Songklanakarin J. Sci. Technol.
39 (2), 237-243, Mar. - Apr. 2017

http://www.sjst.psu.ac.th

Original Article

Beneficial effects of young coconut juice on preserving neuronal cell density, lipid, renal and liver profiles in ovariectomized rats. A preliminary study.

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Received: 4 March 2016; Revised: 4 May 2016; Accepted: 20 May 2016

Abstract

Our previous study showed that young coconut juice (YCJ) at a high dose of 100 mL/kgBW had many health benefits e.g. it delayed Alzheimer's pathologies, preserved neuronal cells, accelerated wound healing and prevented osteoporosis. However, such a large dose of YCJ over a period of time started to have unfavourable side effects e.g. the deposition of glycogen in the liver. Therefore, our aim in the present study was to investigate the lowest neuroprotective dose of YCJ that would cause the least side effects for long-term consumption by postmenopausal women, using ovariectomized (ovx) rats as a model for postmenopausal women. Three lower doses of YCJ (10, 20 and 40 mL/kg body weight) were applied. The results clearly showed that the OY10 group was the best dose to help to preserve neuronal cells in both the hippocampus and the prefrontal cortex with cell numbers being higher than for the ovx group at various degrees of significance in each brain region. After 10 weeks of treatment, the circulating levels of BUN, creatinine, cholesterol, triglyceride, HDL, LDL, AST, ALT, ALP, total protein, albumin, calcium and phosphorus of the OY10 group were not significantly different from those of the sham and ovx groups. This study has confirmed that feeding YCJ had beneficial effects on the serum lipid profile, and maintained liver and renal functions for up to 10 weeks after administration. YCJ consumption at 10 mL/kgBW/day for 10 weeks, however, did increase body weight and serum glucose when compared with the control groups. Therefore, supplementation with YCJ in postmenopausal women with a diabetic condition should only be allowed under supervision by a physician.

Keywords: young coconut juice, neuronal cell density, lipid, kidney, liver

1. Introduction

Our previous study showed that young coconut juice (YCJ) containing beta-sitosterol (Ratanaburee *et al.*, 2014) at a high dose of 100 mL/kgBW delayed Alzheimer's pathologies (Radenahmad *et al.,* 2011) and prevented neuronal cell death (Radenahmad *et al.,* 2009). We have also shown that YCJ accelerated wound healing (Radenahmad *et al.,* 2012; 2015), and prevented osteoporosis (Morii *et al.,* 2015; Radenahmad *et al.,* 2014; Suwanpal *et al*., 2011; Yusuh *et al.*, 2010). However, such a large dose of YCJ feeding for 5 weeks started to have unfavourable side effects e.g. the deposition of

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glycogen in the liver (Payanglee *et al.,* 2016).

With this background, 3 lower doses of YCJ were used to find the optimal neuroprotective dose to be used for hormone replacement therapy (HRT) treatment using ovx rats as a model for postmenopausal women. Therefore, our aim in the present study was to investigate the lowest neuroprotective dose of YCJ that would cause the least side effects for long-term consumption by postmenopausal women using ovariectomized (ovx) rats as a model.

2. Materials and Methods

2.1 YCJ preparation

A large volume of young coconut juice (*Cocos nucifera* L., Arecaceae) was collected from Khlong Hoi Khong district, Hat Yai, Songkhla, Thailand, then dried, and the powder form kept at -30°C until used. This powder was freshly reconstituted and prepared for oral intake every day. A complete description of YCJ, including its preparation and administration, has been provided in our previous publications (Radenahmad *et al.,* 2006).

2.2 Animals

All animals used were adult eight-month old, female Wistar rats weighing 250-300 g. The rats were housed in a controlled environment at 25±1°C on an illumination schedule of a 12 h light-12 h dark cycle, with unrestricted access to standard pellet food and water. The study was approved by the Committee on Animal Care, and was carried out in accordance with the Guiding Principles for the Care and Use of Research Animals promulgated by the Prince of Songkla University.

2.3 Experimental design

The rats were randomly divided into 7 groups (10 rats per group). The first group consisted of the control baseline rats (BL), the second consisted of the sham-operated rats receiving vehicle (SW) and the third group consisted of the ovx rats receiving vehicle (reverse osmosis water) (OW), and the fourth consisted of the ovx rats injected with exogenous estrogen (2.5 μg/kg BW of estradiol benzoate, EB) three days a week (OB). The fifth, the sixth and the seventh groups consisted of ovx rats that received YCJ at 10 (OY10), 20 (OY20) and 40 (OY40) mL/kg BW/day, respectively. In this study, the administration of EB and YCJ was started one week after performing ovariectomy. At the end of the experimentation, rats were sacrificed and the brains were removed and processed into paraffin blocks for cresyl violet staining.

2.4 Microscopic analysis by quantitative histomorphometry

Counting of the neuronal cells was performed by two independent observers. An eyepiece micrometer was mounted on a light microscope, and counting was made at x40 magnification. Data were expressed as the number of neurons per mm^2. Readings from both observers were then added and the average was determined. The mean ± SEM was used to compare the seven groups.

2.5 Blood biochemistry and serum estradiol

Whole blood was allowed to clot and then centrifuged at 3000 g for 15 minutes to obtain serum and kept at -20°C until the determination of serum lipid, protein, renal and liver function markers. Serum levels of glucose, total cholesterol, triglycerides, HDL, LDL, AST (aspartate aminotransferase), ALT (alanine aminotransferase), ALP (alkaline phosphatase), total protein, albumin, BUN, creatinine, phosphorus and calcium were determined using the Modular P800 Roche Diasnotic, Thailand and for estradiol (E2) using the chemiluminescent immunoassay (CIA) technique (ECLIA, Modular E 170C, Estradiol II 03000079 122, Roche, Germany).

2.6 Statistical analysis

The data were analyzed by calculating a mean value and the standard error of the mean (mean ± SEM). Statistical analysis was performed using the Kruskal-Wallis and the Mann-Whitney U-tests. Values of $p < 0.05$ were considered statistically significant.

3. Results and Discussion

3.1 Histopathological assessment of neuronal cells

Cresyl violet was used to demonstrate the Nissl substance in the neurons and cell nuclei (Figure 1). In the present study, the number of neuronal cells in CA1, CA2, CA3 of hippocampus (HP) and prefrontal cortex (PF) of the ovx rats that received YCJ at 10, 20 and 40 mL/kgBW/day were not significantly different from those of the baseline and sham groups (Table 1). Among the three doses of YCJ (10, 20 and 40 mL/kgBW/day), however, OY10 seemed to be the best dose for a neuroprotective effect on the neuronal cell density. Therefore, YCJ preserved neuronal cell density in the HP and PF, the brain regions responsible for improved learning and memory in ovx rats, a model for postmenopausal women. Immunohistochemistry for specific neurotransmitters, and estrogen receptors of both types (ERα and ERβ) had also been studied to confirm these phenomena. That manuscript has been separately prepared owing to the large amount of data.

3.2 Body weight (BW) (Table 2)

After ovx for 10 weeks, the body weight of OW group was significantly higher than those of the SW and OB groups. The body weight of the OB group was not significantly different from those of the SW group. The present results indicated

Table 1. Numbers of neuronal cells (pyramidal and glial cells)stained by cresyl violet (mean±SEM) in the hippocampal areas: CA1, CA2 and CA3; and prefrontal cortex (PF) of the 7 groups examined, n = 10 per group. PF = pre-frontal cortex. BL = Baseline group, SW = Sham-operated group, OW = Ovariectomized group, OB = Ovariectomized group receiving estradiol benzoate, OY10 = Ovariectomized rats receiving YCJ 10 mL/kgBW/day, OY20 = Ovariectomized rats receiving YCJ 20mL/kgBW/day, OY40 = Ovariectomized rats receiving YCJ 40 mL/kgBW/day. [a] $p<0.05$ compared with the SW group, [b]$p<0.05$ compared with OB group,[c] $p<0.05$ compared with the OY20 group.

Groups \ Areas	Neuronal cell numbers/mm^2			
	CA1	CA2	CA3	PF
BL (n=10)	48.73±2.79	45.40±5.16	46.52±2.34	74.54±3.50
SW (n=10)	44.84±0.90	39.08±2.23	45.72±1.51	72.32±4.06
OW (n=10)	45.42±1.84	37.17±2.34	45.77±1.52	75.32±3.38
OB (n=10)	46.23±2.17	38.77±1.56	46.14±2.22	87.78±6.34[a]
OY10 (n=10)	44.57±1.36	37.06±1.91	45.46±0.82	75.30±4.13
OY20 (n=10)	43.26±1.15	37.79±2.27	44.07±1.59	70.38±4.94
OY40 (n=10)	44.25±1.55	42.15±1.81[c]	41.44±1.53	69.01±3.77[b]

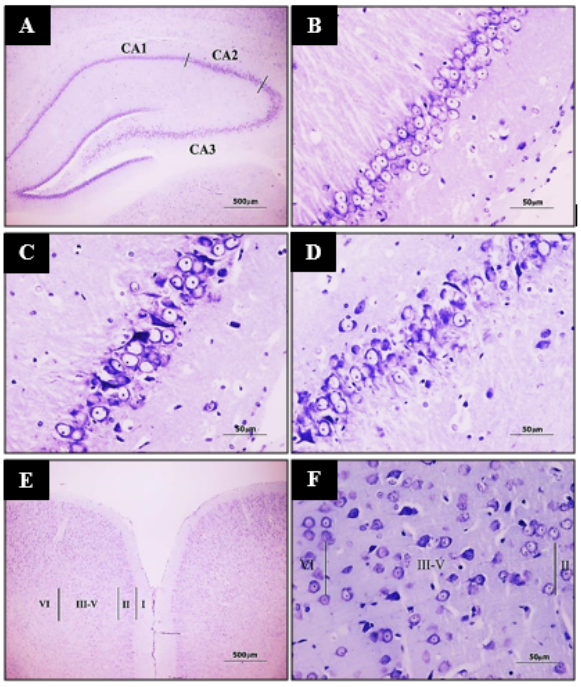

Figure 1. Histology of hippocampus (CA1, CA2, CA3) and prefrontal cortex (PF):
Expression of cresyl violet positive neurons in CA1, CA2, CA3 of hippocampus and prefrontal cortex (PF). A = overview of hippocampal areas divided into 3 parts: CA1, CA2 and CA3. B = CA1. C = CA2. D = CA3. E = overview of prefrontal cortex showing layer I-VI. F = higher magnification of prefrontal cortex areas II-VI, A & E: x4 magnification. B, C, D & F: x40 magnification.

Table 2. Body weight (g) of the 7 groups examined, n = 10 per group. BL = Baseline group, SW = Sham-operated group, OW = Ovariectomized group, OB = Ovariectomized group receiving estradiol benzoate, OY10 = Ovariectomized rats receiving YCJ 10 mL/kgBW/day, OY20 = Ovariectomized rats receiving YCJ 20 mL/kgBW/day, OY40 = Ovariectomized rats receiving YCJ 40 mL/kgBW/day. [aa]$p<0.01$ compared with the BL group, [bb]$p<0.01$ compared with the SW group, [cc]$p<0.01$ compared with the OW group, [dd]$p<0.01$ comparison between those at the commencement of the study and the day that rats were terminated.

Groups \ Days	Body weight (g)	
	Started	Finished
BL (n=10)	261.55±5.14	261.55±5.14
SW (n=10)	284.00±4.69	301.09±2.49[aa, dd]
OW (n=10)	300.73±5.80	344.00±7.79[aa, bb, dd]
OB (n=10)	289.18±7.08	309.45±8.84[aa, cc]
OY10 (n=10)	278.45±3.84	324.82±6.69[aa, bb, dd]
OY20 (n=10)	273.09±5.48	325.00±5.03[aa, bb, dd]
OY40 (n=10)	278.64±4.81	334.55±5.94[aa, bb, dd]

that the deficiency of estrogen in the OW group might be one of the factors that could cause the increase of body weight and the supplementation with estrogen by injection with EB in the OB group could maintain the body weight. This was supported by Rosa *et al.* (2009) who found that estrogen

treatment modulated iron metabolism that was involved with adiposity in the adipose tissue of ovx rats. The body weight of all 3 OY groups (OY10, OY20, OY40) was lower than those of the OW group even though the significance was not detected. Nevertheless, the body weight of 3 OY groups was higher than the SW and OB groups, this might imply that supplement with YCJ at 10, 20 and 40 mL/kgBW was not enough to reduce/to maintain the body weight.

3.3 % Organ weight/Body weight (BW) (Table 3)

3.3.1 % brain weight/BW

The %age brain/BW of the SW group was significantly lower than that of the BL group indicating that older rats had a lower brain weight. Furthermore, the %age brain/BW of the OW group was significantly lower than that of the BL and SW groups and indicated that not only aging but also estrogen deficiency influenced the brain weight. This was confirmed by the %age brain/BW of the OB group as follows: when ovx rats were injected with EB 3 times per week (OB group), the %age brain/BW of the OB group could be restored back to the normal control level that is, the %age brain/BW of the OB group was not significantly different from that of the SW group. The %age brain/BW of all three OY groups was significantly lower than those of the BL and SW groups indicated that supplemention of the ovx rats with 10, 20 and 40 mL/kgBW/day (OY10, OY20 and OY40) did not restore the brain weight.

3.3.2 % uterine weight/BW

In contrast to the % age brain/BW, the % uterine weight/BW of the SW group was not significantly different from that of the BL group. After ovx for 10 weeks, the% uterine weight/BW of the OW group was significantly lower than that of either the BL or SW groups. This indicated that estrogen deficiency influenced uterine weight. The % uterine weight/BW of OB group was significantly lower than those of the BL and SW groups and was significantly higher than that of the OW group. This indicated that injection with 2.5 μg of EB 3 times per week in the ovx rats could increase the uterine weight in the ovx rats; nevertheless that did not restore the uterine weight back to the level in the control groups (BL and SW groups). The % uterine weight/BW of the OY groups was significantly lower than those of the BL, SW and the OB groups accept that of the OY40 group. The %uterine weight/BW of the OY40 group was significantly higher than that of the OY20 group. The histology of the uterus (Figure 2) also showed no lesions in any of the three doses of YCJ supplement (OY10, OY20, OY40). These data implied that feeding with YCJ with the 3 different doses (OY10, OY20 and OY40) did not cause uterine hyperplasia/ hypertrophy in the ovx rats.

3.3.3 % ovarian weight/BW

In the same way as for the % uterine weight/BW, the % ovarian weight/BW of SW group was not significantly

Table 3. %age organ weight/body weight (BW)
The %age of brain, uterus, ovaries, liver and kidney weight were calculated per body weight of the 7 groups examined, n = 10 per group. BL = Baseline group, SW = Sham-operated group, OW = Ovariectomized group, OB = Ovariectomized group receiving estradiol benzoate, OY10 = Ovariectomized rats receiving YCJ 10 mL/kgBW/day, OY20 = Ovariectomized rats receiving YCJ 20mL/kgBW/day, OY40 = Ovariectomized rats receiving YCJ 40 mL/kgBW/day. [a, aa]$p<0.05$, $p<0.01$ compared with the BL group, respectively, [b, bb]$p<0.05$, $p<0.01$ compared with the SW group, respectively, [c, cc]$p<0.05$, $p<0.01$ compared with the OW group,respectively, [d]$p<0.05$ compared with OB group, [e]$p<0.05$ compared with the OY10 group, [f]$p<0.05$ compared with the OY20 group.

Groups \ Parameters	% Organ weight/Body weight (BW)				
	% Brain Weight/BW	% Uterine Weight/BW	% Ovarian Weight/BW	% Liver Weight/BW	% Kidney Weight/BW
BL (n=10)	0.50±0.01	0.17±0.02	0.08±0.01	2.74±0.08	0.55±0.01
SW (n=10)	0.44±0.0[aa]	0.16±0.02	0.09±0.01	2.64±0.11	0.57±0.02
OW (n=10)	0.40±0.01[aa, bb]	0.04±0.01[aa, bb]	-	2.43±0.08[a]	0.49±0.01[aa, bb]
OB (n=10)	0.44±0.01[a]	0.10±0.02[a, b, cc]	-	2.49±0.07[a]	0.52±0.02[b]
OY10 (n=10)	0.41±0.01[aa, bb]	0.05±0.01[aa, bb, d]	-	2.37±0.09[aa]	0.50±0.01[a, bb]
OY20 (n=10)	0.40±0.01[aa, bb]	0.04±0.00[aa, bb, d]	-	2.58±0.08	0.54±0.01[c, e]
OY40 (n=10)	0.40±0.01[aa, bb]	0.06±0.01[aa, bb, f]	-	2.45±0.07[a]	0.52±0.01[bb]

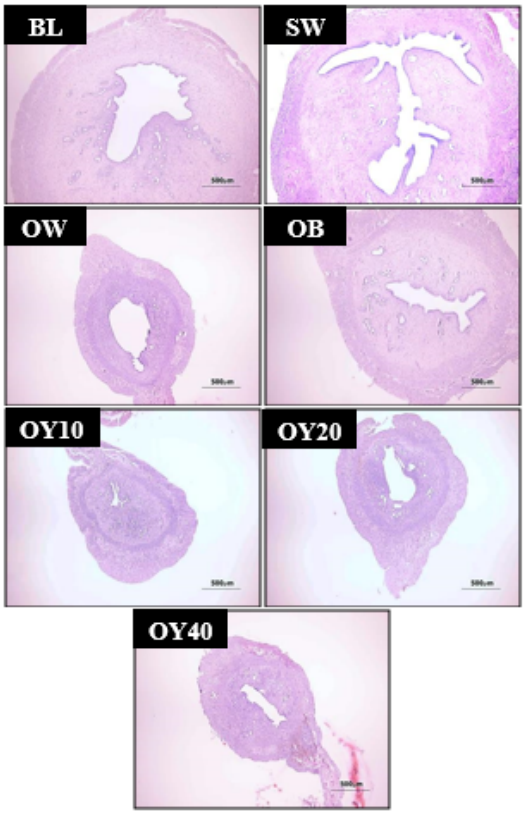

Figure 2. Histology of uterus:
Haematoxylin-eosin staining of uterine tissue of 7 groups examined, paraffin sections, 5 µm thick, x4 magnification. BL = Baseline group, SW = Sham-operated group, OW = Ovariectomized group, OB = Ovariectomized group receiving estradiol benzoate, OY10 = Ovariectomized rats receiving YCJ 10 mL/kgBW/day, OY20 = Ovariectomized rats receiving YCJ 20mL/kgBW/day, OY40 = Ovariectomized rats receiving YCJ 40 mL/kgBW/day.

different from that of the BL group. That might imply that the reason why the % uterine weight/BW between those 2 groups was not significantly different was because the uterine functions depend on estrogen produced by the ovaries.

3.3.4 % liver weight/BW:

That % liver weight/BW of the OW, OY10 and OY40 groups was significantly lower than that of BL group. Neither of YCJ feeding groups (OY groups) was significantly different from that of the SW or OB groups. This implies that feeding YCJ did not increase liver weights, so the 3 different doses of YCJ supplementation (10, 20 and 40 mL/kgBW) did not cause any harm to the liver.

3.3.5 % kidney weight/BW

Just as for the %age of liver weight/BW, the %age of the kidney weight/BW of the YCJ feeding groups was not higher than those of control groups (BL and SW groups). This implies that feeding YCJ did not increase kidney weight, so YCJ supplementation did not cause any harm to the kidney.

3.4 Serum chemical analysis

The serum levels of cholesterol, HDL, LDL, triglyceride, BUN, creatinine, total protein, albumin, AST, ALT, ALP, calcium and phosphorus of the OY10 group were not significantly different when compared with the SW group (Table 4). Among the 3 doses of YCJ feeding, the ovx rats receiving YCJ at 10 mL/kg BW/day had all the parameters except the glucose level close to the SW group. These data are consistent with the antioxidant and hepatoprotective effect studied by Loki and Rajamohan (2003), or the hypolipidemic properties investigated by Sandhya and Rajamohan (2008), improved the lipid profile (Sandhya & Rajamohan, 2006) and prevented renal dysfunction (Preetha *et al.*, 2013). The active compounds of YCJ that could influence lipid levels included a free amino acid L-arginine, ascorbic acid, and minerals such as calcium, magnesium, and potassium. Among these, L-arginine could play a major role (Sandhya & Rajamohan, 2006). Another possibility that could explain the lowering effect of the lipid parameters by YCJ were the results from phytosterols, specifically β-sitosterol, that is a major compound of phytosterols of YCJ (Ratanaburee *et al.* 2014; Rujiralai & Sitaruno, 2009). Furthermore, another possibility explanation of decreased serum triglyceride in rats fed with coconut water may be the increased activity of a lipoprotein lipase in the heart and adipose tissue (Sandhya & Rajamohan 2008). (Please see details in "discussion" of Radenahmad *et al.,* 2016).

4. Conclusions

In summary, this preliminary study has illustrated that YCJ fed at 10 mL/kg body weight per day, which is equal to the consumption of one coconut per day, was the optimal neuroprotective dose to preserve neuronal cell density in the HP and PF, the brain areas that are involved with spatial memory and novel object recognition. This dose did not affect the serum level of calcium or of phosphorus; the female reproductive organs including the uterus and ovaries, or other vital organs including the brain, liver and kidney. However, it did increase the glucose serum level and body weight. Therefore, supplementation with YCJ in postmenopausal women with a diabetic condition should only be allowed under supervision by a physician.

Acknowledgements

We thank Dr. Brian Hodgson at Faculty of Pharmaceutical Science, Prince of Songkla University, for correction and improvement of the manuscript. This work was supported by the government budget of Prince of Songkla University, GRANT CODE: SCI580566S.

Table 4. Serum chemical analysis (mean±SEM) of the 7 group examined, n = 10 per group. BL = Baseline group, SW = Sham-operated group, OW = Ovariectomized group, OB = Ovariectomized group receiving estradiol benzoate, OY10 = Ovariectomized rats receiving YCJ 10 mL/kgBW/day, OY20 = Ovariectomized rats receiv-ing YCJ 20mL/kgBW/day, OY40 = Ovariectomized rats receiving YCJ 40 mL/kgBW/day. BUN = blood urea nitrogen, HDL = high density lipoprotein cholesterol, LDL = low density lipoprotein cholesterol, AST = aspartate aminotransferase, ALT = alanine aminotransferase, ALP = alkaline phosphatase. [a, aa]$p<0.05$, $p<0.01$ compared with the BL group, respectively, [b]$p<0.05$ compared with the SW group, [c]$p<0.05$ compared with the OW group, [d]$p<0.05$ compared with the OB group, [e]$p<0.05$ compared with the OY10 group.

	Serum chemical analysis						
Groups / Parameters	BL (n=10)	SW (n=10)	OW (n=10)	OB (n=10)	OY10 (n=10)	OY20 (n=10)	OY40 (n=10)
Glucose (mg/dL)	166±11.32	120.18±5.68[aa]	120.45±9.31[aa]	113.36±11.72[aa]	134.73±8.62[b]	116.82±10.72[aa]	132.27±8.95[a]
BUN (mg%)	27.58±1.11	25.73±0.87	20.82±1.58[aa, b]	23.55±1.21[a]	23.73±1.45[a]	27.27±1.95[c]	25.18±1.07[c]
Creatinine (mg%)	0.78±0.05	0.79±0.05	0.8±0.08	0.81±0.08	0.95±0.14	0.83±0.09	0.82±0.07
Cholesterol (mg%)	50.42±3.33	61.64±5.18	69.82±6.16[aa]	59.64±3.19	69.27±4.43[aa]	71.45±6.66[a]	84.55±7.17[aa, b, d]
HDL (mg%)	24.67±1.63	34.36±2.14[a]	35.27±2.04[a]	32.27±1.33[a]	35.82±2.07[a]	37.91±2.78[a]	34.36±1.12[aa, d]
LDL (mg%)	13.25±1.54	15.82±3.52	23.18±4.23	13.91±1.97	22.27±3.21[aa]	22.82±3.96	33.73±5.68[aa, b, d]
Triglyceride (mg%)	98±11.28	83.09±9.10	85.73±10.94	106.55±23.38	81.27±9.39	94.73±10.28	92.64±12.23
AST (U/L)	477.17±223.65	279.82±43.34	300.18±23.07	364.82±25.28	343.00±24.77	303.18±19.10	253.64±37.26[d, e]
ALT (U/L)	223.67±67.63	128.09±15.79	115.91±12.15[a]	109.91±7.79[a]	109.91±6.25[a]	114.27±10.83[a]	128.36±17.63
ALP (U/L)	145.92±21.49	218.00±51.84	167.64±14.28	141.91±16.60	158.91±17.23	163.18±22.32	160.82±19.71
Total protein (g%)	8.93±0.25	8.22±0.18[a]	7.88±0.18[aa]	7.90±0.32[a]	7.90±0.18[a]	7.58±0.17[aa, b]	7.95±0.19[a]
Albumin (g%)	5.12±0.15	4.09±0.27[aa]	3.89±0.23[aa]	3.92±0.33[a]	3.75±0.23[aa]	3.75±0.22[aa]	3.99±0.27[aa]
Calcium (mg/dL)	9.90±0.08	10.11±0.19	10.08±0.30	9.98±0.29	10.12±0.28	9.94±0.23	9.98±0.16
Phosphorus (mg/dL)	5.85±0.33	5.72±0.46	6.01±0.46	6.80±0.46	6.00±0.33	6.14±0.44	6.12±0.40

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