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Vitor Grigio

Efeitos do óleo de coco na adrenal de gerbilos

São José do Rio Preto
2023

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Dissertação apresentada como parte dos requisitos para obtenção do título de Mestre em Biociências, área de concentração em Biologia Estrutural e Funcional, junto ao Programa de Pós-Graduação em Biociências, do Instituto de Biociências, Letras e Ciências Exatas da Universidade Estadual Paulista "Júlio de Mesquita Filho", Campus de São José do Rio Preto.

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À minha família que sempre me apoiou e acreditou em meus sonhos.

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“A ciência nos dá a melhor aproximação que o ser humano conseguiu para compreender o mundo, o que nos deu melhores meios para modificá-lo, faltando apenas mais sabedoria.”

(GILSON VOLPATO, 2019, p. 4)

RESUMO

A adrenal é uma importante glândula endócrina responsável pela síntese de diversos hormônios esteroides como glicocorticoides, mineralocorticoides, precursores de andrógenos e estrógenos. O colesterol utilizado durante a esteroidogênese para produção desses hormônios esteroides é derivado das gorduras ingeridas na alimentação. A quantidade e qualidade desses lipídios interferem na produção hormonal e o excesso de consumo de lipídios pode impactar negativamente nesse processo. Além da gordura ingerida na alimentação, atualmente, vem crescendo o consumo de óleos como fitoterápicos como é o caso do óleo de coco. Dessa forma, o objetivo do estudo foi verificar os efeitos da ingestão prolongada de óleo de coco na adrenal de gerbilos. Para isso, gerbilos da Mongólia, machos, com 3 meses de idade, foram divididos em três grupos experimentais (n=12): um grupo controle intacto que não recebeu tratamento, um grupo controle de gavagem, que foram gavados com 0,1 ml de água diariamente, e um grupo tratado com óleo de coco, que recebeu 0,1 ml de óleo de coco diariamente. Os animais foram tratados durante 12 meses e eutanasiados com 15 meses de idade. Depois de coletados, os órgãos foram submetidos a análises morfológicas, morfométricas, imuno-histoquímicas para identificação de receptores, enzimas, proliferação e morte celular, além das análises hormonais e de Western Blotting. Os resultados obtidos mostraram que o consumo prolongado de óleo de coco promoveu uma redução no acúmulo de grânulos de lipofuscina nas células do córtex da adrenal. Por outro lado, o óleo ocasionou aumento da área das células e da espessura da zona reticulada e do acúmulo de gotículas lipídicas, além de reduzir a quantidade de enzimas estereidogênicas como a CYP17, 3BHSD e 17BHSD. Foi observado também que o óleo aumenta a expressão dos receptores de estrógeno do tipo alfa. Essas alterações nos permitem concluir que as alterações na dieta lipídica podem ocasionar modificações na morfofisiologia da adrenal e consequentemente impactar na funcionalidade da glândula.

Palavras–chave: Óleo de coco. Esteroidogênese. Dieta lipídica. Adrenal. Gerbilo da Mongólia.

ABSTRACT

The adrenal is an important endocrine gland responsible for the synthesis of various steroid hormones such as glucocorticoids, mineralocorticoids, androgen precursors, and estrogens. The cholesterol used during steroidogenesis to produce these steroid hormones is derived from the fats ingested in the diet. The quantity and quality of these lipids interfere with hormone production, and excessive consumption of lipids can negatively impact this process. Besides the fat ingested in the diet, currently, the consumption of oils such as coconut oil has been increasing. Thus, the objective of this study was to verify the effects of prolonged coconut oil intake on the adrenal of gerbils. For this purpose, 3-month-old male Mongolian gerbils were divided into three experimental groups (n=12), an intact control group that received no treatment, a gavage control group, which were received, orally, 0.1 ml of water daily, and a coconut oil treated group, which received 0.1 ml of coconut oil daily. The animals were treated for 12 months and euthanized at 15 months of age. After being collected, the organs were submitted to morphological, morphometric, immunohistochemistry analyses to identify receptors, enzymes, proliferation, and cell death, as well as hormonal and Western Blotting analyses. The results obtained showed that prolonged coconut oil consumption promoted a reduction in the accumulation of lipofuscin granules in adrenal cells. On the other hand, the oil caused an increase in cell area and cross-linked zone thickness and lipid droplet accumulation, and reduced the amount of steroidogenic enzymes such as CYP17, 3BHSD, and 17BHSD. It was also observed that the oil increases the expression of estrogen receptors of the alpha type. These changes allow us to conclude that changes in the lipid diet may cause changes in adrenal morphophysiology and consequently impact the functionality of the gland.

Keywords: Coconut oil. Steroidogenesis. Lipid diet. Adrenal. Mongolian gerbil.

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LISTA DE ABREVIATURAS E SIGLAS

AR	Receptor de andrógeno
CYP17	Citocromo P450 17A1
CYP19	Citocromo P450 19A1
CYTB5	Citocromo B5
CO	Óleo de coco
ERα	Receptor de estrógeno tipo alfa
ERβ	Receptor de estrógeno tipo beta
GC	Controle gavagem
DHEA	Deidroepiandrosterona
DHEAS	Sulfato de deidroepiandrosterona
HE	Hematoxilina-eosina
HPA	Hipotálamo-Hipófise-Adrenal
IC	Controle intacto
PBS+NP40	Tampão fosfato-salino com NP40
PBSt	Tampão fosfato-salino com tween
PHH3	Phospho-histona H3
StAR	Proteína reguladora aguda da esteroidogênese
ZF	Zona fasciculada
ZG	Zona glomerulosa
ZR	Zona reticulada
3BHSD	3β-hidroxiesteroide desidrogenase
17BHSD	17β-hidroxiesteroide desidrogenase

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1 Introdução

1.1 Morfologia da Adrenal

As adrenais, ou também chamadas de suprarrenais, são glândulas endócrinas, presentes em par, localizadas acima dos rins. Histologicamente, são divididas em medula, a região central da glândula, e o córtex, formando uma camada periférica, e que pode ser dividido em três zonas concêntricas: zona glomerulosa (ZG), zona fasciculada (ZF) e zona reticulada (ZR) (Figura 1). Cada uma dessas regiões secreta diferentes tipos de hormônios, como os mineralocorticoides, glicocorticoides e hormônios gonadocorticodes que tem funções vitais para a homeostasia do organismo, o que evidencia a importância da glândula (PIGNATTI; FLÜCK, 2021).

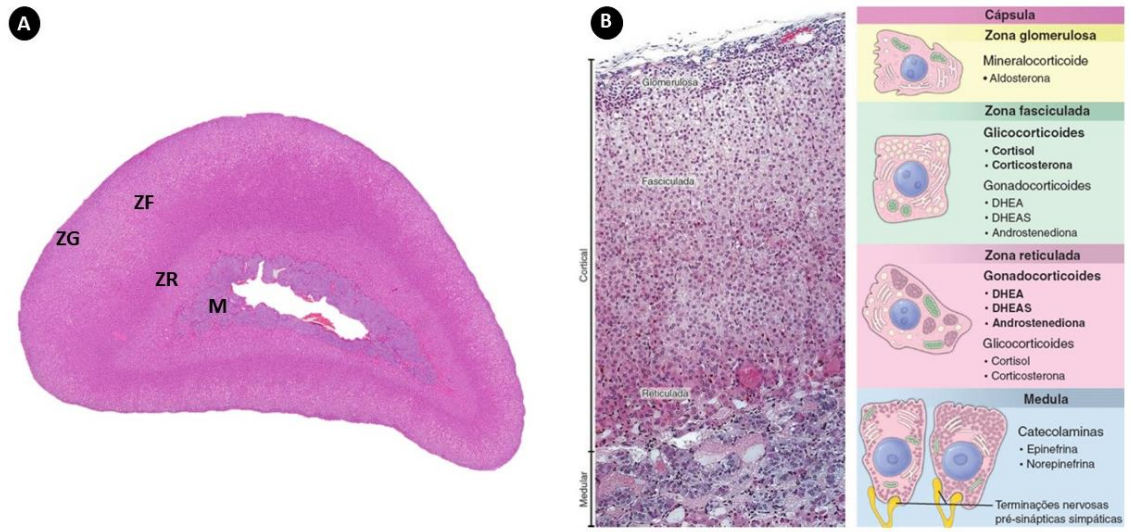

Figura 1: Morfologia da adrenal. A glândula é dividida em medula (M) e córtex que por sua vez é divido em três zonas distintas a zona glomerulosa (ZG), fasciculada (ZF) e reticulada (ZR) (A). As diferentes regiões da adrenal possuem organização e morfologia celular distintas e são responsáveis pela produção de diferentes hormônios (B). Fonte: Figura adaptada de (ROSS; PAWLINA, 2021).

A região da medula é formada por tecido conjuntivo, diversos capilares sanguíneos e células cromafins, responsáveis pela secreção de catecolaminas, como norepinefrina e epinefrina. A liberação desses hormônios produzidos na medula estimula a glicogenólise, aumento da pressão arterial, dilatação de vasos, aumento da frequência

cárdica e respiratória e diversas outras alterações no organismo que preparam o corpo para uma situação de resposta de “luta ou fuga” (BECHMANN et al., 2021).

A zona glomerulosa, a mais periférica do córtex, é formada por células piramidais ou colunares, organizadas em cordões em formato de arco e atuam na secreção de mineralocorticoides, como aldosterona, que regula a homeostasia de sódio, do potássio e no equilíbrio hídrico, permitindo controlar a excreção de água pelos rins, o que é de extrema importância, principalmente, para animais que vivem em situação de estresse hídrico (KEMPPAINEN; BEHREND, 1997). A zona intermediaria do córtex, chamada de zona fasciculada, formada por células grandes e poliédricas dispostas em cordões retilíneos, cuja principal secreção são os glicocorticoides, como o cortisol e o corticosterona, que regulam o metabolismo de glicose e de ácidos graxos, através de processos como a gliconeogênese e glicogênese (PIGNATTI; FLÜCK, 2021). Por fim, a zona mais interna do córtex, zona reticulada, com células pequenas, dispostas em cordões anastomosados e com grande quantidade de grânulos de lipofuscina, cuja principal secreção são os hormônios gonadocorticoides (androgênios suprarrenais), como a desidroepiandrosterona (DHEA), sulfato de desidroepiandrosterona (DHEAS) e androstenediona, que atuam no desenvolvimento de características sexuais secundárias e como precursores de outros hormônios como testosterona e estrogênio que atuam na regulação dos órgãos reprodutivos (GALLO-PAYET; BATTISTA, 2014).

1.2 Esteroidogênese na Adrenal

Os hormônios esteroides, produzidos na adrenal, são sintetizados a partir do colesterol através do processo de esteroidogênese e a maior parte desse colesterol é obtido através da dieta (MILLER; AUCHUS, 2011). Inicialmente, a proteína reguladora aguda da esteroidogênese (StAR) é responsável por mobilizar o colesterol para dentro da mitocôndria, onde ele é clivado pela enzima CYP11, e convertido em pregnenolona. A partir dessa conversão a pregnenolona pode dar origem a diversos hormônios como a aldosterona, o cortisol, a testosterona, a androstenediona, o estradiol e o DHEA, através de uma série de enzimas que possuem especificidade nas diferentes zonas do córtex (TURCU; AUCHUS, 2015).

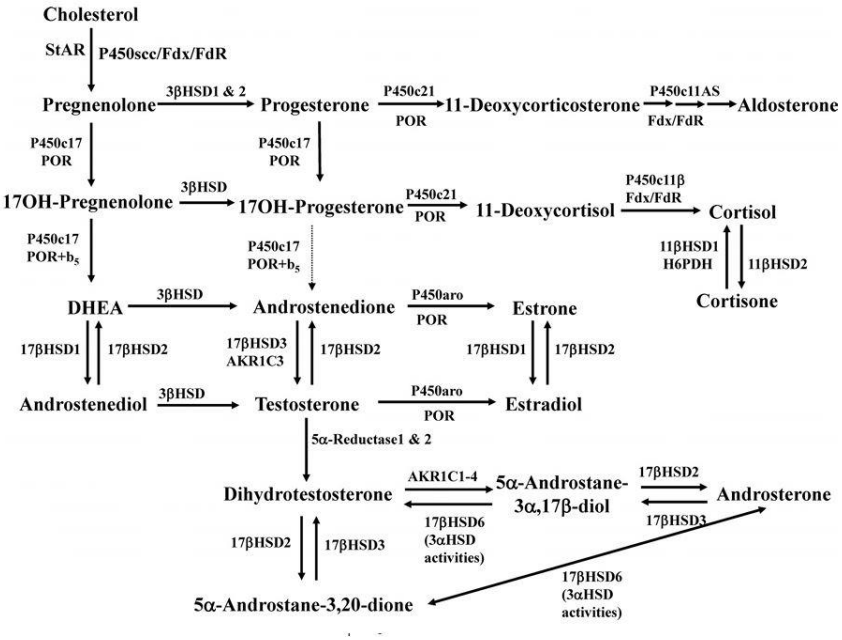

Figura 2: Esquema da esteroidogênese da adrenal. As principais enzimas envolvidas no processo e os principiais hormônios produzidos. Fonte: Adaptado de MILLER; AUCHUS, 2011.

Grande parte dessas enzimas são da família das Citocromos P450 e das hidroxiesteroide desidrogenase (Figura 2). Como a 3β- hidroxiesteroide desidrogenase (3BHSD) que converte pregnenolona em progesterona, 17α-hidroxipregnenolona em 17α-hidroxiprogesterona, DHEA em androstenediona e androstenediol em testosterona. Dessa mesma família afaz parte a 17β-hidroxiesteroide desidrogenase (17BHSD) que atua na interconversão de androstenediona em testosterona, DHEA em androstenediol, estrona em estradiol entre outras reações. Outra enzima com papel fundamental na esteroidogênese é 17α-hidroxilase (CYP17) que atua principalmente na conversão da pregnenolona e progesterona em 17α-hidroxipregnenolona e 17α-hidroxiprogesterona, respectivamente (MILLER; AUCHUS, 2011). Além dessas enzimas, há outras enzimas como a citocromo B5 (CYTB5) que não atua diretamente nas conversões dos precursores da esteroidogênese mas tem um papel importante no processo, regulando o funcionamento das enzimas CYP17 e 3BHSD e consequentemente regulando a produção hormonal da glândula (STORBECK et al., 2015).

O colesterol utilizado durante a síntese dos hormônios esteroides, é em grande parte obtido a partir da alimentação, dessa forma, a quantidade e a qualidade de gorduras ingeridas pelo indivíduo podem impactar na esteroidogênese (ZHAO; ZHAO; ZHU, 2017). Estudos com camundongos já demostraram que uma dieta com alto teor de gordura interfere na concentração de corticosterona circulante no corpo, além de causar alterações

no eixo hipotálamo-hipófise-adrenal (HPA), influenciando na produção hormonal (AUVINEN et al., 2012).

1.3 Óleo de Coco

O óleo de coco é extraído do fruto da *Cocos nucifera*, uma planta da família Palmae, e vem sendo utilizado há muitos anos, principalmente na cultura oriental, como um fitoterápico. Seu consumo vem aumentando nos últimos anos, também, pela suposta propriedade de auxiliar perda de peso, muito difundido nos meios de comunicação (RODRIGUES, 2012). Em 2010 foi produzido no mundo mais de 3,5 milhões de toneladas de óleo de coco e grande parte dessa produção é exportada para o Estados Unidos e União Europeia, que são grandes consumidores do óleo de coco, e que apresentam, respectivamente, uma média de consumo de 1,28 kg e 1,3 kg per capita por ano (EYRES et al., 2016).

Este óleo contém alto nível de ácidos graxos saturados e também apresenta características únicas como sabor e odor suave, alta resistência à rancificação, fácil digestão e absorção, além de uma faixa estreita de temperatura de fusão (MARINA et al., 2009). Alguns dos principais ácidos graxos presentes no óleo são o ácido láurico, o mirístico, o palmítico, o esteárico, o oleico e o linoleico (ASSUNÇÃO et al., 2009). É também um óleo rico em polifenóis que possuem ações anti-inflamatórias já comprovadas (ZAKARIA et al., 2011).

O consumo de óleo de coco possui benefícios, como efeitos antiulcerogênico, antinociceptivo, anti-inflamatório, antimicrobiano e hepatoprotetor (INTAHPHUAK; KHONSUNG; PANTHONG, 2010; YEAP et al., 2015). A grande quantidade de polifenóis presente no óleo auxiliou a reversão de hepatoesteatose, em estudos realizados com ratos (NARAYANANKUTTY et al., 2018). O tratamento com óleo de coco também manteve níveis normais de colesterol e dos demais parâmetros lipídicos nos tecidos e no sangue dos animais testados, mas aumentou a concentração de colesterol HDL (NEVIN; RAJAMOHAN, 2004).

Experimentos realizados com ratos mostraram que a administração de óleo de coco aumentou o nível das enzimas antioxidantes, preveniu a peroxidação dos lipídios, e reduziu a quantidade de radicais livres no organismo, o que pode estar associado aos componentes antioxidantes presentes em maior quantidade, em comparação a outros

óleos (NEVIN; RAJAMOHAN, 2005, 2008). Efeitos semelhantes, *in vitro*, foram também encontrados por Illam e colaboradores (2017) que verificaram que o tratamento com o óleo restaura, a níveis próximos do normal, diversas enzimas associadas ao estresse oxidativo (ILLAM; NARAYANANKUTTY; RAGHAVAMENON, 2017).

O óleo de coco possui efeitos em diversos órgãos e tecidos como no sistema cardiovascular prevenindo doenças coronarianas, no controle de glicemias e do diabetes tipo 2. Além disso, possui efeitos na saúde da pele e cabelos no tratamento de dermatites e protegendo os pelos de danos causados pelo calor. No sistema esquelético, há evidências que mostram que o óleo auxilia na saúde dos ossos e protege dos efeitos da osteoporose (WALLACE, 2018).

Especificamente na adrenal, o óleo de coco, em ratos, foi capaz de retornar a parâmetros próximo do normal o peso da adrenal e a produção de corticosterona, que foram elevados experimentalmente pelo estresse (YEAP et al., 2015). Mas ainda não foram esclarecidos os efeitos do óleo na morfofisiologia e esteroidogênese da glândula.

1.4 Gerbilo da Mongólia

O gerbilo da Mongolia (*Meriones unguiculatus*) é um modelo experimental já amplamente utilizado em estudos de biologia reprodutiva (RUIZ et al., 2023), especialmente no estudo da próstata (CASTRO et al., 2021; CUSTODIO et al., 2008; QUINTAR et al., 2017).

A adrenal do gerbilo possui um alto peso relativo quando comparada ao outros roedores o que índica a importância da glândula para a espécie (MALENDOWICZ, 1984). Para o estudo da glândula adrenal, o gerbilo da Mongólia tem se apresentado como um modelo animal promissor, por possuir a morfologia do tecido da adrenal muito semelhante à dos primatas, apresentando as três zonas do córtex bem definidas e aparentes (KADIOGLU; HARRISON, 1975; MALENDOWICZ, 1984), diferentes de outros roedores, como ratos e camundongos, que não possuem a zonas bem delimitadas, possuem diferenças na enzimas estereidogênicas, como a CYP17 e não produzem hormônios androgênicos na adrenal (GALAC; WILSON, 2015) . Além dessas diferenças o gerbilo secreta os principais hormônios adrenocorticais, como o cortisol, o que diferencia a espécie dos demais roedores utilizados como modelos experimentais, e a

sugere como um modelo experimental adequado aos estudos da biologia da adrenal (RUIZ et al., 2023).

1.5 Justificativa

O consumo excessivo de lipídios causa alterações na morfologia da adrenal, como aumento da glândula e das zonas do córtex (TOPAL et al., 2019). Além disso, a dieta com alto teor de gordura e carboidratos provoca lesões na adrenal de gerbilos e altera principalmente a zona fasciculada que é responsável pela síntese de glicocorticoides (BELLAHRECHE et al., 2022).

Dietas com alto teor de lipídios estão cada vez mais presente no cotidiano das pessoas com o aumento do consumo de alimentos industrializados e altamente processados, mas também com o aumento da ingestão de óleos vegetais utilizados como alimentos funcionais ou como possíveis fitoterápicos. O óleo de coco é um exemplo desses alimentos que teve seu consumo aumentado nos últimos anos (RODRIGUES, 2012). O consumo prolongado desse óleo pode ter impactos na morfofisiologia de diversos órgãos e tecidos, inclusive da adrenal, pelo aumento da disponibilidade lipídica no organismo. Assim, se faz necessário investigar os efeitos desse consumo a fim de se obter parâmetros para avaliar os benefícios e riscos desse tipo de suplementação.

2 Objetivos

O objetivo da dissertação foi testar os efeitos do consumo prolongado do óleo de coco na adrenal de gerbilos da Mongólia.

2.1 Objetivos específicos

I- Identificar alterações na morfologia da adrenal causada pela ingestão prolongada do óleo de coco;

II- Avaliar se o óleo de coco causa alterações em enzimas estereidogênicas da adrenal;

III- Avaliar se o óleo de coco provoca alterações na expressão dos receptores de andrógeno e de estrógenos da adrenal.

3 Artigo a ser submetido para publicação

3.1 Title

Coconut oil consumption impacts adrenal gland morphology and steroidogenesis in Mongolian gerbils.

3.2 Introduction

The adrenal gland is an important endocrine gland for regulating vital functions in the body such as stress response, ions balance, and sexual maturation (PIGNATTI; FLÜCK, 2021). Excessive lipid consumption causes changes in the morphology of the adrenal gland, altering the thickness of the cortex zones (TOPAL et al., 2019). High-fat diets fats cause injury to the adrenal gland and alter the zona fasciculata that is responsible for glucocorticoid synthesis (BELLAHRECHE et al., 2022) and cause a stimulation of the hypothalamic-pituitary-adrenal axis, inducing increased production of glucocorticoids, which cause changes in the adrenal gland and the stress process triggered by the gland (SHIN et al., 2010; TANNENBAUM et al., 1997).

High-fat diets are increasingly present in people's daily lives with the increase in consumption of highly processed foods, and the increase in the intake of vegetable oils used as functional foods or as possible phytotherapic. This is the case of coconut oil, extracted from the fruit of the *cocos nucifera*, which has had its consumption increased in recent years and having several beneficial effects described as an anti-inflammatory, cardioprotective antioxidant, hepatoprotective among others (DEEN et al., 2021; INTAHPHUAK; KHONSUNG; PANTHONG, 2010), however, it is rich in saturated fatty acids increasing the availability of lipids in the body. Coconut oil consumption in the adrenal has been shown experimentally to reduce gland weight and corticosterone production which were elevated (YEAP et al., 2015). However, the effects of prolonged consumption of this oil on the morphophysiology and steroidogenesis of the gland have not yet been understood.

The study aimed to test the effects of prolonged coconut oil consumption on the adrenal gland of Mongolian gerbils. The Mongolian gerbil was used as an experimental model because of the morpho-physiological similarities that its adrenal gland has with the

primates adrenal gland and the facility of maintenance under experimental conditions (RUIZ et al., 2023).

3.3 Methodology

3.3.1 Animals and experimental design

Were used 36 adult (3 months old) male Mongolian gerbils (*Meriones unguiculatus*) from the Animal Breeding Center of the Institute of Biosciences, Humanities and Exact Sciences (IBILCE/UNESP). During the whole experiment, the animals were kept in polysulfone isolators, with controlled temperature (23ºC) and luminosity (12h light/12h dark). Water and feed were offered *ad libitum*. All procedures adopted with the animals were performed according to the rules of the National Council of Control of Animal Experimentation (CONCEA, Brazil) and approved by the local Ethics Committee on the Use of Animals (CEUA/IBILCE/UNESP), under number 230/2021.

The animals were randomly distributed into three experimental groups (n=12): Intact Control (IC) which the animals were not subjected to any type of treatment; Gavage Control (GC) in which the animals were gavaged with 0.1 ml/day of water for 12 months; Coconut Oil (CO) in which the animals received via gavage 0.1 ml/day of coconut oil (Nature Corp LTDA, Sorocaba, Brazil) for 12 months. The dose of coconut oil used was equivalent to 13 ml for an adult male (80kg) (REAGAN-SHAW; NIHAL; AHMAD, 2008). All animals were euthanized at 15 months of age with an anesthetic overdose (3mg/kg xylazine hydrochloride and 10mg/kg ketamine hydrochloride) followed by decapitation for blood collection. The blood was centrifuged and stored at -80°C for further analysis. The adrenal glands were removed and weighed, some were fixed in 4% paraformaldehyde for 24h (n=6/group) and some adrenal glands were frozen in liquid nitrogen (n=6/group) and stored at -80º C.

3.3.2 Morphological and morphometric analyses

The adrenal glands fixed in paraformaldehyde were submitted to histological processing. For this, the organs were dehydrated in a gradual ethanol series, diaphanized in xylol, embedded in paraffin Histosec (Merck) in a Leica Semi Enclosed System

(TP1020, Leica Biosystems), and then sectioned 4 μm thickness. The sections of the central region of the organ, in which it is possible to observe the three zones of the cortex and the medulla, were stained with Hematoxylin and Eosin (HE) and Gömöri's Trichrome for morphological and morphometric analyses. The slides were photographed under a light microscope (Olympus BX60) at 400x magnification and measurements of the thickness of the capsule, glomerulosa zone, fasciculate zone, and reticular zone were made with the aid of Image Pro-Plus 6.0 software (Media Cybernetics). For this, 35 measurements of each zone per animal were taken, totaling 210 measurements per group. Then, still using the slides stained with HE, the area of the cell and its nucleus in each zone of the adrenal cortex was measured. For this, the QuPath software (Version 0.4.2) was used and the area of at least 2000 cells per zone of each animal was measured. Also using this software, the number of cells per area in each zone was measured. For this, the number of cells counted was divided by the area occupied.

The histological sections were also submitted to Gomöri-Halmi Trichrome staining to identify the lipofuscin granules (TOLOSA et al., 2003). To quantify the granules, 15 fields per animal were photographed at 400x magnification, totaling 90 fields per group, and with the help of Image J software (SCHNEIDER; RASBAND; ELICEIRI, 2012) the relative area (%) marked by staining was measured.

3.3.3 Lipid Droplet Analysis

Frozen adrenals were sectioned on a cryostat (6 μm) then stained with Oil Red O, counterstained with hematoxylin and the slides mounted with glycerin (MEHLEM et al., 2013). They were photographed under a light microscope (Olympus BX60) at 400x magnification, 15 fields per slide, totaling 90 fields per group, and quantified the area (%) marked by the dye with the Image J software.

3.3.4 Immunohistochemistry analyses

For immunohistochemistry analyses, the sections were deparaffinized and rehydrated. Peroxidase blockade was performed with hydrogen peroxide baths (5%) diluted in methanol before and after antigen retrieval. Antigen retrieval was performed using citrate or tris-EDTA buffer for 40 minutes at 98°C. Nonspecific protein blocking was performed with skim milk powder (5%) in PBS for 40 minutes. Between steps, the

sections were washed with PBS+NP40 or PBSt. The slides were incubated with primary antibodies overnight at 4°C and diluted according to their specificities: phospho-histone H3 (PHH3, rabbit polyclonal, 1:75, Ser10, 9701, Cell Signaling), active/cleaved caspase-3 (rabbit polyclonal, 1: 50, NB100-56113, Novus Biological), androgen receptor (anti-AR, mouse monoclonal, sc-7305, 1:50, Santa Cruz Biotechnology), estrogen receptor alpha (anti-ERα, mouse monoclonal, sc-8005, 1: 50, Santa Cruz Biotechnology), estrogen receptor beta (anti-ERβ rabbit polyclonal, PA1-310B, 1:50, Invitrogen, Thermo Fisher), aromatase (CYP19, mouse monoclonal, sc-74176, 1:50, Santa Cruz Biotechnology), 17BHSD (17β-HSD (M-174), rabbit polyclonal, sc-32872, 1: 50, Santa Cruz Biotechnology), 3BHSD (HSD3B2, rabbit polyclonal, E-AB-15114, 1:50, Elabscience), CYP17 (CYP17A1, rabbit polyclonal, E-AB-11124, 1:50, Elabscience), Cytochrome b5 (CYTB5, rabbit polyclonal, E-AB- 14924, 1:50, Elabscience). The slides were then incubated with postprimary antibody and polymer (NovolinkTM polymer detection system 1, Leica Biosystems Newcastle Ltd.) for one hour each at room temperature. Finally, they were developed with 3,3-diaminobenzidine tetrachlorhydrate (DAB) (NovolinkTM DAB, RE7270-CE, Leica Biosystems) and counterstained with hematoxylin. For each immunohistochemistry reaction, one section was incubated without a primary antibody to obtain a negative control of the reaction.

For quantification of the immunohistochemistry analyses, positive cells were counted throughout the adrenal cortex using one histological section per animal (n=6/group) and calculating the percentage of positive cells. This was done using the pathological analysis software QuPath (Version 0.4.2).

3.3.5 Western blotting

To quantify the expression of receptors, their isoforms, and steroidogenic enzymes, Western blotting was performed. The frozen adrenal glands were submitted to the process of protein extraction with extraction buffer containing protease inhibitor (Protease Inhibitor Cocktail, P8340, Sigma Aldrich, St. Louis, MO, USA). The samples were subjected to electrophoresis in SDS page gel and transferred to a nitrocellulose membrane (Amersham Protram, 10,600,003, GE Healthcare, Darmstadt, Germany) (LEONEL et al., 2020). The membranes were incubated with skimmed milk for 1 hour at room temperature; then were incubated with primary antibodies, AR (anti-AR, mouse monoclonal, sc-7305, 1:300, Santa Cruz Biotechnology) ER-α (anti-ERα, mouse

monoclonal, sc-8005, 1:1000, Santa Cruz Biotechnology), ER-β (anti-ERβ rabbit polyclonal, PA1-310B, 1:500, Invitrogen, Thermo Fisher), CYP19 (anti-CYP19, mouse monoclonal, sc-74176, 1:500, Santa Cruz Biotechnology), CYTB5 (anti-CYTB5, rabbit polyclonal, E-AB- 14924, 1:1000, Elabscience) or GAPDH (mouse monoclonal MB374 Millipore, 1:2000) diluted in skimmed milk 1% in TBSt, overnight at 4ºC, and incubated with secondary antibodies (mouse Anti-mouse IgG, HRP-linked Antibody, #7076, 1:2000, Cell Signaling or Anti-rabbit IgG, HRP-linked Antibody, #7074, 1:2000, Cell Signaling) for 2 hours at room temperature. The membranes were incubated with Luminol-Enhacer solution (Cod. XLS142L, Cyanagen, Italy) and peroxide solution (Cod. XLS142P, Cyanagen, Italy). Finally, the membranes were revealed in an imaging system machine (ChemiDoc MP, BioRad, Hercules, CA, USA). The density of the bands and the molecular weight of isoforms were quantified using Image Lab software (Bio-Rad Laboratories, Inc.).

3.3.6 Hormonal analyses

Serum hormone levels of testosterone, estradiol, and dehydroepiandrosterone sulfate (DHEAS) were quantified using commercial ELISA kits (testosterone 52151; 17-β-estradiol 52041 and S-DHEA 52181, IBL International). Frozen serum (n=6) was used and analyses were performed in duplicates. The protocol was done according to the manufacturer's manual and the readings were performed in a microplate reader (Epoch™ Multi-Volume Spectrophotometer System- BioTek Instruments).

3.3.7 Statistical analyses

Statistical analyses were performed in GraphPad Prism 5.0 software. The data were subjected to the Kolmogorov-Smirnov test to analyze the normality of the distribution. Then, parametric data were subjected to the ANOVA test followed by Tukey's test, and non-parametric data were analyzed by the Kruskal-Wallis test followed by Dunn's test. Data were presented with the mean and standard deviation and the significance level adopted was 5% ($p \leq 0.05$).

3.4 Results

3.4.1 Morphological and morphometric data

The biometric data, thicknesses of the cortex zones, and cell volume are shown in Table 1. The biometric data showed that coconut oil treatment did not change the weight of the animals or the adrenals. The morphological structure of the zones, capsule and adrenocortical cells was not altered by prolonged coconut oil consumption (Figure 1), but the morphometric analyses showed an increase in the thickness of the zona glomerulosa in the animals of the gavage control group (58.77 ± 9.75) which did not occur in the coconut oil treated group (38.20 ± 4.19) and the intact control (38.33 ± 2.54). In the zona reticularis, coconut oil promoted an increase in thickness (828.0 ± 83.0) that did not occur in the control groups (CI: 775.3 ± 182.7 and CG: 410.3 ± 25.9) (Table 1).

Table 1: Biometric and morphometric data of adrenal cortex. The data were expressed as mean followed by standard deviation[1].

	IC	**GC**	**CO**
Body weight (g)	95.75 ± 11.07	95.00 ± 12.4	86.17 ± 11.0
Right adrenal gland (g)	0.021 ± 0.005	0.019 ± 0.002	0.020 ± 0.004
Left adrenal gland (g)	0.024 ± 0.006	0.020 ± 0.002	0.023 ± 0.005
Capsule thickness (µm)	9.18 ± 1.32	9.34 ± 1.26	8.11 ± 1.06
Zona glomerulosa thickness (µm)	38.33 ± 2.54^{a}	58.77 ± 9.75^{b}	38.20 ± 4.19^{a}
Zona fasciculata thickness (µm)	260.1 ± 21.1	242.6 ± 31.5	247.8 ± 15.2
Zona reticularis thickness (µm)	775.3 ± 182.7^{a}	410.3 ± 25.9^{b}	828.0 ± 83.0^{c}
Cell area zona glomerulosa (µm^2)	88.95 ± 3.52^{a}	102.1 ± 4.01^{b}	92.86 ± 4.16^{a}
Cell area zona fasciculata (µm^2)	185.1 ± 13.4	178.5 ± 15.0	173.9 ± 19.1
Cell area zona reticularis (µm^2)	106.5 ± 5.4^{a}	93.2 ± 8.3^{a}	118.7 ± 11.5^{b}
Nucleus area zona glomerulosa (µm^2)	21.53 ± 1.3	22.86 ± 1.1	22.20 ± 1.3
Nucleus area zona fasciculata (µm^2)	24.78 ± 0.6	27.37 ± 1.6	25.01 ± 1.7
Nucleus area zona reticularis (µm^2)	26.30 ± 1.3	25.51 ± 0.7	26.80 ± 2.8

[1] Data were subjected to ANOVA statistical tests followed by Tukey's test where $a \neq b \neq c$. IC: Intact control group; GC: gavage control group; CO: coconut oil treated group.

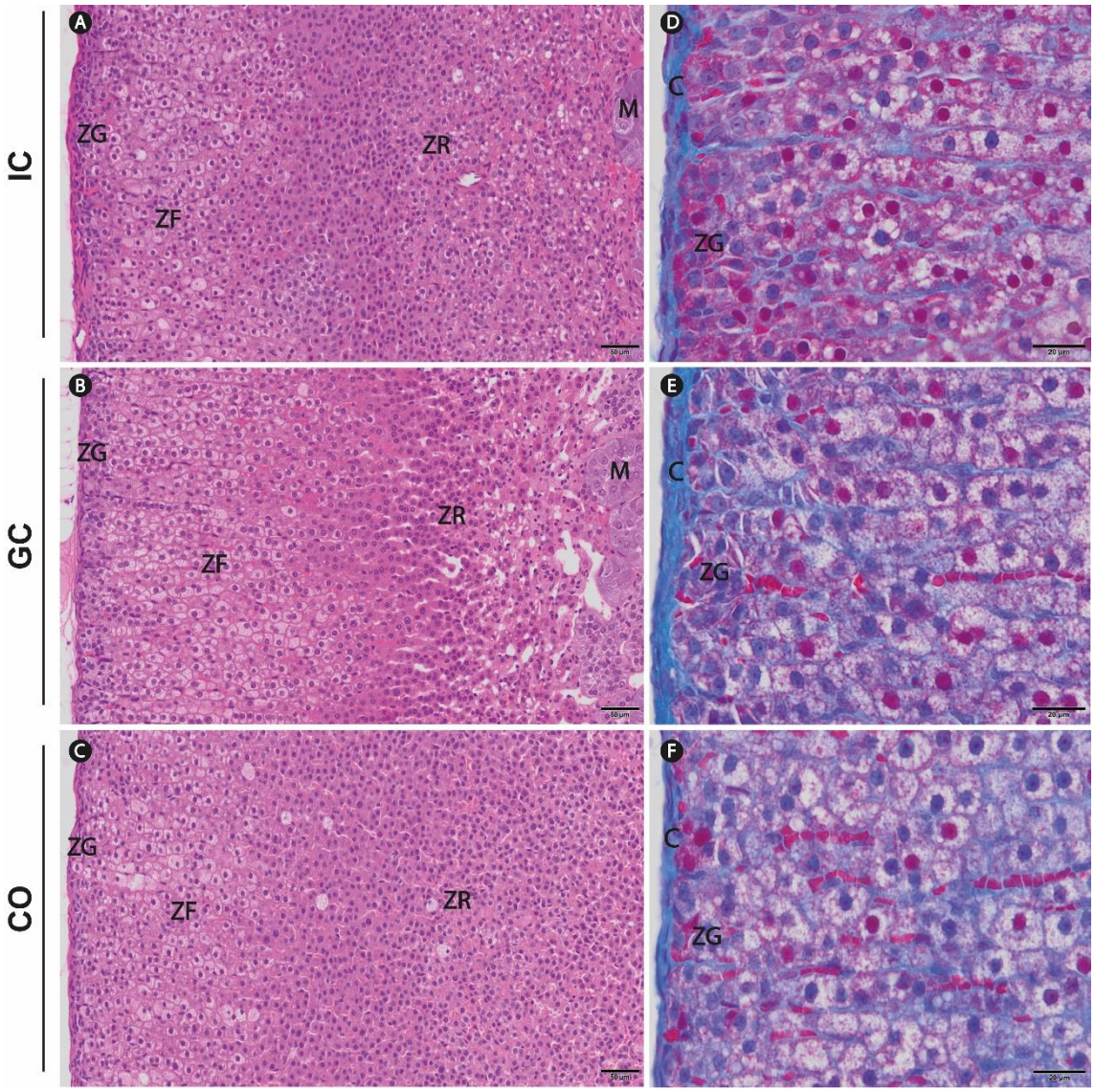

Figure 1: Morphology of the adrenal cortex. Photomicrographs of adrenal cortex stained with hematoxylin-eosin (A-C) and Gomori's trichrome (D-F). Intact control (IC) (A, D), gavage control (GC) (B, E), and coconut oil treated group (CO) (C, F). Capsule (C), zona glomerulosa (ZG), zona fasciculata (ZF), zona reticularis (ZR) and medulla (M). 200x (A-C) and 400x (D-F) magnification.

In addition to the change in zone thicknesses, the morphometric data (Table 1) showed that the cell area of the ZG increased in the GC (102.1 ± 4.01) group when compared to the IC (88.95 ± 3.52) and CO (92.86 ± 4.16) groups. In the ZF, there were no changes in zone thickness or cell size. In the ZR, the consumption of coconut oil promoted an increase in cell area (118.7 ± 11.5), which did not occur in the control groups (IC: 106.5 ± 5.4 and GC: 93.2 ± 8.3). The size of the nuclei did not vary in any of the three zones of the adrenal cortex (Table 1).

The morphological analyses also showed the accumulation of lipofuscin granules which is high in the adrenal cells of the IC group (18.32 ± 5.7) and decreased significantly in the animals of the GC group (4.12 ± 1.4) and even more in the animals that received coconut oil (1.84 ± 1.6) (Figure 2 A-D). It was also observed that coconut oil promoted an increase in lipid droplets (44.9 ± 4.3) in adrenal cortex cells that was not observed in control groups (IC: 34.34 ± 2.9 and GC: 36.72 ± 5.9) (Figure 2 E-H).

A Pearson correlation analysis between cell area and amount of lipid droplets was performed in the CO group and showed that there is a positive correlation ($r = 0.8999$) between cell enlargement and lipid droplet accumulation.

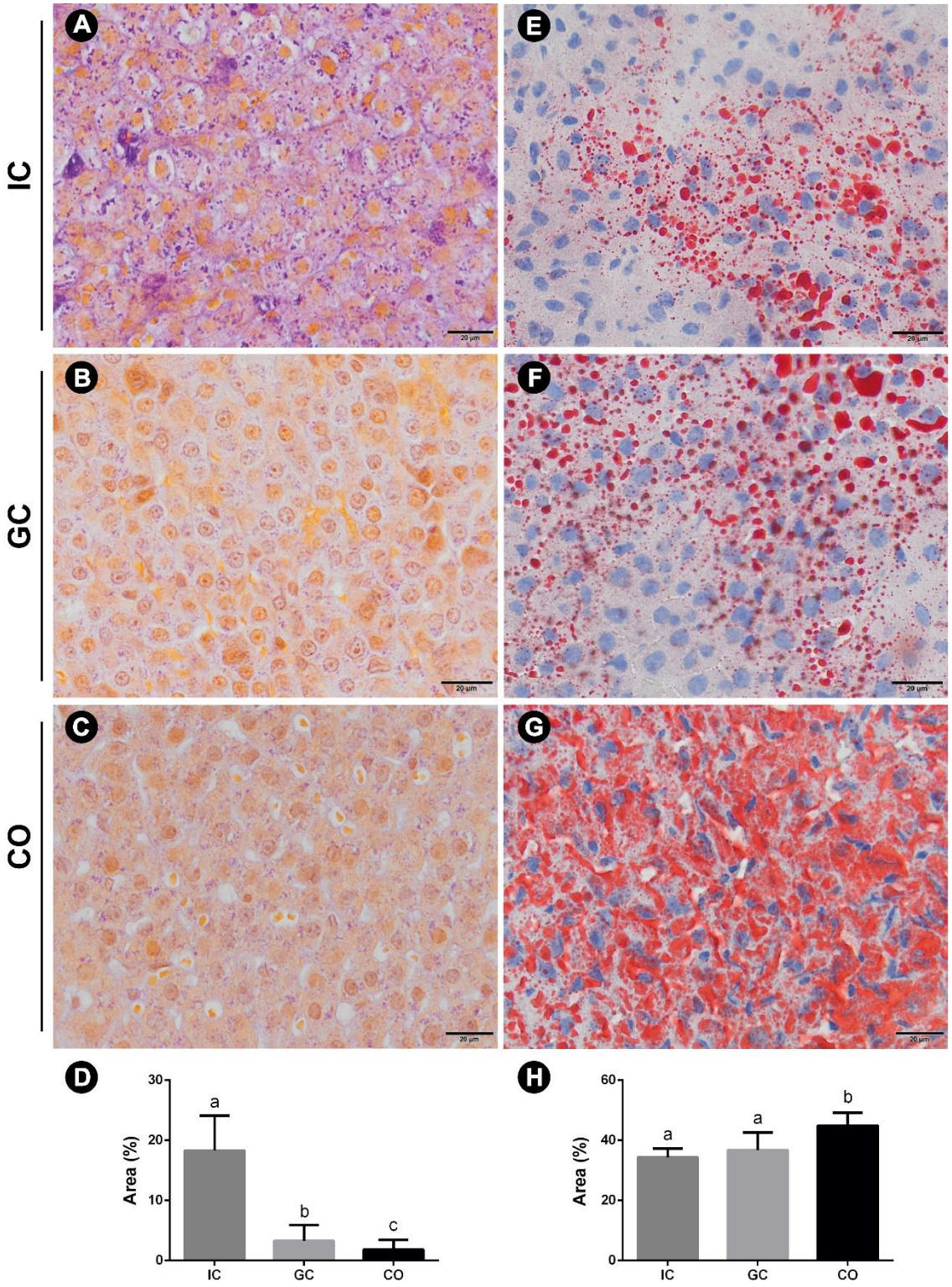

Figure 2: Accumulation of lipofuscin granules and lipid droplets in the adrenal cortex. Photomicrographs of the adrenal cortex stained with Gomori-Halmi trichrome (A-C) and Oil Red O (E-G). Intact control (IC) (A, E), gavage control (GC) (B, F), and coconut oil treated group (CO) (C, G). 400x magnification. Mean and standard deviation of area (%) containing lipofuscin granules (D) and area (%) containing lipid droplets (H). Values were subjected to ANOVA statistical test followed by Tukey's test which $a \neq b \neq c$.

3.4.2 Proliferation and cell death

Immunohistochemistry for PHH3 labeling showed that there was no change in the percentage of cells positive for this immunolabeling (Figure 3 A-D). The same was observed in the immunohistochemistry reaction for the identification of Caspase-3, in which there were no changes in the number of positive cells after treatment with coconut oil (Figure 3 E-H).

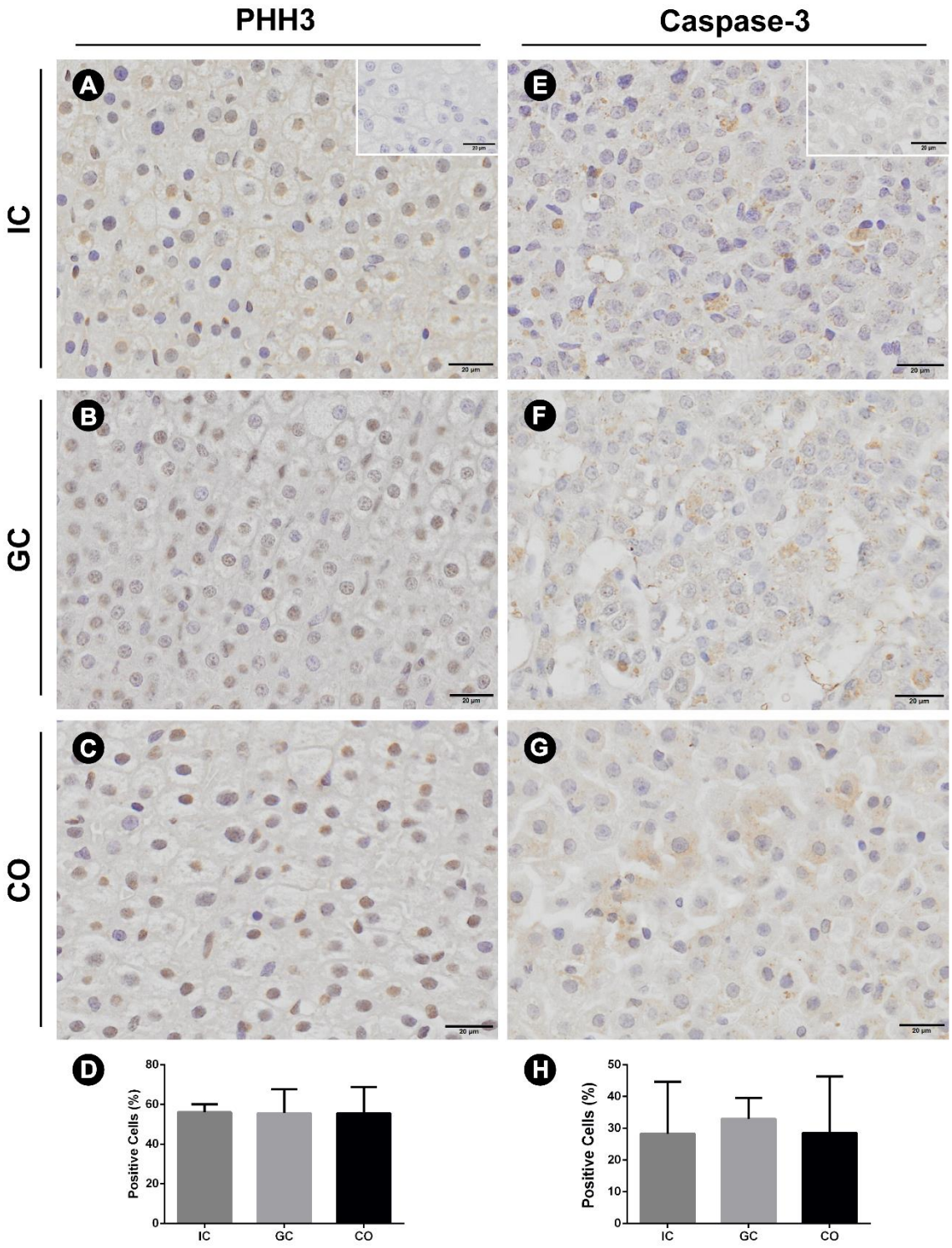

Figure 3: Proliferation and cell death of the adrenal cortex. Photomicrographs of adrenal subjected to immunohistochemistry for cell proliferation (PHH3) (A-C) and cell death (caspase-3) (E-G) staining. Intact control (IC) (A, E), gavage control (GC) (B, F), and coconut oil treated group (CO) (C, G). Insert with the negative control (A, E). 400x magnification. Mean and standard deviation of positive cells (%) for PHH3 immunoreaction (D) of positive cells (%) for caspase-3 immunoreaction (H). Values were subjected to ANOVA statistical test followed by Tukey's test.

3.4.3 Receptor expression

Androgen receptor (AR) and estrogen receptor (ER-α and ER-β) expression were quantified using immunohistochemistry for the quantification of labeled cells and Western blotting for protein quantification. The immunohistochemistry analysis for AR showed that there was no significant change in the number of cells expressing AR between the experimental groups (Figure 4 A-D). However, Western blotting showed that there was a decrease in the amount of AR expressed in the adrenal of animals in the GC group when compared with the other experimental groups (Figure 4 N).

Coconut oil treatment promoted an increase in cells expressing ER-α, identified by immunohistochemistry (Figure 4 E-H), and western blotting showed that this increased expression occurred in two isoforms of ER-α, the 68 kDa and the 47 kDa (Figure 4 P, Q). ER-β had no changes in the number of cells labeled by immunohistochemistry (Figure 4 I-L) and neither in the number of receptors expressed by these cells (Figure 4 O).

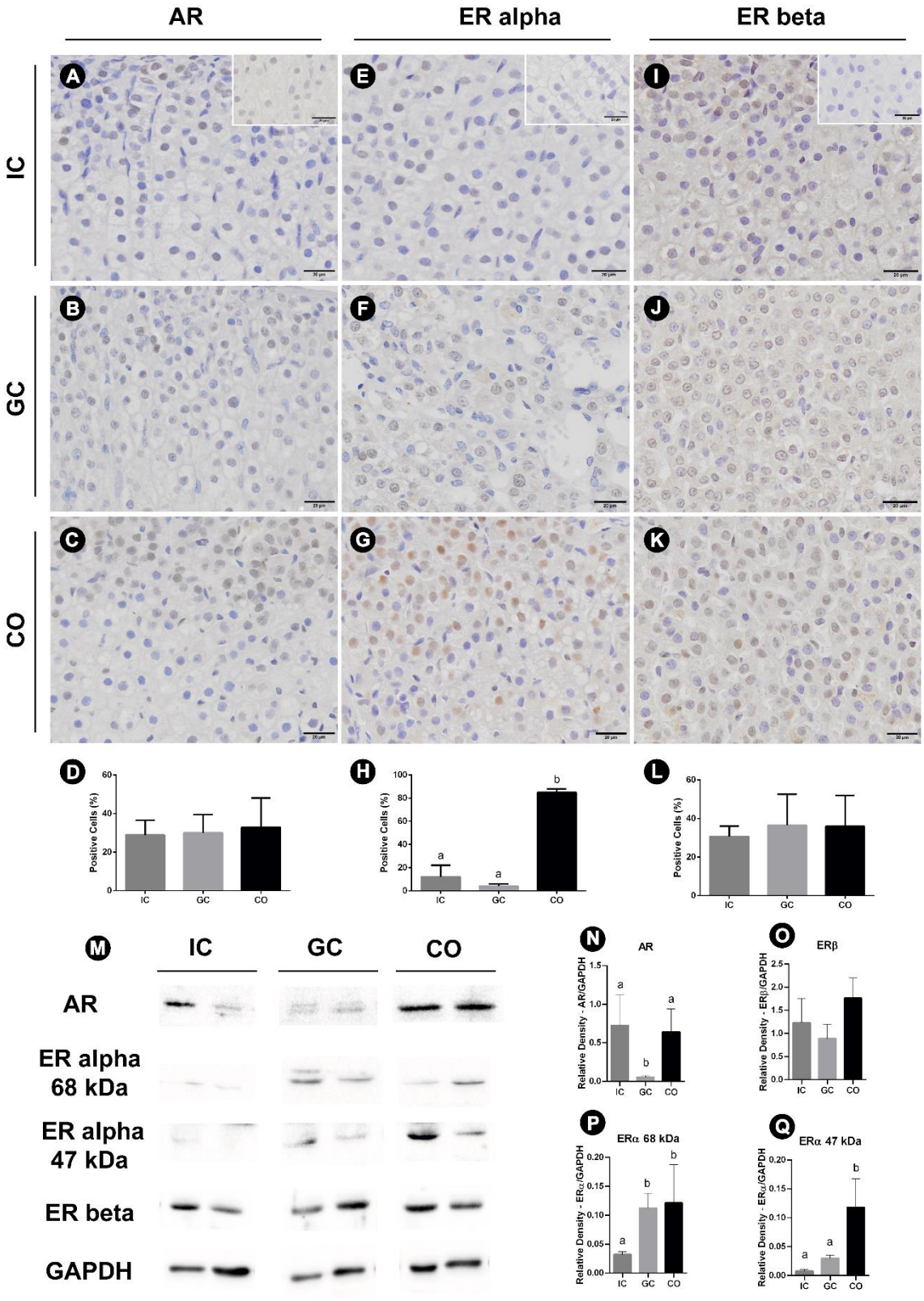

Figure 4: Receptor expression. Photomicrographs of the adrenal cortex subjected to immunohistochemistry for androgen receptor (AR) labeling (A-D), estrogen receptor alpha (ER-α) (E-H), and estrogen receptor beta (ER-β) (I-L). Intact control (IC) (A, E, I), gavage control (GC) (B, F, J), and coconut oil treated group (CO) (C, G, K). Inserts with the negative control

from immunohistochemistry (A, E, I). 400x magnification. Mean and standard deviation of positive cells (%) for AR immunoreaction (D), of positive cells (%) for ER-α immunoreaction (H), and of positive cells (%) for ER-β immunoreaction (L). Representative blots for AR, ER-α 68 KDA and 47 kDa, and ER-β (M). Relative density of AR(N), ER-β (O), ER-α 68 kDa (P), and ER-α 47 kDa (Q). Endogenous control was performed by GAPDH expression. Relative density of AR and ER-α 68 KDA values were subjected to the Kruskal-Wallis test followed by Dunn's test and the other data were subjected to ANOVA statistical test followed by Tukey's test which $a \neq b$.

3.4.4 Steroidogenic enzymes expression

To understand the effects of coconut oil on steroidogenesis in the adrenal cortex, the expression of some key enzymes in the steroidogenesis process was analyzed. Immunohistochemistry analysis showed that CYP17, 3BHSD, and 17BHSD enzymes were decreased by coconut oil consumption (Figure 5 A-L).

The immunohistochemistry for the CYP19 enzyme showed an increase in the GC group of cells expressing the enzyme (Figure 5 M-P), besides increasing the total quantity of the enzyme demonstrated by Western blotting (Figure 5 V), and this increase did not occur in the animals receiving coconut oil.

Finally, the CYTB5 enzyme showed a decrease in the CG group when analyzed by the number of cells labeled by immunohistochemistry reaction (Figure 5 Q-T) but did not show significant differences in Western blotting analysis (Figure 5 W).

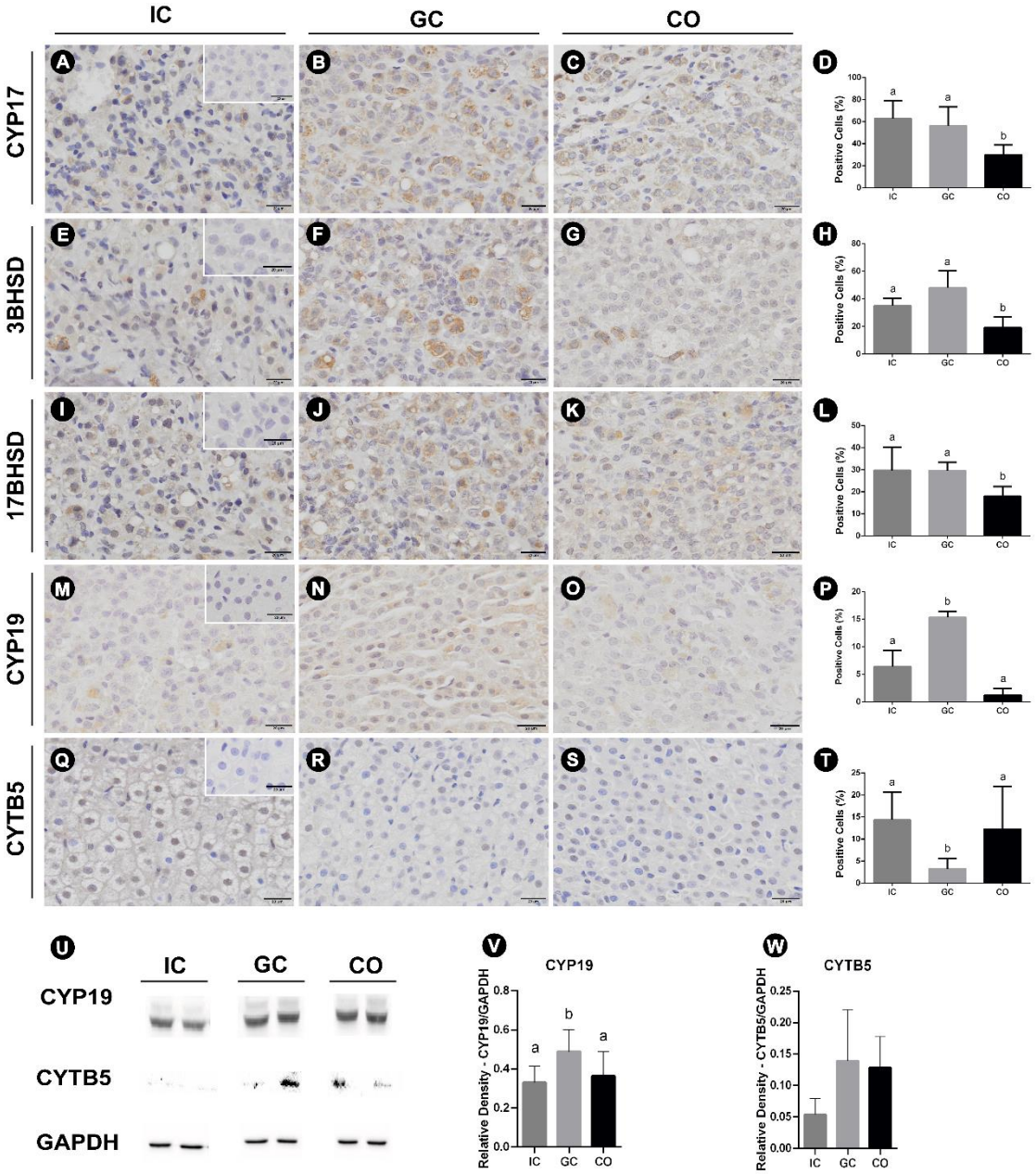

Figure 5: Expression of steroidogenic enzymes. Photomicrographs of adrenal subjected to immunohistochemistry for labeling of CYP17 (A-C), 3BHSD (E-G), 17BHSD (I-K), CYP19 (M-O) and CYTB5 (Q-S). Intact control (IC) (A, E, I, M, Q), gavage control (GC) (B, F, J, N, R), and coconut oil treated group (CO) (C, G, K, O, S). Insert with the negative control of the immunohistochemistry reaction (A, E, I, M, Q). 400x magnification. Mean and standard deviation of positive cells (%) for immunoreaction of CYP17 (D), 3BHSD (H), 17BHSD (L), CYP19 (P), and CYTB5 (T). Representative blots of CYP19 and CYTB5 (U). Relative density of CYP19 (V) and CYTB5 (W). Endogenous control was performed by GAPDH expression. Positive cells for immunoreaction of CYTB5 values were subjected to the Kruskal-Wallis test followed by Dunn's test and the other data were subjected to ANOVA statistical test followed by Tukey's test which $a \neq b$.

3.4.5 Hormone levels

Serum testosterone, estradiol, and DHEAS levels were quantified with an ELISA assay. Coconut oil consumption did not alter the level of any of the three hormones in the serum of the animals (Table 2).

Table 2: Serum hormone levels. The data were expressed as mean followed by standard deviation[1].

	IC	**GC**	**CO**
Estradiol (pg/mL)	35.6 ± 9.4	59.4 ± 33.9	40.2 ± 17.9
Testosterone (ng/mL)	1.6 ± 0.3	1.7 ± 0.4	1.8 ± 0.5
DHEAS (µg/mL)	0.14 ± 0.03	0.11 ± 0.03	0.13 ± 0.01

[1] Data were subjected to ANOVA statistical tests followed by Tukey's test. IC: Intact control group; GC: gavage control group; CO: coconut oil treated group.

3.5 Discussion

The present study showed the effects that prolonged consumption of coconut oil can cause in the adrenal gland. Daily intake of the oil promoted changes in gland morphometry, altered the expression of estrogen receptors and enzymes associated with the steroidogenesis process, and reduced the accumulation of lipofuscin granules.

Among the morphological changes, the coconut oil did not alter the ZG and ZF, however, it promoted an increase in the thickness of the ZR. The ZR is a zone responsible for the synthesis of androgen hormones and their precursors such as DHEA, DHEAS, and androstenedione (DUMONTET; MARTINEZ, 2021). This increase in the ZR suggests a change in amount and functional activity of the cells in the zone. However, when analyzing immunohistochemistry for PHH3, a marker that indicates cell proliferation in the adrenal gland (DUREGON et al., 2014), we observed that there is no increase in the cell proliferation rate in the adrenal of animals that received coconut oil, which would not explain the increase in the ZR by the increased amount of cells. Similarly, the labeling for caspase-3, which indicates the process of cell death (ALMEIDA; MATOS; NEVES, 2007), showed no changes between the groups. Thus, the increase in the ZR was not caused by the change in the number of cells, but by the increase in the size of these cells

that was evidenced by morphometric analysis, showing that the consumption of coconut oil increases the cell area of the ZR.

Coconut oil also promoted an increase in lipid droplets in adrenocortical cells. Lipid droplets are an important reserve of energy for the cell and of cholesterol for the synthesis of steroid hormones (YU et al., 2018). The high amount of lipid droplets may be associated with the higher intake of coconut oil-derived fatty acids considering that this increase only occurred in the CO group. This accumulation of lipids can be associated with increased adrenocortical cell volume, which may explain the increased thickness of the ZR observed. Lipid droplets store esterified cholesterol which is used as the main precursor of steroid hormones (SHEN; AZHAR; KRAEMER, 2016). In addition to acting as a lipid store, recent studies have demonstrated that there are steroidogenic enzymes near the droplet, which make them important sites of steroidogenesis in adrenal cells (YU et al., 2018). Thus, the increase of droplets in the adrenals of coconut oil-treated animals may impact the morphology of the zones and cause changes in the steroidogenesis process of the gland.

During aging, the adrenal undergoes an accumulation of lipofuscin granules in its cells, a result of the cellular senescence process that is intensified by steroid synthesis (GAO et al., 2021). This accumulation of lipofuscin may even be associated with the production of adenomas (ANGELOUSI et al., 2017). Coconut oil treatment led to a reduction in lipofuscin granules, which indicates a delay or decrease in the cellular senescence process. This reduction in lipofuscin granule accumulation may be associated with an increase in lipid droplets. Considering that lipid droplets act as cellular buffers preventing lipid damage and cellular stress (JARC; PETAN, 2019) and consequently reducing the accumulation of lipofuscin granules.

Regarding hormone receptors, AR in the adrenal has an important role in zonal development and a protective role against age-related degeneration of adrenocortical cells (GANNON et al., 2019). In the gavage control group the expression of this receptor was reduced and the coconut oil maintained normal levels of AR. ER alpha, on the other hand, is associated with adrenal function and DHEA synthesis, however, it has already been described that in adrenocortical carcinomas there is an increase in the expression of this receptor that acts in the maintenance and proliferation of the carcinoma (FELIZOLA et al., 2013). In the animals of the CO group, although the presence of carcinomas was not identified, there was a large increase in ER-alpha. This receptor imbalance may trigger a

favorable environment for the establishment of proliferative disorder with the potential for the establishment of carcinomas caused by coconut oil consumption, which may occur over a longer period of consumption. On the other hand, the ER-beta, which when unbalanced may also indicate tumorigenic processes in the adrenal (DE CREMOUX et al., 2008) was not altered by coconut oil consumption.

Coconut oil treatment reduced the presence of CYP17, 3BHSD, and 17BHSD enzymes in the adrenal cortex. The CYP17 enzyme acts in the process of steroidogenesis by catalyzing the hydroxylation of pregnenolone and progesterone, forming precursors of DHEA and androstenedione (MILLER; AUCHUS, 2011). On the other hand, 3BHSD is an enzyme responsible for several actions in the steroidogenesis process, such as the conversion of pregnenolone into progesterone, 17α-hydroxypregnenolone into 17α-hydroxyprogesterone, DHEA into androstenedione, and androstenediol into testosterone. Finally, the 17BHSD enzyme is responsible for the synthesis of androstenedione, testosterone, DHEA, and estradiol (MILLER; AUCHUS, 2011). Thus, the reduction of these enzymes may impact steroidogenesis and consequently the hormone production of the gland. Studies with rats have shown that the consumption of oils can decrease the expression of steroidogenic enzymes in the testes (MCVEY et al., 2008) as the decrease of 17BHSD in Leydig cells caused by a high lipid diet (PINTO-FOCHI et al., 2016) and this was observed in the present study, however in the steroidogenesis of the adrenal. These enzymatic changes may be explained by altered plasma fatty acid composition and the ability of some fatty acids to inhibit enzymes involved in lipid metabolism (MCVEY et al., 2008). However, these mechanisms still need to be better studied to understand the cellular processes that cause the enzymatic decrease associated with the ingestion of oils.

The CYP19 enzyme increased in the animals of the gavage control group, but in the animals of the CO group, this increase was not observed. This enzyme is responsible for estrogen biosynthesis and a high expression of this enzyme is observed in adrenal tumors (MOREAU et al., 2009). The data demonstrate that gavage promoted an unbalance in the expression of CYP19, which may have been caused by the stress of manipulation, however, coconut oil minimized this process avoiding the alteration. CYTB5, on the other hand, plays an important role in the regulation of steroidogenesis, modulating the activities of key enzymes such as CYP17 and 3BHSD (STORBECK et al., 2015). During aging, CYTB5 expression tends to decrease which may impact adrenal hormone production (DHARIA et al., 2005). However, in the present study, only the

animals in the GC group showed this decrease which may again be related to gavage stress. However, coconut oil maintained CYTB5 enzyme expression at normal levels.

Despite all the enzymatic changes related to coconut oil consumption, the amount of testosterone, estradiol, and DHEAS were not altered. This can be explained by the fact that the quantification of these hormones is systemic and not specific to the adrenal. Thus, even if the enzymes are altered, the amounts of hormones in the body are not changed, because they can be synthesized in other organs or steroidogenic pathways.

In conclusion, the results obtained showed that increased dietary lipids, as coconut oil consumption, can cause changes in adrenal morphological and morphometric parameters, reduce steroidogenic enzymes, imbalance estrogen receptors, and consequently alter the functionality of the gland.

4 Conclusões

O presente estudo mostrou que o consumo de óleo de coco, por longo período, promoveu alterações na morfologia da adrenal, aumentando a área celular e a espessura da zona reticulada. Além disso, o óleo de coco provocou um aumento de gotículas lipídicas nas células adrenocorticais.

O consumo prolongado do óleo de coco reduziu a expressão de enzimas estereidogênicas como a CYP17, 3BHSD e 17BHSD, além de ocasionar um aumento dos receptores de estrógeno do tipo alfa.

Dessa forma, podemos concluir que o aumento de lipídios na dieta, como o óleo de coco, ocasiona alterações na morfofisiologia da adrenal e que podem impactar na funcionalidade da glândula.

Referências

ALMEIDA, H.; MATOS, L.; NEVES, D. Caspase-3 and Bcl-2 expression in aging in adrenal zona reticularis after dexamethasone administration. **Annals of the New York Academy of Sciences**, v. 1119, n. 1, p. 190–195, 2007.

ANGELOUSI, A. et al. Lipofuscin Accumulation in Cortisol-Producing Adenomas with and Without PRKACA Mutations. **Hormone and Metabolic Research**, v. 49, n. 10, p. 786–792, 2017.

ASSUNÇÃO, M. L. et al. Effects of dietary coconut oil on the biochemical and anthropometric profiles of women presenting abdominal obesity. **Lipids**, v. 44, n. 7, p. 593–601, 2009.

AUVINEN, H. E. et al. The effects of high fat diet on the basal activity of the hypothalamus-pituitary-adrenal axis in mice. **Journal of Endocrinology**, v. 214, n. 2, p. 191–197, 2012.

BECHMANN, N. et al. Adrenal medulla development and medullary-cortical interactions. **Molecular and Cellular Endocrinology**, v. 528, n. November 2020, p. 111258, 2021.

BELLAHRECHE, Z. et al. High-fat/high-carbohydrate-diet short- and middle-term effects on gerbil adrenal zona fasciculata histology. **Tissue and Cell**, v. 75, n. August

2021, p. 101729, 2022.

CASTRO, N. F. DA C. et al. Therapeutic effects of β-caryophyllene on proliferative disorders and inflammation of the gerbil prostate. **Prostate**, v. 81, n. 12, p. 812–824, 2021.

CUSTODIO, A. M. G. et al. Aging effects on the mongolian gerbil female prostate (Skene's paraurethral glands): Structural, ultrastructural, quantitative, and hormonal evaluations. **Anatomical Record**, v. 291, n. 4, p. 463–474, 2008.

DE CREMOUX, P. et al. Expression of progesterone and estradiol receptors in normal adrenal cortex, adrenocortical tumors, and primary pigmented nodular adrenocortical disease. **Endocrine-Related Cancer**, v. 15, n. 2, p. 465–474, 2008.

DEEN, A. et al. Chemical composition and health benefits of coconut oil: an overview. **Journal of the Science of Food and Agriculture**, v. 101, n. 6, p. 2182–2193, 2021.

DHARIA, S. et al. Effects of aging on cytochrome B5 expression in the human adrenal gland. **Journal of Clinical Endocrinology and Metabolism**, v. 90, n. 7, p. 4357–4361, 2005.

DUMONTET, T.; MARTINEZ, A. Adrenal androgens, adrenarche, and zona reticularis: A human affair? **Molecular and Cellular Endocrinology**, v. 528, n. December 2020, p. 111239, 2021.

DUREGON, E. et al. Comparative diagnostic and prognostic performances of the hematoxylin-eosin and phospho-histone H3 mitotic count and Ki-67 index in adrenocortical carcinoma. **Modern Pathology**, v. 27, n. 9, p. 1246–1254, 2014.

EYRES, L. et al. Coconut oil consumption and cardiovascular risk factors in humans. **Nutrition Reviews**, v. 74, n. 4, p. 267–280, 2016.

FELIZOLA, S. J. A. et al. Estrogen-related receptor α in normal adrenal cortex and adrenocortical tumors: Involvement in development and oncogenesis. **Molecular and Cellular Endocrinology**, v. 365, n. 2, p. 207–211, 2013.

GALAC, S.; WILSON, D. B. Animal Models of Adrenocortical Tumorigenesis. **Endocrinology and Metabolism Clinics of North America**, v. 44, n. 2, p. 297–310, 2015.

GALLO-PAYET, N.; BATTISTA, M. C. Steroidogenesis-adrenal cell signal transduction. **Comprehensive Physiology**, v. 4, n. 3, p. 889–964, 2014.

GANNON, A. L. et al. Androgen receptor signalling in the male adrenal facilitates X-zone regression, cell turnover and protects against adrenal degeneration during ageing. **Scientific Reports**, v. 9, n. 1, p. 1–16, 2019.

GAO, X. et al. Cellular senescence in adrenocortical biology and its disorders. **Cells**, v. 10, n. 12, p. 1–26, 2021.

ILLAM, S. P.; NARAYANANKUTTY, A.; RAGHAVAMENON, A. C. Polyphenols of virgin coconut oil prevent pro-oxidant mediated cell death. **Toxicology Mechanisms and Methods**, v. 27, n. 6, p. 442–450, 2017.

INTAHPHUAK, S.; KHONSUNG, P.; PANTHONG, A. Anti-inflammatory, analgesic, and antipyretic activities of virgin coconut oil. **Pharmaceutical Biology**, v. 48, n. 2, p. 151–157, 2010.

JARC, E.; PETAN, T. Lipid droplets and the management of cellular stress. **Yale Journal of Biology and Medicine**, v. 92, n. 3, p. 435–452, 2019.

KADIOGLU, D.; HARRISON, R. G. The ultrastructure of the adrenal cortex of the Mongolian gerbil (M. unguiculatus). **Journal of Anatomy**, v. 120, n. 1, p. 179–189, 1975.

KEMPPAINEN, R. J.; BEHREND, E. N. Adrenal physiology. **The Veterinary clinics of North America. Small animal practice**, v. 27, n. 2, p. 173–186, 1997.

LEONEL, E. C. R. et al. Impact of perinatal bisphenol A and 17β estradiol exposure: Comparing hormone receptor response. **Ecotoxicology and Environmental Safety**, v. 188, n. November, p. 109918, 2020.

MALENDOWICZ, L. K. Sex differences in adrenocortical structure and function. XVI. Stereological and karyometric studies on the cortex of the suprarenal gland of intact adult male and female Mongolian gerbils (Meriones unguiculatus). **J. Anat.**, v. 139, n. 3, p. 525–533, 1 abr. 1984.

MARINA, A. M. et al. Chemical properties of virgin coconut oil. **Journal of the American Oil Chemists' Society**, v. 86, n. 4, p. 301–307, 2009.

MCVEY, M. J. et al. Effects of dietary fats and proteins on rat testicular steroidogenic enzymes and serum testosterone levels. **Food and Chemical Toxicology**, v. 46, n. 1, p. 259–269, 2008.

MILLER, W. L.; AUCHUS, R. J. The molecular biology, biochemistry, and physiology of human steroidogenesis and its disorders. **Endocrine Reviews**, v. 32, n. 1, p. 81–151, 2011.

MOREAU, F. et al. Aromatase expression in the normal human adult adrenal and in adrenocortical tumors: Biochemical, immunohistochemical, and molecular studies. **European Journal of Endocrinology**, v. 160, n. 1, p. 93–99, 2009.

NARAYANANKUTTY, A. et al. Virgin coconut oil reverses hepatic steatosis by restoring redox homeostasis and lipid metabolism in male Wistar rats. **Journal of the Science of Food and Agriculture**, v. 98, n. 5, p. 1757–1764, 2018.

NEVIN, K. G.; RAJAMOHAN, T. Beneficial effects of virgin coconut oil on lipid parameters and in vitro LDL oxidation. **Clinical Biochemistry**, v. 37, n. 9, p. 830–835, 2004.

NEVIN, K. G.; RAJAMOHAN, T. Virgin coconut oil supplemented diet increases the antioxidant status in rats. **Food Chemistry**, v. 99, n. 2, p. 260–266, 2005.

NEVIN, K. G.; RAJAMOHAN, T. Influence of virgin coconut oil on blood coagulation factors, lipid levels and LDL oxidation in cholesterol fed Sprague-Dawley rats. **European e-Journal of Clinical Nutrition and Metabolism**, v. 3, n. 1, p. 1–8, 2008.

PIGNATTI, E.; FLÜCK, C. E. Adrenal cortex development and related disorders leading to adrenal insufficiency. **Molecular and Cellular Endocrinology**, v. 527, p. 111206, 2021.

PINTO-FOCHI, M. E. et al. A high-fat diet fed during different periods of life impairs steroidogenesis of rat Leydig cells. **Reproduction**, v. 152, n. 6, p. 795–808, 2016.

QUINTAR, A. A. et al. The mongolian gerbil (Meriones unguiculatus) as a model for inflammation-promoted prostate carcinogenesis. **Cell Biology International**, v. 41, n. 11, p. 1234–1238, 2017.

RODRIGUES, A. Óleo de Coco–Milagre para Emagrecer ou Mais um Modismo? **Abeso**, n. 56, p. 3, 2012.

ROSS, M.; PAWLINA, W. **Histologia: Texto e Atlas**. 8. ed. Rio de Janeiro: Guanabara Koogan, 2021.

RUIZ, T. F. R. et al. The Mongolian Gerbil as a Useful Experimental Model in Reproductive Biology. **Reproductive Sciences**, n. 0123456789, 2023.

SCHNEIDER, C. A.; RASBAND, W. S.; ELICEIRI, K. W. NIH Image to ImageJ: 25 years of image analysis. **Nature Methods**, v. 9, n. 7, p. 671–675, 2012.

SHEN, W. J.; AZHAR, S.; KRAEMER, F. B. Lipid droplets and steroidogenic cells. **Experimental Cell Research**, v. 340, n. 2, p. 209–214, 2016.

SHIN, A. C. et al. Chronic exposure to a high-fat diet affects stress axis function differentially in diet-induced obese and diet-resistant rats. **International Journal of Obesity**, v. 34, n. 7, p. 1218–1226, 2010.

STORBECK, K. H. et al. Cytochrome b5 modulates multiple reactions in steroidogenesis by diverse mechanisms. **Journal of Steroid Biochemistry and Molecular Biology**, v. 151, p. 66–73, 2015.

TANNENBAUM, B. M. et al. High-fat feeding alters both basal and stress-induced hypothalamic- pituitary-adrenal activity in the rat. **American Journal of Physiology - Endocrinology and Metabolism**, v. 273, n. 6 36-6, p. 1168–1177, 1997.

TOLOSA, E. M. C. DE et al. **Manual de técnicas para histologia: normal e patológica**. 1. ed. São Paulo: Manole, 2003.

TOPAL, F. et al. Effect of consuming high-fat diet on the morphological parameters of adrenal gland. **Bratislava Medical Journal**, v. 120, n. 08, p. 593–600, 2019.

TURCU, A. F.; AUCHUS, R. J. Adrenal Steroidogenesis and Congenital Adrenal Hyperplasia. **Endocrinology and Metabolism Clinics of North America**, v. 44, n. 2, p. 275–296, 2015.

VOLPATO, G. **Ciência da Filosofia à Publicação**. 7. ed. [s.l: s.n.].

WALLACE, T. C. Health Effects of Coconut Oil—A Narrative Review of Current Evidence. **Journal of the American College of Nutrition**, v. 38, n. 2, p. 97–107, 2018.

YEAP, S. K. et al. Antistress and antioxidant effects of virgin coconut oil in vivo. **Experimental and Therapeutic Medicine**, v. 9, n. 1, p. 39–42, 2015.

YU, J. et al. The Adrenal Lipid Droplet is a New Site for Steroid Hormone Metabolism. **Proteomics**, v. 18, n. 23, p. 1–12, 2018.

ZAKARIA, Z. A. et al. In vivo antinociceptive and anti-inflammatory activities of dried and fermented processed virgin coconut oil. **Medical Principles and Practice**, v. 20, n. 3, p. 231–236, 2011.

ZHAO, J. L.; ZHAO, Y. Y.; ZHU, W. J. A high-fat, high-protein diet attenuates the negative impact of casein-induced chronic inflammation on testicular steroidogenesis and sperm parameters in adult mice. **General and Comparative Endocrinology**, v. 252, p. 48–59, 2017.