

# The Effects of Hyperlipidemia on Implant Osseointegration in the Mouse Femur

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A high-fat (HF) diet inducing hyperlipidemia has been associated with the pathophysiology of major diseases, such as atherosclerosis and osteoporosis. A HF diet has significant adverse effects on bone, including lower bone density, volume, and strength. Statins, drugs that lower serum cholesterol levels have beneficial effects on bone metabolism. Since the host's bone quantity, quality, and healing potential play a crucial role in osseointegration of dental implants, we hypothesized that hyperlipidemia may negatively affect implant osseointegration. In the present study, we evaluated the effects of hyperlipidemia on implant osseointegration in mice. Atherosclerosis susceptible C57BL/6J male mice were randomly placed on a control chow or a HF diet. After 12 weeks on the diet, each mouse received a titanium implant in the proximal metaphysis of the femur. The animals were humanely killed at 4 or 8 weeks after the implant surgery. Results showed that the mice fed a HF diet had significantly increased implant loss as well as decreased formation and strength of bone-to-implant interface. These results support the hypothesis that a HF diet can significantly compromise osseointegration, causing poor outcome in dental implant therapy.

**Key Words:** *high-fat diet, hyperlipidemia, dental implant, osseointegration*

## INTRODUCTION

The atherogenic or high-fat (HF) diet induces hyperlipidemia, characterized by an elevation of lipids in the bloodstream. Hyperlipidemia is widespread in our society, with total cholesterol levels above 200 mg/mL for over 45.0% of people 20 years of age or older.<sup>1</sup> HF diet is associated with the pathophysiology of major diseases, including atherosclerosis and osteoporosis.<sup>2-4</sup> Interestingly, both hyperlipidemia and atherosclerosis have been linked to periodontal disease.<sup>5-7</sup>

A HF diet has significant adverse effects on bone health, leading to lower bone mineral density and to higher risk of osteoporosis and bone fracture.<sup>8,9</sup> Statins, HMG-CoA reductase inhibitors that lower cholesterol levels, have beneficial effects on bone metabolism by inducing bone formation and mineral density as well as decreasing the risk of hip fractures.<sup>10-13</sup> At the cellular level, osteoblasts are capable of oxidizing low-

density lipoproteins, possibly increasing the local concentration of oxidized reactive products in bone milieu.<sup>14</sup> Oxidative stress generated in hyperlipidemic conditions inhibits the differentiation of bone cells.<sup>15,16</sup> Statins enhance osteoblastic differentiation and mineralization<sup>17</sup> and suppress osteoclastogenesis.<sup>18</sup> Furthermore, around implants, statins increase osteogenesis, suppress osteoclast formation, and increase bone volume.<sup>19</sup>

The effects of hyperlipidemia on bone health may also interfere with dental implant therapy since the host's bone quantity, quality, and healing potential play an important role in osseointegration.<sup>20-22</sup> Currently, the role of hyperlipidemia in implant osseointegration is unknown. Because of the deleterious effects of hyperlipidemia in bone, we hypothesized that hyperlipidemia negatively affects implant osseointegration. The present study evaluates implant osseointegration in hyperlipidemic mice at 4 and 8 weeks after implant placement.

## MATERIALS AND METHODS

### Mice and diets

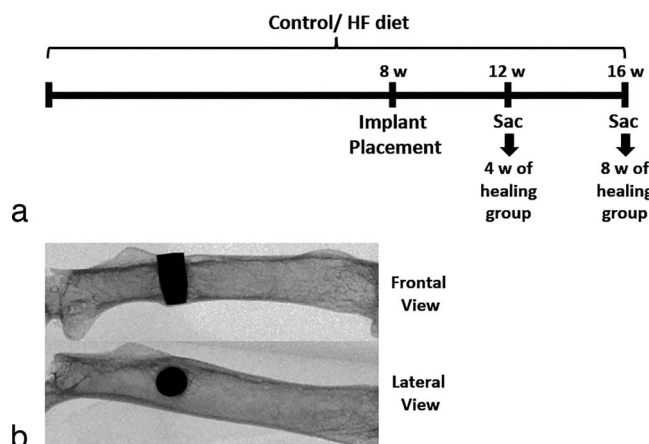
Four-week-old C57BL/6J male mice (atherosclerosis susceptible strain, The Jackson Laboratories, Bar Harbor, Maine) were

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**FIGURE 1.** (a) Schematic diagram of the timeline for the diet and implant placement. HF = high-fat; W = weeks; Sac = humanely killed. (b) X-ray images showing the implant placement location (distal to the third trochanter) on a frontal and lateral view.

randomly divided into 2 groups: control chow diet and an atherogenic HF diet (TD 90221, Harlan Teklad, Madison, Wis; including 1.25% cholesterol, 15.8% fat, and 0.5% cholate).<sup>9,23,24</sup> Animals were started on the diet 12 weeks prior to the implant placement (Figure 1a). The experimental protocols were reviewed and approved by the Chancellor's Animal Research Committee of the University of California, Los Angeles (UCLA).

#### Implant placement

Under general gas anesthesia, an incision in the lateral aspect of the femur was created, and a pilot hole was made using a 1/2 mm round carbide burr with high speed. The sterile custom-made cylindrical smooth-type tapered titanium implants (1 × 2 mm) were placed by push-fit in the diaphysis of the left femur, perpendicular to its long axis (bicortically) (Figure 1b), and the wound was sutured. Animals were given antibiotics (sulfamethoxazole and trimethoprim oral suspension, USP; 850 µg/170 µg per mL) for 7 days. The animals were then humanely killed at 4 or 8 weeks after implant placement (Figure 1a).

#### Serum analysis

Blood was collected by heart puncture, and serum was sent to the UCLA Division of Laboratory Animal Medicine to analyze levels of cholesterol, triglyceride, and glucose.

#### Evaluation of implant osseointegration

At necropsy, femurs were procured and fixed for 48 hours in 10% formalin. The femurs were scanned by micro-computerized tomography (SkyScan 1172, Kontich, Belgium).<sup>25</sup> The entire implant site was selected as the region-of-interest.

All quantitative measurements were performed on the midsection of the implant, and analysis was conducted on its proximal, distal, lateral, and medial sides. The bone-to-implant contact (BIC) was considered the sum of all regions of contact between bone (2 coronal and 2 sagittal) and implant (µm)/ whole implant length from the first to the last BIC (µm) × 100, and measured using the Dolphin 11.0 imaging software (Chatsworth, Calif).

#### Implant push-in test

The implant push-in test was performed as described by Ogawa et al.<sup>26</sup> In brief, femurs were embedded in autopolymerizing resin in a custom-made mold, in which the bottom flat surface was parallel to the top surface of the implant. The Instron machine (Instron 5544 Electro-mechanical Testing System, Instron, Canton, Mass) contained a steel-pushing rod (0.8-mm diameter) that applied a load on the implant at a speed of 1 mm/min, while simultaneously recording values. The push-in value was determined as the breakpoint load, which is the maximum load prior to a drop in the load-displacement curve.<sup>26</sup>

#### Statistical analysis

Two-tailed Student *t* test was performed to compare the effects of the chow diet and the HF diet. Results are presented as the mean ± SEM of measurements, considering statistically significant values of *P* < .05.

### RESULTS

#### High-fat diet

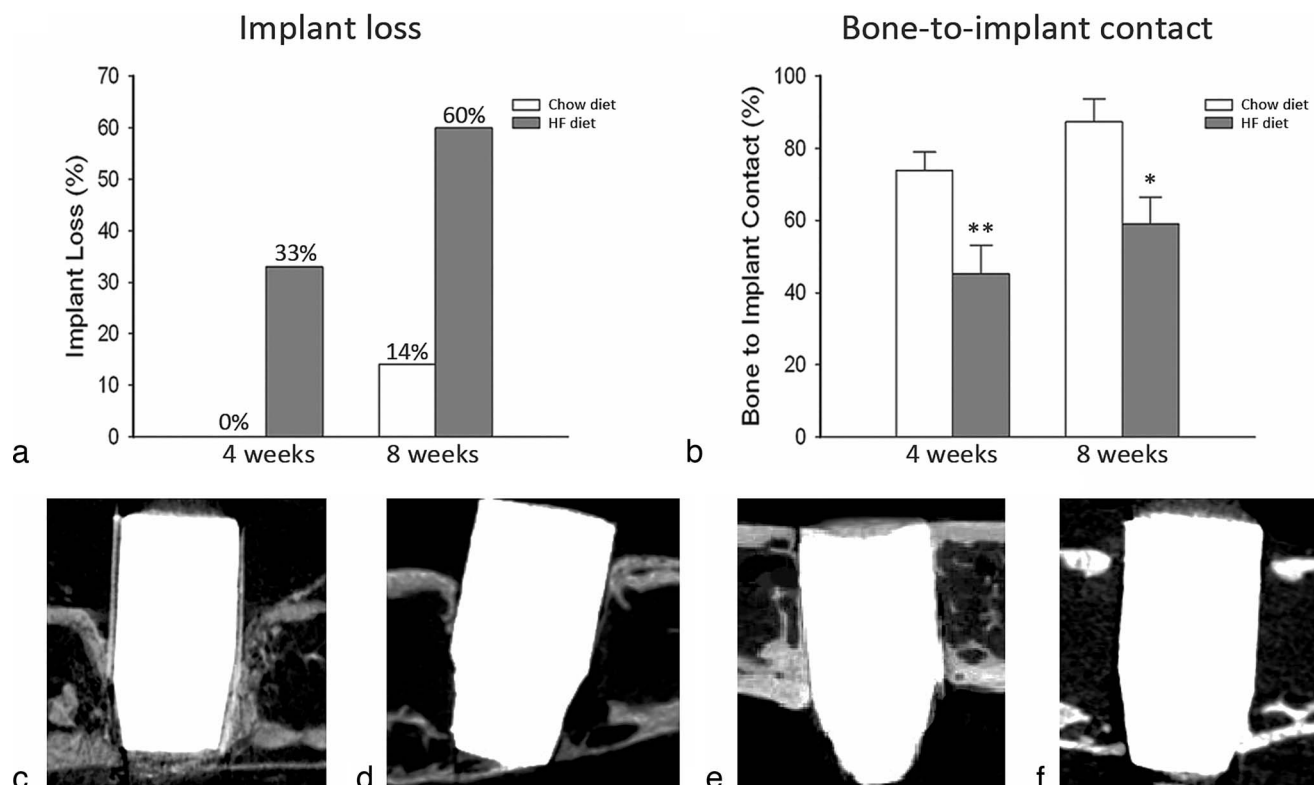
Serum levels of total cholesterol, triglycerides, and glucose were measured. The HF diet significantly increased the total cholesterol levels twofold at both time points (*P* < .0001) (Table 1). The HF diet had an opposite effect on the triglyceride levels, which was significantly higher in the chow diet (*P* < .001) (Table 1). Glucose was also higher in the chow diet, but only in the 4-week group (*P* < .05) (Table 1).

TABLE 1

Effects of the high-fat (HF) diet on serum cholesterol (n ≥ 9/group), triglycerides (n ≥ 8/group), and glucose (n ≥ 8/group) (values are mean ± SEM in mg/dL)

	4 Weeks		8 Weeks	
	Control Diet	HF Diet	Control Diet	HF Diet
Cholesterol	102.67 ± 3.97	206.30 ± 14.00****	113.33 ± 3.27	223.70 ± 10.71****
Triglycerides	150.25 ± 13.15	64.00 ± 6.17****	142.38 ± 15.23	71.40 ± 9.89***
Glucose	299.20 ± 30.79	210.00 ± 14.02*	230.78 ± 8.05	244.20 ± 22.43

Significant difference when compared to respective control: \**P* < .05; \*\**P* < .01; \*\*\**P* < .001; \*\*\*\**P* < .0001.



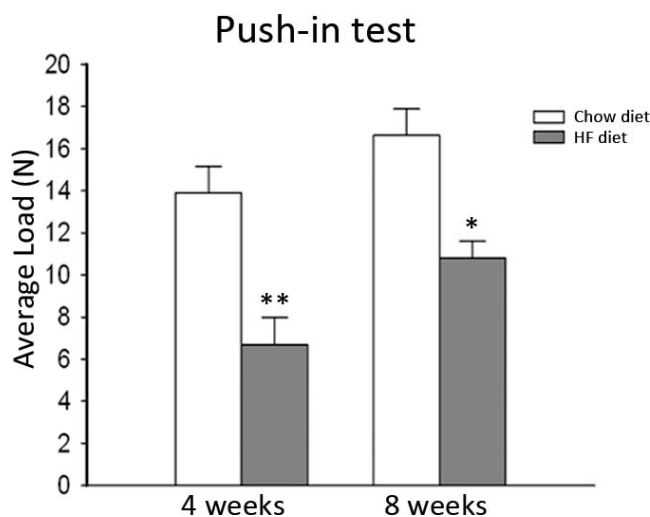
**FIGURE 2.** Effects of the high-fat (HF) diet on implant osseointegration at 4 and 8 weeks after implant placement. (a) Percent of implant loss at 4 weeks and 8 weeks after implant placement ( $n \geq 6$ /group). (b) Percent of bone-to-implant contact throughout in the entire implant length ( $n \geq 6$ /group). Significant difference when compared to respective control: \* $P < .05$ , \*\* $P < .01$ . (c–f) Representative micro-computerized tomography images of the implant in each group: (c) 4-week chow diet, (d) 4-week HF diet, (e) 8-week chow diet, (f) 8-week HF diet. No statistically significant differences were found when comparing chow diet and HF diet groups within the respective time points.

### Hyperlipidemia and osseointegration

The timeline of the diet and implant placement as well as the location of the implant are shown in Figure 1. After 4 or 8 weeks of healing, mice were euthanized and scanned using micro-CT to determine the remaining percentage of osseointegrated implants. The percentage of implants lost was greater in the HF groups compared to their respective control groups (chow diet) at both time points (Figure 2a).

To determine whether there were differences in BIC in the control vs the HF diet groups, micro-CT analysis was performed. BIC was significantly higher in the chow diet mice as compared to the HF diet mice at the respective time point ( $P < .01$  at 4 weeks and  $P < .05$  at 8 weeks) (Figure 2b). However, no statistically significant difference was found when comparing the BIC from the 4 and 8 week time points within the respective diet groups (chow diet at 4 weeks compared to chow diet at 8 weeks and HF diet at 4 weeks compared to HF diet at 8 weeks) (Figure 2c through f).

Biomechanical evaluation of osseointegration, with the push-in test, revealed that the HF diet group required a lower load to break the bone-to-implant interface compared to the chow diet, in both time points ( $P < .01$  at 4 weeks and  $P < .05$  at 8 weeks) (Figure 3). A statistically significant difference was also found when comparing the average load between HF diet mice



**FIGURE 3.** Load (force) necessary to break the bone-to-implant interface ( $n \geq 3$ /group). Significant difference when compared to respective control: \* $P < .05$ . No statistically significant differences were found when comparing chow diet and high-fat diet groups within the respective time points.

at 4 and 8 weeks ( $P < .05$ ) However, no statistical difference was observed between the chow diet mice at 4 and 8 weeks.

### DISCUSSION

Successful osseointegration is a major determinant of implant success. The lack of osseointegration is a major concern for patients, especially when another implant placement cannot be easily performed. The ultimate consequence of the failure to obtain osseointegration is the loss of the implant. It was demonstrated that the degree of bone loss before implant placement is a significant factor that causes implant loss.<sup>27</sup> Osseointegration is also critical since 36% of the patients who had lost a prior implant lost a second one in another occasion.<sup>28</sup>

The results of the present study show that a high-fat diet significantly reduced implant osseointegration and bone volume in the femurs of mice. Implant loss increased, and load necessary to break the bone-to-implant interface decreased in mice on a HF diet, which has been shown to significantly reduce bone density and mineral content, thus altering morphologic and mechanical properties.<sup>23,24,29</sup> More than one mechanism can account for the deleterious effects of a HF diet in the osseointegration shown in the present study. At a cellular level, hyperlipidemic conditions lead to inhibition of osteogenic signaling,<sup>4</sup> decrease of the formation of mature osteoblasts, a higher expression of molecular markers of bone remodeling,<sup>24</sup> enhancement of osteoclast differentiation and activity,<sup>2</sup> and the increase of bone resorption.<sup>24,30</sup> Moreover, hyperlipidemia significantly impairs bone healing, thus decreasing bone surface and volume.<sup>9</sup>

The present study also demonstrated that a HF diet decreases the bone-to-implant interface. The peri-implant bone tissue formation and mineralization by osteoblasts is similar to the process of bone fracture repair. During fracture repair, osteoblasts deposit a collagenous matrix.<sup>31</sup> It has been demonstrated that a HF diet disrupts collagen processing<sup>23</sup> as well as orientation, causing loss of local alignment,<sup>9</sup> which may reduce mechanical integrity and quality of bone. The HF diet-induced loss of bone strength, bending stiffness, and fracture toughness<sup>9,29</sup> may also be attributable to inhibitory effects on differentiation<sup>15</sup> and mineralization of osteoblasts and stimulatory effects on differentiation and resorption of osteoclasts.<sup>2</sup>

Testing orthopedic or dental implants in mouse femoral bones to study bone-implant interaction, microstructure, composition, and bone remodeling accompanies some limitations. Although bone remodeling in resorption cavities in mice has been shown to be similar to the Haversian remodeling in larger animals, mouse cortical bones do not undergo Haversian remodeling as humans do.<sup>32</sup> Femurs also have a larger marrow space in comparison to jaws, causing less remodeling to take place in femurs compared with jaws. Lack of load on the implant should be also considered a limitation because dental implants in general are osseointegrated to withstand loading pressure.<sup>33</sup> However, there are also advantages of using mice as a model to study bone regeneration and development of drug therapies. These include availability of a wide variety of mouse models with genetic mutations and cost effectiveness in handling and drug consumption.<sup>34</sup>

In conclusion, the present in vivo study demonstrates that

hyperlipidemia significantly increases implant loss and decreases the formation and strength of the bone-to-implant interface in the mouse femur. Human clinical correlation is required to determine the effects of hyperlipidemia on dental implant survival and success. No large clinical studies exist to evaluate hyperlipidemia in dental implant failure. Studies that evaluate patients with coronary artery disease and implant failure include mostly patients with treated hyperlipidemic conditions by cholesterol-lowering medications.<sup>35</sup> However, this study is important as we continuously attempt to identify medical risk factors associated with implant success, including bone strength and bone-to-implant contact. However, increased implant failure, decreased osseointegration, and poor mechanical strength suggest that untreated hyperlipidemia may be a risk factor in this implant model system.

### ABBREVIATIONS

BIC: bone-to-implant contact  
HF: high fat

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### REFERENCES

1. Kuklina EV, Yoon PW, Keenan NL. Trends in high levels of low-density lipoprotein cholesterol in the United States, 1999–2006. *JAMA*. 2009; 302:2104–2110.
2. Tintut Y, Morony S, Demer LL. Hyperlipidemia promotes osteoclastic potential of bone marrow cells ex vivo. *Arterioscler Thromb Vasc Biol*. 2004;24:e6–e10.
3. Corwin RL, Hartman TJ, Maczuga SA, Graubard BI. Dietary saturated fat intake is inversely associated with bone density in humans: analysis of NHANES III. *J Nutr*. 2006;136:159–165.
4. Huang MS, Morony S, Lu J, et al. Atherogenic phospholipids attenuate osteogenic signaling by BMP-2 and parathyroid hormone in osteoblasts. *J Biol Chem*. 2007;282:21237–21243.
5. Lowe GD. The relationship between infection, inflammation, and cardiovascular disease: an overview. *Ann Periodontol*. 2001;6:1–8.
6. Solomon DH, Avron J, Canning CF, Wang PS. Lipid levels and bone mineral density. *Am J Med*. 2005;118:1414.
7. Fentoglu O, Bozkurt FY. The bi-directional relationship between periodontal disease and hyperlipidemia. *Eur J Dent*. 2008;2:142–146.
8. Corwin RL. Effects of dietary fats on bone health in advanced age. *Prostaglandins Leukot Essent Fatty Acids*. 2003;68:379–386.
9. Piri F, Lu J, Ye F, et al. Adverse effects of hyperlipidemia on bone regeneration and strength. *J Bone Miner Res*. 2012;27:309–318.
10. Mundy G, Garrett R, Harris S, et al. Stimulation of bone formation in vitro and in rodents by statins. *Science*. 1999;286:1946–1949.
11. Edwards CJ, Hart DJ, Spector TD. Oral statins and increased bone-mineral density in postmenopausal women. *Lancet*. 2000;355:2218–2219.
12. Meier CR, Schlienger RG, Kraenzlin ME, Schlegel B, Jick H. Statin drugs and the risk of fracture. *JAMA*. 2000;284:1921–1922.
13. Montagnani A, Gonnelli S, Cepollaro C, et al. Effect of simvastatin treatment on bone mineral density and bone turnover in hypercholester-

- olemic postmenopausal women: a 1-year longitudinal study. *Bone*. 2003;32:427–433.
14. Brodeur MR, Brissette L, Falstra L, Ouellet P, Moreau R. Influence of oxidized low-density lipoproteins (LDL) on the viability of osteoblastic cells. *Free Radic Biol Med*. 2008;44:506–517.
  15. Mody N, Parhami F, Sarafian TA, Demer LL. Oxidative stress modulates osteoblastic differentiation of vascular and bone cells. *Free Radic Biol Med*. 2001;31:509–519.
  16. Almeida M, Ambrogini E, Han L, Manolagas SC, Jilka R. Increased lipid oxidation causes oxidative stress, increased peroxisome proliferator-activated receptor-gamma expression, and diminished pro-osteogenic Wnt signaling in the skeleton. *J Biol Chem*. 2009;284:27438–27448.
  17. Maeda T, Matsunuma A, Kawane T, Horiuchi N. Simvastatin promotes osteoblast differentiation and mineralization in MC3T3-E1 cells. *Biochem Biophys Res Commun*. 2001;280:874–877.
  18. Ayukawa Y, Yasukawa E, Moriyama Y, et al. Local application of statin promotes bone repair through the suppression of osteoclasts and the enhancement of osteoblasts at bone-healing sites in rats. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod*. 2009;107:336–342.
  19. Moriyama Y, Ayukawa Y, Ogino Y, et al. Local application of fluvastatin improves peri-implant bone quantity and mechanical properties: a rodent study. *Acta Biomater*. 2010;6:1610–1618.
  20. Fedele S, Sabbah W, Donos N, Porter S, D'Aiuto F. Common oral mucosal diseases, systemic inflammation, and cardiovascular diseases in a large cross-sectional US survey. *Am Heart J*. 2011;161:344–350.
  21. Gaetti-Jardim EC, Santiago-Junior JF, Goiato MC, Pellizer EP, Magro-Filho O, Jardim Junior EG. Dental implants in patients with osteoporosis: a clinical reality? *J Craniofac Surg*. 2011;22:1111–1113.
  22. Olivares-Navarrete R, Raines AL, Hyzy SL, et al. Osteoblast maturation and new bone formation in response to titanium implant surface features are reduced with age. *J Bone Miner Res*. 2012;27:1773–1783.
  23. Parhami F, Tintut Y, Beamer WG, Gharavi N, Goodman W, Demer LL. Atherogenic high-fat diet reduces bone mineralization in mice. *J Bone Miner Res*. 2001;16:182–188.
  24. Sage AP, Lu J, Atti E, et al. Hyperlipidemia induces resistance to PTH bone anabolism in mice via oxidized lipids. *J Bone Miner Res*. 2011;26:1197–1206.
  25. Aghaloo TL, Kang B, Sung EC, et al. Periodontal disease and bisphosphonates induce osteonecrosis of the jaws in the rat. *J Bone Miner Res*. 2011;26:1871–1882.
  26. Ogawa T, Ozawa S, Shih JH, et al. Biomechanical evaluation of osseous implants having different surface topographies in rats. *J Dent Res*. 2000;79:1857–1863.
  27. Berglundh T, Persson L, Klinge B. A systematic review of the incidence of biological and technical complications in implant dentistry reported in prospective longitudinal studies of at least 5 years. *J Clin Periodontol*. 2002;29(suppl 3):197–212; discussion 232–293.
  28. Roos-Jansaker AM, Lindahl C, Renvert H, Renvert S. Nine- to fourteen-year follow-up of implant treatment. Part I: implant loss and associations to various factors. *J Clin Periodontol*. 2006;33:283–289.
  29. Ionova-Martin SS, Do SH, Barth HD, et al. Reduced size-independent mechanical properties of cortical bone in high-fat diet-induced obesity. *Bone*. 2010;46:217–225.
  30. Lange J, Barz T, Ekkernkamp A, Klötting I, Follak N. Gene expression profile in bone of diabetes-prone BB/OK rats fed a high-fat diet. *Genes Nutr*. 2013;8:99–104.
  31. Marco F, Milena F, Gianluca G, Vittoria O. Peri-implant osteogenesis in health and osteoporosis. *Micron*. 2005;36:630–644.
  32. Nunamaker DM. Experimental models of fracture repair. *Clin Orthop Relat Res*. 1998;(355 suppl):S56–S65.
  33. Brunski JB. Biomechanical factors affecting the bone-dental implant interface. *Clin Mater*. 1992;10:153–201.
  34. Histing T, Garcia P, Holstein JH, et al. Small animal bone healing models: standards, tips, and pitfalls results of a consensus meeting. *Bone*. 2011;49:591–599.
  35. Moy PK, Medina D, Shetty V, Aghaloo TL. Dental implant failure rates and associated risk factors. *Int J Oral Maxillofac Implants*. 2005;20:569–577.