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CARBONATED BEVERAGES AFFECT LEVELS OF ANDROGEN RECEPTOR AND TESTOSTERONE SECRETION IN MICE

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Abstract

Objectives. This work aimed to study the influences of carbonated beverages (CBs) on the testis growth and the expression levels of androgen receptor (AR) of mice.

Methods. Two experimental groups of 30 mice each PEP-1 and PEP-2 drank 50% and 100% Pepsi-Cola, respectively for 15 days. Other 2 experimental groups of 30 mice each COC-1 and COC-2 drank 50% and 100% Coca-Cola, respectively for 15 days. The control group (CG) of 30 mice drank water. Bilateral testes were harvested aseptically on days 0, 5, 7, 10, 13 and 15. Real-time PCR and Western blot were implemented to detect levels of androgen receptor (AR) mRNA and protein in testis tissues.

Results. Testes masses of PEP-2, COC-1 and COC-2 were greater than those of PEP-1 and CG ($P < 0.05$). On day 15, testis longitudinal diameter (TLD) of CBs-treated mice was increased as compared to CG. TLD, testes transverse diameters (TTD) and AR proteins levels of PEP-2 and COC-2 were increased in comparison with CG ($P<0.05$). Serum testosterone concentrations of PEP-2 were higher than that of COC-1 and CG ($P < 0.05$). Levels of AR mRNAs of four CBs-treated mice were increased by 60.18%, 67.26%, 65.93% and 78.76%.

Conclusions. A high concentration of Coca-Cola and Pepsi-Cola could raise TLD and TDD, enhance testosterone secretion, and increase serum EGF concentrations.

Keywords: Epidermal growth factor, Carbonated soft drinks, Androgen receptor.

INTRODUCTION

Coca-Cola (Coke) and Pepsi-Cola are two main carbonated beverages (CBs) drunk by numerous consumers over the world. CBs consumption may lead to obesity, cardiovascular diseases and type 2 diabetes mellitus (1). CBs increased weight body of rats after consuming over one year (2). Epidemiologic studies evaluated the association between caffeine and fertility (3), which indicated that health damage effects of CBs on adolescents and children were serious. Previous studies also suggested that CBs affected fertility (4). Massive consumption of coffee or caffeine increased testosterone production (5). Coca-Cola could induce incidence of malignant mammary tumors in Sprague–Dawley rat (6). However, many reports were questionnaire and prospective investigations based on the local populations in a region. Some reports even showed the opposite (7). So far there has been little documentation regarding correlation between CBs consumption and reproduction function and fertility in humans and animals (8). CBs have been associated with alterations in estradiol and other hormones which may affect ovulation, the length of the follicular or luteal phase (9). The exact mechanisms that carbonated beverages affected the fertility are undetermined. Comparative experimental studies are scanty (6, 10).

Our preliminary study showed that administration of Coca-Cola and Pepsi-Cola for 25 days could decrease ovaries mass, reduce the ovarian cortex thickness, as well as affect follicles development (11). Besides, cola and Pepsi-Cola could inhibit burn repair and physics strength of the healed tissues of the burned rabbits (12). Therefore, drinking carbonated beverages may threaten reproductive success (13).

Androgens play an important role in sexual differentiation and secondary characteristics formation of the male mammals. Androgen exerts its physiological functions by combining its specific androgen receptor (AR) in target tissues (14). Testosterone, a crucial androgen, has an important role on forming puberty and maintain normal male libido. Currently, it remains undetermined whether long-term intake of CBs influences the synthesis and secretion of testosterone (15).

The present study aimed to investigate the effects of carbonated beverages (CBs, including Coca-Cola and Pepsi-Cola) on the testis development and expression levels of androgen receptor (AR) of mice, and also to

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provide scientific bases for the rational consumption of CBs, and prevent prostate dysfunction and cancer.

MATERIALS AND METHODS

Animals

150 non-copulating male mice (Mus musculus), body weight of 20.84 ± 2.45g, were bought from Lanzhou University [License No. SCXK (Gansu) 2005-0007]. They were allocated into 5 groups: Pepsi-Cola 1 (PEP-1), Pepsi-Cola 2 (PEP-2), Coca-Cola 1 (COC-1), Coca-Cola 2 (COC-2) and the control group (CG, n=30). Mice were raised in mice cages that were equipped with automatic water dispensers under the same conditions kept at 30% to 50% humidity and 22℃-24℃. Water was provided ad libitum. Mice were freely fed with daily ration feeds (Lanzhou Taihua Feed Co., China). Each mouse was weighed every day. The experiment was begun after an adaptation period of 7 days.

Animal treatments and sample collection

Pure Pepsi-Cola and Coca-Cola were purchased from the monopolized store; 50% Pepsi-Cola (Coca-Cola) was prepared by releasing one liter (1 L) of pure Coca-Cola (Pepsi-Cola) with 1 liter of tap water (1:1 v/v). Mice in PEP-1 and PEP-2 drank freely 50% and pure (100%) Pepsi-Cola, respectively for consecutive 15 days. Animals in COC-1 and COC-2 drank freely 50% and pure (100%) Coca-Cola, respectively for consecutive 15 days. Mice in the control group (CG) drank freely tap water. Bilateral testes were aseptically collected from each mouse on days 0, 5, 7, 10 and 15, respectively. Blood was harvested from the jugular vein of each mouse killed by neck dislocation. Serum was isolated from blood by centrifugation at 3000 × g for 10 min, and then stored at -20℃ until analysis. Animal treatment was permitted by the Experiment Animal Care and Use Committee of Gansu province, China.

Measurements of testis mass, testis longitudinal and transverse diameter

The mass of each testis was measured using an electronic balance. The average was determined on the basis of both left and right values. The extraneous tissues and fats on the testis surface were removed using sterile scissors. The testis longitudinal diameter (TLD) and testis transverse diameter (TTD) of each testis were accurately measured with a vernier caliper.

Measurements of serum testosterone and epidermal growth factor (EGF)

The contents of serum testosterone and EGF were measured using the mice ELISA kit (Shanghai Qiaodu Biological Technology Co., Ltd, Shanghai, China), as per instruction manual (Qiaodu biotechnology corporation, Shanghai, China). The detection level was 0.40pg/mL. Each assay was done in triplicate.

Determination of expression levels of AR mRNA

Total RNAs were extracted from the testis samples (16), then reversely transcribed into cDNA with the superscriptTM first strand synthesis system for RT-PCR (Invitrogen, Beijing, China).

Appropriate primers of the androgen receptor (AR, GenBank No: X59592.1) were designed and synthesized by Sangon Biotech Co., Ltd. (Shanghai, China). Only those primers showing dimmer-free reactions were used for further analysis.

The sequences of androgen receptor primers (Gene ID: NM_013476.4) were forward primer: 5′- aagagacgaggaggcaggata-3′ and reverse primer: 5′- gccgggaggtgctatgttag-3′ (17, 18); mouse beta-actin (NM_007393) forward primer sequence: 5′-gtatgcctctggtcgtacca-3′ and Reverse primer primer sequence: 5′-ttgctgacaggatgcagaag-3′; mouse glyceraldehyde-3-phosphate dehydrogenase (GAPDH, NM_008084.2) forward primer sequence: 5′-cttcaacagcgacactcactct-3′ and reverse primer sequence: 5′- ccaccaccctgttgctgta-3′. The expression level of androgen receptor mRNA was determined using qRT-PCR. The level of every group was assessed by the $2^{-\Delta\Delta CT}$ method and normalized to beta-actin of CG on day 0. Each sample was executed in triplicate.

Western blot analysis of androgen receptor protein in testes

Western blot was performed so as to evaluate the expression levels of androgen receptor (AR) protein in testis tissues after Pepsi-Cola and Coca-Cola treatment. Summarily, the polyvinylidene fluoride membranes (PVDF) were incubated with Anti-AR antibody (Sigma, diluted 1:500) and β-actin (1:1000, ab8227, USA) polyclonal antibodies at 4 ℃ overnight, before being exposed to the appropriate secondary antibody (1:2000) for 1 h. Monoclonal antibody (1:10 k) of mouse anti-β-actin was used as a control. The integrated optical densities (OD) of image bands were determined using Quantity One software (Bio-Rad, Hercules, CA, USA). Expression of AR protein was measured as grey value ratio between target band and β-actin band. The negative control was set without primary antibody.

Statistical analyses

All variables of three groups complied with the assumptions for an one-way ANOVA using SPSS v. 20.0. Then, Tukey's post-hoc tests were done to compare pair wise differences after significant differences were identified. Significance level was P<0.05.

RESULTS

The testes weights of PEP-2, COC-1 and COC-2 were significantly increased on day 15 (P<0.05) as compared to PEP-1 and CG (Table 1). The outcomes demonstrated a high dose of Pepsi-Cola or Coca-Cola could promote testis growth and development.

Data in Table 2 showed that TLD values of four experimental groups were elevated as compared to CG on days 10 and 15. TLDs of COC-2 and PEP-2 were significantly greater than that of CG. On day 15, TTD values of COC-2 and PEP-2 were also larger than that of CG, COC-1 and PEP-1 groups. The results indicated that higher doses of Pepsi-Cola and Coca-Cola could enhance the testis longitudinal and transverse diameter, and then they promoted testis growth of mice.

As shown in Figure 1A, the concentrations of serum testosterone in all mice were enhanced after the Pepsi-Cola and Coca-Cola treatment. Serum testosterone concentration of PEP-2 was higher than that of other four groups. On days 10 and 15, there were significant differences between PEP-2 and CG. Serum testosterone concentration of COC-2 was also higher than that of CG (P<0.05). The outcomes indicated that high doses of Pepsi-Cola and Coca-Cola could improve testosterone secretion of male mice.

Data in Figure 1B showed that the serum concentrations of epidermal growth factor (EGF) of treatment groups were slightly increased as compared to CG on days 10 and 15. EGF concentrations of PEP-2 and COC-2 were significantly higher than CG (P<0.05) on day 15,. The results demonstrated a high dose of Pepsi-Cola and Coca-Cola could raise EGF activity.

Expression levels of AR mRNAs in mouse testes were detected using qRT-PCR so as to assess the FRBI influences on s of AR mRNAs. As shown in Figure 2, expressions of AR mRNAs in four treatment groups were increased. On day 15, increments of COC-1, COC-2, PEP-1 and PEP-2 mice were 60.18%, 67.26%, 65.93% and 78.76% (P<0.05) on the bases of the CG level. Pepsi-Cola and Coca-Cola could enhance expressions of AR mRNAs in the testes of mice.

Expression levels of androgen receptor (AR) proteins in CBs-treated mice were increased (Fig. 3) in comparison with CG. On day 15, AR proteins levels of PEP-2 and COC-2 mice were increased as compared to CG (P<0.05). A high concentration of Pepsi-Cola and Coca-Cola could accelerate expressions of AR protein in the testes of mice.

Table 1. Changes of testis weights of mice (mg)

Group	3d	5d	7d	10d	15d
COC-1	73±15	54±6	90±10^{*b}	77±15	98±7
COC-2	62±11	64±9	89±9^{*b}	89±10	97±10
PEP-1	80±14	60±12	49±8^{a}	78±12	85±6
PEP-2	65±12	61±10	91±5^{*b}	79±11	99±16
CG	71±11	60±8	66±6	76±14	86±7

Data are the average of both left and right testis weights. * P<0.05 when compared to control group; ** P<0.01 when compared to control group. The same superscript letters in the same column mean that there was no significant difference. The different superscripts mean that there was significant difference between groups, of which adjacent superscript (such as ab, bc) indicate the difference was significant (P<0.05), while interval superscript (such as ac, bd) show the difference was highly significant (P<0.01).

DISCUSSION

Currently, carbonated beverages (CBs), are daily consumed by many people worldwide (3, 19). CBs have been a deleterious key factor along with their consumption increase. The overweight, obesity, diabetes and endocrine disorders are associated with massive consumption of CBs (2, 20). CBs harmfully affect reproduction functions (21, 22). A survey of 2500 men indicated that the sperms

Table 2. Testis longitudinal diameter (TLD) and testis transverse diameter (TTD) (μm)

Group	Testis longitudinal diameter			Testis transverse diameter		
	5d	10d	15d	5d	10d	15d
COC-1	225.7±35.6	257.3±25.1	288.1±27.3	54.3±6.2	77.4±15.2	83.0±7.3^{a}
COC-2	221.1±30.2	289.6±20.7*	297.0±26.2*	64.9±9.2	82.4±10.2	97.1±10.6^{*b}
PEP-1	226.5±39.7	267.8±12.5	285.5±26.8	60.6±12.3	78.2±12.1	85.7±6.3^{a}
PEP-2	224.3±33.6	279.6±31.9*	299.5±36.2*	61.6±10.5	83.0±11.3	99.6±16.8^{*b}
CG	220.2±41.9	247.6±34.1	268.6±37.7	60.2±8.6	76.6±14.5	86.6±7.2

Data are the average of both left and right testis masses of 5 mice (sample collection from 5 mice in every time). * P<0.05 when compared to control group; ** P<0.01 when compared to control group. The same superscript letters in the same column mean that there was no significant difference. The different superscripts mean that there was significant difference between groups, of which adjacent superscript (such as ab, bc) indicate the difference was significant (P<0.05), while interval superscript (such as ac, bd) show the difference was highly significant (P<0.01).

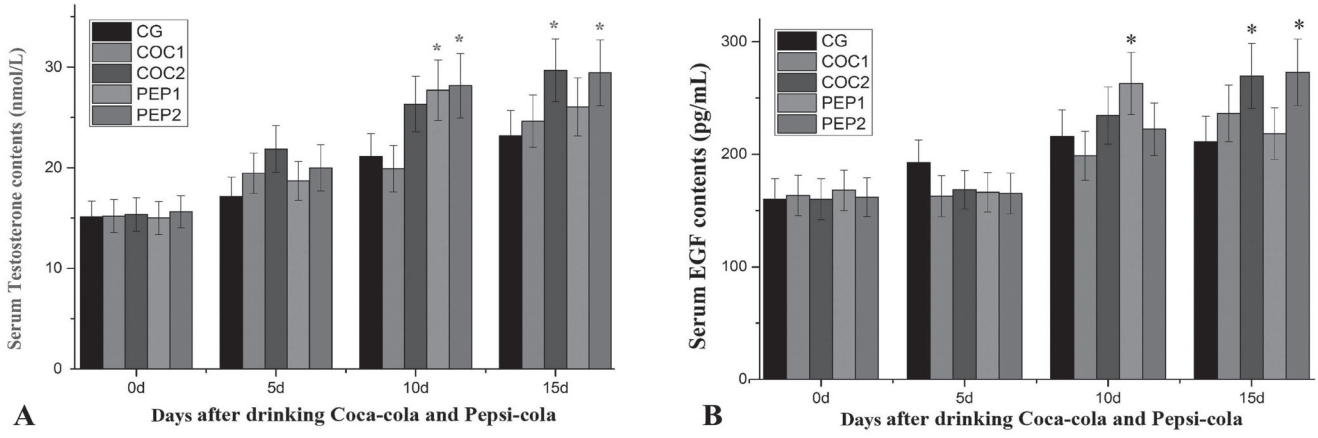

Figure 1. Changes in serum testosterone and epidermal growth factor (EGF) concentrations in mice (sample collection from 5 mice in every time, the same for the following figures). Testosterone concentration of PEP-2 and COC-2 groups were higher than that of CG. Serum EGF concentrations in treatment groups were increased as compared to CG on days 10 and 15. A. Serum testosterone concentrations; B. Serum epidermal growth factor (EGF) concentrations. * P<0.05 as compared to CG.

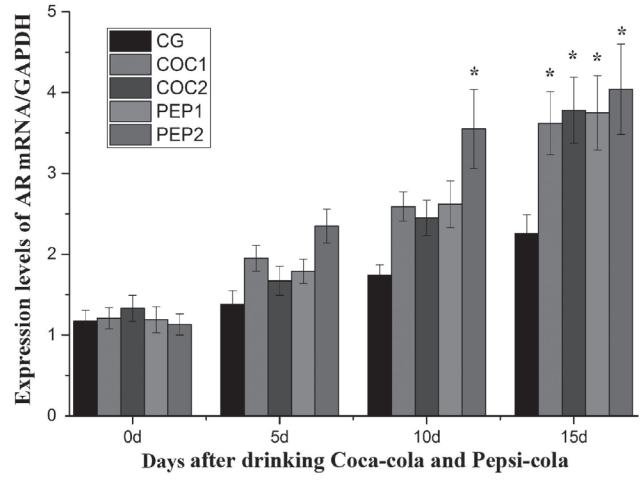

Figure 2. Expression levels of AR mRNAs in testes. * P<0.05 as compared to CG. On day 15, the increments of AR mRNAs in COC-1, COC-2, PEP-1 and PEP-2 mice were 60.18%, 67.26%, 65.93% and 78.76% on the bases of the CG level.

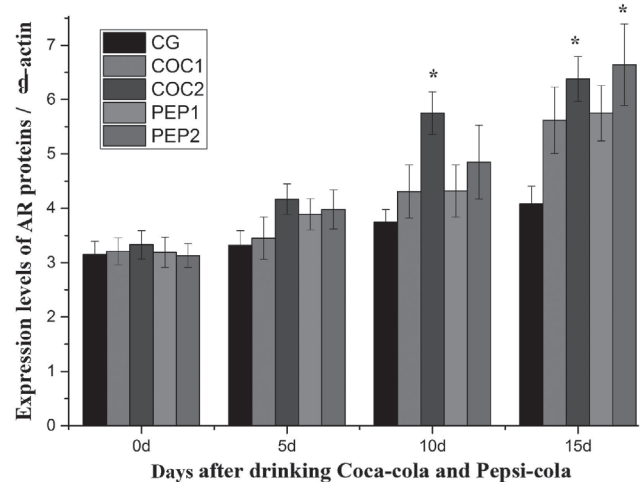

Figure 3. Levels of AR proteins in mouse testes. On day 15, AR proteins levels of PEP-2 and COC-2 mice were increased in comparison with CG. * P<0.05 as compared to CG; **P<0.01 as compared to CG.

quantity was reduced by 30% after men drank 1 liter cola beverage every day as compared to those without drinking any CSD. The oxidants and additives in CBs result in proteins oxidation, cell damages (15) and reduce sperm motility (23).

In our study, we found that testes masses of PEP-2, COC-1 and COC-2 groups were increased in comparison with PEP-1 and CG (P<0.05) on day 15. The testes longitudinal and transverse diameters become larger as compared to CG. Our initial experiments demonstrated that Pepsi-Cola and Coca-Cola could reduce ovarian weight, inhibited the development of follicles and oocytes. Pepsi-Cola and Coca-Cola obviously reduced pregnancy rate and fetus numbers of female mice, such affected reproduction behaviors of female mice (11). Our results revealed serum EGF contents of COC-2 and PEP-2 were higher than that of CG. These findings demonstrated high doses of Coca-Cola and Pepsi-Cola could enhance EGF activity and testosterone secretion. These are in agreement with our previous study in rabbits (12). However, the effects have to be deeply studied. Its mechanisms also have to be further explained in other mammals in the future (5).

Androgen and its receptor (AR) affect the development of normal prostate and prostate cancer (24). In the present study, we investigated the effects of Coca-Cola and Pepsi-Cola on androgen receptor (AR) expression levels in the mouse testes. The findings indicated AR mRNA and protein levels of the CBs-treated mice were strikingly increased when compared

to CG normal level. Nowadays, scarce document is reported on this subject (25). Such, these findings still need to be validated in the future (18).

In conclusion, drinking Coca-Cola and Pepsi-Cola could promote testis development, enhance testosterone secretion, increase serum EGF concentrations, also accelerated expressions of AR protein in the mice testes. Our findings provided the scientific bases and for fully understanding CBs effects and their mechanism on development and reproduction functions of humans, but also benefit to prevent prostate dysfunction and cancer.

Conflict of interest

The authors declare that they have no conflict of interest.

Acknowledgements

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Ethical Approval

The research protocol was approved by the Experiment Animal Care and Use Committee of Gansu province, the People's Republic of China. All mice were treated as per ethical rules.

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