


The Influence of Dietary Salt on the Osseointegration of Implants in Aging Rats

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Abstract

Introduction The bone-implant interface has been studied extensively, but only few papers focused on the nutritional aspects that may affect bone quality, especially salt intake.

Objective To study the osseointegration of implants in salt-loaded rats with low mineral bone content.

Methods A total of 60 4-month-old male rats were divided in 2 groups ($n = 30$), being these groups divided in 2 periods, (2 and 4 months). The control group received a normal diet, while the test group received a diet supplemented with 1% sodium chloride (NaCl). Implants were placed in the tibia of both groups. A total of 15 animals of each group were sacrificed at the 2nd month of the experiment, while the remaining animals were sacrificed at the 4th month.

Results No statistically significant difference was found in food intake between the groups on any experimental period, but a statistically significant difference was found in the liquid intake in the saline group in both periods. For all groups, osseointegration was observed in both groups. The mean percentage of osseointegration in the cortical bone, in the trabecular bone, and in the total osseointegrated surface between the control ($46.38 \pm 16.17\%$) and saline ($49.13 \pm 11.52\%$) groups at 2 months was not statistically different ($p = 0.61$). The total osseointegration areas of the control ($53.98 \pm 12.06\%$) and saline ($51.40 \pm 13.01\%$) groups at the 4th month of the study were not statistically ($p = 0.61$).

Conclusion Ingestion of salt did not affect directly the osseointegration process during the period of the experiment. The results suggest that mineral losses may not affect the achievement of good osseointegration in aging rats.

Keywords

- ▶ osseointegration
- ▶ sodium chloride
- ▶ bone density
- ▶ bone-implant interface

Introduction

Many epidemiological studies about oral health status in populations have demonstrated a direct relationship between the number of edentulous people and their age, as well as an inverse relationship between the number of teeth in mouth and age,^{1,2} which would mean an increase in the needs for dental implants in the elderly.³

The osseointegration concept and its application in clinical dentistry have been widely used in modern dentistry, especially for edentulous people. According to Bränemark, osseointegration can be defined as a structural and functional direct connection between a live bone and an implant surface under functional load.⁴ Schroeder clearly demonstrated the direct anchorage between the implant surface and the bone.⁵ The interface between bone and implant has been considered the

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key point for osseointegration. Many studies have been made to analyze its structure in the macroscopic, microscopic, and ultrastructural levels.⁶

The literature related to the indication of implants in patients with low bone quality is controversial, specifically in relation to osteoporosis.^{7,8} In spite of the fact that osteoporosis results in a loss of bone mass, especially in long bones, the repairing and healing process may occur normally.⁹

Nutrition deficiency, especially calcium (Ca) deficiency, has been considered one of the risk factors for bone loss in humans.¹⁰ The typical Western diet is rich in proteins, in salt, and in processed foods, which could contribute to the increased incidence of osteoporosis.¹¹ In many Western countries, the ingestion of salt is considered high (~10 g/day), and a moderate reduction in salt intake (to 4 g/day) would lower blood pressure and Ca excretion.¹² The effects of sodium are explained by the fact that sodium and Ca have a shared transport mechanism in the renal proximal tubules. Any increase in the filtering of one ion would lead to an increase in the excretion of the other.¹³⁻¹⁵ Several authors agree that the high ingestion of sodium chloride (NaCl) causes an increase in the loss of Ca through urine, and that it reduces the mineral composition of the bone, thus affecting the balance of Ca in the organism.¹⁶⁻¹⁹ The increase in the consumption of salt also produces an increase in the urinary excretion of hydroxyproline, indicating an increase of the bone resorption process.²⁰⁻²²

During the aging process, some physiological modifications occur in the mouth of the elderly individual. Loss of gustative capacity is one of them, and it has been related to an increase in sugar and salt consumption by the elderly.²³ Low salivary flow and xerostomia are other factors that are associated with an increase in the taste preferences for salty and acid foods by the elderly. Galili demonstrated that animals with induced xerostomia preferred a salty diet.²⁴ It is also well-known that elderly people are a group that consumes a very high amount of medications. A great proportion of these medications have as side effects xerostomia or a decrease in the salivary flow,²⁵ which might be responsible for an increased NaCl intake in the elderly group of the population.

Another issue, which should be pointed out, is that nutritional status is related to oral health status. Many investigations have demonstrated a clear relationship between masticatory efficiency and nutritional deficiency.^{26,27} Epidemiological data showed that many aged people have a poor oral health status that results in a reduction in the masticatory capacity.²⁸⁻³⁰ The altered taste perception influences the preferences for salty food. Elderly people, especially those in a poor health status, are more likely to eat a salty diet.

Researches have been made to relate the mineral density of the bone and bone resorption as a consequence of the ingestion of salt. The bone density of the femur, of the tibia, and of the vertebrae in mice has been investigated.³¹ Mice fed with a high-salt diet presented a decrease of 5.5% in the bone density of different bones. Similar results in terms of reduction of the Ca content in the bone were found in other studies.^{21,32} Chan et al clearly demonstrated in rats that the Ca metabolism and its concentration in the bone suffered alterations when the

animals were submitted to different levels of salt ingestion. They concluded that the high consumption of salt causes an increase in the loss of Ca and a reduction of the content of Ca in the bone.³² Although the relationship between salt intake and Ca excretion has been reported, the influence of different nutrients, especially NaCl, on the osseointegration process, was not found in the literature.

The objective of the present investigation is to analyze the effects of high NaCl ingestion on the osseointegration process around titanium implants in an aging rats model experiment.

Material and Methods

Experimental Animals and Study Design

Few animal models are available to study the effect of novel implant surface modifications in osteoporotic conditions. Laboratory rats comply with several practical advantages, including the reliability of several methods for rapid induction of osteoporotic conditions.^{33,34} Therefore, Wistar rats were used in the present study.

A total of 60 4-month-old male rats were obtained from a biological research institute. All of the animals were housed under conditions of controlled temperature ($23 \pm 2^\circ\text{C}$) and artificial light (12-hour light/dark) cycle.

The animal research model of the present study follows the guidelines and international principles of biomedical research in animals (World Health Organization-United Nations Educational, Scientific and Cultural Organization [WHO-UNESCO]),³⁵ and has been approved by the scientific and ethical committee of São Lucas Hospital of Pontifícia Universidade Católica of Rio Grande do Sul.

The rats were randomly divided in 2×4 groups (60 days and 120 days groups)³⁶ of 15 animals each, and they were all fed with a normal diet of standard pellets (Nuvital Nutrientes Ltda., Colombo, PR, Brazil) containing 1% of NaCl and 0.45% of Ca. Group A (control) received the normal diet and distilled water, both *ad libitum*. Group B (experimental) received the normal diet and a saline solution (1% NaCl),^{37,38} both also *ad libitum*. We have shown in a previous study that rats submitted to the same saline solution had a reduction in the bone mineral content in the tibia.³⁹ Both liquid ingestion and food consumption were measured weekly. The animals were weighted at the beginning of the experiment and in the day of the sacrifice.

Implants and Surgical Procedures

Screw-shaped implants made of commercially pure titanium were machined in an identical manner (titanium content, 99.66%; Conexão Sistemas de Prótese, São Paulo, SP, Brazil). The total length of each implant was 3.5 mm; the thread diameter was 1.8 mm, and the pitch was 1 mm. The implants were cleaned and sterilized by the manufacturer.

The rats were anesthetized by abdominal administration of 0.1 ml/kg of a 1:1 mixture of ketamine chloride (Ketamina; Virbac, São Paulo, SP, Brazil) and tiazine (Rompun; Bayer, São Paulo, SP, Brazil). The animals were then divided equally into test (salt solution) and control (water) groups with 2 and 4 months of duration of the experiment.

An implant was placed in the proximal metaphysis of the tibia in each animal 60 days after the saline solution was initiated. After general anesthesia, the bone surface of the tibia was exposed by an incision ~10 mm long. Under profuse saline irrigation, the implantation hole was drilled at a low rotation speed; the implant was then inserted into the hole.

A total of 15 rats of each group were sacrificed by decapitation under anesthesia (described above) at 60 and 120 days after the implantation, and the implants were removed along with bone tissue and fixed in 10% buffered formaldehyde.

Sample Preparation and Analysis

The specimens were dehydrated and embedded in Technovit 7100 glycol methacrylate resin (Kulzer Technik, Wehrleim, Germany). One sagittal undecalcified ground section was prepared along the axis of the implant. Cuts were made using the method described by Padilha et al.⁴⁰ Each section was stained with 1% toluidine blue and inspected under a B201 light microscope (Olympus, Tokyo, Japan).⁴¹

Photographs of each specimen were taken with a B201 stereomicroscope (Olympus, Tokyo, Japan) in 50x magnification using a conventional Kodak ULTRA 135, ASA 100 film (Kodak, Rochester, NY, USA). Afterwards, the photographs were digitized with an Epson Expression 636 desk scanner (Epson, Suwa, Japan).

The implant-bone contacts were morphometrically evaluated according to the following primary parameters (► Fig. 1):

- Total cortical bone contact (mm): the total amount of cortical bone in contact with the implant (► Fig. 1, A + B; C + D; E + F; G + H).
- Total trabecular bone contact (mm): the total amount of trabecular bone in contact with the implant (► Fig. 1, K + L).
- Length around the implant (mm): length around the implant in the bone tissue.
- Total bone contact length (mm): the total amount of bone (cortical + trabecular) in contact with the implant.
- Bone contact rate (%), calculated as follows: (total contact length/length around the implant) x 100. The same was applied for cortical and trabecular contact rate.

The primary parameters were measured from the digital images using the UTHSCSA Image Tool, version 2.02 automated imaging analytic system (Wilcox et al, University of Texas Health Science Center, San Antonio TX, USA).

The statistical analysis was conducted using the t-test for differences between the groups, and analysis of variance (ANOVA) to see the differences of the variables among the groups during the experimental period. The level of significance was $p < 0.05$.

Results

During the experimental period, the animals were weighted at the day of the sacrifice. The mean weight of the control group was 395.33 g (± 32.73), while in the experimental group the mean weight was 403.38 g (± 31.56). No statistically significant difference was found between the groups.



Fig. 1 Scheme of cortical and trabecular bone measures

All of the implant sites were mechanically tested and were found to be clinically stable. Also, no clinical findings of adverse reactions or of inflammation could be observed around the implants.

The animals did not show health complications in the surgical site during the experiment.

Histologic Evaluation

For all groups, areas of osseointegration in cortical and trabecular bone around the implant were seen on both the control and saline groups (► Fig. 2). The implant-bone contacts were morphometrically measured and expressed in percentages. ► Table 1 shows that the mean percentage of osseointegration in both the control and saline groups were 38.70% and 38.11% in the cortical bone, and 7.69% and 11.02% in the trabecular bone, respectively, and that the total osseointegrated surface ($46.38 \pm 16.17\%$) and ($49.13 \pm 11.52\%$) at 2 months was not statistically different between the two groups ($p = 0.61$).

At 4 months after the insertion of the implant, the results showed that the mean percentage of osseointegration in both the control and saline groups were 46.92% and 41.18% in the cortical bone and 7.06% and 10.22% in the trabecular bone, respectively, and that the total osseointegrated implant surface ($53.98 \pm 12.06\%$) and ($51.40 \pm 13.01\%$) was not

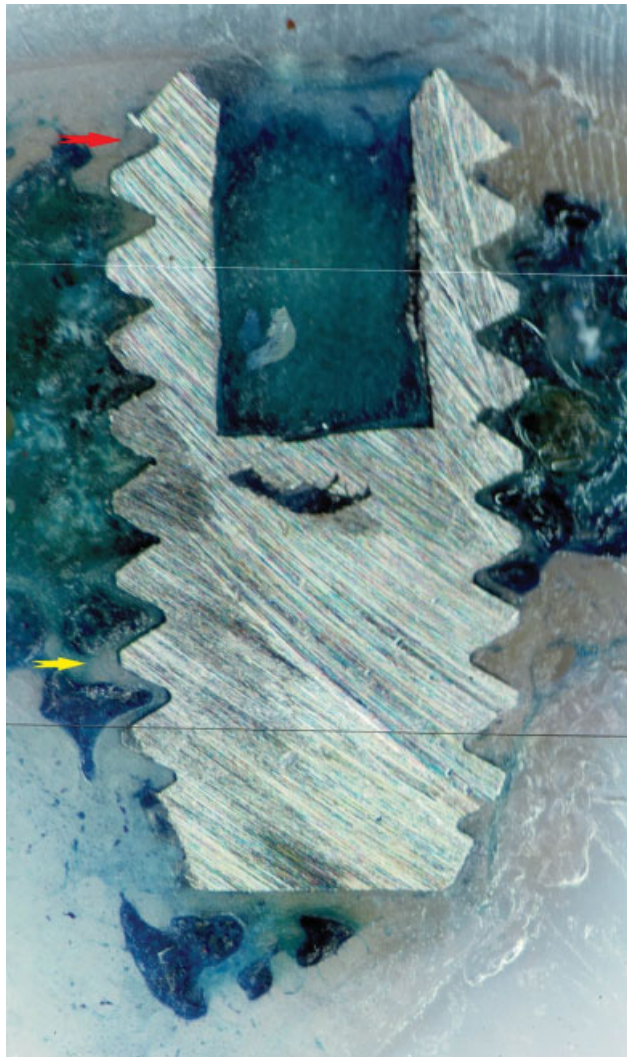


Fig. 2 Osseointegration areas in the cortical bone (red arrow) and in the trabecular bone (yellow arrow).

Table 1 Mean and standard deviation of the cortical bone, of the trabecular bone, and of the total osseointegrated implant surface (in percentages) of the control and saline groups at 2 months

	Control	Saline	<i>p-value</i>
	Mean ± SD <i>n</i> = 15	Mean ± SD <i>n</i> = 15	
Cortical bone	38.70 ± 17.50	38.11 ± 12.13	0.92
Trabecular bone	7.69 ± 10.50	11.02 ± 10.81	0.42
Total osseointegrated implant surface	46.38 ± 16.17	49.13 ± 11.52	0.61

t-test ($p < 0.05$).

statistically significant the difference between the two groups ($p = 0.61$) as can be seen in **Table 2**.

Table 3 shows a comparison of the osseointegration process between the 2 time periods (2 and 4 months) of the control group. It can be observed that an increase in the osseointegration area occurred in the cortical bone (38.70 to 46.92%) and in the total osseointegrated implant surface

Table 2 Mean and standard deviation of the cortical bone, of the trabecular bone, and of the total osseointegrated implant surface (in percentages) of the control and saline groups at 4 months

	Control	Saline	<i>p-value</i>
	Mean ± SD <i>n</i> = 15	Mean ± SD <i>n</i> = 15	
Cortical bone	46.92 ± 12.47	41.18 ± 14.70	0.30
Trabecular bone	7.06 ± 5.67	10.22 ± 11.54	0.40
Total osseointegrated implant surface	53.98 ± 12.06	51.40 ± 13.01	0.61

t-test ($p < 0.05$).

Table 3 Mean and standard deviation of the cortical bone, of the trabecular bone, and of the total osseointegrated implant surface (in percentages) of the control and saline groups at 2 and 4 months

Osseointegration areas	2 months Mean ± SD	4 months Mean ± SD	<i>p-value</i>
Cortical bone			
Control	38.70 ± 17.50	46.92 ± 12.47	0.18
Saline	38.11 ± 12.13	41.18 ± 14.70	0.56
Trabecular Bone			
Control	7.69 ± 10.50	7.06 ± 5.68	0.85
Saline	11.02 ± 10.81	10.22 ± 11.54	0.85
Total osseointegrated implant surface			
Control	46.38 ± 16.17	53.98 ± 12.06	0.18
Saline	49.13 ± 11.52	51.40 ± 13.01	0.64

Analysis of variance ($p < 0.05$).

(46.38 to 53.98%) with time, although this is not statistically significant ($p = 0.18$).

A similar pattern is observed in the saline group with time (2 and 4 months). The cortical bone contact (38.11 to 41.18%) and the total osseointegrated implant surface (49.13 to 51.40%) increased, but there was not a statistically significant difference ($p = 0.64$).

In both groups (control and saline), the total osseointegration improved with time, but the difference between the two time periods was not statistically significant.

The ANOVA test was applied to see differences on the percentages of cortical bone, trabecular bone, and total osseointegrated surface between the groups during the experimental period. No statistically significant difference was found.

Discussion

Although the bone-implant interface has been studied extensively, a very small number of papers have been concerned with the nutrition factors that may affect bone quality, both prior to the implant placement, as well as during the healing period.

The quality of the bony bed has been evaluated by the necessary torque to remove the implant, but many factors are known to affect bone quality.⁴² Among these factors, nutrition deficiency, and especially calcium deficiency, has been considered a risk factor for bone loss in humans.¹⁰

The present study was conducted to obtain information regarding osseointegration of implants in rats with low mineral content induced by a dietary salt ingestion. Bone reaction after the placement of implants with time have been examined through the use of rat tibia at 60 and 120 days.

The body weight and the food consumption of the rats did not show a statistically significant difference between the control and experimental groups at different times. In relation to the liquid, the experimental group drank significantly more than the control group. These results show that the experimental animals had an even higher ingestion of salt through the saline solution. Since both groups had no difference in the amount of Ca intake from the food, this suggests that Ca losses from bone may not be replaced in the experimental group. According to our previous study, the ingestion of a salty diet can affect adversely the incorporation of bone mineral in rats receiving normal dietary Ca for a period of 4 months.³⁹ Other studies also suggested that, in salt loaded rats, the lower bone Ca content is believed to have resulted from an increased urinary loss of Ca that was not compensated by a higher intestinal absorption.^{13,20}

In long-term studies of the salt effect on bone, Cohen et al believe that an adaptive process in Ca homeostasis might take place.⁴³ It has also been demonstrated elsewhere that the ingestion of NaCl for a long time did not affect the mineral content of the tibia in rats.³⁹

Although the ingestion of salt can reduce the bone mineral content in the tibia of rats, we observed in the present study that the ingestion of salt did not affect directly the osseointegration process. Only a slight difference in bone contact between the implant and the new trabecular bone area is observed in the saline group at 60 and 120 days. This result could be expected due to the difficulties in measuring this osseointegration area. In spite of this fact, salt ingestion did not affect significantly the trabecular bone contact with the implant at 2 and 4 months after the implantation. When the same groups were compared with time, a statistically significant difference between groups was not found in relation to trabecular osseointegration areas. There was not a statistically significant difference for total osseointegration. On the other hand, a study done with osteoporotic-induced rats showed that a lower maxillary bone quality can reduce early bone formation on implant surfaces.⁴⁴

In relation to the cortical bone contact with the implant, the results show that there was no statistically significant difference between the two groups at different times of the experiment. This suggests that mineral losses caused by high salt ingestion may not affect the achievement of good osseointegration, even if an implant is applied in a situation of lower bone quality. In contrast, Yamazaki found that in rats submitted to ovariectomy, a decrease in bone mass causes a reduction in the contact area between implant and bone, and may also cause a reduction in the supporting ability of the

implant due to the thinning of the surrounding trabecular bone.⁷

The healing process after the placement of implants into the tibiae of rats is known to reach completion ~ 56 days after the implantation.³⁶ However, the effect of mineral losses caused by salt ingestion with time on bone remodeling around the implants has not been clear. In the present work, we have not found a statistically significant difference in a long-term study between the control and experimental group in terms of osseointegration. We have also found that new methods have been developed to improve the success rate of clinical dental implants in rat models with osteoporosis.⁴⁵

Giro et al performed a systematic review focused on failure rates and percentages of osseointegration of dental implants in healthy and osteoporotic subjects and found that definitive conclusions regarding the impact of osteoporosis on dental implant therapy cannot be made at this time, and that it could be suggested that osteoporotic subjects can receive dental implant therapy.⁴⁶ Another retrospective study comparing women with different osteoporosis status concluded that the diagnosis of osteopenia or of osteoporosis did not contribute to an increased risk of implant failure.⁴⁷ Our study in animal models contributes with the idea that osteoporosis is not a clinical contraindication for dental implant treatment.

Conclusions

Despite the fact that a saline diet can affect the bone mineral content in the tibia of rats, the results demonstrated that there was no difference in osseointegration between the control and saline groups. To better elucidate the relationship between the pathologic changes in bone and in bone reactions around the implants due to salt intake, it is necessary to investigate in more details the effects of aging on the remodeling process of bone in the osseointegration site.

Conflicts of Interests

The authors have no conflicts of interests to disclose.

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