Original Article

The Investigation of Bone-Implant Connection and New Bone Formation in Fasting and High-Fatty Diet Rats

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ABSTRACT

Background: Hyperlipidemia caused by a high-fat diet (HFD) has many adverse effects on the cardiovascular system, including vascular problems. In addition, a HFD also has significant adverse effects on bone health. **Aim:** The aim of this study is to examine bone-implant osteointegration and new bone formation in peri-implant defects in fasting and high-fatty diet applied rats. **Materials and Methods:** In this study, 28 female Sprague Dawley rats were used. The rats were divided into four groups, with seven rats in each group: the control group on a normal diet (Group 1) (n = 7), the fasted group (Group 2) (n = 7), the high-fatty diet (HFD) group (Group 3) (n = 7), and the fasted and HFD group (Group 4) (n = 7). Titanium implants with a diameter of 2.5 mm and a length of 4 mm were placed in the right tibia bones of the subjects, and a bone graft corresponding to 2 mm of the implant length was placed in the bone defect applied to the neck region. All rats that continued the administered diet for 12 weeks were sacrificed at the end of the experiment period. The implants and surrounding bone tissue were surgically removed and subjected to biomechanical analysis to assess bone-implant osteointegration and peri-implant new bone formation. **Results:** It was determined that there was no statistically significant difference between the rats in the control group and the other three groups in terms of bone-implant osteointegration and peri-implant new bone formation ($P > 0.05$). **Conclusion:** As a result of this study, it was determined that fasting or maintaining a HFD does not adversely affect bone-implant osteointegration or peri-implant new bone formation in the tibias of rats.

KEYWORDS: *Bone augmentation, fasting, guided bone regeneration, high-fatty diet, implant, osteointegration*

INTRODUCTION

Atherogenic or high-fatty diets (HFD) cause hyperlipidemia, characterized by elevated lipids in the bloodstream.[1] Atherosclerosis, vascular calcification, and osteoporosis have become important health problems in today's aging population. Hyperlipidemia caused by

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an HFD has many adverse effects on the cardiovascular system, including vascular problems.[2,3] Additionally, both hyperlipidemia and atherosclerosis have been associated with periodontal diseases.[4]

An HFD also has significant adverse effects on bone health, leading to lower bone mineral density and a higher risk of osteoporosis and bone fractures.[2,5] Statins, cholesterol-lowering HMG-CoA reductase inhibitors, have beneficial effects on bone metabolism by stimulating bone formation and mineral density, thereby reducing the risk of hip fractures.[6] Osteoblasts can undergo oxidation with low-density lipoproteins, leading to increased local concentrations of oxidized reactive products in the bone environment.[7] Oxidative stress, occurring in hyperlipidemic conditions, inhibits bone cell differentiation.[8]

According to data from an epidemiological study, hyperlipidemia was detected in 63% of osteoporotic patients. In addition, several studies have reported that obesity is a risk factor for osteoporosis in humans. Epidemiological studies have reported a statistically significant inverse relationship between serum cholesterol levels and bone mineral parameters and density, regardless of age and body mass index. Experimental animal studies have also associated HFD consumption with a decrease in bone mineral content and density. Oxidized lipids induce osteoclastogenesis while reducing parathyroid hormone levels and bone morphogenetic protein-2 signaling. Experimental studies have reported that hyperlipidemia causes bone loss in mice.[1,3]

While evidence suggesting that reducing overall food consumption can extend the mammalian lifespan emerged in the early 1900s, reducing food intake without causing malnutrition has been widely used in experimental models to delay the aging process. Results of studies in model organisms suggest that reducing calorie intake, also known as dietary restriction, and changes in diet composition (e.g., protein content) or timing of food intake (e.g., intermittent fasting) may be beneficial in the prevention of chronic diseases, including cancer.[9-15]

Food deprivation, calorie restriction, periodic starvation, or moderate food deprivation can increase the ability to fight diseases in vertebrates.[9] Experimental studies have shown that starvation in rodents strengthens the immune system, reduces inflammation, and is an effective method for longevity.[10-13] A sensitive homeostatic balance occurs in energy consumption during starvation.[14] The balance between energy stores and the energy spent is achieved by the delicate balance between the carbohydrate, lipid, and protein metabolisms. Exposure to starvation shows itself with an alarming picture in the body and encourages the body to form acute phase proteins, which have the potential to protect the body from oxidative stress and take part in cell regeneration. In addition, short-term fasting (72h–96h) combined with chemotherapy has been shown to have powerful and beneficial effects in the treatment of various types of cancer.[14,15]

Dental implant-supported dentures are a widely used and scientifically accepted treatment option for both partial and complete edentulism. However, factors such as the patient's systemic condition, bone quality, implant characteristics, and smoking habits are crucial for the success of dental implant treatment. Although there have been isolated studies on the effects of HFD and starvation, their effects on bone-implant integration remain incompletely investigated and controversial.[3,10-13,16]

The aim of this study is to investigate the effects of an HFD and starvation on the osteointegration of titanium implants in the tibial bones of rats and new bone formation after guided bone regeneration around the implant.

Materials and Methods

Animal and study design

All surgical and experimental procedures were performed at the Firat University Experimental Research Center. Approval for the study was obtained from the Firat University Animal Experiments Ethics Committee (Elazig/Turkiye) (2019/112). The rats used for the experiment were obtained from the same center. All animal experiments were performed in accordance with the Helsinki Declaration for the care and welfare of animals. A total of 28 female Sprague Dawley rats with an average age of 1 year were used in the study. To ensure standardization, vaginal smear tests were performed to synchronize their estrus cycles. The mean body weight of the rats on the first day of the experiment was 230–250 grams. The rats were housed in plastic cages with a 12-hour dark and 12-hour light cycle, and they had free access to food and water according to their diet.

Cavities were opened in the corticocancellous bone in the metaphyseal part of the right tibia bones of the rats. Titanium implants (Implance Dental Implant Systems, AGS Medical Corporation, Istanbul, Turkiye) with a diameter of 2.5 mm and a length of 4 mm were placed in the cavities. While opening the implant cavities, cavities with a 4 mm diameter were also opened in the neck region, corresponding to 2 mm of the implant length. After the placement of the bone graft in the circumferential bone defect formed, the rats were randomly divided into four study groups as follows:

Control group (n = 7): After the surgical implant placement procedures, the subjects in this group were given a normal diet during the 12-week experiment period with no additional procedures.

Fasted group (n = 7): After the surgical implant placement procedures, the subjects in this group were fasted for 3 days a week during the 12-week experiment period. Subjects were released on water intake.

HFD group (n = 7): After the surgical implant placement procedures, the subjects in this group were given a HFD during the 12-week experiment period with no additional procedures.

Fasting group on a HFD (n = 7): After the surgical implant placement procedures, the subjects in this group were given a fatty diet during the 12-week experiment period and were additionally fasted for 3 days a week.

The number of rats required for the experimental setup was determined and calculated by power analysis. The deviation was 8%, type 1 error (α) was 0.05, and type 2 error (β) (power = 0.80), and when the rats were divided into groups, it was calculated that there should be at least seven rats in each group for the experimental setup.

Surgical procedure

General anesthesia was induced with intramuscular injections of ketamine hydrochloride (50 mg/kg, Ketasol, Richter Pharma AG, Wels, Austria) and xylazine (5 mg/kg, Rompun, Bayer, Germany). All surgical procedures were performed under sterile conditions. After the rats were anesthetized, the surgical area was washed with povidone iodine and shaved. A 15 mm incision was made at the right tibial crest to expose the corticocancellous part of the tibial metaphyseal bone [Figure 1]. Implant sockets were created using appropriate burs in saline perfusion. Cavities were opened with a diameter of 4 mm in the upper half of the implant length and 2.5 mm in the lower half of the implant length [Figure 2]. Titanium implants (Implance Dental Implant System, AGS Medical Corporation, Istanbul, Turkey) with a 4 mm length and 2.5 mm diameter with resorbable blast material surfaces were placed in the metaphysis of the tibial bone, and primary stabilization was achieved.[17] [Figure 3] The cavity formed around the implant was filled with xenogeneic bone graft. Following the placement of the titanium implants, the flap was returned to its original position. Fascia, subcutaneous tissue, and skin were sutured with 4–0 polyglactin sutures. All subjects received intramuscular injections of antibiotics (50 mg/kg penicillin) and analgesics (0.1 mg/kg tramadol hydrochloride) for postoperative 3 days in order to prevent pain and infection. All surgical procedures were performed atraumatically by the same investigator.

Animal feeding procedure

The rats in the control and dietary restriction applied groups were fed with standard feed. The rats in the HFD and HFD with exposure to starvation applied groups were fed with a feed containing 40% fat-based calories.[18] Normal diet content: 200 g/kg casein, 615 g/kg starch, 80 g/kg corn oil, and 105 g/kg cellulose. Fatty diet content: 200 g/kg casein, 145 g/kg starch, 150 g/kg sucrose, 400 g/kg beef tallow, and 105 g/kg cellulose. Diets were prepared by mixing all the materials in a mixing machine and turning them into pellets. The feeds turned into pellets were dried and given to the subjects.[18]

Biomechanical analysis

The experimental setup was completed after 12 weeks. In general, fatal or non-fatal complications (such as wound formation or wound infection) were not encountered during the trial period. At the end of the experiment period, the rats were euthanized under deep anesthesia and the titanium implants were removed along with the surrounding bone tissue. The excised tissues were fixed in a 10% formalin solution. A rapid assessment was made to avoid possible dehydration of the samples. The samples were placed in polymethylmethacrylate blocks before analysis. The implants were fixed as blocks with a reverse torque device. Each implant was fixed to the digital torque tool (Mark-10, NY, USA) for evaluation [Figure 4]. The implant-bone block, which was made into a single block, was placed in the device chamber to measure the reverse torque. Reverse torque was measured by gradually increasing the forces. The application was completed when the implants returned to the bone socket. When the force was balanced, the highest value (Newton/cm) obtained on the reverse torque meter display was automatically recorded.

Statistical analysis

The SPSS 23.0 for Windows program (IBM SPSS Statistics for Windows, Armonk, NY, USA) was used for statistical analysis. The data for each group were expressed as mean ± min-max. The Shapiro–Wilk test was used to evaluate whether the data showed normal distribution. The data were found not to be normally distributed, and differences between groups were determined using the Kruskal–Wallis test. The Mann–Whitney U test was used to determine the group from which the difference originates in pairwise comparisons. Significance was evaluated at the $P < 0.05$ level.

Results

Biomechanical analysis results of bone-implant connection (BIC) for all groups are shown in Table 1. Although there was no statistically significant difference between the rats in the control group and the other three groups in terms of bone-implant osteointegration, it was found to be close to significance ($P > 0.05$). It was observed that the BIC value in the fasted group was numerically higher than in the control group. In addition, the BIC value was significantly lower in the groups that were on a HFD and those exposed to starvation compared to the control group.

Figure 1: Incision in tibial crest and elevation of the soft tissues for reaching the corticocancellous part of tibial bone

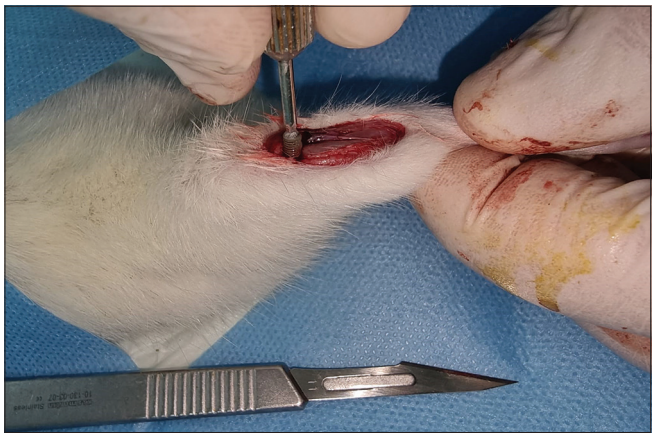

Figure 3: Insertion of the resorbable blast material surface implants into the bone cavities

Discussion

The aim of this study was to investigate the effects of a HFD and fasting on implant osseointegration and new bone formation after guided bone regeneration around the implant in rat tibias using biomechanical methods.

Successful osteointegration is the main determinant of implant success. Lack of osteointegration is a major concern, especially in patients with challenging implant placements and systemic problems like diabetes and metabolic bone diseases, patients with type 4 bone tissue with low bone mineral content and density, etc. The degree of existing bone loss prior to implant placement has been shown to be an important factor in implant success.[19] In a previous study, 36% of patients who had lost a previous implant and experienced bone loss also

Figure 2: Creation of implant cavities with serum perfusion in corticocancellous part of the metaphysical part of tibia

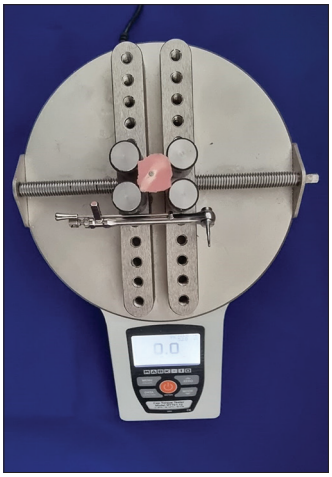

Figure 4: Biomechanical analysis of the osseointegration of the implants with reverse torque after experimental protocols (Mark 10, NY, USA)

Table 1: Biomechanical bone-implant connection (BIC) levels of the groups. *P*=0,052

Parameter	Groups	*n*	Mean (Newton/cm)	Std. Dev.	Min-Max (Newton/cm)	*P**
BIC	Graft Controls	7	2,14	0,54	1,60-3,20	>0,05
	Graft Fasting	7	2,46	0,83	1,60-3,90	
	Graft HFD	7	1,53	0,55	0,90-2,40	
	Graft HFD Fasting	7	1,66	0,70	0,90-2,80	

HFD=High-fat diet, SD=Standard deviation. *Kruskal–Wallis test (Nonparametric data)

lost their second implant in the same area.[20] Guided bone regeneration applications are a frequently used treatment option in the treatment of peri-implant bone tissue loss and peri-implant bone tissue insufficiency.[1-3,19,20]

Adipose tissue is not an inert organ whose sole function is to store energy; it also has metabolic functions such as the secretion of proteins involved in bone metabolism. In fact, a HFD has a large effect on bone metabolism regardless of the weight of the subject that is mainly dependent on endocrine stimulus rather than mechanical stimulus.[21,22] Many mechanisms have been proposed to explain HFD-induced osteoclastogenesis, including elevated levels of the proinflammatory cytokines IL-1 and Tumor necrosis factor, derived from adipose tissue macrophages in the blood, increased (Receptor activator of NF-κB ligand) expression in bones and decreased expression of the anti-osteoclastogenic cytokine IL-10.[23-25] Although the evaluation of the data maintained from this study showed no statistical significance, the results were close to significance. It was determined that the biomechanical data obtained especially in the HFD applied groups were low when compared to the control group. In a study by Dundar *et al*. in which the effects of a HFD and restraint stress on osteointegration were examined, it was reported that histologically, a HFD negatively affected BIC levels compared to the controls.[16]

It has been reported in experimental studies that exposure to starvation could have positive effects on the treatment of cancer (melanoma, glioma, breast cancer, prostate cancer, lung cancer, leukemia).[14] The body reacts to food deprivation by increasing the breakdown of proteins. Proteolytic pathways release amino acids during starvation and use them for energy, which is compensated by the formation of new protein molecules in the body. As a result, the body is given a new opportunity for regeneration.[14] The relationship between prolonged life expectancy and short-term hunger is thought to include changes in energy production and use, suppression of oxidative stress, reduction of insulin resistance, inflammatory response, and changes in communication between cells and organs.[14,15] According to the present study, the biomechanical data obtained from the fasted group being higher than the control group at a numerical level can be evaluated in terms of the positive relationship between a fasting diet and bone metabolism.

In previous studies, rats fed with an atherogenic HFD not only became hyperlipidemic but also showed significantly reduced bone mineral content and reduced bone volume in both femoral and tibial bones. For example, Lac *et al*.[26] stated that during the early growth period (35 days), rats fed with a HFD had lower bone mineral density and exhibited a negative correlation between visceral fat and bone mineral density compared to a normal diet-fed control group. Lu *et al*.[27] reported in an experimental study on young male rats that bone mineral content levels and the trabecular bone area were significantly reduced in the HFD group compared to control groups. Pirih *et al*.[2] reported that oxidized lipids and/or hyperlipidemia adversely affect the mechanical strength of bone and also impair bone regeneration. Micro-computed tomography and histological results showed significantly impaired bone regeneration in men with a HFD. It was also found that the cortical bone volume fraction (bone volume/tissue volume) was significantly reduced in the femoral bones of the subjects in the HFD group compared to the control group. Keuroghlian *et al*.[1] reported that in the femurs of atherosclerosis-susceptible C57BL/6J male rats fed with a HFD showed a decrease in the amount and strength of bone-implant fusion and a significant increase in implant loss. These results support the hypothesis that a HFD could decrease osteointegration and cause negative results in dental implant treatments. In contradiction to the literature, although a statistically significant difference between the BIC levels of the groups was not found in this study, a significant difference at the numerical level was found in the BIC levels of the HFD group. The reason why there was no statistical difference may be due to the number of subjects.

Hirasawa *et al*.[28] stated that the HFD also increased osteoblast apoptosis. The increased level of low-density lipoprotein in rats is directly proportional to the increase in type 1 collagen C-terminal telopeptide (CTX), a marker of osteoclastic bone resorption. Parhami *et al*.[29] found that oxidized lipids inhibit the differentiation of osteoblasts. Sage *et al*.[30] reported that lipid oxidation products compromised the adverse effects of a HFD on bone tissue. Contrary to the literature, the present results show that BIC did not decrease in either the HFD-fed rats or the rats exposed to periodic fasting compared to rats fed a normal diet during the 3-month feeding period. However, it is striking that the BIC data of the rats in the fasting group were found to be numerically higher than the controls.

Although reverse torque analysis cannot be used clinically, it is used to evaluate initial implant stability and bone-implant fusion in animal and laboratory studies. When the implant in the bone is rotated with a reverse torque, the connection between the bone and the implant is broken, allowing the bone-implant fusion to be evaluated in terms of force. Reverse torque analysis is an objective evaluation method of bone-implant fusion. Reverse torque analysis allows the evaluation

of the entire bone around the implant. In histological analysis, evaluation can only be made on a specific and very thin section (6 μ). In our study, the reverse torque test was used to evaluate the effect of postoperative fasting diet and a fatty diet combined with a fasting diet on osseointegration, according to the literature.[31]

Conclusion

With the present study, it was concluded that hyperlipidemia and starvation did not have a significant effect on bone-implant osteointegration and new bone formation around the implant in rat tibias. Correlation with clinical studies is necessary to clearly understand the effects of hyperlipidemia and starvation on dental implant survival and success. It can be stated that the present study aims to determine the medical risk factors associated with implant success, including new bone formation and bone-implant fusion, could contribute to the literature.

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Conflicts of interest

There are no conflicts of interest.

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