

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 neoformation³⁻⁵ 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 postoperative, 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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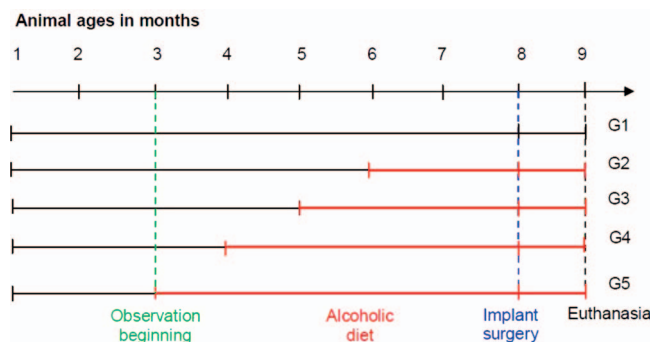


FIGURE 1. Timeline of experimental procedures.

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 \times 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 μ m) 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,

	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).

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.

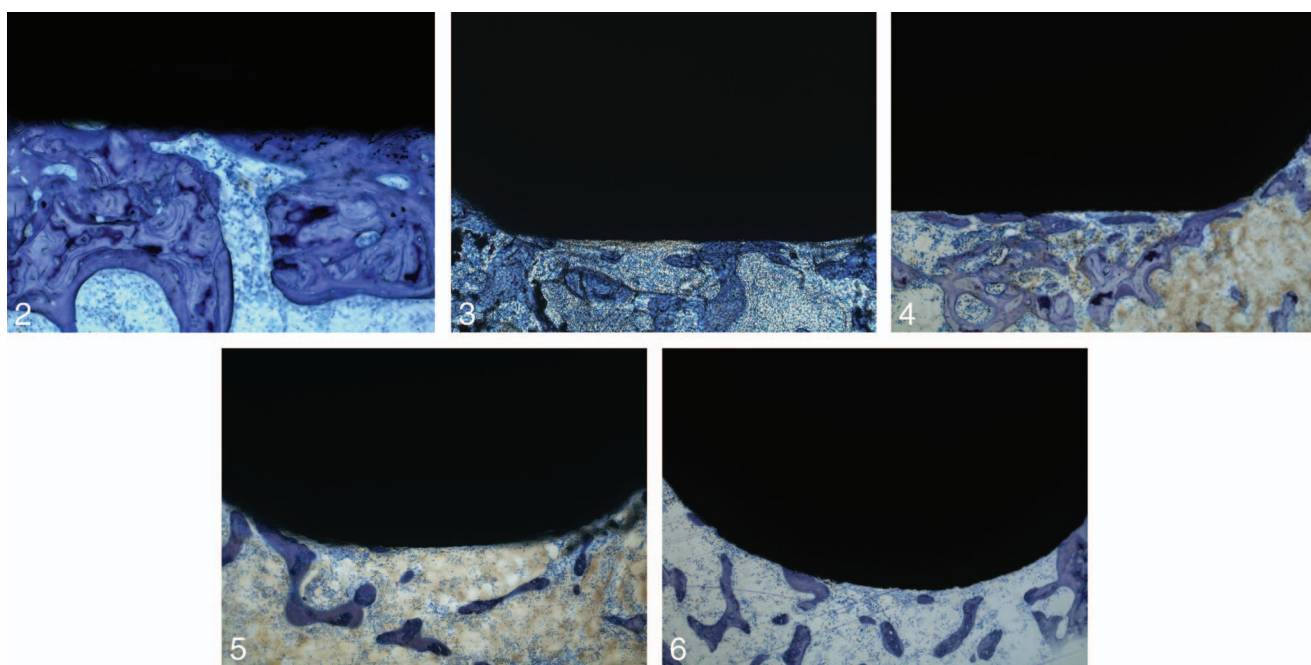
Serum levels of calcium and phosphate are shown in Table 3. There is no significant difference in serum calcium level among groups; however, the serum phosphate level was significantly lower in G1 (Mann-Whitney *U* test).

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 al¹³ 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*		

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

osseointegration. In 2004, Koo et al¹¹ 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 metabolism 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,

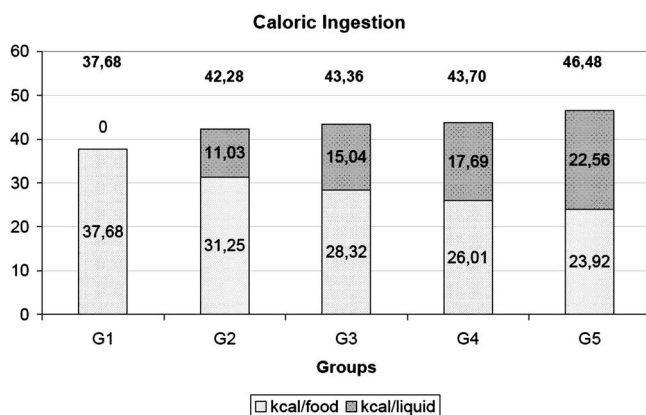


FIGURE 7. Graphic presenting caloric intake from alcoholic solution, food, and both (in bold).

	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 5
Spearman's correlation test among quantitative variables

		Mesiocervical	Mesio-apical	Apical	Disto-apical	Distocervical	Weight gain	Calcium	Phosphate	Total calories	Calories from food
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%	100.0%	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 alcoholics⁹ 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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REFERENCES

1. Andrade AG, Anthony JC, Silveira CM. *Álcool e suas consequências: uma abordagem multiconceitual*. Barueri, Brazil: Minha Editora; 2009.
2. Fleming M, Mihic SJ, Harris RA. Etanol. In: Lazo JS, Parker KL, Goodman AG, eds. *As bases farmacológicas da terapêutica*. Rio de Janeiro: McGraw-Hill; 2010:527-541.
3. Sampson HW. Effect of alcohol consumption on adult and aged bone: a histomorphometric study of the rat animal model. *Alcohol Clin Exp Res*. 1999;23:1416-1417.
4. Turner RT, Sibonga JD. Effects of alcohol use and estrogen on bone. *Alcohol Res Health*. 2001;25:276-281.
5. Chakkalakal DA. Alcohol-induced bone loss and deficient bone repair. *Alcohol Clin Exp Res*. 2005;29:2077-2090.
6. Torricelli P, Fini M, Giavaresi G, et al. Chronic alcohol abuse and endosseous implants: linkage of in vitro osteoblast dysfunction to titanium osseointegration rate. *Toxicol*. 2008;243:138-144.

7. Confavreux CB. Bone: from a reservoir of minerals to a regulator of energy metabolism. *Kidney Int.* 2011;79:S14–S19.
8. Keiver K, Duggal S, Simpson ME. Alcohol administration results in a prolonged decrease in blood ionized calcium levels in the rat. *Alcohol.* 2005;37:173–178.
9. Shiber JR, Mattu A. Serum phosphate abnormalities in the emergency department. *J Emerg Med.* 2002;23:395–400.
10. Colnot C, Romero DM, Huang S, et al. Molecular analysis of healing at a bone-implant interface. *J Dent Res.* 2007;86:862–867.
11. Koo S, König Júnior B, Mizusaki CI, Allegrini Júnior S, Yoshimoto M, Carbonari MJ. Effects of alcohol consumption on osseointegration of titanium implants in rabbits. *Implant Dent.* 2004;13:232–237.
12. Galindo-Moreno P, Fauri M, Ávila-Ortiz G, Fernández-Barbero JE, Cabrera-Léon A, Sánchez-Fernández E. Influence of alcohol and tobacco habits on peri-implant marginal bone loss: a prospective study. *Clin Oral Implants Res.* 2005;16:579–586.
13. Deco CP, Marchini AMPS, Bárbara MAM, Vasconcellos LMR, Rocha RF, Marchini L. Negative effects of alcohol intake and estrogen deficiency combination on osseointegration in a rat model. *J Oral Implantol.* 2011;37:633–639.
14. Viera-Negrón YE, Ruan WH, Winger JN, Hou X, Sharawy MM, Borke JL. Effect of ovariectomy and alendronate on implant osseointegration in rat maxillary bone. *J Oral Implantol.* 2008;34:76–82.
15. Donath K, Breuner G. A method for the study of undecalcified bones and teeth with attached soft tissues. *J Oral Pathol.* 1982;11:318–326.
16. Alghamdi H, Anand PS, Anil S. Undersized implant site preparation to enhance primary implant stability in poor bone density: a prospective clinical study. *J Oral Maxillofac Surg.* 2011;69:e506–e512.
17. Lieber CS. Relationship between nutrition, alcohol use and liver disease. *Alcohol Res Health.* 2003;27:220–231.
18. Bunout D. Nutritional and metabolic effects of alcoholism: their relationship with alcoholic liver disease. *Nutrition.* 1999;15:583–589.
19. Cornier M, Gayles EC, Bessesen DH. The effects of chronic alcohol consumption on energy balance in rats. *Metabolism.* 2001;5:787–791.
20. Tujague J, Keer WC. Metabolic effects: energy intake estimates of respondent-measured alcoholic beverages. *Alcohol Alcohol.* 2009;44:34–41.
21. McDonald JT, Margen S. Wine versus alcohol in human nutrition I. Nitrogen and calorie balance. *Am J Clin Nutr.* 1976;29:1093–1103.
22. Santolaria F, González-Reimers E, Pérez-Manzano JL, et al. Osteopenia assessed by body composition analysis is related to malnutrition in alcoholic patients. *Alcohol.* 2000;22:147–157.
23. Baran DT, Bryant C, Robson D. Alcohol-induced alterations in calcium metabolism in the pregnant rat. *Am J Clin Nutr.* 1982;36:41–45.
24. Moe SM. Disorders involving calcium, phosphorus, and magnesium. *Prim Care.* 2008;35:215–237, v–vi.
25. Ryback RS, Eckardt MJ, Pautler CP. Clinical relationships between serum phosphorus and other blood chemistry values in alcoholics. *Arch Intern Med.* 1980;140:673–677.
26. Elisaf M, Milionis H, Siamopoulos KC. Hypomagnesemic hypokalemia and hypocalcemia: clinical and laboratory characteristics. *Miner Electrolyte Metab.* 1997;23:105–112.
27. McDonald JT, Margen S. Wine versus alcohol in human nutrition III. Calcium, phosphorus, and magnesium balance. *Am J Clin Nutr.* 1979;32:823–833.
28. Adler AJ, Fillipone EJ, Berlyne GM. Effect of chronic alcohol intake on muscle composition and metabolic balance of calcium and phosphate in rats. *Am J Physiol.* 1985;249:E584–E588.
29. Fukumoto S. Disorders of phosphate metabolism. *Rinsho Byori.* 2010;58:225–231.
30. Chavel SM, Taraszka KS, Schaffer JV, Lazova R, Schechner JS. Calciphylaxis associated with acute, reversible renal failure in the setting of alcoholic cirrhosis. *J Am Acad Dermatol.* 2004;50:S125–S128.
31. Spaia S. Phosphate binders: sevelamer in the prevention and treatment of hyperphosphataemia in chronic renal failure. *Hippokratia.* 2011;15:S22–S26.