Negative Effects of Alcohol Intake and Estrogen Deficiency Combination on Osseointegration in a Rat Model

Camila Porto de Deco, DDS, MSD
Adriana Mathias Pereira da Silva Marchini, DDS
Mary Anne Moreira Bárbara, DDS, MSD
Luana Marotta Reis de Vasconcellos, DDS, MSD, PhD
Rosilene Fernandes da Rocha, PhD
Leonardo Marchini, DDS, MSD, PhD

Alcohol intake and estrogen deficiency can both affect bone physiology and have shown to have an adverse effect on dental implant therapy. However, the combination of both factors on osseointegration is unknown. The aim of this study was to evaluate osseointegration in rats fed with alcohol and presenting induced estrogen deficiency. Ninety-six female rats were divided according to diet and hormonal condition into 6 groups as follows: group Sh-W: sham (simulated ovariectomy) control, food and water ad libitum; group Sh-Et: sham, food and 20% ethanol solution ad libitum; group Sh-Su: sham, food and sucrose solution controlled to ensure an isocaloric diet in relation to Sh-Et; group Ov-W: ovariectomy, food and water ad libitum; group Ov-Et: ovariectomy, food and 20% ethanol solution ad libitum; and group Ov-Su: ovariectomy, food and sucrose solution controlled to ensure an isocaloric diet as Ov-Et. The groups were subdivided according to time of euthanasia: 30 and 45 days after placement of implants. Implant surgery was performed 1 month after ovariectomy or sham. After euthanasia, the femurs were removed and evaluated by histomorphometry. Groups Ov-Et and Ov-Su showed the lowest percentage of bone-to-implant contact. The combination of alcohol intake and estrogen deficiency, and the combination of estrogen deficiency and reduced ingestion of food can negatively affect osseointegration in rats.

Key Words: ethanol, osseointegration, dental implants, estrogen deficiency
importance of studying the health impacts of alcohol consumption in Brazil and other countries, as it is a common habit worldwide.

From the standpoint of oral health, alcohol consumption seems to have a negative effect on treatment with implants, compromising patients’ oral rehabilitation.\(^2,3\) This is likely due to the fact that alcohol consumption alters bone metabolism, reducing osteoblast proliferation\(^4\) and increasing osteoclast activity.\(^2\)

Implant therapy is indicated for people who have lost teeth, but implant placement is more difficult when there has been excessive residual ridge resorption.\(^5\) One possible factor contributing to extensive residual ridge resorption is osteoporosis,\(^6\) a common disease affecting more than 50% of women and 20% of men 50 years of age and older.\(^7\) In women, osteoporosis is mainly caused by estrogen deficiency. The decision to place an implant, which is frequently applied at this age, must take into account the presence of osteoporosis.\(^5,8-10\)

Considering (a) the high incidence of alcohol consumption,\(^1\) (b) the high incidence of osteoporosis,\(^7\) and (c) the reportedly negative impact of alcohol consumption\(^2,3\) and osteoporosis\(^5,8\) on osseointegration, it was hypothesized that the combination of alcohol consumption and estrogen deficiency could negatively affect osseointegration. To test this hypothesis, the present study evaluated the percentage of bone-to-implant contact in rats with estrogen deficiency and alcohol consumption.

**Materials and Methods**

**Animals**

The study was approved by the ethics committee of the São José dos Campos School of Dentistry, State University of São Paulo (UNESP) (Protocol 030 /2007 – PA/CEP).

Ninety-six 3-month-old female rats (Rattus norvegicus, albino Wistar strain) were used. Nine of them died during the experiment (final \(n = 87\)). Three animals died during the anesthesia, 4 died or were euthanized due to ovariotomy complications, and 2 were euthanized because of femur fracture during the implant surgery. They were housed at room temperature, fed a controlled diet, and weighed regularly.

The rats were divided into 6 experimental groups according to their dietary and hormonal conditions as follows: group Sh-W (\(n = 15\)): sham (simulated ovariotomy), food and water ad libitum; group Sh-Et (\(n = 12\)): sham, food and 20% ethanol solution ad libitum;\(^2,8\) group Sh-Su (\(n = 15\)): sham, food and sucrose solution controlled to ensure an isocaloric diet\(^11-14\) as Sh-Et; group Ov-W (\(n = 16\)): ovariotomy, food and water ad libitum; group Ov-Et (\(n = 14\)): ovariotomy, food and 20% ethanol solution ad libitum; and group Ov-Su (\(n = 15\)): ovariotomy, food and sucrose solution controlled to ensure an isocaloric diet as Ov-Et.

The dietary conditions were applied for 60 or 75 days, a period that is reported sufficient to detect bone effects.\(^2,12,14,15\) Each group was divided in half to receive a diet for 60 or 75 days.

The 20% ethanol solution was obtained from an absolute ethanol (Eclíbrar, Santo Amaro, São Paulo, Brazil) dilution in water. The sucrose (Vetec Química Fina Ltda, Rio de Janeiro, Rio de Janeiro, Brazil) solution was obtained by water dilution.

**Anesthesia**

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

**Ovariectomy and sham**

The rats of groups Ov-W, Ov-Et, and Ov-Su underwent ovariotomy to promote osteo-
The rats of groups Sh-W, Sh-Et, and Sh-Su were subjected to sham surgery to promote surgical stress. All groups received antibiotics via a single intramuscular injection after surgery.

**Implant surgery**

All rats received implants 30 days after ovariectomy or sham surgery. The implants, which were made of commercially available titanium alloy (Ti4V6Al), were cylindrical in shape (2 mm length × 2 mm diameter). The external surfaces were machine cut and then acid etched. All the implants were sterilized individually before placement.

The implants were placed at the distal epiphysis of the femur in each animal of each group. Left or right femurs were implanted randomly, according to a routinely used implant surgery protocol.

**Euthanasia**

After an osseointegration period of 30 or 45 days, the rats were euthanized (Figure 1) with an excessive dose of anesthetic. In groups Ov-W, Ov-Et, and Ov-Su the ovariectomy was confirmed through the visualization of ovary absence and uterus atrophy. In groups Sh-W, Sh-Et, and Sh-Su, normal ovaries and uteruses were seen. Femurs were removed and prepared for histologic evaluation.

**Histologic preparation and histomorphometric analysis**

Evaluation of the bone-implant response and determination of osseointegration was conducted by the undecalcified bone method, 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 evaluated, making a total of approximately 30 slices per experimental group. The slices were stained with toluidine blue (Figure 2).

The percentage of direct bone-to-implant contact was evaluated in the area between the implant surface and cortical bone, using image analysis software (ImageJ version 1.31 for Windows, National Institutes of Health).

**Statistical analysis**

A significance level of .05 was adopted for all tests applied in this study. Generalized linear models were used to evaluate the experimental groups and euthanasia time and their correlation with the percentage of bone-to-implant contact. Tukey multiple comparison test was used to compare experimental groups. Pearson correlation was used to check for possible correlations between percentage of contact, weight variation, and food consumption. Levene test was used to determine the homogeneity of variance.

**Results**

There was no difference between the subgroups, 30 or 45 days of osseointegration,
concerning percentage of bone-to-implant contact or any other outcome. Considering this result, they were grouped together for analysis.

The entire sample presented a significant weight gain from the beginning to the end of the experimental procedures \((P < .001)\). The initial mean weight was 277.5 ± 3.75 g, while the final mean weight was 325.8 ± 7.46 g, with no difference among the groups.

The percentage of bone-to-implant contact was smaller in the groups Ov-Et and Ov-Su when compared with the other groups \((P < .001)\), as indicated in Figure 3. There was no significant statistical difference between Ov-Et and Ov-Su \((P = .966)\).

Total ingested calories (calories from solid food and calories from ingested liquids) was higher for groups Ov-Et and Ov-Su than for other groups \((P = .003\) and \(.017\), respectively), as seen in Figure 4 and Table 1. However, this difference was not statistically significant between Ov-Et and Ov-Su (Table 1).

**DISCUSSION**

The results suggest that osseointegration is likely to be impaired by the combination of alcohol and estrogen deficiency.
of estrogen deficiency and alcohol intake, thus confirming the previously proposed hypothesis.

Some authors\textsuperscript{21–23} postulate that alcohol intake may have a positive impact on bone metabolism in women with postmenopausal osteoporosis (osteoporosis caused by estrogen deficiency). According to these authors, alcohol intake could cause a reduction in bone metabolism which increases in response to reduced estrogen. However, the findings of this study indicate that the group that had estrogen deficiency associated with excessive (20% of available liquid) alcohol intake (group Ov-Et) had statistically lower percentages of bone-to-implant contact in rats, leading to impairment of osseointegration.

However, there is no consensus about the dosage that would promote the positive effect proposed by the aforementioned authors.\textsuperscript{21–23} Furthermore, some authors\textsuperscript{24} speculate that this effect could be caused by other components found in alcoholic beverages and not by alcohol per se.

Another study\textsuperscript{2} evaluated osseointegration in animals subjected to alcohol intake and found that the alcohol group had a smaller percentage of bone-to-implant contact than the control group. The present study did not confirm the findings of those authors, as the results presented here did not show any difference between the non-ovariectomized and alcohol treated (Sh-Et) and the non-ovariectomized and non-alcohol-treated groups (Sh-Su and Sh-W). This discrepancy can be attributed to methodologic differences. The cited study\textsuperscript{2} used screw-type implants in rabbit tibia, while this study used plain implants in rat femur. However, the most important difference is probably the period of alcohol intake. In the above mentioned study,\textsuperscript{2} the period of alcohol intake was 7 or 15 days longer than in the present study. Although the present study did not find significant differences linked to the period of ethanol intake after implant surgery, it seems reasonable to assume that extended periods of alcohol intake before implant placement could cause some difference. Studies involving longer periods of alcohol intake should be undertaken to elucidate these results.

A previous evaluation\textsuperscript{17} of osseointegration of titanium implants in ovariectomized rats found no statistically significant difference in the percentage of bone-to-implant contact between ovariectomized and sham groups, considering cortical bone. Other studies\textsuperscript{8,18} also evaluated the interaction between osseointegration and osteoporosis and found similar results. The present study also found no difference between ovariectomized non-alcohol and sham groups.

Other researchers\textsuperscript{5} have found significant differences between osteoporotic and non-osteoporotic animals in terms of percentage of bone-to-implant contact. A possible explanation for their results is the method they used to induce osteoporosis. While the present study and others\textsuperscript{8,17,18} induced osteoporosis by ovariectomy (promoting estrogen deficiency), the cited study\textsuperscript{5} induced osteoporosis by daily intramuscular glucocorticoid injections. The manner in which estrogen and glucocorticoids induce bone loss is complex and not fully understood. Glucocorticoids inhibit the expression of important genes for bone formation, including genes

<table>
<thead>
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<th>Groups</th>
<th>Sh-W</th>
<th>Sh-Et</th>
<th>Sh-Su</th>
<th>Ov-W</th>
<th>Ov-Et</th>
</tr>
</thead>
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<td>.009*</td>
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<tr>
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<tr>
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<tr>
<td>Ov-Su</td>
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<td>.142</td>
<td>.913</td>
<td>&lt;.001*</td>
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</tr>
</tbody>
</table>

\textsuperscript{1}Sh-W indicates sham, food and water; Sh-Et, sham, food and ethanol; Sh-Su, sham, food and sucrose; Ov-W, ovariectomy, food and water; Ov-Et, ovariectomy, food and ethanol; Ov-Su, ovariectomy, food and sucrose.

\textsuperscript{*}Statistically significant difference (Tukey multiple comparisons).
that translate to collagen A1, transforming growth factor β, fibronectin, and insulin-like growth factor. The glucocorticoid-mediated bone reabsorption mechanism has not yet been fully established; however, as in estrogen reduction, it includes increased production of receptor activator of NFkB ligand in association with reduced production of osteoprotegerin, resulting in increased osteoclast recruitment and survival. However, the decrease in bone formation is greater than that noted in postmenopausal osteoporosis. The effects of glucocorticoids on bone are fast, perhaps faster than the effects of estrogen on bone.

The ambiguous results obtained so far in animal experiments and clinical studies do not allow for conclusions to be drawn concerning the extent to which osteoporosis, caused by estrogen deficiency, influences implant osseointegration. That is why osteoporosis is currently considered a risk factor, though not an impediment to implant therapy. However, the present study clearly demonstrates that the combination of alcohol intake and estrogen deficiency has a negative impact on osseointegration in rats. Similar to the Ov-Et group (ovariectomized and alcohol-treated), the Ov-Su group (ovariectomized and food and sucrose solution controlled to ensure an isocaloric diet like Ov-Et) also presented impaired osseointegration. Group Ov-Su was not fed with alcohol but was ovariectomized and had lower food consumption as did group Ov-Et. This appears to indicate that the association of estrogen reduction and nutritional deficiency resulting from lower food intake could play an important role in impairing osseointegration, rather than alcohol intake per se. The reduced food consumption seen in groups Sh-Et and Ov-Et was due to the calories of ethanol providing part of the calorific needs of the animals.

Nutritional deficiency resulting from alcohol intake has already been reported earli-

er and can be explained by the anorexic effect of ethanol. Considering this hypothesis, further studies involving the control of key nutrients should be undertaken to gain a deeper understanding of the effect of alcohol intake on osseointegration in rats.

The present study demonstrates that alcohol intake associated with estrogen deficiency and estrogen deficiency combined with reduced ingestion of food can impair osseointegration in rats.

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