UNIVERSIDADE DE SÃO PAULO
FACULDADE DE ODONTOLOGIA DE BAURU

EVER ELIAS MENA LAURA

Impact of metformin on periodontal response to orthodontic forces in type 1 and 2 diabetic rats

Impacto da metformina na resposta periodontal à forças ortodônticas em ratos diabéticos tipo 1 e 2

BAURU
2019

EVER ELIAS MENA LAURA

Impact of metformin on periodontal response to orthodontic forces in type 1 and 2 diabetic rats

Impacto da metformina na resposta periodontal à forças ortodônticas em ratos diabéticos tipo 1 e 2

Tese constituída por artigos apresentada a Faculdade de Odontologia de Bauru da Universidade de São Paulo para obtenção do título de Doutor em Ciências no Programa de Ciências Odontológicas Aplicadas, na área de concentração Biologia Oral.

Orientador: Prof. Dr. Gerson Francisco de Assis

BAURU
2019

Ever Elias Mena Laura
Impact of metformin on periodontal response to orthodontic forces in type 1 and 2 diabetic rats/ Ever Elias Mena Laura. – Bauru, 2019.
48p.; il; 30cm.

Tese (Doutorado) – Faculdade de Odontologia de Bauru. Universidade de São Paulo

Orientador: Prof. Dr. Gerson Francisco de Assis

Comitê de Ética da FOB-USP
CEEPA Nº 33/2013
Data: 26/05/2014
CEEPA Nº 06/2016
Data: 26/08/2016

FOLHA DE APROVAÇÃO

DEDICATÓRIA

Dedico este trabalho à minha família, em especial a meus pais Eleuterio e Inés, seguindo seus passos sempre.

AGRADECIMENTOS

Deus se agrada quando vê que os Seus filhos são gratos.

Mas graças a Deus, que nos dá a vitória por meio de nosso Senhor Jesus Cristo.

1 Coríntios 15:57

Agradecimentos especiais aos meus queridos pais ***Eleuterio*** e ***Inés*** pelo animo confiança, incentivo e apoio incondicional com meus objetivos, os melhores. Também aos meus irmãos ***Ruben, Renzo, Milka, Esther, Jhonatan, Benjamin*****,** e às minhas cunhadas ***Alejandra*** e ***Mabili*** e cunhado ***Noel*** pelo apoio, amizade e companheirismo continuo.

Ao meu orientador Prof. Dr. ***Gerson Francisco de Assis***, quem se mostra sempre comprometido nas atividades dos seus orientados. Recebi colaboração constante desde o meu mestrado, e agradeço bastante por esse apoio, amizade, tolerância e compreensão. Saiba que foi um exemplo para mim na forma que orienta aos alunos, e também na forma que lida com as atividades da graduação. Agradeço pois o Prof. tem contribuído bastante na minha formação e crescimento pessoal.

À Dra. ***Tânia Mary Cestari***, pessoa bastante dedicada, detalhista, criteriosa e bastante profissional. Quando crescer, quem sabe, chego lá. Agradeço por ter participado na minha orientação desde o meu mestrado. Espero continuar trabalhando e contando com a sua ajuda.

Ao **Prof. Dr.** ***Rumio Taga*** pela amizade e apoio sinceros. O seu conhecimento na nossa área teve muito valor para entender nossas pesquisas e ter segurança com os nossos conceitos. O Prof. também é um exemplo a seguir.

Ao Prof. Dr. ***Gustavo Garlet***, pela amizade, colaboração e exemplo.

À Profa Dra. ***Camila Rodini***, pela amizade, carisma e colaboração no laboratório.

À secretária ***Teresa*** (Tê) por inúmeras colaborações. Sempre prestativa e eficiente, fazendo sempre mais do que pedimos. Essa alegria contagiante faz do trabalho um ambiente agradável.

Às técnicas ***Daniele Ceolin*** (Dani) e ***Patrícia Germino*** (Paty) pela amizade e em especial pela colaboração no processamento das amostras e confecção das minhas lâminas.

Aos técnicos do Biotério Central da FOB-USP ***Erasmo***, ***Luis, Richard, e Elias*** pelo apoio técnico e amizade.

Agradecimentos especiais a ***Aline Dionizio, Heloisa Pereira*** e à técnica ***Clarice*** da UNESP de Rio claro pelas colaborações com os experimentos.

Aos dois alunos de Iniciação científica: ***Luan Macena, Rodrigo Almeida*** e ***Carol Cestari*** pela amizade e colaboração em todos os experimentos avaliações até a fase final.

À ***Dona Divina*** pelos serviços que presta no nosso laboratório.

Aos caros colegas e amigos do laboratório de histologia: ***Daniela Santos Pereira*****,** agradeço pela sua amizade, e por inúmeras colaborações nas pesquisas e disciplinas desde o mestrado; ***Paulinha Sanches*** pela sua amizade e colaboração nas disciplinas, continue assim; ***Ricardo Arantes***, parceiro do laboratório pela paciência e amizade em todo esse tempo; ***Cláudia Biguetti (Claudinha)*** alemã da amizade, agradeço pelo seu apoio, conselhos, colaborações, e convivencia na Histoband ; ***Franco Cavalha*** pela amizade e convivência na Histoband; ***Vinicius Tagliavini*** pela boa amizade e convivência; ***Carlos Eduardo Repeke*** (Cadu) e ***Daiane*** pela amizade; ***Carol Francisconi*** pelo apoio e amizade; Aos demais amigos do laboratório e da faculdade ***Rodrigo Buzo, Angélica, Suelen Paini, Michelle Soriani, Natalia Lopes, Rafael Ortiz, Priscila Colavitepela, Carol Rocha, Lucas Cambiaghi, Barbara Mansano, Luciana Pereira*****,** ***Bruna Bighetti*****,** ***Andreia Espindola*** obrigado a vocês por terem feito do laboratório um ambiente muito agradável.

Ao pessoal da fonoaudiologia turma XXX, em especial a ***Julia Angélico, Nayra Covas, Tainá, Camila e Viviane,*** obrigado pela amizade e torcida.

Agradecimentos muito especiais a: Família Kennerly Herrera (**Péricles, Cilma, Laís, Paulinha**)**,** Família Gaspar (**Jaime, Joelma, Joao, Julia**), Família Contelli (**Xavier, Ismênia, Dani, Joao**), **Jeffrey Rojas Paulus, Kevin Gutnaz**. **Prof. Dr. Paulo S. S. Santos** e **Marcinha** e família. Obrigado pela convivência.

E aos amigos da Bolívia: **Ainda Cosme**, **Tatiana Mallea**, **Nico Quispe**, **Eymi Cazas**, **Reyna Aguilar, Rodrigo Abuná**, **Jonathan Delgadillo** e **Geraldine Vargas** pelo apoio na distância.

A todos meus sinceros agradecimentos, de alguma forma contribuíram na execução deste trabalho e para o crescimento pessoal. O mais importante em nossas conquistas é sabermos reconhecer o valor de todos aqueles que estiveram conosco.

...Muito obrigado!

AGRADECIMENTOS INSTITUCIONAIS

À Faculdade ***de Odontologia de Bauru da Universidade de São Paulo*** (FOB – USP).

À ***Fundação de Amparo à Pesquisa do Estado de São Paulo*** (FAPESP), órgão de fomento viabilizou o desenvolvimento deste trabalho. (Auxilio Regular FAPESP Processo: 2014/07080-2).

Ao ***Prof. Dr. Vahan Agopyan***, digníssimo Reitor da Universidade de São Paulo.

Ao ***Prof. Dr. Pedro Vitoriano Oliveira***, digníssimo secretário Geral da Universidade de São Paulo.

Ao ***Prof. Dr. Carlos Ferreira dos Santos***, digníssimo Diretor da Faculdade de Odontologia de Bauru da Universidade de São Paulo.

Ao ***Prof. Dr. Guilherme dos Reis Pereira Janson***, digníssimo Vice-Diretor da Faculdade de Odontologia de Bauru da Universidade de São Paulo.

Ao ***Prof. Dr. José Roberto Pereira Lauris***, digníssimo Prefeito do Campus da Faculdade de Odontologia de Bauru da Universidade de São Paulo.

À ***Profa. Dra. Izabel Regina Fischer Rubira Bullen***, digníssima Coordenadora do Programa de Pós-Graduação em Ciências Odontológicas Aplicadas e Presidente da Comissão de Pós-Graduação na área de Estomatologia e Biologia Oral, da Faculdade de Odontologia de Bauru da Universidade de São Paulo.

“O que fazemos em vida, ecoa na eternidade”

— Gladiador (2000)

ABSTRACT

Objectives: We studied the periodontal response to orthodontic forces in type 1 diabetic rats (T1D) treated with insulin and metformin and type 2 diabetic rats (T2D) treated exclusively with metformin (MET). **Materials and methods:** In article 1, T1D was induced by single injection of streptozotocin (STZ), whereas in article 2, T2D was induced by 90 days of high fat diet (HFD) with a single and low dose administration of STZ. In both articles 1 and 2 as soon as diabetes was induced in rats, an orthodontic appliance was installed to move the right upper first molar mesially for periods of 0, 3, 7, and 14 days. Samples were analyzed by micro-CT histomorphometry and immunohistochemistry for TRAP + cells. **Results:** Diabetic induction with STZ on the one hand, and HDF plus STZ on the other resulted in pathognomonic signs of T1D (hyperglycemia) and T2D (increase in body mass, insulin resistance, glucose intolerance and hyperglycemia), respectively. The addition of MET decreased blood glucose to values close to NG and better than insulin alone in T1D. In T2D, MET significantly reduced blood glucose, insulin tolerance and glucose tolerance. During orthodontic movement (OTM), T1D and T2D led to greater mesial movement, mesial inclination, mesial rotation (mesioversion), periodontal ligament spacing associated to a larger number of TRAP+ cells, and bone resorption surfaces (ORS) which were significantly reduced by MET on T2D and MET added to insulin on T1D. T2D presented maxillary osteoporosis or reduced BV / TV and BA / TA before OTM, but in T1D this occurred during OTM, however these effects were counteracted by MET. Yet, a different pattern of OTM occurs in T1D and T2D due to different bone density, and extrusion versus intrusion presented in T1D and T2D, respectively. **Conclusion:** The addition of metformin to insulin in T1D or single administration in T2D reduces the adverse effects on periodontal tissues during orthodontic movement in type 1 and 2 diabetic rats.

Keywords: Metformin; Type 1 diabetes; Type 2 diabetes; Orthodontic tooth movement; Periodontium.

RESUMO

Objetivos: Nós estudamos a resposta periodontal às forças ortodônticas em ratos diabéticos tipo 1 (T1D) tratados com metformina (MET) adicionada à insulina, e ratos diabéticos tipo 2 (T2D) tratados exclusivamente com metformina. **Materiais e métodos**: No artigo 1 a T1D foi induzida por injeção única de estreptozotocina (STZ), enquanto que no artigo 2 a T2D foi induzida por alimentação rica em gordura (HFD) por 90 dias e administração de dose única e baixa de STZ. Em ambos artigos 1 e 2 assim que a diabetes foi induzida nos ratos, um aparelho ortodóntico foi instalado para movimentar o primeiro molar superior direito mesialmente por períodos de 0, 3, 7, e 14 dias. As amostras foram analisadas por micro-CT histomorfometria e imunohistoquímica para células TRAP+. **Resultados**: A indução diabética, com STZ por um lado, e HDF mais STZ por outro, resultou em signos patognomônicos da T1D (hiperglicemia) e T2D (aumento da massa corporal, resistência insulina, intolerância à glicose e hiperglicemia), respectivamente. A adição de MET diminuiu a glicemia a valores próximos do NG e melhor do que só insulina na T1D. Na T2D a MET reduziu significantemente a glicemia, tolerância à insulina e tolerância à glicose. Durante o movimento ortodôntico (MO), T1D e T2D levaram a maior movimento mesial, inclinação mesial, rotação mesial (mesioversão), espaçamento do ligamento periodontal associado a maior numero de células TRAP+ e superfícies de reabsorção óssea (ORS) os quais foram significantemente reduzidos pela MET na T2D e a adição de MET à insulina na T1D. A T2D apresentou osteoporose maxilar ou BV/TV e BA/TA reduzido antes do MO, mas no T1D, isso ocorreu durante o MO, porem esses efeitos foram contrariados pela MET. Ainda um diferente padrão de MO ocorre na T1D e T2D pela diferente densidade óssea, e extrusão versus a intrusão apresentadas na T1D e T2D, respectivamente. **Conclusão**: A adição de metformina à insulina na T1D ou administração única na T2D reduzem os efeitos adversos no periodonto durante o movimento ortodôntico em ratos diabéticos tipo 1 e 2.

Palavras-Chave: Metformina; Diabetes tipo 1; Diabetes tipo 2; Movimento dentário ortodôntico; Periodonto.

ACRONYMS AND ABBREVIATIONS

ANOVA	Annalise of variance
BMD	Bone mineral density
BV	Bone volume
BV/TV	Bone volume / total volume
BA/TA	Bone area / total area
CEEPA	Ethics Committee FOB-USP
cm	Centimeter
DB or DBR	Disto-buccal root
EDTA	Ethylenediamine tetraacetic acid
FBGL	Fasting blood glucose levels
Fig	Figure
g	Gram
GTT	Glucose tolerance test
HA	Hyaline areas
HE	Hematoxylin – eosin
HFD	High fat diet
ITT	Insulin tolerance test
kg	kilogram
MET	Metformin
mg	Milligram
Micro-CT	Microcomputed tomography
mL	Milliliter
mm	Millimeter
mm^2	Squared millimeter
NG	Normoglycemic
OTM	Orthodontic tooth movement
ORS	Osteoclastic resorption surface
PDL	Periodontal ligament
STZ	Streptozotocin
T1D	Type 1 diabetes
T2D	Type 2 diabetes
TRAP	Tartrate-resistant acid phosphatase

TABLE OF CONTENTS

1 INTRODUCTION

1 INTRODUCTION

DM prevalence is increasing worldwide. It reaches 37% of population in some regions (Kharroubi and Darwish, 2015). More specifically, according to de International Diabetes Federation, current global prevalence of diabetics is 425 million, they are likely to be 592 million by 2035 people, and 90% of them are Type 2 (Idf, 2019). Diabetes mellitus (DM) comprehends a certain group of metabolic diseases characterized by hyperglycemia however the most common are type 1 and 2 (T1D and T2D, respectively). T1D patients displays marked hyperglycemia. It represents a common endocrine and metabolic condition with absolute insulin deficiency especially seen in children. As consequence they display clinical polyuria, polydipsia, polyphagia, weight loss, and blurred vision (Who, 2019). T1D or insulin-dependent diabetes is also synonym of "autoimmune T1D" because T1D-associated autoantibodies produces autoimmunity causing loss of pancreatic islet β-cells in majority of the patients (Roep and Peakman, 2012), besides, mutations in more than one gene, as well as environmental factors are also related to its pathoethiology (WHO, 2019). T2D, formerly noninsulin-dependent diabetes, is more frequent in adult population and primarily related to lifestyle factors, genetics and other environmental factors (Kharroubi and Darwish, 2015). Nonetheless, T2D is increasing in children, adolescents, and younger adults due to the rising obesity, physical inactivity, and energy-dense diets (Goyal and Jialal, 2019a). Accordingly, T2D is associated to insulin-resistance, a process in which insulin secretion is uncapable to maintain euglycaemia and leads to beta cell dysfunction Additionally, obesity is linked to T2D for it contributes to insulin resistance by elevating the levels of circulating free fatty acids which in turn inhibit glucose uptake, glycogen synthesis and glycolysis. Those processes are later compensated by an increase in insulin production and secretion (Carvalheira *et al.*, 2002) which will eventually lead to pancreatic β-cells dysfunction, inappropriate glucose response and glucose intolerance (Cerf, 2013; Goyal and Jialal, 2019b).

Nonetheless, the sequence of successions and the nature of signals derived from the insulin resistant tissues to induce an appropriate beta-cell response remains unclear in the literature. (Cerf, 2013; Goyal and Jialal, 2019b). Concomitantly, adipose tissue, acts as an endocrine organ that secretes a large number of factors related to immune cell functions. Besides, adipocytes increase causes adipose tissue hypoxia and subsequent chronic inflammation (Ye *et al.*, 2007). In rodents and humans, this tissue is infiltrated by neutrophils and later by macrophages which are also positively correlated with insulin resistance (Talukdar *et al.*, 2012). Once diabetes is stablished, different detrimental effects on tissues are produced and those depend on inherent factors from both T1D and T2D.

In odontology there is well stablished bidirectional relationship between periodontitis and T1D or T2D, i.e. periodontitis itself worsening hyperglycemia and vice versa. Therefore, periodontium is brittle under diabetic condition and these detrimental effects have a significant impact in dental therapies such as orthodontics. During orthodontic tooth movement (OTM), the periodontium, a reach in cells and vascularized connective tissue, undergo an aseptic-like inflammation (Li *et al.*, 2018) in where equilibrated soft and hard tissues remodeling occurs whenever a correct amount of force is applied. Accordingly, in normoglycemic individuals, pressure site generation by mechanical stimulus promotes alveolar bone resorption due to osteoclastic activity and, in the tension site, bone matrix deposition and mineralization by osteoblastic activity (Hadjidakis and Androulakis, 2006; Krishnan and Davidovitch, 2006; Wise and King, 2008). In vitro, periodontal ligament cells undergoing compression force increase the production of factors related to osteoclast activity and survival then promoting alveolar bone resorption (Cao *et al.*, 2014; Yi *et al.*, 2016). On the tension side fibroblast experiment tension and then releases cytokines related to osteoblast activity and bone formation (Meikle, 2006). All these event change during diabetes however clinical and laboratorial studies are scarce. Regarding T1D, although a morphological

description of detrimental effects on periodontal tissues is been reported early (Holtgrave and Donath, 1989), few reports haven presented to date. Among them (Villarino *et al.*, 2011) confirmed previous reports, also (Braga *et al.*, 2011) presented a more comprehensive molecular pathway involved. However, there are orthodontic and periodontal important clinical factors to be approached such as the tooth movement pattern, periodontal width or thickness, also alveolar bone height and volume. What is more, there is almost no knowledge of what occurs ins such conditions under treatment. According to (Jonasson *et al.*, 2018), alveolar bone is unique capable of following teeth's movements; this bone formed around teeth during eruption and their PDL, thus, the longer the teeth after eruption, the larger the alveolar process. In a mature maxilla, different densities are found in the alveolar process (Jonasson *et al.*, 2018), thus, different turnover rates (Parfitt, 2013), however, how diabetes or diabetic treatment affects these factors are not fully understood.

Metformin (MET), a biguanide and an oral anti-hyperglycemic agent is the first therapy of choice in T2 diabetics. Although some side effects exist (Dujic *et al.*, 2016), it is effective because it promotes insulin sensibility, slows the release of glucose stored in the liver and reduces glucose absorption in the gut (Hostalek *et al.*, 2015). MET enhances insulin action, improves glycemic control, then leads to reduce insulin dose requirement as well as weight gain. Nonetheless, MET adjunctive therapy is not formally prescribed in T1D as in T2D is (Degeeter and Williamson, 2016), and whether to add metformin to insulin therapy in T1D is under debate . (Beysel *et al.*, 2018). In the article 1, we approached the use of MET as an adjunctive therapy of insulin in T1D rats undergoing OTM. In young Wistar rats, T1D was induced by Streptozotocin, then and orthodontic appliance was placed between the scissors and the first upper molar (M1) in order to move it mesially. Four groups that properly simulate clinical conditions related to diabetic patients were established: A control Normoglycemic (NG); diabetic (T1D); Insulin treated diabetics (I-T1D); and the proposed

MET plus Insulin treated diabetics (IM-T1D). To understand whether MET adjunction to Insulin has any effect on periodontal behavior while submitted to orthodontic forces, several analyzes were performed. Accordingly, 3D spatial position changes of the M1 or tooth movement pattern was approached by micro-CT; periodontal tissues events and remodeling were approached by micro-CT and histomorphometry to evaluate periodontal spacing, alveolar bone volume fraction and presence of hyaline tissue; finally, osteoclastic activity as the presence of positive trap cells were also recorded. All these variables may give a comprehensive panorama of the PDL behavior of T1D diabetics that are submitted to OTM, also to antidiabetic therapy such as insulin or insulin plus MET.

In regard to T2D subjected to OTM, periodontal tissues may also be frail. T2D involves other diseases such as obesity and osteoporosis, therefore, the present chronic inflammation (Talukdar *et al.*, 2012) can directly influence the periodontal response related to force loading. To date few laboratorial studies approached T2D effects on tooth movement and all of them point periodontal response to be altered during orthodontic tooth movement (Plut *et al.*, 2015; Sun *et al.*, 2017; Gomes *et al.*, 2018). Regarding treatment, (Sun *et al.*, 2017) reported MET use in T2 animals subjected to OTM by histologic means. They pointed the outcomes as evidence that MET reverses T2D deleterious effects in such conditions. Those assumptions were based on higher OTM rate recorded manually in silicone models and cellular activity recorded in histological sections. In the article 2 we present a more complete scene of the T2D and MET effects on OTM. For that purpose, T2D was induced by high fat diet (HFD), a modified form from AIN-93 standard diet, associated to streptozotocin for it resembles T2D development similar to humans (Vatandoust *et al.*, 2018); therefore, rats underwent glucose tolerant test (GTT) and insulin tolerant test (ITT) to conferee prediabetes and insulin resistance (IR) presence in these animals. As T2D was stablished, an experimental group was treated by MET daily (M-T2D) and other groups were untreated (T2D) and

normoglycemic (NG). Outcomes after 14 days of OTM were evaluated by micro-CT and histology. The impact of both T2D and MET on periodontal tissues during orthodontic tooth movement is not fully understood. Although orthodontics is commonly performed in young patients, adult population seeking for orthodontic treatment is growing and dentists have to face proportionally the adult-related systemic diseases such as T2D (Almadih *et al.*, 2018).

Orthodontic studies related to diabetes are scarce. Additionally, it seems that there exists a tendency of generalization for both T1D and T2D among orthodontic review studies (Najeeb *et al.*, 2017; Almadih *et al.*, 2018; Chauhan *et al.*, 2018). Thus, we aimed to study metformin effect on periodontal tissues during orthodontic tooth movement in T1D and T2D rats by micro-CT, histology and immunohistology for TRAP.

2 ARTICLES

2 ARTICLES

This thesis comprises two articles:

ARTICLE 1 – Metformin as an add-on to insulin improves periodontal response during orthodontic tooth movement in type 1 diabetic rats

ARTICLE 2 – Metformin therapy to prevent periodontal breakdown after orthodontic forces in type two diabetic rats. A micro-CT, Histomorphometric and immunohistochemical evaluation

2.1 ARTICLE 1 – *"This is the peer reviewed version of the following article: [***Metformin as an add-on to insulin improves periodontal response during orthodontic tooth movement in type 1 diabetic rats***], which has been published in final form at [*https://aap.onlinelibrary.wiley.com/doi/abs/10.1002/JPER.18-0140*]. This article may be used for non-commercial purposes in accordance with Wiley Terms and Conditions for Use of Self-Archived Versions."*

Metformin as An Add-On to Insulin Improves Periodontal Response During Orthodontic Tooth Movement in Type 1 Diabetic Rats

Ever Elias Mena Laura, DDS, MSc *, Tania Mary Cestari, BS, PHD *,
Rodrigo Almeida, BMSc, MSc †, Daniela Santos Pereira, BMSc, MSc *
Rumio Taga, DDS, PHD, Professor *,
Gustavo Pompermaier Garlet, DDS, MSc, PHD, Professor *,
Gerson Francisco de Assis, BS, MSc, PHD, Professor *

Filiations and degrees:

* Department of Biological Sciences, School of Dentistry of Bauru, São Paulo University, Bauru, São Paulo, Brazil

† Department of Bioprocess and Biotechnology School of Pharmaceutical Sciences, Universidade Estadual Paulista – UNESP, Araraquara, São Paulo, Brazil

Address correspondence to:

Ever Elias Mena Laura, PHD student

Department of Biological Sciences, School of Dentistry of Bauru

São Paulo University, Bauru, São Paulo, Brazil

Email: everlauramena@live.com; Phone number: +55 14 991036705

Words: 3989**; Nº figures:** 5**; Nº tables:** 1**; References:** 50

Running title: Metformin plus insulin in periodontal response to OTM in T1D

One sentence summary: Metformin plus insulin therapy ameliorates glycemic control and the periodontal tissue response to orthodontic forces in T1D rats.

ABSTRACT

Background: Type 1 diabetes (T1D) is associated with delayed tissue healing and bone loss. Periodontal tissues during tooth movement (OTM) in T1D and under diabetic treatment are poorly understood. We aimed to study the effect of metformin as an add-on to insulin therapy on periodontal structures during OTM in T1D rats.

Methods: Rats were divided into normoglycemic (NG, n=20) and **s**treptozotocin-induced diabetic groups that were untreated (T1D, n=20), treated with insulin (I-T1D, n=20), or treated with insulin plus metformin (IM-T1D, n=20). After 7 days of treatment, the first right upper molar (M1) was moved mesially. At day 14, the pattern of OTM and the periodontal tissues were analyzed by micro-CT, histomorphometry and immunohistochemistry for TRAP.

Results: In T1D, major osteoclastogenic activity and bone loss versus other groups were confirmed by a greater TRAP-positive cell number and reabsorption surface on both the pressure and tension sides for 14 days ($p<0.01$). Additionally, we observed low bone volume density. Metformin plus insulin resulted in a daily insulin dose reduction and major glycemic control versus I-T1D. Although no significant differences were observed between I-T1D and IM-T1D, the tooth displacement and inclination, periodontal ligament thickness and alveolar bone density on the pressure side in IM-T1D were similar to that of NG ($p>0.05$).

Conclusion: Antidiabetic treatment reduces severe periodontal damage during applied orthodontic force in T1D untreated rats. Metformin as an add-on to insulin therapy resulted in glycemic control and a periodontal tissue response to orthodontic forces that was similar to that of normoglycemic rats.

KEYWORDS: Diabetes Mellitus Type 1, Insulin, Metformin, Orthodontic Tooth Movement, Alveolar Bone Loss

2.2 ARTICLE 2 –

Metformin therapy to prevent periodontal breakdown after orthodontic forces in type two diabetic rats. A micro-ct, histomorphometric and immunohistochemical evaluation

Ever Mena Laura https://orcid.org/0000-0002-5494-6569

Luan Pereira Macena https://orcid.org/0000-0002-9798-9390

Ana Carolina Cestari Bighetti https://orcid.org/0000-0001-9177-368X

Daniella Pereira Catanzaro https://orcid.org/0000-0002-9902-5464

Tania Mary Cestari https://orcid.org/0000-0003-1287-5974

Ricardo Quirico Pinheiro Machado https://orcid.org/0000-0002-5675-3162

Gustavo Pompermaier Garlet https://orcid.org/0000-0002-5071-8382

Rumio Taga https://orcid.org/0000-0002-5593-0999

Gerson Francisco de Assis https://orcid.org/0000-0001-8225-3164

ABSTRACT

Aim: We aimed to study evaluate the effects of metformin (MET) on the periodontal response to orthodontic forces in type two diabetic (T2D) rats. **Materials and methods:** Eighty-four Wistar rats were divided in normoglycemic (NG, n = 28), type two diabetics (T2D, n= 28) induced by high fat diet and low dose of streptozotocin, and T2D treated with MET 150mg/kg (M-T2D, n = 28). T2D was confirmed by fasting blood glucose ≥ 180 mg/dL. Glucose tolerant and insulin resistance tests were also conducted. After 25 days of T2D induction, orthodontic mesial tooth movement (OTM) was conducted for periods of 0, 3, 7, and 14 days. Samples were analyzed by micro-CT, histomorphometry and immunohistochemistry for TRAP. **Results:** Alveolar bone volume fraction (BV/TV and BA/TA) is reduced in T2D versus M-T2D and NG before OTM. During OTM, significant higher OTM, mesial tipping, mesial rotation, intrusion, periodontal thickness spacing and loss of alveolar BV/TV and BA/TA was found in T2D versus M-T2D and NG. It is accompanied by higher resorption areas and TRAP cells number on pressure side. **Conclusion:** Metformin therapy prevents from T2D increased osteoclastic resorption activity, reduced alveolar bone density, periodontal spacing and altered tooth movement patterns during orthodontic tooth movement.

3 DISCUSSION

3 DISCUSSION

Metformin effects as and adjunctive therapy to insulin in T1D, and as single treatment in T2 was studied during orthodontic treatment. Force loading on cells and tissues produces osteoclastic reabsorption of the alveolar bone and remodeling of periodontal tissues, necessary events to produce successful orthodontic tooth movement (Nishijima *et al.*, 2006). In both studies we found that MET use promotes good glycemic control then protective effects on periodontal tissues undergoing tooth movement.

In this study we confirmed T1D and T2D model in rats displaying a different course of diabetes. On the one hand Streptozotocin administration to induce T1D lead to higher glycaemia (450 ± 105mg/dl), water and food intake accompanied by body mass reduction in a short period of time as it occurs in humans. Similar clinical adverse effects are pathognomonic of T1D individuals (Who, 2019). We found that MET adjunction to insulin significantly reversed this clinical condition better than insulin alone and close to that of NG individuals. On the other hand, HFD to induce T2D was administered for 105 days (from young adult age to adult age), and only after, a low dose of Streptozotocin was administered to slightly elevate glycaemia. In this type of diabetes, rats underwent body mass gain or obesity, insulin resistance and eventually hyperglycemia (less than 350mg/dl) just as T2D develops in humans (Carvalheira *et al.*, 2002; Srinivasan *et al.*, 2005; Cerf, 2013). In this study, T2D was significantly related to alveolar bone osteoporosis prior to orthodontic force application, accordingly, osteoporosis is a frequent T2D adverse effect (Anaforoglu *et al.*, 2009; Arikan *et al.*, 2012; Walsh and Vilaca, 2017). MET treatment efficiently controlled hyperglycemia achieving NG levels at the end of the experiments. Additionally, MET ameliorated the glucose tolerance and insulin tolerance since the beginning of treatment and it is also seen in current studies (Horakova *et al.*, 2019)). Significant alveolar BV/TV and BA/TA recover was only achieved at 14 days of OTM. In this context, whether MET benefit

osteoporosis or even reduces fragility to fractures in T2D remains controversial (Jeyabalan *et al.*, 2012; Mccarthy *et al.*, 2016). Nonetheless, this study supports for MET amelioration g alveolar bone density.

In normoglycemic animals, clinical parameters including body mas, water did not change significantly along the experiments. In the article 2, NG animals showed a small gradual increase of body mass until the end of the experiments, however it is concomitant to rat aging and entering to adult life (Nistiar *et al.*, 2012). During OTM the three tooth movement phases occurred: an initial phase with initial tooth displacement within the periodontal ligament, creating a widened tension side and a narrowed pressure side; a lag phase where no tooth movement is observed; and a post lag phase where a gradual accelerated tooth movement occurs (Krishnan and Davidovitch, 2006; Wise and King, 2008). Those phases where according to the experimental periods in our OTM model. At day 0, NG showed alveolar bone integrity, compact cortical with Sharpey fibers and a periodontal ligament composed of dense organized collagen fibers perpendicular / oblique to the surface of the alveolar bone, and richly cellular and vascular. PDL thickness appeared uniform, ranging from 35 to 90um, and BV/TV showed no alterations. At 3 days, *initial OTM phase*, higher PDL spacing and fibers stretching were evident on tension side, alveolar bone resorption also occurred. On the pressure side, alveolar bone surface was proximate to the dental root, narrowing PDL thickness and forming hyalinized and reabsorption areas. Between 3 and 7 days, a *lag phase* with almost no significant changes were observed. In the second experiment, hyaline areas persisted until day 7, still no BA/TA or BV/TV reduction was recorded or observed in both articles. Between 7 and 14 days, *post lag phase*, PDL underwent periodontal spaces recovery with reorganization of vessels and collagen fibers on both sides. On tension side, in NG it was accompanied with bone formation on alveolar bone surface. In some cases of NG (2/14) and M-T2D (3/14) and T2D (6/14) groups, root resorption

(cementum and dentin) were also evident. In this regard, root resorption is not evaluated in both articles and it may represent a limitation in both studies.

T1D significantly weakens periodontal tissues so that orthodontic forces lead to a high periodontal breakdown, associated to hyaline areas presence as it is observed in previous studies (Holtgrave and Donath, 1989; Villarino *et al.*, 2011). Besides, we found altered tooth movement pattern, periodontal spacing and loss of alveolar bone density. Such changes were not previously described. In the present study, MET addition to insulin clearly ameliorates clinical parameters of T1D animals. MET helps to regain body mass and to reduce glycaemia proximate to that of normoglycemic. Previous studies suggested that MET ameliorates insulin action in peripheral tissues then reduces insulin needs (Faichney and Tate, 2003; Hostalek *et al.*, 2015). However, controlling glycaemia in T1D remains difficult because of the risk of suffering hypoglycemic crisis (Sayarifard *et al.*, 2017) and coma among insulin users (Wright, 2003); therefore, different levels of hyperglycemia persist among T1D patients. Regarding OTM, the resulting periodontal breakdown is clearly prevented by MET addition. PDL showed to be brittle in such T1D condition, then as consequence, tooth movement pattern was altered. However, MET treatment prevented from those movements leading to a tooth movement pattern proximate to that of NG. MET addition also reduced the bone loss (BV/TV or BA/TA) observed in T1D. Whether MET has a direct effect on bone tissue is still under debate, however MET is related to higher osteoblastic activity among laboratorial studies (Jang, Kim, Bae*, et al.*, 2011; Jang, Kim, Lee*, et al.*, 2011; Mai *et al.*, 2011). In this regard, this study supports for MET having an osteogenic effect or at least related to higher alveolar bone volume fraction than insulin treated or untreated T1D.

Resembling T1D, T2D produced adverse effect on PDL tissues during OTM. The higher amount of tooth mesial displacement that occurs in both T1 and T2D versus NG, is not accompanied by bone formation in the tension side and just by tissues breakdown. Hence,

clinically, higher T1D or T2D tooth movement may represent a signal of unfavorable OTM in which adverse effects are happening. Orthodontic treatment planning studies regarding time of treatment and treatments goals to be achieved in diabetic patients are still scarce. Higher PDL spacing and OTM patterns is only been approached in an osteoporotic model study (Xu *et al.*, 2013). Some differences between T1D and T2D were found in the tooth movement pattern and alveolar bone density. The tooth intrusion instead of T1D extrusion may be explained by the brittleness of an osteoporotic alveolar bone in T2D (Parfitt, 2013). Accordingly, molar intrusion is been pointed as the most difficult tooth movement among orthodontics (Ayadi *et al.*, 2018). Osteoporotic bone entails higher turnover (Parfitt, 2013), thus, tooth intrusion were possible just because factors such as direction of the force, low resistance of tissues and high alveolar turnover gathered together in T2D. MET reduced the T2D deleterious effects on periodontium during OTM. Following orthodontic force loading, alveolar bone density continues to reduce along with periodontal spacing and an altered tooth movement pattern. However, in the MET treated group BV/TV as well as BA/TA recovered at 14 days. We found single MET treatment being enough to recover the detrimental effects of T2D on periodontal tissues subjected to OTM close to NG however most of these effects were seen at 14 days of OTM where MET therapy reached 39 days. Still molecular pathways are needed to understand cellular related activity. MET restoring bone loss after OTM in diabetics is supported by the PDL thickness reduction in tension side and BV/TV recovery.

4 CONCLUSIONS

4 CONCLUSIONS

This study supports that:

- T1D and T2D have different harmful effects on periodontal tissues undergoing OTM.
- In T1D animals, force loading goes beyond the adaptive capacity of tissues. In addition, this causes cell death, extensive areas of tissue hyalinization and alveolar bone loss leading to a different tooth movement pattern.
- Metformin as an adjunctive to insulin therapy, promotes insulin sparing, better glycemic control and closer to NG values.
- MET plus Insulin avoids different tooth movement patterns and ameliorates the PDL response, reduces tissue damage and increases the PDL recovery better than insulin alone and similar to NG.
- HFD plus STZ in young adults wistar rats successfully resembled T2D development similar to that of humans; induction resulted in obesity, insulin resistance and maxillae osteoporosis.
- During OTM, T2D results in an increase of TRAP cells, resorption areas and reduced bone volume fraction, leading to altered tooth movement patterns.
- Metformin therapy achieves glycemic control, and tooth movement pattern proximate to that of normoglycemic individuals; it is accompanied with less bone loss and periodontal spacing.

REFERENCES

REFERENCES

ALMADIH, A. et al. Orthodontic Treatment Consideration in Diabetic Patients. **J Clin Med Res,** v. 10, n. 2, p. 77-81, Feb 2018. ISSN 1918-3003 (Print)1918-3011 (Electronic). Available at: < http://dx.doi.org/10.14740/jocmr3285w >.

ANAFOROGLU, I. et al. Prevalence of osteoporosis and factors affecting bone mineral density among postmenopausal Turkish women with type 2 diabetes. **Journal of Diabetes and its Complications,** v. 23, n. 1, p. 12-17, 1// 2009. ISSN 1056-8727. Available at: < http://www.sciencedirect.com/science/article/pii/S1056872707000694 >. Accessed on: 2009/2//.

ARIKAN, S. et al. Insulin Resistance in Type 2 Diabetes Mellitus May Be Related to Bone Mineral Density. **Journal of Clinical Densitometry,** v. 15, n. 2, p. 186-190, 4// 2012. ISSN 1094-6950. Available at: < http://www.sciencedirect.com/science/article/pii/S1094695011002162 >. Accessed on: 2012/6//.

AYADI, I. et al. [Orthodontic intrusion using mini-screws]. **Orthod Fr,** v. 89, n. 4, p. 397-410, Dec 2018. ISSN 0078-6608 (Print)
0078-6608.

BEYSEL, S. et al. The effects of metformin in type 1 diabetes mellitus. **BMC endocrine disorders,** v. 18, n. 1, p. 1-1, 2018. ISSN 1472-6823. Available at: < https://www.ncbi.nlm.nih.gov/pubmed/29338714 >.Available at: < https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5771191/ >.

BRAGA, S. M. G. et al. Effect of diabetes on orthodontic tooth movement in a mouse model. **European Journal of Oral Sciences,** v. 119, n. 1, p. 7-14, Feb 2011. ISSN 0909-8836. Available at: < <Go to ISI>://WOS:000286381000002 >.

CAO, H. et al. Force-induced Adrb2 in periodontal ligament cells promotes tooth movement. **J Dent Res,** v. 93, n. 11, p. 1163-9, Nov 2014. ISSN 0022-0345. Available at: < http://dx.doi.org/10.1177/0022034514551769 >.

CARVALHEIRA, J. B. C.; ZECCHIN, H. G.; SAAD, M. J. A. Bases moleculares e fisiológicas da resistência à insulina **BIOLOGIA MOLECULAR,** v. 5, n. 1, 2002.

CERF, M. E. Beta cell dysfunction and insulin resistance. **Frontiers in endocrinology,** v. 4, p. 37-37, 2013. ISSN 1664-2392. Available at: < https://www.ncbi.nlm.nih.gov/pubmed/23542897 >.Available at: < https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3608918/ >.

CHAUHAN, N. et al. Diabetes mellitus, a myth in orthodontics? **International Journal of Preventive and Clinical Dental Research,** v. 5, n. 3, p. 24, 2018. ISSN 2349-7319.

DEGEETER, M.; WILLIAMSON, B. Alternative Agents in Type 1 Diabetes in Addition to Insulin Therapy: Metformin, Alpha-Glucosidase Inhibitors, Pioglitazone, GLP-1 Agonists, DPP-IV Inhibitors, and SGLT-2 Inhibitors. **J Pharm Pract,** v. 29, n. 2, p. 144-59, Apr 2016. ISSN 0897-1900.

DUJIC, T. et al. Organic cation transporter 1 variants and gastrointestinal side effects of metformin in patients with Type 2 diabetes. **Diabetic medicine : a journal of the British Diabetic Association,** v. 33, n. 4, p. 511-514, 2016. ISSN 1464-5491
0742-3071. Available at: < https://www.ncbi.nlm.nih.gov/pubmed/26605869 >.Available at: < https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5064645/ >.

FAICHNEY, J. D.; TATE, P. W. Metformin in type 1 diabetes: is this a good or bad idea? **Diabetes Care,** v. 26, n. 5, p. 1655, May 2003. ISSN 0149-5992 (Print)0149-5992. Available at: < http://dx.doi.org/ >.

GOMES, M. F. et al. Effects of the photobiomodulation using different energy densities on the periodontal tissues under orthodontic force in rats with type 2 diabetes mellitus. **Braz Oral Res,** v. 32, p. e61, Oct 25 2018. ISSN 1806-8324.

GOYAL, R.; JIALAL, I. Diabetes Mellitus Type 2. 2019/02/24 2019a. Available at: < https://www.ncbi.nlm.nih.gov/pubmed/ >.

_____. Glucose Intolerance. 2019/04/01 2019b. Available at: < https://www.ncbi.nlm.nih.gov/pubmed/ >.

HADJIDAKIS, D. J.; ANDROULAKIS, II. Bone remodeling. **Ann N Y Acad Sci,** v. 1092, p. 385-96, Dec 2006. ISSN 0077-8923 (Print)
0077-8923.

HOLTGRAVE, E. A.; DONATH, K. [Periodontal reactions to orthodontic forces in the diabetic metabolic state]. **Fortschr Kieferorthop,** v. 50, n. 4, p. 326-37, Aug 1989. ISSN 0015-816X (Print)
0015-816x.

HORAKOVA, O. et al. Metformin acutely lowers blood glucose levels by inhibition of intestinal glucose transport. **Scientific Reports,** v. 9, n. 1, p. 6156, 2019/04/16 2019. ISSN 2045-2322. Available at: < https://doi.org/10.1038/s41598-019-42531-0 >.

HOSTALEK, U.; GWILT, M.; HILDEMANN, S. Therapeutic Use of Metformin in Prediabetes and Diabetes Prevention. **Drugs,** v. 75, n. 10, p. 1071-1094, 2015. ISSN 1179-1950
0012-6667. Available at: < https://www.ncbi.nlm.nih.gov/pubmed/26059289 >.Available at: < https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4498279/ >.

IDF. About diabetes. 2019. Avaialble at: < https://idf.org/52-about-diabetes.html >. Accessed on: 07/01/2019.

JANG, W. G. et al. Metformin induces osteoblast differentiation via orphan nuclear receptor SHP-mediated transactivation of Runx2. **Bone,** v. 48, n. 4, p. 885-93, Apr 1 2011. ISSN 1873-2763.

_____. AMP-activated protein kinase (AMPK) positively regulates osteoblast differentiation via induction of Dlx5-dependent Runx2 expression in MC3T3E1 cells. **Biochem Biophys Res Commun,** v. 404, n. 4, p. 1004-9, Jan 28 2011. ISSN 0006-291x.

JEYABALAN, J. et al. THE ANTI-DIABETIC DRUG METFORMIN HAS NO DELETERIOUS EFFECT ON BONE MASS IN VIVO BUT DOES NOT INDUCE OSTEOGENESIS NOR FRACTURE HEALING. **Osteoporosis International,** v. 23, p. S536-S536, Jul 2012. ISSN 0937-941X. Available at: < <Go to ISI>://WOS:000309261000035 >.

JONASSON, G.; SKOGLUND, I.; RYTHÉN, M. The rise and fall of the alveolar process: Dependency of teeth and metabolic aspects. **Archives of oral biology**, 2018. ISSN 0003-9969.

KHARROUBI, A. T.; DARWISH, H. M. Diabetes mellitus: The epidemic of the century. **World journal of diabetes,** v. 6, n. 6, p. 850-867, 2015. ISSN 1948-9358. Available at: < https://www.ncbi.nlm.nih.gov/pubmed/26131326 >.Available at: < https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4478580/ >.

KRISHNAN, V.; DAVIDOVITCH, Z. E. Cellular, molecular, and tissue-level reactions to orthodontic force. **American Journal of Orthodontics and Dentofacial Orthopedics,** v. 129, n. 4, p. 469.e1-469.e32, 4// 2006. ISSN 0889-5406. Available at: < http://www.sciencedirect.com/science/article/pii/S0889540605011054 >.

LI, Y. et al. Orthodontic tooth movement: The biology and clinical implications. **The Kaohsiung Journal of Medical Sciences,** v. 34, n. 4, p. 207-214, 2018/04/01/ 2018. ISSN 1607-551X. Available at: < http://www.sciencedirect.com/science/article/pii/S1607551X17307647 >.

MAI, Q. G. et al. Metformin stimulates osteoprotegerin and reduces RANKL expression in osteoblasts and ovariectomized rats. **Journal of Cellular Biochemistry,** v. 112, n. 10, p. 2902-2909, 2011. ISSN 0730-2312
1097-4644. Available at: < http://www.embase.com/search/results?subaction=viewrecord&from=export&id=L362632858 >.Available at: < http://dx.doi.org/10.1002/jcb.23206 >.

MCCARTHY, A. D.; CORTIZO, A. M.; SEDLINSKY, C. Metformin revisited: Does this regulator of AMP-activated protein kinase secondarily affect bone metabolism and prevent diabetic osteopathy? **World Journal of Diabetes,** v. 7, n. 6, p. 122-133, Mar 2016. ISSN 1948-9358. Available at: < <Go to ISI>://WOS:000373215200002 >.

MEIKLE, M. C. The tissue, cellular, and molecular regulation of orthodontic tooth movement: 100 years after Carl Sandstedt. **Eur J Orthod,** v. 28, n. 3, p. 221-40, Jun 2006. ISSN 0141-5387 (Print)
0141-5387.

NAJEEB, S. et al. Influence of uncontrolled diabetes mellitus on periodontal tissues during orthodontic tooth movement: a systematic review of animal studies. **Progress in Orthodontics,** v. 18, n. 1, p. 5, 2017/02/06 2017. ISSN 2196-1042. Available at: < https://doi.org/10.1186/s40510-017-0159-z >.

NISHIJIMA, Y. et al. Levels of RANKL and OPG in gingival crevicular fluid during orthodontic tooth movement and effect of compression force on releases from periodontal ligament cells in vitro. **Orthod Craniofac Res,** v. 9, n. 2, p. 63-70, May 2006. ISSN 1601-6335 (Print)
1601-6335.

NISTIAR, F. et al. Age dependency on some physiological and biochemical parameters of male Wistar rats in controlled environment. **Journal of environmental science and health. Part A, Toxic/hazardous substances & environmental engineering,** v. 47, p. 1224-33, 07/15 2012.

PARFITT, A. M. Chapter 36 - Skeletal Heterogeneity and the Purposes of Bone Remodeling: Implications for the Understanding of Osteoporosis. In: ROBERT, M.;DAVID, F., *et al* (Ed.). **Osteoporosis (Fourth Edition)**. San Diego: Academic Press, 2013. p.855-872. ISBN 978-0-12-415853-5.

PLUT, A. et al. Bone remodeling during orthodontic tooth movement in rats with type 2 diabetes. **Am J Orthod Dentofacial Orthop,** v. 148, n. 6, p. 1017-25, Dec 2015. ISSN 0889-5406.

ROEP, B. O.; PEAKMAN, M. Antigen targets of type 1 diabetes autoimmunity. **Cold Spring Harbor perspectives in medicine,** v. 2, n. 4, p. a007781-a007781, 2012. ISSN 2157-1422. Available at: < https://www.ncbi.nlm.nih.gov/pubmed/22474615 >.Available at: < https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3312399/ >.

SAYARIFARD, F. et al. Status of Bone Mineral Density in Children with Type 1 Diabetes Mellitus and Its Related Factors. **Iranian Journal of Pediatrics,** v. 27, n. 4, 2017. Available at: <

http://cdn.neoscriber.org/cdn/serve/313ee/17d5c9e1903aa7f3c7f231191984a5af199ccd73/ijp-27-04-9062.pdf >.

SRINIVASAN, K. et al. Combination of high-fat diet-fed and low-dose streptozotocin-treated rat: a model for type 2 diabetes and pharmacological screening. **Pharmacol Res,** v. 52, n. 4, p. 313-20, Oct 2005. ISSN 1043-6618 (Print)
1043-6618.

SUN, J. et al. Histological evidence that metformin reverses the adverse effects of diabetes on orthodontic tooth movement in rats. **J Mol Histol,** v. 48, n. 2, p. 73-81, Apr 2017. ISSN 1567-2379.

TALUKDAR, S. et al. Neutrophils mediate insulin resistance in mice fed a high-fat diet through secreted elastase. **Nature medicine,** v. 18, n. 9, p. 1407, 2012. ISSN 1546-170X.

VATANDOUST, N. et al. Novel High-Fat Diet Formulation and Streptozotocin Treatment for Induction of Prediabetes and Type 2 Diabetes in Rats. **Advanced biomedical research,** v. 7, p. 107-107, 2018. ISSN 2277-9175. Available at: < https://www.ncbi.nlm.nih.gov/pubmed/30069438 >.Available at: < https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6050973/ >.

VILLARINO, M. E.; LEWICKI, M.; UBIOS, A. M. Bone response to orthodontic forces in diabetic Wistar rats. **Am J Orthod Dentofacial Orthop,** v. 139, n. 4 Suppl, p. S76-82, Apr 2011. ISSN 0889-5406.

WALSH, J. S.; VILACA, T. Obesity, Type 2 Diabetes and Bone in Adults. **Calcified tissue international,** v. 100, n. 5, p. 528-535, 2017. ISSN 1432-0827
0171-967X. Available at: < https://www.ncbi.nlm.nih.gov/pubmed/28280846 >.Available at: < https://www.ncbi.nlm.nih.gov/pmc/articles/PMC5394147/ >.

WHO. About diabetes. 2019. Avaialble at: < https://www.who.int/diabetes/action_online/basics/en/index1.html >. Accessed on: 2019.

WISE, G. E.; KING, G. J. Mechanisms of tooth eruption and orthodontic tooth movement. **J Dent Res,** v. 87, n. 5, p. 414-34, May 2008. ISSN 0022-0345 (Print)
0022-0345.

WRIGHT, J. DIABETES MELLITUS | Chemical Pathology. In: CABALLERO, B. (Ed.). **Encyclopedia of Food Sciences and Nutrition (Second Edition)**. Oxford: Academic Press, 2003. p.1782-1787. ISBN 978-0-12-227055-0.

XU, Y. et al. Periodontal microstructure change and tooth movement pattern under different force magnitudes in ovariectomized rats: an in-vivo microcomputed tomography study. **Am J Orthod Dentofacial Orthop,** v. 143, n. 6, p. 828-36, Jun 2013. ISSN 0889-5406.

YE, J. et al. Hypoxia is a potential risk factor for chronic inflammation and adiponectin reduction in adipose tissue of ob/ob and dietary obese mice. **American Journal of Physiology-Endocrinology and Metabolism,** v. 293, n. 4, p. E1118-E1128, 2007. ISSN 0193-1849.

YI, J. et al. Caffeine may enhance orthodontic tooth movement through increasing osteoclastogenesis induced by periodontal ligament cells under compression. **Arch Oral Biol,** v. 64, p. 51-60, Apr 2016. ISSN 0003-9969. Available at: < http://dx.doi.org/10.1016/j.archoralbio.2015.12.009 >.

APPENDIXES

DECLARATION OF EXCLUSIVE USE OF THE ARTICLE IN DISSERTATION/THESIS

We hereby declare that we are aware of the article "**Metformin as an add-on to insulin improves periodontal response during orthodontic tooth movement in type 1 diabetic rats**" will be included in Thesis of the student **Ever Elias Mena Laura** was not used and may not be used in other works of Graduate Programs at the Bauru School of Dentistry, University of São Paulo.

Bauru, August 30, 2019.

Ever Elias Mena Laura
Author
Signature

Tania Mary Cestari
Author
Signature

Rodrigo Almeida
Author
Signature

Daniela Santos Pereira
Author
Signature

Rumio Taga
Author
Signature

Gustavo Pompermaier Garlet
Author
Signature

Gerson Francisco Assis
Author
Signature

DECLARATION OF EXCLUSIVE USE OF THE ARTICLE IN DISSERTATION/THESIS

We hereby declare that we are aware of the article "**Metformin therapy to prevent periodontal breakdown after orthodontic forces in type two diabetic rats. A micro-CT, Histomorphometric and immunohistochemical evaluation**" will be included in Thesis of the student **Ever Elias Mena Laura** was not used and may not be used in other works of Graduate Programs at the Bauru School of Dentistry, University of São Paulo.

Bauru, August 30, 2019.

Ever Elias Mena Laura
Author
Signature

Luan Pereira Macena
Author
Signature

Ana Carolina Cestari Bighetti
Author
Signature

Daniela Santos Pereira
Author
Signature

Tania Mary Cestari
Author
Signature

Ricardo Quirico Pinheiro Machado
Author
Signature

Gustavo Pompermaier Garlet
Author
Signature

Rumio Taga
Author
Signature

Gerson Francisco de Assis
Author
Signature

ANNEXES

ANNEX 1

Approval of Ethical Committee article 1

Universidade de São Paulo
Faculdade de Odontologia de Bauru

Comissão de Ética no Ensino e Pesquisa em Animais

CEEPA-Proc. Nº 033/2013

Bauru, 26 de maio de 2014.

Senhor Professor,

Em atenção às alterações no projeto de pesquisa denominado **Efeito da Metformina no osso alveolar durante a movimentação ortodôntica em ratos diabéticos induzidos pela Estreptozotocina,** de autoria de Ever Elias Mena Laura, com colaboração de Tania Mary Cestari e Danila Santos Pereira, sob sua orientação foi enviado ao relator para avaliação, quais sejam:

Alteração no título para: *"Influência de drogas antidiabéticas no metabolismo ósseo alveolar durante a movimentação dentária em modelos experimentais de ratos diabéticos tipo 1 e 2"*;

Número total de animais: 180 ratos, divididos em seis grupos experimentais, em 3 períodos experimentais.

Considerando que tais modificações não implicam em impedimentos éticos, o relator emitiu parecer favorável, o que foi aceito *ad referendum* desta Comissão.

Lembramos que qualquer outra alteração que ocorrer na pesquisa, esta Comissão deverá imediatamente comunicada, bem como ao final, um relatório com os resultados obtidos seja enviado para análise ética e emissão de parecer, o qual poderá ser utilizado para fins de publicação científica.

Atenciosamente,

Prof. Dr Gustavo Pompermaier Garlet
Vice-Presidente da Comissão de Ética no Ensino e Pesquisa em Animais

Prof. Dr Gerson Francisco de Assis
Docente do Departamento de Ciências Biológicas

Al. Dr. Octávio Pinheiro Brisolla, 9-75 – Bauru-SP – CEP 17012-101 – C.P. 73
e-mail: mferrari@fob.usp.br – Fone/FAX (0xx14) 3235-8356
http://www.fob.usp.br

ANNEX 1

Approval of Ethical Committee Article 2

Universidade de São Paulo
Faculdade de Odontologia de Bauru

Comissão de Ética no Ensino e Pesquisa em Animais

CEEPA-Proc. Nº 006/2016

Bauru, 31 de agosto de 2016.

Senhor Professor,

Informamos que a proposta intitulada ***Efeito protetor da Metformina no processo de remodelação óssea durante a aplicação de forças ortodônticas em ratos diabéticos tipo-2***, *registrada sob* ***CEEPA-Proc. Nº 006/2016,*** tendo Vossa Senhoria como Pesquisador Responsável, que envolve a utilização de animais pertencentes ao filo Chordata, subfilo Vertebrata (exceto humanos), para fins de pesquisa científica, encontra-se de acordo com os preceitos da Lei nº 11.794, de 8 de outubro de 2008, do Decreto nº 6.899, de 15 de julho de 2009, e com as normas editadas pelo Conselho Nacional de Controle da Experimentação Animal (CONCEA), foi analisada e considerada APROVADA a sua execução nas dependências da FOB-USP, em reunião ordinária da Comissão de Ética no Ensino e Pesquisa em Animais (CEEPA), realizada no dia 26 de agosto de 2016.

Finalidade	*( ) Ensino (X) Pesquisa Científica*
Vigência da autorização:	*Julho/2016 a Novembro/2017*
Espécie/linhagem/raça:	*Rato heterogênico/ Wistar albino*
Nº de animais:	*79*
Peso/Idade	*200g-250g/60 dias*
Sexo:	*Machos*
Origem:	*Biotério Central da PUSP/RP*

Esta CEEPA solicita que ao final da pesquisa seja enviado um Relatório com os resultados obtidos para análise ética e emissão de parecer final, o qual poderá ser utilizado para fins de publicação científica.

Atenciosamente,

Profª Drª Ana Paula Campanelli
Presidente da Comissão de Ética no Ensino e Pesquisa em Animais

Prof. Dr. Gerson Francisco de Assis
Docente do Departamento de Ciências Biológicas

Al. Dr. Octávio Pinheiro Brisolla, 9-75 – Bauru-SP – CEP 17012-901 – C.P. 73
e-mail: ceepa@fob.usp.br – Fone/FAX (0xx14) 3235-8356
http://www.fob.usp.br