UNIVERSIDADE FEDERAL DO RIO GRANDE DO SUL

PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIAS MÉDICAS:

ENDOCRINOLOGIA

TÍTULO

EXPRESSÃO DO INFLAMASSOMA NLRP3 E DO FATOR DE LIPÓLISE CGI-58 NA OBESIDADE

TESE DE DOUTORADO

JAKELINE RHEINHEIMER

Porto Alegre, 09 de Março de 2018.

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Tese de Doutorado apresentada ao Programa de Pós-Graduação em Ciências Médicas: Endocrinologia da Universidade Federal do Rio Grande do Sul (UFRGS) como requisito para obtenção do título de Doutora em Endocrinologia.

Orientadora: Profa. Dra. Daisy Crispim Moreira

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A minha irmã Janyelle, um exemplo de força e superação.

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Sumário

1. LISTA DE ABREVIATURAS DA TESE

ABHD5	*αβ hydrolase domain-containing 5*
ASC	*Associated speck-like protein containing*
ASO	*Antisense oligonucleotides*
ATGL	*Adipose triglyceride lipase*
CGI-58	*Comparative gene identification-58*
DAMPs	*Danger-associated molecular patterns*
DATG	Dieta com alto teor de gordura
DM	Diabetes mellitus
DM2	Diabetes mellitus tipo 2
HbA1c	Hemoglobina glicada
HDL	Lipoproteína de alta densidade
HSL	*Hormone-sensitive lipase*
IC	Intervalo de confiança
IL	Interleucina
IL-1β	Interleucina-1β
IMC	Índice de massa corporal
KD	*Knockdown*
KO	*Knockout*
LPAAT	*Lysophosphatidic acid acyltransferase*
MAGL	*Monoacylglycerol lipase*

MCP-1	Proteína quimiotática de monócitos-1
NLRP3	*Nod-like receptores family pyrin domain containing 3*
NLRs	*Nod-like receptores*
OMS	Organização Mundial da Saúde
PAMPs	*Pathogen-associated molecular pattern*
RC	Razão de chance
RI	Resistência à insulina
RT-qPCR	PCR em tempo real quantitativo
TA	Tecido adiposo
TAS	Tecido adiposo subcutâneo
TAV	Tecido adiposo visceral
TG	Triglicerídeos
TLRs	*Toll-like receptors*
TMR	Taxa metabólica de repouso
TNF	Fator de necrose tumoral

LISTA DE ABREVIATURAS DOS ARTIGOS ORIGINAIS:

3'UTR	*3'Untranslated region*
ABHD5	*αβ hydrolase domain-containing 5*
ASC	*Associated speck-like protein containing*
ASO	*Antisense oligonucleotides*
ATGL	*Adipose triglyceride lipase*
BMI	*Body mass index*
BP	*Blood pressure*
CARD	*Caspase recruitment domain*
CASP-1	*Caspase-1*
CDS	*Chanarin-Dorfman syndrome*
CGI-58	*Comparative gene identification-58*
CI	*Confidence interval*
DAMPs	*Danger-associated molecular patterns*
FFAs	*Free fatty acids*
FFM	*Fat-free mass*
FOXO1	*Forkhead box-containing protein O subfamily-1*
FPG	*Fasting plasma glucose*
HbA1c	*Glycated hemoglobin*
HFD	*High fat diet*
HOMA-IR	*Homeostatic model assessment - insulin resistance*

HPRT1	*Hypoxanthine phosphoribosyl-transferase 1*
HSL	*Hormone-sensitive lipase*
IL	*Interleukin*
IR	*Insulin resistance*
IRS-1	*Insulin receptor substrate-1*
KD	*Knockdown*
KO	*Knockout*
LAGB	*Laparoscopic adjustable gastric banding*
LFD	*Low fat diet*
LPAAT	*Lysophosphatidic acid acyltransferase*
LPS	*Lipopolysaccharide*
MAGL	*Monoacylglycerol lipase*
MAKO	*Macrophage-specific Cgi-58 knockout*
MeSH	*Medical subject headings*
MHO	*Metabolically healthy obese*
MUFA	*Monounsaturated fatty acid*
MUO	*Metabolically unhealthy obese*
NEFAs	*Saturated non-esterified fatty acids*
NF-κB	*Nuclear factor-κB*
NLRP3	*Nod-like receptores family pyrin domain containing 3*
NLRs	*Nod-like receptors*
OR	*Odds ratio*

PAMPs	*Pathogen-associated molecular pattern*
PBMCs	*Peripheral blood mononuclear cells*
PGC-1β	*Peroxisome Proliferator-Activated Receptor-γ Coativator-β*
PLIN1	*Perilipin-1*
PPARγ	*Peroxisome Proliferator-Activated Receptor-γ*
Pre-DM	*Pre-diabetes mellitus*
PRRs	*Pattern-recognition receptors*
REE	*Resting energy expenditure*
RLHs	*Retinoic acid-inducible gene I-like*
ROS	*Reactive oxygen species*
RT-qPCR	*Quantitative real-time PCR*
SAT	*Subcutaneous adipose tissue*
SFA	*Saturated fatty acid*
SOCS3	*Suppressor of cytokine signaling 3*
SVCs	*Stromal vascular cells*
T2DM	*Type 2 diabetes mellitus*
Tg	*Transgenic*
TLRs	*Toll-like receptors*
TNF	*Tumor necrosis factor*
VAT	*Visceral adipose tissue*
WAT	*White adipose tissue*

2. RESUMO

A obesidade é considerada uma epidemia na atualidade, principalmente nos países em desenvolvimento. Indivíduos com obesidade possuem maior risco para desenvolvimento de doenças cardiovasculares, diabetes mellitus tipo 2 (DM2) e alguns tipos de câncer, o que diminui a qualidade e expectativa de vida destes indivíduos. O tecido adiposo (TA) é o sítio anatômico da manifestação dos principais efeitos fisiopatológicos da obesidade. Alterações neste tecido estão implicadas, não somente no desenvolvimento da obesidade, mas também destas outras doenças associadas. Uma das alterações presentes nos indivíduos com obesidade é uma inflamação crônica de baixo grau, associada à produção aumentada de citocinas proinflamatórias. Evidências sugerem que o inflamassoma NLRP3 (*Nod-like receptores family pyrin domain containing 3*) é um dos principais responsáveis pela produção de citocinas proinflamatórias no TA, como a interleucina-1β (IL-1β).

Embora vários estudos tenham relatado uma associação do NLRP3 com obesidade e / ou resistência à insulina (RI); resultados contraditórios também foram relatados por outros estudos. Portanto, realizamos uma revisão sistemática para resumir os resultados de estudos que avaliaram a associação do NLRP3 com obesidade e RI. Dezenove estudos foram incluídos na revisão. Os estudos foram selecionados por apresentarem análises de expressão do *NLRP3* no TA de indivíduos com e sem obesidade ou avaliarem associações entre polimorfismos neste gene e obesidade. Em geral, estudos em humanos indicam que a obesidade e RI estão associadas com a expressão aumentada de *NLRP3* no TA. Estudos em ratos obesos corroboraram essa associação. Além disso, dieta com alto teor de gordura (DATG) aumenta a expressão de *Nlrp3* no TA de camundongos, enquanto que a dieta que restringe calorias diminui sua expressão. Da mesma

forma, o bloqueio do *Nlrp3* em camundongos protege contra a obesidade e RI induzidas por DATG. São escassos os estudos que avaliaram polimorfismos no *NLRP3* e obesidade.

Visto que a obesidade é uma doença multifatorial, com componentes ambientais e genéticos envolvidos no seu desenvolvimento, fatores diversos devem ser analisados, em especial, no TA. O *comparative gene identification-58* (CGI-58) foi primeiramente identificado como um co-ativador da *adipose triglyceride lipase* (ATGL), aumentado a lipólise. Estudos mais recentes também identificaram funções do CGI-58 independentes da ativação da ATGL. O CGI-58 parece atuar como uma *lysophosphatidic acid acyltransferase* (LPAAT), ou seja, possui a capacidade de catalisar a modificação do ácido lisofosfatídico em ácido fosfatídico. Isto representa um passo importante na biossíntese de lipídios neutros e glicerofosfolipídios, gerando moléculas de sinalização que regulam processos inflamatórios e a ação da insulina.

Até o momento, apenas alguns poucos estudos avaliaram a associação entre CGI-58 e obesidade, com resultados inconclusivos. Assim, comparamos a expressão de *CGI-58* no TA subcutâneo (TAS) de indivíduos com diferentes categorias de índice de massa corporal (IMC). Também avaliamos se o *CGI-58* se correlaciona com parâmetros de composição corporal, taxa metabólica de repouso (TMR), RI e perfis lipídicos e glicêmicos. Para tal, utilizamos biópsias de TAS de 67 indivíduos submetidos à cirurgia bariátrica ou cirurgia abdominal eletiva. Os pacientes foram divididos em: Grupo 1 (n = 14; IMC < 27,0 kg/m^2), Grupo 2 (n = 24; IMC 30,0 - 39,9 kg/m^2) e Grupo 3 (n = 29; IMC ≥ 40,0 kg/m^2). A expressão gênica do *CGI-58* foi quantificada usando RT-qPCR. A expressão de *CGI-58* foi diminuída em pacientes do Grupo 3 [mediana de 0,60 (0,45 - 0,85, percentil 25º - 75º)] e Grupo 2 [0,85 (0,50 - 1,23)] em comparação com pacientes do Grupo 1 [1,70 (0,99 - 2,70)] ($p < 0,001$). Valores de *CGI-58* acima da mediana foram associados com menor risco de obesidade, ajustando-se para as covariáveis (RC = 0,036, IC 95% 0,003 - 0,410).

Além disso, a expressão de *CGI-58* foi negativamente correlacionada com parâmetros de composição corporal (IMC, circunferência da cintura, porcentagens de massa gorda e massa magra), TMR, perfil lipídico (colesterol total e triglicerídeos), RI e hemoglobina glicada, enquanto correlacionou-se positivamente com o colesterol HDL. Sendo assim, a expressão de *CGI-58* está diminuída em indivíduos com obesidade, estando associada com um pior perfil metabólico.

De acordo com exposto é possível que NLRP3 e CGI-58 tenham um papel importante no desenvolvimento da obesidade. Parece que estes dois genes atuam de forma inversa, pois o *NLRP3* está aumentado em indivíduos com obesidade enquanto o *CGI-58* está diminuído. De forma interessante, um estudo anterior demonstrou que ratos com bloqueio de *CGI-58* especificamente nos macrófagos e tratados com DATG tiveram inflamação exacerbada e RI devido à ativação do inflamassoma NLRP3 e consequente secreção de IL-1β. No entanto, mais estudo são necessários para esclarecer o papel do CGI-58 na patogênese da obesidade.

3. ABSTRACT

Nowadays, obesity is considered epidemic, especially in developing countries. Individuals with obesity are at greater risk for developing cardiovascular diseases, type 2 diabetes mellitus (T2DM) and some types of cancer, which reduces the quality and life expectancy of these individuals. Adipose tissue (AT) is the anatomical site of the manifestation of the main pathophysiological effects of obesity. Alterations in this tissue are implicated, not only in the development of obesity, but also of these other associated diseases. One of the alterations present in individuals with obesity is a chronic low-grade inflammation, associated with increased production of pro-inflammatory cytokines. Evidence has suggested that the NLRP3 inflammasome (*Nod-like receptors family pyrin domain containing 3*) is one of the main factors involved in the production of pro-inflammatory cytokines in AT, such as interleukin-1β (IL-1β).

Although several studies have reported an association of the NLRP3 inflammasome with obesity and / or insulin resistance (IR); contradictory results have also been reported by other studies. Therefore, we conducted a systematic review to summarize the results of the studies that evaluated the association of NLRP3 with obesity and IR. Nineteen studies were included in the review. Selected studies focused on *NLRP3* expression in the TA of individuals with and without obesity or evaluated associations between polymorphisms in this gene and obesity. Overall, human studies indicate that obesity and IR are associated with increased *NLRP3* expression in AT. Studies in obese mice corroborate this association. Moreover, high fat diet (HFD) increases *Nlrp3* expression in murine AT while calorie-restricted diet decreases its expression. Hence, *Nlrp3* blockade in mice protects against HFD-induced obesity and IR. Few studies analyzed the association of *NLRP3* polymorphisms with obesity. Considering that obesity is a multifactorial

disease, with environmental and genetic components involved in its development, several factors must be analyzed, especially in AT. Initially, comparative gene identification-58 (CGI-58) was identified as a co-activator of adipose triglyceride lipase (ATGL), increasing lipolysis. Recent studies have also identified CGI-58 functions independent of ATGL activation. CGI-58 seems to act as a lysophosphatidic acid acyltransferase (LPAAT), that is, it has the ability to catalyze the modification of lysophosphatidic acid in phosphatidic acid. This represents an important step in the biosynthesis of neutral lipids and glycerol-phospholipids, generating signaling molecules that regulate inflammatory processes and insulin action.

To date, only few studies have evaluated the association between CGI-58 and obesity, with inconclusive results. Thus, we compared *CGI-58* expression among subcutaneous adipose tissue (SAT) of subjects with different body mass index (BMI) categories. We also evaluated if *CGI-58* correlates with body composition parameters, resting energy expenditure (REE), insulin resistance (IR), and lipid and glycemic profiles. To this, SAT biopsies were obtained from 67 individuals who underwent bariatric surgery or elective abdominal surgery. Patients were divided in: Group 1 ($n = 14$; BMI < 27.0 kg/m^2), Group 2 ($n = 24$; BMI 30.0 - 39.9 kg/m^2) and Group 3 ($n = 29$; BMI ≥ 40.0 kg/m^2). *CGI-58* expression was quantified using RT-qPCR. *CGI-58* expression was decreased in patients from Group 3 [median 0.60 (0.45 – 0.85, 25th – 75th percentiles)] and Group 2 [0.85 (0.50 – 1.23)] compared to Group 1 patients [1.70 (0.99 – 2.70)] ($P < 0.001$). *CGI-58* values above median were associated with protection for obesity, adjusting for covariables (OR = 0.036, 95% CI 0.003 – 0.410). Moreover, *CGI-58* expression was negatively correlated with body composition parameters (BMI, waist circumference, fat mass, and fat-free mass), REE, lipid profile (total cholesterol and triglycerides), IR, and glycated hemoglobin, while it was positively

correlated with HDL cholesterol. Therefore, *CGI-58* expression is decreased in patients with obesity, and it was associated with worse metabolic profile.

According to the aforementioned data, it is possible that NLRP3 and CGI-58 might play an important role in the development of obesity. It seems that these two genes act in opposite ways, since NLRP3 is increased in individuals with obesity while CGI-58 is decreased. Interestingly, a previous study demonstrated that macrophage-specific Cgi-58 blockade in AT from mice increased inflammation and IR after HFD, due to the activation of the NLRP3 inflammasome and, consequent secretion of IL-1β. However, additional studies are needed to clarify the role of CGI-58 in the pathogenesis in the obesity.

4. APRESENTAÇÃO DA TESE

Esta tese de doutorado segue o formato proposto pelo Programa de Pós-Graduação em Ciências Médicas: Endocrinologia da Universidade Federal do Rio Grande do Sul, sendo apresentada na forma de uma breve introdução geral sobre o assunto da tese. Na sequência é apresentado um artigo de revisão sistemática, já publicado, que aborda os estudos que analisaram o envolvimento do NLRP3 na obesidade e resistência à insulina. Após, incluímos o artigo original que já foi submetido à revista indexada. Ao final, são apresentadas conclusões e perspectivas relacionadas ao tema da tese.

ARTIGO I: *Current role of the NLRP3 inflammasome on obesity and insulin resistance: A systematic review* (Publicado na revista *Metabolism* em 2017).

ARTIGO II: *CGI-58 gene expression is decreased in the adipose tissue of patients with obesity* **(**Submetido à revista *Gene* em fevereiro de 2018).

5. INTRODUÇÃO

5.1 OBESIDADE

A obesidade é caracterizada por acumulação excessiva ou anormal de gordura no tecido adiposo (TA) e órgãos ectópicos. Ao longo da última década, a prevalência de obesidade no mundo aumentou drasticamente em todos os grupos etários, especialmente em países desenvolvidos (1, 2). Segundo a Organização Mundial da Saúde (OMS), aproximadamente 13% da população mundial tem obesidade e 3,4 milhões de adultos morrem a cada ano como resultado do sobrepeso ou obesidade (3). São também atribuídas à obesidade e ao sobrepeso, 44% dos casos de diabetes mellitus (DM), 23% de doenças isquêmicas do coração e 7-41% de alguns tipos de câncer (1-4). Na população brasileira tem-se utilizado a classificação proposta pela OMS para a obesidade, ou seja, ter o índice de massa corporal (IMC) maior ou igual a 30 kg/m² (3, 5). Estima-se que, no Brasil, 21% da população esteja com obesidade (3).

O crescente aumento da obesidade na população global tem instigado a busca pelo entendimento dos mecanismos patofisiológicos envolvidos nessa doença (3, 6). O tecido adiposo (TA) é o sítio anatômico da manifestação dos principais efeitos fisiopatológicos da obesidade e, portanto, tem sido extensamente estudado (7). Conhecidamente, o adipócito no TA tem a função de armazenar a energia em excesso na forma de triacilglicerol que, em resposta aos requisitos metabólicos do corpo como um todo, pode ser utilizado novamente após hidrólise com liberação de ácidos graxos livres (8).

Na obesidade, é identificado um desequilíbrio entre a energia consumida e a energia gasta, ocorrendo acúmulo excessivo de triglicerídeos e de gordura corporal. O gasto energético que ocorre naturalmente no indivíduo e corresponde ao utilizado para manter o metabolismo celular, o

funcionamento dos órgãos e a manutenção das funções vitais do corpo é chamado de taxa metabólica de repouso (TMR). Este representa cerca de 50 a 70% do gasto energético diário em indivíduos sedentários (10, 12). Os demais gastos energéticos ocorrem por fatores externos como, por exemplo, energia gasta pela atividade física e pela ingesta de alimentos (5, 10).

Nas últimas décadas, o TA tem sido identificado, não somente como um reservatório de energia, mas também como um órgão de funções endócrinas, capaz de secretar várias moléculas bioativas, que são denominadas "adipocinas", em resposta ao estado nutricional sistêmico, resultando em um mecanismo de *feedback* de homeostase metabólica (9). Algumas das adipocinas atualmente conhecidas são a leptina, adiponectina, resistina, visfatina e fator de necrose tumoral (TNF), as quais apresentam funções diferentes e estão relacionadas com a modulação do metabolismo da glicose e lipídios, com a saciedade e com processos anti- ou proinflamatórios no TA (10, 11).

Interessantemente, em indivíduos com obesidade é observada uma inflamação estéril crônica de baixo grau (12, 13). No entanto, os mecanismos envolvidos nesta inflamação ainda precisam ser melhor estudados. O TA é um tecido heterogêneo composto por adipócitos maduros e por células da fração estromal vascular. A fração estromal vascular inclui, entre diversos tipos celulares, pré-adipócitos, fibroblastos, células endoteliais e macrófagos. No TA de indivíduos com obesidade existe um aumento expressivo de células do sistema imune, principalmente macrófagos (14, 15). Uma das hipóteses é que com a ocorrência de hipertrofia do TA, os adipócitos acabam secretando maiores níveis de TNF, o que, por sua vez, estimula a produção da proteína quimiotática de monócitos-1 (MCP-1) por pré-adipócitos e o consequente recrutamento de mais macrófagos para o TA. Isto desencadeia um círculo vicioso de recrutamento de macrófagos e de produção de citocinas inflamatórias, tais como TNF, interleucina (IL)-6 e IL-1β. Além disso, no TA de

indivíduos com obesidade, os macrófagos apresentam uma mudança no seu fenótipo, de macrófagos M2 (anti-inflamatório) para M1 (proinflamatório) (16).

5.2. *NOD-LIKE RECEPTORS FAMILY PYRIN DOMAIN CONTAINING 3 (NLRP3)*

O sistema imune inato é um sistema sofisticado que detecta agentes patogênicos e sinais derivados de estresse celular. As células do sistema imune inato, como os macrófagos, identificam estes fatores exógenos ou endógenos a partir de receptores de reconhecimento padrão (17). Os receptores do tipo Toll-like (do inglês *Toll Like Receptors* - TLRs) são os receptores mais bem conhecidos e identificam sequências moleculares específicas presentes em agentes patogênicos e conservadas durante a evolução, denominadas padrão molecular associado a patógenos (do inglês *Pathogen-associated molecular pattern* - PAMPs) (18). Recentemente, foram identificados os receptores NOD-like (NLRs) que reconhecem além dos PAMPs, padrões moleculares associados ao perigo (DAMPs). O NLRP3 é um dos membros mais bem descritos desta família de receptores (19).

O NLRP3, como os demais NLRs, possui a capacidade de reconhecer DAMPs, tais como proteínas do choque térmico, ceramidas, espécies reativas de oxigênio, ácidos graxos saturados e algumas proteínas da matriz extracelular. O reconhecimento de alguns DAMPs é crucial para a ativação de respostas inflamatórias do tipo estéril não infecciosas, características de processos como trauma e isquemia (20). Alguns DAMPs parecem também estar envolvidos na manutenção da inflamação e resistência à insulina (RI) em indivíduos com obesidade. Na obesidade é notável a existência de metabólitos em excesso, como ácido graxos, ceramidas e palmitato. Em alguns estudos é verificado também uma associação entre o aumento destes metabólitos e o desenvolvimento do DM tipo 2 (DM2) (21, 22).

Os NLRs são receptores citoplasmáticos e que se oligomerizam para formar o inflamassoma, que é um complexo multiproteico composto pela proteína NLR, pelo adaptador *associated speck-like protein containing* (ASC) e pela enzima caspase-1. O inflamassoma NLRP3 controla a produção de citocinas proinflamatórias, IL-1β e IL-18 (21, 23). Vandanmagsar *et. al*. demonstraram que em ratos com obesidade induzida por dieta, as expressões de *IL-1β* e do inflamassoma *NLRP3* no TA visceral (TAV) estão correlacionadas positivamente com o peso corporal e adiposidade, sendo proveniente principalmente dos macrófagos. O mesmo grupo verificou que a perda de peso em indivíduos com obesidade e DM2 melhora a sensibilidade à insulina, o que foi associado a uma redução nos níveis de IL-1β e à redução da expressão de *NLRP3* no TA subcutâneo (TAS) (21). No entanto, Goossens *et. al*. (24) compararam a expressão do *NLRP3* no TAS de homens com obesidade *vs.* indivíduos magros, mas não observaram diferenças entre os dois grupos, embora tenham observado uma correlação positiva entre a expressão de *NLRP3* e inflamação no TAS. Um outro estudo avaliou indivíduos com obesidade classificados como metabolicamente saudáveis ou não-saudáveis (que desenvolveram distúrbios metabólicos), demonstrando um aumento na secreção e expressão de *IL-1β* e na expressão do *NLRP3* no TAV de pacientes não-saudáveis comparados com o grupo metabolicamente saudável (25).

Em camundongos, o bloqueio do gene *NLRP3* e o seu impacto sobre a obesidade, RI e DM2 são divergentes. Stienstra *et. al.* (26) identificaram que o bloqueio do *NLRP3* evitou a obesidade e a IR induzidas por dieta com alto teor de gordura (DATG). Os mesmos autores também observaram um menor tamanho dos adipócitos e infiltração de macrófagos no TAV dos camundongos com bloqueio de *NLRP3* em comparação com os controles. Da mesma forma, Wen *et. al.* (27) demonstraram que com a DATG, os níveis de glicose e insulina no sangue eram significativamente menores em camundongos com o bloqueio do *NLRP3* em comparação com os

camundongos controle. Em contraste, Ringling *et. al.* (28) relataram que os camundongos com bloqueio do *NLRP3* não diferiram dos controles quanto ao ganho de peso, inflamação do TA ou intolerância à glicose após tratamento com dieta ocidental (45% de gordura e 1% de colesterol).

Ensaios clínicos randomizados têm demonstrado que o bloqueio da sinalização da citocina proinflamatória IL-1β por um antagonista do receptor IL-1 recombinante humano leva a redução sustentada da inflamação sistêmica e diminuição dos níveis glicêmico em pacientes com DM2 (29, 30). Atualmente, reconhece-se que o mecanismo de ação de certas terapias anti-inflamatórias utilizadas em algumas doenças pode, em parte, ser atribuída à inibição de NLRP3 (31-33). Alguns compostos de pequenas moléculas (*small-molecule inhibitors*) podem inibir a ativação do NLRP3 *in vitro* (34), como, por exemplo, a glibenclamida (utilizada no tratamento de DM2), que foi capaz de inibir diretamente o NLRP3, embora apenas em altas doses (35).

Fatores endógenos também podem estar envolvidos na redução ou aumento do potencial inflamatório do NLRP3. Alguns estudos demonstraram que polimorfismos no gene *NLRP3* podem alterar a produção ou função do inflamassoma, estando associados a doenças inflamatórias, incluindo a doença de crohn, DM tipo 1, artrite reumatoide, doença celíaca, lúpus eritematoso sistêmico e, mais recentemente, ao DM2 (36-39).

5.3. *COMPARATIVE GENE IDENTIFICATION (CGI-58)*

O gene *comparative gene identification-58* (CGI-58), também conhecido como *αβ hydrolase domain-containing 5* (ABHD5), entrou em evidência quando, em 2001, Lefèvre *et. al.* (40) identificaram em humanos uma mutação neste gene associada à síndrome de Chanarin-Dorfman. Essa síndrome é rara, tem herança autossômica recessiva e é caracterizada pelo acúmulo intracelular anormal de gotículas lipídicas em diversos tecidos (40). Posteriormente, foi

demonstrado que o CGI-58 é um co-ativador da *adipose triglyceride lipase* (ATGL) envolvida na lipólise (41). A lipólise é rigorosamente regulada pelas lipases ATGL, *hormone-sensitive lipase* (HSL) e *monoacylglycerol lipase* (MAGL). No entanto, a ATGL é a enzima inicial na conversão de triglicerídeos (TG) em glicerol e ácidos graxos livres **(Figura 1)**. A interação com a CGI-58 pode aumentar em 30-70% a atividade lipolítica da ATGL em células em cultura, dependendo das condições experimentais (42).

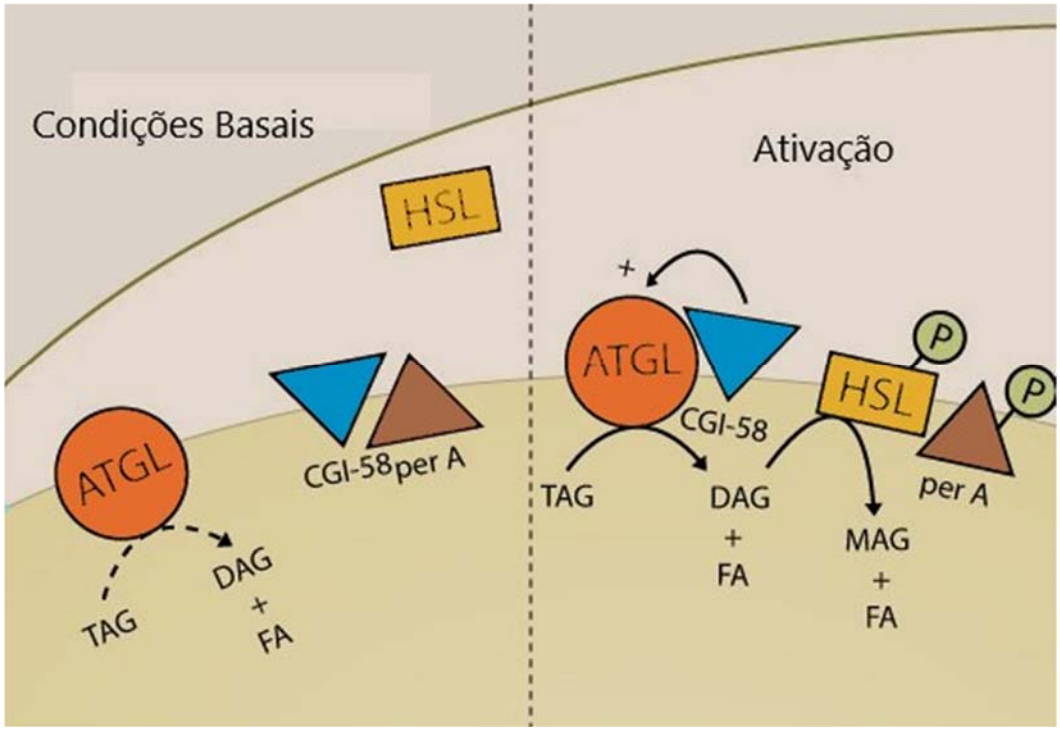

Figura 1: Condições basais: a proteína associada a gotas lipídicas, a perilipina A, funciona como uma barreira que protege a TAG da hidrólise e interage com a proteína CGI-58 na superfície das gotículas lipídicas. Ativação: A ativação da lipólise ocorre pela fosforilação da perilipina A, o que inibi a sua interação com a proteína CGI-58. Assim, o CGI-58 se dissocia para formar um complexo com ATGL. O CGI-58 mostrou ser um co-fator para ATGL, mas não HSL. Abreviaturas: ATGL, *adipose triacylglyceride lipase*; DAG, *diacylglycerol*; FA, *fatty acid*; HSL, *hormone sensitive lipase*; per A, *perilipin* A; TAG, *triacylglycerol*.

Estudos mais recentes identificaram funções do CGI-58 independentes da ativação do ATGL (43-45). Camundongos com deficiência total de *CGI-58* desenvolvem um defeito de barreira da pele grave, independente do ATGL, morrendo logo após o nascimento. Embora a morte prematura tenha impedido uma caracterização fenotípica de camundongos adultos com deficiência global de *CGI-58*, a caracterização de camundongos com deficiência de CGI-58 específica de tecidos revelou novos pontos de vista sobre seu papel no metabolismo lipídico e energético (44). O CGI-58 também parece atuar como uma *lysophosphatidic acid acyltransferase* (LPAAT), ou seja, possui a capacidade de catalisar a modificação do ácido lisofosfatídico em ácido fosfatídico. Isto representa um passo importante na biossíntese de lipídios neutros e glicerofosfolipídios, gerando moléculas de sinalização que regulam processos inflamatórios e a ação da insulina (44, 46).

Embora o CGI-58 seja uma molécula aparentemente promissora a ser estudada, em relação à patogênese da obesidade, poucos estudos avaliaram a expressão de *CGI-58* em seres humanos (**Tabela 1**) (47-49). Karki *et. al.* (47) relataram um aumento na expressão do *CGI-58* em TAS de pacientes com obesidade após perda de peso mediada por cirurgia bariátrica. Neste mesmo estudo, a expressão do *CGI-58* foi correlacionada negativamente com os níveis de hemoglobina glicada (HbA1c). No entanto, em outro estudo não foram observadas diferenças significativas na expressão de *CGI-58* em TAS e TAV de indivíduos com obesidade e controles magros (48). De forma controversa, quando se avaliou a expressão de *CGI-58* em células mononucleares de indivíduos saudáveis foi verificado uma correlação negativa entre a sua expressão e IMC (49).

Também são controversos os resultados dos estudos que avaliaram a expressão de *Cgi-58* no TA de camundongos (**Tabela 1**). Miao *et. al.* (49) relataram a diminuição da expressão de *Cgi-58* em TAS e TAV de camundongos submetidos à dieta com alto teor de gordura (DATG) em

comparação com os controles. Além disso, a expressão de *Cgi-58* em macrófagos derivados do TAV correlacionou-se negativamente com o aumento de peso induzido por DATG (49). Em contraste, Gaidhu *et. al.* (1) observaram um aumento na expressão de *Cgi-58* em TAS e TAV de camundongos tratados com DATG e Badin *et. al.* (50) relataram o aumento da expressão desse gene no músculo esquelético de camundongos tratados com DATG. Já, Kinney *et. al.* (51) não encontraram diferenças nos níveis de proteína Cgi-58 no TA epididimal de camundongos tratados com DATG e nos alimentados com dieta tradicional.

Quatro estudos avaliaram o efeito do bloqueio tecido-específico do *Cgi-58* em camundongos submetidos à DATG ou dieta padrão (43, 52-54) sob o desenvolvimento de obesidade, RI ou DM2. O *knockdown* (KD) de *Cgi-58* no TA epididimal ou fígado, usando *antisense oligonucleotides* (ASO), preveniu o ganho de peso e RI nos camundongos alimentados com DATG; embora, tenha sido associado com um aumento de esteatose hepática e nos níveis de TG (53, 54). Nos camundongos alimentados com dieta normal, o bloqueio do *Cgi-58* não alterou o ganho de peso (53). O *knockout* (KO) de *Cgi-58* em macrófagos de camundongos levou ao aumento da intolerância à glicose, RI e inflamação (43). De maneira interessante, camundongos KO para *Cgi-58* no tecido muscular e submetidos à DATG apresentaram melhora na tolerância à glicose e sensibilidade à insulina em comparação aos camundongos normais submetidos a mesma dieta (52). Esses camundongos KO apresentaram porcentagens similares de massa magra e massa gorda aos camundongos controles. Somente um estudo analisou o efeito da superexpressão do *Cgi-58* no TA de camundongos tratados com DATG *vs*. camundongos alimentados com dieta padrão (55). Os camundongos com superexpressão de *Cgi-58* não foram protegidos contra a obesidade induzida por DATG e apresentaram níveis de TG plasmáticos semelhantes àqueles mostrados pelo grupo controle (55).

Tabela 1: Resumo dos estudos que avaliaram a associação entre a expressão do CGI-58 e obesidade ou RI.

Autor, ano	**Amostras**	**Tecido**	**Resultados**
Badin, 2013	Camundongo CH3 (DATG *vs*. dieta normal).	Músculo esquelético	↑ níveis proteicos de CGI-58 em músculo de camundongos com DATG *vs*. dieta normal.
Brown, 2010	Camundongo C57BL/6 com *Cgi-58* KD (DATG *vs*. dieta normal).	TA epididimal/ fígado	↓ obesidade induzida por DATG em camundongos KD para *Cgi-58*; camundongos KD para *Cgi-58* alimentados com DATG tiveram melhor sensibilidade à insulina que os controles.
Cantley, 2013	Camundongo C57BL/6 com *Cgi-58* KD (DATG *vs*. dieta normal).	TA epididimal/ fígado	↓ ganho de peso e RI em camundongos KD para *Cgi-58* alimentados com DATG.
Caviglia, 2011	Camundongo Tg para *Cgi-58*.	TA perigonodal/ TAS/fígado	↔ camundongos Tg para *Cgi-58* não ficaram protegidos da obesidade induzida por DATG.
Gaidhu, 2010	Camundongo C57BL/6J (DATG *vs*. dieta normal).	TAS/TAV	↑ expressão de *Cgi-58* em TAS e TAV de camundongos alimentados com DATG *vs*. camundongos com dieta normal.
Karki, 2015	Indivíduos com obesidade (antes e após cirurgia bariátrica).	TAS	↑ expressão de *CGI-58* após perda de peso (seguimento de 8 ± 5 meses).
Kinney, 2010	Camundongo C57BL/6J (DATG *vs*. dieta normal).	TA epididimal	↔ níveis proteicos de Cgi-58.

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7. JUSTIFICATIVA E OBJETIVOS

As abordagens atuais para o tratamento da obesidade, incluindo a mudança na dieta e estilo de vida, não foram bem sucedidas na redução da epidemia da obesidade. Indivíduos obesos possuem maior risco de desenvolverem outras doenças relacionadas como, por exemplo, DM2 e doenças isquêmicas do coração. Tanto a própria obesidade como as doenças associadas comprometem a qualidade de vida e a produtividade dos indivíduos afetados, além de requererem elevados custos para seu tratamento. Dessa forma, a identificação dos mecanismos que podem influenciar o grau de obesidade podem nos trazer avanços na prevenção e/ou melhora da qualidade de vida dos indivíduos com essa doença.

Uma baixa inflamação sistêmica é observada em indivíduos com obesidade e algumas doenças associadas são também exacerbadas com esta inflamação. Estudos avaliando o tecido adiposo (TA) tem desvendado diversos papéis que este tecido desempenha. Atualmente, sabe-se que o TA é dinamicamente envolvido na regulação da função celular e na gênese de doenças por meio de complexa rede de sinais endócrinos, parácrinos e autócrinos atuando sobre diversos tecidos. O inflamassoma NLRP3 tem sido associado a diversas doenças inflamatórias, no entanto, com relação à associação deste com obesidade, os estudos ainda parecem ser controversos. Uma revisão sistemática poderia responder de forma mais clara sobre o assunto, pois nesta é utilizado métodos sistemáticos para identificar, selecionar e avaliar criticamente os dados existentes na literatura.

O CGI-58 parece estar envolvido não somente na regulação da lipólise como se achava anteriormente, mas também em rotas envolvidas na inflamação de tecidos, principalmente em macrófagos. Além disso, parece fazer parte de vias de sinalização que atuam na regulação da ação da insulina. E, embora o CGI-58 pareça ser uma molécula promissora a ser estudada na patogênese

da obesidade, poucos estudos avaliaram a expressão de *CGI-58* no TA de indivíduos com e sem obesidade e sua associação com possíveis variáveis metabólicas. Compreender os mecanismos moleculares presentes no TA de indivíduos com obesidade continua sendo um grande desafio médico.

Em vista do exposto, os principais objetivos da presente tese foram:

Objetivos Gerais:

- Sumarizar os resultados dos estudos que avaliaram a associação entre o NLRP3 e obesidade e/ou resistência à insulina através de uma revisão sistemática.

- Comparar a expressão de *CGI-58* no tecido adiposo subcutâneo de indivíduos com diferentes categorias de índice de massa corporal (IMC) e avaliar se a expressão desse gene se correlaciona com diferentes parâmetros de composição corporal, TMR, resistência à insulina e perfis lipídicos e glicêmicos.

8. ARTIGO I

Current role of the NLRP3 inflammasome on obesity and insulin resistance:

A systematic review

Current role of the NLRP3 inflammasome on obesity and insulin resistance: a systematic review

Jakeline Rheinheimer[a,b], Bianca M. de Souza[a,b], Natali S. Cardoso[a], Andrea C. Bauer[a,b], Daisy Crispim[a,b]*

[a] Endocrine Division, Hospital de Clínicas de Porto Alegre. Porto Alegre, Rio Grande do Sul, Brazil.

[b] Post-graduation Program in Endocrinology, Faculdade de Medicina, Universidade Federal do Rio Grande do Sul, Porto Alegre, Rio Grande do Sul, Brazil.

Abstract

NLRP3 inflammasome activation seems to be a culprit behind the chronic inflammation characteristic of obesity and insulin resistance (IR). Nutrient excess generates danger-associated molecules that activate NLRP3 inflammasome-caspase 1, leading to maturation of IL-1β and IL-18, which are proinflammatory cytokines released by immune cells infiltrating the adipose tissue (AT) from obese subjects. Although several studies have reported an association of the NLRP3 inflammasome with obesity and/or IR; contradictory results were also reported by other studies. Therefore, we conducted a systematic review to summarize results of studies that evaluated the association of the NLRP3 with obesity and IR. Nineteen studies were included in the review. These studies focused on *NLRP3* expression/polymorphism analyses in AT. Overall, human studies indicate that obesity and IR are associated with increased *NLRP3* expression in AT. Studies in obese mice corroborate this association. Moreover, high fat diet (HFD) increases *Nlrp3* expression in murine AT while calorie-restricted diet decreases its expression. Hence, *Nlrp3* blockade in mice protects against HFD-induced obesity and IR. *NLRP3* rs10754558 polymorphism is associated with risk for T2DM in Chinese Han populations. In conclusion, available studies strongly points for an association between NLRP3 inflammasome and obesity/IR.

Keywords: *NLRP3* expression; inflammation; obesity; insulin resistance.

1. Introduction

Chronic low-grade inflammation is observed in subjects with obesity and represents a mechanistic link between obesity, insulin resistance (IR) and type 2 diabetes mellitus (T2DM) [1-3]. While several studies suggest that massive expansion of adipose tissue (AT) is an important first step in driving the enhanced inflammatory state, the underlying molecular mechanisms modulating this process are still unclear [4]. A variety of immune cells, including proinflammatory macrophages (M1-like), have been shown to infiltrate the AT and affect its homeostasis by increasing the production of cytokines such as IL-1β, IL-6 and TNF [4-6].

Macrophages and other innate immune cells can induce inflammatory reactions through detection of pathogen- or danger-associated molecular patterns (PAMPs or DAMPs) using a wide range of pattern-recognition receptors (PRRs) [7-9]. Many types of PRRs have been identified so far, including toll-like receptors (TLRs), retinoic acid-inducible gene I-like helicases (RLHs) and nucleotide-binding oligomerization domain-like receptors (NLRs) [7, 10, 11]. The NLR family, pyrin domain-containing 3 (NLRP3) cytosolic protein is certainly the most studied NLR member [11, 12]. Upon activation by PAMPs or DAMPs, NLRP3 interacts with the adapter protein apoptosis-associated speck-like protein (ASC). Then, the caspase recruitment domain (CARD) of ASC binds to the CARD domain on procaspase-1, forming the NLRP3 inflammasome [12-14]. This leads to procaspase-1 self-cleavage, generating the active caspase-1, which induces the conversion of IL-1β and IL-18 immature forms to their active forms that are secreted (**Figure 1**) [12, 14].

Compelling evidence suggests that activation of the NLRP3 inflammasome by DAMPs has a central role in obesity-induced inflammation, IR and T2DM [4, 15-20]. The role of NLRP3 inflammasome in the pathogenesis of obesity was supported by data showing that *Nlrp3*$^{-/-}$ and *Asc*$^{-/-}$ knockout (KO) mice are protected against high fat diet (HFD)-induced

obesity and IR [15, 16, 20, 21]. Moreover, NLRP3 inflammasome/caspase-1 activation seems to be a key regulator of adipocyte differentiation and directs adipocytes toward a more insulin-resistant phenotype [17]. Consistently, caloric restriction and exercise-mediated weight loss in obese subjects with T2DM reduce *NLRP3* and *IL-1β* gene expressions in abdominal subcutaneous AT (SAT), improving insulin sensitivity [15]. However, some studies were not able to find an association between NLRP3 inflammasome and obesity or IR [22-24]. Possible explanations for the contradictory results are differences in animal models analyzed, genetic variants in the *NLRP3* gene influencing its expression, differences in *NLRP3* expression between distinct AT cells, presence of comorbidities in obese patients (such as T2DM), and presence of endogenous suppressors of the inflammasome [4].

Understanding the molecular mechanisms of chronic inflammation in AT remains a major medical challenge [25]. Thus, to further investigate the association between NLRP3 inflammasome and obesity, IR and T2DM, we performed a systematic review of the literature on the subject.

2. Materials and Methods

2.1. Search strategy and eligibility criteria

This systematic review was designed and described in accordance with the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) guidelines [26]. To identify all studies that analyzed associations of *NLRP3* inflammasome with obesity, IR or T2DM, we performed an electronic literature search in PubMed and Embase repositories, without data restriction. The following medical subject headings (MeSH) were used for this search: ("NLRP3") AND ("Obesity" OR "Type 2 diabetes" OR "Insulin Resistance"). The search was completed on February 2017, was restricted to English, Spanish or Portuguese

language papers, and included both human and animal studies. All articles identified were also searched manually to identify other important citations.

Eligibility evaluation was done by title and abstract reviews and when abstracts did not provide enough information, the full text of the paper was retrieved for evaluation. This was performed independently, in a standardized manner, by two investigators (J.R. and N.S.C.). Discordances were resolved between then and, when necessary, a third investigator (D.C.) was consulted. Studies were considered eligible for inclusion if they matched at least one of the following criteria: 1) human studies that analyzed NLRP3 gene and/or protein expressions in AT from subjects with different degrees of obesity, IR or T2DM; 2) human studies that compared frequencies of *NLRP3* polymorphisms between subjects with and without these diseases; 3) studies in mice or rats that compared NLRP3 expressions in AT from lean and obese animals; 4) studies in these animals that analyzed the effect of different diets or bariatric surgery on NLPR3 expressions; and 5) animal studies that analyzed the effect of *NLRP3* knockout (KO), knockdown (KD) or overexpression on obesity, IR or T2DM.

If data were duplicated and had been published more than once, the comprehensive study was chosen for inclusion in the review. Exclusion criteria were as follows: 1) review articles; 2) duplicated articles; 3) studies that did not evaluate obesity, IR or T2DM; 4) studies that did not analyze *NLRP3* gene or protein expressions in AT or the effect of *NLRP3* KO, KD or overexpression on the diseases of interest; and 5) studies that analyzed *NLRP3* expression only after in vitro modifications.

2.2. Data extraction

Data were independently extracted by two investigators (J.R. and B.M.S.) using a standardized abstraction form [27, 28], and consensus was sought in all extracted items.

When an agreement could not be achieved, differences in data extraction were resolved by a third reviewer (D.C.) and by reading the original publication. Data extracted from each study in humans were as follow: 1) characteristics of the studies [including name of first author, publication year, study groups, number of subjects in each group, age, body mass index (BMI), homeostatic model assessment - insulin resistance (HOMA-IR), weight, gender, ethnicity, presence of T2DM, analyzed tissue]; 2) *NLRP3* polymorphism frequencies [including genotype and allele distributions in case and control groups and OR (95% CI)]; 3) gene and protein expressions in AT. For studies in animal models, the following data were extracted from each article: 1) animal model; 2) type of genetic modification (*Nlrp3* KO or transgenic); 3) type of diet administered to the animals (i.e. chow, HFD or other); 4) protein and gene expressions.

3. Results

3.1. Literature search and characteristics of eligible studies

The strategy used to identify and select studies for inclusion in this systematic review is shown in **Figure 2**. A total of 473 possibly relevant articles were retrieved by searching the electronic databases, and 430 of them were excluded during the review of titles and abstracts accordingly to the exclusion criteria mentioned above and shown in **Figure 2**. Forty-three articles therefore appeared to be eligible at this point and had their full texts evaluated. Nevertheless, after careful analysis of the full texts, another 24 studies were excluded because of missing information about NLRP3 expressions or outcomes of interest (n = 19), ineligible designs since NLRP3 expression was evaluated after in vitro interventions (n = 3), or articles with duplicated results (n = 2) (**Figure 2**). Hence, a total of 19 articles [15, 20-24, 29-41] fulfilled the eligibility criteria and were included in the review.

Among these studies, 8 reported *NLRP3* expressions in AT or polymorphism frequencies in humans [22, 23, 29, 31, 32, 37, 38, 40], 9 focused in *Nlrp3* expressions in murine models [20, 21, 24, 33-36, 39, 41], and only 2 analyzed *NLRP3* in both human and murine samples [15, 30] (**Table 1 and 2**). The main characteristics of the 10 studies performed in humans are depicted in the **Supplementary Table 1**. As can be seen in the table, case and control samples varied across studies. Four studies evaluated obese (BMI ≥30 kg/m^2) *vs.* lean (BMI< 25 kg/m^2) patients [22, 29, 30, 40], and 2 studies included obese patients before and after weight loss interventions, namely: bariatric surgery [23] or caloric restriction/ exercise lifestyle modifications [15]. Kursawe *et al*. [32] analyzed obese adolescents with low or high VAT/VAT + SAT ratio, while Bando *et al*. [31] evaluated patients who were submitted to an implantable heart device. Two studies evaluated *NLRP3* polymorphisms between T2DM patients and non-diabetic controls [37, 38]. Sample numbers as well as mean age and body mass index (BMI) widely varied among studies (**Supplementary Table 1**).

Among the 11 studies performed in mice/rats, 4 studies evaluated *Nlrp3* expressions in AT from mice fed with different HFD, calorie-restricted diet or chow diet [15, 30, 33, 35] (**Table 2**). Five studies analyzed the effect of *Nlrp3* ablation in mice fed with chow diet, low fat diet (LFD) and/or HFD [15, 20, 21, 24, 39]. Mocanu *et al*. [36] investigated the effect of bariatric surgery on *Nlrp3* expression in AT from Sprague-Dawley obese rats, and Nagareddy *et al*. [34] analyzed *Nlrp3* expression in lean or Ob/Ob mice fed on a chow diet. Wang *et al.* [41] compared *Nlrp3* and *Casp-1* expressions in epididymal fat from db/db mice and age-matched wild-type (WT) mice.

3.2. Studies that evaluated NLRP3 expressions in relation to obesity, IR and T2DM

Overall, most studies indicate that NLRP3 is upregulated in SAT or VAT from obese patients compared to lean controls [29-32, 40] (**Table 1**). Interestingly, Esser *et al*. [29] showed that *NLRP3* and *IL-1β* expressions were increased in VAT from metabolically unhealthy obese (MUO) patients compared to metabolically healthy obese (MHO) patients, and that *NLRP3* expression positively correlated with IR values. Of note, T2DM was only observed among MUO patients [29]. Accordingly, Kursawe *et al*. [32] reported that *NLRP3*, *ASC*, *CASP-1* and *IL-1β* expressions were increased in obese adolescents with high VAT/VAT+SAT ratio compared to obese adolescents with low ratio. However, Goossens *et al*. [22] did not find any difference in *NLRP3* expression between SAT from 10 obese or 9 lean male subjects. Despite this negative result, possibly explained by a small sample size, these authors observed increased expressions of *CASP-1* and Th1 transcripts (*CTBX21*/*CD3ε*) in SAT from obese compared to lean subjects. Furthermore, *CASP-1* and Th1 transcript expressions were positively correlated with IR and impaired glucose metabolism [22]. The authors concluded that NLRP3 inflammasome and a Th1 shift in the T cell population in SAT may trigger or exacerbate AT inflammatory processes, contributing to IR [22].

Regarding weight loss interventions, Vandanmagsar *et al.* [15] showed that calorie restriction- and exercise-mediated weight loss in obese T2DM patients was associated with a reduction of *NLRP3* and *IL-1β* expressions in SAT as well as with decreased inflammation and improved insulin sensitivity. In contrast, Moschen *et al.* [23] found that *NLRP3* expressions in SAT, VAT and liver from severe obese patients (7 male and 14 female) were not affected by weight loss after 6 months of laparoscopic adjustable gastric banding (LAGB) surgery; although, *IL-1β* expression decreased after the intervention. Weight loss after 6 months widely varied (mean 24.7 ± 8.1, range 18.5 to 50.5), which could be one explanation for the lack of association between *NLRP3* expression and this outcome. In addition, the

authors could not exclude posttranscriptional modifications of the NLRP3 and did not measure *CASP-1* expressions in the analyzed tissues [23].

3.3. Studies that evaluated NLRP3 expressions in animal models

Four studies compared *Nlrp3* expression in AT from mice fed with different diets [15, 30, 33, 35]. Percentages of fat or other components in analyzed diets are shown in **Table 2**. Yin *et al.* [30] found that *Nlrp3* expression was increased in SAT from C57BL/6J mice fed by 3 months with a HFD compared with C57BL/6J control mice; however, Betanzos-Cabrera *et al*. [33] were not able to find any difference in *Nlrp3*, *Asc*, *Casp-1* and *Il-1β* expressions in AT or liver from CD-1 mice fed by 4 months with HFD or chow diet. Finucane *et al.* [35] reported that *Nlrp3*, *Casp-1* and *Il-1β* expressions in AT were increased in C57BL/6J mice treated during 6 months with saturated fatty acid (SFA)-HFD diet compared to mice fed with both monounsaturated fatty acid (MUFA)-HFD and chow diet. Vandanmagsar *et al.* [15] showed decreased *Nlrp3* expression in VAT and SAT from C57BL/6J mice fed with a 40% calorie-restricted diet compared to the chow diet group, and this expression positively correlated with body weight and adiposity in VAT from these animals.

Accordingly to the above-mentioned studies, *Nlrp3* was increased in stromal vascular cells (SVCs) from obese (Ob/Ob) mice compared to C57BL/6J lean mice [34], and the expression of this gene in omental fat from Sprague-Dawley obese rats decreased after 3 months post-bariatric surgery-mediated weight loss [36]. Wang *et al.* [41] reported that *Nlrp3* and *Casp-1* expressions were increased in epididymal fat from db/db mice compared with age-matched C57BL/6J wild-type mice.

Five studies evaluated the effect of *Nlrp3* KO in mice fed with different diets [15, 20, 21, 24, 39]. Stienstra *et al.* [20] reported that *Nlrp3*$^{-/-}$ C57BL/6J mice were protected against HFD-induced obesity and IR and had smaller adipocyte size and macrophage infiltration in

VAT compared to control mice after 4 months of follow-up. Moreover, ablation of *Nlrp3* prevented inflamassome activation in fat depots and liver and enhanced insulin signaling in C57BL/6J mice fed with HFD during 6 weeks [15]. Wen *et al*. [21] showed that under HFD during 3 months, blood glucose and insulin levels were significantly lower in *Nlrp3*$^{-/-}$ C57BL/6J mice compared to wild type mice. In contrast, in mice fed with chow diet, *Nlrp3* KO had no effect on weight gain and glucose homeostasis compared to WT mice [24]. Ringling *et al*. [39] reported that *Nlrp3*$^{-/-}$ C57BL/6J mice were not protected from obesity, AT inflammation or glucose intolerance after 6 months of treatment with a Western diet (45% of fat and 1% of cholesterol). However, *Nlrp3*$^{-/-}$ mice were protected against Western diet-induced aortic stiffening, exhibited smaller cardiomyocytes, and had reduced cardiac fibrosis compared to wild-type mice fed under the same conditions [39].

3.4. Studies that evaluated associations of polymorphisms in the NLRP3 gene and obesity, IR or T2DM

To date, no study has evaluated the association between *NLRP3* polymorphisms and obesity. Two studies compared frequencies of *NLRP3* polymorphisms between T2DM patients and non-diabetic subjects [37, 38]. Zheng *et al*. [37] reported that the G/G genotype of the *NLRP3* rs10754558 (C/G) polymorphism was associated with risk for T2DM in a Chinese Han population (OR= 1.26, 95% CI 1.07-1.75) as well as with higher LDL-cholesterol and IR levels. The *NLRP3* rs4612666 (C/T) polymorphism was not associated with T2DM in this population [37]. Another study performed in the Chinese population investigated the *NLRP3* rs10754558 (C/G), rs7512998 (T/C) and rs12137901 (C/T) polymorphisms, replicating the association between the rs10754558 G/G genotype and T2DM risk (OR= 1.81, 95% CI 1.16-2.83) [38]. The other 2 polymorphisms analyzed were not associated with the disease [38].

4. Discussion

Sterile chronic inflammation is a cornerstone of immune activation in obesity and IR. The molecular mechanisms of this inflammatory state include activation of the NLRP3 inflammasome by over-nutrition [42]. Accordingly, several studies have reported an association of the NLRP3 inflammasome with obesity, IR or T2DM [4, 15, 17-20]; however, contradictory results were reported by other studies [22-24, 33]. Therefore, we performed a systematic review aiming to summarize the results of studies on this subject.

The majority of human studies indicate that obesity is associated with increased *NLRP3* expression in AT [15, 29-32, 40]. Studies in murine models of obesity also corroborate this association [34, 36]. These results are somehow expected since components of the NLRP3 inflammasome activate IL-1β and IL-18, important proinflammatory cytokines released by immune cells infiltrating the AT from obese subjects [4] (**Figure 1**). Due to many functions of IL-1β, its production is tightly regulated, requiring 2 signals [43]. The first signal (called "priming") is provided by glucose, palmitate, ceramide, uric acid or lipopolysaccharide (LPS) [4, 42]. These DAMPs/PAMPs are recognized by TLRs, activating mainly nuclear factor-κB (NF-κB)-dependent signaling pathways that trigger the expression of the inflammasome components, pro-IL-1β and pro-IL-18, which, after translation, remain in the cytoplasm in inactive forms. Then, a second signal leads to NLRP3 inflammasome assembly and activation, leading to caspase-1-dependent cleavage of pro-IL-1β into the active IL-1β [4, 11, 12, 42, 43]. Three models have been suggested to explain the second signal: 1) extracellular ATP inducing K^+ efflux; 2) DAMPs/PAMPs triggering the generation of mitochondrial reactive oxygen species (ROS); and 3) phagocytosed irritants, such as amyloid-β, forming crystalline or particulate structures, which can cause lysosomal rupture and release of lysosomal components, as cathepsin B [12, 43].

NLRP3 inflammasome-activated IL-1β has an important role in the development of obesity-induced IR and T2DM [4, 20]. IL-1β directly inhibits insulin signaling pathways by reducing tyrosine phosphorylation of insulin receptor substrate-1 (IRS-1) and negatively regulating *IRS-1* gene expression; thus, inducing IR [44]. Hence, in animal studies, lack of IL-1β protects against AT inflammation and IR upon HFD feeding, consequently improving glucose homeostasis [21, 45]. In humans, increased IL-1β levels correlated with T2DM development [46], and the blockade of this cytokine signaling improved glycemic control whereas reducing markers of systemic inflammation [47]. Noteworthy, *NLRP3* and *IL-1β* expressions seem to be increased in VAT from MUO patients compared to MHO patients, with *NLRP3* expression positively correlating with IR values [29]. Moreover, *NLRP3* and *IL-1β* expressions decreased in SAT from T2DM patients after 1 year of calorie restriction- and exercise-mediated weight loss [15].

Elevated circulating levels of free fatty acids (FFAs) have been shown to be an important hallmark of obesity, IR and T2DM [48, 49]. Dietary FFAs are recognized DAMPs that activate the NLRP3 inflammasome [4, 12, 18, 21, 50], establishing a mechanistic link between nutrient excess and inflammation. Saturated FFAs (palmitate) can act both as first or second signals necessary for inflammasome activation [4, 21]. Moreover, Wen *et al*. [21] showed that palmitate can lead to inflammasome activation and IL-1β release through a newly identified AMPK-autophagy-ROS signaling pathway. Increased palmitate levels also induce intracellular accumulation of ceramide, which is another potent activator of the NLRP3 inflammasome in macrophages, providing a continuous supply of DAMPs for inflammasome activation [11, 15]. Studies in macrophages and animal models also shows that oxidized LDL and cholesterol crystals trigger inflammasome activation [12]. Importantly, unsaturated FFAs (oleate and linoleate) and omega-3 fatty acids prevented activation of NLRP3 in human macrophages [51, 52].

In agreement with this background, most studies included also indicate that HFD increases *Nlrp3* expression in AT from mice while calorie-restricted diet seems to decrease the expression of this gene [15, 30, 35]. For that reason, the ablation of *Nlrp*3 in murine models seems to be able to protect against HFD-induced obesity and IR and also decrease blood glucose and insulin levels [15, 20, 21]. In contrast with these studies, Betanzos-Cabrera *et al*. [33] did not found any difference in *NLRP3* expression in liver or AT from CD-1 mice fed with HFD or chow diet during 4 months. In addition, Ringling *et al*. [39] reported that *NLRP3*$^{-/-}$ C57BL/6J mice were not protected against Western diet-induced weight gain, AT inflammation and glucose intolerance. Weight gain also does not seem to be affected by *Nlrp3*$^{-/-}$ in mice fed with a chow diet [24]. These contradictory results might be explained by different type of diets used (including amount of fat and type of fat), duration of feeding, and differences in animal models studied, such as mice with different background or varied degrees of metabolic disease or obesity.

Polymorphisms in the *NLRP3* gene have been associated with several diseases, such as Crohn's disease [53], rheumatoid arthritis [54], systemic lupus erythematosus [55], aspirin-induced asthma [56], HIV infection [57], and celiac disease and type 1 diabetes [58]. Only 2 studies evaluated the association between *NLRP3* polymorphisms and T2DM, both suggesting the association of the rs10754558 (C/G) polymorphism with risk for this disease [37, 38]. Zheng *et al*. [37] also reported that the rs10754558 G allele was associated with increased IR and LDL levels compared to patients carrying the C/C genotype. This polymorphism is located in the 3′UTR of the *NLRP3* gene, and the G allele seems to increase *NLRP3* mRNA stability, consequently increasing its expression [56]. Until now, no study has evaluated the association between *NLRP3* polymorphisms and obesity. Thus, further studies are necessary to confirm if *NLRP3* polymorphisms are associated with T2DM and/or obesity in different ethnicities.

In conclusion, this systematic review indicates that obesity is associated with increased *NLRP3* expression in AT from humans and mice. Hence, the blockade of this gene in murine models is able to protect against HFD-induced obesity and IR; although this might not reflect what happens in human since murine models do not always mimics human pathophysiology. Additional studies need to evaluate the association of *NLRP3* polymorphisms with these diseases.

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Conflict of interest

All authors declare no conflict of interest.

Author's contributions

J.R. conceived and designed the study, collected and analyzed data, and wrote the manuscript; B.M and N.S.C, collected and analyzed data; A.C.B. contributed to figure creation, the discussion, and reviewed the manuscript. DC conceived and designed the study, analyzed data, and wrote the manuscript. All authors read and approved the final manuscript.

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Legends of figures:

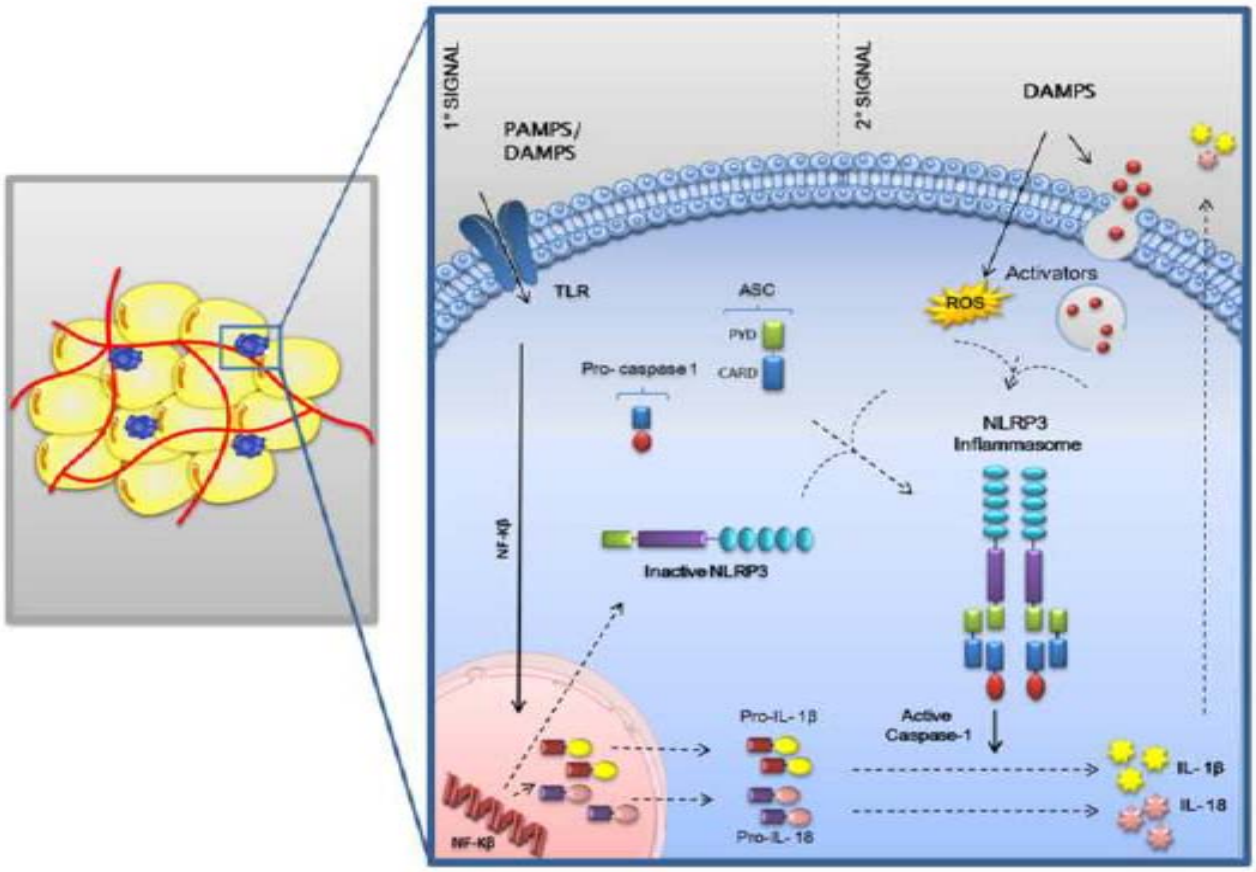

Figure 1. NLRP3 inflammasome activation. Two signals are necessary to activate NLRP3 inflamassome. In obese patients, macrophages present in adipose tissue are activated upon exposure to pathogen-associated molecular patterns (PAMPs) or danger-associated molecular patterns (DAMPs) acting mainly through toll-like receptors (TLRs). This first signal activates NF-κB in the nucleus, leading to transcription of inactive NLRP3, pro-IL-1β and pro-IL-18, which, after translation, remain in the cytoplasm in its inactive forms. The second signal, that includes reactive oxygen species (ROS) generation and lysosomal rupture, among others, activates the NLRP3 inflammasome by facilitating the oligomerization of the inactive NLRP3, the apoptosis-associated speck-like protein (ASC), and the procaspase-1. This

activated complex, in turn, catalyzes the conversion of procaspase-1 to caspase-1, which will promote the conversion of pro-IL-1β into IL-1β and pro-IL-18 into IL-18.

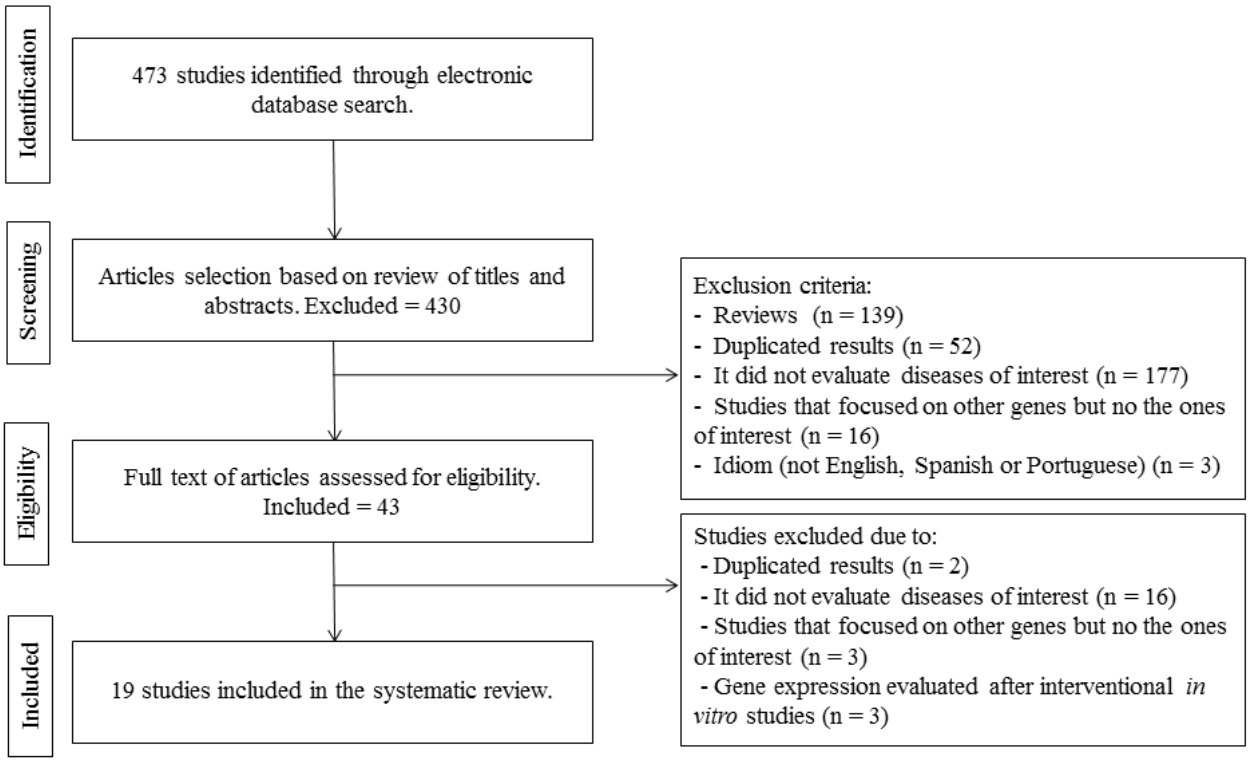

Figure 2. Flowchart illustrating the search strategy used in the systematic review

Table 1. Associations between *NLRP3* expressions and obesity or IR in human.

1st author, year (Ref)	Samples	Tissue	Results
Bando, 2015 [31]	Patients who had implanted a heart device.	SAT	↑ *NLRP3* expression in patients with obesity, dyslipidemia and/or DM. *NLRP3* correlated with BMI (+) and adiponectin levels (-).
Esser, 2013 [29]	MUO and MHO patients *vs*. lean subjects.	SAT/VAT	↑ *NLRP3* in VAT from MUO patients *vs*. MHO and lean subjects; *NLRP3* correlated with IR (+).
Goossens, 2012 [22]	Obese patients *vs*. lean subjects.	SAT	↔ *NLRP3* was similar between groups.
Kursawe, 2016 [32]	Obese adolescents.	SAT	↑ *NLRP3* in SAT from adolescents with high VAT/VAT+SAT ratio *vs*. low ratio group.
Moschen, 2011 [23]	Severely obese patients (before *vs*. after LAGB-surgery).	SAT/VAT/ liver	↔ *NLRP3* was not affected by weight loss.
Serena, 2016 [40]	Obese *vs*. lean subjects.	ASCs	↑ *NLRP3* expression in ASCs from obese and/or T2DM subjects *vs*. lean subjects.
Vandanmagsar, 2011 [15]	Obese T2DM patients (before *vs*. after 1 year-weight loss);	SAT	↓ *NLRP3* in SAT from T2DM patients after weight loss, which associated with improved insulin sensitivity;

Yin, 2014 [30]	Lean *vs*. obese women;	SAT	↑ *NLRP3* in SAT of obese *vs*. lean women;

ASCs = Adipose derived-stem cells; AT = Adipose tissue; BMI = Body mass index; DM = diabetes mellitus; IR = insulin resistance; LAGB = Laparoscopic adjustable gastric banding; MHO = Metabolic healthy obese; MUO = Metabolically unhealthy obese; SAT = Subcutaneous AT; T2DM = Type 2 DM; VAT = Visceral AT.

Table 2. Associations between *NLRP3* expressions and obesity or IR in mice.

1st author, year (Ref)	Samples	Tissue	Results
Betanzos-Cabrera, 2012 [33]	CD-1 male mice (HFD *vs*. chow diet). HFD: 50% saturated fat, 32% carbohydrate, 18% protein (4 months).	Liver/AT	↔ *Nlrp3* was similar between groups.
Finucane, 2015 [35]	C57BL/6 male mice (SFA-HFD *vs*. MUFA-HFD *vs*. chow diet). SFA-HFD: 45% palmitic acid; MUFA-HFD: 45% oleic acid (24 weeks).	AT	↑ *Nlrp3* in SFA-HFD mice *vs*. MUFA-HFD or chow diet mice.
Jin, 2011 [24]	*Nlrp3*$^{-/-}$ male mice (chow diet).	-	↔ Weight gain was similar between *Nlrp3*$^{-/-}$ mice and WT mice.
Mocanu, 2015 [36]	Sprague-Dawley obese rats (RYGB- vs. sham-surgery). HFD: 60% fat (12 weeks).	SAT/omental/ mesenteric AT	↓ *Nlrp3* in omental AT from the RYGB group *vs*. controls (90 days after surgery). *Nlrp3* correlated with changes in body weight (+).

Nagareddy, 2014 [34]	Lean C57BL/6J *vs*. Ob/Ob male mice.	SVCs/VAT	↑ *Nlrp3* in SVCs from Ob/Ob *vs*. lean mice.
Ringling, 2016 [39]	*Nlrp3*$^{-/-}$ male C57BL/6J mice (Western diet *vs*. chow diet). Western diet: 44.9% fat, 35.1% carbohydrate, 20% protein (24 weeks).	White and brown AT	↔ *Nlrp3*$^{-/-}$ did not protect against western diet induced-weight gain, -AT inflammation, and -glucose intolerance.
Stienstra, 2011 [20]	*Nlrp3*$^{-/-}$ C57BL/6 male mice (HFD *vs*. LFD). HFD: 45% fat; LFD: 10% fat (4 months).	VAT	↓ HFD-induced obesity and IR in *Nlrp3*$^{-/-}$ mice. ↓ adipocyte size and macrophage infiltration in VAT from in *Nlrp3*$^{-/-}$ mice.
Vandanmagsar, 2011 [15]	C57BL/6 female mice (chow *vs*. 40% calorie-restricted diet); *Nlrp3*$^{-/-}$ male mice [HFD (60% fat) *vs*. chow diet; 6 weeks].	VAT	*Nlrp3* expression in murine VAT correlated with body weight (+); ↑ insulin signaling in *Nlrp3*$^{-/-}$ HFD-mice.
Wang, 2015 [41]	db/db *vs.* WT C57BL/6J male mice	epididymal fat	↑ *Nlrp3* in db/db *vs*. WT mice.

Wen, 2011 [21]	*Nlrp3*$^{-/-}$ C57BL/6 male mice (HFD) HFD: 40% fat (12 weeks).	Plasma	↓ blood glucose and insulin levels in *Nlrp3*$^{-/-}$ HFD-mice *vs*. WT mice.
Yin, 2014 [30]	C57BL/6J male mice [HFD (60% fat) *vs*. chow diet; 12 weeks].	epididymal fat	↑ *Nlrp3* in fat from HFD *vs*. chow diet mice.

AT = Adipose tissue; HFD = High fat diet; IR = insulin resistance; LFD = Low fat diet; MUFA = Monounsaturated fatty acids; RYGB = Roux-en-Y Gastric Bypass; SAT = Subcutaneous AT; SFA = Saturated fatty acids; SVCs = Stromal vascular cells; VAT = Visceral AT; WT = wild type.

Miao, 2014	Camundongo com *Cgi-58*$^{-/-}$ MaKO (DATG *vs*. dieta normal).	Fígado/TA epididimal	↑ RI, intolerância à glicose e inflamação em camundongos com *Cgi-58*$^{-/-}$ MaKO alimentados com DATG *vs*. dieta normal.
Miao, 2015	Camundongo C57BL/6 (DATG *vs*. dieta normal); Indivíduos saudáveis.	TAS/TAV (camundongo); CMSP (humanos)	↓ *Cgi-58* in TAS e TAV de camundongos alimentados com DATG *vs*. controles. Expressão de *Cgi-58* em macrófagos do TA correlacionou-se com ganho de peso em camundongos alimentados com DATG (-); *CGI-58* em CMSP de humanos correlacionou-se com IMC (-).
Steinberg, 2007	Indivíduos magros *vs*. indivíduos com obesidade.	TAS/TAV	↔ *CGI-58* TAS e TAV.
Xie, 2015	Camundongo com *Cgi-58*$^{-/-}$ (mCgi58-KO) *vs*. controle (DATG).	TA/fígado/ coração /músculos	↑ tolerância à glicose e sensibilidade à insulina em camundongos *mCgi58-KO vs*. camundongos controles; ↔ distribuição de massa magra e gorda em camundongos mCgi58-KO e controles.

ASO = *antisense oligonucleotides*; TA = tecido adiposo; IMC = índice de massa corporal; DATG = dieta com alto teor de gordura; RI = resistência à ação de insulina; KD = *knockdown*; MaKO = *knockout* macrofágos-específico para *Cgi-58*; mCgi58-KO = *knockout* músculo-específico para *Cgi-58*; CMSP = células mononucleares do sangue periférico; TAS = TA subcutâneo; Tg = transgênico; TAV = TA visceral.

9. ARTIGO II

CGI-58 gene expression is decreased in the adipose tissue of patients with obesity

***CGI-58* gene expression is decreased in the adipose tissue of patients with obesity**

Jakeline Rheinheimer[a,b], Bianca Marmontel de Souza[a,b], Natali Silva Cardoso[a], Cristiane Bauermann Leitão[a,b], Milene Moehlecke[c], Andrea Carla Bauer[b], Daisy Crispim[a,b]*

[a] Endocrine Division, Hospital de Clínicas de Porto Alegre. Porto Alegre, Rio Grande do Sul, Brazil.

[b] Postgraduation Program in Medical Sciences: Endocrinology, Faculdade de Medicina, Universidade Federal do Rio Grande do Sul, Porto Alegre, Rio Grande do Sul, Brazil.

[c] Department of Endocrinology, Faculdade de Medicina, Universidade Luterana do Brasil, Canoas, Rio Grande do Sul, Brazil.

ABSTRACT

The protein comparative gene identification 58 (CGI-58) markedly enhances adipose triglyceride lipase (ATGL)-mediated lipolysis. To date, only few studies have evaluated the association between CGI-58 and obesity, with inconclusive results. Thus, we compared *CGI-58* expression in subcutaneous adipose tissue (SAT) of subjects with different body mass index (BMI) categories. We also evaluated if *CGI-58* correlates with body composition parameters, resting energy expenditure (REE), insulin resistance (IR), and lipid and glycemic profiles. SAT biopsies were obtained from 67 individuals who underwent bariatric surgery or elective abdominal surgery. Patients were divided in: Group 1 (n = 14; BMI < 27.0 kg/m^2), Group 2 (n = 24; BMI 30.0 - 39.9 kg/m^2) and Group 3 (n = 29; BMI ≥ 40.0 kg/m^2). All subjects underwent physical and laboratory evaluations. *CGI-58* expression was quantified using RT-qPCR. *CGI-58* expression was decreased in patients from Group 3 [median 0.60 (0.45 – 0.85, 25th – 75th percentiles)] and Group 2 [0.85 (0.50 – 1.23)] compared to Group 1 patients [1.70 (0.99 – 2.70)] (P < 0.001). *CGI-58* values above median were associated with lower risk for obesity, adjusting for covariables (OR = 0.036, 95% CI 0.003 – 0.41). Moreover, *CGI-58* expression was negatively correlated with body composition parameters (BMI, waist circumference, fat mass, fat-free mass), REE, lipid profile (total cholesterol and triglycerides), IR, and HbA1c, while it was positively correlated with HDL cholesterol. In conclusion, *CGI-58* expression is decreased in patients with obesity, and it was associated with worse metabolic profile.

Keywords: Obesity; CGI-58, Gene Expression; Adipose Tissue.

1. Introduction

Obesity results from a chronic imbalance between energy intake and expenditure, and it is associated with increased prevalence of metabolic diseases such as systemic inflammation, insulin resistance (IR) and dyslipidemia, which can predispose to type 2 diabetes mellitus (T2DM) and cardiovascular diseases (1, 2). In adipose tissue, excess of energy is stored inside cytosolic lipid droplets as triglycerides. During fasting or exercise, the stored fat is mobilized for utilization as free fatty acids (FFAs) and glycerol via lipolysis (3, 4). Lipolysis in white adipose tissue (WAT) is an orderly and regulated process that confers to WAT the capacity to adapt to various nutritional and physiological conditions (5). The complete hydrolysis of triglycerides is mediated by the orchestrated activation of adipose triglyceride lipase (ATGL), hormone-sensitive lipase (HSL), and monoacylglycerol lipase (3, 4).

There is compelling evidence that the protein comparative gene identification 58 (CGI-58), also known as α/β hydrolase domain-containing protein 5 (ABHD5), markedly enhances ATGL-mediated lipolysis (6-8). Loss-of-function mutations in the human *CGI-58* gene cause the Chanarin-Dorfman syndrome (CDS), a rare autosomal recessive disease where triglycerides accumulate in lipid droplets from several tissues, causing ichthyosis, which can be accompanied by liver steatosis, cardiomyopathy, ataxia, hearing loss, and mental retardation (9, 10). Although the molecular basis of ATGL activation by CGI-58 remains unclear, it seems that in basal conditions, CGI-58 is repressed by its binding to perilipin-1 (PLIN1) on the surface of lipid droplets. Upon hormonal stimulation of lipolysis, PLIN1 is phosphorylated by protein kinase A, releasing CGI-58, which then activates ATGL (8).

Although CGI-58 is a recognized co-activator of ATGL-mediated lipolysis, to date, only few studies have evaluated the association of this protein with obesity and IR (11-18), showing inconclusive results. *Cgi-58* gene expression in visceral adipose tissue (VAT)-derived macrophages negatively correlated with high fat diet (HFD)-induced weight gain in mice (14). However, mice overexpressing *Cgi-58* were not protected against HFD-induced obesity (11). Intriguingly, *Cgi-58* blockade in adipose

tissue, liver or muscle prevented high fat diet (HFD)-induced obesity and/or IR in mice (12, 15, 18), while *Cgi-58* blockade in adipose tissue-derived macrophages (MaKO) increased IR after HFD (13). In humans, only few studies have evaluated *CGI-58* expression in adipose tissue from patients with obesity and lean subjects, with inconclusive results (16, 19-21).

Taking into account that little is known about the association between CGI-58 and obesity, we therefore aimed to compare *CGI-58* expression in subcutaneous adipose tissue (SAT) from subjects with different body mass index (BMI) categories. We also evaluated if *CGI-58* expression correlates with different parameters of body composition, resting energy expenditure (REE), IR, and lipid and glycemic profiles.

2. Methods

2.1. *Study subjects*

This case-control study comprised 67 patients recruited at Hospital de Clínicas de Porto Alegre (HCPA) between August 2013 and August 2016. Patients were divided into 3 groups according to BMI categories, as follows: 1) 14 lean patients with BMI between 18.5 and 27.0 kg/m^2 (Group 1); 2) 24 patients with BMI 30.0 – 39.9 kg/m^2 (Group 2: obesity degrees 1 or 2), and 3) 29 patients with BMI ≥ 40.0 kg/m^2 (Group 3: obesity degree 3). Of note, obesity was classified following WHO guidelines (22) and also as proposed by Lipschitz *et al*. (23) for elderly patients (subjects without obesity: BMI < 27.0 kg/m^2). Biopsies of SAT were collected during elective laparoscopic abdominal surgeries (Groups 1 and 2) or during an open Roux-en-Y gastric bypass surgery (Group 3). Eligible participants had to be 18-years old or older. Exclusion criteria were: presence of systemic infection, impaired thyroid function, any acute inflammatory disease, cancer, current treatment with systemic corticosteroids, pregnancy or use of any medication known to influence REE. Clinical, anthropometric, body composition, REE, and

biochemical and hormonal measurements were assessed preoperatively. The study protocol was approved by the Ethical Committee of HCPA, and written informed consent was obtained from all patients prior to enrollment.

2.2. *Clinical, body composition, biochemical and hormonal measurements*

A standard questionnaire was used to collect information regarding socio-demographic status, lifestyle habits (physical activity, smoking status, and alcohol consumption), and actual and previous health history and use of medications. All patients underwent physical and laboratory evaluations. Briefly, they were weighted unshod, wearing light clothes and their height was measured. BMI was calculated as weight (kg)/height^2 (meters). Body composition [fat mass and fat-free mass (FFM)] was evaluated using dual-energy absorptiometry X-ray (DXA; Lunar Prodigy Advance; GE Medical Systems, Madison, WI, USA). REE was assessed by an open-circuit indirect calorimetry (Korr Medical Technologies, Model 7100, Salt Lake City, UT, USA). Respiratory data were collected over a 30-minute period, in the morning, after 12 hours of fasting.

Blood pressure (BP) was measured in sitting position, on the left arm, after a 5-min rest, with a digital sphygmomanometer Onrom (HEM-705CP). The mean of two measurements taken 1 min apart was used to calculate systolic and diastolic BP. Systemic arterial hypertension was defined as blood pressure (BP) ≥ 140/90 mmHg in two occasions or use of antihypertensive medications. Diagnosis of T2DM and pre-diabetes (pre-DM) was established according to the American Diabetes Association guidelines.

Fasting blood samples were taken from all patients before surgery for laboratory analyses. Total plasma cholesterol, HDL cholesterol and triglycerides were measured using enzymatic methods, and LDL cholesterol was calculated with the Friedewald equation. Fasting plasma glucose (FPG) levels were determined using the glucose oxidase method. Glycated hemoglobin (HbA1c) measurements were

performed by high performance liquid chromatography of ion-exchange (Variant II Turbo; Bio-Rad, USA). Serum insulin was quantified by radioimmunoassay (Elecsys R Systems 1010/2010/ modular analysis E170 - ROCHE), and IR was estimated using the homeostasis model assessment (HOMA-IR) index = fasting insulin (μUI/ml) x fasting plasma glucose (mmol/L) / 22.5 (24). Leptin and adiponectin levels were quantified in plasma using ELISA kits (Thermo Fisher Scientific; DE, USA), following the manufacturer's instructions. Serum 25-hydroxi vitamin D [25(OH)D] levels were measured using a chemiluminescent assay (Architect System, Abbott Ireland Diagnostic Division; Longford, Ireland).

2.3. *Quantification of CGI-58 expression in adipose tissue by real-time PCR*

Immediately following SAT biopsy collection, samples were placed in RNAlater solution (Thermo Fisher Scientific) and stored at -80°C until gene expression analyses. Total RNA was extracted from 100 mg of SAT using mirVana™ miRNA Isolation Kit (Thermo Fisher Scientific), according to the manufacturer's instructions. Concentration and quality of RNA samples were assessed using a NANODROP 2000 spectrophotometer (Thermo Fisher Scientific). Only RNA samples with adequate purity ratios (A260/A280 = 1.9-2.1) were used for subsequent analyses. In addition, RNA integrity and purity were checked in agarose gels containing GelRed Nucleic Acid Gel Stain (Biotium, Inc., Hayward, CA, USA). The mean RNA concentration [± standard deviation (SD)] isolated from SAT was 80 ± 31 ng/μL.

Reverse transcription of 250 ng of RNA into cDNA was carried out using the SuperScript VILO Master Mix (Thermo Fisher Scientific), following the manufacturers protocol for the random primer method. Then, cDNA was amplified by quantitative real-time PCR (RT-qPCR). RT-qPCR experiments were performed in a 7500 Fast Real-Time PCR System Thermal Cycler (Thermo Fisher Scientific), using 1 μL of 20X TaqMan Gene Expression Assays (Thermo Fisher Scientific) for *CGI-58* (ID: HS01104373_m1) or for the reference gene hypoxanthine phosphoribosyl-transferase 1 (*HPRT1*; ID

HS99999909_m1), 10 µL of 2X TaqMan Universal Master Mix II (Thermo Fisher Scientific), and 1µL of cDNA template (diluted to 100 ng/µl), in a total volume of 20 µL. Each sample was assayed in triplicate, and a negative control was included in each experiment. Quantification of *CGI-58* mRNA was performed by relative quantification using the comparative ΔΔCq method, and expressed relative to the *HPRT1* gene (25). The ΔΔCq method calculates changes in gene expression as relative fold differences (n-fold changes) between an experimental and an external calibrator sample (25).

2.4. *Statistical analyses*

Normal distribution of variables was checked using the Shapiro-Wilk test. Variables with normal distributions are shown as mean ± SD. Variables with skewed distribution were log-transformed before analyses and are shown as median (25^{th} –75^{th} percentiles). Categorical data are shown as percentage. Clinical and laboratory characteristics and *CGI-58* expressions were compared among groups by using unpaired Student's t-test, one-way ANOVA or χ^2 tests, as appropriate. Correlations between quantitative variables were calculated using Pearson's correlation coefficients. Multivariate logistic regression analysis was performed to assess the independent association of *CGI-58* gene expression with presence of obesity (Groups 2 + 3) as well as to control for possible confounding factors whenever a statistically significant association was found in univariate analyses or based on biological relevance. All statistical analyses were performed using SPSS version 21.0 (IBM SPSS Statistics, Chicago, IL, USA), and P values < 0.05 were considered statistically significant.

3. Results

3.1. *Sample description*

Sixty-seven patients were analyzed, most of them female (71.6%), white (82.1%), and with a mean age of 46.2 ± 13.9 years old. Anthropometric, body composition, REE, clinical and laboratory characteristics of patients according to BMI categories are shown in Table 1. Patients from Group 3 had increased HbA1c levels, HOMA-IR values and prevalence of hypertension compared to patients from Group 1. HDL levels were decreased in Group 3 patients compared to Group 1, while insulin levels were similarly increased in Group 2 and 3 patients in comparison with Group 1 subjects. Moreover, triglyceride levels were differently distributed among the three study groups, while FPG, REE, fat mass and FFM were increased in patients from Group 3 compared to the other two groups (Table 1).

3.2. *CGI-58 expression in SAT from patients with different body mass indexes*

CGI-58 expressions according to BMI categories are depicted in Figure 1. Patients from Group 2 and 3 showed decreased *CGI-58* expressions in SAT compared to Group 1 patients [Group 3: 0.60 median (0.45 – 0.85, 25^{th} –75^{th} percentiles); Group 2: 0.85 (0.50 – 1.23); Group 1: 1.70 (0.99 – 2.70) n-fold changes; $P < 0.001$] (Figure 1). *CGI-58* expression was similar between white and non-white subjects [0.76 (0.48 – 1.28) *vs*. 0.91 (0.74 – 1.45); P = 0.420]. Of note, when we re-classified Group 1 excluding those elderly patients with BMI between 25.0 to 27.0 kg/m^2 (n = 6), *CGI-58* expressions remained downregulated in patients with obesity (Groups 2 and 3: same values as above) compared to normal weight subjects [2.48 (1.67 – 3.42); $P < 0.001$)].

Next, we divided patients above and below median *CGI-58* values. Interesting, 75% (n = 21) of Group 3 patients had *CGI-58* expression below the median value (0.83 n fold changes) compared to 48% (n = 12) of Group 2 patients and to only 7.1% (n = 1) of Group 1 patients (P trend < 0.001). After logistic regression analysis, *CGI-58* values above median were independently associated with lower risk for obesity, after adjustment for gender, ethnicity, presence of hypertension and T2DM (OR = 0.036, 95% CI 0.003 – 0.412; P = 0.007).

3.3. Correlations between CGI-58 expression in SAT and clinical, body composition, REE and laboratory characteristics

Correlation analyses between *CGI-58* expressions and body composition, REE, clinical and laboratory characteristics are shown in Table 2. *CGI-58* expression was negatively correlated with BMI (r = -0.432, P < 0.001), waist circumference (r = -0.600, P < 0.001), fat mass (r = -0.380, P = 0.003), FFM (r = -0.488, P < 0.001), and REE (r = -0.373, P = 0.002). Regarding lipid profile, *CGI-58* expression was negatively correlated with triglycerides (r = -0.585, P < 0.001) and cholesterol levels (r = -0.315, P = 0.050), while was positively correlated with HDL levels (r = 0.250, P = 0.046). Negative correlations were also found between *CGI-58* expression and HbA1c levels (r = -0.421, P < 0.001), insulin levels (r = -0.332, P = 0.009), and HOMA-IR (r = -0.317, P = 0.013) (Table 2). These correlations were also analyzed only in patients with obesity (Groups 2 + 3) and, in this case, *CGI-58* expression kept negatively correlated with waist circumference (r = -0.324, P = 0.018), FFM (r = -0.415, P = 0.002), HbA1c levels (r = -0.427, P < 0.001) and triglyceride levels (r = -0.386, P = 0.005) (Table 2).

3.4. *Correlation between CGI-58 expressions and circulating adipokines and vitamin D*

Adiponectin is an adipokine with remarkable insulin sensitizing properties as well as anti-inflammatory and antiatherogenic actions (26). Leptin is another important adipokine and controls lipid metabolism through inhibition of lipogenesis and stimulation of lipolysis (26, 27). Vitamin D also seems to be involved with regulation of lipogenesis/lipolysis and reduction of inflammation (28). Therefore, we also investigated correlations among *CGI-58* expression and adiponectin, leptin and vitamin D levels.

As shown in **Table 2**, *CGI-58* expression in SAT showed a positive correlation with adiponectin (r = 0.250, P = 0.050) and vitamin D (r = 0.366, P = 0.004) values. No correlation was found between

leptin levels and *CGI-58* expression. In patients with obesity, *CGI-58* expression was positively correlated with vitamin D levels (r = 0.399, P = 0.004) (**Table 2**).

4. Discussion

CGI-58 is a lipid droplet-associated protein that has an important role in mediating intracellular fat hydrolysis by acting as a coactivator of the ATGL (6-8). Despite this, little is known about the association of CGI-58 with obesity and its related metabolic disorders. Of note, the associations of ATGL and HSL lipolytic enzymes with obesity are well established (16, 19, 21, 29-32). In this study, we showed that *CGI-58* expression in SAT was decreased in patients with obesity compared to lean subjects. Accordingly, *CGI-58* values above the median observed in our sample were independently associated with lower risk for obesity.

To our knowledge, only few previous studies have evaluated *CGI-58* expressions in humans (14, 16, 19-21). In agreement with our data, *CGI-58* was downregulated in SAT of subjects with obesity in comparison with lean subjects (20, 21). Moreover, Karki *et al*. (19) showed that *CGI-58* expression in SAT of patients with severe obesity (degree 3) increased after bariatric surgery-mediated weight loss. However, Steinberg *et al.* (16) reported that patients with obesity and lean subjects had similar *CGI-58* expressions in SAT and VAT. Interestingly, Miao *et al.* (14) found that *CGI-58* expression in peripheral blood mononuclear cells (PBMCs) was negatively correlated with BMI in healthy subjects.

Studies in mice have shown inconclusive results (11-15, 18, 33-38). Two studies showed decreased *Cgi-58* expressions in WAT from mice fed with HFD compared to mice fed with chow diet (control group) (14, 38). Bae *et al*. (37) also observed decreased *Cgi-58* expression in VAT of HFD mice compared to control mice after 8 weeks of treadmill exercise. In contrast, Gaidhu *et al.* (33) found that *Cgi-58* expression was increased in VAT and SAT of HFD mice compared to control mice. Another

study did not show any significant difference in Cgi-58 protein levels in epididymal adipose tissue of HFD- and chow diet-mice (34).

A number of studies have evaluated the effect of tissue-specific *Cgi-58* knockout or knockdown using anti-sense oligonucleotides (ASO) in mice fed with HFD or chow diet (12, 13, 15, 18, 35, 36). Total body *Cgi-58* knockout was not investigated since mice lacking *Cgi-58* die shortly after birth due to a skin barrier defect (39). *Cgi-58* blockade with ASO in epididymal adipose tissue and liver prevented HFD-induced weight gain and IR; although it was associated with increased hepatic steatosis and triglyceride levels (12, 18, 36). In chow fed mice, depletion of *Cgi-58* did not alter weigh gain (18).

Cgi-58 knockout (*Cgi-58*$^{-/-}$) in macrophages induced glucose intolerance, IR and proinflammatory activation of adipose tissue-macrophages in HFD mice but not in mice fed with chow diet (13). Nevertheless, Goeritzer *et al*. (35) found that deletion of *Cgi-58* in macrophages did not affect glucose tolerance and atherosclerosis in mice fed with a western type diet. Of note, *Cgi-58*$^{-/-}$ macrophages were skewed to a pro-inflammatory M1 phenotype in culture, accumulated intracellular triglycerides-rich lipid droplets and had decreased phagocytic capacity compared to control mice (35). In contrast, muscle-specific *Cgi-58* knockout improved glucose tolerance and insulin sensitivity in HFD mice, despite causing increased intramyocellular lipid accumulation (15). *Cgi-58*$^{-/-}$ animals were similar to control mice regarding lean mass and fat mass percentages (15). *Cgi-58* overexpression in adipose tissue of HFD and chow diet mice had no effect on HFD-induced obesity and plasma triglyceride levels (11). These contradictory results may be explained by different functions of CGI-58 in fat lipolysis, tissues analyzed, murine models, and percentage of fat in diet. Furthermore, there is a possibility that CGI-58 might have ATGL-independent functions in different tissues (13, 14, 40, 41).

In this context, Yang *et al*. (40) reported that macrophage-specific *Cgi-58* transgenic mice fed with HFD diet showed lower serum levels of proinflammatory cytokines (IL-1β, IL-6, TNF, and MCP-1) and better mitochondrial function compared to control mice. *Cgi-58* overexpression in macrophages also induced PPAR-γ expression (40), which has anti-inflammatory properties in macrophages,

increasing insulin sensitivity (42, 43). In accordance with these data, Miao *et al.* (13) showed that macrophage-specific *Cgi-58* knockout exacerbated HFD-induced inflammation and IR in mice by inhibiting PPAR-γ-sustained mitochondrial function; thus, causing reactive oxygen species (ROS)-dependent NLRP3 inflammasome activation with consequent increase in IL-1β levels. More recently, Miao *et al*. (14) demonstrated that chronic over-nutrition increased circulating levels of saturated non-esterified fatty acids, suppressing Cgi-58 in murine macrophages. Cgi-58 deficiency activated the NLRP3 inflammasome to augment IL-1β secretion, which induced *SOCS3* upregulation, reduced IRS2-AKT signaling and activated FOXO1. Then, activated FOXO1 was able to bind to the IL-1β promoter to increase pro-IL-1β expression in a feedback loop, further enhancing systemic inflammation and IR (14).

CGI-58 was also reported to possess lysophosphatidic acid acyltransferase (LPAAT) activity, suggesting a potential role of this protein in the generation of the phosphatidic acid (PA) (41, 44). PA is a signaling lipid that regulates triglyceride metabolism, insulin sensitivity and immunity. Thus, the generation of PA by CGI-58 in response to inflammatory stimuli may play a critical role in maintaining the balance between systemic inflammation and insulin action (41). Lord *et al*. (41) also showed that CGI-58 is needed for TH1 cytokine signaling in liver, which might explain why decreased *CGI-58* expression causes severe hepatic lipid accumulation yet paradoxically improves hepatic insulin action. Recently, the same research group showed that CGI-58 regulates hepatic triglyceride and diacylglycerol (DAG) storage via a mechanism distinct from ATGL co-activation (36). Interestingly, in WAT, ATGL-mediated lipolysis requires *CGI-58* expression, demonstrating that CGI-58 is a specific co-activator of ATGL in WAT under physiological conditions. Hence, CGI-58 may have different functions on lipid metabolism, insulin action and inflammation depending on the tissue (36).

The present study also shows that *CGI-58* expression in SAT from patients with obesity and lean patients was negatively correlated with different anthropometric and body composition parameters (BMI, waist circumference, fat mass, FFM), REE, lipid profile (total cholesterol and triglyceride levels),

IR, and HbA1c levels, while it was positively correlated with HDL cholesterol and adiponectin levels. In obese patients, *CGI-58* expression negatively correlated with waist circumference, FFM, HbA1c and triglyceride levels. These results are biologically plausible considering that CGI-58 acts on lipid metabolism, PPAR-γ-sustained mitochondrial function, insulin action and inflammation. Considering that patients with severe obesity (Group 3) usually have higher FFM and REE (45, 46), the inverse correlation between *CGI-58* and these characteristics may be indirect since *CGI-58* is decreased in patients with obesity (Table 1). Accordingly to our results, Karki *et al.* (19) also found a negative correlation between *CGI-58* expression and HbA1c levels after bariatric surgery-mediated weight loss. Additional studies are needed to better describe these associations in different populations.

For the first time, this study showed a positive correlation between *CGI-58* expression and vitamin D levels. This association is also plausible considering that vitamin D has acknowledged anti-inflammatory properties and seems to be involved in lipogenesis and lipolysis (28). Furthermore, many studies have reported changes in vitamin D status with BMI changes, with patients with obesity having the lowest levels (47). It is worth noting that two case report studies showed that children with CDS, the autosomal recessive syndrome caused by loss-of-function mutations in the *CGI-58* gene, had decreased vitamin D levels (48, 49), which is in line with our results. Moreover, Jefferson *et al*. (50) found that treatment of myotubes with calcitriol, the active vitamin D metabolite, increased *CGI-58* and *ATGL* gene expressions compared to vehicle-treated myotubes. It remains to be determined if the association between *CGI-58* expression and vitamin D levels is causal or a secondary association due to the role of CGI-58 on obesity and lipid metabolism.

This study has few limitations. First, the number of patients included in each group is relatively small to perform stratification analyses; however, our demonstration of significant correlations even with this sample size makes our data more compelling. Second, our results are limited to SAT depot. We acknowledge that VAT may be more metabolically active than SAT; nevertheless, VAT biopsies were not possible during laparoscopic abdominal surgeries, which was the case for lean subjects and

patients with obesity degree 2. Last, since little is known about the influence of drugs on *CGI-58* expression, we cannot exclude the possibility that use of different medications among groups might affect the expression of this gene in SAT.

In conclusion, *CGI-58* expression is decreased in SAT from patients with obesity. Accordingly, *CGI-58* expression seems to be negatively correlated with body composition parameters, REE, lipid profile, IR, and HbA1c levels, while it is positively correlated with HDL cholesterol, adiponectin and vitamin D levels. These results strengthen the role of CGI-58 on lipid metabolism, inflammation, and insulin action.

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Conflict of interest

The authors declare no conflict of interest.

Contributors

J.R. designed the study, performed data collection and analysis, interpreted the data, and wrote the manuscript. N.S.C. helped with data acquisition and analysis, and edited the manuscript. B.M.S., C.B.L, M.M. and A.C.B helped with data analysis and interpretation and edited the manuscript. D.C. helped with study design, data analysis and interpretation and wrote the manuscript. All authors approved the final version of the manuscript.

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Table 1

Anthropometric, body composition, resting energy expenditure, clinical and laboratory characteristics of patients included in the study.

	Group 1 (n = 14)	Group 2 (n = 24)	Group 3 (n = 29)	P value
Age (years)	50.5 ± 19.4	47.0 ± 13.8	43.4 ± 10.0	0.285
Ethnicity (% non-white)	35.7	16.0	10.7	0.131
Gender (% male)	42.9	32.0	17.9	0.206
BMI (kg/m^2)	23.6 ± 3.1 [a]	33.9 ± 3.5 [b]	48.1 ± 7.6 [c]	< 0.001
HOMA-IR	4.9 (3.1 – 9.9) [a]	9.1 (4.7 – 18.9) [ab]	11.6 (7.2 – 19.8) [b]	0.025
HbA1c (%)	5.5 ± 0.7 [a]	5.8 ± 0.8 [ab]	6.4 ± 1.3 [b]	0.016
FPG (mg/dL)	129.7 ± 38.4 [a]	119.4 ± 26.3 [a]	148.0 ± 52.7 [b]	0.049
Insulin (μIU/mL)	17.2 (9.5 – 23.1) [a]	31.0 (16.8 – 49.8) [b]	35.3 (21.0 – 46.2) [b]	0.015
REE (kcal/d)	1460 ± 402 [a]	1795 ± 616 [a]	2223 ± 646 [b]	0.001
Fat mass (kg)	41.6 ± 9.1 [a]	49.2 ± 12.0 [a]	58.0 ± 9.4 [b]	< 0.001
Fat-free mass (kg)	43.9 ± 9.5 [a]	51.7 ± 12.6 [a]	66.0 ± 20.7 [b]	< 0.001
Systolic BP (mmHg)	129.0 ± 28.7	129.1 ± 14.4	132.6 ± 14.2	0.727

Diastolic BP (mmHg)	73.2 ± 12.4	76.2 ± 11.5	81.9 ± 10.3	0.053
Hypertension (%)	35.7 [a]	60.0 [ab]	78.6 [b]	0.024
Total cholesterol (mg/dL)	159.7 ± 35.9	187.9 ± 51.5	181.1 ± 37.4	0.183
Triglycerides (mg/dL)	56.5 (31.2 – 99.5) [a]	94.0 (60.0 –149.2) [b]	130.0 (103.2 – 250.2) [c]	< 0.001
HDL-cholesterol (mg/dL)	46.4 ± 12.7 [a]	42.4 ± 9.6 [ab]	37.8 ± 9.6 [b]	0.045
LDL-cholesterol (mg/dL)	152.9 ± 41.7	165.6 ± 48.8	146.7 ± 38.1	0.312
T2DM (%)	14.3 [ab]	8.0 [a]	35.7 [b]	0.037
Pre-diabetes (%)	7.1	8.0	14.3	0.682

Data are expressed as mean ± SD, median (25th - 75th percentiles), or %. P values were obtained with χ^2 tests or ANOVA, followed by post-hoc tests as appropriate. Variants with equal letters do not differ significantly in the statistical tests; those denoted with different letters were statistically different. BMI, body mass index; BP, blood pressure; FPG, fasting plasma glucose; HbA1c, glycated hemoglobin; HOMA-IR, homeostasis model assessment-insulin resistance; REE, rest energy expenditure; T2DM, type 2 diabetes mellitus.

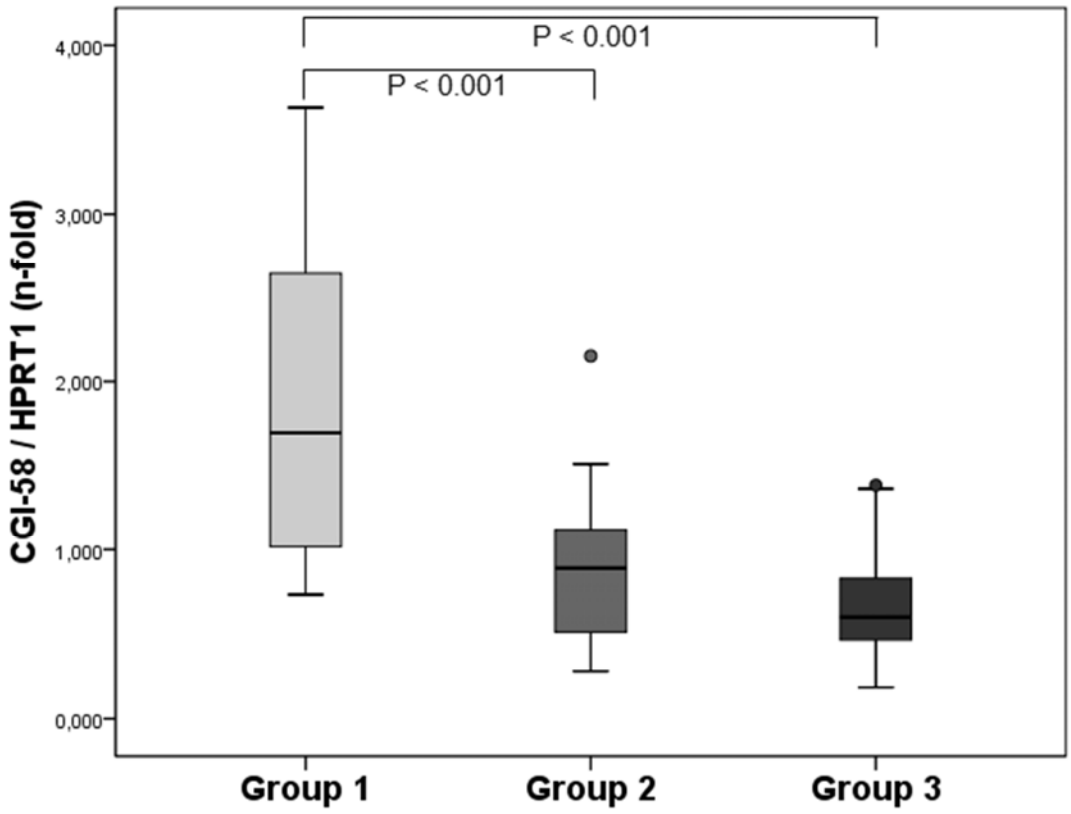
Table 2

Correlations between *CGI-58* expressions and anthropometric, body composition, resting energy expenditure, clinical and laboratory characteristics.

	CGI-58 expression All groups (n = 67)	*CGI-58* expression in groups 2 + 3 (patients with obesity, n = 53)
	r / P value	*r* / P value
Age (years)	-0.125 / 0.353	-0.145 / 0.302
BMI (kg/m^2)	-0.432 / < 0.001	-0.111 / 0.472
Waist circumference (cm)	-0.600 / < 0.001	-0.324 / 0.018
Fat mass (kg)	-0.380 / 0.003	-0.091 / 0.535
Fat-free mass (kg)	-0.488 / < 0.001	-0.412 / 0.002
HOMA-IR	-0.317 / 0.013	-0.263 / 0.062
HbA1c (%)	-0.421 / < 0.001	-0.427 / < 0.001
FPG (mg/dL)	-0.093 / 0.459	-0.101 / 0.427
Insulin (µIU/mL)	-0.332 / 0.009	-0.260 / 0.065
REE (kcal)	-0.373 / 0.002	-0.293 / 0.095
Total cholesterol (mg/dL)	-0.315 / 0.050	-0.243 / 0.086
Triglycerides (mg/dL)	-0.585 / < 0.001	-0.386 / 0.005
HDL-cholesterol (mg/dL)	0.250 / 0.046	0.179 / 0.205
LDL-cholesterol (mg/dL)	-0.078 / 0.560	-0.104 / 0.471
Leptin (µg/ml)	-0.236 / 0.070	0.211 / 0.158
Adiponectin (µg/ml)	0.250 / 0.050	0.198 / 0.172
Vitamin D (ng/ml)	0.366 / 0.004	0.399 / 0.004

BMI, body mass index; FPG= fasting plasma glucose; HbA1c, glycated hemoglobin; REE, rest energy expenditure; HOMA-IR, homeostasis model assessment-insulin resistance.

Figure 1 *CGI-58* gene expressions according to body mass index categories. P values were obtained using One-Way ANOVA with post-hoc Tukey's test. The horizontal line inside each box indicates the median, the top and bottom of the box indicate the interquartile range, whiskers indicate the 5th and 95th percentiles, and circles indicate outlier values. Group 1: Patients with BMI < 27.0 kg/m^2; Group 2: BMI 30.0 – 39.9 kg/m^2; and Group 3: BMI ≥ 40.0 kg/m^2.



10. CONCLUSÕES

A obesidade e sobrepeso têm se tornado endêmicos nos últimas décadas. A obesidade e suas doenças associadas diminuem a qualidade e expectativa de vida dos indivíduos afetados. Dessa forma, identificar os mecanismos e compreender as modificações existentes nos tecidos destes indivíduos, em especial no TA, podem proporcionar novas formas de tratamento para prevenir ou reduzir os danos causados pela obesidade.

De acordo com a nossa revisão sistemática, a maior parte dos estudos em humanos demonstrou um aumento da expressão do *NLRP3* no TA de indivíduos com obesidade em comparação aos indivíduos magros. Nos estudos com modelos murinos de obesidade foi verificada a mesma tendência nos resultados, mas com dados um pouco mais contraditórios. Até o momento, poucos estudos avaliaram a associação entre polimorfismos no gene *NLRP3* e obesidade, RI e/ou DM2, com resultados inconclusivos.

Diferentemente do NLRP3, poucos estudos, até o momento, avaliaram a relação entre o gene *CGI-58* e a obesidade. Interessantemente, demonstramos que a expressão gênica do *CGI-58* está diminuída em SAT de pacientes com obesidade quando comparado com indivíduos magros. Além disso, a expressão *CGI-58* parece estar correlacionada negativamente com os parâmetros de composição corporal, TMR, perfil lipídico, RI e HbA1c, enquanto está positivamente correlacionada com níveis de colesterol HDL, adiponectina e vitamina D. Estes resultados reforçam o papel do *CGI-58* no metabolismo lipídico, inflamação e ação da insulina, trazendo questionamentos adicionais sobre o verdadeiro impacto dessa promissora molécula na saúde/doença.

11. PERPECTIVAS FUTURAS

Como perspectivas futuras, devido aos resultados encontrados na revisão sistemática, consideramos promissor analisar inibidores endógenos do NLRP3, como a expressão de microRNAs (miRNAs), no TA de indivíduos com e sem obesidade. Um estudo, realizado por Bauernfeind *et. al.* (56) identificou que o miR-223 diminui a expressão de *NLRP3* no TA. Dessa forma, temos como perspectiva avaliar a expressão deste miRNA nas amostras de SAT analisadas no presente estudo. Além disso, devido ao pequeno número de estudos que avaliaram a associação entre polimorfismos no gene *NLRP3* e obesidade e suas doenças associadas, consideramos necessário estudar alguns polimorfismos neste gene em amostra de indivíduos com e sem obesidade da nossa população

Em relação ao CGI-58, as perspectivas futuras podem ser inúmeras, pois poucos estudos foram feitos avaliando a relação desta proteína com obesidade, principalmente em humanos. No entanto, parece ser um foco promissor a realização de estudos funcionais que avaliem o impacto do tratamento com calcitriol em células adiposas sob a expressão de *CGI-58*. Sendo também interessante a avalição de genes que são ativados no TA de indivíduos com obesidade, como adipocinas e genes inflamatórios, no contexto de bloqueio do gene *CGI-58* em macrófagos e/ou adipócitos.

12. COLABORAÇÃO EM OUTROS TRABALHOS

Além dos artigos que fazem parte da presente tese, ao longo do período do doutorado foram desenvolvidos, em colaboração, os seguintes manuscritos:

- Use of additives, scaffolds and extracellular matrix components for improvement of human pancreatic islet outcomes in vitro: a systematic review. Lemos NE, Brondani LA, Dieter C, **Rheinheimer J**, Boucas AP, Bauermann CL, Crispim D; Bauer AC. Islets, v. 5 , p. 73-86 , 2017.

- Early reduction of resting energy expenditure and successful weight loss after Roux-en-Y gastric bypass. Moehlecke M, Andriatta BC, **Rheinheimer J**, Maciel T, Manoel R, Crispim D, Bauermann CL. Surgery for Obesity and Related Diseases, v. 13, p. 204-209, 2017.

- Nitric oxide levels in patients with diabetes mellitus: A systematic review and meta-analysis. Assmann TS, Brondani LA, Boucas AP, R**heinheimer J,** Souza BM, Canani LH, Bauer AC, Crispim D. Nitric Oxide (Print), v. 61, p. 1, 2016.

- Human pancreatic islet transplantation: an update and description of the establishment of a pancreatic islet isolation laboratory. **Rheinheimer J,** Bauer AC, Silveiro SP, Estivalet AF, Bouças AP, Rosa AR, Souza BM, Oliveira F, Cruz LA, Brondani LA, Azevedo MJ, Lemos NE, Carlessi R, Assmann TS, Gross JL, Bauermann CL, Crispim D. Archives of Endocrinology and Metabolism, v. 59, p. 161-170, 2015.

- Polymorphisms of the UCP2 Gene Are Associated with Glomerular Filtration Rate in Type 2 Diabetic Patients and with Decreased UCP2 Gene Expression in Human Kidney. Souza BM, Michels M, Sortica D, Bouças AP, **Rheinheimer J**, Buffon MP, Bauer AC, Canani LH, Crispim D. Plos One, v. 10, p. e0132938, 2015.

- The -308G>A Polymorphism of the TNF Gene Is Associated With Proliferative Diabetic Retinopathy in Caucasian Brazilians with Type 2 Diabetes. Sesti LFC, Crispim D, Canani LH, Polina ER, **Rheinheimer J**, Carvalho PS, Gross JL Santos KG. Investigative Ophthalmology & Visual Science, v. 56, p. 1184-1190, 2015.

- The TCF7L2 rs7903146 (C/T) polymorphism is associated with risk to type 2 diabetes mellitus in Southern-Brazil. Assmann TS, Duarte GC, **Rheinheimer J**, Cruz LA, Canani LH, Crispim D. Arq Bras Endocrinol Metabol, v. 9, p. 918-25, 2014.

- Brain Death-Induced Inflammatory Activity in Human Pancreatic Tissue. Rech TH, Crispim D, **Rheinheimer J**, Barkan SS, Osvaldt AB, Grezzana TJM, Kruel CRP, Martini J, Gross JL, Bauermann CL. Transplantation, v. 97, p. 212-219, 2014.

Manuscritos submetidos:

- Brain death-induced inflammatory activity is similar to sepsis-induced cytokine release. Schwarz P, Custódio G, **Rheinheimer J**, Crispim D, Leitão CB, Rech TH.

- Renal effects of exendin-4 in a murine model of brain death. Lemos NE, Dieter C, Carlessi R, **Rheinheimer J**, Brondani LA, Leitão CB, Bauer AC, Crispim D.