Letter to the Editor

Maternal Genistein Intake Can Reduce Body Weight in Male Offspring*

ZHANG Yun Bo[1,†], YAN Jing Dong[2,†], YANG Su Qing[2,†], GUO Ji Peng[1], ZHANG Xiao[1], SUN Xiao Xi[1], NA Xiao Lin[1,#], and DAI Shao Chun[3,#]

The study objectives were to investigate the relationship between early exposure to genistein and obesity in young adulthood and to evaluate changes in reproductive health during puberty and adulthood following *in utero* exposure to genistein. Thirty-two female rats were randomized into four groups; low dose 400 mg genistein/kg diet group (LG), mid-dose 1200 mg genistein/kg diet group (MG), high dose 3600 mg genistein/kg diet group (HG), and control group without genistein diet (CON). Rats were fed genistein at the beginning of pregnancy along with a high-fat diet. Pups were sacrificed at week 4 and week 8 after birth. High performance liquid chromatography (HPLC) results showed a correlation between maternal genistein intake and genistein concentration in pups' plasma. Compared to CON, body weight reduced significantly in male HG group at week 8. No statistical differences were found in plasma estradiol (E2), testosterone (T), interleukin (IL)-6, and C-reactive protein (CRP) levels with early genistein exposure. Furthermore, uterine histopathology showed notable changes in groups HG and MG compared with CON at week 4 and week 8. In conclusion, maternal genistein supplement could reduce body weight in male pups and alter uterine histopathology in female pups.

Research has shown that approximately 25% of all formula-fed infants in the United States are fed soy-based infant formula (SBIF), and over 20 million infants in the United States have been fed soy formula. These formulas contain high levels of estrogenic isoflavonegenistein, raising concerns about acute and/or long-term adverse effects of neonatal genistein on reproductive and other organs. However, in 2009, a report from the National Toxicology Program Center for the Evaluation of Risks to Human Health Reproduction in the United States concluded that there is limited human data to form a definitive conclusion regarding the safety of SBIF.

Soy isoflavones, a subcategory of phytoestrogens, are naturally derived from soy plants. Genistein accounts for approximately two thirds of the soy isoflavone content. Genistein binds to estrogen receptor α but has greater affinity for estrogen receptor β. Much of the concern is associated with the fact that genistein may have potential estrogen-like activities, binding to estrogen receptor and thereby exert a biological effect. Ball et al. found 5 mg/kg of genistein during gestation and lactation could decrease anogenital distance[1]. Other study demonstrated exposure to genistein during gestation and lactation demasculinized the reproductive system in rats[2] and perinatal exposure to genistein altered reproductive development and aggressive behavior in male mice.

Besides estrogenic activity, genistein also has anti-inflammatory effect and down-modulates leptin production in adipocytes, resulting in reduce body weight. Su et al. showed female offspring of rats fed soy protein isolate, which contains abundant genistein significantly lowered body weight and abdominal fat mass[3]. Further investigations demonstrated maternal genistein intake at dose of 250 mg/kg protected mouse offspring from obesity. However, other studies showed different results. One study indicated genistein exposure during early postnatal period favors the development of obesity

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1. Department of Environmental Hygiene, Public Health College, Harbin Medical University, Harbin 150081, Heilongjiang, China; 2. The First Affiliated Hospital of Heilongjiang University of Traditional Chinese, Harbin 150040, Heilongjiang, China; 3. Department of Ultrasonography, Harbin Medical University Cancer Hospital, Harbin 150081, Heilongjiang, China

in females, but not males. Several studies have shown genistein has an effect on body weight and reproductive outcomes, however, there are limited studies demonstrating the impact of early-life genistein exposure on inflammatory markers and other hormone levels.

Adulthood adiposity can be programmed by developmental dietary exposures in experimental animal models and in humans. Maternal diet during pregnancy and breastfeeding may affect metabolic health in infant and their lives. Therefore, the current study aims to determine the effects of genistein exposure on reproductive development and body weight throughout gestation and lactation, and examine the changes in inflammatory markers and hormone levels in blood.

The changes in body weight of male pups in different groups are shown in Table 1. In males, maternal genistein intake did not alter body weight from week 2 to week 4 compared to control group. Nevertheless, body weight decreased significantly in three genistein groups at week 5 when compared to control group (P=0.000 for HG, P=0.000 for MG and P=0.002 for LG), and the decrease continued until the end of the experiment. High dose maternal genistein intake can reduce body weight in male offspring even at their adulthood (P=0.007). Furthermore, low dose genistein (400 mg/kg) appears to increase body weight but the results were not statistically significant. However, maternal genistein intake did not significantly alter body weight at week 8 in female pups (data not shown). In adult, several studies in obese humans and animals suggest genistein exhibit significant anti-obesity effects[4-5]. However, the effects of maternal genistein exposure on body weight in their offspring were not consensus[3,6-7]. In our study, we concluded high dose of genistein treatment could reduce body weight at week 5 in both males and females offspring and at week 8 in males when exposure to genistein was during pregnancy and lactation. Moreover, rats in the high dose genistein group consumed more food compared to the control group (data not shown), which suggests high dose of maternal genistein exposure could decrease food utilization rate even when they become adult. This is a new significant finding from the present study. Obesity, and particularly increased adiposity, are primary risk factors for numerous adult-onset diseases, and the rates of obesity are alarmingly high, both in the developed and developing worlds. Many measures are currently executed to reduce body weight such as exercise, diet, and even pharmacotherapy. Genistein intake during pregnancy and lactation appears to correlate with weight loss-an interesting measure for the increasingly high obesity rate. On the contrary, the side effect of maternal genistein should also be explored. In the present study, a dose-dependent response was observed in three genistein groups; the higher the dose, the lower the body weight. High dose of genistein treatment to dams may be toxic to their offspring resulting in reduced body weight and rate of food utilization. Furthermore, the reason for the difference in body weight changes between males and females should be studied further.

Pups were sacrificed and the organs were weighed at week 4 and week 8. In female rats, uterine and ovary weights increased in the group high and middle dose of genistein supplementation after normalization to body weight at week 4 and week 8 (Table 1. 4 week P=0.000 in HG and MG groups in uterus compared with CON group, P=0.002

Table 1. Effect of Maternal GenisteinIntake on Body Weight in Male Offspring

Group	Body Weight/g						
	2 week	3 week	4 week	5 week	6 week	7 week	8 week
HG	21.63 (18.73-33.94)	33.78 (23.78-47.58)	48.75 (39.32-51.71)	69.80 (61.40-77.15)*	117.90 (109.20-129.50)	174.00 (165.88-199.78)	217.60 (210.50-247.95)*
MG	18.42 (16.27-30.26)	27.31 (24.68-50.09)	40.87 (36.13-52.21)	78.25 (72.30-79.50)*	123.00 (112.05-154.05)	173.90 (165.30-201.10)	217.10 (211.35-245.85)
LG	27.02 (19.83-28.22)	30.24 (28.22-39.47)	41.02 (36.33-51.69)	72.15 (64.95-80.68)*	138.95 (125.80-167.825)	198.15 (177.23-235.50)	248.25 (220.78-296.13)
CON	18.81 (14.69-27.33)	29.26 (25.37-45.29)	46.40 (42.25-64.36)	89.80 (82.43-97.48)	136.65 (120.38-143.55)	196.50 (179.93-204.58)	257.20 (236.75-260.68)

Note. HG, high-dose group, 3600 mg genistein/kg diet; MG, mid-dose group, 1200 mg genistein/kg diet; LG, low-dose group, 400 mg genistein/kg diet; CON, control group without genistein diet. Data are expressed as median and inter-quartile range (25th-75th). Mann Whitney U test was used to compare each treatment group with a single control group. *P<0.008 was interpreted as a significant difference.

in MG group in ovarium compared with CON group). Phytoestrogen genistein, present in soy products, is of interest because *in utero* exposure to genistein caused reproductive problems in our rat model and maternal consumption of soy is prevalent in human populations. Li et al. found that decreased age at vaginal opening, increased length on estrus stage, and accelerated mammary gland development were detected in 100 and 500 ppm genistein-treated groups[8]. In our study, we found genistein could increase uterine weight in female offspring at week 4 and ovary weight at week 8 (Table 2). While other organ's weights such as the brain, liver, spleen, and kidney was not affected (data not shown). In male rats, high dose of genistein did not alter reproductive weight at week 4 and week 8 after normalization to body weight. Furthermore, neither dose of genistein affected other organs weights such as the brain, liver, spleen, and kidney (data not shown).

The pups' uterine pathology in each group with different dose of genistein treated is shown in Figure 1. In week 4, note tall columnar epithelial lining cells in HG group, columnar epithelial lining cells in MG group, and low columnar epithelial lining cells in LG group in uterine endometrium compared with low columnar epithelial lining cells in Control group. The number of follicles was notably increased in HG group compared with Control group. Meanwhile, the changes in the endometrial glandular epithelium in week 8 were similar to week 4. In addition, the secretory vacuole could be found in HG group and MG group in week 8. However, no marked changes were observed in epididymis pathology in male pups at week 4 and week 8. This is in accordance with Yang et al. who found tall columnar endometrial epithelial cells, large cytoplasm in endometrial cells, and vacuolar degeneration in isoflavone group[9].

Table 2. Effect of Maternal Genistein Supplementation on Reproductive Organ/body Weight Ratio in Female Offspring at Week 4 and Week 8

Group	4 week		8 week	
	Uterus	Ovarium	Uterus	Ovarium
HG	0.23 (0.20-0.31)*	0.08 (0.07-0.11)	0.28 (0.26-0.32)	0.12 (0.10-0.13)
MG	0.24 (0.19-0.27)*	0.09 (0.08-0.11)*	0.41 (0.33-0.51)	0.15 (0.09-0.17)
LG	0.12 (0.11-0.16)	0.08 (0.07-0.10)	0.29 (0.21-0.33)	0.12 (0.11-0.15)
CON	0.12 (0.10-0.14)	0.07 (0.06-0.08)	0.32 (0.28-0.36)	0.13 (0.09-0.18)

Note. HG, high-dose group, 3600 mg genistein/kg diet; MG, mid-dose group, 1200 mg genistein/kg diet; LG, low-dose group, 400 mg genistein/kg diet; CON, control group without genistein diet. Data are expressed as median and inter-quartile range (25th-75th). Mann Whitney *U* test was used to compare each treatment group with a single control group. *$P<0.008$ was interpreted as a significant difference.

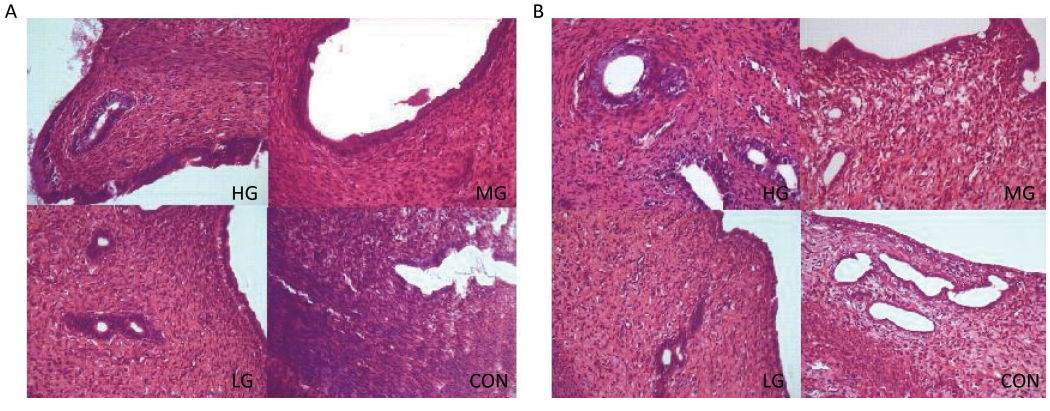

Figure 1. Uterine pathology of female offspring treated with maternal genistein at week 4 (A) and week 8 (B). HG, high-dose group, 3600 mg genistein/kg diet; MG, mid-dose group, 1200 mg genistein/kg diet; LG, low-dose group, 400 mg genistein/kg diet; CON, control group without genistein diet.

The serum concentration of genistein in every group was detected by HPLC. The serum genistein in offspring correlated with maternal genistein intake. Compared with the control group, serum genistein concentrations in HG group and MG group were markedly higher in their offspring (P<0.05). In addition to insulin, estradiol (E2), testosterone (T), interleukin (IL)-6, and C-reactive protein (CRP) serum levels were measured in offspring at week 4 (data not shown) and week 8. Genistein had no effect on these serum levels in both males and females.

Napier et al. reported serum concentrations of E2 and T decreased in male offspring (whose mothers were given isoflavone soy diet) at 22 days of age compared to control, but measurements were equivalent at 35 days, and no differences observed in serum E2 concentrations at 96 days of age but serum T concentrations decreased[10]. This means maternal isoflavone intake had an effect on E2 and T serum level fluctuation in rats. The mechanism of testosterone reduction might be genistein could inhibit testosterone secretion by fetal Leydig cells during early fetal development, within the 'masculinization programming window'. However, no differences were found in E2 and T levels between genistein groups and control group in our research. The reasons for this could be due to the fluctuation of hormone levels affected by maternal genistein intake and the large variation of data.

Increasing evidence has suggested that inflammation may be associated with obesity and metabolic syndrome. In the present study, despite evidence supporting maternal genistein intake during pregnancy and lactation can reduce body weight in male offspring, no changes were found in inflammatory markers IL-6 and CRP serum levels. This suggests that body weight alone does not affect inflammation, but also the type of intervention, abdominal fat, menstrual cycle, drugs, etc.

[†]ZHANG Yun Bo, YAN Jing Dong, and YANG Su Qing contributed equally to this work.

[#]Correspondence should be addressed to NA Xiao Lin, PhD, Tel: 86-451-8750-2839, E-mail: naxiaolin1495@sohu.com; DAI Shao Chun, PhD, Tel: 86-451-8750-29025, E-mail: daishaochun@yeah.net

Biographical notes of the first authors: ZHANG Yun Bo, female, born in 1982, majoring in bioactivities of phytoestrogen isoflavone; YAN Jing Dong, male, born in 1964, Associate Chief Physician, majoring in traditional Chinese medicine; YANG Su Qing, female, born in 1964, Professor, majoring in traditional Chinese medicine.

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REFERENCES

1. Ball ER, Caniglia MK, Wilcox JL, et al. Effects of genistein in the maternal diet on reproductive development and spatial learning in male rats. Horm Behav, 2010; 57, 313-22.
2. Wisniewski AB, Klein SL, Lakshmanan Y, et al. Exposure to genistein during gestation and lactation demasculinizes the reproductive system in rats. J Urol, 2003; 169, 1582-6.
3. Su Y, Shankar K, Simmen RC. Early soy exposure via maternal diet regulates rat mammary epithelial differentiation by paracrine signaling from stromal adipocytes. J Nutr, 2009; 139, 945-51.
4. Sakai T, Kogiso M. Soy isoflavones and immunity. JMI, 2008; 55, 167-73.
5. Merritt JC. Metabolic syndrome: soybean foods and serum lipids. J Nati Med Assoc, 2004; 96, 1032-41.
6. McClain RM, Wolz E, Davidovich A, et al. Reproductive safety studies with genistein in rats. Food Chem Toxicol, 2007; 45, 1319-32.
7. Tousen Y, Umeki M, Nakashima Y, et al. Effects of genistein, an isoflavone, on pregnancy outcome and organ weights of pregnant and lactating rats and development of their suckling pups. J Nutr Sci Vitaminol (Tokyo), 2006; 52, 174-82.
8. Li R, Zhao F, Diao H, et al. Postweaning dietary genistein exposure advances puberty without significantly affecting early pregnancy in C57BL/6J female mice. Reprod Toxicol, 2014; 44, 85-92.
9. Yang Y, Zhang YB, Sheng W, et al. Influence of soy isoflavone on lindane cumulant in sprague-dawley rats. Biomed Environ Sci, 2014; 27, 637-40.
10. Napier ID, Simon L, Perry D, et al. Testicular development in male rats is sensitive to a soy-based diet in the neonatal period. Biol Reprod, 2014; 90, 40.