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The influence of short-term diabetes mellitus and insulin therapy on alveolar bone loss in rats

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Background: It is well known that the multiple direct and indirect consequences of hyperglycemia in diabetic individuals have been linked to a number of abnormal host effector mechanisms that could lead to an increased risk of developing periodontal disease.

Objective: The aim of this study was to investigate the effect of short-term experimental diabetes and insulin therapy on the severity of alveolar bone loss in rats, and the effect of experimental periodontitis on glycemic control.

Methods: Seventy-two male Wistar rats were divided into four groups: group I animals were submitted to dental ligature around lower right first molars (ligated); group II consisted of streptozotocin (STZ)-diabetic, ligated rats; group III represented STZ-diabetic, unligated rats; and group IV consisted of insulin-treated (6 U/day), STZ-diabetic, ligated rats. Blood glucose of all diabetic rats was monitored at regular intervals. Standardized digital radiographs were taken after killing at 7, 15 and 30 days to measure the amount of bone loss about the mesial root surface of the first molar tooth in each rat.

Results: No significant ($p < 0.05$) changes in plasma glucose levels of insulin-treated diabetic rats were found among the different examinations after the beginning of insulin therapy. Rats from group II showed significantly greater increases in mean plasma glucose levels at 15 and 30 days after ligature placement compared with rats from group III ($p < 0.05$). Furthermore, in spite of the significant alveolar bone loss progression that was observed in groups I, II and IV ($p < 0.00001$; two-way ANOVA), no significant differences among these groups regarding the severity of bone loss ($p = 0.77$) and no significant interaction between treatment group and time ($p = 0.81$) were found.

Conclusions: Within the limits of this study, it can be suggested that the severity of periodontal disease was not affected by short-term diabetes, and that experimental periodontitis increased blood glucose levels in uncontrolled diabetic rats.

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Diabetes mellitus is a metabolic disorder characterized by an abnormal regulation of glucose metabolism. The hyperglycemia developed from either a deficiency in insulin production or an impaired cellular sensitivity to insulin, Types 1 and 2 diabetes, respectively, can be associated with systemic disorders including myopathies, neuropathy, macrovascular disease, altered wound healing (1, 2) and with an increased susceptibility to periodontal disease (3).

Several epidemiological studies have showed a higher prevalence and severity

of periodontal disease in patients with diabetes mellitus. The Pima Indians, who have the highest reported prevalence of Type 2 diabetes mellitus in the world (4) were studied in at least four cross-sectional studies, clearly demonstrating that the prevalence of periodontitis is higher in diabetic patients than in non-diabetics (5–8). Patients with Type 1 diabetes have also been studied in controlled cross-sectional studies, showing that periodontal changes were always more severe in diabetics. Furthermore, these studies have indicated that diabetics with poor metabolic control had more severe periodontitis than those who were considered to be well controlled (9, 10). Another finding that comes out of a 2-year controlled study (3) is that type 2 diabetes mellitus subjects had a significantly increased risk of progressive alveolar bone loss compared to non-diabetic subjects.

A number of underlying mechanisms likely account for the association of diabetes with periodontal disease: reduced polymorphonuclear leukocyte function and chemotaxis, reduced collagen synthesis and maturation, increased collagenase activity, and formation of advanced glycation end-products, which can bind to macrophage and monocyte receptors resulting in increased secretion of tumor necrosis factor-α, interleukin-1 and prostaglandin E-2 (PGE_2) (11–14). Collectively, these mechanisms could lead to impaired host resistance to infection, defective wound healing and exaggerated inflammatory response (2), justifying the severe periodontal destruction and tooth loss in diabetic individuals.

Strong evidence exists to support the notion that hyperglycemia is the primary factor responsible for the development of diabetic complications. Indeed, several studies have observed more severe periodontitis in patients with poorly controlled diabetes (15–17) and in non-diabetic patients with hyperglycemia (18, 19). Therefore, it can be suggested that metabolic control may affect the status of periodontal disease. However, many studies have demonstrated that better blood glucose levels could not directly explain the better clinical presentation (20, 21) and the course of pre-existing periodontitis in diabetic patients (22).

Furthermore, many studies provide evidence that inflammatory states may induce a chronic state of insulin resistance, contributing to a risk of poor glycemic control in diabetic patients (23–25). However, other investigations have found no effect on metabolic control after periodontal treatment (16, 26–29). Consequently, no firm conclusions can be drawn about this topic from this collective body of literature. The purpose of the present study was to investigate the effect of short-term experimental diabetes mellitus and insulin therapy on the severity of alveolar bone loss in rats, and the effect of experimental periodontitis on glycemic control.

Material and methods

Seventy-two adult male Wistar rats weighing approximately 200 g each were used in this study. The animals were housed in cages in groups of four per cage, and fed with a standard laboratory diet and given tap water ad libitum. The protocol of this study was in accordance with guidelines approved by the Institutional Experimentation Committee of the School of Dentistry at Araraquara, São Paulo, Brazil. Figure 1 outlines the experimental protocol.

A group of 18 rats was injected with citrate buffer alone (control, group I). The other 54 rats (groups II, III and IV) were fasted for 14 h and diabetes was induced by a single injection of 50 mg/kg body weight streptozotocin (STZ) (Sigma, St. Louis, MO, USA) dissolved in citrate buffer (0.01 M; pH 4.5) into the jugular vein. After the injection, the animals were given free access to water and food. Blood samples were obtained from the tip of the tail of conscious rats from STZ-diabetic groups II, III and IV, between the hours of 6.00 a.m. and 8.00 a.m., 3 days after diabetes induction (Exam 1, Fig. 1). The samples were collected in Eppendorf tubes containing liquaemin sodium (Liquemine®, La Roche Ltd, Basel, Switzerland). After centrifugation, plasma was separated and assessed for level of glucose with the aid of a glucose analyzer (Autoanalyzer, Technicom RA-XT, Bayer, Dublin, Ireland). A plasma glucose level greater than 300 mg/dl confirmed the presence of diabetes. One day after diabetes confirmation, the rats from group IV received subcutaneous injections of NPH (Neutral Protamine Harguerdon) of insulin (Biohulin NU-100, 100 U/ml, Biobrás, Montes Claros, MG, Brazil) diluted in 0.9% NaCl (3 U/twice a day). The other rats from the diabetic groups II and III, and the control animals from group I were treated with saline solution.

After 7 days (Exam 2), blood samples confirmed that the glycemic control was succeeded in the rats from group IV. Then, all the animals from groups I, II and IV underwent anesthetization obtained by intramuscular administration of ketamine (0.08 ml/100 g body weight) (Francotar®, Virbac do Brazil Ind. e Com. LTDA, São Paulo, Brazil). The lower right first molar of each rat received the cotton ligature in a submarginal position to induce experimental periodontitis

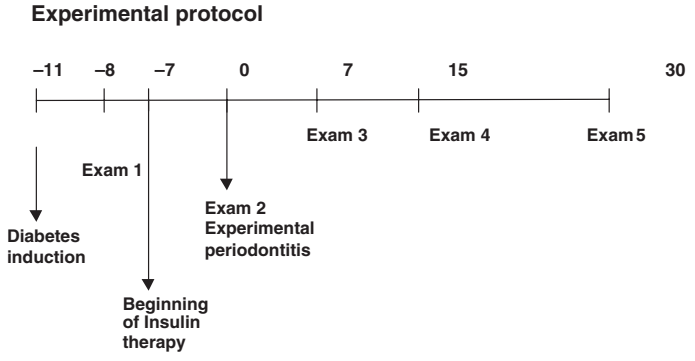

Fig. 1. Experimental protocol.

(30, 31). The STZ-diabetic rats from group III were left unligated.

The animals were killed, in numbers of six rats per group, at 7, 15 and 30 days after ligature placement. At these time-periods, blood samples were taken in order to measure the plasma glucose concentration (mg/dl) of all animals (Exams 3, 4 and 5, respectively, at 7, 15 and 30 days).

The jaws were removed to determine the degree of bone loss. Standardized digital radiographs were obtained with the use of a computerized imaging system, CDR® (Computed Dental Radiography for Microsoft Windows, Shick Technologies, Inc., Dialom Dental Products, USA), that utilizes an electronic sensor instead of X-ray film. Electronic sensors were exposed at 65 kV and 10 mA with time of exposition of 12 impulses/s. The source-to-film distance was always set at 50 cm. The distance between the cemento-enamel junction and the height of alveolar bone was determined for mesial root surfaces of lower right first molars with the aid of the CDR software. Millimeters of bone loss at each radiograph were measured three times by the same examiner, in different days, in order to reduce the variation in the data.

Statistical analyses

A one-way analysis of variance (ANOVA) was used to determine significances of differences among groups of STZ-diabetic rats (groups I, II, III and IV), regarding their plasma glucose measurements three days after STZ injection. The intragroup changes were analyzed by paired *t*-test.

Two-way ANOVA was employed to analyze radiographic data from all four groups, using the Tukey's test for subsequent multiple comparisons. Data are expressed as means ± SEM. Significance level was always set at 95% and all calculations were performed using Statistica 5.1 for Windows (StatSoft Inc., Tulsa, OK, USA).

Results

Plasma glucose concentrations

No statistical differences ($p < 0.05$) were found among groups of STZ-diabetic rats (groups II, III and IV) regarding the initial plasma glucose measurements after STZ injection (Exam 1, Table 1). The mean plasma glucose levels in the STZ-diabetic rats were statistically higher ($p < 0.05$) when compared with the mean plasma glucose levels of control rats (group I) in all exams (Exams 1, 2, 3, 4 and 5) at all experimental periods (7, 15 and 30 days).

There were no statistically significant changes among different exams in the control group (group I) in any experimental period.

At experimental period of 7 days in the uncontrolled diabetic group of rats with ligature (group II), there were no significant differences ($p < 0.05$) among mean plasma glucose levels at the three different exams (Table 1). However, at experimental period of 15 days, the mean plasma glucose level of the fourth exam (640.83 ± 166.22) was statistically greater ($p < 0.05$) than those of the anterior exams (440.83 ± 63.34, 445.17 ± 60.03 and 449.17 ± 108.37, respectively, exams 1, 2 and 3). At experimental period of 30 days a similar behavior was found: the mean plasma glucose level at the fourth exam (716.67 ± 59.35) was greater than those of the first (469.17 ± 38.77), second (538.33 ± 30.91) and third (496.67 ± 55.43) exams. In addition, there was no statistical difference between the fourth and fifth exams.

In the uncontrolled diabetic group of rats without ligature (group III), a trend to increasing blood glucose levels with time was also verified; however, a statistically significant difference was only found at experimental period of 15 days where the mean plasma glucose level of the fourth exam (514.03 ± 26.70) was statistically greater ($p < 0.05$) than that of exam 1 (470.33 ± 60.28). Furthermore, the blood glucose levels in the fourth exam at experimental period of 15 days and in the fourth and fifth exams at

Table 1. Mean serum glucose levels (mg/dl) and standard deviation of rats from groups I, II, III and IV:

		Plasma glucose (mg/dl)				
Group	Period	Exam 1	Exam 2	Exam 3	Exam 4	Exam 5
I	7 ($n = 6$)	131.10 ± 8.70	117.68 ± 4.20	134.87 ± 12.00		
	15 ($n = 6$)	146.21 ± 4.51	161.17 ± 5.93	153.31 ± 9.08	149.13 ± 8.34	
	30 ($n = 6$)	123.63 ± 6.31	126.00 ± 7.84	127.29 ± 6.17	148.70 ± 6.45	141.12 ± 10.81
II	7 ($n = 6$)	510.00 ± 82.04[a]	539.00 ± 33.82[a]	534.00 ± 40.17[a]		
	15 ($n = 6$)	440.83 ± 63.34[a]	445.17 ± 60.03[a]	449.17 ± 108.37[a]	640.83 ± 166.22[abcd]	
	30 ($n = 6$)	469.17 ± 38.77[a]	538.33 ± 30.91[a]	496.67 ± 55.43[a]	716.67 ± 59.35[abcd]	750.83 ± 56.82[abcd]
III	7 ($n = 6$)	506.30 ± 48.50[a]	510.00 ± 46.40[a]	523.70 ± 44.40[a]		
	15 ($n = 6$)	470.33 ± 60.28[a]	490.08 ± 50.20[a]	487.63 ± 57.21[a]	514.03 ± 26.70[abe]	
	30 ($n = 6$)	461.39 ± 42.83[a]	470.50 ± 60.17[a]	456.21 ± 51.33[a]	485.69 ± 24.47[ae]	465.19 ± 15.36[ae]
IV	7 ($n = 6$)	476.67 ± 45.25[a]	78.83 ± 5.93[ab]	82.00 ± 4.55[ab]		
	15 ($n = 6$)	474.17 ± 50.37[a]	73.67 ± 9.30[ab]	69.67 ± 9.72[ab]	70.50 ± 14.34[ab]	
	30 ($n = 6$)	433.33 ± 85.91[a]	67.17 ± 6.34[ab]	66.33 ± 5.88[ab]	67.00 ± 7.85[ab]	66.67 ± 11.86[ab]

[a]Indicates a significant difference, $p < 0.05$, when compared with the corresponding exam at the same experimental period in group I.
[b]Indicates a significant difference, $p < 0.05$, when compared with the exam 1.
[c]Indicates a significant difference, $p < 0.05$, when compared with the exam 2.
[d]Indicates a significant difference, $p < 0.05$, when compared with the exam 3.
[e]Indicates a significant difference, $p < 0.05$, when compared with the corresponding exam at the same experimental period in group II.

experimental period of 30 days in group III were statistically lower than those of group II ($p < 0.05$).

The mean and standard deviation of plasma glucose level (mg/dl) of the insulin-treated diabetic rats (group IV) at the experimental periods of 7, 15 and 30 days are also shown in Table 1. The mean plasma glucose levels 7 days after the beginning of insulin treatment (exam 2) was lower than that of the first exam at all the experimental periods (7, 15 and 30 days), confirming the insulin control of glucose levels. The same behavior was verified in the following exams of group IV (exams 3, 4 and 5). However, no further differences among exams were found in any of the experimental periods after the periodontitis induction.

Radiographic data

Table 2 shows mean radiographic alveolar bone loss for groups I, II, III and IV, at experimental periods of 7, 15 and 30 days. Mean alveolar bone loss in group III (diabetes without experimental periodontitis) was statistically lower when compared with the mean alveolar bone loss in the ligated-animals (groups I, II and IV) in all the experimental periods evaluated. There were no statistically significant differences with regards to the mean alveolar bone loss among experimental periods in the group III.

An increasing alveolar bone loss over the 30-day period was evident, with significant differences between experimental periods of 30 and 7 days ($p = 0.00001$) in the experimental groups I, II and IV. At experimental period of 15 days only the control and insulin-treated diabetic groups showed significant increases in mean bone loss when compared with the experimental period of 7 days ($p = 0.01$ and $p = 0.004$, respectively). However, no significant differences among groups regarding the severity of alveolar bone loss ($p = 0.77$) and no significant interaction between treatment group and time ($p = 0.81$) were found.

Discussion

The association between diabetes mellitus and periodontal disease has been a topic of discussion for many years. Several epidemiological studies have reported a positive correlation between diabetes and the prevalence and severity of periodontal disease (5, 6, 32). In fact, this increased susceptibility of periodontal disease in diabetic subjects could be correlated with the impaired metabolic control of glycemic levels (33).

Besides the reduced polymorphonuclear leukocyte function and its depressed chemotaxis (34, 35), the plausibility of this relationship could be well explained by some indirect consequences of hyperglycemia (2) that could adversely affect host resistance to microbial dental plaque. Elevated blood glucose levels can result in the tissue and plasma accumulation of the irreversible advanced glycation end-products, through the non-enzymatic glycation and oxidation proteins and lipids (36). The presence of advanced glycation end-products has been linked to the development of diabetic complications (37). The interaction of advanced glycation end-products with cell surface binding sites (receptors for advanced glycation end products), which are present on endothelial cells, mononuclear phagocytes and fibroblasts, results in hyperpermeability (38), increased production of vascular cell adhesion molecule-1 (39), up-regulation of the proinflammatory monocyte response, resulting in enhanced production of tumor necrosis factor, interleukin-1 or interleukin-6 (40–42), and decreased production of type I collagen (43). Thus, hyperglycemia can interfere with the risk of developing periodontal destruction by leading to an exaggerated inflammatory response and compromised wound healing.

It is also well known that bone metabolism is impaired in uncontrolled diabetes (44, 45). Sayinalp *et al.* (45) found that following the restoration of good glycemic control in diabetic patients, the serum concentration of osteocalcin, an indicator of osteoblast function, was increased and, in consequence, the osteoblast function re-established. Similarly, in an STZ-diabetic model, Shyng *et al.* (44) showed significantly lower calcein uptake in diabetic rat femurs than that of the control or insulin-treated rat. In fact, low dynamic bone formation and osteoporosis characterize the diabetic-rat model (46, 47).

In the present study, we used STZ, a potent diabetogenic agent that causes selective destruction of pancreatic beta cells, in order to experimentally induce a decreased secretion of insulin. In agreement with findings reported in the literature (11, 48, 49), our results showed an increase in plasma glucose levels in STZ-treated rats.

The model of periodontitis-associated diabetes has been used to study the effects of diabetes on periodontal disease both in rats (50) and mice (11, 51). However, to the best of our knowledge, there have been no studies reported that assess the effects of insulin therapy on alveolar bone of diabetic rats subjected to ligature-induced periodontitis. The objective of our study was to demonstrate that insulin therapy would correct a possible negative effect of STZ-induced diabetes mellitus on the alveolar bone loss of rats subjected to experimental periodontitis.

Recently, a study by Mishima *et al.* (52) demonstrated that insulin treatment of diabetic rats normalized the histometric measures of alveolar bone remodeling in a 24-day experiment. Our

Table 2. Mean radiographic alveolar bone loss (mm) and standard deviation after experimental periodontitis in groups I, II, III and IV in each experimental period:

Experimental period (days)	Mean alveolar bone loss ± SD (mm)			
	Group I	Group II	Group III	Group IV
7 ($n = 6$)	0.72 ± 0.16[a]	0.80 ± 0.23[a]	0.07 ± 0.03	0.71 ± 0.17[a]
15 ($n = 6$)	1.02 ± 0.19[ab]	1.05 ± 0.17[a]	0.13 ± 0.12	1.13 ± 0.17[ab]
30 ($n = 6$)	1.22 ± 0.41[ab]	1.15 ± 0.30[ab]	0.11 ± 0.09	1.27 ± 0.18[ab]

[a]Indicates a significant difference, $p < 0.05$, when compared with group III at the same experimental period.

[b]Indicates a significant difference, $p < 0.05$, using two-way ANOVA, when compared with experimental period of 7 days in the same experimental group.

data showed that daily insulin injection (6 U) lead to decreased levels of plasma glucose, between 60.45 and 86.55 mg/dl, below the levels of non-diabetic rats (group I). However, in spite of the effective glycemic control reached in the present study, our radiographic results revealed no differences among groups of rats subjected to experimental periodontitis (groups I, II and IV, respectively, control, diabetic- and insulin-treated diabetic groups) in any of the experimental periods (7, 15 and 30 days) regarding the mean alveolar bone level measured at the mesial surface of the mandibulary right first molars. The absence of significant increased alveolar bone loss in the hyperglycemic group (group II) when compared to groups I (control) and IV (diabetes with insulin therapy), may be explained by a possible influence of the duration of diabetes on the severity of periodontal disease. The present study was a short-term evaluation (only 1 month) of the effects of hyperglycemia on periodontitis. A longer period of experimental diabetes may be necessary to result in a possible enhanced accumulation of advanced glycation endproducts in the periodontal tissues, and consequently, enhanced effects on periodontal inflammation, as shown by other studies (11, 51). Lalla *et al.* (11) showed significantly increased alveolar bone loss in diabetic mice compared with non-diabetic mice only at 3 and 4 months after diabetes induction, therefore reinforcing the hypothesis of the possible influence of the duration of diabetes on the severity of experimental periodontitis in rats. Actually, it has been shown that patients with long-term diabetes are more likely to develop periodontitis than those who have had the disease for a short time (9, 53–55). In addition, it is stated in a Position Paper of the American Academy of Periodontology (14): 'The incidence of periodontitis increases among diabetic subjects after puberty and as the patient population ages', and 'Periodontal disease may be more frequent and severe in diabetic individuals with more advanced systemic complications'.

Despite the apparent lack of effect of diabetes on the alveolar bone loss found in our present investigation, significant greater increases ($p < 0.05$) in the mean plasma glucose levels were showed in the uncontrolled diabetic rats with ligature (group II) at 15 and 30 days after ligature placement, when compared with those of the uncontrolled diabetic rats without ligature (group III). This finding is in agreement with those of previous studies (17, 56) and would indicate that even in a short term, periodontal disease may complicate the severity of diabetes in rats. By the other hand, insulin therapy seemed to overcome the deleterious effect of experimental periodontal disease on the glycemic control, probably due to the hypoglycemic state caused by the daily injection of 6 U of insulin.

In conclusion, the present study showed that short-term experimental diabetes had no influence on the severity of periodontitis in rats. In addition, it was shown that blood glucose level could be affected by experimental periodontitis in uncontrolled diabetic rats. Future studies should involve greater experimental periods to determine whether a longer duration of diabetes is associated with more significant alveolar bone loss in the same animal model.

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References

1. American Diabetes Association. Expert Committee on the Diagnosis and Classification of Diabetes Mellitus. Committee report. *Diabetes Care* 1997;**20:**1183–1197.
2. Lalla E, Lamster IB, Drury S, Fu C, Schmidt AM. Hyperglycemia, glycoxidation and receptor for advanced glycation endproducts: potential mechanisms underlying diabetic complications, including diabetes-associated periodontitis. *Periodontol 2000* 2000;**23:**50–62.
3. Taylor GW *et al.* Non-insulin dependent diabetes mellitus and alveolar bone loss progression over 2 years. *J Periodontol* 1998;**69:**76–83.
4. Knowler WC, Pettitt DJ, Vasquez B, Rotwein PS, Andreone TL, Permutt MA. Polymorphism in the 5′ flanking region of the human insulin gene. Relationships with noninsulin-dependent diabetes mellitus, glucose and insulin concentrations, and diabetes treatment in the Pima Indians. *J Clin Invest* 1984;**74:**2129–2135.
5. Shlossman M *et al.* Type 2 diabetes mellitus and periodontal disease. *J Am Dent Assoc* 1990;**121:**32–36.
6. Emrich LJ, Shlossman M, Genco RJ. Periodontal disease and insulin-dependent diabetes mellitus. *J Periodontol* 1991; **62:**123–130.
7. Nelson RG *et al.* Periodontal disease and NIDDM in Pima Indians *Diabetes Care* 1990;**13:**836–840.
8. Unal T, Firatli E, Sivas A, Meric H, Oz H. Fructosamine as a possible monitoring parameter in non-insulin dependent diabetes mellitus patients with periodontal disease. *J Periodontol* 1993;**64:**191–194.
9. Thorstensson H, Hugoson A. Periodontal disease experience in adult long-duration insulin-dependent diabetics. *J Clin Periodontol* 1993;**20:**352–358.
10. de Pommereau V, Dargent-Pare C, Robert JJ, Brion M. Periodontal status in insulin-dependent diabetic adolescents. *J Clin Periodontol* 1992;**19:**628–632.
11. Lalla E, Lamster IB, Feit M, Huang L, Schmidt AM. A murine model of accelerated periodontal disease in diabetes. *J Periodont Res* 1998;**33:**387–399.
12. Nishimura F *et al.* Periodontal disease as a complication of Diabetes Mellitus. *Ann Periodontol* 1998;**3:**20–29.
13. Salvi GE, Beck JD, Offenbacher S. PGE_2, IL-1β, and TNF-α responses in diabetic as modifiers of periodontal disease expression. *Ann Periodontol* 1998;**3:**40–50.
14. Position paper. Diabetes and Periodontal diseases. *J Periodontol* 2000;**71:**664–678.
15. Safkan-Seppälä B, Ainamo J. Periodontal conditions in insulin-dependent diabetes-mellitus. *J Clin Periodontol* 1992;**19:**24–29.
16. Seppala B, Seppala M, Ainamo J. A longitudinal study on insulin-dependent diabetes mellitus and periodontal disease. *J Clin Periodontol* 1993;**20:**161–165.
17. Taylor GW *et al.* Severe periodontitis and risk for poor glycemic control in patients with non-insulin-dependent diabetes mellitus. *J Periodontol* 1996;**67:**1085–1093.

18. Katz J, Peretz B, Sgan-Cohen HD, Horev T, Eldad A. Periodontal status by CPITN, and associated variables in an Israeli permanent force military population. *J Clin Periodontol* 2000;**27:**319–324.
19. Katz J. Elevated blood glucose levels in patients with severe periodontal disease. *J Clin Periodontol* 2001;**28:**710–712.
20. Rylander H, Ramberg P, Blohme G, Lindhe J. Prevalence of periodontal disease in young diabetics. *J Clin Periodontol* 1987;**14:**38–43.
21. Kawamura M, Tsurumoto A, Fukuda S, Sasahara H. Health behaviors and their relation to metabolic control and periodontal status in type 2 diabetic patients: a model tested using a linear structural relations program. *J Periodontol* 2001; **72:**1246–1253.
22. Novaes Junior AB, Pereira AL, de Moraes N, Novaes AB. Manifestations of insulin-dependent diabetes mellitus in the periodontium of young Brazilian patients. *J Periodontol* 1991;**62:**116–122.
23. Hallgren R, Lundquist G. Elevated serum levels of pancreatic polypeptide are related to impaired glucose handling in inflammatory states. *Scand J Gastroenterol* 1983;**18:**561–564.
24. Svenson KL, Lundqvist G, Wide L, Hallgren R. Impaired glucose handling in active rheumatoid arthritis: effects of corticosteroids and antirheumatic treatment. *Metabolism* 1987;**36:**944–948.
25. Flier JS. Insulin receptors and insulin resistance. *Annu Rev Med* 1983;**34:**145–160.
26. Aldridge JP, Lester V, Watts TL, Collins A, Viberti G, Wilson RF. Single-blind studies of the effects of improved periodontal health on metabolic control in type 1 diabetes mellitus. *J Clin Periodontol* 1995;**22:**271–275.
27. Smith GT, Greenbaum CJ, Johnson BD, Persson GR. Short-term responses to periodontal therapy in insulin-dependent diabetic patients. *J Periodontol* 1996; **67:**794–802.
28. Christgau M, Palitzsch KD, Schmalz G, Kreiner U, Frenzel S. Healing response to non-surgical periodontal therapy in patients with diabetes mellitus: clinical, microbiological, and immunologic results. *J Clin Periodontol* 1998;**25:**112–124.
29. Grossi SG. Treatment of periodontal disease and control of diabetes: an assessment of the evidence and need for future research. *Ann Periodontol* 2001;**6:**138–145.
30. Johnson JH. Effects of local irritation and dextran and sulfhate administration on the periodontium of the rat. *J Periodont Res* 1975;**10:**332–345.
31. Holzhausen M, Rossa C Jr, Marcantonio E Jr, Nassar PO, Spolidorio DMP, Spolidorio LC. Effect of selective cyclooxygenase-2 inhibition on the development of ligature induced periodontitis in rats. *J Periodontol* 2002;**73:**1030–1036.
32. Grossi SG, Zambon JJ, Ho AW *et al*. Assessment for risk for periodontal disease. I. Risk indicators for attachment loss. *J Periodontol* 1994;**65:**260–267.
33. Bartolucci EG, Parkes RB. Accelerated periodontal breakdown in uncontrolled diabetes. Pathogenesis and treatment. *Oral Surg Oral Med Oral Pathol* 1981; **52:**387–390.
34. Manouchehr-Pour M, Spagnuolo PJ, Rodman HM, Bissada NF. Comparison of neutrophil chemotactic response in diabetic patients with mild and severe periodontal disease. *J Periodontol* 1991; **52:**410–414.
35. McMullen JA, Van Dyke TE, Horoszewics HU, Genco RJ. Neutrophil chemotaxis in individuals with advanced periodontal disease and a genetic predisposition to diabetes mellitus. *J Periodontol* 1981; **52:**167–173.
36. Schmidt AM, Weidman E, Lalla E *et al*. Advanced glycation endproducts induce oxidant stress in the gingiva: a potential mechanism underlying accelerated periodontal disease associated with diabetes. *J Periodont Res* 1996;**31:**508–515.
37. Brownlee M. Glycation products and the pathogenesis of diabetic complications. *Diabetes Care* 1992;**15:**1835–1843.
38. Wautier JL, Zoukourian C, Chappey O *et al*. Receptor-mediated endothelial cell dysfunction in diabetic vasculopathy: soluble receptor for advanced glycation endproducts blocks hyperpermeability. *J Clin Invest* 1996;**97:**238–243.
39. Schmidt AM, Hori O, Chen J *et al*. Advanced glycation endproducts interacting with their endothelial receptor induce expression of vascular cell adhesion molecule-1 (VCAM-1): a potential mechanism for the accelerated vasculopathy of diabetes. *J Clin Invest* 1995;**96:**1395–1403.
40. Schmidt AM, Yan SD, Brett J, Mora R, Stern D. Regulation of mononuclear phagocyte migration by cell surface binding proteins for advanced glycosylation endproducts. *J Clin Invest* 1993;**92:**2155–2168.
41. Schmidt AM, Hasu M, Popov D *et al*. The receptor for advanced glycation endproducts (AGEs) has a central role in vessel wall interactions and gene activation in response to AGEs in the intravascular space. *Proc Natl Acad Sci USA* 1994;**91:**8807–8811.
42. Miyata T, Hori O. Zhang *et al*. The receptor for advanced endproducts (RAGE) mediates the interaction of AGE-β_2-microglobulin with human mononuclear phagocytes via an oxidant-sensitive pathway: implications for the pathogenesis of dialysis-related amyloidosis. *J Clin Invest* 1996;**98:**1088–1094.
43. Owen WF Jr, Hou FF, Stuart RO *et al*. Beta 2-microglobulin modified with advanced glycation end products modulates collagen synthesis by human fibroblasts. *Kidney Int* 1998;**53:**1365–1373.
44. Shyng YC, Devlin H, Sloan P. The effect of streptozotocin-induced experimental diabetes mellitus on calvarial defect healing and bone turnover in the rat. *Int J Oral Maxillofac Surg* 2001;**30:**70–74.
45. Sayinalp S, Gedik O, Koray Z. Increasing serum osteocalcin after glycemic control in diabetic men. *Calcif Tissue Int* 1995; **57:**422–425.
46. Shires R, Teitelbaum SL, Bergfeld MA, Fallon MD, Slatopolsky E, Avioli LV. The effect of streptozotocin-induced chronic diabetes mellitus on bone and mineral homeostasis in the rat. *J Lab Clin Med* 1981;**97:**231–240.
47. Verhaeghe J, van Herck E, Visser WJ *et al*. Bone and mineral metabolism in BB rats with long-term diabetes. Decreased bone turnover and osteoporosis. *Diabetes* 1990;**39:**477–482.
48. Pepato MT, Magnani MR, Kettelhut IC, Brunetti IL. Effect of oral vanadyl sulfate treatment on serum enzymes and lipids of streptozotocin-diabetic young rats. *Mol Cell Biochem* 1999;**198:**157–161.
49. Pepato MT, Keller EH, Baviera AM, Kettelhut IC, Vendramini RC, Brunetti IL. Anti-diabetic activity of Bauhinia forficata decoction in streptozotocin-diabetic rats. *J Ethnopharmacol* 2002;**81:**191–197.
50. Doxey DL, Cutler CW, Iacopino AM. Diabetes prevents periodontitis-induced increases in gingival platelet derived growth factor-B and interleukin-1 beta in a rat model. *J Periodontol* 1998;**69:**113–119.
51. Lalla E, Lamster IB, Feit M *et al*. Blockade of RAGE suppresses periodontitis-associated bone loss in diabetic mice. *J Clin Invest* 2000;**105:**1117–1124.
52. Mishima N, Sahara N, Shirakawa M, Ozawa M. Effect of streptozotocin-induced diabetes mellitus on alveolar bone deposition in the rat. *Arch Oral Biol* 2002;**47:**843–849.
53. Finestone AJ, Boorujy SR. Diabetes mellitus and periodontal disease. *Diabetes* 1967;**16:**336–340.
54. Glavind L, Lund B, Loe H. The relationship between periodontal state and diabetes duration, insulin dosage and retinal changes. *J Periodontol* 1968;**39:**341–347.
55. Hugoson A, Thorstensson H, Falk H, Kuylenstierna J. Periodontal conditions in insulin-dependent diabetics. *J Clin Periodontol* 1989;**16:**215–223.
56. Grossi SG, Genco RJ. Periodontal disease and diabetes mellitus: a two-way relationship. *Ann Periodontol* 1998;**3:**51–61.