Research Article

Effect of High Salt Diet on Bone in Adult Male Albino Rats

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Abstract
Introduction: high salt diet (HSD) is a dangerous food habit. It has many harmful effects on arterial blood pressure, cardiovascular system, and kidney. **Aim of work:** To assess the effects of the high salt diet (HSD) on adult male albino rats' bone. **Materials and methods:** twenty-four adult male albino rats were divided into two groups: Control group and HSD-fed group. **Results:** Administration of HSD (8 %) for seven weeks to male rats resulted in increased food intake, decreased body weight gain, and BMI, as well as osteoporosis. There was increase in all bone resorption parameters and decrease in bone formation parameters. **Conclusion:** Accordingly, the results obtained from the present study revealed that HSD induced osteoporosis.
Keywords: Diet, Bone, dangerous food and blood pressure

Introduction
The skeleton has structural, protective and locomotor functions, and is a storage site for calcium. Bone has two components; 80% cortical bone and 20% trabecular bone. Cortical bone that surrounds the marrow space is dense and solid as it is heavily calcified. It has the main structural and protective role. It has an outer periosteal surface and inner endosteal surface. The periosteum is a fibrous connective tissue sheath that surrounds the outer cortical surface of bone, except at joints where bone is lined by articular cartilage (Neto and Ferreira, 2018). The structural components of bone consist of extracellular matrix and hydroxy-apatite crystals of calcium-phosphate deposited in it. In addition, bone has water in several forms as bulky water in the Haversian canals which tightly bound water in the organic matrix (Alford et al., 2015). Bone cells regulate skeletal metabolism, their progenitors are also help in this process. Bone cells involved in bone regulation are osteoblasts, osteocytes, and osteoclasts. Monocytes, macrophages, and mast cells may also mediate certain aspects of skeletal metabolism (Kini and Nandeesh., 2012)

Many hormones affect bone metabolism as parathormone hormone, calcitonin, vitamin D3, growth hormone, thyroid hormone and as well sex hormones. Multiple nutrients have role in bone development, maintenance and prevention of bone loss. Dietary calcium is one of the most important nutrients for bone integrity. On the other hand, high dietary sodium intake adversely affects bone integrity causing osteoporosis (Whitish, 2004). The common salt (sodium chloride; NaCl) is an important micronutrient added to food for several aspects. It makes taste of food better, preserves the food and improves the appearance of processed foods. Its physiological need for a human is covered by 10–20 mmol/day (0.58–1.16g of NaCl). However, salt intake till now is generally greater and can exceed 10 g/day in many populations which exceeds more than 200mmol/day.

High salt (HS) intake is related to high risk for developing many diseases as hypertension, cardiovascular diseases (CVDs), metabolic syndrome and osteoporosis. So many well developed societies recommended limiting salt intake to 3.75–6 g/day(Lanaspa et al., 2018). Osteoporosis is a bone disease characterized by reduced density and quality of bone resulting in weakened skeleton and increasing the risk of fractures. It is associated with increased morbidity and mortality. The main clinical manifestations are back pain, loss of height, spinal deformity as well as fractures of the vertebrae, hips and wrists. Diagnosis is typically based on the bone mineral density (BMD) measurements or the history of fracture following minimal

trauma. BMD represents the quantity of the mineralized tissue in the bones including both size and density. It is the most important parameter for determining the of bone susceptibility to fractures (Popp et al., 2016). High sodium intake is harmful to the bones as it decreases the reabsorption of renal calcium leading to high urinary calcium excretion(Dar et al., 2018b).

The present study is designed to investigate the effects of high salt diet on bone of adult male albino rates.

Materials and methods

Animals:

A total of 24 adult albino male of Sprague-Dawley strain, weighing between 150 and 200 g, were housed at room temperature with natural dark/light cycles in 6 mesh cages (4rats/cage). The rats had free access to water and commercial rat chow (Nile Company, Egypt) for one week before the start of the experiment for acclimatization. The experimental protocol was documented according to the rules of the animal care and use committee, faculty of Science, Cairo University. The rats were divided into the following two groups (12 rats each):

Control males (CM): Male rats fed normal salt diets containing 0.3% salt.
High salt diet-fed males (HSD-M): Male rats fed high salt diets containing 8% salt.

Experimental protocol:

The HSD (8%) was prepared by adding extra NaCl to the normal salt powdered chow (7.7% NaCl is added weight/weight to the normal 0.3% NaCl which is originally present in the standard rat chow). After that, the powder was mixed with a small amount of water, formed into pellets, and dried overnight in an oven at 60°C (Oloyo et al., 2019). For the studied groups, the food intake was calculated daily for 7 weeks which was the experimental duration. Initial and final body weights were measured to assess body weight gain and body mass index (BMI). Rats were weighed using an electronic balance (FY 2000), the naso-anal length was measured using a strip meter from the nose to the anus, and BMI was calculated according to the formula put by Novlli et al., 2007: BMI = body weight (g) / length^2 (cm^2).

At the end of the 7th week, all rats were subjected to overnight fasting. After that, the rats were sacrificed by decapitation. The blood samples were immediately collected from the jugular veins. The serum samples were separated in 2 ml Eppendorf tubes, and stored at -20 °C until used for estimating the level of serum calcium, phosphorous, alkaline phosphatase, acid phosphatase, and parathormone (PTH).

The hind left limbs of all rats were also dissected. The femurs of each rat were gently removed and cleaned from adhering muscles and soft tissues. They were used to assess femur length, weight, dry weight, fat-free dry weight, ash weight, and bone mineral density (BMD).

Results

Evaluation of the food intake, body weight gain, and BMI:

The data presented in table (1) show that HSD significantly increased the food intake compared to the control groups. On the other hand, the body weight gain and BMI were significantly lower in HSD group compared to the control group.

Table (1): Effect of HSD on food intake, body weight gain, and BMI:

N : 12 / Parameters	Control Male	HSD Male
Food intake (g/day)	16.8 ± 0.72	21.8 ± 1.16[a]
Bodyweight gain (g)	103.8 ± 2.64	51 ± 9.14[a]
% of weight gain	+55.66%	+28.38%[a]
BMI(g/cm^2)	0.607 ± 0.17	0.577 ± 0.205[a]

Data represent mean ± S.E. ***n*:** number of rats in each group. **HSD:** high salt diet. **BMI:** body mass index. **[a]:** significant difference from the control male group, $P < 0.05$.

Evaluation of the serum parameters:

The data presented in table (2) showed that HSD did not significantly affect both serum calcium and phosphorous levels compared to the control group. Regarding the bone turnover markers; alkaline phosphatase and acid phosphatase, HSD significantly increased these parameters compared to the control group. As regards PTH, HSD significantly increased its level compared to the control group.

Table (2): Effect of HSD on the serum parameters:

N:12 / Parameters	Control Male	HSD Male
Calcium (mg %)	8.31± 0.056	8.3 ± 0.07
Phosphorous (mg %)	5.34± 0.039	5.28 ± 0.02
Alkaline phosphatase(U/L)	119.1± 2.01	206.2 ± 4.4[a]
Acid phosphatase (U/L)	26.96±0.69	77.55 ± 2.2[a]
PTH (pg/ml)	28.33± 1.66	57.76 ± 1.5[a]

Data represent mean ± S.E. ***n*:** number of rats in each group. **HSD:** high salt diet.
PTH: parathormone. **[a]:** significant difference from the control male group, $P < 0.05$.

Evaluation of the bone parameters (measured from the left femurs):

The data presented in table (3) showed that HSD did not significantly affect femur length compared to the control group. Regarding bone weight, dry weight, fat-free dry weight, ash weight, and BMD; HSD significantly decreased these parameters compared to the control group.

Table (3): Effect of HSD on the bone parameters measured from the left femurs:

N : 12 / Parameters	Control Male	HSD Male
Femur length(cm)	3.42 ± 0.041	3.33± 0.058
Bone Weight (g)	0.6012±0.027	0.509± 0.014[a]
Dry weight (g)	0.494± 0.0017	0.3086± 0.039[a]
FFDW (g)	0.485± 0.0018	0.393 ± 0.01[a]
Ash weight (g)	0.261 ± 0.164	0.187± 0.002[a]
BMD (g/cm^3)	3.847 ± 0.017	2.99 ± 0.0048[a]

Data represent mean ± S.E. ***n*:** number of rats in each group. **HSD:** high salt diet.
FFDW: fat-free dry weight. **BMD:** Bone mineral density.
[a]: significant difference from the control male group, $P < 0.05$.

Discussion

The results obtained in the present study showed that there is a decrease in weight gain and BMI in spite of increased food intake in the HSD group when compared to the control group. These results are in line with Oloyo et al., 2019 who found that salt intake stimulates appetite, food intake, and body metabolism, which then causes an increase in energy expenditure and leads to a decrease in body weight. This decrease in weight gain may also be related to the increase in body water loss through excessive urination observed during the experiment.

On studying the direct effects of HSD on the bones, the present study demonstrated that HSD induced osteoporosis as evidenced by: (1) Increased levels of PTH as well as bone turnover markers; alkaline phosphatase and acid phosphatase. (2) Decreased bone weight, dry weight, fat-free dry weight, ash weight, and BMD. The HSD-induced osteoporosis comes in line with Fatahi et al., 2018 who reported a positive association between dietary sodium intake and the risk of increased loss of BMD which, in turn, results in osteoporosis.

The HSD-induced osteoporosis can be explained by the increased PTH level. When salt intake is increased, calcium excretion in urine increases. This should be compensated by increasing calcium mobilization from bone and absorption from the intestine via increasing in serum PTH and calcitriol, respectively (Robinson et al., 2019).

The results obtained in the present study showed that HSD did not significantly affect both serum calcium and phosphorous levels compared to the control groups. The insignificant changes of serum calcium level in spite of increased urinary calcium excretion is explained by the consequent increase in PTH and calcitriol levels that resulted in increased bone resorption and intestinal calcium absorption, respectively**.** The normal serum phosphorous level in HSD groups can also be explained by the balance between increasing its intestinal absorption secondary to increased Na^+ level, and decreasing its renal reabsorption secondary to increased PTH level (Vafa et al., 2016).

Conclusion

Administration of HSD (8 %) for seven weeks to male rats resulted in osteoporosis. HSD may produce this effect through increasing calcium loss in urine, which stimulates the excessive secretion of parathormone hormone with its vigorous resorping effects on bone.

Recommendations

- Hypernatremia is a dangerous situation. Dehydration can lead to increased NaCl concentration in the body and therefore around bone cells. Old people are known to lose the appropriate thirst to balance their water intake so they are liable to dehydration and consequently to all hazards of HSD. So the amount of water and salt intake must be taken seriously in old people.
- For renal patients, they must observe their diet and reduce table salt for the prevention of more electrolyte imbalance and kidney injury.

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