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## The Effects of Hyperlipidemia in Implant Osseointegration

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### Abstract

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 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 an HF diet. After 12 weeks on the diet, each mouse received a titanium implant in the proximal metaphysis of the femur. The animals were sacrificed at 4 or 8 weeks after the implant surgery. Results showed that the HF diet fed mice had significantly increased implant loss as well as decreased formation and strength of bone-to-implant interface. These results support the hypothesis that an HF diet can significantly compromise osseointegration, causing poor outcome in dental implant therapy.

### Keywords

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 or older (Kuklina, Yoon et al. 2009). HF diet is associated with the pathophysiology of major diseases, including atherosclerosis and osteoporosis (Tintut, Morony et al. 2004; Corwin, Hartman et al. 2006; Huang, Morony et al. 2007). Interestingly, both hyperlipidemia and atherosclerosis have been linked to periodontal disease (Lowe 2001; Solomon, Avorn et al. 2005; Fentoglu and Bozkurt 2008).

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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 (Corwin 2003; Piri, Lu et al. 2012). 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 (Mundy, Garrett et al. 1999; Edwards, Hart et al. 2000; Meier, Schlienger et al. 2000; Montagnani, Gonnelli et al. 2003). At the cellular level, osteoblasts are capable of oxidizing low-density lipoproteins, possibly increasing the local concentration of oxidized reactive products in bone milieu (Brodeur, Brissette et al. 2008). Oxidative stress generated in hyperlipidemic conditions inhibits the differentiation of bone cells (Mody, Parhami et al. 2001; Almeida, Ambrogini et al. 2009). Statins enhance osteoblastic differentiation and mineralization (Maeda, Matsunuma et al. 2001) and suppress osteoclastogenesis (Ayukawa, Yasukawa et al. 2009). Furthermore, around implants, statins increase osteogenesis, suppress osteoclast formation and increase bone volume (Moriyama, Ayukawa et al. 2010).

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 (Fedele, Sabbah et al. 2011; Gaetti-Jardim, Santiago-Junior et al. 2011; Olivares-Navarrete, Raines et al. 2012). 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 four and eight weeks after implant placement.

## MATERIALS & METHODS

### Mice and diets

Four-week-old C57BL/6J male mice (atherosclerosis susceptible strain, The Jackson Laboratories, Bar Harbor ME) were randomly divided into two groups: control chow diet and an atherogenic HF diet (TD 90221, Harlan Teklad, Madison, WI; including 1.25% cholesterol, 15.8% fat and 0.5% cholate) (Parhami, Tintut et al. 2001; Sage, Lu et al. 2011; Piri, Lu et al. 2012). 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 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 sacrificed 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-computed tomography ( $\mu$ CT; SkyScan 1172; Kontich, Belgium) (Aghaloo, Kang et al. 2011). The entire implant site was selected as the region-of-interest (ROI).

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 (two coronal and two sagittal) and implant ( $\mu$ m)/whole implant length from the first to the last BIC ( $\mu$ m) $\times$ 100, and measured using the Dolphin 11.0 Imaging software (Chatsworth, CA, USA).

### Implant push-in test

The implant push-in test was performed as described by Ogawa *et al* (Ogawa, Ozawa et al. 2000). 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, MA, USA) contained a steel-pushing rod (0.8mm 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 (Ogawa, Ozawa et al. 2000).

### Statistical analysis

Two-tailed Student's *t*-test was performed to compare the effects of the chow diet and the HF diet groups. Results are presented as the mean  $\pm$  SEM of measurements, considering statistically significant values of  $p < 0.05$ .

## RESULTS

### High fat diet

Serum levels of total cholesterol, triglycerides and glucose were measured. The HF diet significantly increased the total cholesterol levels 2-fold at both time points ( $p < 0.0001$ ) (Table 1). The HF diet had an opposite effect in the triglycerides levels, which was significantly higher in the chow diet ( $p < 0.001$ ) (Table 1). Glucose was also higher in the chow diet, but only in the 4-week group ( $p < 0.05$ ) (Table 1).

### Hyperlipidemia and Osseointegration

Timeline of the diet and implant placement as well as 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 implant survival was 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 bone-to-implant contact (BIC) in the control versus the HFD, micro-CT analysis was performed. BIC was significantly higher in the chow diet mice as compared to the HFD at the respective time point ( $p < 0.01$  at 4 weeks and  $p < 0.05$  at 8 weeks) (Figure 2B). However, no statistically significant difference was found when comparing the BIC from 4 and 8 week time points within the respective diet groups (chow diet at 4 weeks compared to chow diet at 8 weeks and HFD at 4 weeks compared to HFD at 8 weeks (Figure 2C–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 ( $p < 0.01$  at 4 weeks and  $p < 0.05$  at 8 weeks) (Figure 3). Statistical significant difference was also found when comparing the average load between high fat mice at 4 and 8 weeks ( $p < 0.05$ ) 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 (Berglundh, Persson et al. 2002). Osseointegration is also critical since 36% of the patients who had lost a prior implant lost a second one in another occasion (Roos-Jansaker, Lindahl et al. 2006).

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 an HF diet, which has been shown to significantly reduce bone density and mineral content, thus altering morphological and mechanical properties (Parhami, Tintut et al. 2001; Ionova-Martin, Do et al. 2010; Sage, Lu et al. 2011). More than one mechanism can account for the deleterious effects of an HF diet in the osseointegration shown in the present study. At a cellular level, hyperlipidemic conditions lead to inhibition of osteogenic signaling (Huang, Morony et al. 2007), decrease of the formation of mature osteoblasts, a higher expression of molecular markers of bone remodeling (Sage, Lu et al. 2011), enhance of osteoclast differentiation and activity (Tintut, Morony et al. 2004), and the increase of bone resorption (Sage, Lu et al. 2011; Lange, Barz et al. 2013). Moreover, hyperlipidemia significantly impairs bone healing, thus decreasing bone surface and volume (Pirih, Lu et al. 2012).

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(Marco, Milena et al. 2005). It has been demonstrated that a HF diet disrupts collagen processing (Parhami, Tintut et al. 2001) as well as orientation, causing loss of local alignment (Pirih, Lu et al. 2012), which may reduce mechanical integrity and quality of bone. The HF diet-induced loss of bone strength, bending stiffness, and fracture toughness(Ionova-Martin, Do et al. 2010; Pirih, Lu et al. 2012) may also be attributable to inhibitory effects on differentiation(Mody, Parhami et al. 2001) and mineralization of osteoblasts and stimulatory effects on differentiation and resorption of osteoclasts(Tintut, Morony et al. 2004).

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(Nunamaker 1998). 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 (Hoshaw, Brunski et al. 1997). 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(Histing, Garcia et al. 2011).

In conclusion, the present *in vivo* study demonstrates that a 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 (Moy, Medina et al. 2005). 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.

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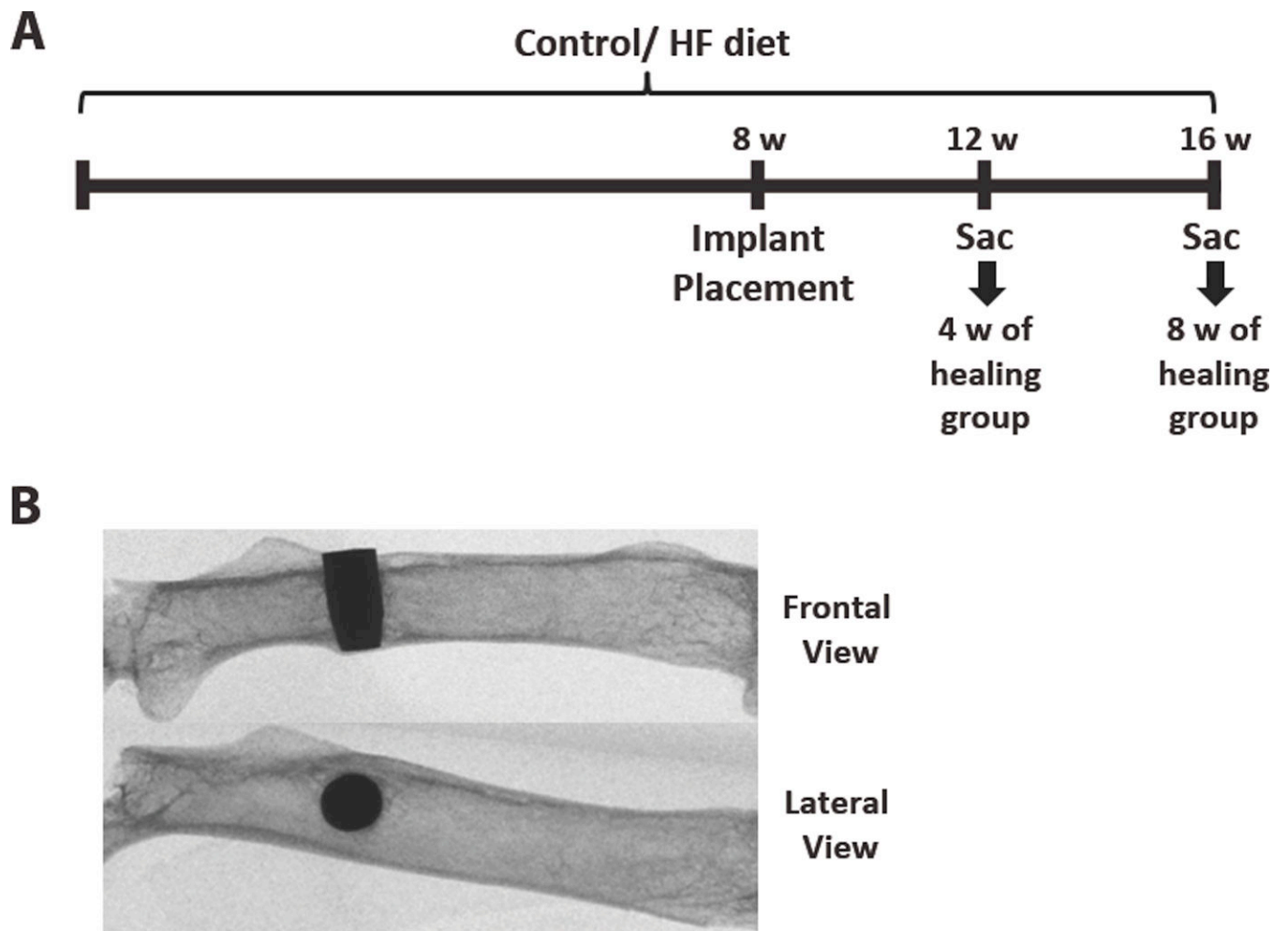
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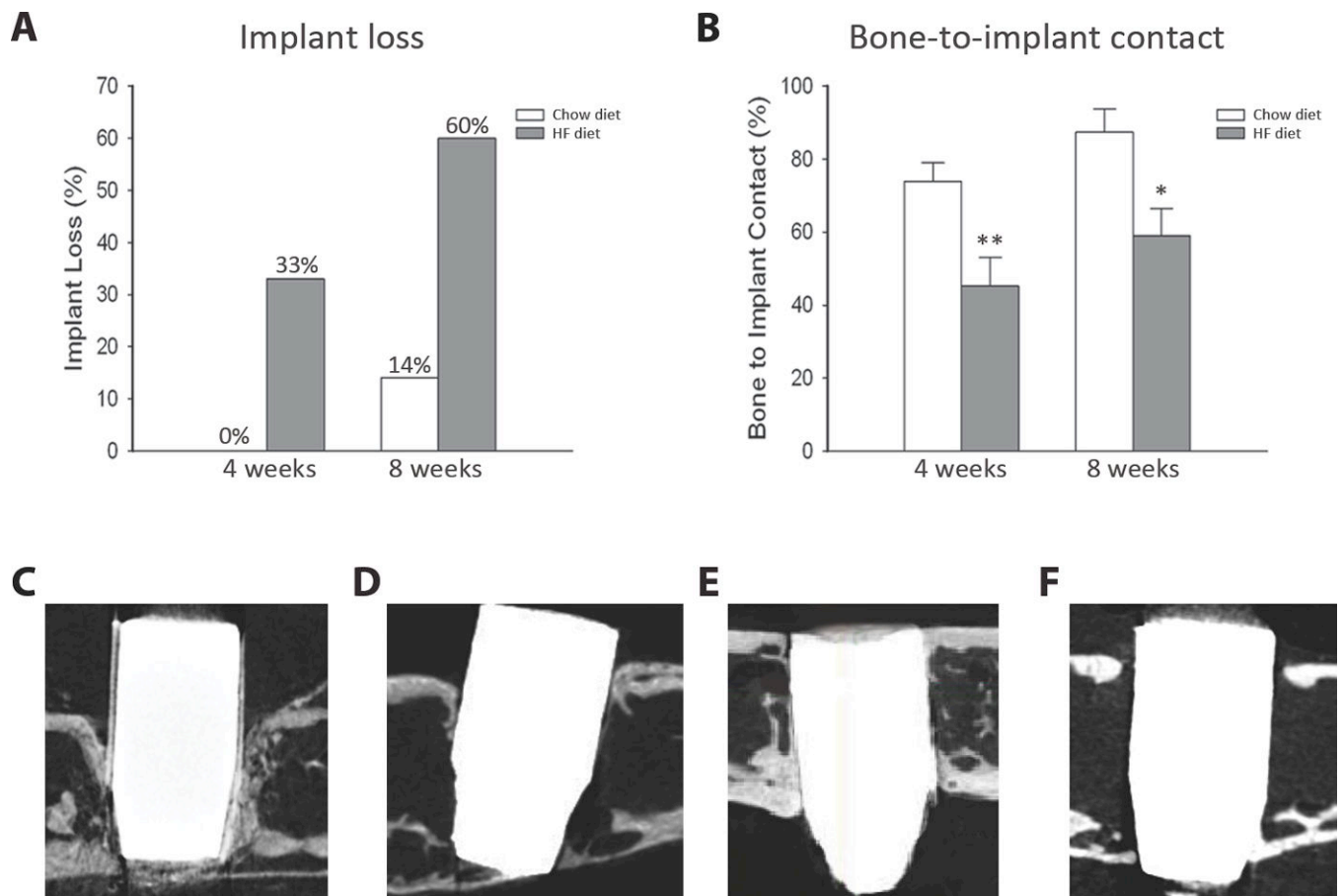




**Figure 1.**

(**A**) Schematic diagram of the timeline for the diet and implant placement. HF = high-fat; W = weeks; Sac = sacrifice. (**B**) X-ray images showing the implant placement location (distal to the 3<sup>rd</sup> trochanter) on a frontal and lateral view.





**Figure 2.**

Effects of the 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 = 6/group).

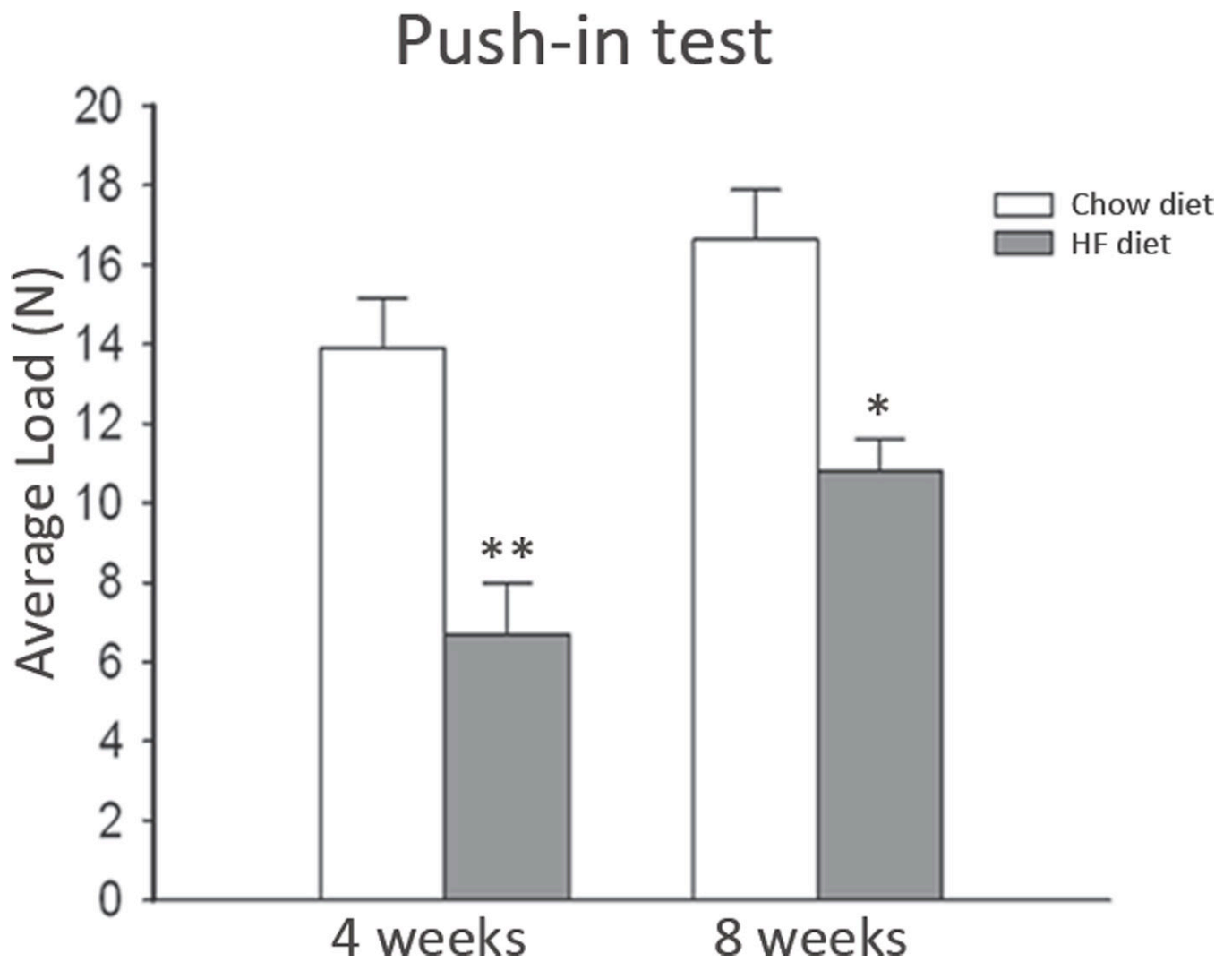
(B) Percent of bone to implant contact throughout in the entire implant length (n = 6/group).

Significant difference when compared to respective control: \* $p < 0.05$ , \*\* $p < 0.01$ . (C–F)

Representative  $\mu$ CT 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.



**Figure 3.**

Load (force) necessary to break the bone to implant interface (n = 3/group). Significant difference when compared to respective control: \* $p < 0.05$ . No statistically significant differences were found when comparing chow diet and HF diet groups within the respective time points.

**Table 1**

Effects of the HF diet on serum cholesterol (n = 9/group), triglycerides (n = 8/group) and glucose (n = 8/group).

(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

Values are mean ± SEM. Significant difference when compared to respective control:

\*  $p < 0.05$ ,

\*\*  $p < 0.01$ ,

\*\*\*  $p < 0.001$ ,

\*\*\*\*  $p < 0.0001$ .