Influence of Sodium Chloride Intake on Some Micro and Macro Elements of Male Albino Rats.

By

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ABSTRACT

This paper examines the effect of different sodium chloride dietary intakes on micro and macro elements of rats. Thirty male albino rats were housed individually in plastic cages and were subdivided into four groups. The first served as normal control. The second received 1 fold sodium chloride(twice the normal) fed diet, the third received 2 folds sodium chloride fed diet and the forth received 3 folds sodium chloride fed diet. The fifth group received 4 folds sodium chloride fed diet. Experiment lasted for 3 weeks of feeding. The results showed that water consumption increase associated with increased salt intake. Changes in relative growth rate were associated with daily food consumption. 1 fold salt feeding enhanced more weight gain. Relative organs weights were approximately not affected except for 3 folds sodium chloride intake. Serum calcium, magnesium and albumin concentrations and hepatic iron content were not affected with different levels of salt feeding. However sera iron, phosphorus, zinc and total protein were increased significantly, especially with high doses of sodium chloride intake. Calcium in bone was decreased significantly with high salt feeding. Iron content of spleen was increased significantly with salt consumption. In conclusion, 1 fold sodium chloride intake enhanced food consumption and weight gain of rats than other high salt levels diets. High salt intake enhanced depletion of bone calcium with elevation of spleen and serum iron and increased serum phosphorus, zinc and total protein content which may increase the risk of red blood cells lyses and osteoporosis.

***Key words:* Sodium chloride, rats, minerals, diet, growth rate, bone, spleen, calcium, iron, phosphorus, zinc, albumin, total protein, salt, serum.**

INTRODUCTION:

Different nutrients have been reported to play a role in bone development, and mineral distribution inside the body (Ilich and Kersteitter, 2000). Among them, dietary high salt intake was found to decrease femoral calcium concentration in rats (Chan *et al.*, 1993). High dietary sodium chloride intake was reported (Greedon and Cashman, 2000; Ho *et al.*, 2001 and Carbone *et al.*, 2003) to decrease renal calcium reabsorption that results in greater urinary calcium excretion. In an important study (Breslau *et al.*, 1982), young adults responded to increased sodium intake with increases in

urinary calcium, serum parathormone, calcitriol and intestinal calcium absorption. In contrast, the increased urinary calcium excretion following an increase in sodium intake in postmenopausal women was not accompanied by increases in serum parathormone or calcitriol or in intestinal calcium absorption (Breslau *et al.*, 1985).

It has been argued (Antonios and MacGregor, 1995; MacGregor, 1996) that a high salt intake is a major risk factor for osteoporosis, based on the following reported observations: Dietary salt claimed to be the main determinant of urinary calcium excretion (McCarron *et al.*, 1981) and negative correlation between urinary sodium excretion and bone density in postmenopausal women (Devine *et al.*, 1995). On the other hand, reference was made to the absence of direct evidence that high sodium intake is an important risk factor for osteoporosis (Lau and Woo, 1994) and also to the low incidence of osteoporosis in south-east Asia, where high salt intake is common, indicating that sodium is not a major risk factor (Draper, 1994). Although recognizing that young adults may adapt to a high sodium intake by a mechanism avoiding bone resorption (Evans and Eastell, 1995). Effect of dietary salt on micro and macro elements has taken little attention. Thus this paper was investigated to examine the influence of different regimen of sodium chloride on some of these elements in male albino rats.

MATERIAL AND METHODS:

Animals:

Thirty male albino rats (*sprgue dawley strain*) 6 weeks old, purchased form the Egyptian Organization for emmunity and Vaccine giza , ARE were housed individually in plastic cages. Rats were housed in a controlled-temperature ($25\pm2^{O}C$) and humidity ((25±2 %) environment, with a 12 hour light / dark cycle and free access to food and tap water. Body weight, food and water intake were determined at week intervals.

Diets preparation:

Basal Balanced diet:

Basal diet prepared form fine ingredients per 100g according to AIN (1993). The diet composed of 14% protein (derived from neutral casein); sunflower oil 10%; salt mixture 4% (camphel 1961); vitamin mixture 1% (Hegested et al., 1941); Dl-methionin 0.3%; choline chloride 0.2% and corn starch up to 100g.

Experimental design:

1- Normal controls: They received basal balanced diet and water *ad libitum.*

2- 1 fold sodium chloride from standered for feeding second group:

3- 2 folds sodium chloride from standered for feeding thrid group:

4- 3 folds sodium chloride from standered for feeding fourth grooup:
5- 4 folds sodium chloride from standered for feeding fifth group:

Animals of different groups were anaesthetized with diethyl ether and rapidly dissected after 3 weeks of feeding.

Sample collection and biochemical analyses:

Blood samples were collected from the inferior vena cava in glass centrifuge tubes, and then centrifuged). Sera were separated in plastic viels and stored at – 20°C in deep freez till further biochemical measurements.

All serum mineral concentrations were determined colorimetrically using Biocon Diagnostic kits, Germany the method of Doumas *et al.*, (1971) for albumin; Gosling (1986) for calcium; Josephson and Gyllensward (1957) for total protein; Mann and Yoe, (1956) for magnesium; Fiske and Subbarow, (1925) for phosphorus and Siedel *et al.*, (1984) for iron.

Serum zinc, bone calcium, liver iron and spleen iron were measured as the method of Rice (1972) using Unicam 929 Analytical Atomic Absorption Spectrometer in Nutrition Institute, Cairo, Egypt.

Statistical analysis:

Data were statistically analyzed by one–way analysis of variance (Anova-Tukey test) using SPSS 10.1 software package. The P values < 0.05 were considered significant.

RESULTS:

Body weight, growth rate and weight gain:

Body weights were approximately not affected with salt level in diet (Table 3). Relative growth rate (RGR) of 1 fold dietary salt was significantly increased by 23.68, 31.11 and 35.75% compared with normal controls after 1, 2 and 3 weeks of feeding. On the other hand RGR of 2 and 3 folds salt fed rats was decreased significantly at 1st week of feeding and remain unchanged by 3rd week of feeding compared with normal controls. RGR of 4 folds salt fed rats was not affected at 1st and 2nd week of feeding and increased significantly by 3rd week of feeding by 29.54% compared with normal controls (Table 4).

The weight gain was increased significantly and gradually with time interval in 1 fold salt fed rats and only at 3 weeks of feeding for 4 folds rats compared with normal controls (Table, 7).

Organs weights and relative organs weights:

liver and spleen relative weights were not affected with salt feeding except for 3 fold salt group (Table 5). A significant were found decrease in relative liver and spleen weights by 20.21 and 17.95% compared with normal controls respectively after 3 weeks of feeding 3 fold NaCl(Table, 6).

Kidney relative weight was increased significantly by 21.6% in 1 fold salt group compared

with normal controls after 3 weeks of feeding. Femur weight was increased significantly in both 2 and 3 fold salt fed rats by 19.08% and 21.71% after 3 weeks of feeding compared with normal controls respectively.

Serum micro and macro elements:

Figs. (1-10) shows the effect of sodium chloride of diet on some serum and tissues nutrients. Salt intake did not affect serum calcium, magnesium and albumin concentrations. On the other hand iron was increased significantly in both 2 fold and 4 fold salts feeding with magnitudes of 41.88% and 20.78% respectively compared with normal controls after 3 weeks of feeding. Phosphorus concentration was increased significantly by 23.75% only in 2 fold group compared with normal controls. With respect to zinc, it was increased significantly by 132.84%, 65.67%, 72.72% and 26.53% in 1, 2, 3 and 4 fold salt feeding groups compared with normal control respectively. Total protein content was elevated significantly by 17.89% and 20.39% in 2 and 3 fold salt group compared with normal controls.

Bone calcium was decreased significantly after 3 weeks of 1, 2, 3, and 4 fold salt groups by 40.75%, 44.64%, 45.82% and 39.52% compared with normal control respectively. Liver iron was not affected with salt in diet, however spleen iron increased significantly by 27.31%, 29.34%, 55.8 %and 34.98% after 3 weeks of 1, 2, 3 and 4 fold salts in diet compared with normal controls.

Food and water intake:

Food intake was decreased significantly in both 2 and 3 fold salt fed rats with magnitudes of 20.63 and 17.46% compared with normal control respectively after 1 week of feeding. After 2 and 3 weeks of feeding food consumption was not significantly affected (Fig. 1).

Water intake was increased significantly in all salt fed groups parallel to the increase in salt of diet (Fig. 2).

DISCUSSION:

The influence of nutrients on body minerals is still poorly understood and uncertain (Cohen and Roe, 2000). The results of the present study indicated that changes in growth rate were associated with that of food intake in different salt fed groups. Coelho *et al.*, (2006) showed that food intake was higher in high salt diet rats. Saric *et al.*, (2005) showed that salt exposed animals had greater water consumption during 2 months period and significantly lowered body weight from week 5 of experiment.

It was reported that the daily water intake of laboratory rats is elevated proportionally as dietary NaCl content is increased up to 25%, without compromising food intake (Camble *et al.*, 1929 and Richter and Mosier, 1954), since dietary NaCl adds to the osmotic load of food, and increased plasma osmolality being known to stimulate thirst

(Gilman, 1937 and Ramsay and Thrasher, 1990). These observations were in line with our results maintained on figures11 and 12. It always seemes likely that increased water intake results form the recurrent stimulation of osmoregulatory thirst. The renal retention of Na^+ ingested in food is believed to stimulate water intake (Ragan *el al.*, 1940).

The present work results found that the lowest weight gain was reported in 2 fold salt group. This may be attributed to the lowest food intake. Many investigators have repeatedly observed that increased dietary salt ingestion by healthy adults resulted by increased urinary calcium loss, which potentially increases the risk of developing osteoporosis (Massey and Whiting, 1996) and kidney stones (Massey and Whiting, 1995).

The salt-induced an increase in urinary calcium is hypothesized to cause a subtle drop in plasma ionized calcium, which stimulates parathyroid hormone release (Goulding *et al.*, 1986; Shortt and Flynn, 1990 and Evans and Eastell, 1995). These observations can explain the slight decrease in serum calcium of 2 and 3 folds of salt intake and the corresponding increase in serum phosphorus which may be as a result of phosphate retention (Ganong, 1997). In line with our results of decreasing bone calcium content significantly in response to salt feeding, studies of the effects of high dietary salt supplementation on bone health in rats have provided evidence of reduced bone calcium content over days of the growing period (Goulding and Gold, 1984) and bone loss due to increased bone resorption rather than decreased bone formation using a radiolabelling technique (Goulding and Gold, 1988). It could be assumed that increased serum calcium occurred on expense of bone calcium content.

Massay (2005) reported that high salt feeding did not affect urinary magnesium. In line with these results, of present work showed that diet salt did not affect serum magnesium.

the results indicated that increased serum and spleen iron content compared with normal control especially in both 2 and 4 folds salt groups.

Results on table 6 showed increased serum zinc content in all salt fed groups. It was reported that increased urinary excretion of zinc occurs in individuals with hypertension (Latner, 1975) which is the case with exposing to high salt feeding (Coelho *et al.*, 2006). The results showed increased serum total protein content especially in 2 and 3 folds salt fed rats. Protein is an important for intact formation of cell membranes and hemoglobin.

The results of present study showed that relative liver, spleen and heart weights were decreased significantly in 3 folds salt fed rats. It could be noticed that although decreased food intake occurs in 2 folds salt fed rats, body weights were increased, this increase in body weights may be

attributed to the increase in water retention which approached 50% over normal control. Studies suggested that visceral osmoreceptors or Na^+- receptors (Morita *et al.*, 1997) in rats detect the osmolality (or Na^+ concentration) of hepatic portal blood and send an afferent signal to the caudal brain stem that contributes to the stimulation of thirst and neurohyophysed hormone secretion (Stricker *et al.*, 2002). Thus rats fed on high slat diet may drink water rapidly after salt feeding, and in amounts for osmoregulation. Coelho *et al.*, (2006) attributed decreased body weights of high salt fed rats, though their food intake increased, to an increase in brown adipose tissue uncoupling protein expression and the consequence higher energy expenditure. High salt feeding elevated plasma T4 (Coelho *et al.*, 2006) which stimulates brown adipose tissue function and energy expenditure (Riberio *et al.*, 2000), leading to a decrease in body weight despite a higher energy intake.

In conclusion, It could be noticed that gradual increase in salt concentration in diet resulted in obvious loss in bone calcium, and increased risk of osteoporosis. It enhanced food and water consumption and weight gain. Salt intake enhanced spleen and serum iron and elevated serum phosphorus, zinc and total protein. It seems that body adapted for high salt intake.

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Table (1): Salt mixture composition (1000g)

Compounds	Amounts
CaCO3	300g
KH2PO4	322g
CaPO4 2H2O	75g
MgSO4 7H2O	102g
NaCl	167g
FeC6H6O7 6H2O	27.5g
KCl	0.9g
Mn SO4 H2O	7.678g
Zn Cl2	0.25g
Cu SO4 5H2O	0.35g
Total	1000g

Table (2): Vitamin mixture composition.

Compounds	Amounts
Vitamin A	400.000(IU)
Vitamin D3	100.000(IU)
Vitamin E	5000(IU)
Vitamin K3	2.5g
Vitamin B1 HCl	1g
Vitamin B2	1g
Vitamin B6 HCl	1g
Calcium pantothenate	3g
Nicotinic acid	4.5g
Folic acid	0.09g
Inisotol	5g
P-amino – Benzoic acid	5g
Vitamin B12	0.00135g
Biotin	0.029g
Dextrose	977.115g

Table(3): Body weights of rats fed varying levels of sodium chloride.

Time interval	Control	1 fold	2 folds	3 folds	4 folds
0 time	151.5 ± 8.8	152 ± 15.21	172.3 ± 28.61	176 [e] ± 16.89	148.3 ±21.6
1 week	178.5 ± 18.89	185.5 ± 11.79	195.8 ± 26.93	201.3 ± 24.44	175.6 ± 9.89
2 weeks	202.3 ± 23.30	218.8 ± 10.42	216.8 ± 26.93	225.6 ± 30.91	201.3 ± 9.20
3 weeks	215.5 ± 26.07	239.1 ± 9.06	235.8 ± 29.92	246.3 ± 33.57	229.5 ± 21.41

Table (4): Effect of feeding with varying levels of dietary sodium chloride feeding on relative growth rate of rats.

Time interval	Control	1 fold	2 folds	3 folds	4 folds
1 week	17.82 ± 1.71	22.04 ± 2.13 [a,c,d]	13.64 ± 1.45 [a,b,e]	14.39 ± 1.53 [a,b,e]	18.42 ± 2.01 [b,c,d]
2 weeks	33.53 ± 3.43	43.96 ± 4.11 [a,c,d,e]	32.81 ± 3.14 [a,b,e]	28.22 ± 2.97 [b,e]	35.73 ± 3.62 [b,d]
3 weeks	42.24. ± 4.16	57.34 ± 5.63 [a,c,d]	48.39 ± 5.12 [b,e]	39.96 ± 3.87 [b,e]	54.72 ± 5.66 [a,c,d]

Table (5): Effect of feeding with varying levels of dietary sodium chloride feeding for 3 weeks on liver, kidney, spleen, femur and heart weights of rats.

	Control	1 fold	2 folds	3 folds	4 folds
Liver	5.97 ± 0.69	6.73 ± 1.23 [d]	6.28 ± 0.77	5.45 ± 0.37 [b]	5.74 ±0.68
Kidney	1.25 ± 0.16	1.52 ± 0.20 [a]	1.40 ± 0.21	1.27 ± 0.12 [b]	1.27 ± 0.09 [b]
Spleen	0.83 ± 0.18	0.81 ± 0.07	0.88 ± 0.11	0.80 ± 0.10	0.79 ± 0.11
Femur	1.52 ± 0.20	1.64 ± 0.15	1.81 ± 0.24 [a]	1.85 ± 0.21 [a]	1.61 ± 0.23
Heart	0.65 ± 0.06	0.73 ± 0.08 [d]	0.71 ± 0.11 [d]	0.52 ± 0.08 [a,b,c,e]	0.63 ± 0.08

Table (6):Effect of feeding with varying levels of sodium chloride feeding for 3 weeks on relative organs weights of rats.

	Control	1 fold	2 folds	3 folds	4 folds
Liver	**2.77 ± 0.28**	**[d] 2.81 ± 0.30**	**2.66 ± 0.25**	**[a,b,c] 2.21 ± 0.24**	**2.50 ±0.26**
Kidney	**0.58 ± 0.06**	**[d] 0.64 ± 0.06**	**0.59 ± 0.06**	**[b] 0.52 ± 0.05**	**0.55 ± 0.06**
Spleen	**0.39 ± 0.04**	**0.34 ± 0.03**	**0.37 ± 0.04**	**[a] 0.32 ± 0.03**	**0.34 ± 0.04**
Femur	**0.71 ± 0.08**	**0.69 ± 0.07**	**0.77 ± 0.04**	**0.75 ± 0.08**	**0.70 ± 0.08**
Heart	**0.30 ± 0.03**	**[d] 0.31 ± 0.03**	**[d] 0.30 ± 0.03**	**[a,b,,c,e] 0.21 ± 0.02**	**[d] 0.27 ± 0.03**

Table (7): Effect of feeding with varying levels of sodium chloride on weight gain (g) of rats.

Time interval	Control	1 fold	2 folds	3 folds	4 folds
1 week	**27 ± 2.62**	**[a,c,d,e] 33 ± 3.33**	**[b] 23 ± 2.12**	**[b] 25 ± 2.21**	**[b] 27 ±3.12**
2 weeks	**51 ± 5.26**	**[a,c,d,e] 66 ± 7.14**	**[b,e] 44 ± 4.46**	**[b] 49 ± 5.18**	**[b] 53 ± 5.14**
3 weeks	**64 ± 6.62**	**[a,c,d] 87 ± 8.97**	**[b,e] 63 ± 6.53**	**[b] 70 ± 8.96**	**[a,c] 81 ± 9.11**

a = Significant difference compared to controls.
b = Significant difference compared to 1 fold salt feeding.
c = Significant difference compared to 2 folds salt feeding.
d = Significant difference compared to 3 folds salt feeding.
e = Significant difference compared to 4 folds salt feeding.

Fig. (1): Effect of feeding with varying levels of sodium chloride on serum calcium concentrations of rats

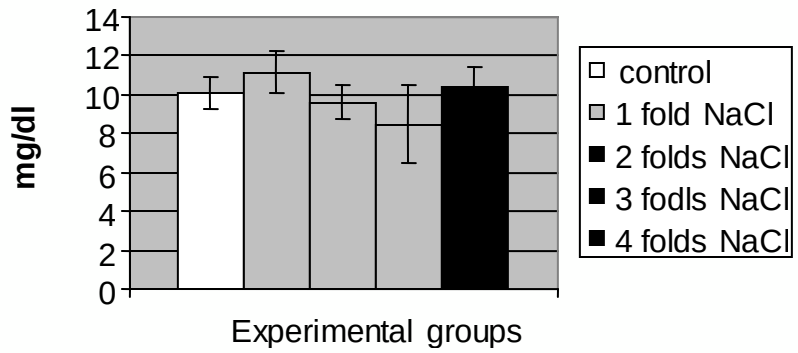


Fig. (2) : Effect of feeding with varying levels of sodium chloride on serum iron concentrations of rats

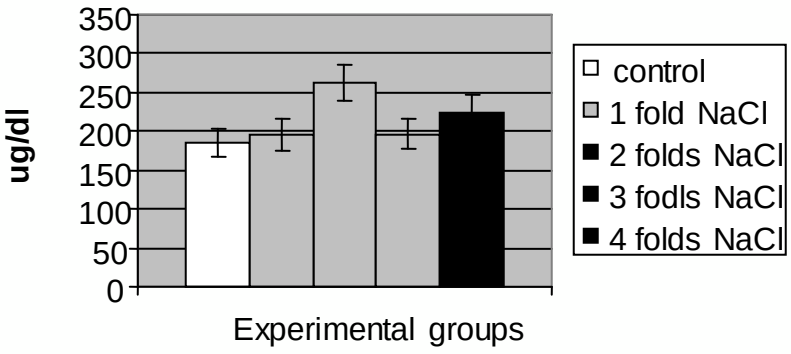


Fig. (3) : Effect of feeding with varying levels of sodium chloride on serum phosphorus concentrations of rats

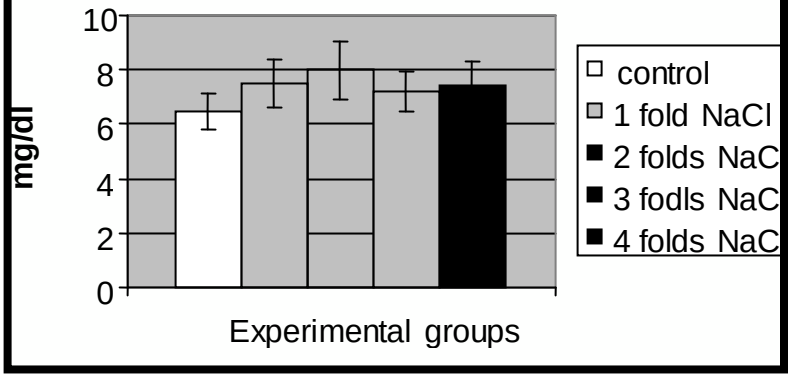


Fig. (4) :Effect of feeding with varying levels of sodium chloride on serum magnesium concentrations .of rats

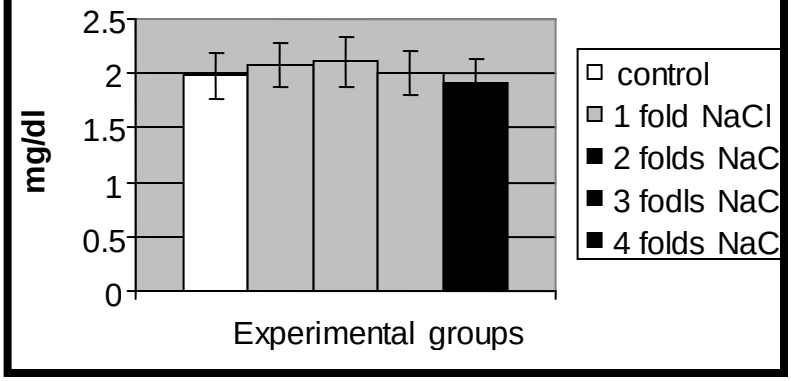


Fig. (5):Effect of feeding with varying levels of sodium chloride on serum zinc concentrations of rats.

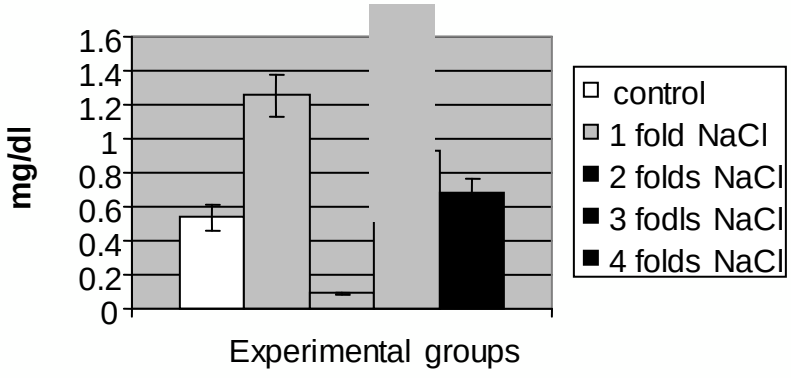
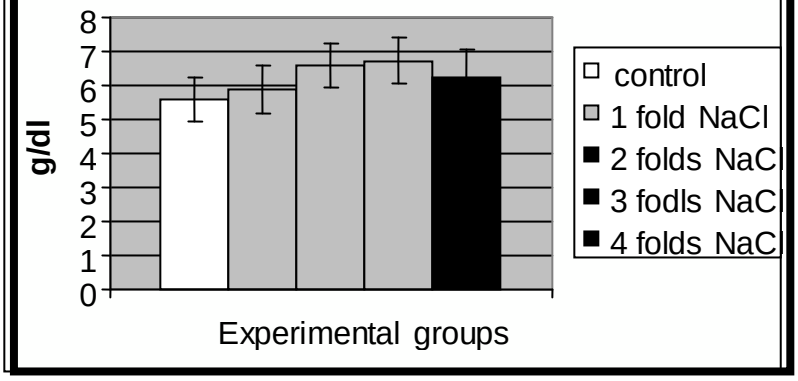


Fig.(6) : Effect of feeding with varying levels of sodium chloride on serum total protein concentrations of rats



z

Fig.(7) :Effect of feeding with varying levels of sodium chloride on serum albumin concentrations of rats

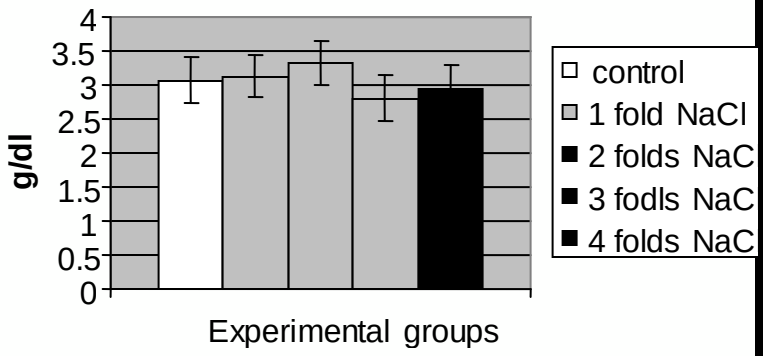


Fig.(8) :Effect of feeding with varying levels of sodium chloride on bone calcium concentrations of rats

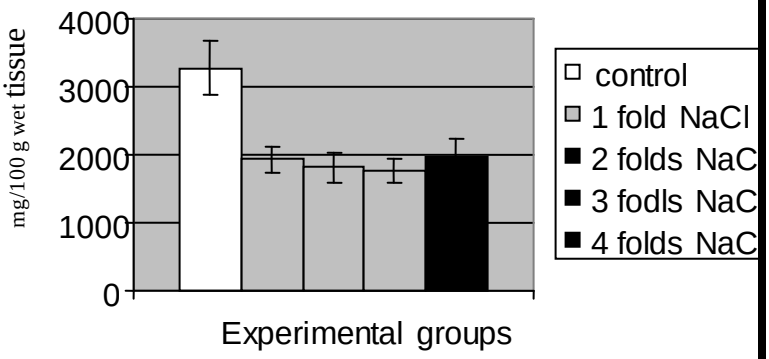


Fig. (9) :Effect of feeding with varying levels of sodium chloride on liver iron concentrations of rats.

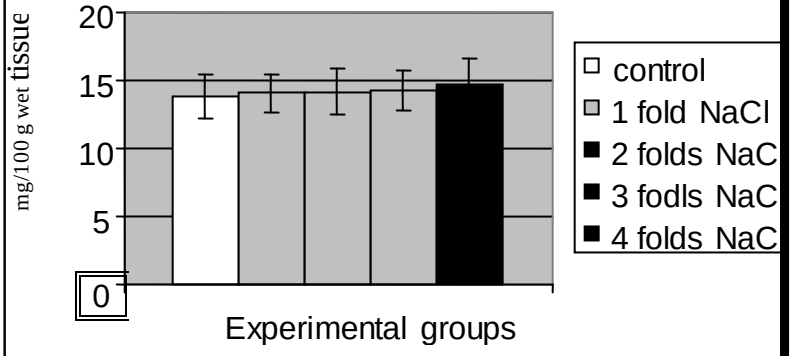


Fig.(10) :Effect of feeding with varying levels of sodium chloride spleen iron concentrations of rats.

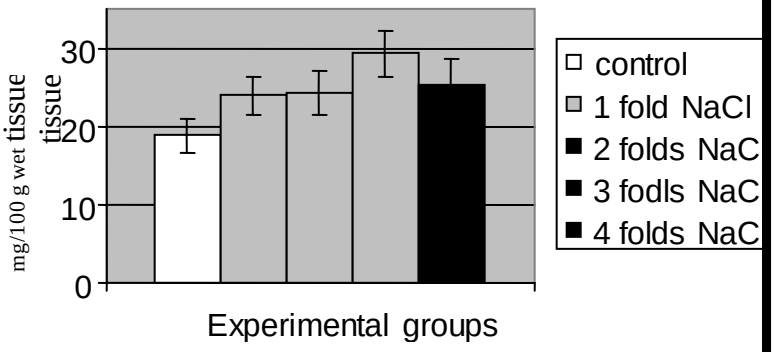


Fig.(11) Daily food intake of rats feeding with varying levels of sodium chloride .

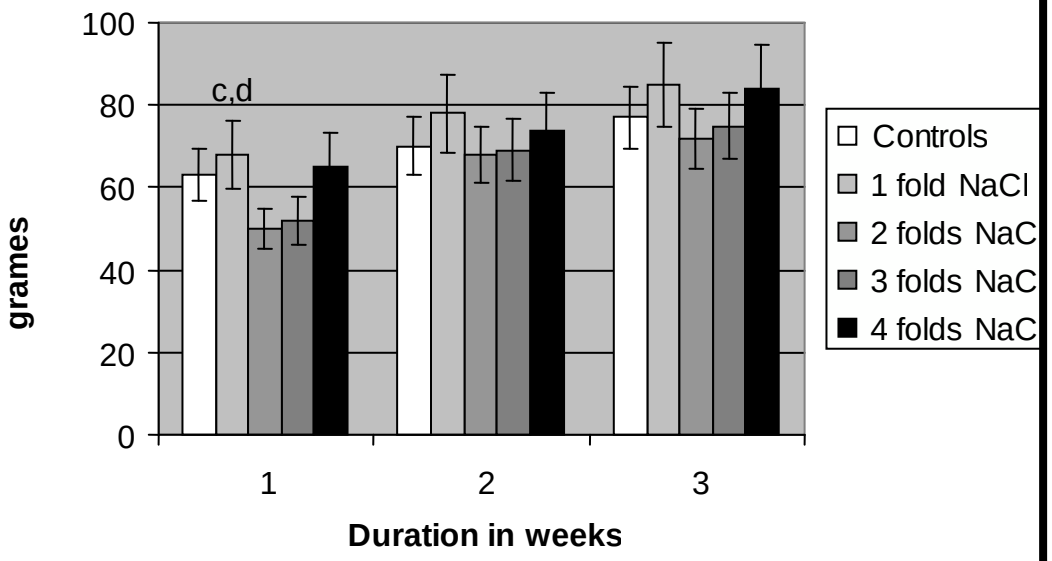
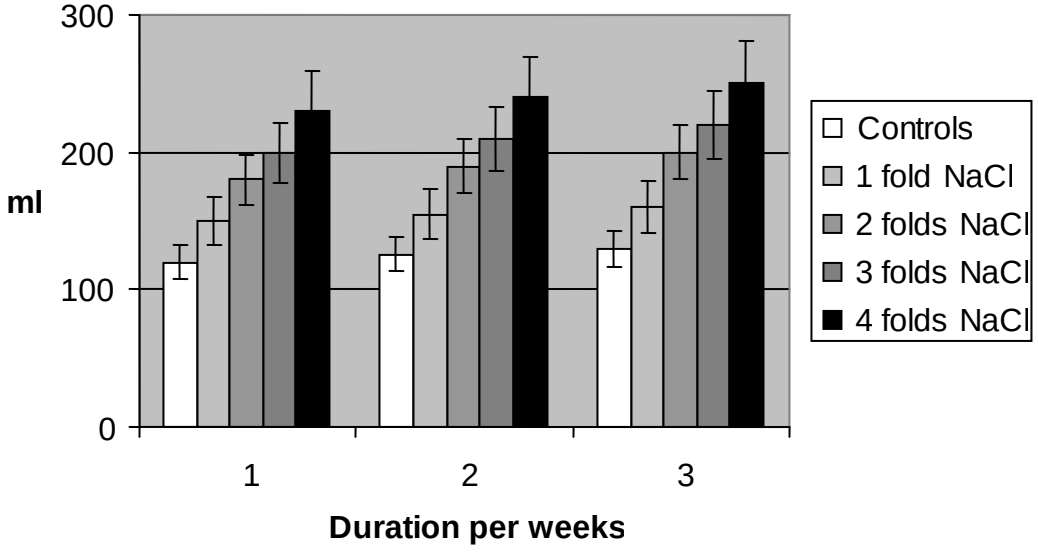


Fig (12)Daily water intake of rats feeding with varying levels of sodium chloride .



تأثير تناول كلوريد الصوديوم على بعض العناصر في ذكور الجرذان البيضاء.

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الملخص العربي

يختبر هذا البحث تأثير تناول جرعات غذائية مختلفة من كلوريد الصوديوم(ملح الطعام)علي بعض العناصر الدقيقة والكبيرة في الجرذان. تم تسكين ثلاثين من ذكور الجرذان البيضاء فرادي داخل أقفاص بلاستيكية، وتم تقسيمهم إلي أربعة مجموعات تجريبية. المجموعة الأولي الضابطة تم تغذيتها بغذاء عياري متزن، تم إعطاء المجموعة الثانية وجبة غذائية تحتوي على ضعف تركيز كلوريد الصوديوم. تم أعطاء المجموعة الثالثة وجبة غذائية تحتوي على أربعة اضعاف من تركيز كلوريد الصوديوم في المجموعة القياسية. هذا وقد أعطيت المجموعة الرابعة غذاء يحتوي على ستة اضعاف تركيز كلوريد الصوديوم. أما المجموعة الخامسة فقد تغذت على غذاء يحتوي على ثمانية اضعاف تركيز كلوريد الصوديوم. ارتبطت التغيرات الحادثة في معدل النمو النسبي بالمتناول اليومي من الغذاء. هذا وقد لوحظ أن التغذية على غذاء مضاعف الملح قد حفز من المكتسب الوزني عن المجموعات التجريبية الأخرى. لم يتأثر وزن الأعضاء النسبي تقريباً فيما عدا عند تناول جرعة مضاعفة ستة مرات من كلوريد الصوديوم.
لم يتأثر تركيز الكالسيوم، الماغنسيوم، والألبيومين في مصل الدم والحديد داخل الكبد مع التغذية بجرعات الملح المختلفة. هذا بينما أرتفع تركيز الحديد، الفوسفور، والزنك والبروتينات الكلية معنويا في مصل الدم ، وخاصة مع تناول الجرعات العالية من كلوريد الصوديوم. انخفض تركيز كالسيوم العظام معنويا مع تناول الأغذية عالية الملح. هذا وقد ارتفع تركيز الحديد داخل الطحال معنويا مع استهلاك الملح.
تخلص هذه الدراسة إلي أن تناول جرعة مضاعفة من كلوريد الصوديوم يحفز من المستهلك الغذائي والوزن المكتسب للجرذان عن باقي الأغذية عالية الملح وان تناول الغذاء عالي الملح يحفز انخفاض كالسيوم العظام، مصحوباً بارتفاع تركيز الحديد في كل من الطحال، مصل الدم وكذلك تركيز الفوسفور والزنك والبروتين الكلي في مصل الدم والذي يمكن أن يرفع من مخاطر الإصابة بهشاشة العظام وتحلل الخلايا الحمراء.