# Extended Periods of Alcohol Intake Negatively Affects Osseointegration in Rats

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The negative effects of chronic and excessive consumption of alcohol on bone metabolism are reported in the literature. Alcoholism causes a reduction in bone quality and delays fracture repair, among other deleterious effects. However, its effect on osseointegration in dental implants is not fully established. The aim of this research was to investigate the influence of prolonged and excessive consumption of alcohol on osseointegration in rats. Thirty-five female rats, 3 months of age, were divided into five groups according to alcohol consumption period: control (no alcohol), and 3, 4, 5, and 6 months of alcohol consumption. All animals received solid food ad libitum. At 8 months of age, all animals received a dental implant in the right femur, and euthanasia was performed 1 month after the implant placement (final n = 27). Quantification of the percentage of bone-implant direct contact was performed by histomorphometry. Serum levels of calcium and phosphate were also measured. The groups that consumed alcohol for longer periods presented decreased percentages of bone-implant direct contact. The difference was higher in implants apical region. Alcohol consumption did not affect serum calcium levels but raised the level of serum phosphate. Alcohol consumption increased caloric intake but also increased weight loss. It was concluded that chronic and excessive consumption of alcohol can impair osseointegration in rats.

*Key Words:* alcohol, osseointegration, dental implants

## INTRODUCTION

Dental implants are considered the treatment of choice for rehabilitation of partially or totally edentulous individuals. Although performed with high rate of success, some factors related to the basic process of osseointegration are not yet fully elucidated. It is unclear how or if some patients' related habits or conditions are able to influence this process. One of these habits is alcoholism.

It is estimated that 2 billion people in the world consume alcoholic beverages.[1] Excessive alcohol consumption affects the economy of the entire society. The quality of life is also affected once this habit becomes a health hazard.[2]

Alcohol has negative effects on various organs and tissues. In bone tissue, it delays the neoformation[3–5] by decreasing the synthetic and proliferative capacity of osteoblasts.[6] Calcium and phosphate, essential minerals in bone composition, could also have their serum levels affected by alcohol consumption.[7–9]

Several studies have shown the effects of alcohol on bone metabolism and repair, but there is little information regarding alcohol consumption in osseointegration of dental implants. Although there are similarities between the processes of bone healing and osseointegration, the latter has peculiarities that

qualify osseointegration as a unique event, requiring specific studies.[13]

Few studies have evaluated the effect of alcohol on osseointegration, and those had short evaluation periods.[11–13] The absence of research in this field, coupled with the growing use of implants and greater alcohol consumption, reinforce the need to study the effects of alcohol on osseointegration. The aim of this paper is to verify the impact of long periods of excessive alcohol consumption on osseointegration in rats. Our hypothesis is excessive and prolonged consumption of alcohol can be harmful to osseointegration. To test this hypothesis, the bone-implant direct contact, as well as the serum calcium and phosphate levels, was evaluated in different periods of alcohol consumption in an animal model.

## MATERIALS AND METHODS

### Animals

Thirty-five female 3-month-old rats (*Rattus norvegicus*, albinus Wistar strain) were used. They were housed at room temperature and fed a controlled diet. Eight animals died during the anesthesia or during postoperatory, resulting in a final n = 27.

The rats were equally divided into 5 experimental groups according to their dietary conditions, as follows: group G1 (final n = 6): food and water ad libitum; G2 (final n = 6): food and 20% alcohol solution ad libitum for 3 months; G3 (final n = 4): food and 20% alcohol solution ad libitum for 4 months; G4 (final n = 6): food and 20% alcohol solution ad libitum for 5 months; and

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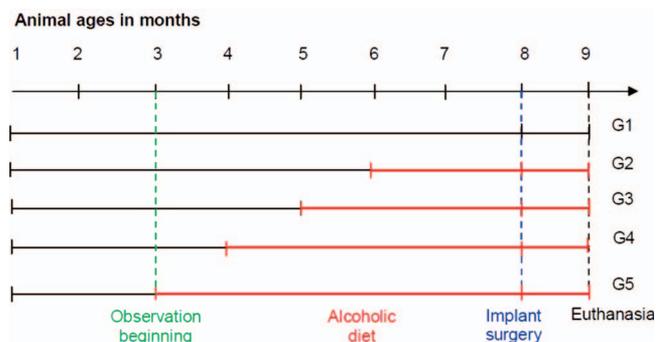* *Corresponding author,* e-mail: leonardo-marchini@uiowa.edu

**Figure 1.** Timeline of experimental procedures.

| TABLE 1 | | | | |
|---|---|---|---|---|
| Percentage of direct bone-to-implant contact, considering the whole implant area | | | | |
| | G1 | G2 | G3 | G4 | G5 |
| Average | 66.8% | 74.4% | 65.1% | 53.8% | 64.2% |
| Median | 78.4% | 82.2% | 66.7% | 53.8% | 69.9% |
| Standard Deviation | 29.6% | 27.8% | 31.0% | 33.1% | 30.8% |
| Q1 | 42.9% | 60.3% | 47.9% | 28.1% | 49.4% |
| Q3 | 87.8% | 100.0% | 100.0% | 78.7% | 89.8% |
| N | 64 | 81 | 68 | 66 | 54 |
| IC | 7.2% | 6.0% | 7.4% | 8.0% | 8.2% |
| P-value | | | .004* | | |

*Indicates a significant difference (Kruskal-Wallis test).

G5 (final n = 5): food and 20% alcohol solution ad libitum for 6 months. Figure 1 shows the timeline of the experiment. The alcohol 20% solution was obtained from an absolute alcohol (Ecibra, Santo Amaro, Brazil) dilution in water.

The study was approved by the ethics committee of the São José dos Campos School of Dentistry, State University of São Paulo, according to the protocol number 028/2009 – PA/CEP.

### Anesthesia

The rats were anesthetized by intramuscular injection of 0.1ml/Kg of 1.25:1 mixture of xylazine chloride (Anasedan-Vetbrands, Jacareí, Brazil) and ketamine chloride (Dopalen-Vetbrands, Jacareí, Brazil), whenever necessary during the procedures described.

### Implant placement

The rats from all groups received implants 30 days before euthanasia. The implants were made of a commercially available titanium alloy (Ti4V6Al) and were cylindrical in shape (2 mm length × 2 mm diameter). The external surfaces were machined and then blasted with aluminum oxide. All implants were sterilized individually before placement and placed in the distal epiphysis of the right femur in each animal of each group, as described earlier.[13]

### Euthanasia

After an osseointegration period of 30 days,[13,14] the rats were euthanized with an excessive dose of anesthetic. The right femurs were removed and prepared for histological evaluation.

### Histologic preparation

Evaluation of the bone-implant morphology was conducted by the undecalcified bone method[13,15] using plastic-embedded (Arkema, São Paulo, Brazil) sections (approximately 80 μm) under a Zeiss Axiophot light microscope (Carl Zeiss, Oberkochen, Germany). Two slices per femur were obtained. These were prepared and stained with toluidine blue.

### Histomorphometric analysis

The percentage of direct bone-to-implant contact was evaluated in the area between the implant surface and cortical bone, using image analysis software (NIH ImageJ version 1.31 for Windows, National Institutes for Health). The bone-implant contacts were measured in the mesiocervical (from cervical border to the center of mesial area of the implant), mesio-apical (from the center of mesial surface to apical implant border), distocervical (from cervical border to the center of distal area of the implant), disto-apical (from the center of distal area to apical implant border), and apical (from the mesial to the distal apical border of the implant) surfaces.

### Serum calcium and phosphate

After the excessive dose of anesthesia, blood was collected from the abdominal aorta and sent to a veterinary laboratory (Diagnovet, São José dos Campos, Brazil) for quantification of serum calcium and phosphate.

### Statistical analysis

Data were not normally distributed and had no homocedasticity, so the comparison tests used were nonparametric (Kruskal-Wallis and Mann-Whitney U tests). The correlation test was used in quantitative variables, and the Spearman correlation test was used to measure the intensity of the correlation. A significance level of 0.05 was adopted for all applied tests.

### RESULTS

The percentage of direct bone-to-implant contact considering the whole implant area is shown in Table 1. G4 and G5 showed the lowest percentage of direct bone-to-implant contact, and G4 was significantly different from all other groups, except from G5 (Mann-Whitney U test).

The percentage of direct bone-to-implant contact was also assessed in the implant areas separately: mesiocervical, mesio-apical, apical, distocervical, and disto-apical. Table 2 shows the results for the apical area. There were also differences among groups: G4 and G5 had the lowest percentage of direct bone-to-implant contact in the apical area. G1 was different from all other groups except G2 (Mann-Whitney U test). There was no significant difference (Mann-Whitney U test) among groups in all other areas (mesiocervical, mesio-apical, distocervical, and disto-apical). Figures 2–6 show representative photomicro-

TABLE 2

Percentage of direct bone-to-implant contact, considering only the apical area of the implants

|  | G1 | G2 | G3 | G4 | G5 |
|---|---|---|---|---|---|
| Average | 72.9% | 54.5% | 46.9% | 37.6% | 40.7% |
| Median | 76.9% | 60.5% | 51.3% | 43.3% | 48.4% |
| Standard deviation | 23.4% | 31.0% | 21.2% | 26.8% | 30.8% |
| Q1 | 67.3% | 28.3% | 31.2% | 11.4% | 19.3% |
| Q3 | 86.9% | 75.6% | 63.8% | 58.1% | 57.9% |
| N | 14 | 16 | 14 | 14 | 11 |
| IC | 12.2% | 15.2% | 11.1% | 14.1% | 18.2% |
| P-value |  |  | .007* |  |  |

*Indicates a significant difference (Kruskal-Wallis test).

graphs of each group's histomorphometric analysis in the apical region.

Serum levels of calcium and phosphate are shown in Table 3. There was no significant difference in serum calcium level among groups; however, the serum phosphate level was significantly lower in G1 (Mann-Whitney $U$ test).

The animals ingested calories from the food and alcohol solutions (except for G1, which had calorie intake provided entirely by food). Figure 7 shows the total amount of calories ingested by each group and the distribution according to the source: alcohol solution or food. G1 had the lowest total caloric intake, and this difference was significant (Mann-Whitney $U$ test). However, this group showed the greatest weight gain (Table 4), but this difference was not significant (Mann-Whitney $U$ Test).

It was possible to observe a strong and inverse correlation between caloric intake from food per day and caloric intake from alcoholic solution per day ($-100\%$, $P < .001$). Calories from food correlated directly and calories from alcohol correlated inversely with bone-implant direct contact in apical area ($-90.0\%$, $P = .037$). A strong and direct correlation was also observed between the level of serum calcium and ingested calories from the alcoholic solution ($100\%$, $P < .001$), and a strong and inverse correlation between the level of serum calcium and ingested calories from food ($-100\%$, $P < .001$). Serum calcium also inversely correlated with bone-implant direct contact in apical area ($-90.0\%$, $P = .037$). A strong and inverse correlation between level of serum phosphate and weight gain ($-90.0\%$, $P = .037$) was also verified. Table 5 shows the correlation tests for quantitative variables.

## DISCUSSION

In 2011, Deco et al[13] evaluated the percentage of bone-implant direct after chronic consumption of alcohol in rats but found no difference between the groups who consumed alcohol and those with an isocaloric diet. The authors attributed this result partly due to the short time of alcohol consumption, which was 10 weeks at maximum. They hypothesized that longer periods would have worse effects on osseointegration. In the present study, groups of longer periods of alcohol consumption were those with the lowest percentage of bone-implant direct contact when all implant areas were evaluated, thus confirming the previously presented hypothesis that excessive and prolonged consumption of alcohol can be harmful to
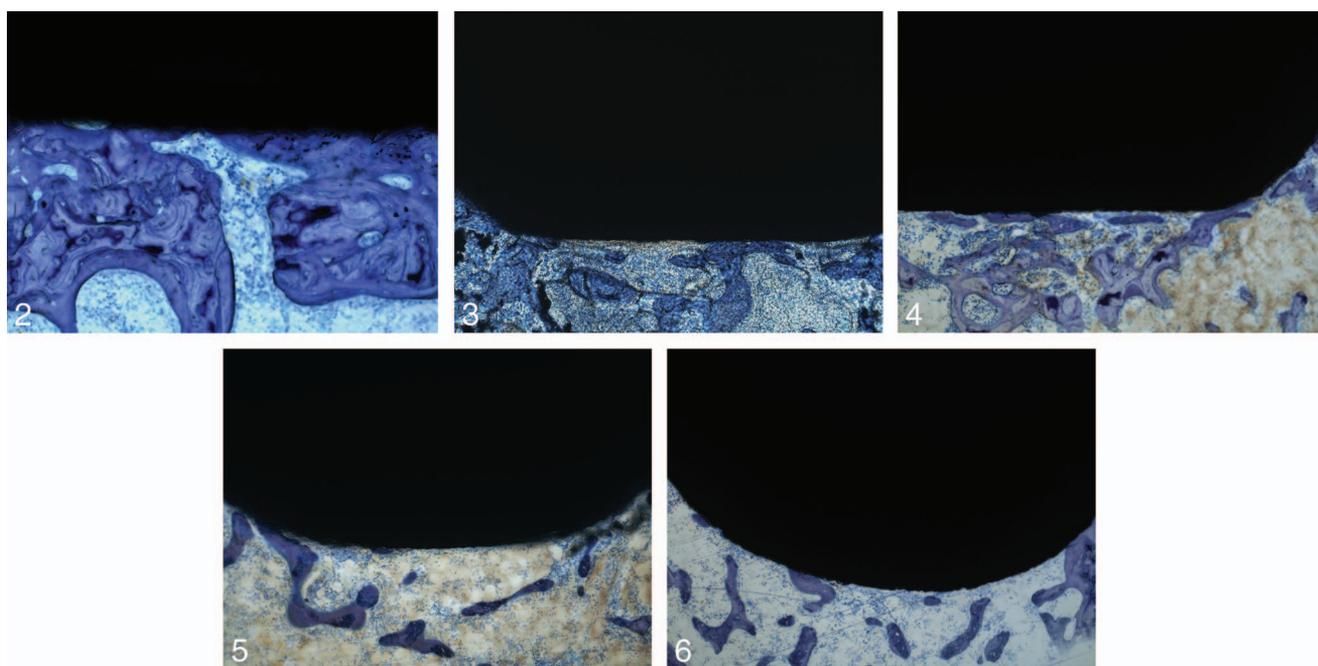


FIGURES 2–6. FIGURE 2. Photomicrograph of the implant in the apical region of Group 1 (food and water ad libitum). FIGURE 3. Photomicrograph of the implant in the apical region of Group 2 (food and 20% alcohol solution ad libitum for 3 months). FIGURE 4. Photomicrograph of the implant in the apical region of Group 3 (food and 20% alcohol solution ad libitum for 4 months). FIGURE 5. Photomicrograph of the implant in the apical region of Group 4 (food and 20% alcohol solution ad libitum for 5 months). FIGURE 6. Photomicrograph of the implant in the apical region of Group 5 (food and 20% alcohol solution ad libitum for 6 months).

| | Calcium | | | | | Phosphorous | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | G1 | G2 | G3 | G4 | G5 | G1 | G2 | G3 | G4 | G5 |
| Average | 9.58 | 9.60 | 9.77 | 9.90 | 10.10 | 6.98 | 9.08 | 11.13 | 8.68 | 11.00 |
| Median | 9.50 | 9.65 | 10.10 | 9.90 | 10.00 | 6.60 | 9.50 | 11.30 | 8.75 | 10.70 |
| Standard deviation | 0.30 | 0.22 | 0.76 | 0.41 | 0.28 | 0.82 | 1.06 | 1.26 | 0.26 | 2.07 |
| Q1 | 9.45 | 9.53 | 9.50 | 9.58 | 9.90 | 6.58 | 8.93 | 10.55 | 8.60 | 10.23 |
| Q3 | 9.63 | 9.73 | 10.20 | 10.23 | 10.20 | 7.00 | 9.65 | 11.80 | 8.83 | 11.48 |
| N | 4 | 4 | 3 | 4 | 4 | 4 | 4 | 3 | 4 | 4 |
| IC | 0.29 | 0.21 | 0.86 | 0.40 | 0.28 | 0.80 | 1.04 | 1.42 | 0.26 | 2.03 |
| P-value | | | .269 | | | | | .009* | | |

**TABLE 3**

**Serum calcium and phosphate**

*Indicates a significant difference (Kruskal-Wallis test).

osseointegration. In 2004, Koo et al[11] also found a difference between the percentage of bone-implant direct contact in animals treated with alcohol, although alcohol consumption was done only for a maximum of 11 weeks.

When evaluated separately, the unique implant region that showed a significant difference to different groups of alcohol consumption was the apical region. The groups of longer periods of alcohol consumption had the worst average, and the control group had the highest. This area did not present a bone-implant direct contact immediately after surgery, due to the prevalence of medular bone. By doing so, this region had more difficulties in bone formation.[16]

Regarding diet, there was a strong and inverse correlation between caloric intake from food per day and the caloric consumption from alcohol per day. This was expected since alcohol provides an energy source (each gram of alcohol provides 7.1 calories). Although alcohol is a source of energy, it does not provide nutrients. The reason the ingestion of large amounts of alcohol provides lower weight gain or even weight loss was discussed earlier.[18,19] This can be caused by alcohol changes in intake, digestion, and absorption of nutrients[17,20] or by increased sweating and heat dissipation through vasodilatation,[21] thereby increasing caloric expenditure. However, the most accepted hypothesis is that the exacerbated consumption of alcohol changes its metabolization via the enzyme alcohol dehydrogenase to microsomal oxidation system alcohol, which

is less efficient and requires more energy expenditure.[17,22] This explanation may also help us understand why calories from food correlated directly and calories from alcohol correlated inversely with bone-implant direct contact in apical area in the present study.

Calcium and phosphate are minerals essential to bone composition.[7] In the present paper, serum levels were measured to determine whether alcohol consumption can affect these components. Only phosphate showed differences between groups, and the control group had the lowest value.

There was a strong and direct correlation between level of serum calcium and ingested calories from the alcoholic solution and a strong and inverse correlation between level of serum calcium and ingested calories from food. The serum calcium increased gradually with the time of alcohol consumption, but the difference was not significant. This result contradicts the literature that shows alcohol consumption lowers serum calcium levels, although the mechanism of action is not yet known.[8] One possible explanation for our results is alcohol may have caused an increased production of parathyroid hormone (PTH),[23] which normalized the level of calcium. One way of restoring the levels of serum calcium by PTH is the absorption of calcium from the bones,[24] which could explain the strong and inverse correlation observed between calcium and bone-implant direct contact in the apical region. If part of the bone calcium was removed to restore the serum levels, it seems possible that this reduction in bone has been harmful to osseointegration. However, this is only a hypothesis since we did not evaluate serum PTH. We also observed a strong and inverse correlation between serum calcium and food intake,
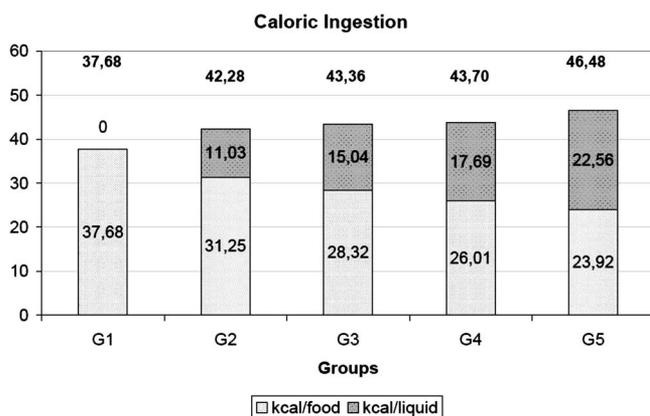


**FIGURE 7.** Graphic presenting caloric intake from alcoholic solution, food, and both (in bold).

| | G1 | G2 | G3 | G4 | G5 |
|---|---|---|---|---|---|
| Average | 57.50 | 54.17 | 42.50 | 45.83 | 45.00 |
| Median | 55.00 | 50.00 | 45.00 | 47.50 | 50.00 |
| Standard deviation | 10.37 | 17.72 | 8.66 | 17.72 | 8.66 |
| Q1 | 51.25 | 41.25 | 41.25 | 36.25 | 45.00 |
| Q3 | 66.25 | 66.25 | 46.25 | 55.00 | 50.00 |
| N | 6 | 6 | 4 | 6 | 5 |
| IC | 8.30 | 14.18 | 8.49 | 14.18 | 7.59 |
| P-value | | | .322 | | |

**TABLE 4**

**Weight gain during the experiment in each group (Kruskal-Wallis test)**

| | | Mesiocervical | Mesio-apical | Apical | Disto-apical | Distocervical | Weight gain | Calcium | Phosphate | Total calories | Calories from food |
|---|---|---|---|---|---|---|---|---|---|---|---|
| **TABLE 5** | | | | | | | | | | | |
| *Spearman's correlation test among quantitative variables* | | | | | | | | | | | |
| Mesio-apical | corr | 50.0% | | | | | | | | | |
| | P-value | .391 | | | | | | | | | |
| Apical | corr | −10.0% | 70.0% | | | | | | | | |
| | P-value | .873 | .188 | | | | | | | | |
| Disto-apical | corr | 70.0% | 10.0% | −10.0% | | | | | | | |
| | P-value | .188 | .873 | .873 | | | | | | | |
| Distocervical | corr | 70.0% | 60.0% | 50.0% | 70.0% | | | | | | |
| | P-value | .188 | .285 | .391 | .188 | | | | | | |
| Weight gain | corr | −30.0% | 10.0% | 60.0% | 30.0% | 30.0% | | | | | |
| | P-value | .624 | .873 | .285 | .624 | .624 | | | | | |
| Calcium | corr | 30.0% | −60.0% | −90.0% | 20.0% | −20.0% | −70.0% | | | | |
| | P-value | .624 | .285 | .037* | .747 | .747 | .188 | | | | |
| Phosphate | corr | 60.0% | 30.0% | −30.0% | −10.0% | 10.0% | −90.0% | 50.0% | | | |
| | P-value | .285 | .624 | .624 | .873 | .873 | .037* | .391 | | | |
| Total calories | corr | 30.0% | −10.0% | −60.0% | −30.0% | −30.0% | −100% | 70.0% | 90.0% | | |
| | P-value | .624 | .873 | .285 | .624 | .624 | <.001* | .188 | .037* | | |
| Calories from food | corr | −30.0% | 60.0% | 90.0% | −20.0% | 20.0% | 70% | −100% | −50.0% | −70.0% | |
| | P-value | .624 | .285 | .037* | .747 | .747 | .188 | <.001* | .391 | .188 | |
| Calories from alcohol | corr | 30.0% | −60.0% | −90.0% | 20.0% | −20.0% | −70% | 100% | 50.0% | 70.0% | −100% |
| | P-value | .624 | .285 | .037* | .747 | .747 | .188 | <.001* | .391 | .188 | <.001* |

*Indicates a significant correlation.

which could happen since animals that ate minor amounts of food were also the ones that consumed more alcohol, leading us to the correlation previously discussed.

Our results showed that serum phosphate was higher in rats that consumed alcohol, and they were the ones who gained less weight, which explains the strong inverse correlation between serum phosphate and weight gain. Changes in serum phosphate may occur in alcoholics[9] who often have hyperphosphatemia,[25,26] although this is not always observed.[27,28] The serum phosphate level is maintained by the intestinal phosphate absorption, renal reabsorption, and balance between intracellular and phosphate in bone tissue. The alteration of the renal reabsorption of phosphate is the most common cause of chronic renal disorders,[29] and advanced alcoholism can cause this problem, culminating in hyperphosphatemia.[30,31]

The present study presented its limitations to be the small number of animals and the absence of assessing the level of blood alcohol. Moreover, the use of absolute alcohol in preparing the alcoholic solutions may also be questioned, since it may contain traces of chemical agents used in its preparation, although it is unlikely that a trace amount could interfere with the results. These conditions should be improved in future research. Moreover, there might be care taken when relating these results to humans because of the differences in bone, metabolism, and diet.

Nevertheless, to the best of our knowledge, this is the first study investigating the influence of prolonged and excessive alcohol consumption in osseointegration. The results showed chronic alcohol consumption had a negative influence on osseointegration. However, it is not possible to establish how much of this loss was due to alcohol and how much was caused by changes in diet that occur secondary to alcoholism. More

studies must be done in order to elucidate these results, including renal evaluation and checking PTH levels.

## ABBREVIATION

PTH: parathyroid hormone

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