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Peri-pubertal high caffeine exposure increases ovarian estradiol production in immature rats

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ABSTRACT

Chronic caffeine consumption exerts a negligible effect on the reproductive organs of normal adult females, but it is not known whether this is also true for children and adolescents. Here, we investigated the effects of high caffeine exposure on sexual maturation and ovarian estradiol production in immature female rats. Immature female SD rats were divided into controls and caffeine groups fed 120 and 180 mg/kg/day for 4 or 8 weeks. There was a significant delay in vaginal opening in the caffeine-fed groups. In addition, serum estradiol levels were elevated in the caffeine-fed animals after 2 and 4 weeks of exposure. Estradiol secretion as well as aromatase expression also increased significantly in the ovarian cells in response to caffeine. These results demonstrate that peripubertal exposure to high caffeine increases estradiol production in the ovary; this may disturb the coordinated regulation of the hypothalamo-pituitary-ovarian axis, thereby interfering with sexual maturation.

1. Introduction

Caffeine is now increasingly available in energy drinks whose unrestricted availability makes them easily accessible by younger children [1–3]. Approximately 75–95% of children and adolescents consume caffeine on a regular basis, and 12% can be classed as 'high chronic' users [3]. In general, daily caffeine consumption for children and adolescents is recommended not to exceed 2.5 mg/kg/day, equating to one small cup of coffee [4]. However, most current energy drinks have about 100 mg of caffeine per serving and a few of them have up to 500 mg per serving [2,5].

It has been reported that caffeine caused a significant increase in estradiol secretion by H295R cells (a human adrenocortical carcinoma cell line) [6]. In addition, perturbation of steroid hormone balance at higher caffeine intakes has been documented in men and male rabbits [7,8]. Similarly, there is suggestive evidence for a positive or negative association between caffeine intake and estrogen levels in postmenopausal [9–11] or premenopausal women [10,12]. As mentioned above, evidence from laboratory studies and human cases has raised concern that high caffeine exposure may alter sex hormone levels and interfere with the endocrine system.

Adolescence is a critical period for the growth and maturation of the reproductive organs, and is characterized by extensive morphological and functional changes [13], suggesting that caffeine might have an even more pronounced impact on the reproductive system than it does in adults. Human studies have generally been of small size and flawed by inaccurate assessment of exposure levels because they relied on self-reporting of dietary data. Furthermore, most studies have been carried out in adults and cannot be generalized to children and adolescents. On the other hand, previous clinical experiments have shown gender differences in cardiovascular response to caffeine [14] as well as in caffeine elimination rate, which even fluctuated across the ovarian cycle in rats and women [15,16]. Although peripubertal caffeine exposure has been shown to affect testosterone production in the testis of immature rats [17], the possibility that it may disturb ovarian steroidogenesis during female puberty has not been examined. Estrogens are potent regulator of physiological responses and play an important role during the female pubertal period. In fact, chemically-induced changes in estrogen production can alter the onset of puberty and ovulatory cycles [18,19]. Furthermore, impairment of the endocrine processes

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Table 1
Effect of caffeine exposure on body weight gain and body fat.

Parameters		Group	Experiment 4 weeks	8 weeks
Weight gain (g)		CT	138.9 ± 14.8	214.7 ± 24.7
		CF1	107.8 ± 12.8***	163.1 ± 18.3***
		CF2	103.5 ± 10.8***	162.0 ± 13.9***
Fat	mass (g)	CT	22.4 ± 6.18	54.9 ± 15.43
		CF1	13.6 ± 5.00**	28.6 ± 10.78**
		CF2	16.6 ± 4.04*	24.9 ± 9.39***
	% of TBM	CT	11.6 ± 3.04	20.8 ± 4.90
		CF1	8.7 ± 3.32	13.6 ± 4.96*
		CF2	10.8 ± 2.28	11.8 ± 4.49**

Values are expressed as mean ± SD of 10 animals per group. Body weight gain (g) = terminal body weight − initial body weight. TBM, total body mass; fat%, total body fat divided by TBM. CT, control; CF1, caffeine 120 mg/kg/day; CF2, caffeine 180 mg/kg/day. *$p < 0.05$, **$p < 0.01$, *** $p < 0.001$ vs. CT.

Table 2
Effect of caffeine on reproductive organ weight.

Experiment		4 weeks			8weeks		
Group		CT	CF1	CF2	CT	CF1	CF2
Ovarian weight (mg)	Absolute	36.7 ± 5.8	21.3 ± 4.7***	20.9 ± 3.9***	39.6 ± 7.4	38.1 ± 7.4	40.0 ± 6.4
	Relative	19.1 ± 3.4	13.1 ± 2.1***	13.2 ± 2.3**	14.8 ± 2.6	17.6 ± 2.9*	18.6 ± 3.0*
Uterine weight (mg)	Absolute	357.9 ± 64.9	265.9 ± 78.4*	362.8 ± 168.0	563.1 ± 173.6	578.6 ± 192.9	503.1 ± 367.4
	Relative	187.8 ± 41.9	168.0 ± 41.4	236.1 ± 102.1	214.6 ± 63.5	279.6 ± 104.5	238.3 ± 175.7
Uterine length (mm)		51.9 ± 10.2	36.4 ± 7.2**	40.4 ± 7.3*	59.3 ± 20.0	58.3 ± 20.9	44.8 ± 15.8*

Values are means ± SD of ten rats per group at each designated time point. Organ weights relative to terminal body weight are presented as relative weight (mg) per 100 g body weight. Ovarian weights are the sum of left and right weights. Uterine length is the sum of the lengths of the two uterine horns. CT, control; CF1, caffeine 120 mg/kg/day; CF2, caffeine 180 mg/kg/day. *$p < 0.05$, **$p < 0.01$, *** $p < 0.001$ vs. CT.

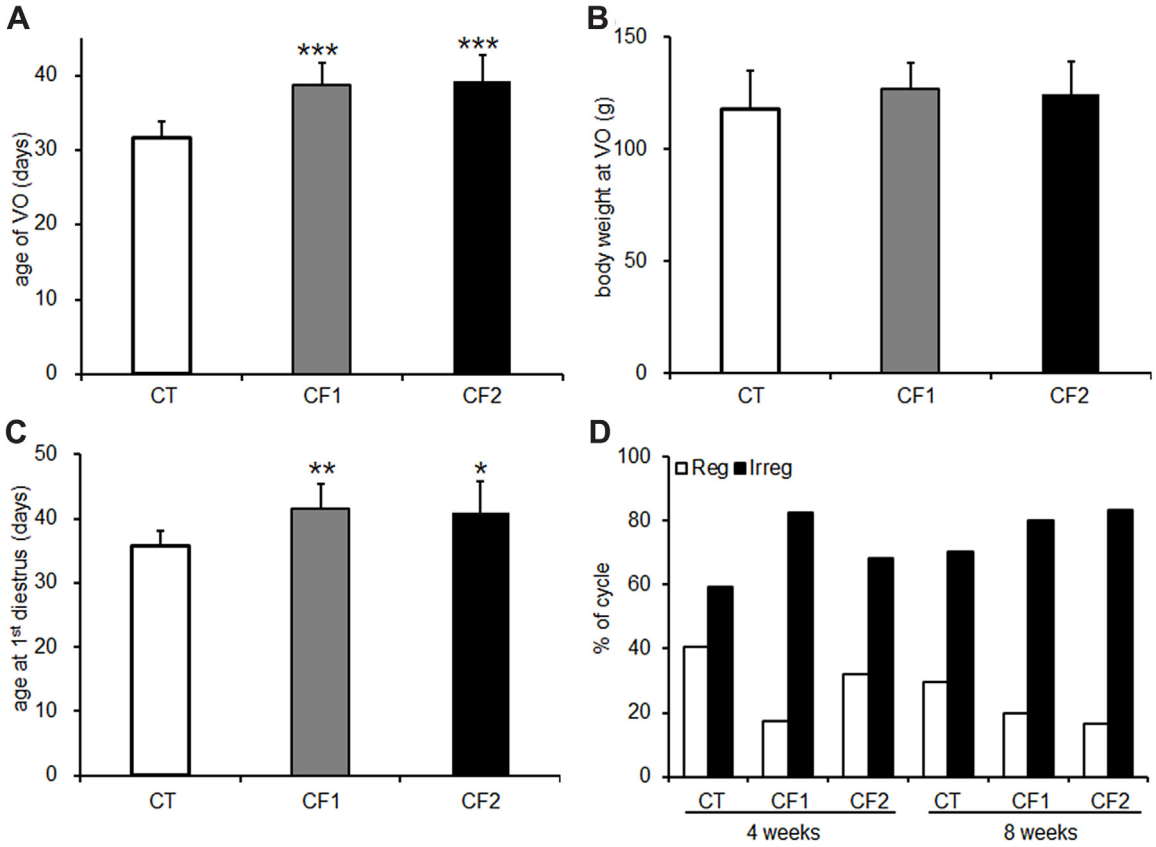

Fig. 1. Effects of peripubertal caffeine exposure on female puberty onset and estrous cycles. Puberty onset was determined by (A) mean age and (B) body weights at vaginal opening, and (C) mean age at first diestrus. Values are expressed as mean ± SD. (D) Proportions (% of total cycles) of regular and irregular estrous cycles after 4 and 8 weeks. The cell types present in vaginal swabs were used to determine the stage of the estrous cycle from vaginal opening to the end of the experiment. Data are presented as mean percentages of regular and irregular cycles in each of the groups. CT, control; CF1, caffeine 120 mg/kg/day; CF2, caffeine 180 mg/kg/day. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ vs. CT.