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Increase of bile acids synthesis and excretion caused by taurine administration prevents the ovariectomy-induced increase in cholesterol concentrations in the serum low-density lipoprotein fraction of Wistar rats

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Abstract

We examined the effect of dietary taurine on the concentrations of serum cholesterol and apolipoprotein in lipoprotein fractions of Six-month-old ovariectomized, which were used as a model of hypercholesterolemia in postmenopausal woman, or sham operated rats. Taurine significantly reduced the serum total and low-density lipoprotein cholesterol concentrations only in the ovariectomized rats. In contrast, taurine significantly lowered the serum apolipoprotein B concentration and serum very low-density lipoprotein-apolipoprotein E concentration only in the sham operated rats. The serum total and high density lipoprotein-apolipoprotein E concentrations were significantly lower in the rats fed taurine than in those fed the control diet regardless of whether they had undergone ovariectomy. The esterified cholesterol level in the liver was significantly lower and the level of hepatic cholesterol 7α-hydroxylase activity was significantly higher in the rats fed taurine than in those fed the control diet. The total bile acids concentration in the feces and intestinal contents of rats fed taurine were significantly higher than those in rats fed the control diet regardless of whether they had undergone ovariectomy. In the sham-rats, taurine accelerated bile acid synthesis and excretion, thereby increasing cholesterol consumption. The increased cholesterol consumption might be compensated by accelerating cholesterol synthesis and/or reducing the synthesis and release of very low-density lipoprotein from the liver. But in the ovariectomized rats, although taurine also accelerated bile acid synthesis and excretion, cholesterol demand might be compensated by excess cholesterol in the blood.

Keywords: Taurine; Lipoprotein; Apolipoprotein B; Bile acids; Cholesterol 7α-hydroxylase; Rat

1. Introduction

Taurine, or 2-aminoethanesulfonic acid, is a sulfur-containing amino acid that is widely distributed in animal tissues, and it has a variety of biological activities [1]. One of the effects of taurine is that it influences the plasma lipid level by changing lipid metabolism. Among animals fed a hypercholesterolemic diet, taurine reduced the serum cholesterol level [2,3,4,5].

The rate of coronary heart disease (CHD) among premenopausal women is lower than that among men. However, after the onset of menopause, the risk of CHD in women increases dramatically due to ovarian hormone deficiency [6,7]. One possible explanation for the relationship between ovarian hormone deficiency and increased risk for CHD is the presence of high blood cholesterol in postmenopausal women [8,9]. Reduced ovarian function in the presence of high blood cholesterol has been implicated as a major risk factor for CHD. Hypercholesterolemia is common among postmenopausal women. Postmenopausal women taking estrogen replacement therapy have a lower serum cholesterol level than those who do not receive estrogen replacement. These data show that a woman's serum cholesterol level is influenced by the status of estrogen.

In previous studies, we demonstrated that taurine had a hypocholesterolemic effect in an estrogen-deficient animal

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model, the ovariectomized (OVX) rat [10,11]. The rat is one of the experimental models generally used to study the effects and mechanisms of the action of estrogen on the plasma cholesterol level [12,13,14,15,16,17], although the rat has notable differences and shortcomings as a model of human cholesterol metabolism. The reason for the presence of hypercholesterolemia in the ovariectomized or estrogen-deficient animal is still unclear, but one report have suggested that there is a relationship between hypercholesterolemia in the ovariectomized animal and lipoprotein metabolism [18]. Clarkson et al. [19] reported that OVX raised the serum low density lipoprotein (LDL) cholesterol concentration in monkeys. Van Lenten et al. [20] reported that the serum LDL-cholesterol concentration of ovariectomized rats was elevated as a result of increased very low density lipoprotein (VLDL) synthesis and secretion. These data suggests that OVX may increase the conversion of VLDL to LDL by increasing the plasma lipoprotein lipase activity simultaneously with increasing VLDL synthesis and secretion in various animals. We were interested in the phenomenon that ovariectomy increases the serum LDL-cholesterol concentration despite the lack of cholesterol feeding. A few reports have examined the effect of taurine on lipoprotein metabolism in high-cholesterol-feeding animals [21,22,23]; however, there has been no report on the effect of taurine in rats with hypercholesterolemia that is not a result of cholesterol feeding, such as in ovariectomized rats.

Many reports have suggested that the taurine administration lowered the serum cholesterol level by increasing bile acids synthesis and excretion. In our previous study, taurine administration increased bile flow and bile acids secretion in ovariectomized rats [11]. In previous studies, taurine administration did not affect the serum cholesterol concentration although it increased bile acids synthesis and excretion, in normal male rats that were fed a cholesterol-free diet, in rats with hypercholesterolemia due to the administration of polychlorinated biphenyl (PCB) [24] or caffeine [25], and in rats with artificial hypothyroidemia [26]. These results may indicate that taurine administration increases bile acids synthesis by stimulating hepatic cholesterol 7α-hydroxylase activity not depending on the condition of cholesterol metabolism but taurine may have a cholesterol-lowering effect only in hypercholesterolemia caused by specific factors. Many researchers reported that newly synthesized cholesterol by 3-hydroxy-3-methyl-3-glutaryl-CoA (HMG-CoA) reductase is major substrate of bile acids synthesis by cholesterol 7α-hydroxylase [27]. But it is difficult to explain the reason why taurine administration decrease serum cholesterol concentration in ovariectomyzed rat or the rat fed hypercholesterolemic diet, if increasing cholesterol synthesis is only way of compensating increasing consumption of cholesterol to synthesize bile acids. We supposed that sparing cholesterol in circulation may be selected as the way of compensating in hypercholesterolemic situation, such as hypercholesterolemic diet feeding or ovariectomy. It is possible that the difference of way of compensating cholesterol shortage may be reflected on the component of the serum lipoproteins. In present study, we examined the effect of taurine administration on the serum lipoprotein and lipids composition of lipoprotein fractions in normal and ovariectomized rats.

Table 1
Composition of control diet and the diet containing 5% taurine

Component	Control diet	Diet with 5% Taurine
	g/kg	
Casein	200	200
Corn oil	50	50
Mineral mixture[a]	40	40
Vitamin mixture[b]	10	10
Sucrose	350	325
α-Corn starch	350	325
Taurine	—	50

[a] Based on AIN-76.
[b] The AIN-76 vitamin mixture used in this study contained 20 g of choline bitartrate/100 g.

2. Methods and materials

2.1. Animals and diets

This study was approved by the Laboratory Animal Care Committee of Ehime University, and the rats were maintained in accordance with the Guidelines for the Care and Use of Laboratory Animals of Ehime University.

Six-month-old, *retired from breeding*, breeder Wistar female rats (Nippon SLC, Shizuoka, Japan) were used in this experiment. The rats were acclimated by feeding a commercial solid diet (MF^{TM}; Oriental Yeast Co., Osaka, Japan) ad libitum for 7 days. The rats were housed in individual cages with screen bottoms of stainless steel in a room maintained at 23 $\pm$ 1°C with a 12-h light-dark cycle (light, 0700–1900 h). After acclimation, the rats were divided into two groups of 12 rats each. Under sodium pentobarbital (Nembutal, Dainippon Pharmaceutical, Osaka, Japan, 30 mg/kg weight, intraperitoneal injection) anesthesia, bilateral ovariectomy (OVX) was performed on the first group of rats (OVX-rats), and a sham operation was performed on the second group of rats (sham-rats). After 8-day-recovery feeding with a commercial solid diet (MF^{TM}; Oriental Yeast Co., Osaka, Japan), the OVX-rats and sham-rats were then divided into groups of 6 rats so that the rats in the two groups had approximately the same body weight. Rats were given free access to either the control diet (C-diet) or diet containing 5% taurine (T-diet) (Table 1) and water for 4 wks. The body weight and level of food intake by weight were recorded daily for each rat in the morning before replenishing the diet.

Feces were collected during the final 3 days of the ex-

perimental period from individual rats, and they were freeze-dried and weighed. The rats were sacrificed by decapitation and a blood sample was collected from the neck at midnight in a non-fasted state on the last day of the experimental period. After the blood was kept at room temperature for at least 30 min, the serum was separated by centrifugation at 1,500×g at 4°C for 10 min, and stored at −50°C until analysis. The liver was immediately removed after blood collection and weighed; a part of the liver was immediately used for determination of the level of activity of hepatic cholesterol 7α-hydroxylase, and the remaining liver was stored at −50°C until other analyses. After the liver was removed, the pylorus and ileocecal junction was ligated and the intestine from the duodenum to anus was removed. After removal, the intestine was weighed with its content (total intestinal weight); the content was drained from the cecocolonic junction into a 50 ml vial with approximately 30 ml deionized water, weighed and freeze-dried. The intestinal wall was flushed with ice-cold saline, blotted on filter paper and weighed (intestinal wall weight). The weight of the intestinal content was calculated as the difference between the total intestine weight and the intestinal wall weight. The carcass was minced and the level of total lipids was measured gravimetrically after extraction by the method of Folch et al. [28]. The level of total protein was determined by the method of Kjeldal et al. [29]

2.2. Lipoprotein fraction

Lipoproteins were isolated by the ultracentrifugation method as modified by Hatch [30]. Briefly, 0.6 ml of serum was added to an ultracentrifuge tube and 0.3 ml of 11.4 g/L sodium chloride solution containing 0.5M EDTA (pH 8.0, d 1.006 g/ml) was layered over the surface. The tubes were capped and centrifuged in a TL-100.2 rotor (Beckman, Fullerton, CA) in a Beckman TL-100 ultracentrifuge at 100,000×g at 12°C for 2.5 h. The VLDL fraction was defined as the 0.15 ml at the top of the tube. The middle layer in the tube (0.15 ml) was removed to avoid contamination by IDL, and the bottom 0.6 ml in the tube was transferred to another tube and mixed with 0.3 ml of d 1.182 g/ml solution (prepared from d 1.006 g/ml by the addition of 249.8 g/L sodium bromide). This was centrifuged at 100,000×g, at 12°C for 2.5 h. The LDL fraction was defined as the 0.15 ml at the top of the tube. The middle layer from in the tube (0.15 ml) was removed to avoid contamination by HDL_1, and the bottom 0.6 ml in the tubes was transferred to another tube and mixed with 0.3 ml of d 1.478 g/ml solution (which had been prepared from the d 1.006 g/ml solution by adding 783.2 g/L sodium bromide) and centrifuged at 100,000×g at 12°C for 4 h. The HDL fraction, which was defined as the 0.15 ml at the top of the tubes, was removed. The remaining 0.75 ml was defined as the VHDL fraction.

The levels of total cholesterol, triglycerides and phospholipids in the serum and lipoprotein fractions were determined by a commercially available kits (Cholesterol E-Test Wako, TG E-test Wako and PL C-test Wako, respectively, Wako Pure chemical, Osaka, Japan). Concentrations of apolipoproteins were estimated by rocket electroimmunoassay [31].

2.3. Determination of the level of hepatic cholesterol 7α-hydroxylase activity

The microsomal fraction of the liver was prepared according to the method of Horio et al [32]. Briefly, fresh liver was homogenized (20% w/v) with ice-cold 1.15% potassium chloride with Potter-Elvehjem homogenizer. The homogenate was centrifuged at 10,000×g at 4°C for 30 min. The post-mitochondrial supernatant was centrifuged at 105,000×g at 4°C for 60 min. The microsomal pellets were used for the determination of the level of cholesterol 7α-hydroxylase activity according to the method of Ogishima and Okuda [33] with minor modifications. Briefly, the microsomal pellet was suspended in 0.1 M potassium phosphate buffer (pH 7.4) containing 0.1 mM EDTA, 20 mM cysteamine-hydrochloride, 5 mM magnesium chloride, 10 mM glucose-6-phosphate, 1 mM NADP and 2,500 unit/L glucose-6-phosphate dehydrogenase (Sigma Chemical, St. Louis, MO) in a final volume of 0.5 ml and incubated at 37°C for 25 min. At the end of the incubation, 50 μl of 6% sodium cholate and 20 unit/L of cholesterol oxidase (Type A, code C00–311, Toyobo, Osaka, Japan) dissolved in 10 mM potassium phosphate buffer (pH 7.4) containing 20% (v/v) glycerol and 1 mM dithiothreitol, were added to the reaction mixture and the mixture was further incubated for 10 min. The reaction was terminated by adding 0.3 ml of methanol, and the mixture was extracted with 3 ml of petroleum ether. The extract was evaporated to dryness, dissolved in100 μl of chloroform, and filtered through a 0.45μm Millipore filter and a Twenty μL of the sample was applied to a normal phase HPLC column (4.6 × 250 mm, S5W 25cm Analytical column; Waters, Milford, MA). The mobile phase was a mixture of n-hexane and isopropanol (82:18, v/v) at 40°C. The flow rate was 1.5 ml/min, and the elution was monitored at 254 nm.

2.4. Lipids analysis

The level of total lipids in the liver tissue was gravimetrically determined after extraction by the method of Folch et al. [28]. The total cholesterol and free cholesterol levels in the liver tissue were measured calorimetrically with the Cholesterol E-Test Wako kit and the Free Cholesterol E-Test Wako kit (Wako Pure Chemical Industries Co., Osaka, Japan), respectively. The level of esterified cholesterol in the liver tissue was estimated as the difference between the total cholesterol level and free cholesterol level in liver. Briefly, lipids were extracted from 500 mg of the liver of a rat with chloroform: methanol (2:1, v/v), according to the method of Folch et al. [28]. After lipid extraction, the

Table 2
Effects of taurine administration and ovariectomy on food intake, body weight gain and food efficiency in rats fed a cholesterol-free diet[a,b]

	Sham[c]		OVX[d]		2-way ANOVA[e]
	C-diet[f]	T-diet[g]	C-diet[f]	T-diet[g]	
Body weight gain (g/28 days)	13 ± 4[b]	8 ± 2[b]	37 ± 6[a]	26 ± 7[a,b]	OVX
Food intake (g/28 days)	359 ± 12[a,b]	341 ± 17[b]	400 ± 13[a]	370 ± 13[a,b]	OVX
Food efficiency[h]	0.037 ± 0.011[b]	0.021 ± 0.004[b]	0.090 ± 0.01[a]	0.066 ± 0.018[a,b]	OVX

[a] Rats underwent ovariectomy (OVX) or a sham operation (sham), followed by 8d-recovery feeding. Then they were given free access to the control diet or 5% taurine diet for 4 wk.
[b] Each value represents the mean ± SEM, n = 6. Values in a row with different superscript letters are significantly different by Tukey's multiple range test.
[c] Sham, sham operated.
[d] OVX, ovariectomized.
[e] Statistical significance of difference among values was analyzed by two-way ANOVA. NS, not significant ($P > 0.05$).
[f] C-diet, control diet, see Table 1.
[g] T-diet, control diet with 5% taurine added.
[h] Expressed as body weight gain per food intake.

volume of the lipid solution was adjusted to 20 ml with the same solution. One mL of this extract was dried under a nitrogen stream, and the residue was mixed with 100 μl of isopropanol containing 100 g Triton X100/L. Twenty μL of this mixture was mixed with 3 ml of aqueous enzyme solution according to the standard procedure of the Cholesterol E-Test Wako kit or the Free Cholesterol E-Test Wako kit (Wako Pure Chemical Industries Co., Osaka, Japan), and the concentration of cholesterol was determined calorimetrically. In our preliminary study, 20 μl of isopropanol containing 100 g Triton X100/L had no effect on the enzymatic reactions.

Bile acids in the feces and in the intestinal content were extracted with a mixture of chloroform: methanol (1:1, v/v) at 70°C for 60 h [34]. The level of bile acids was enzymatically determined by the 3β-hydroxysteroid dehydrogenase assay method of Sheltawy and Losowsky [35] using taurocholic acid as the standard.

Fecal cholesterol and coprostanol were extracted by the method of Terpstra et al. [36], and their levels were determined by gas chromatography. Lyophilized feces (50 mg) were added to a tube containing 700 μl of methanol and 220 μl of sodium hydroxide solution (5 M); 5α-cholestane was used as the internal standard. The mixture was vigorously vortexed and then incubated for 2 h in a shaking water bath of 80°C. Sodium chloride was added after cooling to prevent gel formation, and the neutral sterols were extracted three times with 3 ml of petroleum ether (60−80). The extracts were evaporated to dryness. The sterols were analyzed on a Hewlett-Packard HP 5890 gas chromatograph (Palo Alto, CA) equipped with a flame-ionization detector and a capillary column (DB-1, 30 m, 0.53 mm i.d., 0.3 μm film thickness, J&W scientific, Folsom, CA). A 1 μl sample dissolved in 1 ml of hexane was injected directly into the column. The column temperature was 260°C. Helium gas was used as the carrier at pressure of 138 kPa.

2.5. Statistical analysis

Differences attributable to taurine administration (dietary effect), OVX (operation effect), and their interaction were determined by two-way ANOVA using Super ANOVA (Abacus Concepts Inc., Berkeley, CA). Tukey's multiple range test was used to assess significant differences between variables using Super ANOVA (Abacus Concepts Inc., Berkeley, CA).

3. Results

3.1. Growth and body components

Table 2 shows the body weight gain, food intake and food efficiency of OVX-rats and sham-rats that had been fed the C- or T-diets for 4 weeks. The body weight gain, food intake and food efficiency of OVX-rats were significantly higher than the respective parameter of the sham-rats (2-way ANOVA; operation effect; $p < 0.05$, $p < 0.05$, $p < 0.05$). Taurine administration did not significantly affect these parameters. The level of total lipids and the total lipids/total protein ratio in the body composition of rats fed taurine were significantly higher than the respective level in the rats fed the control diet regardless of whether the rat had undergone OVX (dietary effect; $p < 0.05$, $p < 0.05$) (Table 3).

3.2. Distribution of serum lipids and apolipoprotein among lipoprotein fractions

Table 4 shows the distribution of serum lipids among the lipoprotein fractions of OVX-rats and sham-rats that had been fed the C- or T-diet for 4 weeks. The serum total cholesterol concentration of the rats fed taurine was significantly lower than that of rats fed the control diet, and this

Table 3
Effects of taurine administration and ovariectomy on the body components of rat fed a cholesterol-free diet[a,b]

	Sham[c]		OVX[d]		2-way ANOVA[e]
	C-diet[f]	T-diet[g]	C-diet[f]	T-diet[g]	
Total proteins (g/carcass)	46.1 ± 2.6	45.0 ± 1.3	45.7 ± 1.3	46.2 ± 1.4	NS
Total lipids (g/carcass)	58.2 ± 4.2	53.8 ± 5.4	72.0 ± 4.9	54.3 ± 3.5	T
Total lipids/total proteins	1.27 ± 0.08	1.19 ± 0.09	1.58 ± 0.10	1.19 ± 0.10	T

[a] Rats underwent ovariectomy (OVX) or a sham operation (sham) followed by 8d-recovery feeding. Then, they were given free access to the control diet or 5% taurine diet for 4 wk.
[b] Each value represents the mean ± SEM, n = 6. Values in a row with different superscript letters are significantly different by Tukey's multiple range test.
[c] Sham, sham operated.
[d] OVX, ovariectomized.
[e] Statistical significance of difference among values was analyzed by two-way ANOVA. NS, not significant ($P > 0.05$).
[f] C-diet, control diet, see Table 1.
[g] T-diet, control diet with 5% taurine added.

was mainly due to a significant reduction in the LDL cholesterol concentration (dietary effect; $p < 0.05$). Taurine induced significant reductions in the total and LDL cholesterol concentrations only in the OVX-rats (Tukey's multiple range test ($p < 0.05$, $p < 0.05$)), although the interaction between the dietary and operation effects was not significant by 2-way-ANOVA (total cholesterol: interaction; $p = 0.345031$, LDL cholesterol: interaction; $p = 0.198136$). The serum total phospholipids concentration of the rats fed taurine was significantly lower than that of rats fed the control diet (dietary effect; $p < 0.05$), and this was due mainly to a significant reduction in the LDL phospholipid concentration and partly to a reduction in the VLDL phospholipids concentration. Taurine administration did not affect the total triglycerides concentration, although the VLDL triglycerides concentration of the rats fed taurine was significantly lower than that of rats fed the control diet (dietary effect; $p < 0.05$). OVX significantly increased the total, LDL and

Table 4
Effects of taurine administration and ovariectomy on the distribution of cholesterol, triacylglycerols and phospholipids in the lipoprotein fractions in aged rats fed a cholesterol-free semi-purified diet[a,b]

	Sham[c]		OVX[d]		2-way ANOVA[e]
	C-diet[f]	T-diet[g]	C-diet[f]	T-diet[g]	
Cholesterol					
			mM		
Total	3.49 ± 0.21[b]	3.09 ± 0.18[b]	4.41 ± 0.31[a]	3.61 ± 0.15[b]	T, OVX
VLDL	0.16 ± 0.02	0.13 ± 0.01	0.16 ± 0.02	0.13 ± 0.02	NS
LDL	1.35 ± 0.14[b]	1.19 ± 0.12[b]	1.99 ± 0.20[a]	1.49 ± 0.07[b]	T, OVX
HDL	1.34 ± 0.07	1.33 ± 0.05	1.56 ± 0.10	1.48 ± 0.09	OVX
Triacylglycerols					
Total	2.14 ± 0.52	1.76 ± 0.24	2.21 ± 0.46	1.38 ± 0.07	NS
VLDL	1.45 ± 0.34	1.07 ± 0.12	1.50 ± 0.26	0.81 ± 0.07	T
LDL	0.34 ± 0.08	0.36 ± 0.06	0.36 ± 0.06	0.27 ± 0.02	NS
HDL	0.06 ± 0.00	0.06 ± 0.01	0.07 ± 0.01	0.08 ± 0.01	OVX
Phospholipids					
Total	3.78 ± 0.18[a,b]	3.53 ± 0.25[b]	4.52 ± 0.29[a]	3.55 ± 0.23[b]	T
VLDL	0.25 ± 0.06[a]	0.16 ± 0.02[a,b]	0.21 ± 0.04[a,b]	0.11 ± 0.02[b]	T
LDL	1.04 ± 0.05[a]	0.89 ± 0.09[a]	1.46 ± 0.12[b]	1.02 ± 0.06[a,b]	T, OVX
HDL	1.31 ± 0.08	1.26 ± 0.05	1.37 ± 0.09	1.30 ± 0.06	NS
LP Free	0.88 ± 0.04	0.84 ± 0.05	1.00 ± 0.06	0.84 ± 0.04	OVX

[a] Rats underwent ovariectomy (OVX) or a sham operation (sham) followed by 8d-recovery feeding. Then, they were given free access to the control diet or 5% taurine diet for 4 wk.
[b] Each value represents the mean ± SEM, n = 6. Values in a row with different superscript letters are significantly different by Tukey's multiple range test.
[c] Sham, sham operated.
[d] OVX, ovariectomized.
[e] Statistical significance of difference among values was analyzed by two-way ANOVA. NS, not significant ($P > 0.05$).
[f] C-diet, control diet, see Table 1.
[g] T-diet, control diet with 5% taurine added.

Table 5
Effects of taurine administration and ovariectomy on serum apoproteins in aged rats fed a cholesterol-free semi-purified diet[a,b]

	Sham[c]		OVX[d]		2-way ANOVA[e]
	C-diet[f]	T-diet[g]	C-diet[f]	T-diet[g]	
			mg/L		
Apo A-I	816 ± 42	821 ± 27	887 ± 55	793 ± 38	NS
Apo A-IV	266 ± 69	289 ± 20	291 ± 45	291 ± 21	NS
Apo B	58 ± 2[a]	44 ± 3[b]	60 ± 3[a]	56 ± 2[a]	T, OVX
Total Apo E	753 ± 28[a,b]	665 ± 33[b]	818 ± 23[a]	672 ± 18[b]	T
Apo E-VLDL	55 ± 6[a]	25 ± 4[c]	43 ± 5[a,b]	34 ± 4[bc]	T, T × OVX
Apo E-HDL	643 ± 21	592 ± 47	717 ± 32	605 ± 19	T
LDL-cholesterol/Apo B	0.022 ± 0.002[b]	0.019 ± 0.002[b]	0.032 ±0.003[a]	0.024 ± 0.001[b]	T, OVX

[a] Rats underwent ovariectomy (OVX) or a sham operation (sham) before followed by 8d-recovery feeding. Then they and given free access to the control diet or 5% taurine diet for 4 wk.
[b] Each value represents the mean ± SEM, n = 6. Values in a row with different superscript letters are significantly different by Tukey's multiple range test.
[c] Sham, sham operated.
[d] OVX, ovariectomized.
[e] Statistical significance of difference among values was analyzed by two-way ANOVA. NS, not significant (P > 0.05).
[f] C-diet, control diet, see Table 1.
[g] T-diet, control diet with 5% taurine added.

HDL-cholesterol levels (operation effect; $p < 0.05$, $p < 0.05$, $p < 0.05$), the HDL-triglycerides level (operation effect; $p < 0.05$), and the LDL-phospholipids level (operation effect; $p < 0.05$).

Table 5 shows the levels of serum apolipoproteins in OVX-rats and sham-rats that had been fed the C- or T-diet for 4 weeks. Taurine administration significantly reduced the Apo E-VLDL concentration only in sham-rats (interaction; $p < 0.05$, Tukey's multiple range test; $p < 0.05$). Taurine administration also significantly reduced the Apo B concentration only in the sham-rats (Tukey's multiple range test; $p < 0.05$), although the interaction between the dietary and operation effects was not significant (interaction; $p = 0.068243$). In contrast, taurine administration significantly lowered the Apo E concentration only in the OVX-rats (Tukey's multiple range test; $p < 0.05$), although the interaction between the dietary and operation effects was not significant (interaction; $p = 0.268947$). The serum Apo E-HDL concentration of rats fed taurine was significantly lower than that of rats fed the control diet (dietary effect; $p < 0.05$). Taurine administration significantly reduced the ratio of the serum LDL cholesterol concentration to serum Apo B concentration (LDL-cholesterol/Apo B) only in OVX-rats (Tukey's multiple range test; $p < 0.05$), though the interaction between the dietary and operation effects was not significant (interaction; $p = 0.198136$).

3.3. Liver lipids and hepatic 7α-hydroxylase activity

The level of liver lipids and hepatic cholesterol 7α-hydroxylase activity in OVX- and sham-rats that had been fed the C- or T-diet for 4 weeks are shown in Table 6. Taurine administration and OVX did not affect the liver weight. The level of esterified cholesterol in the liver was significantly lower and the level of hepatic cholesterol 7α-hydroxylase activity was significantly higher in the rats fed taurine in comparison with the respective parameter in rats fed the control diet (dietary effect; $p < 0.05$, $P < 0.05$). The levels of total lipids, free cholesterol and triglycerides in the liver tissue of OVX-rats were significantly higher than the respective level in the liver of the sham-rats (operation effect; $p < 0.05$, $p < 0.05$, $p < 0.05$).

3.4. Feacal and intestinal steroids

Table 7 shows the levels of steroids in the feces and intestinal contents of OVX-rats and sham-rats that had been fed the C- or T-diet for 4 weeks. The total bile acids concentration in the feces and intestinal contents of rats fed taurine were significantly higher than the respective level in rats fed control diet whether or not the rat had undergone OVX (dietary effect; $p < 0.05$). Neither taurine administration nor OVX affected the cholesterol + coprostanol concentration and coprostanol/cholesterol ratio in the feces.

4. Discussion

In the present study, the hypercholesterolemia in the OVX-rats was mainly due to the increase in the LDL-cholesterol concentration (Table 4). In the present study, taurine administration raised the hepatic cholesterol 7α-hydroxylase activity as well as the levels of intestinal and fecal bile acids regardless of whether the rat had undergone OVX (Tables 6 and 7), but taurine significantly reduced the total and LDL-cholesterol concentration only in the OVX-rats (Tukey's multiple range test ($p < 0.05$)), and the interaction between the dietary and operation effects was not

Table 6
Effects of taurine administration and ovariectomy on the liver of aged rats fed a cholesterol-free semi-purified diet[a,b]

	Sham[c]		OVX[d]		2-way ANOVA[e]
	C-diet[f]	T-diet[g]	C-diet[f]	T-diet[g]	
Liver, wet weight (g)	8.5 ± 0.5	8.3 ± 0.4	8.8 ± 0.7	9.0 ± 0.3	NS
Total lipids (mg/Liver)	674 ± 67	779 ± 106	1077 ± 221	1135 ± 180	OVX
Cholesterol (μmol/Liver)					
Free	41.2 ± 2.8	43.4 ± 2.5	60.1 ± 9.9	55.5 ± 6.7	OVX
Ester	8.2 ± 0.7	7.2 ± 0.8	12.4 ± 2.2	7.4 ± 1.3	T
Triglycerides (μmol/Liver)	465 ± 95	583 ± 134	990 ± 278	1044 ± 234	OVX
Phospholipids (μmol/Liver)	188 ± 10	190 ± 7	193 ± 14	194 ± 4	NS
Cholesterol 7α activity (pmol/mg protein/min)	19.1 ± 2.2[a,b]	32.5 ± 5.8[a]	13.7 ± 1.0[b]	25.3 ± 3.3[a,b]	T

[a] Rats underwent ovariectomy (OVX) or a sham operation (sham) followed by 8d-recovery feeding. Then, they were given free access to the control diet or 5% taurine diet for 4 wk.
[b] Each value represents the mean ± SEM, n = 6. Values in a row with different superscript letters are significantly different by Tukey's multiple range test.
[c] Sham, sham operated.
[d] OVX, ovariectomized.
[e] Statistical significance of difference among values was analyzed by two-way ANOVA. NS, not significant ($P > 0.05$).
[f] C-diet, control diet, see Table 1.
[g] T-diet, control diet with 5% taurine added.

significant by 2-way ANOVA (total cholesterol: interaction, $p = 0.345031$; LDL-cholesterol: interaction, $p = 0.198136$, Table 4). In the present study, the serum LDL-cholesterol concentration were each negatively correlated with the hepatic cholesterol 7α-hydroxylase activity only in the OVX-rats (Fig. 1, B). These data may indicate that the serum LDL-cholesterol concentration of the OVX-rats was decreased due to compensating the shortage of cholesterol caused by rising bile acids synthesis. In the present study, OVX significantly increased the LDL-cholesterol/Apo B level and taurine administration inhibited this OVX-induced increase (Table 5). In the sham-rats, taurine administration did not significantly affect the serum LDL-cholesterol/Apo B level (Table 5). This indicates that the LDL in OVX-rats carries an abnormally level of cholesterol, and that taurine normalizes the cholesterol load. Kanazawa and Oike [37] reported that the LDL-cholesterol/Apo B in patients with ischemic heart disease was higher than that in healthy persons. Under normal conditions of serum cholesterol concentrations, a loss of the free cholesterol pool, such as when the consumption of the cholesterol pool increases due to acceleration of bile acids synthesis and excretion, may be compensated by accelerating the synthesis of cholesterol. The compensation of shortage of cholesterol by serum LDL-cholesterol may be specific to hypercholesterolemic situations. In the present study, the hepatic HMG-CoA reductase

Table 7
Effects of taurine administration and ovariectomy on fecal steroids and intestinal contents in aged rats fed a cholesterol-free semi-purified diet[a,b]

	Sham[c]		OVX[d]		2-way ANOVA[e]
	C-diet[f]	T-diet[g]	C-diet[f]	T-diet[g]	
Feces					
Total bile acid (μmol/d)	16 ± 1[b,c]	22 ± 2[a]	15 ± 1[c]	21 ± 2[a,b]	T
Cholesterol + Coprostanol (μmol/d)	6.43 ± 0.33	6.86 ± 0.43	7.73 ± 0.57	7.88 ± 0.47	NS
Coprostanol/Cholesterol	2.25 ± 0.88	1.74 ± 0.74	2.90 ± 0.52	0.93 ± 0.43	NS
Intestinal contents					
Total bile acid (μmol/intestinal contents)	126 ± 8	143 ± 12	127 ± 6	150 ± 6	T

[a] Rats underwent ovariectomy (OVX) or a sham operation (sham) followed by 8d-recovery feeding. Then, they were given free access to the control diet or 5% taurine diet for 4 wk.
[b] Each value represents the mean ± SEM, n = 6. Values in a row with different superscript letters are significantly different by Tukey's multiple range test.
[c] Sham, sham operated.
[d] OVX, ovariectomized.
[e] Statistical significance of difference among values were analyzed two-way ANOVA. NS, not significant ($P > 0.05$).
[f] C-diet, control diet, see Table 1.
[g] T-diet, control diet with 5% taurine added.

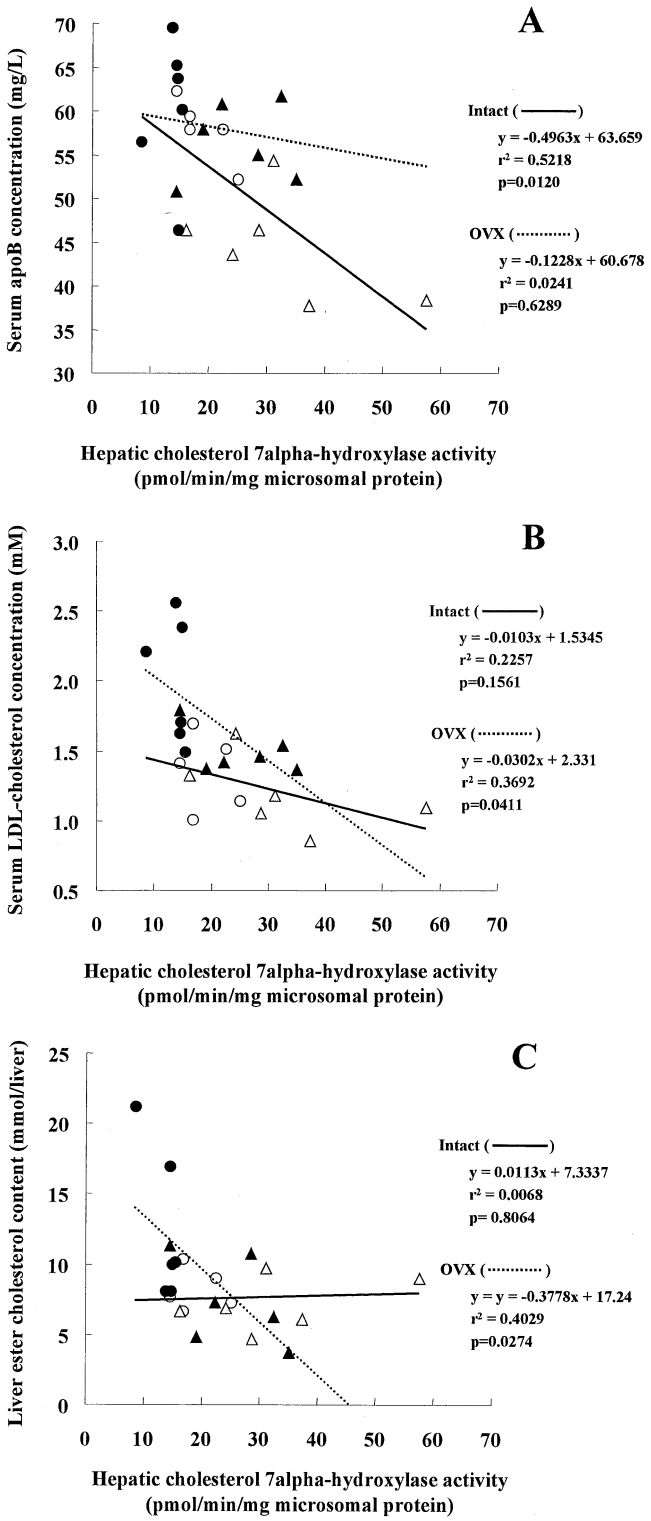

Fig. 1. Relationship between the serum Apo B concentration (A), serum LDL-cholesterol concentration (B) or liver ester cholesterol content (C) and the level of cholesterol 7α-hydroxylase activity in sham-rats fed the diet with 5% taurine (Δ) or control diet (○), and in OVX-rats fed the diet with 5% taurine (▲) or control diet (●) for 4 weeks. The data from the sham-rats and OVX-rats were analyzed separately.

activity was not determined, but Yamori and colleagues [22] reported that taurine administration decreased serum cholesterol concentration and increased bile acids synthesis and excretion but had no effect on hepatic HMG-CoA reductase activity in SHR-SP rats fed a cholesterol diet. A high cholesterol diet feeding causes increase of serum LDL-cholesterol concentration as well as OVX. They also reported that taurine administration increased bile acids synthesis and excretion, and increased HMG-CoA reductase activity, but did not affect serum cholesterol concentration in SHR-SP rats fed a cholesterol-free diet [22]. Bellentani et al. [38] reported that taurine administration increased HMG-CoA reductase activity in normal rats.

The reason that taurine increases the cholesterol 7α-hydroxylase activity is unclear. Stephan et al. [40] reported that taurine administration increased the level of cysteine, the precursor of taurine, and that is raised by taurine administration and cysteine administration raised the level of cholesterol 7α-hydroxylase activity. They hypothesized that cysteine was essential agent which caused increase of cholesterol 7α-hydroxylase activity.

OVX did not raised the serum VLDL-cholesterol concentration (Table 4). Colvin et al. [39] reported that bile acids synthesis was lowered by ovariectomy and that it recovered by administration of several estrogens in monkeys fed a moderately atherogenic diet for 12 weeks. An increased amount of VLDL-cholesterol may be released to the blood when a normal level of bile acids excretion can not be maintained as a result of OVX, i.e., estrogen deficiency. However, in the present study, there were no significant differences in the level of 7α-hydroxylase activity (Table 6) nor bile acids excretion between the Sham- and OVX-rats (Table 7). In the present study, there were no significant interaction between taurine administration and OVX by two-way ANOVA in the hepatic esterified cholesterol concentration (Table 6), but the hepatic esterified cholesterol concentration were each negatively correlated with the hepatic cholesterol 7α-hydroxylase activity only in the OVX-rats (Fig. 1, C). This significant correlation specifically shown in OVX-rats can not be accounted that the shortage of cholesterol caused by accelerating bile acids synthesis was compensated directly by the hepatic esterified cholesterol only in OVX-rats, because the hepatic esterified cholesterol not available for bile acids synthesis and the newly synthesized cholesterol is major substrate of hepatic cholesterol 7α-hydroxylase. The hepatic esterified cholesterol is mainly secreted to the blood as a major component of VLDL. Secretion of VLDL is known to be parallel with hepatic esterified cholesterol concentration. Most part of VLDL is converted to LDL in blood. We suppose that this significant correlation specifically shown in OVX-rats indicates that the esterified cholesterol synthesis and secretion of VLDL was decreased to compensate the shortage of hepatic free cholesterol caused by accelerating bile acids synthesis in OVX-rats. Stephan et al. [40] reported that taurine administration first raises the level of hepatic cho-

lesterol 7α-hydroxylase and then activate LDL-receptors in culture of Hep-G2 cell. LDL-cholesterol mainly delivers to liver as esterified cholesterol through LDL receptor. Increase of LDL clearance may contradict to decrease of esterified cholesterol in OVX-rats fed taurine administration. But LDL clearance is known to be closely correlated with serum LDL cholesterol concentration in vivo. In our previous study, the serum cholesterol concentration of OVX-rats decreased to plateau values until 7 days after taurine administration. In present experiment of 28 days, the LDL clearance may conform to low serum cholesterol concentration though the LDL clearance might be activated by taurine in the earlier period.

In the present study, taurine administration reduced the concentration of VLDL-triglycerides and concentration and phospholipids in the lipoprotein fractions (Table 4). These may reflect a reduction in the flux of LDL and VLDL caused by taurine administration as described above.

In the present study, taurine administration significantly lowered serum Apo B concentration and serum apo E-VLDL concentration in sham-rats but did not affect in the OVX-rats by Tukey's multiple range test though about serum Apo B concentration there was no significant interaction between taurine administration and OVX by 2-way ANOVA (Table 5). Apo B is secreted from liver responding situation of triacylglycerol, cholesterol ester and phospholipids in liver [41]. Apo B secretion from Hep-G2 cell was decreased responding loss of esterified cholesterol synthesis [42], and similar data was reported in experiment using rabbits hepatocites [43]. In the present study, taurine administration significantly lowered the hepatic esterified cholesterol concentration regardless of whether the rat had undergone OVX, and the fact that taurine administration did not significantly affect the hepatic triglycerides concentration (Table 6). The results that had been obtained using the Hep G2 cells and rabbit hepatocites, were reflected in the Sham-rats but not in the OVX-rats in the present experiment. OVX may disorder the response of the Apo B secretion to the hepatic esterified cholesterol. The serum Apo B concentration negatively correlated with the level of hepatic cholesterol 7 alpha-hydroxylase activity only in the sham-rats (Fig. 1, A). The Apo B synthesis and secretion was increased responding to the increase of gene expression of hepatic cholesterol 7α-hydroxylase in transgenic mice expressing cholesterol-7α-hydroxylase [44,45]. The coordinate expression of cholesterol biosynthetic/catabolic enzymes and hepatic VLDL assembly/secretion are mediated at least in part through the sterol response element binding protein (SREBP) transcription factor family [44,45,46] but concerning about OVX-rat, more explanation may be needed.

In the present study, we ascertained that taurine administration decreased the serum LDL cholesterol concentration specifically in the OVX-rats, nevertheless taurine administration increase the bile acids synthesis and excretion in rats whether or not had undergone OVX, and the heapatic cholesterol 7α-hydroxylase activity negatively correlated with the serum LDL cholesterol concentration and the level of esterified cholesterol in the liver specifically in OVX-rats. It is possible that the increase of cholesterol consumption in bile acid synthesis is compensated by sparing cholesterol in circulation in OVX-rats, while by increasing cholesterol synthesis in sham-rat.

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