

Effect of Carbonated Drinks on Uterine Development – An Experimental Study

Gong Zhuandi¹, Wei Suocheng², Wei Limin³, Liang Haoqin⁴, Lai Luju⁵

ABSTRACT

Background and Objective: Consumption of Coca-cola and Pepsi-cola has harmful impact on human health. Currently, scant data is available regarding effects of carbonated beverages on female reproductive system. This study was aimed to investigate the effects of carbonated beverages on uterine development and expression of follicle stimulating hormone receptor in mice model.

Methods: A total of 150 female Kunming mice weighing 20.36 ± 2.28 gm. were divided into five groups with 30 mice in each group. Groups COC-1 and COC-2 mice were given 50% and 100% Coca-cola per oral respectively for 25 days. Groups PEP-1 and PEP-2 mice were given 50% and 100% Pepsi-cola per oral respectively. Control group (CG) mice were given tap-water per oral. Western blotting was done to ascertain the levels of Follicle Stimulating Hormone Receptor (FSHR) protein in ovaries. Follicle Stimulating Hormone (FSH) levels were measured by ELISA kit.

Results: The weight of uterus in the groups COC-2 and PEP-2 was lower than that of CG. Uterine wall and uterine cavities were slightly decreased in Coca-cola and Pepsi-cola groups compared to CG. Uterine mucus was ruffled. Uterus wall thicknesses (UWT) were reduced. In comparison with CG, FSHR protein levels in COC-2 and PEP-2 groups were reduced by 21.14% and 22.80%. Serum FSH levels of COC-1, COC-2 and PEP-1 were less than that of CG. Body weights of the one-week-old mice from COC-2, PEP-1 and PEP-2 groups were increased in COC-2 and PEP-2 groups in comparison with CG ($P < 0.05$).

Conclusion: Oral ingestion of Coca-cola and Pepsi-cola for a longer duration reduces uterine weight, affects the uterine histology and inhibits the uterine development. reduced expression of FSHR protein may be linked with reduced growth and development of new born mice.

KEYWORDS: Coca-cola, Pepsi-cola, Uterus, Follicle Stimulating Hormone Receptor, Mice.

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INTRODUCTION

The carbonated beverages (CB) mainly consist of carbonated water, citric acid, sugar and caffeine. All carbonated beverages are acidic drinks. Currently, there is not only an increase in consumption of the carbonated beverages but its components are found to be harmful and involved in the carcinogenesis as well.¹ The increase in the prevalence rate of obesity, type-II diabetes or metabolic syndrome are related to CBs.^{2,3} Caffeine (including coffee, cocoa and chocolate) have been found to adversely affect the reproduction.^{4,5} According to a study the bodyweight was increased

in the female Sprague-Dawley rats exposed to Coca-cola from 30, 39, or 55 weeks of age.⁶ Numerous studies have investigated the effect of caffeine on fertility in women. However, most of these studies have retrospectively collected information; the results were acquired based on the questionnaire investigation or the prospective analyses of clinical cases or epidemiological observations, all of these make the results susceptible to biases and inconsistent mutually.^{7,8} So far, there has been insufficient quantitative evaluation of the carbonated beverages intake on fertility.² Currently, there is not only increased consumption of the carbonated beverages but its components are found to be harmful factors involved in carcinogenic processes.^{7,9,14} The comparative experimental studies are scarce as well.^{10,11}

The present study aimed to investigate comparatively the effects of Coca-cola and Pepsi-cola on uterine development at the different concentrations along with determination of serum levels of follicle-stimulating hormone receptor (FSHR) and follicle-stimulating hormone (FSH), so as to provide the experimental basis for further study on the effects and mechanisms of CBs on uterine diseases in humans.

METHODS

Animals and Ethics

In order to carry out a sufficiently large experiment and acquire meaningful results, 150 non-cycling Kunming mice (*Mus musculus*), 28 days old having bodyweight 20.44 ± 2.45 gm. were included. All mice were randomly assigned into five groups, each group comprising of $n = 30$ mice. The groups were named as: Pepsi-Cola group 1 (PEP-1), Pepsi-Cola group 2 (PEP-2), Coca-Cola group 1 (COC-1), Coca-Cola group 2 (COC-2) and control groups (CG). Each mouse was accurately weighed every day using an electronic weigh balance. The mice were given commercial diet and water ad libitum. All animal treatment procedures were approved by the Experiment Animal Care and Use Committee of the Gansu province of China.

Animal Treatments and Sample Collection

Pure (100%) Pepsi-Cola and Coca-Cola in 5-liter bottles were purchased from a supermarket in

Lanzhou city of China. Fifty percentage Pepsi-Cola or Coca-Cola was prepared by adding the equivalent volume of tap water into the pure (100%) Pepsi-Cola and Coca-Cola, respectively. They were stored at room temperature.

Mice of group PEP-1 and PEP-2 were allowed to feed freely on 50% and 100% Pepsi-Cola for consecutive 25 days. Similarly, COC-1 and COC-2 group mice ingested freely 50% and 100% Coca-Cola for consecutive 25 days. Mice of the control group (CG) drank freely tap water ad libitum. Another five mice were used as a normal control on day 0.

Five mice were randomly chosen from each group on days 5, 10, 15, 20 and 25. Each mouse was anesthetized by intramuscularly injecting 0.1mg/kg xylazine. Each animal was sacrificed by cervical dislocation. Blood samples and bilateral uterine horns were aseptically harvested on days 5, 10, 15, 20 and 25. Each uterine horn was accurately weighed using an electronic balance. The serum was separated and stored at -20°C . On day 25, two healthy male mice (45 days old) and five mice of each group were kept together for a week so as to mate randomly.

Measurements of Uterine Weight

The uterine weight of each mouse was weighed using an electronic balance on days 0, 5, 10, 15, 20 and 25.¹²

Histological Observations and Measurement

Uterus samples fixed in 10% formaldehyde were embedded with paraffin wax, sliced (5 μm) and stained with hematoxylin and eosin (H&E). The sections were evaluated and photographed under the light microscope and electron microscope. Five sites in each section (5 sections in every group, totaling 125 sites for each group) were measured. Uterine wall thickness (UWT) of each mouse was measured using Images Advanced 3.2 and Image Pro-Plus 2.0.

Western Blot of FSHR Protein

To evaluate the FSHR protein expression in mice uteri following Coca-Cola and Pepsi Cola intake, western blotting was done as per the methods established.^{13,14} The protein expression of FSHR

was determined as the gray value ratio between the FSHR band and β -actin band. The tests were repeated three times. The negative control was also incubated without the primary antibodies.

Serum FSH Detection

Serum concentrations of FSH were detected using ELISA kits for mice. The concentrations were measured strictly according to the manufacturer's instructions. Optical density (OD) of each sample was read with the MK-3 type enzyme-label instrument at 450 nm wave length. The samples were measured in triplicate.

Pregnant Mice and New-Born Mice

The pregnancy rate and duration were calculated. The new-born mice numbers and gender ratio were also determined. Body weight of each new-born mouse was weighed every day using an electronic balance.

STATISTICAL ANALYSIS

Statistical analysis was done using SPSS v. 21.0 (SPSS Inc. Chicago, IL, USA). Data are presented as means \pm SEM. All variables of three groups complied with the assumptions for a one-way ANOVA. When significant differences were identified, supplementary Tukey's *post-hoc* tests were conducted to investigate pair wise differences. *P* value lower than 0.05 was considered to be a significant difference (**P* < 0.05 and ***P* < 0.01).

RESULTS

Uterine Weight

The uterine weights of COC-2 and PEP-2 mice were less than that of the control group (CG) during the

Table-1: Uterine weights of female mice (gm).

Group	0d	5d	10d	15d	20d	25d
CG	19.9 \pm 1.9	23.8 \pm 2.1	25.4 \pm 1.6	27.6 \pm 2.1 ^a	28.7 \pm 2.1 ^a	30.4 \pm 2.1 ^a
PEP-1	20.8 \pm 1.7	23.7 \pm 2.2	26.8 \pm 2.1	26.7 \pm 2.7	27.1 \pm 2.1	28.3 \pm 2.1
PEP-2	19.5 \pm 2.0	22.0 \pm 1.8	23.8 \pm 2.0	25.6 \pm 2.0 ^b	26.7 \pm 2.0 ^b	27.6 \pm 1.6 ^b
COC-1	20.7 \pm 1.3	23.6 \pm 1.2	26.9 \pm 1.8	25.9 \pm 1.8	27.3 \pm 1.7	28.7 \pm 1.6
COC-2	20.4 \pm 1.3	22.1 \pm 1.7	24.1 \pm 1.4	26.1 \pm 1.8	27.0 \pm 1.5	27.9 \pm 1.5 ^b

Note: The different lowercase letter superscripts mean that there was significant difference between experimental group and control group (CG) (*P* < 0.05).

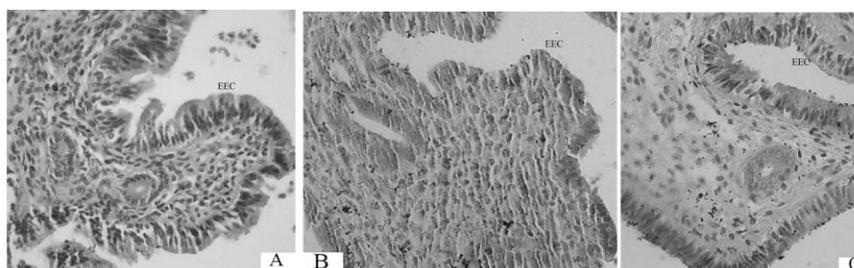


Fig.1: Histopathological changes of uteri in mice ($\times 400$). A, B and C represent CG, COC-2 and PEP-2 groups, respectively. EEC: endometrial epithelial cells.

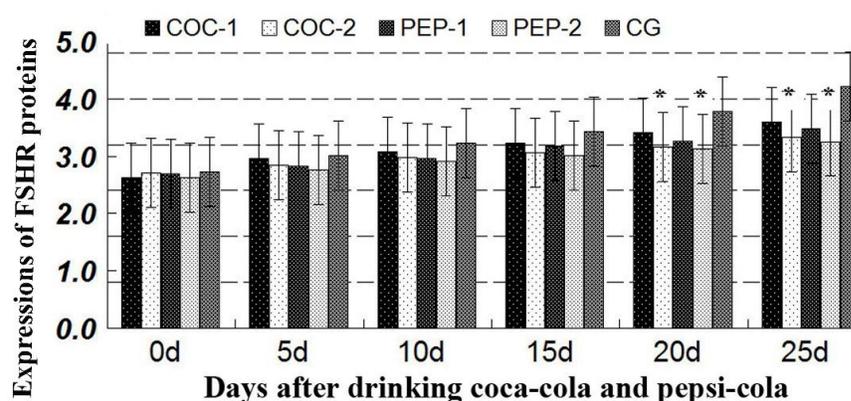


Fig.2: Levels of uterine FSHR proteins. **P* < 0.05 when compared to CG.

whole experiment (Table-1). At Day 15, the uterine weights of all experimental mice were decreased in comparison with CG. On day 25, the uterine weights of COC-2 and PEP-2 were significantly lower than that of CG (*P*, < 0.05). The findings indicated that oral ingestion of high doses of Coca-cola and Pepsi-cola for a longer duration could reduce uterine weights.

Histopathological Evaluation of Uterus

In the control group (CG), the uterine structure of

mice was clear and complete. The columnar cells of uterine glands were arranged regularly (Fig:1A). The endometrium was complete and was in secretory phase.

In the Coca-cola group, the uterine cavities were slightly decreased. The uterine wall mildly became thin (Fig:1B). The cytoplasm of endometrial epithelial cells (EEC) decreased slightly. The epithelium became shortened. The reduction of the endometrial folds and EEC cytoplasm was observed.

In the Pepsi-cola group, the uterine wall thinned noticeably, especially observed in the endometrium in comparison with CG on day 25 (Fig:1C). The uterine cavity became narrow slightly in PEP-2 mice. The cytoplasm of epithelial cells was reduced. The uterine mucous was ruffled. PEP-2 subgroup showed the uterine lumen and their glands were contracted. The reduction of the number of uterine glands was also observed. Less epithelial cells were observed in PEP-2. The results demonstrated that Coca-cola and Pepsi-cola affected the uterine histological structure, and also inhibited the uterine development of mice.

Uterine Wall Thickness (UWT)

The lower UWT was detected in the COC-2 group. However, UWT had no significant difference between the four experimental groups during the experiment.

The results indicated that the administration of Coca-cola and Pepsi-cola had no effects on the uterine wall thickness of mice.

Levels of FSHR Proteins in Uteri

In comparison with CG, FSHR protein levels in all experimental mice decreased during the whole

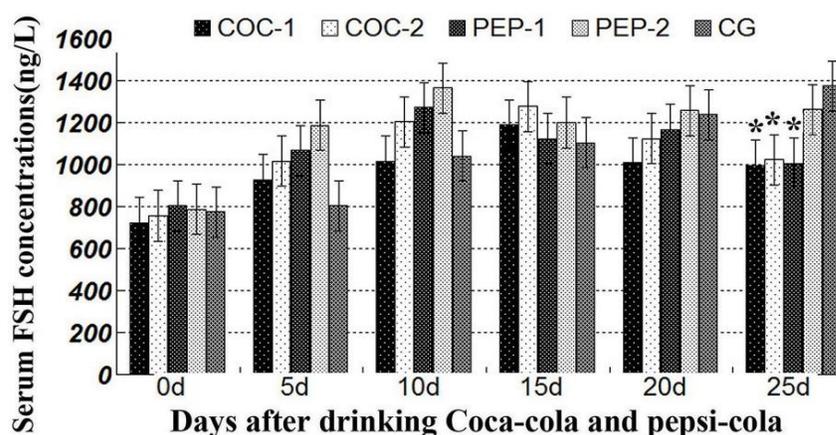


Fig.3: Changes of serum FSH concentrations. * $P < 0.05$ as compared to the control group (CG). ** $P < 0.01$ as compared with CG.

Table-2: Reproduction profiles female mice and new born mice.

Group	Pregnancy Rate (%)	Pregnancy Period (d)	Offspring Numbers	Mean Birth Numbers	Gender Ratio (M:F)	Survival Rate (%)
CG	4 (100.0) ^a	20.00 ± 1.02	52 ^a	10.5 ± 1.1	70.0:30.0 ^a	51 (98.1) ^a
PEP-1	5 (100.0) ^A	20.6 ± 1.5	55 ^B	11.0 ± 1.2	60.0:40.0	53 (96.4)
PEP-2	3 (60.0) ^{bb}	19.67 ± 1.1	28 ^{bc}	9.3 ± 1.0 ^B	44.4:55.6 ^b	26 (92.8)
COC-1	4 (80.0)	0.0 ± 0.8	48	12.0 ± 1.1 ^A	40.0:60.0 ^b	46 (95.8)
COC-2	4 (80.0)	21.0 ± 1.2	37 ^{ba}	9.0 ± 1.6 ^B	47.4:52.6 ^b	33 (89.2) ^b

Note: Compared to control group, the different lowercase letter superscripts mean that there was significant difference between groups ($P < 0.05$). The different capital letter superscripts represent that there was significant difference between experimental groups ($P < 0.05$). Survival rate and body weights of each group were determined on day 7 after offspring birth (or one-week-old).

Table-3: Body weights of new born mice (gm).

Group	0d	3d	5d	7d	10d	12d
CG	1.60 ± 0.09	2.36 ± 0.19	3.42 ± 0.24	4.25 ± 0.49 ^a	5.75 ± 0.30 ^a	6.38 ± 0.34 ^a
PEP-1	1.64 ± 0.13	2.55 ± 0.25	3.74 ± 0.26	4.64 ± 0.17	6.01 ± 0.28	6.89 ± 0.26
PEP-2	1.71 ± 0.10	2.53 ± 0.26	3.64 ± 0.24	4.84 ± 0.45 ^b	6.09 ± 0.31	7.30 ± 0.36 ^{bb}
COC-1	1.74 ± 0.13	2.48 ± 0.26	3.35 ± 0.30	4.05 ± 0.41	5.04 ± 0.32 ^{ba}	6.26 ± 0.24 ^A
COC-2	1.75 ± 0.14	2.93 ± 0.23	3.90 ± 0.33	5.17 ± 1.33 ^b	6.42 ± 0.33 ^{bb}	7.34 ± 0.25 ^{bb}

Note: Compared to control group, the different lowercase letter superscripts mean that there was significant difference between groups ($P < 0.05$). The different capital letter superscripts represent that there was significant difference between experimental groups ($P < 0.05$).

experiment (Fig:2). At days 20 and 25, FSHR protein levels of COC-2 and PEP-2 groups were significantly lower than that of control mice ($P < 0.05$). However, there was no significant difference between groups on other days. These findings demonstrated Coca-cola and Pepsi-cola had inhibitory effects on expressions of FSHR

protein in uteri of mice.

Serum FSH Concentrations

Serum FSH concentrations in four experimental groups were increased slightly in comparison with CG on days 5 and 10 (Fig:3). But, FSH contents of COC-1, COC-2 and PEP-1 were less than that of CG on day 25 ($P < 0.05$). The results indicated that Coca-Cola and Pepsi-Cola inhibited FSH secretions at a late period.

Fertility Index of Female Mice

There were no significant differences in pregnancy duration between all groups (Table-2). The pregnancy rate of PEP-2 mice was clearly lower than that of CG. Average new-born mice numbers were highest in COC-1 and lowest in COC-2 mice. The gender ratio (Male: Female) of new-born mice decreased in experimental mice. Male percentages of COC-1, COC-2 and PEP-2 groups were significantly lower than that of CG ($P < 0.05$). The survival rate of 1-week old mice was reduced in COC-2 when as compared to CG (Table-2) ($P < 0.05$).

The body weights of the new-born mice had no significant difference between all groups on days 0 to 5 (Table-3). On day 7 (1 week old), the body weight of new-born mice in PEP-2 and COC-2 groups were increased in comparison with CG ($P < 0.05$). Besides, the body weights of new-born mice in PEP-2 and COC-2 were higher than that of CG and COC-1 mice on day 12 ($P < 0.05$). The findings demonstrated a high concentration of Pepsi-cola and Coca-cola affected the fertility of female mice as well as growth and development of the new born mice.

DISCUSSION

Carbonated drinks, where the main components are sugar and caffeine, have been one of the crucial factors for diseases due to their increased consumption.¹ A great deal of evidence has accumulated concerning the effects of carbonated drinks (especially caffeine) consumption on reproduction and pre-and postnatal development.^{15,16} Caffeine consumption is one of the many factors that implicated in the reduction of fecundity. It could reduce the fecundity in

women.^{7,17} Epidemiological studies in animals and humans had investigated the association between caffeine ingestion and adverse reproductive/developmental effects. Participants with a higher caffeine intake had lower fecundity than those with a lower intake level.¹⁷ However, different results were reported about caffeine intake and a reduction in fertility.^{2,17} The mechanisms via which caffeine or caffeinated beverages could affect fertility are uncertain. Little is known about the relationship between carbonated beverage consumption and the risk of diseases in human beings.¹⁷

Carbonated drinks may alter hormone (such as estradiol) levels and may lead to pregnancy loss. They have been associated with alterations in the estradiol and other hormones.^{15,19} The current study indicated serum FSH concentrations in experimental mice were slightly increased on days 5 and 10. However, serum FSH of COC-1, COC-2 and PEP-1 was decreased on day 25. Up to date, little information in this aspect has been reported.^{20,21}

In the present study, the results demonstrated that the uterine weights of COC-2 and PEP-2 were significantly lower than that of CG ($P < 0.05$) on days 20 and 25. Uterine wall and uterine cavities were slightly decreased. Endometrial epithelial cells became shortened. The uterine mucous was ruffled. The epithelial cells were decreased in PEP-2. UWT of experimental group mice was lower than CG. These findings still have to be thoroughly explored because similar reports are very scanty. Therefore the accurate effects of carbonated beverages (including Coca-cola and Pepsi-cola) need to be further verified.

FSH can promote the proliferation and differentiation of pre-antral follicles. It also induces growth and maturation of ovarian follicles.²² FSHR has been found to be expressed in uteri. However, it is unknown whether high doses of Coca-cola and Pepsi-cola influence FSH synthesis and FSHR expression in uteri.^{8,23} The current study showed that FSHR protein levels in all experimental mice were decreased throughout the experiment. On days 20 and 25, FSHR protein levels of COC-2 and PEP-2 groups were significantly lower than that of control mice ($P < 0.05$). The results suggest that Coca-cola and Pepsi-cola can enhance FSH synthesis at an early stage and inhibit FSH secretions at a late period. These also inhibited

expression levels of FSHR proteins in mice uteri. The exact mechanisms remain unknown.

Caffeine consumption during pregnancy can have a potentially adverse impact on fetal growth. An epidemiological study indicated a low birth weight or intrauterine growth retardation in infants whose mothers were exposed to caffeine during pregnancy.⁸ In the present study, body weights of the new-born mice in COC-2 and PEP-2 groups were increased on day 7 in comparison with CG. The body weights of COC-1 mice were lower than that of CG. The findings indicated high doses of Coca-cola and Pepsi-cola could affect the growth of the new-born mice.

CONCLUSION

Oral intake of Coca-cola and Pepsi-cola for a longer duration can decrease uterine weights, affect the uterine histological structure, and also inhibited the uterine development. They reduced uterine expressions of FSHR protein, such affected development of the new-born offspring. The findings in this study havelaid a foundation and provided the scientific bases for further research on the effects and mechanisms of Coca-cola and Pepsi-cola on uterine development and reproduction functions in humans. These proteins provide the experimental basis for therapy of the related uterine diseases.

LIMITATIONS OF STUDY

There were a few limitations in the present study. Only two concentrations of Pepsi-cola and Coco-cola were used in the current study with wide difference. Secondly, experimental duration was short.

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CONFLICT OF INTEREST

None to declare.

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Author's Contribution

ZG: Design and acquisition of data.

SW: Conception of data and drafting of article.

LW, HL& LL: Analysis and interpretation of data.

All authors critically revised the manuscript for important intellectual contents and approved the final version.