ACTUALIZACIONES EN OSTEOLOGÍA

Asociación Argentina de Osteología y Metabolismo Mineral

VOL. 15, Nº 3 - septiembre / diciembre 2019

Indizada en SCImago, EBSCO, Latindex, LILACS, SciELO, Scopus & Embase y SIIC Data Bases

A.A.O.M.M.
(Asociación Argentina de Osteología
y Metabolismo Mineral)

ISSN 1669-8975 *(Print)*;
ISSN 1669-8983 *(Online)*
Revista Cuatrimestral
Rosario, Santa Fe, Argentina
www.osteologia.org.ar

ADROMUX®

ACIDO IBANDRONICO 150 mg

El Ibandronato de Gador

TAMBIEN x3 comprimidos recubiertos

- Una toma mensual[1]
- Beneficio a largo plazo[2]
- Reduce el riesgo de fracturas osteoporóticas[3]

PAP Preserfar · PAMI INSSJP · IOMA Instituto de Obra Médico Asistencial · Recetario Solidario

PRESENTACIONES:
Envases con 1 y 3 comprimidos recubiertos conteniendo 150 mg de ácido ibandrónico.

Para más información sobre ADROMUX®, visite www.gador.com.ar

Referencias: **1.** Prospecto Adromux®, Gador S.A., FUR ANMAT: Ene 2012. **2.** Miller P, et al. Efficacy of monthly oral ibandronate is sustained over 5 years: the MOBILE long-term extension study. Osteoporos Int 2012; 23: 1747-1756. **3.** Harris S.T, et al. Ibandronate and the risk of non-vertebral and clinical fractures in women with postmenopausal osteoporosis: result of a meta-analysis of phase III studies. Curr Med Res Opin 2008; 24 (1): 237-245.

Gador Osteología

DOMECQ & LAFAGE

Laboratorios Clínicos

Nuestra Prioridad es el paciente

Más de 60 años en el área de la salud en la Argentina, centrado en el bienestar, en la seguridad del paciente y en la asistencia a la comunidad médica.

Cantidad de **PACIENTES** atendidos por día

1.000

Cantidad de **PRÁCTICAS** informadas mensualmente

190.000

Tiempo de **RESPUESTA** para rutina ambulatoria

24 hs.

Tiempo de **RESPUESTA** para urgencias en el Hosp. Alemán

1 hora

Sedes

Hospital Alemán
Av. Pueyrredón 1640
Tel.: 4827.7000

Recoleta
Paraná 1395
Tel.: 4811.5566

Villa Devoto
Av. Segurola 2127
Tel.: 4566.5734

Villa Urquiza
La Pampa 5017
Tel.: 4523.6936

Villa del Parque
Helguera 2880
Tel.: 4822.1008

Ramos Mejía
Alsina 520
Tel.: 4654.7120

Martínez
Pje. Lamarca 383
Tel.: 4793.3191

www.labdl.com.ar

ACTUALIZACIONES EN OSTEOLOGÍA

Asociación Argentina de Osteología y Metabolismo Mineral

XXXVI REUNIÓN ANUAL

Asociación Argentina de Osteología y Metabolismo Mineral

María Linzoain,
"Confidencias", 2004.
Acrílico sobre lienzo, 60 x 80 cm.
Galería Zurbarán.

VOL. 15, Nº 3
septiembre /diciembre 2019
ISSN 1669-8975 *(Print)*; ISSN 1669-8983 *(Online)*
www.osteologia.org.ar
Rosario, Santa Fe, Argentina

Indizada en SCImago, EBSCO, Latindex, LILACS, SciELO, Scopus & Embase y SIIC Data Bases

ACTUALIZACIONES EN OSTEOLOGÍA

Publicación de la Asociación Argentina de Osteología y Metabolismo Mineral.

VOL. 15, Nº 3
septiembre / diciembre 2019
ISSN 1669-8975 (Print); ISSN 1669-8983 (Online)
www.osteologia.org.ar
Rosario, Santa Fe, Argentina
Aparición: cuatrimestral

Asociación Argentina de Osteología y Metabolismo Mineral
PROPIETARIO: Asociación Argentina de Osteología y Metabolismo Mineral
DOMICILIO LEGAL: 9 de julio 1324, (2000) Rosario, Santa Fe, Argentina
www.aaomm.org.ar / info@aaomm.org.ar

Perfil de la revista

Actualizaciones en Osteología es el órgano científico de la Asociación Argentina de Osteología y Metabolismo Mineral (AAOMM). Actualizaciones en Osteología acepta para su publicación trabajos redactados en español o en inglés, que aborden aspectos clínicos o experimentales dentro de la osteología y el metabolismo mineral que puedan considerarse de utilidad e interés para nuestra comunidad científica. Dichos trabajos habrán de ser inéditos, cumplir los requisitos de uniformidad para el envío demanuscritos y estar comprendidos en algunas de las secciones de la revista (Actualizaciones, Artículos Originales, Comunicaciones Breves, Casuísticas, Editoriales, Cartas al Editor). Los artículos son revisados por pares, expertos nacionales e internacionales.

Los artículos publicados en Actualizaciones en Osteología son indizados en SCImago (Journals and Country Scientific Indicators), EBSCO (EBSCO Host Research Databases), Latindex (Sistema Regional de Información en Línea para Revistas Científicas de América Latina, el Caribe, España y Portugal), LILACS (Literatura Latinoamericana en Ciencias de la Salud), base de datos corporativa del Sistema BIREME (Centro Latinoamericeno y del Caribe de Información en Ciencias de la Salud), SciELO (Scientific Electronic Library Online), Scopus & Embase (Elsevier Bibliographic Databases) y SIIC Data Bases (Sociedad Iberoamericana de Información Científica).

Scope

Actualizaciones en Osteología is the official scientific journal of the Argentinean Association of Osteology and Mineral Metabolism (AAOMM). Actualizaciones en Osteogía publishes manuscripts written in Spanish or English describing clinical and experimental aspects within osteology and mineral metabolism. The articles should be original, meet the uniform requirements for manuscript submission and be comprised in one of the sections of the journal (Original Articles, Review Articles, Short Communications, Case Reports, Editorials, Letters to the Editor). Articles are peer-reviewed by national and international experts in the field.

The articles published in Actualizaciones en Osteología are indexed in SCImago (Journals and Country Scientific Indicators), EBSCO (EBSCO Host Research Databases), Latindex (Regional Information System for Scientific Journals Online of Latin America, the Caribbean, Spain and Portugal), LILACS (Latin American Literature in Health Sciences), BIREME (Latin American and Caribbean Center on Health Sciences), SciELO (Scientific Electronic Library Online), Scopus & Embase (Elsevier Bibliographic Databases) and SIIC data Bases (Iberoamerican Society Scientific Information).

ACTUALIZACIONES EN OSTEOLOGÍA

Publicación de la Asociación Argentina de Osteología y Metabolismo Mineral.

AUTORIDADES DE AAOMM
COMISIÓN DIRECTIVA 2018-2019

Presidente
Dra. Susana Zeni

Vicepresidente
Dr. Lucas Brun

Secretaria
Dra. Graciela Brito

Tesorera
Dra. Mariana Seijo

Vocales
Dra. Carola Bozal
Dr. Adrián Campelo
Dra. Silvana Di Gregorio
Dra. Sara Feldman
Dra. Gabriela Picotto
Dra. Gloria Rovai
Dr. Fernando Saravi
Dra. Ana Wittich

ACTUALIZACIONES EN OSTEOLOGÍA

Vol 15, Nº 3, septiembre / diciembre 2019

ÍNDICE

Actual. Osteol 2019; 15(3): 177-179.
Internet: http://www.osteologia.org.ar

EDITORIAL / ***Editorial***

OSTEOCALCINA Y RESPUESTA AL ESTRÉS AGUDO

Fernando Daniel Saraví*

Instituto de Fisiología, Facultad de Ciencias Médicas, Universidad Nacional de Cuyo. Servicio de Densitometría, Escuela de Medicina Nuclear. Mendoza. Instituto Balseiro, Universidad Nacional de Cuyo, San Carlos de Bariloche, Río Negro. Argentina.

La osteocalcina es la proteína no colágena más abundante de la matriz ósea. Es sintetizada por los osteoblastos (y odontoblastos), donde es carboxilada (glaOC) en una reacción dependiente de vitamina K, y posteriormente secretada, uniéndose a la hidroxiapatita. La osteocalcina puede descarboxilarse en el medio ácido generado por la actividad osteoclástica. Además, como se verá después, puede ser secretada por los osteoblastos sin previa carboxilación (gluOC).

Si bien se reconoce su utilidad como marcador de formación y recambio óseos, tras décadas de investigación aún no resulta claro el papel exacto de la osteocalcina en la mineralización.[1]

Por otra parte, la gluOC tiene diversos efectos sistémicos de tipo hormonal. La pista inicial fue la observación de que el fenotipo de ratones con supresión del gen de osteocalcina incluía exceso de grasa corporal y escasa fertilidad.[2]

Entre los efectos hormonales citados se incluyen la estimulación de la liberación de insulina y de la multiplicación de las células beta pancreáticas.[3] La gluOC también estimula la secreción de adiponectina por los adipocitos, lo que incrementa la sensibilidad tisular a la insulina.[4]

A su vez, la estimulación del receptor de insulina de los osteoblastos es necesaria para la adquisición de una masa ósea normal y para la secreción de osteocalcina.[5] Al menos en parte, el efecto de la insulina puede deberse a la regulación de una fosfatasa osteotesticular (OTP-PCP; gen *Esp*). La anulación del gen *Esp* causa un fenotipo opuesto al de la anulación del gen de la osteocalcina. El eje metabólico osteopancreático ha sido recientemente objeto de revisión en esta revista.[6]

En el músculo, la insulina aumenta la captación de glucosa y ácidos grasos con un efecto neto principalmente anabólico. Contrariamente a lo que ocurre con la secreción de insulina, la secreción de osteocalcina aumenta durante el ejercicio. La osteocalcina también aumenta la captación de glucosa y ácidos grasos, pero estimula su utilización como sustratos energéticos; en otras palabras, un efecto catabólico. Además, la osteocalcina estimula la secreción de interleucina 6 por los miocitos. Esta citocina, a su vez, estimula la secreción de osteocalcina descarboxilada. La expresión del receptor de osteocalcina en el músculo es necesaria para la adaptación al ejercicio y contribuye al mantenimiento de la masa muscular.[7]

E-mail: fernando.saravi@hotmail.es

La evidencia brevemente mencionada sugiere un importante papel del hueso en general, y de la osteocalcina en particular, en la regulación del metabolismo. A ello debe añadirse un posible papel en la reproducción. En efecto, la gluOC estimula la secreción de testosterona por las células de Leydig, mientras que, por el contrario, no afecta la secreción de esteroides ováricos. Los efectos de la gluOC hasta aquí descriptos dependen de un receptor acoplado a proteína G llamado Gprc6a.[8]

Por otra parte, la osteocalcina también influye en el desarrollo del sistema nervioso, que no expresa Gprc6a; no obstante, se ha identificado allí otro receptor llamado Gpr158.[9] Los ratones carentes de osteocalcina muestran conducta pasiva, aumento de la ansiedad y déficit de memoria y aprendizaje. Durante el desarrollo, la gluOC penetra la barrera hematoencefálica y se une a neuronas del rafe dorsal, el área tegmental ventral y el hipocampo. La osteocalcina estimula la síntesis de monoaminas y reduce la síntesis de GABA. Además reduce la apoptosis en el hipocampo.[8]

Todo lo anterior ha llevado a postular una coordinación regulada del desarrollo esquelético, el metabolismo energético, la función muscular y los mecanismos cognitivos, en la cual la osteocalcina tendría un papel central.[2,8]

Con esta hipótesis en mente, es muy interesante, aunque quizá no demasiado sorprendente, que se haya informado recientemente evidencia de la participación de la gluOC en respuesta al estrés agudo.[10] La exposición al estrés agudo en animales y humanos causa un rápido e importante aumento de la gluOC plasmática. Dicho incremento es selectivo para la osteocalcina, ya que no afecta otras moléculas provenientes del hueso. El efecto reflejo, dependiente de la amígdala, parece mediado por fibras nerviosas que liberan glutamato en la proximidad de los osteoblastos. Estos incorporan el neurotransmisor mediante un transportador llamado Glast o Eaat1 y responden liberando gluOC. El efecto parece deberse a la inhibición de la carboxilación de la osteocalcina por inhibición de la enzima carboxilante (gamma-carboxilasa) por el glutamato incorporado.

Las respuestas al estrés observadas en ratones incluyeron aumento del gasto energético, la glucemia, la temperatura corporal y la frecuencia cardíaca, menor resistencia de las vías aéreas y mayor saturación arterial de oxígeno. Estas respuestas al estrés no se observaron en ratones con inactivación del gen de OC o del receptor Gprc6a (pero no del Gpr58).

Curiosamente, la gluOC no modificó la respuesta simpática periférica al estrés. En cambio, la gluOC redujo la descarga parasimpática al corazón y los bronquios y la síntesis periférica de acetilcolina. Estas respuestas requerían la presencia de receptores Gprc6a en neuronas posganglionares parasimpáticas. Por otra parte, el aumento del consumo de energía durante el estrés agudo no disminuyó con la anulación de los receptores Gprc6a ni Gpr158, sugiriendo que tal respuesta es mediada por un tercer receptor aún no identificado.

La inyección de gluOC produjo una respuesta fisiológica similar al estrés agudo. Cabe notar que la respuesta al estrés, dependiente al menos en parte de OC, se presentó también en ratones normales adrenalectomizados, pero no en animales adrenalectomizados con supresión total o parcial del gen de osteocalcina. Los autores citan evidencia de que los humanos con deficiencia de glucocorticoides conservan la capacidad de responder al estrés.

El posible papel de la osteocalcina en la respuesta al estrés agudo enriquece el cambiante panorama de hueso como órgano endocrino y su papel biológico en la adaptación al ambiente, la supervivencia y la reproducción.

Conflictos de intereses: el autor declara no tener conflictos de intereses.

Referencias

1. Lombardi G, Perego S, Luzi L, Banfi G. A four-season molecule: osteocalcin. Updates in its physiological roles. *Endocrine*. 2015; 48:394-404.
2. Wei J, Karsenty G. An overview of the metabolic functions of osteocalcin. *Rev Endocr Metab Disord*. 2015; 16:93-8.
3. Ferron M, Wei J, Yoshizawa T, et al. Insulin signaling in osteoblasts integrates bone remodeling and energy metabolism. *Cell*. 2010; 142:296-308.
4. Lee NK, Sowa H, Hinoi E, et al. Endocrine regulation of energy metabolism by the skeleton. *Cell*. 2007; 130:456-69.
5. Fulzele K, Riddle RC, DiGirolamo DJ, et al. Insulin receptor signaling in osteoblasts regulates postnatal bone acquisition and body composition. *Cell*. 2010; 142:309-19.
6. Battaglino R. El esqueleto como órgano endocrino: funciones metabólicas de la osteocalcina. *Actual Osteol*. 2017; 13:225-32.
7. Moser SC, van der Eerden BCJ. Osteocalcin – a versatile bone-derived hormone. Front *Endocrinol*. 2019; 9:794.
8. Karsenty G. Update on the biology of osteocalcin. *Endocr Pract*. 2017; 23:1270-4.
9. Khrimian L, Obri A, Ramos-Brossier M, et al. Gpr158 mediates osteocalcin's regulation of cognition*. J Exp Med*. 2017; 214:2859-73.
10. Berger JM, Singh P, Khrimian L, et al. Mediation of acute stress response by the skeleton. *Cell Metab*. 2019; 30:1-13.

Actual. Osteol 2019; 15(3): 180-191.
Internet: http://www.osteologia.org.ar

ARTÍCULOS ORIGINALES / *Originals*

INTERCELLULAR MEDIATORS IN BONE REMODELING REGULATION IN THE EXPERIMENTAL RENAL PATHOLOGY

Sergey Pavlov,[1*] Nataliia Babenko,[1] Marina Kumetchko,[1] Olga Litvinova,[1] Natalia Semko,[1] Olga Pavlova[2]

[1] Central Research Laboratory, Kharkiv Medical Academy of Postgraduate Education, Kharkiv, Ukraine. [2] Department of Adolescence Medicine, Kharkiv Medical Academy of Postgraduate Education, Kharkiv, Ukraine.

Abstract

Bone metabolism disorders are characterized by an imbalance of bone resorption and formation in the bone remodeling process. Glucocorticoids that are used to treat kidney diseases exacerbate these disorders. P-selectin and galectin-3 are molecules involved in the sclerotic process in kidney, whereas bone resorption is regulated by the interaction between the nuclear factor activator kappa β receptor (RANK), its ligand (RANKL) and the RANKL decoy receptor osteoprotegerin (OPG).

The aim of this study was to investigate the cellular and molecular mechanisms of disruption of bone remodeling regulation processes, reflected by intercellular mediators (RANKL, OPG, P-selectin and galectin-3) in chronic kidney disease experimental model treated with glucocorticoids.

Rats were divided into four groups of 10 animals each. The first group, the control group, included intact animals. The second group consisted of rats with impaired bone remodeling resulting from chronic kidney disease (experimental group (CKD). The third group was a group of animals with impaired bone remodeling due to exposure to glucocorticoids (experimental group (GCs)). The fourth group consisted of rats with impaired bone remodeling in chronic kidney disease, followed by exposure to glucocorticoids (experimental group (CKD + GCs)). The effects of CKD and glucocorticoid were evaluated biochemically, histologically and by measuring bone density. An enzyme-linked immunoassay was used to measure intercellular mediator levels in the serum.

The bone density in the experimental groups was reduced compared to the control group. RANKL levels in animals of three experimental groups were higher than in intact animals. Serum levels of OPG were higher in CKD and GCs groups than in intact animals. At the same time, in the animals' blood serum of the CKD + GCs group, the levels of OPG were lower, than those in animals from the control group. The levels of galectin-3 in the serum of the experimental groups GCs and CKD + GCs were lower than in intact animals. The serum levels of galectin-3 in animals of the CKD group

**E-mail: cndl@med.edu.ua*

were higher than those in animals from the control group. The levels of P-selectin were lower in the serum of the GCs group than in intact animals. At the same time, the levels of P-selectin were higher in the CKD and CKD + GCs groups, than those in animals from the control group.

In conclusion, the study of the complex system of bone remodeling regulation, which includes many factors and their interactions, may lead to the development of new methods for treating patients with chronic kidney disease in order to prevent osteoporosis in the future.

Keywords: boneremodeling, renalinsufficiency, chronic, glucocorticoids, cytokines.

Resumen

Las enfermedades metabólicas óseas se caracterizan por un desequilibrio en el proceso de remodelación ósea en los que participan mediadores tales como receptor del activador del factor nuclear- kappa- β (RANK), su ligando (RANKL) y la osteoprotegerina (OPG). Los glucocorticoides, frecuentemente empleados en el tratamiento de la enfermedad renal crónica, exacerban este desequilibrio. En la enfermedad esclerótica renal, las moléculas de adhesión celular P-selectina and galectina-3 tienen un rol fundamental.

El objetivo de esta trabajo fue estudiar las alteraciones en los mediadores de la remodelación ósea (RANKL, OPG, P-selectina and galectina-3) en un modelo de enfermedad renal crónica con tratamiento glucocorticoideo.

Rratas Wistar hembras fueron divididos en 4 grupos: control (C); enfermedad renal crónica con afección de la remodelación ósea (ERC); animales con afección de la remodelación ósea expuestos a glucocorticoides (GC); enfermedad renal crónica con afección de la remodelación ósea tratados con glucocorticoides (ERC+GC). Los efectos de la ERC y los GC fueron evaluados bioquímicamente, histológicamente y por medición de la densidad ósea. RANKL, OPG, P-selectina and galectina-3 se cuantificaron en muestras de sangre venosa empleando enzimoinmuno análisis.

En los 3 grupos experimentales la densidad ósea se evidenció reducida y los niveles séricos de RANKL elevados respecto al grupo control. Los niveles de OPG en los grupos ERC y GC fueron superiores mientras que en el grupo ERC+GC menores respecto a los animales controles. Galectina 3 plasmática en GC y ERC+GC se encontró reducida y aumentada en los animales ERC, en comparación con los animales controles. La concentración sérica de P-selectina sérica fue mayor en los grupos ERC y ERC+GC, y menor en los animales GC respecto a los niveles plasmáticos de los animales intactos.

El avance del conocimiento sobre la regulación de la remodelación ósea a través de la interacción de mediadores sistémicos, en un futuro, puede conducir al desarrollo de nuevas estrategias terapéuticas para la prevención de la osteoporosis en pacientes con enfermedad renal crónica.

Palabras clave: remodelación ósea, enfermedad renal crónica, glucocorticoides; citoquinas.

Introduction

Chronic kidney disease (CKD) affects 10-15% of the population worldwide.[1] Reduction in renal function in CKD patients affects a number of interrelated secondary pathophysiological processes, including mineral and bone disorders.[2] The impaired bone metabolism in individuals with kidney function insufficiency determines the need for early detection and prevention of CKD and its associated complications. Thus, it is necessary to search for markers that reflect the presence of pathological changes in the renal tissue and determine their nature.

Glucocorticoids (GCs) are widely used to treat various inflammatory diseases, including kidney disease, due to their anti-inflammatory actions through the suppression of the production of pro-inflammatory cytokines. At the same time, GCs suppress bone formation due to both disruption of the functional activity of osteoblasts, as well as reduction of their number, and impaired precursor differentiation.[3] Unfortunately, our knowledge about molecular regulators that modulate the differentiation and activity of osteoclasts and osteoblasts is still insufficient.[4]

Many intercellular mediators are involved in the processes of bone resorption and formation, as well as in the stages of kidney fibrosis. There may be interdependencies between bone remodeling disorders and kidney pathology, realized through cytokines, which simultaneously affect bone and kidney tissue.[5]

The cytokine system comprising the nuclear factor activator kappa β receptor (RANK), its ligand (RANKL) and the decoy receptor osteoprotegerin (OPG) play key roles in the regulation of bone remodeling. This cytokine system is also actively involved in the regulation of such processes as angiogenesis, neovascularization and remodeling of the vessel wall.[6]

Chronic kidney disease is a consequence of the interstitial extracellular matrix expansion, which leads to nephron loss. Renal tissue remodeling disorder is caused by an imbalance between cell proliferation and apoptosis. The selectin and galectin family of proteins play an important role in these processes. Selectins mediate the migration of inflammatory cells to the renal interstitium, which, in turn, can cause apoptosis and tubular atrophy, and interstitial fibrosis.[7] Galectin-3 is able to trigger apoptosis through the extracellular and mitochondrial pathways, exerting both pro-and anti-apoptotic actions.[8] The aim of this study was to investigate the processes that lead to the regulation of bone remodeling by intercellular mediators (e.g., RANKL, OPG, P-selectin and galectin-3) in experimental chronic kidney disease subsequent exposure to glucocorticoids.

Materials and methods

An experimental study was conducted in four groups of female white Wistar rats aged 9 months and weighing 250 ± 30 g, in accordance with the principles of the European Convention for the Protection of Vertebrate Animals (Strasbourg, 1986) and the rules for working with experimental animals approved by the Bioethics Committee of Kharkiv Medical Academy of Postgraduate Education.

The first group – the control group, included intact animals (n = 10). The second group (n = 10) consisted of rats with an impaired bone remodeling resulting from chronic kidney disease (experimental group (CKD). The third group (n = 10) was a group of animals with an impaired bone remodeling under the influence of glucocorticoids (experimental group (GCs). The creation of a model of experimental bone remodeling disorders under the influence of glucocorticoids was carried out by injecting dexamethasone phosphate at a dose of 6 mg/kg intramuscularly twice a week for a month.[9] The fourth group (n = 10) consisted of rats with an impaired bone remodeling in chronic kidney disease followed by glucocorticoid exposure (experimental group (CKD + GCs). The model

of kidney damage in CKD and CKD + GCs groups was performed by a single injection of 50% glycerol solution at a dose of 10 ml/kg of animal body weight. The development of CKD was controlled in accordance with the methodology of the model's authors.[10] Glomerular filtration rate and morphological changes in kidney tissue were evaluated. Six weeks after the injection of glycerol, animals were injected with dexamethasone phosphate at a dose of 6 mg/kg intramuscularly twice a week for a month.[9] Animals were euthanized by inhalation of chloroform in a confined space. Bone density was measured as the ratio of bone mass (grams) to its volume (cubic centimeters).[11] The femora were separated, cleaned of soft tissues and weighed. Since the study was focused only in changes in bone density, not all organic components of the bone (such as collagen fibrils, components of the bone marrow) were removed before measurement. The error associated with the presence of organic component was considered negligible. The volume of the femur was determined by the displaced fluid volume. For each animal, the average value of the femoral parameters was determined, consisting of the obtained values for the right and left femur. Based on the measurement results, bone density was calculated.

Histology of the kidneys was performed in samples fixed in 10% neutral formalin, and then dehydrated in increasing strength of alcohols (50°, 70° and twice 96°), then alcohol with chloroform was used, then chloroform, followed by paraffin embedding.[12] Sections, 5-7 microns thick, were stained with hematoxylin and eosin, or picric acid/acid fuchsin, following the Van Gieson' s method.

For histological examination, the thoracic and lumbar spine vertebrae of the rats were isolated. The material was fixed in 10% neutral formalin, decalcified in 5% nitric acid, embedded in paraffin according to a conventional technique.[12] Sections, 7-10 #m thick, were stained with hematoxylin and eosin, or picric acid/acid fuchsin, following the Van Gieson' s method.

The sections were visualized using a "Primo Star" were (Carl Zeiss). Photomicrographs of the preparations were obtained using a Microocular digital camera.

Studies of the cytokine level were performed in serum by enzyme immunoassay. Blood samples we collected from the heart. The levels of RANKL were measured using the «ampli-sRANKL» kit (BIOMEDICA, Austria). OPG levels were determined using the «Human Osteoprotegerin Instant» kit (eBioscience, Austria), and the P-selectin levels were determined using the «Human sP-selectin Platinum ELISA» kit (eBioscience, Austria). The levels of galectin-3 were determined using the «Human Galectin-3 Platinum ELISA» kit (eBioscience, Austria).

Resultsarepresentedasmean±SE(standard error of the arithmetic average). Statistical analyses of the results were performed using the Statistica 6.0 software package, using the non-parametric Kruskal-Wallis test for independent samples and correlation analysis. Differences were considered statistically significant with p values < 0.05.

Results

The measured bone density of animals of the experimental groups was significantly reduced compared with control group (Table). The kidneys of experimental rats of CKD and CKD + GCs groups revealed significant structural changes, suggesting the disruption of the excretory function of the organ. Thus, diffuse venous-capillary plethora is noted in all the specimens, blood separation into plasma and uniform elements, erythrostasis is observed in the dilated vessels, which is a manifestation of a disruption of the blood supply to the organ and the rheological properties of the blood (Fig. 1).

The structure and shape of the renal glomeruli are preserved. Also there are glomeruli of a "branched" form, which can be a manifestation of microcirculatory disorders.

Table. Changes in bone density, cytokines and lectins in the control and experimental groups.

Parameter	Control group	Group with impaired bone remodeling in CKD	Group with impaired bone remodeling in GCs	Group with impaired bone remodeling in CKD + GCs
Bone density (g/cm)	1.62 ± 0.032	1.43 ± 0.032*	1.37 ± 0.041*	1.53 ± 0.026*
RANKL, pmol/l	0.131 ± 0.020	0.184 ± 0.041	0.167 ± 0.046	0.158 ± 0.043
OPG, pg/ml	21.588 ± 2.015	28.338 ± 2.431	27.177 ± 5,386	16.588 ± 1.633
Galectin-3, ng/ml	1.151 ± 0.075	1.208 ± 0,095	1.117 ± 0.086	0.592 ± 0.037*
P-selectin, ng/ml	2.231 ± 0.080	2.956 ± 0,060*	1.656 ± 0.107*	3.380 ± 0.062*

* $p < 0.05$ in comparison with the control group

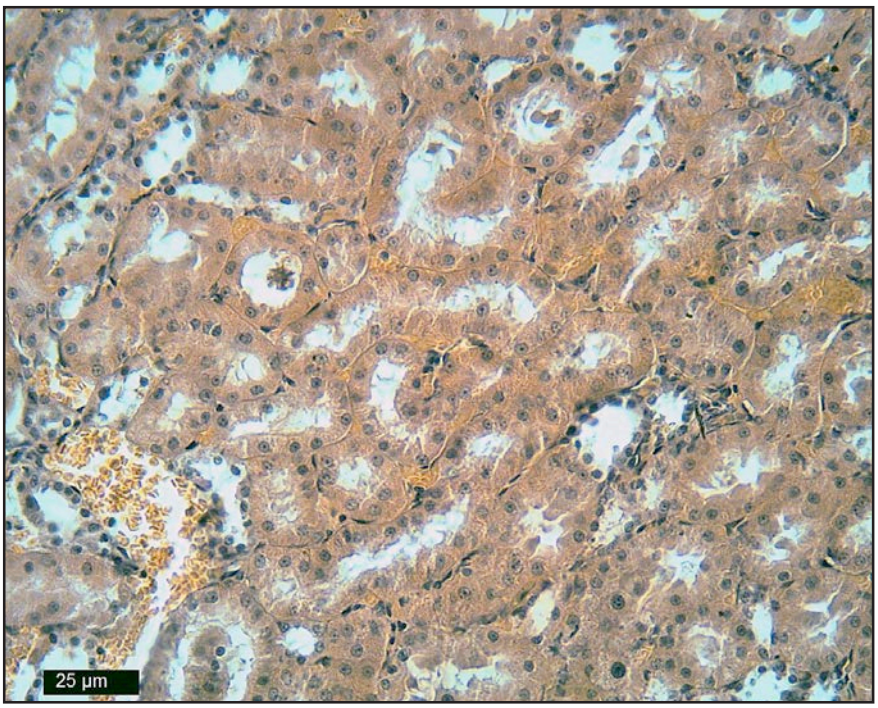

Figure 1. Section of rat renal cortex. Venous-capillary plethora Dystrophy of the epithelium. Hematoxylin and staining.

Polygonal and wrinkled glomeruli with enlarged capsule lumen, indicative of edema and atrophy were observed. Furthermore, nephrosclerosis centers in the cortex were noted. Connective tissue with a large number of fibroblasts with large, brightly colored functionally active nuclei, were seen in a destructively altered renal epithelium, gradually replacing it (Fig. 2).

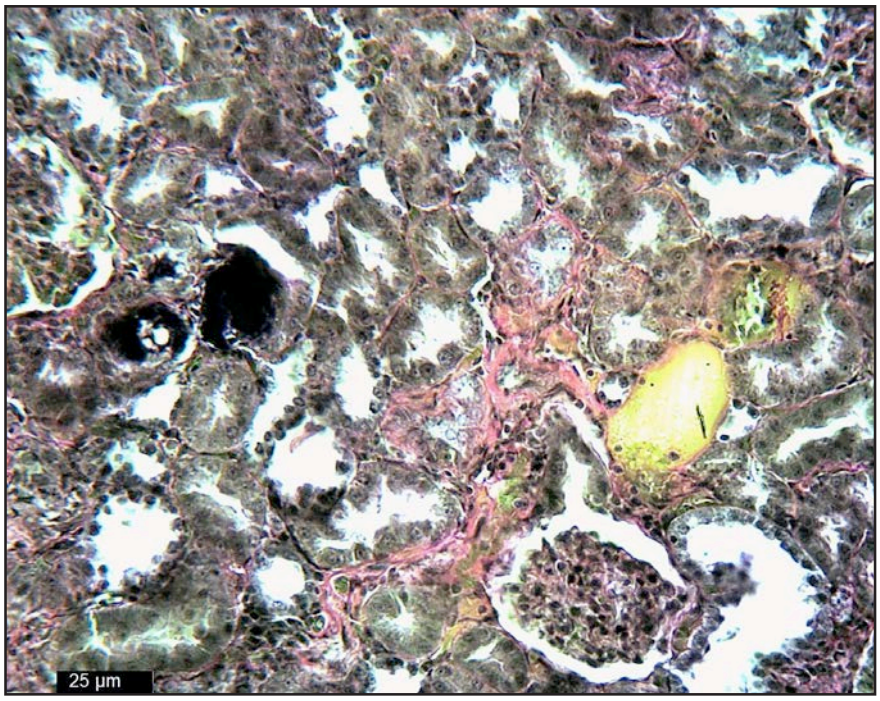

Figure 2. Histological section of the rat renal cortex. There are nephrosclerosis centers. The shape of glomeruli is polygonal. Calcification and colloid-like substance in the lumens of the tubules. Van Gieson `s stain.

Thus, histological examination confirms that a single injection of a glycerol solution in experimental animals of the CKD and CKD + GCs groups leads to dystrophic and necrotic changes in the kidney tubular apparatus, resulting in CKD.

Microscopic examination of histological preparations of vertebral bodies in the control group rats showed a typical structure of bone tissue. Spongy bone consisted of wide anastomosing trabeculae, separated by medullary spaces, which contained red bone marrow. Lacunae with osteocytes were located in the bone tissue of trabeculae and dark blue, slightly wavy cement lines were clearly visible. The cortex, represented by a compact bone, had enough width along the entire length.

Alteration of bone tissue histology was revealed by microscopic examination of the vertebral bodies of rats of three experimental groups (Fig. 3).

In the cancellous bone, these disorders were associated with a decrease in the trabeculae thickness and the trabecular meshwork density reduction the number trabeculae and their contacts with each other and with the cortical plate were decreased. Most of the bone beams were thinned and had uneven edges and blind ends, which indicates the predominance of bone resorption processes. Compared to the control group, in histological preparations of experimental animals osteocytic lacunae of osteocytes containing cells at different stages of necrobiosis, uneven staining of the main substance of bone tissue, basophilia, and thickening of cement lines were noted. The bone marrow contained a significant amount of adipocytes, i.e. it was mixed. The cortical plate of the vertebral bodies was uneven

in width and thinned. The compact bone density reduction process was confirmed by the presence of dilated osteocyte lacunae, vascular channels and single cavities filled with reticulo-fibrous tissue and red bone marrow. There were various sizes of osteocytes with an uneven distribution. Severe basophilia of the lacunae walls part and mosaic-colored in the matrix areas reflected an alteration of the calcification process.

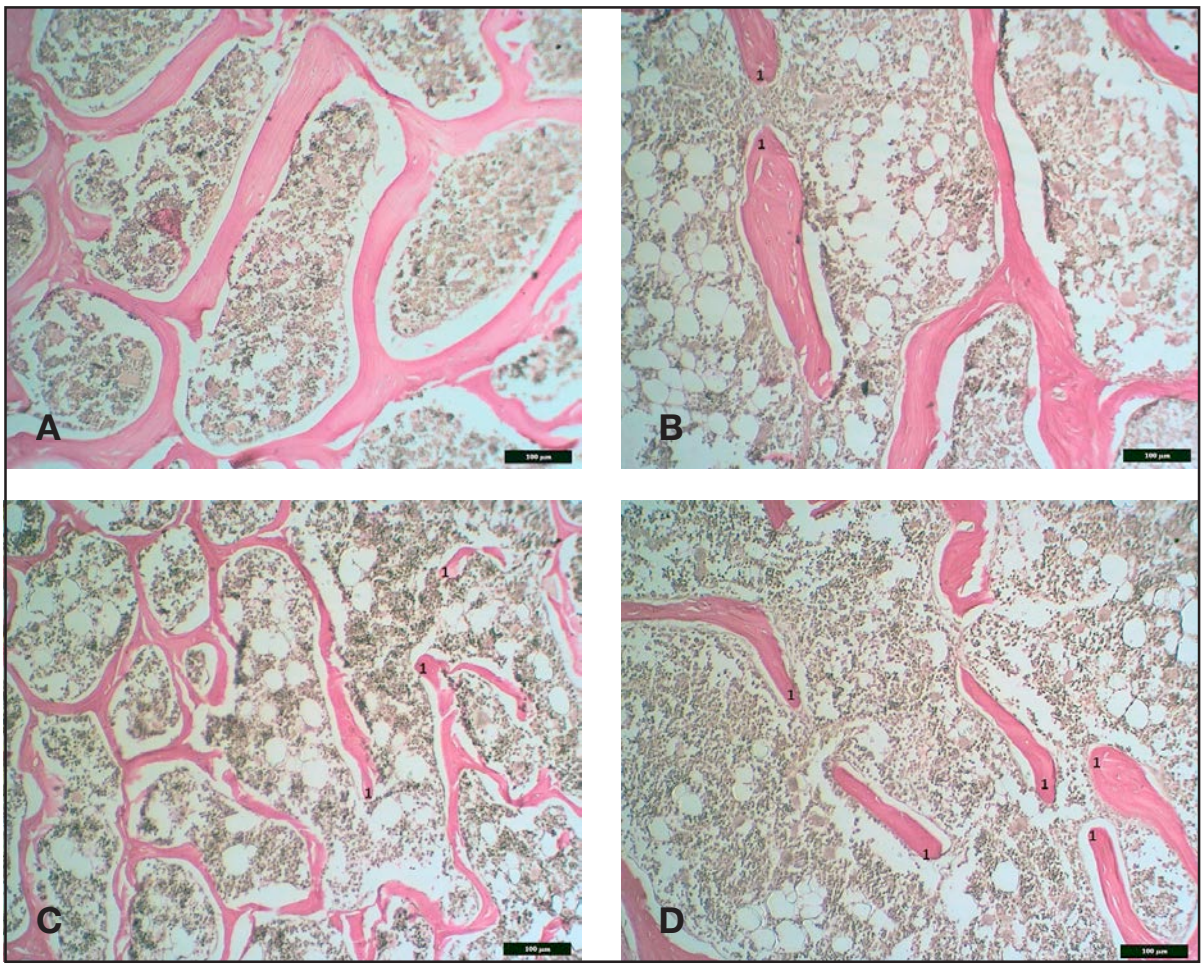

Figure 3. The section of rat lumbar spine vertebrae: **A:** the control group; **B:** group with an impaired bone remodeling in CKD; **C:** group with an impaired bone remodeling in GCs; **D:** group with an impaired bone remodeling in CKD + GCs: 1 – blind ends of trabeculae. Van Gieson staining.

The levels of RANKL in the serum of the three experimental groups were higher than in intact animals, but the difference did not reach statistical significance (Table). The levels of OPG in the serum of the CKD and GCs groups were higher than in intact animals. At the same time, in the serum of the CKD + GCs group, the levels of OPG were lower than those in animals from the control group. The levels of galectin-3 in the serum of the experimental groups GCs and CKD + GCs were lower than in intact animals. The levels of galectin-3 in the serum of the CKD group were higher than those in animals from the control group. The levels of P-selectin in the animals' blood serum of the GCs group

were lower than in intact animals. At the same time, in the serum of the CKD and CKD + GCs groups, the levels of P-selectin were higher than those in animals from the control group (Table).

When conducting a correlation analysis in the control group, a direct strong correlation was found ($r = 0.683$, $p < 0.05$) between the content of RANKL and P-selectin. In the experimental groups of animals, this correlation was not significant. In the control group, strong negative correlations were found between P-selectin and bone density ($r = -0.766$, $p < 0.05$), RANKL and bone density ($r = -0.706$, $p < 0.05$). In the experimental groups, the correlation between the content of P-selectin and bone density changed direction. In the groups of CKD and GCs, the correlation between the content RANKL and bone density was not significant, while in CKD + GCs group the relationship between the content of RANKL and bone density remained inverse ($r = -0.407$, $p > 0.05$).

Discussion

The observed decrease in bone density in animals of three experimental groups compared to bone density in animals of the control group can probably be due to the negative effect of inflammation and GCs on bone metabolism.[13,14] Inflammation modulates bone resorption mainly due to the ability of proinflammatory cytokines to cause imbalances in the RANKL/OPG system, stimulating osteoclastogenesis.[15]

Inanimalsofthe CKDand GCsexperimental groups, serum RANKL and its natural antagonist OPG were increased. RANKL is the main inducer of osteoclast maturation. An increase in RANKL expression leads to bone resorption, which corresponds to a decrease in bone density in these groups. The action of various factors controlling bone resorption is carried out through the influence on the synthesis of RANKL and OPG in osteoblasts, which activates osteoclastogenesis. At the same time, glucocorticoids activate RANKL and inhibit OPG synthesis in osteoblasts. However, in our study, an increase in the concentration of OPG in the serum of animals of the GCs group was observed. Due to the influence of GCs, activation of differentiation of osteoclast progenitor cells can occur, which is characterized by increased expression of both RANKL and its RANK receptor. In response to increasing RANK levels, OPG production is enhanced. An increase in the concentration of serum OPG is also observed in animals of the group with CKD. It has been found that some pro-inflammatory cytokines, such as IL-1, increase the production of RANKL and OPG in osteoblasts.[16] Thus, IL-1, by activating the expression of RANKL on the surface of osteoblasts, regulates bone metabolism, stimulating osteoclastogenesis. On the other hand, this cytokine inhibits the formation of osteoclasts, increasing the production of OPG. Thus, an elevation in the level of serum OPG in animals of the CKD group can be considered as a compensatory reaction to an increase in the activity of osteoclasts.

A significant increase in RANKL and a decrease in OPG in the blood serum of animals of the CKD + GCs group (table) compared to those in intact animals can be caused by the action of GCs simultaneously with the inflammatory effects in CKD. With CKD, there is an increase in the production of pro-inflammatory cytokines, which, in turn, stimulates the expression of RANKL and reduces the production of OPG, which stimulates the differentiation and activation of osteoclasts and helps to reduce bone density. GCs are able to act directly on osteoclasts, prolonging life span and reducing apoptosis of mature osteoclasts, despite the reduction in the number of their precursors,[17] which ultimately contributes to the process of bone resorption. This is confirmed by the inverse relationship found by us between the level of RANKL and bone density in the CKD + GCs experimental group.

Our studies have shown a significant increase in the serum level of P-selectin, a protein that is expressed on the surface of activated endothelial cells and platelets, in the CKD group compared to intact animals (table). The elevation of this lectin appears to be an homeostatic response to inflammation in CKD,[18] the development of which was facilitated by significant platelet activation and endothelial dysfunction. Currently, the role of endothelial dysfunction in the development of many chronic diseases, including CKD, has been demonstrated.[19]

Theinflammatoryresponsein CKDdepends on the presence of both proinflammatory cytokines and adhesion molecules, which ensures the interaction of endothelial cells with circulating leukocytes and then leukocytes with elements of the extracellular matrix, which leads to the accumulation of leukocytes in the inflammatory foci.[14] Uncontrolled leukocyte adhesion is of great importance in the pathogenesis of inflammation. Interacting with ligands on the membrane of circulating leukocytes, P-selectin mediates leukocyte adhesion to the activated endothelium in the process of inflammation. Thus, an increase in P-selectin expression in the CKD animal group is an important sign of endothelial cell activation associated with the development of inflammation in CKD.

A significant decrease in the level of P-selectin was observed in the GCs experimental group. GCs are able to inhibit endothelial expression of proinflammatory mediators, such as cytokines, chemokines, and adhesion molecules,[20] which reflects the level of P-selectin in animals of this group. At the same time, in the CKD + GCs group a significant increase in serum P-selectin level was observed compared to intact animals, which indicates endothelial-platelet dysfunction with CKD when exposed to GCs. Many authors point at to an increased level of P-selectin in CKD, but there is no consensus on the effect of GC on the expression of P-selectin. According to the literature, along with the inhibitory effect of dexamethasone on the expression of this protein,[21] high doses of dexamethasone increase the levels of P-selectin,[22] or do not affect its content.[23] An increase in the level of P-selectin in the CKD + GCs group is probably associated with GCs-induced vascular endothelial dysfunction in addition to the effects of inflammation in CKD.

The found correlations in groups of animals between P-selectin and RANKL may be due to the mutual influence of these intercellular mediators on bone metabolism. The correlations between the content of P-selectin and bone density indicate the complexity and ambiguity of the role of P-selectin in the regulation of bone metabolism and emphasize the involvement of adhesion molecules in bone remodeling processes. Features of the effect of GCs on endothelial function in case of inflammation require further study to develop and improve existing treatment strategies.

An important role in cell proliferation, adhesion, differentiation, angiogenesis, and apoptosis is played by galectin-3. Further, this pleiotropic lectin plays a key role in liver, kidney, lung and myocardial fibrogenesis.[24] Moreover, galectin-3 plays an important role in modulating the immune and inflammatory response.[25]

Galectin-3 can affect bone homeostasis by regulating the function and interaction of osteoblasts and osteoclasts. Previous studies have shown that exogenous recombinant galectin-3 inhibits terminal differentiation of osteoblasts, which may indicate a different or even opposite effect of galectin-3 on osteoblastogenesis depending on its intracellular or extracellular localization.[26] At the same time, galectin-3, expressed on the surface of osteoclasts, is involved in the regulation process of bone resorption. However, data on the effect of this lectin on osteoclastogenesis are ambiguous.[25]

An increase in serum galectin-3 was detected in the group of animals with CKD. This

is consistent with evidence that a decrease in renal function is associated with an increase in the level of this lectin.[27] On the other hand, a decrease in galectin-3 was observed in the GCs and CKD + GCs groups. In the CKD + GCs group, its decrease is even more significant. It should be noted that galectin-3 has a pro-inflammatory effect in acute conditions, while the anti-inflammatory effects of this lectin prevail in chronic inflammatory processes.[28]

At the same time, a decrease in the expression of galectin-3 is induced by GCs, but the intensity of changes in the concentration of this lectin and the time of their appearance depend on the species, the concentration of GCs and the time of their exposure.[29] Probably, such a change in the expression of galectin-3 in the GCs and CKD + GCs groups is associated with the effects of glucocorticoids and the anti-inflammatory effects of galectin-3 in chronic inflammation. The reduction of bone density in the GCs and CKD + GCs groups, together with a decrease in the expression of galectin-3, which inhibits osteoclastogenesis in these groups, suggests a negative feedback mechanism, which might restrain excessive osteoclastogenesis.

Conclusion

Two different effects of the influence of glucocorticoids on the development of the pathological process in case of kidney disease are possible. GCs can be a treatment factor that reduces the intensity of inflammation in the kidneys and, accordingly, the risks of developing osteoporosis due to renal insufficiency. However, at the same time, GCs themselves are a risk factor for osteoporosis. We did not find confirmation of the additive, subtractive, or cumulative effects of GCs acting simultaneously with CKD on metabolic processes in the bone. It was found that these relationships are changing significantly during the development of the pathological process. Further research is required to determine the optimal regimen for using GCs to minimize the activity of the pathological process, both in the kidneys and in the bone.

The studied profile of intercellular mediators and the revealed correlations suggest alterations in the regulatory pathways that lead to abnormalities in osteolytic processes activation with development of inflammation in chronic kidney disease. The imbalance between the levels of RANKL and OPG, resulting from the alteration of the feedback mechanism, contributes to bone resorption and, therefore, leads to altered bone remodeling.

Further studies will assess the role of extracellular mediators in the regulatory mechanisms of bone metabolic disturbances when exposed to glucocorticoids, both in renal diseases and in other chronic pathologies. The study of a complex system of regulation of bone remodeling, which includes many factors and their interactions, in the future may lead to the development of methods for treating patients with chronic kidney disease in order to prevent osteoporosis.

Funding

This study was funded by the Ministry of Health of Ukraine for the state budget.

Conflictos de interés: los autores declaran no tener conflictos de interés.

Recibido: junio 2019
Aceptado: marzo 2020

References

1. Stenvinkel P, Painer J, Kuro-O M, et al. Novel treatment strategies for chronic kidney disease: insights from the animal kingdom. *Nat Rev Nephrol.* 2018; 14(4):265-284.
2. Cherif A, Preciado P, Maheshwari V, et al. A mathematical model of bone remodeling in patients with uremia and metabolic bone diseases. *Nephrol Dial Transplant.* 2018; 33(l):165-166.
3. Zhou H, Cooper MS, Seibel MJ. Endogenous glucocorticoids and bone. *Bone Res.* 2013; 1(2):107-119.
4. Lewiecki EM, Binkley N. What we do not know about osteoporosis. *J Endocrinol Invest.* 2016; 39(5):491-493.
5. Pavlov SB, Kumechko MV, Litvinova OB, Babenko NM, Goncharova AV. Bone regulatory mechanisms destruction in experimental chronic kidney disease. *Fiziol Zh.* 2016; 62(3):54-59.
6. Benslimane-Ahmim Z, Heymann D, Dizier B, et al. Osteoprotegerin, a new actor in vasculogenesis, stimulates endothelial colony-forming cells properties. *J Thromb Haemost.* 2011; 9(4):834-43.
7. Lange-Sperandio B, Cachat F, Thornhill BA, Chevalier RL. Selectins mediate macrophage infiltration in obstructive nephropathy in newborn mice. *Kidney Int.* 2002; 61(2):516-524.
8. Stillman BN, Hsu DK, Pang M, et al. Galectin-3 and galectin-1 bind distinct cell surface glycoprotein receptors to induce T cell death. *J Immunol.* 2006; 176(2):778-789.
9. Liu Y, Chen Y, Zhao H, Zhong L, Wu L, Cui L.. Effects of different doses of dexamethasone on bone qualities in rats. *Sheng Wu Yi Xue Gong Cheng Xue Za Zhi.* 2011; 28(4):737-743, 747 (in Chinese).
10. Kondakov II, Topchii II, Kirienko OM. Influence of glicerol on functional-morphological indicators of kidneys at modelling renal insufficiency in rats. *Ukr J Nephrol Dialysis.* 2013; 3(39):14-20 (in Ukrainian).
11. Podkovkin VG, Ivanov DG, Ivanov GA. The effect of magnetic field on the bone tissue status in rats with high level bone resorption. *Advances in current natural sciences. Biological sciences.* 2008; 7:13-16 (in Russian).
12. Sarkisov DS, Perov JuL, editors. Mikroskopicheskaja tehnika: rukovodstvo dlja vrachej i laborantov. Moscow: Medicina; 1996. 544 p. (in Russian).
13. Messina OD, Somma LF, Tamborenea MI et al. Guías para el diagnóstico, la prevención y el tratamiento de la osteoporosis inducida por glucocorticoides en el adulto. *Actual Osteol.* 2016; 12(2):107-125.
14. Imig JD, Ryan MJ. Immune and inflammatory role in renal disease. *Compr Physiol.* 2013; 3(2):957–976.
15. Redlich K, Smolen JS. Inflammatory bone loss: pathogenesis and therapeutic intervention. *Nat Rev Drug Discov.* 2012; 11(3):234-250.
16. Lambert C, Oury C, Dejardin E, Chariot A, Piette J, Malaise M, Merville MP, Franchimont N. Further insights in the mechanisms of interleukin-1 β stimulation of osteoprotegerin in osteoblast-like cells. *J Bone Miner Res.* 2007; 22(9):1350-1361.
17. Jia D, O'Brien CA, Stewart SA, Manolagas SC, Weinstein RS. Glucocorticoids act directly on osteoclasts to increase their life span and reduce bone density. *Endocrinology.* 2006; 147(12):5592-5599.
18. Lu GY, Xu RJ, Zhang SH, et al. Alteration of circulatory platelet microparticles and endothelial microparticles in patients with chronic kidney disease. *Int J Clin Exp Med.* 2015; 8(9):16704-16708.
19. Drozdz D, Kwinta P, Sztefko K, et al. Oxidative stress biomarkers and left ventricular hypertrophy in children with chronic kidney disease. *Oxid Med Cell Longev.* 2016; 2016:7520231, doi: 10.1155/2016/7520231.
20. Zieliríska KA, Van Moortel L, Opdenakker G, De Bosscher K, Van den Steen PE. Endothelial response to glucocorticoids in inflammatory diseases. *Front Immunol.* 2016; 7:592.
21. Xiping Z, Jun F, Jie Z, et al. Influence of dexamethasone on the expression levels of P-se-

lectin protein in multiple organs of rats with severe acute pancreatitis. *Inflamm Res.* 2010; 59(1):31-39.

22. Jilma B, Cvitko T, Winter-Fabry A, Petroczi K, Quehenberger P, Blann AD. High dose dexamethasone increases circulating P-selectin and von Willebrand factor levels in healthy men. *Thromb Haemost.* 2005; 94(04):797-801.
23. Xia L, Pan J, Yao L, McEver R. A proteasome inhibitor, an antioxidant, or a salicylate, but not a glucocorticoid, blocks constitutive and cytokine-inducible expression of P-selectin in human endothelial cells. *Blood.* 1998; 91(5):1625-1632.
24. Li LC, Li J, Gao J. Functions of galectin-3 and its role in fibrotic diseases. *J Pharmacol Exp Ther.* 2014; 351(2):336-343.
25. Iacobini C, Fantauzzi CB, Pugliese G, Menini S. Role of galectin-3 in bone cell differentiation, bone pathophysiology and vascular osteogenesis. *Int J Mol Sci.* 2017; 18(11):2481.
26. Nakajima K, Kho DH, Yanagawa T, et al. Galectin-3 inhibits osteoblast differentiation through notch signaling. *Neoplasia.* 2014; 16(11):939-949.
27. Rebholz CM, Selvin E, Liang M, et al. Plasma galectin-3 levels are associated with the risk of incident chronic kidney disease. *Kidney Int.* 2018; 93(1):252-259.
28. Pugliese G, Iacobini C, Pesce CM, Menini S. Galectin-3: An emerging all-out player in metabolic disorders and their complications. *Glycobiology.* 2015; 25(2):136-150.
29. Dabelic S, Goreta SS, Dumic J. Galectin-3 in macrophage-like cells exposed to immunomodulatory drugs. *Biochim Biophys Acta.* 2006; 1760(4):701-709.

Actual. Osteol 2019; 15(3): 192-204.
Internet: http://www.osteologia.org.ar

ARTÍCULOS ORIGINALES / *Originals*

RABBIT GROWTH PLATE MORPHOLOGY IN TEMPORARY BILATERAL BLOCKING

Mykola Korzh,[1] Victor Rokutov,[2] Dmytro Iershov,[2] Nataliya Ashukina,[1] Valentyna Maltseva,*[1] Sergey Khmyzov[1]

[1] Sytenko Institute of Spine and Joint Pathology, National Academy of Medical Science of Ukraine, Kharkiv, Ukraine. [2] Dnipropetrovsk Clinical Medical Center for Mother and child named by prof. Rudnev, Dnipro city, Ukraine.

Abstract

Blocking of the growth plate (GP) using plates with screws (tension band plating) is a modern method used to correct deformities and moderate leg length discrepancy in growing children. Determining the duration of temporary bilateral blocking without the occurrence of irreversible changes of GP is of paramount importance important.

Methods: Two-month-old Californian breed male rabbits (n=30) were exposed to bilateral blocking of the distal GP of the right femur locking plates with screws for 3, 5, and 7 weeks. The fixators were removed after 5 and 7 weeks in 18 rabbits and 3 weeks after that, animals were sacrificed. The contralateral limb was used as a control. Histological, histomorphometric, and X-ray analyses were performed.

Results: During GP blocking, its height gradually decreased. This decreased was more pronounced after 7 weeks. Destructive changes progressed with an increase in the blocking duration. Three weeks after discontinuation of the bilateral blocking that lasted 5 weeks, the height of the GP significantly increased 1.2 times on the lateral side and 1.9 times on the medial side ($p<0.001$) compared to the control. When blocking was discontinued after 7 weeks, the structure of the GP was partially restored after 3 weeks, the height of GP significantly increased 1.2 times on the lateral side, and 1.07 times on the medial side ($p<0.01$) compared to the control.

Conclusion: Restoration of the structural-functional features of the GP after the removal of the plates depends on the duration of temporary bilateral blocking, which must be taken into account in the clinical setting.

Key words: temporary bilateral blocking, rabbit, guided growth, tension band plating, histology.

**E-mail: maltseva.val.evg@gmail.com*

Resumen

El bloqueo de la placa de crecimiento (PC) utilizando placas con tornillos (banda de tensión) es un método moderno utilizado para corregir deformidades y alteraciones moderadas en la longitud de las piernas en niños en crecimiento. Es de suma importancia determinar cuál debe ser la duración del bloqueo bilateral temporal sin que ocurran cambios irreversibles en la PC.

Métodos: Conejos machos de raza californiana de dos meses de edad (n = 30) fueron expuestos al bloqueo bilateral de la PC distal colocando placas del fémur derecho con tornillos durante 3, 5 y 7 semanas. Los fijadores fueron retirados después de 5 y 7 semanas en 18 de los conejos, y 3 semanas después los animales fueron sacrificados. La extremidad contralateral se utilizó como control. Se realizaron análisis histológicos, histomorfométricos y de rayos X.

Resultados: Durante el bloqueo de la PC, su altura disminuyó gradualmente. Esta disminución fue más pronunciada después de 7 semanas. Los cambios destructivos se incrementaron a medida aumentaba la duración del bloqueo. Tres semanas después de la interrupción del bloqueo bilateral que duró 5 semanas, la altura de la PC aumentó significativamente 1.2 veces en el lado lateral y 1.9 veces en el lado medial (p <0.001) en comparación con el control.

Conclusión: La restauración de las características funcionales estructurales de la PC después de la extracción de las placas depende de la duración del bloqueo bilateral temporal, lo que debería tenerse en cuenta en el tratamiento clínico de estas alteraciones.

Palabras clave: *bloqueo bilateral temporal, crecimiento guiado, placas de bandas de tensión, histología.*

Introduction

The concept of "guided growth" is widely used in pediatric orthopedics and represents the influence on the functioning of bone the growth plates. There are various methods of growth plate blocking (permanent epiphysiodesis, stapling, PETS (percutaneous epiphysiodesis using transphyseal screws) technique, and temporary tension band plating). Tension band plating for temporary bilateral blocking is a modern method used to correct coronal and sagittal deformations of lower limb bones (unilateral blocking)[1-3] and moderate (2-5 cm) leg length discrepancies (bilateral blocking) in growing children.[3-5] The advantage of this method, compared to the use of staples, is a less rigid growth plate blocking, which reduces the risk of damage to the growth plate.[6] The rate of complications for this method is less than 10%.[4] In a multicentre study, the results of treatment of frontal deformities (varus and valgus) and leg length discrepancy in 126 children using plates with screws were analyzed. The authors noted complications in 20 (18%) patients, most often associated with implant failure (i.e.: migration and fracture) in 10% of the patients. The second most frequent complication was associated with premature closure of growth plate in 5 % of the patients.[7] The authors noted that the use of growth plate blocking in patients just before the onset of skeletal maturity is a complex issue, and careful monitoring throughout the whole treatment period prevents most of the complications.

In another similar multicentre study involving 537 patients, the importance of the

patient's age for the successful correction of deformity was also emphasized, and the following complications were found: breakage of screws – 0.53% and limitation of movements – 1.12%.[8] The time of the removal of the fixators is also an important factor of achieving optimal correction. In particular, in a retrospective analysis of 94 patients, it has been shown that an increase in a fixator lifespan by ≥ 6 months increases the risk of hypercorrection (odds ratio, 19.2; 95% confidence interval, 5.2-71.4; p<0.005).[9]

Growth acceleration after unilateral growth plate blocking using plates with screws is one of the features that occurs after this procedure, especially in younger patients with high growth potential (younger than 14 years).[6] However, this phenomenon is not well understood.[10] In an experimental study in rabbits, which had a plate in the proximal tibia for 3 weeks, growth acceleration of the medial side was identified histologically 2 weeks after removal of the plate. Growth arrest was discovered 3 weeks after the plate was removed, when the columnar structure in the growth plate was restored.[10]

Consequently, determining the age for the initiation of application and the duration of the growth plate blocking without the occurrence of irreversible changes is definitely an important issue, the solution of which will increase the efficacy of the application of this method.

The aim of study was to evaluate the morphological changes of the distal growth plate of the femur of rabbits during and after temporary bilateral blocking using locking plates with screws.

Materials and methods

Animals

The experiments were carried out on two-month-old Californian breed male rabbits (n=30), with an average body weight of 1.5 ± 0.2 kg, in the experimental biological clinic of the Sytenko Institute of Spine and Joint Pathology, Ukraine. The animals were kept in separate cages, with a 12-hour light period, and provided with a complete diet and water *ad libitum*. The experimental model was validated by N. Mast et al.[11] and consists of a temporary bilateral blocking of the distal growth plate of the femur using locking plates with screws (temporary bilateral blocking). During work with the animals, the requirements of the European Convention for the protection of vertebrate animals used for experimental and other scientific purposes,[12] and the Law of Ukraine "On the protection of animals against cruel treatment"[13] were observed. The protocol for experiments on animals was approved by the Bioethics Committee at the Sytenko Institute of Spine and Joint Pathology (No. 116 dated 25.03.2013, No. 154 dated 27.06.2016).

Experimental design

All rabbits were exposed to temporary bilateral blocking.[14] The contralateral limb was used as a control.

The animals were randomly divided into two groups. The first group (n = 18) was used to study the morphological structure of the epiphyseal cartilage after its bilateral blocking for 3, 5, and 7 weeks. In the second group (n = 12), removal of the fixators was performed under intravenous anesthesia, following temporary bilateral blocking, which lasted for 5 and 7 weeks. Three weeks after that, the animals were sacrificed to further study the morphological structure of the epiphyseal cartilage. After preliminary sedation with a combination of ketamine (35 mg/kg) and xylazine (5 mg/kg), the animals were euthanized by intravenous injection of a lethal dose of phenobarbital (150 mg/kg). Later, both femoral bones were removed to be used for histological analyzes.

Surgical intervention technique

The animals underwent surgical intervention in aseptic conditions under intravenous anesthesia with a combination of ketamine (35 mg/kg) and xylazine (5 mg/kg). The skin of the lower extremities was shaved and treated

with a betadine solution three times. Linear longitudinal incisions of the skin up to 1.5 cm in length were performed along the medial and lateral surfaces in the region of distal epimethaphysis of the femur. Extraperiosteal fixation, first of the medial side and then the lateral side of the distal growth plate of the femur was performed using blocking plates with two screws. One screw was introduced into the epiphysis (Fig. 1A), and the other into the femoral metaphysis (Figure 1B). The location of the screws was controlled by X-rays (Fig. 1). The length of the screws fixing the plate did not exceed half the transverse size of the proximal femur in the frontal plane. Additionally, a Kirschner wire was introduced into the distal part of the operated and contralateral femoral bone[14] (Fig. 1C).

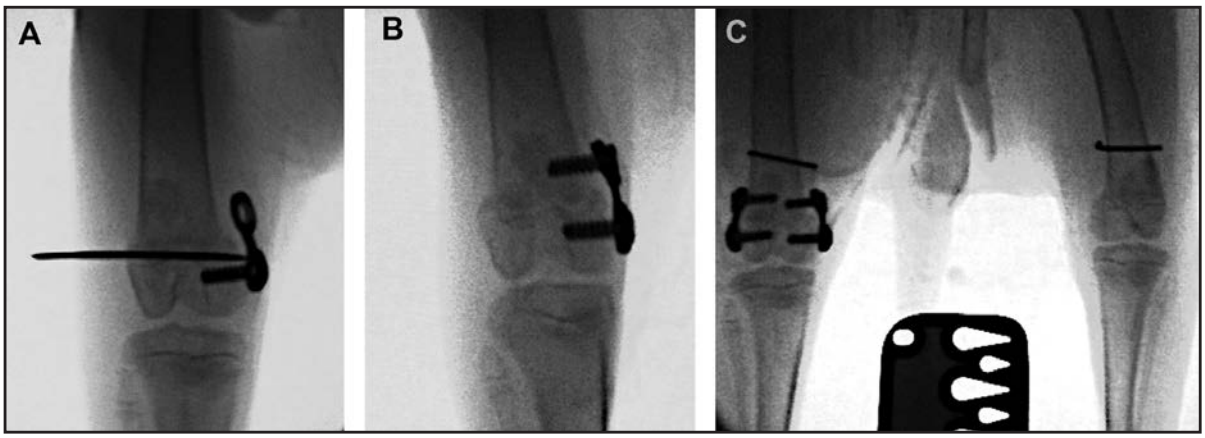

Figure 1. X-ray control during surgery of the bilateral blocking of the rabbit femur: one screw was introduced into the medial epiphysis (A), the other into the medial femoral metaphysis (B). Bilateral blocked limb and contralateral limb with Kirschner wire in the distal part of the femurs (C).

In the preoperative period and for 2 days after the operation, antibiotic prophylaxis with Cephazolin (5 mg×kg/day) was performed. In addition, the animals received treatment of the postoperative wounds with antiseptic solutions for the first 3 days following the operation.

Histological analysis

The rabbit femoral bones were fixed in 10% neutral formalin; after decalcification in 10% formic acid, the screws were removed and the distal parts were cut out. The material was dehydrated and poured into paraffin. Longitudinal histological 5-6 μm thick sections (7 of each sample) were stained with hematoxylin and eosin. The analysis of the obtained histological preparations was carried out under a light microscope Olympus BX-63 equipped with a digital camera DP73 (Olympus).

During histological analysis of epiphyseal cartilage, structural features of the resting, proliferating and hypertrophic zones were evaluated (Figure 2). The resting zone was determined to be between the subchondral bone and the region where chondrocytes are located in columns. The proliferation zone consisted of columns of chondrocytes, and the hypertrophic zone consisted of enlarged cells located between the proliferating zone and the primary spongiosa. Such delineation into zones is used according to previously conducted histomorphometry studies of epiphyseal cartilage in animals.[15] In addition, the condition of the primary spongiosa zone was analyzed.

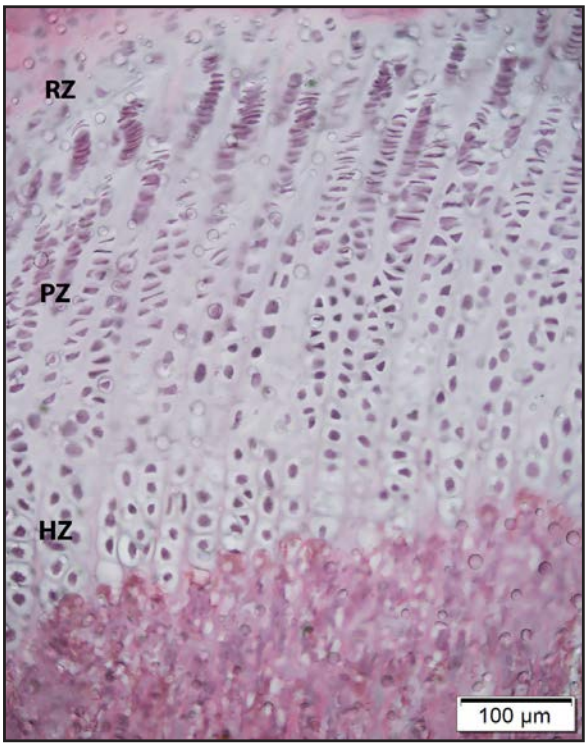

Figure 2. Histology of growth plate of a control rabbit. RZ-resting zone; PZ-proliferating zone; HZ-hypertrophic zone. Hematoxilin and eosin staining.

Histomorphometric study

The height of the epiphyseal cartilage in the medial, lateral and middle sides of the experimental (operated) and control limbs (measured in 7 longitudinal sections, 3 measurements for each medial, lateral and middle side) was measured using the software CellSens Dimension 1.8.1 (2013) for the microscope Olympus BX-63 (200×). Medial and lateral sides were determined to be at a distance of 2.4 mm from the central axis of the bone.

X-ray

The radiography (anteroposterior projection) of the hind limbs of rabbits was performed 1, 2 and 3 weeks after plate removal. The mechanical Lateral Distal Femoral Angle (mLDFA) was measured on each radiograph using the "Angle" software.

Statistical analysis

The obtained indicators are presented as mean ± standard deviation (SD). Paired t-test was used for compare mean values blocked and contralateral (control) limbs at the same period. Unpaired t-test was used for compare mean values of independent samples. One-way ANOVA was performed with Bonferroni correction for multiple comparisons with duration of bilateral blocking as factor. The difference between the mean values was considered statistically significant for $p<0.05$. The IBM SPSS Statistics 20 software was used during the analysis.

Results

Histological analysis

Three weeks after the bilateral blocking, it was found that the growth plate characteristic zones were preserved, but the histoarchitectonics was slightly disturbed from the medial and lateral sides. These changes manifested through the appearance of cell-free areas with slightly basophilic coloration and some disorganization of the columns in the proliferating zone. The resting zone was represented mainly by one (in minor areas – two) layer of elongated chondrocytes. In the hypertrophic zone, throughout the whole growth plate area of the operated limb, chondrocytes of round shape, shadow cells, and empty lacunae were observed. In the primary spongiosa, bone trabeculae located parallel to each other were identified at the medial and lateral sides, but their areas were slightly smaller than the middle part and the control limb. The overall height of the growth plate was irregular: it was slightly lower on the lateral and medial sides, but not significantly different when compared to the control limb, and it was decreased in the middle area by 1.1 times ($p<0.05$) (Table 1).

Five weeks after the bilateral blocking, structural disorders were identified on the entire territory of the epiphyseal cartilage, with a greater manifestation of disturbances in the medial and lateral sides than the middle side. Additional findings include

Table 1. Growth plate height (μm) measurements at different period after bilateral blocking.

Side of growth plate	Time of blocking (weeks)						paired t-test weeks			one-way ANOVA	
	3		5		7						
	blocked limb	control limb	blocked limb	control limb	blocked limb	control limb	3	5	7	blocked limb	control limb
Lateral	153.78±8.61	163.14±3.68	197.75±11.45	316.50±8.87	175.03±3.29	365.67±7.38	ns	p<0.001	p<0.001	p<0.01	p<0.001
Middle	119.86±3.63	134.54±1.71	172.07 ± 6.37	239.67±7.19	146.00±2.99	307.27±5.54	p<0.05	p<0.001	p<0.001	p<0.001	p<0.001
Medial	183.07±9.57	192.06±4.79	186.08 ± 7.97	306.48±9.58	204.36±5.85	409.04±6.92	ns	p<0.001	p<0.001	ns	p<0.001

Paired t-test; difference between the mean values of blocked and control limbs in the similar sides at the same period was considered statistically significant for p<0.05. one-way ANOVA test with the Bonferroni correction; difference between the mean values of limbs in the similar sites at the 3, 5 and 7 weeks was considered statistically significant for p<0.05.

absence of a resting zone in the isolated sites, chaotic location of the chondrocyte columns in the proliferating zone, the presence of single cells, shadow cells, and sites of cell-free matrix (Figure 3A). The hypertrophic zone was intermittent; the density of chondrocytes on the preserved sites was low. The invasion of blood vessels into the zone was noticed, with bone tissue forming around these blood vessels. The primary spongiosa zone was represented by fine-meshed bone trabeculae, which differed from the characteristic structure defined in the control limb. The histomorphometric analysis showed a decrease in the height of the growth plate compared to the control limb in all investigated sides: lateral – by 1.6 times (p<0.001); medial – by 1.6 times (p<0.001); middle – by 1.4 times (p<0.001) (Table 1).

Seven weeks after the bilateral blocking, marked destructive changes were detected throughout distal epiphyseal cartilage (Fig. 3C). There was an intermittency of the resting zone with the formation of isogenic groups of cells and areas with a slightly basophilic matrix free from cells that spread to the subchondral bone. In the proliferating zone, the columnar structure of the chondrocytes was disturbed, and the number of cells in columns was reduced. Areas of the matrix without cells were found occasionally. The border with the hypertrophic zone was not clear. The hypertrophic zone was absent at some sites, and the proliferating zone transformed into the primary spongiosa zone where bone trabeculae were located chaotically. A distinctive feature was the expansion of inter-trabecular spaces, in which cyst-like masses were observed. The height of the growth plate was uniformly significantly lower (p<0.001) compared to the control limb: by 2.1 times on the lateral side; by 2.0 on the medial side; and by 2.0 on the middle side (Table 1).

According to one-way ANOVA, it was found that after the bilateral blocking, the height on all sides of the growth plate varies unequally. The height of the middle of the growth plate increased by 1.4 times between weeks 3 and 5 of bilateral blocking, and decreased by 1.2 times between weeks 5 and 7. The height of the lateral side of the growth plate increased by 1.3 times between weeks 3 and 5 of bilateral blocking, but lacked significant changes between weeks 5 and 7. In the blocked limb, the height of the medial side of the growth plate did not differ at any time. In the control limbs, the height of the growth plate increased uniformly in all measured zones during the observation period.

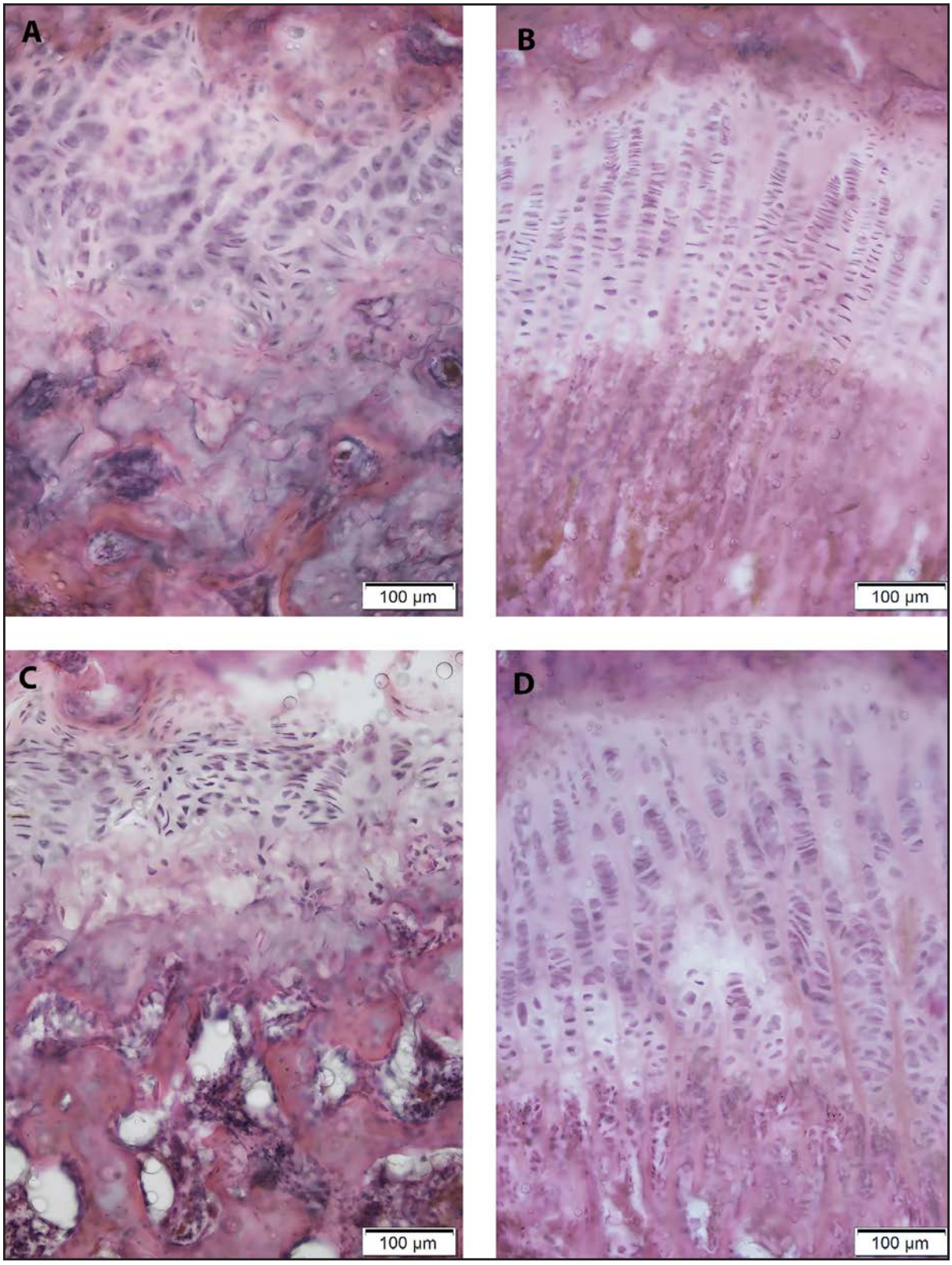

Figure 3. Histological analysis of distal femoral growth plate of the rabbits. Destructive changes in the growth plate after 5 (A) and 7 (C) weeks of bilateral blocking using blocked plates and restoration structure of growth plate in 3 weeks after the discontinuation of 5- (B) or 7-week-long temporary bilateral blocking (D). The appearance of new short chondrocyte columns in proliferative zone (B and D). Hematoxilin and eosin staining.

Three weeks after the 5-week-long temporary bilateral blocking, the epiphyseal cartilage of the operated limb was continuous (Figure 3B). The resting, proliferating and hypertrophic zones were clearly observed. However, the resting zone was intermittent, especially in the middle side, and contained isogenic groups of up to 4 round-shaped chondrocytes. The areas of the cell-free matrix, which were found in all zones of the epiphyseal cartilage, were characterized by mosaic coloration–from slightly basophilic to sharply eosinophilic. In the proliferating zone, the columns of chondrocytes were apparent, some of which were not parallel to each other, and at different angles to the limb axis. From the medial side, there were shorter columns that contained between 8 and 12 cells. They began from the resting zone and ended, not reaching the hypertrophic zone. The structure of the chondrocytes, located in the columns of the proliferating zone of both extremities, was typical – they had a triangular shape, flattened nuclei and a basophilic coloration. The hypertrophic zone did not differ from the control limb in terms of its structure. In the primary spongiosa zone of both limbs, bone trabeculae were located parallel to the bone axis.

According to the results of the histomorphometric analysis, the height of the epiphyseal cartilage was uneven: significantly higher than the values of the control limb on the lateral (by 1.2 times) and medial (by 1.9 times) sides ($p<0.001$), without changes in the middle side (Table 2).

Table 2. Growth plate height (μm) measurements in 3 weeks after temporary bilateral blocking.

Side of growth plate	Investigation time (weeks)				paired t-test		unpaired t-test	
	5-week-long temporary bilateral blocking		7-week-long temporary bilateral blocking		weeks			
	blocked limb	control limb	blocked limb	control limb	5	7	blocked limb	control limb
Lateral	362.34±5.80	295.15±4.62	359.93±12.49	321.51±6.41	p<0.001	p<0.01	ns	p<0.01
Middle	265.29±5.80	261.79±6.27	251.34±6.62	262.75±6.18	ns	ns	ns	ns
Medial	530.68±24.14	284.92±5.41	389.27±7.53	362.87±4.56	p<0.001	p<0.01	p<0.001	p<0.001

Paired t-test; difference between the mean values of blocked and control limbs in the similar sides at the same period was considered statistically significant for $p<0.05$. Unpaired t-test; difference between the mean values of limbs in the similar sites at the 5-week-long and 7-week-long temporary bilateral blocking period was considered statistically significant for $p<0.05$.

Three weeks after the 7-week-long temporary bilateral blocking, continuous epiphyseal cartilage was observed on the longitudinal histological sections of the operated limb (Figure 3D). In contrast with the control limb and the limb that underwent 5-week-long temporary bilateral blocking, the resting zone was almost impossible to identify. In the proliferating zone from the medial and lateral sides, the columnar structure of chondrocytes was disturbed: isolated cells, shadow cells, and columns of differing heights were observed. Cell-free areas with uneven coloration of the matrix were found throughout the whole length of the epiphyseal cartilage of the experimental limb. However, they were much less common in the control limb. The hypertrophic zone was rather similar to the control limb by structure. Invasion of blood vessels from the primary

spongiosa was observed. In the primary spongiosa zone, most of the bone trabeculae that were observed were parallel to the axis of the bone. However, they were much shorter than the ones in the control limb, and some had a looped structure. According to the results of the histomorphometric analysis, it was determined that on the middle side, the height of the growth plate did not differ from the value in the control limb, but on the lateral and medial sides it had increased by 1.2 and 1.07 times respectively ($p < 0.01$) (Table 2).

According to one-way ANOVA, three weeks after the plate removal flollowing the 7-week-long temporary bilateral blocking, the height of the growth plate on the medial side was 1.4 times less than the value after 5-week-long temporary bilateral blocking. In the middle and lateral sides of the growth plate, no differences were found between groups with different blocking periods. In the control limbs, the height of the growth plate increased during the observation period in the lateral and medial sides, without changes in the middle side.

X-ray

There were no differences in the mLDFA in the operated limb after 1, 2, and 3 weeks after the plates' removal when compared with the control for all periods of measurement (Fig. 4). Earlier, we obtained similar data when measuring the mLDFA while implementing bilateral blocking for 3, 5, and 7 weeks.[16]

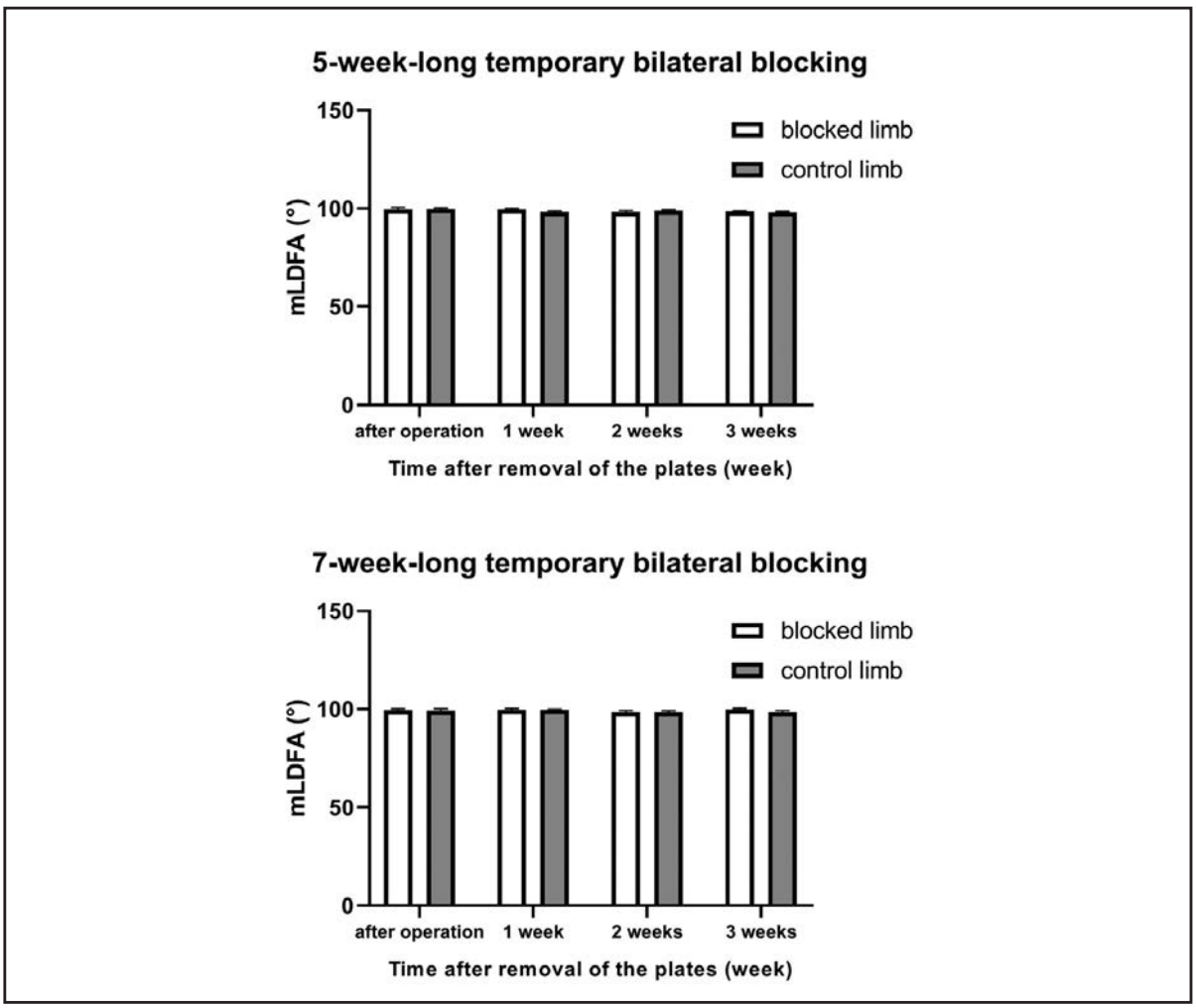

Figure 4. Analysis of the mechanical lateral distal femoral angle (mLDFA) 1-3 weeks after the discontinuation of the 5- or 7-week-long temporary bilateral blocking. There were no statistically significant difference between the blocked and the control limbs for all terms (paired t-test).

Discussion

The method of temporary unilateral blocking of the growth plate of long bones with the purpose of correction of the femoral or tibial deformities in children using plates with screws was adopted relatively recently – since 2007.[17] Therefore, the number of clinical trials, especially for the analysis of follow-up results, is limited. Identified complications, in particular the premature closure of the growth plate, prompted us to conduct an experimental study to determine the possibility of restoring the functionality of the growth plate after removal of the plates.

We found that 3, 5 and 7 weeks after the temporary bilateral blocking, the height of the epiphyseal cartilage and the primary spongiosa zone gradually decreased compared to the control limb, indicating a delay in the longitudinal growth of the bone. We noted that growth was suspended in the medial side of the growth plate after 3 weeks, in the lateral – after 5 weeks, and after 7 weeks in the middle side. Destructive changes (histoarchitectonics disorders, changes in cell density, etc.) had progressed with the increase of the blocking duration.

Three weeks after the 5-week-long temporary bilateral blocking, complete restoration of the morphological structure of the epiphyseal cartilage, including the primary spongiosa zone, took place. An increase in the height of the growth plate on both sides in the operated limb (by 1.2 times on the lateral side, by 1.9 times on the medial side) was established, whereas after 5 weeks of bilateral blocking, the height of the epiphyseal cartilage decreased over its entire length, compared with the control limb (Table 1-2). In a similar study in rabbits, which used unilateral growth plate blocking of the distal femoral bone with nonabsorbable filament and screws for 4 weeks, restoration of the length of the blocked bone after incision of a non-absorbable filament was established after 4 weeks.[18] In another study, during an experiment on rabbits, a growth rebound was detected in the proximal tibia region 2 weeks after the removal of the plate, which was used to block the growth plate for 3 weeks, on the side where the plate was located.[10] In our study, the growth plate was blocked over longer periods (5 and 7 weeks) compared to Martínez GS. et al.[18] and Corominas-Frances L. et al.[10] However, we also found a significant increase in the growth plate on both sides after removing the plates in both groups; whereas the maximum increase in growth was identified on the medial side in the group with a shorter blocking period (5 weeks).

In our experiment, when we removed the plates and discontinued the bilateral blocking of the distal growth plate of the rabbit femoral bone after 7 weeks, the structure of the epiphyseal cartilage was partially restored in 3 weeks, but did not become completely identical to the control limb, which we associate with an increase in the blocking time. During this process, the growth of the bone in length due to the functioning of the growth plate occurred, as evidenced by the characteristic structure of the hypertrophic and primary spongiosa zones. The height of the epiphyseal cartilage in the operated limb was greater in comparison with the control one: after removing the blocked plates, it was 1.2 times larger on the lateral side, and 1.07 times larger on the medial side ($p<0.01$) (Table 2).

Growth of the limb in length occurs due to the activity of different zones of the epiphyseal cartilage, mainly due to the degree of hypertrophy of the chondrocytes (40-50%), and only 10% depends on the proliferation of the chondrocytes.[19] One of the reasons for this is that the hypertrophied chondrocytes become precursors of about 50% of the osteoblasts, which are subsequently involved in the endochondral bone formation.[20,21] In our study, starting from week 5 of blocking, the hypertrophic zone almost disappeared; presumably this is explained by the discovered decrease in the height of the epiphyseal

cartilage in weeks 5 and 7 of blocking compared to the control limb.

In addition, the identified disorders in the primary spongiosa zone can also be related precisely to the disorders in the process of hypertrophy of chondrocytes. In experimental studies of the effect of compression on the epiphyseal cartilage of the proximal part of the tibia of rabbits (aged 13 weeks), reduction in expression of collagen types II and X was identified in week 6 of the experiment, which may indicate a decrease in the number of proliferative and hypertrophied chondrocytes, respectively, due to compression.[22] In addition, the proliferation of the chondrocytes in the resting zone is affected by hypertrophied chondrocytes through the secretion of Indian hedgehog. That is, in the absence of hypertrophied chondrocytes, the proliferation of the chondrocytes in the resting zone is also affected.[23] At the same time, 3 weeks after the discontinuation of the 5- or 7-week-long temporary bilateral blocking, the restoration of the structure of the proliferating zone and the appearance of new short chondrocyte columns have been established.

In our study, the age of the animals at the beginning of the experiment was 8 weeks. It is known that the maximum rate of growth of the femur of rabbits occurs in the first 4 weeks of life and at weeks 8-10 it slows down and then reaches a plateau. Also, the growth rates of the right and left femoral bones are not significantly different.[24] In the distal part of the femur of the rabbit, the growth plate is completely closed at the age of 19-24 weeks.[25] That is, in our study, 3 weeks after the 7-week-long temporary bilateral blocking, the age of the animals was 18 weeks, and the bone growth was almost complete. However, an increase in the growth plate height was established 3 weeks after the 7-week-long temporary bilateral blocking compared with the control limb. This may indicate that when the method of temporary bilateral blocking is used, restoration of the growth plate function may be possible even in the case of prolonged growth inhibition.

According to our previous X-ray study, the mLDFA after the bilateral blocking was implemented for 3, 5 and 7 weeks did not differ from the mLDFA in the control bone[16]. Histologically, we had established that the suppression of growth occurred earlier on the medial side than on the lateral and middle sides. After the removal of the plates, the greatest increase of the height of the growth plate occurred on the medial side. This phenomenon possibly compensates for the growth suppression caused by temporary bilateral blocking. However, it did not lead to bone deformation, which we confirmed by measuring the mLDFA, which did not differ from the mLDFA in the control bone.

Temporary bilateral blocking leads to the development of structural abnormalities in the growth plate, which cause inhibition of its function. Restoration of the structural-functional features of the growth plate after the removal of the plates depends on the blocking period, which must be taken into account in the clinical setting.

Conflicto de intereses: los autores declaran no tener conflicto de intereses.

Recibido: agosto 2019
Aceptado: enero 2020

References

1. Bouchard M. Guided growth: novel applications in the hip, knee, and ankle. *J Pediatr Orthop* 2017; 37:S32-S36.
2. Heflin JA, Ford S, Stevens P. Guided growth for tibia vara (Blount's disease). *Medicine (Baltimore)* 2016; 95:e4951.
3. Vogt B, Schiedel F, Rödl R. Guided growth in children and adolescents. Correction of leg length discrepancies and leg axis deformities. Orthopade 2014; 43:267-284.
4. Ruzbarsky JJ, Goodbody C, Dodwell E. Closing the growth plate: A review of indications and surgical options. *Curr Opin Pediatr* 2017; 29:80-86.
5. Stevens PM. The role of guided growth as it relates to limb lengthening. *J Child Orthop* 2016; 10:479-486.
6. Zajonz D, Schumann E, Wojan M, et al. Treatment of genu valgum in children by means of temporary hemiepiphysiodesis using eight-plates: short-term findings. *BMC Musculoskelet Disord* 2017; 18:456.
7. Joeris A, Ramseier L, Langendörfer M, et al. Paediatric lower limb deformity correction with the eight plate. *J Pediatr Orthop B* 2017; 26:441-8. doi:10.1097/bpb.0000000000000397.
8. Danino B, Rödl R, Herzenberg JE, et al. Guided growth: preliminary results of a multinational study of 967 physes in 537 patients. *J Child Orthop* 2018; 12:91-96. doi:10.1302/1863-2548.12.170050.
9. Lawing C, Margalit A, Ukwuani G, Sponseller PD. Predicting late follow-up and understanding its consequences in growth modulation for pediatric lower limb deformities. *J Pediatr Orthop* 2019; 39:295-301.
10. Corominas-Frances L, Sanpera I, Saus-Sarrias C, Tejada-Gavela S, Sanpera-Iglesias J, Frontera-Juan G. Rebound growth after hemiepiphysiodesis. An animal-based experimental study of incidence and chronology. *Bone Joint J* 2015; 97-B:862-868.
11. Mast N, Brown NA, Brown C. Validation of a genu valgum model in a rabbit hind limb. *J Pediatr Orthop* 2008; 28:375-80.
12. Council of Europe. European Convention for the protection of vertebrate animals used for experimental and other scientific purposes (1986, Mar 18) http://www.worldlii.org/int/other/treaties/COETSER/1986/1.html
13. Verkhovna Rada of Ukraine. The law of Ukraine on the protection of animals from cruel treatment (2006, Jun 2) https://zakon.rada.gov.ua/laws/show/3447-15
14. Khmyzov SO, Rokutov VS, Yershov DV, Makedonsky IO. X-ray assessment of the growth plate functioning after cessation of its temporary bilateral blocking by different types of plates: an experimental study. *Trauma* 2019; 20:67-72.
15. Tomaszewski R, Gap A, Wiktor L. Histological evaluation in autologous growth plate chondrocyte grafting in rabbits. *J Cytol Histol* 2017; 8:472.
16. Khmyzov SO, Rokutov VS, Iershov DV. Development of distal metaepiphysis of the femur in conditions of temporary bilateral blocking of the growth zone (experimental study). *Ortop Travmatol Protez* 2017; 3:48-53.
17. Stevens PM. Guided growth for angular correction: a preliminary series using a tension band plate. *J Pediatr Orthop* 2007; 27:253-9.
18. Martínez GS, Baar AZ, Ibañez AL, Vergara PG, Carmona MC, Drago SP. Assessment of femoral physeal activity after transitory hemiepiphysiodesis using screws and nonabsorbable filament. *J Pediatr Orthop* 2014; 34:208-212.
19. Stokes IAF. Mechanical effects on skeletal growth. *J Musculoskelet Neuronal Interact* 2002; 2:277-80.
20. Park J, Gebhardt M, Golovchenko S, et al. Dual pathways to endochondral osteoblasts: a novel chondrocyte-derived osteoprogenitor cell identified in hypertrophic cartilage. *Biol Open* 2015; 4:608-621.
21. Yang L, Tsang KY, Tang HC, Chan D, Cheah KS. Hypertrophic chondrocytes can become osteoblasts and osteocytes in endochondral bone formation. *Proc Natl Acad Sci U S A* 2014; 111:12097-12102.

22. Bries AD, Weiner DS, Jacquet R, et al. A study in vivo of the effects of a static compressive load on the proximal tibial physis in rabbits. *J Bone Joint Surg Am* 2012; 94:e1111-10.
23. Mizuhashi K, Ono W, Matsushita Y, et al. Resting zone of the growth plate houses a unique class of skeletal stem cells. *Nature* 2018; 563:254-258.
24. Rudicel S, Lee KE, Pelker RR. Dimensions of the rabbit femur during growth. *Am J Vet Res* 1985; 46:268-9.
25. Kaweblum M, Aguilar MDC, Blancas E, et al. Histological and radiographic determination of the age of physeal closure of the distal femur, proximal tibia, and proximal fibula of the New Zealand white rabbit. *J Orthop Res* 1994; 12:747-9.

Actual. Osteol 2019; 15(3): 205-213.
Internet: http://www.osteologia.org.ar

ARTÍCULOS ORIGINALES / *Originals*

EFFECT OF FERMENTED MILK WITH KEFIR GRAINS ON THE *IN VITRO* DEMINERALIZATION OF BOVINE TOOTH ENAMEL

María E. Chulibert,[1,2] Alejo Ferrer,[2] Karina E. Koch,[2] Alfredo Rigalli[1,2,3]*

[1] Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET). [2] Laboratorio de Biología Ósea. Facultad de Medicina, Universidad Nacional de Rosario. [3] Consejo de Investigaciones Universidad Nacional de Rosario. Santa Fe, Argentina.

Abstract

The dental caries is a progressive destruction of the teeth tissue due to the disbalance in the normal molecule interactions between the enamel and the biofilm, which alters the demineralization-remineralization process. Milk fermentation produces casein-phosphopeptides with proved remineralizing capacity of the enamel. The presence of these peptides in fermented milk with kefir grains has been described. The purpose of this work was to evaluate *in vitro* the capacity of milk kefir to prevent the demineralization of dental enamel.

Bovine incisors (n=68, 17 per group) were treated for 72 h with different solutions: I: artificial saliva at pH 7.2 , II: demineralizing solution at pH 4.5, III: supernatant of kefir fermented milk at pH 4.5, IV: milk supernatant at pH 4.5. The effects of treatments were evaluated by the change in the weight of the specimens, calcium concentration in the solution and by scanning electron microscopy (SEM) of the enamel. Kefir milk supernatant prevented the demineralization process, that was evidenced by a change in weight and calcium concentration that were not different from group I, although the pH was 4.5. In contrast, group IV showed a decrease in weight and an increase in calcium concentration, compared with group I (one way ANOVA, $p<0.05$). Images of SEM agree with the values of weight and calcium concentration. These results indicate that kefir milk supernatant has a protective effect on enamel demineralization *in vitro*.

Key words: kefir, demineralization, tooth enamel.

**E-mail: arigalli@unr.edu.ar*

Resumen

La caries dental es una patología debido a un desequilibrio en las interacciones moleculares normales entre el esmalte y la biopelícula, que altera el proceso de desmineralización-remineralización. La fermentación de la leche produce fosfopéptidos de caseína con probada capacidad remineralizante del esmalte, y se ha descripto la presencia de estos péptidos en la leche fermentada con granos de kéfir. El propósito de este trabajo fue evaluar in vitro la capacidad del kéfir de leche para prevenir la desmineralización del esmalte dental.

Sesenta y ocho incisivos bovinos (17 por grupo) fueron tratados durante 72 h con diferentes soluciones: I: saliva artificial, pH 7.2, II: solución desmineralizante, pH 4.5, III: sobrenadante de leche fermentada con kefir, pH 4.5, IV: sobrenadante de leche, pH 4.5. El proceso de desmineralización se evaluó mediante el cambio en el peso de las muestras, la concentración de calcio en la solución y microscopía electrónica de barrido (SEM) del esmalte. El sobrenadante de leche fermentada con kéfir impidió el proceso de desmineralización, que se evidenció por un cambio en el peso y la concentración de calcio que no discreparon del grupo I, a pesar de haber tenido un pH de 4.5. En contraste, el grupo IV mostró una disminución en el peso y un aumento en la concentración de calcio, en comparación con el grupo I (ANOVA a un criterio, $p<0.05$). Las imágenes SEM concuerdan con los cambios en el peso y la concentración de calcio en los grupos estudiados. Los datos obtenidos demuestran que el sobrenadante de la leche tratada con kéfir tiene un efecto protector sobre la desmineralización del esmalte in vitro, inducida por el pH ácido.

Palabras clave: *kefir, desmineralización, esmalte dental.*

Introduction

Oral health is an essential part of the overall health and therefore affects the total well-being of people. Dental caries is a disease with high prevalence and public health costs worldwide, despite the use of fluoride and other preventive methods.[1,2] Dental erosion is a multifactorial condition influenced by three main factors: chemical, biological and behavioral. The erosive potential of erosive agents like acidic drinks or foodstuffs depends on chemical factors such as pH, mineral content, clearance on tooth surface, calcium chelation properties, etc.[3]

It is well known that milk and milk products are rich in calcium and phosphorus ions and that they have a high buffering capacity. In addition, milk and its derivatives are a source of biopeptides with beneficial health activity.[4] Fermentation of milk leads to the production of lactic acid and the resulting fall in pH inhibits growth of many pathogenic organisms.[5]

Caseinophospeptides (CPP) are peptidic fractions derived from milk caseins with anticariogenic activity due to the ability to stabilize calcium phosphate on enamel, thus preventing demineralization and promoting remineralization. Most CPP contain a sequence with three phosphoserine residues, followed by two glutamic acids. Negative side chains corresponding to phosphate groups are responsible for binding to minerals, especially calcium.[6]

CPP have become of great interest in the dental field since they can associate to calcium phosphate on the tooth surface to form a pool of calcium and phosphate ions that maintains a state of saturation surrounding the enamel. As a consequence, demineralization is inhibited and the remineralization of enamel is increased.[7] Most of the *in vitro* and *in situ* studies showed strong evidence of CPP bioactivity in the oral cavity.[8]

Kefir milk also known as kefir, originally

from the Caucasus Mountains, is one of the oldest fermented milk. Fermentation is carried out by kefir grains, which contain a varied microbiota composed of lactic bacteria and yeasts and, together with the polysaccharide kefiran,[9] form a symbiotic community that confer unique properties to this beverage.[10]

The consumption of kefir milk has been shown to be as effective as sodium fluoride in the reduction of *Streptococcus mutans* in saliva, supporting the use of modified dairy products for anticariogenic purposes. The presence of phosphopeptides in kefir milk has been recently described and the binding affinity to calcium was confirmed for one of them, but it is estimated that other sequences of the 62 identified of the phosphopeptides could also have mineral binding properties.[11] In addition, kefir milk can be prepared and maintained easily at home, and it constitutes a high calcium and low lactose content food.[12]

To our knowledge, until now there were no studies demonstrating the protective effect of kefir milk on dental demineralization.

The aim of this study was to evaluate the concomitant effect of the kefir supernatant in the presence of acid that arises from milk fermentation.

Materials and methods

For these studies, freshly extracted bovine teeth, which were obtained from animals slaughtered in *CTC* slaughter-house from *Puerto Vilelas* (Province of Chaco, Argentina) were used. Teeth free from caries and enamel defects from bovines that are not older than 3 years old (68 specimens) were included in this study. Crowns were polished with a circular brush with nylon bristles mounted on hand piece and then they were rinsed with distilled water. Teeth were immersed in 5 % formalin for one week at 4°C.

A cross-section at the height of tooth neck was performed to separate the coronal portion from the root with a diamond blade mounted on hand piece with plenty of cooling steady stream of water. The cervical area, the cutting area and the duct of the coronal portion were covered with acid-resistant varnish, leaving only the adamantine tissue exposed. The crowns were stored in saline solution for one week.

This work has been approved by the Ethical Committee of the School of Medicine of Rosario National University.

Treatments

Samples were divided at random in 4 groups and, an adaptation of the method proposed by Ferrazzano to produce enamel erosion was used.[13] The adaptation of the method included: incubation at 4ºC instead of 37ºC and the use of bovine teeth instead of human teeth. The reason for the first modification is that we propose to use samples containing live microorganisms (group III, see below), and at 37ºC the microbial growth and metabolism could not be controlled under our experimental conditions. The second modification is due to the impossibility to obtain the necessary number of human teeth with the adequate quality to carry out the experiment. Each tooth was immersed in 3 ml of solution. Tubes were kept at 4°C for 72 h and at the end of the experiment, measurements were performed on the teeth and the solutions. The following groups were studied:

Group I: samples were immersed in artificial saliva with the following composition: p-hydroxymethylbenzoate (2.00 g/l), sodium carboxymethylcellulose (10.0 g/l), KCl (8.38 mmol/l), $MgCl_2$ (0.29 mmol/l), $CaCl_2$ (1.13 mmol/l), K_2HPO_4 (4.62 mmol/l), KH_2PO_4 (2.40 mmol/l), pH 7.2.

Group II: samples were immersed in demineralizing solution with the following composition: lactic acid (0.1 mol/l), sodium carboxymethylcellulose (0.2 g/l), and pH 4.5. These samples acted as demineralizing control groups.

Group III: samples were treated with supernatant of milk treated with kefir grains,

after centrifugation at 5000 g and 8ºC for 5 min (see below preparation of supernatant from kefir milk). Solutions of Group III had a pH of 4.5 as the consequence of the fermentation process of milk carbohydrate by the kefir grains.

Group IV: samples were immersed in the supernatant obtained from the treatment of milk with lactic acid to obtain a pH of 4.5, and 5 min of centrifugation at 5000 g at 8º C.

Preparation of supernatant from kefir milk

Commercial skimmed milk with 110 mg/100 ml of Ca and desiccated kefir (Prama®, Argentina) was used in the experiments described in this paper. Kefir grains were added at a ratio 5 g per 100 ml of pasteurized milk, 3 % lipid content. The mixture was left fermenting for 24 h at room temperature. The final pH of the solution was measured and if it was higher than 4.5, it was adjusted to such value by adding lactic acid. After milk fermentation for 24 h, it was centrifuged at 5000 g at 8°C for 10 min (Refrigerated centrifugal HERMLE Z 323 K model, Germany). With this procedure, the supernatant was obtained and the precipitate formed by the insoluble fraction was discarded. The supernatant was preserved for the treatment of Group III.

Evaluation Methods

Quantitative Analysis

The effect of solutions on demineralization and remineralization processes was measured through the change in weight and calcium concentration of solutions, after incubation with the crowns. Before and after treatments, the samples were dried until constant weight at 30°C. Each sample was weighted before (t0) and after (tf) the treatment with the solutions. A high precision scale of 0.1 mg error was used for this measurement (Mettler, Switzerland). The difference between weight at (tf) and (t0) in each specimen was calculated. A positive value was interpreted as mineral gain, whereas a negative value was interpreted as predominance of the demineralization process over the remineralization one. Similarly, before (t0) and after (tf) treatment, calcium concentration was measured in the solutions and the difference between tf and t0 was calculated. A positive value of the difference in calcium concentration was interpreted as predominance of the demineralization process over remineralization.

Calcium concentration in the solutions was measured before and after the treatments by atomic absorption spectroscopy with an AROLAB MK II equipment (Metrolab, Buenos Aires, Argentina), using acetylene flame:air in a 1.5:2 ratio. The results were expressed in µg of Ca released or captured during the experiment.

The pH measurements were performed with pH meter Methrom 632.

Qualitative Analysis

Scanning electron microscope (SEM) images were obtained from three specimens of each group. They were placed on platens with vestibular side up and were metalized with a thin layer of gold, by electrical metalization (Sputtering Denton Vacuum Desk II, Moorestown, United States) and were observed by SEM (JEOL JEOL 5800LV. Tokyo, Japan- Electronic Microscopy Service –UNNE). Images were captured digitally with a magnification of x1000 and x3000. (Digitizer Gatan model 788 Digiscan II Pleasanton, United States). Only x3000 images are shown in the result section of this manuscript.

Statistical analyses: statistical analyses were performed using the stat, base and agricolae packages of software R 3.4.4.[14]

Results

Qualitative analysis by SEM indicates that the treatment with kefir milk supernatant has a protective effect against pH induced demineralization. The SEM images of the different treatments are described below.

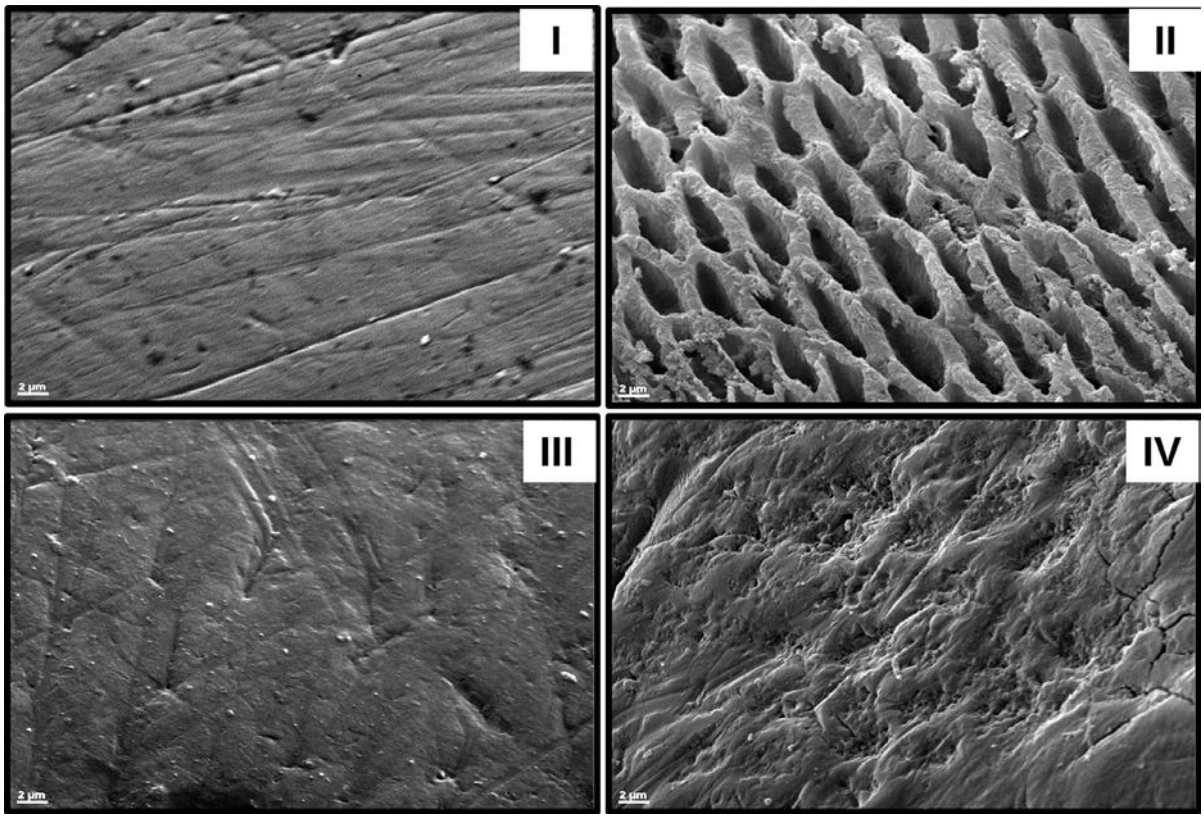

Figure 1: surface of the tooth enamel after 72 h incubation with different solutions. I) healthy enamel of group I, after treatment with artificial saliva, pH 7.2; II) demineralized enamel of group II, after treatment with lactic acid solution at pH 4.5; III) treated with supernatant of kefir milk at pH 4.5; IV) demineralized enamel of group IV: treated with supernatant of milk obtained by treatment of milk with lactic acid, pH 4.5. Images are displayed at 3000x.

SEM evaluation of group I (Fig. 1 I) showed a healthy enamel surface, regular and with uniform texture.

The SEM image of group II (Fig. 1 II) showed the surface micromorphology of artificial lesions created in positive control. The formation of a characteristic honeycomb structure in the demineralized enamel, which exhibits substantial lesions following a specific "as etching" pattern is evident. The core of the prisms was dissolved leaving prominent peripheral margins.

In the SEM images of samples of group III (Fig. 1 III), demineralized areas were not observed. A relatively smooth and even surface, appearing as covered with a mineral deposit is described, possibly this mineral sediment completely filled the previously created lesions. This group showed images of the adamantine surface of relatively smooth appearance and with no undercutting by demineralization.

In the SEM images of group IV (Fig. 1 IV), a heterogeneous surface with large and irregular erosions were observed.

Significant difference was found in weight among treatments applied to bovine tooth enamel (ANOVA, $p < 0.05$). Groups II and IV had a negative weight difference, which implies demineralization of tooth enamel, while groups I

and III had a positive weight difference, meaning that there was no demineralization of tooth enamel. When a post test was performed, a significant difference was found between group I and II, group III differs from group II, and group IV differs from group I. The change in the teeth weight in different groups are consistent with the images obtained by SEM.

Table 1: difference in weight (mg) between the final and initial weight of specimens. At least one equal superscript letter between two columns indicates non-significant differences. * Indicates differences respect Group I, # Indicates differences respect group p<0.05. One way ANOVA, post test LSD.test. Data are shown as mean ± standard deviation.

Group I	Group II	Group III	Group IV
-8.7±5.4	334±534 *	20,3±42,1 #	40,2±65,9 *

A significant difference was found for the difference in calcium among treatments (ANOVA, p <0.05). The difference in calcium concentration in group I, indicates that calcium is not lost by the treatment with artificial saliva. Although groups II, III and IV had a positive calcium difference, group III had the lowest value of calcium lost. This value was not different from group I. On the other hand, groups II and IV differ from group I (LSD test, p <0.05).

Table 2: difference between final an initial calcium concentration (mg/dl). Negative values indicate Ca uptake by the specimen, and a positive values indicate calcium lost from teeth. * Indicates differences respect Group I, # Indicates differences respect group p<0.05. One way ANOVA, post test LSD.test. Data are shown as mean ± standard deviation.

Group I	Group II	Group III	Group IV
5.1±9.9	-2.3±5.1 *	2.8±5.3 #	-0.2±5.8 *

Discussion

The dental caries is a process that implies a lack of balance in the normal molecule interactions between the tooth surface and the adjacent microbial biofilm. If the loss of minerals occurs at a higher speed than the corresponding mineral deposition, it has the potential to lead to enamel cavitation and side effects in dentin and pulp, ending with the localized destruction of tooth hard tissue.[15]

Scientific evidence has shown that the enamel gradual demineralization is normal due to the loss of both calcium and phosphate ions. At the same time, the enamel is remineralized thanks to saliva maintenance, mineral balance, and oral pH. Nevertheless, this balance can be affected, leaning mainly for demineralization.[16]

The enamel remineralization, which occurs physiologically in the oral environment, can be fostered by remineralizing agents or other systems that favor this action. This process has been known for more than a hundred years, but only in recent decades its therapeutic role has been accepted for the control of dental caries.[17]

New enamel remineralizing agents are being considered for the management of patients at high caries risk and the treatment of subclinical lesions and mild white spot lesions, since they provide an alternative to the use of fluoride and the use of dental sealant type materials.[18]

Fermented milk presents a number of beneficial health properties and it is considered a functional food. These beneficial properties can be attributed to the microorganisms that are used in the elaboration of the fermented milk, and to the different products released during the fermentation process.[19] Numerous peptidic fractions with bioactive properties have been isolated from fermented milk.[20]

Biological active peptides are those that exert, additionally to their nutritional aminoacid supply, a physiological effect. These are inactive inside the precursor protein sequence and can be released *in vitro* or *in vivo* by enzymatic hydrolysis.[21]

The data reported in this paper show that supernatant of kefir milk does not have demineralizing effect on enamel *in vitro*. This result is consistent with other studies that indicate a link between dairy consumption and the reduction of dental caries. This effect is mainly attributed to several factors: tooth remineralization, inhibition of bacterial colonization and biofilm inhibition.[22]

Dairy products are the most recognized food group with anti-caries activity.[23] Using human and animal *in vitro* models, the anti-cariogenic activity of milk products was attributed to direct chemical effects of CPP, calcium and phosphate.[24]

The results of this paper indicate that the demineralization of enamel in the presence of the supernatant of fermented milk with kefir grains was significantly reduced *in vitro*. These results were confirmed by SEM micrographs of group I and III where the samples exhibited less surface changes, compared to samples from groups II and IV. Further, our findings are consistent with the proposed mechanism for CPP anti-cariogenic, action, which is associated to CPP interaction with the ACP (amorphous calcium phosphate) on the tooth surface, forming nano-complexes (CPP-ACP) that are incorporated into the dental plaque and stick to the dental surface, acting as calcium and phosphate reservoirs. These nanoparticles of CPP and calcium phosphate, are capable of capturing the excess of free ions and maintain an atmosphere of supersaturation of these ions with respect to enamel, which prevents demineralization and promote remineralization.[25] These peptides have recently been identified in kefir milk, and the calcium binding capability of one of them has been confirmed.[26] Therefore, future research should focus on *in vivo* studies and epidemiological effects of the consumption of functional food in the reduction or elimination of dental caries.

The encouraging results obtained in group III could promote research for the development of preventive measures against dental caries, based on the use of active ingredients derived from food (kefir, yogurt, and cheese) that offer the advantage of being completely biocompatible, easy to obtain, nontoxic, and less expensive than pharmacological treatments.

This work shows that dairy products like kefir milk represent a system capable of preventing demineralization of enamel in its early stages, which strengthen the physiological mechanisms of protection. The identification and characterization of these peptides, being naturally derived from milk, on one hand would allow their use to develop commercial products for oral application

without adverse effects and, on the other hand, would add another functional food feature to kefir, which consumption could be recommended to people suffering from tooth demineralization with therapeutic purposes. Presumably, CPP content in kefir milk is greater than in milk due to proteolytic activity of microorganisms contained in this drink.[27]

Moreover, consumption of probiotic products containing live microorganisms improves oral health. Recently, a pilot study has shown that consumption of kefir is as effective as sodium fluoride in reducing the load of *Streptococcus mutans*. These results support the use of modified milk with anti-cariogenic goal.[28]

It is concluded that kefir milk has a protective effect on enamel demineralization, even at a low pH values. Therefore, although the consumption of kefir is not a method of treatment, it provides a prevention method valid against early enamel demineralization when physiological protection mechanisms are insufficient. It is important to highlight that kefir milk can be prepared at home, as kefir grains are available at food markets.

Conflicto de intereses: los autores declaran no tener conflicto de intereses.

Recibido: noviembre 2019
Aceptado: febrero 2020

References

1. Hamissi J, Ramezani GH, Ghodousi A. Prevalence of dental caries among high school attendees in Qazvin, Iran. *J Ind.an Soc Pedod Prev Dent* 2008; 26 Suppl 2:S53-5.
2. Cochrane NJ, Cai F, Huq NL, Burow MF, Reynolds EC. New approaches to enhanced remineralization of tooth enamel. *J Dent Res* 2010; 89: 1187-97.
3. Olivan SRG, Sfalcin RA, Fernandez RAM, et al. Preventive efect of the remineralizing materials on dental erosion lesions by speckle technique: an vitro analysis. *Photodiagnosis Photodyn Ther* 2020; 7:101655.
4. Reynolds EC, Cai F, Shen P, Walker GD. Retention in plaque and remineralization of enamel lesions by various forms of calcium in a mouthrinse or sugar-free chewing gum. *J Dent Res* 2003; 82:206–211.
5. Southgate DA: Milk and milk products, fats and oils; in Garrow JS, James WPT, Ralph A (eds): Human Nutrition and Dietetics. Edinburgh, Churchill Livingstone, 2000, pp 375–383.
6. Moynihan P. Foods and factors that protect against dental caries. *Nutr Bull* 2000; 25:281-6.
7. Reynolds EC, Cai F, Shen P, Walker GD. Retention in plaque and remineralization of enamel lesions by various forms of calcium in a mouthrinse or sugar-free chewing gum. *J Dent Res* 2003; 82:206–211.
8. Walker G, Cai F, Shen P, et al. Increased remineralization of tooth enamel by milk containing added casein phosphopeptide-amorphous calcium phosphate. *J Dairy Res.* 2006; 73(1):74-8.
9. Beshkova D, Simova ED, Simov, ZI, Frengova GI, Spasov ZN. Pure cultures for making kefir. Food Microbiol. 2002; 19:537-544.
10. Ghasempour M, Sefdgar SA, Moghadamnia AA, Ghadimi R, Gharekhani S, Shirkhani L. Comparative study of kefr yogurt-drink and sodium fluoride mouth rinse on salivary mutans streptococci. *J Contemp Dent Pract.* 2014, 1; 15(2):214-7.
11. EbnerJ, Arslan AA, Fedorova M, Hoffmann

R, Küçükçetin A, Pischetsrieder M. Peptide profiling of bovine kefir reveals 236 unique peptides released from caseins during its production by starter culture or kefir grains. *J Proteomics.* 2015, 117:41-57.

12. Fina BL, Brun LR, Rigalli A. Increase of calcium and reduction of lactose concentration in milk by treatment with kefir grains and eggshell. *Int J Food Sci Nutr.* 2016; 67(2):133-40.
13. Ferrazzano GF, Cantile T, Quarto M, Ingenito A, Chianese L, Addeo F. Protective effect of yogurt extract on dental enamel demineralization in vitro. *Aust Dental J* 2008; 53(4):314–319.
14. R Core Team (2018). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.
15. Pitts NB, Stamm JW. International Consensus Workshop on Caries Clinical Trials (ICW-CCT) - Final consensus statements: Agreeing where the evidence leads. *J Dent Res.* 2004; 83:(Spec Iss C)125-128.
16. Acosta MG, Rodríguez D, Nazar I. El fosfato de calcio fosfopéptido amorfo y su camino en la remineralización. *Oral.* 2013; 14(45):1007-1010.
17. Gutiérrez B, Planells P. Actualización en odontología mínimamente invasiva: remineralización e infiltración de lesiones incipientes de caries. *Cient Dent* 2010; 7(3):183-191.
18. Castellanos 2013. La remineralización del esmalte bajo el entendimiento actual de la caries dental. *Univ Odontol.* 2013; 32(69): 49-59.
19. Leroy F, De Vuyst L. Lactic acid bacteria as functional starter cultures for the food fermentation industry. *Trends Food Sci Technol.* 2004; 15(2): 67-78.
20. Nagpal R, Behare P, Rana R, Kumar A, Kumar M, Arora S, Yadav H. Bioactive peptides derived from milk proteins and their health beneficial potentials: an update. *Food Func.* 2011; 2(1), 18-27.
21. Beshkova D, Simova ED, Simov, ZI, Frengova GI, Spasov ZN. Pure cultures for making kefir. *Food Microbiol.* 2002; 19: 537-544.
22. Merritt J, Qi F, Shi W: Milk helps build strong teeth and promotes oral health. *J Calif Dent* Assoc 2006; 34:361–366.
23. Aimutis WR: Bioactive properties of milk proteins with particular focus on anticariogenesis. *J Nutr* 2004; 134:989S-995S.
24. Reynolds EC: Remineralisation of enamel subsurface lesions by casein phosphopeptides-stabilized calcium phosphate solutions. *J Dent Res* 1998; 76:1587-1595.
25. Cross KJ, Huq NL, Palamara JE, Perich J W, Reynolds EC: Physicochemical characterization of casein phosphopeptideamorphous calcium phosphate nanocomplexes. *J Biol Chem* 2005; 280:15362-15369.
26. EbnerJ, Arslan AA, Fedorova M, Hoffmann R, Küçükçetin A, Pischetsrieder M: Peptide profiling of bovine kefir reveals 236 unique peptides released from caseins during its production by starter culture or kefir grains. *J Proteomics* 2015; 117:41-57.
27. Beermann C, Hartung J: Physiological properties of milk ingredients released by fermentation. *Food Funct* 2013; 4:185-199.
28. Ghasempour M, Sefdgar SA, Moghadamnia AA, Ghadimi R, Gharekhani S, Shirkhani L: Comparative study of kefir yogurt-drink and sodium fluoride mouth rinse on salivary *Streptococcus mutans*. *J Contemp Dent Pract* 2014; 15:214-7.

Actual. Osteol 2019; 15(3): 214-224.
Internet: http://www.osteologia.org.ar

ARTÍCULOS ORIGINALES / ***Originals***

RELACIÓN ENTRE NIVELES DE VITAMINA D Y PERFIL LIPÍDICO EN EMBARAZADAS DE ALTO RIESGO

Evangelina Giacoia,[1] María Verónica Ledesma,[1]* Silvia Cabrera,[2] Katherine Grisales Rave,[1] Patricia Rodríguez,[3] Viviana Bacchini[1]

[1] Servicio de Endocrinología y Metabolismo, Hospital Nacional Prof. Alejandro Posadas. [2] Especialista en Estadística para Ciencias de la Salud, H.I.G.A. Prof. Dr. Ramón Carrillo. [3] Servicio de Bioquímica, Sección Endocrinología, Hospital Nacional Prof. Alejandro Posadas. Buenos Aires, Argentina.

Resumen

En la Argentina, las embarazadas presentan alta prevalencia (80%) de hipovitaminosis D y de sobrepeso u obesidad (27,4%). Ambas condiciones pueden aumentar la morbimortalidad materno-fetal. Bajos niveles de vitamina D se han relacionado con aumento del colesterol total, LDL, triglicéridos (Tg) y descenso de HDL. Objetivo: evaluar los niveles de 25-hidroxivitamina D (25OHD) y su relación con el perfil lipídico en pacientes embarazadas de alto riesgo. Materiales y métodos: estudio de corte transversal entre septiembre de 2016 y abril de 2017. Se excluyeron pacientes que recibieron suplementos de vitamina D, con disfunción tiroidea no compensada, malabsorción, insuficiencia cardíaca, renal o hepática y dislipidemia familiar. Niveles circulantes de 25OHD < 30 ng/ml se consideraron hipovitaminosis. Resultados: se evaluaron 86 embarazadas de 29,3 ± 7,1 años durante la semana 28 ± 6,5. El IMC pregestacional fue 28,3 ± 6,5 kg/m^2 y la ganancia de peso 7 ± 4,3 kg. Perfil lipídico: colesterol total 240 ± 54 mg/dl; LDL 156 ± 54 mg/dl; HDL 66 ± 15 mg/dl; Tg 204 ± 80 mg/dl. La media de 25OHD fue de 23,8 ± 9 ng/ml, con una prevalencia de hipovitaminosis D de 77,9 %. Las pacientes con hipovitaminosis D presentaron mayores valores de colesterol total y LDL ($p < 0,05$), con tendencia no significativa a presentar mayores valores de Tg. Conclusión: en embarazadas de alto riesgo se observó una alta prevalencia de hipovitaminosis D, asociada con mayores concentraciones de colesterol total y LDL.

Palabras clave: embarazo de alto riesgo, vitamina D, colesterol, hipercolesterolemia, triglicéridos.

**E-mail: veroledesma86@hotmail.com*

Abstract

RELATIONSHIP BETWEEN VITAMIN D LEVELS AND LIPID PROFILE IN HIGH RISK PREGNANT WOMEN

In Argentina, pregnant women have a high prevalence (80 %) of hypovitaminosis D and overweight/obesity (27.4%), conditions that can increase maternal-fetal morbidity and mortality. Low levels of 25-hydroxyvitamin D (25OHD) have been linked to an increase in total cholesterol, LDL cholesterol, triglycerides (TG) and a decrease in HDL cholesterol. Objective: to evaluate the levels of vitamin D and its relationship with the lipid profile in high risk pregnant patients. Materials and methods: cross-sectional study between September 2016 and April 2017. Patients who received vitamin D supplements or had non-compensated thyroid dysfunction, malabsorption, heart failure, renal or hepatic failure, or familial dyslipidemia were excluded. Hypovitaminosis D was defined as a circulating level of 25OHD < 30 ng/ml.

Results: We assessed 86 women of 29.3 ± 7.1 years during pregnancy week 28 ± 6.5. Pre-gestational BMI was 28.3 ± 6.5 kg/m^2. Their weight gain was 7 ± 4.3 kg. Lipid profile: total cholesterol 240 ± 54 mg/dl; LDL cholesterol 156 ± 54 mg/dl; HDL cholesterol 66 ± 15 mg/dL; TG 204 ± 80 mg/dl. The mean 25OHD level was 23.8 ± 9 ng/ml, with a 77.9 % prevalence of hypovitaminosis D. Patients with hypovitaminosis D had higher values of total cholesterol and LDL cholesterol (p <0.05), and a non-significant trend toward higher triglyceridemia. Conclusion: A high prevalence of hypovitaminosis D, associated with high total and LDL cholesterol was found in high risk pregnant women.

Key words: *high risk pregnant women, vitamin D, cholesterol, hypercholesterolemia, triglycerides.*

Introducción

Actualmente se conocen efectos beneficiosos de la vitamina D más allá de los relacionados con el metabolismo fosfocálcico, conocidos como acciones no clásicas, dentro de las cuales se encuentran las relacionadas con los sistemas inmunitario y cardiovascular, y el metabolismo lipídico.[1,2]

La hipovitaminosis D representa a nivel mundial un problema de salud pública, especialmente en mujeres embarazadas. Según Holick y col., la mayoría de las embarazadas tienen deficiencia o insuficiencia de vitamina D.[1,3-6] Se estima que un 40 a 98% de las mujeres embarazadas en todo el mundo tienen concentraciones de 25-hidroxivitamina D (25OHD) menores de 20 ng/ml y un 15 a 84%, concentraciones menores de 10 ng/ml.[7]

Según la Guía Argentina de Vitamina D, en un estudio realizado en embarazadas de hospitales de la ciudad de Buenos Aires en primavera y verano, el 88% presentaban niveles de 25OHD $\leq$ 30 ng/ml (hipovitaminosis D).[8] Otro estudio informó que el 27,4% de las mujeres argentinas embarazadas en Buenos Aires tenían sobrepeso u obesidad gestacional.[9] La hipovitaminosis D y el exceso de peso pueden aumentar la morbimortalidad materno-fetal mediante: diabetes gestacional, hipertensión arterial, preeclampsia, parto prematuro y mayor indicación de cesárea. El riesgo de hipertensión en el embarazo, macrosomía y cesárea tienen relación directa con el índice de masa corporal (IMC) elevado.[10]

Durante el embarazo normal se produce un aumento fisiológico en los niveles de lípidos, triglicéridos (Tg) y colesterol total, a medida que avanza la edad gestacional. Sin embargo, los altos niveles de colesterol o Tg maternos se asocian con parto prematuro, hipertensión inducida por el embarazo, preeclampsia y ma-

crosomía.[11] La obesidad es un factor de riesgo relacionado con la deficiencia de vitamina D, posiblemente asociado a su acumulación en el tejido adiposo.[12]

Los niveles más bajos de 25OHD en mujeres embarazadas con sobrepeso u obesidad con alto riesgo de diabetes gestacional se asocian con dislipidemia (elevación de Tg y colesterol total),[13] perfiles inflamatorios y adipocinas subóptimos, y alteración en el metabolismo de la glucosa. Estas asociaciones podrían explicarse por la adiponectina de alto peso molecular, la cual disminuye en estados de hipovitaminosis D, generando mayor estado inflamatorio con tendencia a disglucemia, insulinorresistencia y dislipidemia, con resultados adversos como diabetes gestacional y parto pretérmino.[7]

La deficiencia de vitamina D se ha relacionado también con factores de riesgo cardiometabólicos que incluyen obesidad, resistencia a la insulina, hipertensión, dislipidemia, así como diabetes tipo 2 y enfermedad cardiovascular. Con respecto a la dislipidemia, la vitamina D parece actuar sobre el receptor de vitamina D para prevenir la formación de células espumosas; reducir la absorción de colesterol LDL acetilado; promover la formación de partículas de HDL y regular los niveles de apolipoproteína A-1 en suero, todo lo cual mejora el transporte de colesterol y los lípidos en general.[14]

Nuestro objetivo fue evaluar niveles de 25OHD y su relación con el perfil lipídico en pacientes embarazadas de alto riesgo, considerando como hipótesis que la insuficiencia de 25OHD se asocia con menores concentraciones de HDL y mayores niveles de LDL y Tg.

Pacientes y métodos

Se realizó un estudio de corte transversal de embarazadas de alto riesgo, definido por la presencia de diabetes gestacional, hipertensión inducida por embarazo o disfunción tiroidea o diabetes pregestacional que asistieron al Servicio de Endocrinología durante el período septiembre de 2016-abril de 2017. Criterios de inclusión: pacientes mayores de edad que acudieron al Consultorio de Alto Riesgo de Endocrinología, con historias clínicas completas.

Se excluyeron las pacientes que recibieron suplementos de vitamina D, que tenían disfunción tiroidea no compensada (hipotiroidismo con TSH mayor de 10 µUI/ml y hormonas tiroideas bajas o hipertiroidismo con TSH inhibida con hormonas tiroideas elevadas, con tratamiento específico o sin él), malabsorción, insuficiencia cardíaca, renal o hepática, dislipidemia familiar y cualquier etiología que generara déficit de vitamina D.

Las variables estudiadas fueron: edad (años), semana de embarazo, estación del año, índice de masa corporal (IMC) pregestacional, calculado dividiendo los kilogramos de masa por el cuadrado de la estatura en metros (kg/m^2), y ganancia de peso en kg. Se obtuvieron muestras de sangre entre las 8 y las 9 horas, con 12 horas de ayuno previo. Se midió colesterol total, colesterol HDL, colesterol LDL y Tg en mg/dl por colorimetría Cobas Roche®. La 25OHD (ng/mL) fue medida por quimioluminiscencia (LIAISON®) de DiaSorin con coeficiente de variación intraensayo menor de 8% e interensayo de 13,2%.

Las voluntarias que participaron de este estudio firmaron consentimiento informado y el estudio fue aprobado por el Comité de Ética hospitalario.

De las 90 pacientes embarazadas evaluadas, se incluyeron 86. Cuatro pacientes fueron excluidas del estudio, una por falta de datos en la historia clínica y tres por patología tiroidea no compensada (TSH mayor de 10 µUI/ml). Tuvieron una edad de 29,3 ± 7,1 años y se encontraban en la semana gestacional 28 ± 6,5.

De las pacientes incluidas con alto riesgo, 37 tenían diabetes gestacional, 7 hipertensión inducida por embarazo, 32 disfunción tiroidea (25 hipotiroidismo, 7 hipertiroidismo), 7 diabetes gestacional más hipotiroidismo y 3 diabetes gestacional más hipertensión.

Los niveles plasmáticos de 25OHD se clasificaron en 3 categorías teniendo en cuenta las directrices de la Endocrine Society 2011:[1]

1. Suficiente ≥ 30 ng/ml
2. Insuficiente 21 a 29 ng/ml
3. Deficiente <20 ng/ml.

Se consideró con hipovitaminosis D a aquellas pacientes con insuficiencia o deficiencia de vitamina D (valores de 25OHD menores de 30 ng/ml).[15]

Los valores de referencia del perfil lipídico fueron determinados según criterios de percentiles cuando hubo elevación de las concentraciones de colesterol total, LDL y Tg por encima del percentil 95 y niveles de HDL por debajo del percentil 5 para la edad gestacional.[16] A fin de determinar dichos percentiles se utilizó como modelo el trabajo de Ywaskewycz Benítez y col.:[17] percentil 95 de colesterol según el trimestre (primero, segundo y tercero), total, 230, 290 y 231 mg/dl; LDL 134, 191 y 230 mg/dl y Tg de 158, 257 y 371 mg/dl, respectivamente. El percentil 5 para HDL se consideró 38,2, 42,7 y 40,1 mg/dl según el trimestre.[17]

Se evaluó IMC pregestacional para establecer el estado nutricional previo a la gestación. El peso pregestacional se adquirió de datos de historia clínica obstétrica.

En la República Argentina, de acuerdo con las Recomendaciones en Nutrición para los equipos de salud 2012, se clasificó el IMC por edad gestacional utilizando la Gráfica de IMC versus edad gestacional, tomando el IMC al momento de la consulta. Se clasificó como *Adecuada*: el área delimitada por las curvas -1 y +1 SD; *Baja* por debajo de -1 SD; *Elevada* mayor de 1 SD.[18]

Análisis estadístico

Para el análisis estadístico se utilizó el programa SPSS 23.0®. Se calcularon media y mediana. Como medida de dispersión se utilizó el desvío estándar (SD) para la media y el rango para la mediana. Se evaluó la normalidad de las variables a través de las pruebas de Kolmogorov y Shapiro. Las variables categóricas fueron informadas en porcentajes. Para la comparación de las variables categóricas se utilizó el test de Fisher y para las variables continuas *t* de Student, ANOVA o Kruskal-Wallis según tipo y números de grupos para comparar. Antes de la realización del ANOVA se evaluó la homocedasticidad de los grupos y la distribución. Cuando el test de ANOVA fue significativo, se realizó análisis *post-hoc* con la prueba de Scheffé. Para la correlación de las variables se utilizó el coeficiente de Pearson. Se consideró significativa una $p<0,05$.

Resultados

En el análisis de las 86 embarazadas de alto riesgo (diabetes gestacional, enfermedades tiroideas e hipertensión arterial) incluidas no se discriminó por subgrupos de causa de riesgo.

La media del IMC pregestacional fue 28,3 ± 6,5 kg/m^2. Presentaron bajo peso (IMC <18,5 kg/m^2) el 2% (n = 2), peso normal (IMC 18,5 a 24,9 kg/m^2) el 35% (n = 30), sobrepeso (IMC 25,0 a 29,9 kg/m^2) el 30% (n = 26) y obesidad (IMC ≥ 30,0 kg/m^2) el 33% (n = 28). De nuestras pacientes, 63% (n = 54) tuvieron sobrepeso u obesidad. La media de 25OHD fue de 23,8 ± 9 ng/ml. La prevalencia de hipovitaminosis D (deficiencia + insuficiencia) fue del 77,9%.

Los valores medios del perfil lipídico fueron colesterol total 240 ± 54 mg/dl, LDL 156 ± 54 mg/dl, HDL 66 ± 15 mg/dl y Tg 204 ± 80 mg/dl.

Según los valores de LDL se evaluaron los niveles de vitamina D y se obtuvo que dentro de la categoría de deficiencia de 25OHD presentaron LDL con una media de 177 ± 54 mg/dl, insuficiencia de 25 OHD con valores de LDL con una media de 142 ± 45 mg/dl y suficiencia de 25OHD con LDL con una media de 154 ± 60 mg/dl ($p = 0,028$). En el análisis *post-hoc* del colesterol LDL se encontraron diferencias entre el grupo de déficit con el de insuficiencia ($p = 0,01$) y en menor medida con el de suficiencia ($p = 0,07$).

Según los valores de colesterol total, se evaluaron niveles de vitamina D y se obtuvo que

Tabla 1. Características de la población de mujeres embarazadas de alto riesgo según los niveles de 25OHD.

		NIVELES DE 25OHD (ng/ml)							
		Total	≤ 20		21-29		≥ 30		P valor #
		n	n	%	n	%	n	%	
Edad materna (años)	< 25	27	7	25,9	11	40,7	9	33,3	ns
	25-29	11	4	36,4	6	54,5	1	9,1	
	30-34	23	7	30,4	12	52,2	4	17,4	
	> 35	25	12	48,0	8	32,0	5	20,0	
IMC pregestacional (kg/m²)	< 18,5	2	1	50,0	-	-	1	50,0	ns
	18,5 a 25	30	12	40,0	10	33,3	8	26,7	
	25 a 30	26	9	34,6	10	38,5	7	26,9	
	> 30	28	8	28,6	17	60,7	3	10,7	
IMC para edad gestacional	Baja	5	3	60,0	1	20,0	1	20,0	ns
	Adecuada	45	17	37,8	14	31,1	14	31,1	
	Elevada	36	10	27,8	22	43,0	4	22,1	

#Se utilizó prueba de Fisher. Al no haber diferencia entre los grupos no se realizó prueba post-hoc.

Tabla 2. Niveles de 25OHD según características de la muestra y perfil lipídico.

	NIVELES DE 25OHD (ng/ml)								
	Total (n=86)		≤ 20 (n=30) Grupo 1		21-29 (n=37) Grupo 2		≥ 30 (n=19) Grupo 3		P
	Media	SD	Media	SD	Media	SD	Media	SD	
Edad materna (años)	29,3	7,1	30,4	6,8	29,4	6,9	27,6	7,9	ns[1]
Semanas de embarazo	28,5	6,5	28,9	6,15	28,5	5,9	29,7	7,1	ns[1]
IMC pregestacional (kg/m²)	28,3	6,5	27,1	6,6	29,5	4,8	26,8	6,5	ns[2]
Ganancia de peso (kg)	7,0	4,3	7,0	5,7	6,8	2,9	7,3	4,4	ns[2]
Colesterol total (mg/dl)	240	54	265	55	222	45	237	57	0,005*[2]
LDL (mg/dl)	156	54	177	54	142	45	154	60	0,028#[2]
HDL (mg/dl)	66	15	66	14	68	14	66	20	ns[2]
Tg (mg/dl)	204	80	218	87	186	72	216	84	ns[2]

** Diferencias entre grupos 1-2 y 1-3 (Análisis post-hoc: Prueba de Scheffé).*

 # Diferencias entre grupos 1-2 (Análisis post-hoc: Prueba de Scheffé).

[1]Se utilizo prueba de Kruskal-Wallis. [2] Se utilizo test de ANOVA.

dentro de la categoría de deficiencia de 25OHD presentaron colesterol total con una media de 265 ± 55 mg/dl, insuficiencia de 25OHD con una media de 222 ± 45 mg/dl y suficiencia de 25OHD con una media de 237±57 mg/dl (p = 0,005). En el análisis de comparaciones múltiples hubo diferencias significativas entre la media de colesterol total entre los grupos déficit comparados con el de insuficiencia (p = 0,002) y suficiencia (p = 0,03). También hubo una tendencia hacia mayores valores de Tg en la hipovitaminosis D, pero sin alcanzar significación estadística.

En cuanto al análisis de la relación entre niveles de 25OHD y el perfil lipídico se halló que las pacientes con deficiencia e insuficiencia presentaron mayores valores de colesterol total (Figura 1) y LDL (Figura 2).

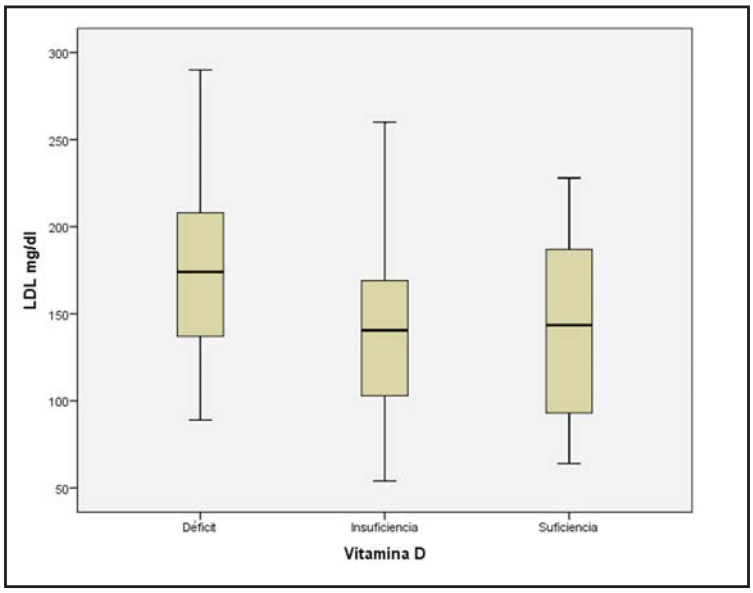

Figura 1. Colesterol LDL según niveles de vitamina D (p = 0,028 según ANOVA)**.**

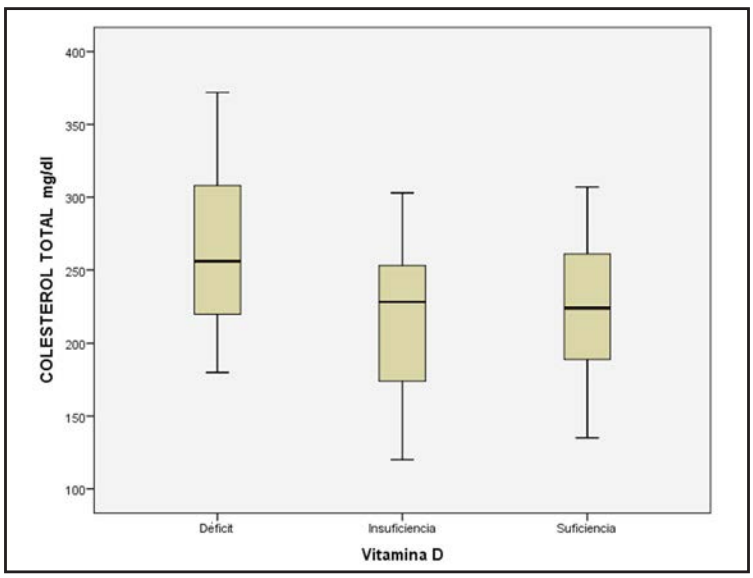

Figura 2. Colesterol total según niveles de vitamina D (p = 0,005 según ANOVA).

Al relacionar IMC pregestacional con niveles de 25OHD se encontró que, dentro de la categoría de deficiencia de 25OHD, presentaron IMC de 27,1 ± 6,6 kg/m^2. En insuficiencia de 25OHD, el IMC fue de 29,5 ± 4,8 kg/m^2 y en suficiencia de 25OHD fue de 26,8 ± 6,5 kg/m^2, sin diferencia estadísticamente significativa (véase Tabla 2).

Según IMC versus edad gestacional al momento de la consulta, se evaluaron los niveles de vitamina D y se obtuvo que, dentro de la categoría ganancia baja (n = 5), presentó hipovitaminosis D el 80%, con 25OHD de 23,4 ± 16,6 ng/ml; en ganancia adecuada (n = 45) presentó hipovitaminosis D el 69 %, con 25OHD de 23,5 ± 9,0 ng/ml. En ganancia elevada (n = 36) presentó hipovitaminosis D el 71%, con 25OHD de 23,0 ± 9,0 ng/ml; con valor de p > 0,05 en la comparación de los tres grupos (test de Kruskal- Wallis) y de ganancia adecuada versus alta (test *t* de Student).

La hipovitaminosis D fue más prevalente en mujeres con sobrepeso u obesidad (46,4 %) en comparación con las mujeres con peso normal y bajo (27,5 %).

Al comparar la época del año cuando se realizó la determinación de 25OHD, se obtuvo que la 25OHD en primavera fue de 20,6 ± 8,4 ng/dl, en verano de 24,0 ± 8,5 ng/dl y en otoño de 25,1 ± 10,9 ng/dl, con p >0,05 en la comparación de los niveles de vitamina D con relación a los meses del año. Cabe notar que no se realizaron determinaciones durante el invierno.

Discusión

Durante el embarazo se producen cambios fisiológicos en el metabolismo de hidratos de carbono, así como también en el asociado a los lípidos (colesterol, HDL, LDL, Tg) que equilibran las demandas de energía del feto y preparan a la madre para el parto y la lactancia. La tendencia al incremento en las concentraciones lipídicas en la mujer embarazada, resultan entonces adaptaciones normales para el desarrollo fetal. Pero existen múltiples factores que pueden relacionarse con un incremento mayor (patológico), el cual se asocia a mayor riesgo cardiovascular, como por ejemplo tabaquismo, sedentarismo, sobrepeso y bajos niveles de vitamina D.[5,14,19-21]

El sobrepeso y la obesidad se asocian a niveles bajos de 25OHD tanto en mujeres embarazadas como no embarazadas. La exposición intrauterina a bajos niveles de 25OHD puede traer consecuencias en la descendencia, como aumento de la resistencia a la insulina y mayor porcentaje de grasa corporal, lo cual durante la gestación se relaciona con anomalías cardiometabólicas en la descendencia.[19,21]

En individuos con obesidad, la ingesta dietaria y la exposición al sol pueden influir parcialmente en los niveles de 25OHD, pero el secuestro en el tejido adiposo parece ser el mecanismo principal.[5,12,19] En estados de hipovitaminosis D disminuye la adiponectina de alto peso molecular, generando un mayor estado inflamatorio con tendencia a disglucemia, insulinorresistencia y dislipidemia, con resultados adversos como la diabetes gestacional y el parto pretérmino. Se cree que el mecanismo por el cual la vitamina D puede aumentar la adiponectina es a través de la supresión del gen TNF-α y el sistema renina-angiotensina del tejido adiposo. La vitamina D puede reducir el riesgo de diabetes gestacional al elevar el calcio intracelular, que es vital para la glucólisis de las células β y la señalización de glucosa, o al actuar en el receptor de vitamina D para regular el receptor de insulina y facilitar la oxidación y el transporte de glucosa basales y mediados por insulina.[7]

La deficiencia materna de 25OHD es un problema importante de salud pública. Su prevalencia en mujeres embarazadas varía entre 18 y 84%.[22,23] En un estudio realizado en Buenos Aires, República Argentina, por Oliveri y col., el 88% de las embarazadas estudiadas presentaban niveles de 25OHD menores de 30 ng/ml, en primavera-verano.[4]

En cuanto a la relación de patologías gestacionales de riesgo con la 25OHD, está

descripto que la hormona estimulante de la tiroides y los niveles de glucosa en sangre en embarazadas se correlacionan negativamente con los niveles de 25OHD. Las embarazadas con diabetes gestacional tienen un metabolismo anormal de la insulina y mayor proporción de disfunción tiroidea asociada. Un estudio realizado en China evaluó niveles de 250HD en embarazadas de alto riesgo con diabetes gestacional e hipotiroidismo subclínico. Comparó 100 gestantes con estas patologías con un grupo control de 100 gestantes sanas. Los niveles de 25OHD en el grupo de observación fueron más bajos que los del grupo de control (27,86 ± 7,35 ng/ml versus 39,25 ± 8,90 ng/ml; P < 0,01). El 75% de las mujeres embarazadas del grupo de riesgo tuvieron hipovitaminosis D (menor de 30 ng/ml).[24] En nuestro trabajo, la prevalencia de hipovitaminosis D en mujeres embarazadas de alto riesgo (con diabetes gestacional, enfermedades tiroideas o hipertensión arterial) fue de 77,9%, similar a lo descripto en la bibliografía. No se encontraron trabajos relacionados donde se hayan estudiado las mismas características que en nuestra muestra.

El objetivo que nos planteamos al realizar nuestro estudio fue principalmente relacionar si en embarazadas de alto riesgo existe relación entre el perfil lipídico y la vitamina D; más específicamente si el colesterol LDL y el colesterol total aumentan en situaciones donde la 25OHD disminuye.

Aumentar los niveles de 25OHD plasmáticos (exposición solar, descenso de peso, lácteos fortalecidos), mejoraría la síntesis de 1,25-dihidroxivitamina D, aumentando de esta forma la absorción intestinal de calcio y fósforo. Esto mejoraría la sensibilidad a la insulina y la relación HDL/LDL, mejorando el perfil metabólico de esta población sensible de embarazadas. Existen varios estudios que demuestran una asociación positiva de 25OHD con colesterol HDL, y negativa con LDL y Tg. Niveles suficientes de 25OHD mejorarían el perfil lipídico, al disminuir la síntesis de colesterol por inhibición de la actividad de la β-hidroxi-β-metilglutaril-coenzima A reductasa. La absorción de calcio por la 25OHD tendría un efecto indirecto en reducir los niveles de Tg, al actuar a nivel hepático.[21,23,25,26]

Un estudio aleatorizado, doble ciego, controlado con placebo, realizado por Asemi y col., evaluó el efecto de la suplementación con 25OHD en el metabolismo glucémico y lipídico en 54 embarazadas con diabetes gestacional, encontrándose una reducción estadísticamente significativa en los valores de glucemia y colesterol LDL. De dicho análisis se concluye que niveles suficientes de vitamina D mejorarían perfiles metabólicos, inflamación y biomarcadores de estrés oxidativo, por lo que estaría indicada su suplementación. El tratamiento con vitamina D en mujeres con diabetes gestacional mejoró la glucemia, el colesterol total y las concentraciones de colesterol LDL, pero no influyó en el colesterol HDL ni en los Tg.[23]

Los niveles circulantes de 25OHD y ácidos grasos omega-3 resultarían ser menores en mujeres con diabetes gestacional que en gestantes sanas. En un ensayo clínico aleatorizado, doble ciego, controlado con placebo, realizado en 140 mujeres con diabetes gestacional, se evaluó la relación de la sustitución con vitamina D y ácidos omega-3 con el perfil lipídico y glucémico. Encontraron mejoría en el control glucémico y reducciones significativas de los triglicéridos séricos y en las concentraciones de VLDL.[26]

En el estudio realizado por Haidari y col. se compararon los niveles de vitamina D entre un grupo de embarazadas con diabetes gestacional y otro con normoglucemia. Los niveles séricos de 25OHD fueron significativamente más bajos en el grupo con diabetes gestacional; también hubo una correlación negativa significativa entre 25OHD y glucemia en ayunas e IMC pregestacional.[27]

Se realizaron estudios de niveles de 25OHD en embarazadas con hipertensión gestacional. La forma biológicamente activa de la vi-

tamina D, 1,25-dihidroxivitamina D, puede suprimir la biosíntesis de renina y la proliferación de células del músculo liso vascular, modulando la producción de citocinas y regulando la transcripción de genes vinculados a la invasión placentaria. En una muestra de 117 pacientes con hipertensión gestacional encontraron que el 78,9% tenían hipovitaminosis D.[28,29] En otro estudio, cuyo objetivo fue determinar los efectos de la suplementación con vitamina D en el perfil lipídico y glucémico de mujeres embarazadas con hipertensión arterial o sin ella, se encontró significativa reducción en el colesterol total, LDL y Tg, junto con un incremento en niveles de HDL.[30]

Sobre la base de la bibliografía analizada no pudimos relacionar los indicadores de embarazo de alto riesgo con los niveles bajos de 25OHD, quizá por el escaso número de embarazadas incluidas.

Limitaciones del estudio: se evaluó el nivel de 25OHD solo una vez, y esto puede no reflejar el estado de vitamina D a largo plazo. Además, el número de pacientes estudiadas no basta para extrapolar estos resultados a la población.

Conclusión

La prevalencia de hipovitaminosis D analizada en la muestra de pacientes embarazadas de alto riesgo fue del 77,9%. Las pacientes con menores niveles de 25OHD presentaron mayores niveles de colesterol total y LDL.

El conocimiento de la relación entre niveles de vitamina D y el perfil lipídico pueden ser de ayuda para intervenir a fin de prevenir morbilidades asociadas. Es de suma importancia asegurar desde inicios del embarazo, e idealmente desde la preconcepción, niveles adecuados (≥ 30 ng/ml) de vitamina D, así como también un índice de masa corporal adecuado, para lograr modificaciones saludables en el perfil lipídico, además de los ya conocidos en relación con el metabolismo fosfocálcico.[1,2,20] Pero se necesitan más estudios para confirmar nuestros resultados.

Conflicto de intereses: los autores declaran no tener conflicto de intereses.

Recibido: mayo de 2019
Aceptado: marzo de 2020

Referencias

1. Holick M, Binkley N, Bischoff-Ferrari H, et al. Evaluation, treatment, and prevention of vitamin D deficiency: an Endocrine Society Clinical Practice Guideline. *J Clin Endocrinol Metab* 2011; 96:1911-30.
2. Pramyothin P, Holick M. Vitamin D supplementation. *Curr Opin Gastroenterol* 2012; 28:139-50.
3. Plantalech L. Mapa de Hipovitaminosis D en Argentina. *Actual Osteol* 2005; 1:11-5.
4. Oliveri B, Parisi M, López L, Brito G, Zeni S, Fernández C. Calcium and vitamin D nutritional status during pregnancy (Abstract). *J Bone Miner Res* 2009; 24(Suppl 1).
5. Dror D, Allen L. Vitamin D inadequacy in pregnancy: biology, outcomes, and interventions. *Nutr Rev* 2010;68:465-77.
6. van Schoor N, Lips P. Worldwide vitamin D status. *Best Pract Res Clinl Endocrinol Metab* 2011; 25:671-80.
7. Mousa A, Abell S, Shorakae S, et al. Relationship between vitamin D and gestational diabetes in overweight or obese pregnant women may be mediated by adiponectin. *Mol Nutr Food Res* 2017; 61:1700488.

8. Sánchez A, Oliveri B, Mansur J, et al. Guía de la Federación Argentina de Sociedades de Endocrinología sobre diagnóstico, prevención y tratamiento de la hipovitaminosis D. *Actual Osteol* 2015; 11:151-71.
9. Bolzán A, Dupraz S, Piaggio L, Rolón M, Guadalupe M. Segunda encuesta antropométrica de embarazadas de la ciudad de Buenos Aires, Argentina 2010. *Rev Bras Saúde Mater Infant* 2011; 11:455-61.
10. Minjarez Corral M, Rincón-Gómez I, Morales-Chomina YA, Espinosa-Velasco MJ, Zárate A, Hernández-Valencia M. Ganancia de peso gestacional como factor de riesgo para desarrollar complicaciones obstétricas. *Perinatol Reprod Hum* 2014; 28:159-66.
11. Vrijkotte T, Krukziener N, Hutten B, Vollebregt K, van Eijsden M, Twickler M. Maternal lipid profile during early pregnancy and pregnancy complications and outcomes: The ABCD Study. *J Clin Endocrinol Metab* 2012; 97:3917-25.
12. Wortsman J, Matsuoka L, Chen T, Lu Z, Holick M. Decreased bioavailability of vitamin D in obesity. *Am J Clin Nutr* 2000; 72:690-93.
13. Al-Ajlan A, Krishnaswamy S, Alokail M, et al. Vitamin D deficiency and dyslipidemia in early pregnancy. *BMC Pregnancy Childbirth* 2015*;* 15:314.
14. Mousa A, Naderpoor N, de Courten M, Scragg R, de Courten B. 25-hydroxyvitamin D is associated with adiposity and cardiometabolic risk factors in a predominantly vitamin D-deficient and overweight/obese but otherwise healthy cohort. *J Steroid Biochem Mol Biol* 2017; 173:258-64.
15. Hollis B. Circulating 25-hydroxyvitamin D levels indicative of vitamin D sufficiency: implications for establishing a new effective dietary intake recommendation for vitamin D. *J Nutr* 2005; 135:317-22.
16. Feitosa A, Barreto L, Silva I, Silva F, Feitosa Filho G. Impact of the use of different diagnostic criteria in the prevalence of dyslipidemia in pregnant women. *Arq Bras Cardiol* 2017; 109:30-8.
17. Ywaskewycz Benítez LR, Bonneau G, Castillo Rascón M, et al. Perfil lipídico por trimestre de gestación en una población de mujeres adultas. *Rev Chil Obstet Ginecol* 2010; 75:227-33.
18. Ministerio de Salud de la Nación. Nutrición y Embarazo. Recomendaciones en Nutrición para los equipos de salud – Dirección Nacional de Maternidad e Infancia. Buenos Aires: Ministerio de Salud; 2012.
19. Hrudey E, Reynolds R, Oostvogels A, Brouwer I, Vrijkotte T. The association between maternal 25-hydroxyvitamin D concentration during gestation and early childhood cardio-metabolic outcomes: is there interaction with pre-pregnancy BMI. *PLoS One 2015*; 10: p.e0133313.
20. Narchi H, Kochiyil J, Zayed R, et al. Maternal vitamin D status throughout and after pregnancy. *J Obstet Gynaecol* 2010; 30:137-42.
21. Lepsch J, Eshriqui I, Farias D, et al. Association between early pregnancy vitamin D status and changes in serum lipid profiles throughout pregnancy. *Metabolism* 2017; 70:85-97.
22. Dawodu A, Wagner CL. Mother-child vitamin D deficiency: an international perspective. *Arch Dis Child* 2007; 92:737-40.
23. Asemi Z, Hashemi T, Karamali M, Samimi M, Esmaillzadeh A. Effects of vitamin D supplementation on glucose metabolism, lipid concentrations, inflammation, and oxidative stress in gestational diabetes: a double-blind randomized controlled clinical trial. *Am J Clin Nutr* 2013; 98:1425-32.
24. Zhou X, Li Z, Li B, Guo S, Yao M. Expression and clinical significance of serum 25-OH-D in pregnant women with SCH (Subclinical Hypothyroidism) and GDM (Gestational Diabetes Mellitus). *Pak J Med Sci* 2018; 34:1278-82.
25. Jorde R, Grimnes G. Vitamin D and metabolic health with special reference to the effect of vitamin D on serum lipids. *Prog Lipid Res* 2011; 50:303-12.
26. Jamilian M, Samimi M, Ebrahimi F, et al. The effects of vitamin D and omega-3 fatty acid co-supplementation on glycemic control and lipid concentrations in patients with gestational diabetes. *J Clin Lipidol* 2017; 11:459-68.

27. Haidari F, Jalali MT, Shahbazian N, Haghighizadeh MH, Azadegan E. Comparison of serum levels of vitamin D and inflammatory markers between women with gestational diabetes mellitus and healthy pregnant control. *J Family Reprod Health* 2016; 10:1-8.
28. Arshiya K, Meenakshi S. An evaluation of association of vitamin D insufficiency with gestational hypertension in pregnant women. *Int J Reprod Contracept Obstet Gynecol* 2018; 7:3109-12.
29. Magnus M, Miliku K, Bauer A, et al. Vitamin D and risk of pregnancy related hypertensive disorders: mendelian randomisation study. *BMJ* 2018; 361:k2167.
30. Sonuga A, Asaolu M, Sonuga O. Effects of vitamin D supplementation on lipid profile and plasma glucose of preeclamptic women in Ibadan, Nigeria. *Open Access Library J* 2018; 5:e4410.

Actual. Osteol 2019; 15(3): 225-236.
Internet: http://www.osteologia.org.ar

REPORTE DE CASOS / *Case Report*

SINUS FLOOR ELEVATION USING A NEW BOVINE BONE GRAFTING MATERIAL. CASE REPORT AND BONE GRAFTING MATERIALS UPDATE

Gretel G. Pellegrini,[1*] Andrea S. Mattiuzzi,[3] Miguel A. Pellegrini,[1] Luis A. Corso,[2] Cintya P. Contreras Morales,[2] Elizabeth Arandia Osinaga,[2] Susana N. Zeni[1,3]

[1]Laboratorio de Osteoporosis y Enfermedades Metabólicas Óseas. Instituto de Inmunología, Genética y Metabolismo (INIGEM). Facultad de Farmacia y Bioquímica-Hospital de Clínicas ¨José de San Martín". CONICET-Universidad de Buenos Aires. [2]Cátedra de Clínica de Operatoria y Prótesis II. Facultad de Odontología. Universidad de Buenos Aires. [3]Cátedra de Bioquímica General y Bucal. Facultad de Odontología. Universidad de Buenos Aires. Buenos Aires, Argentina.

Abstract

Bone grafting is important to preserve the alveolar bone ridge height and volume for dental implant placement. Even though implant-supported overdentures present highly successful outcomes, it seems that a great number of edentulous individuals have not pursued implant-based rehabilitation. The cost of the treatment is one of the reasons of discrepancy between highly successful therapy and its acceptance. Therefore, the development of biomaterials for bone grafting with comparable characteristics and biological effects than those renowned internationally, is necessary. In addition, domestic manufacture would reduce the high costs in public health arising from the application of these biomaterials in the dental feld. The purpose of this clinical case report is to provide preliminary clinical evidence of the efficacy of a new bovine bone graft in the bone healing process when used for sinus floor elevation.

Keywords: bovine bone graft, new bone formation, sinus augmentation, osteoconduction.

Resumen

El uso de injertos óseos es importante para preservar la altura y el volumen de la cresta alveolar para la colocación de implantes dentales. Si bien las sobredentaduras implanto-soportadas presentan resultados altamente exitosos, la mayoría de las personas desdentadas no han sido rehabilitadas mediante implantes dentales. Uno de los principales motivos por los cuales los pacientes no aceptan este tipo de tratamiento, altamente exitoso, es el elevado costo del mismo. Por ello, es necesario el desarrollo de biomateriales de injerto óseo con características y efectos biológicos comparables a los reconocidos internacionalmente. Asimismo, la fabricación nacional reduciría los altos costos en Salud Pública derivados de la aplicación de estos biomateriales en el campo dental. El objetivo de esta comunicación es presentar un caso clínico a fin de proporcionar evidencia preliminar acerca de la eficacia de un nuevo injerto de hueso bovino en el proceso de cicatrización ósea en el levantamiento del piso del seno maxilar.

Palabras clave: *hueso bovino, neoformación ósea, osteoconducción, elevamiento del piso del seno maxilar.*

**E-mail: gp2571@cumc.columbia.edu*

Introduction

Bone grafting implantation is the main treatment modality for bone defect repair and reconstruction.[1] In oral and maxillofacial areas, bone grafting aims to replace the volumetric bone loss that frequently occurs by systemic pathologies, periodontal defects, and tooth loss.[2]

The mechanisms underlying bone healing promoted by a bone graft are osteogenesis (osteo-differentiation and subsequent new bone formation by donor cells derived from the host or graft), osteoinduction (induction of undifferentiated and pluripotent cells to develop osteogenesis into the bone-forming cell lineage), and osteoconduction (the ability to support the attachment of osteoblast and osteo-progenitor cells, and the migration and ingrowth of these cells within the three dimensional architecture of the graft),[3,4] in combination or alone.[5]

Bone grafting materials are classifed as autografts (derived from the same individual), allografts (derived from a different individual from the same species), xenografts (derived from a different species), and alloplasts (derived from synthetic sources).[6] Autografts are the 'gold standard' in the reconstruction of bone defects due to their osteoconductive as well as osteoinductive properties.[7] Although they present excellent biological outcomes, they also have a number of drawbacks. In this regard, the use of autografts increases the operative time due to graft harvest, increases the donor site morbidity and, increases the graft resorption. In addition, they represent a big challenge for the operator since they need to be molded and have limited availability, especially in the pediatric population.[8] Allografts are typically obtained from human corpses and require to be processed before being used.[9,10] Allograft bone is available as cortical, cancellous, corticocancellous forms, or as demineralized bone matrix. It can be processed as mineralized or demineralized, fresh, fresh-frozen, or freeze-dried forms.[11,12] The benefts of allografts include their availability in different shapes and sizes. This is particularly advantageous since avoids donor site morbidity.[13] The major disadvantages of allografts are the potential for disease transmission and graft rejection. In order to decrease the risk of transmitting infectious diseases, allografts need to be treated. The techniques employed include treatment with hypotonic solutions, acetone, ethylene oxide or gamma irradiation that may eliminate cellular and viral particles.[14] However, these processes eliminate the bone cells and denature proteins present in the graft altering the osteoconductive and osteoinductive properties and eliminating the osteogenic properties.[15] In addition, allografts are capable to induce immunological reactions that interfere with the bone healing process leading to rejection of the graft.[13,16-18]

Synthetic bone grafts are osteoconductive and have been shown to integrate to bone.[19] There are many available synthetic graft materials, including bioactive glasses, a- and b-tricalcium phosphate (TCP), and synthetic hydroxyapatite.[19] Ideally, a synthetic bone graft should be biocompatible and cause minimal fbrotic changes.[20] Bioactive glass or "bioglasses" have been widely used as bone substitutes because of their ability to join and integrate to the bone tissue, forming a layer of active apatite on the surface, with similar characteristics to bone.[21] These biomaterials are resorbable and dissolution of their products (soluble silicon and calcium) upregulates seven families of osteoblastic genes promoting osteogenesis.[21,22] Among synthetic materials, synthetic hydroxyapatite, a crystalline phase of calcium phosphate found naturally in the mineral of bone, exhibits initial mechanical rigidity and structure, and demonstrates osteoconductive as well as angiogenic properties *in vivo*.[20] The synthetic hydroxyapatite, is a biocompatible and osteoconductive material due to its physicochemical characteristics.[23] This material al-

lows keeping the space flled extremely well, providing a physical matrix for the deposition of new bone. For these reasons, synthetic hydroxyapatite has high success in the felds of biology, medicine and dentistry

Due to the great popularity of dental implant surgery, the demand for alveolar ridge reconstruction, including sinus augmentation and immediate implant procedure, increased. This new trend in dentistry for implants boosted the development of new grafting materials. Ideally, a bone graft should be biocompatible, biodegradable, osteoconductive, osteoinductive, structurally similar to bone, easy to use, and cost-effective.[5] Within these parameters, a growing number of bone graft alternatives are commercially available and frequently used in dentistry.

In this regard, xenografts, frequently derived from bovine, porcine and coral sources[5], are a suitable alternative. Bovine bone is one of the most popularly used xenografts. This source material is desirable because it is readily available an inexpensive. However, bovine bone grafts require proper preparation to avoid risks such as transmission of zoonoses.[24] Several studies have shown that organic or inorganic matrix derived from bovine bone is biocompatible and osteoconductive.[24, 25] These important biological properties allow the apposition of newly formed bone by osteoprogenitor cells and the partial remodeling by osteoclasts and osteoblasts of the host.[26] Moreover, the large interconnecting pore volume and its composition encourage the formation and ingrowth of new bone at the implantation sites.

Different types of bone grafts are available in the international market. However, it is essential to have a wide variety of them to improve the competitiveness of each product in terms of quality, commercial value and clinical use. Therefore, the development of biomaterials for bone grafting produced by domestic manufactures, with comparable characteristics and biological effects than those well-known internationally, is necessary in order to reduce the high costs in public health arising from the application of these biomaterials in the dental feld.

Synergy Bone Matrix (SBM) (Odontit Implant Systems, Argentine) is a bovine bone graft material manufactured in Argentina, approved by the ANMAT (National Administration of Drugs, Foods and Medical Devices, Argentina) and the FDA (Food and Drug Administration, United States). SBM consists of sterile biocompatible anorganic porous bone mineral matrix for use in periodontal, oral and maxillofacial surgery. It is produced by removal of organic components from bovine bone. Therefore, SBM provides a supportive structure for osteoconduction. The presence of pores in Synergy is of great importance for repairing bone defects.

Even though there is evidence about the osteoconductive properties of SBM in experimental models in rats,[27] to date, there is no clinical evidence in the literature about the use of SBM in sinus floor elevation. The purpose of the present clinical case report is to provide clinical evidence of the effcacy of this new bovine bone graft in the healing process of alveolar bone when used for sinus floor elevation.

Case report

A 54-year-old female patient was referred to the Department of Clinical Operative and Prosthesis II, Dental School, University of Buenos Aires, Buenos Aires, Argentina for rehabilitation of her edentulous maxilla. Radiographic and cone beam computed tomography (CBCT) exhibited severe atrophy in the posterior region of the maxilla (Figure 1). The medical history did not reveal any systemic disease and the patient did not report to be under any medication. The patient aimed to rehabilitate the upper arch with a fxed implant-supported prosthesis. The proposed treatment plan was divided in two stages. The frst stage included the confection of a

complete upper denture, as well as, a surgical and radiological stent, and the reconstruction of the posterior maxillary alveolar ridge. The second stage, after 6 months, consisted in the placement of 4 dental implants in the posterior maxilla. All clinical procedures were conducted under the patient's written informed consent.

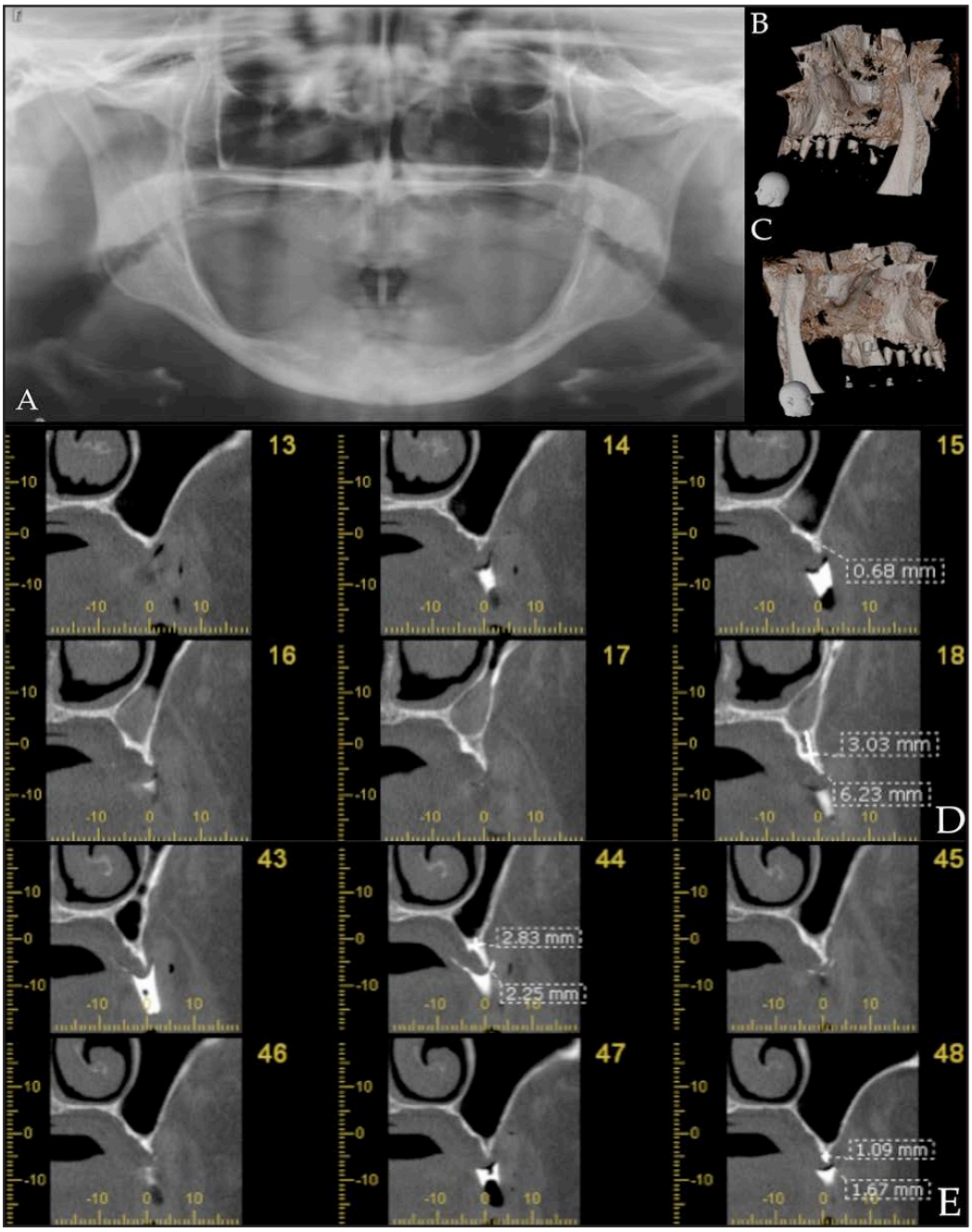

Figure 1. Pre-operative diagnostic images. All images show a dramatic loss of bone in the upper left and right maxilla. A: Panoramic X-ray showing edentulus maxilla and mandible. B, C. 3-D reconstruction of the left (B) and right (C) maxilla with the surgical stent. D, E: Coronal cut from a cone beam computed tomography scan from the left (D) and right (E) maxilla.

Sinus elevation surgery and guided tissue regeneration

The bilateral sinus elevation procedure was performed using the technique previously described by Tatum.[28] Briefly, after anesthesia with infltrative local carticaine hydrochloride 4% with adrenaline 1:100.000 (Totalcaína Forte, Microsules Bernabó, Argentina), a mucoperiosteal flap was elevated with releasing vertical incisions. Once exposed the buccal wall of the remaining alveolar process and the anterolateral wall of the Highmore antrum, a surgical stent was used to locate the lateral window. An oval osteotomy was performed with high-speed handpiece and a round diamond bur under copious irrigation with saline, leaving a "bone island", in the lateral wall of the sinus, attached to the Schneider membrane (Figure 2). This fragment of bone was then turned medially and positioned towards the sinus floor. The sinus membrane was then elevated across the floor and up the medial wall. A bilateral guided bone regeneration procedure was performed using the bovine bone grafting material SBM. In order to adjust the consistency and handling characteristics of SBM, it was mixed with sterile saline (0.9% Sodium Chloride) (Figure 2C).

The size of the granules was 350- 840 #m. The graft was covered with a resorbable collagen membrane (BioCollagen, Bioteck, Italy). Finally, the flap was repositioned and sutured without tension. The patient was instructed to perform oral hygiene and to rinse twice a day during 7 days with chlorhexidine digluconate 0.12% for disinfection of the surgical wound. Amoxicillin-clavulanate 875 mg was prescribed twice a day for 7 days and 500 mg of naproxen was administered every 8-12 hours for 5 days to control postoperative pain. Soft diet was also recommended. The sutures were removed after 7 days. CBCT scans and panoramic x-rays were obtained pre-operative, 6 months after stage 1 and 4 months after stage 2. A biopsy of each treated area was taken with a trephine bur during the implant placement surgery.

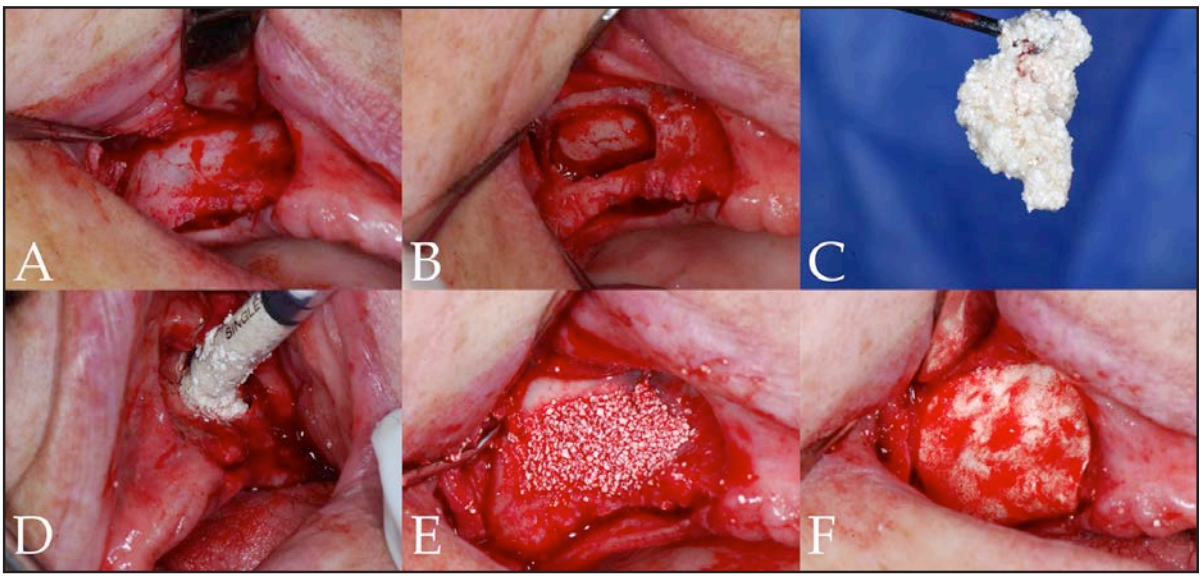

Figure 2. Sinus elevation surgery and guided tissue regeneration. A: elevation of mucoperiosteal flap. B: Oval osteotomy and "bone island" in the lateral wall of the sinus attached to the Schneider membrane. C: Synergy Bone Matrix (SBM). D, E: Placement of SBM for guided bone regeneration. F. The graft was covered with a resorbable collagen membrane.

During the frst surgical stage, a post-operative follow-up 7 days after the procedure revealed that the edges of the flap wounds faced each other and there were no signs of dehiscence or inflammation. The patient did not report any discomfort, pain or inflammation of the treated areas. The post-operative CBCT, taken 6 months after this surgery, exhibited an increase of 10.7 mm and 10.8 mm in the height of the alveolar crest, and an increase in the alveolar crest width of 3.5 mm and 2.8 mm in the right and left side, respectively (Figure 3). Six months after the sinus lift surgery dental implants were placed in the areas that received the bone graft (stage 2). Dental implants in the areas grafted achieved primary stability, indicating that there was an accurate bone quality after the placement of the bone graft. Consistent with the digital imaging fndings, histological evaluation of the bone samples retrieved during the implant surgery revealed that SBM particles were osteoconductive. All particles were surrounded by new bone formation (Figure 4). There were fbro-angiogenic and fbrous areas associated to SBM, as well as gradual regression of associated fbrosis. The bone formation pattern was lamellar and trabecular, and the presence of osteoblast at the surface of the trabeculae, as well as osteocytes, was also observed. There were no signs of inflammation or bone sequestrae.

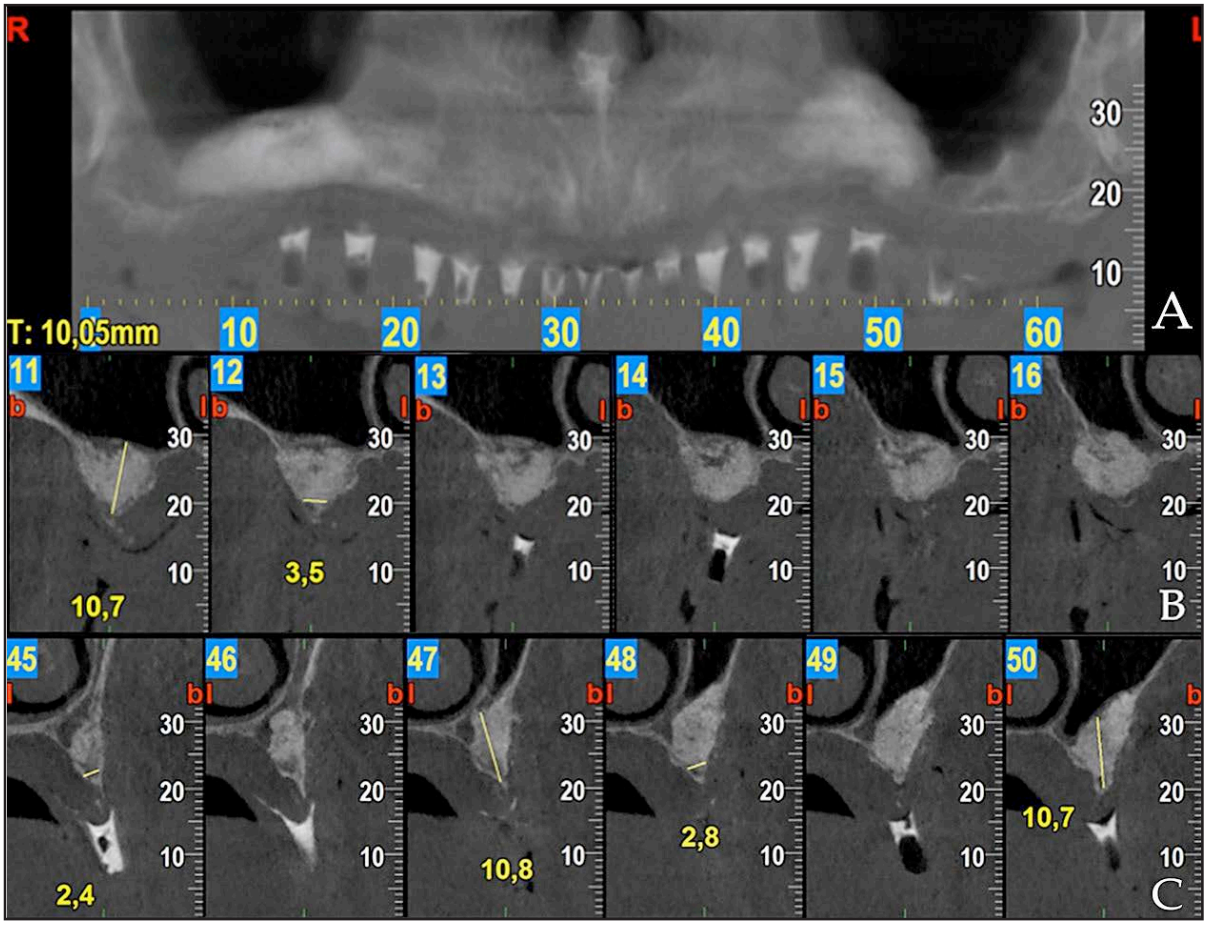

Figure 3. Post-operative CBCT (6 months after the sinus elevation surgery). A, B, C: There was an increase of 10.7 mm and 10.8 mm in the height of the alveolar crest, and an increase in the alveolar crest width of 3.5 mm and 2.8 mm in the right and left side, respectively.

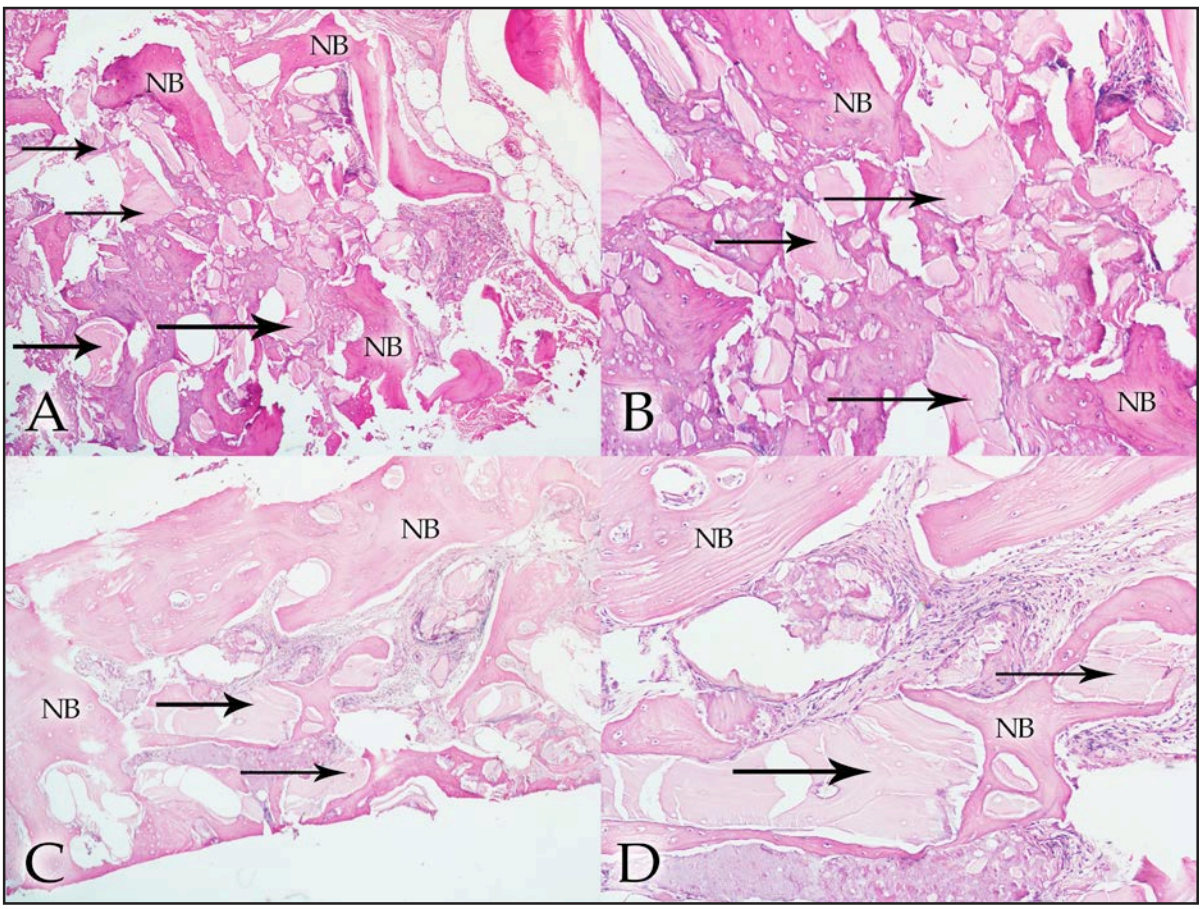

Figure 4. Histological evaluation of the areas grafted with Synergy Bone Matrix (SBM) at 4x, 10x or 20x magnification and stained with Hematoxylin-Eosin. New bone formation surrounding each particle was observed in right (A, B) and left (C, D) grafted sinus. Black arrows indicate SBM particles. NB: new bone formation.

Post-operative 4 month control digital images showed implant osseointegration (Figure 5). No peri-implant radiolucencies were observed. The regenerated bone gain by the graft placement in both sides was preserved (Figure 5). Clinical assessment of the dental implants did not exhibit mobility of the implants and a solid-deaf sound when performing percussion tests showed proper bone healing. The patient did not report pain; there was no leakage of purulent material or signs of inflammation. In addition, the grafted bone presented the similar density than the perisinusal bone at both sides.

Discussion

This is the frst study that provides clinical and histological evidence of the effcacy of SBM, a new bovine bone graft manufactured in Argentina, in the healing process of alveolar bone when used for sinus floor elevation. Similarly to what our group observed in experimental studies in rats[27], the results of the present report provide evidence for the biocompatibility and osteoconductive properties of SBM. Bone graft implantation is the main treatment modality for bone defect repair and reconstruction.[1] In this sense, demineralized bovine bone, offers excellent biocompatibil-

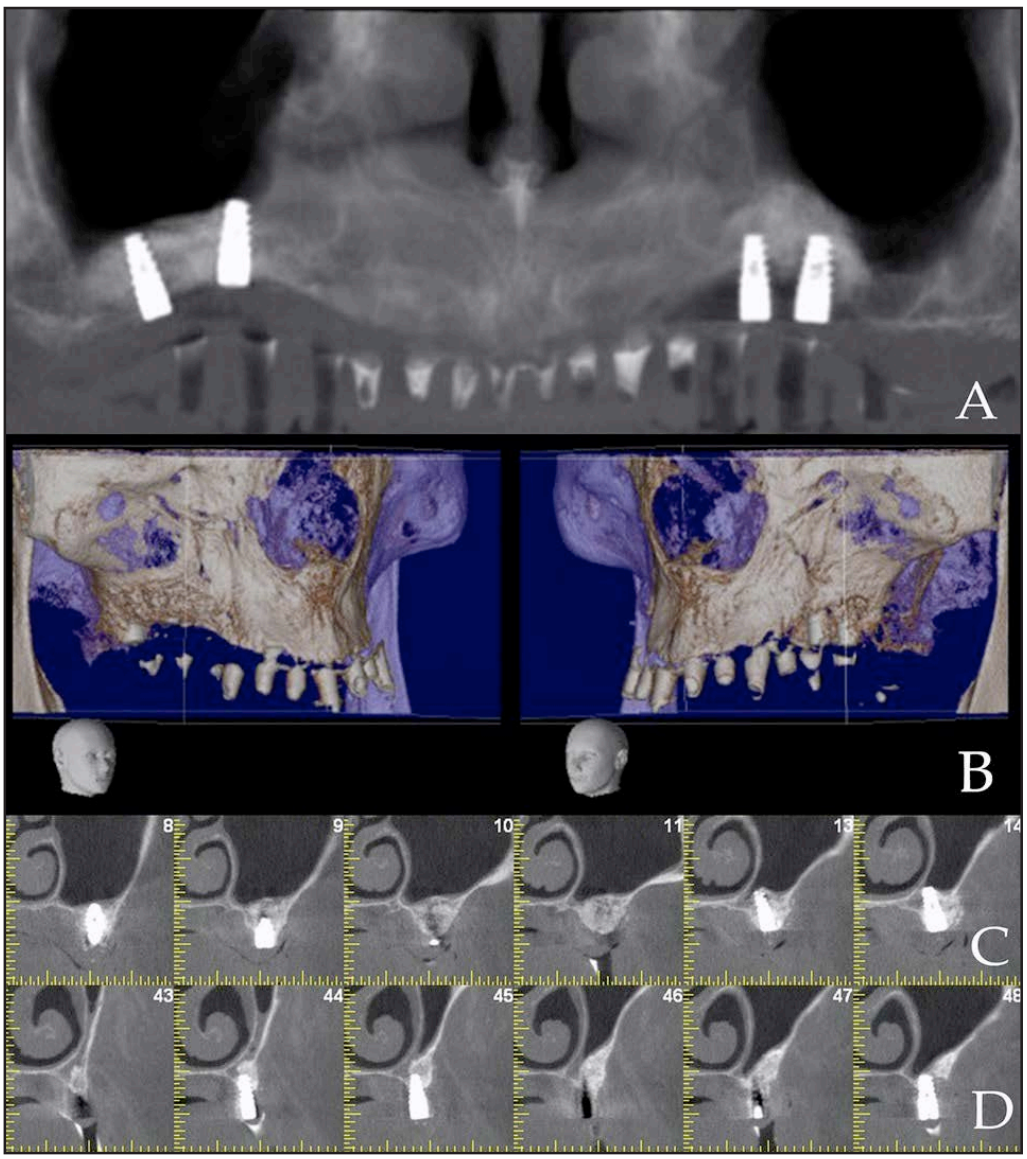

Figure5. Post-dental implant placement diagnostic images. All images show bone gain in both sides of the maxilla that persisted after the placement of dental implants. A: Panoramic X-ray from a CBCT showing the increase in alveolar bone height and dental implants on the right and left side. B: reconstruction of the left and right maxilla with the surgical stent. C, D: coronal cut from a CBCT scan from the left (C) and right (D) maxilla.

ity and physicochemical properties due to its mineral similarity with the host tissues.[29]

SBM is an anorganic bovine bone xenograft indicated for bone defects flling due to their osteoconductive properties. In experimental models, the bone defect above a critical size requires a scaffold to guide bone repair. Deproteinized bovine bone mineral is osteoconductive and provides excellent biocompatibility because it has similar physicochemical characteristics to that of the mineral component of the original bone.[30] These two important biological properties allow apposition of new bone formed by osteoprogenitor

cells located in the host tissue. It is noteworthy that bovine bone inorganic-phase not only promotes the deposition of calcium and phosphate ions, but also it is partially remodeled by osteoclasts and osteoblasts of the host.[25] In addition, the large interconnecting pore volume and its composition encourage the formation and ingrowth of new bone at the implantation sites.

Bone is a dynamic tissue that undergoes remodeling. Bone remodeling is a coupled process that starts with osteoclastic bone resorption followed by osteoblastic bone formation.[31] The osteoclastic resorption of the graft is affected by the particle size as well as the composition and porosity of the material.

Initially, once the graft material is placed, it suffers osteoclastic bone resorption followed by bone formation by osteoblastic action. The porosity of the particles enhances new bone formation by allowing the migration and proliferation of osteoblast and mesenchymal cells.[32] In addition, the microporosity of the particles is believed to enhance ionic exchange with body fluids.[32] This characteristic allows each particle of SBM to serve as a 3-D scaffold in which osteoblast and osteoprogenitor cells migrate and form bone. Consistent with this, we reported active osteogenesis in experimental models using SBM, as evidenced by the presence of bone surfaces covered by osteoblasts around the implanted bone grafts and the formation of mature Haversian systems.[33] Moreover, after 4 weeks, the collagen fbers were replaced by mature bone.[33]

The loss of teeth in the posterior area of the maxilla leads to adverse consequences on masticatory function and occlusal balance. These outcomes negatively results in psychophysical conditions associated with temporomandibular joint and muscle diseases. A frequent problem in oral rehabilitation with implant-supported prostheses in the posterior maxilla is the lack of bone volume associated with alveolar ridge resorption or maxillary sinus pneumatization.[34] The reabsorption of the alveolar bone, adjacent to the floor of the maxillary sinus, may be aggravated by the increase in osteoclastic activity that originates in the periosteum of Schneider's membrane after tooth loss, due to the absence of osteogenesis normally stimulated by the functional load on the bone. In this sense, the bone volume is limited due to the pneumatization of the maxillary sinus on one hand, and the loss of height and width of the alveolar process, on the other. The maxillary sinus floor elevation technique is used to increase the bone volume in that area. This technique consists in elevating the membrane of the floor of the maxillary sinus, and flling the intermediate space with bone substitutes[28] to promote bone formation.[35] The results of this procedure can be affected by the surgical techniques used: simultaneous placement versus delayed implantation of the implant, use of barrier membranes on the lateral window, graft material selection and surface characteristics and length and width of the implants. Depending on the type of graft, the particles are partially reabsorbed and replaced by the patient's own bone during the healing time.[36]

In agreement with Shirmohammadi et al. and Wallace et al. on sinus augmentation utilizing Bio-Oss (BO) as bone graft,[37,38] the case report presented here evidences the effcacy of SBM in the bone healing process, showing osteoconductive properties when used as a grafting material for sinus lift elevation. In this respect, biopsies of the grafted areas showed that SBM particles were surrounded by vital new bone, without evidence of inflammation and bone sequestrae after 6 months of implantation. We neither observed inflammation nor thickening of the repaired Schneiderian membrane.

The use of bone grafts is important to preserve the alveolar bone ridge height and volume indispensable for dental implant placement. Despite the highly successful outcomes for the implant-supported overdentures, it seems that a majority of edentulous individu-

als have not pursued implant-based rehabilitation. Among the reasons cited for this discrepancy between highly successful therapy and its acceptance is the cost of the treatment.[38]

Even though additional comparative studies with greater number of patients and histomorphometric analysis are needed to assess the survival of implants placed in sinuses grafted with SBM, the present case report indicates that SBM is effcient to increase the bone volume of the alveolar crest.

Acknowledgments

The authors would like to thank School of Dentistry, Department of Clinical Operative and Prosthesis II, University of Buenos Aires, Buenos Aires, Argentina, for their assistance in the use of the facilities.

This research was partially funded by National Council of Scientifc and Technical Research (CONICET)-University of Buenos Aires. Institute of Immunology, Genetics and Metabolism (INIGEM). School of Pharmacy and Biochemistry- Clinical Hospital "José de San Martín", Buenos Aires, Argentina and Odontit Implant Systems, Argentina. Synergy Bone Matrix and Bio-Oss were kindly provided by Odontit Implant Systems, Argentina.

Conflicto de intereses: los autores declaran no tener conflicto de intereses.

Recibido: mayo de 2019
Aceptado: diciembre de 2019

References

1. Petite H, Viateau V, Bensaid W et al. Tissue engineered bone regeneration. Nat Biotechnol. 2000; 18:959-63.
2. Fuentes Fernández R, Bucchi C, Navarro P, Beltrán V, Borie E. Bone grafts utilized in dentistry: an analysis of patients' preferences. BMC Med Ethics. 2015; 16:71.
3. Wang WH, Yeung KWK. Bone grafts and biomaterials substitutes for bone defect repair: a review. Bioact Mater. 2017; 2:224-47.
4. Albrektsson T, Johansson C. Osteoinduction, osteoconduction and osseointegration. Eur Spine J. 2001; 10:96-101.
5. Oryan A, Alidadi S, Moshiri A, Maffulli N. Bone regenerative medicine: classic options, novel strategies, and future directions. Journal of Orthopaedic Surg Res. 2014; 9:18.
6. Laurell L, Gottlow J. Guided tissue regeneration update. Int Dental J. 1998; 48: 386-339.
7. Athanasiou VT, Papachristou DJ, Panagopoulos A, Saridis A, Scopa CD, Megas P. Histological comparison of autograft, allograft-DBM, xenograft, and synthetic grafts in a trabecular bone defect: an experimental study in rabbits. Med Sci Monit. 2010; 16: 24-31.
8. Rogers GF, Greene AK. Autogenous bone graft: basic science and clinical implications. J Craniofac Surg. 2012; 23:323-327.
9. Olate S, Rabelo de Oliveira G, Jaimes M, Albergaria-Barbosa JR. Osseous recovery in implant insertion and pre implant reconstructions. Int J Morphol. 2007; 25:649-57.
10. Grover V, Kapoor A, Malhotra R, Sachdeva S. Bone allografts: a review of safety and effcacy. Indian J Dent Res. 2011; 22:496.
11. Bostrom MP, Seigerman DA. The clinical use of allografts, demineralized bone matrices, synthetic bone graft substitutes and osteoin-

ductive growth factors: a survey study. HSS J. 2005; 1:9-18.
12. Zimmermann G, Moghaddam A. Allograft bone matrix versus synthetic bone graft substitutes. Injury.2011; 42:16-21.
13. Gomes KU, Carlini JL, Biron C, Rapoport A, Dedivitis RA. Use of allogeneic bone graft in maxillary reconstruction for installation of dental implants. J Oral Maxillofac Surg. 2008; 66:2335-8.
14. Muller MA, Frank A, Briel M, et al. Substitutes of structural and non-structural autologous bone grafts in hind foot arthrodeses and osteotomies: a systematic review. BMC Musculoskelet Disord. 2012; 14:59.
15. Keating JF, McQueen MM. Substitutes for autologous bone graft in orthopaedic trauma. J Bone Joint Surg. 2001; 83:3-8.
16. Moshiri A, Oryan A. Role of tissue engineering in tendon reconstructive surgery and regenerative medicine: current concepts, approaches and concerns. Hard Tissue. 2012; 1:11.
17. Oryan A, Alidadi S, Moshiri A. Current concerns regarding healing of bone defects. Hard Tissue. 2013; 2:13.
18. Parikh SN. Bone graft substitutes: past, present, future. J Postgrad Med. 2002; 48:142-8.
19. Moore WR, Graves SE, Bain GI. Synthetic bone graft substitutes. ANZ J Surg. 2001; 71: 354-61.
20. Appleford MR, Oh S, Oh N, Ong JL. In vivo study on hydroxyapatite scaffolds with trabecular architecture for bone repair. J Biomed Mater Res. 2009; 89:1019-27.
21. Hench LL, Xynos ID, Polak JM. Bioactive glasses for in situ tissue regeneration. J Biomater Sci Polym Ed. 2004; 15:543-62.
22. Hench LL. The story of bioglass. J Mater Sci Mater Med. 2006; 17:967-78.
23. Parizi AM, Oryan A, Shafei-Sarverstani Z, Bigham-Sadegh A. Effectiveness of synthetic hydroxyapatite versus Persian Gulf coral in an animal model of long bone defect reconstruction. J OrthopTraumatol. 2013; 14:259-68.
24. Molly L, Vandromme H, Quirynen M, Schepers E, Adams JL, Van Steenberghe D. Bone formation following implantation of bone biomaterials into extractions sites. J Periodontal. 2008; 79:1108-15.
25. Wenz B, Oesch B, Horst M. Analysis of the risk of transmitting bovine spongiform encephalopathy though bone grafts derived from bovine bone. Biomaterials. 2001; 22:1599-1606.
26. Accorsi-Mendoça T, Zambuzzi WF, Bramante CM, et al. Biological monitoring of xenomaterial for grafting: an evaluation in critical- sized calvarial defects. J Mater Sci Mater Med. 2011; 22:997-1004.
27. Pellegrini GG, Gonzalez-Ghaves M, Orzuza R, Zeni SN. Preliminary study on the biocompatibility and osteoconductive properties of a new bovine bone graft. *Actual. Osteol.* 2017; 13:116-24.
28. Tatum HJr. Maxillary and sinus implant reconstruction. Dent Clin North Am. 1986; 30:227-9.
29. Jarcho M. Calcium phosphate ceramics as a hard tissue prosthetics. Clin Orthop Relat Res. 1981; 157:259-78.
30. Petite H, Viateau V, Bensaïd W, et al. Tissue engi- neered bone regeneration. Nat Biotechnol. 2000; 18:959-63.
31. Kenkre JS, Bassett J. The bone remodeling cycle. Ann Clin Biochem. 2018; 55: 308-27.
32. Figueiredo M, Henriques J, Martins G, Guerra F, Judas F, Figueiredo H. Physicochemical Characterization of Biomaterials Commonly Used in Dentistry as Bone Substitutes- Comparison with Human Bone. J Biomed Mater Res B Appl Biomater. 2010; 92: 409-19.
33. Pellegrini G, Gonzales Chaves M, Orzuza R, Zeni SN. Preliminary study on the biocompatibility and osteoconductive properties of a new bovine bone graft.Actual. Osteol. 2017; 13: 116-24.
34. Chiapasco M, Zaniboni M. Methods to treat the edentulous posterior maxilla: implants with sinus grafting. J Oral Maxillofac Surg. 2009; 67:867-71.
35. Block MS, Kent JN. Sinus augmentation for dental implants: the use of autogenous bone. J Oral Maxillofac Surg. 1997; 55:1281-6.
36. Schulze-Späte U, Dietrich T, Wu C, Wang K, Hasturk H, Dibart S. Systemic vitamin D

supplementation and local bone formation after maxillary sinus augmentation - a randomized, double-blind, placebo-controlled clinical investigation. Clin Oral Implants Res. 2016; 27:701-6.

37. Shirmohammadi A, Roshangar L, Chitsazi MT, Pourabbas R, Faramarzie M, Rahmanpour N. Comparative study on the effcacy of anorganic bovine bone (Bio-Oss) and nanocrystalline hydroxyapatite (Ostim) in maxillary sinus floor augmentation. Int Sch Res Notices. 2014; Article ID 967091.
38. Wallace SS, Froum SJ, Cho SC et al. Sinus augmentation utilizing anorganic bovine bone (Bio-Oss) with absorbable and nonabsorbable membranes placed over the lateral window: histomorphometric and clinical analyses. Int J Periodontics Restorative Dent. 2005; 25:551-9.

Actual. Osteol 2019; 15(3): 237-241.
Internet: http://www.osteologia.org.ar

ACTUALIZACIONES EN OSTEOLOGÍA

Vol 15, Nº 1, mayo / agosto 2019

ÍNDICE

ACTUALIZACIONES EN OSTEOLOGÍA

Vol 15, Nº 2, mayo / agosto 2019

ÍNDICE

ACTUALIZACIONES EN OSTEOLOGÍA

Vol 15, Nº 3, septiembre / diciembre 2019

ÍNDICE

INSTRUCCIONES PARA LOS AUTORES

El envío de un artículo a **Actualizaciones en Osteología** es considerado como una declaración tácita de que no ha sido enviado a evaluar al mismo tiempo o aceptado para su publicación en otro medio. En las directrices para la preparación de manuscritos, **Actualizaciones en Osteología** sigue los requisitos del Comité Internacional de Editores de Revistas Médicas (ICMJE) en la versión más reciente disponible en http://www.icmje.org.

Los manuscritos deben ser preparados usando Word, hoja A4 con márgenes de al menos 20 mm, espacio simple, en tipografía Arial 10 u otra de tamaño similar. El manuscrito -**en español o en inglés-** debe enviarse por correo electrónico a **actualizaciones@osteologia.org.ar**. Las páginas deben estar numeradas consecutivamente empezando por la que incluye el título. Abreviaturas y símbolos: sólo se deberán utilizar abreviaturas estándares, evitando su uso en el título y en el resumen.
*Los manuscritos que no se ajusten a los requisitos de **Actualizaciones en Osteología**, incluidos su organización, estructura y figuras serán devueltos a los autores sin revisión.*

La **primer página** debe contener: (a) Título del trabajo en español e inglés, (b) título abreviado para el encabezado de página, (c) nombre completo de los autores -subrayado el apellido-, (d) nombre de las instituciones en la cual se desempeña cada autor, (e) dirección de correo electrónico de un autor, (f) sección de la revista a la que corresponde el artículo y (g) conflicto de intereses.

Las secciones de la revista son: ***Artículos Originales, Actualizaciones, Comunicaciones Breves, Casuísticas, Editoriales, Cartas al Editor.***
Los **Artículos Originales** deben ser divididos en Introducción, Materiales y Métodos, Resultados y Discusión. Los títulos deben estar escritos en letra negrita. La extensión máxima del texto recomendado es de 5.000 palabras y hasta 5 figuras y 5 tablas. Se sugiere no incluir más de 50 referencias. Las **Actualizaciones** tienen una extensión máxima recomendada de 6.000 palabras y hasta 5 figuras y 5 tablas. Se sugiere no incluir más de 60 referencias. Para las **Comunicaciones Breves** y **Casuísticas** se sugiere un máximo de 3.000 palabras de extensión y hasta 4 figuras y 4 tablas. Se sugiere no incluir más de 30 referencias. La **Casuística** deben contener las siguientes secciones: Introducción, Caso Clínico y Discusión.
Para cualquier tipo de artículo mencionado anteriormente, se debe incluir un **resumen en español y en inglés** sin incluir tablas o figuras, cada uno con una extensión máxima de 250 palabras. Además, se requieren de 3 a 6 palabras clave en inglés y español.

AUTHOR GUIDELINES

Submission of a manuscript to "**Actualizaciones en Osteología"** is regarded as a tacit declaration that has not been submitted at the same time or accepted for publication elsewhere. In the guidelines for the preparation of manuscripts, "**Actualizaciones en Osteología"** follows the requirements of the International Committee of Medical Journal Editors (ICMJE) in the most recent version available in http://www.icmje.org.

Manuscripts should be prepared using Word on A4 paper with margins of at least 20 mm, simple spacing, in letter font type Arial 10, or other of a similar size. The manuscript -**in Spanish or in English**- should be submitted by email to **actualizaciones@osteologia.org.ar**. Pages must be consecutively numbered starting with the title page. Units of measurement: metric units should be used, with decimal points. Abbreviations and Symbols: only standard abbreviations should be used, avoiding them in the title and abstract.
*Manuscripts that do not conform to **"Actualizaciones en Osteología"** requirements, including requirements for manuscript organization, format, and figure will be returned to the authors without review.*

The **first page** must contain: (a) title of the work in Spanish and English, (b) abbreviated title for running head, (c) complete name of the authors -the latter must be underlined-; (d) name of the institutions in which they work, (e) address and email of the corresponding author, (f) section of the journal to which paper corresponds; (g) conflict of interest.

The journal sections include: ***Original Articles, Reviews, Brief Communications, Case Reports, Editorials, Letters to the Editor.***
Original Articles should be divided into Introduction, Materials and Methods, Results and Discussion. Titles must be written in bold type. The recommended maximum text extension is 5,000 words and up to 5 figures and 5 tables will be accepted. It is suggested not to include more than 50 references. ***Reviews*** have a recommended maximum text extension of 6,000 words and up to 5 figures and 5 tables will be accepted. It is suggested not to include more than 60 references. ***Brief Communications*** and ***Case Reports*** should have a recommended maximum of 3,000 words of text extension and up to 4 figures and 4 tables will be accepted. It is suggested not to include more than 30 references. *Case Reports* should be divided into Introduction, Clinical Case and Discussion.
For all type of article described previously, a **250-word *Abstract* in Spanish and in English**, not including tables or figures, must also be included. Also, 3 to 6 key words in English and Spanish are required. Authors who are not fluent in Spanish and,

En los **Artículos Originales** y **Casuísticas** detallar la solicitud de consentimiento informado. Además se solicita se indiquen las normas y directrices éticas y los métodos estadísticos utilizados (Originales).
Para las **Cartas al editor** y **Editoriales** se sugiere un máximo de 1.000 palabras y se admitirán para las Cartas al Editor hasta 2 figuras o tablas. Se sugiere no incluir más de 10 referencias. **Agradecimientos**: la ayuda técnica, el apoyo financiero y las contribuciones que no justifican la autoría se pueden enumerar en este ítem. **Conflicto de intereses**: los autores deben revelar cualquier relación financiera que podría conducir a un conflicto de intereses en relación con el artículo publicado.

Las **referencias** deben ser numeradas consecutivamente. Usar números en superíndices para indicar las referencias en el texto. Para las referencias seguir los siguientes ejemplos:
1. Revistas: Todos los autores serán incluidos si son seis o menos; si hay más de seis, el tercero será seguido de "et al". Los títulos de las revistas deben abreviarse de acuerdo con el estilo usado en el Index Medicus (disponible en http://www.nlm.nih.gov). Los nombres de las revistas deben ir en itálica. Ejemplo: T Diab, Wang J, S Reinwald, Guldberg RE, Burr DB. Efectos de la combinación de tratamiento de raloxifeno y alendronato en las propiedades biomecánicas de hueso vertebral. *J Bone Miner Res* 2011; 26: 270-6.
2. Capítulo de libro: Rigalli A. Eutanasia. En: Rigalli A, Di Loreto VE (eds). Experimental Surgical Models in the Laboratory Rat. Boca Raton, Florida: CRC Press, 2009, p. 31-2.
3. Sitios Web: Organización Mundial de la Salud (OMS). The Stop TB Web Alert. (2000, Dec 6) http://www.stoptb.org/updates/index.html

Las **Tablas** con sus respectivos títulos explicativos se presentarán al final del manuscrito numeradas en números arábigos. Deben ser indispensables y comprensibles por sí mismas. No se utilizarán líneas verticales entre las columnas y sólo se emplearán líneas horizontales en los siguientes casos: parte superior de la tabla, parte inferior del encabezado de la tabla y final de la tabla. En el texto manuscrito se indicará la ubicación aproximada con la leyenda "Insertar Tabla aquí".

Las **Figuras** deben ser presentadas separadamente del texto. El manuscrito sólo incluirá una leyenda explicativa. El formato requerido de imágenes es ".jpg o .tif" en calidad no menor de 300 dpi de resolución. No se aceptarán imágenes en archivos de *Word* ni *Power Point*. En las micrografías se debe indicar la escala o el aumento que se usó. Tener en cuenta que en la versión *on line* la imagen se mostrará a color, mientras que en la versión impresa se observará en escala de grises. En el manuscrito se indicará la ubicación aproximada con la leyenda "Insertar Figura aquí".

therefore, are not able to include the abstract and keywords in this language, can submit the manuscript without them.
In **Original papers** and **Case Reports** record informed consent by patients. Ethical standards and guidelines followed will be indicated and statistical methods will be described (in Originals).
Letters to the editor and ***Editorials*** have a recommended maximum of 1000 words and for Letters to the editor up to 2 figures or tables will be accepted. It is suggested not to include more than 10 references. **Acknowledgments:** technical assistance, financial support, and contributions that do not justify authorship may be listed. **Conflict of interest:** authors must disclose any financial relationship that could lead to a conflict of interest in relation to the published article.

References should be numbered consecutively. Use superscript numerals for references in the text. References should be mention according to the following examples:
1. Journals: All authors will be included if they are six or less; if more than six, the third one will be followed by "et al". The titles of journals should be abbreviated according to the style used in Index Medicus (also available in http://www.nlm.nih.gov). The names of journals should be in italics. Example: Diab T, Wang J, Reinwald S, Guldberg RE, Burr DB. Effects of the combination treatment of raloxifene and alendronate on the biomechanical properties of vertebral bone. *J Bone Miner Res* 2011; 26:270-6.
2. Books chapter: Rigalli A. Euthanasia. In: Rigalli A, Di Loreto VE (eds). Experimental Surgical Models in the Laboratory Rat. Boca Raton, Florida: CRC press, 2009, p. 31-2.
3. Web sites: World Health Organization (WHO). The Stop TB Web Alert. (2000, Dec 6) http://www.stoptb.org/updates/index.html

Tables and its legends will be presented al the end of the manuscript numbered in Arabic numerals. They should be indispensable and comprehensible by themselves. No vertical lines between columns and horizontal lines will be used, except in general three lines: one separating the Table title, another for the headings of the rest, and the last one indicating the end of the Table. The manuscript text will indicate the approximate location with the legend "Insert Table here".

Figures should be submitted separately from the text. The manuscript text will only include an explanatory legend. The required format of images is ".jpg or .tif" no less than 300 dpi resolution. Images in Word files will not be accepted. In the micrographs the scale or magnification used must be indicated. *Take into account that while in the online version the image is shown in color, in the printed version it will be presented in grayscale.* The manuscript text will indicate the approximate location with the legend "Insert Figure here".

Todos los artículos publicados en **Actualizaciones en Osteología** están sujetos a revisión por pares. La revisión se hace por un editor y al menos dos revisores con amplia experiencia en el tema. La identidad de los autores y revisores se mantiene confidencial. El editor devolverá a los autores, sin pasar por el proceso de arbitraje aquellos manuscritos que no se ajusten a las normas de preparación o que no coincidan con los propósitos de **Actualizaciones en Osteología**. El tiempo de evaluación dura entre 30 y 60 días. El autor será notificado por correo electrónico de la aceptación (con o sin correcciones) o rechazo del manuscrito. La decisión es definitiva sin posibilidad de apelar. El Comité Editorial se reserva el derecho de introducir, con conocimiento de los autores, todos los cambios de redacción necesarios de acuerdo a reglas gramaticales y de diseño. Los trabajos aceptados serán devueltos a los autores con las modificaciones solicitadas y se les concederá un período no mayor de 30 días para el envío de la versión corregida. Después de la aceptación y el diseño del artículo, los autores dispondrán de 72 horas para revisar la prueba de galera.

POLÍTICAS EDITORIALES

Actualizaciones en Osteología es una revista de Acceso Abierto.

Costo de publicación: La publicación de artículos no tiene costo con excepción de las imágenes a color en caso de que esto sea requerido por los autores.

Derechos de autor. Actualizaciones en Osteología es la publicación oficial de la Asociación Argentina de Osteología y Metabolismo Mineral (AAOMM) que posee los derechos de autor de todo el material publicado en dicha revista.

Responsabilidades éticas. Cuando se describan experiencias en seres humanos, los autores deberán indicar si los procedimientos realizados siguieron las normas éticas de un comité institucional o regional en la experimentación con seres humanos, y de acuerdo con la Asociación Médica Mundial y la Declaración de Helsinki. Los autores también enviarán el modelo de consentimiento informado utilizado para el estudio. Cuando se describen experimentos con animales, se debe indicar si se siguieron los lineamientos de un Comité o Consejo sobre el cuidado y uso de animales de experimentación.

Duplicación/publicación redundante. Los autores son responsables de asegurar que el manuscrito -incluyendo todos los datos, figuras y tablas- no ha sido publicado anteriormente. Además, es responsabilidad de los autores asegurar que el manuscrito no ha sido, ni será, sometido a evaluación por otra revista mientras está bajo revisión por Actualizaciones en Osteología. Los artículos originales que proporcionan nuevos datos de los estudios que han sido objeto de publicaciones anteriores deben evitar la superposición de los mismos y los autores deberán enviar información sobre todas las publicaciones previas al Editor responsable.

All articles published in **Actualizaciones en Osteología** are subject to peer review. The review is made by an Editor and at least two reviewers with extensive experience in the particular subject. The identity of the authors and reviewers is kept confidential. The Editor will return to the authors, without going through the arbitration process those manuscripts that do not conform to the standards of preparation or that do not match the purposes and orientation of **Actualizaciones en Osteología.** Evaluation time takes between 30 and 60 days. The corresponding author will be notified by email on the acceptance (with or without corrections) or rejection of the manuscript. This decision is final. The Editorial Committee reserves the right to introduce, to the authors' knowledge, all editorial changes required by grammatical rules and layout needs. Accepted papers will be returned with modifications to authors for any corrections and they will be granted a return period of not more than 30 days. After acceptance and layout, authors will be awarded 72 hours to review the galley proof.

EDITORIAL POLICIES

Actualizaciones en Osteología is an Open Access Journal.

Page Fees: No charge for publication is required except for color figures if requested by authors.

Copyright. Actualizaciones en Osteología is the official journal of the Argentinean Association of Osteology and Mineral Metabolism (AAOMM), which holds copyright to all material published in the Journal.

Ethical responsibilities. When experiments performed in humans are described, authors must indicate whether the procedures performed followed ethical standards of the (institutional or regional) committee on human experimentation, and in accordance with the World Medical Association and the Declaration of Helsinki. Authors must also send the informed consent model used for the study. When animal experiments are described, it should be indicated whether guidelines of an institution or research council on care and use of laboratory animals were followed.

Duplicate/Redundant Publication. The corresponding author is responsible for ensuring that the manuscript -including all data, figures, tables, and supplementary materials- has not been previously reported or published. Further, it is the responsibility of the corresponding author to ensure that the manuscript has not been, and will not be, submitted to another journal while under review by Actualizaciones en Osteología. Original Articles providing new data from studies that have been the subject of previous publications must avoid data overlap and authors must provide information on all previous publications to the Editor-in-Chief.

A.A.O.M.M.

(Asociación Argentina de Osteología
y Metabolismo Mineral)