholesterol and triacylglycerols

Liver total lipids were determined according to Folch *et al.* (1957). Liver TC concentration was determined using the same kit as for serum TC following extraction of liver samples with methanol–chloroform (2:1, v/v). Liver triacylglycerol concentration was determined using a kit provided by Randox Co. (kit #TR1697) after extraction with methanol–chloroform (2:1, v/v).

Analysis of faecal neutral steroids

Faecal steroids were obtained from dried faeces according to Folch *et al.* (1957) using the following procedures (Schneider *et al.* 2000). An aliquot of dried faeces (100 mg) was extracted with methanol–chloroform (2:1, v/v) containing 5α-cholestane (Sigma-Aldrich, St. Louis, MO, USA) and the sample saponified in 2 ml methanolic KOH for 2 h at $50°C$. After cooling to ambient temperature and adding 2·0 ml deionized water, the lipids were extracted into 5 ml hexane. The sample was derivatized before GC by adding 100 µl pyridine (Fluka, Buchs, Switzerland), followed by 50 µl Sylon BTZ (Supelco, Bellefonte, PA, USA). GC analyses were carried out using a CP-3800 with a DB-1 capillary column (30 m, 0·25 mm inner diameter) (Varian, Palo Alto, CA, USA). The temperature programme started at $90°C$; after an initial hold of 1 min, the oven was programmed to $285°C$ (30 min) at a rate of $15°C$/min. H_2 was used as carrier gas.

Faecal bile acids

Faeces were extracted by the method of Setchell *et al.* (1983), and the extracted solutions were used to determine bile acids concentration (Ausbio Laboratories Ltd, Beijing, China) enzymatically.

Semi-quantitative RT-PCR

Total RNA was purified using Trizol (Invitrogen, Carlsbad, CA, USA) and reverse-transcribed using a Reverse Transcriptase kit (#A3500; Applied Promega, Madison, WI, USA). RNA encoding 3-hydroxy-3-methylglutaryl (HMG)-CoA reductase, cholesterol 7α-hydroxylase (CYP7A1: cytochrome P450, family 7, subfamily A, polypeptide 1), ATP-binding cassette transporter 1 (ABCA1), liver X receptor α (LXR-α) and glyceraldehyde-3-phosphate dehydrogenase (GAPDH; used as an invariant control) were analysed by semi-quantitative RT-PCR. The primer sequences are listed in Table 3. PCR was carried out as follows: denature at $94°C$ for 5 min for the first cycle, and then cycles consisting of $94°C$ for 1 min, annealing at $60°C$ for 1 min, and extension at $72°C$ for 2 min. Twenty-five cycles were performed for GAPDH and CYP7A1, thirty cycles for LXR-α, thirty-five cycles for

Table 3. Primer sequences used in mRNA quantification by RT-PCR

	Sense	Antisense	Reference
ABCA1	5′-GTG AAC TTT GCC AAG GAC CA-3′	5′-AGG CTA CAA AGG CAC TGC C-3′	Qiu *et al.* (2001)
CYP7A1	5′-GCC GTC CAA GAA ATC AAG CAG T-3′	5′-TGT GGG CAG CGA GAA CAA AGT-3′	Fukushima *et al.* (2000)
HMG-CoA	5′-GCG TGC AAA GAC AAT CCT GGA G -3′	5′-GTT AGA CCT TGA GAA CCC AAT G -3′	Fukushima *et al.* (2000)
LXR-α	5′-GAG TTG TGG AAG ACA GAA CCT CAA-3′	5′-GGG CAT CCT GGC TTC CTC-3′	Steffensen *et al.* (2003)
GAPDH	5′-GCC ATC AAC GAC CCC TTC ATT-3′	5′-CGC CTG CTT CAC CAC CTT CTT-3′	Fukushima *et al.* (2000)

ABCA1, ATP-binding cassette transporter 1; CYP7A1, cytochrome P450, family 7, subfamily A, polypeptide 1; HMG-CoA, 3-hydroxy-3-methylglutaryl CoA; LXR-α, liver X receptor α; GAPDH, glyceraldehyde-3-phosphate dehydrogenase.

HMG-CoA reductase and twenty-six cycles for ABCA1. Amplification products were electrophoresed on a 1·5 % agarose gel and the relative quantity of mRNA was estimated by densitometry scanning with X-rays (Smart view 2002 program; Shanghai FURI Science and Technology Co. Ltd., Shanghai, China).

Statistical analysis

Values are presented as means and standard deviations and, where appropriate, data were tested by one-way ANOVA using the LSD procedure of the SPSS10.0 package (SPSS Inc, Chicago, IL, USA). Individual comparisons were made by least-squares means. Differences with $P < 0.05$ were considered significant.

Results

Food intake and body weight

Supplementation with different TE levels did not affect food intake or BW gain and food efficiency of the rats (Table 4).

Serum lipids

In Sprague–Dawley rats supplemented with 1·5 and 3·0 g TE/kg BW, serum TC concentrations were significantly reduced by 20 % ($P < 0.01$) and 14 % ($P < 0.05$), respectively (Table 5). Serum LDL-C and HDL-C levels were reduced by 26 % ($P = 0.017$) and 12·5 % ($P = 0.052$) in rats supplemented with 1·5 g TE/kg BW. There was no difference in HDL-C:TC among groups. The largest reductions in serum LDL-C and TC were found in rats supplemented with 1·5 g TE/kg BW.

Liver total lipids, total cholesterol and triacylglycerols

Relative liver weight of the rats supplemented with 1·5 g TE/kg BW was reduced significantly ($P < 0.05$). Liver total lipids, TC and triacylglycerol concentrations were reduced in particular in

the livers of rats supplemented with 1·5 and 3·0 g TE/kg BW (Table 6).

Faecal bile acids and neutral steroids excretion

Daily faecal output was not different between groups. Both faecal total bile acids concentration (μmol/g) and daily faecal bile acids excretion (μmol/d) were significantly higher ($P < 0.05$) in rats supplemented with 1·5 and 3·0 g TE/kg BW (Table 7)

Cholesterol, coprostanol and coprostanone were the main neutral steroids in rat faeces. Faecal concentrations and daily excretion of total neutral steroids were both increased significantly ($P < 0.01$) in rats supplemented with 1·5 and 3·0 g TE/kg BW (Table 7). Faecal cholesterol and coprostanol daily excretion were increased significantly by 172·6 % and 99·7 %, respectively, in rats supplemented with 1·5 g TE/kg BW, and by 81·4 % and 61·4 % in rats supplemented with 3·0 g TE /kg BW (all $P < 0.01$). Furthermore, in rats supplemented with 1·5 g TE/kg BW, the faecal coprostanone excretion was also significantly higher ($P < 0.05$) than in rats without TE supplement. Dietary cholesterol apparently absorbed was lowered by supplementation with TE.

mRNA levels

Although RT-PCR is less sensitive to determine mRNA levels than real-time PCR and other methods, it is also used because of its simplicity and low cost. In the present study, we chose this method to compare the mRNA levels between groups. The results indicated that liver mRNA levels of ABCA1, CYP7A1, HMG-CoA reductase and LXR-α could be quantified by RT-PCR. Hepatic CYP7A1 mRNA concentration was up-regulated significantly in rats supplemented with TE ($P = 0.062$, 0·000 and 0·013 at 0·75, 1·5 and 3·0 g TE/kg BW, respectively). LXR-α was also up-regulated in rats supplemented with 3·0 g TE/kg

Table 4. Effects of Chinese soft-shelled turtle whole egg powder (TE) on body weight (BW) and food intake in rats

(Mean values with their standard deviations for ten animals per group)

	0 g TE/kg BW		0·75 g TE/kg BW		1·5 g TE/kg BW		3·0 g TE/kg BW	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Weight (g)								
Week 0	168·4	6·4	164·4	4·5	163·9	6·4	166·5	6·6
Week 24	660·7	106·0	659·6	95·7	645·2	60·3	651·3	62·3
Weight gain (g)	492·3	95·1	495·6	87·4	482·1	91·5	498·1	62·3
Food intake (g)	3187·3	103·4	3101·3	109·2	2993·3	144·8	3013·8	156·2
Food efficiency (%)	16·8	0·05	16·3	0·07	16·2	0·07	16·5	0·06

For details of diets and procedures, see p. 314.

Table 5. Effects of Chinese soft-shelled turtle whole egg powder (TE) on serum lipids in rats

(Mean values with their standard deviations for ten animals per group)

	0 g TE/kg BW		0·75 g TE/kg BW		1·5 g TE/kg BW		3·0 g TE/kg BW	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
TC (mmol/l)								
Week 0	1·87	0·25	1·89	0·18	1·88	0·17	1·86	0·19
Week 24	3·09	0·41	2·75	0·51	2·47**	0·31	2·65*	0·33
HDL-C (mmol/l)	1·27	0·21	1·19	0·22	1·11	0·13	1·17	0·19
LDL-C (mmol/l)	1·22	0·25	1·05	0·36	0·90*	0·11	1·03	0·18
HDL-C:TC	0·42	0·06	0·44	0·08	0·45	0·04	0·45	0·05

BW, body weight; TC, total cholesterol; HDL-C, HDL-cholesterol; LDL-C, LDL-cholesterol.
Mean values were significantly different from those of the other groups: *$P<0.05$, **$P<0.01$.
For details of diets and procedures, see p. 314 of proofs.

BW only ($P=0.016$; Fig. 1). Transcription of other genes was not affected (data not shown).

Discussion

TE was well tolerated by the Sprague–Dawley rats and did not affect food intake, growth, body or organ weights even at the high dose of 3·0 g/kg BW. TE supplementation exerted a significant cholesterol-lowering effect, especially at the doses of 1·5 and 3·0 g/kg BW. Moreover, the serum cholesterol change induced by TE has not been described previously.

In the present study, using Sprague–Dawley rats as animal models, we demonstrated the cholesterol-lowering effect of TE. In the rats, supplementation with TE for 24 weeks significantly reduced serum TC and LDL-C levels, and liver lipid and cholesterol concentrations, compared with the control group without TE supplement. A number of studies using different animal models have demonstrated the cholesterol-lowering effects of soya protein, fish protein and white lupin seed protein. Several mechanisms have been shown to explain this effect of soya protein. Enhanced faecal steroid excretion, which is the major route for cholesterol excretion from the body (Huff & Carroll, 1980), may be one of these mechanisms. In the present study, TE (1·5 and 3·0 g/kg BW) increased the excretion of steroids, including bile acids and neutral steroids, resulting in significant lowering of serum cholesterol levels. We believe that the TE supplement modified cholesterol metabolism because the faecal steroid concentrations were increased but not the total faecal output.

Methionine has been shown to elevate serum cholesterol concentration (Sugiyama *et al.* 1986). However, methionine supplementation to a soya diet did not abolish the cholesterol-lowering effects of soya protein relative to casein (Kern *et al.* 2002), suggesting that some factor other than methionine may be responsible at least in part for the cholesterol-lowering effect of soya protein. However, it was suggested that the higher methionine:glycine in casein may be responsible for the elevation in serum cholesterol (Kritchevsky *et al.* 1982), and glycine supplementation to a casein-based diet lowered serum cholesterol concentration in rats (Sugiyama *et al.* 1986). In the present experiment, the methionine:glycine was 0·70 in TE. It is lower than that in casein (2·3) and soya protein supplemented with methionine (1·2; Kern *et al.* 2002). It was also suggested that the increased serum cholesterol level that occurs with casein feeding was caused by the high lysine:arginine in casein (Berge *et al.* 1984; Madsen *et al.* 1998). The lysine:arginine in TE is 1·19, lower than that in casein (1·8), slightly higher than that in fish protein (1·1; Wergedahl *et al.* 2004) and soya protein (0·8), favouring a cholesterol-lowering effect by TE. Thus, the amino acid composition of TE may contribute, at least in part, to its cholesterol-lowering effect.

CYP7A1 is a liver-specific enzyme that catalyses the rate-limiting step in the classic pathway of bile acid synthesis responsible for the conversion of cholesterol to bile acids (Russell & Setchell, 1992; Kramer *et al.* 2003), which is the primary mechanism for the removal of cholesterol from the body. This mechanism plays an important role in regulation of bile acid biosynthesis and cholesterol homeostasis. Spady *et al.* (1995) demonstrated that over-expression of exogenous *CYP7A1* genes effectively reduced the plasma cholesterol level in hamsters fed a low- or high-fat diet. Yokogoshi *et al.* (1999) found that taurine supplementation reduced the rat blood level of cholesterol and induced CYP7A1 activity and *CYP7A1* gene expression. Moreover, serum cholesterol level was negatively correlated with CYP7A1 mRNA level. In the present

Table 6. Liver total lipids, total cholesterol and triacylglycerols in rats supplemented with Chinese soft-shelled turtle whole egg powder (TE) for 24 weeks

(Mean values with their standard deviations for ten animals per group)

	0 g TE/kg BW		0·75 g TE/kg BW		1·5 g TE/kg BW		3·0 g TE/kg BW	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Relative liver weight (g/kg BW)	37·45	7·9	36·71	7·2	31·76*	4·4	33·94	2·9
Total lipids (g/g wet liver)	0·4048	0·04	0·3825	0·05	0·3253*	0·04	0·3494*	0·03
Total cholesterol (μmol/g wet liver)	61·35	20·38	55·73	19·44	41·02	14·79	31·60*	11·63
Triacylglycerols (μmol/g wet liver)	45·70	7·29	40·91	8·02	31·84*	7·61	32·47*	8·03

BW, body weight.
Mean values were significantly different from those of the other groups: *$P<0.05$.
For details of diets and procedures, see p. 314 of proofs.

Table 7. Faecal total bile acids, cholesterol, coprostanol and coprostanone excretion by rats supplemented with Chinese soft-shelled turtle whole egg powder (TE) for 24 weeks

(Mean values with their standard deviations for ten animals per group, unless indicated otherwise)

	0 g TE/kg BW		1·5 g TE/kg BW		3·0 g TE/kg BW	
	Mean	SD	Mean	SD	Mean	SD
Cholesterol intake (μmol/d)	756		738		761	
Faecal output (g dry weight/d)	3·6		3·8		3·5	
Faecal bile acids excretion†						
(μmol/g dry faeces)	16·91	3·81	21·37*	4·80	21·12*	2·12
(μmol/d)	60·84		81·21		73·92	
Faecal total neutral steroids excretion‡						
(μmol/g dry faeces)	39·58	2·29	96·13**	4·08	73·83**	4·76
(μmol/d)	142·49		365·29		258·41	
Faecal cholesterol excretion‡						
(μmol/g dry faeces)	28·86	2·26	74·55**	3·51	55·73**	
(μmol/d)	103·90		283·29		195·06	
Faecal coprostanol excretion‡						
(μmol/g dry faeces)	10·08	1·19	19·07**	1·06	16·73**	2·18
(μmol/d)	36·29		72·47		58·56	
Faecal coprostanone excretion‡						
(μmol/g dry faeces)	0·64	0·28	2·51*	0·16	1·37	0·58
(μmol/d)	2·30		9·53		4·80	
Faecal total steroids excretion§ (μmol/d)	203·33		446·50		332·33	
Cholesterol apparent absorption‖ (μmol/d)	552·67		291·5		428·67	
Dietary cholesterol apparently absorbed¶ (%)	73·10		39·50		56·33	

BW, body weight.

Mean values were significantly different from those of the control (0 g TE/kg BW) group: *$P<0.05$, **$P<0.01$.

† The number of samples determined was eight.

‡ The number of samples determined was six.

§ Faecal total steroids excretion = faecal total neutral steroids excretion + faecal bile acids.

‖ Cholesterol apparent absorption = cholesterol intake − total steroid excretion.

¶ Percentage dietary cholesterol apparently absorbed = 100 × (cholesterol intake − total steroids excretion)/cholesterol intake.

For details of diets and procedures, see p. 314 of proofs.

study, liver CYP7A1 mRNA level was up-regulated by TE supplementation. This may be the mechanism whereby TE could reduce serum cholesterol.

LXR-α is an oxysterol-activated receptor. In the present study, TE supplementation significantly increased liver LXR-α mRNA level at the dose of 3·0 g/kg BW. Activated LXR-α could induce expression of a small set of target genes that includes murine *CYP7A1*, which results in increased synthesis of primary bile acids (Edwards *et al.* 2002). LXR-α knockout mice fed a high-cholesterol diet accumulate hepatic cholesterol as a result of an inability to induce expression of CYP7A1, the enzyme controlling the rate-limiting step in bile acid synthesis (Peet *et al.* 1998). There may be some special factors in TE that can activate the expression of LXR-α.

TE is a new food resource that contains abundant phosphatidylcholine (2610 mg/100 g) and PUFA (fatty acid composition is given in Table 1), which are both cholesterol-lowering factors in food, but the cholesterol content is low (850 mg/100 g). Although phosphatidylcholine plays an important role in intestinal lipid absorption by enhancing micellar lipid solubility and providing the surface coat for the formation of chylomicrons, numerous *in vitro* studies have shown that phosphatidylcholine inhibits cholesterol uptake. Jiang *et al.* (2001) reported that egg phosphatidylcholine could markedly lower the lymphatic absorption of cholesterol *in vivo*. In the present study, TE decreased cholesterol absorption and then increased cholesterol faecal excretion, which might have arisen because of the large content of phosphatidylcholine in TE. Studies in rats and other animal models (Grundy, 1986; Kris-Etherton *et al.* 1999; Lopez-Miranda *et al.* 2000) have suggested that dietary MUFA have a potential lipid-lowering

effect. There are many studies (e.g. Willett *et al.* 1995) that support the positive effects of the intake of fish oil (mainly rich in PUFA) on lowering blood lipids. TE is a kind of traditional food in which MUFA and PUFA are both abundant. It is our future work to determine which acts as the cholesterol-lowering agent.

In summary, the present study demonstrates that TE has serum cholesterol-lowering effects in Sprague–Dawley rats. This effect is mediated by enhancing bile acid synthesis and faecal excretion of neutral and acidic steroids. However, because cholesterol
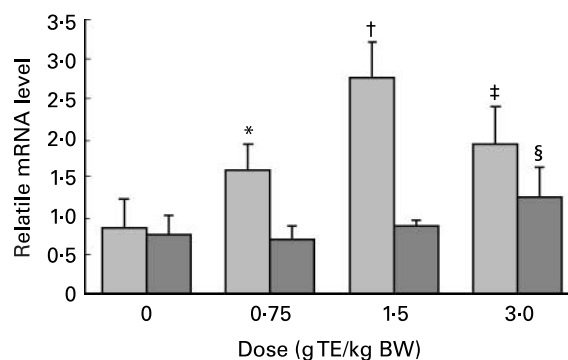

Fig. 1. Hepatic concentration of cytochrome P450, family 7, subfamily A, polypeptide 1 (▦) and liver X receptor α (▦) in rats supplemented with Chinese soft-shelled turtle whole egg powder (TE) at different doses (BW, body weight) for 24 weeks. mRNA levels were normalized to the values of glyceraldehyde-3-phosphate dehydrogenase. Values are means with their standard deviations shown by vertical bars for three animals per group. Mean values were significantly different from those of the control (0 g TE/kg BW) group: *$P=0.062$; †$P=0.000$; ‡$P=0.013$; §$P=0.016$.

metabolism in rats is very different from that in human and other species, future studies will be conducted in man. The mechanisms by which TE lowers plasma cholesterol need to be further characterized and the special functional factors in TE need to be identified.

Acknowledgements

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