Original

Calcium, Phosphorus and Oxygen Around Implant at Early Osseointegration in Hyperlipidemic Rats

Qibo Liu^{1,2)*}, Zhifeng Wang^{1,3)*}, Xiaofei Dong⁴⁾, Hui Wang⁵⁾ and Jing Lan^{1,2)}

¹⁾ Shandong Provincial Key Laboratory of Oral Tissue Regeneration, School of Stomatology, Shandong University, Jinan, Shandong, China

²⁾ Department of Prosthodontics, School of Stomatology, Shandong University, Jinan, Shandong, China

³⁾ Department of Pediatric Dentistry, School of Stomatology, Shandong University, Jinan, Shandong, China

⁴⁾ The State Key Laboratory Breeding Base of Basic Science of Stomatology (Hubei- MOST) & Key Laboratory of Oral Biomedicine Ministry of Education, School & Hospital of Stomatology, Wuhan University, Wuhan, Hubei, China

⁵⁾ Department of Dental Implantology, Affiliated Stomological Hospital of Suzhou University, Suzhou, Jiangsu, China

(Accepted for publication, December 22, 2018)

Abstract: The study was to observe dental implant osseointegration at early stage and analysis the influence of the ratio of calcium and phosphorus variation on osseointegration in hyperlipidemic rats. Wistar rats were randomly divided into control group with normal diet and experimental group with high fat diet. 8 weeks later, serum lipid levels was detected and titanium implants were placed into bilateral femurs. At day1, 3, 5 after implant, rats were sacrificed and 5 mm bone with implants was obtained. Hard tissue slices and methylene blue-acid fuchsin staining were performed to observe the interface of bone-implant. And energy dispersive X-ray spectrometer (EDS) was used to measure the percentage of oxygen and the ratio of calcium and phosphorus. All rats in experimental group were successfully induced into hyperlipidemic status. Histological results revealed less osteoblasts and trabecular bone, but more osteoclasts and trabecular space in experimental group than those in control group. Higher content of oxygen and lower ratio of calcium and phosphorus were also observed in experimental group than those in control group ($P < 0.05$). Hyperlipidemia could hinder implant osseointegration at early stage after implantation. This inhibition might be closely related to higher content of oxygen and lower ratio of calcium and phosphorus.

Key Words: Bone healing, Hyperlipidemia, Implant osseointegration, Oxygen, Ratio of calcium and phosphorus

Introduction

Hyperlipidemia is characterized with elevated lipids in bloodstream, which is caused by atherosclerosis or high-fat (HF) diet. Hyperlipidemia is closely related to osteoporosis, which usually involves alveolar bone¹⁾. And mice with HF diet demonstrate significant increased implant failure, decreased bone formation and compromising strength of implant-bone interface²⁾. In recent years, the morbidity of hyperlipidemia is increasing. In America, 12.1% of adults had high total cholesterol (TC) and 18.5% with low high-density lipoprotein (LDL) cholesterol during 2011-2014³⁾.

With the survive rate of dental implant is high, it becomes common prosthetics in the world. The successful osseointegration is determined the long-term survive rate. The studies about the influence of hyperlipidemia on implant osseointegration is still at an initial stage. Some studies have indicated that hyperlipidemia inhibits osteoblastogenesis and bone metabolism through hindering alkaline phosphatase activity and mineralization^{4,5)}, which were markers of osteoblast differentiation. Hyperlipidemia also impaired bone regeneration and mechanical strength^{6,7)}.

Oxygen (O) is necessary in cell metabolism and bone repair by regulating proliferation and differentiation of bone marrow stem cells (BMSCs), chondrocytes and osteoblasts⁸⁻¹¹⁾. O participates oxidation-reduction reaction to produce reactive oxygen species (ROS). High lipid level could increase oxidative stress by overproduction of ROS¹²⁾ that bone healing process between the implant-bone interface was suppressed¹³⁻¹⁵⁾. Except O, calcium (Ca) and phosphorus (P) are also important components in bone mineralization and bone formation¹⁶⁻¹⁹⁾. Ca has been proved to be crucial in implant osseointegration, involving platelet adhesion, orientation and conformation of proteins on the interface^{20,21)}. Low concentration of P is released from bone, which simultaneously lead to the release of Ca because of an unfavorable ratio of Ca to P (Ca/P)²²⁾. Interaction between Ca and P could affect bone metabolism and development^{23,24)}. The Ca/P was positively related to bone density, strength and quality²⁵⁾. Maintaining an adequate Ca and P with appropriate ratio is of crucial importance for implant-bone interface stability and good osseointegration. But the effect of hyperlipidemia on O, Ca and P is still unknown.

The poor implant osseointegration at implant-bone interface at the early stage after implantation resulted in the majority of dental implants failure^{26,27)}. In this study, we observed implant osseointegration at implant-bone interface of hyperlipidemic rats from histological slices. Meanwhile, O, Ca and P in tissue were also assayed using EDS.

*Qibo Liu and Zhifeng Wang are co-first authors.

Correspondence to: Dr. Jing Lan, Shandong Provincial Key Laboratory of Oral Tissue Regeneration, Department of Prosthodontics, School of Stomatology, Shandong University, No.44-1 Wenhua Road West, Jinan, China;
Fax: 86-531-88382923; Email: lanjing@sdu.edu.cn

Figure 1. Six microzones analyzed by EDS. (A) The selected area is in the middle part of the implant 1/3. (B) A line, perpendicular to the long axis of implants, was drawn and then “1~6” six points at 10µm intervals along the implant in this line was chosen, which was also called six microzones. The midpoints of “3” and “4” were around the edge of implants.

Figure 2. The serum levels of two groups. The rats in experimental group whose serum levels of TC were about 2 times more than rats in control group and levels of TG and LDL were significantly higher than those of control group were defined as successful hyperlipidemic rats.

Materials and Methods

The study was approved by the ethics committee of Shandong University Dental Hospital, and informed consents were obtained (protocol number GD201615).

Animals

36 adult male Wistar rats were obtained from Shandong University Experimental Animal Center (Ji'nan, China). All experimental protocols were guided and approved by Institutional Animal Care and Use Committee of School of Stomatology of Shandong University (Ji'nan, China). The rats were randomly divided into control group fed with normal diet and experimental group with HF diet during whole experiment. HF diet was purchased from Beijing Ke'aoxieli Co., Ltd (Beijing, China).

8 weeks later, endocanthion vein blood specimens were collected to monitor serum TC, Triglyceride (TG), high-density lipoprotein (HDL) and LDL.

Implantation and samples preparation

All rats were anesthetized with chloral hydrate (0.3 ml/100 g). After regular skin preparation and disinfection, a 1cm vertical incision was made near the epiphyseal of femur and blunt dissection of femoral rectus and femoral muscles to fully expose bone. The implant hole was prepared by 1.2 mm pilot drill, which rotated at 800 revolutions per minute and cooled with saline. Titanium (Ti) implants (Shuangyang®

Figure 3. Hard tissue slices and staining. The red dying area was trabecular bone, the dark blue area was osteoblasts and osteoclasts, the black area was the implant, and the blue violet area was collagen fibers. (A) Control group at day 1 (× 400 fold). (B) Experimental group at day 1 (× 400 fold). (C) Control group at day 3 (× 400 fold). (D) Experimental group at day 3 (× 400 fold). (E) Control group at day 5 (× 400 fold). (F) Experimental group at day 5 (× 400 fold). It can be seen that the trabecular bone (red area) of the experimental group arranged disorder especially at day 5; while the blue-stained area around the implant was more heavy, meaning that the collagen content was higher. (Methylene blue staining, Bar = 20 µm).

Table 1. The serum TC, TG, HDL and LDL of two groups

	TC	TG	HDL	LDL
Control group	1.26±0.24	0.79±0.20	0.80±0.15	0.25±0.05
Experimental group	2.43±0.26*	1.35±0.31*	1.17±0.20*	0.44±0.13*

(mean ± SD mmol/l, n=18)

The rats in experimental group whose serum levels of TC were about 2 times more than rats in control group and levels of TG and LDL were significantly higher than those of control group were defined as successful hyperlipidemic rats.

*Significant difference between experimental and control group ($P < 0.05$).

Table 2. The percentage of O at day 1, 3, 5 after implantation

microzone	C1d	E1d	C3d	E3d	C5d	E5d
4	72.76±2.58	79.29±3.11*	72.33±2.71	78.93±3.07*	72.17±3.09	78.73±2.61*
5	63.01±2.59	69.73±3.22*	63.09±2.56	69.81±3.28*	63.14±2.64	69.92±3.12*
6	61.30±2.51	67.80±3.16*	61.46±2.83	68.36±3.19*	62.04±2.82	68.66±3.00*

“C” means control group, “E” experimental group. C1d indicates the control group 1 day after implantation, E3d indicates the experimental group 3 days after implantation, and so on.

*:Significant differences between experimental and control group at the same time ($P < 0.05$).

Table 3. The ratio of Ca/P at day 1, 3, 5 after implantation

microzone	C1d	E1d	C3d	E3d	C5d	E5d
4	1.20±0.09	1.02±0.06*	1.29±0.10	1.09±0.07*	1.38±0.11	1.17±0.07*
5	1.57±0.14	1.30±0.08*	1.51±0.14	1.26±0.06*	1.48±0.12	1.23±0.09*
6	1.65±0.12	1.40±0.09*	1.60±0.13	1.34±0.09*	1.56±0.13	1.31±0.08*

“C” means control group, “E” experimental group. C1d indicates the control group 1 day after implantation, E3d indicates the experimental group 3 days after implantation, and so on.

*:Significant differences between experimental and control group at the same day ($P < 0.05$).

Figure 4. Differences between osteoblasts, osteoclasts, trabeculae and collagen fibers at day 1, 5 after implantation. Quantitative analysis of slices, there were more trabecular bone (B) and osteoblasts (A), less osteoclasts (A) and collagen fibers (B) in control group than those in experimental group at day 1, 5 after implantation. * $P < 0.05$.

self-tapping screw, 1.5 mm wide and 2.5 mm long, Zhangjiagang city, China) were placed into bilateral femurs distal to epiphyseal. Mucosa and muscles were sutured with 4-0 thread and skin with 5-0. 200000 u Cefazolin sodium was administrated in 3 days after surgery.

At day 1, 3, 5 after implantation, the rats from each group were sacrificed by overdosed chloral hydrate. 5 mm bones around implants were dissected and fixed in 4% paraformaldehyde, dehydrated in ascending series of ethanol and embedded with resin.

Hard tissue slices and staining

The samples were cut and grinded into 30 μ m thick sections

(E400CS, EXAKT Vertriebs GmbH, Germany), then methylene blue staining was performed. Inverted microscopy was used to observe and photograph the interface of implant-bone. Image-Pro Plus software was used to analyze the area of trabecular bone and the number of osteoblasts in the original photograph at day1, 5 after implantation and made a histogram.

Micro-composition analysis

Other samples of each group were used to measure concentrations of O, Ca and P at six microzones, and calculate percentage and ratio with energy disperse X-ray spectrometer (EDS). Six microzones were

Figure 5. The percentage of O at day 1, 3, 5 after implantation. In both groups, the percentage of O increased a little with time at microzone 5 and 6. Percentage of O in experimental group was significantly higher than that in control group at microzone 4, 5, and 6 (* $P < 0.05$).

Figure 6. The ratio of Ca/P at day 1, 3, 5 after implantation. Ca/P increased slightly from day 1 to day 5 in both groups at microzone 4. Ca/P in experimental group was significantly lower than that in control group at microzone 4, 5, and 6 (* $P < 0.05$).

marked “1–6” at 10 μ m intervals and perpendicular to the medium 1/3 of implants (Fig. 1). “3” and “4” were around the edge of implant.

Statistical analysis

Data were expressed as mean $\pm$ standard deviation (SD) and analyzed by SPSS software 13.0. Comparisons were analyzed with One-way ANOVA and t-test. $P < 0.05$ was considered statistically significant difference.

Results

Establishment of hyperlipidemic rats

TC level in experimental group was about twofold than that in control group ($P < 0.05$), and also TG, HDL and LDL levels were significant higher than those of control group ($P < 0.05$) (Table 1, Fig. 2). All rats in experimental group were successfully induced to hyperlipidemic rats.

Analysis of hard tissue slices

At day 1, 3 and 5 after implantation, there were crescent-shaped trabecular bone, bone remodeling caused by osteoclast phagocytosis, and gradually increased osteoblasts, osteoclasts and collagen fibers. There is a large amount of osteoclasts distribution, with three or more nuclei in control group (Fig. 3A). In experimental group at day 1, 3, 5 (Fig. 4B, D and F), trabecular bone arranged disorder, and collagen fibers were less around the implant.

Through the quantitative analysis of the slices, there were more trabecular bone (Fig. 4B) and osteoblasts (Fig. 4A), less osteoclasts (Fig. 4A) and collagen fibers (Fig. 4B) in control group than those in experimental group at day 1, 5 after implantation. It can be seen that there is a statistically significant difference between the control group and the experimental group (* $P < 0.05$).

Micro-composition analysis

In both groups, the percentage of O increased a little with time at microzone 5 and 6, but decreased at microzone 4 ($P > 0.05$) (Table 2, Fig. 5). Percentage of O in experimental group was significantly higher than that in control group at microzone 4, 5, and 6 ($P < 0.05$).

Ca/P increased slightly from day 1 to day 5 in both groups at microzone 4, but decreased at microzone 5 and 6 ($P > 0.05$) (Table 3, Fig. 6). Ca/P in experimental group was significantly lower than that in control group at microzone 4, 5, and 6 ($P < 0.05$).

Ca, P and O could not be detected in microzone 1, 2 and 3.

Discussion

In this study, we tested the influences of hyperlipidemia on dental implants osteointegration historically in rats at early stages (1st, 3rd, 5th day post-surgery). In our results, osseointegration at early stage was hindered in hyperlipidemic rats and this inhibition might be closely related to higher content of oxygen and lower ratio of calcium and phosphorus than normal rats.

Osteoblasts, which play a key role in osseointegration process, secrete bone matrix with type I collagen as its main component. The deposition of calcium and phosphorus on collagen fibers forms hydroxyapatite to mineralize bone matrix. Osteoblasts are surrounded by newly forming tissue to become bone cells, which in turn produce new bone tissue²⁸). Ca and P ions around the implant produce an initiating stimulation for osteoblasts resulting in an increasing of the amount of synthetic collagen and ultimately promotes the formation of new bone²⁹). In this study, we found that although the number of osteoblasts and trabecular bone around the two groups increased with time, the composition of bone cells and trabecular bone was less in hyperlipidemia group. There were more osteoclasts and trabecular space than the control group.

HF diet could induce the changes of bone morphological and mechanical properties, such as bone density and content of bone mineral, and affect implant osseointegration²). The osseointegration is similar to bone fracture healing. During fracture healing, osteoblasts deposit collagenous matrix. The first day after implantation, mesenchymal cells, pre-osteoblasts and osteoblasts adhered to implant surface and began to produce collagen fibrils, but in the coming days, osteoblasts began to deposit collagen matrix directly on the early formed cement line/lamina limitans layer described on the implant surface³⁰). Hyperlipidemia disrupted collagen processing as well as orientation, causing loss of local alignment, which might reduce mechanical integrity and quality of bone

and impair bone healing³¹⁾.

Epidemiological data showed that HF diet could increase lipoprotein levels and their oxidative products³²⁾, enhancing oxidative stress¹²⁾. Intracellular oxidative stress was the initial factor for changing of bone metabolism³¹⁾. Oxidative stress not only led to inhibition of osteogenic signaling pathway, but also decreased formation of mature osteoblasts and expression of molecular markers of bone remodeling³³⁻³⁵⁾. Serum lipid levels had an negative correlation with the bone mineral content, bone mineral density and mass³⁶⁾. It was confirmed that oxidative stress induced by ROS at implant-bone interface greatly reduced cells growth onto the implant surface and biological functions of osteoblasts, which would hinder bone repair at early^{13, 15)} and later stage, even lead to implant failure^{14, 37)}. Higher content of O in experimental group induced overproduction of ROS, which decreased osteoblasts and new bone formation in hyperlipidemia.

Ca had principal roles at almost every stage of bone repair process: from the formation of the provisional matrix to mineralization and bone remodeling³⁸⁾. Studies *in vitro* showed that increased concentrations of Ca and P ions in the medium contributed to proliferation and differentiation of pre-osteoblasts and MSCs to osteoblasts, leading to new bone formation³⁹⁻⁴¹⁾. Ti implant surfaces modified with Ca ions stimulated osteoblasts attachment, proliferation and differentiation, and improved implant osseointegration⁴²⁾. In addition, Ca and P ions-co-immobilization onto Ti implant could stimulate the response of pre-osteoblasts, with greater alkaline phosphatase activity and bone-like nodule formation⁴³⁾.

A normal range of Ca/P will lead to optimal bone mineralization and bone formation^{17, 22, 44)}. The Ca/P of native bone hydroxyapatite (HA) is 1.67 in human, so most bone related bioactive materials aims at this criteria. An optimal range of Ca/P was from 1.57 to 1.71, which are closer to mature bone HA, could be beneficial to bone mineralization and formation. Furthermore at early calcification of nodules, 1.35 was good for bone mineralization⁴⁵⁾. While in this study, Ca/P was 1.1~1.3 in experimental group and 1.3~1.6 in control group, and Ca/P in experimental group was lower than that in control group. Ca/P in control group is more beneficial for bone mineralization and osteoblast differentiation at early stage.

In conclusion, hyperlipidemia hindered implant osseointegration in rats at early stage after implantation. This inhibition might be modulated by overproduction of oxidative stress and low Ca/P, which further affected osteoblast differentiation, bone mineralization and formation.

Acknowledgements

This work was supported by the National Natural Science Foundation of China [grant numbers 81671025] and the Department of Science and Technology of Shandong Province [grant number 2015GSF118186].

Conflict of Interest

There are no conflicts of interest in this study.

References

- Guiglia R, Di Fede O, Lo Russo L, Sprini D, Rini GB and Campisi G. Osteoporosis, jawbones and periodontal disease. *Med Oral Patol Oral Cir Bucal* 18: e93-99, 2013
- Keuroghlian A, Barroso ADV, Kirikian G, Bezouglaia O, Tintut Y, Tetradis S, Moy P, Pirih F and Aghaloo T. The effects of hyperlipidemia on implant osseointegration in the mouse femur. *J Oral Implantol* 41: E7-E11, 2015
- Carroll MD, Fryar CD and Kit BK. Total and high-density lipoprotein cholesterol in adults: United States, 2011-2014. *NCHS Data Brief* 1-8, 2015
- Choukroun J, Khoury G, Khoury F, Russe P, Testori T, Komiyama Y, Sammartino G, Palacci P, Tunali M and Choukroun E. Two neglected biologic risk factors in bone grafting and implantology: high low-density lipoprotein cholesterol and low serum vitamin D. *J Oral Implantol* 40: 110-114, 2014
- Pelton K, Krieder J, Joiner D, Freeman MR, Goldstein SA and Solomon KR. Hypercholesterolemia promotes an osteoporotic phenotype. *Am J Pathol* 181: 928-936, 2012
- Huang X, Wang Z, Li D, Huang Z, Dong X, Li C and Lan J. Study of microRNAs targeted Dvl2 on the osteoblasts differentiation of rat BMSCs in hyperlipidemia environment. *J Cell Physiol* 2017
- Pirih F, Lu J, Ye F, Bezouglaia O, Atti E, Ascenzi M-G, Tetradis S, Demer L, Aghaloo T and Tintut Y. Adverse effects of hyperlipidemia on bone regeneration and strength. *J Bone Miner Res* 27: 309-318, 2012
- Lu C, Saless N, Wang X, Sinha A, Decker S, Kazakia G, Hou H, Williams B, Swartz HM, Hunt TK, Miclau T and Marcucio RS. The role of oxygen during fracture healing. *Bone* 52: 220-229, 2013
- Wagegg M, Gaber T, Lohanatha FL, Hahne M, Strehl C, Fangradt M, Tran CL, Schoenbeck K, Hoff P, Ode A, Perka C, Duda GN and Buttgerit F. Hypoxia promotes osteogenesis but suppresses adipogenesis of human mesenchymal stromal cells in a hypoxia-inducible factor-1 dependent manner. *PLoS One* 7: 2012
- Merceron C, Vinatier C, Portron S, Masson M, Amiaud J, Guigand L, Cherel Y, Weiss P and Guicheux J. Differential effects of hypoxia on osteochondrogenic potential of human adipose-derived stem cells. *Am J Physiol Cell Physiol* 298: C355-364, 2010
- Holzwarth C, Vaegler M, Gieseke F, Pfister SM, Handgretinger R, Kerst G and Muller I. Low physiologic oxygen tensions reduce proliferation and differentiation of human multipotent mesenchymal stromal cells. *BMC Cell Biol* 11: 11, 2010
- Brodeur MR, Brissette L, Falstra L, Ouellet P and Moreau R. Influence of oxidized low-density lipoproteins (LDL) on the viability of osteoblastic cells. *Free Radic Biol Med* 44: 506-517, 2008
- Ueno T, Ikeda T, Tsukimura N, Ishijima M, Minamikawa H, Sugita Y, Yamada M, Wakabayashi N and Ogawa T. Novel antioxidant capability of titanium induced by UV light treatment. *Biomaterials* 108: 177-186, 2016
- Callaway DA and Jiang JX. Reactive oxygen species and oxidative stress in osteoclastogenesis, skeletal aging and bone diseases. *J Bone Miner Metab* 33: 359-370, 2015
- Lee YH, Bhattarai G, Park IS, Kim GR, Kim GE, Lee MH and Yi HK. Bone regeneration around N-acetyl cysteine-loaded nanotube titanium dental implant in rat mandible. *Biomaterials* 34: 10199-10208, 2013
- Olgun O and Aygun A. Nutritional factors affecting the breaking strength of bone in laying hens. *World Poultry Sci* 72: 821-832, 2016
- Acar DB, Kavuncuoglu S, Cetinkaya M, Petmezci E, Dursun M, Korkmaz O and Altuncu EK. Assessment of the place of tubular reabsorption of phosphorus in the diagnosis of osteopenia of prematurity. *Turk Pediatri Ars* 50: 45-50, 2015
- Mohammadi H, Hafezi M, Nezafati N, Heasark S, Nadernezhad A, Ghazanfari SMH and Sepantafar M. Bioinorganics in bioactive calcium silicate ceramics for bone tissue repair: bioactivity and biological properties. *J Ceram Sci Technol* 5: 1-12, 2014
- Habibovic P and Barralet JE. Bioinorganics and biomaterials: bone repair. *Acta Biomater* 7: 3013-3026, 2011

20. Gupta S and Reviakine I. Platelet activation profiles on TiO₂: effect of Ca²⁺ binding to the surface. *Biointerphases* 7: 28, 2012
21. Marie PJ. The calcium-sensing receptor in bone cells: a potential therapeutic target in osteoporosis. *Bone* 46: 571-576, 2010
22. Christmann V, Gradusen CJ, Kornmann MN, Roeleveld N, van Goudoever JB and van Heijst AF. Changes in biochemical parameters of the calcium-phosphorus homeostasis in relation to nutritional Intake in very-low-birth-weight infants. *Nutrients* 8: 2016
23. Shapiro R and Heaney RP. Co-dependence of calcium and phosphorus for growth and bone development under conditions of varying deficiency. *Bone* 32: 532-540, 2003
24. Coats AM, Zioupos P and Aspden RM. Material properties of subchondral bone from patients with osteoporosis or osteoarthritis by microindentation testing and electron probe microanalysis. *Calcif Tissue Int* 73: 66-71, 2003
25. Kourkoulis N, Balatsoukas I and Tzaphlidou M. Ca/P concentration ratio at different sites of normal and osteoporotic rabbit bones evaluated by Auger and energy dispersive X-ray spectroscopy. *J Biol Phys* 38: 279-291, 2012
26. Palma-Carrio C, Maestre-Ferrin L, Penarrocha-Oltra D, Penarrocha-Diogo MA and Penarrocha-Diogo M. Risk factors associated with early failure of dental implants. A literature review. *Med Oral Patol Oral Cir Bucal* 16: e514-517, 2011
27. Esposito M, Hirsch JM, Lekholm U and Thomsen P. Biological factors contributing to failures of osseointegrated oral implants. (II). Etiopathogenesis. *Eur J Oral Sci* 106: 721-764, 1998
28. Sage AP, Lu J, Atti E, Tetradis S, Ascenzi MG, Adams DJ, Demer LL and Tintut Y. Hyperlipidemia induces resistance to PTH bone anabolism in mice via oxidized lipids. *J Bone Miner Res* 26: 1197-1206, 2011
29. Xin L, Jamie G, Jinxiu L, Sidney I, Ivo K, David R, Demer LL and Yin T. Roles of parathyroid hormone (PTH) receptor and reactive oxygen species in hyperlipidemia-induced PTH resistance in preosteoblasts. *J Cell Biochem* 115: 179-188, 2013
30. Marco F, Milena F, Gianluca G and Vittoria O. Peri-implant osteogenesis in health and osteoporosis. *Micron* 36: 630-644, 2005
31. Ionova-Martin SS, Do SH, Barth HD, Szadkowska M, Porter AE, Ager JWR, Ager JW, Jr., Alliston T, Vaisse C and Ritchie RO. Reduced size-independent mechanical properties of cortical bone in high-fat diet-induced obesity. *Bone* 46: 217-225, 2010
32. Laurila A, Cole SP, Merat S, Obonyo M, Palinski W, Fierer J and Witztum JL. High-fat, high-cholesterol diet increases the incidence of gastritis in LDL receptor-negative mice. *Arterioscler Thromb Vasc Biol* 21: 991-996, 2001
33. Dong X, Wang Z, Wang H and Lan J. The research of dishevelled-2 in dental implant osseointegration of hyperlipidemic rats. *Int J Oral Maxillofac Implants* 33: 351-356, 2018
34. Maziere C, Salle V, Gomila C and Maziere JC. Oxidized low density lipoprotein enhanced RANKL expression in human osteoblast-like cells. Involvement of ERK, NFkappaB and NFAT. *Biochim Biophys Acta* 1832: 1756-1764, 2013
35. Almeida M, Ambrogini E, Han L, Manolagas SC and Jilka RL. 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* 284: 27438-27448, 2009
36. Wu S and De Luca F. Role of cholesterol in the regulation of growth plate chondrogenesis and longitudinal bone growth. *J Biol Chem* 279: 4642-4647, 2004
37. Koerdts S, Siebers J, Bloch W, Ristow O, Kuebler AC and Reuther T. Role of oxidative and nitrosative stress in autogenous bone grafts to the mandible using guided bone regeneration and a deproteinized bovine bone material. *J Cranio Maxill Surg* 42: 560-567, 2014
38. Anitua E, Prado R, Orive G and Tejero R. Effects of calcium-modified titanium implant surfaces on platelet activation, clot formation, and osseointegration. *J Biomed Mater Res A* 103: 969-980, 2015
39. Welldon KJ, Findlay DM, Evdokiou A, Ormsby RT and Atkins GJ. Calcium induces pro-anabolic effects on human primary osteoblasts associated with acquisition of mature osteocyte markers. *Mol Cell Endocrinol* 376: 85-92, 2013
40. Barradas AM, Fernandes HA, Groen N, Chai YC, Schrooten J, van de Peppel J, van Leeuwen JP, van Blitterswijk CA and de Boer J. A calcium-induced signaling cascade leading to osteogenic differentiation of human bone marrow-derived mesenchymal stromal cells. *Biomaterials* 33: 3205-3215, 2012
41. Khoshniat S, Bourguin A, Julien M, Petit M, Pilet P, Rouillon T, Masson M, Gatiús M, Weiss P, Guicheux J and Beck L. Phosphate-dependent stimulation of MGP and OPN expression in osteoblasts via the ERK1/2 pathway is modulated by calcium. *Bone* 48: 894-902, 2011
42. Anitua E, Pinás L, Murias A, Prado R and Tejero R. Effects of calcium ions on titanium surfaces for bone regeneration. *Colloids Surf B Biointerfaces* 130: 173-181, 2015
43. Sunarso, Toita R, Tsuru K and Ishikawa K. Immobilization of calcium and phosphate ions improves the osteoconductivity of titanium implants. *Mater Sci Eng C Mater Biol Appl* 68: 291-298, 2016
44. De Curtis M and Rigo J. Nutrition and kidney in preterm infant. *J Matern Fetal Neonatal Med* 25 Suppl 1: 55-59, 2012
45. Bonucci E. Bone mineralization. *Front Biosci (Landmark Ed)* 17: 100-128, 2012