Impact of diabetes mellitus and metabolic control on bone healing around osseointegrated implants: removal torque and histomorphometric analysis in rats

Key words: bone density, dental implant, diabetes mellitus, histology

Abstract

Objectives: To evaluate bone healing around dental implants with established osseointegration in experimental diabetes mellitus (DM) and insulin therapy by histomorphometric and removal torque analysis in a rat model.

Materials and methods: A total of 80 male Wistar rats received a titanium implant in the tibiae proximal metaphysis. After a healing period of 60 days, the rats were divided into four groups of 20 animals each: a 2-month control group, sacrificed at time (group A), a diabetic group (group D), an insulin group (group I), and a 4-month control group (group C), subdivided half for removal torque and half for histomorphometric analysis. In the D and I groups the DM was induced by a single injection of 40 mg/kg body weight streptozotocin (STZ). Two days after DM induction, group I received subcutaneous doses of insulin twice a day, during 2 months. Groups C and D received only saline. Two months after induction of DM, the animals of groups D, C and I were sacrificed. The plasmatic levels of glucose (GPL) were monitored throughout the experiment. Evaluation of the percentages of bone-to-implant contact and bone area within the limits of the implant threads was done by histomorphometric and mechanical torque analysis. Data were analyzed by ANOVA at significant level of 5%.

Results: The GPL were within normal range for groups A, C and I and higher for group D. The means and standard deviations (SD) for histomorphometric bone area showed significant difference between group D (69.34 ± 5.00%) and groups C (78.20 ± 4.88%) and I (79.63 ± 4.97%). Related to bone-to-implant contact there were no significant difference between the groups D (60.81 ± 6.83%), C (63.37 ± 5.88%) and I (66.97 ± 4.13%). The means and SD for removal torque showed that group D (12.91 ± 2.51 Ncm) was statistically lower than group I (17.10 ± 3.06 Ncm) and C (16.95 ± 5.39 Ncm).

Conclusions: Diabetes mellitus impaired the bone healing around dental implants with established osseointegration because the results presented a lower percentage of bone area in group D in relation to groups C and I resulting in a lowest torque values for implant removal. Moreover, insulin therapy prevents the occurrence of bone abnormalities found in diabetic animals and osseointegration was not compromised.

The success of the dental implants is related to the osseointegration that is defined as a direct bone-to-implant contact without interposition of any other tissue [Brañemark et al. 1969]. To achieve and maintain osseointegration, the health, oral hygiene of the patient and non administration of certain medications are the key aspects to be evaluated before the implant planning [Adell et al. 1990, Sakakura et al. 2003, 2006, 2007]. One chronic metabolic disorder that impairs the osseointegration is diabetes mellitus (DM) that affects bone healing. DM is characterized by hyperglycemia due to insufficient insulin action (type 2) or impaired insulin secretion (type 1) according to The Expert Committee on the Diagnosis & Classification of Diabetes Mellitus, 1997. The majority of experimental studies show that effect of type 1 DM on the implant osseointegration...
has been associated with impaired osseous wound and bone healing, decreased bone density and increased fracture risk and increased susceptibility to periodontal disease [Taylor et al. 1998; Lalla et al. 2000].

The biological effect of diabetes on osseointegration has been evaluated by clinical and experimental studies [Holzhausen et al. 2004; de Morais et al. 2009; Fiorellini et al. 1999, Kotsovilis et al. 2006]. The new bone formation around the dental implant was more immature and slower in diabetic rats [Retzepi & Donos 2010]. Moreover, there was a decrease related to bone-to-implant contact. On the other hand, two studies found similarity between controls and diabetic rats as showed in a review [Retzepi & Donos 2010]. Furthermore, insulin therapy is an option for diabetes. Regarding the maintenance of osseointegration in diabetic animals, one study showed that insulin-treated rats have more bone-to-implant contact compared with uncontrolled group over a 4-month period but this study does not include control group with no disease [Kwon et al. 2005].

Previous studies concerning bone metabolism and metabolic control in experimental diabetes have shown that insulin therapy is able to keep diabetic bone metabolism similar to healthy controls [de Morais et al. 2009, Shyng et al. 2001; Follik et al. 2004]. Moreover, insulin therapy has improved the implant osseointegration in experimental diabetic as demonstrated in a review [Retzepi & Donos 2010]. In a recent study, de Morais et al. [2009] evaluated the effect of DM and insulin therapy on bone density using digital subtraction radiography and showed that DM impaired bone density around osseointegrated implants and insulin therapy can maintain bone density similar to the control animals.

Besides qualitative histological analysis and histomorphometric parameters used to evaluate the osseointegration, the influence of DM on the biomechanical properties of dental implants need to be considered. Margonar et al. [2003] evaluated the influence of diabetes and insulin therapy using the removal torque of implants in rabbits. Insulin therapy did not change the torque values in diabetic animals, but the DM had a negative influence on mechanical retention of implants and bone-to-implant contact when compared to control group. We were able to find only one study that was related to the above, that deals with experimental diabetes and metabolic control using insulin therapy and removal torque to evaluate implants osseointegration. This is an important issue considering the increasing number of diabetic patients that are under insulin therapy that need dental rehabilitation such as dental implant.

The aim of this study was to evaluate the impact of DM and metabolic control on bone healing around dental implants with established osseointegration by removal torque and histomorphometric analysis in rats.

**Materials and methods**

**Sample**

A total of 80 male Wistar rats, aged 120 days, weighing approximately 280 g each, were maintained in the animal facilities of the Araraquara Dental School, with controlled temperature, humidity, and light exposure. Throughout the experimental period rats were housed in plastic cages, fed by a standard laboratory diet and given water ad libitum. The study protocol was conducted according to the recommendations of the Brazilian College of Animal Experimentation (COBEA) and the protocol was approved by the local Institutional Experimentation Committee for Animal Care and Use [protocol 27/2003].

**Experimental protocol**

A total of 80 implants were placed in 80 tibiae of adult Wistar rats after a 2-week acclimatization period. After a bone-healing period of 60 days, which was necessary to obtain the osseointegration of the implants, the animals were randomly divided into four groups of 20 animals each and subdivided into 10 rats for removal torque analysis and 10 rats for histomorphometric analysis. A 2-month control group was sacrificed at time (group A) 2 months of implant healing, to obtain a standard of implant osseointegration. The remaining 60 rats continued the experiment for another 2 months and were randomly allocated to three groups: Diabetes mellitus was induced using 40 mg/kg Streptozotocin in a diabetic group (group D) and an insulinc group (group I). During 2 months, group I received subcutaneous doses of a total 8.5 IU insulin twice a day. The 4-month control group (group C) and group D received only saline solution (NaCl 0.9%). After 2 months, the animals of groups D, C and I were sacrificed [Fig. 1].

**Implant surgery**

The surgical implant placement was performed as previously described [de Morais et al. 2009]. Initially, general anesthesia was done by intramuscular injections of a combination of ketamine hydrochloride [Agener Uniao Ltda, Sao Paulo, SP, Brazil] at a concentration of 0.08 ml/100 g and xylazine hydrochloride [Rompum, Bayer SA, Sao Paulo, SP, Brazil] at 0.04 ml/100 g of body weight. Next, the rats were submitted to a trichotomy in the inner leg and after disinfection with iodine solution, a 2 cm incision was performed on the internal side of the right hind leg, just below the knee, and the tibial metaphysis was exposed by blunt dissection. Then, the osteotomy was performed using a progressive sequence of drills under profuse saline irrigation. One machined-surface implants, of 4.0 mm length and 2.2 mm diameter (AS Technology, Sao Jose dos Campos, SP, Brazil) were placed in the right leg of each animal, until the screw thread had been completely introduced into the cortical bone. The soft tissues were replaced and sutured in separate layers [Vicryl Ethicon 5.0, Johnson Prod., Sao Jose dos Campos, Brasil]. In the immediate postoperative period, a single intramuscular injection of antibiotics, 0.1 ml/kg body weight of a mixture of penicillin and streptomycin [Pentabiotics®, Wyeth-Whitell Ltda, Sao Paulo, Brasil] was administered.

**Induction of diabetes mellitus**

The induction of diabetes mellitus was done as previously described [de Morais et al. 2009]. Briefly, the diabetic group (group D) and insulin group (group I) fasted for 16 h and diabetes was induced by a single injection of 40 mg/kg body weight streptozotocin (STZ) (Sigma, St. Louis, MO, USA) dissolved in citrate buffer [0.01 M; pH 4.5] into the penile vein. One day after diabetes induction, blood samples were obtained from the animals tip tail. 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 by the glucose-oxidase method using a Hitachi U 1100 spectrophotometer, [Hitachi Ltd., Tokyo, Japan]. Plasma glucose levels greater than 300 mg/dl were considered diabetic [Mitruk & Rawlsley 1981]. Five blood samples were taken during the experiment (exam 1 – before implant placement; exam 2 – prior to induction of diabetes, 60 days after implant surgery; exam 3 – 1 day after induction; exam 4 – during the treat-
ment period, 90 days after implant surgery, exam 5 – at the time of sacrifice, 120 days after implant surgery) to control the glucose plasma level (GPL).

Insulin therapy
One day after diabetes confirmation, the animals from group 1 received subcutaneous doses of NPH (Neutral Protamine Hagedorn) insulin (Biohulin NU-100, 100 U/ml) diluted in 0.9% NaCl (5.5 IU at 6 p.m. and 3.5 IU at 6 a.m.) as previously described (de Morais et al. 2009). Groups C and D received only saline by the same route.

Body weight
The body weight was obtained three times in all animals at the following periods: before implant surgery, 60 days after implant surgery, and at the sacrifice, using a digital analytical scale (AS) as previously described (de Morais et al. 2009).

Histomorphometric procedures
After the animals were sacrificed by deepening anesthesia, the machined-surface implants with surrounding tissue in each tibia of 10 rats from each group were removed and fixed in 4% neutral formalin for 48 h. Nondecalcified sections were prepared by a technique previously described by Dohnath & Breuner (1982). The specimens were dehydrated using increasing concentrations of ethanol, and were then embedded in glycolmethacrylate-based resin (Technovit 7200 VLC, Heraeus Kulzer, Wehrheim, Germany). The blocks were cut into 100-μm-thick sections by aiming the center of the implant diameter along its long axis using a cutting-grinding unit (EXAKT Apparatebau GmbH & Co., Nordersted, Germany). A single section was obtained from each implant. The sections were reduced to a final thickness of 30 μm by grinding and polishing using an EXAKT microgrinding unit. The sections were stained in 1% toluidine blue (Sigma) and the area between the threads occupied by bone tissue obtained from the square pixels and pixels, respectively, were converted to percentage values. To evaluate the bone near to the implant, two histomorphometric variables were analyzed as stated below.

1) The linear extension of the implant featuring bone tissue in contact with the tita-
nium of the implant, between the initial three threads of the implant in the mesial and distal region
2) Area between the threads, occupied by bone tissue, according to the methodology used by Sennerby et al. (1992), among the initial three threads of the implant in the mesial and distal region

Analysis of removal torque of implants
Analysis of the removal torque was made after the animals' sacrifices. The tibia on the remaining 40 rats was dissected to expose the implant and the cover screws and attach to a torque meter (Tohnichi, Model 15-BTG-N, Tokyo, Japan) with a scale range of 3–24 Ncm and divisions of 0.05 Ncm was used for the test. A wrench was adapted to the implant head to apply a counterclockwise movement to remove the implant, and the maximal torque value nec-
essary for manual removal of each implant was measured in Newton centimeters (Ncm).

Data analysis
The variation in weight between the animals was compared three times by a one-way anal-

Table 1. Mean and standard deviation of weight (g) for the groups A: (2 months control group); C: (4 months control group); D: (diabetic) and I: (insulin) during the three experimental times: implant placement (initial), 2 and 4 months

<table>
<thead>
<tr>
<th>Groups</th>
<th>Weight (g)</th>
<th>2 months</th>
<th>4 months</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group A</td>
<td>312.00 ± 43.73**</td>
<td>417.86 ± 22.67**</td>
<td></td>
</tr>
<tr>
<td>Group C</td>
<td>320.28 ± 34.64***</td>
<td>414.07 ± 26.81***</td>
<td>517.64 ± 36.84**</td>
</tr>
<tr>
<td>Group D</td>
<td>302.78 ± 22.80***</td>
<td>411.78 ± 22.01***</td>
<td>299.78 ± 55.80***</td>
</tr>
<tr>
<td>Group I</td>
<td>335.71 ± 22.10**</td>
<td>426.14 ± 14.69**</td>
<td>528.71 ± 23.60***</td>
</tr>
</tbody>
</table>

*Significant difference between group D and both groups C and I at 4 months (P < 0.05).
**Significant intra-group differences between time periods (P < 0.05).

Results
Clinical assessments
After 2 months of the implant surgery, the implants of all groups showed adequate healing, with no implant loss and infection during this period. However, after a period of 2 months after the induction of diabetes mellitus, the animals of the group D had a significantly lower body weight in relation to groups C and I, which showed a significant gain in weight over the time (Table 1).

Glucose plasma levels
With 40 mg/kg body weight STZ, all animals in D and I groups developed experimental DM and maintained a high glucose level above 350 mg/dL, minimum value to be considered diabetic. No statistically significant differences at a significant level of 5% in GPLs were found among groups in the initial exams (exams 1 and 2). In relation to group I, there was a significant decrease in GPLs after insulin administration (exams 4 and 5). The group D showed elevated blood glucose levels (hyperglycemia) statistically significant during the experimental period of the DM induction in relation to groups C and I (Table 2).

Histomorphometric analysis
Linear extension of bone-implant contact
The histomorphometric results related to linear bone-implant contact showed that time had a positive effect on bone-implant contact, determined by the significant superiority of the group C toward group A. In group D, the bone-implant contact was higher than that in Group A but with no statistically significant difference. In addition to these results, it was...
observed that in the group I the contact between bone and implant remained similar to the group C and statistically superior to the group A. Observing the period of 4 months, the percentage of bone-implant contact showed no statistical differences between groups, however, the group D showed the lowest value, probably indicating a slower bone turnover in relation to groups I and C (Table 3).

**Bone area**

The average percentage of bone area between the initial three mesial and distal threads of the implant in group D was significantly lower in relation to the other groups C and I considering the period of 4 months. When we analyzed the influence of time it was possible to observe a small increase in bone area in groups C, and I related to group A, without significant differences. However, in the group D, this was not observed, there was a small reduction in bone area in relation to group A (Table 3).

**Histological description**

In this study, implants can be considered osseointegrated because in all groups was observed direct contact, partial or total, between bone and implant. In this experimental model, the rat tibia has two cortical bones, upper and lower, and an interposed bone marrow. The cortical bone was visible above the bone remodeling near the implant region in all groups. Group A had lower bone fill between the threads and less contact between bone and implant brought by granulation tissue (Fig. 2a, b). On the other hand, in the period of 4 months, the presence of a greater bone fill between the threads of the implant and increased contact between the bone and the implant was evident, especially for groups C (Fig. 3a, b) and I (Fig. 4a, b). The bone fill in group D showed differences in comparison to other groups. There was increased bone fill between the threads of the implant in group D than in group A, however, the bone in group D was less compact compared to groups C and I, i.e., there were spaces with connective tissue brought in with bone tissue (Fig. 5a, b).

In the medullary portion, especially in 4 months groups, was observed a bone formation between the implants threads. This result suggests that there was a remodeling intramedullary and a osteoconductive stimulus of the implant oxide layer and also by the displacement of the bone fragments during the implant installation. In the inferior cortical bone, there was a large bone-implant contact, as well as the presence of a mineralized and compact bone in all groups [A, C, D and I].

**Removal torque implant**

The removal torque of the implant was affected by the DM. The results showed that the removal torque in group D was significantly lower than group I and C, but without

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**Table 2. Mean and standard deviation of glucose plasma levels (mg/dl) for all groups during the experiment (before implant surgery – exam 1; before induction of diabetes – exam 2; after induction – exam 3; 30 days after diabetes induction – exam 4; and at the time of sacrifice – exam 5)**

<table>
<thead>
<tr>
<th>Exam</th>
<th>Groups</th>
<th>Exam 1</th>
<th>Exam 2</th>
<th>Exam 3</th>
<th>Exam 4</th>
<th>Exam 5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group A</td>
<td>133.63 ± 11.55</td>
<td>108.72 ± 15.33</td>
<td>123.54 ± 18.95</td>
<td>93.50 ± 16.86</td>
<td>127.35 ± 14.39</td>
<td></td>
</tr>
<tr>
<td>Group C</td>
<td>136.92 ± 16.75</td>
<td>110.85 ± 10.42</td>
<td>106.86 ± 13.15</td>
<td>422.33 ± 45.14</td>
<td>494.66 ± 58.77</td>
<td></td>
</tr>
<tr>
<td>Group D</td>
<td>127.22 ± 17.24</td>
<td>107.11 ± 27.22</td>
<td>510.00 ± 34.00</td>
<td>64.00 ± 6.13</td>
<td>75.11 ± 11.75</td>
<td></td>
</tr>
</tbody>
</table>

*Significant difference among groups at the same experimental period. (P < 0.05)
†Significant intra-group differences, when compared with exam 1. (P < 0.05)
‡Significant intra-group differences, when compared with exam 2. (P < 0.05)
§Significant intra-group differences, when compared with exam 3. (P < 0.05)

**Table 3. Mean and standard deviation of linear bone-to-implant contact and bone area between the group A (2-month control group, sacrificed at time) and groups D (diabetic) and C (4-month control group), sacrificed at 4 months period**

<table>
<thead>
<tr>
<th>Groups</th>
<th>Bone-implant Contact (%)</th>
<th>Bone Area (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group A</td>
<td>53.93 ± 9.31</td>
<td>73.65 ± 4.31</td>
</tr>
<tr>
<td>Group C</td>
<td>63.37 ± 5.88</td>
<td>78.20 ± 4.88</td>
</tr>
<tr>
<td>Group D</td>
<td>60.81 ± 6.83</td>
<td>69.34 ± 5.00</td>
</tr>
<tr>
<td>Group I</td>
<td>66.97 ± 4.13</td>
<td>79.63 ± 4.97</td>
</tr>
</tbody>
</table>

*Significant difference related to group A (P = 0.014985)
†Significant difference related to group D (P = 0.0005518)
percentage of bone filling the implant threads and the bone-to-implant contact. 10× magnification. [b] Enlarged image of the cortical bone in group D showing bone remodeling. You can see the dividing line between the preexisting bone and the newly formed bone. 20× magnification.

Fig. 4. Histological sections of bone in the cervical threads of the implant in group I. [a] Note the bone filling on the threads and the bone-to-implant contact. 10× magnification. [b] The complete bone filling of the implant thread containing compact and mineralized bone in group I. 20× magnification.

Table 4. Median, mean, standard deviation, and range of removal torque of the implants among the groups

<table>
<thead>
<tr>
<th>Groups</th>
<th>Median</th>
<th>Mean/Standard deviation</th>
<th>Torque Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group A</td>
<td>13.5</td>
<td>13.13 ± 2.65</td>
<td>9.0–17.5</td>
</tr>
<tr>
<td>Group C</td>
<td>16.0</td>
<td>16.95 ± 5.39</td>
<td>10.5–29.5</td>
</tr>
<tr>
<td>Group D</td>
<td>13.0</td>
<td>12.91 ± 2.51†</td>
<td>7.0–15.5</td>
</tr>
<tr>
<td>Group I</td>
<td>17.5</td>
<td>17.10 ± 3.06*†</td>
<td>12.0–21.0</td>
</tr>
</tbody>
</table>

†Significant difference related to group D (P < 0.05)
‡Significant difference related to group A (P < 0.05)
*Significant difference related to group C (P < 0.05)

and significantly less contact between the bone and the implant. On the other hand, insulin therapy was able to keep similar results to a control group [C]. This deleterious effect observed in diabetic animals may have occurred because the DM affects bone repair [Devlin et al. 1996] promoting reduction on the osseous matrix formation [Spanheimer et al. 1988], modifying protein synthesis [Grandini et al. 1990], increasing the time required for mineralization of the osteoid matrix [Follak et al. 2004]; reducing the osseous turnover [Shyng et al. 2001], reducing the expression of transcription factors that regulate the osteoblastic differentiation [Lu et al. 2003], reducing the number of osteoblasts and osteoclasts producing metabolic changes [Dixit & Ekstrom 1987], and resulting in reduction on collagen waste [Santana et al. 2003] and osteocalcin [Dixit & Ekstrom 1987]. These factors may explain the bone commitment around implants in diabetic animals [Fiorellini et al. 1999], as observed in this study. However, our findings show less intensity of the observed parameters, probably because the bone was evaluated after healing, over a period free from influences of DM and insulin therapy, introduced after 60 days of osseointegration, unlike most studies that assessed the influence of disease and treatment at the beginning of osseointegration.

In concordance with another study (Kwon et al. 2005), significant differences between bone marrow-to-implant contacts were observed starting at 2 months in both groups: diabetics and insulin. On the other hand, the analysis were made in different areas of bone and it is known that DM exerts greater influence on bone marrow. Also, in relation to the percentage of bone-to-implant contact in diabetic animals, DM does not prevent the bone formation and mineralization. These findings could be explained because in diabetic rats the new bone formation continue, but to a lesser extent, whereas osteosclerosis and mineralization occurs more slowly and proportional to the amount of bone formation existent [Goodman & Hori 1984].

In relation to bone area, the literature shows controversial results. Bone formation was impaired in diabetic animals when compared to healthy ones [Takeshita et al. 1997]. Nevins et al. (1998) have shown similar results for bone area and bone formation that were higher at 14 days and lower at 28 days when compared to healthy animals, however, in another study the diabetic had a higher bone formation [McCracken et al. 2006]. Our study showed a negative effect of high level rate of blood glucose on osseointegration that is in agreement with other studies [Nevins et al. 1998; Giglio et al. 2000; McCracken et al. 2000; Siqueira et al. 2003].

The descriptive histological results of this study are in agreement with other studies who suggest that DM may be associated with a reduced, immature, and less organized bone formation around implants as an incomplete and delayed response in bone repair of the implant in diabetic rats compared to non-diabetic rats [Nevins et al. 1998; McCracken et al. 2000, 2006; Ottoni & Chopard 2004; Kotsovilis et al. 2006]. Therefore, the histomorphometric results of this study showed that after 2 months of bone repair after induction of DM, there was a tendency to decrease bone density around the dental implants in the presence of DM.

The insulin administration could restore blood glucose levels, correct some of the bone changes caused by diabetes mellitus and maintain similar bone density [Devlin et al. 1996; Shyng et al. 2001; Follak et al. 2004]. Our study is in agreement with these findings because the administration of insulin restored the normal glucose levels in all diabetic animals (Fiorellini et al. 1999), as observed in this study. However, our findings show less intensity of the observed parameters, probably because the bone was evaluated after healing, over a period free from influences of DM and insulin therapy, introduced after 60 days of osseointegration, unlike most studies that assessed the influence of disease and treatment at the beginning of osseointegration.

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analysis performed in this experiment and the results of group I were similar to those of group C.

The success of insulin therapy in our study can be explained: by standardizing dose of insulin, confirmed in the pilot study, the animal spent most of their time under normal glucose levels. However, others studies did not show the strict control of GPLs, perhaps by the dose of insulin or amount of administration (Goodman & Hori 1984; Margonar et al. 2003). Moreover, the insulin therapy was started 2 days after induction of DM and probably did not cause irreversible damage to these animals. A long-term DM can cause systemic reversible or irreversible damage to the body and a delayed insulin treatment can partially rectify the damage, as shown by Hoskins & Scott [1984] that did not have full recovery of renal damage with insulin.

Some authors noted that bone response in the insulin group is higher than in controls, although not statistically significant, and suggested that the type of drug such as STZ could influence the bone formation around implants (McCacken et al. 2006). This fact was observed in the work of Siqueira et al. [2003]. These authors used alloxan to induce DM in rats and found a lower bone formation around the implants justified by the use of the drug. On the other hand, in our study the animals were induced with STZ and showed similar results between insulin and control group. This condition is probably linked to the initial period of insulin treatment and maintenance of glucose control, avoiding irreversible damage to the body by DM than the type of drug used for induction.

Bone density and bone-to-implant contact in group I were similar to group C. Also, our results are in agreement to the study of Siqueira et al. [2003] that found similar results regarding bone formation around the implants in animals treated with insulin compared to healthy ones. However, Fiorelli et al. [1999] showed recovery in bone density, but bone-to-implant contact remained lower compared to the healthy animal.

The results of implant removal torque in this study showed that DM impaired the retention of the implants, because group D had the lowest removal torque value in the period of 4 months. There was statistically significant difference between the groups D related to group I and C, and this finding is in agreement with other study [Lekholm 1993] that showed in diabetic animals a smaller force to remove the implants.

Unlike the findings of Margonar et al. [2003], insulin treatment was able to correct the effects of DM related to mechanical retention. The resistance of the implants on removal torque can be correlated to the contact extension of bone tissue to the implant and the degree of bone mineralization. Studies have shown that the reverse torque values increase in the major periods and the more bone-to-implant contact greater is the resistance to reverse torque [Carlsson et al. 1989; Cordioli et al. 2000; Schou et al. 2003]. Furthermore, bone strength in areas of repair is committed in diabetic animals, as reported in the study of Donath & Breuner [1982] that showed recovery in bone mineral density, but bone-to-implant contact and bone area, less bone-to-implant contact had a positive relationship to the torque, i.e., group D had lower bone area, less bone-to-implant contact and lower retention of the implant in the bone. Related to group I all these parameters were higher than in group D and very similar to those in group C.

In conclusion, experimental induction of DM seems to affect the bone tissue around osseointegrated implants, because there was a tendency for negative results, in the bone-to-implant contact and bone area, consequently the lowest torque values for implant removal. Insulin therapy prevented the occurrence of bone abnormalities found in diabetic animals and osseointegration was not compromised in this experimental group which was similar to group C.

Acknowledgements

This study was supported by the Research Foundation of Sao Paulo State (FAPESP), Grant # 04/07931-0 and Brazilian Federal Agency for Evaluation of Graduate Education (CAPES) for the Scholarship. We thank Engineer Sidival Dias from AS Technology, Sao Jose dos Campos, Brazil, for supplying the titanium micro-implant, Ivy Kiemle Trindade Suedam, DDS, PhD, for helping in this research.


