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ALTERAÇÕES NO CONTROLE AUTÔNOMO DO FUNCIONAMENTO CARDIOVASCULAR EM ADULTOS COMO RESULTADO DO HIPOTIREOIDISMO GESTACIONAL DURANTE A VIDA FETAL

JULIANA MILAN ALVES

UBERLÂNDIA

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Dissertação apresentada ao Programa de Pós-Graduação em Ciências da Saúde da Faculdade de Medicina da Universidade Federal de Uberlândia, como requisito parcial para a obtenção do título de Mestre em Ciências da Saúde.

Área de concentração: Fisiopatologia de doenças e agravos à saúde.

Orientador: Prof. Dr. Guilherme Morais Puga

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Juliana Milan Alves

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Presidente da banca (orientador): Prof. Dr. Guilherme Morais Puga

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DEDICATÓRIA

A meus pais, que tudo me deram,
A minha irmã, que tudo me ensinou

RESUMO

Introdução: Atualmente, sabe-se que estímulos internos e externos ao ambiente intrauterino durante o desenvolvimento fetal podem ter importantes consequências fisiológicas na vida adulta do indivíduo. Vários estudos têm mostrado que diferentes doenças na idade adulta podem ter sua origem nas primeiras fases da vida. A diminuição dos níveis de hormônio tireoidiano materno, como nos casos de hipotireoidismo gestacional (GHT) e durante a lactação, está relacionada a problemas no desenvolvimento cardíaco fetal e doenças cardiovasculares de longo prazo. No entanto, os mecanismos por trás desses efeitos ainda não são totalmente compreendidos. **Objetivos:** Portanto, o objetivo deste trabalho foi avaliar as consequências cardiovasculares na vida adulta causadas pelo GHT durante os períodos de gestação e lactação. **Materiais e métodos:** Nós investigamos isso medindo a pressão arterial, frequência cardíaca, função barorreflexa e controle autonômico cardiovascular de ratos Wistar adultos que foram submetidos a GHT fetal e neonatal. **Resultados:** Como resultados, descobrimos que a prole de ratas com hipotireoidismo induzido durante a gravidez e / ou lactação apresentou ganho de peso significativamente menor nos primeiros 90 dias após o nascimento. Esses ratos também apresentaram aumento tanto da pressão arterial média quanto da pressão arterial sistólica, além de aumento do índice simpático-vagal quando comparados ao grupo controle. Além disso, apresentaram redução da função barorreflexa diante de um desafio farmacológico, mostrando que têm predomínio da função simpática tanto no coração quanto nos vasos. **Conclusão**: Em conclusão, nossos resultados mostraram que a deficiência de hormônio tireoidiano materno pode produzir alterações crônicas na função cardiovascular na vida adulta da prole.

Palavras-chave: pressão arterial, barorreflexo, frequência cardíaca, hipotireoidismo gestacional, doenças cardiovasculares, índice simpatovagal.

ABSTRACT

Introduction: Currently, it is known that internal and external stimuli to the intrauterine environment during fetal development can have important physiological consequences in the individual's adult life. Several studies have shown that different diseases in adulthood may have their origin in the early stages of life. A decrease in maternal thyroid hormone levels, as in gestational hypothyroidism (GHT) cases and during the lactation period, is related to problems in fetal heart development and long-term cardiovascular diseases. However, the mechanisms behind these effects are not yet fully understood. **Objectives:** Therefore, the aim of this work was to evaluate the cardiovascular consequences in adulthood caused by GHT during gestation and lactation periods. **Material and methods:** We investigated this by measuring the arterial pressure, heart rate, baroreflex function and autonomic cardiovascular control of adult Wistar rats that underwent fetal and neonatal GHT. **Results:** As results, we found that the offspring of rats with induced hypothyroidism during pregnancy and/or lactation showed significantly less weight gain in the first 90 days after birth. These rats also presented an increase in both mean arterial pressure and systolic blood pressure, in addition to an increase of the sympathovagal index when compared to the control group. **Conclusion:** In conclusion, our results showed that maternal thyroid hormone deficiency can chronically produce changes in cardiovascular function in the adult life of the offspring.

Keywords: arterial pressure, baroreflex, heart rate, gestational hypothyroidism, cardiovascular diseases, sympathovagal index.

LISTA DE ILUSTRAÇÕES

LISTA DE ABREVIATURAS E SIGLAS

AVEh	Acidente Vascular Encefálico Hemorrágico
AVEi	Acidente Vascular Encefálico Isquêmico
DE-9	Nono Dia Embrionário
eHHT	Eixo Hipotálamo-Hipófise-Tireoide
FCI	Frequência Cardíaca Intrínseca
GHT	Hipotireoidismo Gestacional
hCG	Gonadotrofina Coriônica
HGE	Hipotireoidismo Gestacional Experimental
HT	Hormônios Tireoidianos
IAM	Infarto Agudo do Miocárdio
ICC	Insuficiência Cardíaca Crônica
MMT	2mercapto-1-metimazol
PA	Pressão Arterial
PTU	6-propil-2-tiouracil
SRAA	Sistema Renina-Angiotensina-Aldosterona
T3	Triiodotironina
T4	Tiroxina / Tetraiodotironina
TRH	Hormônio Liberador de TSH
TSH	Tireotrofina / Hormônio Estimulante da Tireoide

LISTA DE ABREVIATURAS E SIGLAS ARTIGO

BI	Baroreflex Index
BP	Blood Pressure
CEUA	Animal Research and Ethics Committee
CONCEA	National Council for The Control of Animal Experimentation
CONTROL	Offspring Treated with Water During the Mother's Gestation Period
DAB	Double Autonomic Blockade
DAP	Diastolic Arterial Pressure
GHT	Gestational Hypothyroidism
HF	High Frequency
HR	Heart Rate
I.M.	Intramuscular
I.P.	Intraperitoneal
I.V.	Intravenous
iHR	Intrinsic Heart Rate
LF	Low Frequency
MAP	Mean Arterial Pressure
MMI	Methylmercaptoimidazole
NO	Nitric Oxide
OMG	Offspring Treated with Methimazole During the Mother's Gestation Period
OMGL	Offspring Treated with Methimazole During the Mother's Pregnancy And Lactation Period
OML	Offspring Treated with Methimazole During the Mother's Lactation Period
PAP	Pulsatile Blood Pressure
REBIR	Rodent Biotery Network
SAP	Systolic Arterial Pressure
SEM	Standard Error of The Mean
T3	Triiodothyronine
T4	Tetraiodothyronine
TH	Thyroid Hormones
TR	Th Specific Receptors
TRH	Hypothalamic Thyrotrophin Releasing Hormone
UFU	Federal University of Uberlandia
VSI	Vagal Sympathetic Index

SUMÁRIO

1 INTRODUÇÃO

O desenvolvimento fetal envolve uma formação bem ordenada, portanto, as respostas adaptativas geralmente resultam em alterações irreversíveis na estrutura e função de tecidos (unidades funcionais, tipos celulares e número de células alterados), o que pode ser comprovado na vida adulta, resultando em maior susceptibilidade a doenças (GLUCKMAN; HANSON, 2004; LANGLEY-EVANS, 2006; NIJLAND; FORD; NATHANIELSZ, 2008), conceito chamado de *programming fetal* (BARKER, 1993).

Em ratos, até o oitavo dia embrionário, os fetos tem como fonte materna os HT. Apenas a partir do nono dia embrionário (DE-9) é possível observar o surgimento da glândula tireoide, devido ao fato de que a partir deste momento, a glândula já é capaz de concentrar tireoglobulina e captar íons iodeto. Estudos demonstraram que mesmo pequenas deficiências de iodo materno são capazes de diminuir os níveis de T4 do feto (KLEIN; OJAMAA, 2001).

Ratas grávidas podem ser submetidas ao hipotireoidismo gestacional experimental (HGE) de 3 principais formas: tireoidectomia seguida por tratamento com drogas antitireoidianas; administração de 2mercapto-1-metimazol (MMT); e administração de 6-propil-2-tiouracil (PTU). Sendo os dois últimos métodos realizados ainda durante a gestação e/ou lactação (SALA-ROCA, 2002; ZAMONER; PESSOA-PUREUR, 2011; ARGUMEDO; SANZ; OLGUÍN, 2012).

O hipotireoidismo gestacional (HG) é uma das disfunções endócrinas mais comuns durante a gestação (SHAN; TENG, 2019). Os hormônios tireoidianos (HT) da mãe são essenciais para o feto, até que sua própria glândula seja totalmente funcional. Seus níveis anormais podem trazer consequências graves para a gestante, além dos possíveis danos para o desenvolvimento fetal, levando a prejuízos da saúde ao longo da vida desse indivíduo. (SANTISTEBAN; BERNAL, 2005). O hipotireoidismo é caracterizado por níveis insuficientes de HT circulantes. Manifesta-se de uma forma primária, a qual é mais prevalente na população ou de forma secundária, decorrente de doença hipotalâmica ou hipofisária (WOEBER, 2020).

Alguns estudos *in vivo* indicam que a prole de animais expostos ao hipotireoidismo em ambiente intrauterino é propensa a ter disfunções cardíadas, porém os mecanismos por trás desse impacto ainda precisam ser elucidados (GHANBARI et al., 2015). Estes estudos mostram que dois principais conceitos surgiram para explicar como os HT exercem suas funções cardiovasculares: função direta no miocárdio; e, interações diretamente no sistema nervoso autônomo, principalmente no sistema simpático. A partir destes efeitos diretos e indiretos, algumas consequências patofisiológicas podem ser encontradas, como contratilidade ou

relaxamento miocárdico alterado, alterações na pressão de pulso, redução do débito cardíaco, fração de ejeção diminuída, bradicardia e redução do volume diastólico final (KLEIN; OJAMAA, 2001).

2 FUNDAMENTAÇÃO TEÓRICA

2.1 Hemodinâmica

A homeostasia do organismo depende de uma perfusão tecidual adequada e constante que atenda a demanda metabólica de cada região. A manutenção do fluxo sanguíneo corporal é garantida pelas estratégias locais de perfusão sanguínea, pelo débito cardíaco e mecanismos regulatórios. Dessa forma, existe um sincronismo extremamente elegante com as fases do ciclo cardíaco (sístole e diástole). A PA é o resultado do débito cardíaco pela resistência vascular periférica. O débito cardíaco por sua vez depende de mais três variáveis: o volume diastólico final, a contratilidade miocárdica e frequência cardíaca. Esses parâmetros são continuamente ajustados ao longo do dia, para que a PA possa permanecer dentro de limites aceitáveis, mesmo após a realização de diferentes atividades que possam alterar o fluxo sanguíneo (DAMPNEY et al., 2002). A disfunção mesmo de pequenas partes do grande sistema de controle da PA pode acarretar em diversas doenças cardiovasculares importantes (GUYENET, 2006).

Dentre os mecanismos regulatórios da PA, estão os mecanismos a curto prazo, que são ativados dentro de segundos a minutos, como a atividade central autonômica e os barorreceptores periféricos que detectam rapidamente mudanças bruscas nos níveis pressóricos; e os mecanismos a longo-prazo, que depende da ação conjunta de hormônios e do sistema nervoso simpático, ambos são extremamente necessários para garantir a perfusão ideal de tecidos e órgãos (DAMPNEY et al., 2002; IRIGOYEN et al., 2001; TAYLOR et al., 2014). Os barorreceptores são mecanorreceptores que detectam distensões vasculares quando a PA aumenta ou diminui. Quando essa sensibilidade é comprometida, há correlação com o aumento da mortalidade em pacientes com insuficiência cardíaca crônica (ICC) e após infarto agudo do miocárdio (IAM), além do aumento da incidência de acidentes vasculares encefálicos hemorrágicos (AVEh) e isquêmicos (AVEi) (LA ROVERE et al., 2001; 2009; TAYLOR et al., 2014).

2.2 Glândula Tireoide

A glândula tireóide regula diversos processos metabólicos importantes no organismo através da produção de seus hormônios. O desenvolvimento estrutural da glândula tireóide se

inicia na terceira semana de vida intra-uterina do embrião, sendo a primeira glândula endócrina a se desenvolver em seres humanos (SENESE et al., 2014). O hormônio tetraiodotironina (T4) é o principal produto da secreção total da tiréoide e age como pró-hormônio para formação do triiodotironina (T3), o hormônio biológicamente ativo (BOELAERT; FRANKLYN, 2005; SENESE et al., 2014). Sabe-se que apenas 20% do T3 disponível na circulação é secretado diretamente pela própria glândula (BOELAERT; FRANKLYN, 2005; SENESE et al., 2014). A regulação da produção dos hormônios tireoidianos (HT) é realizada pela ação tireotrofina sérica (TSH) sintetizada pela hipófise anterior em resposta ao hormônio liberador de TSH (TRH), que por sua vez é secretado pelo hipotálamo. Os HTs regulam funções celulares através de atividade em receptores nucleares ou também em receptores não-nucleares, como na membrana plasmática, citoplasma e algumas organelas (SENESE et al., 2014). Os processos regulados pelos HTs incluem o desenvolvimento de órgaos e tecidos, crescimento corporal e metabolismo. Diversos estudos já demonstraram os efeitos da deficiência de iodo ou de distúrbios nos níveis de HTs, no desenvolvimento fetal, na infância e na vida adulta (BRAVERMAN; COOPER, 2012). No coração, a Triiodotironina (conhecido como T3), é o HT que medeia os efeitos mais importantes, os quais são: aumento da força e velocidade de contração sistólica e velocidade do relaxamento diastólico; reduz a resistência vascular, assim como tônus coronário; e, aumenta angiogênese arteriolar coronária (KLEIN; OJAMAA, 2001).

A tireoide sofre alterações importantes durante o período gestacional. No primeiro trimestre da gestação, a gonadotrofina coriônica (hCG) estimula diretamente a glândula tireoide a produzir mais hormônios T3 e T4, levando ao decaimento dos níveis do hormônio estimulante da tireoide (TSH), devido ao feedback negativo do eixo hipotálamo-hipófise-tireoide (eHHT). Além dos estímulos do hCG, há também elevação dos níveis de hormônios tireoidianos maternos por influencia da elevação dos níveis de estrógenos da mulher, o que ocorre durante toda a gestação (GLINOER, 1999). Levando em consideração que o feto utiliza os hormonios tireoidianos da mãe enquanto sua propria glandula não está completamente formada e funcional (SANTISTEBAN; BERNAL, 2005), o Ministerio da Saúde do Brasil (2014) recomenda que a ingestão de iodo pela mãe seja adaptada. Normalmente, recomenda-se que pelo menos 150ug/dia de iodo façam parte da dieta diária de indivíduos normais. Para gestantes, a ingestão diária precisa ser dobrada. E em ratos, a glândula madura está instalada e com plena função apenas no 17º dia de gestação. No entanto, os hormônios da tireoide são secretados apenas no 20º dia (FISHER et al., 1977).

Para induzir o hipotireoidismo durante a gestação, alguns medicamentos antitireoidianos podem ser usados, ao invés da tireoidectomia. Um desses medicamentos é o

metilmercaptoimidazol (metimazol, MMI, Sigma-Aldrich, Saint Louis, MO, EUA), inibidor da tireoperoxidase, que atua evitando a oxidação central do iodo e, assim, a incorporação de mesmo à tireoglobulina (Abuid e Larsen, 1974), inibindo diretamente a síntese de T4 e, conseqüentemente, de T3 (Mookadam et al., 2004).

2.3 *Programming Fetal*

Durante o período de rápido crescimento, o desenvolvimento do embrião ou do feto está extremamente susceptível a influências de alterações no ambiente materno. Estímulos ambientais adversos podem perturbar os processos de proliferação e diferenciação celulares levando a mudanças no curso normal da maturação de órgãos e tecidos. Como exemplos destes estímulos adversos estão: fatores nutricionais (MCMILLEN, 2005), agentes estressores psicológicos e fisiológicos (LAZINSKI et al., 2008) e exposição materna a substâncias químicas tóxicas, estilo de vida e intervenções médicas durante a gravidez (PADMANABHAN et al., 2016), além de possíveis desequilíbrios na sinalização endócrina entre mãe e feto (SECKL, 2004; WEAVER et al., 2004). Em 1912 foi demonstrado pela primeira vez a influência dos hormônios tireoidianos no desenvolvimento de vertebrados. O experimento consistia em alimentar girinos com tecido tireoideo dissecado de cavalos para avaliar o desenvolvimento destes animais. Como resultado, estes girinos desenvolviam-se em sapos precocemente (GUDERNATSCH, 1912).

No contexto de doenças programadas durante a gestação, a hiperglicemia também é objeto de estudo. Estudos demonstram que o estado hiperglicêmico durante a gestação pode trazer consequências para a prole, como desenvolvimento de hipertensão, disfunção de barorreflexo e hiperativação do Sistema-Renina-Angiotensina-Aldosterona (SRAA) (WICHI et al., 2005). Além disso, a obesidade e diabetes também traz alterações do fenótipo da prole na vida adulta. Como evidencia, pesquisas mostram que crianças filhas de mães obesas e diabéticas, possuem duas vezes mais chance de desenvolver as mesmas doenças na vida adulta (BONEY et al., 2005). A placenta pode ser a responsável por expressar proteínas responsaveis por estresse oxidativo, inflamação e apoptose em grávidas obesas (OLIVA et al., 2012).

Se descompensado, o hipotireoidismo gestacional acarreta em consequências graves para a gestante e para o feto, como hipertensão, hemorragia pós-parto, anemia, disfunção cardíaca ventricular, aborto espontâneo, morte fetal, baixo peso ao nascer e desenvolvimento cerebral anormal da criança (HADDOW et al., 1999; ALMEIDA et al., 2007). O disturbio metabolico pode afetar diretamente o feto pois suas celulas trofoblasticas expressam transportadores e receptores dos hormonios tireoidianos, sendo necessários para placentação

adequada, o que é de extrema importancia para garantir nutrição fetal (BARBER et al., 2005; AGHAJANOVA et al., 2011; CARTER, 2012; GUTTMACHER; MADDOX; SPONG, 2014).

Ainda existem poucos estudos relevantes buscando elucidar as consequências do hipotireoidismo materno na saúde cardiovascular de indivíduos adultos (Heikkinen et al., 2017). Inúmeros estudos têm investigado a influência do hipotireoidismo experimental sobre o comportamento materno e suas repercussões na prole. Repercussões estas, focadas sobre o comportamento ingestivo, perfil metabólico e até mesmo sobre a regulação sensorial nociceptiva.

3 OBJETIVO GERAL

O objetivo deste estudo foi avaliar as repercussões cardiovasculares do hipotireoidismo gestacional na prole adulta.

3.1 Objetivos Específicos

Avaliar os efeitos do hipotireoidismo materno sobre:

a. O peso corpóreo na prole adulta;
b. Parâmetros basais da frequência cardíaca na prole adulta;
c. Parâmetros hemodinâmicos basais (pressão arterial média, sistólica e diastólica) na prole adulta;
d. Atividade intrínseca e extrínseca do controle autonômico cardíaco tônico:
    - Frequência cardíaca intrínseca;
    - Controle autonômico da frequência cardíaca.
e. Atividade do controle reflexo cardíaco:
    - Sensibilidade barorreflexa espontânea.

4 ARTIGO

CHANGES IN THE AUTONOMIC CONTROL OF CARDIOVASCULAR FUNCTIONING IN ADULTHOOD AS A RESULT OF GESTATIONAL HYPOTHYRODISM DURING FETAL LIFE

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ABSTRACT: Currently, it is known that internal and external stimuli to the intrauterine environment during fetal development can have important physiological consequences in the individual's adult life. Several studies have shown that different diseases in adulthood may have their origin in the early stages of life. A decrease in maternal thyroid hormone levels, as in gestational hypothyroidism (GHT) cases and during the lactation period, is related to problems in fetal heart development and long-term cardiovascular diseases. However, the mechanisms behind these effects are not yet fully understood. Therefore, the aim of this work was to evaluate the cardiovascular consequences in adulthood caused by GHT during gestation and lactation periods. We investigated this by measuring the arterial pressure, heart rate, baroreflex function and autonomic cardiovascular control of adult Wistar rats that underwent fetal and neonatal GHT. As results, we found that the offspring of rats with induced hypothyroidism during pregnancy and/or lactation showed significantly less weight gain in the first 90 days after birth. These rats also presented an increase in both mean arterial pressure and systolic blood pressure, in addition to an increase of the sympathovagal index when compared to the control group. Also, they presented a reduction on the barorreflex function in face of a pharmacological challenge, showing that they have a predominance of sympathetic function both in heart and in vessels. In conclusion, our results showed that maternal thyroid hormone deficiency can chronically produce changes in cardiovascular function in the adult life of the offspring.

Keywords: arterial pressure, baroreflex, heart rate, gestational hypothyroidism, cardiovascular diseases, sympathovagal index.

1. Introduction

Fetal period and the respective conditions for intrauterine maturation are closely correlated with physiological dynamics and with the risk of developing diseases during adulthood (Langley-Evans, 2006). Several studies support the hypothesis that intrauterine gestational conditions may set the pathophysiology of many diseases diagnosed during adulthood, such as diabetes, hyperlipidemia, obesity and cardiovascular diseases, especially arterial hypertension. Following on from this growing body of evidence, 'fetal programming' concept emerged at 90's (Godfrey and Barker, 2001; Shan and Teng, 2019).

During the period of rapid growing, embryo or fetal development is susceptible to external/environmental influences reflecting maternal life. A diverse range of stimuli may unsettle cell proliferation and differentiation processes, thus modifying the regular paths underlying the adequate maturation of organs and tissues. Fetal development mainly depends on the genetic apparatus (York et al., 2014) and on aspects related to nutritional, hormonal and metabolic challenges at this intrauterine phase (Tzanetakou, 2011). In the light of this aspects, thyroid hormones (TH) are pivotal to the development and growing throughout the lifetime (Bourguignon and Parent, 2010).

Thyroid gland and the respective TH are protagonists in the regulation of metabolism. The development of this glandular tissue is noticed from the third week of embryos' life. In humans, is the first of the body's endocrine glands to develop (Senese et al., 2014), and in rats, the mature gland is in place and with full function only at the 17th day of gestation. However, thyroid hormones are only secreted on the 20th day (Fisher et al., 1977). Tetraiodothyronine (T4) is the main output thyroid hormone and acts as a pro-hormone used as substrate for triiodothyronine (T3) production, being the latter in charge of the TH effects. However, it is known that only 20% of the available systemic T3 is secreted daily by the gland (Boelaert & Franklyn, 2005; Senese et al., 2014). The HT regulation results from feedback loops among thyroid, hypophysis and hypothalamus. Hypophyseal thyrotrophin produced by adenohypophysis in response to hypothalamic thyrotrophin releasing hormone (TRH) reaches systemic circulation (TRH). This hormonal axis regulates TH, which bind to target receptors located at nucleus, cell membranes and in organelles (Senese et al., 2014). Among the main effects displayed by TH at peripheral level, it is worth highlighting organ and tissues trophism and metabolism. Literature comprises the effects of iodine and HT unbalances on fetal, childhood and adulthood periods (Braverman & Cooper, 2012). Converse to found during hyperthyroidism, hypothyroidism is responsible for low metabolic rates that reflect TH deficiency, as result from low TH outputs from thyroid (Biondi and Klein, 2004). While the mild levels of hypothyroidism are likely subclinical, the severe forms display hallmarking symptoms (Biondi and Klein, 2004). TH specific receptors (TR) are expressed in cardiomyocytes, mainly its TRα isoform. Changes in thyroid function are linked to cardiac morphophysiological alterations cardiac trophism and function such, diastolic dysfunction, reduction in ejection and other features likely found in cardiac hypertrophy and failure. Vascular changes also count: TH alterations are able to affect vascular stiffness and vasomotion, thus modifying peripheral resistance and tissue perfusion (Biondi and Klein, 2004; Grais and Sowers, 2014; Udovcic et al., 2018).

Hypothyroidism is frequent during human pregnancy (Shan and Teng, 2019). Gestational hypothyroidism (GHT) is a clinical condition whose incidence reaches 2% to 5% (approximately 200 out of 9,000) of pregnant women (Teng et al., 2013). Besides impacting pregnant health, this low TH levels of GHT may affect fetal development, which may evoke latter effect upon subject's health throughout the life after birth. Transporters and receptors for TH are expressed in trophoblastic cells of the embryo. TH adequate levels are required for an ideal placentation, since it is needed for fetal nutrition, gas exchange and metabolite excretion (Aghajanova et al., 2011; Barber et al., 2005; Carter, 2012; Guttmacher et al., 2014). Mother's TH are necessary for an adequate fetal development, in special during the period that the gland is yet absent or immature (Santisteban and Bernal, 2005).

The effects of gestational hypothyroidism upon fetal health was assessed in different organs and functions of humans and experimental models (Chattergoon et al., 2012; Karbalaei et al., 2013). Reductions in serum TH levels during intrauterine life of rodents results in lower weight gain and cardiac diseases in the offspring life (Ghanbari et al., 2015; Thornburg, 2011). Another study found that GHT is able to affect proteins related to cardiac inotropy 30 days after birth (Chizzonite and Zak, 1984). Ghanbari and coworkers found alterations in cardiac ventricular pressure dynamics and in the measures of contractility and relaxation during GHT (Ghanbari et al., 2015). However, whether these autonomic components would contribute to these cardiovascular consequences remained to be unraveled. Therefore, we next investigated cardiovascular consequences during adulthood caused by GHT. To achieve this aim, we measured arterial pressure, heart rate, spontaneous baroreflex function and autonomic control of heartbeat of adult rats that underwent fetal GHT.

In order to induce hypothyroidism during gestation, some antithyroid drugs can be used, instead of performing thyroidectomy. One of this drugs is the methylmercaptoimidazole (methymazole, MMI, Sigma-Aldrich, Saint

Louis, MO, USA) and it inhibits thyroperoxidase, which acts by preventing the central oxidation of iodide and, thus, the incorporation of iodine into thyroglobulin (Abuid and Larsen, 1974), directly inhibiting the synthesis of T4 and, consequently, that of T3 (Mookadam et al., 2004).

2. Materials and methods

2.1. Animals and induction of gestational hypothyroidism

The animals were obtained from the Rodent Biottery Network (REBIR) of the Federal University of Uberlândia (UFU) and kept in isolated box under light/dark cycle control (12/12h) and room temperature (23±2 °C). The animals had free access to water and food. Female Wistar rats (~ 200 g) had their estrous cycle monitored daily through a vaginal smear. Once the proestrus phase was detected, adult males (~ 300 g) were placed in cages for mating at night (2 females / male). The following day, the females were returned to their original boxes and subjected to a vaginal smear to check for a possible pregnancy, due to the presence of sperm. The pregnancy rats were separated into individual boxes and their male offspring rats divided into the following groups: *a*. Control (offspring treated with water during the mother's gestation period, n = 10), *b*. OMG (offspring treated with methimazole during the mother's gestation period, n = 8), *c*. OML (offspring treated with methimazole during the mother's lactation period, n = 9) and *d*. OMGL (offspring treated with methimazole during the mother's pregnancy and lactation period, n = 10). It is important to note that the number of animals varied according to the experimental protocols used. For the induction of gestational hypothyroidism (Ahmed et al., 2010; Ahmed et al., 2012), pregnant rats received 0.02% methylmercaptoimidazole (methymazole, MMI, Sigma-Aldrich, Saint Louis, MO, USA) in the drink water. The MMI offer period was according to the experimental groups. After delivery, the litter was adapted to only eight animals/ mother until the weaning period (21 days post-natal). Offspring's treated with MMI at different times during pregnancy and breastfeeding were compared with the corresponding control offspring. All procedures are in accordance with the Animal Research and Ethics Committee (CEUA) of the Federal University of Uberlândia (Protocol 071/18), which works in accordance with the rules of the National Council for the Control of Animal Experimentation (CONCEA) and the international guiding principles for biomedical research involving animals. All efforts were made to minimize the suffering of the animals and to reduce the number of animals used.

2.2. Surgical procedure and acquisition of biological data

At 90 days, the offspring were submitted to anesthesia with ketamine (100 mg / kg, i.p) and xylazine (14 mg / kg, i.p.) and then catheterization of the left femoral artery and vein was performed, as described by Oliveira (2018) for the registration of cardiovascular parameters, drug administration respectively. After catheterization surgeries, Pentabiotic (200 mg / kg; Fort Dodge, Brazil, i.m.) and Flunixin (2.5 mg / kg, i.m.) were administered intramuscularly to prevent possible infections and pain. After 24 hours of catheterization, the non-anesthetized animals were connected to a data acquisition system in which the arterial catheter was connected to a PA transducer "Disposable BP Transducer (no stopcock) MLT0699" (ADInstruments, Colorado Springs, CO, USA) , connected to a signal amplifier "Bridge Amp FE221" (ADInstruments, Colorado Springs, CO, USA) and connected to an analog / digital converter "PowerLab 1766 8/35" (ADInstruments, Colorado Springs, CO, USA) with communication to a computer containing the "LabChart v.7.3.8" interface software (ADInstruments, Colorado Springs, CO, USA). Pulsatile blood pressure (PAP), mean arterial pressure (MAP), systolic blood pressure (SBP), diastolic blood pressure (DBP) and heart rate (HR) were recorded simultaneously. MAP was calculated from PAP as 1/3 of SBP plus 2/3 of DBP), whereas SBP and DBP were calculated as the average of the maximum and minimum PAP peak respectively. HR was calculated by averaging pulse intervals (PI) in milliseconds multiplied by one minute in milliseconds or 60,000 ms. The software captured the PA signals with a sampling frequency of 1000 Hz. All variables were recorded simultaneously for a period of 15 minutes for analysis of hemodynamic values (MAP, SAP, DAP and HR) and to study the variability of HR and PAS. At the end of this baseline registration period, the animals were submitted to pharmacological challenges to study the autonomic components and reflex activity of the baroreceptor.

2.3. Heart Rate, Systolic blood pressure and Spontaneous Baroreflex Activity

From the baseline resting records of 15 minutes of BP and pulse interval, we determined a period of 10 minutes without artifacts for analysis in the CardioSeries software (v2.4, http://sites.google.com/site / cardioseries). The program provides analysis in the frequency domain, allowing adjustments in the interpolation rate (10 Hz), segment length (512 points) and the limits of the frequency bands (VLF-0.00 to 0.20 / LF-0, 20 to 0.75 / HF-0.75

to 3.00) for HR and BP variability. We also performed through the software the analysis of the sequence method that consists of evaluating stretches of beats in sequence where they describe ascending ramps (increases PA and PI) or descending ramps (decreases PA and PI) allowing adjustments on the minimum change in BP (0.5 mmHg), minimum change in PI (we use 0.5 ms), minimum cycles in sequence (3 beats), beat delay rate (0 beats) and r index for inclusion (0.80). We evaluated and used the HR variability from the PI, the BP from the SBP and the baroreflex gain analyzed by the sequence method. After selecting the record section, manual filtering was performed to eliminate premature ectopic beats and artifacts. The calculations were automatically performed by the Cardioseries software and the results were made available in text boxes with the nomenclature and the unit. Later, they were grouped and treated for graphic production.

2.4. Baroreflex Study

The first pharmacological challenge was the baroreflex sensitivity test using Phenylephrine (α1-adrenergic agonist; 6 μg / kg; Sigma Aldrich, St. Louis, Missouri, USA; i.v.) to promote increased BP and reflex bradycardia, and Nitroprusside of Sodium (nitric oxide donor; 32 μg / kg; Sigma Aldrich, St. Louis, Missouri, USA; i.v.), to promote BP reduction and reflex tachycardia. The drugs were administered with a 5 min time interval between administrations, so that there was time to observe the pharmacological response and to prevent interference from the responses of one drug to the responses of the next drug. At the end of the records, the data were analyzed by extracting values referring to ΔMAP and ΔHR (calculated by the difference of the mean of 30" before and the peak response after pharmacological administration) and the Baroreflex Index (calculated by the quotient of ΔHR by ΔMAP).

2.5. Study of Autonomic Tone

The second challenge was to assess sympathovagal activity and was assessed by double autonomic blockade (DAB) using methylatropine (cholinergic antagonist; 3 mg / kg; Sigma Aldrich, St. Louis, Missouri, USA; iv) to evaluate only the sympathetic activity on the heart, and Metoprolol (β1-adrenergic antagonist; 5 mg / kg; Sigma Aldrich, St. Louis, Missouri, USA; iv) to assess only parasympathetic activity on the heart. Drugs were administered with a 15 min time interval between administrations. This, so that there was time to observe the pharmacological response and, after the combination of the two drugs, the intrinsic heart rate (IHR) was acquired. The sequence of intravenous drug administration was altered between the 24 and 48 hour records. More specifically, the vagal sympathetic index (VSI) was calculated using the ratio between baseline HR and iHR.

2.6. Statistical analysis

The data were presented as means ± SEM. Statistical analyzes were performed using the Prism software (v.8; GraphPad Software, Inc., San Diego CA, USA). All results were statistically evaluated by the one-way and/or two-way ANOVA variance test followed by the Tukey post-test. In all cases, a significant difference was considered for values of $p < 0.05$.

3. Results

The newborn body weight was not different when compare the experimental groups to control (Control: 7.7±0,35 g, vs OMG: 7.08±0,30 g, vs OML: 7.7±0,51 g, vs OMGL: 6.44±0,24 g, Figure 1). After 90 days, the OMGL group showed less weight gain when compared to the control and OML group (Control: 355.5±10,19 g, vs OMG: 325,0±4,5 g, vs OML 334.7±6,03 g, vs OMGL: 312,23±3,31 g, Figure 1). In Fig. 2A, it is shown that all experimental groups showed no changes in baseline heart rate levels when compared to control group (Control: 398.7±10,9 bpm, vs OMG: 399.5±12.2 bpm, vs OML: 417.5±10.5 bpm, vs OMGL: 390.2±6.0 bpm). Differently, increases in basal MAP (Control: 106.3±1.3 mmHg, vs OMG: 117.7±2.23 mmHg, vs OML: 110.8±2.16 mmHg, vs OMGL: 118.2±1.8 mmHg, Fig. 2B) and SAP (Control: 138.9±1.7 mmHg vs OMG: 147.8±2.0 mmHg vs OML: 141.7±2.9 mmHg vs OMGL: 150.1±2.0 mmHg, Fig. 2C) was evidenced on groups OMG and OMGL. DAP (Control: 92.8±2.1 mmHg vs OMG: 101.9±1.40 mmHg vs OML: 100.6±4.30 mmHg vs OMGL: 100.3±2.5 mmHg, Fig. 2D) was not different in experimental groups, when compared to Control rats.

In the figure 3, we wanted to evaluate the participation of autonomic component on cardiac regulation. For this, we focus our interest on the behavior of heart rate after vagal blockade with atropine sulfate (3 mg / kg), followed of the sympathetic blockade with methoprolol (5 mg / kg). These procedures make possible to evidence the vagal and sympathetic effects and tonus on the heart and intrinsic heart rate. The Figure 3A shows that the sympathovagal index in OMG and OMGL animals presented a significantly increased then compared to Control animals (Control: 1.03±0.02, vs OMG: 1.24±0.02, vs OMGL, 1.30±0.03, $p<0,05$), while in the OML group there

were no differences in relation to the control (Control: 1.03±0.02, vs. OML: 1.05±0.02, p<0,05). About the intrinsic heart rate (IHR) we observed that there was no difference between the experimental groups (Control: 356.9 ± 8.10 bpm, vs OMG: 358.2 ± 11.5 bpm, vs OML: 359.9 ± 8.3 bpm, vs OMGL: 327 ± 4.5 bpm; Figure 3B). Analyzing the baroreflex index (BI; (ΔHR/ΔMAP, bpm/mmHg) by means of the bradycardia and tachycardia response after infusion of phenylephrine and sodium nitroprusside, we observed a reduction in BI only after the phenylephrine infusion (Control: -2.04±0.15 bpm/mmHg vs OMG: -1.61±0.10 bpm/mmHg vs OML: -1.85±0.09 bpm/mmHg vs OMGL: -1.05±0.12 bpm/mmHg, Figure 4A). The tachycardia response to sodium nitroprusside infusion was not different when compared the experimental groups to control.

As shown in Figure 5, OMGL rats showed differences in the power of the LF band (normalized units, Control: 29.56±1.94 *vs* OMGL: 40.5±1.43; Fig. 5A, p<0,05) and HF band (normalized units, Control: 70.40±1.9 *vs* OMGL: 59.5±1.4; Fig. 5B, p<0,05) of interval pulse spectrum, as well as in LF/HF (Control: 0.41±0.04 *vs* OMGL: 0.63±0.04; Fig. 5D, p<0,05) when compared to control or other experimental groups. However, the HF band (absolute units, Control: 4.22±0.58 *vs* OMGL: 4.21±0.76; Fig. 5C) is not different. The power of the LF band of SAP spectrum was found higher in OMGL rats when compared the other experimental groups (Control: 5.30±0.15 mmHg2 vs OMG: 6.89±0.4 mmHg2, OML: 5.9±0.5 mmHg2 vs OMGL: 8.29±0.4 mmHg2; Fig. 6E) while the effectiveness of the baroreflex (BEI) has not been modified.

4. Discussion and conclusions

The results obtained in this study showed that thyroid hormone deficiency, especially during intrauterine life and the 21-day lactation period of the offspring, can permanently produce changes in cardiovascular function in the adult life of the offspring. We also observed that the body weight gain was lower starting from 60 days of life in all groups analyzed.

All of these changes suggest that hypothyroidism during these phases can alter the functioning of one or more organs of this system (ie, heart, vessels and / or autonomic nervous system). Regarding weight gain, our results corroborate previous findings, where children of mothers who received propylthiouracil (PTU) during pregnancy did not obtain similar weight gain when purchased from the respective control groups (Behzadi and Ganji, 2005; Rohani et al., 2009; Sawin et al., 1998). Observing the hemodynamic results, we demonstrated that OMG and OMGL rats with 90 days of age have high mean arterial and systolic blood pressure, however, the baseline levels of diastolic blood pressure and heart rate were not altered. It has been shown that decreased thyroid function promotes changes in cardiovascular function, thus compromising cardiac contractility, cardiac output, peripheral vascular resistance, and cardiac chronotropic function. (Kisso et al., 2008). It is important to emphasize that this study evaluated the chronic effects of hypothyroidism in female rats, with the addition of propylthiouracil (PTU). In 2012, Santos et al., evaluated for the first time the cardiovascular changes produced by the deficiency of maternal thyroid hormones in adult offspring. In this study, the authors showed systemic arterial hypertension associated with bradycardia at rest, as well as an increase in the low-frequency component of systolic blood pressure by analyzing the spectral component of blood pressure, demonstrating an increase in sympathetic tone in the vessels. This characterized a justification for the increase in blood pressure levels. Our findings are compatible with this study, however, we added new information to the literature, as, for the first time, we demonstrated that in addition to the increase in the baseline pressure response, there is an increase in cardiac sympathetic tonic activity and a decrease in baroreflex sensitivity in OMG and OMGL rats.

Other important information was evidenced with the increase of the low frequency component (sympathetic) and decrease of the high frequency component (parasympathetic) as well as the LF / HF ratio that represents the sympathovagal balance. These data were confirmed by the double autonomic block. All these observed responses would justify the hemodynamic changes found. In this sense, it has been reinforced that HT deficiency leads to reduced sensitivity and responses to catecholamines, which together promote the reduction of the chronotropic and inotropic function of the heart (Arioglu et al., 2010). It is known that T3 acts directly on cardiomyocytes, altering cardiac function, regardless of its action on peripheral vessels (Danzi and Klein, 2012; Morkin, 1993). In our study, we did not observe changes in baseline heart rate levels, possibly due to the reestablishment of hormone levels as adults. Other characteristics of T3 are modular and / or determine vascular relaxation. This effect has been linked to increased expression of a critical enzyme for the synthesis of an important gas vasodilator factor, nitric oxide (NO) (ie, nitric oxide synthase; NOS) (McAllister et al., 2005; Quesada et al., 2002). On the other hand, the lack of T3 leads to increased peripheral vascular resistance due to reduced NOS expression, which can contribute to the genesis of hypertension (Moulakakis et al., 2008). In conclusion, we demonstrated for the first time that experimental hypothyroidism, during pregnancy and lactation, produces different responses when we analyze each experimental treatment situation separately. In addition to the

hemodynamic changes described in previous studies, we observed that there were changes in the autonomic balance in baseline conditions as well as in situations of pharmacological challenges. In addition to tonic changes, we have shown changes in the reflex component of blood pressure.

5. Conflicts of interest

The authors declare that they have no conflicts of interest.

6. Acknowledgments

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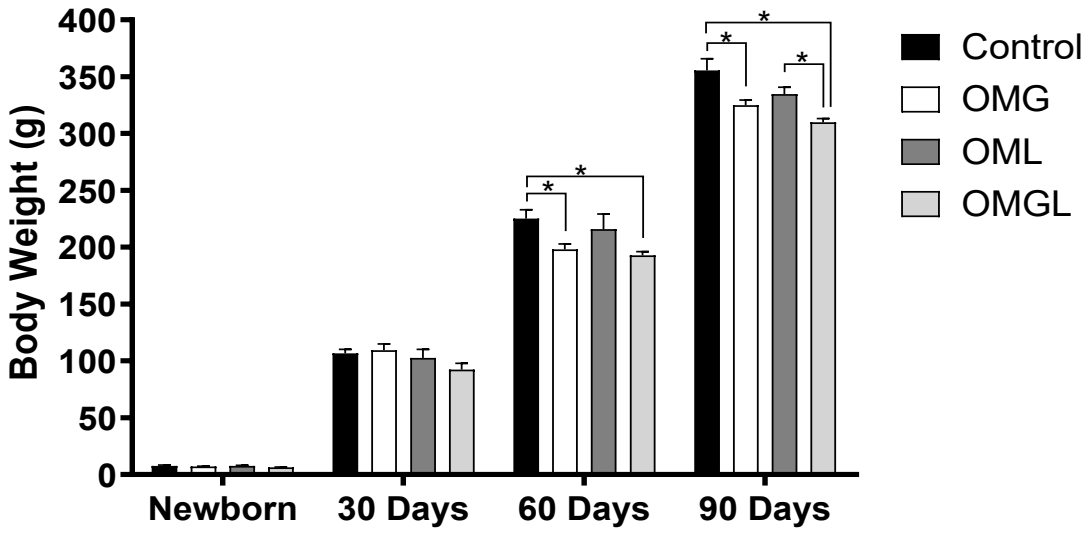

Fig. 1. Effect of gestational hypothyroidism (GHT) on the body weight of offspring (at newborn, 30, 60 and 90 days old). Offspring treated with water during the mother's gestation period (Control, n = 8), offspring treated with methimazole during the mother's gestation period (OMG, n = 6), offspring treated with methimazole during the mother's lactation period (OML, n = 8) and offspring treated with methimazole during the mother's pregnancy and lactation period (OMGL, n = 8). Data presented as means ± SEM, (*) $p<0,05$. One-way and two-way ANOVA was used followed by the Tukey post-test.

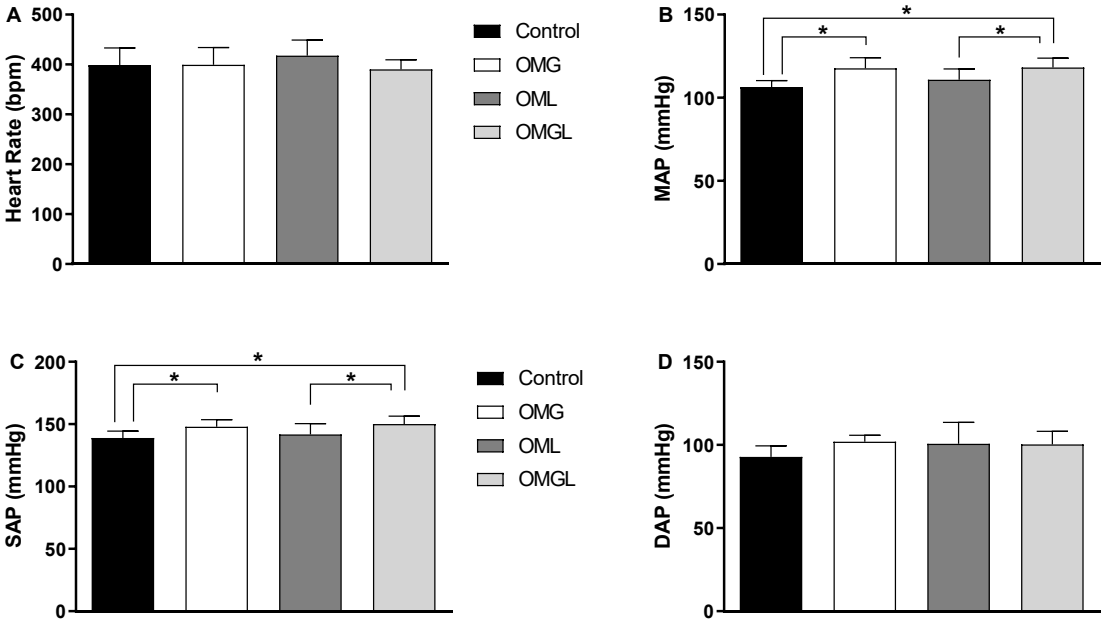

Fig. 2. Effect of gestational hypothyroidism (GHT) on the mean arterial pressure (MAP, A), heart rate (HR, B), systolic arterial pressure (SAP, C) and diastolic arterial pressure (DAP, D) of 90 day old offspring. Offspring treated with water during the mother's gestation period (Control, n = 10), offspring treated with methimazole during the mother's gestation period (OMG, n = 8), offspring treated with methimazole during the mother's lactation period (OML, n = 9) and offspring treated with methimazole during the mother's pregnancy and lactation period (OMGL, n = 10). Data presented as means ± SEM, (*) $p<0,05$. One-way ANOVA was used followed by the Tukey post-test.

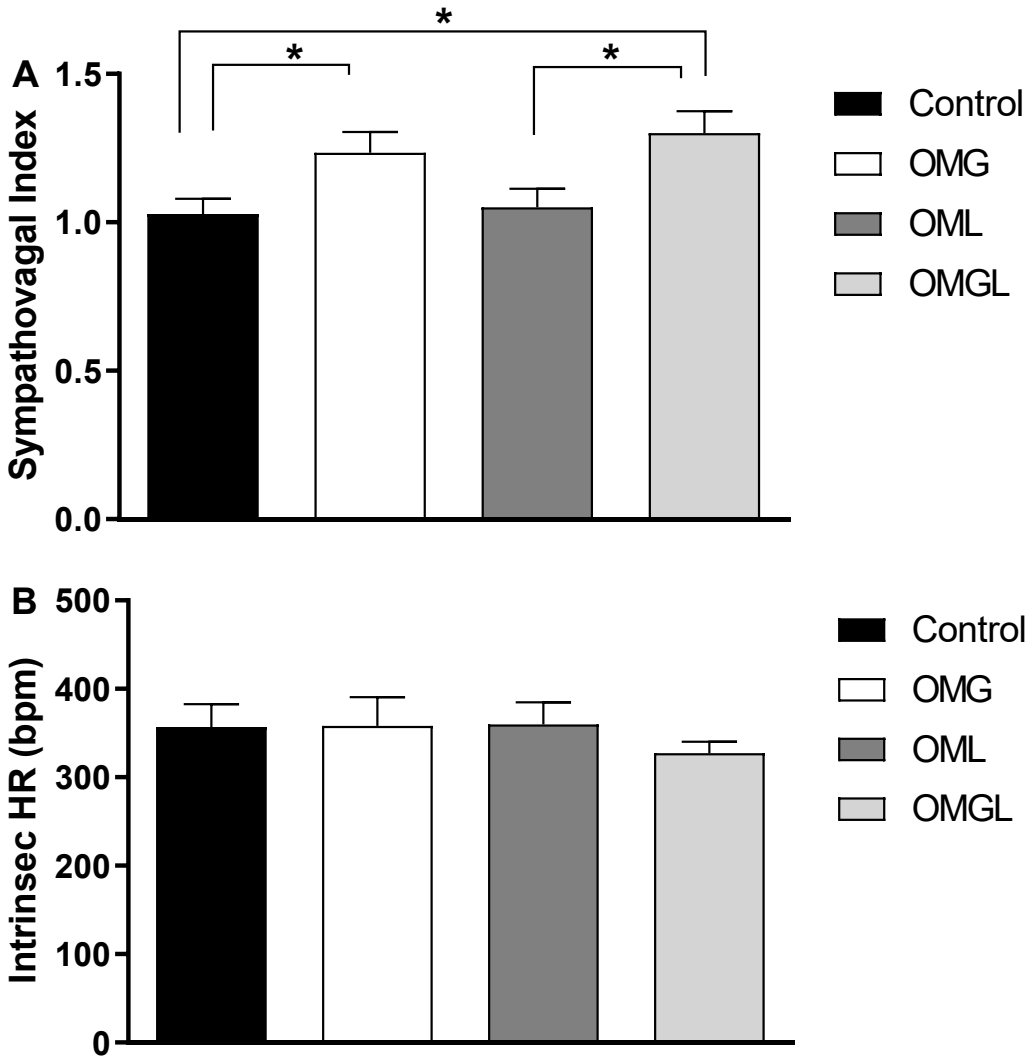

Fig. 3 Effect of gestational hypothyroidism (GHT) on the intrinsic heart rate (IHR, A) and simpa-vagal index (SVI, B) of 90 day old offspring. Offspring treated with water during the mother's gestation period (Control, n = 10), offspring treated with methimazole during the mother's gestation period (OMG, n = 8), offspring treated with methimazole during the mother's lactation period (OML, n = 9) and offspring treated with methimazole during the mother's pregnancy and lactation period (OMGL, n = 8). Data presented as means ± SEM, (*) p<0,05. One-way ANOVA was used followed by the Barlett's tes and the Tukey post-test.

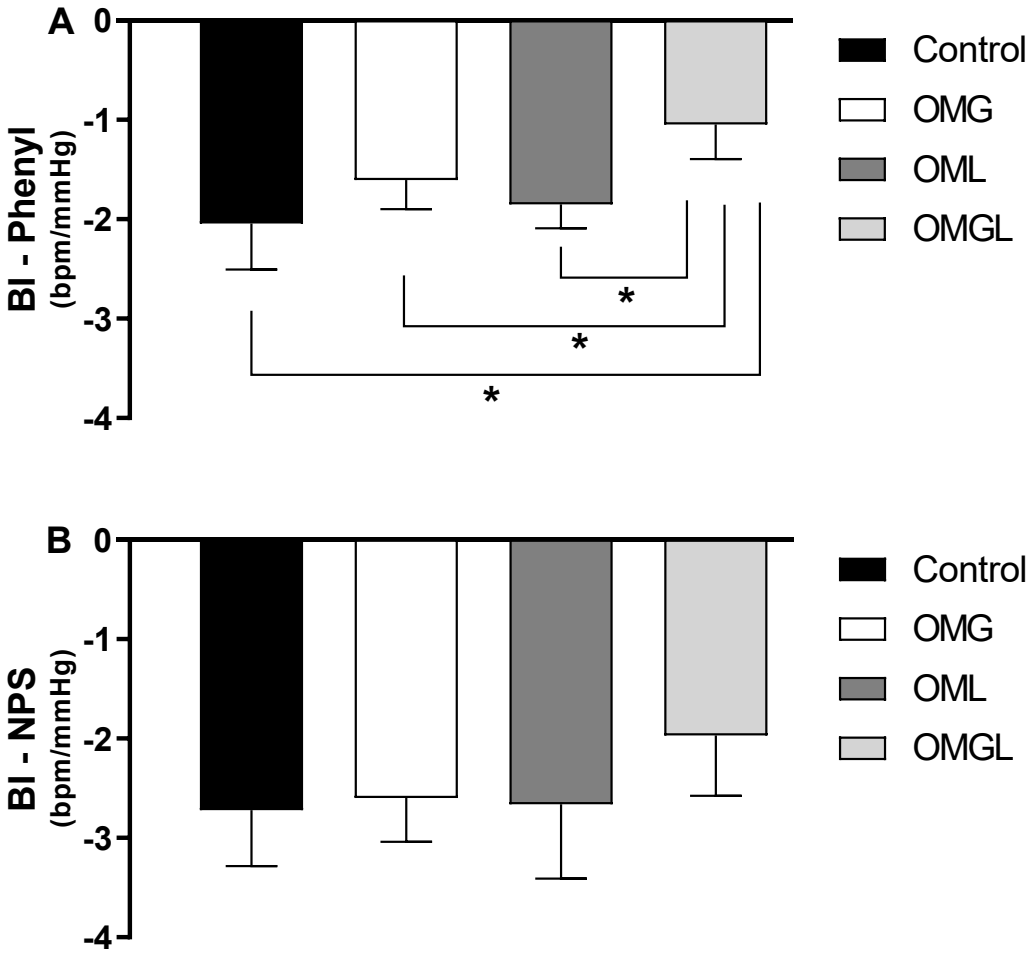

Fig. 4 Effect of gestational hypothyroidism (GHT) on baroreflex index (BI-Phenyl, A and BI-Nitro, B) of 90 day old offspring after drugs administration. Offspring treated with water during the mother's gestation period (Control, n = 10), offspring treated with methimazole during the mother's gestation period (OMG, n = 8), offspring treated with methimazole during the mother's lactation period (OML, n = 8) and offspring treated with methimazole during the mother's pregnancy and lactation period (OMGL, n = 9). Data presented as means ± SEM, (*) $p<0,05$. One-way ANOVA was used followed by the Barlett's tes and the Tukey post-test.

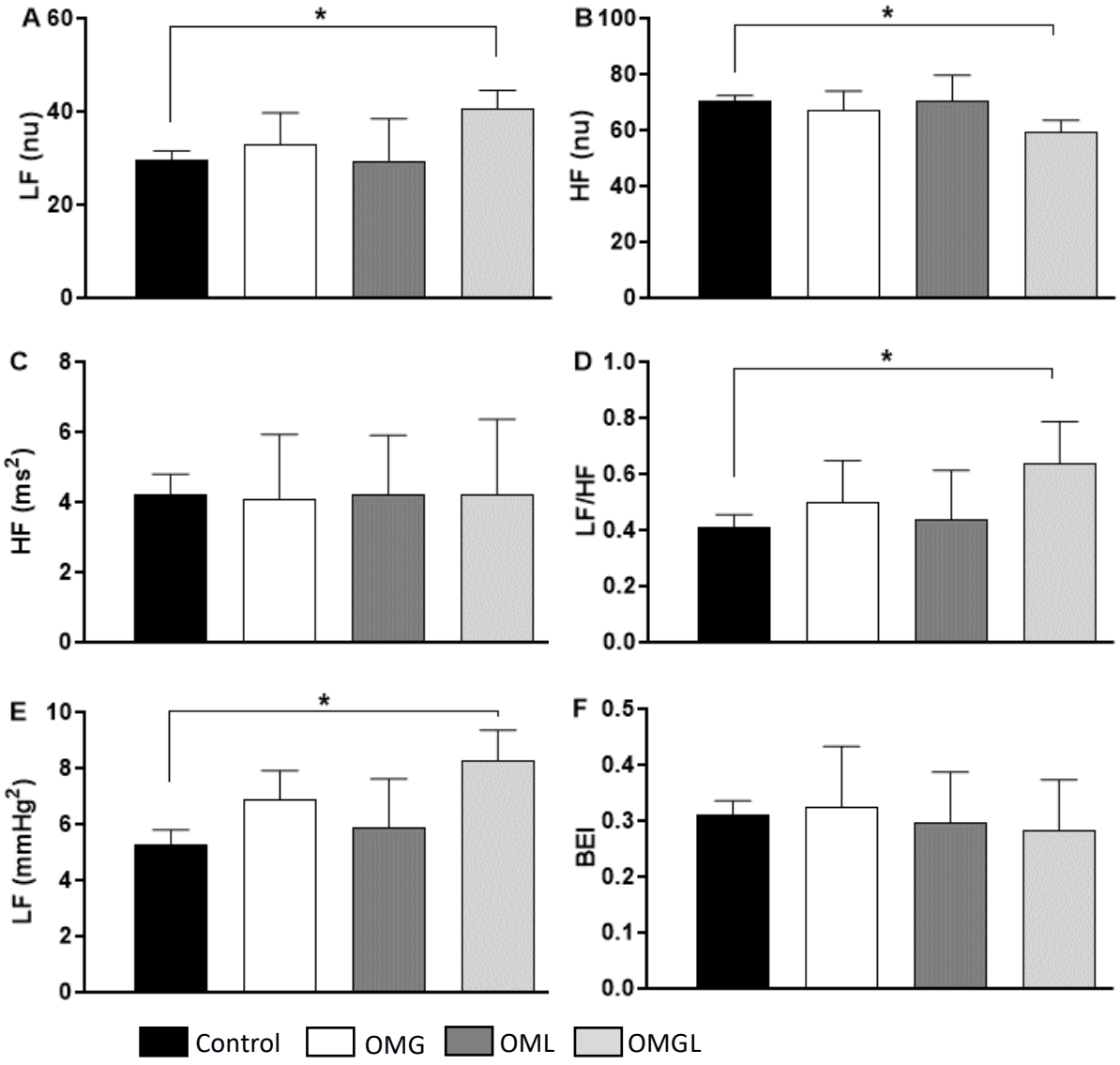

Fig. 5 Effect of gestational hypothyroidism (GHT) on heart rate's (HR) and systemic blood pressure's (SBP) variability of 90 day old offspring. Offspring treated with water during the mother's gestation period (Control, n = 9), offspring treated with methimazole during the mother's gestation period (OMG, n = 7), offspring treated with methimazole during the mother's lactation period (OML, n = 9) and offspring treated with methimazole during the mother's pregnancy and lactation period (OMGL, n = 8). Data presented as means ± SEM, (*) $p<0,05$. One-way ANOVA was used followed by the Barlett's test and the Tukey post-test.

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