

Mandibular bone density and calcium content affected by different kind of stress in mice

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Abstract

Objectives: Stress is considered to affect many body and mental functions. This leads to activation of the hypothalamic-pituitary-adrenal axis and the adrenomedullary sympathetic system resulting to increased glucocorticoid release. Corticosteroids are known to cause systemic bone loss. The aim of the study was to investigate the role of different kinds of stress on the mandible bone mass of Wistar mice. **Methods:** 75 male Wistar mice were divided into three groups (n=25 each). The animals of group C were submitted to stress by electroshock with 22-45 volts for a duration of 4 seconds each minute for one hour each day. Group B was submitted to isolation stress and group A was the control group. The duration of the experiment was 137 days. **Results:** The adrenals weight was increased (group C vs group A, $p<0.001$; group B vs group A $p<0.05$), while urine hydroxyproline was reduced under stress. The calcium content of the mandible and the ratio between calcium content and mandible volume was decreased ($p<0.05$ for both groups). **Conclusions:** Mandibular bone mass was affected by different kinds of stress and may represent a considerable parameter for the diagnosis, prevention and treatment of bone mass deficiency.

Keywords: Mandible, Bone Density, Calcium Stress, Mice

Introduction

Stress may be considered as a process that affects many bodily, mental and psychological functions^{1,2}. The central components of the stress response are the hypothalamic-pituitary-adrenal axis and the sympathetic nervous system³⁻¹¹.

Adrenal hypertrophy, gastric ulceration, and thymolymphatic dystrophy are the classical triad of the stress noted many decades ago by Selye^{12,13}.

Cortisol reduces the utilization of amino acids for protein formation in muscle cells. Stress resulting in cortisol excess can lead to progressive protein loss, muscle weakness and atrophy, and bone mass loss through increased calcium excretion and less calcium absorption¹⁴. Three mechanisms are involved

in bone loss induced by corticosteroids: First, physiological changes; second, behavioral distortion of eating, drinking, exercise and sleep habits; third, anxiety, depression, loss of social roles and social isolation, further promoting the stress cycle¹⁵.

Bone resorption can be assessed using the urinary biomarker hydroxyproline¹⁶. This is a useful tool for assessing fracture risk at hip, humerus and other bones, as well as for monitoring the effectiveness of osteoporosis therapy as it provides important information about the antiremodeling efficacy of various treatments, particularly the antiresorptive medications¹⁷.

It has been reported that impaired mineralization of the mandible, as shown by low ratios of calcium and inorganic phosphorus to hydroxyproline, occur in rhesus monkeys submitted to postcranial immobilization¹⁸. Moreover, it is documented that osseointegration of titanium implants may be affected by corticosteroid treatment.

In recent years, an interrelationship between systemic loss and resorption of the alveolar bone has been observed. The degree of alveolar bone loss increases with age, and this may be partly related to systemic conditions that also encourage the development of osteoporosis. The relationship between the mandible and primary osteoporosis was further established by clinical studies¹⁹⁻²². Although the alveolar bone loss is independently influenced by local and systemic factors, including osteoporosis, a cross-sectional study in post-menopausal women, evaluated the influence of oral infection

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Groups Xmean±SD	Control (groupA)	Isolation stress (groupB)	Stress electrical stimuli (group C)
Food g/day	8,1±0,5*	6±0,2	6,5±0,6
Water ml/day	14,8±0,9#	12,2±0,7	10,6±0,4
*A/B and A/C p<0,05 and # A/C p<0.05			

Table 1. Food and water consumed per day.

	Group A	Group B	Group C
Body weight (g)	40,74±0,7	36,54±0,87	37,927±0,83
Adrenals absolute Weight mg ± SE	5,95±0,16	4,87±0,18	6,54±0,1**
Adrenals weight/body weight mg/g ± SE	148±0,3	132±0,5	167±0,6**
White cells %	44,2±3,7	69,3±2,7*	42,5±3,3
**p<0,001 *p<0,05			

Table 2. Body and Adrenals weight on day of sacrifice.

and age in association with osteoporosis and oral bone loss²³. Apart from systemic conditions alveolar bone loss can be precipitated by a combination of local factors (periodontal diseases) that increase periodontal alveolar bone loss rates²⁴.

Stress-induced hypercortisolism and the excess of released glucocorticoids also directly affect bones, inhibiting osteoblastic activity and causing osteoporosis²⁵. The main effect of glucocorticoids on bone is inhibition of osteoblast function, leading to linear decrease in bone formation²⁶.

It has been also referred that systemically, corticosteroids treatment reduces circulating levels of estrogen and modestly increases parathyroid hormone levels, while at local level, decreases insulin-like growth factor I (IGF-I) production, induce IGF-I resistance and increases nuclear factor kappaB ligand production by osteoblasts²⁷. These alterations inhibit new bone formation and stimulate bone resorption with a net loss of bone, resulting to decreased bone mineral density, osteoporosis and increased fracture-risk.

Weinstein et al.²⁸ recently demonstrated that treatment with dexamethasone decreased the number of osteoclast progenitors in bone marrow, whereas the total number of osteoclasts on the bone surface was increased by the promotion of osteoclast survival via the prevention of apoptosis. Steroid treatment significantly decreased the bone mineral content (BMC) and bone mineral density (BMD) of the femur (metaphysis and diaphysis) and also significantly lowered the cortical thickness²⁹.

Recent studies indicate that jaws may serve as an index for BMD and the mandible panoramic radiography measures, may be useful tools in identifying postmenopausal women or subjects at risk for osteoporosis with low skeletal BMD³⁰⁻³³, high bone turnover rate³⁴, or high incidence of osteoporotic fractures³⁵.

The aim of the study was to investigate the role of different kind of stress in the mandible bone mass of Wistar mice.

Control (group A)	Isolation stress (group B)	Stress electrical stimuli (group C)
16± 2,3	56,5±4,7*	68,7±3,5)*
*A/B and A/C p<0,05		

Table 3. Corticosterone concentration µg/dl (Xmean±SD).

Material and methods

75 male Wistar mice aged 2.5 months were divided into three groups (n=25 each). The animals were housed under a daily cycle of 12 h darkness and 12 h light, a constant temperature of 21±1°C. The experiment duration was 137 days.

The animals of group A were used as the control group while the animals of group B were submitted to isolation stress. The animals of group C were exposed to chronic stress by electroshock with 22-45 volts for duration of 4 seconds per minute for one hour on each day. A special cage with floor bars and “static scrambler” circuit inducing electrical stimulus was used for this (Ugo Basile automatic reflex conditioner Cat. No.7530.)

During the study all animals had access in standard rodent chow and water *ad libitum*. The food contained albumins 18%, fats 3-7%, ash 5%, Ca 1.5-2%, P04 0.8-1%, total celluloses 6%.

The animals were weighed every 10 days. Urine levels of hydroxyproline were measured on Day 60 and Day 90 day, using the Hypernosticon kit (Organon-Teknika, Boxtel, Holland)³⁶ and the animal motor activity was evaluated with a special appliance (Ugo Basile activity cage Cat. No. 7420) on Day 58 and Day 90.

Groups Xmean±SD	Control (group A)	Isolation stress (group B)	Stress electrical stimuli (group C)
Body weight (g)	40,74±0,7	36,54±0,87	37,927±0,83
Mandible abs. weight (mg)	55,291±0,96	52±1,6	51,96±0,88
Ratio Mand.weight/body weight (mg/g)	2,71±0,04	2,8±0,07	2,7±0,06
Volume mand. cm ³	24,42±1,2	26,36±1,41	22,93±1,14
Specific weight mg/ cm ³	2,26±0,8	1,97±1,13	2,26±0,77
Absolut Ca ⁺⁺ mg/dl	480±1,9	423±0,94*	411,53±0,82*
Ratio Ca ⁺⁺ mand/ mand. volume mg/dl/ cm ³	17,14±0,862	16,79±0,96	12,14±0,092*
Ratio Ca ⁺⁺ mand/ mand. Weight1 mg/dl/mg	7,57±0,211	8,11±0,32	8,25±0,1142*
Hydroxyproline mg/24h	0,451±0,07	0,335±0,071	0,353±0,044
Ca/HO-Pr	1064,3±27,14	1262,69±13,24	1165,81±18,64
*p<0,05			

Table 4. Bone Parameters during stress influence.

Animals were anesthetized by ether in special cages and sacrificed on Day 137. Blood was collected from the right atrium by heparinized test tubes, stored on ice and centrifuged at 3000 g for 10 minutes at 4 degrees Celsius for the determination of blood corticosterone levels. Corticosterone concentration in plasma was measured by radioimmunoassay ¹²⁵I- Radioassay. (ImmuChem Co Carson City CA)³⁷. Animals were sacrificed between 8.00 h and 10.00 in the morning. The animals of groups B and A were taken from the housed cages and sacrificed immediately while group C was sacrificed 1 hour after the electrical stimuli.

The incisors of the mandible were removed because of their increased contents in calcium that could influence the calcium estimation of the jaw. Adrenal glands and mandible were isolated, removed and weighed. White blood cells count was determined. Mandible volume and calcium content were measured. After isolating the mandible from soft tissues, it was placed in special porcelain cups and was heated to 600 degrees Celsius for 24 hours. Thereafter, 30 ml of HCl solution (6N) was added to the resulting bone ash. Bone calcium content was measured by atomic absorption spectroscopy, which determines the presence of metals in liquid samples (Perkin Elmer A Analyst 700).

The animals were housed and cared according to the "Guide for the Care and Use of Experimental animals"³⁸.

Results

Compared to group A, both stressed groups had significantly lower food consumption, whereas the difference in water intake was lower only in group C (Table 1). Differences in mobility were not statistically significant. Body weight was similar between groups on the day of sacrifice, but the stressed animals had a slower increase of body weight during the experimentation period (Table 2). Compared to baseline, the final weight was increased by 10% in group C, 15% in group B and 17% in group A. A statistically significant difference (p<0.01) was observed between the mean weight of groups B and A during the last 10 days.

The absolute weight of the adrenals and the ratio between adrenal weight and body weight was statistically significantly in-

creased under stress condition in group C (electrical stimuli) in comparison to the control group (p<0.001). The percentage of white cells (lymphocytes) was increased in group B while no difference was observed in group C (Table 2). Plasma corticosterone levels of group B and C were increased in comparison to group A (Table 3).

Urine collection for the determination of hydroxyproline excretion was performed in special cages (5 mice per cage in order to collect enough urine). No statistically significant differences between the groups were found, but the ratio between calcium and hydroxyproline was higher in the stressed groups (Table 4).

Stress affects the weight of the jaw. A non statistically significant decrease of the mandible absolute weight in Group B and C was observed. The mandible volume was decreased in group C (electrical stimuli) and increased in the isolation group compared to the control (no statistically significant). In addition no difference in the mandible specific weight between the groups was observed. The absolute calcium concentration of the mandible was decreased in stressed groups in comparison to control (C vs A, B vs A, p<0.05).

The ratio between calcium content and mandible volume was statistically significantly decreased in group C compared to the control, while the ratio calcium versus mandible weight was increased under stress but statistically significant only in group C.

Discussion

Osteoporosis, the most common degenerative disease³⁹ in developed countries, typically exhibits reduced bone mass resulting from imbalanced bone remodelling with a net increase in bone resorption. Epidemiological studies implicate major depression as one of the most important medical conditions that contribute to reduced BMD and increased incidence of osteoporosis⁴⁰.

A large number of studies have observed an increased activity of the adrenal cortex during stress. Many investigators of various medical fields have documented steroid-induced osteoporosis, but these results are not fully disseminated among dental professionals^{24,41-43}.

Glucocorticoids and sympathetic agonists are considered as inhibitors of bone formation and inducers of bone mass reduction⁴⁴⁻⁴⁶.

According to the results (Table 2) it is obvious that both kind of stress procedures increase animal adrenals weight, which is an established index of enhanced corticosteroids levels in systematic circulation, as a response to stress stimuli and as it is referred by other investigators and as it is even described to promote an excess of injuries because of stress⁴⁷⁻⁴⁸.

In Table 4 it is shown that although stress influences the bone mass quality of the mandible, no differences in mandible weight/body weight ratio is observed. This may occur because both body weight and mandible absolute weights are reduced by stress, so as the expressed ratio can not reveal these alterations. The observed decrease in body weight of the experimental groups may be attributed to stress enhanced corticosteroids release. Similar results are demonstrated in animals under prednisone treatment, which may serve as an experimental model for osteoporosis⁴⁹.

Moreover a large number of studies demonstrated a decrease of the mandible size in length and height under glucocorticoid treatment, that may reflect a diminution of the bone weight^{50,51}. Similar changes of mandibular sizes are observed in population with osteoporosis induced fracture⁵².

The mandible volume was decreased in experimental animals under electrical stimuli. Probably the electrical stimuli inducing intense stress condition and further corticoids release resulted in reduction of the bone mineral content. This is in agreement with the findings of Kozai et al⁵³ where repeated prednisone treatment produced similar changes in the mandible such as decreased cortical bone mineral content, cortical thickness, stress/strain index and tissue bone volume. The immobilization stress and the low-mineral diet leads to decreased mandible BMC^{54,55}. In addition, other studies show a highly significant correlation between total body bone mineral content assessed by absorptiometry (Dichromatic Bone Densitometer Model 2600; Norland Co., WI, USA) and the total body ash weight of rats demonstrating the reduced bone density in osteoporotic rats⁵⁶. The above stressed animals had limited food intake in relationship to the controls (Table 1), which is in accordance with the study of the restricted mineral diet^{57,58}. On the other hand the observed increased animal mobility under stress may be helpful to prevent further bones injuries.

In contrast, the ratio between calcium content and mandible weight was increased in the stressed groups. Similar results are reported by various investigators, who observed increased bone mass parameters in asthmatic subjects undergoing corticosteroids therapy, where long term inhaled budesonide led to a small enhancement or no alteration of bone calcium content⁵⁷⁻⁵⁹. Besides it is suggested that even a long duration of corticosteroids administration does not necessarily affect the calcium content of various bones⁶⁰.

On the other hand an excess of catabolism occurs during stress since as it is shown in Table 4 urine hydroxyproline levels are decreased in both stressed groups¹⁸. In contrast the ratio between calcium and hydroxyproline is increased in the

stressed groups and it can be suggested that it reflects a decreased bone organic fraction⁶¹.

Although hydroxyproline was decreased in both stressed groups without statistical difference, this discrepancy from the control may serve as an index of the osseous tissue catabolism and can explain that since the bone is deprived from organic substitute it contains relatively more calcium per bone unit. Furthermore it is suggested that minimal trauma fractures may occur in patients treated with glucocorticoids at higher bone mineral density than is seen with other primary or secondary causes of osteoporosis⁶².

It may be concluded that mandible bone mass is affected by different kinds of stress and may serve as a parameter for the diagnosis, prevention and treatment of bone mass deficiency for better osteoporosis incidence estimation and management in the general population^{63,64}.

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