

Sex Difference of Adenine Effects in Rats: Renal Function, Bone Mineral Density and Sex Steroidogenesis

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Abstract. Adenine is widely used in clinical field, however, an excess of adenine is harmful. It is known that the feeding of an adenine-rich diet induces renal failure and decreases bone mineral density (BMD) and the serum testosterone level in male rats. However, there is little information about the influence of adenine on female animals. We compared the effects of adenine treatment between male and female rats. Young male and female rats were administered adenine adjusted with distilled water (6 mg/ml, 50 mg/ml and 100 mg/ml) for 8 weeks (3 times/week, 8–16 week old). In male rats, renal failure was induced by 100 mg/ml adenine treatment and renal dysfunction was induced by 50 mg/ml adenine treatment. Bone loss and the reduction of the testosterone level were also caused by both concentrations of adenine. However, the serum testosterone level and BMD in male rats were decreased by 6 mg/ml adenine treatment by which renal dysfunction was not caused. It is suggested that adenine directly affected bone metabolism and sex steroidogenesis in male animals, not through altering renal dysfunction. In female rats, conversely, renal dysfunction was induced only in the 100 mg/ml group, which was somewhat different from the observation in male rats. The serum 17-beta estradiol level and the BMD in female rats were not affected by adenine treatment at all. In conclusion, there is a significant difference of the effects of adenine, which is commonly contained in medicine and general foods, on steroidogenesis and renal function between male and female rats.

Key words: Adenine, Bone loss, Sex difference, Testosterone, 17 beta-HSD

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ADENINE is a common chemical substance, and a constituent of nucleic acid in the living body. Adenine is clinically used to increase the leukocyte production in patients with leukopenia; however, undesirable side effects such as hyperuricemia, urolithiasis and renal failure are known to occur. Adenine-induced renal impairment was reported by Bendich *et al.* [1] and Philips *et al.* [2]. A new animal model of chronic renal failure induced by adenine-rich diet was reported by Yokozawa *et al.* [3]. Adenine phosphoribosyltransferase (APRT)

is a ubiquitously expressed enzyme that catalyzes the synthesis of adenosine monophosphate from adenine and 5-phosphoribosyl-1-pyrophosphate [4]. Adenine is produced endogenously as a by-product of the polyamine pathway and by the reaction of adenosine with *S*-adenosylhomocysteine hydrolase [5, 6]. In the absence of functional APRT, adenine is oxidized to 8-hydroxyadenine by xanthine dehydrogenase, and then oxidized to 2,8-dihydroxyadenine (DHA) [7]. The sparingly soluble nature of DHA results in the excretion of DHA crystals in the urine and, frequently, the deposition of DHA stones in the kidneys. Feeding of adenine-rich diet induces renal failure by the increase of DHA in male rats [8]. On the other hand, Okada *et al.* reported the reversibility of adenine-induced renal failure in the discontinuation of the

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adenine diet [9]. Chronic renal failure is associated with severe complications, including ectopic calcification of soft tissues, secondary hyperparathyroidism, and renal osteodystrophy (ROD). It is commonly documented that renal insufficiency is responsible for gonadal impairment. However, Adachi *et al.* [10] reported that adenine-induced renal failure rats differ from 5/6 nephrectomized rats in the causes of the decrease of testicular testosterone synthesis. Negi [11] reported about ROD in adenine-induced chronic renal failure male rats, but discontinuation of the adenine diet showed recovery in the bone mineral density (BMD) of the femur in the affected rats.

Therefore, we thought that the inhibition of testosterone synthesis and the lower value of the BMD are caused by adenine independently of renal impairment. In our previous study of adenine administration to the male rats, the decrease of the serum testosterone level and the decrease of BMD were shown without renal failure in low dose adenine. However, the research on the influence of adenine in female rats, including renal failure, is scarce. In this study, we investigated the influence of the adenine treatment to female rats, and discussed the sex difference towards adenine treatment.

Materials and Methods

Animals and treatments

This study was designed so as not to give any extra pain to the animals according to the Japanese Law on the Protection of Animals and the ethics committee regulations of the Suzuka University of Medical Science. Young male and female rats (both 7 weeks old) of the Sprague Dawley strain were purchased from CLEA Japan, Inc. (Tokyo, Japan). They were housed under controlled lighting conditions (12 hours light, 12 hours dark) and temperature (22 ± 2 celcius). They were given free access to food (CE-2: CLEA Japan, Inc.) and water. The animals were used for the study after a 1-week acclimation period. During the acclimation period, the BMD of their lumbar (L5 and L6) and femur (left and right) were measured with dual energy X-ray absorptiometry (DCS-600R: ALOKA CO., LTD, Tokyo, Japan). Male rats and female rats were divided into 4 groups, each based on mean lumbar BMD. Three concentrations (6 mg/ml, 50 mg/ml and 100 mg/ml) of adenine (Wako Pure Chemical Industries, Ltd.,

Osaka, Japan) were adjusted with distilled water. In the normal group, the rats were not given any treatment. In the adenine-treated groups, the rats were administered adenine in a quantity of 2 ml/kg body weight for 8-weeks (3 times/week). Although the experiment time was different between the male rats and female rats, the experimental conditions were the same.

Measurement of BMD, urinalysis data and serum chemistry and hormone

The body weight of each rat was measured at the beginning of the study and every week during the study. On the 4th and 8th week (12 and 16 weeks old), the rats were anesthetized with pentobarbitalsodium (Nembutal: Dainippon Pharmaceutical Co., Ltd., Osaka, Japan) and their BMD measured. On the 8th week, urine was collected in metabolic cage for 24 hours (food was not given), and then the blood was collected after anesthesia with diethyl-ether (Wako Pure Chemical Industries, Ltd., Osaka, Japan). The urine volume, the levels of creatinine, calcium and inorganic phosphate in urine were measured. The serum levels of creatinine, testosterone (in male rats, RIA method) and 17-beta estradiol (in female rats, RIA method) were measured.

Statistical analysis

Vertical bars represent the mean \pm standard deviation (SD) in the figures and tables. Statistical differences between the normal group and each adenine-treated group were determined by one-way ANOVA followed by Dunnett's test, and $P < 0.05$ was taken to indicate significance.

Results

Table 1 indicates the increase of body weight. On the 8th week, the 50 mg/ml and 100 mg/ml male groups had significantly lower weight gain than the normal group. In the female rats, increase of body weight with adenine treatment was roughly similar to that in the male rats; however, the 50 and 100 mg/ml groups were inhibited in increase of body weight. There was no weight gain between the 4th and 8th week in both the male and female 100 mg/ml group. Urinalysis data are shown in Fig. 1 (a–d: male rats, e–h: female rats). The 50 mg/ml and 100 mg/ml male

Table 1. Increase of body weight (g) in normal and adenine-treated male and female rats since the start of the experiment

group (n)	2nd week	4th week	8th week
male			
normal (7)	19 ± 7	118 ± 17	214 ± 30
6 mg/ml (9)	19 ± 8	106 ± 26	183 ± 36
50 mg/ml (9)	14 ± 9	101 ± 25	163 ± 40*
100 mg/ml (9)	7 ± 14	91 ± 22	95 ± 43***
female			
normal (9)	21 ± 2	58 ± 8	95 ± 17
6 mg/ml (8)	20 ± 4	56 ± 10	89 ± 14
50 mg/ml (10)	20 ± 4	50 ± 4*	79 ± 10*
100 mg/ml (9)	21 ± 10	42 ± 11**	38 ± 19***

Data are expressed as the mean ± SD, and the differences between the normal group and each adenine-treated group were evaluated for significance using one-way ANOVA followed by Dunnett's test (*P<0.05, **P<0.01 and ***P<0.001). In the adenine-treated groups, rats were administered adenine in a quantity of 2 ml/kg body weight for 8-weeks (3 times/week, 8–16 week old).

Table 2. Serum levels of creatinine (Cr), testosterone (T) and 17 beta-estradiol (E₂) in 8th week

group	male		female	
	Cr (mg/dl)	T (ng/ml)	Cr (mg/dl)	17 beta-E ₂ (pg/ml)
normal	0.47 ± 0.05 (7)	1.93 ± 0.30 (7)	0.72 ± 0.11 (9)	34.5 ± 7.1 (9)
6 mg/ml	0.51 ± 0.09 (9)	1.10 ± 0.61 (9)**	0.73 ± 0.09 (8)	37.3 ± 7.9 (8)
50 mg/ml	1.02 ± 0.23 (9)***	0.82 ± 0.45 (9)**	0.70 ± 0.05 (10)	34.1 ± 10.8 (10)
100 mg/ml	3.15 ± 0.46 (6)***	0.14 ± 0.07 (6)***	0.94 ± 0.15 (9)**	33.8 ± 7.0 (9)

Data are expressed as the mean ± SD, and the differences between the normal group and each adenine-treated group were evaluated for significance using one-way ANOVA followed by Dunnett's test (**P<0.01 and ***P<0.001). () represents the number of animals. In the adenine-treated groups, rats were administered adenine in a quantity of 2 ml/kg body weight for 8-weeks (3 times/week, 8–16 week old).

Table 3. The mean bone mineral density of lumbar (L5 and L6) and femur (left and right) in each group (mg/cm²)

group	male		female	
	lumbar	femur	lumbar	femur
normal	193 ± 6 (7)	240 ± 9 (7)	185 ± 11 (9)	223 ± 7 (9)
6 mg/ml	175 ± 13 (9)**	227 ± 10 (9)*	184 ± 11 (8)	224 ± 11 (8)
50 mg/ml	187 ± 9 (9)	226 ± 9 (9)**	193 ± 13 (10)	225 ± 9 (10)
100 mg/ml	176 ± 7 (9)**	206 ± 10 (9)***	183 ± 8 (9)	220 ± 6 (9)

Data are expressed as the mean ± SD, and the differences between the normal group and each adenine-treated group were evaluated for significance using one-way ANOVA followed by Dunnett's test (*P<0.05, **P<0.01 and ***P<0.001). () represents the number of animals. In the adenine-treated groups, rats were administered adenine in a quantity of 2 ml/kg body weight for 8-weeks (3 times/week, 8–16 week old).

groups had significantly increased urine volumes compared with the normal group (Fig. 1-a). The excretion of creatinine (Cr) and inorganic phosphate (P) in the 100 mg/ml male group were significantly lowered compared with those in the normal group (Fig. 1-b and 1-d), while the excretion of calcium (Ca) was signifi-

cantly increased (Fig. 1-c). The adenine-treated female rats showed somewhat different urinalysis data compared to the male rats. As shown in Fig. 1-e to 1-h, the excretion of Cr in the 100 mg/ml female group was similar to that in the 100 mg/ml male group, which was lower compared to the normal group. However, the

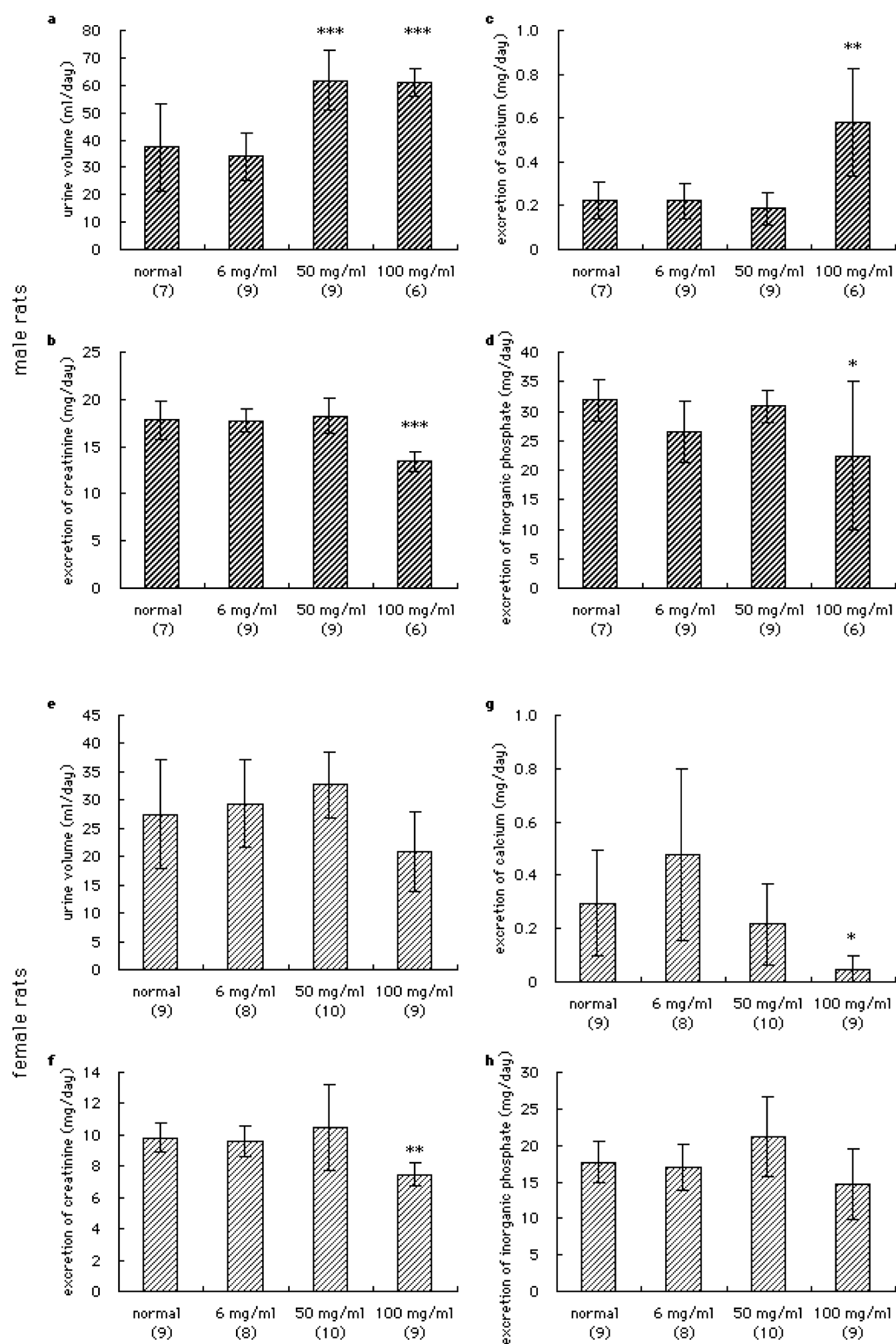


Fig. 1. Urinalysis data of urine volume, creatinine, calcium and inorganic phosphate in male rats (a-d) and female rats (e-h). () represents the number of animals. Data are expressed as the mean \pm SD, and the differences between the normal group and each adenine-treated group were evaluated for significance using one-way ANOVA followed by Dunnett's test (* $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$). In the adenine-treated groups, rats were administered adenine in a quantity of 2 ml/kg body weight for 8-weeks (3 times/week, 8-16 week old).

urine volume and excretion of P in female rats were not different among each group. Furthermore, the excretion of Ca in the 100 mg/ml adenine-treated female rats decreased compared to that in normal female rats (Fig. 1-g).

As shown in Table 2, the 50 mg/ml and 100 mg/ml male groups were significantly higher in their serum Cr level than the normal group and the 100 mg/ml male group was considerably higher than the 50 mg/ml male group. In the serum Cr level of female rats, only the 100 mg/ml group was the significantly higher than the normal group. As for the serum concentration of testosterone (Table 2), the 6 mg/ml and 50 mg/ml male groups were significantly lower than the normal group, and the 100 mg/ml group showed considerably lower value. Conversely, female rats showed no difference in their serum 17 beta-estradiol (E_2) level among each group. The BMD on 8th week (16 weeks old) is shown in Table 3. The 6 mg/ml and 100 mg/ml male group showed a lower spinal BMD compared to the normal group. The femoral BMD decreased in accordance with the reduction of serum testosterone levels. Conversely, female rats showed no difference in the BMD among each group. The sex difference to adenine administration was clearly indicated in these results.

Discussion

Yokozawa *et al.* [3, 8, 12, 13] reported that the long-term feeding of adenine-rich diets to male rats produced metabolic abnormalities resembling renal failure in human and DHA nephrolithiasis. In our results, excess adenine, namely 100 mg/ml, caused renal failure in male rats. The 50 mg/ml adenine-treated male rats showed renal dysfunction with an increase in urinary volume and in the serum Cr level. The renal insufficiency groups showed lower serum concentration of testosterone. However, we noticed that the testosterone (T) level was reduced in the 6 mg/ml adenine-treated male rats, but they showed no influence on the renal function in this study. According to the testicular histology report using both stepwise-nephrectomized and adenine-treated renal failure male rats, abnormality of the testicular tissue was not detected [14]. However, the serum T level was reduced in the adenine-induced uremic rats, and interestingly, testosterone reduction in such animals occurred via the inhibition of testosterone synthesis enzyme (17 beta-hydroxysteroid dehydro-

genase), and it is distinct from nephrectomized animals [10, 15, 16].

So far, several distinct isoenzymes of 17 beta-hydroxysteroid dehydrogenase (17 beta-HSD) have been characterized and cloned [17–22]. Type 1 17 beta-HSD (17 beta-HSD1) is a cytosolic nicotinamide adenine dinucleotide phosphate (NADPH)-dependent enzyme, which preferentially metabolizes estrone (E_1) to estradiol (E_2). Type 2 17 beta-HSD is a nicotinamide adenine dinucleotide (NAD)-dependent microsomal enzyme, which carries out the reverse reaction of 17 beta-HSD1 and oxidizes E_2 to E_1 . Type 3 17 beta-HSD (17 beta-HSD3) is an NADPH-dependent microsomal enzyme, which preferentially catalyzes the conversion of androstenedione to T in the testes. According to the report on tryptophan-niacin metabolism in adenine-induced renal failure male rats, the conversion ratio of tryptophan to niacin was lowered in the adenine group, and the contents of NAD and NADP in liver, kidney and blood were also lowered in the adenine group [23]. NADPH related to 17 beta-HSD3 is the reduced form of NADP, and it is thought that the decrease in NADP is the causes of reduction of 17 beta-HSD3 activity.

The followings are considered to be the factors that lead to the reduction of BMD due to renal failure: Secondary hyperparathyroidism, a decrease of the synthesis of active vitamin D and an abnormality metabolism of calcium. However, in this study, BMD was reduced in the 6 mg/ml adenine-treated male rats, which indicated no influence on renal function. It is thus suggested that the reduction of the T level and the reduction of BMD on low dose adenine were caused directly by adenine, and not by renal failure.

Androgens are known to stimulate bone formation and play an important role in the maintenance of bone status [24–28], and more recent studies have suggested that testosterone may have significant effects on osteoblast development and activity *in vivo* [29, 30]. Actually, a clinical trial report [31] suggested that dihydrotestosterone was not essential for the beneficial effects of testosterone on BMD in older men. In female rats, the serum level of 17-beta E_2 and BMD were not affected by adenine treatment at all, with or without renal dysfunction. Our study defined the sex difference in the influence of adenine treatment. Therefore, we guess that the reduction of testosterone synthesis by adenine in male rats leads to decreased BMD.

Adenine may have no influence on tryptophan-

niacin metabolism in female rats, unlike the case in male rats [23]. On the other hand, according to the report on steroidogenesis in metyrapone treated male rats, the serum T level and the specific activity of 17 β -HSD in the testes were significantly decreased, while the serum E₂ level was not markedly altered compared to control [32]. Only the testosterone synthesis might be possibly influenced by the adenine, such as in the above-mentioned report [32]. The testosterone synthesis pathway is relatively simple, while the estrogen synthesis pathway has various bypaths. This may be the reason the male rats indicated a more severe effect due to adenine compared with the female rats.

This study could shed light on the effect of adenine on bone metabolism through sex hormone synthesis. Adenine is commonly contained in medicines and gen-

eral foods. These findings suggest that sex differences should be fully taken into account in considering the response to general medicine, including the occurrence of adverse events. Further understanding is necessary of the fundamentals of Gender Specific Medicine.

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