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# Miso (Japanese soybean paste) was protective against hypertension compared to same amount of sodium chloride diet in Dahl salt-sensitive rats

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## Abstract

The purpose of this study was to compare the effects of Miso and sodium chloride (NaCl) on blood pressure in both sexes Dahl and SD rats. The systolic and diastolic blood pressures were measured at 2, 4, 8 and 12 weeks of treatment with a Miso diet including 2.3%NaCl or diets with 2.3% and 1.9% NaCl or normal diet (MF diet, Oriental Yeast Co) with 0.3% NaCl diet. The rats were autopsied after 12 weeks on a diet. Diastolic blood pressure in male Dahl rats was significantly increased by the 2.3% NaCl diet as compared with the MF group ($P<0.01$) or Miso group ($P<0.05$) from 4 weeks of treatment. After that diastolic and systolic blood pressures in both high NaCl groups were significantly increased when compared with the MF or Miso group. Systolic blood pressure in female Dahl rats on 2.3% NaCl was significantly increased from 8 weeks after treatment. Nephropathy was observed in both sexes of Dahl rats but not SD rats. These results show that blood pressure is not increased by the Miso diet.

Key words: Miso, NaCl, hypertension, rat

## Introduction

A traditional ingredient of the Japanese diet, Miso, is fermented from

soybeans, rice, wheat, or oats. It contains vitamins, microorganisms, salts, minerals, plant proteins, carbohydrates, and fat. It is used on a daily basis as a flavor in soup and solid food in Japan and other parts of Asia and remains an essential ingredient for Japanese-style cooking. We have described chemopreventive effects of Miso against intestinal injury in X-irradiated mice (1) and it has also been reported to reduce the occurrence of liver (2,3) and gastric tumors (4), as well as development of aberrant crypt foci (ACF) in a dose-dependent manner in experimental animals (5). However, the effects of soy beans and related foodstuffs on cancer risk are complex (6). Recently, our experimental studies provided evidence that long-term fermented Miso is quite effective in aiding the recovery of stem cells in the small intestinal crypts after irradiation (7), and in decreasing the development of azoxymethane -induced ACF (8) and colon cancers (9), and also pulmonary adenocarcinomas induced by dipropanolnitrosamine (10). We also reported that the glandular stomach adenocarcinoma incidence induced by *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine was significantly decreased in Miso fed groups as compared to stomach cancer on a diet with an equivalent NaCl without Miso (11).

Epidemiologic studies have observed a relationship between dietary NaCl

intake and blood pressure (12-16). The National Nutrition Survey shows that approximately one-eighth of the dietary salt intake in the Japanese diet comes from Miso (17). Therefore, the source of the dietary salt in Japanese diet, which is very different from that in Western countries, could be the target for a salt reduction strategy. Low-sodium soy sauce and miso are generally considered to have less taste. Kanda et al (18) reported miso-soup intake at two bowls per day or over was protective against hypertension during four-year follow-up. From the viewpoint of hypertension prevention, it is interested the association between the level of blood pressure and lifestyle parameters. In the present study, we investigated the effects of Miso with NaCl as compared to sodium chloride (NaCl) without Miso, one of its main constituents, on the blood pressure in sodium chloride sensitive Dahl rats.

## Materials and Methods

### Animals

Dahl (Dahl S/Jr Sea) salt-sensitive male rats, 5 weeks of age at the commencement of the study, were purchased from Seac Yoshitomi, Ltd (Yoshitomi, Japan). Similar Sprague-Dawly (SD) rats (Crj:CD(SD) IGS) which was the original strain of Dahl salt-sensitive rats, were obtained from

Charles River (Hino Japan). The rats were housed five to a polycarbonate cage under constant conditions of temperature (24±2℃) and relative humidity (55±10%), with a 12:12-hour light-dark cycle. The animals were maintained according to the "Guide for the Care and Use of Laboratory Animals" established by Hiroshima University. All were given free access to a commercial diet (MF; Oriental Yeast Co., Tokyo, Japan) alone or with added Miso. Ten percent w/w of dry red Miso (180-days fermentation, Miso Central Institute, Tokyo, Japan) and 2.3% or 1.9% sodium chloride (Wako Chemical special grade) were added to commercial rodent diet (MF, Oriental Yeast, Co, Tokyo) and pelleted. Normal tap water was provided *ad libitum*. Diet and drinking water consumption were measured during the eleventh week of the experiment. Five rats of each sex and strain were placed in experimental groups that were fed Miso including 2.3% NaCl, 1.9% NaCl, 2.3% NaCl or MF diet (0.3% NaCl). The Miso and MF groups were both replicated once.

The animals' blood pressures were determined by the tail-cufff methods, using a programmed sphygmomanometer (BP-98E, Softron Co. Ltd., Tokyo). The unanaesthetized rats were restrained for 5 min using a temperature-controlled warming holder (37℃) designed for rats, before the blood pressure was measured between 13:00 to 15:00 at 24℃ room

temperature. Blood pressure and body weight were measured at one, two, four, eight and twelve weeks after the beginning of the experiment.

Autopsies were performed and bodies and major organs were weighted and were fixed in 10% buffered formalin. Histological evaluation was performed by routine procedures with H&E and Azan staining.

Statistical significance was determined with the Dunnett's method for multiple comparisons using logarithmic transformation and the $X^2$-test.

**Results**

During the eleventh week, the intake diet of the Miso and NaCl diets were tended to be significant greater than MF diet. (Tables 1) The intake of drinking water by Dahl rats of both sexes and by female SD rats in group given Miso, 1.9% NaCl and 2.3% NaCl were significantly increased as compared with the MF groups ($P<0.01$).

Dahl rats were significantly heavier than SD rats at the start of the experiment (Fig 1,2). At the end of experiment male Dahl rats body weights were significantly higher than the SD males (Fig. 1).

The systolic blood pressure of Dahl male rats of both NaCl groups were

significantly increased from 8 weeks on the experiment as compared with rats on Miso and MF diets (Fig. 3a). Systolic blood pressure in SD male rats was increased from 8 weeks on 3.3% NaCl and after 4 weeks on 1.9% NaCl (Fig. 3a). Diastolic blood pressures in male Dahl rats on the 2.3% NaCl diets were increased from 4 weeks of treatment, and from 8 weeks on 1.9% NaCl (Fig. 3b). Systolic blood pressure in the Dahl female rats on 2.3% NaCl was significantly increased as compared with other groups from 8 weeks after treatment (Fig. 4a). In SD female rats, systolic blood pressure in 2.3%NaCl groups was increased 4 and 8 weeks when compared with the MF group, and 12 weeks when compared with the Miso group. In female SD rats, diastolic blood pressure in the 2.3% NaCl group was increased form 8 weeks (Fig. 4b).

Final body weight in all Dahl rats were significantly decreased than those of SD MF rats. Spleen and testis relative weights (organ weight/body weight x 100) in Dahl male rats were significantly greater than those in SD male rat (Table 2), kidney and heart in Miso and NaCl groups of Dahl male rats, liver in Miso and 2.3% NaCl group, and adrenal in Miso and 1.9% NaCl. Spleen weight in 2.3% NaCl and relative weight in 2.3% and 1.9% NaCl, heart weight and relative weights in Miso and 1.9% NaCl, and adrenal relative weights of Dahl male rats were significantly heavier than those Dahl MF diet

group. In SD male rats, liver weights in Miso and 1.9% NaCl groups, and kidney and heart in 1.9% NaCl group were significantly increased as compared with MF value.

In female spleen weight and relative heart weight in MF value of Dahl groups were significantly heavier than SD group value, and liver except MF value was opposite (Table 3). Heart weights in Miso and 2.3% NaCl, liver and kidney weight 2.3% NaCl, and relative liver, kidney and heart weights in Miso and 2.3% NaCl groups of Dahl rats were significantly heavier than those Dahl MF value. Liver in Miso and both NaCl groups and kidney weight in 1.9% NaCl group of SD rats were significantly heavier than those in MF group of SD rats. Relative spleen weight in Miso and NaCl groups were significantly lighter than that in MF value.

Histological examination

Casts regeneration and cell infiltration in kidney were observed in Dahl rats. Sclerosis and hydronephrosis in kidney were also observed 2, and 1 case in respectively, in 1.9% of NaCl group. In liver basophilic appeared in all groups. Arteritis in liver also were observed each one case in Miso, 2.3% NaCl and 1.9% of male Dahl groups and in heart of male Dahl 2.3% NaCl

group. In SD groups there were no histological changes.

**Discussion**

In this experiment a Miso diet with 2.3% of NaCl and MF did not increased blood pressure in Dahl rats whereas the MF diet with 2.3% NaCl increased it. In the 1960s, Dahl selectively bred rats for sensitivity to the hypertensive effects of high-salt diet from SD rats (19). Besides fulminant hypertension, Dahl salt sensitive rats on the high-salt diet develop progressive proteinurea (20) and severe renal vascular and glomerular lesions and associated renal tubular lesions (21). Sterzel et al (20) showed that 5 to 6-week-old Dahl salt sensitive rats have proteinurea associated with segmental retraction of podocyte foot process. The early phase of hypertension was not associated with an overall loss of renal function, lower number of glomeruli, or glomerular hypertension. On a low-salt diet, these with still develop hypertension and renal damage with age (22), but the onset and progression is slower compared with their response on a high-salt diet. Dahl et al also reported that the ultimate blood pressure levels were similar in both sexes on a high salt diet, while the males generally developed hypertension more rapidly

than the females (19). In the present data systolic blood pressure in Dahl female rats of 1.9% NaCl group was not increased as compared with 2.3% NaCl group from 8 weeks after treatment, there were no glomerular lesions in all groups and also systolic and diastolic blood pressures in Dahl rats were increased but not in SD rats. It is good agreement with previous reports and our findings.

In epidemiological data, He et al demonstrated that dietary soybean protein reduced blood pressure in a randomized, double-blind, controlled trial in Chinese adults (23). Several small clinical trials have reported inconsistent findings regarding the effect of soybean protein on blood pressure (24-26). Washburn and colleagues (25) compared the effect of 20 g of soybean protein containing 34 mg of phytoestrogens given either in 1 dose or in 2 doses with that of 20 g of complex carbohydrates on cardiovascular disease risk factors and menopausal symptoms among 51 women in a randomized, controlled trial. They observed a significant reduction in diastolic blood pressure in the twice-daily soybean protein diet compared with the carbohydrate control diet. Burke and colleagues (26) examined the effects of soybean protein on 24-hour ambulatory blood pressure among 41 treated hypertensive patients in a randomized, controlled trial. He also documented that blood pressure

reduction associated with soybean dietary protein was greater than that of the carbohydrate control (23). Conventionally, increased intake of complex carbohydrate has been recommended as a replacement for saturated fat intake to reduce cardiovascular risk (27). Soy based diet attenuated the development of hypertension both in female and male SHR rats (28). These findings suggested that increased intake of soybean protein may play an important role in prevention and treatment of hypertension.

Nakamura et al (29) conducted a double-blind, randomized placebo-controlled study to evaluate the effects of 6-week diet containing approximately 25% to 20% low-sodium soy sauce and Miso (an approximately 10% reduction of total dietary salt intake) in Japanese on blood pressure. The changes in blood pressure observed in the experiment were not significant. However, in those aged 40 years and older, a 6.4mm Hg net reduction in diastolic blood pressure with no significant change in systolic blood pressure was noted in the low-sodium intake group. And Nakamura et al reported that a 6-week trial of low-sodium soy sauce and Miso resulted in a reduction in urinary salt of 0.7g/day in the intervention group and the net difference of urinary salt excretion between the intervention group and the controls was 1.4g/day. They concluded that an approximately 10 %

reduction of dietary salt intake affected diastolic blood pressure in middle aged or older people. When one consumes 13g dietary salt daily, the estimated salt intake from soy sauce will change from 3.3g to 2.4g and that from Miso will change from 1.6 to 1.3 by replacing the seasoning with the low-sodium type, and thus one can achieve 1.2g reduction of daily dietary salt intake. Dietary reduction of NaCl on soy sauce rather than Miso may be used to reduced diastolic blood pressure. On the other hands, Kanda et al performed a study of the association of lifestyle parameters with the future risk of hypertension in normotensive subjects. They administtered a baseline questionnaire and performed a four-year follow-up of 445 normotensive Japanese at 35 to 89 years of age. In 60 to 69 year old subjects, changes of blood pressure during four years were negatively correlated with boiled rice intake in men and with Japanese tea intake in women (18). Multiple logistic regression analysis revealed that Miso-soup intake of two or more bowls per day was protective against hypertension during the follow-up ($p < 0.05$). These results indicate that the nature and amounts of food intake is important in the prevention of hypertension in the elderly. It also prevents increased blood pressure when compared with rats fed the equivalent NaCl concentration without Miso in men and animals. However, the mechanism

of these important effects was unknown. Further studies are needed to elucidate the mechanism and effective materials in Miso.

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Figure legends

Fig. 1. Body weights in male rats throughout experiment. (mean±SD) [a]: Significantly different from SD MF value (P<0.05); [b]: Significantly different from SD MF value (P<0.01)

Fig. 2. Body weights in female rats throughout experiment. (mean±SD) [a]: Significantly different from SD MF value (P<0.05); [b]: Significantly different from SD MF value (P<0.01)

Fig. 3a. Systolic blood pressure in male rats. (mean±SD); *:Significantly different from same strain MF (P<0.05); **:Significantly different from same strain MF (P<0.01); [a]: Significantly different from same strain Miso (P<0.05); [b]: Significantly different from same strain Miso (P<0.01)

Fig. 3b. Diastolic blood pressure in male rats. (mean±SD); *:Significantly different from same strain MF (P<0.05); **:Significantly different from same strain MF (P<0.01); [a]: Significantly different from same strain Miso (P<0.05); [b]: Significantly different from same strain Miso (P<0.01)

Fig. 4a. Systolic blood pressure in female rats. (mean±SD); *:Significantly different from same strain MF (P<0.05); **:Significantly different from same strain MF (P<0.01); [a]: Significantly different from same strain Miso (P<0.05); [b]: Significantly different from same strain Miso (P<0.01)

Fig. 4b. Diastolic blood pressure in female rats. (mean±SD); *:Significantly different from same strain MF (P<0.05); **:Significantly different from same strain MF (P<0.01); [a]: Significantly different from same strain Miso (P<0.05); [b]: Significantly different from same strain Miso (P<0.01)

Table 1 Intake of drinking water and diet during the eleventh experimental week

| Male rats | | | | | |
|---|---|---|---|---|---|
| Strain | Treatment | NaCl concentration (%) | Used animals | Drinking water ml/day/rat | Diet g/day/rat |
| Dahl | Miso | 2.3 | 10 | 61.1±11.9** | 18.5±1.9* |
| Dahl | NaCl | 2.3 | 5 | 81.2±3.9** | 23.8±7.5 |
| Dahl | NaCl | 1.9 | 5 | 54.0±4.4** | 17.3±2.6 |
| Dahl | MF(commercial diet) | 0.3 | 10 | 37.2±8.6 | 16.1±2.9 |
| SD | Miso | 2.3 | 10 | 57.4±7.8 | 20.2±2.6 |
| SD | NaCl | 2.3 | 5 | 55.4±4.1 | 20.3±1.2 |
| SD | NaCl | 1.9 | 5 | 45.0±5.4 | 21.2±2.3 |
| SD | MF(commercial diet) | 0.3 | 10 | 51.5±6.9 | 19.8±1.0 |
| Female rats | | | | | |
| Group | Treatment | NaCl concentration (%) | Used animals | Drinking water ml/day/rat | Diet g/day/rat |
| Dahl | Miso | 2.3 | 10 | 36.6±3.1** | 13.4±1.6 |
| Dahl | NaCl | 2.3 | 5 | 50.1±13.0** | 14.3±1.8 |
| Dahl | NaCl | 1.9 | 5 | 34.0±2.4** | 12.7±1.5 |
| Dahl | MF(commercial diet) | 0.3 | 10 | 26.6±3.5 | 12.9±0.9 |
| SD | Miso | 2.3 | 10 | 35.4±5.8[a] | 16.4±1.5 |
| SD | NaCl | 2.3 | 5 | 41.2±8.4[a] | 32.5±16.7 |
| SD | NaCl | 1.9 | 5 | 40.3±5.2[a] | 27.2±22.5 |
| SD | MF(commercial diet) | 0.3 | 10 | 25.1±2.3 | 14.1±1.4 |

(mean±SD)
*::Significantly different from Dahl MF value ($P<0.05$)
**:Significantly different from Dahl MF value ($P<0.01$)
[a]: Significantly different from SD MF value ($P<0.01$)

Table 2 Body and organ weights and relative weights (organ weight/body weight x 100) in male rats

| Group | BW | Spleen | Liver | Kidney | Testis | Heart | Adrenal |
|---|---|---|---|---|---|---|---|
| Dahl Miso(2.3%NaCl) | 418±12d | 1.04±0.10[d] | 13.90±0.85 | 3.12±0.24 | 3.60±0.17[d] | 1.50±0.18[b,c] | 0.069±0.010 |
| | | (0.25±0.02[d]) | (3.32±0.16[c]) | (0.73±0.05[d]) | (0.86±0.05[d]) | (0.36±0.03[b,d]) | (0.017±0.003[a,d]) |
| Dahl NaCl(2.3%) | 425±13c | 1.13±0.08[a,d] | 14.32±0.48 | 3.70±0.76[b,d] | 3.63±0.09[c] | 1.46±0.06 | 0.057±0.008 |
| | | (0.27±0.02[b,d]) | (3.37±0.15[c]) | (0.93±0.20[b,d]) | (0.85±0.02[d]) | (0.34±0.01[d]) | (0.014±0.002) |
| Dahl NaCl(1.9%) | 415±17d | 1.12±0.11[d] | 13.56±0.54 | 3.14±0.15 | 3.54±0.21 | 1.64±0.10[b,d] | 0.073±0.022[c] |
| | | (0.27±0.03[b,d]) | (3.27±0.08) | (0.75±0.04[a,d]) | (0.85±0.04[d]) | (0.40±0.03[b,d]) | (0.018±0.005[a,d]) |
| Dahl MF(0.3%NaCl) | 423±20c | 1.00±0.06[d] | 13.61±0.97 | 2.78±0.26 | 3.50±0.18 | 1.41±0.10 | 0.057±0.011 |
| | | (0.23±0.01[d]) | (3.15±0.18) | (0.64±0.05) | (0.81±0.05[d]) | (0.31±0.03) | (0.013±0.002) |
| SD Miso(2.3%NaCl) | 478±48b | 0.77±0.12 | 15.27±1.70[a] | 3.18±0.51 | 3.51±0.34 | 1.54±0.16 | 0.061±0.009 |
| | | (0.16±0.02) | (3.19±0.15) | (0.68±0.10) | (0.74±0.10) | (0.31±0.03) | (0.013±0.001) |
| SD NaCl(2.3%) | 446±44 | 0.71±0.07 | 13.90±1.69 | 2.97±0.13 | 3.14±0.16 | 1.38±0.06 | 0.048±0.004 |
| | | (0.16±0.01) | (3.12±0.22) | (0.64±0.06) | (0.71±0.09[a]) | (0.31±0.02) | (0.011±0.002) |
| SD NaCl(1.9%) | 495±17b | 0.81±0.08 | 16.63±1.43[b,c] | 3.45±0.39[b,d] | 3.41±0.28 | 1.51±0.07[a,c] | 0.060±0.013 |
| | | (0.16±0.02) | (3.36±0.26) | (0.67±0.04) | (0.69±0.07[b]) | (0.30±0.01) | (0.012±0.002) |
| SD MF(0.3%NaCl) | 475±45a | 0.75±0.08 | 14.82±1.64 | 2.79±0.35 | 3.26±0.28 | 1.35±0.11 | 0.056±0.011 |
| | | (0.16±0.02) | (3.12±0.23) | (0.60±0.07) | (0.69±0.07[b]) | (0.29±0.03) | (0.012±0.002) |

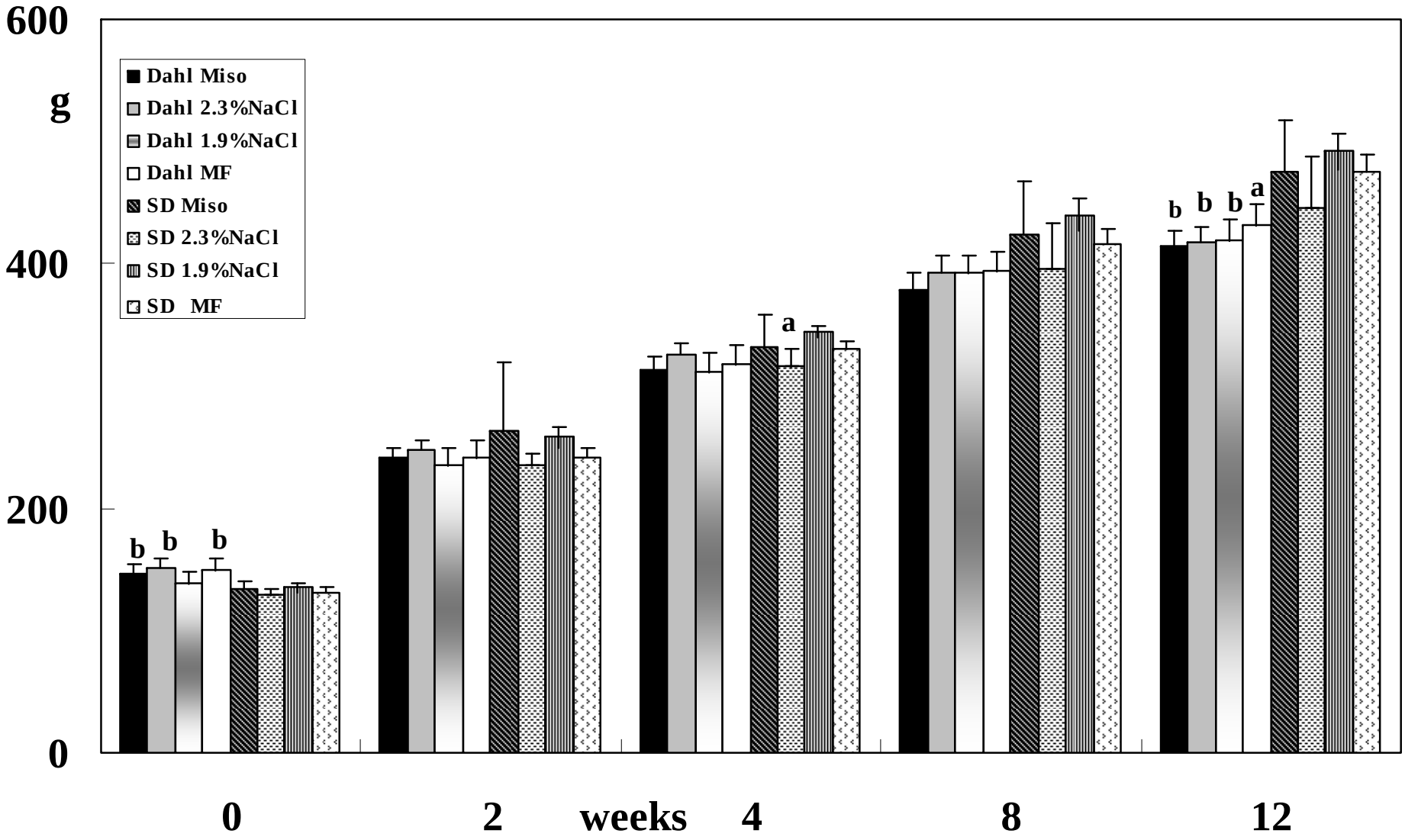
(mean±SD)
a: Significantly different from Dahl MF value (P<0.05)
b: Significantly different from Dahl MF value (P<0.01)
c: Significantly different from SD MF value (P<0.05)
d: Significantly different from SD MF value (P<0.01)

Table 3 Body and organ weights and relative (organ weight/body weight x 100 ) weights in female rats

| Group | BW | Spleen | Liver | Kidney | Ovary | Uterus | Heart | Adrenal |
|---|---|---|---|---|---|---|---|---|
| Dahl Miso (2.3%NaCl) | 268±13 | 0.68±0.05[d] | 8.36±0.80 | 1.81±0.18 | 0.15±0.04 | 0.54±0.14 | 0.99±0.09[a] | 0.076±0.010 |
| | | (0.25±0.02) | (3.11±0.23[b]) | (0.69±0.06[b,d]) | (0.057±0.014) | (0.20±0.05) | (0.37±0.04[b,d]) | (0.028±0.004) |
| Dahl NaCl(2.3%) | 274±7 | 0.78±0.07[d] | 8.96±0.75[b] | 2.23±0.46[b,d] | 0.13±0.02 | 0.56±0.06 | 1.00±0.07[a] | 0.073±0.007 |
| | | (0.28±0.02[a]) | (3.27±0.22[b]) | (0.77±0.08[b,d]) | (0.049±0.006) | (0.20±0.02) | (0.36±0.02[a.d]) | (0.027±0.002) |
| Dahl NaCl(1.9%) | 272±17 | 0.68±0.06[d] | 8,23±0.40 | 1.87±0.14 | 0.13±0.02 | 0.51±0.06 | 0.95±0.12 | 0.074±0.017 |
| | | (0.25±0.01) | (3.03±0.14) | (0.68±0.03) | (0.049±0.009) | (0.19±0.03) | (0.35±0.05[d]) | (0.028±0.007) |
| Dahl MF (0.3%NaCl) | 273±12 | 0.69±0.04[d] | 7.67±0.68 | 1.63±0.11 | 0.14±0.04 | 0.50±0.09 | 0.88±0.08 | 0.073±0.009 |
| | | (0.25±0.01) | (2.81±0.18) | (0.61±0.03) | (0.052±0.016) | (0.18±0.03) | (0.32±0.02) | (0.027±0.004) |
| SD Miso (2.3%NaCl) | 309±22[b,d] | 0.50±0.05[b] | 8.96±0.78[b,c] | 1.79±0.19 | 0.16±0.03 | 0.56±0.08 | 0.90±0.09 | 0.069±0.011 |
| | | (0.16±0.02[d]) | (2.90±0.18) | (0.60±0.04) | (0.051±0.010) | (0.18±0.03) | (0.29±0.02) | (0.023±0.004) |
| SD NaCl(2.3%) | 317±34[b,d] | 0.52±0.06[b] | 9.54±1.32[b,d] | 1.85±0.20 | 0.17±0.05 | 0.54±0.08 | 0.90±0.08 | 0.066±0.013 |
| | | (0.16±0.01[c]) | (3.02±0.41) | (0.60±0.06) | (0.053±0.011) | (0.17±0.03) | (0.28±0.02) | (0.021±0.005) |
| SD NaCl(1.9%) | 309±34[a,c] | 0.47±0.03[b] | 9.22±0.69[b,c] | 1.94±0.15[a,c] | 0.16±0.03 | 0.64±0.23 | 0.89±0.07 | 0.069±0.013 |
| | | (0.15±0.01[d]) | (2.99±0.13) | (0.64±0.06) | (0.053±0.008) | (0.21±0.06) | (0.29±0.02) | (0.023±0.006) |
| SD MF (0.3%NaCl) | 276±33 | 0.53±0.13[b] | 7.97±1.18 | 1.64±0.23 | 0.15±0.02 | 0.55±0.18 | 0.83±0.07 | 0.066±0.011 |
| | | (0.19±0.03) | (2.88±0.18) | (0.60±0.07) | (0.054±0.010) | (0.21±0.09) | (0.30±0.03) | (0.024±0.004) |

(mean±SD)
a: Significantly different from Dahl MF value (P<0.05)
b: Significantly different from Dahl MF value (P<0.0b)
c: Significantly different from SD MF value (P<0.05)
d: Significantly different from SD MF value (P<0.01)

**Fig. 1. Body weight in male rats**



# Fig. 2. Body weights in female rats

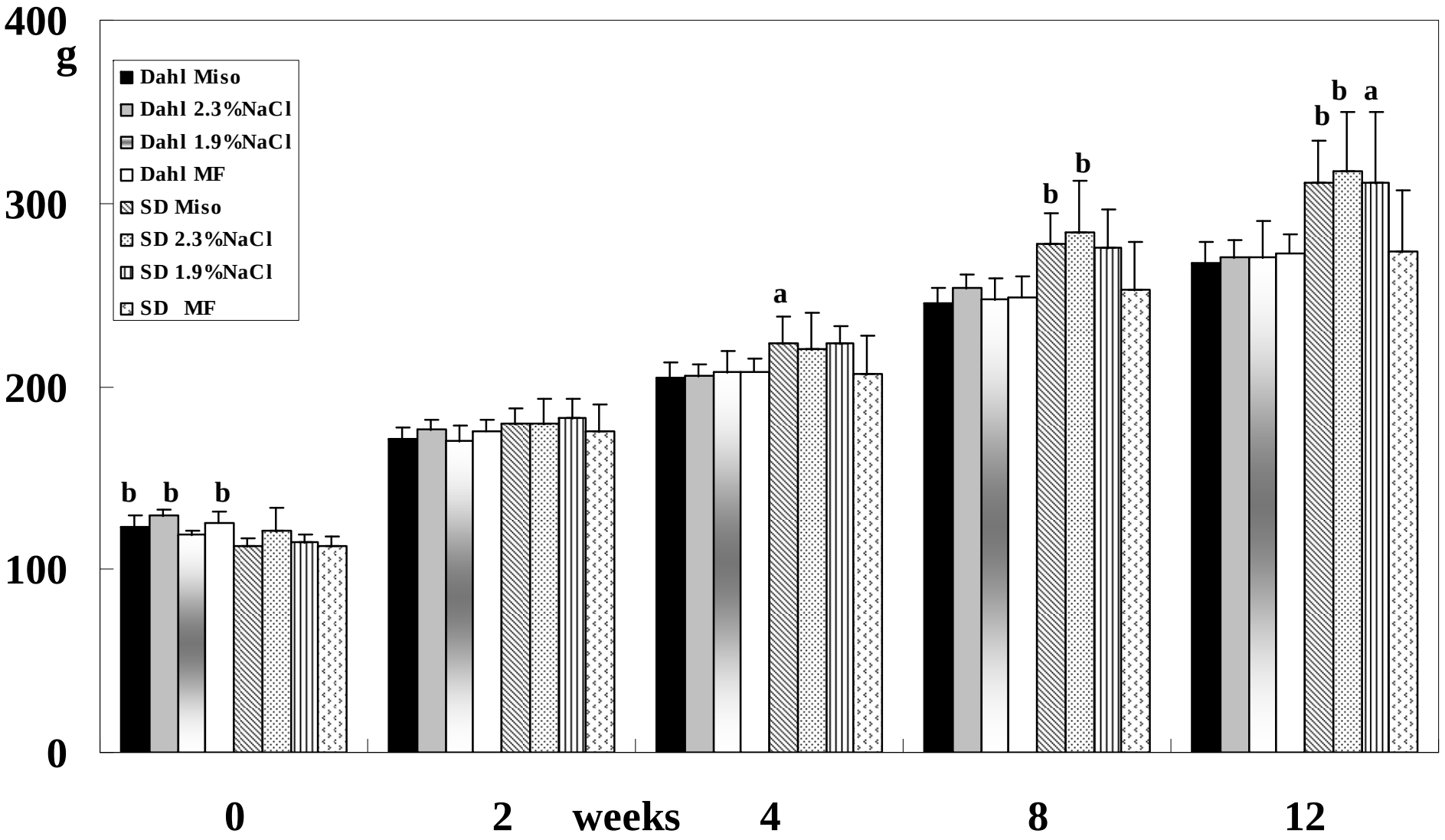


**Fig. 3a. Systolic blood pressure in male rats**

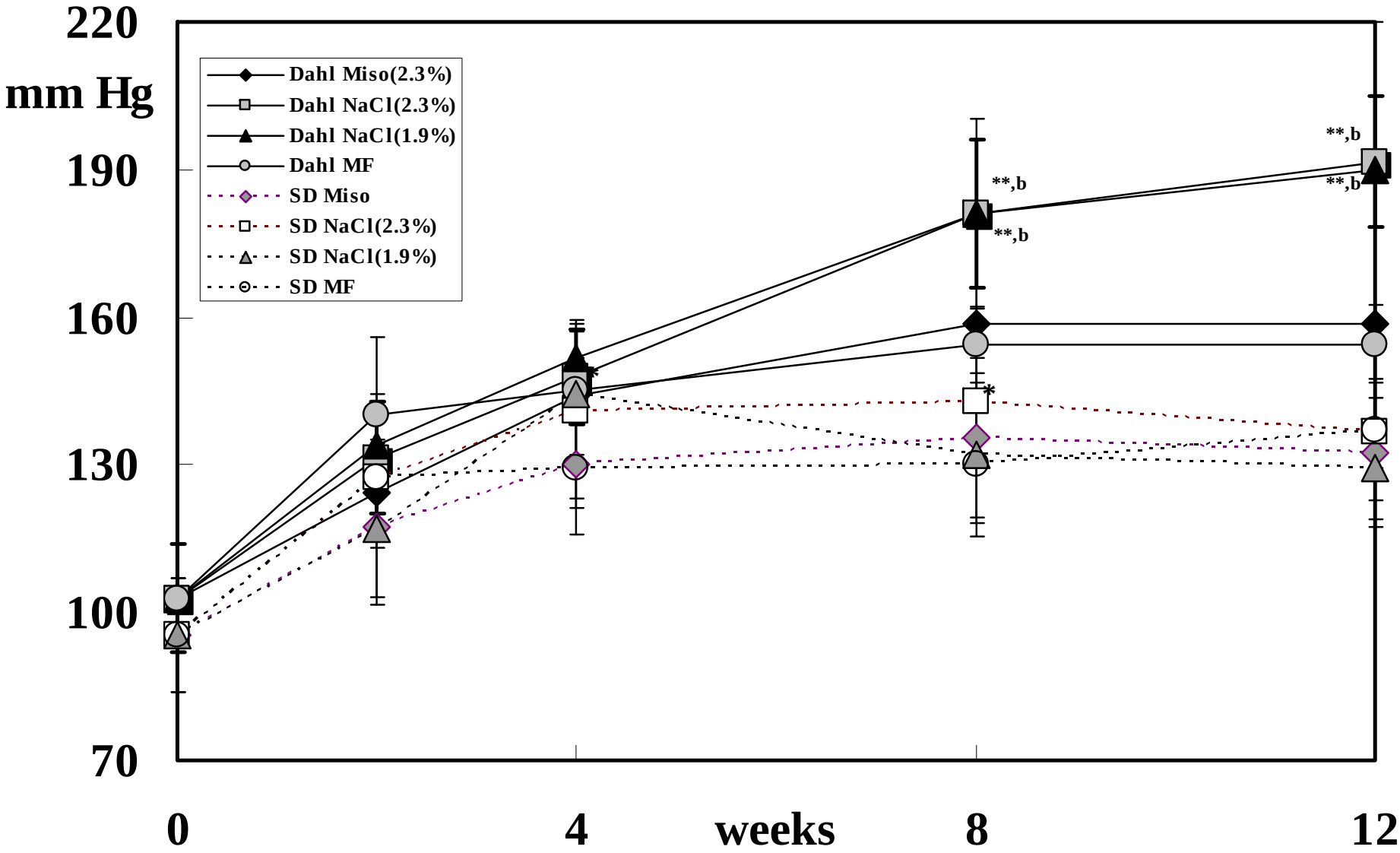


**Fig. 3b. Diastolic blood pressure in male rats**

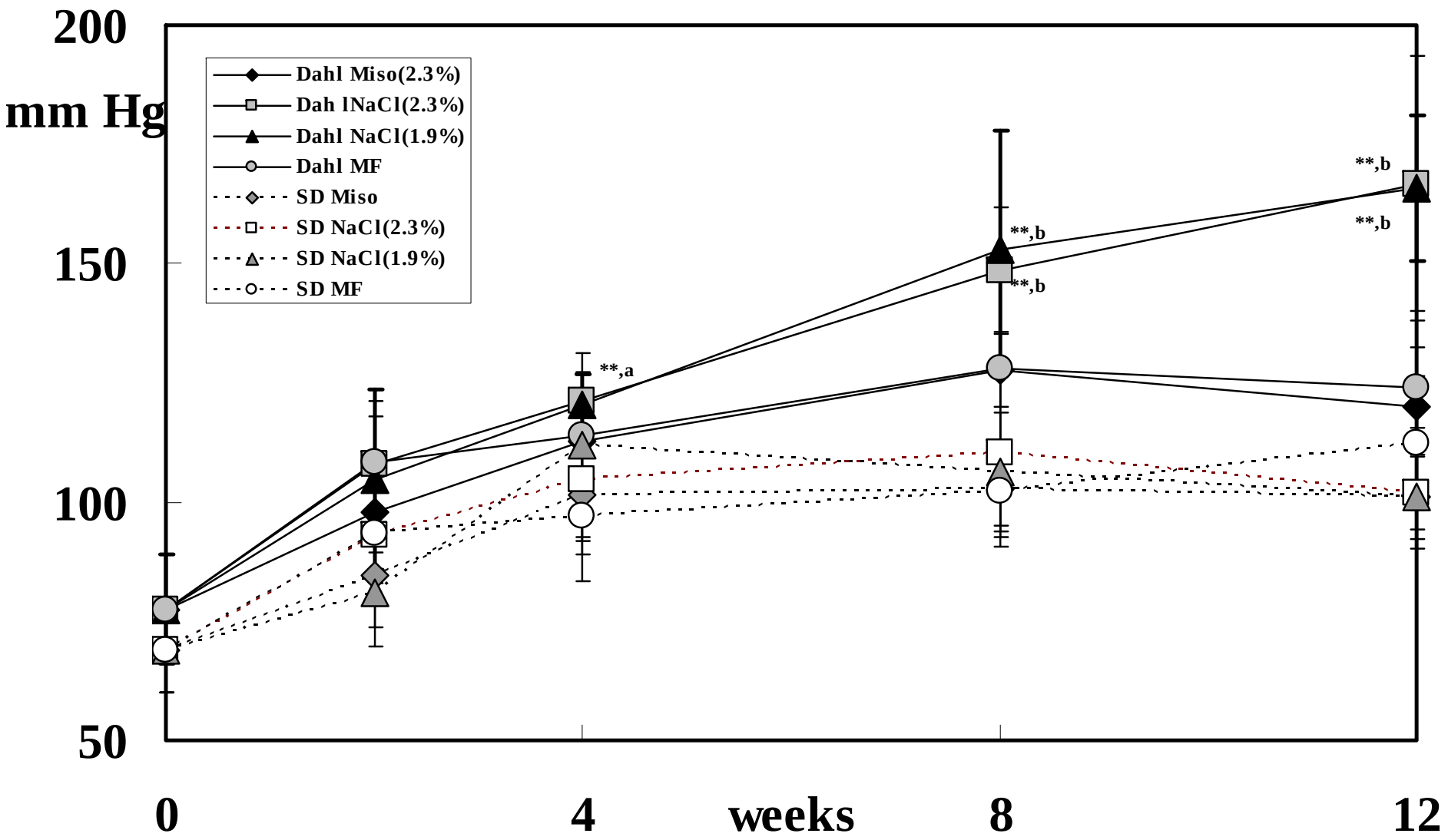


**Fig. 4a. Systolic blood pressure in female rats**

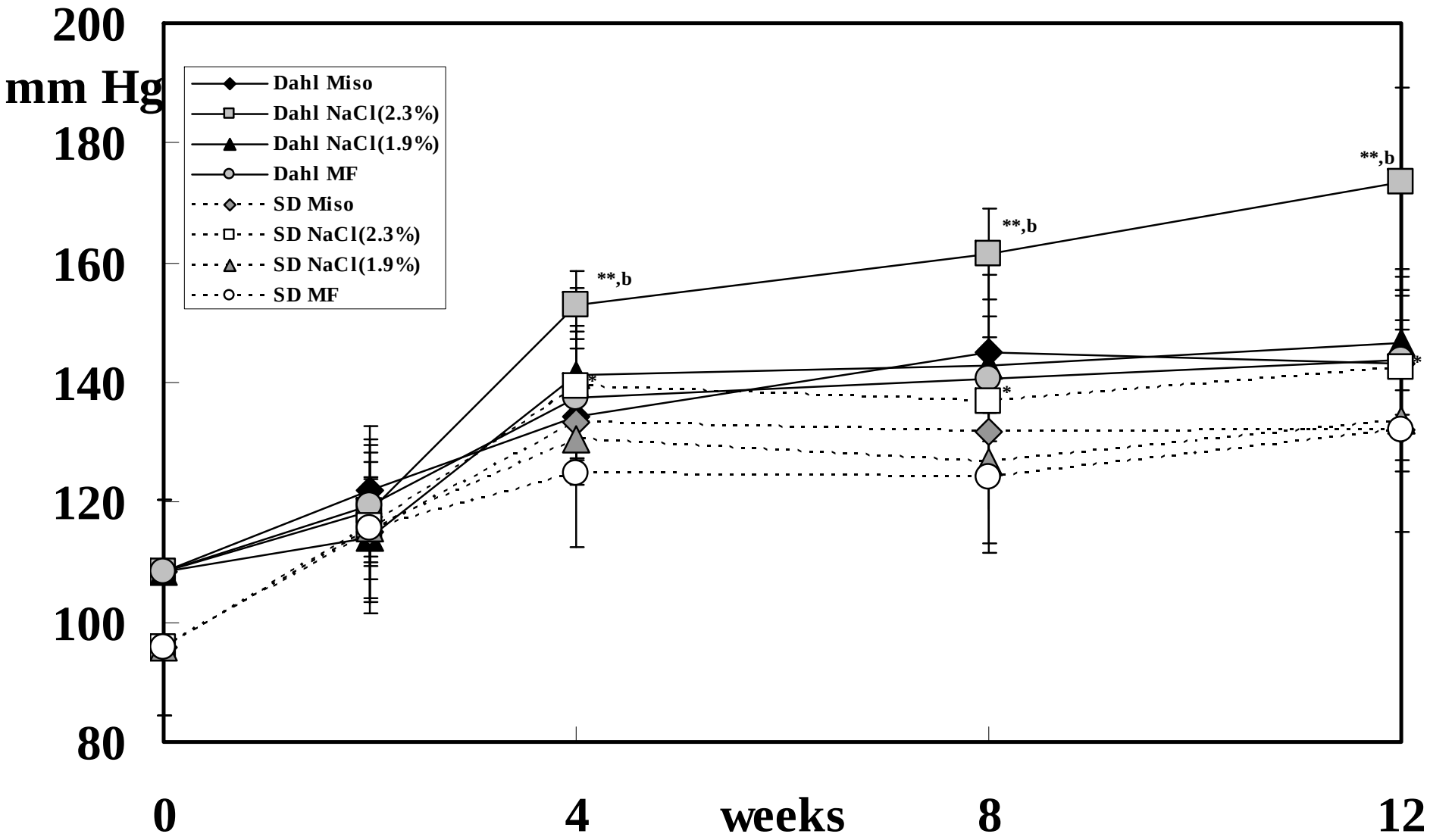


**Fig. 4b. Daistolic blood pressure in female rats**