Original Article

Dietary Calcium Decreases Plasma Cholesterol Level only in Female but not in Male Hamster Fed a High Cholesterol Diet *

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Abstract

Objective To investigate the effect of dietary calcium on plasma lipoprotein profile in castrated and ovariectomized hamsters.

Methods Male, castrated, female and ovariectomized hamsters (*n*=36 each group) were randomly divided into three sub-groups (*n*=12) and fed one of the three diets containing 0, 2, and 8 g calcium per kg diet for a period of six weeks. Changes in plasma lipoprotein profile were monitored at the end of week 0, 3 and 6.

Results Plasma total cholesterol (TC), non-high density lipoprotein cholesterol (non-HDL-C), triacylglycerols (TG) and TC/HDL-C were decreased only in intact female and ovariectomized hamsters. In contrast, three levels of dietary calcium had no effect on lipoprotein profiles in both intact male and castrated hamsters.

Conclusion Beneficial modification of lipoprotein profile by dietary calcium was gender-dependent at least in hamsters.

Key words: Calcium; Cholesterol; Triacylglycerols; Fecal sterols

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INTRODUCTION

Dietary calcium supplementation has been shown to decrease plasma total cholesterol (TC) in rabbits[1], hamsters[2], rats[3-4], and pigs[5], and in humans[6]. In menopausal women, calcium supplementation not only decreased low-density lipoprotein cholesterol (LDL-C) but also increased the high-density lipoprotein cholesterol (HDL-C) level[7]. Dietary calcium appeared to be more effective in reducing plasma TC in women than in men[6-7]. We have previously investigated the effect of dietary calcium on plasma TC in ovariectomized hamsters and, findings showed that the plasma TC level was dose-dependently decreased with increase of the calcium level in diet[8].

Benefits associated with calcium supplementation have been clearly demonstrated in menopausal women given a calcium supplement[6-7]. However, the studies on supplementation of calcium in

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andropausal men and male animals are lacking. And the objective of the present study was therefore to adopt castrated hamsters as a model of andropausal men to investigate the effect of dietary calcium on plasma TC compared with of the same effect on ovariectomized hamsters.

MATERIALS AND METHODS

Diets

Diet ingredients were commercially obtained from Harlan Teklad (Madison, WI, USA) except for lard, which was purchased from a local market. Cholesterol, DL-methionine, cholesterol and calcium dibasic phosphate were obtained from Sigma Chemical (St. Lois, MO, USA). Three diets were prepared. The first diet contained no added calcium (Ca-0), which was a mixture of the following ingredients (g/kg diet): cornstarch, 508; casein, 242; lard, 50; sucrose, 119; calcium-deficient mineral mix, 40; vitamin mix, 20; DL-methionine, 1; cholesterol, 1. The two experimental diets were similarly prepared by adding 5 grams of $CaHPO_4$ (equivalent to 2 g Ca/kg diet; Ca-2), and 20 grams of $CaHPO_4$ (equivalent to 8 g Ca/kg diet; Ca-8), respectively, into Ca-0 diet. Each diet was mixed with a gelatin solution (20 g/L) in a ratio of 200 g diet per liter and then was cut into pieces of approximately 10 g cubes and stored and frozen at -20 °C.

Hamsters

Male, castrated, female and ovariectomized Golden Syrian hamsters (*n*=36 each group) were obtained from the Laboratory Animal Services Centre, The Chinese University of Hong Kong. Each group was randomly divided into three sub-groups (*n*=12) fed one of the three diets namely Ca-0, Ca-2, and Ca-8, respectively. Two hamsters were housed per cage in wire-bottomed cages at 23 °C in an animal room with 12-hour light-dark cycle. All hamsters were acclimatized to the Ca-8 diet for a period of 2 weeks and subsequently maintained on their respective diets for additional six weeks. Diets and water were available *ad libitum*, with any uneaten food being weighed and replaced with fresh food daily. Body weights were measured and the fecal samples per cage were collected weekly. Blood (about 0.5 mL) was collected from the retro-orbital sinus into a heparinized capillary tube at the end of week 0, 3, and 6 following food deprivation for 14 h over night and light anaesthesia, by using a mixture of ketamine, xylazine and saline (v/v/v; 4:1:5). The blood was centrifuged at 1000 g for 10 min, and the plasma was collected and stored at -20 °C until analysis. At the end of week 6, all hamsters were killed by carbon dioxide suffocation. The livers were removed, washed in saline, and weighed. All tissue samples were flash frozen in liquid nitrogen and stored at -80 °C until analysis. The protocols were approved and conducted in accordance with the guidelines set by the Animal Experimental Ethical Committee, The Chinese University of Hong Kong.

Plasma Lipoproteins

Plasma TC and total triacylglycerols (TG) were measured by using the enzymatic kits from Infinity (Waltham, MA, USA.) and Stanbio Laboratories (Boerne, TX, USA), respectively. Before HDL-C was measured, LDL and very low-density lipoprotein (VLDL) were first precipitated with phosphotungstic acid and magnesium chloride by using a commercial kit (Stanbio Laboratories). HDL-C in the supernatant was determined similarly as it was for TC. LDL-C (Strictly speaking Non-HDL-C) was calculated by deducting HDL from TC.

Calcium in Plasma and Diet

The AOAC Official Method 968.08 (AOAC International, 1995) was used to measure calcium in diet[8]. After the sample was heated in a furnace at 550 °C for 4 hr and cooled at room temperature, it was dissolved in 10 mL of 3 mol/L HCl solution followed by first being boiled for 10 min and then cooling and being diluted to a volume of 100 mL. The measurement solution was then prepared by two-fold dilution with addition of 1% lanthanum oxide (AAS grade, Sigma) to reduce phosphate interference. The solution was quantified in a Varian Spectra-800 atomic absorption spectrophotometer with the standard solution of calcium (Sigma) as a reference. Instrumental conditions were set at a pump flow rate, 3.0 mL/min; wavelength, 422.7 nm; lamp current, 10 mA; slit width, 0.5 nm; and air/C_2H_2 flow ratio, 3 to 1. Plasma calcium concentration was measured by using a commercial kit (Sigma).

Liver Cholesterol

Hepatic cholesterol concentration was quantified as we previously described[9-10]. Stigmastanol was used as an internal standard, and methanol-chloroform mixture (2:1, v/v) was used to extract total liver lipids. The liver lipids were then mildly saponified and the cholesterol was converted into its trimethylsilyl-ether (TMS) derivative before the GC analysis.

Fecal Neutral and Acidic Sterols

Fecal total sterols in the feces were analyzed as we previously described[9-10]. Stigmastanol and hyodeoxycholic acid were added into the fecal samples as internal standards for quantification of fecal neutral and acidic sterols, respectively. After being mildly hydrolyzed, the fecal neutral sterols were extracted into cyclohexane and were converted into their TMS derivatives. The acid sterols in the bottom aqueous layer were saponified, extracted and converted into their TMS derivatives. The analyses of individual neutral and acidic sterol TMS derivatives were performed in a fused silica capillary column (SAC-5, 30 m ×0.25 mm, i.d.; Supelco Bellefonte, PA, USA) by using a Shimadzu GC-14 B Gas-Liquid Chromatograph equipped with a flame ionization detector (Kyoto, Japan).

Statistics

The two-way analysis of variance (ANOVA) followed by post hoc LSD test on SigmaStat Advisory Statistical Software (SigmaStat Version 14.0, SPSS Inc., Chicago, USA) was used for statistical analyses. Significance was defined as *P*-value less than 0.05.

RESULTS

Food Intake, Body, and Organ Weight

Calcium had no significant effect on the final body weights of intact female, intact male, ovariectomized and castrated hamsters within each group of (Table 1). Similarly, no significant effect of dietary calcium on food intakes was seen among each group.

Table 1. Food Intake and Body Weights of Hamsters Fed Diets Containing no Added Calcium (Ca-0) and Two Experimental Diets Supplemented with 2 g (Ca-2), and 8 g (Ca-8) Ca Per kg Diet

Gender	Intact				Ovariectomized			
	Ca-0	Ca-2	Ca-8	*P* Value	Ca-0	Ca-2	Ca-8	*P* Value
Female								
Food intake (g/day)	11.83±2.53	12.70±8.66	11.81±10.08	0.5	12.33±1.03	12.72±0.93	11.78±1.56	0.42
Initial body weight (g)	101.36±9.24	106.36±10.27	104.55±9.07	0.47	120.80±11.65	127.50±9.41	125.40±14.69	0.4
Final body weight (g)	136.36±12.67	137.73±14.55	125.91±15.78	0.13	135.00±10.66	140.20±12.22	133.60±12.27	0.12
Male								
Food intake (g/day)	10.53±1.04	10.75±1.17	10.68±1.24	0.93	9.82±15.37	10.37±13.44	10.55±14.15	0.64
Initial body weight (g)	112.70±7.20	114.10±5.39	115.50±6.11	0.6	128.00±8.41	128.00±10.82	130.00±10.00	0.81
Final body weight (g)	125.00±8.37	128.20±13.47	127.00±13.58	0.82	127.73±8.61	134.93±12.74	137.87±16.43	0.25

Note. ANOVA was used to detect the dose effect of Ca on food intake and body weights. Significance was defined as *P*-value less than 0.05.

Quantification of Calcium in Diet and Plasma

The actual calcium concentration in diets of Ca-0, Ca-2, and Ca-8 groups was 0.4, 2.3, and 8.3 g/kg, respectively. With regard to the intact female hamsters, plasma calcium concentrations at the end of Week 6 increased with increase of the dietary calcium level (range: 12.6-15.3 mg/dL) (Table 2). Similarly, plasma calcium in the ovariectomized hamsters increased from 12.9 to 15.1 mg/dL with the increasing calcium in diet (Table 2). In the intact male hamsters, the plasma calcium concentration increased from 9.6 to 13.4 mg/dL while in the castrated hamsters, it increased from 13.4 to 16.5 mg/dL in response to the increasing levels of calcium in diet (Table 3).

Plasma TC, HDL-C, TG, and LDL-C/HDL-C in Ovariectomized Hamsters

Effect of dietary calcium on lipoprotein profile was different in female hamsters versus male hamsters. In intact female and ovariectomized hamsters, lipoprotein profile was similar at the beginning of week 1. At the end of Week 6, plasma TC, LDL-C, TG, and TC/HDL-C were decreased while HDL-C was increased in a dose-dependent manner (Table 2). In contrast, three levels of dietary calcium had no effect on lipoprotein profiles in both intact male and castrated hamsters (Table 3).

Table 2. Change in Plasma Total Cholesterol (TC), Total Triacylglycerols (TG), High-density Lipoprotein Cholesterol (HDL-C), Low-density Lipoprotein Cholesterol (LDL-C) and the Ratio of TC to HDL-C in Female and Ovariectomized Hamsters Fed Diets Containing no Added Calcium (Ca-0) and Two Experimental Diets Supplemented with 2 g (Ca-2), and 8 g (Ca-8) Ca Per kg Diet

	Intact				Ovariectomized			
	Ca-0	Ca-2	Ca-8	*P* Value	Ca-0	Ca-2	Ca-8	*P* Value
Week 0								
TC	157.84±37.05	158.91±33.85	158.30±19.83	1	223.59±34.53	224.88±52.59	222.76±38.06	0.99
TG	94.62±44.88	92.73±37.79	109.86±36.50	0.55	82.45±26.96	82.45±44.16	78.53±29.92	0.95
HDL-C	79.17±12.15	80.64±10.44	76.72±8.52	0.68	120.50±20.74	121.94±11.12	120.21±17.72	0.97
LDL-C	78.67±28.11	78.27±29.92	81.57±17.30	0.95	103.10±18.75	104.07±50.46	102.55±24.71	0.99
TC:HDL-C	1.98±0.32	1.98±0.39	2.07±0.25	0.74	1.86±0.14	1.85±0.40	1.85±0.16	0.99
Week 3								
TC	231.50±48.59	210.91±19.39	202.41±25.82	0.15	241.22±18.71^{a}	238.39±21.08^{ab}	221.27±25.01^{b}	0.08
TG	147.58±40.39^{a}	129.55±32.73^{ab}	99.91±18.42^{b}	0.01	115.00±21.28	124.39±34.46	101.88±31.90	0.2
HDL-C	78.24±15.02	80.29±12.77	84.87±7.48	0.44	84.03±10.31	89.67±11.91	90.66±9.04	0.28
LDL-C	152.35±41.24^{a}	130.62±22.98^{ab}	117.53±22.96^{b}	0.04	156.39±16.64^{a}	149.36±17.52^{a}	131.86±23.03^{b}	0.02
TC:HDL-C	2.95±0.47^{a}	2.68±0.43^{ab}	2.39±0.27^{b}	0.01	2.88±0.29^{a}	2.69±0.29^{ab}	2.48±0.26^{b}	0.01
Week 6								
TC	236.65±43.34^{a}	205.19±30.33^{ab}	188.82±25.12^{b}	0.01	241.42±26.69^{a}	231.62±20.68^{ab}	205.74±31.43^{b}	0.03
TG	120.26±27.58^{a}	105.32±23.55^{ab}	93.86±14.15^{b}	0.04	140.00±33.10	130.13±33.54	109.22±36.11	0.15
HDL-C	89.03±6.04^{b}	88.56±6.20^{b}	96.77±5.50^{a}	<0.01	99.60±13.43^{a}	100.64±10.02^{ab}	110.35±7.92^{b}	0.07
LDL-C	146.85±41.58^{a}	116.62±31.17^{b}	99.86±23.21^{b}	0.01	142.22±33.92^{b}	130.98±17.13^{ab}	102.69±32.10^{a}	0.02
TC:HDL-C	2.64±0.44^{a}	2.33±0.39^{ab}	2.05±0.25^{b}	0.01	2.48±0.53^{a}	2.31±0.21^{ab}	1.91±0.28^{b}	0.02
Serum Ca(mg/dL)	12.64±0.90^{c}	13.82±0.75^{b}	15.32±1.17^{a}	<0.01	12.92±0.90^{c}	13.82±0.42^{b}	15.13±0.61^{a}	<0.01
Cholesterol in Lver (mg/g)	33.51±6.55^{a}	26.71±7.63^{b}	25.39±4.43^{b}	0.12	46.82±7.92^{a}	38.75±11.42^{b}	37.16±9.38^{b}	0.13

Note. ANOVA followed by post hoc LSD test was used to detect the significant differences among the treatments. The dose effect of calcium was considered significant if *P* value < 0.05. ^{a,b,c}Means at the same row within the same groups with different superscript letters differ significantly, *P*<0.05. Serum Ca was measured at the end of week 6.

Table 3. Change in Plasma Total Cholesterol (TC), Total Triacylglycerols (TG), High-density Lipoprotein Cholesterol (HDL-C), Low-density Lipoprotein Cholesterol (LDL-C) and the Ratio of TC to HDL-C in Male and Castrated Hamsters Fed Diets Containing no Added Calcium (Ca-0) and Two Experimental Diets Supplemented with 2 g (Ca-2), and 8 g (Ca-8) Ca Per kg Diet

	Intact				Castrated			
	Ca-0	Ca-2	Ca-8	*P* Value	Ca-0	Ca-2	Ca-8	*P* Value
Week 0								
TC	196.14±46.71	199.77±43.50	197.95±35.03	0.98	203.61±30.77	202.12±36.74	202.67±33.60	0.99
TG	155.73±63.43	174.48±55.03	212.98±79.21	0.18	112.85±42.69	113.21±56.80	106.18±51.20	0.91
HDL-C	81.68±14.59	93.95±10.96	94.50±16.85	0.09	114.82±18.28	113.15±15.56	105.79±13.36	0.26
LDL-C	112.26±38.01	147.85±41.12	103.45±23.70	0.84	88.79±21.77	88.97±23.88	96.88±23.69	0.55
TC:HDL-C	2.37±0.37	1.94±0.39	2.11±0.25	0.21	1.79±0.21	1.78±0.15	1.91±0.18	0.1

(Continued)

	Intact				Castrated			
	Ca-0	Ca-2	Ca-8	*P* Value	Ca-0	Ca-2	Ca-8	*P* Value
Week 3								
TC	160.88±19.86	166.53±26.14	178.00±28.20	0.31	205.33±32.57	209.59±29.77	202.86±31.59	0.84
TG	104.38±21.36^{b}	139.31±46.32^{a}	164.02±41.94^{a}	0.01	120.67±28.88	122.06±29.30	122.98±21.68	0.97
HDL-C	78.93±10.79	87.74±12.67	85.20±11.30	0.24	109.96±15.01	112.43±13.75	116.52±11.24	0.43
LDL-C	81.95±9.65^{ab}	75.02±23.38^{b}	92.80±19.13^{a}	0.12	96.47±33.53	95.33±12.18	93.51±18.88	0.95
TC:HDL-C	2.02±0.05^{ab}	1.86±0.21^{b}	2.04±0.16^{a}	0.06	1.93±0.35	1.86±0.13	1.84±0.21	0.63
Week 6								
TC	164.11±23.89	178.94±17.84	179.00±25.88	0.26	197.15±43.38	188.55±18.04	199.45±17.40	0.56
TG	108.60±24.20	100.62±27.43	119.08±33.48	0.36	111.18±22.71	115.74±35.11	118.21±25.49	0.79
HDL-C	49.07±8.29	52.07±13.79	52.87±11.60	0.74	113.12±16.59	116.03±14.55	120.84±18.84	0.45
LDL-C	119.64±24.52	131.88±18.98	126.13±24.99	0.4	73.02±19.80	72.51±17.21	76.22±16.99	0.86
TC:HDL-C	3.48±0.81	3.64±0.75	3.50±0.82	0.63	1.66±0.21	1.61±0.16	1.62±0.19	0.79
Serum Ca mg/dL)	9.56±0.77^{c}	12.28±0.94^{b}	13.37±0.88^{a}	<0.01	13.42±1.60^{c}	15.36±1.01^{b}	16.47±0.90^{a}	<0.01
Cholesterol in Liver (mg/g)	46.82±7.92	38.75±11.42	38.91±11.70	0.20	47.56±7.30	48.87±18.37	45.47±10.50	0.84

Note. ANOVA followed by post hoc LSD test was used to detect the significant differences among the treatments. The dose effect of calcium was considered significant if *P* value < 0.05. ^{a,b,c}Means at the same row within the same groups with different superscript letters differ significantly, *P*<0.05. Serum Ca was measured at the end of week 6.

Cholesterol Balance

Total intake of cholesterol was compared with the excretion of neutral and acidic sterols (Tables 4 and 5). Cholesterol retention was calculated by difference between the intake and excretion of both neutral and acidic sterols. The apparent cholesterol absorption is defined as cholesterol retention/cholesterol intake. Results demonstrated that apparent cholesterol absorption was dose-dependently decreased with the increasing dietary calcium levels only in the ovariectomized hamsters, while the similar decreasing trend was also observed in the intact female hamsters. However, no statistical significance was found (Table 4). In contrast, apparent cholesterol absorption remained unchanged with the increasing dietary calcium levels in both intact male and castrated hamsters (Table 5).

DISCUSSION

Effect of dietary calcium has been the subject of extensive investigations whether past or present. It has been shown that calcium supplementation beneficially models the lipoprotein profile in hamsters[2], rats[3], pigs[5], and humans[7]. One earlier study examined the association between daily calcium intake and plasma lipoprotein profile in 235 men and 235 women and demonstrated an inverse correlation between dietary calcium intake and plasma TC[6]. Reid investigated the effect of calcium citrate supplementation (1 g/day) on fasting serum lipoprotein in menopausal women and found, that calcium supplement increased HDL-C level while it decreased LDL-C:HDL-C ratio[11]. In view of popularity of calcium supplementation among postmenopausal women, we have previously demonstrated that dietary calcium dose-dependently decreased plasma TC, LDL-C, TC/HDL-C, and TG levels whereas it increased dose-dependently plasma HDL-C level in ovariectomized hamsters, a model to study the cholesterol metabolism in postmenopausal women[8]. The present study is the first of its kind to investigate the effect of dietary calcium on lipoprotein profile in intact male and castrated hamsters compared with their corresponding intact female and ovariectomized hamsters. Results from the study clearly demonstrated that plasma TC, TG, and TC/HDL-C were dose-dependently decreased in female and ovariectomized hamsters while they remained unchanged in intact male and castrated hamsters with the increasing dietary calcium in diets, indicating that effect of dietary calcium on plasma lipoprotein profile was gender-dependent.

Table 4. Cholesterol Balance in Female and Ovariectomized Hamsters Fed Diets Containing no Added Calcium (Ca-0) and two Experimental Diets Supplemented with 2 g (Ca-2), and 8 g (Ca-8) Ca per kg Diet

(mg/hamster/day)	Intact				Ovariectomized			
Week 6	Ca-0	Ca-2	Ca-8	*P* Value	Ca-0	Ca-2	Ca-8	*P* Value
Cholesterol Intake (mg)	10.79±0.73	12.42±1.26	11.70±2.28	0.53	12.68±2.31	12.98±0.74	10.98±1.18	0.26
Fecal Neutral Sterols (mg)								
Coprostanol	0.46±0.57	0.73±0.32	1.01±0.44	0.31	0.34±0.28^{b}	1.02±0.42^{ab}	1.34±0.77^{a}	0.08
Coprostanone	0.06±0.02	0.11±0.09	0.08±0.04	0.49	0.07±0.01^{b}	0.12±0.06^{ab}	0.17±0.04^{a}	0.01
Cholesterol	0.89±0.61	0.59±0.25	0.73±0.46	0.74	0.39±0.26^{b}	0.88±0.50^{ab}	1.05±0.24^{a}	0.05
Dihydrocholesterol	0.23±0.13	0.52±0.42	0.53±0.31	0.33	0.20±0.09^{b}	0.31±0.07^{ab}	0.45±0.10^{a}	0.02
Campersterol	0.07±0.02	0.08±0.02	0.07±0.02	0.75	0.06±0.02^{b}	0.11±0.06^{ab}	0.15±0.04^{a}	0.03
Total (mg)	1.70±0.47^{b}	2.03±0.73^{ab}	2.41±0.69^{a}	0.32	1.05±0.48^{b}	2.44±0.75^{a}	3.16±0.69^{a}	0.01
Fecal Acidic Sterols (mg)								
Lithocholic Acid	0.86±0.74	0.49±0.47	1.14±0.58	0.42	0.20±0.10^{b}	0.54±0.18^{ab}	1.00±0.76^{a}	0.12
Deoxycholic Acid	0.21±0.23	0.21±0.18	0.39±0.58	0.79	0.06±0.04^{c}	0.19±0.22^{bc}	0.54±0.24^{a}	0.02
Chenodeoxycholic Acid	0.19±0.09^{b}	0.32±0.19^{ab}	0.91±0.52^{a}	0.07	0.02±0.01^{c}	0.09±0.05^{b}	0.22±0.04^{a}	<0.01
Cholic Acid	0.17±0.09	0.22±0.12	0.49±0.51	0.45	0.12±0.07	0.07±0.02	0.12±0.06	0.55
Ursodeoxycholic Acid	0.17±0.10	0.35±0.24	0.43±0.09	0.14	0.45±0.25	0.57±0.37	0.70±0.35	0.57
Total (mg)	1.60±1.24^{b}	1.59±1.16^{b}	3.37±1.25^{a}	0.15	0.85±0.44^{b}	1.46±0.47^{b}	2.57±0.77^{a}	0.01
Cholesterol Retained (mg)	7.54±1.89^{ab}	8.80±2.98^{a}	5.91±1.82^{b}	0.29	10.78±2.06^{a}	9.08±1.67^{a}	5.25±1.96^{b}	0.01
Apparent Cholesterol Absorption (%)	69.79±15.87^{a}	69.69±18.10^{a}	49.85±6.21^{b}	0.14	85.18±6.27^{a}	69.74±10.36^{a}	47.24±13.41^{b}	<0.01

Note. ANOVA followed by post hoc LSD test was used to detect the significant differences among the treatments. The dose effect of calcium was considered significant if *P*-value < 0.05. ^{a,b,c}Means at the same row within the same groups with different superscript letters differ significantly, *P*<0.05.

Table 5. Cholesterol Balance in Male and Castrated Hamsters Fed Diets Containing no Added Calcium (Ca-0) and Two Experimental Diets Supplemented with 2 g (Ca-2), and 8 g (Ca-8) Ca Per kg Diet

(mg/hamster/day)	Intact				Ovariectomized			
Week 6	Ca-0	Ca-2	Ca-8	*P* Value	Ca-0	Ca-2	Ca-8	*P* Value
Cholesterol Intake (mg)	12.50±0.51	12.14±0.29	11.83±2.76	0.86	9.38±0.52^{b}	10.42±0.71^{a}	10.81±0.84^{a}	0.02
Fecal Neutral Sterols (mg)								
Coprostanol	0.49±0.40	0.57±0.37	0.63±0.47	0.87	0.47±0.21	1.08±0.79	0.80±0.42	0.23
Coprostanone	0.02±0.01	0.02±0.01	0.02±0.01	0.97	0.02±0.01	0.02±0.01	0.02±0.01	0.56
Cholesterol	0.95±0.52	0.89±0.44	0.66±0.33	0.55	0.48±0.24	0.43±0.39	0.60±0.31	0.71
Dihydrocholesterol	0.22±0.07	0.27±0.08	0.21±0.12	0.51	0.20±0.08	0.31±0.20	0.20±0.07	0.36
Campersterol	0.04±0.01	0.06±0.02	0.04±0.02	0.17	0.03±0.01^{b}	0.04±0.01^{ab}	0.07±0.03^{a}	0.09
Total (mg)	1.71±0.57	1.80±0.57	1.56±0.78	0.83	1.20±0.48	1.88±1.16	1.68±0.77	0.46
Fecal Acidic Sterols (mg)								
Lithocholic Acid	0.02±0.01	0.02±0.01	0.02±0.01	0.93	0.02±0.01	0.02±0.01	0.03±0.03	0.67
Deoxycholic Acid	0.78±0.40	1.06±0.24	0.65±0.38	0.29	0.50±0.25	0.44±0.41	0.59±0.27	0.78
Chenodeoxycholic Acid	0.21±0.08	0.28±0.09	0.17±0.09	0.28	0.21±0.10	0.33±0.21	0.24±0.14	0.54
Cholic Acid	0.04±0.02^{b}	0.07±0.00^{a}	0.04±0.02^{ab}	0.06	0.04±0.01^{b}	0.02±0.01^{c}	0.07±0.02^{a}	<0.01
Ursodeoxycholic Acid	0.09±0.06	0.08±0.01	0.10±0.06	0.83	0.04±0.01	0.07±0.04	0.06±0.02	0.14
Total (mg)	1.14±0.49	1.51±0.34	0.99±0.54	0.32	0.80±0.34	0.88±0.56	0.99±0.43	0.79
Cholesterol Retained (mg)	9.79±0.76	9.20±0.27	9.49±2.46	0.9	7.38±0.76	7.65±2.22	8.14±1.32	0.72
Apparent Cholesterol Absorption (%)	78.46±7.15	75.82±2.53	79.54±8.74	0.79	78.74±8.09	72.76±17.94	75.34±10.40	0.77

Note. ANOVA followed by post hoc LSD test was used to detect the significant differences among the treatments. The dose effect of calcium was considered significant if *P*-value < 0.05. ^{a,b,c}Means at the same row within the same groups with different superscript letters differ significantly, *P*<0.05.

The underlying mechanism by which dietary calcium dose-dependently decreased plasma TC only in intact female and ovariectomized hamsters while it had no effect in intact male and female hamsters remains unknown. We speculate that it may be attributed to gender difference in calcium absorption. It has been shown that the calcium absorption in male and female mice is different[12]. The present result was in agreement with that of Cashman & Flynn, who found male rats had a higher calcium absorption from meal than female rats[13]. The present results also demonstrated that the apparent cholesterol absorption decreased with the increasing dietary calcium in diet in intact female and ovariectomized hamsters, leading to a greater excretion of fecal calcium. This greater calcium excretion in intact female and ovariectomized hamsters was accompanied by a greater excretion of fecal bile acids (Table 4) because the unabsorbed calcium in the intestine could bind bile acids to form a precipitate and thus increase the excretion of bile acids[14]. It is known that excessive cholesterol is eliminated via its conversion to bile acids followed by its excretion into bile. When the excretion of fecal bile acids increased in intact female and ovariectomized hamsters with the increasing dietary calcium, a corresponding reduction in plasma TC was expected.

In summary, we found from this study that dietary calcium favourably modified plasma lipoprotein profile by decreasing plasma TC, TC/HDL-C and TG levels and increasing the plasma HDL-C level in intact female and ovariectomized but not in intact male and castrated hamsters. It was concluded that dietary calcium-induced reduction of plasma TC was gender-dependent, possibly mediated by a gender difference in calcium absorption and bile acid excretion.

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