Journal of
PERIODONTAL RESEARCH

J Periodont Res 2011; 46: 691–696

JOURNAL OF PERIODONTAL RESEARCH
doi:10.1111/j.1600-0765.2011.01391.x

Effects of induced diabetes and the administration of aminoguanidine in the biomechanical retention of implants: a study in rats

R. P. Guimarães[1], P. A. D. de Oliveira[2], A. M. S. D. Oliveira[2]

[1]Pontifícia Universidade Católica de Minas Gerais (PUC-MG), Santa Efigênia, Brasil and [2]Pontifícia Universidade Católica de Minas Gerais, Faculdade de Odontologia, Coração Eucarístico, Belo Horizonte, Brasil

Guimarães RP, de Oliveira PAD, Oliveira AMSD. Effects of induced diabetes and the administration of aminoguanidine in the biomechanical retention of implants: a study in rats. J Periodont Res 2011; 46: 691–696. © 2011 John Wiley & Sons A/S

Background and Objective: The present study aimed to assess the effects of induced diabetes and the administration of aminoguanidine in the biomechanical retention of implants in rats.

Material and Methods: Thirty-six rats were randomly divided into six groups: group 1, healthy rats (no aminoguanidine); group 2 and group 3, healthy rats receiving 10 and 20 mg/kg of aminoguanidine daily, respectively; group 4, diabetic rats (no aminoguanidine); and group 5 and group 6, diabetic rats receiving 10 and 20 mg/kg of aminoguanidine daily, respectively. In each rat an implant was inserted in the femur. After 28 d of healing, the rats were killed. The implants were removed by applying a counter-torque, and the maximum force required for the rupture of the bone–implant interface was recorded using an analog torque meter. The data were evaluated using analysis of variance and the Student's *t*-test.

Results: In the healthy groups, no statistically significant difference could be observed in the average counter-torque values for implant removal, whereas in the diabetic groups, a daily dose of 20 mg/kg of aminoguanidine raised the counter-torque values to the values found in healthy rats.

Conclusion: The administration of 20 mg/kg of aminoguanidine daily in diabetic rats raised the biomechanical retention of the implants to the level observed in the healthy rat group.

Rodrigo P. Guimarães, Masters in Implantology, Pontifícia Universidade Católica de Minas Gerais (PUC-MG), Av. Brasil, 283, Sl. 1701, Santa Efigênia, B.H-M.G, Brasil
Tel: (55) (31)3241 5547
Fax: (55) (31)3241 5547
e-mail: rportog@gmail.com

Key words: aminoguanidine; diabetes mellitus; osseointegration

Accepted for publication May 18, 2011

The harmful effects of hyperglycemia are routinely attributed to a heterogeneous group of molecules called advanced glycation end-products (AGEs), among other factors. The biological concepts that explain the effects of diabetes in osseointegration are related to the effect of AGEs on the formation of bone cells and protein, which can produce an unfavorable environment for osseointegration (1,2). The biological aspects compromised by the formation of AGEs must undergo detailed analyses in implant dentistry (3,4). Diabetes mellitus has generally been considered as a counter-indication to the use of dental implants (5), although cross-sectional studies performed in humans have demonstrated that the survival rate of implants in individuals whose diabetes is well-controlled is similar to that in nondiabetic individuals (6–8),

Aminoguanidine has shown the capacity to inhibit the formation of AGEs from collagen and the basal membranes, both *in vivo* and *in vitro*,

thus overcoming the development of experimental diabetic retinopathy (9) and thereby preventing other systemic complications of hyperglycemia, such as cardiovascular, renal and bone-metabolism pathologies (3).

Studies that investigate the effects of the diabetic state in tissue healing show substantial damage in the repair mechanism of hard and soft tissues caused by qualitative and quantitative changes in the components involved in tissue healing (2,3). However, greater clarification regarding the consequences of hyperglycemic changes in the physiology of peri-implant tissues of diabetic individuals is still needed. The forecast of the increased prevalence of individuals with diabetes in the world (10,11) and the rise in the life expectancy of the population justify new lines of research in search of a better understanding of peri-implant repair in diabetic patients. The present study assessed the effects of induced diabetes and the administration of aminoguanidine in the biomechanical retention of implants in rats.

Material and methods

The present experiment used 36 Wistar rats (average age 5 mo), kept under a 12-h light/12-h dark cycle, with access to food and water *ad libitum*, during the observation period. This project was approved by the Animal Research Ethics Committee of the Universidade Federal de Minas Gerais (UFMG).

For ease of understanding of the methodological developments in this study, Fig. 1 shows a temporal scale of the experiments performed.

In this study the rats were randomly divided into six groups: group 1, healthy rats with no administration of aminoguanidine; group 2, healthy rats administered 10 mg/kg of aminoguanidine daily; group 3, healthy rats administered 20 mg/kg of aminoguanidine daily; group 4, diabetic rats with no administration of aminoguanidine; group 5, diabetic rats administered 10 mg/kg of aminoguanidine daily; and group 6, diabetic rats administered 20 mg/kg of aminoguanidine daily.

Diabetes mellitus was induced in groups 4, 5 and 6 by means of an intraperitoneal injection of alloxan monohydrate (Sigma Chemical Co.®, St Louis, MO, USA) dissolved in saline solution to a concentration of 1 g/50 mL and administered at a dose of 84 mg/kg. Groups 1, 2 and 3 were administered 1 mL of the saline solution only through intraperitoneal injection (12). The blood glucose levels were assessed in blood samples collected from the caudal artery of the rats (13,14). One drop of blood was collected on a receiver tape (Blood Glucose Test Strip; Abbott Diabetes Care Ltd.®, Witney, Oxon, UK), which was attached to the glucose digital reader (Optium X-ceed; Abbott Diabetes Care Ltd.®). The glycemia was evaluated at baseline, at 10 d after induction, at the time of surgery (14 d post-induction) and on the day the rats were killed (28 d after the insertion of the implants). Blood glucose levels of > 250 mg/dL were considered to be indicators of a diabetic state: the blood glucose levels of rats in the control group were < 250 mg/dL throughout the experiment, whereas the rats in the test groups had blood glucose levels of > 250 mg/dL.

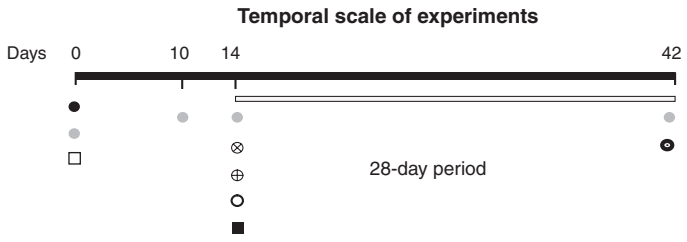

Fig. 1. Temporal scale of experiments. ●, Application of 1 mL of saline solution in groups 1, 2, and 3 (12); □, induction of *diabetes mellitus* in groups 4, 5 and 6 (12); ●, glycemic test (13,14); ⊗, surgery for the insertion of implants (14); ⊕, start of the daily administration of aminoguanidine in groups 2, 3, 5 and 6 (3); ■, start of the daily administration of the saline solution in groups 1 and 4 (3); ○, administration of benzylpenicillin (14); ●, death (14).

Surgical technique

The rats were anaesthetized by intramuscular injection, using a solution of 1 mL of ketamine chlorohydrate (Vetanarcol®; Laboratório Konig S.A., Avellaneda, Argentina) (50 mg) mixed with 1 mL of xylazine (Dopaser®; Laboratório Calier S.A, Barcelona, Spain) (200 mg) at a proportion of 0.2 mL/100 g of weight (14).

A horizontal incision was performed on the antero-medial portion of the femur and the muscles were moved aside by gentle dissection. An implant bed was prepared using a 1.3-mm-diameter drill (NEODENT®; Curitiba, Paraná, Brasil) under constant irrigation with saline solution. The brace was positioned perpendicular to the longitudinal axis of the bone, and the cortical perforation was performed at a low rotation. A self-threading screw of commercially pure titanium, degree IV (NEODENT®), machined, with a length of 4 mm and a diameter of 1.5 mm, was inserted using a manual screwdriver (NEODENT®). The incision was closed using a 4.0 silk suture (Biosut Ltda.®, Belo Horizonte, Minas Gerais, Brasil) with simple isolated sutures, and a single dose of 0.06 mL/kg of benzylpenicillin was administered via intramuscular injection (14). After the surgical procedure, the rats received an analgesic, paracetamol (10 mg/kg of weight), every 8 h in drinking water, which was administered orally over a 2-d period (14).

Application of aminoguanidine

Groups 2, 3, 5 and 6 were treated daily, for 28 d, with aminoguanidine bicarbonate salt (Aminoguanidine Bicarbonate salt; Sigma Chemical Co.®), administered intraperitoneally. In groups 2 and 5, on the day of implant placement, 10 mg/kg of aminoguanidine was administered, while in groups 3 and 6, 20 mg/kg of aminoguanidine was administered. The different dilutions of aminoguanidine were prepared freshly each day, before administration. Groups 1 and 4 received a daily intraperitoneal dose of saline solution for 28 d.

Torque force for the removal of implants

The rats were killed 28 d after implant placement, using an overdose of ketamine chlorohydrate (300 mg/kg). The femurs were surgically removed (14) and placed in a bench vise (Modelo 1200ATG-N-S; Tohnich, Tokyo, Japan). A screwdriver (NEODENT®), specially made for the screw, was adapted to a torque meter, and the torque force required for the rupture of the bone–implant interface was measured in N/cm (15).

Statistical analysis

The normality and homogeneity of the data were tested by means of the Shapiro–Wilk and Bartlett tests, respectively. To study the response variable and the counter-torque force, the analysis of variance and the comparison of averages were performed using the Student's *t*-test. The results were considered significant at $p < 0.05$. All tests were carried out using SAS 6.4 software (Statistical Analysis System; SAS Institute Inc., Cary, NC, USA) (16).

The Lillieofors and Bartlett tests were applied to study the responses of the variation in glucose levels and weight to verify assumptions concerning the normal status of the data and the homogeneity of the variance, respectively. The glucose level response, when analyzed using the Student's *t*-test, agreed with these assumptions, while the variation in weight response, when analyzed using the Kruskall–Wallis test, disagreed with these assumptions. The results were considered significant at $p < 0.05$.

Results

Table 1 and Fig. 2 demonstrate that when aminoguanidine was not administered, the average counter-torque value (N/cm) obtained in the healthy group of rats was statistically higher than the average obtained for the group of diabetic rats ($p < 0.05$). In the groups of healthy rats, there was no significant difference among the counter-torque values ($p > 0.05$). In the groups of diabetic rats, the administration of 20 mg/kg of aminoguanidine raised the average value of the counter-torque force to a level similar to those obtained for the groups of healthy rats ($p > 0.05$).

Table 1. Effect of aminoguanidine on the counter-torque values in healthy and diabetic rats

Aminoguanidine dosage (mg/kg, daily)	Counter-torque values (N/cm)	
	Healthy rats	Diabetic rats
0	2.24 ± 0.5769^{aA}	1.18 ± 0.1483^{bB}
10	2.22 ± 0.8280^{aA}	1.88 ± 0.5019^{aAB}
20	2.77 ± 0.7280^{aA}	2.54 ± 0.6580^{aA}

Data are given as mean ± SE.
Values followed by lowercase letters across the rows and by capital letters down the columns differ significantly ($p < 0.05$; Student's *t*-test).

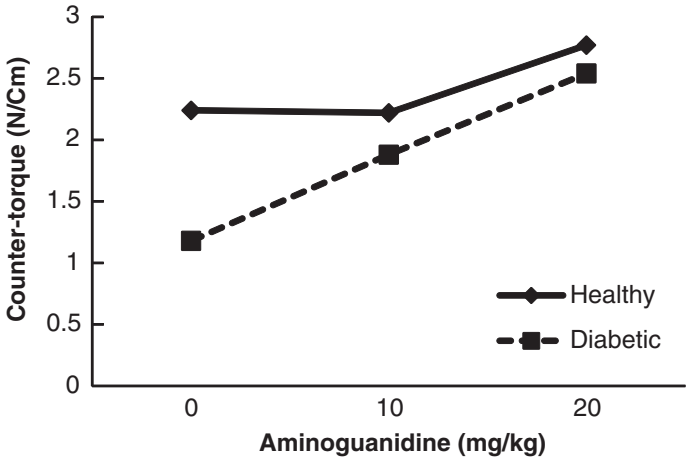

Fig. 2. Variation in the average values of counter-torque (N/cm) according to aminoguanidine dosage and the health condition of the rats.

Table 2 and Fig. 3 show the variation in blood glucose levels. It represents the blood glucose level of the sample taken on the day of implant surgery minus the blood glucose level of the sample taken on the day the rats were killed. In the healthy rats, significant differences were observed among the groups regarding treatment or no treatment with aminoguandine and the different doses used. In the groups of rats with induced diabetes, however, a significant difference was found in the glucose variations among the rats that received 10 mg/kg aminoguanidine compared with those that received 20 mg/kg and with those

Table 2. Effect of aminoguanidine on the variation in blood glucose levels, in healthy and diabetic rats, between the day of implant surgery and the day of death

Aminoguanidine dosage (mg/kg, daily)	Blood glucose level (mg/dL)	
	Healthy rats	Diabetic rats
0	-71.57 ± 19.09^{aA}	9.00 ± 10.14^{bB}
10	-14.42 ± 6.33^{aB}	-71.60 ± 39.23^{aA}
20	-21.12 ± 15.34^{aAB}	$+12.25 \pm 27.28^{aB}$

Data are given as mean ± SE.
Values followed by lowercase letters across the rows and by capital letters down the columns differ significantly ($p < 0.05$; Student's *t*-test).

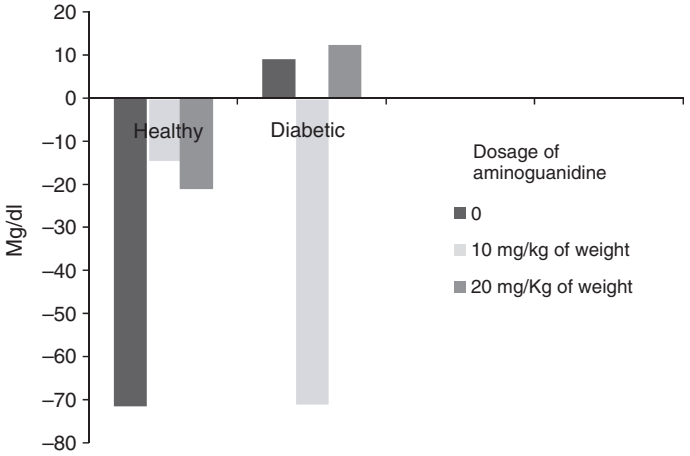

Fig. 3. Variation in blood glucose level (mg/dL) according to the health condition of the rats and the dosage of aminoguanidine administered.

that did not receive the medication. This suggests that 10 mg/kg aminoguanidine may well alter the glucose levels in rats with induced diabetes, whereas at 20 mg/kg, the blood glucose levels presented variations that were comparable with the group in which the aminoguanidine had not been administered.

Table 3 shows the effect of different doses of aminoguanidine on the variation in weight, of healthy and diabetic rats, between the day of surgery and the day of death. In the groups of healthy rats, weight gain was observed, regardless of the application or not of different doses of aminoguanidine, but the differences between groups were not significantly different. A statistically significant difference was observed among the groups of diabetic rats. Weight gain in the rats was observed in those that did not receive aminoguanidine and in those in which aminoguanidine was applied daily at 10 mg/kg, compared with the rats that received 20 mg/kg of aminoguanidine, which, on average, presented a weight loss during the experiment. This finding suggests that aminoguanidine may well affect the weight of rats with diabetes. However, when the groups of diabetic rats were compared with the groups of healthy rats, no statistical difference was observed, which suggests that the application of aminoguanidine at the different doses and in the presence or not of a hyperglycemic state does not appear to have influenced the weight variable in these rats.

Figure 4 represents the difference between the rat blood glucose concentration on the day of surgery and on the day of death, at specific doses of aminoguanidine. It could be observed that in the groups of healthy rats (groups 1, 2 and 3), the glycemia levels remained below 250 mg/dL in all rats. The same goal was achieved among the rats with induced diabetes, where the glycemia levels remained above the pre-established limit.

Table 3. Effect of aminoguanidine on the variation in weight, of healthy and diabetic rats, between the day of implant surgery and the day of death

Aminoguanidine dosage (mg/kg, daily)	Weight (g)	
	Healthy rats	Diabetic rats
0	72.85 ± 5.21^{aA}	66.00 ± 7.48^{aA}
10	41.42 ± 9.61^{aA}	40.00 ± 11.82^{aAB}
20	51.25 ± 4.79^{aA}	-72.50 ± 22.12^{aB}

Data are given as mean ± SE.
Values followed by lowercase letters across the rows and by capital letters down the columns differ significantly ($p < 0.05$; Kruskall–Wallis test).

Discussion

Many studies associate complications from the diabetic state with the formation and accumulation of AGEs in the tissues (17–19), highlighting their negative effect on the various stages of the process of peri-implant bone repair, which begins with adhesion and cell growth, the deposit of the bone matrix, and alteration of the DNA and nuclear proteins, in turn producing an unfavorable environment for osseointegration (5,20–23).

Aminoguanidine is a recently researched drug that has demonstrated the capacity to inhibit the formation of AGEs and consequently to minimize the damage caused by the hyperglycemic state, as shown in studies on the evaluation of angiogenesis (9), in the prevention of diabetic retinopathy (17) and in peri-implant healing (3).

The present study evaluated the effects of aminoguanidine in the biomechanical retention of implants inserted in the femur of mice with induced diabetes. It was observed that the daily administration of 20 mg/kg of aminoguanidine in the group of diabetic rats resulted in average counter-torque forces that were statistically comparable with those of the healthy rat groups, thus demonstrating that, at this concentration, aminoguanidine raised the average counter-torque values to the levels of those in the healthy rat groups. By contrast, in the healthy rat groups, aminoguanidine resulted in no change in the counter-torque force, regardless of the dosage.

Our findings corroborate the findings of Margonar *et al.* (15), who used the biomechanical counter-torque test to evaluate the influence of diabetes in tissue repair and reported statistically lower reverse torque values for the removal of implants in diabetic rats with replacement of insulin, compared with the nondiabetic control group. Other researchers (24,25) have also used the biomechanical counter-torque test as a study method.

Through histomorphometric analysis, many experiments using mice with diabetes induced by streptozotocin or alloxan have demonstrated a level of implant bone contact that is statistically

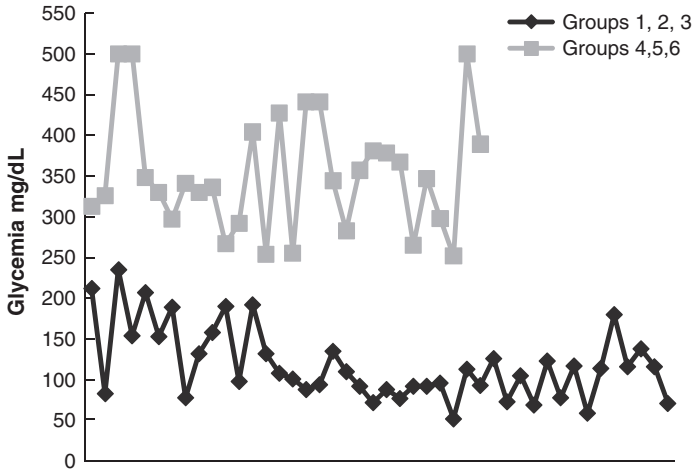

Fig. 4. Glycemia variation in healthy and diabetic rats.

lower in diabetic rats compared with healthy controls (3,4,14,26). Based on these findings, it can be understood that in the present study the presence of AGEs in the peri-implant tissues negatively affected the peri-implant bone repair in diabetic rats. This finding suggests that the formation of AGEs causes changes in the extracellular matrix (1), leading to a reduction in the formation of collagen (2,27,28) and competition with bone-formation proteins. As a result, a reduction in the secretion of alkaline phosphate by osteoblasts and in the serum levels of osteocalcin could be observed. In this manner, the accumulation of AGEs in the tissues may well compromise peri-implant bone repair in the formation of blood clots, in cellular migration and differentiation, in deposition and mineralization of the osteoid and in the maintenance of osteointegration (2). Consequently, a lesser counter-torque force was needed to break the implant–bone interface in diabetic rats who had not received aminoguanidine, compared with the healthy controls.

The results of the present study are in accordance with those reported from one single study that evaluated the effect of aminoguanidine on peri-implant healing in mice with induced diabetes (3) The present study demonstrated that the administration of 20 mg/kg of aminoguanidine in mice with induced diabetes resulted in an increase of the counter-torque force necessary to break the bone–implant contact at levels that are statistically equal to those measured for the healthy rat group.

Potential treatment strategies for complications resulting from AGE links to cells include preventing the formation of links and breaking links already existing. Aminoguanidine is a prototype of a therapeutic agent aimed at preventing the formation of AGEs through its reaction with α,β dicarbonil mixtures, which are responsible for the cross-links with diverse proteins, thus preventing the formation of these products (29). However, in addition to preventing the formation of AGEs, aminoguanidine, when used systematically, shows the capacity to inhibit the D-amino acid oxidase (DAO) and the NO synthase, which, according to Nilsson (30) could, *in vitro*, lead to serious vascular and respiratory side effects. This is caused by the accumulation of histamine in the blood and by the change in the production of nitrous oxide, which is capable of affecting the immunological response, neuron transmission and vascular control.

The diabetic state (a serum level of glucose > 250 mg/dL) used in the present investigation was achieved in a predictable manner and maintained during the entire experimental period. The average glucose values fluctuated between 252 and 441 mg/dL. These values are similar to those reported in other studies: > 300 mg/dL in the studies of Takeshita *et al.* (27,31), Iyama *et al.* (32), Ottoni and Chopard (14) and Kwon *et al.* (1), and > 350 mg/dL in the study of Nevis *et al.* (33). Lower concentrations of glucose, of > 180 mg/dL and of > 200 mg/dL, were used in the studies of Giglio *et al.* (34) and Siqueira *et al.* (26), respectively. The serum levels of glucose were not given in the study of McCraken *et al.* (28).

The results of the present study demonstrate that the application of different doses of aminoguanidine, and in the presence or not of the hyperglycemic state, do not appear to influence the weight of rats. However, the application of aminoguanidine at a dose of 10 mg/kg can alter the blood glucose levels in rats with induced diabetes.

It should be noted that the evaluation method used in the present study to determine the bone/implant contact (i.e. the counter-torque) represents a methodological limitation. It should also be emphasized that the use of aminoguanidine in an attempt to minimize the harmful effect of hyperglycemia in peri-implant bone repair is a theme that has not been explored in depth in the literature. Thus, further studies are required to enhance the understanding of its mechanism of action and/or probable side effects.

Conclusion

The osseointegration process was negatively affected within the model of diabetes induced by alloxan. The administration of aminoguanidine had no effect on the counter-torque force required to remove implants in the healthy rat groups, whereas the administration of 20 mg/kg of aminoguanidine in diabetic rats raised the average values of the counter-torque force needed to rupture the bone–implant interface to those of the healthy rat group.

References

1. Kwon PT, Rahman SS, Kim DM, Kopman JA, Karimbux NY, Fiorellini JP. Maintenance of osseointegration utilizing insulin therapy in a diabetic rat model. *J Periodontol* 2005;**76**(4)**:**621–662.
2. Fiorellini JP, Nevins ML. Dental implant considerations in the diabetic patient. *Periodontol 2000* 2000;**23:**73–77.

3. Kopman JA, Kim DM, Rahman SS, Arandia JA, Karimbux NY, Fiorellini JP. Modulating the effects of diabetes on osseointegration with aminoguanidine and doxycycline. *J Periodontol* 2005;**76**(4)**:**614–620.
4. Kotsovilis S, Karoussis IK, Fourmousis I. A comprehensive and critical review of dental implant placement in diabetic animals and patients. *Clin Oral Implants Res* 2006;**17:**587–599.
5. Fiorellini JP, Chen PK, Nevins M, Nevins ML. A retrospective study of dental implants in diabetic patients. *Int J Periodontics Restorative Dent* 2000;**20**(4)**:** 367–373.
6. Balshi TJ, Wolfinger GJ. Dental implants in the diabetic patient: a retrospective study. *Implant Dent* 1999;**8:**355–359.
7. Fazard P, Anderson l, Nyberg J. Dental implant treatment in diabetic patients. *Implant Dent* 2002;**2:**262–267.
8. Abdulwassie H, Dhanrajani PJ. Diabetes mellitus and dental implants: a clinical study. *Implant Dent* 2002;**11**(1)**:**83–85.
9. Teixeira AS, Andrade SP. Glucose-induced inhibition of angiogenesis in the Rat sponge Granuloma is prevented by aminoguanidine. *Life Sci* 1999;**61**(8)**:**655–662.
10. Amos AF, McCarty DJ, Zimmet P. The rising global burden of diabetes and its complications: estimates and projections to 2010. *Diabet Med* 1997;**14:**1–85.
11. Peppa M, Uribarri J, Vlassara H. Glucose, advanced glycation end products, and diabetes complications: what is new and what works. *Clinical Diabetes* 2003;**21**(4)**:** 186–187.
12. Pontes Andersen CC, Flyvbjerg A, Buschard K, Holmstrup P. Relationship between periodontitis and diabetes: lessons from rodent studies. *J Periodontol* 2007;**78**(7)**:**1265–1275.
13. Pablos AB. *Avaliação da influência das drogas antiinflamatórias não esteroidais sobre a reparação óssea no diabetes experimental* [Dissertação]. São Paulo: Universidade de São Paulo, 2003.
14. Ottoni CE, Chopard RP. Histomorphometric evaluation of new bone formation in diabetic rats submitted to insertion of temporary implants. *Braz Dent J* 2004; **15**(2)**:**87–92.
15. Margonar R, Sakakura CE, Holzhausen M, Pepato MT, Alba RC, Marcantonio E. The influence of diabetes mellitus and insulin therapy on biomechanical retention around dental implants: a study in rabbits. *Implant Dent* 2003;**12**(4)**:**333–339.
16. Statistical Analysis System Institute. *SAS/STAT®*: User's Guide. Statistics Version 6.4. Cary: Statistical Analysis System Institute, 1996:168.
17. Hammes HP, Martin S, Federlin K, Geisen K, Brownlee M. Aminoguanidine treatment inhibits the development of experimental diabetic retinopathy. *Proc Natl Acad Sci* 1991;**88:**11555–11558.
18. Beisswenger PJ, Makita Z, Curphey TJ, *et al*. Formation of immunochemical advanced glycation end products precedes and correlates with early manifestations of renal and retinal disease in diabetes. *Diabetes* 1995;**44:**824–829.
19. Vlassara H, Striker LJ, Teichberg S, Fuh H, Li YM, Steffes M. Advanced glycation endproducts induce glomerular sclerosis and albuminuria in normal rats. *Proc Natl Acad Sci* 1994;**91:**11704–11708.
20. Schmidt AM, Yan SD, Yan SF, Stern DM. The biology of the receptor for advanced glycation end products and its ligands. *Biochim Biophys Acta* 2001;**1498:** 99–111.
21. Vlassara H. The AGE receptor in the pathogenesis of diabetic complications. *Diabetes Metab Res Rev* 2001;**17:**436–443.
22. Southerland JH, Taylor GW, Offenbacher S. Diabetes and periodontal infection: making the connection. *Clinical Diabetes* 2005;**23**(4)**:**171–178.
23. Katayama Y, Akatsu T, Yamamoto M, Kugai N, Nagata N. Role of nonenzymatic glycosylation of type I collagen indiabetic osteopenia. *J Bone Miner Res* 1996;**11:**931–937.
24. Buser D, Nydegger T, Hirt HP, Cochran DL, Nolte LP. Removal torque values of titanium implants in the maxilla of miniature pigs. *Int J Oral Maxillofac Implants* 1998;**13:**611–619.
25. Giro G, Sakakura CE, Gonçalves D, Pereira RM, Marcantonio E Jr, Orrico SR. Effect of 17 β- Estradiol and alendronate on the removal torque of osseointegrated titanium implants in ovariectomized rats. *J Periodontol* 2007; **78**(7)**:**1316–1321.
26. Siqueira JT, Cavalher-Machado SC, Arana-Chavez VE, Sannomiya P. Bone formation around titanium implants in the rat tibia: role of insulin. *Implant Dent* 2003;**12**(3)**:**242–249.
27. Takeshita F, Iyama S, Ayukawa Y, Kido MA, Murai K, Suetsugu T. The effects of diabetes on the interface between hydroxyapatite implants and bone in rat tibia. *J Periodontol* 1997;**68:**180–185.
28. McCracken MS, Aponte-Wesson R, Chavali R, Lemons JE. Bone associated with implants in diabetic and insulin-treated rats. *Clin Oral Implants Res* 2006; **17:**494–500.
29. Tournalley PJ. Use of aminoguanidine (Pimagedine) to prevent the formation of advanced glycation endproducts. *Arch Biochem Biophys* 2003;**419:**31–40.
30. Nilsson BO. Biological effects of aminoguanidine: an update. *Inflamm Res* 1999; **48**(10)**:**509–515.
31. Takeshita F, Murai K, Iyama S, Ayukawa Y, Suetsugu T. Uncontrolled diabetes hinders bone formation around titanium implants in rat tibiae. A light and fluorescence microscopy, and image processing study. *J Periodontol* 1998;**69**(3)**:**314–320.
32. Iyama S, Takeshita F, Ayukawa Y, Kido MA, Suetsugu T, Tanaka T. Study regional distribution of bone formed around hydroxyapatite implants in the tibial of streptozotocin induced diabetics rats using multiple fluorescent labeling and confocal laser scanning microscopy. *J Periodontol* 1997;**68**(12)**:**1169–1175.
33. Nevins ML, Karimbux NY, Weber HP, Giannobile WV, Fiorellini JP. Wound healing around endosseous implants in experimental diabetes. *Int J Oral Maxillofac Implants* 1998;**13:**620–629.
34. Giglio MJ, Giannunzio G, Olmedo D, Guglielmotti MB. Histomorphometric study of bone healing around laminar implants in experimental diabetes. *Implant Dent* 2000;**9:**143–149.