

UNIVERSIDADE ESTADUAL PAULISTA
“JÚLIO DE MESQUITA FILHO”
FACULDADE DE MEDICINA

Tawana Pascon

**Impacto do exercício de natação em rata prenhe
diabética no desenvolvimento do esmalte,
microdureza, e volume dos dentes da ninhada**

*Impact of diabetic pregnant rat swimming exercise on enamel
development, microhardness, and volume of offspring's teeth*

Tese apresentada à Faculdade
de Medicina, Universidade
Estadual Paulista “Júlio de
Mesquita Filho”, Campus de
Botucatu, para obtenção do
título de Doutora em
Tocoginecologia.

Orientadora: Prof.^a Emérita Marilza Vieira Cunha Rudge

Coorientadores: Dr. Diego Giroto Bussanelli

Dr^a. Juliana Ferreira Floriano

Botucatu

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Coorientador: Diego Girotto Bussaneli

Coorientador: Juliana Ferreira Floriano

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Programação fetal.

Tawana Pascon

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Orientadora: Prof^ª. Emérita Marilza Vieira Cunha Rudge

Comissão Examinadora para Exame Geral de Qualificação:

Prof^ª. Emérita Marilza Vieira Cunha Rudge
Faculdade de Medicina de Botucatu – FMB - UNESP

Prof^ª. Dr^ª. Rita de Cássia Loiola Cordeiro
Faculdade de Odontologia de Araraquara - FOA - UNESP

Prof. Dr. Carlos Izaias Sartorão Filho
Fundação Educacional do Município de Assis – FEMA

Prof. Dr. Manuel Restrepo
Faculdade de Odontologia - Universidad CES

Prof^ª. Dr^ª. Patrícia de Souza Rossignoli
Faculdade de Filosofia e Ciências – FFC - UNESP

Botucatu, 27 de fevereiro de 2023.

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Epígrafe

“A dúvida é o princípio da sabedoria”

Aristóteles

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Lista de Abreviações

Português e Inglês

LISTA DE ABREVIACÕES EM PORTUGUÊS

Ca	Calcio
CAPES	Coordenação de Aperfeiçoamento de Pessoal de Nível Superior
CEP	Comitê de Ética em Pesquisa
CEUA	Comissão de Ética no Uso de Animais
CIDPN	Centro de Investigação do Diabetes Perinatal
DED	Desenvolvimento do esmalte dentário
DDE	Defeito no Desenvolvimento do Esmalte
DM	<i>Diabetes mellitus</i>
DMG	<i>Diabetes mellitus</i> gestacional
Dr.	Doutor
Dr^a.	Doutora
FAPESP	Fundação de Amparo à Pesquisa do Estado de São Paulo
FEMA	Fundação Educacional do Município de Assis
FFC	Faculdade de Filosofia e Ciências
FMB	Faculdade de Medicina de Botucatu
FOA	Faculdade de Odontologia de Araraquara
FOB	Faculdade de Odontologia de Bauru
GJ	Glicemia de jejum
IDE	Índice de defeitos de esmalte
IU	Incontinência urinária
IU-EG	Incontinência urinária específica da gestação
LACIFI	Laboratório de Ciências Fisiológicas
LIT-UroGo	Laboratório de Investigação Translacional em Uroginecologia e Obstetrícia

MAP	Músculos do assoalho pélvico
MEV	Microscopia Eletrônica de Varredura
MICRO CT	Microtomografia
Prof.	Professor
Prof^a.	Professora
PTH	Paratormônio
TCLE	Termo de Consentimento Livre e Esclarecido
TOTG	Teste oral de tolerância à glicose
UNESP	Universidade Estadual Paulista
UNIMAR	Universidade de Marília
USP	Universidade de São Paulo

LISTA DE ABREVIACES EM INGLS

%	Percentage
ACOG	American College of Obstetricians and Gynecologists
ADA	American Diabetes Association
BMI	Body mass index
CONCEA	Council for Control of Animal Experimentation
DED	Dental enamel development
DDE	Developmental defects of enamel
DOHaD	Developmental origins of adult health and disease
EDS	Energy Dispersive Spectroscopy
FOAD	Fetal origin of adult disease
GDM	Gestational <i>Diabetes Mellitus</i>
NCD	Non-communicable diseases
OGTT	Oral glucose tolerance test
PDRC	Perinatal Diabetes Research Center
SE	Swimming exercise
SEM	Scanning Electron Microscope
SD	Standard deviation
STZ	Streptozotocin

Resumo da Tese

Pascon, T. Impacto do exercício de natação em rata prenhe no desenvolvimento do esmalte, microdureza e volume dos dentes da ninhada. 2023. 141f. Tese (Doutorado) – Faculdade de Medicina de Botucatu, Universidade Estadual Paulista, Brasil.

Introdução: 1) Durante o desenvolvimento intrauterino o feto é vulnerável a diversos fatores, principalmente por via materna. O esmalte dentário é considerado um marcador biológico sensível para algumas condições, é um tecido sem células vivas e não pode ser reparado depois de danificado. Os defeitos de desenvolvimento do esmalte (DDE) podem sugerir a associação com fatores sistêmicos ou locais e programação fetal. Portanto, este estudo teve como objetivo projetar e desenvolver o diagrama com a cronologia do desenvolvimento do esmalte dentário (DED) para possibilitar a investigação dos fatores de risco intrauterinos e repercussões da programação fetal da DDE com base no conceitual do *Diamater Study Group*. 2) Estudos epidemiológicos e em modelos animais mostraram que a hiperglicemia altera o processo de desenvolvimento do dente, afetando a erupção e a mineralização do dente e isso inclui o desenvolvimento do esmalte do dente. Ressalta-se que a organização do esmalte, dentina, cemento e polpa dentária é comum tanto para humanos quanto para roedores. O Diabetes Mellitus Gestacional (DMG) foi associado a efeitos adversos do DDE na prole. Identificar fatores associados a alterações no esmalte do descendente, bem como desenvolver estudos com propostas de intervenção, para que medidas preventivas e terapêuticas possam ser investigadas e adotadas no futuro a fim de minimizar as consequências negativas do DMG no desenvolvimento do esmalte dentário. O objetivo deste estudo foi analisar o impacto do exercício de natação de ratas grávidas no desenvolvimento do esmalte, microdureza e volume dos dentes dos filhotes. **Métodos e Resultados:** 1) O “diagrama conceitual do DED

Diamater” foi desenvolvido considerando a cronologia de formação do esmalte na dentição decídua e permanente com início na vida intrauterina. Os dentes foram diferenciados por grupo de dentes, tipo de dentição e por porcentagem de formação de esmalte dentário por superfície do dente. Os períodos do início até a formação completa do esmalte foram considerados e relacionados com o período intrauterino, nascimento e pós-natal. **2)** Para induzir o modelo de ratos, fêmeas Wistar recém-nascidas receberam no primeiro dia de vida injeção subcutânea de STZ (Sigma®). Ratos não diabéticos receberam injeção subcutânea de tampão citrato. Todas as ratas recém-nascidas foram mantidas com suas mães até o final do período de lactação (21 dias). Após este período, as ratas mães foram eutanasiadas por injeção de tiopental sódico (Thiopentax® - 80 mg/kg). Os recém-nascidos do sexo feminino foram mantidos até a idade adulta. O protocolo de exercício de natação foi baseado em estudos anteriores e considerado um protocolo de exercício de intensidade moderada. A primeira sessão de treino iniciou com 20 minutos, aumentando progressivamente 10 minutos/dia até 60 minutos. As ratas sedentárias foram expostas à água diariamente por 15 minutos, a uma profundidade de 10 cm na temperatura da água $31\pm 1^{\circ}\text{C}$ por 6 dias/semana, do dia 0 até o dia 20 gestacional, visando não promover adaptações fisiológicas a partir da prática de exercícios. Todos os ratos recém-nascidos foram mantidos com suas mães até o final do período de lactação (21 dias). Para este estudo foram utilizados os dentes da prole: foram extraídos quatro incisivos de cada animal de cada grupo (dentes incisivos superiores e inferiores). Todas as amostras foram identificadas e armazenadas em formaldeído 10%. Para avaliar o volume do esmalte, todos os dentes foram escaneados em um sistema de micro-CT. Para a avaliação da microestrutura, as amostras foram avaliadas por meio de Microscopia Eletrônica de Varredura. As amostras foram avaliadas quanto à presença ou ausência de fraturas, quebras na “ponta”, rugosidade, arranhões e ondulações. Para a avaliação

da microestrutura, as amostras foram avaliadas por meio de Microscopia Eletrônica de Varredura. Avaliação da microdureza. Para cada animal de todos os grupos, quatro incisivos foram avaliados. As superfícies retificadas dos dentes foram polidas com pastas de polimento de 6 l e 1 l. A microdureza dos dentes foi testada usando um indentador Vicker. O volume do esmalte não apresentou diferença entre os grupos. No entanto, um maior volume de esmalte foi demonstrado nos dentes inferiores quando comparados aos dentes superiores nos quatro grupos. O grupo Dsed apresentou maior número de alterações em relação a fraturas, fissuras, rugosidade, formação de esmalte e a presença concomitante de opacidade difusa e demarcada. Aumento significativo na concentração de Na foi observado no grupo NDex em comparação com o grupo NDsed, enquanto nenhuma diferença foi encontrada nas concentrações de Mg, Fe, C, O, Ca, P, Cl e Al entre os grupos. Houve diminuição da microdureza nos grupos Dsed e Dex em relação ao grupo NDex. O grupo Dex apresentou redução dos efeitos deletérios observados no grupo Dsed e resultados semelhantes aos grupos ND (NDsed e NDex). Os grupos diabéticos e não diabéticos exercitados apresentaram aspecto de consolidação da fissura. Nosso estudo sobre a distribuição de elementos no esmalte de descendentes de dentes de ratos diabéticos também pode ser importante para a compreensão dos processos de mineralização do esmalte, determinando como os elementos alteram as propriedades dos materiais e explorando a relação entre o binômio gravidez e descendência. Os achados deste estudo demonstram o impacto negativo do Diabetes em ratas prenhes no esmalte dentário, principalmente no grupo sem intervenção com exercício de natação. **Conclusão: 1)** O diagrama conceitual do DED Diamater foi desenvolvido e permite visualizar a inter-relação do DED com a vida intrauterina até a idade pós-natal de quatro anos. O diagrama apresenta o grupo de dentes, tipo de dentição, superfície dentária e evolução do DED, indicando o início de acordo com a semana gestacional e ano de vida que

completa a formação do esmalte dentário. A ferramenta pode direcionar intervenções preventivas e terapêuticas em DDE que podem interferir positivamente na saúde bucal das crianças mais tarde na vida e nas gerações futuras. **2)** Os resultados sustentam que não houve diferença entre os grupos na análise do volume do esmalte. Não praticar natação e ter diabetes durante a gravidez teve maior impacto negativo em relação à ocorrência de fraturas, fissuras, alterações de rugosidade, formação de esmalte e a presença concomitante de opacidade difusa e demarcada. O diabetes em geral causou uma diminuição na microdureza do esmalte em comparação ao grupo não diabético e o exercício de natação foi capaz de reverter as fissuras.

Palavras-chave: feto, diabete, programação fetal, esmalte dentário, gravidez, parto, puerpério, exercício, natação, ratas

Thesis Abstract

Pascon, T. Impact of pregnant rat swimming exercise on enamel development, microhardness, and volume of offspring's teeth 2023. 141p. Thesis (Doctorate) - Botucatu Medical School (FMB), São Paulo State University (UNESP), Brazil.

Background: **1)** During intrauterine development the fetus is vulnerable to various factors, mainly affected via maternal. Dental enamel is considered a sensitive biological marker for some conditions, it's a tissue with no living cells and cannot be repair after damaged. The developmental defects of enamel (DDE) may suggest the association with systemic or local factors and fetal programming. Therefore, this study aimed to design and develop the diagram with the chronology of the DED to enable investigation of the intrauterine risk factors and fetal programming repercussions of DDE based on Diamater Study Group conceptual. **2)** Epidemiological and animal model studies have shown that hyperglycemia alters the tooth development process by affecting tooth eruption and mineralization and this includes tooth enamel development. It is noteworthy that the organization of enamel, dentin, cementum and dental pulp is common to both humans and rodents. Gestational Diabetes Mellitus (GDM) was associated with adverse effects of DDE on offspring. To identify factors associated with changes in the descendant's enamel, as well as to develop studies with intervention proposals, so that preventive and therapeutic measures can be investigated and adopted in the future in order to minimize the negative consequences of GDM on the development of dental enamel. The aim this study was analyze of Impact of pregnant rat swimming exercise on enamel development, microhardness, and volume of offspring's teeth. **Methods and Results:** **1)** The "DED Diamater's conceptual diagram" was developed considering of the chronology enamel formation in the deciduous and permanent dentition with start in intrauterine life. The teeth were differentiated by group of teeth, type of dentition and by percentage of dental enamel formation per surface of the tooth. The periods to the beginning until

complete enamel formation was considered and was related with the intrauterine, birth and postnatal period. **2)** To induce rats' model, newborn female Wistar received in the first day of life subcutaneously injection of STZ (Sigma®). Non-diabetic rats received subcutaneous injection of citrate buffer. All female newborn rats were maintained with their mothers until the end of the lactation period (21 days). After this period, the mother rats were euthanized by sodium thiopental injection (Thiopentax® - 80 mg/kg). The female newborns were maintained until adulthood. The swimming exercise protocol was based on previous studies and considered to be a moderate intensity exercise protocol. The first training session started with 20 minutes, progressively increasing 10 minutes/day until 60 minutes. The sedentary rats were exposed to water daily for 15 minutes, at a depth of 10 cm at water temperature $31\pm 1^{\circ}\text{C}$ for 6 days/week, from gestational day 0 until gestational day 20, aiming not to promote physiological adaptations from exercise practice. All newborn rats were maintained with their mothers until the end of the lactation period (21 days). For this study the offspring teeth was used: four incisors of each animal of each group were extracted (upper and lower incisors teeth). All samples were identified and stored in formaldehyde 10%. In order to evaluate enamel volume, all teeth were scanned in a micro-CT system. For the evaluation of the microstructure, the samples were evaluated by means of Scanning Electron Microscopy. The samples were evaluated for the presence or absence of fractures, breaks at the "tip", roughness, scratches and dimples. For the evaluation of the microstructure, the samples were evaluated by means of Scanning Electron Microscopy. Microhardness evaluation. For each animal of all groups, four incisors were evaluated. Ground surfaces of teeth were polished using 6l and 1l polishing pastes. Teeth microhardness was tested using a Vicker indenter. The enamel volume showed no difference between the groups. However, a greater enamel volume was demonstrated in the lower teeth when compared with the upper teeth in the four groups. The Dsed group showed a greater number of alterations in relation to fractures, fissures,

roughness, enamel formation and the concomitant presence of diffuse opacity and as demarcated. A significant increase in Na concentration was observed in the NDex group compared with NDsed group, while no difference was found in the Mg, Fe, C, O, Ca, P, Cl e Al concentrations between groups. There was a decrease in microhardness in the Dsed and Dex groups compared to the NDex group. The Dex group showed a reduction in the deleterious effects observed in the Dsed group and similar results to the ND groups (NDsed and NDex). The exercised diabetic and non-diabetic groups showed an appearance of fissure consolidation.

Our study on the distribution of elements in the enamel of offspring of the diabetic rats' teeth may also be important for the understanding of the enamel mineralization processes, determining how elements change the properties of the materials, and exploring the relationship between binomial pregnancy and offspring. The findings in this study demonstrate the negative impact of Diabetes in pregnant rats on dental enamel, especially the group without intervention with swimming exercise. **Conclusion: 1)** DED Diamater's conceptual diagram was developed and makes it possible to view the interrelation of the DED with intrauterine life to four years postnatal age. The diagram present of the group of teeth, type of dentition, tooth surface and evolution of DED, indicating the onset according to gestational week and year of life that completes the formation of dental enamel. The tool may to target preventive and therapeutic interventions in DDE that can positively interfere with children's oral health later life and in future generation. **2)** The results support that there was no difference between groups in the analysis of enamel volume. Not performing swimming exercises and having diabetes during pregnancy had a greater negative impact in relation to the occurrence of fractures, fissures, changes in roughness, enamel formation and the concomitant presence of diffuse and demarcated opacity. Diabetes in general caused a decrease in enamel microhardness compared to the non-diabetic group and swimming exercise

was able to reverse fissures.

keywords: fetus, diabetes, fetal programming, dental enamel, pregnancy, birth, postpartum period, exercise, swimming, rats

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Seção 1

Trajetória Acadêmica

Iniciei a formação acadêmica em odontologia na Universidade de Marília em 2005 e, ao concluir em 2009, a Odontopediatria estava definida como área de interesse de trabalho. Entre 2010 e 2012, realizei curso de especialização em Odontopediatria na UNESP em Araçatuba que foi, indubitavelmente, a diretriz para o interesse em ampliar o interesse de atuação clínica e associar a pesquisa científica. Agregando conhecimento e complementando a atuação, cursei entre 2010 e 2013 a especialização em ortodontia na Universidade de Marília, e desta formação, o conhecimento aprofundado sobre formação dental e do esmalte desde o período intrauterino.

Ao longo de boa parte da minha vida acompanhei cada resultado obtido das investigações do Grupo de Pesquisa “Diabetes e Gravidez: Clínico e Experimental” *Diamater Study Group*, do qual minha mãe faz parte desde 2002. No final de 2014, como sempre de forma entusiasmada, ela falou sobre a “tríade” que havia se formado em torno da Hiperglicemia gestacional, miopatia diabética gestacional e incontinência urinária gestacional, e do projeto ambicioso projeto Temático de investigar ampla e profundamente esta associação.

Entre as investigações proposta neste Temático, me despertou especial atenção, as análises bioquímicas como cálcio, calcitonina, paratormônio e vitamina D, por serem variáveis relacionadas a formação do esmalte dentário desde a fase intrauterina. Neste momento, todo conhecimento adquirido na graduação e especializações se agrupou, e

surgiu a seguinte pergunta da dissertação de mestrado: se o meio materno está alterado, e a formação dental e do esmalte se inicia na vida intrauterina, este pode gerar repercussões negativas, porque o desenvolvimento dental e do esmalte depende da cascata destas mesmas variáveis bioquímicas.

Em 2015, iniciei no grupo de pesquisa participando das reuniões e discussões e paralelamente construindo o projeto de mestrado. Fazer parte de grupo de estudos com tantos anseios em relação à pesquisa abriu meus horizontes para um mundo novo. Entre 2016 e 2018, cursei o mestrado sob orientação da Profa. Marilza Rudge, que culminou com a dissertação intitulada “Associação do *Diabetes Mellitus* Gestacional com os Defeitos de Desenvolvimento de Esmalte na Dentição da Criança”. Os resultados obtidos e publicados (<https://doi.org/10.1371/journal.pone.0211771>), abriram novas linhas de investigação e nos impulsionaram a ampliar e aprofundar o conhecimento e desta forma propusemos a condução, de forma translacional, deste doutorado.

Ingressei no Programa em Tocoginecologia como aluna regular de doutorado em 2018 com proposta de estudo translacional. Entre 2018 e 2022 cursei 10 disciplinas, coletei dados e colaborei na elaboração de trabalhos apresentados em eventos, sendo 1 pôster em evento nacional como autora principal (Anexo 1) e 3 internacionais como coautora em 2018

e 2019 (Anexo 2-4). Sou autora de 12 artigos, sendo um deles como autora principal (Anexo 5) e outros 11 como autora ou como membro do *Diamater Study Group/The Perinatal Diabetes Research Group* (Anexos 6-16), conforme orientado e recomendado pela Capes.

Como dentista, meu interesse maior foi verificar a repercussão da hiperglicemia materna na dentição da criança a curto e longo prazo. Inicialmente a proposta clínica do estudo translacional foi o estudo de coorte prospectiva, com objetivo de analisar a associação da hiperglicemia gestacional materna e os níveis sanguíneos maternos do cálcio, calcitonina, paratormônio, vitamina D, vitamina A, cortisol e fósforo, no defeito do desenvolvimento de esmalte na dentição decídua do descendente. Contudo, pela situação pandêmica, não foi possível realizar os exames bucais e coletar as imagens dos dentes das crianças. Utilizamos estratégias para obter de alguma forma as imagens, mas não obtivemos sucesso. Este estudo será continuado tão logo a situação sanitária permita as atividades presenciais.

Considerando a viabilidade e factibilidade de realizar análises em modelo animal com proposta na etapa *bed to bench* de intervenção (preventiva ou minimizadora) com exercícios, e com o intuito de verificar o impacto do exercício em ratas prenhes diabéticas no desenvolvimento do esmalte da ninhada, de forma translacional, esta tese foi desenvolvida. O estudo pré-clínico teve como objetivo realizar *screening* no esmalte da dentição da ninhada sob a ótica do Volume do Esmalte por meio da

Microtomografia (Micro CT) com realização dos escaneamentos (*Scans*), por meio do software de reconstrução NRecon e do software para as medidas CTan, da Microdureza e da Microscopia Eletrônica de Varredura (MEV).

Esta tese tem o propósito de ampliar e aprofundar o conhecimento sobre as repercussões maternas nos filhos de mães diabéticas e verificar o efeito da terapêutico com exercícios sobre o Defeito no Desenvolvimento do Esmalte (DDE). A proposta, é inédita e colabora na elucidação de fatores de risco para o desfecho e terapêutica do DDE de filhos de mães com DMG.

As aprovações do Comitê de Ética em Pesquisa (CEP) e Comissão de Ética no Uso de Animais (CEUA) referentes a esta Tese estão apresentadas nos Anexos 17 e 18, respectivamente.

Cumprir a etapa até aqui, foi processo de muitos desafios; conciliei as atividades da pós-graduação, consultório, filhos, família e pessoais, contudo foi de extrema gratificação e com valioso amadurecimento.

Seção 2

Contextualização

Avanços do conhecimento sobre a repercussão da exposição materna a hiperglicemia no desenvolvimento do esmalte dentário do descendente

Os Grupos de Pesquisa “Diabetes e Gravidez: Clínico e Experimental” e *Diamater Study Group* realizam pesquisas multicêntricas nacionais e internacionais relacionadas à gravidez complicada pelo diabetes; e de forma intrincada investiga repercussões e resultados maternos e fetais, perinatais e neonatais dos descendentes destas mulheres (1-8). Nos últimos quase 50 anos, os dois grupos juntos realizaram estudos clínicos e pré-clínicos. Importantes lacunas do conhecimento foram preenchidas ao longo destes anos. Estes resultados interferiram direta e profundamente em diferentes condutas e manejos obstétricos e clínicos com benefício à mulher, ao feto e ao recém-nascido (9-16). A internacionalização das pesquisas se evidencia com estudos pré-clínicos, que tomaram importante proporção com estudos sobre as repercussões a curto e longo prazo do diabetes no desenvolvimento fetal embrionário (17-24).

O *diabetes mellitus* gestacional (DMG) (25,26) está associado a um risco aumentado de complicações para o binômio materno-fetal durante a gravidez, bem como no período após o nascimento (27-29). A literatura tem reforçado que a hiperglicemia gestacional de intensidade variada, independentemente do diagnóstico de DMG, está envolvida na determinação do peso ao nascimento (30,31), macrosomia fetal,

hipoglicemia neonatal, hiperinsulinismo fetal (28), aumento dos índices de mortalidade fetal e perinatal (1,13), malformações congênitas (32), prematuridade e síndrome do desconforto respiratório (28). No entanto, os efeitos do diabetes materno no desenvolvimento dos dentes e os mecanismos subjacentes não foram completamente investigados (33).

Estudos epidemiológicos e de modelos animais mostraram que a hiperglicemia altera o dente em seu processo de desenvolvimento, e pode afetar a erupção e a mineralização do dente (34-36); e isto inclui o desenvolvimento do esmalte dentário.

Demanda bioquímica na gestação e repercussão no feto e neonato

Durante a gestação normoglicêmica, cerca de 250 gramas por dia de Cálcio (Ca) é transferido da mãe para o feto no 3º trimestre gestacional (37), sendo que os níveis de Ca são mais elevados no feto do que na mãe (38), e concomitante há aumento do funcionamento das glândulas paratireoides. Esse estado de hiperparatireoidismo relativo funciona como uma compensação homeostática para melhorar os estoques de Ca materno enviado para o feto (37).

O Ca tem intrincada relação com minerais, vitaminas e hormônios, demonstrando interdependência em cascata para sua manutenção e transferência. O paratormônio (PTH), hormônio secretado pela paratireoide (39), age nos osteoclastos que, por ação enzimática, reabsorvem a matriz

e solubilizam o Ca. O PTH, portanto, tem função primordial no *turnover* ósseo, isto é, no equilíbrio entre aposição e reabsorção, na manutenção do Ca em torno de 8,9 a 10 mg/dL e na absorção de Ca no intestino (39). O PTH mantém os níveis de Ca, aumentando a reabsorção intestinal e diminuindo a excreção urinária (40). A maioria do Ca "economizado" durante a gestação (cerca de 30 g) é transportada ativamente através da placenta e, principalmente no terceiro trimestre, depositada no esqueleto fetal.

A calcitonina é hormônio secretado pela tireoide, que apresenta como principal efeito participar do metabolismo do Ca. A secreção desse hormônio é controlada, basicamente, pela concentração de Ca, ou seja, os níveis de calcitonina aumentam ou diminuem diretamente proporcional ao aumento ou diminuição do Ca (37).

Como o PTH e a calcitonina não atravessam a barreira placentária, ao nascimento, pelos altos níveis de calcitonina, PTH e vitamina D neonatal dos fetos das mães diabéticas, ocorre hipocalcemia devido à interrupção da transferência materno-fetal de Ca (37,41).

A vitamina D, indiretamente, está relacionada na regulação do Ca extracelular e no fluxo deste mineral dentro das células beta. A secreção de insulina é processo mediado pelo Ca, portanto, alterações na regulação de seu fluxo podem ser prejudiciais à função secretória das células pancreáticas e também para ação propriamente dita da insulina. Dessa forma, tanto níveis inadequados de Ca, quanto níveis deficientes de

vitamina D, podem alterar o balanço entre os compartimentos intracelular e extracelular de Ca, afetando a liberação normal de insulina (42).

Além de repercutir em diferentes variáveis maternas e do neonato o Ca, calcitonina, PTH e vitamina D são indispensáveis para a saúde do organismo em toda sua complexidade, entre elas a estruturação dos ossos e dentes (43).

Desenvolvimento dentário na vida intrauterina e após o nascimento

O primeiro sinal morfológico da formação dentária ocorre durante a 4ª e 6ª semana intrauterina quando há o aparecimento da lâmina dentária primária (42,44). Todas as fases do desenvolvimento dentário se processarão a partir desta lâmina dentária primária (45).

Do ponto de vista embriológico o dente é órgão misto derivado de duas camadas, o ectoderma que formará a coroa, e o mesoderma que dará origem a dentina, cemento e polpa. Os dentes decíduos comumente denominados “de leite”, iniciam sua formação na 7ª semana de vida intrauterina e o esmalte de todos eles, está usualmente completo por volta do primeiro ano de vida. Todos os dentes decíduos estão geralmente erupcionados por volta de 24 e 36 meses e a estrutura radicular desses dentes está geralmente completa por volta dos três anos (42,44).

A odontogênese refere-se ao processo embriológico que dá origem ao germe dentário (42,45). A cronologia da formação do germe dental pode

ser observada na Figura 1.

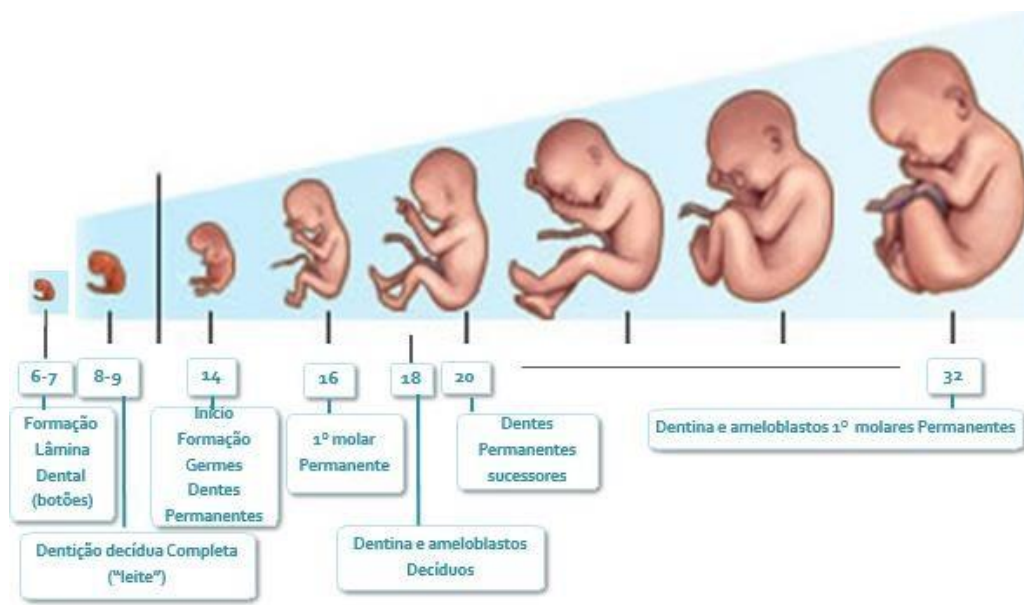


Figura 1: Desenvolvimento embrionário do germe dental. FONTE: adaptado de Bolland et al.,2008 (46).

A formação do esmalte e da dentina acontecem simultaneamente, e ambos os processos começam a partir de uma linha que formará a junção amelodentinária (47) e são descritos em fases: pré-secretora, fase secretora e fase de maturação (43). Na fase pré-secretora, ao lado da junção amelodentinária referente ao esmalte estão os ameloblastos que secretam a matriz orgânica. Os ameloblastos diferenciam-se, mudam de polaridade e desenvolvem extenso aparato de síntese proteica (43,47). Na fase secretora, pela expressão genética programada, à medida que secretam as proteínas da matriz extracelular, os ameloblastos se afastam do centro do órgão dentário (48); e nesta fase os cristais de hidroxapatita são depositados na matriz orgânica e formam placas finas alongadas. É

nesta frente de mineralização que se inicia a deposição mineral. Após o depósito da camada de esmalte aprismático, os ameloblastos secretores desenvolvem nas suas porções terminais processo em forma de cone (47). À medida que os ameloblastos secretam as proteínas do esmalte, os cristais de hidroxiapatita continuam a crescer em comprimento, mas crescem muito pouco em largura e em espessura (43,48), sendo que, comprimento final dos cristais depende de quanto tempo os ameloblastos secretam (47).

A quantidade de esmalte depositado em um dia varia de acordo com fatores sistêmicos e podem formar padrões distintos de linhas incrementais que são reproduzidas fielmente no esmalte de todos os dentes em formação num determinado momento (43).

Distúrbios durante a fase secretora da amelogênese resultarão em esmalte patologicamente fino ou hipoplásico, devido ao insuficiente alongamento dos cristais. Os distúrbios sistêmicos ocorridos nestes neonatos parecem atuar no mecanismo de armazenamento de minerais, que também parece afetar diretamente a mineralização dos tecidos dentários (47). Quando ocorre distúrbio no metabolismo do Ca, como por exemplo em gestação complicada pelo diabetes, podem predispor a alterações nos dentes decíduos (49). O Ca transferido da mãe para o feto é depositado principalmente sob a forma de hidroxiapatita e a maior taxa de acúmulo de Ca ocorre no período de deposição e concomitante com parte do período de calcificação dos dentes decíduos (50).

A mineralização ou calcificação dentária compreende a deposição de sais minerais, principalmente Ca e fósforo, sobre a matriz tecidual anteriormente formada. A deposição inicia-se sobre as pontas das cúspides e bordos incisais dos dentes, e continua com aposições concêntricas e sucessivas sobre os pequenos pontos de origem. Os dentes decíduos iniciam a sua calcificação entre a 14^a e 18^a semana intrauterina, com início nos incisivos centrais e término no 2º molar (51). Os ápices dos dentes decíduos fecham geralmente um ano após a erupção do dente e geralmente ocorre entre o ano e meio e três anos da criança (42).

Devido a sua composição, o esmalte é rígido e duro (52), e com presença de diversos componentes químicos no esmalte dentário, como: cálcio, água, dióxido de carbono, magnésio, sódio, potássio e fósforo (53,54).

Alterações no esmalte em relação às suas propriedades físicas, mecânicas e químicas podem afetar permanentemente a dentição decídua e permanente, dependendo do momento em que o dente ficou exposto ao agente agressor.

O esmalte dental é considerado importante marcador biológico sensível a uma série de eventos e situações, pois não contém células vivas e não pode reparar danos (55). Esta condição permite a definição da ocorrência do evento causador (55) e tem sido utilizada como um indicativo de padrões de saúde (56) e qualidade de vida, seja em aspectos gerais (57) ou em situações específicas (58).

Defeitos do desenvolvimento do Esmalte e *Diabetes Mellitus* Gestacional

Alterações recorrentes são os defeitos de desenvolvimento de esmalte (DDE) e geralmente refletem distúrbio ocorrido durante determinado período da amelogênese durante a gestação, sendo que, somente os dentes em formação no período em que se deu o distúrbio serão afetados. Portanto, com o conhecimento da cronologia de mineralização dentária, pode-se estimar em qual período a mineralização do esmalte foi afetada na gestação (47).

Vários índices clínicos têm sido usados para registrar os defeitos de desenvolvimento de esmalte, tais como: o índice Al-Alousi, o índice de defeitos de desenvolvimento de esmalte (Índice DDE), índice de fluorose da superfície dentária, o índice de defeitos de esmalte (IDE) (59), e o mais preconizado e utilizado cientificamente, o índice modificado de defeitos de desenvolvimento de esmalte (Índice DDE modificado) (60). Embora estes índices permitam a classificação dos DDE, não foram encontrados guias que correlacionassem os defeitos observados pelos índices com o período gestacional de seu desenvolvimento. Desta forma tornou-se importante desenvolver um diagrama conceitual com modulação dinâmica da programação DDE fetal, desde as fases iniciais da vida, durante o período gestacional, até o final da formação do período dentário.

Os estudos disponíveis enfocam o *diabetes mellitus* tipo II, e os

estudos em animais têm resultados heterogêneos, que são inconclusivos. Além disso, a maioria dos estudos originais sobre a prole da mãe com DMG não incluiu dados sobre DDE

A literatura demonstrou em estudo com animais que, além dos fatores genéticos, traumáticos e hormonais podem afetar a amelogênese e o processo de desenvolvimento ao afetar a erupção e a mineralização do dente e isto inclui o desenvolvimento do esmalte dentário (61,62). Vale ressaltar que a organização do esmalte, dentina, cemento e polpa dentária é comum tanto para os humanos quanto para os roedores (63).

Estudo recente de Pascon et. al (2019) (64) com objetivo de avaliar as consequências a longo prazo da exposição intrauterina à hiperglicemia em DDE nos descendentes entre 3-12 anos após o nascimento, concluiu que o DMG foi associado aos efeitos adversos do DDE na prole. Estes resultados estabeleceram as bases para novos estudos afim de determinar o impacto do DMG no risco de DDE a longo prazo. As oportunidades para melhorar os efeitos perinatais e os efeitos intergeracionais do DMG em longo prazo existem no nível de prevenção, triagem e tratamento do DMG e intervenções pós-natal de seus descendentes. É necessário avançar ainda mais para melhorar os resultados em longo prazo para os descendentes expostos ao meio materno alterado pela hiperglicemia, e a compreensão dos fatores de risco é essencial.

Identificar fatores associados às alterações do esmalte do descendente como também, desenvolver estudos com propostas de

intervenção, para que medidas preventivas e terapêuticas possam ser investigadas e futuramente adotadas de forma a minimizar as consequências negativas do DMG no desenvolvimento do esmalte dental.

Os estudos de acompanhamento em longo prazo são desafiadores, particularmente em relação ao custo e ao planejamento em longo prazo. Além disso, ao mesmo tempo em que se esforça para aperfeiçoar continuamente o controle glicêmico antes e durante a gravidez é, sem dúvida, importante melhorar os resultados a curto e longo prazo para as crianças de mães com DMG.

Impacto da COVID-19 na pesquisa clínica

Neste sentido, a proposta inicial desta tese incluiu três diferentes abordagens de pesquisa na mesma temática: 1) pesquisa clínica com o objetivo de analisar a repercussão da hiperglicemia gestacional materna no DDE da dentição decídua da criança e associar os níveis maternos das variáveis bioquímicas Ca, calcitonina, PTH e vitamina D com DDE; 2) desenvolver o Diagrama que facilitasse a correlação do período gestacional com a fase de formação de esmalte dental do descendente e 3) pesquisa experimental para avaliar a repercussão do exercício em ratas prenhes com diabetes moderado sobre o esmalte dos dentes da ninhada.

Inesperadamente a proposta clínica teve importante impacto negativo do Covid-19 no desenvolvimento da pesquisa e meios para superá-lo. Desafio que exigiu muitas adaptações e estratégias para

desenvolver pesquisa foi a pandemia do Covid-19 e seus efeitos na sociedade. O efeito foi devastador para humanidade e refletiu no desenvolvimento das pesquisas e no acesso aos serviços de saúde. Isso afetou substancialmente nossa pesquisa clínica. Os meios para superar este desafio na parte clínica da pesquisa incluíram as visitas virtuais e telemedicina, considerando que estas desempenham papel fundamental na realização de pesquisas e na prestação de cuidados aos pacientes na sociedade. Embora todo esforço tenha sido realizado pela equipe Diamater e pela doutoranda, o sucesso foi parcial. Cumprimos o compromisso do cronograma com a coleta de sangue dos grupos de mães DMG e não-DMG, contudo, não foi possível avaliar com rigor científico a dentição decídua da criança. Depois de discussão com a equipe, tomamos a decisão de aguardar o retorno da “normalidade” das condições sanitárias para retomarmos a proposta clínica inicial após a conclusão deste doutorado.

A presente Tese, inédita, integra de modo complementar o Projeto Temático Diamater, segue a linha da dissertação de mestrado desenvolvida pela doutoranda, agora com os objetivos a saber:

1) Projetar e desenvolver o diagrama com a cronologia do desenvolvimento do esmalte dentário (DED) para possibilitar a investigação dos fatores de risco intrauterinos e repercussões da programação fetal da DDE, com base no conceitual do *Diamater Study Group*.

2) Analisar o impacto do exercício de natação das ratas prenhes diabéticas no desenvolvimento do esmalte dentário da ninhada em sua morfologia, estrutura, e propriedades físicas e químicas.

Os resultados obtidos até o momento da Qualificação desta Tese, estão apresentados em 1 artigo, completo, e resultados preliminares do artigo 2.

1 - Fetal and postnatal programming of dental enamel development: the DED Diamater's conceptual diagram

2 – Impact of diabetic pregnant rat swimming exercise on enamel development, microhardness, and volume of offspring's teeth.

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Seção 3
Artigos

Artigo 1

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Fetal and postnatal programming of dental enamel development: the

DED Diamater's conceptual diagram

Tawana Pascon^{1‡}, MD, Angelica Mercia Pascon Barbosa^{1,2‡}, PhD, Fabiane Affonso Pinheiro^{1*}, PhD, Caroline Baldini Prudencio^{1*}, PhD, Carlos Izaias Sartorao-Filho^{1,3*}, PhD, Sthefanie Kenickel Nunes^{1*}, PhD, Gabriela Azevedo Garcia^{1,4*}, BSc, Rafael Guilen de Oliveira¹, MD, Vinicius Krieger Costa Nogueira^{5*}, PhD, Diego Giroto Bussaneli^{5●}, PhD, Marilza Vieira Cunha Rudge^{1●}, PhD, Diamater Study Group[#]

‡ TP and AMPB are the first authors of this work.

* These authors contributed equally to this work.

● MVCR and DGB are the last authors of this work.

[#]Diamater Study Group - Rudge MVC, Barbosa AMP, Calderon IMP, Souza FP, Lehana T, Graeff CFO, Rodrigues MRK, Felisbino SL, Barbosa WF, Bossolan G, Corrente JE, Nunes HRC, Abbade JF, Rossignoli PS, Pedroni CR, Járrmy-Di Bella ZI, Uchôa SMM, Duarte MAH, Mareco EA, Sakalem ME, Martinho NM, Bussaneli DG, Orlandi MIG, Pascon C, Dangiô TD, Rudge CVC, Piculo F, Prata GM, Avramidis RE, Carvalho CNF, Magyori ABM, Nava GTA, Caldeirão TCD, Shetty RHL, Reyes DRA, Alves FCB, Marcondes JPC, Takemoto MLS, Prudencio CB, Pinheiro FA, Sartorao-Filho CI, Quiroz SBCV, Pascon T, Nunes SK, Catinelli BB, Reis FVDS, Oliveira RG, Costa SMB, Menezes MO, Santos NJ, Enriquez EMA, Takano L, Carr AM, Garcia GA, Iamundo LF, Bassin HCM, Barbosa VP, Jacomin M, Silva AJB, Lourenço IO, Marostica de Sá J, Caruso IP, Rasmussen LT, Nogueira VKC, Ribeiro-Paes JT, França DCH, Bastos HVM, Heliodoro MLA, Kuroda MN, Carvalho HL, Mosele FC, Oliveira MA, Chaves MR, Laverde EA

¹São Paulo State University (UNESP), Botucatu Medical School, Department of Gynecology and Obstetrics, Botucatu, São Paulo, Brazil.

²São Paulo State University (UNESP), School of Philosophy and Sciences, Department of Physiotherapy and Occupational Therapy, Marília, São Paulo, Brazil.

³Educational Foundation of the Municipality of Assis (FEMA), Medical School, Assis, Brazil.

⁴São Paulo State University (UNESP), Faculty of Sciences, Bauru, São Paulo, Brazil.

⁵São Paulo State University (UNESP), School of Dentistry, Department of Morphology and Pediatric Dentistry, Araraquara, São Paulo, Brazil.

Corresponding Author: Marilza Vieira Cunha Rudge

Department of Gynecology and Obstetrics, Botucatu Medical School, UNESP

Distrito de Rubião Jr s/n 18.618-000, Botucatu, São Paulo, Brazil

Phone: +55 (14) 3880-1631, email: m.rudge@unesp.br

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Abstract

Background: During intrauterine development, the fetus is vulnerable to various factors, mainly affected by the maternal interface. Dental enamel is considered a sensitive biological marker for some conditions; it's a tissue with no living cells and cannot be repaired after damage. Therefore, the developmental defects of enamel (DDE) may suggest an association with systemic or local factors and fetal programming. Therefore, this study aimed to design and develop the diagram with the chronology of dental enamel development (DED) to enable investigation of the intrauterine risk factors and fetal programming repercussions of DDE based on the Diamater Study Group concept.

Methods and results: The “DED Diamater’s conceptual diagram” was developed considering the chronology of enamel formation in the deciduous and permanent dentition starting in intrauterine life. The teeth were differentiated by the group of teeth, type of dentition, and by percentage of dental enamel formation per surface of the tooth. The periods from the beginning until complete enamel formation were considered and related to the intrauterine, birth, and postnatal periods.

Conclusion: DED Diamater’s conceptual diagram was developed and made it possible to view the interrelation of the DED with intrauterine life to four years postnatal age. The diagram presents the group of teeth, type of dentition, tooth surface, and evolution of DED, indicating the onset according to gestational week and year of life that completes the formation of dental enamel. The tool may target preventive and therapeutic interventions in DDE that can positively interfere with children's oral health later in life and in future generations.

Keywords: fetus, fetal programming, dental enamel, pregnancy, birth, postpartum period, intrauterine

Introduction

Conditions during fetal and postnatal life significantly impact adult cardiovascular, metabolic, and neurological health. This is named fetal programming or the developmental origins of adult health and disease (DOHaD) hypothesis (1–3).

The DOHaD represents an important tool for long-term disease and death and an essential theoretical shift in bioarchitecture. The interpreted dental indicators provide a framework to explore the variables associated with frailty and hidden heterogeneous risk (4–6). However, understanding the pathophysiological mechanisms of how this phenomenon is activated and established requires further investigation.

Adult-onset diseases, including development defects of enamel (DDE), can originate from early life. Tooth enamel remains a significant public health challenge. A previous study highlighted major changes in dental enamel development (DED) of mothers with historic Gestational Diabetes Mellitus (GDM), suggesting fetal programming or DOHaD caused by a hyperglycemic intrauterine environment, including higher prevalence of DDE and a higher proportion of teeth with DDE in offspring of GDM mothers (7).

Moreover, a higher proportion of demarcated opacity and hypoplasia in deciduous dentition and a higher proportion of diffuse opacity in permanent dentition may be considered the profile of fetal programming of DDE caused

by GDM (7). Tooth enamel does not remodel once formed during early life and follows an incremental and rhythmical pattern of growth and development (8–10).

However, studies exploring the DOHaD must consider the developmental timing and frequency at which stressors are experienced, as well as cultural and environmental factors that may alleviate or exacerbate physiological constraints related to surviving early life stress (5).

There are several reports on tooth development, starting in intrauterine life (11), extending through gestation, and continuing in the postnatal period. Cellular and genetic molecular sequential networks control it at specific times and places (12,13), namely amelogenesis. Interestingly, the process mentioned above involved the secretion stage into the enamel protein matrix (14,15) and the maturation stage into the mineral's incorporation. Thus, the crystals increase in thickness and width, giving enamel physical and mechanical properties, such as porosity and hardness (16). However, it is required to clarify that the beginning of tooth enamel formation does not co-occur in all teeth; all primary teeth and two groups of permanent teeth begin enamel formation in intrauterine life (8,14,17-19).

Dental enamel protects the dental crown from masticatory efforts while protecting the dentin-pulp complex vitality against chemical, physical and thermal injuries (15). Thus, any disturbances that occur during the intrauterine environment can result in DDE (20). Additionally, the DDE analysis may be helpful for individual and populational diagnosis, suggesting

an association with systemic or local factors. This condition allows the definition of the occurrence of the causative event (21). It has been used as an indicator of health standards (22) and quality of life, either in general aspects (23) or in specific situations (24).

An extensive literature search for assessing a schematic figure of DED did not refer to any specific instrument for evaluating DED's fetal and postnatal conceptual framework. Therefore, due to the need for a fully developed tool, we created a new mechanism to demonstrate the conceptual framework of DED with each period of intrauterine life up to 4 years old. Therefore, this study aimed to design and develop a conceptual diagram with dynamic modulation of fetal DDE programming, from the early stages of life, during the gestational period until the end of the dental period formation.

This brief tool, wide sense, is too detailed with valuable insights for researchers and clinicians to enhance their ability to carry out a prenatal insult diagnosis to DOHaD. This establishes an investigation window of the possible risk factors and fetal programming repercussions to target preventive and therapeutic new interventions that can positively interfere with children's oral health and future generations (22).

Methods

Diamater is a scientific acronym of an interdisciplinary research committee (group) to study the interaction between pregnancy, GDM, and long-term

maternal and offspring outcomes. The difficulties we have found in the full description of DDE in offspring of GDM mothers, the available literature (8,19,25-30), and previous experience in the design and validity of a questionnaire (31) allow us to create the Diamater conceptual diagram of DED (7). This tool was elaborated to facilitate the visual interrelationship between DDE and the corresponding period to the DED stage.

There were three phases in developing the Diamater conceptual diagram of DED: item selection, item development, and content validity.

The first phase, item selection, the chronology enamel formation aspects were considered as follows. A preliminary version (α - conceptual diagram) was elaborated according to a period of life, including intrauterine life (gestational weeks), at birth and postnatal period; type of dentition; dental groups, and percentage of dental enamel formation per surface.

In item development's second phase, an internal analysis was performed inside the Diamater group, and an intermediate version (β - conceptual diagram) was elaborated. The content of this intermediate version includes detailed specifications on the item selected as a period of life, including intrauterine life (gestational weeks), at birth and the postnatal period (monthly in the first year and annually until the fourth year); type of dentition (deciduous and permanent); dental groups (incisors, canines, premolars, and molars); and percentage of dental enamel formation per surface (buccal, lingual, mesial, distal and occlusal or incisal).

In the third phase, content validity, the figure resultant from phase 2 was

sent to 3 Ph.D. level academic experts in dentistry by email, one with clinical expertise (RCLC), one with research expertise (DGB), and another layman on the theme (TNP). Each candidate item was assessed by clinical relevance, organization and understanding of things, and adequacy as an instrument for evaluating the influence of intrauterine.

The final version of the DED Diamater conceptual diagram was defined after review and suggestions from 3 external volunteer evaluators. The final version of the “DED Diamater conceptual diagram” was divided into three periods, with deciduous and permanent dentition, with all teeth considered, and the percentage of dental enamel formation at intrauterine life, birth, and postnatal period. The layout was designed to differentiate all dental characteristics and fetal and postnatal.

Results

The final version of the DED Diamater conceptual diagram is shown in Figure 1. The diagram presents three distinct blocks differentiated by time considering intrauterine life, from the 14th week, birth and postnatal period until the 4th year of life. Each period is differentiated by colors and the lines and columns are properly identified, which allows the visualization of the DED in each period of life. Teeth are shown by dental arch, tooth group, type of dentition and by surface. The start of dental enamel formation in intrauterine life, the percentage of dental enamel formation at birth to complete enamel formation after birth can be seen in the columns at each

stage described.

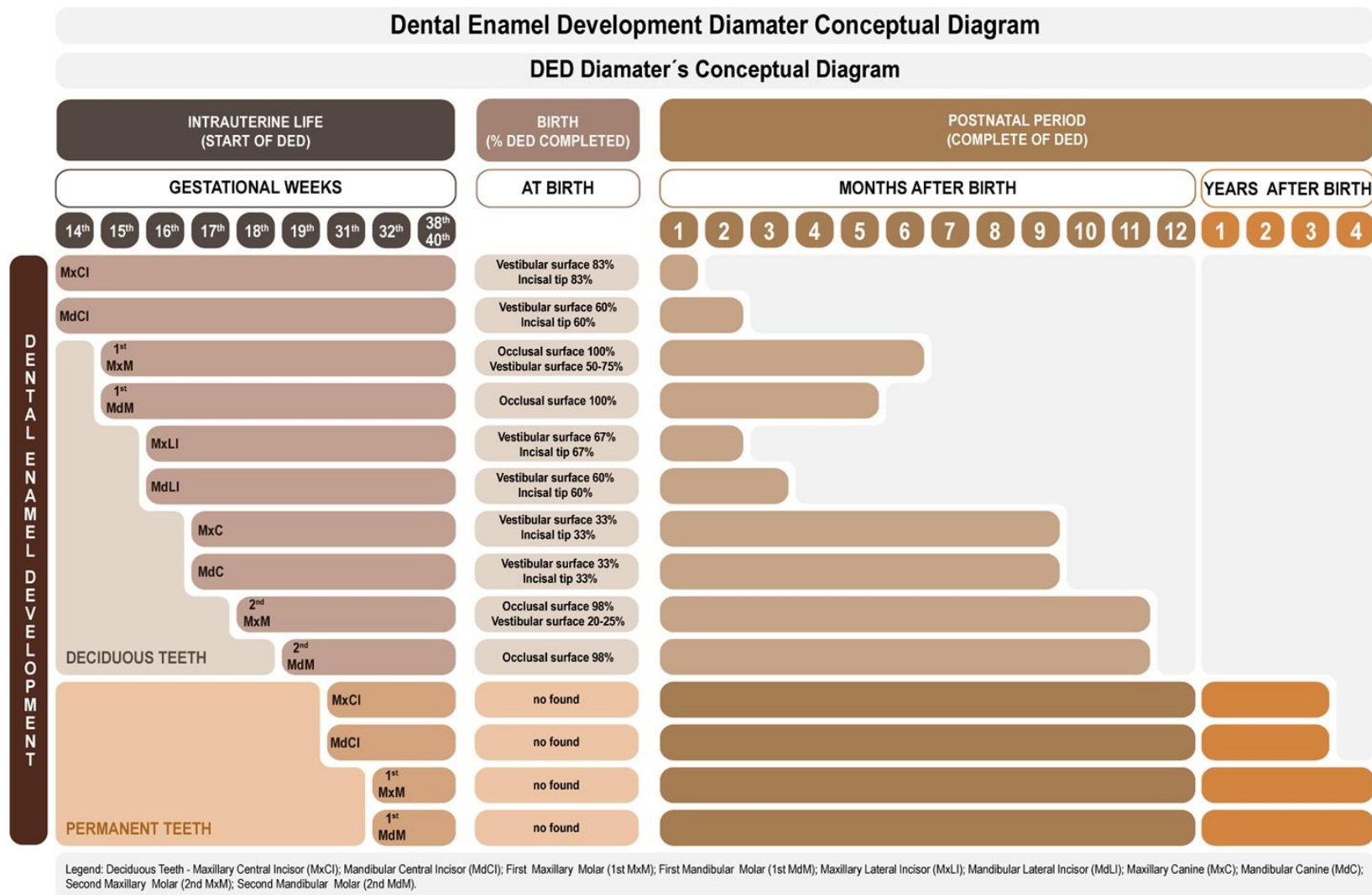


Figure 1: Dental Enamel Development Diamater's Conceptual Diagram.

Discussion

The interrelationship of the DED with intrauterine life to four years postnatal age presented in DED Diamater's conceptual diagram is a tool can be helpful for clinicians and researchers when investigating possible etiologic factors and in fetal programming directing the anamnesis to a specific stage of development since the clinical appearance of DDE hardly indicates its etiology, but can suggest when it occurred (22), establishing an investigation window that may to target preventive and therapeutic interventions futures that can positively interfere with children's oral health and in the future generation.

DDE can negatively affect oral health and dental esthetics (20), cause tooth sensitivity, modify (32), make anesthesia difficult (33), and risk factor for cavities and erosion in children's teeth (34), and this is a significant problem that requires public health care. The fetal origin of adult disease hypothesis postulates that a stressful in-utero environment can negatively affect fetal programming, potentially leading to chronic disease in later life. Factors known to impact fetal programming include the timing, intensity, duration, and nature of the external stressor during pregnancy. As such, dynamic modulation of fetal programming is heavily involved in shaping health throughout life, possibly by influencing metabolic parameters (35). This study presents the diagram as a basis for case studies or extensive investigations.

Study limitations

Limitations in our data should be considered. For example, the design presents only some of the present all the dents in its diagram, although it was not the objective, however future studies, which intend to identify risk factors for DDE in teeth that start enamel formation after birth, should be carried out.

Conclusion

In summary, the DED Diamater Conceptual Diagram as a clinical instrument establishes a dental profit from the 14th weeks of intrauterine life until the fourth year of postnatal life, displaying the gestational week of onset and the year of life that completes the formation of dental enamel. In this context, the DED Diamater conceptual diagram provides the ability to identify the prenatal and postnatal life-long exposure to DOHaD. Also, this tool needs to be placed on public health prevention strategies and therapeutic initiatives to positively interfere with children's oral health late life and the generations. Further the interesting for opportunity of a novel focus of research, and we hope to have provided not only a knowledge base but also some conceptual ideas for future studies.

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Artigo 2

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Impact of diabetic pregnant rat swimming exercise on enamel development, microhardness, and volume of offspring's teeth

T. Pascon^{1‡}, A.M.P. Barbosa^{1,2‡}, A.M. Carr¹, C.B. Prudencio^{1*}, B.B. Catinelli¹, V.P. Barbosa³, J.F. Floriano¹, G.A. Garcia^{1,5*}, R.G. Oliveira¹, M.A.H. Duarte⁶, L.C. Conti⁶, D.G. Bussaneli^{4•}, M.V.C. Rudge^{1•}, DiamaterStudy Group[#]

‡ TP and AMPB are first authors on this work.

* These authors contributed equally to this work.

• MVCR and DGB are last authors on this work.

[#]Diamater Study Group - Rudge MVC, Barbosa AMP, Calderon IMP, Souza FP, Lehana T, Graeff CFO, Rodrigues MRK, Felisbino SL, Barbosa WF, Bossolan G, Corrente JE, Nunes HRC, Abbade JF, Rossignoli PS, Pedroni CR, Járrmy-Di Bella ZI, Uchôa SMM, Duarte MAH, Mareco EA, Sakalem ME, Martinho NM, Bussaneli DG, Orlandi MIG, Pascon C, Dangiô TD, Rudge CVC, Piculo F, Prata GM, Avramidis RE, Carvalho CNF, Magyori ABM, Nava GTA, Caldeirão TCD, Shetty RHL, Reyes DRA, Alves FCB, Marcondes JPC, Takemoto MLS, Prudencio CB, Pinheiro FA, Sartorao Filho CI, Quiroz SBCV, Pascon T, Nunes SK, Catinelli BB, Reis FVDS, Oliveira RG, Costa SMB, Menezes MO, Santos NJ, Enriquez EMA, Takano L, Carr AM, Garcia GA, Iamundo LF, Bassin HCM, Barbosa VP, Jacomin M, Silva AJB, Lourenço IO, Marostica de Sá J, Caruso IP, Rasmussen LT, Nogueira VKC, Ribeiro-Paes JT, França DCH, Bastos HVM, Heliodoro MLA, Kuroda MN, Carvalho H.L., Mosele FC, Oliveira MA, Chaves MR, Laverde EA

¹São Paulo State University (UNESP), Botucatu Medical School, Department of Gynecology and Obstetrics, Botucatu, São Paulo, Brazil.

²São Paulo State University (UNESP), School of Philosophy and Sciences, Department of Physiotherapy and Occupational Therapy, Marília, São Paulo, Brazil.

³Marília University (UNIMAR), Medical School, Marília, São Paulo, Brazil.

⁴São Paulo State University (UNESP), School of Dentistry, Department of Morphology and Pediatric Dentistry, Araraquara, São Paulo, Brazil.

⁵São Paulo State University (UNESP), Faculty of Sciences, Bauru, São Paulo, Brazil.

⁶Department of Restorative Dentistry, Endodontics and Dental Materials, Bauru School of Dentistry, University of São Paulo (USP), Bauru, São Paulo, Brazil.

Corresponding Author:

M.V.C. Rudge

Department of Gynecology and Obstetrics, Botucatu Medical School (FMB – UNESP), Botucatu, SP, Brazil.

E-mail: m.rudge@unesp.br

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Abstract

Gestational diabetes mellitus was associated with adverse effects of the enamel developmental defects on offspring. The aim of this study was analyzed of Impact of pregnant rat swimming exercise on enamel development, microhardness, and volume of offspring's teeth. The pregnant rats were submitted to the swimming exercise protocol with moderate intensity exercise protocol. Offspring teeth was used: four incisors of each animal of each group were extracted. Teeth were evaluated in relation to enamel volume, microstructure, presence or absence of fractures, breaks at the "tip", roughness, fissures, scratches and dimples, and microhardness. The enamel volume showed no difference between the groups. The Dsed group showed a greater number of alterations in relation to fractures, fissures, roughness, enamel formation and the concomitant presence of diffuse opacity and as demarcated. Increase in Na concentration was observed in the NDex group compared with NDsed group, while no difference was found in the others concentrations. There was a decrease in microhardness in the Dsed and Dex groups compared to the NDex group. The Dex group showed a reduction in the deleterious effects observed in the Dsed group and similar results to the ND groups. The exercised diabetic and non-diabetic groups showed an appearance of fissure consolidation. The results support that there was no difference between groups in the analysis of enamel volume. Not performing swimming exercises and having diabetes during pregnancy had a greater negative impact in relation to the occurrence of fractures, fissures, changes in roughness, enamel formation and the concomitant presence of diffuse and demarcated opacity. Diabetes in general caused a decrease in enamel microhardness compared to the non-diabetic group and swimming exercise was able to reverse fissures.

Keywords: dental enamel, diabetic, pregnant rats, offspring, swimming

exercise.

Introduction

Maternal gestational diabetes mellitus (GDM) has a huge impact on fetal programming. Bearing in mind that children born from GDM women carry an increased risk for future illness such as developmental defects of enamel (DDE) (Kobayashi et al. 2018) between 3 and 12 years (Pascon et al. 2019), a translational study should be performed. Such study from bed to bench would deepen the knowledge not only about enamel development, microhardness, and volume in a hyperglycemic intrauterine condition but also about the control of such aberrant maternal environment through swimming exercise (SE) (Pascon et al. 2019; Shashikadze et al. 2021).

GDM has recently reached epidemic proportions worldwide and dysregulation of glucose metabolism is found in up to 15-25% of pregnancies (Aisa et al. 2019; Shashikadze et al. 2021).

There is a rapidly expanding literature to support the impact of *in utero* environment on adult health and the health of future generations (Hopkins and Cutfield 2011; Ruszała et al. 2022).

Additionally, the conditions during fetal or early postnatal development influence the individual's risk of developing non-communicable diseases in later life Developmental Origins of Health and Disease (DOHaD) paradigm (Yessoufou and Moutairou 2011). Previous study demonstrates that fetal exposure to GDM seems to stimulate a negative impact on dental enamel

(Tolomeu et al. 2022).

Further, compelling evidence support the idea that DDE negatively affect oral health and aesthetics, cause tooth sensitivity, malocclusion (Hong et al. 2009) resulting in anesthesia difficulties because of the hypersensitivity (Jälevik and Norén 2000). All these damaged regions may cause vulnerability to carious lesions, erosion, abrasion or even post-eruptive breakdowns in children's teeth (Seow et al. 2011) impacting in adult life.

As a consequence, interventions to optimize maternal, fetal, and child health are extremely important in order to prevent adult non-communicable diseases (NCD) (Hales and Gluckman 2001; Hanson et al. 2011).

Currently, hyperglycemia during pregnancy is thought to be one of the most important determinants of aberrant fetal development (Chen et al. 2017).

The environment in utero and in early neonatal life may induce a permanent response in the fetus and the newborn, leading to enhanced susceptibility to later diseases. These effects have been considered as transgenerational and are probably due to an epigenetic transmission mediated by the mother (Aerts and Van Assche 2006; Poston 2010).

Offspring of mothers with GDM have thinner enamel of incisors, with less volume and lower mineral density compared with offspring who have not been exposed prenatally to a hyperglycemic environment. These data have provided evidence linking maternal diabetes with enamel morphogenesis in offspring (Chen et al. 2017).

A new mechanism through which Apex1 (Apurinic/apyrimidinendonuclease) mediates the tooth development in maternal diabetes-affected offspring, and indicates a direct link between maternal diabetes and epigenetic silencing of genes such as *Oct4* and *Nanog*. The results of this study also suggest novel targets for strategies to prevent or treat tooth hypoplasia in addition to valuable information for tissue engineering to regenerate teeth (Chen et al. 2017).

These observations mold the fetal origin of adult disease (FOAD) hypothesis and subsequently the developmental origins of health and disease (DOHaD) framework whereby adverse events in early life are shown to impact health across the life course via the process now preferentially termed 'developmental programming' (Vickers 2022).

In clinical practice, the maternal diabetes and the consequent fetal hyperinsulinism should be avoided. Early detection of gestational diabetes and strict metabolic control are essential (Hod et al. 2015).

There is considerable evidence that exercise during a healthy pregnancy has positive effects on the mother and the fetus, as well as possibly in the forthcoming childhood. Exercise, as a supplement to routine care, significantly improves postprandial glycemic control, reducing fasting blood glucose for pregnant women with GDM and may even delay the need for insulin use (Colberg et al. 2013; Harrison et al. 2016; Taylor 2018). Undoubtedly, regular and scheduled exercise remains of vital importance

for the appropriate glucose control for pregnant women with GDM and it is proven to lead to improved pregnancy outcomes with fewer negative side effects (Halvatsiotis et al. 2020). Exercise, as an effective strategy to improve glucose homeostasis, can lower blood glucose levels and improve insulin sensitivity during pregnancy (Harrison et al. 2016).

The use of dietary interventions, exercise and medication, create a perfect environment of preventing and managing diabetes in a pregnancy. It proves to be required for better outcomes of pregnancy, and to ensure health of the mother and the newborn. In addition, there are increasing numbers of studies disclosing the beneficial role of exercise in humans, as it can prevent and even reverse the course to disease development (Halvatsiotis et al. 2020).

Either the American College of Obstetricians and Gynecologists (ACOG) or Canadian Guidelines for Physical Activity in Pregnancy agree that exercise during a healthy pregnancy has positive effects on the mother, fetus, as well as in childhood (Mottola et al. 2018).

SE interference with this “developmental program” in pregnancy of diabetic rats may be an interesting and innovative strategy. SE can be understood as a lifestyle change to restore the normality of the maternal environment. A rapidly expanding literature indicates that animal models are valuable tools for understanding the pathogenesis of developmental programming and developing therapeutic interventions for DOHaD-related diseases.

(Hopkins and Cutfield 2011; McMillen and Robinson 2005; McMullen and Mostyn 2009; Ojeda et al. 2008). Epidemiologic and animal model studies have shown that hyperglycemia during pregnancy alters the tooth development process, affecting tooth eruption and mineralization (Lal et al. 2008; Villarino et al. 2005; Yeh et al. 2012).

Maternal exercise reverses the detrimental effects of maternal high-fat feeding on offspring metabolism of female mice, vanishing the harmful effects of a maternal high-fat diet on glucose tolerance and hepatocyte glucose metabolism in female offspring (Stanford et al. 2017).

Morphological evaluation (Cremonini et al. 2021) turned toward the direction opposite to that previously stated. Our previous study confirmed the reversed diabetic-induced myopathy through SE intervention during the whole pregnancy in diabetic rats (Catinelli et al. 2022).

These results strongly suggest the influence of controlled maternal hyperglycemic environment in mothers by SE. However, none of these studies examined physical abnormalities of neonatal dental enamel of MHP mothers submitted to SE during the whole pregnancy. There is a growing need to identify if similar SE in rats' model of diabetic myopathy move backward the enamel changes in offspring. Emerging evidence indicates that animal models are valuable tools for understanding the pathogenesis of developmental programming and developing therapeutic interventions for DOHaD-related diseases (Hsu and Tain 2021; McMillen and Robinson

2005; McMullen and Mostyn 2009; Ojeda et al. 2008).

However, the effects of maternal diabetes on tooth development, and the associated influence of SE have not been thoroughly investigated. Therefore, this study aimed to evaluate the impact of diabetic pregnant rat swimming exercise on enamel development, microhardness, and volume of offspring's teeth.

Materials and Methods

This current project corresponds to the translational part of the Diamater Study, a cohort Thematic Project with the financial support of São Paulo Research Foundation (FAPESP 2016/01743-5), a state research foundation in Brazil. The Diamater cohort project has been developed to investigate biomarkers of the deleterious effects in women with GDM (Rudge et al. 2020). According to the requirement to develop innovative treatments for gestational diabetes maternal and offspring negative repercussions, the translational project with swimming was developed.

Ethics and Animals

All animal experiments were approved by the Institutional Animal Care and Use Committee, Faculty of Philosophy and Sciences, São Paulo State University (UNESP), in accordance with the Brazilian Council for Control of Animal Experimentation (CONCEA) (protocol number 007/2016). The study

is reported in accordance with the ARRIVE guidelines. Female and male Wistar rats obtained from ANILAB were housed in a facility with constant temperature ($22\pm 2^{\circ}\text{C}$) and humidity ($55\pm 5\%$) on a controlled 12h light–12h dark cycle with food and water ad libitum, in individual plastic cages during all experimental protocol. The experimental sequence is shown in Figure 1.

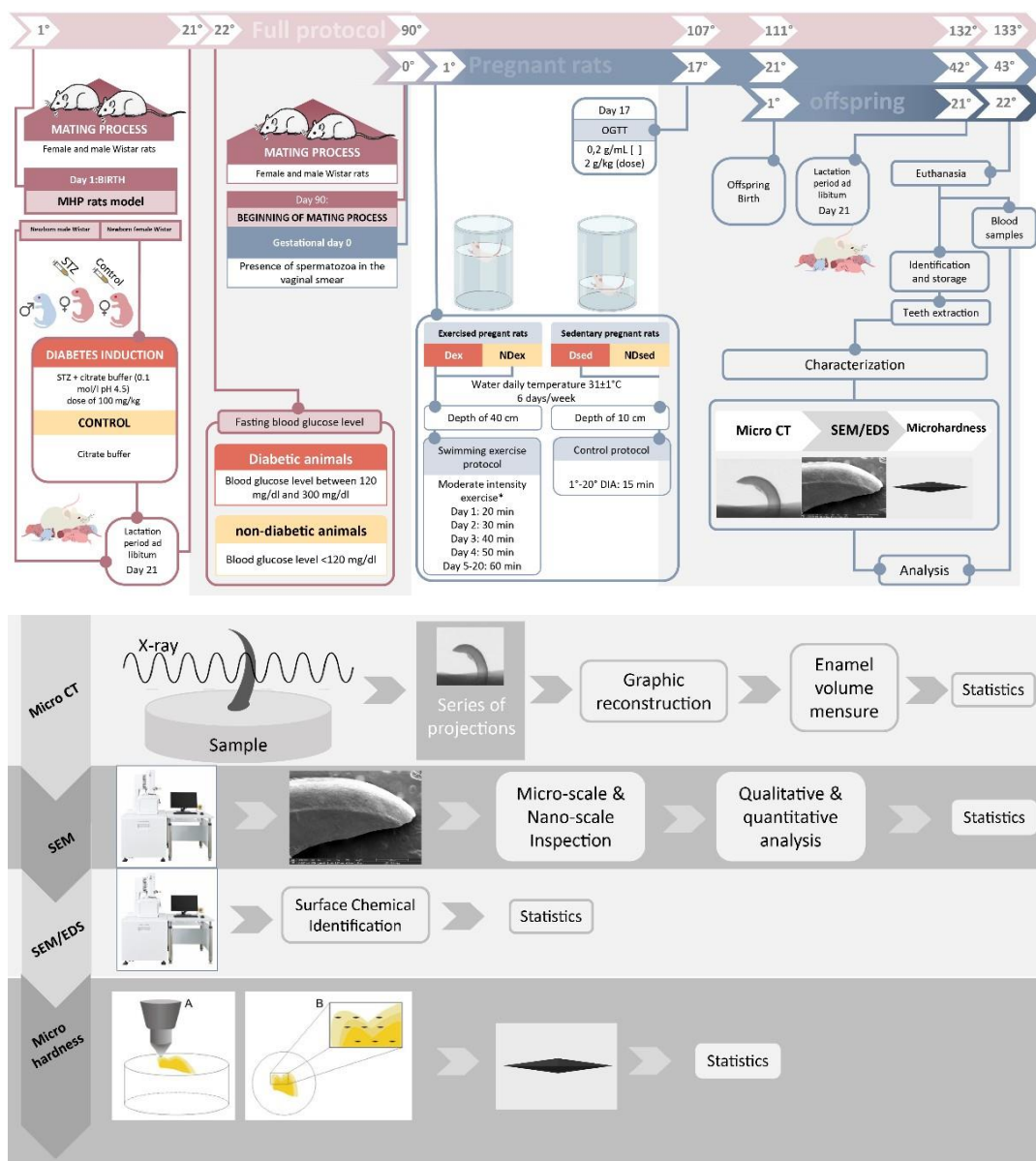


Figure 1 – Study design. Author: Diamater Study Group.

Generation of MHP rats' model

The MHP model is the same as previous reports (Damasceno et al. 2013; Damasceno et al. 2014; Marini et al. 2017; Vesentini et al. 2018; Vesentini et al. 2020). To induce MHP rats' model, newborn female Wistar received in the first day of life subcutaneously injection of streptozotocin - STZ (Sigma®) diluted in citrate buffer (0.1 mol/l pH 4.5) in a dose of 100 mg/kg. Non-diabetic rats received subcutaneous injection of citrate buffer (0.1 mol/l pH 4.5) (Damasceno et al. 2008). All female and male newborns rats were maintained with their mothers until the end of the lactation period (21 days). After this period, the mother rats were euthanized by sodium thiopental injection (Thiopentax® - 80 mg/kg). The female newborns were maintained until adulthood. Fasting blood glucose level was determined in adult life, and used for inclusion or exclusion criteria in the study. Diabetic animals should present blood glucose level between 120 mg/dl and 300 mg/dl, and non-diabetic animals blood glucose level <120 mg/dl (Nascimento 2014).

Mating process

At approximately day 90 of age, four diabetic and non-diabetic female rats were housed overnight with one adult male rat. The presence of spermatozoa in the vaginal smear was considered gestational day 0 (Volpato et al. 2009). After, rats were housed in individual cages until 21^o day of pregnancy.

Experimental Groups

On gestational day 0, female rats were randomly allocated into four experimental groups according to sedentary lifestyle or swimming exercise: Non-Diabetic Sedentary (NDsed) (n=12), Non-Diabetic Exercised (NDex) (n=10), Diabetic Sedentary (Dsed) (n=14) and Diabetic Exercised (Dex) (n=14). Dex is the study group and the other three (NDsed, NDex and Dsed) are control groups.

Swimming exercise protocol

The swimming exercise protocol was based on previous studies (Damasceno et al. 2004; Rudge and Calderon 2005; Rudge et al. 2005) and considered to be a moderate intensity exercise protocol (Damasceno et al. 2004). The exercised animals were exposed to water daily temperature $31\pm 1^{\circ}\text{C}$ for 6 days/week, from gestational day 0 until gestational day 20 on a pool at a depth of 40 cm at the water. The first training session started with 20 minutes, progressively increasing 10 minutes/day until 60 minutes. The sedentary rats were exposed to water daily for 15 minutes, at a depth of 10 cm at water temperature $31\pm 1^{\circ}\text{C}$ for 6 days/week, from gestational day 0 until gestational day 20, aiming not to promote physiological adaptations from exercise practice.

Oral Glucose Tolerance Test (OGTT)

On a gestational day 17, an OGTT was performed to confirm the glucose intolerance in diabetics (Catinelli et al. 2022; Damasceno et al. 2008). Fasting glycemia and at 10, 20, 30, 60 and 120 minutes after administration of an intragastric glucose solution (0.2 g/ml) in a dose of 2.0 g/kg were measured. Diabetes diagnosis was confirmed with two or more blood glucose measurements >140 mg/dl (Catinelli et al. 2022; de Campos et al. 2007).

Lactation period and sample collection

All newborn rats were maintained with their mothers until the end of the lactation period (21 days). After this period, the mother rats were euthanized by sodium thiopental injection (Thiopentax® - 80 mg/kg). The newborns were maintained until adulthood (21 days of age) and after this period the newborns were also euthanized as proceeded with their mothers. The offspring's heads and blood were collected and stored in – 4° C until teeth extraction process. It is important to highlight that other female pregnant rats and offspring samples were analyzed in different projects.

Teeth extraction and preparation

For this study the offspring teeth was used: four incisors of each animal of each group were extracted (upper and lower incisors) by Luer alveolotome.

All samples were identified and stored in formaldehyde 10%.

Enamel volume evaluation

In order to evaluate enamel volume, all teeth were scanned in a micro-CT system (SkyScan 1174; Bruker-microCT, Kontich, Belgium). The scanning parameters were set at 50 kV x-ray source at 800 uA, 1.00° rotation step, 360° of rotation, a voxel size of 22.9 - um and resolution 1.024 x 1.304. Then, images were reconstructed with the NRecon software (NRecon v.1.6.3, Bruker-microCT) and enamel volume was calculated with CTan software (Bruker-microCT). Teeth that fractured during extraction were excluded from the study.

Morphological and chemistry evaluation

Sample morphology were characterized by a field-emission Scanning Electron Microscope (SEM), model JEOL JSM-7500F using a PC-SEM v 2,1,0,3 software coupled with secondary electron detector, backscattered electron detector and Energy Dispersive Spectroscopy – EDS (Thermo Scientific, Ultra Dry model, with NSS 2.3 software). The EDS was used to identify the surface chemical. Three images were obtained for each sample. The presence of fractures and roughness was evaluated.

Microhardness evaluation

For each animal of all groups, four incisors were evaluated. Ground surfaces of teeth were polished using grit 600 and 1200 Waterproof Paper Silicon Carbide. Based on Vieira et al. (2005), teeth microhardness was tested using a Vicker indenter (10 g per 10 seconds). The average value for all enamel indentations was also calculated (average enamel). The location of the indentations was based on a previous study that performed microhardness in mice teeth (DenBesten et al. 2001). The calculation of the microhardness on each point was done by using the equation below:

$$HV = \frac{1.854P}{d_1^2}$$

where P is applied load (kg), d1 is diameter of the indentation (mm), and HV is Vicker's microhardness (kg/mm²). Extensive attrition and enamel loss in the tooth samples from the 100 ppm A/J strain mice prevented microhardness testing; therefore, those enamel samples were not analyzed in this study.

Statistical analysis

GraphPad Prism® v.8.0 software was used to analyze the data. Data are expressed as mean $\pm$ standard deviation (SD). Comparisons of measurements among groups were performed by two-way ANOVA followed by Tukey's multiple comparison tests. For all statistical comparisons, $p < 0.05$ was considered statistically significant.

Results

The experimental design included three offspring control groups, i.e., NDsed (n=12), NDex (n=10), and Dsed (n=14) allowing the analysis of the SE intervention in offspring dental enamel in the authentic study offspring group: the Dex (n=14). A total of 200 teeth were analyzed (100 upper and 100 lower). The total number of teeth analyzed considering the Ndsed group was 48 (24 upper and 24 lower), 40 in the NDex group (20 upper and 20 lower), 56 in the Dsed group (28 upper and 28 lower) and 56 in the Dex group (28 upper and 28 lower). For each analysis, different amounts of teeth were used considering their needs. The OGTT showed that Dsed and Dex groups

had two or more blood glucose measurements > 140 mg/dl. These data confirm that the SE practice during pregnancy did not promote changes in blood glucose levels.

Enamel volume evaluation

For enamel volume analysis, a total of 146 teeth were analyzed (Dex=37; Dsed=36; Ndex=36; Ndsed=37). The data showed no difference between groups in the enamel volume (Fig.2 A). However, a greater enamel volume was demonstrated in the lower teeth when compared to the upper teeth in the four groups. The NDsed group demonstrated that the enamel volume of the lower teeth was greater compared to the upper teeth of the NDex, Dsed and Dex groups. The enamel volume on the lower teeth was greater in the NDex group compared to the upper teeth in the Dsed and Dex groups. In the Dsed group, the enamel volume of the lower teeth was greater compared to the upper teeth in the Dex group. The comparisons showed significance $p<0.0001$ (Fig. 2 B).

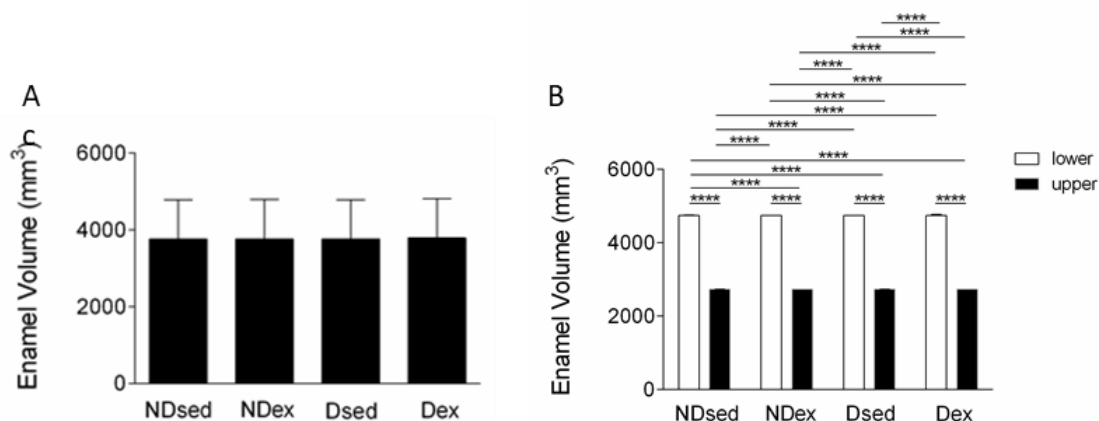


Figure 2 – Enamel volume analysis between groups (A) and between upper and lower teeth (B). * $p < 0,05$; ** $p < 0,01$; *** $p < 0,001$; **** $p < 0,0001$.

Morphological

Morphological analysis was described based on the characteristics of NDsed group. This is a study with unpublished analyzes in which the qualitative method is not well established in the literature. Therefore, prior to the comparative analyses, three experienced specialists in the field, blindly identified and listed all the characteristics of the enamel of all teeth in the four groups and built the base of the variables to be scored.

Subsequently, two of the examiners individually evaluated each group and recorded all the characteristics seen in each of the teeth in each group. When there was disagreement, the third examiner, blindly, defined the divergent characteristic. After completing the analysis of all groups, a double check was performed to ensure that the launch was absolutely correct. For morphological analysis of the enamel, five upper and five lower teeth of each group were analyzed, adding up to 40 teeth in total.

Incisal edge fracture (lower magnification).

The NDsed group showed fractures, while the NDex group showed a small fracture in the upper and lower teeth. However, a large fracture was observed in the upper teeth and a medium fracture in the lower teeth in the Dsed group, while medium fractures were observed in upper teeth and small fractures in lower teeth in the Dex group. The Dsed group presented fractures with a higher degree of severity in relation to the other groups and the Dex presented a similar result in lower teeth when compared with the NDex (Fig 3).

Fissures (lower magnification)

The NDsed group showed no fissures in the teeth. Both exercised groups (NDex and Dex) showed no fissures in the upper teeth. The NDex group presented fissures with horizontal, linear and narrow characteristics in upper and lower teeth, and linear characteristics in the lower teeth. The Dsed showed vertical, bifurcated, linear, narrow fissures in upper and lower teeth. The Dex group showed linear, narrow and non-linear fissures only in the lower teeth. It was observed that Dex presented fissures similar to the NDex group, while the Dsed group had the highest number of fissures and the only one with fissures in upper teeth (Fig 3).

Fissures (higher magnification)

The images at higher magnification showed horizontal, linear, parallel fissures in upper and lower teeth in NDsed group. In the NDex group, the absence of fissures in the upper teeth and horizontal, linear, strict and consolidated fissures in the lower teeth were demonstrated. The Dsed group showed fissures with all the characteristics analyzed in the upper and lower teeth. The Dex group showed horizontal, vertical, linear, parallel and narrow fissures in upper and lower teeth. It was observed that the NDex group was the only one that presented absence of fractures in upper teeth. The NDsed group showed fissures in upper and lower teeth with all the characteristics analyzed. Comparing Dex with the other groups, there was similarity with the NDsed group (Fig 3).

Enamel formation (lower magnification)

The Dsed, NDex and Dex groups showed characteristics of a brushed appearance in upper and lower teeth. NDsed and Dex showed a mosaic and fish scale appearance. Dsed was the only group that presented only a disorganized appearance in enamel formation, while the Dex, NDex and NDsed groups showed similar characteristics regarding enamel formation (Fig 3).

Enamel formation (higher magnification)

All groups had a uniform appearance over most of the tooth enamel area. The NDsed, Dsed and Dex groups showed irregular enamel formation in some parts of the enamel, which was not observed in the Dsed group (Fig. 3).

Roughness (higher magnification)

The NDsed and Dex groups showed mild to moderate roughness, the NDex group showed moderate roughness and the Dsed group showed roughness between smooth and light. These data showed that the Dsed group was the only one to present a smooth surface. Dex group showed similarity with the NDsed and NDex groups (Fig. 3).

Deposition (higher magnification)

Regarding enamel deposition, it was observed that all groups had uniform deposition on upper and lower teeth. The NDex and Dex groups presented enamel overlapping in some areas, showing similarity between exercised groups (Fig. 3).

Cavitation (higher magnification)

There was no enamel cavitation in all groups (Fig. 3).

Coloring (higher magnification)

The uniform aspect was observed in both diabetic groups (Dsed and Dex). The Dsed group showed demarcated and diffuse opacity in upper and lower teeth and Dex group showed diffuse opacity in upper and lower teeth (Fig. 3).

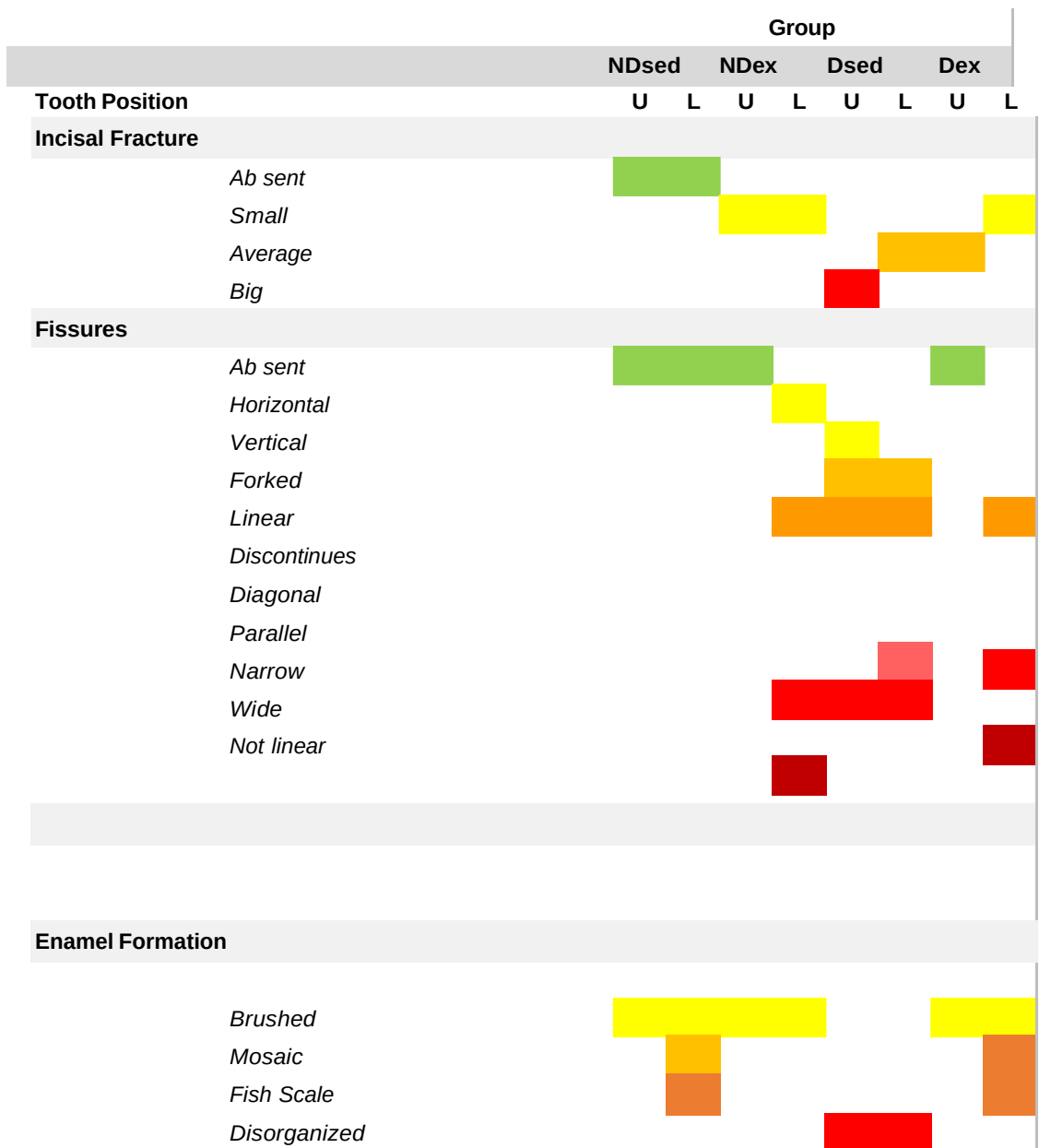


Figure 3 – Enamel morphological characteristics of all teeth in the four groups and built the base of the variables to be scored. Legend: Non-Diabetic Sedentary (NDsed) (n=12), Non-Diabetic Exercised (NDsed) (n=10), Diabetic Sedentary (Dsed) (n=14) and Diabetic Exercised (Dex) (n=14). Upper (U) and Lower (L).

Figure 3 - to be continued.



Figure 3 – Enamel morphological characteristics of all teeth in the four groups and built the base of the variables to be scored. Legend: Non-Diabetic Sedentary (NDsed) (n=12), Non-Diabetic Exercised (NDsed) (n=10), Diabetic Sedentary (Dsed) (n=14) and Diabetic Exercised (Dex) (n=14). Upper (U) and Lower (L).

Chemistry evaluation

To the chemistry evaluation 53 teeth were used (Dex = 12; Dsed = 16; NDex = 12; NDsed = 12). The results of the comparison between the surface chemical identification groups are showed in the Figure 3. The following biochemical variables were identified on the enamel surface: calcium (Ca), oxygen (O), potassium (P), carbon (C), iron (Fe), sodium (Na), magnesium (Mg) and aluminum (Al).

A significant increase in Na concentration was observed in the NDex group compared to the NDsed group and an increase in Na concentration in the Dex group compared to the Dsed and NDsed groups. The biochemical variables Cl, C, O, Ca, P, Mg, Fe and Al showed no differences between groups (Fig.4).

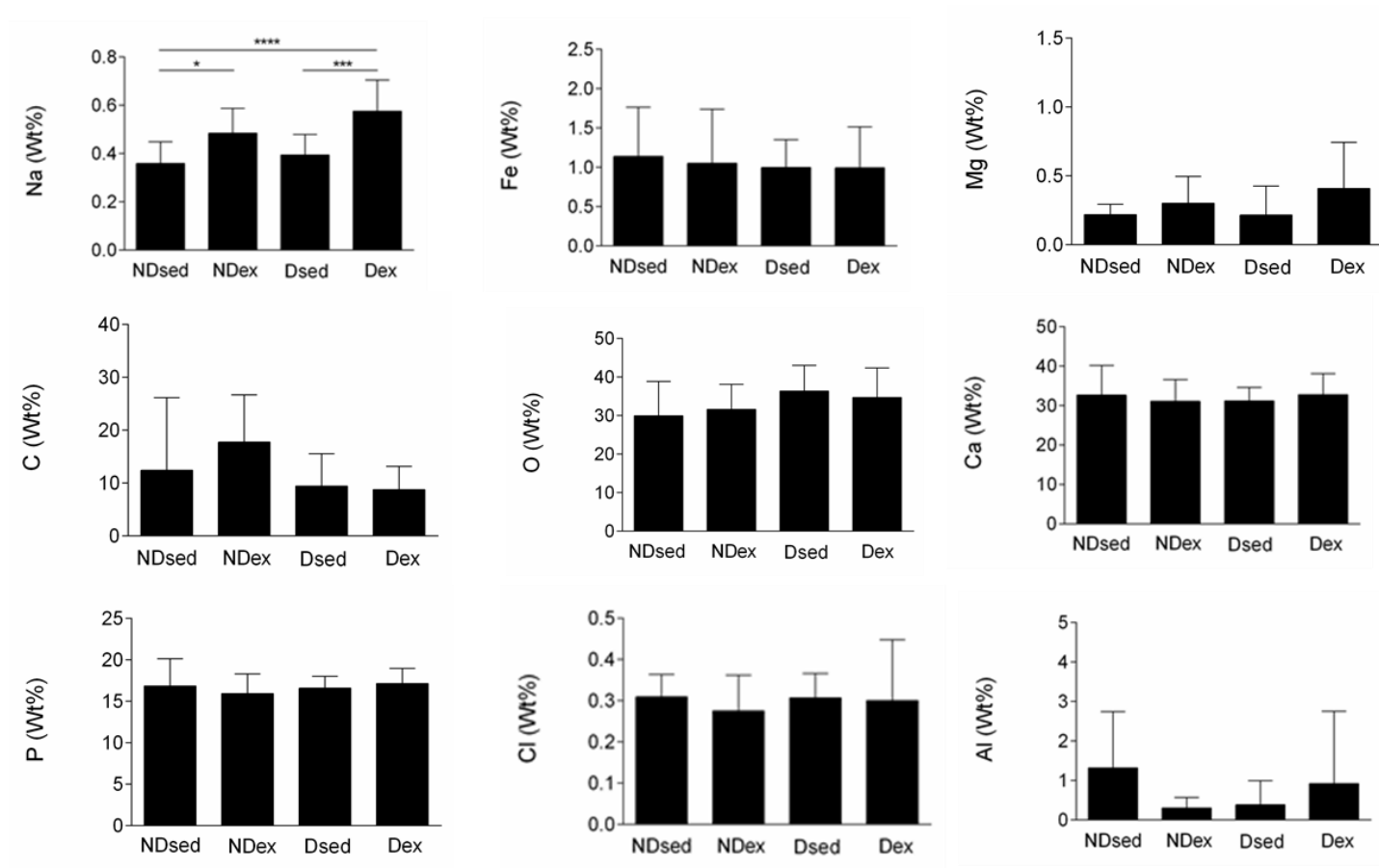


Figure 4 – Analysis of enamel surface chemical between groups. Legend: Non-Diabetic Sedentary (NDsed), Non-Diabetic Exercised (NDex), Diabetic Sedentary (Dsed) and Diabetic Exercised (Dex). * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$.

The data showed no difference between groups in the Cl, C, O, Ca, P and Al concentrations in the upper and lower teeth. The Dex group showed increased concentration of Mg in the upper teeth in the compared to the lower teeth in the Dsed group and an increased concentration of Fe in the upper teeth compared to the lower teeth in the NDsed group. Also, there was an increase in Na concentration in the lower and upper teeth in the Dex group compared to the lower and upper teeth in the NDsed group. In addition, increased Na concentration was observed in the lower teeth in the Dex group compared to the lower teeth in the Dsed group and an increase in the concentration of Na in the lower teeth in the Dex group compared to the upper teeth in the Dsed group (Fig 5).

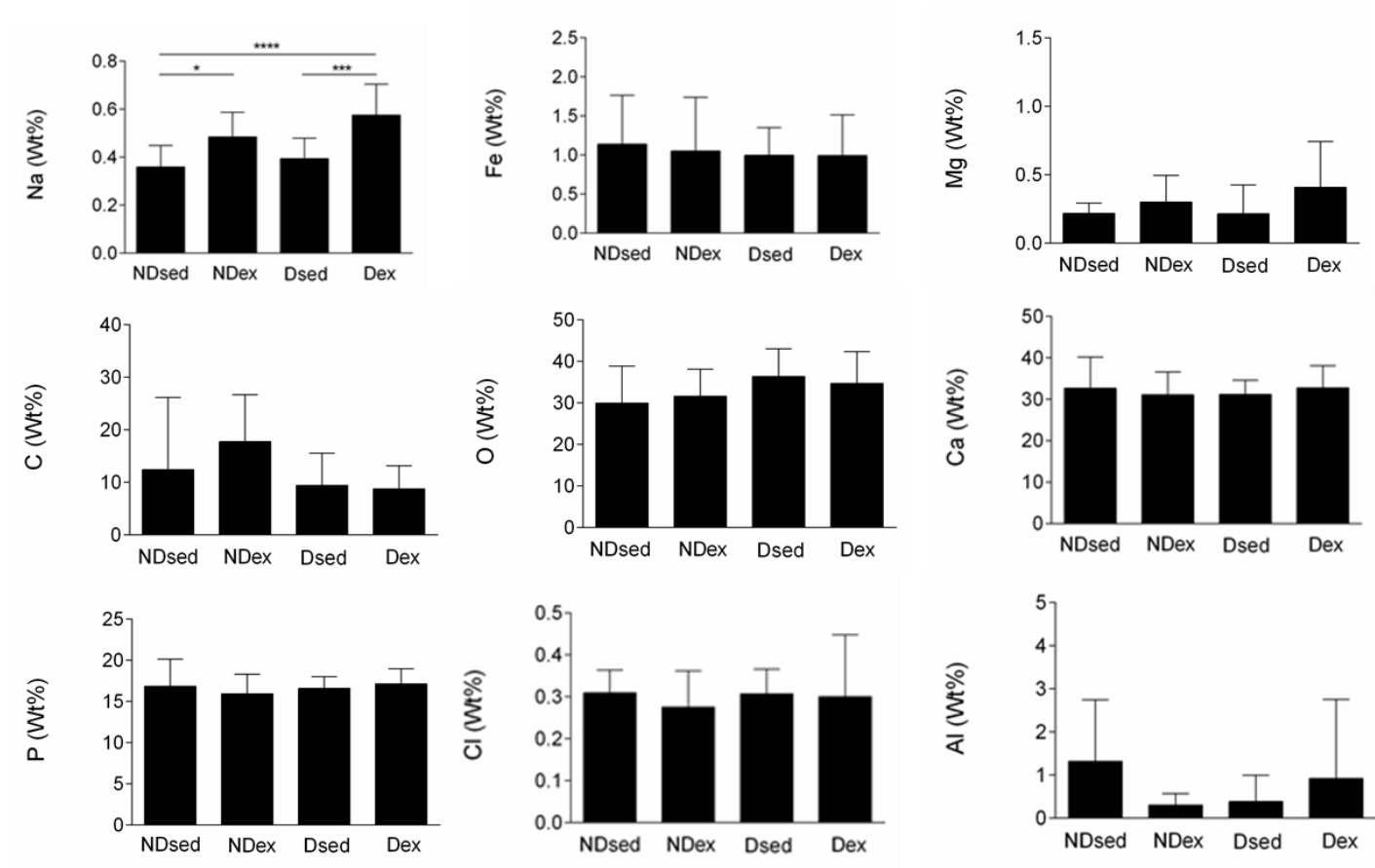


Figure 5 – Analysis of enamel surface chemical between the arches (upper and lower teeth). Legend: Non-Diabetic Sedentary (NDsed), Non-Diabetic Exercised (NDsed), Diabetic Sedentary (Dsed) and Diabetic Exercised (Dex). * $p < 0,05$; ** $p < 0,01$; *** $p < 0,001$; **** $p < 0,0001$.

Microhardness evaluation

To the microhardness evaluation a total of 76 teeth were analyzed (Dex = 16; Dsed = 20; NDex = 20; NDsed = 20). The results showed a decrease in microhardness in the Dsed and Dex groups compared to the NDex group ($p < 0.01$ and $p < 0.05$, respectively) and a decrease in microhardness in the upper teeth of the Dsed and Dex groups compared to the upper teeth in the NDex group ($p < 0.05$). Regarding the microhardness of the lower teeth, there was no difference between groups (Fig. 6).

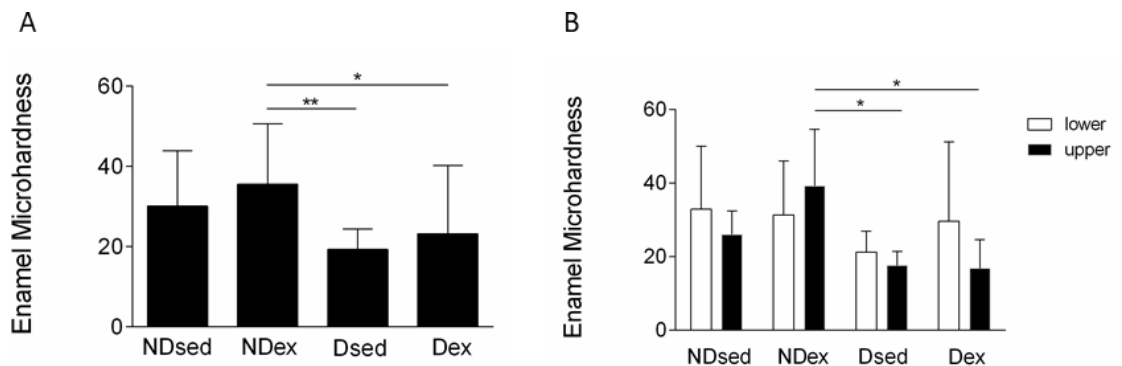


Figure 6 – Comparison of enamel microhardness between groups (A) and between upper and lower teeth within a group and between groups (B). Legend: Non-Diabetic Sedentary (NDsed), Non-Diabetic Exercised (NDex), Diabetic Sedentary (Dsed) and Diabetic Exercised (Dex). * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$.

Discussion

The enamel volume showed no difference between the groups. However, a greater enamel volume was demonstrated in the lower teeth when compared with the upper teeth in the four groups. The Dsed group showed a greater number of alterations in relation to fractures, fissures, roughness, enamel formation and the concomitant presence of diffuse opacity and as demarcated.

A significant increase in Na concentration was observed in the NDex group compared with NDsed group, while no difference was found in the Mg, Fe, C, O, Ca, P, Cl e Al concentrations between groups. There was a decrease in microhardness in the Dsed and Dex groups compared to the NDex group. The Dex group showed a reduction in the deleterious effects observed in the Dsed group and similar results to the ND groups (NDsed and NDex). The exercised diabetic and non-diabetic groups showed an appearance of fissure consolidation.

Our study on the distribution of elements in the enamel of offspring of the diabetic rats' teeth may also be important for the understanding of the enamel mineralization processes, determining how elements change the properties of the materials, and exploring the relationship between binomial pregnancy and offspring.

The findings in this study demonstrate the negative impact of Diabetes in pregnant rats on dental enamel, especially the group without intervention

with swimming exercise. In-vivo study demonstrated too that Diabetes negatively affects tooth enamel and dentine microhardness and enamel was influenced much more negatively and rapidly compared with dentine in diabetic groups (Saghiri et al., 2022).

The enamel organ exercises intense cellular control over increases of Ca and P concentration during the formation and maturation stages of amelogenesis (SASAKI, DEBARI, HIGASHI, 1984) and what draws our attention considering the heavy role of Ca in the development of enamel, is the fact that Ca did not present different values between the group with and without swimming exercises or with and without diabetes.

Systematic review with the aimed to verify the association between GDM and Developmental Defects of Enamel (DDE) demonstrated that children of mothers who had GDM presented a greater likelihood of general DDE (OR = 2.72; 95% CI: 1.66-4.44), MIH (OR = 3.14; 95% CI: 1.20-8.25) and hypoplasia (OR = 2.17; 95% CI: 1.36-3.46). No association was found between HPSM and GDM (OR = 0.60; 95% CI: 0.17-2.20) (Tolomeu et. al, 2022). Although the present study was carried out in an animal model, the negative impact of diabetes on dental enamel is increasingly clear and confirmed, although the swimming exercise reduced or reversed several alterations that were observed in the diabetic group without swimming exercise.

Regarding the results in which the marked opacity and diffuse opacity were

observed in the Dsed group and the diffuse opacity observed in the Dex group, we consider that the swimming exercise was able to avoid the occurrence of the marked opacity. Study in animal model showed that the enamel and dentine thickness were significantly reduced (hypoplasia) and there was a significant reduction of the rate of dentine mineral apposition and formation, that confirm the detrimental influence on the formation of enamel and dentine in the early growth stage (Arhatari, Andrewartha, White, 2014). It is noteworthy that understanding morphological changes and mineral content of tooth hard tissues may influence dental treatment.

Pits and fissures are important structures inside teeth that only have a width of about 50–200 micrometers. The deep enfolding of the enamel in these pits and fissures causes difficulties in maintaining proper oral hygiene which makes it common to develop dental caries in these areas (González-Sotelo et al., 2022; Arhatari, Andrewartha, White, 2014; Cochrane, et al., 2010). The imaging of dental pits and fissures provides obvious visual evidence to help in promotion of oral health and to improve personal tooth care in preventive treatment protocols (Arhatari, Andrewartha, White, 2014), thus, our findings suggest that swimming exercise in rats Diabetic pregnant women or rats without diabetes were able to reverse fissures once we observed healing or filling of the fissures.

The main limitations in this study were reduced access to micro-CT equipment (highly demanded because of the wide range of materials

analysis it can perform, since it is able to modulate the amount of X-rays), time spent for scanning, reconstruction and analysis of the images, among others; and though the number of samples per group was reduced, the micro-CT scanning per sample and study stage was comprehensive. However, additional studies are recommended in relation to enamel alterations in offspring of the diabetic's rats.

Clinical significance: Even within the limitation of this animal model study, the results observed in this enamel analysis indicate that offspring of gestational diabetic mothers may be more susceptible to dental caries and teeth size discrepancies. The enamel alteration compromises appropriate stress transfer from enamel to dentin, contributing to the odds of fracture and cavitation. Enamel fracture over a dentin lesion may happen as one of the normal pathways to caries progression and may act as a confounding factor during clinical diagnostic decisions (Neves et al., 2015). Dental problems should be handled carefully and their diabetic condition monitoring is of prime importance, especially during early stage of tooth development (Abbassy et al., 2015). Studies with randomized clinical trials with swimming exercises are strongly indicated to confirm, in the human model, the positive effect of exercise observed in the animal model.

Conclusion

The results support that there was no difference between groups in the

analysis of enamel volume. Not performing swimming exercises and having diabetes during pregnancy had a greater negative impact in relation to the occurrence of fractures, fissures, changes in roughness, enamel formation and the concomitant presence of diffuse and demarcated opacity. Diabetes in general caused a decrease in enamel microhardness compared to the non-diabetic group and swimming exercise was able to reverse fissures.

Acknowledgments

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Data availability

The datasets used and/or analyzed during the present study are available from the corresponding author on request.

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Seção 5

Perspectivas Acadêmicas e Científicas

Pensar nas perspectivas nesta fase final do doutorado, me fez rever todo o processo vivido até aqui e me estimulou ainda mais a cumprir esta etapa que é tão tensa e que exige dedicação, às vezes, até além da nossa capacidade. A formação no mestrado e doutorado, fortaleceram e ampliaram a perspectiva de iniciar a carreira docente e de pesquisadora.

Para a continuidade da busca do conhecimento e na colaboração para melhorar a assistência à criança pela ótica da gestação, a proposta é permanecer como colaboradora do *Diamater Study Group* e desenvolver o Pós-Doutorado na mesma linha de pesquisa desta trajetória e continuar colaborando com o Grupo.

A carreira docente sempre esteve como meta a ser atingida e na mesma semana que conclui a escrita desta tese para defesa, tive a grata surpresa de ser contratada como docente na UNIMAR, instituição que cursei a graduação, para ministrar disciplina em odontopediatria.

Espero poder levar o conhecimento e principalmente beneficiar diretamente as crianças, ao longo desta nova etapa da minha vida.

Seção 6
Diameter Study Group

Pesquisadores Nacionais

Profa. Emérita Marilza Vieira Cunha Rudge
Profa. Titular Iracema de Mattos Paranhos Calderon
Profa. Dra. Angélica Mérica Pascon Barbosa
Profa. Dra. Fátima Pereira de Souza
Prof. Titular Carlos Frederico de Oliveira Graeff
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Prof. Dr. Edson Assunção Mareco
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Profa. Titular Maeli Dal Pai
Profa. Dra. Selma Maria Michelin Matheus
Profa. Dra. Ana Karina Cristiuma De Luca
Profa. Dra. Daisy Maria Fávero Salvadori
Prof. Dr. Rondinelli Donizetti Herculano
Prof. Dr. Spencer Luiz Marques Payao
Prof. Dr. Heber Lombardi de Carvalho
Prof. Dr. João Tadeu Ribeiro Paes

Pesquisadores Internacionais

PhD Adonis Hijas
PhD Luis Sobrevia Luarte
PhD Bary Berghmans
PhD Rob de Bie
PhD Costanza Emanuelli
PhD Baerbel Junginger
PhD Antonio Musàro
PhD Lehana Thabane

Apoio à Pesquisa

Rita de Cássia Athanázio
Cinthia Scolástico Cecílio

Pesquisadores Associados

PhD Raghavendra Hallur Lakshmana Shetty
Dra. Fernanda Cristina Bérghamo Alves
Dra. Maíra Libertad Soligo Takemoto
Dra. Juliana Ferreira Floriano
Dra. Fernanda Piculo
Dra. Gabriela Marini Prata
Dra. Cibebe Vieira Cunha Rudge
Dra. Talita Costa Domingues
Dra. Caroline Baldini Prudencio
Dra. Fabiane Affonso Pinheiro
Dr. David Rafael Abreu Reyes
Dr. João Paulo de Castro Marcondes
Dr. Fabio Joly Campos
Dr. Ícaro Putinhon Caruso
Dr. Lucas Trevizani Rasmussen
Dr. Vinícius Krieger Costa Nogueira
Dr. Carlos Isaías Sartorão Filho
Dr. Nilton José dos Santo
Dra. Sthefanie Kenickel Nunes
Dr. Guilherme Thomaz de Aquino Nava
Ma. Carolina Neiva Frota de Carvalho
Ma. Sofia Beatriz Carolina Vega Quiroz
Ma. Tawana Pascon
Ma. Bruna Bologna Catinelli
Ma. Fabiana Vieira Duarte de Souza Reis
Me. Rafael Guilen de Oliveira
Ma. Sarah Maria Barneze Costa
Ma. Isabella Otenio de Lourenço
Ma. Jéssica Maróstica de Sá
Ma. Raissa Escandiusi Avramidis
Ma. Adriely Bittencourt Morgenstern Magyori
Me. Luiz Takano
Ma. Danielle Cristina Honório França
Ma. Aline Medolago Carr
Ma. Francielle Caroline Mosele
Eusebio Mario Amador Enriquez
José Vitor Freitas Melo (*in memoriam*)
Gabriela Azevedo Garcia
Luana Fávaro Iamundo
Henrique Caetano Mingoranci Bassin
Carolina Pascon Marques
Michele Jacomin
Ana Julia Bimbatti Silva
Maiara Isabele Gonçalves Orlandi
Tatiana Daniele Dangiό
Vitória Pascon Barbosa
Melissa Nahomi Kuroda
Isabela Cristina Soares
Henrique Vieira Miranda Bastos
Edith Anais Laverde
Mathias Rennó Chaves
Michele Aline de Oliveira





Seção 7

Finanças

Anexo 1

Apresentação de trabalho em evento nacional



Digitalizado com CamScanner

Anexo 2
Apresentação de trabalho em evento internacional



Digitalizado com CamScanner

Anexo 3
Apresentação de trabalho em evento internacional



Digitalizado com CamScanner

Anexo 4
Apresentação de trabalho em evento internacional



Anexo 5

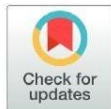
RESEARCH ARTICLE

Prenatal exposure to gestational diabetes mellitus increases developmental defects in the enamel of offspring

Tawana Pascon¹, Angélica M. P. Barbosa^{1,2*}, Rita C. L. Cordeiro³, Diego G. Bussanelli³, Caroline B. Prudencio¹, Sthefanie K. Nunes¹, Fabiane A. Pinheiro¹, Grasiela Bossolan¹, Leandro G. Oliveira¹, Iracema M. P. Calderon¹, Gabriela Marini⁴, Marilza V. C. Rudge¹

1 Department of Gynecology and Obstetrics, São Paulo State University (UNESP), Botucatu Medical School, Botucatu, São Paulo, Brazil, **2** Department of Physiotherapy and Occupational Therapy, São Paulo State University (UNESP), School of Philosophy and Sciences, Marília, São Paulo, Brazil, **3** Department of Pediatric Dentistry and Orthodontics, São Paulo State University (UNESP), Araraquara School of Dentistry, Araraquara, São Paulo, Brazil, **4** Health Sciences Center, University of the Sacred Heart (USC), Bauru, São Paulo, Brazil

* angelicapascon@gmail.com



Abstract

Background and objective

Gestational diabetes mellitus (GDM) is associated with short- and long-term maternal and perinatal repercussions. Our objective was to evaluate the long-term consequences of intra-uterine exposure to hyperglycemia on Developmental Defects of Enamel (DDE) in offspring.

Results

Overall, 50 children of women with GDM and 250 children of normoglycemic women participated, the latter serving as controls. Children were examined at the age between 3 and 12 years. In addition to physical examination, two independent observers examined and rated photographs to identify specific types of DDE in a blinded fashion. Among offspring of mothers with GDM, rates of DDE (all types combined) and hypoplasia (specific type) were significantly higher ($p < 0.001$, $p = 0.04$), in comparison to offspring of normoglycemic mothers. Considering only the affected teeth (1060 in GDM category; 5499 in controls), rates of DDE (all types combined) were significantly higher for total teeth ($p < 0.001$) and deciduous teeth ($p < 0.001$), but not permanent teeth. In specific types of DDE involving deciduous teeth, rates of demarcate opacity were significantly higher ($p < 0.001$; canine and 2nd mandibular molars) and hypoplasia ($p < 0.001$; 2nd maxillary molars and 2nd mandibular molars). In permanent teeth, the rate of diffuse opacity in association with GDM was significantly higher ($p < 0.001$; maxillary central incisors and 1st maxillary molars).

Conclusion

GDM was associated with the adverse effects of DDE on offspring. This study lays the foundation for future studies to determine the impact of GDM on long-term risk of DDE.

OPEN ACCESS

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Anexo 6

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CLINICAL ARTICLE

Neurourology
Urodynamics ICS  WILEY

Pelvic floor muscle dysfunction at 3D transperineal ultrasound in maternal exposure to gestational diabetes mellitus: A prospective cohort study during pregnancy

Fabiane A. Pinheiro¹ | Carlos I. Sartorão Filho^{1,2}  | Caroline B. Prudencio¹  |
Sthefanie K. Nunes¹  | Tawana Pascon¹ | Ragavendra L. S. Hallur^{1,3} |
Luis Takano^{1,2} | Eusébio M. A. Enriquez¹ | Bruna B. Catinelli¹ |
Aline M. Carr¹ | Baerbel Junginger⁴  | Marilza V. C. Rudge¹  |
Angélica M. P. Barbosa^{1,5}  | The Diamater Study Group

¹Postgraduate Program on Tocogynecology, Botucatu Medical School (FMB), São Paulo State University (UNESP), Botucatu, São Paulo State, Brazil

²Department of Medical Health, Medical School, Fundação Educacional do Município de Assis (FEMA), Assis Municipality Educational Foundation (FEMA), Assis, São Paulo State, Brazil

³Center for Biotechnology, Pravara Institute of Medical Sciences (Deemed to be University), Loni, Maharashtra, India

⁴Department of Gynecology, Pelvic Floor Center Charité, Charité University Hospital, Berlin, Germany

⁵Department of Physiotherapy and Occupational Therapy, School of Philosophy and Sciences, São Paulo State University (UNESP), Marília, São Paulo State, Brazil

Correspondence

Angélica M. P. Barbosa, PT, PhD,
Department of Physiotherapy and
Occupational Therapy, School of
Philosophy and Sciences, São Paulo State
University (UNESP), Marília, SP, Brazil.
Email: angelicapascon@gmail.com

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Estado de São Paulo,
Grant/Award Numbers: FAPESP/2016/
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Abstract

Aim: This study aimed to assess, for the first time, the dynamic morphometry of pelvic floor muscles (PFM) using three-dimensional transperineal ultrasound (3D-TPUS) and its progression at two-time points of gestation between women with and without gestational diabetes mellitus (GDM), and whether the PFM dysfunction is connected to GDM.

Methods: The study comprised 83 consecutive pregnant women with ($n = 38$) and without ($n = 45$) GDM screened at 24–30 and 38–40 weeks of gestation. 3D-TPUS and a mobility test were used to quantify PFM dynamic morphometry during maximum contraction and the Valsalva maneuver.

Results: When compared to the control group, GDM women had no significant variations in all levator hiatal dimensions at 24–30 weeks of gestation. Meanwhile, women with GDM experienced an increase in levator hiatal area (LHa) ($p < 0.000$) during PFM contraction and enlargement in LHa ($p < 0.001$) during Valsalva maneuver ($p = 0.010$) at 38–40 weeks of gestation.

Fabiane A. Pinheiro, Carlos I. Sartorão Filho, Marilza V. C. Rudge, and Angélica M. P. Barbosa contributed equally to this study.

Fabiane A. Pinheiro and Carlos I. Sartorão Filho are co-first authors.

Caroline B. Prudencio, Sthefanie K. Nunes, Tawana Pascon, Ragavendra L. S. Hallur, Luis Takano, Eusébio M. A. Enriquez, Bruna B. Catinelli, Aline M. Carr, and Baerbel Junginger contributed equally to this study.

Marilza V. C. Rudge and Angélica M. P. Barbosa are last authors of this study.

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Anexo 7

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BioMedical Engineering
OnLine

RESEARCH

Open Access



Viability of ex-vivo myography as a diagnostic tool for rectus abdominis muscle electrical activity collected at Cesarean section within a diamater cohort study

David R. A. Reyes^{1†}, Angelica M. P. Barbosa^{1,2†}, Floriano F. Juliana¹, Quiroz B. C. V. Sofia¹, Sarah M. B. Costa¹, Raghavendra L. S. Hallur^{1,3}, Eusebio M. A. Enriquez¹, Rafael G. Oliveira¹, Patricia de Souza Rossignoli², Cristiane Rodrigues Pedroni², Fernanda C. B. Alves¹, Gabriela A. Garcia¹, Joelcio F. Abbade¹, Carolina N. F. Carvalho¹, Luis Sobrevia^{1,4,5,6,7}, Marilza V. C. Rudge^{1*†}, Iracema I. M. P. Calderon^{1†} and The Diamater Study Group

[†]David R. A. Reyes and Angelica M. P. Barbosa are the first authors

[†]Marilza V. C. Rudge and Iracema I. M. P. Calderon are the last

Abstract

Background: *Ex-vivo* myography enables the assessment of muscle electrical activity response. This study explored the viability of determining the physiological responses

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The Diamater Study Group

M. V. C. Rudge, A. M. P. Barbosa, I. M. P. Calderon, F. P. Souza, T. Lehana, C. F. O. Graeff, C. G. Magalhães, R. A. A. Costa, S. A. M. Lima, M. R. K. Rodrigues, S. L. Felisbino, W. F. Barbosa, F. J. Campos, G. Bossolan, J. E. Corrente, H. R. C. Nunes, J. F. Abbade, P. S. Rossignoli, C. R. Pedroni, Á. N. Atallah, Z. I. Jármay-Di Bella, S. M. M. Uchôa, M. A. H. Duarte, E. A. Mareco, M. E. Sakalem, N. M. Martinho, D. G. Bussaneli, M. I. G. Orlandi, C. Pascon, T. D. Dangiô, C. V. C. Rudge, F. Piculo, G. M. Prata, R. E. Avramidis, C. N. F. Carvalho, A. B. M. Magyori, G. T. A. Nava, T. C. D. Caldeirão, R. H. L. Shetty, D. R. A. Reyes, F. C. B. Alves, J. P. C. Marcondes, M. L. S. Takemoto, C. B. Prudencio, F. A. Pinheiro, C. I. Sartorao Filho, S. B. C. V. Quiroz, T. Pascon, S. K. Nunes, B. B. Catinelli, F. V. D. S. Reis, R. G. Oliveira, S. M. B. Costa, M. O. Menezes, N. J. Santos, E. M. A. Enriquez, L. Takano, A. M. Carr, G. A. Garcia, L. F. Iamundo, H. C. M. Bassin, V. P. Barbosa, M. Jacomin, A. J. B. Silva, I. O. Lourenço, J. Marosticadesá, I. P. Caruso, L. T. Rasmussen, V. K. C. Nogueira, J. T. Ribeiro-Paes, D. C. H. França, H. V. M. Bastos, M. L. A. Heliodoro, M. N. Kuroda, H. L. Carvalho.

ing pregnancy [1–5]. As a striated muscle, the main focus of research has been on contractile muscle cells [6]. RAM has synergistic contractile activity with the pelvic floor



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scientific reports



OPEN

Reversal of diabetic-induced myopathy by swimming exercise in pregnant rats: a translational intervention study

Bruna B. Catinelli^{1,37}, Patrícia S. Rossignoli^{2,37}, Juliana F. Floriano¹, Aline M. Carr¹, Rafael G. de Oliveira¹, Nilton J. dos Santos^{3,4}, Lara C. C. Úbeda⁵, Maria Angélica Spadella⁶, Raghavendra L. S. Hallur^{1,7}, Luis Sobrevia^{1,8,9,10,11}, Sérgio L. Felisbino³, Iracema M. P. Calderon¹, Angélica M. P. Barbosa^{1,2,38}, Marilza V. C. Rudge^{1,38} & The Diamater Study Group^{1*}

Gestational diabetes mellitus (GDM) plus rectus abdominis muscle (RAM) myopathy predicts long-term urinary incontinence (UI). Atrophic and stiff RAM are characteristics of diabetes-induced myopathy (DiM) in pregnant rats. This study aimed to determine whether swimming exercise (SE) has a therapeutic effect in mild hyperglycemic pregnant rats model. We hypothesized that SE training might help to reverse RAM DiM. Mild hyperglycemic pregnant rats model was obtained by a unique subcutaneous injection of 100 mg/kg streptozotocin (diabetic group) or citrate buffer (non-diabetic group) on the first day of life in Wistar female newborns. At 90 days of life, the rats are mated and randomly allocated to remain sedentary or subjected to a SE protocol. The SE protocol started at gestational day 0 and consisted of 60 min/day for 6 days/week in a period of 20 days in a swim tunnel. On day 21, rats were sacrificed, and RAM was collected and studied by picosirius red, immunohistochemistry, and transmission electron microscopy. The SE protocol increased the fiber area and diameter, and the slow-twitch and fast-twitch fiber area and diameter in the diabetic

The Diamater Study Group

M. V. C. Rudge¹, A. M. P. Barbosa^{1,2}, I. M. P. Calderon¹, L. Sobrevia^{1,8,9,10,11}, F. P. Souza¹², B. Berghmans¹³, L. Thabane¹⁴, B. Junginger¹⁵, C. F. O. Graeff¹⁶, C. G. Magalhães¹⁷, R. A. Costa¹, S. A. M. Lima¹⁸, M. R. Kron-Rodrigues¹⁹, S. L. Felisbino³, W. Barbosa²⁰, F. J. Campos¹⁷, G. Bossolan¹, J. E. Corrente²¹, H. R. C. Nunes¹⁷, J. Abbade¹⁷, P. S. Rossignoli², C. R. Pedroni², A. N. Atallah²², Z. I. K. J. Di Bella²³, S. M. M. Uchoa²⁴, M. A. H. Duarte²⁵, E. A. Mareco²⁶, M. E. Sakalem²⁷, N. Martinho^{28,29}, L. S. R. Hallur^{1,7}, D. R. A. Reyes¹, F. C. B. Alves¹, J. P. C. Marcondes¹, C. B. Prudencio¹, F. A. Pinheiro¹, C. I. Sartorão Filho¹, S. B. C. V. Quiroz¹, T. Pascon¹, S. K. Nunes¹, B. B. Catinelli¹, F. V. D. S. Reis¹, R. G. Oliveira¹, S. Barneze¹, E. M. A. Enriquez¹, L. Takano¹, A. M. Carr¹, A. B. M. Magyori¹, L. F. Iamundo¹, C. N. F. Carvalho¹, M. Jacomin¹, R. E. Avramidis¹, A. J. B. Silva², M. I. G. Orlandi¹, T. D. Dangi¹, H. C. M. Bassin¹, M. L. S. Takemoto¹, T. D. Caldeirão¹, N. J. Santos^{3,4}, I. O. Lourenço¹², J. Marostica de Sá¹², I. P. Caruso¹², L. T. Rasmussen³⁰, G. A. Garcia¹, G. T. A. Nava³¹, C. P. Marques³², D. G. Bussaneli³³, V. K. C. Nogueira³³, C. V. C. Rudge³², F. Picolo³⁴, G. M. Prata³⁵ & V. P. Barbosa³⁶

Netherlands. ³⁷These authors contributed equally: Bruna B. Catinelli and Patrícia S. Rossignoli. ³⁸These authors jointly supervised this work: Angélica M. P. Barbosa and Marilza V. C. Rudge. *A list of authors and their affiliations appears at the end of the paper. ³⁵email: marilzarudge@gmail.com

Anexo 9



International Journal of
Molecular Sciences



Article

Transcriptomic Profiling of Rectus Abdominis Muscle in Women with Gestational Diabetes-Induced Myopathy: Characterization of Pathophysiology and Potential Muscle Biomarkers of Pregnancy-Specific Urinary Incontinence

Fernanda Cristina Bergamo Alves ¹, Rafael Guilen de Oliveira ¹, David Rafael Abreu Reyes ¹, Gabriela Azevedo Garcia ², Juliana Ferreira Floriano ¹, Raghavendra Hallur Lakshmana Shetty ^{1,3}, Edson Assunção Mareco ⁴, Maeli Dal-Pai-Silva ⁵, Spencer Luiz Marques Payão ⁶, Fátima Pereira de Souza ⁷, Steven S. Witkin ^{8,9}, Luis Sobrevia ^{1,10,11,12,13,14}, Angélica Mércia Pascon Barbosa ^{1,15,†}, Marilza Vieira Cunha Rudge ^{1,*,†} and Diamater Study Group [‡]

¹ Department of Gynecology and Obstetrics, Botucatu Medical School (FMB), São Paulo State University (UNESP), Botucatu 18618-687, Brazil

² Botucatu Medical School, Botucatu, São Paulo State University (UNESP), Botucatu 18618-687, Brazil



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have reported that pelvic floor muscle disorders are due to a GDM-induced-myopathy (GDiM) of the pelvic floor muscle and rectus abdominis muscle (RAM). We now describe the transcriptome profiling of the RAM obtained by Cesarean section from GDM and non-GDM women with and without pregnancy-specific urinary incontinence (PSUI). We identified 650 genes in total, and the differentially expressed genes were defined by comparing three control groups to the GDM with PSUI group (GDiM). Enrichment analysis showed that GDM with PSUI was associated with decreased gene expression related to muscle structure and muscle protein synthesis, the reduced ability of muscle fibers to ameliorate muscle damage, and the altered the maintenance and generation of energy through glycogenesis. Potential genetic muscle biomarkers were validated by RT-PCR, and their relationship to the pathophysiology of the disease was verified. These findings help elucidate the

Anexo 10



OPEN ACCESS

EDITED BY
Eric Siegel,
University of Arkansas for Medical
Sciences, United States

REVIEWED BY
Luís Mercado,
University of Arkansas for Medical
Sciences, United States
Diana Escalona-Vargas,
University of Arkansas for Medical
Sciences, United States

*CORRESPONDENCE
Angélica Mércia Pascon Barbosa
angelicapascon@gmail.com

[†]These authors share first authorship

[‡]These authors share last authorship

[§]These authors have contributed
equally to this work

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Gestational diabetes is associated with alteration on pelvic floor muscle activation pattern during pregnancy and postpartum: Prospective cohort using electromyography assessment

Caroline Baldini Prudencio^{1†}, Sthefanie Kenickel Nunes^{1†},
Fabiane Affonso Pinheiro^{1§}, Carlos Izaías Sartório Filho^{1§},
Guilherme Thomaz de Aquino Nava^{2§},
Sauro Emerick Salomoni³, Cristiane Rodrigues Pedroni^{4§},
Marilza Vieira Cunha Rudge^{1†}, Angélica Mércia Pascon Barbosa^{1,4*†}
and Diamater Study Group

¹São Paulo State University (Unesp), Postgraduate Program on Tocogynecology, Botucatu Medical School, Botucatu, Brazil, ²Human Development and Technologies, Institute of Biosciences, São Paulo State University (UNESP), Rio Claro, Brazil, ³Independent Researcher, Hobart, TAS, Australia, ⁴São Paulo State University (Unesp), Botucatu, Brazil

Diamater study group

Rudge MVC, Barbosa, AMP, Calderon IMP, Souza FP, Berghmans B, Thabane L, Junginger B, Graeff CFO, Magalhães CG, Costa RA, Lima SAM, Kron-Rodrigues MR, Felisbino S, Barbosa W, Campos FJ, Bossolan G, Corrente JE, Nunes HRC, Abbade J, Rossignoli PS, Pedroni CR, Atallah AN, Di Bella ZIKJ, Uchoa SMM, Hungaro MA, Mareco EA, Sakalem ME, Martinho N, Hallur LSR, Reyes DRA, Alves FCB, Marcondes JPC, Prudencio CB, Pinheiro FA, Sartório Filho CI, Quiroz SBCV, Pascon T, Nunes SK, Catinelli BB, Reis FVDS, Oliveira RG, Barneze S, Enriquez EMA, Takano L, Carr AM, Magyori ABM, Iamundo LF, Carvalho CNF, Jacomin M, Avramidis RE, Silva AJB, Orlandi MIG, Dangi TD, Bassin HCM, Melo JVF, Takemoto MLS, Menezes MD, Caldeirão TD, Santos NJ, Lourenço IO, Marostica de Sá J, Caruso IP, Rasmussen LT, Garcia GA, Nava GTA, Pascon C, Bussaneli DG, Nogueira VKC, Rudge CVC, Piculo F, Prata GM, Barbosa VP.

Anexo 11

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ORIGINAL ARTICLE



Relaxin-2 during pregnancy according to glycemia, continence status, and pelvic floor muscle function

Caroline Baldini Prudencio¹ · Sthefanie Kenickel Nunes¹ · Fabiane Affonso Pinheiro¹ · Carlos Isaías Sartorão Filho¹ · Flávia Ignácio Antônio² · Guilherme Thomaz de Aquino Nava³ · Marilza Vieira Cunha Rudge¹ · Angélica Mércia Pascon Barbosa^{1,2} · Diamater Study Group

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Abstract

Introduction and hypothesis To investigate relaxin-2 concentration comparing gestational diabetes mellitus (GDM) and non-GDM patients during pregnancy according to urinary incontinence (UI) and pelvic function status.

Methods This is a cross-sectional study evaluating 282 pregnant women from 24 weeks of gestation. The participants were divided into two groups, non-GDM and GDM, according to American Diabetes Association's diabetes mellitus gestational threshold. In addition, according to subanalysis, both groups were subdivided according to the presence of pregnancy-specific urinary incontinence: non-GDM continent, non-GDM incontinent, GDM continent, and GDM incontinent. All participants filled in questionnaires on clinical, obstetric, and urinary continence status (International Consultation on Incontinence Questionnaire-Short Form, ICIQ-SF, and Incontinence Severity Index, ISI), followed by pelvic floor muscle evaluation by the PERFECT scheme in which strength, endurance, and speed of contractions were evaluated.

Results Serum relaxin-2 concentrations were significantly lower in pregnant women with pregnancy-specific urinary incontinence in both non-GDM and GDM patients, but GDM showed the lowest concentration. In addition, the stratification of the groups according to pelvic floor muscle strength showed that pregnant patients with GDM and modified Oxford scale 0–2 had significantly lower levels than those who were non-GDM and GDM with Modified Oxford Scale 3–5. Relaxin-2 level was much lower in GDM incontinent pregnant women with MOS 0–2 compared to the other three groups.

Conclusions Lower relaxin-2 concentration was associated with the presence of pregnancy-specific urinary incontinence, but the combination of GDM, pregnancy-specific urinary incontinence, and low pelvic floor muscle strength showed the lowest levels of relaxin-2 compared to the other three groups.

Keywords Gestational diabetes ·

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Diamater Study Group

Calderon IMP, Souza FP, Berghmans B, de Bie R, Thabane L, Junginger B, Graeff CFO, Magalhães CG, Costa RA, Lima SAM, Kron-Rodrigues MR, Felisbino S, Barbosa W, Campos FJ, Bossolan G, Corrente JE, Nunes HRC, Abbade J, Rossignoli PS, Pedroni CR, Atallah AN, Di Bella ZIKJ, Uchoa SMM, Hungaro MA, Mareco EA, Sakalem ME, Martinho N, Hallur LSR, Reyes DRA, Alves FCB, Marcondes JPC, Quiroz SBCV, **Pascon T**, Catinelli BB, Reis FVDS, Oliveira RG, Barneze S, Enriquez EMA, Takano L, Carr AM, Magyori ABM, Iamundo LF, Carvalho CNF, Jacomin M, Avramidis RE, Silva AJB, Orlandi MIG, Dangiό TD, Bassin HCM, Melo JVF, Takemoto MLS, Menezes MD, Caldeirão TD, Santos NJ, Lourenço IO, Marostica de Sá J, Caruso IP, Rasmussen LT, Garcia GA, Pascon C, Bussaneli DG, Nogueira VKC, Rudge CVC, Piculo F, Prata GM, Barbosa VP.

✉ Angélica Mércia Pascon Barbosa
angelicapascon@gmail.com

¹ Postgraduate Program on Tocogyn, University (UNESP), São Paulo, Brazil

² School of Rehabilitation Sciences, University of Ottawa, Ottawa, Canada

³ Department of Physical Education, of Rio Claro, São Paulo State University (UNESP), São Paulo, Rio Claro, Brazil

atrophy, thinning, disorganization, and co-localization of fast

Anexo 12

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BMC Pregnancy and Childbirth

STUDY PROTOCOL

Open Access



Study protocol to investigate biomolecular muscle profile as predictors of long-term urinary incontinence in women with gestational diabetes mellitus

Marilza V. C. Rudge^{1*}, Fátima P. Souza², Joelcio F. Abbade¹, Raghavendra L. S. Hallur¹, João Paulo C. Marcondes¹,
Fernanda Piculo^{1,3}, Gabriela Marini^{1,4}, Giovana Vesentini¹, Lehana Thabane^{5,6}, Steven S. Witkin^{7,8},
Iracema M. P. Calderon¹, Angélica M. P. Barbosa^{1,9}, and The Diamater Study Group

Abstract

Background: Pelvic floor muscles (PFM) atrophy, co-localization of fast and slow PFM or RAM hyperglycemic-related myo investigated. This study aims to assess th incontinence (PS-UI) 12 months after the RAM hyperglycemic myopathy will be e

Methods: The Diamater is an ongoing of Diabetes Research Centre (PDRC), Botuc Diagnosis of GDM and PS-UI will be ma in the study will occur at the time of C- months postpartum. Study groups will be plus PS-UI; and (4) Non-GDM without PS myopathy at 12 months after C-section. squeeze pressure, 3D pelvic floor ultraso be analyzed for ex-vivo contractility, mo hyperglycemic myopathy. Additional va educational level, ethnicity, body mass i therapy.

Discussion: To our knowledge, this will be the first study to use PFM physical and biomolecular muscle properties for the assessment of cause-effect relationships. This study may reveal previously undetermined com-

Keywords: Gestational diabetes mellitus, Hyperglycemic myopathy, Pelvic floor muscles, Rectus abdominis muscles, Urinary incontinence, Proteomics, Collagen, Electromyography, Transmission electron microscopy

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The Diamater Study Group: Rudge MV, Barbosa AMP, Calderon IMP, Souza FP, Abbade JF, Hallur LSR, Piculo F, Marini G, Vesentini G, Thabane L, Palma MS, Graeff CFO, Arni RK, Herculano RD, Salvadori DF, Mateus S, Dal Pai Silva M, Magalhães CG, Costa RA, Lima SAM, Felisbino SL, Barbosa W, Atallah A, Girão MJB, Di Bella Z, Uchoa SM, Payão S, Hijas A, Berghman B, De Bie R, Sobrevia L, Junginger B, Alves FCB, Rossignoli PS, Prudencio CB, Orlandi MIG, Gonçalves MI, Nunes SK, Catinelli BB, Quiroz S, Sarmento BV, Pinheiro FA, Sartório CI, Lucas RR, Reyes DRA, Quiroz SBCV, Enriquez EMA, Oliveira RG, Floriano JF, Marcondes JPC, Barneze S, Dangio TD, Pascon T, Rossignoli P, Freitas JV, Takano L, Reis F, Caldeirão TD, Fernandes JN, Carr AM, Gaitero MVC, Corrente JE, Nunes HRC, Candido AF, Sarmento, Costa SMB, Dangio TD, Pascon T, Melo JVF, Takano L, Reis FVDS, Caldeirão TD, Carr AM, Garcia GA.

* Correspondence: marilzarudge@gmail.com

¹Department of Gynecology and Obstetrics, Botucatu Medical School (FMB), São Paulo State University (UNESP), CEP18618-687, São Paulo, Brazil

Full list of author information is available at the end of the article



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RESEARCH ARTICLE

Alterations in the structural characteristics of rectus abdominis muscles caused by diabetes and pregnancy: A comparative study of the rat model and women

Giovana Vesentini^{1,2*}, Angélica M. P. Barbosa^{1,3}, Débora C. Damasceno², Gabriela Marini^{1,2,4}, Fernanda Piculo^{1,2}, Selma M. M. Matheus^{1,5}, Raghavendra L. S. Hallur^{1,2}, Sthefanie K. Nunes^{1,2}, Bruna B. Catinelli^{1,2}, Claudia G. Magalhães^{1,2}, Roberto Costa^{1,2}, Joelcio F. Abbade^{1,2}, José E. Corrente^{1,6}, Iracema M. P. Calderon^{1,2}, Marilza V. C. Rudge^{1,2}, The DIAMATER Study Group[¶]

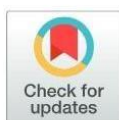
1 Perinatal Diabetes Research Center, University Hospital, Botucatu Medical School, Univ Estadual Paulista_UNESP, Botucatu, São Paulo, Brazil, **2** Department of Gynecology and Obstetrics, São Paulo State University (UNESP), Botucatu, São Paulo, Brazil, **3** Department of Gynecology and Obstetrics, São Paulo State University (UNESP), Botucatu, São Paulo, Brazil, **4** Department of Gynecology and Obstetrics, São Paulo State University (UNESP), Botucatu, São Paulo, Brazil, **5** Department of Gynecology and Obstetrics, São Paulo State University (UNESP), Botucatu, São Paulo, Brazil, **6** Department of Gynecology and Obstetrics, São Paulo State University (UNESP), Botucatu, São Paulo, Brazil

[¶] Membership of the group is available at gi.vesentini@hotmail.com

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****The Diamater Study Group:** Rudge MVC (Universidade Estadual Paulista), Barbosa AMP (Universidade Estadual Paulista), Calderon IMP (Universidade Estadual Paulista), Souza FP (Universidade Estadual Paulista), Hijas A (Case Western Reserve University), Sobrevia L (Pontificia Universidad Catolica de Chile), Palma MS (Universidade Estadual Paulista), Graeff CFO (Universidade Estadual Paulista), Arni RK (Universidade Estadual Paulista), Herculano RD (Universidade Estadual Paulista), Salvadori DF (Universidade Estadual Paulista), Matheus S (Universidade Estadual Paulista), Dal Pai Silva M (Universidade Estadual Paulista), Magalhães CG (Universidade Estadual Paulista), Costa RA (Universidade Estadual Paulista), Lima SAM (Universidade Estadual Paulista), Emanuel C (Imperial College London), Felisbino S (Universidade Estadual Paulista), Barbosa W (Universidade Estadual Paulista), Atallah A (Universidade Federal de São Paulo), Girão MJB (Universidade Federal de São Paulo), Di Bella Z (Universidade Federal de São Paulo), Uchoa SM (Universidade Católica de Pernambuco), Payão S (Faculdade de Medicina de Marília), Berghman B (Maastrich University), De Bie R (Maastrich University), Junginger B (Charité Universitätsmedizin Berlin), Alves FCB (Universidade Estadual Paulista), Rossignoli PS (Universidade Estadual Paulista), Abbade J (Universidade Estadual Paulista), Prudencio CB (Universidade Estadual Paulista), Orlandi MIG (Universidade Estadual Paulista), Gonçalves MI (Universidade Estadual Paulista), Nunes SK (Universidade Estadual Paulista), Catinelli BB (Universidade Estadual Paulista), Sarmento BV (Universidade Estadual Paulista), Pinheiro FA (Universidade Estadual Paulista), Sartório CI (Universidade Estadual Paulista), Quiroz SBCV (Universidade Estadual Paulista), Reyes DRA (Universidade Estadual Paulista), Enriquez EMA (Universidade Estadual Paulista), Oliveira RG (Universidade Estadual Paulista), Floriano JF (Universidade Estadual Paulista), Marcondes JPC (Universidade Estadual Paulista), Costa SMB (Universidade Estadual Paulista), Dangió TD (Universidade Estadual Paulista), Pascon T (Universidade Estadual Paulista), Hallur LSR (Universidade Estadual Paulista), Melo JVF (Universidade Estadual Paulista), Takano L (Universidade Estadual Paulista), Reis FVDS (Universidade Estadual Paulista), Caldeirão TD (Universidade Estadual Paulista), Carr AM (Universidade Estadual Paulista), Garcia GA (Universidade Estadual Paulista), Rabadan GB (Universidade Estadual Paulista), Bassin HCM (Faculdade de Medicina de Marília), Suyama KS (Universidade Estadual Paulista), Damasceno LN (Universidade Estadual Paulista), Takemoto MLS (Universidade Estadual Paulista), Menezes MD (Universidade Estadual Paulista), Bussaneli DG (Universidade Estadual Paulista), Nogueira, VKC (Universidade Estadual Paulista), Lima PR (Universidade Estadual Paulista), Lourenço IO (Universidade Estadual Paulista), Marostica de Sá J (Universidade Estadual Paulista), Megid RA (Universidade Estadual Paulista), Caruso IP (Universidade Estadual Paulista), Rasmussen LT (Faculdade de Medicina de Marília), Prata GM (Universidade do Sagrado Coração), Piculo F (Faculdades Integradas de Bauru), Vesentini G (Universidade Estadual Paulista), Arantes MA (Universidade Estadual Paulista), Ferraz GAR (Universidade Estadual Paulista), Camargo LP (Universidade Estadual Paulista), Kron MR (Universidade Estadual Paulista), Corrente JE (Universidade Estadual Paulista), Nunes HRC (Universidade Estadual Paulista).



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Abstract

Background

In the present study, we evaluated the structural characteristics of the rectus abdominis muscle (RAM) in women with gestational diabetes mellitus (GDM).

Method

Newborns female (weight) subcutaneous fat, glycemic pregnancy and the RAM tissue and those diagnosed with GDM were recruited, and RAM thickness was measured.

Results

No statistically significant differences were found between the groups.

Anexo 14

DIABETES RESEARCH AND CLINICAL PRACTICE 166 (2020) 108315

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International Diabetes Federation

DIABETES RESEARCH AND CLINICAL PRACTICE

Deleterious effects of gestational diabetes mellitus on the characteristics of the rectus abdominis muscle associated with pregnancy-specific urinary incontinence

Giovana Vesentini^{a,b}, Angélica M.P. Barbosa^c, Juliana F. Floriano^{a,b}, Sérgio L. Felisbino^d, Sarah M.B. Costa^{a,b}, Fernanda Piculo^{a,b}, Gabriela Marini^{a,b,e}, Sthefanie K. Nunes^{a,b}, David R.A. Reyes^{a,b}, João P.C. Marcondes^{a,b}, Raghavendra L.S. Hallur^{a,b}, Ariane L. Rozza^d, Cláudia G. Magalhães^{a,b}, Roberto Costa^{a,b}, Joelcio F. Abbade^{a,b}, José E. Corrente^f, Iracema M.P. Calderon^{a,b}, Selma M.M. Matheus^g, Marilza V.C. Rudge^{a,b,*}, The Diamater Study Group¹

^a Perinatal Diabetes Research Center, University Hospital, Botucatu Medical School, Univ Estadual Paulista UNESP, São Paulo State, Brazil

^b São Paulo State University (UNESP), Botucatu Medical School, São Paulo State, Brazil

^c São Paulo State University (UNESP), School of Philosophy, São Paulo State, Brazil

^d São Paulo State University (UNESP), Institute of Biology, São Paulo State, Brazil

^e Universidade Sagrado Coração, Department of Health Sciences, São Paulo State, Brazil

^f São Paulo State University (UNESP), Institute of Biology, São Paulo State, Brazil

^g São Paulo State University (UNESP), Institute of Biology, São Paulo State, Brazil

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ABSTRACT

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**** The Diamater Study Group:** Rudge Mvc, Barbosa, AMP, Caldeiron IMP, Souza FP, Hijas A, Sobrevia L, Palma MS, Graeff CFO, Arni RK, Herculano RD, Salvadori DF, Mateus S, Dal Pai Silva M, Magalhães CG, Costa RA, Lima SAM, Emanuelli C, Felisbino SI, Barbosa W, Atallah A, Girão MJB, Di Bella Z, Uchoa SM, Payão S, Berghman B, De Bie R, Junginger B, Alves FCB, Rossignoli PS, Abbade J, Prudencio CB, Orlandi MIG, Gonçalves MI, Nunes SK, Catinelli BB, Quiroz S, Sarmento BV, Pinheiro FA, Sartorão CI, Quiroz SBCV, Reyes DRA, Enriquez EMA, Oliveira RG, Floriano JE, Marcondes JPC, Sarmento, Costa SMB, Dangiô TD, **Pascon T**, Hallur LSR, Melo JVF, Takano L, Reis FVDS, Caldeirão TD, Carr AM, Garcia GA, Rabadan GB, Bassin HCM, Suyama KS, Damasceno LN, Takemoto MLS, Menezes MD, Bussaneli DG, Nogueira, VKC, Lima PR, Lourenço IO, Marostica de Sá J, Megid RA, Caruso IP, Rasmussen LT, Prata GM, Piculo F, Vesentini G, Arantes MA, Ferraz GAR, Camargo LP, Kron MR, Corrente JE, Nunes HRC.

* Corresponding author at: Univ Estadual Paulista Botucatu, SP 13618-970, Brazil.

E-mail address: marilzarudge@gmail.com (M.V.C.).

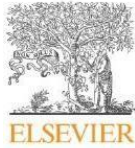
¹ DIAMATER Study Group Department of Gynecology, São Paulo State, Brazil.

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Anexo 15

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Altered maternal metabolism during mild gestational hyperglycemia as a predictor of adverse perinatal outcomes: A comprehensive analysis[☆]



Marilza Vieira Cunha Rudge^{a,b,*}, Angélica Mercia Pascon Barbosa^{b,c}, Luis Sobrevia^{b,d,e,f}, Rafael Bottaro Gelaleti^b, Raghavendra Lakshmana Shetty Hallur^b, João Paulo Castro Marcondes^b, Daisy Maria Fávero Salvadori^g, Caroline Baldini Prudêncio^b, Claudia Garcia Magalhães^a, Roberto Costa^a, Joelcio Francisco Abbade^a, José Eduardo Corrente^h, Iracema de Mattos Paranhos Calderon^{a,b}, The Perinatal Diabetes Research Group¹

^a Department of Gynecology and Obstetrics, Botucatu Medical School (FMB), São Paulo State University (UNESP), CEP18618-687, São Paulo, Brazil

^b Graduate Program in Gynecology, Obstetrics, and Mastology, Botucatu Medical School (FMB), São Paulo State University (UNESP), São Paulo, Brazil

^c Department of Physiotherapy and Occupational Therapy, School of Philosophy and Sciences, Marília, São Paulo State University (UNESP), São Paulo, Brazil

^d Cellular and Molecular Physiology Laboratory (CMPL), Department of Obstetrics, Division of Obstetrics and Gynaecology, School of Medicine, Faculty of Medicine, Pontificia Universidad Católica de Chile, Santiago 8330024, Chile

^e Department of Physiology, Faculty of Pharmacy, Universidad de Sevilla, Sevilla E-41012, Spain

^f University of Queensland Centre for Clinical Research (UQCCR), Faculty of Medicine and Biomedical Sciences, University of Queensland, Herston, QLD 4029, Queensland, Australia

^g Department of Pathology, Botucatu Medical School (FMB), São Paulo State University (UNESP), São Paulo, Brazil

^h Biostatistics Department, Bioscience Institute, São Paulo State University (UNESP), São Paulo, Brazil

¹ M.V. Rudge, I.M.P. Calderon, A.P. Barbosa, J. Abbade, R.A.A. Costa, C.G. Magalhães, D.F. Salvadori, R. Gelaleti, R.L.S. Hallur, J.P. Marcondes, J.F. Floriano, D.R.A. Reyes, L. Sobrevia, C.B. Prudêncio, F. Pículo, G. Marini, G. Vesentini, G. Morceli, C.A. Negrato, H.D. Prazeres, S. Molina, M. Arantes, A.C. Cavassini, L. Kerche, A.K.C. De Luca, S. Corrêa-Silva, E. Bevilacqua, J.B. Moreli, L. Pietro, S. Daher, S. Fabio, A.C. Honório-França, A.A. Queiroz, C.C.P. Hara, C.A.L. Boraschi, T.A.V.L. Pauletti, L. Jovanovic, A. Dias, A.N. Atallah, M.D. Ramos, M.A.M. Brasil, C.V.C. Rudge, A. Tristão, U. Del Nero, M. Mendonça, S.S. Witkin, C.I. Sartório Filho, S.K. Nunes, F.A. Pinheiro, S.V. Quiroz, T. Pascon, T.D. Caldeirão, A.P. Oliveira, B.F. Nicolosi, C.V. Bolognani, D.L.G. Fagundes, I.C.F. Llanos, J.M. Vernini, L.B.S.M. Reis, M.P. Sirimarco, N.M. Basso, N.A.Q. Maquesim, S.A.L.C. Silva, S.C. Silva, T.T. Scudeller, W. Ayach, A.P.M. Almeida, B.F.C.A. Nicolosi, C.P. Lima, D. Luminoso, F.C. Vasconcellos, G.A.R. Ferraz, H. Migliolaro, L.P. Camargo, M.L.S. Macedo, M.R.K. Rodrigues and P.H.O. Anézio.

Placenta
Cytokines
Apoptosis
Natural killer cells
Inflammatory mediators
IRS-1 gene
Gene expression
Moderator analysis

Uncensored relationship between maternal excessive fetal growth, also supplying moderate analysis in relation to hyperglycemia, MGH is associated with an increased incidence of hypertension, metabolic syndrome, persistent insulin resistance after pregnancy, and high risk of developing type 2 diabetes mellitus (T2DM) after pregnancy. Structural changes and functional abnormalities resulting from MGH were observed in placenta. The fully adjusted model concluded that the predictor variable (MGH), which creates a complex environment for the fetus, has a direct effect on excessive birth weight and produces a z-score for ratios of birth weight to gestational age of ≥ 2 . Maternal age, pre-pregnancy BMI, number of previous pregnancies, numbers of prenatal visits, and 1 h OGTT are moderator variables that impact MGH and excessive fetal growth. These results show that maternal MGH has some characteristics associated with similar long-term T2DM development and similar adverse perinatal results to those of gestational diabetes mellitus (GDM) mothers, making it an intermediate maternal and placental marker between normoglycemic and GDM mothers.

[☆] This article is part of a Special Issue entitled: Membrane Transporters and Receptors in Pregnancy Metabolic Complications edited by Luis Sobrevia.

* Corresponding author at: Department of Gynecology and Obstetrics, Botucatu Medical School (FMB), São Paulo State University (UNESP), Botucatu 18618-687, São Paulo, Brazil.

E-mail address: marilzarudge@gmail.com (M.V.C. Rudge).

¹ M.V. Rudge, I.M.P. Calderon, A.P. Barbosa, J. Abbade, R.A.A. Costa, C.G. Magalhães, D.F. Salvadori, R. Gelaleti, R.L.S. Hallur, J.P. Marcondes, J.F. Floriano, D.R.A. Reyes, L. Sobrevia, C.B. Prudêncio, F. Pículo, G. Marini, G. Vesentini, G. Morceli, C.A. Negrato, H.D. Prazeres, S. Molina, M. Arantes, A.C. Cavassini, L. Kerche, A.K.C. De Luca, S. Corrêa-Silva, E. Bevilacqua, J.B. Moreli, L. Pietro, S. Daher, S. Fabio, A.C. Honório-França, A.A. Queiroz, C.C.P. Hara, C.A.L. Boraschi, T.A.V.L. Pauletti, L. Jovanovic, A. Dias, A.N. Atallah, M.D. Ramos, M.A.M. Brasil, C.V.C. Rudge, A. Tristão, U. Del Nero, M. Mendonça, S.S. Witkin, C.I. Sartório Filho, S.K. Nunes, F.A. Pinheiro, S.V. Quiroz, T. Pascon, T.D. Caldeirão, A.P. Oliveira, B.F. Nicolosi, C.V. Bolognani, D.L.G. Fagundes, I.C.F. Llanos, J.M. Vernini, L.B.S.M. Reis, M.P. Sirimarco, N.M. Basso, N.A.Q. Maquesim, S.A.L.C. Silva, S.C. Silva, T.T. Scudeller, W. Ayach, A.P.M. Almeida, B.F.C.A. Nicolosi, C.P. Lima, D. Luminoso, F.C. Vasconcellos, G.A.R. Ferraz, H. Migliolaro, L.P. Camargo, M.L.S. Macedo, M.R.K. Rodrigues and P.H.O. Anézio.

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REGISTERED REPORT PROTOCOL

Effectiveness of the pelvic floor muscle training on muscular dysfunction and pregnancy specific urinary incontinence in pregnant women with gestational diabetes mellitus: A systematic review protocol

Angélica Mércia Pascon Barbosa^{1‡*}, Eusebio Mario Amador Enriquez^{2‡}, Meline Rossetto Kron Rodrigues^{3‡}, Caroline Baldini Prudencio^{2‡}, Alvaro Nagib Atallah^{4‡}, David Rafael Abreu Reyes^{2‡}, Raghavendra Lakshmana Shetty Hallur^{2‡}, Sthefanie Kenickel Nunes^{2‡}, Fabiane Affonso Pinheiro^{2‡}, Carlos Isaías Sartório Filho^{2‡}, Gabriela Lopes Piemonte Andrade^{5‡}, Bary Berghmans^{6‡}, Rob de Bie^{6‡}, Silvana Andréa Molina Lima^{7‡}, Marilza Vieira Cunha Rudge^{2‡}, The Diamater Study Group¹

¹ Department of Physiotherapy and Occupational Therapy, School of Philosophy and Sciences, São



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OPEN ACCESS

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The Diamater Study Group: Rudge MVC, Barbosa, AMP, Caldeiron IMP, Souza FP, Berghmans B, de Bie R, Thabane L, Junginger B, Graeff CFO, Magalhães CG, Costa RA, Lima SAM, Kron-Rodrigues MR, Felisbino S, Barbosa W, Campos FJ, Bossolan G, Corrente JE, Nunes HRC Abbade J, Rossignoli PS, Pedroni CR, Atallah AN, Di Bella ZIKJ, Uchoa SMM, Hungaro MA, Mareco EA, Sakalem ME, Martinho N, Hallur LSR, Reyes DRA, Alves FCB, Marcondes JPC, Prudencio CB, Pinheiro FA, Sartório CI, Quiroz SBCV, **Pascon T**, Nunes SK, Catinelli BB, Reis FVDS, Oliveira RG, Barneze S, Enriquez EMA, Takano L, Carr AM, Magyori ABM, Iamundo LF, Carvalho CNF, Jacomin M, Avramidis RE, Silva AJB, Orlandi MIG, Dangió TD, Bassin HCM, Melo JVF, Takemoto MLS, Menezes MD, Caldeirão TD, Santos NJ, Lourenço IO, Marostica de Sá J, Caruso IP, Rasmussen LT, Garcia GA, Nava GTA, Pascon C, Bussanelli DG, Nogueira VKC, Rudge CVC, Piculo F, Prata GM.

Abstract

Background

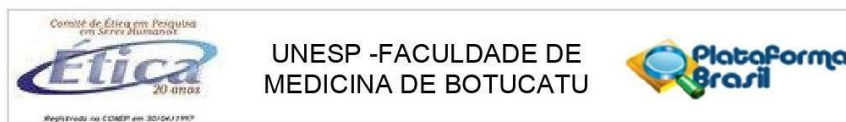
There is ample evidence that gestational diabetes mellitus has a direct influence on urinary incontinence and pelvic floor muscles. There are no standardized pelvic floor muscle exercise programs in the literature for the physiotherapy and differ in the type of exercise, intensity, type and duration of application, and the frequency and duration of treatment sessions. The aim of this systematic review will be to investigate that Pelvic Floor Muscle Training can prevent and/or decrease the pregnancy specific urinary incontinence in women with gestational diabetes mellitus or gestational hyperglycemia.

Methods

We will perform a systematic review according to the Cochrane methodology of Randomized Controlled Trials. An overall search strategy will be developed and adapted for Embase, MEDLINE, LILACS, and CENTRAL databases, with the date of consultation until

Anexo 17

Aprovação do projeto pelo CEP



PARECER CONSUBSTANCIADO DO CEP

DADOS DO PROJETO DE PESQUISA

Título da Pesquisa: Projeto Mãe: Estudo de coorte prospectivo: nova tríade gestacional (hiperglicemia, incontinência urinária e perfil clínico, molecular e ômico da miopatia hiperglicêmica) na predição de incontinência. Pesquisa translacional com biodevice para regeneração muscular em ratas com diabetes

Subprojeto 1: Relação dos achados eletromiográficos dos músculos do assoalho pélvico com os níveis de relaxina ao longo da gestação e após o parto de mulheres com hiperglicemia gestacional e incontinência urinária específica da gestação (Orientador: Marilza Vieira Cunha Rudge/ Orientando: Caroline Baldini Prudencio/ Nível: Doutorado)

Subprojeto 2: Ultrassonografia Tridimensional do assoalho pélvico de mulheres com hiperglicemia gestacional 16-18 meses após a gestação (Orientador: Marilza Vieira Cunha Rudge/ Orientando: Carlos Izaías Sartorão Filho/ Nível: Doutorado);

Subprojeto 3: Coorte prospectiva da tríade hiperglicemia gestacional, incontinência urinária específica da gestação e disfunção muscular do assoalho pélvico, avaliada pela ultrassonografia funcional, como preditora da incontinência urinária e disfunção muscular 6-18 meses após o parto (Orientador: Marilza Vieira Cunha Rudge/ Orientando: Fabiane Affonso Pinheiro/ Nível: Doutorado)

Pesquisador: Marilza Vieira Cunha Rudge

Área Temática:

Versão: 2

CAAE: 82225617.0.0000.5411

Instituição Proponente: Departamento de Ginecologia e Obstetrícia

Patrocinador Principal: FUNDACAO DE AMPARO A PESQUISA DO ESTADO DE SAO PAULO

DADOS DO PARECER

Número do Parecer: 2.511.990

Apresentação do Projeto:

Trata-se de um projeto temático com 03 subprojetos.

Projeto Mãe: Estudo de coorte prospectivo: nova tríade gestacional (hiperglicemia, incontinência urinária e perfil clínico, molecular e ômico da miopatia hiperglicêmica) na predição de

Endereço: Chácara Butignolli, s/n	CEP: 18.618-970
Bairro: Rubião Junior	
UF: SP	Município: BOTUCATU
Telefone: (14)3880-1609	E-mail: cep@fmb.unesp.br

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incontinência. Pesquisa translacional com biodevice para regeneração muscular em ratas com diabetes

Subprojeto 1: Relação dos achados eletromiográficos dos músculos do assoalho pélvico com os níveis de relaxina ao longo da gestação e após o parto de mulheres com hiperglicemia gestacional e incontinência urinária específica da gestação (Orientador: Marilza Vieira Cunha Rudge/ Orientando: Caroline Baldini Prudencio/ Nível: Doutorado)

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Subprojeto 3: Coorte prospectiva da tríade hiperglicemia gestacional, incontinência urinária específica da gestação e disfunção muscular do assoalho pélvico, avaliada pela ultrassonografia funcional, como preditora da incontinência urinária e disfunção muscular 6-18 meses após o parto (Orientador: Marilza Vieira Cunha Rudge/ Orientando: Fabiane Afonso Pinheiro/ Nível: Doutorado)

No estudo clínico, as participantes serão selecionadas entre março 2016 e dezembro de 2019 no Centro de Investigação do Diabetes Perinatal da FMB UNESP, no Ambulatório de Gestão de Risco do Hospital Materno Infantil da FAMEMA e no Hospital Regional de Assis.

O total de participantes será de 160 considerando as quatro coortes que serão constituídas. Será realizada análise do perfil bioquímico, obtido pelas análises sanguíneas dos níveis de CCL7, Relaxina, Ca, PTH, calcitonina, vitamina D, glicose e insulina; nos MAPs, serão realizados a Avaliação Funcional do Assoalho pélvico (AFA), Eletromiografia (EMG) e Ultrassonografia (US), nos MRA serão realizados EMG e US, no momento da cesárea a contratilidade ex vivo obtida pelo Miógrafo, e os perfis morfológico (ultraestrutura das fibras musculares e da matriz extracelular), molecular (Expressão Gênica e Proteica das fibras musculares e da matriz extracelular), organóide, Ômico (transcriptômica, proteômica e metabolômica). Para o estudo experimental, serão utilizadas ratas Wistar prenhes, divididas em grupos (n=20 cada) não diabéticas e diabéticas, que serão submetidas à instalação do biodevice de biomembrana de látex revestida de células-tronco mesenquimais, implantados na musculatura estriada uretral e MRA. O estudo de Revisão Sistemática seguirá as normas e será registrado na Colaboração Cochrane.

Objetivo da Pesquisa:

Analisar a influência da associação entre HGG (DMG e HGL), "IUEG" no perfil da "MHG" (morfológico, funcional, bioquímico, molecular e ômico) dos MRA e MAPs. Hipótese nula correspondente: Não há associação entre HG gestacional, "IUEG" com o perfil da "MHG"

Endereço: Chácara Butignolli, s/n		CEP: 18.618-970
Bairro: Rubião Junior		
UF: SP	Município: BOTUCATU	
Telefone: (14)3880-1609	E-mail: cep@fmb.unesp.br	



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(morfológico, funcional, bioquímico, molecular e ômico).Objetivo 2: Analisar a tríade HGG, "IUEG" e perfil da "MHG" (morfológico, funcional, bioquímico, molecular e ômico) do MRA e do MAPs, com incidência de IU 6-12 meses PP e perfil morfológico, funcional e bioquímico, dentro das quatro coortes constituídas pela combinação entre HGG e "IUEG".Hipótese nula correspondente: O risco de IU 6-12 meses PP e o perfil morfológico, bioquímico e funcional do MRA e MAPs não sofrem efeito da tríade HGG, "IUEG" e perfil da "MHG" (morfológico, funcional, bioquímico, molecular e ômico) do MRA e do MAPs em mulheres com HGG.Objetivo 3: Biodevice Avaliar o efeito do biodevice de látex de seringueira com CTMs na regeneração do músculo estriado uretral e MRA no PP de ratas com diabetes moderado, no perfil morfológico, microvascular, molecular e ômico. Hipótese nula correspondente: Não há efeito estatisticamente significativo do uso do biodevice com CTMs sobre a miopatia diabética.Objetivo 4: Revisão Sistemática: Determinar a melhor evidência científica, por meio da revisão sistemática, sobre efetividade dos exercícios sobre a DMAP e "IUEG" em gestantes HGs.

Avaliação dos Riscos e Benefícios:

Descritos no estudo e no TCLE.

Riscos: EMG, US, AFA, dinamometro: Desconforto e dor leves na região vaginal Coleta sangue: dor, desconforto, hematoma local (solução, compressão e gelo) Retalho de músculo: este procedimento não oferece riscos adicionais aos já considerados ao realizar a cirurgia cesariana Benefícios: - Que o conjunto das análises morfológicas, funcionais, bioquímicas, moleculares e o mapeamento ômico caracterizem a "MHG" relacionada à "IUEG" em gestantes HGs em dois estádios de gravidade da miopatia diferenciados pelo tipo de distúrbio hiperglicêmico (HGL e DMG). B - Que a tríade caracterizada por HGG, "IUEG" e perfil (morfológico, funcional, bioquímico, molecular e ômico) da "MHG" do MRA e MAPs seja preditora de IU 6-12 meses PP. C - Que o conjunto das análises morfológicas, funcionais, bioquímicas, moleculares e o mapeamento ômico definam marcadores úteis no diagnóstico e em futuras terapias. D - Que a revisão sistemática evidencie a primeira linha de intervenção preventiva ou reabilitadora para a DMAP e "IUEG" em gestantes HGs.

E - Que a "IUEG" em gestantes HGs seja incluída de modo inovador como intercorrência clínica na gestação diabética e com HGL e represente nova ferramenta na definição do impacto materno de longo prazo do DMG e HGL. F - Espera-se que a "IUEG" em gestantes HGs seja considerada problema de saúde pública pela imbricação de duas patologias com elevada prevalência (IU e DMG), que tem impacto no custo em saúde e que uma (HGG-DMG ou HGL), por sua ação sobre os músculos (miopatia), se confirme como a base fisiopatológica da ocorrência da outra (IU). G - Que

Endereço: Chácara Butignolli, s/n

Bairro: Rubião Junior

UF: SP

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Município: BOTUCATU

CEP: 18.618-970

E-mail: cep@fmb.unesp.br

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seja confirmada a potencialidade da utilização do biodevice de biomembrana de látex como suporte para CTMs, para regeneração da musculatura estriada uretral e no MRA PP cesárea de ratas com diabetes moderado para futuros estudos nas fases pré-clínica e clínica. Esta abordagem translacional proposta trará esta pesquisa para a fronteira do conhecimento na área de Saúde da Mulher, uma vez que estão sendo aplicadas metodologias de análise que se complementam na abordagem de problemática de interesse médico/social.

Comentários e Considerações sobre a Pesquisa:

Pesquisa seguindo os preceitos éticos, com documentação adequada ao estudo apresentada.

Considerações sobre os Termos de apresentação obrigatória:

Apresentados TCLE e também autorizações para um estudo temático e multicentrico, e armazenamento de material em Biobanco.

Recomendações:

Aprovação, sem necessidade de envio à CONEP.

Conclusões ou Pendências e Lista de Inadequações:

Não há.

Considerações Finais a critério do CEP:

Conforme deliberação do Colegiado em reunião extraordinária do Comitê de Ética em Pesquisa da FMB/UNESP, realizada em 22 de fevereiro de 2018, o projeto encontra-se APROVADO, sem necessidade de envio à CONEP.

O Comitê de Ética em Pesquisa, no entanto, informa que ao final da execução da pesquisa, seja enviado o "Relatório Final de Atividades", na forma de "Notificação", via Plataforma Brasil.

Atenciosamente,

Comitê de Ética em Pesquisa da Faculdade de Medicina de Botucatu - UNESP

Este parecer foi elaborado baseado nos documentos abaixo relacionados:

Tipo Documento	Arquivo	Postagem	Autor	Situação
Informações Básicas do Projeto	PB_INFORMAÇÕES_BÁSICAS_DO_PROJETO_951323.pdf	06/02/2018 16:21:03		Aceito
Outros	EAP_Marilza_Rudge.pdf	18/01/2018 17:27:08	Marilza Vieira Cunha Rudge	Aceito

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Bairro: Rubião Junior **CEP:** 18.618-970
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Telefone: (14)3880-1609 **E-mail:** cep@fmb.unesp.br

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Outros	Fluxograma_Tematico_CEP.pptx	26/11/2017 19:18:14	Marilza Vieira Cunha Rudge	Aceito
Outros	HRA.pdf	26/11/2017 19:09:17	Marilza Vieira Cunha Rudge	Aceito
Outros	termodeautorizacao_HRA.pdf	26/11/2017 19:01:53	Marilza Vieira Cunha Rudge	Aceito
Outros	coparticipacao_assis_municipio.jpeg	26/11/2017 18:53:44	Marilza Vieira Cunha Rudge	Aceito
Folha de Rosto	folhaderosto_tematico.pdf	26/11/2017 17:47:28	Marilza Vieira Cunha Rudge	Aceito
Outros	SUBPROJETO_3.doc	05/10/2017 23:41:43	Marilza Vieira Cunha Rudge	Aceito
Outros	SUBPROJETO_2.docx	05/10/2017 23:41:04	Marilza Vieira Cunha Rudge	Aceito
Projeto Detalhado / Brochura Investigador	Projeto_mae.docx	05/10/2017 23:39:04	Marilza Vieira Cunha Rudge	Aceito
Outros	Projeto_mae_Subprojetos.docx	05/10/2017 23:37:51	Marilza Vieira Cunha Rudge	Aceito
Orçamento	orcamento_tematico.pdf	05/10/2017 23:36:42	Marilza Vieira Cunha Rudge	Aceito
Outros	SUBPROJETO_1.doc	05/10/2017 22:54:11	Marilza Vieira Cunha Rudge	Aceito
TCLE / Termos de Assentimento / Justificativa de Ausência	TCLE_tematico.docx	05/10/2017 22:48:47	Marilza Vieira Cunha Rudge	Aceito
Outros	Co_participacao_FFC.pdf	21/09/2017 17:02:46	Marilza Vieira Cunha Rudge	Aceito
Outros	Co_participacao_Famema.jpeg	21/09/2017 17:02:11	Marilza Vieira Cunha Rudge	Aceito
Outros	Co_participacao_Araraquara.pdf	21/09/2017 17:00:09	Marilza Vieira Cunha Rudge	Aceito
Outros	Co_participacao_Bauru.pdf	21/09/2017 16:59:28	Marilza Vieira Cunha Rudge	Aceito
Outros	Co_participacao_RioPreto.pdf	21/09/2017 16:58:52	Marilza Vieira Cunha Rudge	Aceito
Outros	Co_participacao_RioClaro.pdf	21/09/2017 16:57:58	Marilza Vieira Cunha Rudge	Aceito

Situação do Parecer:

Aprovado

Necessita Apreciação da CONEP:

Não

Endereço: Chácara Butignolli, s/n
Bairro: Rubião Junior **CEP:** 18.618-970
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Telefone: (14)3880-1609 **E-mail:** cep@fmb.unesp.br

Anexo 18 Aprovação pela CEUA



UNIVERSIDADE ESTADUAL PAULISTA
"JÚLIO DE MESQUITA FILHO"
Campus de Marília
CEUA – Comissão de Ética no Uso de Animal

FLS.	27
PROC. Nº	1183/2016
RUBRICA	

CERTIFICADO 007/2016

Certificamos que a proposta intitulada "ANÁLISE MORFOLÓGICA E MOLECULAR DO MÚSCULO RETO ABDOMINAL E PERIURETRAL DE RATAS PRENHES COM DIABETE MODERADO SUBMETIDAS A PROGRAMA DE NATAÇÃO", registrada sob o protocolo nº 1183/2016, sob a responsabilidade de Angélica Mércia Pascon Barbosa, 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 de Experimentação Animal (CONCEA), e foi aprovada pela COMISSÃO DE ÉTICA NO USO DE ANIMAIS (CEUA) da FFC – UNESP do Campus de Marília, em reunião de 22/09/2016.

Finalidade	() Ensino (X) Pesquisa Científica
Vigência da autorização	De 09/2016 a 08/2017
Espécie/linhagem/raça	Rato/ Wistar
Nº de animais	90
Peso/Idade	400g/110-120 dias
Sexo	F
Origem	Biotério da Faculdade de Medicina de Marília

Marília, 22 de setembro de 2016.

Dra. Luciana Pinato
Presidente da CEUA-FFC da UNESP-
Campus de Marília



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Universidade Estadual Paulista
Faculdade de Medicina de Botucatu

Fone: (14) 3880-1608/3880-1609
E-mail Secretaria:
ceua@fmb.unesp.br/graziela@fmb.unesp.br/sara.sampaio@fmb.unesp.br
Rua: João Buttignoli, s/nº - Distrito Rubião Junior - Botucatu/SP
CEP: 18.618-970



Instituída na Faculdade de Medicina através da Portaria do Diretor nº 30 de 26/04/99

Comissão de Ética no Uso de Animais

Ofício nº 020/2019 – CEUA – FMB/UNESP

Botucatu, 08 de agosto de 2019.

Prezada Senhora,

Em atendimento ao documento enviado, solicitando a Inclusão de coleta de sangue, extração de dentes e amostras do tecido muscular, referente ao Projeto de Pesquisa (**Protocolo CEUA 1234/2017**) – **Título:** “Coorte prospectiva da tríade hiperglicemia gestacional,” incontinência urinária específica da gestação” e perfil (morfológico, funcional, bioquímico, molecular e ômico) da “miopatia hiperglicêmica gestacional” como preditora da incontinência urinária e disfunção muscular 6-12 meses pós-parto; e pesquisa translacional do impacto do biodevice de látex de seringueira com células-tronco mesenquimais na regeneração muscular pós-parto de ratas diabética.”, aprovado em 26/10/2017, informo que a solicitação foi **APROVADA** por essa Comissão, porém a coleta de sangue deve ser limitada a **800 µl** por semana.

Não há nenhum impedimento ético para o envio do sangue e do tecido muscular coletado para o exterior. Entretanto, ressalto que aprovação na CEUA não exclui a necessidade de realização dos outros trâmites legais necessários para envio de amostra biológica para serem analisadas em outros países.

Adicionalmente a aprovação na CEUA não prevê a inclusão de nenhum animal adicional aos já solicitados no projeto de pesquisa **CEUA 1234/2017**.

Atenciosamente,

Profa. Assis. Bertha Furian Polegato

Presidente da Comissão de Ética no Uso de Animais

Faculdade de Medicina de Botucatu – UNESP

Ilustríssima Senhora

Professor Titular Marilza Vieira Cunha Rudge
Departamento de Ginecologia e Obstetrícia
Faculdade de Medicina de Botucatu – UNESP