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**ASSOCIAÇÃO ENTRE AS CONCENTRAÇÕES DE MAGNÉSIO, ZINCO E
SELÊNIO E MARCADORES METABÓLICOS DOS FENÓTIPOS OBESIDADE
METABOLICAMENTE SAUDÁVEL E OBESIDADE METABOLICAMENTE NÃO
SAUDÁVEL**

Teresina - PI

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KYRIA JAYANNE CLÍMACO CRUZ

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Tese apresentada ao Programa de Pós-Graduação em Alimentos e Nutrição da Universidade Federal do Piauí como requisito para obtenção do título de Doutor em Alimentos e Nutrição.

Orientadora

Profa. Dra. Dilina do Nascimento Marreiro

Coorientador

Prof. Dr. Emídio Marques de Matos Neto

TERESINA

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Banca examinadora:

Presidente: Profa. Dra. Dilina do Nascimento Marreiro

1º Examinador: Profa. Dra. Silvia Maria Franciscato Cozzolino

2º Examinador: Profa. Dra. Fabiana Poltronieri

3º Examinador: Profa. Dra. Luciana Rossi Marques

4º Examinador: Dr. Vladimir Costa Silva

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*“O meu corpo e o meu coração
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força do meu coração e a minha
herança para sempre”.*

Salmos 73.26

*“Mil poderão cair ao seu lado, dez
mil a sua direita, mas nada o
atingirá”.*

Salmos 91.7

RESUMO

CRUZ, K. J. C. **Associação entre as concentrações de minerais (magnésio, zinco e selênio) e marcadores metabólicos dos fenótipos obesidade metabolicamente saudável e obesidade metabolicamente não saudável.** 2019. Tese - Programa de Pós-graduação em Alimentos e Nutrição, Universidade Federal do Piauí, Teresina-PI.

INTRODUÇÃO: Estudos epidemiológicos identificaram um subconjunto da população obesa com fenótipo metabólico benéfico, conhecido como obesidade metabolicamente saudável. Por outro lado, quando ocorre disfunção do tecido adiposo e acúmulo de gordura intra-abdominal, o indivíduo obeso apresenta fenótipo metabolicamente não saudável. Atualmente, pesquisadores têm mostrado interesse pela avaliação de micronutrientes nos dois tipos de fenótipos da obesidade.

OBJETIVO: Avaliar a associação entre as concentrações de minerais (magnésio, zinco e selênio) e marcadores metabólicos dos fenótipos obesidade metabolicamente saudável e obesidade metabolicamente não saudável.

MÉTODOS: Foi conduzido estudo transversal com 140 mulheres, que foram distribuídas em três grupos: grupo experimental I (obesas metabolicamente saudáveis, n=35), grupo experimental II (obesas metabolicamente não saudáveis, n=28) e grupo controle (mulheres saudáveis, n=77). Foram aferidos peso corporal, estatura e circunferências da cintura, do quadril e do pescoço. Índice de massa corpórea, relação cintura-quadril, relação cintura-estatura e índices de adiposidade foram calculados. As determinações das concentrações séricas de glicose e insulina de jejum foram realizadas pelos métodos enzimático colorimétrico e quimioluminescência, respectivamente. As análises das concentrações plasmáticas de hemoglobina glicada foram conduzidas por meio do método de cromatografia de troca iônica. Os lipídios séricos foram analisados segundo método enzimático colorimétrico. Os índices HOMA-IR (*Homeostasis Model Assessment Insulin Resistance*), HOMA- β , índices de Castelli I e II foram calculados. A pressão arterial foi aferida. A avaliação das concentrações de cortisol sérico e leptina plasmática foi realizada pelo método de eletroquimioluminescência e radioimunoensaio, respectivamente. As concentrações plasmáticas de TBARS (substâncias reativas ao ácido tiobarbitúrico) foram determinadas seguindo a metodologia descrita por Ohkawa, Ohishi e Yagi (1979), com adaptações. A atividade das enzimas superóxido dismutase eritrocitária e glutatona peroxidase eritrocitária foi avaliada em analisador bioquímico automático. As concentrações séricas dos hormônios TSH, T3 e T4 livres foram determinadas por quimioluminescência. A análise da ingestão de energia, macronutrientes magnésio, zinco e selênio foi realizada por meio do registro alimentar de três dias, utilizando o *software* Nutwin, versão 1.5. As concentrações plasmáticas, eritrocitárias e urinárias dos minerais foram determinadas em espectrômetro de emissão óptica com plasma acoplado indutivamente. **RESULTADOS:** Os valores de insulina de jejum, HOMA-IR, HOMA- β , triglicerídeos, VLDL, HDL-colesterol, índice de Castelli I e pressão arterial sistólica foram semelhantes entre o grupo controle e as mulheres obesas metabolicamente saudáveis e estas ainda tinham valores reduzidos destes parâmetros em relação às obesas não saudáveis, com exceção do HDL-colesterol, que se encontrava elevado. Ambos os grupos dos fenótipos obesos possuíam valores elevados de TBARS e leptina e reduzidos da enzima superóxido dismutase, quando comparados ao grupo controle. No entanto, somente as obesas não saudáveis mostraram valores elevados da fração não-HDL-colesterol e do índice de Castelli II, em relação às participantes eutróficas. As pacientes obesas apresentaram concentrações plasmáticas e

eritrocitárias reduzidas dos minerais e valores urinários elevados, em relação ao grupo controle, independente do fenótipo saudável ou não saudável. Houve correlação significativa entre as concentrações dos minerais e marcadores metabólicos dos fenótipos metabolicamente saudáveis e não saudáveis. **CONCLUSÃO:** O estudo revela a influência da deficiência nestes minerais em distúrbios metabólicos presentes na obesidade, a exemplo da resistência à insulina, dislipidemias, estresse oxidativo e alterações nos hormônios tireoideanos.

Palavras-chave: Obesidade. Obesidade metabolicamente saudável. Magnésio. Zinco. Selênio.

ABSTRACT

CRUZ, K. J. C. **Association between mineral concentrations (magnesium, zinc and selenium) and metabolic markers of metabolically healthy obesity and metabolically unhealthy obesity phenotypes.** 2019. Thesis – Postgraduate Program in Food and Nutrition, Federal University of Piauí, Teresina-PI.

INTRODUCTION: Epidemiological studies have identified a subgroup within the obese population who displays the benefit metabolic phenotype, and is known as metabolically healthy obesity. On the other hand, the obese individual displays metabolically unhealthy phenotype when there is adipose tissue dysfunction and intra-abdominal fat accumulation. Currently, researches for assessing biomarkers of micronutrients concentrations in both two obesity-related phenotypes have been carried out. **AIM:** To evaluate the association between mineral concentrations (magnesium, zinc and selenium) and metabolic markers of metabolically healthy obesity and metabolically unhealthy obesity phenotypes. **METHODS:** This was a cross-sectional study involving 140 women. The participants were divided into three groups: experimental group I (metabolically healthy obese women, n = 35), experimental group II (metabolically unhealthy obese women, n = 28) and control group (eutrophic women, n = 77). This study is within the project entitled “Impact of minerals on endocrine metabolic disorders”. Body weight, height, waist circumference, hip circumference and neck circumference were measured. Body mass index, waist-hip ratio, waist-height ratio and some adiposity indices were calculated. Analyses of fasting glucose and serum insulin concentration were carried out using the colorimetric enzymatic method and chemiluminescence method, respectively. Analysis of glycated hemoglobin was carried out using the ion exchange chromatography method. Serum lipids were analyzed using the colorimetric enzymatic method. HOMA-IR index (Homeostasis Model Assessment Insulin Resistance), HOMA- β index, Castelli's risk index I and II were calculated. Analysis of serum cortisol concentration and plasma leptin level were performed using the electrochemiluminescence method and radioimmunoassay method, respectively. Plasma concentration of TBARS was determined using the method proposed by Ohkawa; Ohishi e Yagi (1979), with adaptations. Erythrocyte glutathione peroxidase and erythrocyte dismutase superoxide activities were analyzed using an automatic biochemical analyzer. Serum TSH, T3 and T4 concentrations were evaluated using the chemiluminescence method. The three-day food record method was used for the analysis of the dietary intake of energy, macronutrients, magnesium zinc and selenium. Dietary intake was calculated using the software NutWin, version 1.5. Elemental analyses of the plasma, erythrocyte and urinary minerals levels were performed on an inductively coupled plasma spectrometer - Optical Emission Spectrometry. **RESULTS:** Serum insulin, HOMA-IR, HOMA- β , triglycerides, VLDL, HDL cholesterol, Castelli's risk index I and arterial systolic pressure were similar between control group and metabolically healthy obese women; however, metabolically healthy obese showed lower values of these parameters than unhealthy obese, except HDL cholesterol, which was higher. The both obese phenotypes groups showed higher values of TBARS and leptin, and lower superoxide dismutase activity than control group. However, only metabolically unhealthy obese showed higher values of non-HDL cholesterol and Castelli's risk index II than eutrophic women. Obese patients presented lower plasma and erythrocyte mineral concentrations, and higher urinary mineral levels than control group, independently of healthy or unhealthy phenotypes. Statistically significant correlation

was found between parameters of mineral concentrations and metabolic markers of metabolically healthy and metabolically unhealthy phenotypes. **CONCLUSION:** This study shows the influence of magnesium, zinc and selenium deficiencies on obesity-associated metabolic disorders, such as insulin resistance, dyslipidemias, oxidative stress and thyroid hormonal dysfunction.

Keywords: Obesity. Metabolically Healthy Obesity. Magnesium. Zinc. Selenium.

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LISTA DE ABREVIATURAS E SIGLAS

ACC1 - Acetil CoA carboxilase

AdipoQ - Adiponectina

AGRP - Proteína relacionada ao gene Agouti

AHA - *American Heart Association* (Associação Americana do Coração)

AKT - Proteína quinase B

ApoB - Apolipoproteína B

Apo E - Apolipoproteína E

ASK-1 - Quinase reguladora de sinais apoptóticos 1

ASP - Proteína estimuladora de acilação

Ca - Cálcio

CaSR - Receptor sensível ao cálcio extracelular

CC - Circunferência da cintura

C/EBPs - Proteínas ligantes ao amplificador CCAAT

CHO - Carboidratos

CT - Colesterol total

Cu - Cobre

DGAT1 - Diacilglicerol aciltransferase 1

F - Feminino

Fe - Ferro

FAS - Ácido graxo sintase

FOXO1 - *Forkhead box protein O1*

GLP-1 - Peptídeo semelhante ao glucagon 1

GLUT-4 - Transportador de glicose tipo 4

GPJ - Glicose plasmática de jejum

GSK-3 - Glicogênio sintase quinase 3

HDL - Lipoproteína de alta densidade

HIF-1 α - Fator 1 α induzido por hipóxia

HMG-CoA redutase - 3-hidroxi-3-metilglutaril-CoA redutase

HOMA-IR - *Homeostasis Model Assessment Insulin Resistance* (modelo de avaliação homeostático de resistência insulínica)

H₂O₂ - Peróxido de hidrogênio

IDF - *International Diabetes Federation* (Federação Internacional de Diabetes)

IGF-1 - Fator de crescimento semelhante à insulina tipo 1

IKK - Quinase do inibidor do fator nuclear kappa B

IKK β - Quinase beta do inibidor do fator nuclear kappa B

IL-1 β - Interleucina 1 β

IL-10 - Interleucina 10

IL-6 - Interleucina 6

IMC - Índice de massa corpórea

INS - Insulina de jejum

IRAP - Aminopeptidase responsiva à insulina

IRS - Substrato do receptor de insulina

IRS-1 - Substrato 1 do receptor de insulina

8-iso-PGF2 α - 8-iso-prostaglandina F2 α

JNK - *Janus* quinase

LCAT - Lecitina:colesterol aciltransferase

LDL - Lipoproteína de baixa densidade

M - Masculino

MAPK - Proteína quinase ativada por mitógeno

MCP-1 - Proteína quimiotática de monócitos-1

MIF - Fator inibitório da migração de macrófagos

MTF-1 - Fator de transcrição regulamentar de metal 1

NADPH oxidase - Nicotinamida adenina dinucleotídeo fosfato oxidase

NCEP-ATP III - *National Cholesterol Education Program - Adult Treatment Panel III*
(Programa Nacional de Educação em Colesterol III Painel de Tratamento de Adultos)

NF- κ B - Fator nuclear kappa B

NGF - Fator de crescimento nervoso

NHANES III - *The Third National Health and Nutrition Examination Survey* (Terceira Pesquisa de Exames de Saúde e Nutrição Nacional)

NMDA - N-metil-D-aspartato

NMDAR - Receptor N-metil-D-aspartato

NO - Óxido Nítrico

NPY - Neuropeptídeo Y

Nrf2 - Fator nuclear eritróide 2 relacionado ao fator 2

OMS - Organização Mundial da Saúde

ObMS - Obesidade metabolicamente saudável

ONOO⁻ - Peroxinitrito

PA - Pressão arterial

PBMC - Células mononucleares do sangue periférico

PCR - Proteína C reativa

PEDF - Fator derivado do epitélio pigmentado

PG - Prostaglandina

PGRN - Progranulina

PI3K - Fosfatidilinositol 3-quinase

PIP3 - Fosfatidilinositol 3,4,5-trifostato

PPAR- α - Receptor alfa ativado por proliferadores de peroxissomas

PPAR- γ - Receptor gama ativado por proliferadores de peroxissomas

PTEN - Fosfatase homóloga à tensina

PTPase - Proteína tirosina fosfatase

PTPase 1B - Proteína tirosina fosfatase 1B

QUICKI - *Quantitative Insulin-Sensitivity Check Index* (Índice quantitativo de verificação de sensibilidade à insulina)

SCD-1 - Estearoil-CoA dessaturase-1

SOD - Superóxido dismutase

SREBP-1c - Proteína de ligação ao elemento regulador de estero 1c

STAT - Transdutor de sinal e ativador da transcrição

TBARs - Substâncias reativas ao tiobarbitúrico

TG - Triglicerídeos

TGF- β - Fator de transformação do crescimento β

TLR - Receptor *toll-like*

TNF- α - Fator de necrose tumoral α

VEGF - Fator de crescimento endotelial vascular

Vigitel - Vigilância de fatores de risco e proteção para doenças crônicas por inquérito telefônico

VLDL - Lipoproteínas de muito baixa densidade

ZAG - Zinco- α 2-glicoproteína

ZIP - *Zrt-and Irt-like protein*

ZnT - *Zinc transporter*

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Objetivos

*Associação entre as concentrações de minerais (magnésio, zinco e selênio) e marcadores metabólicos dos
fenótipos obesidade metabolicamente saudável e obesidade metabolicamente não saudável*

Kyria Jayanne Clímaco Cruz

3 OBJETIVOS

3.1 Objetivo Geral

Avaliar a associação entre as concentrações de minerais (magnésio, zinco e selênio) e marcadores metabólicos dos fenótipos obesidade metabolicamente saudável e obesidade metabolicamente não saudável.

3.2 Objetivos Específicos

- Caracterizar os fenótipos de mulheres obesas metabolicamente saudáveis e não saudáveis;
- Estimar o consumo alimentar de energia, macronutrientes, magnésio, zinco e selênio e sua adequação na dieta;
- Determinar as concentrações plasmáticas, eritrocitárias e urinárias de magnésio, zinco e selênio;
- Caracterizar a adiposidade geral e visceral;
- Determinar parâmetros do controle glicêmico, risco cardiovascular e do estresse oxidativo;
- Analisar as concentrações séricas de leptina, cortisol e hormônios tireoidianos.

Metodologia

*Associação entre as concentrações de minerais (magnésio, zinco e selênio) e marcadores metabólicos dos
fenótipos obesidade metabolicamente saudável e obesidade metabolicamente não saudável*

Kyria Jayanne Clímaco Cruz

4 METODOLOGIA

Estudo de natureza transversal envolvendo mulheres com obesidade ou peso normal, na faixa etária entre 20 e 50 anos de idade. Esta pesquisa faz parte de um projeto maior intitulado “Impacto de minerais em distúrbios endocrinometabólicos”. A definição da amostra do estudo foi baseada na amostragem por conveniência, sendo que as participantes com obesidade foram recrutadas a partir da demanda espontânea de ambulatórios clínicos da cidade de Teresina – PI, e as eutróficas por meio de chamadas públicas, em mídia digital e eventos de educação nutricional, e ambulatórios clínicos.

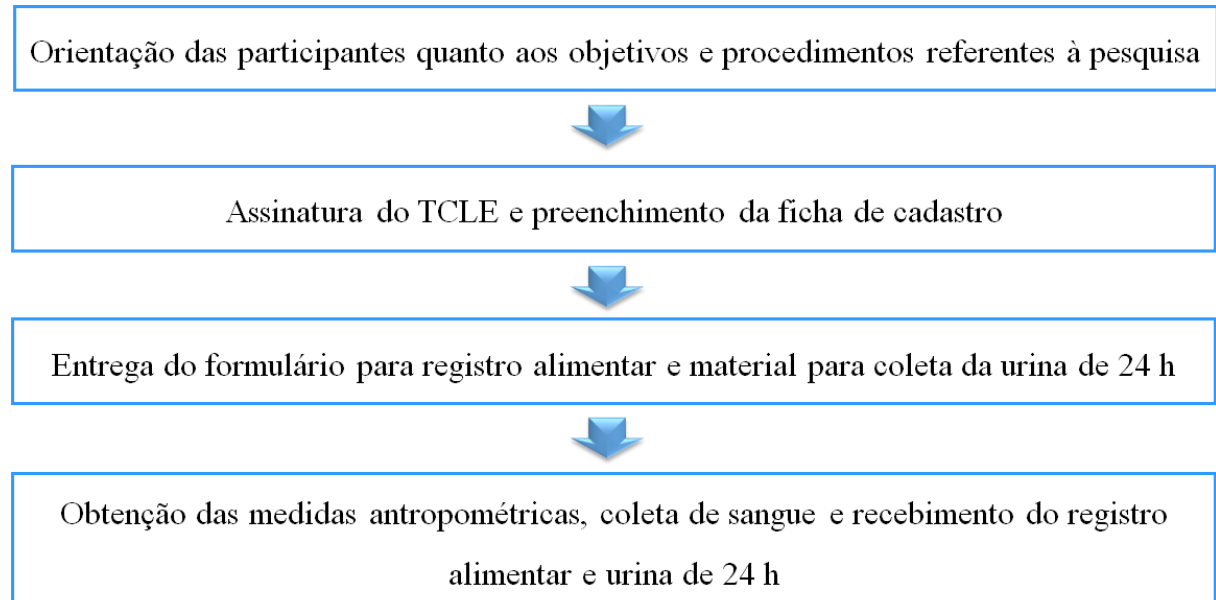
As participantes foram selecionadas por meio de entrevista, com os seguintes critérios de inclusão: idade entre 20 e 50 anos, índice de massa corpórea (IMC) a partir de 35,0 kg/m² (grupos experimental I e II) e entre 18,5 e 24,9 kg/m² (grupo controle), ausência de diabetes mellitus, doença renal crônica, câncer ou infecções recentes; não estar gestante ou lactante; não estar participando de outro estudo clínico; não fazer uso de suplemento vitamínico-mineral e/ou medicamentos que possam interferir no estado nutricional relativo aos minerais e nos distúrbios metabólicos avaliados; não fumantes; não ingerir álcool de forma crônica. Estas informações foram autorrelatadas pelas participantes.

O projeto foi aprovado pelo Comitê de Ética em Pesquisa da Universidade Federal do Piauí, sob número de parecer 2.014.100, conforme prevê a Resolução 466/12 do Conselho Nacional de Saúde (CNS) (BRASIL, 2012). Todas as participantes assinaram um termo de consentimento livre e esclarecido (APÊNDICE A) do estudo, elaborado de acordo com a “Declaração de Helsinki III”, capítulo 50, parágrafos 50.20/27, que trata da proteção dos participantes e orienta procedimentos referentes às pesquisas que necessitam de experiências com humanos. Em seguida, foi preenchida uma ficha de cadastro, após receberem informações detalhadas sobre a pesquisa com linguagem adequada, conforme estabelecido pela Resolução 466/12 do Conselho Nacional de Saúde (BRASIL, 2012).

Em seguida, foram entregues formulário para o registro alimentar e material para coleta da urina de 24 horas, bem como foram agendadas datas para obtenção das medidas antropométricas, coleta de sangue e entrega do registro alimentar e urina

de 24 horas. As atividades realizadas com as participantes do estudo estão esquematizadas na figura 7.

Figura 7 - Fluxograma das atividades realizadas com as participantes do estudo.



As metodologias utilizadas para avaliação antropométrica, estimativa do consumo alimentar e análise dos parâmetros bioquímicos estão apresentadas nos capítulos 1 e 2, que tratam dos artigos originados desta tese.

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Resultados e Discussão

Capítulo 1

*Associação entre as concentrações de minerais (magnésio, zinco e selênio) e marcadores metabólicos dos
fenótipos obesidade metabolicamente saudável e obesidade metabolicamente não saudável*

Kyria Jayanne Clímaco Cruz

Artigo Científico: Associação entre as concentrações de magnésio, zinco e selênio e marcadores metabólicos dos fenótipos obesidade metabolicamente saudável e obesidade metabolicamente não saudável

Periódico: *Nutrients* (Artigo a ser submetido)

Qualis: A1 na área Ciência dos Alimentos

Fator de impacto: 4,196



Associação entre as concentrações de magnésio, zinco e selênio e marcadores metabólicos dos fenótipos obesidade metabolicamente saudável e obesidade metabolicamente não saudável

Kyria Jayanne Clímaco Cruz; Ana Raquel Soares de Oliveira; Jennifer Beatriz Silva Morais; Stéfany Rodrigues de Sousa Melo; Loanne Rocha dos Santos; Larissa Cristina Fontenelle; Suelem Torres de Freitas; Gilberto Simeone Henriques; Silvana Auxiliadora Bordin da Silva; Carla Soraya Costa Maia; Francisco Erasmo de Oliveira; Carlos Henrique Nery Costa; Emídio Marques de Matos Neto; Dilina do Nascimento Marreiro

Resumo

Estudos epidemiológicos têm identificado um subconjunto da população obesa com fenótipo metabólico benéfico, conhecido como obesidade metabolicamente saudável. Por outro lado, quando ocorre disfunção do tecido adiposo, o indivíduo obeso apresenta fenótipo metabolicamente não saudável. Pesquisadores têm mostrado interesse pela avaliação de micronutrientes nos dois fenótipos de obesidade. O objetivo do presente estudo foi avaliar a associação entre as concentrações de minerais (magnésio, zinco e selênio) e marcadores metabólicos dos fenótipos obesidade metabolicamente saudável e não saudável. Foi conduzido estudo transversal com 112 mulheres, que foram distribuídas em três grupos: grupo experimental I (obesas metabolicamente saudáveis, n=35), grupo experimental II (obesas metabolicamente não saudáveis, n=28) e grupo controle (mulheres saudáveis, n=77). Foram aferidos peso, altura e circunferências da cintura, do quadril e do pescoço. Índice de massa corpórea, relação cintura-quadril, relação cintura-estatura e índices de adiposidade foram calculados. Foram conduzidas as determinações da glicose de jejum, hemoglobina glicada, insulina de jejum e dos parâmetros do risco cardiovascular (lipídios séricos e pressão arterial). Os índices HOMA-IR (*Homeostasis Model Assessment Insulin Resistance*), HOMA- β , índices de Castelli I e II foram calculados. As análises de cortisol sérico, leptina plasmática, concentrações plasmáticas de TBARS (substâncias reativas ao ácido tiobarbitúrico), hormônios tireoidianos, atividade das enzimas superóxido dismutase eritrocitária e glutathione peroxidase eritrocitária foram realizadas. A análise da ingestão de energia, macronutrientes, magnésio, zinco e selênio e a determinação das concentrações plasmáticas, eritrocitárias e urinárias destes minerais foram conduzidas. As pacientes obesas apresentam concentrações plasmáticas e eritrocitárias reduzidas e valores urinários elevados dos minerais magnésio, zinco e selênio, independente do fenótipo saudável ou não saudável. A deficiência nestes minerais está associada aos distúrbios metabólicos presentes na obesidade, a exemplo da resistência à insulina, dislipidemias, estresse oxidativo e alterações nos hormônios tireoidianos.

Palavras-chave: Obesidade. Obesidade metabolicamente saudável. Magnésio. Zinco. Selênio.

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Introdução

A obesidade é uma doença crônica caracterizada pelo acúmulo excessivo de gordura corporal (WHO, 2016). Estudos epidemiológicos têm identificado um subconjunto da população obesa com fenótipo metabólico benéfico, conhecido como obesidade metabolicamente saudável. Este fenótipo é caracterizado por perfil inflamatório favorável, sensibilidade à insulina, pressão arterial normal e ausência de dislipidemia (DURWARD et al., 2012; MARRA et al., 2019; WILDMAN et al., 2008; ZHANG et al., 2016).

Por outro lado, o fenótipo obesidade metabolicamente não saudável é caracterizado pela presença de distúrbios metabólicos, a exemplo da inflamação crônica de baixo grau, resistência à insulina e estresse oxidativo. Estes distúrbios parecem ser decorrentes da disfunção do tecido adiposo e acúmulo de gordura intra-abdominal, que ocorre quando a capacidade de expansão do tecido adiposo subcutâneo de indivíduos obesos é excedida (BLÜHER, 2009; BRAY, 2018; GOOSSENS, 2017).

A literatura também tem evidenciado a existência de deficiências nutricionais em indivíduos obesos, em particular, os minerais magnésio, zinco e selênio. Estes nutrientes exercem funções anti-inflamatória, antioxidante e sensibilizadora da ação da insulina. Assim, a deficiência nestes micronutrientes tem repercussões metabólicas importantes que contribuem para exacerbar os distúrbios metabólicos presentes nesses pacientes (CRUZ et al., 2014; OLIVEIRA et al., 2015; MORAIS et al., 2017).

Atualmente, pesquisadores têm mostrado interesse pela avaliação de micronutrientes em obesos metabolicamente saudáveis, que parecem apresentar concentrações séricas elevadas de minerais, como o magnésio, em relação aos pacientes obesos não saudáveis. Dessa forma, sugere-se que estes nutrientes podem contribuir para a prevenção de distúrbios metabólicos, como o estresse oxidativo, inflamação, dislipidemias e resistência à insulina, minimizando assim a manifestação destas desordens no grupo metabolicamente saudável (GUERRERO-ROMERO; RODRÍGUEZ-MORÁN, 2013; STENZEL et al., 2018). No entanto, as evidências sobre o tema ainda são escassas na literatura publicada.

Portanto, o objetivo do presente estudo foi avaliar a associação entre as concentrações de minerais (magnésio, zinco e selênio) e marcadores metabólicos dos

Associação entre as concentrações de minerais (magnésio, zinco e selênio) e marcadores metabólicos dos fenótipos obesidade metabolicamente saudável e obesidade metabolicamente não saudável

fenótipos obesidade metabolicamente saudável e obesidade metabolicamente não saudável, na perspectiva de encontrar possíveis contribuições das funções fisiológicas exercidas pelos minerais na proteção contra distúrbios associados à obesidade.

Métodos

Foi conduzido estudo transversal com 112 mulheres, que foram distribuídas em três grupos: grupo experimental I (mulheres com obesidade metabolicamente saudáveis, n=35), grupo experimental II (mulheres com obesidade metabolicamente não saudáveis, n=28) e grupo controle (mulheres saudáveis, n=77). As participantes foram recrutadas da demanda espontânea de ambulatório particular da cidade de Teresina-Piauí.

Para diagnosticar a obesidade metabolicamente saudável, foram considerados os critérios NCEP-ATP III (2005) associados aos valores de HOMA-IR (*Homeostasis Model Assessment Insulin Resistance*) e de hemoglobina glicada. As mulheres foram consideradas metabolicamente saudáveis quando possuíam até dois parâmetros alterados, dentre os seguintes: circunferência da cintura > 88 cm, pressão arterial sistólica ≥ 130 mmHg ou diastólica ≥ 85 mmHg, glicemia de jejum ≥ 100 mg/dL, triglicerídeos ≥ 150 mg/dL, HDL-colesterol < 50 mg/dL, HOMA-IR > 2,71 e hemoglobina glicada $\geq 5,7\%$ (MATTHEWS et al., 1985; ADA, 2017).

As participantes foram selecionadas por meio de entrevista, com os seguintes critérios de inclusão: idade entre 20 e 50 anos, índice de massa corpórea (IMC) a partir de 35,0 kg/m² (grupos experimental I e II) e entre 18,5 e 24,9 kg/m² (grupo controle), ausência de diabetes *mellitus*, doença renal crônica, câncer ou infecções recentes; não estar gestante ou lactante; não estar participando de outro estudo clínico; não fazer uso de suplemento vitamínico-mineral e/ou medicamentos que possam interferir no estado nutricional relativo aos minerais e nos distúrbios metabólicos avaliados; não fumantes; não ingerir álcool de forma crônica. Estas informações foram autorrelatadas pelas participantes.

O estudo foi aprovado pelo Comitê de Ética da Universidade Federal do Piauí, Brasil, sob número 2.014.100. Todas as participantes que aceitaram participar da pesquisa assinaram um termo de consentimento livre e esclarecido, conforme prevê a Resolução 466/12 do Conselho Nacional de Saúde (CNS) (BRASIL, 2012).

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Avaliação do Estado Nutricional

O peso corporal, estatura, circunferência da cintura, do pescoço e quadril das participantes foram aferidos. O IMC foi calculado a partir do peso da participante do estudo dividido por sua estatura elevada ao quadrado, e a classificação do estado nutricional foi conduzida seguindo as recomendações da Organização Mundial de Saúde (WHO, 2000). Os valores de referência utilizados para circunferência da cintura e do pescoço foram os recomendados pela Organização Mundial de Saúde (WHO, 2008) e Ben-Noun et al. (2004), respectivamente.

Coleta do Material Biológico

Amostras de 30 mL de sangue venoso foram coletadas no período da manhã, entre 7 e 9 horas, estando as participantes em jejum mínimo de 12 horas. Para a coleta de urina de 24h, foram fornecidos frascos desmineralizados, que foram pesados antes e após a coleta em balança semianalítica para a determinação do volume urinário a partir da densidade. Após esse procedimento, foram retirados 8 mL de urina e distribuídos em 4 microtubos de polipropileno, e conservados em *freezer* a -20°C para análise posterior dos minerais zinco e selênio. O volume remanescente foi acidificado com ácido clorídrico a 3 mol/L e homogeneizado para evitar a precipitação do magnésio na urina e para melhor preservação do mineral (ELIN, 1987). Em seguida, foram retirados 4 mL de urina e distribuídos em 2 microtubos de polipropileno, os quais foram conservados em *freezer* a -20°C.

Determinação do Controle Glicêmico

As concentrações séricas da glicose de jejum foram analisadas por meio do método enzimático colorimétrico, utilizando-se os kits *Labtest*. Os valores de glicose de jejum entre 75 e 99 mg/dL foram considerados normais, segundo os critérios definidos pela *American Diabetes Association* (ADA, 2017). As concentrações séricas de insulina foram determinadas segundo o método de quimioluminescência, considerando como padrão de referência os valores entre 6 e 27 µU/mL.

A análise das concentrações plasmáticas de hemoglobina glicada foi conduzida pelo método de cromatografia de troca iônica. Os valores abaixo de 5,7% para a hemoglobina glicada foram considerados normais, segundo a *American Diabetes Association* (ADA, 2017).

Os valores de HOMA-IR foram calculados por meio da seguinte fórmula: $(\text{insulina } [\mu\text{U/mL}] \times \text{glicose } [\text{mmol/L}]) / 22,5$. O índice HOMA- β foi calculado usando o programa *HOMA Calculator*, versão 2.2.3 (*HOMA Calculator*, 2017). Valores de HOMA-IR acima de 2,71 ou do HOMA- β acima de 1,8 indicaram um quadro de resistência à insulina (MATTHEWS et al., 1985; WALLACE et al., 2004).

Avaliação do Risco Cardiovascular

Aferição da Pressão Arterial

A aferição da pressão arterial foi realizada com aparelho digital de pulso da marca G-Tech, modelo BP3AF1-3, após a participante ficar dez minutos em repouso. Foram considerados como padrão de referência os valores de pressão sistólica menores que 130 mmHg e pressão diastólica, menores que 85 mmHg (ANDRADE et al., 2010).

Determinação do Perfil Lipídico

As concentrações séricas de colesterol total, LDL-colesterol, HDL-colesterol e triglicérides foram determinadas segundo método enzimático colorimétrico, em analisador bioquímico automático Cobas Integra (*Roche Diagnostics*, Brasil), utilizando kits *Roche*. A fração LDL-colesterol foi calculada de acordo com a fórmula de Friedwald et al. (1972): $\text{LDL-c} = \text{CT} - \text{HDL-c} - \text{TG}/5$, sendo válida para valores de triglicérides até 400 mg/dL. A fração não-HDL-colesterol foi calculada por meio da diferença entre os valores de colesterol total e HDL-colesterol. Os valores para lipídios séricos definidos na Atualização da Diretriz Brasileira de Dislipidemias e Prevenção da Aterosclerose – 2017 foram utilizados como padrão de referência (FALUDI et al., 2017).

Relação Cintura-quadril e Relação Cintura-estatura

A relação cintura-quadril foi calculada a partir da circunferência da cintura da participante dividido pela circunferência do quadril (WHO, 1998), e a relação cintura-estatura foi calculada dividindo-se a circunferência da cintura pela estatura (PITANGA; LESSA, 2006).

Índices de Adiposidade Corporal

O índice de conicidade foi calculado usando a seguinte fórmula: circunferência da cintura [cm]/(0,109 $\sqrt{\text{peso corporal [kg]}}$ / estatura [m]) (PITANGA; LESSA, 2006). O índice de formato corporal foi calculado por meio da fórmula: circunferência da cintura [m]/ (IMC^{2/3} x $\sqrt{\text{peso corporal [kg]}}$ / estatura [m]) (KRAKAUER; KRAKAUER, 2012).

O índice de circularidade corporal foi calculado usando a fórmula: 364,2 – (365,5 x excentricidade [e]), sendo $e = \sqrt{1 - (\text{circunferência da cintura [cm]} / 4\pi) / (0,5 \times \text{estatura [cm]})}$ (THOMAS et al., 2013). O índice de adiposidade corporal foi calculado usando a seguinte fórmula: ((circunferência do quadril [cm]/estatura [m]^{1,5}) -18) (BERGMAN et al., 2011). O índice de adiposidade visceral foi calculado usando a presente fórmula sexo específica: (circunferência da cintura [cm]/36,58 + [1,89 x IMC])x(triglicerídeos [mmol/L]/0,81) x (1,52/HDL-c [mmol/L]) para mulheres (AMATO et al., 2010).

Índices de Castelli I e II

Para determinação do risco de doença cardiovascular, também foram utilizados os índices de Castelli I e II, que correspondem à razão entre o colesterol total e o HDL-colesterol e a razão entre LDL-colesterol e HDL-colesterol, respectivamente. Os valores de referência são: índice de Castelli I $\leq 4,3$ e índice de Castelli II $\leq 2,9$ (CASTELLI; ABBOT; MCNAMARA, 1983).

Determinação de Marcadores do Estresse Oxidativo

As concentrações plasmáticas das substâncias reativas ao ácido tiobarbitúrico (TBARS) foram determinadas de acordo com o método proposto por Ohkawa; Ohishi; Yagi (1979). A calibração da curva foi preparada utilizando as concentrações de 0,5 a 8,0 nmol/mL do reagente padrão 1,1,3,3-tetraetoxipropeno. A absorbância foi medida com espectrofotômetro *Bel Photonics UV/Vis*, modelo 1102, em comprimento de onda de 532nm.

A determinação da atividade das enzimas glutathione peroxidase eritrocitária e superóxido dismutase eritrocitária foi realizada em analisador bioquímico automático (*Labmax 240*); utilizando-se, respectivamente, kit *Ransel 505* (Laboratório *Randox*), de acordo com metodologia proposta por Paglia e Valentine (1967) e kit *Ransod* (Laboratório *Randox*), conforme metodologia recomendada pelo fabricante. A concentração de hemoglobina do hemolisado foi determinada para o resultado final ser expresso em unidades de enzima por grama de hemoglobina (U/g Hg). Os valores de referência utilizados para atividade da glutathione peroxidase e superóxido dismutase foram, respectivamente, 27,5 a 73,6 U/gHg e 1.102 a 1.601 U/gHg, propostos pela *Randox*.

Determinação das Concentrações de Cortisol Sérico e Leptina Plasmática

A avaliação da concentração de cortisol sérico foi realizada segundo o método de eletroquimioluminescência, sendo adotado como padrão de referência valores entre 6,23 – 18,01 µg/dL no turno da manhã (NIEMAN et al., 2008). A determinação da concentração plasmática de leptina foi realizada de acordo com o método de radioimunoensaio, utilizando kit Elisa.

Determinação dos Hormônios Tireoidianos

As concentrações séricas dos hormônios TSH (hormônio estimulante da tireoide), T3 e T4 livres foram determinadas pelo método de quimioluminescência, utilizando kits específicos e seguindo as instruções do fabricante (*Abbott*, Estados Unidos). Foram consideradas eutiroidianas as pacientes que apresentaram valores de

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TSH, T3 e T4 livres nos seguintes intervalos de referência, respectivamente: 0,40-5,0 µUI/mL, 1,71-3,71 pg/mL e 0,70-1,50 ng/mL.

Análise da Ingestão Dietética dos Minerais (Magnésio, Zinco e Selênio)

O método do registro alimentar de três dias foi utilizado para análise da ingestão dietética de energia, macronutrientes e minerais, sendo calculada por meio do programa *Nutwin*, versão 1.5 (ANÇÃO et al., 2002). Os valores de EAR (*Estimated Average Requirement*) recomendados pelas DRIs (*Dietary Reference Intakes*) foram utilizados como padrão de referência para verificar adequação na ingestão destes nutrientes (IOM, 1997, 2001, 2005, 2000).

Os valores de referência adotados para os minerais foram: 255 mg de magnésio/dia para mulheres com idade de 19 a 30 anos e 265 mg de magnésio/dia para aquelas com mais de 30 anos (IOM, 1997); 6,8 mg de zinco/dia (IOM, 2001); 45µg de selênio/dia e 1 µg de selênio/kg/dia (IOM, 2000; KIPP et al., 2015).

Os valores da ingestão de energia, macronutrientes e minerais foram inseridos na plataforma online *Multiple Source Method* (MSM), versão 1.0.1, para ajustes de variabilidade intrapessoal e interpessoal, corrigida por técnicas de modelagem estatística, bem como para estimativa do consumo alimentar habitual desses nutrientes, por meio de análise de regressão logística (HAUBROCK et al., 2011; LAUREANO et al., 2016; MSM, 2011; SOUVEREIN et al., 2011). Este programa pode ser acessado por meio do *website* MSM (<https://nugo.dife.de/msm/>).

Os valores dietéticos de macronutrientes e minerais também foram ajustados em relação à energia por meio do método residual, evitando distorções geradas por diferenças no consumo energético. Após verificar a normalidade da distribuição dos dados, os valores de ingestão foram ajustados em relação à energia pelo cálculo do nutriente (FISBERG et al., 2005; JAIME et al., 2003; WILLETT; STAMPFER, 1986).

Avaliação dos Parâmetros Bioquímicos dos Minerais (Magnésio, Zinco e Selênio)

Para análise das concentrações plasmáticas dos minerais, o plasma foi separado do sangue total por centrifugação (CIENTEC® 4K15, São Paulo, Brasil) a 1831xg durante 15 minutos a 4 °C. Em seguida, o plasma foi extraído com pipeta

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automática e acondicionado em microtubos de polipropileno, sendo posteriormente conservados a -20 °C.

Para separação dos eritrócitos, foram utilizados os métodos propostos por Whitehouse et al. (1982). A massa eritrocitária foi lavada 3 vezes com 5 mL de solução salina isotônica (NaCl a 0,9%), sendo cuidadosamente homogeneizada por inversão e centrifugada (CIENTEC® 4K15, São Paulo, Brasil) a 2493xg por 10 minutos a 4 °C e o sobrenadante aspirado e descartado. Após a última centrifugação, a solução salina foi descartada e a massa eritrocitária extraída foi cuidadosamente transferida para microtubos, que foram mantidos à temperatura de – 20°C para análise posterior.

As análises das concentrações plasmáticas, eritrocitárias e urinárias foram realizadas no Laboratório de Espectrometria de Emissão Atômica - Embrapa (Centro Nacional de Pesquisa de Milho e Sorgo), localizado em Sete Lagoas – Minas Gerais. A análise elementar dos minerais foi realizada em espectrômetro de plasma indutivamente acoplado - Espectrometria de Emissão Óptica com uma configuração de vista axial e um nebulizador V-Groove (720 ICP/OES, Varian Inc., Califórnia, Estados Unidos).

Os valores de referência adotados foram: 0,75 a 1,05 mmol/L para magnésio plasmático (TOPF; MURRAY, 2003), 1,65 a 2,65 mmol/L para magnésio eritrocitário, 3,00 a 5,00 mmol/dia para magnésio urinário (TIETZ, 1995), 75 a 110 µg/dL para zinco plasmático (GIBSON, 2005), 40 a 44 µg/gHb para zinco eritrocitário (GUTHRIE; PICCIANO, 1994), 300-600 µg/24 horas para zinco urinário (GIBSON, 2005), 80 a 95 µg/L para selênio plasmático (para maximização da atividade da glutathione peroxidase plasmática) e valor $\geq$ 65 µg/L (para atividade ótima das enzimas deiodinases) (THOMSON, 2004), e 0,18 a 0,55 µg/gHb para selênio eritrocitário (VITOUX; ARNAUD; CHAPPUIS, 1999).

Análise Estatística

Tratamento a dados faltantes

Considerando o número de dados ausentes no banco de dados, sendo 4,8% no grupo controle e 15,8% no grupo de obesas, optou-se por realizar um processo de imputação de dados, com o objetivo de “completar” o banco e possibilitar a análise

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com todos os indivíduos do estudo. O método imputação única foi utilizado para o grupo de variáveis com dados ausentes menores que 15%, e a imputação múltipla, quando houve mais de 15% de perda de dados, segundo Harrel Jr. (2001).

As imputações única e múltipla foram realizadas pelo método *Predictive Mean Matching* (PMM), o qual é indicado para variáveis quantitativas. Para imputação única por meio deste método, foram conduzidas três etapas (LITTLE, 1988): (1) estimação de um modelo de regressão, sendo que a variável de interesse (a imputar) foi a variável resposta e as variáveis recolhidas restantes foram as explicativas; (2) estimação do valor da variável de interesse para os sujeitos com o dado omissos; (3) emparelhamento do valor da variável de interesse predito, para os sujeitos com o dado omissos, com o valor ajustado mais próximo (feito a partir do cálculo da distância euclidiana). No caso em que houve mais de um valor ajustado com distância igual à distância mínima encontrada, foi escolhido aleatoriamente o valor a imputar, dentre os que sofreram empate.

A imputação múltipla por PMM foi baseada nos mesmos princípios que a anteriormente descrita, exceto que em vez de se gerar apenas um valor, foram gerados vários. Depois de realizadas as imputações múltiplas, as estimativas gerais para os valores foram obtidas pela média dos valores imputados ($m=5$).

Como os métodos de imputação usados neste estudo são baseados em análise de regressão linear, foi necessário definir variáveis que seriam usadas para imputação. Sob a suposição de que os dados ausentes têm mecanismo de não-resposta MAR (*Missing at Random*), variáveis explicativas que tinham uma relação mais próxima com as variáveis respostas foram incluídas nos modelos.

Para realizar as imputações únicas e as imputações múltiplas, foram utilizadas a função criada por Andreozzi (2013) e o pacote *Multivariate Imputation by Chained Equations* (MICE), respectivamente. Ambos os processos foram realizados no programa R (R Development Core Team, 2019).

Análise dos Dados

A pressuposição de normalidade das variáveis foi avaliada por meio do teste de Kolmogorov-Smirnov e teste Shapiro-Wilk. Para comparação entre os grupos, foram aplicados os testes ANOVA ou Kruskal-Wallis, dependendo da normalidade

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dos dados. Foram utilizados os testes *post-hoc Tukey e Dunn*. A análise de correlação entre as variáveis foi conduzida por meio dos coeficientes de *Pearson* ou *Spearman*. Todos os testes foram considerados significativos quando $p < 0,05$.

Resultados

Os valores médios e desvios padrões da idade e dos parâmetros antropométricos utilizados na avaliação do estado nutricional das participantes deste estudo estão apresentados na tabela 1. Houve diferença significativa entre os grupos para todos os parâmetros avaliados, exceto idade.

Tabela 01 - Valores médios e desvios padrões da idade e dos parâmetros antropométricos das participantes do estudo. Teresina-PI, 2019.

Parâmetros	Controle (n=77) Média ± DP	ObMS (n=35) Média ± DP	ObMNS (n=28) Média ± DP	p
Idade (anos)	35,22 ± 7,95	32,29 ± 7,47	36,82 ± 8,70	0,095
Peso corporal (kg)	56,19 ± 5,59 ^a	106,71 ± 12,79 ^b	107,80 ± 19,13 ^b	<0,001*
Estatura (m)	1,58 ± 0,06 ^a	1,62 ± 0,06 ^b	1,59 ± 0,05 ^{a,b}	0,011*
IMC (kg/m ²)	22,39 ± 1,64 ^a	40,61 ± 4,85 ^b	42,50 ± 7,05 ^b	<0,001*
CC (cm)	74,67 ± 5,18 ^a	112,87 ± 10,90 ^b	116,71 ± 14,72 ^b	<0,001*
CQ (cm)	96,42 ± 5,20 ^a	129,72 ± 7,34 ^b	133,23 ± 14,83 ^b	<0,001*
CP (cm)	31,83 ± 2,09 ^a	39,93 ± 2,97 ^b	40,25 ± 3,47 ^b	<0,001*

*ANOVA e teste de *Tukey* ou teste *Kruskal-Wallis* e teste de *Dunn* ($p < 0,05$). Quando p-valor < 0,05, os valores na mesma linha com diferença estatística significativa estão representados por letras diferentes e sem diferença estatística significativa por letras iguais. ObMS = obesas metabolicamente saudáveis; ObMNS = obesas metabolicamente não saudáveis; DP = desvio padrão; IMC = índice de massa corporal; CC = circunferência da cintura; CQ = circunferência do quadril; CP = circunferência do pescoço.

Na tabela 02, verifica-se que as mulheres obesas apresentaram valores superiores dos índices de adiposidade corporal, em relação ao grupo controle. No entanto, as obesas metabolicamente saudáveis possuíam valores inferiores da relação cintura-estatura, índice de circularidade corporal e índice de adiposidade visceral, quando comparadas às não saudáveis.

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Tabela 02 - Valores médios e desvios padrões dos índices de adiposidade corporal das participantes do estudo. Teresina-PI, 2019.

Parâmetros	Controle	ObMS	ObMNS	p
	(n=77)	(n=35)	(n=28)	
	Média ± DP	Média ± DP	Média ± DP	
RCQ	0,78 ± 0,05 ^a	0,87 ± 0,07 ^b	0,88 ± 0,06 ^b	<0,001*
RCE	0,47 ± 0,04 ^a	0,69 ± 0,07 ^b	0,73 ± 0,09 ^c	<0,001*
ICON	1,15 ± 0,06 ^a	1,28 ± 0,08 ^b	1,30 ± 0,09 ^b	<0,001*
IAC	30,59 ± 2,77 ^a	45,01 ± 5,13 ^b	48,41 ± 7,72 ^b	<0,001*
IFC	0,074 ± 0,003	0,075 ± 0,005	0,076 ± 0,005	0,116
ICC	4,26 ± 0,58 ^a	6,78 ± 1,03 ^b	7,35 ± 1,17 ^c	<0,001*
IAV	3,87 ± 1,67 ^a	4,72 ± 2,33 ^b	8,28 ± 5,04 ^c	<0,001*

*ANOVA e teste de *Tukey* ou teste *Kruskal-Wallis* e teste de *Dunn* ($p < 0,05$). Quando p-valor < 0,05, os valores na mesma linha com diferença estatística significativa estão representados por letras diferentes e sem diferença estatística significativa por letras iguais. ObMS = obesas metabolicamente saudáveis; ObMNS = obesas metabolicamente não saudáveis; DP = desvio padrão; RCQ = relação cintura-quadril; RCA = relação cintura-estatura; ICON = índice de conicidade; IAC = índice de adiposidade corporal; IFC = índice de formato corporal; ICC = índice de circularidade corporal; IAV = índice de adiposidade visceral.

A tabela 03 apresenta os marcadores metabólicos avaliados nas participantes do estudo. Os valores de insulina de jejum, HOMA-IR, HOMA- β , triglicerídeos, VLDL, HDL-colesterol, índice de Castelli I e pressão arterial sistólica foram semelhantes entre o grupo controle e as mulheres obesas metabolicamente saudáveis, no entanto estas apresentaram valores reduzidos destes parâmetros em relação às pacientes não saudáveis, com exceção do HDL-colesterol, que se encontrava elevado.

Ambos os grupos dos fenótipos obesos apresentaram valores elevados de TBARS e leptina e reduzidos da enzima superóxido dismutase, quando comparados ao grupo controle. No entanto, somente as obesas não saudáveis mostraram valores elevados da fração não-HDL-colesterol e do índice de Castelli II, em relação às participantes eutróficas.

Tabela 03 - Valores médios e desvios padrão de marcadores metabólicos das participantes do estudo. Teresina-PI, 2019.

Parâmetros	Controle (n=77) Média ± DP	ObMS (n=35) Média ± DP	ObMNS (n=28) Média ± DP	p
GPJ (mg/dL)	80,82 ± 9,40	80,60 ± 8,36	89,68 ± 21,41	0,089
INS (μU/mL)	10,69 ± 3,45 ^{a,b}	9,59 ± 2,55 ^a	12,93 ± 5,50 ^b	0,019*
HbA1c (%)	5,04 ± 0,53	5,03 ± 0,38	5,44 ± 0,93	0,054
HOMA-IR	2,15 ± 0,81 ^{a,b}	1,91 ± 0,59 ^a	3,06 ± 2,26 ^b	0,016*
HOMA-β	1,34 ± 0,43 ^a	1,20 ± 0,32 ^a	1,66 ± 0,77 ^b	0,018*
CT (mg/dL)	191,12 ± 28,50	193,63 ± 24,15	198,14 ± 27,27	0,503
TG (mg/dL)	116,10 ± 35,17 ^a	120,26 ± 28,68 ^a	168,71 ± 53,63 ^b	<0,001*
VLDL (mg/dL)	23,22 ± 7,03 ^a	24,11 ± 5,80 ^a	33,75 ± 10,75 ^b	<0,001*
HDL-c (mg/dL)	55,69 ± 10,93 ^a	53,14 ± 8,90 ^a	43,14 ± 11,55 ^b	<0,001*
LDL-c (mg/dL)	112,21 ± 26,29	116,37 ± 21,84	121,25 ± 24,18	0,244
Não-HDL (mg/dL)	135,43 ± 29,43 ^a	140,49 ± 23,46 ^{a,b}	155,00 ± 24,61 ^b	0,006*
IC I	3,56 ± 0,86 ^a	3,74 ± 0,75 ^a	4,95 ± 1,68 ^b	<0,001*
IC II	2,12 ± 0,73 ^a	2,28 ± 0,67 ^{a,b}	3,07 ± 1,22 ^b	<0,001*
PAS (mmHg)	117,03 ± 12,70 ^a	116,23 ± 15,47 ^a	126,32 ± 11,62 ^b	0,001*
PAD (mmHg)	81,83 ± 11,07 ^a	76,85 ± 9,47 ^b	83,61 ± 8,65 ^a	0,005*
TBARS (nmol/mL)	1,32 ± 0,49 ^a	2,13 ± 0,47 ^b	2,07 ± 0,44 ^b	<0,001*
GPx (U/gHb)	43,01 ± 10,91	43,27 ± 13,00	42,70 ± 11,70	0,943
SOD (U/gHb)	2926,33 ± 601,57 ^a	2239,63 ± 466,71 ^b	2468,10 ± 632,03 ^b	<0,001*
Leptina (ng/mL)	20,12 ± 11,86 ^a	107,23 ± 55,98 ^b	116,06 ± 64,93 ^b	<0,001*
Cortisol (μg/dL)	12,78 ± 5,42	12,52 ± 5,71	11,38 ± 6,33	0,387
T3 livre (pg/mL)	2,25 ± 0,62	2,08 ± 0,29	2,09 ± 0,38	0,115
T4 livre (ng/mL)	1,13 ± 0,27	1,05 ± 0,20	1,05 ± 0,23	0,218
TSH (μUI/mL)	2,69 ± 1,37	3,28 ± 1,68	2,88 ± 1,87	0,235

*ANOVA e teste de *Tukey* ou teste *Kruskal-Wallis* e teste de *Dunn* (p<0,05). Quando p-valor < 0,05, os valores na mesma linha com diferença estatística significativa estão representados por letras diferentes e sem diferença estatística significativa por letras iguais. ObMS = obesas metabolicamente saudáveis; ObMNS = obesas metabolicamente não saudáveis; DP = desvio padrão; GPJ = glicose plasmática de jejum; INS = insulina de jejum; HbA1c = hemoglobina glicada; HOMA-IR = *Homeostasis Model Assessment Insulin Resistance*; HOMA-β = *Homeostasis Model Assessment β*; CT= colesterol total;

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TG = triglicerídeos; VLDL = lipoproteína de muito baixa densidade; HDL = lipoproteína de alta densidade; LDL = lipoproteína de baixa densidade; não-HDL = colesterol não HDL; IC I = índice de Castelli I; IC II = índice de Castelli II; PAS = pressão arterial sistólica; PAD = pressão arterial diastólica; TBARS = substâncias reativas ao ácido tiobarbitúrico; GPx = glutatona peroxidase; SOD = superóxido dismutase; TSH = hormônio estimulante da tireoide.

No grupo das mulheres obesas não saudáveis, foi verificada correlação significativa entre: IMC e as concentrações de hemoglobina glicada ($r = 0,406$; $p = 0,032$) e leptina ($r = 0,516$; $p = 0,005$); circunferência do quadril e o níveis de hemoglobina glicada ($r = 0,381$; $p = 0,046$) e leptina ($r = 0,412$; $p = 0,029$); índice de adiposidade visceral e os valores de triglicerídeos ($r = 0,780$; $p < 0,001$), VLDL ($r = 0,780$; $p < 0,001$), HDL-colesterol ($r = -0,571$; $p = 0,001$) e índice de Castelli I ($r = 0,508$; $p = 0,006$); índice de conicidade e as concentrações de T3 livre ($r = -0,395$; $p = 0,038$) e T4 livre ($r = 0,411$; $p = 0,030$); índice de formato corporal e T4 livre ($r = 0,450$; $p = 0,016$); relação cintura-estatura e leptina ($r = 0,391$; $p = 0,039$); índice de adiposidade corporal e os níveis séricos de leptina ($r = 0,430$; $p = 0,022$) e atividade da superóxido dismutase ($r = -0,389$; $p = 0,041$).

Quanto ao consumo alimentar, verificou-se que os valores médios de energia foram superiores nas dietas das obesas metabolicamente saudáveis em relação ao grupo controle, o que não foi evidenciado para o grupo de obesas não saudáveis. A dieta destas apresentou valores reduzidos do percentual de contribuição dos carboidratos e elevados de proteínas, quando comparadas às dietas das participantes eutróficas (Tabela 04).

As pacientes obesas não saudáveis ainda ingeriram zinco em quantidade superior do que aquelas consumidas pelas obesas saudáveis e grupo controle. No entanto, quando os valores de ingestão dietética dos minerais foram expressos considerando o peso corporal das mulheres, observou-se que as obesas, independente do seu fenótipo, ingeriram menor quantidade de magnésio, zinco e selênio que as eutróficas (Tabela 04).

Tabela 04 - Valores médios e desvios padrão da ingestão dietética de energia, macronutrientes e minerais (magnésio, zinco e selênio) das participantes do estudo. Teresina-PI, 2019.

Parâmetros	Controle (n=77) Média ± DP	ObMS (n=35) Média ± DP	ObMNS (n=28) Média ± DP	p
Energia (kcal)	1632,88 ± 373,23 ^a	1774,04 ± 236,40 ^b	1623,68 ± 277,17 ^{a,b}	0,029*
CHO (%)	50,75 ± 7,28 ^a	49,85 ± 5,13 ^{a,b}	48,14 ± 4,20 ^b	0,008*
PROT (%)	20,14 ± 3,50 ^a	20,91 ± 3,46 ^{a,b}	22,10 ± 2,45 ^b	0,028*
LIP (%)	29,12 ± 5,23	29,23 ± 3,03	29,77 ± 2,69	0,794
Mg (mg/dia)	177,34 ± 32,09	181,57 ± 27,74	185,80 ± 14,88	0,058
Mg (mg/kg/dia)	3,19 ± 0,64 ^a	1,72 ± 0,30 ^b	1,76 ± 0,26 ^b	<0,001*
Zn (mg/dia)	11,31 ± 3,15 ^a	11,68 ± 2,79 ^a	13,33 ± 2,31 ^b	<0,001*
Zn (mg/kg/dia)	0,20 ± 0,06 ^a	0,11 ± 0,03 ^b	0,13 ± 0,03 ^b	<0,001*
Se (µg/dia)	73,54 ± 25,39	76,62 ± 16,86	80,15 ± 16,11	0,105
Se (µg/kg/dia)	1,31 ± 0,45 ^a	0,73 ± 0,18 ^b	0,76 ± 0,18 ^b	<0,001*

*ANOVA e teste de *Tukey* ou teste *Kruskal-Wallis* e teste de *Dunn* ($p < 0,05$). Quando p -valor $< 0,05$, os valores na mesma linha com diferença estatística significativa estão representados por letras diferentes e sem diferença estatística significativa por letras iguais. ObMS = obesas metabolicamente saudáveis; ObMNS = obesas metabolicamente não saudáveis; DP = desvio padrão; CHO = carboidrato; PROT = proteína; LIP = lipídio; Mg = magnésio; Zn = zinco; Se = selênio.

A tabela 05 mostra que as pacientes obesas apresentaram concentrações plasmáticas e eritrocitárias reduzidas dos minerais e valores urinários elevados, em relação ao grupo controle, independente do fenótipo saudável ou não saudável.

As tabelas 06 e 07 mostram os resultados da análise de correlação linear simples entre as concentrações dos minerais (magnésio, zinco e selênio) e marcadores metabólicos das mulheres obesas metabolicamente saudáveis e não saudáveis, respectivamente.

Tabela 05 - Valores médios e desvios padrão de parâmetros bioquímicos dos minerais (magnésio, zinco e selênio) das participantes do estudo. Teresina-PI, 2019.

Parâmetros	Controle (n=77) Média ± DP	ObMS (n=35) Média ± DP	ObMNS (n=28) Média ± DP	p
Mg plasmático (mmol/L)	0,91 ± 0,11 ^a	0,59 ± 0,09 ^b	0,62 ± 0,10 ^b	<0,001*
Mg eritrocitário (mmol/L)	2,30 ± 0,24 ^a	1,58 ± 0,13 ^b	1,57 ± 0,13 ^b	<0,001*
Mg urinário (mmol/dia)	3,45 ± 1,67 ^a	6,08 ± 2,37 ^b	6,15 ± 1,78 ^b	<0,001*
Zn plasmático (µg/dL)	89,41 ± 12,66 ^a	68,55 ± 5,19 ^b	68,23 ± 5,69 ^b	<0,001*
Zn eritrocitário (µg/gHb)	42,63 ± 3,72 ^a	39,19 ± 5,49 ^b	39,24 ± 5,00 ^b	<0,001*
Zn urinário (µg/dia)	192,37 ± 92,87 ^a	402,37 ± 208,37 ^b	295,89 ± 95,94 ^b	<0,001*
Se plasmático (µg/L)	78,89 ± 9,16 ^a	58,59 ± 4,89 ^b	57,99 ± 4,49 ^b	<0,001*
Se eritrocitário (µg/gHb)	0,38 ± 0,08 ^a	0,25 ± 0,02 ^b	0,25 ± 0,03 ^b	<0,001*
Se urinário (µg/dia)	42,53 ± 4,61 ^a	65,09 ± 7,34 ^b	66,86 ± 6,57 ^b	<0,001*

*ANOVA e teste de *Tukey* ou teste *Kruskal-Wallis* e teste de *Dunn* ($p < 0,05$). Quando p-valor $< 0,05$, os valores na mesma linha com diferença estatística significativa estão representados por letras diferentes e sem diferença estatística significativa por letras iguais. ObMS = obesas metabolicamente saudáveis; ObMNS = obesas metabolicamente não saudáveis; DP = desvio padrão; Mg = magnésio; Zn = zinco; Se = selênio.

Tabela 06 – Correlação linear simples entre as concentrações dos minerais (magnésio, zinco e selênio) e marcadores metabólicos das mulheres obesas metabolicamente saudáveis. Teresina-PI, 2019.

Parâmetros	Mg dietético	Mg plasmático	Mg eritrocitário	Mg urinário	Zn dietético	Zn plasmático	Zn eritrocitário	Zn urinário	Se dietético	Se plasmático	Se eritrocitário	Se urinário
GPJ	-0,010	-0,042	0,123	-0,105	-0,014	-0,052	-0,066	-0,286	-0,069	0,300	-0,010	-0,203
INS	0,304	-0,029	0,053	0,057	0,284	0,251	0,424*	0,030	-0,053	0,050	0,265	0,284
HbA1c	-0,029	0,132	-0,076	-0,144	0,297	-0,109	-0,379*	-0,137	0,148	0,069	-0,111	-0,338*
HOMA-IR	0,277	-0,023	0,076	-0,030	-0,087	0,217	0,316	0,006	-0,066	0,090	0,207	0,125
HOMA-β	0,309	-0,022	0,056	0,024	-0,118	0,241	0,394*	0,010	-0,053	0,078	0,247	0,235
CT	-0,142	0,021	0,168	-0,006	-0,141	0,264	0,240	0,185	-0,005	-0,182	0,382*	-0,069
TG	-0,334	0,010	0,188	0,258	-0,069	0,268	0,085	0,185	-0,169	-0,076	0,115	0,001
VLDL	-0,340*	0,004	0,188	0,258	-0,060	0,269	0,082	0,194	-0,167	-0,073	0,112	0,009
HDL-c	-0,208	-0,211	0,195	-0,053	-0,080	0,148	0,170	0,192	0,121	-0,375*	0,348*	0,349*
LDL-c	0,018	0,108	0,056	-0,054	-0,107	0,160	0,174	0,074	-0,010	-0,030	0,251	-0,221
Não-HDL	-0,067	0,101	0,099	0,013	-0,114	0,216	0,183	0,117	-0,051	-0,046	0,261	0,117
IC I	0,133	0,193	-0,098	0,065	0,069	0,036	-0,077	-0,064	-0,143	0,295	-0,165	-0,347*
IC II	0,045	0,288	-0,109	-0,035	0,058	0,022	-0,065	-0,074	-0,119	0,210	-0,144	-0,344*
PAS	-0,027	0,132	-0,213	-0,208	-0,039	0,088	0,130	0,166	0,371*	-0,199	0,084	0,058
PAD	-0,234	0,185	-0,150	0,150	-0,093	0,126	0,240	0,329	0,448*	-0,132	0,185	0,125
TBARS	0,171	0,227	-0,004	-0,088	0,069	-0,089	-0,248	-0,387*	0,298	0,068	-0,076	-0,338*
GPx	0,109	0,047	0,317	-0,131	-0,258	0,130	0,511*	-0,093	-0,079	-0,207	0,578*	-0,032
SOD	-0,104	-0,148	0,373*	-0,390*	-0,066	-0,083	0,306	0,132	0,414*	-0,188	0,440*	0,274
Leptina	0,293	0,052	-0,018	-0,061	0,190	0,157	0,055	-0,213	-0,067	0,129	0,004	-0,040
Cortisol	0,053	-0,110	-0,083	0,103	0,007	-0,120	-0,178	-0,139	-0,213	0,281	-0,195	-0,048
T3 livre	-0,410*	-0,284	0,310	-0,034	-0,083	0,265	0,454*	0,310	0,068	-0,208	0,451*	0,009
T4 livre	-0,249	-0,297	0,310	-0,101	-0,083	0,265	0,454	0,160	0,068	-0,105	0,415	0,009
TSH	0,263	0,399*	0,128	-0,140	-0,006	-0,204	-0,158	-0,243	0,022	0,308	-0,109	0,050

* Coeficiente de correlação linear de *Pearson* ou *Spearman* ($p < 0,05$).*Associação entre as concentrações de minerais (magnésio, zinco e selênio) e marcadores metabólicos dos fenótipos obesidade metabolicamente saudável e obesidade metabolicamente não saudável*

Tabela 07 – Correlação linear simples entre as concentrações dos minerais (magnésio, zinco e selênio) e marcadores metabólicos das mulheres obesas metabolicamente não saudáveis. Teresina-PI, 2019.

Parâmetros	Mg dietético	Mg plasmático	Mg eritrocitário	Mg urinário	Zn dietético	Zn plasmático	Zn eritrocitário	Zn urinário	Se dietético	Se plasmático	Se eritrocitário	Se urinário
GPJ	0,202	0,088	-0,059	0,110	0,070	-0,085	-0,103	0,216	-0,080	0,225	0,144	0,173
INS	0,049	-0,067	-0,018	0,297	0,171	-0,051	0,042	0,529*	0,143	-0,186	0,259	0,049
HbA1c	0,155	0,081	-0,006	0,237	-0,033	-0,137	-0,165	0,214	0,118	-0,160	0,260	0,065
HOMA-IR	0,093	-0,033	-0,031	0,261	0,164	-0,097	-0,005	0,464*	0,100	-0,046	0,277	0,107
HOMA-β	0,054	-0,049	-0,032	0,288	0,166	-0,088	0,013	0,511*	0,088	0,401*	0,045	-0,015
CT	0,113	-0,118	0,146	0,006	0,149	0,115	0,048	0,104	-0,165	-0,366	0,188	0,402*
TG	0,274	0,264	-0,080	0,144	-0,235	0,144	-0,363	-0,078	-0,210	-0,128	-0,243	0,060
VLDL	0,279	0,245	-0,062	0,143	-0,218	0,151	-0,340	-0,079	-0,219	-0,132	-0,234	0,062
HDL-c	-0,201	0,060	0,029	0,040	0,488*	-0,046	-0,031	0,019	0,223	-0,146	0,115	-0,135
LDL-c	0,099	-0,257	0,168	-0,076	0,005	0,085	0,220	0,143	-0,197	-0,285	0,261	0,490*
Não-HDL	0,219	-0,145	0,157	-0,012	0,030	0,149	0,094	0,106	-0,244	-0,337	0,154	0,509*
IC I	0,178	-0,082	0,009	0,012	-0,454*	0,071	0,030	0,125	-0,367	-0,093	-0,068	0,459*
IC II	0,180	-0,208	-0,021	0,016	-0,356	0,030	0,058	0,180	-0,349	-0,143	0,005	0,515*
PAS	-0,221	-0,250	-0,160	-0,080	0,404*	-0,302	-0,168	-0,013	-0,138	-0,315	-0,43	0,105
PAD	-0,058	-0,006	-0,077	0,093	0,232	-0,217	-0,264	-0,019	-0,044	-0,057	-0,106	-0,229
TBARS	-0,120	-0,123	-0,039	0,002	-0,041	-0,284	-0,070	0,158	0,247	0,091	-0,045	0,048
GPx	-0,133	-0,400*	-0,100	-0,111	-0,028	<0,001	0,334	-0,061	-0,087	0,065	0,526*	-0,008
SOD	0,347	0,231	0,083	0,162	-0,104	0,177	0,151	0,127	0,065	0,061	0,154	-0,255
Leptina	-0,002	-0,297	0,140	0,295	-0,028	-0,060	0,029	0,237	0,119	0,104	0,137	0,239
Cortisol	-0,122	-0,018	-0,062	0,307	-0,181	0,338	-0,029	-0,008	-0,076	0,160	-0,051	0,279
T3 livre	-0,320	-0,043	0,114	0,002	-0,145	-0,186	0,004	0,238	-0,007	-0,383*	-0,081	0,007
T4 livre	-0,042	-0,068	0,129	0,065	0,085	-0,171	0,207	-0,056	0,151	0,226	0,212	-0,052
TSH	0,270	0,151	0,221	-0,056	-0,268	-0,303	-0,386*	-0,401*	-0,371	-0,100	-0,331	-0,298

* Coeficiente de correlação linear de *Pearson* ou *Spearman* ($p < 0,05$).*Associação entre as concentrações de minerais (magnésio, zinco e selênio) e marcadores metabólicos dos fenótipos obesidade metabolicamente saudável e obesidade metabolicamente não saudável**Kyria Jayanne Clímaco Cruz*

Discussão

No presente estudo, foram avaliadas as concentrações dos minerais magnésio, zinco e selênio em mulheres obesas com fenótipos metabolicamente saudável ou não saudável, bem como foi investigada a existência de associação entre estes micronutrientes e marcadores metabólicos destes fenótipos.

As mulheres obesas metabolicamente saudáveis avaliadas neste estudo apresentaram parâmetros do controle glicêmico, perfil lipídico e risco cardiovascular em valores semelhantes ao grupo controle, e ainda reduzidos em relação às pacientes não saudáveis. Resultados semelhantes são encontrados na literatura atual (ECKEL et al., 2015; LIN et al., 2017), a qual mostra perfil metabólico benéfico em indivíduos obesos saudáveis.

Por outro lado, as participantes obesas em sua totalidade, independente do fenótipo, possuíam alterações em marcadores do dano oxidativo, caracterizadas pelo aumento nos níveis plasmáticos de TBARS e redução na atividade da enzima superóxido dismutase eritrocitária, o que revela a presença do estresse oxidativo em ambos os grupos de mulheres obesas avaliadas. Sobre tal resultado, é oportuno chamar atenção para a influência da adiposidade corporal, em particular da visceral, na manifestação deste distúrbio metabólico. Este fato pode ser ratificado pela correlação negativa encontrada nesse estudo entre índices de adiposidade corporal e a atividade da superóxido dismutase nas mulheres obesas não saudáveis.

O estudo também revelou correlação positiva significativa entre alguns índices de adiposidade e os valores de hemoglobina glicada, triglicerídeos, VLDL, índice de Castelli I e leptina, bem como correlação negativa entre estes índices e as concentrações de HDL-colesterol nas mulheres obesas não saudáveis. Assim, verifica-se que o aumento na adiposidade corporal está associado ao desenvolvimento de um perfil metabólico desfavorável e ao aumento no risco cardiovascular nestas pacientes.

Nessa discussão, deve-se destacar que as mulheres obesas, quer seja metabolicamente saudável ou não saudável, apresentaram deficiências nutricionais, caracterizadas pelas concentrações plasmáticas e eritrocitárias reduzidas dos minerais magnésio, zinco e selênio. Sobre a deficiência em magnésio, a ingestão dietética reduzida no mineral e sua excreção urinária elevada podem ser fatores

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contribuintes para a redução nas suas concentrações sanguíneas nas pacientes obesas participantes deste estudo.

Quanto às concentrações de zinco das participantes do estudo, um fator que pode ter favorecido a redução em suas concentrações plasmáticas e eritrocitárias foi a excreção urinária, a qual encontrava-se elevada nas mulheres obesas quando comparadas com as eutróficas. Tal achado pode ser justificado pela presença de um descontrole metabólico nestas pacientes, a exemplo da intolerância à glicose, que induz aumento na excreção urinária de zinco.

Nesse contexto, pesquisas em indivíduos com diabetes *mellitus* tipo 2 têm mostrado que a hiperinsulinemia, hiperglicemia e resistência à insulina estão associadas ao aumento na excreção urinária de zinco, pois a glicosúria resultante da hiperglicemia crônica presente nestes pacientes influencia no transporte ativo do mineral nas células tubulares renais (BANDEIRA et al., 2017; FREITAS et al., 2017). Este fato foi confirmado no presente estudo por meio da correlação positiva significativa verificada entre alguns parâmetros glicêmicos (insulina, HOMA-IR e HOMA- β) e o zinco urinário, sugerindo a influência do descontrole glicêmico na hiperzincúria observada nas mulheres obesas avaliadas.

O selênio teve valores de suas concentrações plasmáticas e eritrocitárias reduzidos nas mulheres obesas em relação ao grupo controle, isso pode ser decorrente do estresse oxidativo presente nessas pacientes. Os dados existentes na literatura mostram que a produção excessiva de espécies reativas de oxigênio na obesidade requer uma demanda elevada de nutrientes antioxidantes, o que contribui para a redução do mineral no organismo (COMINETTI et al., 2011; FERNÁNDEZ-SÁNCHEZ et al., 2011).

Um aspecto relevante é que alguns pesquisadores recomendam ingestão dietética de 1 μ g de selênio por kg de peso corporal ao dia para manutenção do estado nutricional adequado relativo ao mineral em indivíduos adultos (KIPP et al., 2015). No entanto, as participantes obesas ingeriram quantidade inferior desse nutriente, o que também pode ter contribuído para a redução nas concentrações plasmáticas e eritrocitárias de selênio nessas pacientes.

A deficiência nos minerais magnésio, zinco e selênio, verificada nas obesas participantes do nosso estudo, sugere repercussões metabólicas importantes, pois foi verificada associação significativa entre a redução do magnésio eritrocitário e a

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diminuição na atividade da enzima superóxido dismutase nas obesas saudáveis. Os valores reduzidos de zinco eritrocitário também foram associados com prejuízo na atividade da enzima glutathione peroxidase eritrocitária neste grupo de obesas,

Além disso, o estudo revela correlação positiva significativa entre os valores dietéticos e eritrocitários de selênio e a atividade da superóxido dismutase nas mulheres obesas saudáveis, e ainda entre as concentrações eritrocitárias de selênio e a atividade da glutathione peroxidase eritrocitária em ambos os grupos de obesas. Estes resultados sugerem a influência da deficiência nesses micronutrientes no comprometimento da atividade de enzimas do sistema de defesa antioxidante, algo que favorece o desenvolvimento do estresse oxidativo na obesidade.

Nessa discussão, deve-se ressaltar que os valores reduzidos dos minerais em estudo, bem como a sua associação negativa com marcadores do estresse oxidativo sugerem prejuízo na contribuição desses nutrientes para redução do estresse oxidativo associado a obesidade, independente do fenótipo metabólico.

Neste estudo, foi observada ainda associação entre a redução do magnésio na dieta das mulheres obesas saudáveis e o aumento nas concentrações séricas de VLDL, bem como entre os valores reduzidos de selênio eritrocitário e de HDL-colesterol. O zinco dietético também foi associado positivamente com HDL-colesterol e negativamente com índice de Castelli I nas obesas não saudáveis. Estes dados indicam que a deficiência nos minerais magnésio, zinco e selênio contribuem para a manifestação de dislipidemias, aumentando o risco cardiovascular em obesos, independente do seu fenótipo metabólico.

Vale mencionar que, nas mulheres obesas não saudáveis, a excreção urinária elevada de selênio foi associada com aumento nos valores de colesterol total, LDL-colesterol, fração não-HDL e índices de Castelli I e II. Tal resultado pode ser justificado pelo fato de que o aumento no selênio urinário induz a redução deste mineral no plasma dessas pacientes, comprometendo suas funções e, portanto, aumentando o risco cardiovascular.

Outro ponto importante é que a deficiência em zinco e selênio, presentes nas mulheres obesas, parece comprometer o metabolismo dos hormônios tireoidianos, considerando a atuação do zinco como cofator e do selênio como componente estrutural das deiodinases, selenoproteínas envolvidas na conversão de T4 em T3 (SCHWEIZER; STEEGBORN, 2015; SEVERO et al., 2019). Assim, embora não

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havendo diferença entre os grupos para as concentrações séricas destes hormônios, foi verificada correlação positiva entre as concentrações eritrocitárias de zinco e selênio e os valores de T3 livre, sugerindo a importância dos minerais na função tireoidiana das pacientes.

A deficiência em magnésio também parece ser importante para o metabolismo dos hormônios tireoidianos, pois a síntese e secreção dos hormônios na pituitária e na glândula tireoide são processos dependentes da formação de AMPc (adenosina 3',5'-monofosfato cíclico) e, portanto, do complexo ATPase-Mg²⁺ (HSU et al., 1984). No nosso estudo, foi encontrada correlação positiva significativa entre as concentrações plasmáticas do mineral e os níveis séricos de TSH nas mulheres obesas, sugerindo que a redução do magnésio plasmático pode diminuir as concentrações deste hormônio.

Em relação às repercussões da deficiência em minerais no controle glicêmico das mulheres obesas avaliadas, o presente estudo evidenciou correlação significativa entre a redução do zinco eritrocitário e a diminuição dos valores de insulina de jejum e HOMA-β e aumento nas concentrações plasmáticas de hemoglobina glicada. A literatura já tem dados consistentes que evidenciam a participação deste mineral na secreção de insulina e manutenção da homeostase glicêmica. Assim, a deficiência em zinco está associada à intolerância à glicose e resistência à insulina na obesidade (AHN et al., 2014; CHIMIENTI, 2013; LI, 2014; OLECHNOWICZ et al., 2018; VASHUM et al., 2014).

A partir da análise dos resultados de associação conduzida neste estudo, permite-se inferir que a condição de deficiência nutricional relativa aos minerais (magnésio, zinco e selênio) presente em mulheres obesas compromete as funções fisiológicas de proteção contra desordens metabólicas, exercidas por estes minerais na obesidade. Dessa forma, identifica-se a necessidade de prováveis intervenções no sentido de corrigir estes aspectos nutricionais e, conseqüentemente, minimizar a manifestação de tais distúrbios.

Algumas limitações do presente estudo podem ser mencionadas, a exemplo do desenho transversal, número amostral de mulheres obesas e ausência da determinação das concentrações séricas da proteína C reativa para diagnóstico dos fenótipos metabolicamente saudável e não saudável. Um ponto forte do estudo é que

esta é a primeira pesquisa a associar as concentrações dos minerais magnésio, zinco e selênio com marcadores metabólicos destes fenótipos.

Conclusão

As mulheres obesas metabolicamente saudáveis apresentam perfil metabólico benéfico, enquanto que as pacientes não saudáveis possuem descontrole metabólico. O estudo revela que as obesas não saudáveis ingerem teor de zinco em quantidade superior as saudáveis e grupo controle, enquanto que a ingestão de selênio e magnésio não mostra diferença entre os grupos.

As pacientes obesas apresentam concentrações plasmáticas e eritrocitárias reduzidas e valores urinários elevados dos minerais magnésio, zinco e selênio, independente do fenótipo saudável ou não saudável. A deficiência nestes minerais associa-se à distúrbios metabólicos presentes na obesidade, a exemplo da resistência à insulina, dislipidemias, estresse oxidativo e alterações nas concentrações dos hormônios tireoidianos.

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Capítulo 2

*Associação entre as concentrações de minerais (magnésio, zinco e selênio) e marcadores metabólicos dos
fenótipos obesidade metabolicamente saudável e obesidade metabolicamente não saudável*

Kyria Jayanne Clímaco Cruz

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Kyria Jayanne Clímaco Cruz

**Association Between Calcium/Magnesium Ratio and Insulin Resistance in
Obese Women**

Calcium/Magnesium Ratio and Insulin Resistance

¹Kyria Jayanne Clímaco Cruz; ²Ana Raquel Soares de Oliveira; ³Jennifer Beatriz Silva
Morais; ⁴Stéfany Rodrigues de Sousa Melo; ⁵Loanne Rocha dos Santos; ⁶Larissa
Cristina Fontenelle; ⁷Suelem Torres de Freitas; ⁸Gustavo Santos de Sousa; ⁹Vladimir
Costa Silva; ¹⁰Carlos Henrique Nery Costa; ¹¹Gilberto Simeone Henriques; ¹²Emídio
Marques de Matos Neto; ¹³Dilina do Nascimento Marreiro

¹ PhD Student, Department of Nutrition, Federal University of Piauí, Campus Minister
Petrônio Portela, Ininga, Teresina, Piauí, Brazil; email: kyriajayanne@hotmail.com

² PhD Student, Department of Nutrition, Federal University of Piauí, Campus Minister
Petrônio Portela, Ininga, Teresina, Piauí, Brazil; email: ana_luizamo@hotmail.com

³ PhD Student, Department of Nutrition, Federal University of Piauí, Campus Minister
Petrônio Portela, Ininga, Teresina, Piauí, Brazil; email:
jenniferbeatriz.morais@gmail.com

⁴ PhD Student, Department of Nutrition, Federal University of Piauí, Campus Minister
Petrônio Portela, Ininga, Teresina, Piauí, Brazil; email: stefany.rsm@gmail.com

⁵ PhD Student, Department of Nutrition, Federal University of Piauí, Campus Minister
Petrônio Portela, Ininga, Teresina, Piauí, Brazil; email: loanners@gmail.com

⁶ PhD Student, Department of Nutrition, Federal University of Piauí, Campus Minister
Petrônio Portela, Ininga, Teresina, Piauí, Brazil; email: larissacris.f@hotmail.com

⁷ Dean of Planning and Institutional Development, Federal University of Pará, Belém,
Pará, Brazil; email: suelem.tf@hotmail.com

⁸ Master Faculty of Medical Sciences, State University of Piauí, Teresina, Piauí,
Brazil; email: gss@gustavosantos.org

⁹ PhD, Federal University of Piauí; email: vladimir.costa@gmail.com

¹⁰ PhD, Federal University of Piauí; email: chcosta@gmail.com

¹¹ PhD, School of Nursing, Federal University of Minas Gerais, 6627 Pres. Antônio
Carlos Ave. Pampulha, Belo Horizonte, Minas Gerais, Brazil; email:
gilberto.simeone@gmail.com

¹² PhD, Department of Physical Education, Federal University of Piauí, Campus Minister Petrônio Portela, Ininga, Teresina, Piauí, Brazil; email: emidiomatos@gmail.com

¹³ PhD, Department of Nutrition, Federal University of Piauí, Campus Minister Petrônio Portela, Ininga, Teresina, Piauí, Brazil; email: dilina.marreiro@gmail.com

Corresponding author: PhD. Dilina do Nascimento Marreiro

Address: Rua Hugo Napoleão, 665, Ed. Palazzo Reale, Aptº. 2001, Jóquei, CEP 64048-320, Teresina, Piauí, Brazil

E-mail: dilina.marreiro@gmail.com

Telephone: +55 (86) 98845-9778

Fax: +55 (86) 32371812

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Abstract

The aim of this study was to evaluate the association between Ca/Mg ratio and insulin resistance in obese women. This was a cross-sectional study involving 128 women. The participants were divided into two groups: obese women, n = 62 and eutrophic women, n = 66. The three-day food record method was used for the analysis of the dietary magnesium and calcium intake. Elemental analyses of the magnesium and calcium were performed on an inductively coupled plasma spectrometer. Ca/Mg ratio in diet, plasma and urine were calculated. Analyses of fasting glucose, serum insulin concentration and glycated hemoglobin were carried out. Index homeostasis model assessment insulin resistance (HOMA-IR) and HOMA- β index were calculated. Obese women ingested a low dietary magnesium, and they presented reduced plasma and erythrocyte magnesium concentrations and elevated urinary magnesium excretion. Dietary calcium intake and plasma calcium concentration were also reduced in obese patients, and urinary calcium excretion were higher than in eutrophic group. Ca/Mg ratio in diet, plasma and urine in obese women were elevated. Statistically significant correlation was found between dietary magnesium and plasma calcium, erythrocyte magnesium and dietary calcium, urinary magnesium and urinary calcium in obese participants. Statistically significant correlation was also found between dietary magnesium and dietary, erythrocyte magnesium and urinary calcium in control group. Ca/Mg ratio seems to be not relevant on glycemic control in the obese women

Associação entre as concentrações de minerais (magnésio, zinco e selênio) e marcadores metabólicos dos fenótipos obesidade metabolicamente saudável e obesidade metabolicamente não saudável

considering the inexistence of association among these variables. Therefore, further researches should be conducted to clarify the importance of Ca/Mg ratio on glycemic control in obesity.

Keywords: calcium, magnesium, insulin resistance, metabolism, obesity.

Introduction

Obesity is characterized by excessive fat accumulation that is the main contributing factor for the development of metabolic disorders in obese individuals. Excessive adipose tissue accumulation, in particular the intra-abdominal adipose tissue, is associated with increased secretion of proinflammatory cytokines, thus favoring the development of low-grade chronic inflammation and insulin resistance ^{1,2}.

Minerals have been researched in order to clarify mechanisms linking minerals with obesity-associated metabolic disorders. Magnesium is an important mineral in cellular energy metabolism, gene expression for antioxidant enzymes and regulation of insulin secretion and insulin action ^{3,4}.

However, obese individuals are magnesium-deficient. As previously reported, plasma, erythrocyte and/or urinary magnesium concentrations are lower in obese individuals than in non-obese individuals ⁵⁻⁷. Magnesium metabolism is regulated by calcium because calcium competes with magnesium for the same carriers in the apical membrane of human renal cells and in the brush-border membrane of human intestinal cells. Therefore, excessive dietary intake of calcium may reduce magnesium intestinal absorption and excessive concentrations of calcium may reduce magnesium renal reabsorption. Thereby, excess calcium may induce magnesium deficiency, thus impairing magnesium functions, for example improvement in insulin sensitivity ⁸⁻¹¹.

Studies have showed the importance of the increased calcium/magnesium (Ca/Mg) ratio on development of metabolic disorders ¹²⁻¹⁴. Rosanoff ¹⁵ verified a rising Ca/Mg intake ratio from food in American adults. Ziniewicz et al. ¹⁶ evaluated the relationship between serum magnesium and calcium concentrations and glycemic control parameters at birth, and they verified newborns with high Ca/Mg ratio presented low levels of insulin.

Although there is consistent evidence in the published literature about the importance of magnesium and calcium physiologic functions for glycemic control, it is important to evaluate the influence of Ca/Mg ratio on insulin resistance because the data still are scarce. Accordingly, the aim of this study was to evaluate the association between Ca/Mg ratio and insulin resistance in obese women.

Methods

This was a cross-sectional study involving 128 women aged between 20 and 50 years old. The participants were divided into two groups: case group (obese women, $n = 62$) and control group (eutrophic women, $n = 66$). Participants were recruited from the spontaneous demand of clinical outpatient in the city of Teresina - Piauí.

Participants were selected according to the following inclusion criteria: body mass index (BMI) between 18.5 and 24.9 kg/m^2 (control group) or a BMI equal to or greater than 35 kg/m^2 (obese women), absence of smoking, absence of pregnancy or lactation, not participating in another clinical study; having no diagnosis of diabetes mellitus, cancer, chronic renal failure, or inflammatory bowel diseases, and not using of vitamin and mineral supplements and/or medicines that could affect the calcium and magnesium levels. Such information was self-reported by the participants.

Study was approved by the Research Ethics Committee of the Federal University of Piauí, Brazil, under the number 2.014.100. All participants provided informed consent after clarification about the research, according to norms of the National Research Council contained in the Resolution 466/12 ¹⁷.

Nutritional Status Assessment

BMI was calculated based on the weight of the participant divided by their height squared. Classification of nutritional status was performed according to the recommendations of the World Health Organization ¹⁸. The measurement of the waist circumference was performed with a flexible and non-stretchable tape measure (Seca®, São Paulo, Brazil), and the classification was based on the reference values established by the World Health Organization ¹⁹.

Evaluation of Dietary Magnesium and Calcium Intake

The three-day food record method was used for the analysis of the dietary magnesium and calcium intake. Dietary magnesium and calcium intake were calculated using the software "NutWin", version 1.5²⁰. Estimated Average Requirement (EAR), in the Dietary Reference Intakes (DRIs), was used as a reference to verify the adequacy of the dietary magnesium and calcium intake^{21,22}.

Dietary intake data of magnesium and calcium were imported into the Multiple Source Method (MSM) program, version 1.0.1 for adjustments of intrapersonal and interpersonal variability, corrected by statistical modelling techniques. The usual dietary intake of magnesium and calcium was estimated with MSM by a logistic regression model. This program can be accessed through the MSM website which can be reached at <https://nugo.dife.de/msm/>²³⁻²⁶.

Dietary intake data of magnesium and calcium also were energy-adjusted by the residual method, reducing the influence of differences in energy consumption on the dietary magnesium and calcium intake. Dietary intake data of magnesium and calcium were energy-adjusted by nutrient calculation after verifying the normality of the data distribution²⁷⁻²⁹.

Assessment of Biochemical Parameters of Magnesium

Samples of 12 ml of venous blood were collected between 7 and 9 AM, and the participants have been fasting for at least 12 hours. The collected blood was distributed in three tubes: one with 30% sodium citrate as anticoagulant for magnesium and calcium analysis (4 ml of blood), another with ethylene diaminetetraacetic acid (EDTA) as anticoagulant for glycated hemoglobin analysis (4 ml of blood), and another without anticoagulant for the assessment of fasting glucose and serum insulin (4 ml of blood).

Plasma was separated from the whole blood by centrifugation at 1831xg during 15 minutes at 4°C (Centrifuge CIENTEC® 4K15). After that, plasma was extracted with an automatic pipette, kept into demineralized polypropylene microtubes and stored at -20°C for later analysis.

Methods proposed by Whitehouse et al. ³⁰ were used to separate erythrocyte and to analyze magnesium. Red cell mass was washed with 5 mL of isotonic saline solution and it was slowly homogenized by inversion and centrifuged at 2493xg for 10 minutes at 4°C (CIENTEC ® 4K15). Supernatant was aspirated and discarded. The procedure described was performed three times to remove the contaminants from erythrocytes (platelets and leukocytes). After the last centrifugation, the saline solution was discarded and red cell mass was carefully extracted using an automatic pipette. Red cell mass was transferred to demineralized polypropylene microtubes which were maintained at -20°C for later analysis.

Demineralized 24-hour collection containers were provided for 24-hour urine collection. These containers were weighed before and after the collection in a semi-analytical balance intending to determine the urinary volume from the density. Six milliliter (6 ml) of urine were distributed in polypropylene microtubes previously demineralized for calcium determination. The remaining volume was acidified with hydrochloric acid at 3 mol/l and homogenized to avoid the precipitation of the magnesium in the urine and also to preserve the magnesium. After this procedure, 6 ml of urine were distributed in polypropylene microtubes previously demineralized. Urine was stored in a freezer at -20°C for later analysis ³¹.

Elemental analyses of the magnesium and calcium were performed on an inductively coupled plasma spectrometer - Optical Emission Spectrometry with an axial view configuration and a V-Groove nebulizer (720 ICP / OES, Varian Inc., California, United States). The reference values adopted were 0.75 to 1.05 mmol/l for plasma magnesium ³², 1.65 to 2.65 mmol/l for erythrocyte magnesium, 3.00 to 5.00 for mmol/day for urinary magnesium ³³, 2.12 to 2.65 mmol/l for plasma calcium ³⁴, and lower values than 4.0 mg/kg/day for urinary calcium ³⁵.

Assessment of Ca/Mg Ratio

Ca/Mg ratios in diet, plasma and urine were calculated using the formulas:

$$\frac{\text{Ca}}{\text{Mg}} \text{ dietary ratio} = \frac{\text{dietary Ca intake (mg/day)}}{\text{dietary Mg intake (mg/day)}}$$

$$\frac{\text{Ca}}{\text{Mg}} \text{ plasma ratio} = \frac{\text{plasma Ca (mmol/l)}}{\text{plasma Mg (mmol/l)}}$$

$$\frac{\text{Ca}}{\text{Mg}} \text{ urinary ratio} = \frac{\text{urinary Ca (mmol/day)}}{\text{urinary Mg (mmol/day)}}$$

Determination of Glycemic Control

Analysis of fasting glucose was performed using the colorimetric enzymatic method and Labtest kits. Values between 75 and 99 mg/dl were considered normal, according to the criteria defined by the American Diabetes Association ³⁶. Serum insulin concentration was determined using the chemiluminescence method, considering as a standard of reference the values between 6 and 27 µU/ml. Analysis of glycated hemoglobin was carried out using the ion exchange chromatography method. Lower values than 5.7% were considered normal ³⁶.

Index homeostasis model assessment insulin resistance (HOMA-IR) was calculated from the concentrations of fasting glucose and fasting insulin. HOMA-β index was calculated using the HOMA Calculator version 2.2.3 program. (HOMA Calculator, 2017). HOMA-IR values greater than 2.71 or HOMA-β values greater than 1.8 have meant insulin resistance ^{37, 38}.

Statistical Analysis

Treatment of missing data

During the process of database consolidation, we observed that the percentage of missing data for all observations was low (8.35%), however the variables with missing data had an average of 20.97% in control group. Thus, a missing-data imputation process was implemented to “complete” the database and to enable analysis with all study subjects.

A simple imputation method was used for the variables groups with missing data lower than 15%, and a multiple imputation method was used for the variables groups with missing data higher than 15%, according to Harrel Jr. ³⁹.

Simple and multiple imputation methods were carried out by Predictive Mean Matching (PMM) method. This imputation method was chosen because it is indicated to impute quantitative variables.

Simple imputation from PMM was conducted in three steps ⁴⁰: (1) estimation of regression model; the response variable was the variable of interest (to be imputed) and the others variables were explanatory; (2) estimation of variable of interest value for the subjects with missing data, and (3) pairing the predicted value of the variable of interest with nearest adjusted value for the subjects with missing data (we calculate the Euclidean distances). The value corresponding to the nearest adjusted value was impute. If there is more than one adjusted value with distance equal to the minimum distance found, so the imputed value is chosen randomly among the equal values.

Multiple imputation from PMM is based on the same principles of the simple imputation method, but many values are generated, not only one. After the multiple imputations have been made, the general estimates for the values were obtained by the mean of the values imputed ($m=5$).

Imputation methods were based on linear regression analysis; thus it was necessary to define variables which were used in imputation process. Explanatory variables with a closer relationship with the response variables were included in models, considering that the missing data were missing at random (MAR).

A function created by Andreozzi ⁴¹ was used for simple imputations, and the multivariate imputation by chained equations (MICE) software was used for multiple imputations. Imputation process was performed in R program ⁴².

Data analysis

Kolmogorov-Smirnov test was performed to check the normality assumption of the variables. Comparisons between the means of continuous variables were performed using the Student's t-test for normally distributed variables, whereas the Mann-Whitney test was performed for non-normally distributed variables. Pearson chi-square test was performed to compare categorical variables. Spearman's Rho (ρ) was performed for analysis of correlations between the variables. A p value < 0.05 was considered to be significant.

Canonical correlation analysis was performed to check associations between mineral group (dietary magnesium intake, plasma magnesium, erythrocyte magnesium, urinary magnesium, dietary calcium intake, plasma calcium and urinary calcium) and glycemic control parameters group (fasting glucose, serum insulin and glycated hemoglobin). Data were normalized to have means equal to zero and standard deviations equal to one in order to reduce the influence of a set of high values variables on another set of low values variables. HOMA-IR and HOMA- β were not included in canonical correlation analysis due to multicollinearity detected.

Wilks' lambda (F approximation to the distribution) was performed to evaluate significance of canonical roots. Explained variance was calculated by squared canonical correlation (canonical R²). Canonical charges and cross canonical charges were estimated.

Logistic regression models were performed to evaluate association between HOMA-IR and magnesium parameters (dietary magnesium intake, plasma magnesium, erythrocyte magnesium and urinary magnesium) and Ca/Mg ratio, separately, with each variable categorized into quartiles with the lowest quartile as the referent category. The final model with dietary magnesium intake was adjusted for age, dietary calcium intake, plasma calcium and urinary calcium, whereas the final model with Ca/Mg ratio was adjusted for age.

Results

Mean values and standard deviations of age and anthropometric parameters used to evaluate the nutritional status of obese women and control group are presented in Table 1. Statistically significant difference ($p < 0.05$) between obese and control group's body weight, height, BMI and waist circumference was found.

Mean values and standard deviations of the glycemic control parameters of the obese women and control group are shown in Table 2. There were no differences in fasting glucose, serum insulin, glycated hemoglobin, HOMA-IR and HOMA- β between the two groups ($p < 0.05$).

There were differences in parameters of magnesium and calcium studied between the two groups ($p < 0.05$), except dietary calcium intake and Ca/Mg intake ratio (Table 3).

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Statistically significant association between obesity and deficiency of erythrocyte magnesium, and between obesity and reduced plasma calcium was found ($p < 0.001$). Furthermore, statistically significant association between obesity and increased urinary excretion of magnesium and calcium was found ($p < 0.001$; Pearson chi-squared test).

Statistically significant correlation was found between dietary magnesium and plasma calcium ($r = -0.331$; $p = 0.009$), erythrocyte magnesium and dietary calcium ($r = -0.468$; $p < 0.001$), urinary magnesium and urinary calcium ($r = 0.426$; $p < 0.001$) in obese participants. Statistically significant correlation was also found between dietary magnesium and dietary calcium ($r = 0.291$; $p = 0.018$), erythrocyte magnesium and urinary calcium ($r = -0.439$; $p < 0.001$) in control group (Table 4).

Statistically significant correlation was found between Ca/Mg intake ratio and HbA1c ($r = -0.295$; $p = 0.020$), urinary calcium and serum insulin ($r = 0.342$; $p = 0.007$), urinary calcium and HOMA-IR ($r = 0.256$; $p = 0.045$), urinary calcium and HOMA- β ($r = 0.324$; $p = 0.010$), urinary calcium and HbA1c ($r = -0.295$; $p = 0.020$) in obese participants (Table 5). There was statistically significant correlation only between urinary calcium and fasting glucose ($r = -0.266$; $p = 0.031$) in control group.

First canonical function was significant ($p = 0.009$), and correlation (0.644) between variable sets of minerals and of control glycemic was expressive. Urinary calcium received a greater canonical charge (0.934) than did others parameters of the minerals in the construction of the first mineral canonical variable. Glycated hemoglobin (-0.638) had the greatest impact in the variable sets of glycemic control parameters (Table 6).

Logistic regression analysis did not show significant association between magnesium status and HOMA-IR, and between Ca/Mg ratio and HOMA-IR.

Discussion

In this study, the assessment of magnesium and calcium status was conducted, and the association between Ca/Mg ratio and insulin resistance was evaluated. Plasma and erythrocyte magnesium concentrations were lower in obese women than in non-obese women and lower than the reference values. Additionally, significant association between obesity and reduced erythrocyte magnesium

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concentrations was found. Similar results were reported by Farhangi et al.⁵, Farhangi et al.⁴³ e Rodriguez-Morán, Guerrero-Romero ⁴⁴ that also found reduced serum magnesium in obese patients.

There are several contributing factors for the magnesium deficiency in obese patients. In this study, the reduced dietary intake of magnesium may have contributed to reduced plasma and erythrocyte magnesium concentrations because the dietary intake of magnesium was lower than the dietary reference intakes (Estimated Average Requirement - EAR). Household Budget Survey (2008-2009) showed that the Brazilian population in general do not have habits of eating magnesium containing-food source, for example leafy green vegetables and nuts.

Elevated urinary magnesium excretion verified in obese women in this study may also justify magnesium deficiency. Therewithal, there was significant association between obesity and elevated urinary magnesium excretion, thus evidencing the altered magnesium homeostasis in obese patients. Therefore, the reduced dietary intake of magnesium and elevated urinary magnesium excretion may together explain reduced plasma and erythrocyte magnesium concentration in obese women.

Calcium can also regulate magnesium homeostasis. In this study, correlation analysis showed significant negative correlation between dietary calcium intake and erythrocyte magnesium concentration in obese women. Accordingly, we suggest that the increase of the dietary intake of calcium may contribute for reduction of the erythrocyte magnesium concentration, thus favoring magnesium deficiency.

This result may be justified by the fact that the homeostatic regulating system of calcium and magnesium involve the same transporters on intestinal and renal cells, for example Calcium Sensing Receptor (CaSR) and Transient Receptor Potential Melastatin 7 (TRPM7). Thus, calcium and magnesium antagonize each other in many physiological activities ^{12, 45}.

Moreover, significant negative correlation between dietary magnesium intake and plasma calcium concentrations in obese women was found. This result shows that the increase of the dietary intake of magnesium may contribute for reduction of the plasma calcium levels, thus evidencing that magnesium also influences on calcium metabolism.

Studies have shown the biochemical relevance of the Ca/Mg intake ratio and Ca/Mg serum ratio for physiological activities of many tissues in healthy and sick

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individuals ^{12, 45}. In this study, Ca/Mg intake ratio was higher in obese women than recommended values by population-based cohort studies conducted in China that was value between 1.7 and 2.8 ¹². Durlach ⁴⁶ also recommend the maintenance of a Ca/Mg intake ratio close to 2 for optimal health in the adult population.

In our study, Ca/Mg plasma ratio and Ca/Mg urinary ratio were higher in obese women than in eutrophic women. About the increased Ca/Mg plasma ratio, it is emphasized that inflammation, oxidative stress and insulin resistance are the main metabolic disorders associated with imbalance between magnesium and calcium in the blood compartments ^{12, 47-49}.

However, there was no significant correlation between Ca/Mg plasma ratio and Ca/Mg urinary ratio and glycemic control parameters in this study. This result may be explained by the fact that obese women presented adequate glycemic control, and others physiological variables may have regulated the glycemic control parameters in obese women in our study.

In this study, significant negative correlation between Ca/Mg intake ratio and glycated hemoglobin was also found in obese women. This result is different of the study's hypothesis and incompatible with the evidence found in the published literature. Because the increase of Ca/Mg intake ratio is associated with glycemic decontrol and disease mortality ^{12, 50}. Haenni et al. ⁵¹, for example, have shown that changes in insulin sensitivity index were inversely correlated to changes in the Ca/Mg serum ratio between ($r = -0.27$, $p < .008$). Thus, further studies about this topic should be carried out.

Another important point is the hypocalcemia verified in obese women in this study which may be due to reduced dietary calcium intake and elevated urinary calcium excretion. About the urinary calcium excretion, researches with animals and humans have shown that the hyperinsulinemia and insulin resistance are associated with hypercalciuria due to calciuretic effect of insulin, thus favoring reduction of plasma calcium ⁵²⁻⁵⁵. This fact is confirmed in our study because significant positive correlation between insulin and urinary calcium levels, HOMA-IR and urinary calcium levels, and HOMA- β and urinary calcium were found in obese women.

In this discussion, it is emphasized that hypocalcemia and reduced dietary calcium intake may contribute to the increase in intracellular calcium concentration. Excessive intracellular calcium accumulation induces the development of obesity-

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associated chronic inflammation and oxidative stress ⁵⁶. However, measurement of intracellular calcium concentration was not conducted in our study which is a limitation of this study.

Another limitation is the fact that gene expression for CaSR and TRPM7 receptors was not performed. A strength of our study is that this research was the first that associated the Ca/Mg ratio in diet, plasma and urine with insulin resistance in obese women.

Conclusion

Obese women in this study ingest a low dietary magnesium, and they present reduced plasma and erythrocyte magnesium concentrations and elevated urinary magnesium excretion. Dietary calcium intake and plasma calcium concentration are also reduced in obese patients, and urinary calcium excretion are higher than in eutrophic group. Ca/Mg ratio in diet, plasma and urine in obese women are elevated.

Simple linear correlation analysis shows calcium seems to influence magnesium homeostasis, being that the increased dietary calcium intake may be associated to reduced erythrocyte magnesium concentration.

Moreover, the Ca/Mg ratio seems to be not relevant on glycemic control in the obese women considering the inexistence of association among these variables. Therefore, further researches should be conducted to clarify the importance of Ca/Mg ratio on glycemic control in obesity.

Authors' contributions

Cruz KJC, Oliveira ARS, Morais JBS, Santos LR, Melo SRS, Fontenelle LC have participated to the redaction and the review of the manuscript; Oliveira ARS, Cruz KJC, Morais JBS, Santos LR, Melo SRS, Fontenelle LC, Freitas ST, Santos GS, Silva VC, have participated to generation, collection, assembly, analysis and/or interpretation of data; Costa CHN, Henriques GS, Matos EMN and Marreiro DN had supervised the paper, participated in the redaction and the review of the paper.

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Table 1 - Mean values and standard deviations of age, body weight, height, body mass index and waist circumference of obese women and control group.

Parameters	Obese women (n=62) Mean $\pm$ SD	Control group (n=66) Mean $\pm$ SD	p
Age (years)	33.95 $\pm$ 8.76	35.98 $\pm$ 7.92	0.157
Body weight (kg)	103.91 $\pm$ 10.30	56.04 $\pm$ 5.69	< 0.001*
Height (m)	1.61 $\pm$ 0.06	1.58 $\pm$ 0.07	0.036*
BMI (kg/m ²)	40.26 $\pm$ 3.37	22.36 $\pm$ 1.69	< 0.001*
WC (cm)	111.97 $\pm$ 9.36	74.72 $\pm$ 5.30	< 0.001*

*Values significantly different between obese women and control groups, Student's t test or Mann-Whitney test ($p < 0.05$). BMI = body mass index; WC = waist circumference; SD = standard deviations.

Table 2 - Mean values and standard deviations of glycemic control parameters of obese women and control group.

Parameters	Obese women (n=62) Mean $\pm$ SD	Control group (n=66) Mean $\pm$ SD	p
Fasting glucose (mg/dL)	83.65 $\pm$ 12.92	79.68 $\pm$ 8.53	0.147
Serum insulin (μ U/mL)	11.13 $\pm$ 3.89	9.51 $\pm$ 2.01	0.080
HbA1c (%)	5.17 $\pm$ 0.49	5.01 $\pm$ 0.56	0.198
HOMA-IR	2.37 $\pm$ 1.13	1.88 $\pm$ 0.46	0.063
HOMA- β	1.42 $\pm$ 0.53	1.19 $\pm$ 0.25	0.071

Student t test ($p > 0.05$). HbA1c = glycated hemoglobin; HOMA-IR = Homeostasis Model Assessment Insulin Resistance; HOMA- β = Homeostasis Model Assessment β ; SD = standard deviations.

Reference values: Fasting Glucose = 75 to 99 mg / dL; Serum insulin = 6 to 27 μ U / mL; HbA1 <5.7; HOMA-IR < 2.71; HOMA- β < 1.8.

Table 3 - Mean values and standard deviations of parameters of magnesium and calcium of obese women and control group.

Parameters	Obese women (n=62)	Control group (n=66)	p
	Mean $\pm$ SD	Mean $\pm$ SD	
Dietary Mg (mg/day)	185.92 $\pm$ 26.35	177.93 $\pm$ 30.48	0.011*
Plasma Mg (mmol/l)	0.61 $\pm$ 0.11	0.92 $\pm$ 0.10	<0.001*
Erythrocyte Mg (mmol/l)	1.57 $\pm$ 0.12	2.31 $\pm$ 0.24	<0.001*
Urinary Mg (mmol/day)	6.02 $\pm$ 1.96	3.45 $\pm$ 1.60	<0.001*
Dietary Ca (mg/day)	542.30 $\pm$ 52.54	548.25 $\pm$ 108.03	0.690
Plasma Ca (mmol/l)	1.94 $\pm$ 0.18	2.46 $\pm$ 0.20	<0.001*
Urinary Ca (mg/kg/day)	5.45 $\pm$ 2.56	2.96 $\pm$ 0.92	<0.001*
Ca/Mg intake ratio	2.98 $\pm$ 0.56	3.14 $\pm$ 0.70	0.098
Ca/Mg plasma ratio	3.28 $\pm$ 0.61	2.71 $\pm$ 0.39	<0.001*
Ca/Mg urinary ratio	2.50 $\pm$ 1.32	1.44 $\pm$ 0.75	<0.001*

* Significantly different values between obese patients and control group, Student t test ($p < 0.05$). Mg = magnesium; Ca = calcium; SD = standard deviations. Reference values: Dietary magnesium: EAR = 255 mg/day, for females aged between 20 and 30 years, and 265 mg/day for females aged between 31 and 50 years (IOM, 1997); Plasma magnesium = 0.75-1.05 mmol/l^a; Erythrocytes magnesium = 1.65-2.65 mmol/l^b; Urinary Magnesium = 3,00-5,00 mmol/day^b. ^aTopf and Murray (2003); ^bTietz (1995). Dietary calcium: EAR = 800 mg/day, for females aged between 19 and 50 years (IOM, 2011); Plasma calcium = 2.12 to 2.65 mmol/l (Madeo et al., 2018). Urinary calcium = <4,0 mg/kg/day (Hodgkinson; Pyrah, 1958).

Table 4 – Simple linear correlation analysis between magnesium and calcium parameters in obese patients and control group.

Parameters	Obese women						Control group					
	Dietary Ca		Plasma Ca		Urinary Ca		Dietary Ca		Plasma Ca		Urinary Ca	
	r	p	r	p	r	p	r	p	r	p	r	p
Dietary Mg	0.237	0.063	-0.331	0.009*	-0.209	0.102	0.291	0.018*	0.006	0.960	-0.118	0.344
Plasma Mg	0.099	0.446	-0.130	0.314	-0.184	0.151	-0.053	0.671	-0.035	0.779	0.068	0.588
Erythrocyte Mg	-0.468	<0.001*	0.115	0.375	0.205	0.109	0.219	0.077	-0.036	0.773	-0.439	<0.001*
Urinary Mg	0.069	0.595	0.220	0.085	0.426	<0.001*	0.055	0.662	-0.077	0.539	0.168	0.178

Pearson correlation coefficient or Spearman correlation coefficient (p <0.05). Mg = magnesium; Ca = calcium.

Table 5 - Simple linear correlation analysis between magnesium and calcium parameters and glycemic control parameters in obese patients.

Variáveis	Fasting glucose		Serum insulin		HbA1c		HOMA-IR		HOMA-β	
	r	p	r	p	r	p	r	p	r	p
Dietary Mg	0.104	0.422	0.089	0.493	0.208	0.105	0.092	0.477	0.094	0.467
Plasma Mg	0.050	0.700	0.006	0.965	0.110	0.393	0.028	0.828	0.021	0.874
Erythrocyte Mg	0.043	0.739	0.051	0.691	0.097	0.453	0.044	0.731	0.038	0.770
Urinary Mg	-0.104	0.420	0.106	0.412	0.052	0.687	0.056	0.665	0.095	0.464
Dietary Ca	-0.024	0.852	-0.199	0.121	-0.021	0.868	-0.166	0.198	-0.188	0.143
Plasma Ca	-0.169	0.190	0.008	0.948	-0.227	0.076	0.007	0.958	-0.001	0.996
Urinary Ca	-0.060	0.643	0.342	0.007*	-0.292	0.021*	0.256	0.045*	0.324	0.010
Ca/Mg intake ratio	-0.200	0.120	-0.193	0.133	-0.295	0.020*	-0.196	0.128	-0.197	0.125
Ca/Mg plasma ratio	-0.094	0.469	0.027	0.832	-0.184	0.152	0.012	0.926	0.014	0.916
Ca/Mg urinary ratio	0.051	0.692	0.143	0.268	-0.193	0.136	0.128	0.320	0.143	0.268

Pearson correlation coefficient or Spearman correlation coefficient (p <0.05). Mg = magnesium; Ca = calcium; HbA1c = glycated hemoglobin; HOMA-IR = Homeostasis Model Assessment Insulin Resistance; HOMA-β = Homeostasis Model Assessment β.

Table 6 - Canonical correlation between minerals and parameters of control glycemic in obese women.

Canonical function	Canonical correlation	Canonical R ²	Fa	GL ₁	GL ₂	p
1	0.644	0.415	1.99	21	150	0.009
2	0.346	0.120	0.78	12	106	0.666
3	0.204	0.042	0.47	5	54	0.798

Canonical charges and cross canonical charges for the canonical function 1

Parameters	Canonical charges	Cross canonical charges
Dietary Mg (mg/day)	-0.255	-0.164
Plasma Mg (mmol/l)	-0.169	-0.109
Erythrocyte Mg (mmol/l)	-0.055	-0.036
Urinary Mg (mmol/day)	-0.046	-0.030
Dietary Ca (mg/day)	-0.256	-0.165
Plasma Ca (mmol/l)	0.309	0.199
Urinary Ca (mg/day)	0.934	0.602
Parameters of control glycemic		
Fasting glucose (mg/dL)	-0.121	-0.078
Serum insulin (μU/mL)	0.454	0.293
HbA1c (%)	-0.638	-0.411

Wilks' Lambda multivariate test. Fa: Approximate F; GL1: degrees of freedom from the samples; GL2: degrees of freedom regarding error. Mg = magnesium; Ca = calcium; HbA1c = glycated hemoglobin.

Capítulo 3

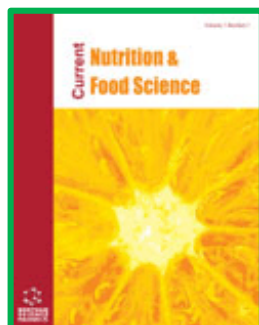
*Associação entre as concentrações de minerais (magnésio, zinco e selênio) e marcadores metabólicos dos
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Associação entre as concentrações de minerais (magnésio, zinco e selênio) e marcadores metabólicos dos fenótipos obesidade metabolicamente saudável e obesidade metabolicamente não saudável

Kyria Jayanne Clímaco Cruz

Hypomagnesemia in obese subjects: evidence of systematic review and meta-analysis

Hypomagnesemia in obese

Kyria Jayanne Clímaco Cruz¹, Ana Raquel Soares de Oliveira¹, Suelem Torres de Freitas², Gilberto Simeone Henriques³, Dilina do Nascimento Marreiro¹

¹Department of Nutrition, Federal University of Piaui, Campus Minister Petronio Portela, Teresina, Piaui, Brazil

² Dean of Planning and Institutional Development, Federal University of Para, Belem, Para, Brazil

³Department of Nutrition, Federal University of Minas Gerais, Belo Horizonte, Minas Gerais, Brazil

Corresponding author: Professor Dr Dilina do Nascimento Marreiro

Mailing adress: Street Hugo Napoleao, 665, apartment 2001, Joquei, Zip code: 64048-320, Teresina, Piaui, Brazil

e-mail: dilina.marreiro@gmail.com

Telephone: + 55 86 988459778

Fax: + 55 86 32371812

Hypomagnesemia in obese subjects: evidence of systematic review and meta-analysis

Hypomagnesemia in obese

ABSTRACT

Background: Magnesium deficiency is a global nutritional problem that seems to influence obesity-associated metabolic disorders, because magnesium plays an important role in the prevention and treatment of many diseases. **Objective:** We conducted a systematic review and meta-analysis to evaluate the relationship between plasma magnesium concentrations and obesity. **Methods:** A systematic review and meta-analysis of case-control studies was conducted. Relevant studies were identified from a literature search using electronic databases. **Results:** Ten case-control studies were evaluated in this meta-analysis. Results demonstrated that obese individuals presented lower plasma magnesium concentration than healthy individuals (standardized mean difference [SMD] = -0.44, 95% confidence interval = -0.88 to -0.01). In subgroup analyses, there were differences in plasma magnesium concentration between obese and healthy individuals according to study location, gender, case age, control age, method for assessment of magnesium concentration and study quality. Furthermore, meta-regression analyses showed that the source of heterogeneity of magnesium levels among studies was control age. **Conclusion:** Evidence of this systematic review and meta-analysis show hypomagnesemia in obese individuals of both genders.

Keywords: obesity, magnesium, human, observational study, review.

1 Introduction

Obesity is characterized by abnormal or excessive fat accumulation that is a risk factor for other chronic diseases, including diabetes mellitus, cardiovascular disease and cancer. Obese individuals have metabolic disorders, such as chronic inflammation, oxidative stress and insulin resistance, which contributing to alterations in minerals concentration in body [1,2].

Studies have showed lower magnesium concentration in plasma of obese individuals than eutrophic individuals [3,4]. This reduction may be due to inadequate dietary magnesium intake. Household Budget Survey [5] showed that the Brazilian population in general do not have habits of eating magnesium containing-food source, such as whole grains, spinach, nuts and legumes. Oliveira *et al.* [6] and Morais *et al.* [7] found lower dietary magnesium intake in obese group than the dietary reference intakes (Estimated Average Requirement - EAR) [8].

Magnesium deficiency is a global nutritional problem that seems to influence obesity-associated metabolic disorders, because magnesium plays an important role in the prevention and treatment of many diseases. Magnesium is cofactor of ATPase-Mg enzyme, therefore, it is essential in energetic metabolism. Furthermore, magnesium induces insulin action and the expression of antioxidant enzyme genes [9,10].

Therefore, we conducted a systematic review and meta-analysis to evaluate the relationship between plasma magnesium concentrations and obesity.

2 Methods

Recommendations of Guidelines for Meta-Analyses and Systematic Reviews of Observational Studies – MOOSE were followed to perform this review [11]. This study was registered in the International prospective register of systematic reviews – PROSPERO (CRD42017065694).

2.1 Data Sources and Study Selection

We accessed articles in Pubmed, Cochrane Library, Scopus and ScienceDirect databases, from March to December 2017, searching studies published from 1999 to 2017. Two authors (KC and AO) independently conducted the literature search. Disagreements between the two investigators were resolved by consensus. Patient, exposition, comparison, and outcomes (PECO) strategy was used for definition of research guiding question [12]. Keywords “magnesium”, “obesity” and “human”, and your synonymous were used in search for articles. Descriptors crossing “(Magnesium or Mg^{2+}) and (Obesity or Obese) and (Humans or Man, Modern or Modern Man or Man or Homo sapiens or Human)” was used in literature search.

2.2 Inclusion and Exclusion Criteria

Published articles were included in this meta-analysis according the following criteria: (1) case-control studies examining the association between plasma magnesium concentration and obesity, (2) human study published in a peer-reviewed journal, (3) studies that enrolled adults aged 18 years old and older, (4) studies that enrolled obese individuals without related diseases (this was verified in the exclusion criteria of the studies), (5) studies that provided mean values and standard deviations (SD) of plasma magnesium concentration, and (6) articles that were written in English or Spanish and published in their entirety, without a publication year limit.

We tried to include all available studies, including contact with authors of articles not published in their entirety and searching in reference lists of obtained articles. Exclusion criteria in this systematic review and meta-analysis were the following: (1) articles that did not satisfy inclusion criteria and (2) some publication types, such as animal study, reviews, case-report and meta-analysis. Study selection details are shown in **Figure 1**.

2.3 Data Extraction and Quality Assessment

Two investigators (KC and AO), co-authors of the present study, independently extracted the data from the original reports. The following information were extracted: first author's family name, year of publication, country, age, gender, sample size, data on levels of magnesium, method for assessment of magnesium concentration and selection location of control group. Disagreements between the two investigators were resolved by consensus.

Methodological quality of studies was assessed using the Newcastle-Ottawa Quality-Assessment Scale for case-control studies [13]. This scale present nine items that were grouped into three major categories. Scale includes risk assessment bias of these topics: selection of patients (definition of case and control, representativeness of the cases, and selection of controls), comparability of cases and controls on the basis of the design or analysis, and exposure. A final score of 7 or more was indicative of a high-quality study.

2.4 Statistical Analysis

The data of interest, presented as continuous values (mean value and SD), were used to obtain the standardized mean differences (SMDs) and 95% confidence interval (CI) of plasma magnesium concentration of obese and eutrophic individuals [14].

Cochran's Q-statistic and I-squared test were performed to test heterogeneity between the studies. I-squared value greater than 50% or a p value of the Q-test less than 0.05 were indicative of high heterogeneity among the included studies. Our protocol proposed the pooling of SMDs for the meta-analysis using a random effects model [15].

Subgroup meta-analyses were conducted according to the pre-specified study-level characteristics. Sources of heterogeneity were location, sex, age, method of magnesium assessment and study quality. Heterogeneity was also assessed by comparing the results from studies grouped according to the pre-specified study-level characteristics using meta-regression. We also conducted sensitivity analyses to

evaluate the influence of each study on the results. Publication bias was evaluated using Egger's test [16] and Begg's test [17]. In the presence of publication bias, the p values for Egger's test and Begg's test were lower than 0.05. All statistical analyses were performed using R software [18].

3 Results

3.1 Selected Articles

We found 15,241 articles in the search conducted in the four mentioned databases. Of these, ten articles identified in PubMed, one in Cochrane, ten in Scopus, and one in Science Direct were considered eligible for inclusion. After removing duplicates articles and studies no published in their entirety, ten articles were finally included in this systematic review and meta-analysis (**Figure 1**).

3.2 Characteristics of studies included

Table 1 shows the characteristics of studies included in this review and quality assessment results. Studies were published from 1999 to 2017. Studies were conducted in four different countries (Italy, Brazil, Iran, Mexico), with male and female individuals. A total of 749 obese participants and 627 controls were enrolled. Mean age of obese participants was 37.98 years, and of healthy individuals was 36.78 years.

Different assay methods for plasma magnesium concentration were used. Six studies used colorimetric assay, three studies used flame atomic absorption spectrophotometry, and one study used direct current plasma emission spectrometer. Methodological quality of studies varied from 5 to 8 points (median of 5 points).

3.3 Quantitative Data Synthesis

Figure 2 shows meta-analysis results. Obese participants presented lower plasma magnesium concentration than eutrophic individuals (SMD = -0.44, 95% confidence interval = -0.88 to -0.01). There was high heterogeneity among studies (I^2

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= 92.5%, $p < 0.001$) (Figure 2). No publication bias was detected with Egger's test ($p = 0.50$) or Begg's test ($p = 0.86$).

3.4 Subgroup, Meta-Regression, and Sensitivity Analyses

Figure 3 shows subgroup and meta-regression analysis results. Subgroup analysis showed that plasma magnesium concentration was lower in obese individuals than control group, particularly in studies conducted in Mexico, studies which included female and male individuals, obese individuals with age higher than 37.98 years, or healthy individuals with age higher than 36.78 years. This result was also found in the studies that used colorimetric method of magnesium assay and in the studies with high methodological quality.

Meta-regression analysis revealed that age of healthy individuals ($p = 0.047$) influenced plasma magnesium concentration in obese and healthy individuals (**Figure 3**). Sensitivity analyses showed that no single study had an effect on pooled SMD (**Figure 4**).

4 Discussion

In this systematic review and meta-analysis, we evaluated ten case-control studies that analyzed plasma magnesium concentration in obese individuals. Meta-analysis demonstrated that obese participants had lower plasma magnesium concentration than healthy individuals (SMD = -0.44, 95% confidence interval = -0.88 to -0.01). This result may be due to reduced dietary magnesium intake in obese group. Five studies included in this meta-analysis showed that the dietary magnesium intake was lower in obese group than the dietary reference intakes (EAR) [3,4,6, 7, 20]. Guerrero-Romero et al. [22] verified that overweight and obese individual intake lower amount of magnesium than healthy individual.

Insulin resistance can also lead to hypomagnesemia in obese patients. Studies show that magnesium reabsorption in distal convoluted tubule and in loop of Henle is reduced in individuals with insulin resistance, such as individuals with obesity and type

2 diabetes mellitus. Thus, insulin resistance can induce urinary magnesium excretion and, consequently, hypomagnesaemia [25,26,27,28,29,30].

Hypomagnesemia may exacerbate chronic inflammation, insulin resistance and oxidative stress in obese individuals. Reduction of magnesium in extracellular compartment induces an increase in intracellular calcium concentration. Excessive intracellular calcium accumulation increases the release of substance P and the production of cytokines pro-inflammatory. Magnesium deficiency also enhances the recruitment of phagocytic cells, which leads to the generation of reactive oxygen species. Hypomagnesemia also impairs insulin receptor tyrosine kinase activity, thus inhibiting activation of phosphatidylinositol protein 3-kinase enzyme and protein kinase B or Akt, proteins involved in insulin signaling pathway [31,32,33].

In our subgroup analyses, there were differences in plasma magnesium concentration between obese and healthy individuals according to study location, gender, case group age, control group age, method for assessment of magnesium concentration and study quality. Furthermore, meta-regression analyses showed that the source of heterogeneity of magnesium levels among studies was control group age.

From the country-stratified analysis, we observed that studies conducted in Mexico showed the lowest plasma magnesium concentrations. About this result, it is noted the studies of Morales-Ruán et al. [34], Cruz-Góngora et al. [35] and Mejía-Rodríguez et al. [36]. These researchers measured magnesium serum concentrations in 5,060 Mexican children, 2,447 Mexican adolescents and 5,410 Mexican adults, respectively. They verified low serum magnesium in 22.6% of children, 37.6% of adolescents, 36.3% of female adults, and 31.0% of male adults. Some authors explained this result due to an insufficient dietary intake of magnesium, high intake of antagonists of the absorption of magnesium (fiber, tannins and phytates), and low intake of enhancers of magnesium absorption as such vitamin C in Mexican population [35,36].

When the subgroup analysis was performed according to case group age and control group age, we observed lower plasma magnesium concentrations in obese individuals and healthy individuals with age greater than the mean age of each group. It is emphasized that bone provides a large exchangeable pool to buffer acute changes

in serum magnesium concentration, and the magnesium content of bone decreases with age, and bone magnesium store is not completely bioavailable during magnesium deprivation [37]. Furthermore, magnesium deficiency in the elderly may be related to an inadequate magnesium intake, a decreased magnesium absorption and/or increased urinary magnesium loss, or multiple drug use [38].

Analysis stratified by magnesium concentration assessment methods revealed lower plasma magnesium concentrations when colorimetric assays was used. Different results were verified by Scalabrini; Ribeiro [39] that conducted a study with objective to compare colorimetric assays and flame atomic absorption spectrophotometry for determination of magnesium. Samples measured by both methods showed no difference between the results. However, colorimetric method uses reference range with higher magnesium values than atomic absorption spectrophotometry method. Thus, a same value may be considered low in colorimetric assay, and normal in spectrophotometry assay. We also found that the high-quality studies showed lower plasma magnesium concentrations. This result may be related to fact that these studies are more careful, minimizing potential biases.

Some systematic reviews and meta-analyses have been performed to investigate the association between magnesium intake and serum concentrations with metabolic syndrome. Findings these meta-analyses suggest an inverse association between magnesium and metabolic syndrome. These results show the importance of magnesium in the protection of obesity-associated diseases [40, 41,42].

A strong feature of this meta-analysis is that the studies were carefully selected according to a predetermined search strategy to include all available case-control studies. There are also potential limitations of this study that should be mentioned: high heterogeneity among studies included, and the possibility that relevant articles were not included because they were published in languages other than English or Spanish or they were not indexed in the electronic databases accessed in this study.

5 Conclusion

Evidence of this systematic review and meta-analysis show hypomagnesemia in obese individuals of both genders. We suggest that new studies be carry out to clarify magnesium status in population with the same lifestyle.

Conflict of Interest

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Authors' Contributions

KJCC, ARSO, STF, GSH and DNM designed the research; KJCC and ARSO conducted the research; KJCC, ARSO, STF, GSH and DNM analyzed data; STF performed statistical analysis; KJCC, ARSO, STF, GSH and DNM wrote paper; DNM had primary responsibility for final content. All authors read and approved the final manuscript.

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Table 1. Characteristics of studies included.

Study	Country	Group	Age	Sample size	Gender	BMI	Plasma Mg (mmol/l)	Method of Mg assay	Selection location of control group	NOS Score
Corica <i>et al.</i> [19].	Italy	Obese	39.2 ± 2.4	19	F/M (13/6)	36.00 ± 1.50	1.24 ± 0.04	DCPES	not described	5
		Controls	34.3 ± 1.4	12	F/M (8/4)	22.10 ± 0.73	1.23 ± 0.06			
Cruz <i>et al.</i> [20]	Brazil	Obese	37.4 ± 6.8	55	F	35.28 ± 2.60	0.81 ± 0.06	FAAS	not described	5
		Controls	35.8 ± 7.6	59	F	22.03 ± 1.56	0.79 ± 0.06			
Faranghi <i>et al.</i> [3]	Iran	Obese	32.5 ± 7.7	42	F	32.92 ± 2.00	0.85 ± 0.10	Colorimetric	not described	5
		Controls	31.4 ± 6.8	40	F	22.63 ± 3.10	0.95 ± 0.25			
Faranghi <i>et al.</i> [4]	Iran	Obese	30 (17-51)	42	F	32.95 ± 3.35	0.87 ± 0.11	Colorimetric	Advertisement with letters	8
		Controls	31 (19-45)	40	F	23.40 ± 4.12	0.97 ± 0.27			
Guerrero-Romero; Rodríguez-Móran [21]	Mexico	Obese	38.4 ± 13.4	100	F/M (75/25)	33.60 ± 3.60	0.74 ± 0.16	Colorimetric	not described	6
		Controls	37.6 ± 12.6	138	F/M (101/37)	21.80 ± 2.00	0.99 ± 0.12			
Guerrero-Romero <i>et al.</i> [22]	Mexico	Obese	42.1 ± 1.0	321	F/M	35.00 ± 5.00	0.78 ± 0.12	Colorimetric	Public invitation	8
		Controls	40.7 ± 11.9	146	F/M	22.60 ± 2.00	0.82 ± 0.14			
Morais <i>et al.</i> [7]	Brazil	Obese	34.7 ± 7.6	31	F	36.50 ± 2.17	0.76 ± 0.08	FAAS	not described	5
		Controls	35.4 ± 8.6	52	F	21.90 ± 1.66	0.78 ± 0.07			
Oliveira <i>et al.</i> [6]	Brazil	Obese	36.3 ± 7.0	65	F	35.68 ± 2.66	0.78 ± 0.08	FAAS	not described	5
		Controls	35.7 ± 7.6	66	F	21.98 ± 1.62	0.77 ± 0.07			

Associação entre as concentrações de minerais (magnésio, zinco e selênio) e marcadores metabólicos dos fenótipos obesidade metabolicamente saudável e obesidade metabolicamente não saudável

Rodríguez - Hernandez <i>et al.</i> [23]	Mexico	Obese	46.1 ± 12.5	20	F/M (10/10)	30.90 ± 3.90	0.78 ± 0.20	Colorimetric	ambulatory	5
		Controls	46.3 ± 7.1	20	F/M (10/10)	21.40 ± 2.60	0.86 ± 0.16			
Rodríguez- Móran, Guerrero- Romero [24]	Mexico	Obese	43.1 ± 12.4	54	F/M	33.90 ± 3.60	0.67 ± 0.16	Colorimetric	households visits	7
		Controls	39.6 ± 14.6	54	F/M	22.90 ± 1.50	0.83 ± 0.12			

Legend: BMI – Body mass index; Mg – Magnesium; NOS - Newcastle-Ottawa Scale; F – Female; M – Male; DCPES - Direct current plasma emission spectrometer; FAAS - Flame atomic absorption spectrophotometry.

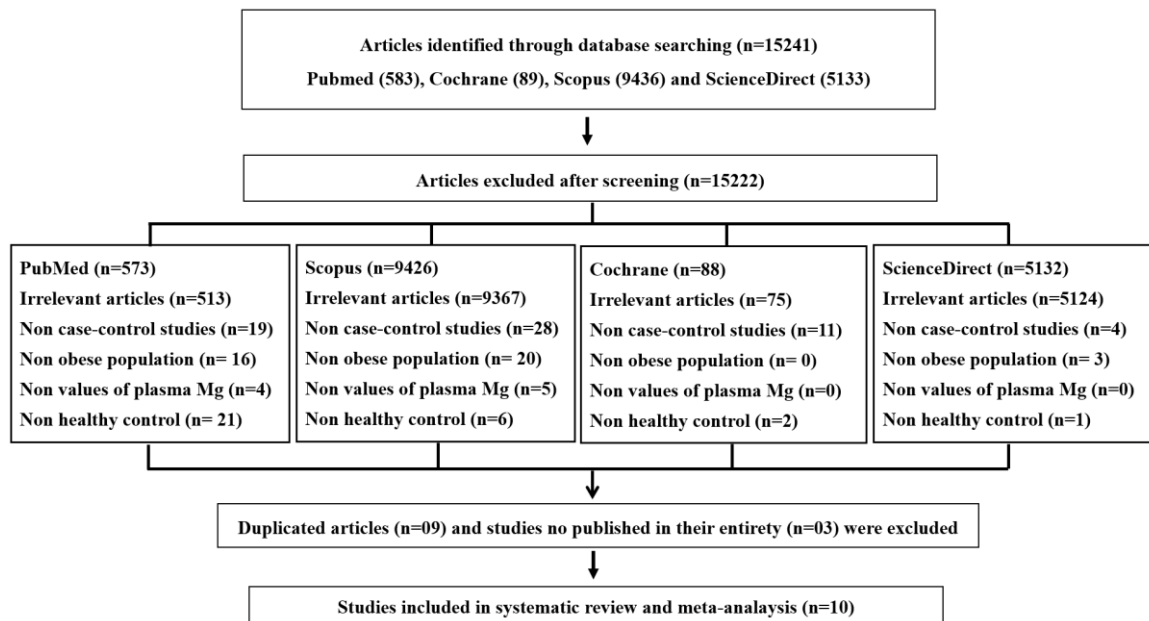
Figure 1. Flow diagram of the study selection.

Figure 2. Forest plots of studies of plasma magnesium concentration in obese participants and control group.

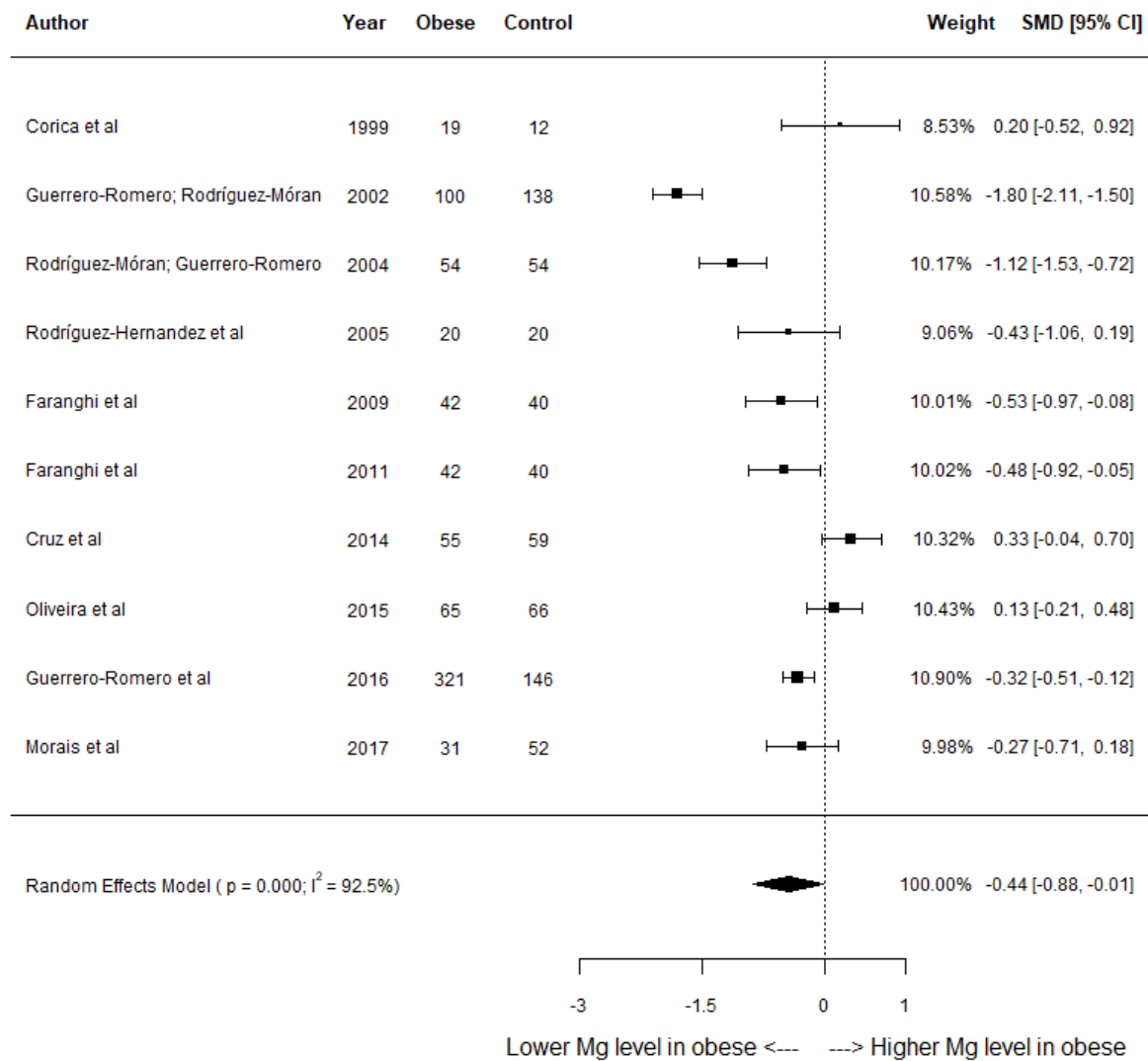
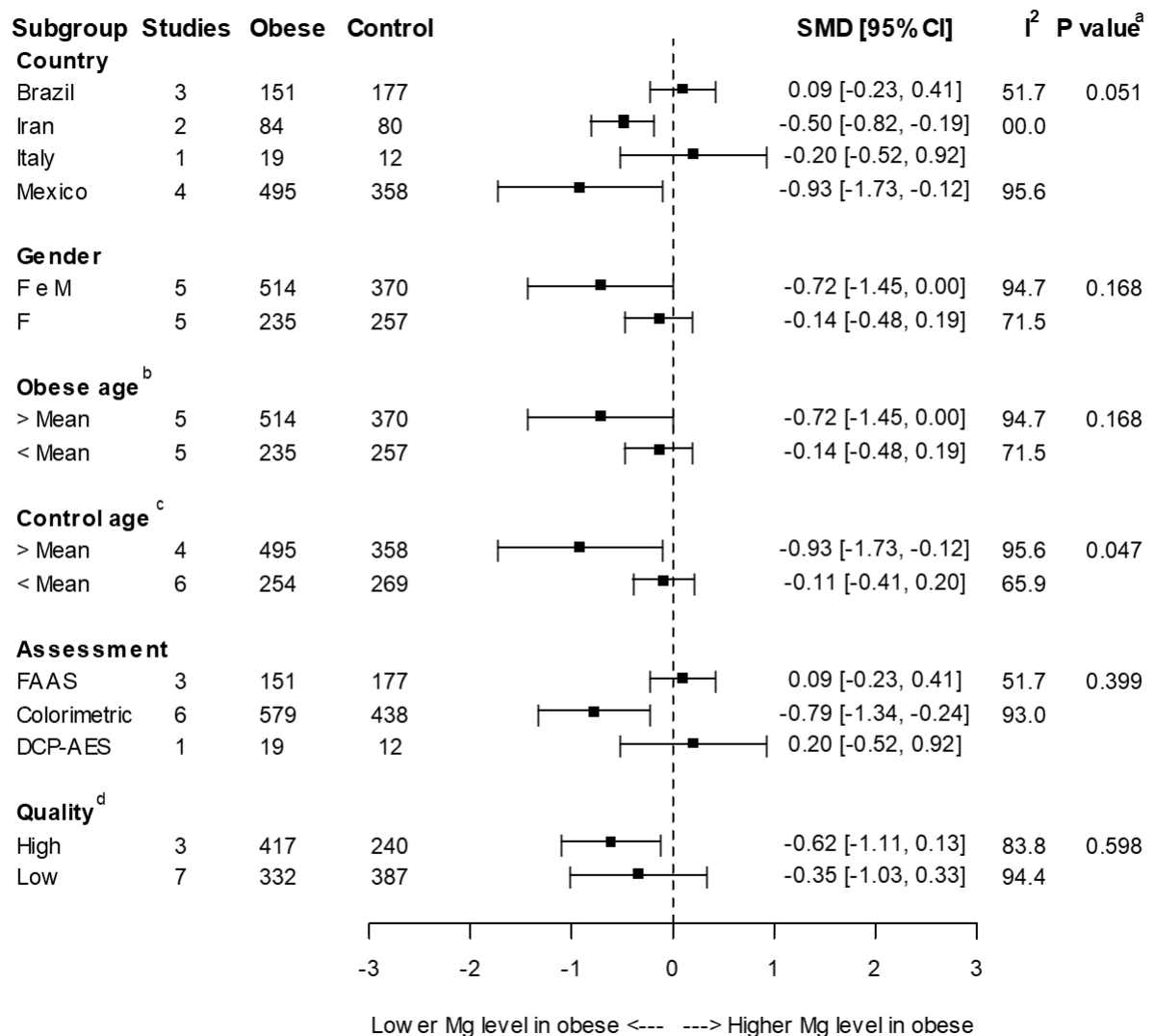
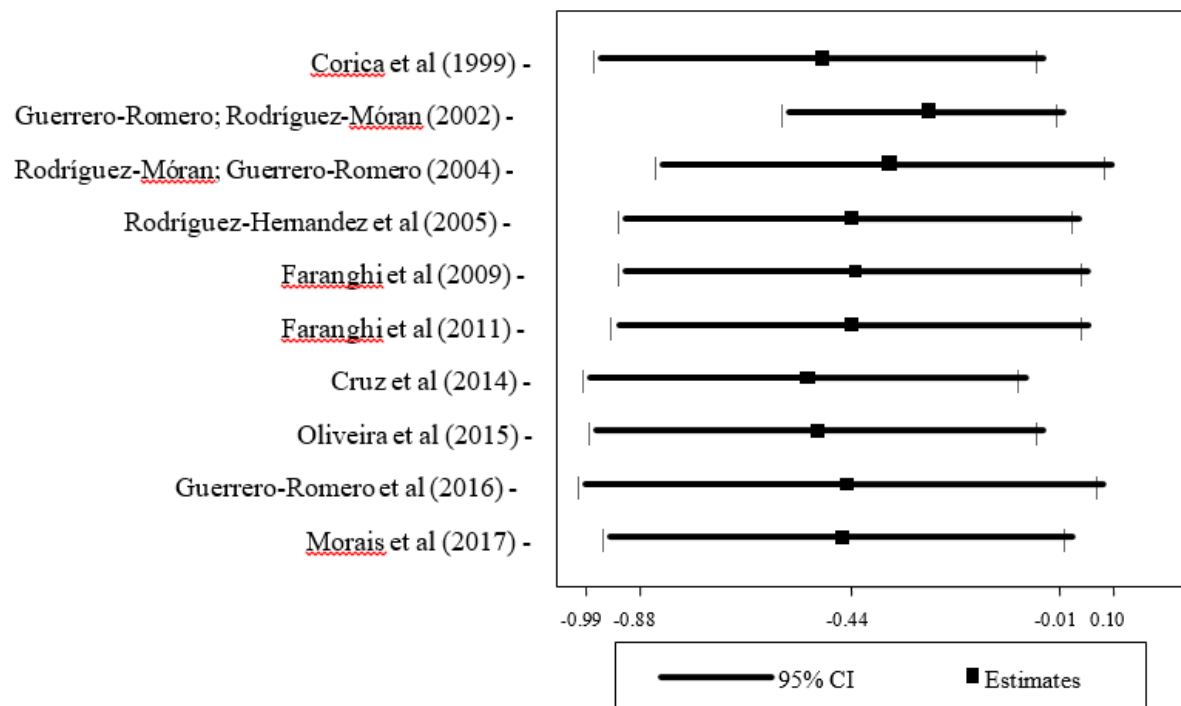


Figure 3. Subgroup and meta-regression analysis results derived from random-effect models of plasma magnesium concentration according to different studies.



Legend: ^ap values from meta-regression of each covariate: country, gender, case age, control age, degree of obesity, assessment method and study quality; ^bmean age of obese participants was 37.98 years; ^c mean age of healthy individuals was 36.78 years; ^d study quality was assessed using the Newcastle-Ottawa Scale.

Figure 4. Forest plot for the sensitivity analysis in meta-analysis.

Legend: Name of each study indicates that it was omitted in the analysis.

Capítulo 4

*Associação entre as concentrações de minerais (magnésio, zinco e selênio) e marcadores metabólicos dos
fenótipos obesidade metabolicamente saudável e obesidade metabolicamente não saudável*

Kyria Jayanne Clímaco Cruz

Artigo Científico: *The Effect of Zinc Supplementation on Insulin Resistance in Obese Subjects: a Systematic Review*

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The Effect of Zinc Supplementation on Insulin Resistance in Obese Subjects: a Systematic Review

Kyria Jayanne Climaco Cruz¹ • Jennifer Beatriz Silva Morais¹ •
Ana Raquel Soares de Oliveira¹ • Juliana Soares Severo¹ •
Dilina do Nascimento Marreiro¹



The Effect of Zinc Supplementation on Insulin Resistance in Obese Subjects: a Systematic Review

Kyria Jayanne Clímaco Cruz¹ · Jennifer Beatriz Silva Morais¹ ·
 Ana Raquel Soares de Oliveira¹ · Juliana Soares Severo¹ ·
 Dilina do Nascimento Marreiro¹

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Abstract Obesity is a chronic disease characterized by excessive accumulation of body fat and the presence of metabolic disorders such as insulin resistance. In this sense, zinc is an important nutrient that stimulates insulin secretion and increases sensitivity to insulin. The aim of this study was to investigate the effect of zinc supplementation on insulin resistance in obese subjects through a systematic review of the available clinical trials. The search for articles was conducted using the PubMed, SciVerse Scopus, SciVerse ScienceDirect, and Cochrane databases, on May 25, 2016, by two authors independently. The recommendations of the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) were followed in the conduct of this review. The Cochrane Collaboration tool was used to assess the risk of bias of the trials included in this review. After screening of the articles, six clinical trials were included in this systematic review. The scientific evidence presented in this systematic review shows that zinc supplementation improves insulin resistance in obese individuals of both sexes.

Keywords Obesity · Zinc · Insulin resistance

Introduction

Obesity is a highly prevalent chronic disease, with complex and multifactorial etiology, characterized by excessive accumulation of body fat and the presence of metabolic disorders

such as insulin resistance, chronic low-grade inflammation, and oxidative stress [1–3].

Currently, researches are being conducted with a view to investigate the role of minerals in protection against metabolic disorders associated with obesity. Zinc, in particular, is an important inducer of insulin secretion, a sensitizer of insulin action, and an anti-inflammatory and antioxidant agent [4, 5].

Regarding the participation of zinc in the signaling pathway of insulin action, it is emphasized that this nutrient stimulates the phosphorylation of the β subunit of the insulin receptor and promotes activation of phosphatidylinositol protein 3-kinase and protein kinase B or Akt, increasing transport for glucose inside cells [5–8].

The literature has shown that obese patients have hypozincemia, which appears to contribute to the development of insulin resistance [9, 10]. The reduction in serum and erythrocyte concentrations of this mineral observed in these individuals may be due to changes in zinc homeostasis [11, 12].

With regard to the possible factors contributing to changes in zinc homeostasis in obesity, the chronic inflammation alters the expression of metallothionein and Zip-14 zinc transporter protein, which promotes the redistribution of plasma mineral content in the fat, liver, and muscle, contributing to hypozincemia in obese individuals [11–15].

However, recent studies show that supplementation with zinc can restore the expression of their carrier proteins modified by obesity, making the mineral available for function [16]. Thus, supplementation with zinc may contribute to the control of metabolic disorders associated with this disease, including insulin resistance [4].

Therefore, the aim of this study was to corroborate the influence of zinc supplementation upon insulin resistance in obese subjects, through a systematic review of clinical trials available in the literature.

✉ Dilina do Nascimento Marreiro
 dilina.marreiro@gmail.com

¹ Department of Nutrition, Federal University of Piauí, Campus Minister Petrônio Portela, Ininga, Teresina, Piauí, Brazil

Methods

Data Sources and Study Selection

The search for articles was conducted in the PubMed, Scopus, ScienceDirect, and Cochrane databases, on May 25, 2016, by two authors (KC and JM), independently. Research conducted by these authors was compared and equivalence verified in the search and selection of articles.

The patient, intervention, comparison, and outcomes (PICO) strategy was used for the guiding questions of this review setting. The keywords “zinc,” “obese,” “obesity,” “insulin resistance,” “insulin sensibility,” and “clinical trial” were used in the literature. The following descriptors crossings were used in the search for articles: “zinc and obes * and clinical trial”, “zinc and (insulin resistance or insulin sensitivity) and clinical trial”, “obes * and zinc and supplementation”, “obes * and zinc (insulin resistance or insulin sensitivity) “and“ zinc and supplementation and (insulin resistance or insulin sensitivity)”.

This systematic review included clinical trials realized with human subjects, published in English or Spanish, without a publication year limit that evaluated the effect of zinc supplementation on insulin resistance in obese individuals without restrictions of gender, age, or ethnicity. Case studies, cross-sectional studies, case-control studies, review articles, and animal research were not

included in this review. The study selection details are shown in Fig. 1.

Relevant information collected from the articles studied in this review included authors, year of publication, place of conducting the survey, sample size, sex and age of the study population, dose and duration of zinc supplementation, control group or placebo, and evaluation methods for insulin resistance or insulin sensitivity.

Assessment of Risk of Bias

The recommendations of the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) [17] were followed in the conduct of this review, in which a flow diagram was included to demonstrate how many publications were identified and screened for eligibility and how many publications were excluded and why. The Cochrane Collaboration tool was used to assess the risk of bias of the trials included in this review [18].

Results

Selected Articles

We found 875 articles in the search conducted in the four mentioned databases. Of these, five articles identified in

Fig. 1 Flow diagram of the study selection. Legend: Search 1: zinc and obes* and clinical trial; search 2: zinc and (insulin resistance or insulin sensitivity) and clinical trial; search 3: obes* and zinc and supplementation; search 4: obes* and zinc and (insulin resistance or insulin sensitivity); search 5: zinc and supplementation and (insulin resistance or insulin sensitivity)

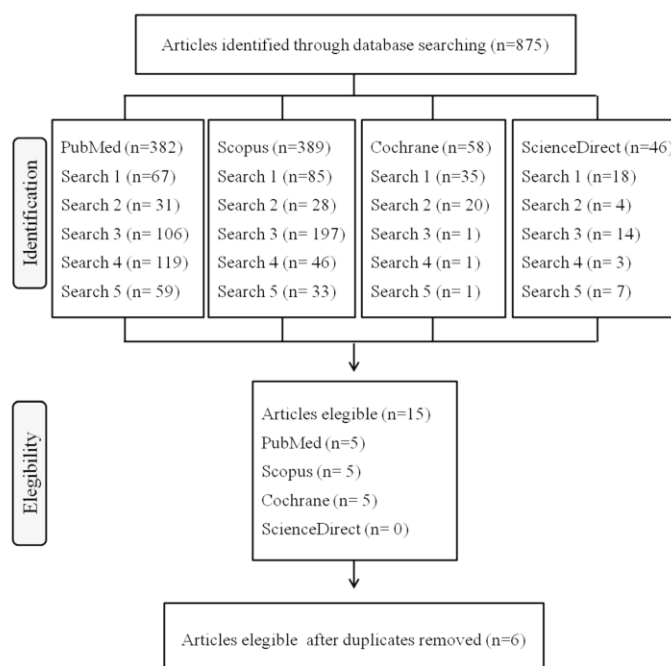


Table 1 Methodological quality of included studies measured by the Cochrane Collaboration tool

	Gómez-García et al. [20]	Marreiro et al. [21]	Hashemipour et al. [22]	Kelishadi et al. [4]	Kim; Lee [19]	Payahoo et al. [23]
Random sequence generation (selection bias)	Y	Y	Y	Y	N	Y
Allocation concealment (selection bias)	Y	Y	Y	Y	N	Y
Blinding of participants and personnel (performance bias)	Y	Y	Y	Y	N	Y
Blinding of outcome assessment (detection bias)	N	N	Y	Y	N	N
Incomplete outcome data (attrition bias)	N	N	N	N	N	N
Selective outcome reporting (reporting bias)	N	N	N	N	Y	N
Other bias	N	N	N	N	N	N

Legend: Y yes; N no

PubMed, five in Scopus, and five in Cochrane were considered eligible for inclusion. After removing duplicates, six articles were finally included in this systematic review.

The assessment of the risk of bias of the trials included in this review is presented in Table 1. It was found that the authors had used techniques to avoid potential biases such as of performance, detection, friction, and information, with the exception of Kim and Lee's study [19], in which techniques to avoid some of these biases were not identified.

Description of Studies

Table 2 shows the characteristics of the clinical trials included in this review. The studies were conducted with children and adults, of both genders, in four different countries. Supplemental zinc doses ranged from 20 to 100 mg per day, in the form of elemental zinc, zinc sulfate, zinc amino acid chelate, and zinc gluconate. Three studies conducted supplementation for 4 weeks and three for 8 weeks. The methods

used for evaluation of insulin resistance and insulin sensitivity were the euglycemic-hyperinsulinemic clamp, the homeostasis model assessment index for insulin resistance (HOMA-IR), and the insulin sensitivity index.

The Effect of Zinc Supplementation on Insulin Resistance in Obese Subjects

Of the six studies included in this review, four found an improvement in insulin resistance in obese subjects after supplementation with zinc. Marreiro et al. [21] performed supplementation with 30 mg of zinc amino acid chelate for 30 days in 28 obese women, aged between 25 and 45 years, and found a reduction in serum insulin and HOMA-IR values (Table 2).

Hashemipour et al. [22] supplemented 30 obese children (6–10 years) with 20 mg per day of elemental zinc for 8 weeks and observed a reduction in serum glucose and insulin and HOMA-IR values. Kelishadi et al. [4] also conducted a study on 30 obese children aged between 6 and 10 years, and found

Table 2 Characteristics of clinical trials included in this review

Authors, publication year	Location	Participants (n)		Gender	Age (year)	Supplementation		Assessment of insulin resistance/insulin sensitivity
		Supplement	Control			Dose	Duration	
Gómez-García et al. [20]	Mexico	7	7	M	21–30	100 mg/day—zinc sulfate	4 weeks	Euglycemic-hyperinsulinemic clamp
Marreiro et al. [21]	Brazil	28	28	F	25–45	30 mg/day—zinc amino chelate	4 weeks	HOMA-IR
Hashemipour et al. [22]	Iran	30	30	F/M	6–10	20 mg/day—elemental zinc	8 weeks	HOMA-IR
Kelishadi et al. [4]	Iran	30	30	F/M	6–10	20 mg/day—elemental zinc	8 weeks	HOMA-IR
Kim; Lee [19]	Korea	20	20	F	18–28	30 mg/day—zinc gluconate	8 weeks	HOMA-IR and insulin sensitivity index
Payahoo et al. [23]	Iran	30	30	F/M	18–45	30 mg/day—zinc	4 weeks	HOMA-IR

HOMA-IR Homeostasis model assessment for insulin resistance

that supplementation with 20 mg per day of elemental zinc for 8 weeks reduced serum concentrations of glucose and insulin and HOMA-IR index values.

Payahoo et al. [23] evaluated the effect of supplementation with 30 mg per day of zinc in men and obese adult women for 4 weeks and observed reduction in HOMA-IR values, showing the beneficial effect of the intervention on improvement of insulin resistance in patients.

Gómez-García et al. [20] and Kim and Lee [19] found no improvement in insulin resistance after zinc supplementation. Gómez-García et al. [20] found that 100 mg per day of zinc sulfate for 30 days did not affect sensitivity to insulin. Kim and Lee [19] showed that supplementation with 30 mg per day of zinc gluconate for 8 weeks in obese women between 18 and 28 years of age did not alter the HOMA-IR values or the insulin sensitivity index.

Discussion

In this systematic review, we evaluated six clinical trials with a view to investigating the effect of zinc supplementation on insulin resistance in obese individuals. Most experiments showed beneficial effects of intervention with zinc supplements in this metabolic disorder associated with obesity.

An important point to note in this discussion concerns discrepancies in the supplementation doses, type of supplement used, bioavailability, and duration of supplementation. These factors may have influenced the results of some parameters used to evaluate insulin resistance. In addition, surveys were conducted in different ethnic groups, age, and gender, which may have contributed to the observed differences.

It is worth mentioning the meta-analysis of Capdor, Foster, and Samman [6], which also evaluated the effects of zinc supplementation on glycemic markers in humans and concluded that the intervention can reduce glucose and glycated hemoglobin concentrations, thus contributing to glycemic control in chronic metabolic disease, especially in diabetic and obese subjects.

It is noteworthy that different molecular mechanisms are involved in zinc acting as a nutrient sensitizer of insulin action. This mineral inhibits the protein tyrosine phosphatase 1B enzyme that catalyzes the dephosphorylation of the β -subunit of the insulin receptor; thus, zinc promotes the phosphorylation of this hormone receptor, an essential step for insulin to function properly [24].

Zinc also contributes to the activation of the Akt protein, promoting the translocation of glucose transporter 4 (GLUT4), and glucose uptake by cells, since this mineral inhibits the action of the phosphatase enzyme phosphatidylinositol 3-kinase (PTEN), which promotes the dephosphorylation of phosphatidylinositol 3,4,5-triphosphate

(PIP3), a metabolite that acts as a second messenger in the insulin signaling pathway [8].

Another important action of zinc relates to the fact that the mineral is a structural component of the insulin-regulated aminopeptidase (IRAP) and activates the phospholipase enzyme 3-kinase, which promotes maintenance of GLUT4 concentration in appropriate amounts in the cell and translocation of this carrier to the plasma membrane for subsequent glucose uptake, contributing to glycemic control in obesity [8, 25].

Two clinical trials included in this systematic review found that zinc supplementation did not influence insulin resistance in obese individuals. Here, it is appropriate to mention the small sample size of these studies, which may have limited the significance of the observed effects of the intervention on the markers evaluated. Associated with this, a limitation suggested by Kim and Lee [19] relates to the fact that the authors have not controlled their analyses for potential confounding factors such as diet and physical activity.

A strong feature of this review is that the studies were carefully selected according to a predetermined search strategy for inclusion of clinical trials with impartial perspective in this systematic review. A limitation that should be mentioned is the possibility that relevant articles were not included because they were published in languages other than English or Spanish or were not indexed in the electronic databases accessed in this study.

Conclusion

The nutritional status regarding zinc of the obese patients is altered and might be considered risk factor for development of metabolic disorders associated with this disease. The scientific evidence presented in this systematic review shows that zinc supplementation appears to contribute to improvement of insulin resistance in obese individuals of both sexes.

Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

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Capítulo 5

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Zinc and Insulin Resistance: Biochemical and Molecular Aspects

Kyria Jayanne Clímaco Cruz¹ • Ana Raquel Soares de Oliveira¹ • Jennifer Beatriz Silva Morais¹ • Juliana Soares Severo¹ • Priscyla Maria Vieira Mendes¹ • Stéfany Rodrigues de Sousa Melo¹ • Gustavo Santos de Sousa² • Dilina do Nascimento Marreiro¹ 



Zinc and Insulin Resistance: Biochemical and Molecular Aspects

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Abstract

Studies have shown the participation of minerals in mechanisms involved in the pathogenesis of insulin resistance. Zinc, in particular, seems to play an important role in the secretion and action of this hormone. Therefore, the aim of this review is to understand the role of zinc in increasing insulin sensitivity. We conducted a search of articles published in the PubMed and ScienceDirect database selected from March 2016 to February 2018, using the keywords “zinc,” “insulin,” “insulin resistance,” “insulin sensitivity,” and “supplementation.” Following the eligibility criteria were selected 53 articles. The scientific evidences presented in this review show the importance of zinc and their carrier proteins in the synthesis and secretion of insulin, as well as in the signaling pathway of action of this hormone. Zinc deficiency is associated with glucose intolerance and insulin resistance; however, the effectiveness of the intervention with the zinc supplementation is still inconclusive.

Keywords Zinc · Insulin resistance · Insulin sensitivity · Insulin secretion · Supplementation

Introduction

Insulin resistance is characterized by reduced sensitivity of the peripheral tissues to the action of this hormone, particularly in the adipose tissue, muscle, and liver. This metabolic disturbance is associated with several diseases such as obesity, type 2 diabetes mellitus, metabolic syndrome, non-alcoholic fatty liver disease, cirrhosis, and polycystic ovary syndrome [1–4].

In this context, various factors are related with the development of insulin resistance, such as excess body fat (particularly the accumulation of intra-abdominal fat), chronic inflammation, oxidative stress, and endocrine disorders such as thyroid dysfunction and changes in cortisol homeostasis [3, 5–7].

In this regard, studies have shown the role of minerals in mechanisms involved in sensitivity to insulin. Zinc, in particular, has been a mineral of great interest for researchers, for its

performance in inducing insulin secretion and insulin sensitivity. This micronutrient is required in the formation and crystallization of insulin, stimulation of phosphorylation of the β subunit of this hormone receptor, activation of the kinase 3 phosphatidylinositol enzyme, and induction of the translocation of glucose transporter 4 (GLUT4) [8–10].

Therefore, considering the complexity of insulin resistance and the high prevalence of this metabolic disorder in several diseases, as well as the likely participation of zinc in protection against resistance to this hormone, this review aims to understand the role of zinc in increasing insulin sensitivity.

Methods

This is a narrative review based on a bibliographical survey of articles in PubMed and ScienceDirect databases, without limit for the year of publication, selected from March 2016 to February 2018. The keywords used in the search were “zinc,” “insulin,” “insulin resistance,” “insulin sensitivity,” and “supplementation.” The descriptors were used alone or combined using the boolean operators “AND” and “OR.”

Studies that presented relevant aspects of the participation of zinc and its carrier proteins in mechanisms underlying the secretion and action of insulin were included. Only studies in English were included. Articles were selected for originality

✉ Dilina do Nascimento Marreiro
dilina.marreiro@gmail.com

¹ Department of Nutrition, Federal University of Piauí, Ministro Petrônio Portella Campus, Ininga, Teresina, Piauí, Brazil

² Faculty of Medical Sciences, State University of Piauí, Teresina, Piauí, Brazil

and relevance, considering the accuracy of the experimental design and adequacy of the sample size. Classic works and recent articles were preferentially used. Dissertations, theses, and articles in which only a summary was available, and those duplicated in different databases were excluded.

Then, we proceeded to the analysis of the included articles, starting with reading the titles, followed by summaries, and later the full text. The application of the exclusion criteria was performed at all stages, always by consensus of the reviewers. The literature included the following types of studies: experimental studies, clinical trials, cross-sectional studies, case control studies, and review articles. At the end, we selected 53 articles.

Zinc: Participation in the Secretion of Insulin

Several studies have shown the participation of zinc in protection against insulin resistance. Zinc deficiency appears to be associated with glucose intolerance caused by impairments in insulin secretion and action, whereas supplementation with zinc increases the concentration of this hormone in the β pancreatic cells and stimulates insulin secretion, as well as promotes improvement in insulin sensitivity [11–17].

Regarding the role of zinc in insulin secretion from pancreatic β cells, this trace element acts in the crucial steps of formation and crystallization of this hormone. It is worth mentioning that within the secretory granules present in pancreatic β cells, insulin undergoes a process of maturation and it is coupled with two zinc ions to form the hexameric complex

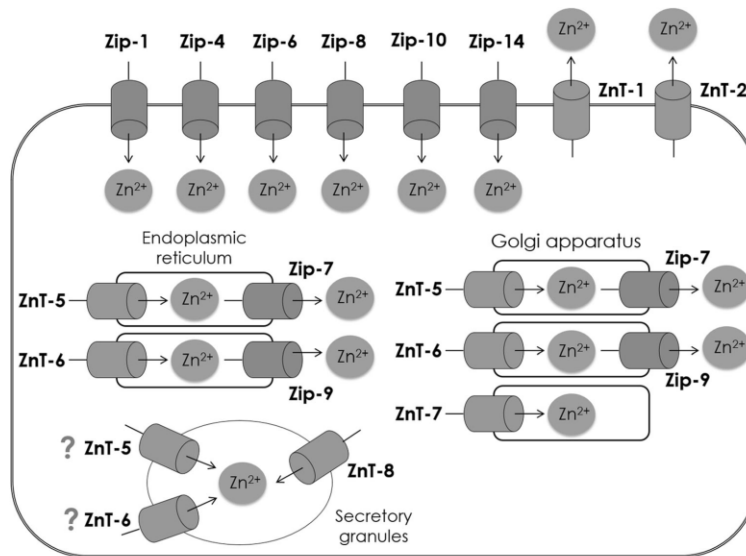
zinc₂-insulin₆, necessary for secretion of this hormone [18, 19].

Cooper-Capetini et al. [15] investigated the effect of zinc supplementation on glucose homeostasis and pancreatic β cell function in an animal model of insulin resistance. These researchers verified that glucose tolerance, HOMA-IR index (homeostasis model assessment-insulin resistance), and glucose-stimulated insulin secretion were significantly improved by ZnCl₂ supplementation in high fat-fed mice by a mechanism that enhances β cell function.

Studies have shown the expression of zinc carrier proteins in β pancreatic cells (Fig. 1), and that these transporters of zinc affect the secretion of insulin [20–22]. Cai et al. [22] examined all members (ZNT1–10) of the ZNT family in pancreatic islets and in β cell lines of humans and mice and there were no substantial differences in the expression of nine ZNT proteins in the human and mouse islets and β cells with exception of ZNT3, which was only detected in human β cells, but not in mouse β cells. Moreover, these researchers found that ZNT2 was localized on the cell surface of both human and mouse β cells, suggesting a role of ZNT2 in direct export of zinc out of the β cell.

Wijesakara et al. [23] evaluated the effect of zinc transporter protein ZNT-8 in pancreatic β cells and found that ZNT-8 gene knockout mice showed glucose intolerance, reduced concentration of intracellular zinc, atypical insulin granules, few secretory granules, increased levels of pro-insulin, and reduction in the first phase of insulin secretion. This indicates the importance of this protein in processing, crystallization, storage, and secretion of insulin and in glucose metabolism.

Fig. 1 Localization of zinc carrier proteins in pancreatic β cell. Legend: Zn²⁺—zinc



ZNT-8 protein transports zinc from the cytoplasm of β pancreatic cells to insulin secretory granules, favoring the formation of zinc-insulin hexamer, which is essential for the secretion of this hormone. Studies show that single nucleotide polymorphisms in the SLC30A8 gene are associated with an increased risk of diabetes mellitus type 2. In conditions of zinc deficiency, whereby there is a reduction in the expression of the gene encoding ZNT-8 in human pancreatic islets and in glucose-stimulated insulin secretion, and an increase in expression of this protein restores the secretion of this hormone [24–26].

ZNT-6 protein transports cytoplasmic zinc to the Golgi apparatus and other vesicular compartments and appears to be involved in pro-insulin metabolism and insulin secretion; however, the underlying mechanism is still unclear [18, 21]. Fukunaka et al. [27] demonstrated, in experiment with cell cultures, that ZNT-6 forms a heterodimer with ZNT-5 and transports zinc to secretory granules. Thus, ZNT-6 and ZNT-5 appears to be important in secretory processes, like for example, the secretion of insulin.

Another protein important is ZNT-7, responsible for the transport of the mineral from the cytoplasm to the Golgi complex of β pancreatic cells, influencing the insulin formation process. Huang et al. [28] demonstrated that overexpression of ZNT-7 in the islet of Langerhans in the mouse pancreas promotes an increase in insulin mRNA expression by modulating the activity of the metal responsive transcription factor-1 (MTF-1), and overexpression of ZNT-7 stimulates the synthesis and secretion of this hormone.

Zinc transporter ZNT-3 is also expressed in β pancreatic cells, and under conditions of hyperglycemia and deficiency of this mineral, an increase in its expression occurs. Deletion of the ZNT-3 gene (ZNT-3^{-/-}) reduces expression of the gene coding for insulin, affects the secretion of this hormone and increases fasting glucose in mice during streptozotocin-induced β cell stress. However, the mechanisms involved in the action of ZNT-3 carrier remain unknown [29].

Concerning the role of ZIP proteins in the metabolism of insulin, it is worth mentioning that the protein ZIP-10 carrier is expressed in the plasma membrane of α and β pancreatic cells. In a zinc deficiency situation, ZIP-10 is translocated to the membrane of intracellular vesicles, and ZIP-10 promotes an efflux of zinc of these vesicles into the cytoplasm of these cells [30–32].

Carrier proteins ZIP-6, 7, and 8 are expressed in various organelles, and ZIP-6 and 8 are located in the plasma membrane and ZIP-7 in the endoplasmic reticulum and Golgi complex [33]. Bellomo et al. [34] have shown that an increase in blood glucose in mice changes zinc homeostasis, inducing the expression of genes Zip-6, 7, and 8 and favoring increased mineral content in pancreatic β cells. Thus, the ZIP-6 and 8 carriers may operate by recycling zinc released during the insulin secretory process.

Liu et al. [35] showed that downregulation of ZIP-6 and 7 carriers in human and mouse islets β pancreatic cells reduces

cytosolic zinc content, impairing insulin secretion stimulated by glucose. Moreover, the authors showed that ZIP-6 protein interacts with the peptide glucagon receptor 1, protecting pancreatic β cells from apoptosis.

Thus, the carriers ZIP-6 and 8 located in the plasma membrane of β pancreatic cells, contribute to the uptake of plasma zinc. Moreover, these proteins act by recycling the mineral secreted with insulin to be reused in the synthesis of the hormone in these cells. ZIP-7, in turn, functions in releasing zinc from the endoplasmic reticulum and Golgi apparatus to the cell cytoplasm, while maintaining the homeostasis of the mineral during maturation and secretion of insulin [33].

Zinc: Participation in the Action of Insulin

Regarding the role of zinc in signaling pathways involved in insulin action, this mineral acts via different molecular mechanisms (Fig. 2). This mineral stimulates phosphorylation of the β subunit of the insulin receptor and promotes the activation of phosphatidylinositol 3 kinase and protein kinase B or Akt, thereby enhancing glucose transport into the cells [9, 10, 36, 37].

Accordingly, Bellomo et al. [38] demonstrated the presence of binding sites for zinc in protein tyrosine phosphatase (PTPase) 1B, an enzyme that regulates insulin action by catalyzing the dephosphorylation of the β subunit of its receptor. Thus, the nutrient deactivates this enzyme, thereby increasing the phosphorylation of insulin receptor.

Zinc also inhibits the action of phosphatase and tensin homolog (PTEN), an enzyme that promotes the dephosphorylation of phosphatidylinositol 3,4,5-triphosphate (PIP3), and it inhibits activation of the protein Akt signaling pathway of insulin. Moreover, this mineral induces directly the action of enzymes phosphatidylinositol 3 kinase and protein Akt. Thus, zinc promotes translocation of GLUT4 and the subsequent glucose uptake by cells (Fig. 2) [10].

Associated with this, zinc acts on the structural components involved in glucose transport and ensure their responsiveness to insulin-regulated aminopeptidase (IRAP) molecule, which is necessary to maintain the concentration of GLUT4 in appropriate amounts in the adipose and muscle cells (Fig. 2) [39].

Zinc stimulates the phosphorylation of glycogen synthase kinase 3 (GSK-3) and transcription factor forkhead box protein O1 (FoxO1), similar to the action of insulin (Fig. 2). The phosphorylation of GSK-3 serine residues inhibits its action, favoring the dephosphorylation and activation of glycogen synthase, involved in glycogen synthesis. Phosphorylation of FoxO1 induces translocation from the nucleus to the cytoplasm and inhibits its action to stimulate the expression of gluconeogenic genes. Thus, zinc induces the storage of glucose as glycogen and inhibits the production of this monosaccharide, thereby contributing to glucose homeostasis [10, 40].

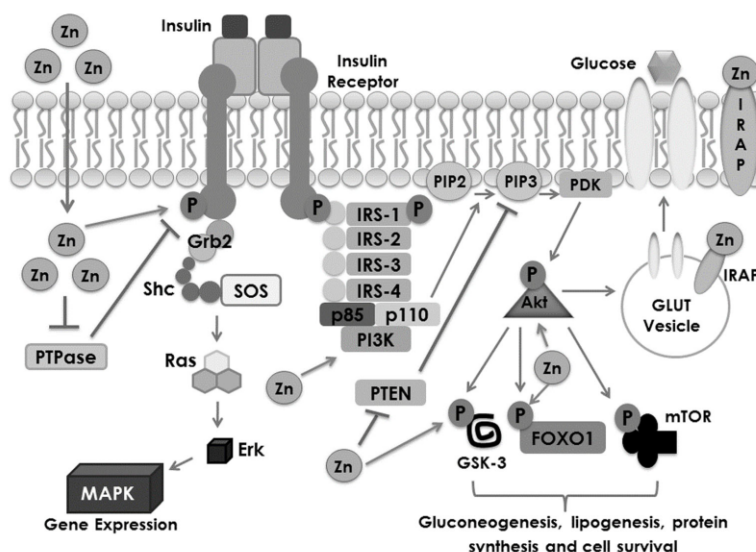


Fig. 2 Molecular mechanisms involved in the participation of zinc in the insulin signaling pathway. Legend: Zn—zinc; PTPase—protein tyrosine phosphatase; Grb2—growth factor receptor-bound protein 2; SOS—son of sevenless protein; Erk—extracellular signal regulated kinases; MAPK—mitogen-activated protein kinases; IRS—insulin receptor substrate; PI3K—phosphoinositide 3-kinase; PIP2—

phosphatidylinositol 4,5-bisphosphate; PIP3—phosphatidylinositol 3,4,5-trisphosphate; PDK—phosphoinositide dependent kinase; PTEN—phosphatase and tensin homolog; Akt—protein kinase B; GSK-3—glycogen synthase kinase 3; FOXO1—forkhead box protein O1; mTOR—mechanistic target of rapamycin; GLUT—glucose transporter; IRAP—insulin-regulated aminopeptidase

Accordingly, Wu et al. [41] demonstrated that zinc can increase glucose uptake by myocytes by modulating the action of insulin signaling pathway proteins. These researchers found that the mineral favored activation of Akt enzyme and GLUT4 translocation and stimulated the phosphorylation of GSK-3 β protein, promoting glucose uptake and glycogen synthesis, respectively.

Another important action of zinc with respect to modulation of transcription of the insulin receptor gene is via zinc finger proteins, which contain three zinc fingers needed for binding. The binding of these proteins is required to enable the expression of the gene coding for the insulin receptor. The zinc finger protein 407 regulates glucose uptake by insulin by increasing levels of messenger RNA of Glut4 and stimulating its transcription, favoring increased concentration of GLUT4 in adipocytes of mice [42].

ZNT-7 also acts in peripheral tissues, stimulating the signaling pathway of insulin action. This zinc transporter is expressed in muscle cells of mice, while deletion of the ZNT-7 gene (ZNT-7 $^{-/-}$) favors reduction of the expression of mRNA of the insulin receptor, the insulin receptor substrate 2 (IRS-2), and Akt protein. Moreover, overexpression of ZNT-7 increases the expression of IRS-2 mRNA, phosphorylation of this substrate, and Akt protein and stimulates glucose uptake in muscle cells [43]. In this context, Tepasomdech et al.

[44] demonstrated that ZNT-7 deficiency contributes to the manifestation of insulin resistance, as it reduces the insulin-stimulated phosphorylation of Akt protein in the subcutaneous adipose tissues of mice, affecting the glucose uptake in this tissue. Zinc transporter ZIP-7 is also involved in glycemic control in the muscle cells of mice, because the inhibition of its expression compromises the phosphorylation of Akt protein and glycogen synthesis in these cells [45].

ZIP-14 protein is located in the plasma membrane of adipocytes, hepatocytes, and pancreatic β cells, and it transports plasma zinc into the cytoplasm of these cells. The expression of this protein is increased during inflammation, thus favoring the “sequestration” of zinc in the liver and adipose tissue. Furthermore, the carrier affects glucose homeostasis, as the deletion of the Zip-14 gene (Zip-14 $^{-/-}$) favors an increase in phosphorylation of the insulin receptor, phosphatidylinositol 3-kinase, and Akt as well as improves the transport of glucose, reduces fasting glucose, activates lipogenesis, inhibits lipolysis, and increases the concentration of serum insulin and hepatic glucose [28, 46, 47].

Aydemir et al. [48] demonstrated that zinc is distributed to multiple sites in hepatocytes of mice through translocation of ZIP14 from plasma membrane to endosomes, and verified that endosomes from Zip14 knockout mice were zinc-deficient. Thus, ZIP-14 is important to zinc cellular distribution. This

study also verified that ZIP14-mediated zinc transport contributes to regulation of endosomal insulin receptor activity and glucose homeostasis in hepatocytes.

A systematic review and meta-analysis of the effects of zinc supplementation in patients with diabetes demonstrated that zinc supplementation has beneficial effects on glycaemic control and promotes healthy lipid parameters [17]. Other meta-analysis of randomized placebo-controlled supplementation trials in humans verified reduction in glucose concentrations and tendency for a decrease in glycated hemoglobin (HbA1c) following zinc supplementation, suggesting that zinc may contribute to the management of hyperglycemia in individuals with chronic metabolic disease [36].

Considering the important functions performed by zinc in the secretion and action of insulin, several studies have been conducted with an aim to investigate the effect of supplementation with this mineral in people with resistance to insulin. Ranasinghe et al. [49] and Islam et al. [50], for example, evaluated the effect of zinc supplementation in elderly and adult diabetic individuals. Ranasinghe et al. [49] verified that the intervention with 20 mg of elemental zinc for 12 months reduced fasting plasma glucose concentrations, oral glucose tolerance test and HOMA-IR index, with significant improvement in β cell function. Islam et al. [50] verified that supplementation with 30 mg of zinc sulfate for 24 weeks improves fasting plasma glucose concentrations, function of β cells and insulin sensitivity.

Some studies also have evaluated the effect of zinc supplementation in obese individuals [8, 51–53]. Payahoo et al. [51] supplemented 60 adult obese individuals with 30 mg of zinc gluconate or placebo for 4 weeks, and zinc supplementation reduced concentrations of serum insulin, and HOMA-IR index. However, some researchers did not verify benefic effect of zinc supplementation on glycemic control parameters [53].

Conclusions

The scientific evidences presented in this review show that zinc and its carrier proteins are involved in insulin synthesis and secretion as well as in the molecular signaling pathways underlying the action of this hormone. This nutrient acts to modulate transcription of the gene coding for the insulin receptor via zinc finger proteins. Thus, zinc deficiency is associated with glucose intolerance and insulin resistance; however, the effectiveness of the intervention with the zinc supplementation is still inconclusive.

Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no conflict of interest.

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Capítulo 6

*Associação entre as concentrações de minerais (magnésio, zinco e selênio) e marcadores metabólicos dos
fenótipos obesidade metabolicamente saudável e obesidade metabolicamente não saudável*

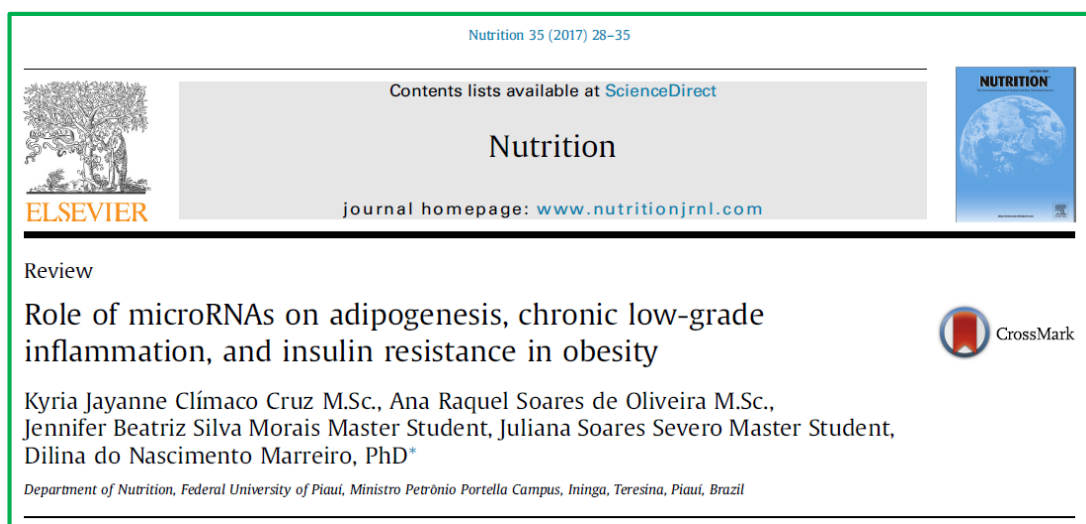
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Review

Role of microRNAs on adipogenesis, chronic low-grade inflammation, and insulin resistance in obesity



Kyria Jayanne Clímaco Cruz M.Sc., Ana Raquel Soares de Oliveira M.Sc.,
Jennifer Beatriz Silva Morais Master Student, Juliana Soares Severo Master Student,
Dilina do Nascimento Marreiro, PhD*

Department of Nutrition, Federal University of Piauí, Ministro Petrônio Portella Campus, Ininga, Teresina, Piauí, Brazil

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ABSTRACT

The aim of this review was to convey updated information on the role of microRNAs in adipogenesis, chronic low-grade inflammation, and insulin resistance in obesity. Obesity is a chronic disease characterized by the presence of metabolic disorders (e.g., low-grade chronic inflammation), which contributes to the manifestation of insulin resistance. Diverse molecular mechanisms have been implicated in the development of these disorders, and microRNAs stand out as a contributing factor. They are a class of noncoding RNAs that regulate the expression of genes by inducing cleavage of mRNAs or via inhibition of protein translation. It is important to point out that obese individuals show alterations in the expression of microRNAs favoring manifestation of the metabolic disorders present in these patients, and these alterations may be reversed by the loss of weight. Therefore, microRNAs may be regarded as potential biomarkers of obesity-related disorders. Further studies on this topic may advance the understanding of the molecular basis of obesity, including the participation of microRNAs in the pathogenesis of this disease.

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Introduction

Obesity is a chronic disease characterized by excessive accumulation of body fat that may be harmful to health and may increase mortality, as it is associated with key metabolic disorders, such as low-grade chronic inflammation and insulin resistance (IR) [1–4].

Studies have been carried out to clarify the molecular mechanisms involved in the development of these metabolic disorders, with emphasis on participation of microRNAs in the regulation of adipocyte differentiation, production of proinflammatory cytokines, and expression of signaling proteins of the insulin pathway [5].

MicroRNAs constitute a class of noncoding RNAs that regulate the expression of genes by inducing cleavage of messenger RNAs (mRNAs) or inhibition of protein translation. The literature

shows alterations in expression of microRNAs in obese individuals; these changes seem to contribute to the manifestation of metabolic disorders in this patient population [6,7].

In this regard, a study conducted with obese children showed alterations in the blood plasma concentrations of microRNAs (downregulation of miR-221 and miR-28-3p, and upregulation of miR-486-5p, -486-3p, -142-3p, -130b, and -423-5p), with a significant association between the microRNAs assessed and the body mass index (BMI), percentage of body fat, waist circumference (WC), body fat distribution, IR index, C-reactive protein (CRP), and plasma concentrations of lipids [8].

Similarly, Pescador et al. [9] analyzed the serum profile of obese individuals' microRNAs hoping to identify biomarkers of obesity-related disorders and confirmed high expression of microRNAs miR-138, -15b, and -376a, demonstrating the potential of these microRNAs in predicting obesity.

Another important characteristic in this sense is the effect of body weight reduction on the expression of microRNAs in obese patients. A study by Ortega et al. [7] demonstrated that the changes in expression of obesity-induced microRNAs were reversed due to loss of weight after bariatric surgery, which was not observed after treatment with a hypocaloric diet.

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* Corresponding author. Tel.: +86 999 15019; fax: +86 323 71812.

E-mail address: Dilina.marreiro@gmail.com (D. do N. Marreiro, PhD).

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In view of the complexity of molecular events involved in the pathogenesis of obesity, with emphasis on the participation of microRNAs in the manifestation of metabolic disorders related to this disease (which have been regarded as potential clinical targets for prevention and/or treatment of obesity), the objective of this review was to convey updated information on the role of these molecules in the control of these disorders.

MicroRNAs and obesity

MicroRNAs are noncoding RNA molecules 18 to 25 nucleotides long that employ posttranscriptional effects to influence the expression of genes because they bind to a sequence of bases located in the 3' untranslated region (3'UTR) of a target mRNA [5,6].

The complete canonical pairing of bases of mature microRNAs with the 3'UTR region of their target mRNAs promotes mRNA cleavage, and this mechanism of action is rare in mammals. The imperfect pairing of the bases favors blockage of protein translation via recruitment of GW182 proteins, which interact with cytoplasmic deadenylases, enzymes that catalyze deadenylation of the target mRNA, that is, removal of the poly-A tail [10–13].

The specificity of microRNAs is determined chiefly by a sequence of nucleotides called the seed sequence, which contains ~8 base pairs. The small number of base pairs in this region allows this microRNA to regulate the expression of diverse genes by means of its binding to various mRNAs [14].

MicroRNAs play an important role in various biological processes, including proliferation and differentiation of adipocytes, showing an association with the metabolic disorders present in obese individuals (e.g., low-grade chronic inflammation and IR) [5,12]. Ortega et al. [7] verified alterations in the blood plasma concentrations of microRNAs in obese individuals and found them to be upregulated in those with morbid obesity.

Li et al. [15] evaluated the profile of genes and microRNAs expressed in the adipose tissue of obese individuals and compared this profile with that in nonobese people. The authors observed differences in the expression of 16 microRNAs and 192 genes, in addition to verifying the existence of 3002 possible pairwise interactions among them.

Wu et al. [16] analyzed the profile of microRNAs in obese patients with type 2 diabetes mellitus (T2DM); an altered expression was demonstrated in these patients. The researchers detected high levels of the mRNA of microRNAs miR-152 and -17 and reduced levels of the miR-138 involved in the manifestation of IR and inflammation in obese patients. Additionally, they observed reduced plasma concentrations of miR-593 in patients with diabetes. Thus, the authors proposed the use of microRNAs for prediction of obesity and T2DM.

Some research has evidenced the association between body weight loss and relevant microRNA expression in dietary and exercise interventions. Flowers et al. [17] conducted a systematic review of quasiexperimental, cross-sectional design studies, of which 12 reported an association between circulating microRNAs and exercise. However, only two of the studies reported changes on microRNAs associated with diet and weight loss, with downregulation of miR-16, -22, -140, -142, -27 b, -154, -409, -433, -224, -376b, and upregulation of miR-130b, -221, -183, -542, -223, -935, and -4772.

MicroRNAs and adipogenesis

Body fat deposits are composed of variable quantities of two types of adipose tissue. White adipose tissue (WAT) stores

energy in the form of triacylglycerol (TG) and is related to chronic inflammation and oxidative stress, whereas brown adipose tissue (BAT) dissipates the energy in the form of heat, by means of the uncoupling protein 1 (UCP1), which can be regarded as an alternative treatment of obesity [18–21].

It is important to point out that the expression of microRNAs in obese individuals is altered mainly in the adipose tissue, as it's also influenced by this type of tissue. Oger et al. [22] evaluated the expression of microRNAs in adipose tissue, liver, and musculoskeletal tissue of obese rats and confirmed downregulation of miR-200 a and -200 b and an increased level of miR-342-3p and 335-5p only in the epididymal WAT of these animals. Furthermore, it was demonstrated that these microRNAs are differentially expressed in the vascular stromal fraction of WAT.

As for participation of microRNAs in the regulatory mechanisms of adipogenesis, Bork et al. [23] confirmed that miR-371 increases adipogenic differentiation of mesenchymal stromal cells because it stimulates the expression of adiponectin and fatty acid-binding protein 4 (FABP4). Shi et al. [24] reported that rats fed a high-fat diet and obese individuals show increased expression of miR-148a, which stimulates adipogenic differentiation of mesenchymal stromal cells by inhibiting the Wnt1 gene.

MiR-378 a-3p also stimulates adipogenesis in obese rats, and an increased expression of this microRNA in the adipose tissue of these animals was confirmed. Scientific evidence shows that miR-378 a-3p inhibits the mitogen-activated protein kinase 1 (MAPK1), increasing the size and number of adipocytes [25]. Ishida et al. [26] confirmed that miR-378 reduces the expression of adiponectin in the adipose tissue of obese rats; they found as well that tumor necrosis factor (TNF)- α increases the expression of this microRNA.

MiR-30c promotes differentiation of adipocytes, because it increases the expression of CCAT/enhancer-binding protein α (C/EBP α) and/or peroxisome proliferator-activated receptor gamma (PPAR γ), which induce adipogenesis. This microRNA works by inhibiting the translation of two target molecules, plasminogen activator inhibitor 1 (PAI-1) and activin receptor-like kinase 2 (ALK2) [27].

During differentiation of adipocytes in obesity, there is an increase in the expression of miR-375, which stimulates this process because it increases mRNA levels of transcription factors C/EBP α and PPAR γ , activates fatty acid-binding protein aP2, and induces accumulation of TGs. The mechanism of action of this microRNA involves a reduction in the expression of extracellular signal-regulated kinases 1/2 (ERK1/2) [28].

MiR-146b also promotes adipogenesis: It works by downregulating sirtuin 1 (SIRT1), a molecule that plays an essential role in mobilization of fatty acids of adipose tissue via deacetylation of transcription factors, including forkhead box protein O1 (FOXO1) [29].

Chen et al. [30] detected increased expression of miR-146b in the visceral and subcutaneous adipose tissue of overweight and obese individuals, in addition to demonstrating that this microRNA suppresses the gene of Krüppel-like factor 7 (KLF7), favoring the secretion of insulin by β cells of the pancreas and expression of adipogenic transcription factors (e.g., C/EBP α and PPAR γ).

On the other hand, miR-135 a-5p inhibits the differentiation of preadipocytes and adipogenesis, and its expression is reduced during these processes. This microRNA works by inhibiting the adenomatous polyposis coli protein (APC), which favors the translocation of β -catenin to the nucleus and activation of cyclin D1 and C-myc, stimulating the Wnt/ β -catenin signaling pathway, which is known to negatively regulate adipogenesis [31].

Table 1
MicroRNA that regulate adipogenesis in obesity

MicroRNA	Expression in obesity	Molecular pathway	Biological system	References
miR-200 a/200 b	Downregulation	Adipocyte differentiation	Obese mouse	[22]
miR-342-3 p	Upregulation	Adipose tissue expansion	Obese mouse	[22]
miR-335-5 p	Upregulation	Pathophysiological functions of WAT Modulation of Bmp7 in preadipocytes	Obese mouse	[22]
miR-371	Upregulation	Increased expression of Adiponectin increased expression of FABP4	Mesenchymal stromal cells	[23]
miR-148 a	Upregulation	Adipocyte differentiation Inhibition of <i>Wnt1</i> expression	Mouse	[24]
miR-378 a-3 p	Upregulation	Inhibition of MAPK1	Obese mouse	[25]
miR-378	Upregulation by TNF- α	Reduced expression of adiponectin (WAT) Inhibition of Pde1B (BAT)	Obese mouse	[26,35]
miR-30 c	Upregulation	Increased expression of C/EBP α /PPAR γ Inhibition of PAI-1/ALK2	Human multipotent adipose-derived stem cells	[27]
miR-375	Upregulation	Increased expression of C/EBP α /PPAR γ Reduced expression of ERK1/2	3 T3-L1 preadipocytes	[28]
miR-146 b	Upregulation	Inhibition of <i>SIRT1</i> expression <i>FOXO1</i> deacetylation	Visceral and subcutaneous adipose tissue (obese and overweight individuals)	[29,30]
miR-135 a-5 p	Downregulation	Inhibition of adipocyte differentiation Inhibition of APC gene expression	3 T3-L1 adipocytes	[31]
miR-302 a	Downregulation	Inhibition of adipocyte differentiation Inhibition of PPAR γ expression	3 T3-L1 adipocytes	[32]
miR-27	Downregulation	Inhibition of adipocyte differentiation Inhibition of PPAR γ expression	3 T3-L1 preadipocytes	[33]
miR-130	Downregulation	Inhibition of adipocyte differentiation Inhibition of PPAR γ expression	Human primary preadipocytes and HeLa cells	[34]
miR-26 a/26 b	Upregulation	Adipocyte differentiation in BAT	Human multipotent adipose-derived stem cells	[36]

ALK2, activin receptor-like kinase-2; APC, adenomatous polyposis coli; BAT, brown adipose tissue; C/EBP α , CCAT/enhancer-binding protein α ; ERK1/2, extracellular signal-regulated kinases 1/2; FOXO1, forkhead box protein O1; KLF7, Kruppel-like factor 7; MAPK1, mitogen-activated protein kinase 1; PAI-1, plasminogen activator inhibitor-1; Pde1 B, phosphodiesterase enzyme; PPAR γ , peroxisome proliferator-activated receptor gamma; SIRT1, sirtuin 1; WAT, white adipose tissue

Another important microRNA in the regulation of adipogenesis is miR-302a. Jeong, Kang, and Koh [32] showed that miR-302a inhibits the differentiation of adipocytes by means of its binding to the 3'UTR region of PPAR γ mRNA, reducing the expression of this receptor. MiR-27a and miR-130 also inhibit the process of differentiation of adipocytes because these microRNAs reduce the PPAR γ expression. The reduced expression of these microRNAs seems to be related to the dysfunction of adipose tissue in obesity [33,34].

Regarding the microRNAs that act by modifying adipogenesis and the expression of proteins in BAT, Pan et al. [35] reported that miR-378 also stimulates adipogenesis and inhibits the phosphodiesterase enzyme Pde1b, and they verified increased expression of this microRNA in this tissue. Therefore, miR-378 increases thermogenesis and energy output, which may contribute to prevention or reduction of obesity.

Scientific evidence shows that miR-26a and miR-26b play essential roles in regulation of differentiation of adipocytes in BAT. These microRNAs induce signaling pathways involved in energy dissipation, activate UCP1, and alter the morphology of mitochondria, favoring formation of BAT; their actions were found to be mediated by inhibition of the ADAM17 gene [36]. Song et al. [37] confirmed that miR-26b stimulates proliferation and differentiation of adipocytes, suggesting that this microRNA may be a target for a therapeutic intervention in obesity.

As for the regulation of the lipid metabolism in obese individuals, it has been reported that miR-33a and miR-33b influence the homeostasis of blood plasma lipids in these patients. The molecular mechanism of action of these microRNAs is related to suppression of genes *SREBF1* and *ABCA1*. The *SREBF1* gene encodes sterol regulatory element-binding protein 1 (*SREBP1*), which activates fatty acid synthase enzymes (FASNs), stearoyl-CoA desaturase (SCD), and acetyl-CoA carboxylase 1 (ACC1). The *ABCA1* gene in turn mediates the efflux of cholesterol

to apolipoprotein A-I (apoA-I) and contributes to the formation of nascent high-density lipoprotein particles, which are essential for the biogenesis of high-density lipoprotein cholesterol and the reverse transport of cholesterol. Thus, miR-33a and miR-33b may be regarded as potential targets for prevention or treatment of obesity-related cardiovascular diseases [38]. Table 1 shows microRNAs that regulate adipogenesis in obesity.

Corroborating the evidence of microRNA participation in adipogenesis, animal experiments have demonstrated that inhibition of key enzymes involved in miRNA biogenesis, such as ribonuclease 3 and endoribonuclease Dicer, suppressed the differentiation of human mesenchymal stem cells into adipocytes, impaired lipogenesis, and downregulated expression of adipocyte markers such as PPAR γ , FAS, and FABP [39].

MicroRNAs and low-grade chronic inflammation

Diverse microRNAs favor the infiltration of macrophages into adipose tissue and polarization of these cells from the anti-inflammatory (M2) phenotype to the proinflammatory (M1) phenotype, contributing to the manifestation of low-grade chronic inflammation and IR in obese individuals. MicroRNA 124a induces the expression of chemokine ligand 2 (*CCL2*), promoting adhesion of monocytes to the vascular wall and formation of atheroma plaques. MicroRNAs 17, 20a, and 106a also stimulate the infiltration by macrophages by inhibiting the expression of the gene coding for signal-regulatory protein α (*SIRP- α*) [40–42].

As for the polarization of macrophages, the key phenomenon is the role of miR-223 in inducing the M2 phenotype of macrophages; inhibition of this microRNA favors activation and infiltration of M1 macrophages into adipose tissue, thus contributing to obesity-related chronic inflammation. Kilic et al. [43] corroborated a reduction in the expression of miR-223 and miR-143 in

obese individuals and those with morbid obesity compared with a control group.

MiR-125b also influences the polarization of macrophages because it reduces the expression of interferon regulatory factor 4 (*IRF4*), decreasing the number of antiinflammatory M2 macrophages [40,44,45]. This microRNA additionally works by stimulating the adipogenic differentiation and accumulation of TGs in adipose tissue by increasing the mRNA levels of PPAR γ , C/EBP α , fatty acid-binding protein 4 (FABP4), and lipase of lipoproteins [46].

It is worth mentioning the participation of miR-145 in the manifestation of low-degree chronic inflammation in obese individuals. Lorente-Cebrián et al. [47] demonstrated that this microRNA stimulates the release of glycerol and secretion of TNF- α by differentiated adipocytes. The increased production of this cytokine is mediated by the activation of subunit p65 of nuclear factor (NF)- κ B. MicroRNA 145 also reduces the expression of protease ADAM17, favoring an increase in the fraction of TNF- α bound to the membrane, its most active biological form. The activation of lipolysis by this microRNA results from the increase of phosphorylation of serine residues of the hormone-sensitive lipase enzyme and the reduction in the mRNA levels of phosphodiesterase 3 B, stimulating the release of glycerol and fatty acids by adipose tissue.

Obesity-related chronic inflammation may induce the expression of some microRNAs (e.g., miR-146a, -215, and -497). As a negative feedback mechanism, miR-497 inhibits kinase β inhibitor of NF- κ B (IKK β), reducing phosphorylation of inhibitor of NF- κ B (I κ B) and hampering the nuclear translocation and action of the transcription factor NF- κ B, which decreases the production of proinflammatory cytokines and, consequently, alleviates metabolic disorders in obese patients [48].

Scientific evidence has revealed that mature adipocytes of obese individuals show increased expression of miR-146b derived from the stimulatory action of proinflammatory cytokines TNF- α and interleukin (IL)-6. This microRNA in turn regulates the inflammatory process by influencing the signaling pathway of the transcription factor NF- κ B [49]. Zhu et al. [50] confirmed that the expression of miR-335 is also increased by TNF- α , IL-6, leptin, and resistin in mature adipocytes of patients with obesity.

Gu et al. [51] showed an elevation of miR-199 a-3 p expression level in mature human adipocytes (visceral) compared with preadipocytes, which was higher in visceral adipose deposits from obese individuals. Additionally, some adipocytokines such as TNF- α , IL-6, and leptin, as well as free fatty acids, significantly induced miR-199 a-3 p expression in mature human adipocytes, whereas resistin had the opposite effect. This result showed that miR-199 a-3 p may influence chronic low-grade inflammation in obesity.

Studies have shown reduced expression of microRNAs 221 and 222 in adipogenesis. Nevertheless, this expression is found to be increased in the adipose tissue of obese rats, and this process seems to be induced by TNF- α [52,53].

MicroRNAs 221 and 222 perform a relevant function in the manifestation of chronic inflammation in obesity because they inhibit proliferation of endothelial cells and angiogenesis, thus contributing to hypoxia in the tissues, which favors production of proinflammatory adipokines and stimulates the expression of miR-27 involved in the development of IR [5].

Meerson et al. [54] observed the existence of a positive correlation among the expression of the miR-221 in the adipose tissue, BMI, and serum concentrations of glucose and insulin. As for the mechanisms involved in the contribution of this

microRNA to the manifestation of IR in obese patients, Meerson et al. demonstrated that miR-221 induces the expression of fatty acid synthase, increasing the plasma concentration of fatty acids and decreasing the expression of receptor 1 of adiponectin and Ets-1 protein, favoring hypoxia and inflammation in adipose tissue.

In contrast, Chou et al. [55] reported that differentiated adipocytes of obese patients show reduced expression of miR-221, when compared with slim individuals. Additionally, these researchers demonstrated that this microRNA contributes to prevention of obesity-related low-grade chronic inflammation by inhibiting the mRNA expression of TNF- α .

The literature shows reduced expression of miR-132 in the visceral adipose tissue and in blood plasma of obese individuals compared with nonobese people [56,57]. In contrast, Sangiao-Alvarellos et al. [58] observed increased expression of miR-132 in the hypothalamus of rats fed a high-fat diet as compared with animals that received a normal-calorie diet, and this increase was reversed by the treatment with leptin.

In this regard, Strum et al. [59] demonstrated that miR-132 stimulates the production of IL-8 and monocyte chemoattractant protein 1 (MCP-1) in human preadipocytes and in vitro differentiated adipocytes via induction of the translocation and activation of NF- κ B. These effects are mediated by the binding of this microRNA to the 3'UTR of silent information regulator 1 (SirT1) mRNA, thus causing translational suppression of SirT1.

Mazloom et al. [60] demonstrated that miR-21 expression is decreased in peripheral blood mononuclear cells (PBMCs) of obese individuals and its reduced expression appears to be associated with increased secreted cytokine level. It is noteworthy that the downregulation of miR-21 expression in PBMCs of obese individuals leads to increased secretion of proinflammatory cytokines by a mechanism involving the upregulation of tumor suppressor programmed cell death protein 4, proinflammatory protein that is one of the transcriptional targets of NF- κ B. Table 2 shows microRNAs that regulate chronic low-grade inflammation in obesity.

MicroRNAs and insulin resistance

MicroRNAs also participate in the manifestation of IR. Ortega et al. [61] showed increased blood plasma concentrations of miR-222 in patients with T2DM, and these concentrations positively correlated with fasting glycemia and the proportion of glycated hemoglobin. The authors also confirmed a positive association between the microRNA concentration and morbid obesity.

Esguerra et al. [62] analyzed microRNA profiles of diabetic rats and confirmed an increase in miR-132 expression in the pancreatic islets of these animals compared with the control group. They also demonstrated that hyperglycemia may regulate the expression of this microRNA both in patients with diabetes and in healthy individuals. An important characteristic is the participation of miR-132 in regulation of cyclic AMP response element-binding protein (CREB), a key transcription factor in the glucose metabolism [63,64].

Regarding miR-320, it is important to point out that this microRNA participates in mechanisms behind the manifestation of insulin resistance in obesity. Ling et al. [65] proved that insulin-resistant adipocytes show increased expression of miR-320, observing as well that this microRNA inhibits the expression of subunit p85 of the phosphatidylinositol 3-kinase enzyme, inhibiting the signaling pathway of this hormone.

In this regard, Tang et al. [66] showed that miR-320 may regulate glycolysis because it inhibits the expression of

Table 2
MicroRNA that regulate chronic low-grade inflammation in obesity

MicroRNA	Expression in obesity	Molecular pathway	Biological system	References
miR-124 a	Downregulation	Increased CCL2 expression	Synoviocytes derived from surgical specimens	[41]
miR-17/20 a/106 a	–	Adhesion of monocytes to the vascular wall	Human myeloblastic cell lines HL-60 and U937	[42]
miR-223/143	Downregulation	Macrophage infiltration Inhibition of SIRP α expression Induced M2 macrophages	Monocyte/macrophage cell line THP-1 Morbidly obese/obese/overweight and control subjects	[43]
miR-125 b	Upregulation	Reduces expression of IRF4 Adipocyte differentiation C/EBP α /PPAR γ /FABP-4 and LPL expression increased	293 T cells, RAW264.7 cells, and BMMs 3 T3-L1 preadipocytes	[44,46]
miR-145	–	Stimulates release of glycerol and secretion of TNF- α Activation of NF- κ B	Subcutaneous WAT obtained from healthy men and women	[47]
miR-497	Upregulation	Reduces expression of ADAM17 Inhibition of NF- κ B Decreased production of proinflammatory cytokines	3 B4.1 and C6 TA4 cells	[48]
miR-146 b	Upregulation	Regulated inflammatory process Influenced signaling pathway of NF- κ B	Human visceral preadipocytes	[49]
miR-146 a/146 b/215/335/497	Upregulation	Inflammation enhances expression of these miR	3 B4.1 and C6 TA4 cells Human visceral preadipocytes Visceral adipocytes	[48–50]
miR-221	Upregulation/ Downregulation	Inhibited proliferation of endothelial cells and angiogenesis Hypoxia Inhibited the mRNA of TNF- α	3 T3-L1 adipocytes Adipose tissues from obese and lean women	[53,55]
miR-222	Upregulation	Inhibited proliferation of endothelial cells and angiogenesis Hypoxia	C57 BL6 J wild-type male mice	[52]
miR-132	Downregulation/ Upregulation	Stimulated production of IL-8 and MCP-1 Activation of NF- κ B Translation suppression of SirT1	Paired omental and subcutaneous adipose tissue samples from obese patients Primary human adipose-derived stem cells Male Wistar rats	[57–59]

CCL2, chemokine ligand 2; C/EBP α , CCAT/enhancer-binding protein α ; FABP-4, fatty acid binding protein 4; IL, interleukin; IRF4, interferon regulatory factor 4; LPL, lipase of lipoproteins; MCP-1, monocyte chemoattractant protein 1; NF, nuclear factor; PPAR γ , peroxisome proliferator-activated receptor gamma; SIRP α , signal-regulatory protein α ; TNF, tumor necrosis factor

phosphofructokinase, a rate-limiting enzyme of the glycolytic pathway. Those authors also confirmed that during oxidative stress, protein Ets-1 inhibits the expression of miR-320, resulting in an increase of this enzyme's activity.

On the other hand, Feng and Chakrabarti [67] reported that hyperglycemia inhibits the expression of miR-320 in endothelial cells and in the renal cortex of diabetic rats. This microRNA in turn inhibits the upregulation of vasoactive factors endothelin 1 and glucose-induced vascular endothelial growth factor in these cells, possibly via modulation of the activation of MAPKs.

MicroRNAs 29a, 29b, and/or 29c also play a relevant role in the insulin signaling pathway and in the glucose metabolism. He et al. [68] confirmed that this microRNA inhibits accumulation of insulin-stimulated glucose by inhibiting phosphorylation of insulin 1 receptor's substrate and the kinase B protein or Akt, and these effects may be mediated by inhibition of insulin-induced gene 1 (*INSIG-1*) and caveolin 2. Those authors also found an increase in the miR-29 expression in the liver and adipose and musculoskeletal tissues of diabetic rats and demonstrated that hyperglycemia and hyperinsulinemia are determinants of the induction of the paralogs miR-29a and miR-29b.

Liang et al. [69] assessed the role of miR-29 in the regulation of hepatic production of glucose and the glycemic levels in diabetic rats and found that the increased hepatic expression of this microRNA improves hyperglycemia because it inhibits the glucose production in hepatocytes. These effects were found to be mediated by the suppression of glucose-6-phosphatase genes and peroxisome proliferator-activated receptor-gamma coactivator-1 α (*PGC-1 α*).

Recent research shows that microRNAs 9, 451 and 696 also play an important role in hepatic gluconeogenesis and glucose homeostasis. Yan et al. [70] demonstrated that hepatic miR-9 expression is decreased in obese mice. The authors found that DNA hypermethylation contributes to downregulation of miR-9 expression in the obese mouse liver, leading to upregulation of *FOXO1* expression and gluconeogenesis.

Zhuo et al. [71] showed that miR-451 is elevated in the liver tissues of dietary and genetic mouse models of diabetes. The authors found that overexpression of this microRNA inhibits hepatic gluconeogenesis, alleviates hyperglycemia, and improves glucose tolerance, and they identified glycerol kinase enzyme as a direct target of miR-451. Thus, this study suggests that miR-451 is a potential therapeutic target against hyperglycemia in diabetes.

Fang et al. [72] found an inverse correlation between miR-696 and *PGC-1 α* protein levels in vivo and in vitro, and they verified that miR-696 directly recognizes a specific location within the 3'-UTR of *PGC-1 α* transcripts, inhibiting of *PGC-1 α* translation in the liver. In this way, the overexpression of miR-696 led to the downregulation of phosphoenolpyruvate carboxykinase (PEPCK), rate-limiting enzyme of gluconeogenesis activated by *PGC-1 α* . These results suggest that miR-696 plays a significant role in hepatic gluconeogenesis and IR.

MiR-20 a-5 p, a member of miR-17 family, regulates glycogenesis in hepatocytes; however, it is downregulated in the liver of diabetic mice. p63 protein was identified as target of miR-20 a-5 p. This protein might directly bind to p53, thereby regulating phosphatase and tensin homolog (PTEN)

Table 3
MicroRNA that participate on insulin resistance in obesity

MicroRNA	Expression in obesity	Molecular pathway	Biological system	References
miR-222	Upregulation	Increased fasting glycemia and glycated hemoglobin	Type 2 Diabetes patients	[61]
miR-132	Upregulation	Regulated CREB expression	Diabetic mouse	[62]
miR-320	Upregulation	Inhibits expression of PI3K and PFK	3 T3-L1 preadipocytes	[65]
miR-29 a/29 b/29 c	Upregulation	Inhibits collection of insulin-stimulated glucose Reduction of phosphorylation of IRS1 and Akt Inhibition of INSIG-1 and caveolin 2	3 T3-L1 adipocytes	[68]
miR-125 a-3 p	Upregulation	Positively associated with JNK Negatively associated with IR and PI3 K	Adipose tissue of obese individuals	[69]
miR-144	Upregulation	Inhibition of IRS1 expression	Type 2 diabetes patients	[79]
miR-103/107	Upregulation	Inhibition of caveolin 1	Obese mouse	[80]

Akt, kinase B protein; CREB, cyclic AMP response element-binding protein; INSIG, insulin-induced gene; IR, insulin receptor; IRS1, insulin 1 receptor's substrate; JNK, C-Jun N-terminal kinase; PFK, phosphofructokinase; PI3K, phosphoinositide 3-kinase

expression and in turn participating in glycogenesis. Thus, the authors suggest that miR-20 a-5 p contributes to hepatic glycogen synthesis through targeting p63 to regulate p53 and PTEN expression [73].

MiR-152 also regulates hepatic glycogenesis by targeting PTEN. However, the expression of this microRNA is downregulated in the liver of diabetic mice and mice fed a high-fat diet. In vivo and in vitro results suggested that inhibition of miR-152 expression induces impaired glycogenesis in hepatocytes [74].

MiR-194 is important to regulate glucose metabolism. However, Latouche et al. [75] verified that expression of this microRNA is reduced in rat model and in human with prediabetes and established diabetes. Inhibition of miR-194 in skeletal muscle cells induced an increase in glucose uptake, glycogen synthesis, and glycolysis. These researchers suggest that miR-194 could be downregulated in patients with early features of diabetes as an adaptive response to facilitate tissue glucose uptake and metabolism in a situation of IR.

Zhou et al. [76] found that miR-106b, miR-27a, and miR-30d play crucial roles in the regulation of glucose metabolism. It was demonstrated that overexpression of these microRNAs in insulin-resistant cells decreased glucose consumption and glucose uptake, and reduced the expression of glucose transporter 4 (GLUT4), mitogen-activated protein kinase 14 (MAPK 14) and phosphatidylinositol 3-kinase (PI3 K) regulatory subunit β .

As for the participation of miR-125 a-3 p in the pathophysiology of obesity, Yeh et al. [77] found increased expression of this microRNA in the adipose tissue of obese individuals, being positively associated with the expression of proinflammatory enzyme c-Jun N-terminal kinase (JNK) and negatively with insulin receptor and phosphatidylinositol 3-kinase, confirming the importance of miR-125 a-3 p in the regulation of the insulin signaling pathway.

MiR-181 b plays an essential role in regulating endothelial function in WAT. Sun et al. [78] found reduced expression of this microRNA in adipose tissue endothelial cells of obese mice, and rescue of miR-181 b expression improved glucose homeostasis and insulin sensitivity. The authors demonstrated that systemic intravenous delivery of miR-181 b accumulated in adipose tissue endothelial cells, enhanced insulin-mediated Akt phosphorylation at Ser473, and reduced endothelial dysfunction, an effect that shifted macrophage polarization toward an M2 anti-inflammatory phenotype in epididymal WAT. It is important to highlight that PHLP2, a phosphatase that dephosphorylates Akt at Ser473, is a novel target of miR-181b.

Researchers have shown that diverse microRNAs play an important role in the pathogenesis of T2DM [79]. Karolina et al. [79] detected altered expression of various microRNAs and demonstrated an increase in the expression of miR-144, which inhibits the insulin signaling pathway through translational inhibition of insulin receptor substrate 1 (IRS-1) in these patients.

A study by Trajkovski et al. [80] demonstrated that obese rats show increased expression of miR-103 and miR-107, which downregulate caveolin 1, an essential regulator of insulin receptor. Therefore, these microRNAs suppress the insulin signaling pathway and the glucose metabolism, predisposing these animals to development of T2DM. Table 3 shows microRNAs that participate in IR in obesity.

Conclusions

Several lines of scientific evidence show that microRNAs are involved in molecular mechanisms underlying the manifestation of obesity-related metabolic disorders (e.g., alterations in adipogenesis, low-grade chronic inflammation, and IR). Therefore, they can be regarded as possible biomarkers of obesity-related disorders. Thus, further studies on this topic may advance the understanding of the molecular basis of obesity, including the involvement of microRNAs in the pathogenesis of this disease.

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Considerações Finais

6 CONSIDERAÇÕES FINAIS

O Capítulo 1 traz dados de um estudo original que evidenciam que as mulheres obesas metabolicamente saudáveis apresentam perfil metabólico favorável, enquanto que as não saudáveis possuem descontrole metabólico, caracterizado pela presença de distúrbios, a exemplo da resistência à insulina, estresse oxidativo e alterações no perfil lipídico.

O estudo também mostra que as mulheres obesas apresentam concentrações plasmáticas e eritrocitárias reduzidas dos minerais e valores urinários elevados, independente do fenótipo saudável ou não saudável. Além disso, indica que a condição de deficiência nutricional relativa aos minerais parece comprometer suas funções na proteção contra as desordens metabólicas avaliadas.

A partir do Capítulo 2 desta tese, pode-se concluir que as mulheres obesas ingerem baixa quantidade de magnésio e cálcio, apresentam concentrações plasmáticas dos minerais e níveis eritrocitários de magnésio reduzidas, bem como excreção urinária elevada desses micronutrientes. As razões Ca/Mg na dieta, plasma e urina se encontram elevadas nas mulheres obesas.

Os resultados sugerem que o cálcio parece influenciar a homeostase do magnésio, sendo que o aumento na ingestão dietética de cálcio está associado à redução no magnésio eritrocitário. No entanto, a razão Ca/Mg parece não ser relevante para o controle glicêmico evidenciado nas mulheres obesas, considerando a inexistência de correlação entre estas variáveis. Portanto, mais estudos são necessários para esclarecer a importância da razão Ca/Mg no controle glicêmico na obesidade.

A revisão sistemática e metanálise apresentada no Capítulo 3 desta tese mostra a presença de hipomagnesemia em indivíduos obesos de ambos os sexos. A revisão sistemática descrita no Capítulo 4, por sua vez, demonstra a existência de alterações no estado nutricional relativo ao zinco em pacientes obesos, o que pode ser considerado fator de risco para o desenvolvimento de desordens metabólicas associadas a esta doença. Por outro lado, a suplementação com zinco parece contribuir para melhora da resistência à insulina em indivíduos obesos de ambos os sexos.

Por meio da revisão narrativa constante no Capítulo 5, pode-se concluir que o zinco e suas proteínas transportadoras estão envolvidos na síntese e secreção da insulina, bem como nas vias moleculares de sinalização da ação deste hormônio. O zinco regula ainda a transcrição do gene codificante para o receptor de insulina por meio das proteínas dedos de zinco.

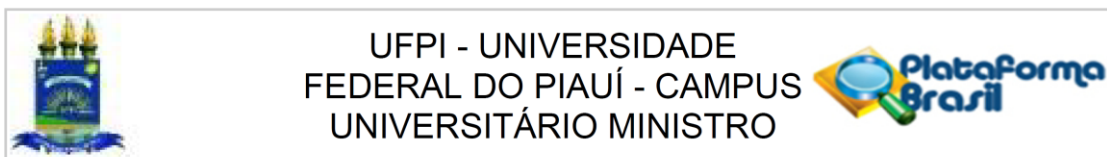
O Capítulo 6 mostra que os microRNAs participam de mecanismos moleculares envolvidos na manifestação de desordens metabólicas na obesidade, podendo, portanto, serem utilizados como possíveis biomarcadores destes distúrbios. No entanto, novos estudos sobre o tema são necessários para avançar no entendimento da base molecular da obesidade, incluindo o envolvimento dos microRNAs na patogênese desta doença.

Anexos

*Associação entre as concentrações de minerais (magnésio, zinco e selênio) e marcadores metabólicos dos
fenótipos obesidade metabolicamente saudável e obesidade metabolicamente não saudável*

Kyria Jayanne Clímaco Cruz

ANEXO A – PARECER CONSUBSTANCIADO DO CEP-UFPI



PARECER CONSUBSTANCIADO DO CEP

DADOS DO PROJETO DE PESQUISA

Título da Pesquisa: Impacto de minerais em distúrbios endócrino-metabólicos

Pesquisador: Dilina do Nascimento Marreiro

Área Temática:

Versão: 1

CAAE: 66921117.7.0000.5214

Instituição Proponente: Universidade Federal do Piauí - UFPI

Patrocinador Principal: Financiamento Próprio

DADOS DO PARECER

Número do Parecer: 2.014.100

Apresentação do Projeto:

Estudo de natureza transversal, analítico, com caso e controle, envolvendo 300 mulheres, na faixa etária entre 20 e 50 anos de idade. As participantes do estudo serão distribuídas em três grupos: grupo experimental I (obesas bariátricas, n=100), grupo experimental II (obesas não bariátricas, n=100) e controle (eutróficas, n=100). Serão realizadas avaliação antropométrica, coleta de material biológico e registro alimentar. A avaliação antropométrica será realizada por meio do índice de massa corpórea, da circunferência da cintura e do pescoço, e da relação cintura-quadril. A análise da ingestão de macronutrientes, zinco, magnésio e selênio será realizada por meio do registro alimentar de três dias, utilizando o programa Nutwin. A análise dos minerais no plasma, eritrócito e urina será realizada por espectrometria de emissão óptica com plasma acoplado indutivamente. As concentrações séricas de glicose e insulina serão determinadas por meio do método enzimático colorimétrico e quimioluminescência, respectivamente. A resistência à insulina será avaliada por meio do cálculo do índice HOMA-IR. A avaliação das concentrações de cortisol sérico e urinário será realizada segundo o método de quimioluminescência. As concentrações séricas de colesterol total, LDL-colesterol, HDL-colesterol e triglicérides serão determinadas segundo método enzimático colorimétrico, por analisador bioquímico automático. A determinação da concentração sérica de zinco-2-glicoproteína será realizada de acordo com o método ELISA do tipo sanduíche. Serão analisados os hormônios TSH, T3 e T4 livres, e T3 e T4 totais pelo método

Endereço: Campus Universitário Ministro Petronio Portella - Pró-Reitoria de Pesquisa

Bairro: Ininga

CEP: 64.049-550

UF: PI

Município: TERESINA

Telefone: (86)3237-2332

Fax: (86)3237-2332

E-mail: cep.ufpi@ufpi.edu.br



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Continuação do Parecer: 2.014.100

de imunoensaio por quimioluminescência. Os anticorpos tireoideanos antitireoperoxidase (TPOAb) e antitireoglobulina (TgAb) serão determinados pelo método de quimioluminescência. As concentrações plasmáticas do malondialdeído serão determinadas pela produção das substâncias reativas ao ácido tiobarbitúrico (TBARS). A atividade da enzima superóxido dismutase eritrocitária e glutatona peroxidase eritrocitária serão determinadas nos eritrócitos pelo método in vitro e da oxidação da glutatona reduzida por um hidroperóxido, respectivamente. A determinação da atividade da enzima catalase será realizada conforme metodologia descrita por Takahara et al. A análise de isoprostanos urinários será determinada por ELISA. A determinação da concentração plasmática de TNF-, IL-6, IL-1, IL-18, IL-10, TGF-, adiponectina e leptina será realizada de acordo com o método Luminex xMAP. A concentração sérica da proteína C reativa será realizada por imunoturbidimetria. A creatinina sérica e a creatininúria de 24h serão determinadas pelo método Lustosa-Basques. Serão coletadas amostras de tecido adiposo visceral e subcutâneo das pacientes obesas durante o intraoperatório de cirurgia bariátrica para investigar o padrão de expressão dos marcadores Akt, IRS, GLUT4, PI3K, AMPK, leptina, adiponectina, zinco-2-glicoproteína, Selenoproteína P, TRPM7, ATGL, LHS, CD36, FAS, UCP, PPAR-, SREBP-1c, Receptores B3-adrenérgicos, NFkB, metalotioneína, Zip 14, TNF-, IL-6, IL-1, IL-18, IL-10, TGF-, deiodinases, receptores de hormônios tireoidianos e de glicocorticoides, 11-HSD1, superóxido dismutase, glutatona peroxidase e catalase.

Objetivo da Pesquisa:

Objetivo Primário:

- Avaliar o impacto de minerais em distúrbios endócrino-metabólicos em mulheres obesas.

Objetivo Secundário:

1. Determinar as concentrações de zinco, magnésio e selênio plasmático, eritrocitário e urinário;
2. Estimar o consumo alimentar de energia, macro e micronutrientes e sua adequação na dieta;
3. Determinar as concentrações de cortisol, cortisona, ACTH, zinco-2-glicoproteína, adiponectina, leptina, proteína C reativa, hormônios e anticorpos tireoidianos, TNF-, IL-6, IL-1, IL-18, IL-10 e TGF-, substâncias reativas ao ácido tiobarbitúrico e isoprostanos;
4. Avaliar o perfil lipídico, o controle glicêmico, a resistência à insulina e a função renal;
5. Determinar a atividade da enzima superóxido dismutase, glutatona peroxidase e catalase;
6. Quantificar a expressão de genes codificantes para Akt, IRS, GLUT4, PI3K, AMPK, leptina,

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Bairro: Ininga **CEP:** 64.049-550
UF: PI **Município:** TERESINA
Telefone: (86)3237-2332 **Fax:** (86)3237-2332 **E-mail:** cep.ufpi@ufpi.edu.br

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Associação entre as concentrações de minerais (magnésio, zinco e selênio) e marcadores metabólicos dos fenótipos obesidade metabolicamente saudável e obesidade metabolicamente não saudável

Kyria Jayanne Clímaco Cruz



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adiponectina, zinco-2-glicoproteína, Selenoproteína P, TRPM7, ATGL, LHS, CD36, FAS, UCP, PPAR-, SREBP-1c, Receptores B3-adrenérgicos, NFkB, metalotioneína, Zip 14, TNF-, IL-6, IL-1, IL-18, IL-10, TGF-, deiodinases, receptores de hormônios tireoidianos e de glicorticoides, 11-HSD1, superóxido dismutase, glutationaperoxidase e catalase.

Avaliação dos Riscos e Benefícios:

Risco: a participante poderá sentir leve desconforto, ocorrência de eventuais hematomas, dor e sangramento e, em casos mais raros, infecção. A fim de minimizar esses riscos, a coleta será realizada por profissional de enfermagem experiente, utilizando materiais estéreis e descartáveis, e a equipe estará preparada para auxiliar a participante diante de qualquer intercorrência.

Benefícios:

As pacientes terão acesso aos resultados dos seus exames bioquímicos, que serão fornecidos após a realização dos mesmos pelos pesquisadores, além de orientação nutricional.

Comentários e Considerações sobre a Pesquisa:

Pesquisa relevante para o processo ensino/aprendizado.

Considerações sobre os Termos de apresentação obrigatória:

Todos os termos de apresentação foram anexados.

Recomendações:

Sem Recomendações.

Conclusões ou Pendências e Lista de Inadequações:

Projeto apto a ser iniciado pois encontra-se elaborado em consonância com a Resolução Nº 466/2012.

Considerações Finais a critério do CEP:

Este parecer foi elaborado baseado nos documentos abaixo relacionados:

Tipo Documento	Arquivo	Postagem	Autor	Situação
Informações Básicas do Projeto	PB_INFORMAÇÕES_BÁSICAS_DO_PROJETO_885107.pdf	10/04/2017 16:26:00		Aceito

Endereço: Campus Universitário Ministro Petronio Portella - Pró-Reitoria de Pesquisa
Bairro: Ininga **CEP:** 64.049-550
UF: PI **Município:** TERESINA
Telefone: (86)3237-2332 **Fax:** (86)3237-2332 **E-mail:** cep.ufpi@ufpi.edu.br



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Continuação do Parecer: 2.014.100

Outros	cartadeencaminhamento.pdf	10/04/2017 16:24:27	Dilina do Nascimento Marreiro	Aceito
Outros	curriculo.pdf	10/04/2017 16:21:58	Dilina do Nascimento Marreiro	Aceito
Declaração de Pesquisadores	declaracaodospesquisadores.pdf	10/04/2017 15:59:50	Dilina do Nascimento Marreiro	Aceito
TCLE / Termos de Assentimento / Justificativa de Ausência	TCLEmorbidas.pdf	10/04/2017 15:57:18	Dilina do Nascimento Marreiro	Aceito
TCLE / Termos de Assentimento / Justificativa de Ausência	TCLE.pdf	10/04/2017 15:57:03	Dilina do Nascimento Marreiro	Aceito
Projeto Detalhado / Brochura Investigador	projeto comite deetica.docx	21/03/2017 14:52:18	Dilina do Nascimento Marreiro	Aceito
Orçamento	ORCAMENTO.docx	21/03/2017 14:50:30	Dilina do Nascimento Marreiro	Aceito
Cronograma	CRONOGRAMA.docx	21/03/2017 14:49:18	Dilina do Nascimento Marreiro	Aceito
Outros	Urina.docx	21/03/2017 14:48:58	Dilina do Nascimento Marreiro	Aceito
Outros	Registro alimentar.docx	21/03/2017 14:47:58	Dilina do Nascimento Marreiro	Aceito
Outros	Ficha de cadastro.docx	21/03/2017 14:45:56	Dilina do Nascimento Marreiro	Aceito
Outros	termo de confidencialidade.jpeg	21/03/2017 14:43:39	Dilina do Nascimento Marreiro	Aceito
Folha de Rosto	folhaderosto.pdf	21/03/2017 14:39:44	Dilina do Nascimento Marreiro	Aceito

Situação do Parecer:

Aprovado

Necessita Apreciação da CONEP:

Não

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Continuação do Parecer: 2.014.100

TERESINA, 12 de Abril de 2017

Assinado por:
Herbert de Sousa Barbosa
(Coordenador)

Endereço: Campus Universitário Ministro Petronio Portella - Pró-Reitoria de Pesquisa
Bairro: Ininga **CEP:** 64.049-550
UF: PI **Município:** TERESINA
Telefone: (86)3237-2332 **Fax:** (86)3237-2332 **E-mail:** cep.ufpi@ufpi.edu.br

ANEXO B – ARTIGO RESULTANTE DA DISSERTAÇÃO DE MESTRADO

Biol Trace Elem Res (2014) 160:305–310
DOI 10.1007/s12011-014-0044-2

Influence of Magnesium on Insulin Resistance in Obese Women

Kyria Jayanne Clímaco Cruz · Ana Raquel Soares de Oliveira · Denise Pereira Pinto ·
Jennifer Beatriz Silva Morais · Fabiana da Silva Lima · Célia Colli ·
Francisco Leonardo Torres-Leal · Dilina do Nascimento Marreiro

Received: 19 May 2014 / Accepted: 9 June 2014 / Published online: 2 July 2014
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Abstract The present study evaluated the influence of magnesium on insulin resistance in obese women. A case-control study involving 114 women on the age between 20 and 50 years old, divided into two groups: control (eutrophic women, $n=59$) and case (obese women, $n=55$). The analysis of magnesium intake was carried out through the 3-day food record and also NutWin software version 1.5. The plasma, erythrocyte, and urinary magnesium concentrations were determined by flame atomic absorption spectrophotometry. The determinations of serum glucose and serum insulin were performed by enzymatic colorimetric method and chemiluminescence, respectively. The insulin resistance was assessed by homeostasis model assessment insulin resistance (HOMA-IR). The mean values of magnesium intake were lower than those recommended, without difference between groups ($p>0.05$). All the patients who were evaluated showed adequate mean concentrations of magnesium in the plasma and erythrocyte. The urinary excretion of this mineral was lower than the reference values in both groups and did not show significant difference ($p>0.05$). The values of serum glucose, serum insulin, and HOMA-IR were higher in obese women compared to the control group. A negative correlation was

observed between erythrocyte magnesium and glycemic parameters ($p<0.05$). Obese patients take in foods with low dietary magnesium content, and they show hypomagnesuria as a compensatory mechanism to keep the plasma concentration of this mineral in adequate levels. The correlation between the erythrocyte magnesium concentration and the parameters of glycemic control suggests the influence of this mineral on the index of insulin resistance in obese women.

Keywords Obesity · Magnesium · Insulin resistance · Nutritional status

Introduction

In recent years, with the discovery that the white adipose tissue has a great ability to secrete adipokines, its endocrine role has been very prominent in the pathogenesis of obesity. This way, the deregulated production of adipokines seems to establish a chronic low-grade inflammatory state, which contributes to the manifestation of insulin resistance and cardiovascular complications [1, 2].

Insulin resistance is mainly characterized by the changes in the glucose metabolism resulting from the inhibition of the signaling pathway of this hormone action. So, several factors are related to the development of this metabolic disturbance in obese people, being that the excess of corporal fat is the main cause, particularly the accumulation of intra-abdominal fat [2, 3].

Hormonal and biochemical disorders found in obesity have been the target of researches that aim to clarify the mechanisms involved in the pathogenesis of this illness. However, recently, researchers have also shown great interest about the influence of minerals in the appearance of these disorders. Magnesium, in a particular way, is important because it plays a primordial function in the energy metabolism [4, 5].

K. J. C. Cruz · A. R. S. de Oliveira · D. P. Pinto · J. B. S. Morais ·
D. do Nascimento Marreiro (✉)
Department of Nutrition, Federal University of Piauí, Campus
Minister Petrônio Portela, Ininga, Teresina, Piauí, Brazil
e-mail: dilina.marreiro@gmail.com

F. da Silva Lima · C. Colli
Department of Food and Experimental Nutrition, Faculty of
Pharmaceutical Sciences, University of São Paulo, Butantã, São
Paulo, São Paulo, Brazil

F. L. Torres-Leal
Department of Physiology and Biophysics, Federal University of
Piauí, Campus Minister Petrônio Portela, Ininga, Teresina, Piauí,
Brazil

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*Associação entre as concentrações de minerais (magnésio, zinco e selênio) e marcadores metabólicos dos
fenótipos obesidade metabolicamente saudável e obesidade metabolicamente não saudável*

Kyria Jayanne Clímaco Cruz

Apêndices

*Associação entre as concentrações de minerais (magnésio, zinco e selênio) e marcadores metabólicos dos
fenótipos obesidade metabolicamente saudável e obesidade metabolicamente não saudável*

Kyria Jayanne Clímaco Cruz

APÊNDICE A - FICHA DE CADASTRO DAS PARTICIPANTES DA PESQUISA



MINISTÉRIO DA EDUCAÇÃO E CULTURA – MEC
UNIVERSIDADE FEDERAL DO PIAUÍ – UFPI
PRÓ-REITORIA DE PESQUISA E PÓS-GRADUAÇÃO

Campus Universitário Ministro Petrônio Portela, Bloco 06 – Bairro Ininga
Cep: 64049-550 – Teresina-PI – Brasil – Fone (86) 3215-5863 – Fone/Fax (86)3215-5560

IDENTIFICAÇÃO

Nº Formulário: _____

Nome: _____

Data: ____/____/____ DN: ____/____/____ Idade: _____ anos

Endereço: _____

Bairro: _____ Cidade: _____

Telefone: _____ Celular 1: _____ Celular 2: _____

Ocupação: _____ Número de pessoas em casa: _____

HISTÓRIA CLÍNICA

a) Tempo de obesidade: _____

b) Fumante? _____ Grávida ou Amamentando? _____

c) Menopausa: Sim () Não () Se sim, há ____ anos.

d) Uso de medicamentos: Sim () Não () Quais? _____

e) Consumo de Bebidas alcoólicas: Sim () Não () Frequência? _____

f) Uso de Suplementos: Sim () Não () Quais? _____

g) Presença de doenças: Sim () Não () Qual? _____

() Diabetes () DRC () Doença Hepática () DCV () Câncer () Disf. Tireoide

f) Pratica exercício físico? Sim () Não () Tipo de exercício físico: _____

PARÂMETROS ANTROPOMÉTRICOS E BIOQUÍMICOS

Peso (kg)	
Estatura (m)	
IMC (kg/m²)	
Circunferência da Cintura (cm)	
Circunferência do Quadril (cm)	
Circunferência do Pescoço (cm)	
Volume Urinário (mL)	

APÊNDICE B - TERMO DE CONSENTIMENTO LIVRE E ESCLARECIDO



MINISTÉRIO DA EDUCAÇÃO E CULTURA
UNIVERSIDADE FEDERAL DO PIAUÍ
PRÓ-REITORIA DE PESQUISA E PÓS-GRADUAÇÃO
Campus Universitário Ministro Petrônio Portella - Bairro Ininga – Teresina/PI
CEP: 64049-550 - Fone (86) 3215 5437.

TERMO DE CONSENTIMENTO LIVRE E ESCLARECIDO

Título do projeto: *Distúrbios Endócrino-Metabólicos em Mulheres Obesas Mórbidas*

Pesquisador responsável: Dra. Dilina do Nascimento Marreiro

Instituição/Departamento: UFPI/ Departamento de Nutrição

Pesquisadores participantes: Dra. Dilina do Nascimento Marreiro, Kyria Jayanne Clímaco Cruz, Ana Raquel Soares de Oliveira, Jennifer Beatriz Silva, Larissa Cristina Fontenelle, Loanne Rocha dos Santos, Stéfany Rodrigues de Sousa Melo.

Telefone para contato (inclusive a cobrar): (86) 99911-7297 (tim)/ (86) 99430-2397

Você está sendo convidada para participar, como voluntária, em uma pesquisa. Você precisa decidir se quer participar ou não. Por favor, não se apresse em tomar a decisão. Leia cuidadosamente o que se segue e pergunte ao responsável pelo estudo sobre qualquer dúvida que tiver. Este estudo será conduzido pelas Doutorandas Kyria Jayanne Clímaco Cruz, Ana Raquel Soares de Oliveira, Jennifer Beatriz Silva Moraes, Larissa Cristina Fontenelle, Loanne Rocha dos Santos, Stéfany Rodrigues de Sousa Melo, sob orientação da Profa. Dra. Dilina do Nascimento Marreiro. Após ser esclarecida sobre as informações a seguir, no caso de aceitar fazer parte do estudo, assine este documento, que está em duas vias. Uma delas é sua e a outra é do pesquisador responsável. Em caso de recusa, você não será penalizada. Em caso de dúvida, você pode procurar o Comitê de Ética em Pesquisa da Universidade Federal do Piauí pelo telefone (086) 3237-2332.

Associação entre as concentrações de minerais (magnésio, zinco e selênio) e marcadores metabólicos dos fenótipos obesidade metabolicamente saudável e obesidade metabolicamente não saudável

Kyria Jayanne Clímaco Cruz

DESCRIÇÃO DA PESQUISA

Esta pesquisa tem por objetivo avaliar o estado nutricional relativo ao magnésio, selênio e zinco e sua relação com distúrbios endócrino-metabólicos em mulheres obesas mórbidas. Para tanto, a participante será submetida à coleta de sangue venoso para análise do zinco, magnésio, selênio, colesterol total, HDL-colesterol, triglicerídeos, TSH, T3 e T4 livres, T3 e T4 totais, anticorpos tireoideanos antitireoperoxidase e antitireoglobulina, zinco- α 2-glicoproteína, leptina, glicose, insulina, cortisol, cortisona, selenoproteína P, adiponectina, proteína C reativa, TNF- α , IL-6, IL-1 β , IL-1Ra, IL-18, IL-10 e TGF- β , substâncias reativas ao ácido tiobarbitúrico, isoprostanos, creatinina sérica, malondialdeído, bem como à coleta de urina para determinação de zinco, magnésio e selênio urinário, cortisol urinário e creatininúria de 24h e, além disso, será determinado o consumo alimentar por meio de registros alimentares. Não será realizada entrevista gravada ou filmada.

Será também quantificado a expressão dos genes codificantes para: Akt, IRS, GLUT4, PI3K, AMPK, leptina, receptor de leptina, adiponectina, zinco- α 2-glicoproteína, selenoproteína P, selenoproteína S, SELENBP1, TRPM7, ATGL, LHS, CD36, FAS, UCP, PPAR- γ , CEBP α , SREBP-1c, receptores B3-adrenérgicos, NF-kB, MAPK8, metalotioneína, Zip 14, TNF- α , IL-6, IL-1 β , IL-1Ra, IL-18, IL-10, TGF- β , deiodinases, receptores de hormônios tireoidianos e de glicorticoides, 11 β -HSD1, 11 β -HSD2, NADPH oxidase, Nrf2, superóxido dismutase, glutational peroxidase e catalase; quantificar a concentração de zinco, magnésio e selênio no tecido adiposo, bem como quantificar a expressão de microRNAs envolvidos nos distúrbios metabólicos na obesidade.

Ao participar da pesquisa, a participante não sofrerá nenhum prejuízo, no entanto, as perguntas presentes na ficha de cadastro e no registro alimentar, poderá trazer possível constrangimento da participante. Além disso, em vista da coleta do material biológico requerido para realização desta pesquisa, a participante poderá sentir leve desconforto, ocorrência de eventuais hematomas, dor e sangramento e, em casos mais raros, infecção. A fim de minimizar esses riscos, a coleta será realizada por profissional de enfermagem experiente, utilizando materiais estéreis e descartáveis, e a equipe estará preparada para auxiliar a participante diante de qualquer intercorrência.

As participantes do estudo terão como benefícios os resultados dos exames bioquímicos das amostras de sangue e de urina, que serão fornecidos após a realização dos mesmos, e orientação nutricional. Em qualquer etapa do estudo, você terá acesso aos profissionais responsáveis pela pesquisa para esclarecimento de eventuais dúvidas. Se você tiver alguma consideração ou dúvida sobre a ética da pesquisa, entre em contato com o Comitê de Ética em Pesquisa (CEP) da Universidade Federal do Piauí (Campus Universitário Ministro Petrônio Portela, Bairro Ininga, Pró Reitoria de Pesquisa – PROPESQ, CEP: 64.049-550, Teresina, Piauí, Brasil; telefone: (86) 3237-2332.

Se você concordar em participar do estudo, seu nome e identidade serão mantidos em sigilo. A menos que requerido por lei ou por sua solicitação, somente o pesquisador, a equipe do estudo, Comitê de Ética independente e inspetores de agências regulamentadoras do governo (quando necessário) terão acesso a suas informações para verificar as informações do estudo. O projeto terá duração de quatro anos, com término previsto para o ano de 2024.

Nomes e assinaturas dos pesquisadores

Kyria Jayanne Clímaco Cruz _____

Ana Raquel Soares de Oliveira _____

Jennifer Beatriz Silva Moraes _____

Larissa Cristina Fontenelle _____

Loanne Rocha dos Santos _____

Stéfany Rodrigues de Sousa Melo _____

Dilina do Nascimento Marreiro _____

CONSENTIMENTO DA PARTICIPAÇÃO DA PESSOA COMO SUJEITO

Eu, _____
 ___, RG _____, CPF _____, abaixo assinado,
 concordo em participar do estudo “*Impacto de minerais em distúrbios endócrino-metabólicos*”, como sujeito. Tive pleno conhecimento das informações que li ou que foram lidas para mim, descrevendo o estudo. Discuti com as Doutorandas Kyria Jayanne Clímaco Cruz, Ana Raquel Soares de Oliveira, Jennifer Beatriz Silva Moraes, Larissa Cristina Fontenelle, Loanne Rocha dos Santos e Stéfany Rodrigues de Sousa Melo sobre a minha decisão em participar desse estudo. Ficaram claros para mim quais serão os propósitos do estudo, os procedimentos a serem realizados, seus desconfortos e riscos, as garantias de confidencialidade e de esclarecimentos permanentes. Ficou claro também que minha participação é isenta de despesas. Concordo, voluntariamente, em participar deste estudo e poderei retirar o meu consentimento a qualquer momento, antes ou durante o mesmo. A retirada do consentimento ao estudo não acarretará penalidades ou prejuízos.

Teresina

___/___/___

 Assinatura da participante

Presenciamos a solicitação de consentimento, esclarecimentos sobre a pesquisa e aceite do sujeito em participar.

Testemunhas (não ligadas à equipe de pesquisadores):

Nome: _____

Assinatura: _____

Nome: _____

Assinatura: _____

Observações complementares _____

APÊNDICE C - REGISTRO ALIMENTAR

Registro diário de sua alimentação

Número do Formulário: _____

- ❖ Nós vamos avaliar sua alimentação. Pedimos que anote neste registro alimentar **tudo** que você **comer e beber** durante todo o dia e à noite. Você deverá anotar todos os alimentos consumidos, sendo 2 dias alternados durante a semana e um dia no final de semana, totalizando 3 dias.
- ❖ Durante o preenchimento deste registro alimentar, alguns aspectos são importantes:
 1. Preencher logo **após o consumo** do alimento;
 2. Especificar as **marcas** dos alimentos industrializados;
 3. Procurar identificar o **tamanho** das frutas, vegetais, pedaços de carne, ou a **quantidade** que cada alimento é consumido, bem como o tipo de preparação (**frito, cozido, assado**) e caso haja molho, que tipo foi utilizado.
 4. Diferenciar qual o **utensílio** é usado, por exemplo:
 1. Colher – de chá, de sobremesa, de sopa, de servir ou concha
 2. Xícara – de chá ou de café
 3. Copo – grande ou de requeijão (americano)
 4. Prato de sobremesa
- 5. **Consumo mensal de óleo e sal da família:**



REGISTRO ALIMENTAR DE 3 DIAS - Nº _____

1º DIA

NÚMERO DO FORMULÁRIO: _____ Data ____/____/____

Segunda () Terça () Quarta () Quinta () Sexta () Sábado () Domingo ()

REFEIÇÕES (Hora)	ALIMENTOS	QUANTIDADES (Medidas caseiras)	MARCA

REGISTRO ALIMENTAR DE 3 DIAS - Nº _____

2º DIA

NÚMERO DO FORMULÁRIO: _____ Data ____/____/____

Segunda () Terça () Quarta () Quinta () Sexta () Sábado () Domingo ()

REFEIÇÕES (Hora)	ALIMENTOS	QUANTIDADES (Medidas caseiras)	MARCA

REGISTRO ALIMENTAR DE 3 DIAS - Nº _____

3º DIA

NÚMERO DO FORMULÁRIO: _____ Data ____/____/____

Segunda () Terça () Quarta () Quinta () Sexta () Sábado () Domingo ()

REFEIÇÕES (Hora)	ALIMENTOS	QUANTIDADES (Medidas caseiras)	MARCA

APÊNDICE D – INSTRUÇÕES ÀS PARTICIPANTES QUANTO À OBTENÇÃO DE URINA DE 24 HORAS

Para se obter exatidão do resultado, é muito importante que a urina seja coletada cuidadosamente. A obtenção da urina será realizada em frasco plástico de 5 litros, previamente desmineralizado, sem conservante e deve ser mantida REFRIGERADA até o horário da entrega.

A urina deverá ser obtida da seguinte forma: pela manhã, ao acordar, a paciente irá esvaziar a bexiga (desprezar a primeira urina, ou seja, não deverá guardá-la no frasco) e marcar rigorosamente este horário. Daí por diante, coletar todas as urinas do dia e da noite, integralmente a cada micção, colocando-as no frasco de coleta e mantido em geladeira (não congelar) entre as micções.

No dia seguinte coletar a 1ª urina da manhã, no mesmo horário que desprezou a 1ª urina do dia anterior e no mesmo frasco completando assim o período de 24 horas.

ATENÇÃO: Todo o material será fornecido pelo pesquisador.

APÊNDICE E – ARTIGOS PUBLICADOS COMO COAUTOR DURANTE O PERÍODO DO DOUTORADO

Periódico/ Ano	Título	Autores
Antioxidants/ 2017	Zinc and Oxidative Stress: Current Mechanisms	Dilina Marreiro, Kyria Cruz , Jennifer Morais, Jéssica Beserra, Juliana Severo, Ana Raquel Oliveira.
Biological Trace Element Research/ 2017	Magnesium Status and Its Association with Oxidative Stress in Obese Women	Jennifer Morais, Juliana Severo, Ana Raquel Oliveira, Kyria Cruz , Thaline Dias, Régina Célia de Assis, Célia Colli, Dilina Marreiro.
Biological Trace Element Research/ 2017	Role of Magnesium in Oxidative Stress in Individuals with Obesity	Jennifer Morais, Juliana Severo, Loanne dos Santos, Stéfany Melo, Raisia Santos, Ana Raquel Oliveira, Kyria Cruz , Dilina Marreiro.
Nutrition/ 2017	Effect of magnesium supplementation on insulin resistance in humans: A systematic review	Jennifer Morais, Juliana Severo, Geórgia Alencar, Ana Raquel Oliveira, Kyria Cruz , Dilina Marreiro, Betânia Freitas, Cecília Carvalho, Maria do Carmo Martins, Karoline Frota.
Revista da Associação Médica Brasileira/ 2017	Hypomagnesemia and its relation with chronic low-grade inflammation in obesity	Ana Raquel Oliveira, Kyria Cruz , Juliana Severo, Jennifer Morais, Taynáh Freitas, Rogério Araújo, Dilina Marreiro.
Alimentação Humana/ 2017	Effect of dietary calcium and vitamin D on cortisol concentrations in morbidly obese women.	Jéssica Beserra, Larissa Rodrigues, Priscilla Silva PCR, Ana Raquel Oliveira, Kyria Cruz , Jennifer Morais, Juliana Severo, Dilina Marreiro, Luana Martins.

Associação entre as concentrações de minerais (magnésio, zinco e selênio) e marcadores metabólicos dos fenótipos obesidade metabolicamente saudável e obesidade metabolicamente não saudável

Kyria Jayanne Clímaco Cruz

Nutrición Clínica Dietética Hospitalaria/ 2017	Dietary intake of vitamin D and its relation to insulin resistance in obese women	Loanne dos Santos, Amanda Braz, Alana Lima, Stéfany Melo, Raisal Santos, Jennifer Moraes, Juliana Severo, Kyria Cruz , Ana Raquel Oliveira, Luana Martins, Dilina Marreiro.
Nutrire/ 2017	Role of vitamin D in insulin resistance in obese individuals	Loanne dos Santos, Alana Lima, Amanda Braz, Stéfany Melo, Jennifer Moraes, Juliana Severo, Ana Raquel Oliveira, Kyria Cruz , Dilina Marreiro.
International Journal for Vitamin and Nutrition Research/ 2019	No Difference in Magnesium Intake between Obese Women and Healthy Controls	Jennifer Moraes, Taynâh Freitas, Juliana Severo, Ana Raquel Oliveira, Kyria Cruz , Betânia Freitas, Célia Colli, Dilina Marreiro.
Biological Trace Element Research/ 2019	Association Between Cortisol, Insulin Resistance and Zinc in Obesity: a Mini- Review	Jennifer Moraes, Juliana Severo, Jéssica Beserra, Ana Raquel Oliveira, Kyria Cruz , Stéfany Melo, Ginivaldo do Nascimento, George de Macedo, Dilina Marreiro.
International Journal for Vitamin and Nutrition Research/ 2019	The Role of Zinc in Thyroid Hormones Metabolism	Juliana Severo, Jennifer Moraes, Taynâh Freitas, Ana Letícia Andrade, Mayara Feitosa, Larissa Fontenelle, Ana Raquel Oliveira, Kyria Cruz , Dilina Marreiro.