UNIVERSIDADE ESTADUAL DE MONTES CLAROS

Thaísa Soares Crespo

Efeitos da gastrectomia vertical com e sem omentectomia no metabolismo, inflamação e expressão do sistema renina-angiotensina no tecido adiposo de ratos obesos.

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UNIVERSIDADE ESTADUAL DE MONTES CLAROS
PROGRAMA DE PÓS-GRADUAÇÃO EM CIÊNCIAS DA SAÚDE

Unimontes

MESTRANDA: THAÍSA SOARES CRESPO

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“Viver é muito perigoso...
Porque aprender a viver é que é o viver mesmo...
Travessia perigosa, mas é a da vida.
Sertão que se alteia e abaixa...
O mais difícil não é um ser bom e proceder honesto,
dificultoso mesmo, é um saber definido o que quer,
e ter o poder de ir até o rabo da palavra”.

Guimarães Rosa, 1956

RESUMO

A obesidade é uma doença crônica manifestada pelo excesso de tecido adiposo decorrente de hiperplasia e/ou hipertrofia dos adipócitos. Ocorre devido a um desequilíbrio entre o ganho e o gasto energético, onde o balanço energético positivo, de maneira cumulativa e prolongada, gera a doença. Apresenta etiologia multifatorial, com dimensões psicossociais e biológicas consideráveis e que vêm adquirindo proporções alarmantes. É considerada um dos principais problemas de saúde pública da sociedade moderna. A obesidade, sobretudo a visceral, está associada com aumento da produção de citocinas pró-inflamatórias, gerando um processo inflamatório crônico e resistência insulínica, contribuindo para o desencadeamento do *diabetes mellitus* tipo II (DM2) e suas consequentes complicações micro e macrovasculares. Além disto, o tecido adiposo representa local de produção de componentes do sistema renina-angiotensina (SRA). A hipertrofia dos adipócitos aumenta a secreção de alguns destes componentes, podendo gerar hipertensão arterial e outras alterações cardiometabólicas, com repercussão na morbimortalidade da população mundial. A cirurgia bariátrica, além de tratar com eficiência a obesidade, tem efeitos terapêuticos no DM2 e dislipidemia. Dentre as técnicas cirúrgicas, a gastrectomia vertical (GV), inicialmente indicada como primeira etapa de tratamento para pacientes de alto risco ou com obesidade extrema, tem sido aventada como opção única e definitiva, devido a resultados satisfatórios quanto à perda de peso e melhora do perfil metabólico. Com a intenção de reduzir o tecido adiposo visceral, a ressecção do omento maior tem sido proposta durante a realização das cirurgias bariátricas. Há controvérsias se a inclusão da omentectomia como parte da técnica cirúrgica traria benefício metabólico adicional aos pacientes obesos. Este trabalho teve como objetivo avaliar os efeitos da GV em ratos machos, com obesidade induzida por dieta, no intuito de ampliar o conhecimento desta técnica sobre os efeitos metabólicos, na inflamação e na expressão do SRA no tecido adiposo. Avaliou também se a omentectomia associada à GV incrementaria os efeitos benéficos na perda de peso, no perfil metabólico e na expressão tecidual de marcadores inflamatórios. Os ratos foram tratados durante oito semanas com dieta padrão (STD) ou dieta hiperlipídica (HFD) e distribuídos em seis grupos compostos de 10 animais, cada: STD e laparotomia (STD+L), STD e gastrectomia vertical (STD+GV), STD e gastrectomia vertical com omentectomia (STD+GVO), HFD e laparotomia (HFD+L), HFD e gastrectomia vertical (HFD+GV) e HFD e gastrectomia vertical com omentectomia (HFD+GVO). Foi avaliado o

perfil corporal, glicídico e lipídico e feito análises da expressão gênica de marcadores inflamatórios (fator de necrose tumoral-alfa/TNF-alfa e interleucina-6/IL-6) e de componentes do SRA (enzima conversora de angiotensina/ECA, enzima conversora de angiotensina II/ECA2 e angiotensinogênio/AGT) no tecido adiposo branco. Os dados foram analisados pelo *GraphPad Prism* 5.0 e 6.0, utilizando teste t de Student, testes *Two-Way* ANOVA e *One-Way* ANOVA e pós-teste de Bonferroni. A GV levou à perda de peso, diminuição da adiposidade, redução dos níveis plasmáticos de glicose, peptídeo-C, insulina e colesterol total. Com a realização da GV houve diminuição da expressão no tecido adiposo branco do TNF-alfa, IL-6, AGT e ECA e aumento da expressão de ECA2. Nos animais submetidos à GV com omentectomia também houve redução do peso corporal, melhora do perfil metabólico e diminuição da expressão tecidual de TNF-alfa, porém sem diferença estatisticamente significativa com relação aos grupos nos quais foi realizada apenas a GV. O trabalho demonstrou efeitos benéficos da GV sobre a perda ponderal, perfil metabólico, inflamação e na expressão tecidual de componentes do SRA podendo, dessa forma, ser considerada uma opção terapêutica no tratamento da obesidade, DM2 e suas comorbidades. Neste estudo, não foi demonstrado efeito benéfico adicional com a associação da omentectomia à GV no perfil glicídico, lipídico e na expressão tecidual de marcadores inflamatórios.

Palavras-chave: Obesidade. Cirurgia Bariátrica. Omento. Sistema Renina-Angiotensina. Citocinas.

ABSTRACT

Obesity is a chronic disease caused by the excess of adipose tissue due to the hyperplasia and/or hypertrophy of the adipocytes. It occurs due to an imbalance between the energy intake and expenditure, where the positive energetic balance, in a cumulative manner, results in disease. The obesity presents a multifactorial etiology, with considerable psychosocial and biological dimensions and has been acquiring alarming proportions. It is considered one of the major public health problems in the modern society. The obesity, especially the visceral, is associated with the increased production of proinflammatory cytokines, resulting in a chronic inflammatory state along with insulin resistance, with a significant increase of diabetes mellitus type II (DM2) and its consequent micro and macrovascular complications. Moreover, the adipose tissue represents an important site for the production of components of the renin-angiotensin system (RAS). The hypertrophy of the adipocytes increases the secretion of some of these components, which may cause arterial hypertension and other cardiometabolic alterations with impact on the morbidity and mortality of the population. The bariatric surgery, in addition to effectively treat obesity, also has therapeutic effects on DM2 and dyslipidemia. Among the surgical techniques, the sleeve gastrectomy (SG) initially indicated as the first step of the treatment for patients with high risk or with severe obesity, has been suggested as an only and definitive option due to its satisfactory results concerning the weight loss and improvement of the metabolic profile. Intended to reduce the visceral adipose tissue, the resection of the great omentum has been proposed during the performance of bariatric surgeries. However, there are controversies if the inclusion of the omentectomy as a part of the bariatric surgery would bring an additional metabolic benefit to the obese patients. In this context, this study aimed to evaluate the SG effects in male rats, with obesity induced by diet, in order to expand the knowledge of this technique on the metabolic effects, inflammation and expression of the RAS components in the adipose tissue. This study also evaluated if the omentectomy associated with SG would increment the benefic effects regarding the weight loss, metabolic profile improvement and in the tissue expression of inflammatory markers. The rats were treated during eight weeks with standard diet (STD) or high-fat diet (HFD) and divided into six groups (n=10/group): STD and laparotomy (STD+L), STD and sleeve gastrectomy (STD+SG), STD and sleeve gastrectomy with omentectomy (STD+SGO), HFD and laparotomy (HFD+L), HFD and sleeve gastrectomy (HFD+SG) and HFD and sleeve

gastrectomy and omentectomy (HFD+SGO). It was evaluated the body, glycemic and lipid profile and performed analysis of the inflammatory markers (tumor necrosis factor-alpha/TNF-α and interleukin-6/IL-6) and RAS components (angiotensin I converting enzyme/ACE, angiotensin I converting enzyme II/ACE2 and angiotensinogen/AGT) expression in the white adipose tissue. The data were analyzed in the *Graph Pad Prism* 5.0 and 6.0 program, applying the Student's t-test, *Two-Way* ANOVA and *One-Way* ANOVA followed by Bonferroni post-hoc test. The SG led to a significant weight loss, decrease in the adiposity and plasma levels of glucose, C-peptide, insulin and total cholesterol. The performance of SG was associated with a decreased expression of TNF-α, IL-6, AGT and ACE along with an increased expression of ACE2. The animals submitted to SG along with omentectomy also presented weight loss, improvement of the metabolic profile and decrease of the tissue expression of TNF-α, but with no statistically significant differences when compared to the groups of animals submitted to the SG alone. This study showed the benefic effects of the SG on the weight loss, inflammation and in the tissue expression of the RAS components in the white adipose tissue, allowing to be thus considered a therapeutic option to the treatment of obesity, DM2 and its comorbidities. Additionally, in the present study it was not established additional benefic effects of the association between omentectomy and SG in the glycemic and lipid profile and also in the tissue expression of inflammatory markers.

Keywords: Obesity. Bariatric Surgery. Omentum. Renin-Angiotensin System. Cytokines.

APRESENTAÇÃO

Esta dissertação segue a formatação preconizada pelo Programa de Pós-Graduação em Ciências da Saúde (PPGCS) – Unimontes / Montes Claros - MG, que recomenda a apresentação de uma primeira seção com a introdução e os objetivos do trabalho, a revisão de literatura e a metodologia.

Uma segunda seção apresenta os produtos (artigos redigidos seguindo normas do periódico escolhido, incluindo lista de referências utilizadas especificamente em cada artigo).

A terceira seção é composta por considerações finais e/ou conclusões e referências das citações utilizadas na introdução, revisão de literatura e metodologia.

Anexos e Apêndices são incluídos após as referências.

Maiores detalhes sobre a formatação e normatização adotadas pelo PPGCS podem ser obtidos no endereço eletrônico www.ppgcs.unimontes.br.

SUMÁRIO

1 INTRODUÇÃO

1.1 Obesidade

A obesidade é uma doença manifestada pelo excesso de tecido adiposo decorrente de hiperplasia e/ou hipertrofia dos adipócitos. Ocorre devido a um desequilíbrio entre o ganho e o gasto energético, onde o balanço energético positivo, de maneira cumulativa e prolongada, gera a doença (1, 2). É uma doença crônica, com prevalência crescente e que vem adquirindo proporções alarmantes em nível global, sendo considerada a pandemia do século XXI (3). Atinge tanto adultos quanto crianças, sendo atualmente destacada como a mais frequente desordem nutricional nos países ocidentais, determinando morbidade maior do que as relacionadas às doenças infecciosas e à desnutrição. É um desafio e um dos principais problemas de saúde pública da sociedade moderna tanto nos países desenvolvidos quanto nas regiões em desenvolvimento no mundo (4, 5).

A obesidade tem etiologia multifatorial. É importante destacar que a exposição a fenômenos ambientais da hipermodernidade (impacto ambiental obesogênico) está fortemente associada com o sobrepeso e a obesidade. Dentre estes fenômenos, podem-se citar fatores relacionados à inatividade física e comportamentos dietéticos, sociais e culturais obesogênicos (6). Os fatores dietéticos (incremento da densidade energética da dieta e aumento crescente de consumo de ácidos graxos e açúcares) e de restrição de atividade física são desencadeantes e agravantes do sobrepeso e obesidade (5, 7, 8). Outro fator importante é a redução do número de horas de sono. Existem evidências de que há relação da restrição do sono e ruptura do ritmo circadiano com desordens metabólicas tais como obesidade, resistência insulínica e diabetes (9, 10). Embora causas monogênicas da obesidade possam ocorrer, como, por exemplo, uma mutação no gene ou receptor da leptina, evidências demonstram que fatores individuais de suscetibilidade biológica, entre muitos outros, interagem na etiologia da patologia e são capazes de ativar ou silenciar genes envolvidos na patogênese da obesidade (11). Estudos envolvendo o genoma humano demonstram associação entre muitos polimorfismos genéticos com a adipogênese, mas os mecanismos pelos quais estas alterações genéticas determinam a

obesidade não são bem compreendidos (12). Estes polimorfismos, juntamente com fatores epigenéticos, poderiam explicar a grande diversidade de manifestação da obesidade entre os indivíduos. Como causas epigenéticas, pode-se citar a microbiota intestinal, estresse e exposição a substâncias consideradas como desreguladoras endócrinas (13). Estudos demonstram a importância da microbiota na gênese da obesidade e suas consequentes desordens metabólicas. A dieta poderia induzir o aumento da prevalência de bactérias obesogênicas, as quais poderiam estar associadas com aumento do peso corporal e, até mesmo, distúrbios neuropsiquiátricos (14-16).

O índice de Massa Corporal (IMC), relação entre peso (em quilogramas/kg) e a altura (em metros ao quadrado/m²), é indicado na classificação da obesidade. De acordo com esse índice, os adultos são classificados em grupos: 18 até 24,9 – Normal; 25 a 29,9 – Sobrepeso; 30 a 34,9 – Obeso grau I; 35 a 39,9 – Obeso grau II; maior do que 40 – Obeso grau III. Portanto, um indivíduo é considerado obeso quando essa relação é igual ou superior a 30 kg/m². Obesidade extrema ou severa ocorre quando esse índice é igual ou superior a 40 kg/m² (1). Dados de estudos epidemiológicos demonstram uma correlação direta entre excesso de tecido adiposo visceral com aumento do risco de doenças cardiometabólicas. Entretanto, uma mensuração precisa do tecido adiposo abdominal requer exames radiológicos de alto custo. Por isso, a medida da circunferência abdominal é recomendada como marcador importante da quantidade de tecido adiposo abdominal (subcutâneo e visceral), podendo identificar pacientes com maior risco cardiometabólico (17).

Em 2014, segundo a Organização Mundial de Saúde (OMS), 39% dos adultos acima de dezoito anos no mundo estavam com sobrepeso (IMC: 25 a 29,9 kg/m²) (39% dos homens e 40% das mulheres) e 13% estavam obesos (IMC $\geq$ 30 kg/m²) (11% dos homens e 15% das mulheres). Então, aproximadamente dois bilhões de adultos no mundo estão com sobrepeso e, destes, mais de meio bilhão são obesos. Segundo a OMS, a prevalência de sobrepeso e obesidade é mais alta no continente americano, com 61% de sobrepeso em ambos os sexos e 27% de obesidade (18). Se as tendências recentes continuarem, em 2030, mais de 57,8% da população mundial adulta (3,3 bilhões de pessoas) poderá estar com sobrepeso ou obesidade (19). A obesidade na infância também é um dos mais sérios desafios na saúde pública do século XXI. O problema é global e está afetando também os países de baixa e média renda,

especialmente em ambientes urbanos. A prevalência tem aumentado em ritmo alarmante (3). Globalmente, em 2013, o número de crianças com menos de cinco anos com excesso de peso, foi estimado em mais de 42 milhões. Cerca de 31 milhões delas vivem em países em desenvolvimento (18). No Brasil, em 2013, segundo o Ministério da Saúde, 51% da população acima de 18 anos estava com sobrepeso (IMC: 25 a 29,9 kg/m²) e 17% com obesidade (IMC $\geq$ 30 kg/m²) (20). O sobrepeso e a obesidade acarretam uma grande sobrecarga econômica ao sistema de saúde e à sociedade, deixando evidente a necessidade de intervenções preventivas e terapêuticas (21).

A obesidade é uma condição complexa, com dimensões sociais, biológicas e psicossociais consideráveis, acarretando um risco aumentado de aparecimento e agravamento de várias doenças crônicas, além de prejuízo na qualidade de vida. Quanto mais tempo o indivíduo se mantém obeso, maior é a possibilidade de ocorrerem doenças e complicações (5). As complicações cardiovasculares principais são hipertensão arterial sistêmica, hipertrofia ventricular esquerda com ou sem insuficiência cardíaca, doença aterotrombótica e doença coronariana (4, 22). As complicações endócrino-metabólicas correlacionadas com obesidade são hiperuricemia com ou sem gota, resistência à insulina, hiperinsulinemia, maior predisposição ao *diabetes mellitus* tipo II (DM2), intolerância à glicose e dislipidemia com aumento do colesterol total, de triglicerídeos e de lipoproteína de baixa densidade (LDL) e redução de lipoproteínas de alta densidade (HDL) (22-24). Do ponto de vista osteoarticular, a obesidade pode predispor a artroses, osteoartrites, epifisiólise da cabeça femoral, *genu valgum* e coxa vara. Pode estar associada com alterações ginecológicas e obstétricas como infertilidade, distúrbios menstruais, doença hipertensiva gestacional, diabetes gestacional e doença tromboembólica durante a gravidez (5). Com relação ao trato gastrointestinal, a obesidade favorece o aumento da prevalência de litíase biliar, coledocolitíase, esteatose hepática, esteatohepatite e cirrose hepática (22, 25). Estudos demonstram que existe associação significativa entre obesidade e diversos tipos de neoplasias, como, por exemplo, neoplasia de cólon, de mama, endométrio, rim e esôfago. Estes dados, e o aumento da tendência mundial de obesidade, sugerem que o excesso de ingestão calórica pode ser a maior causa evitável de câncer em não fumantes (26, 27). Com relação ao aparelho respiratório, há a tendência à hipóxia devido ao aumento da demanda ventilatória, aumento do esforço respiratório, diminuição da eficiência da musculatura respiratória, diminuição da reserva

funcional e dos volumes pulmonares, microatelectasias, hipopneia e apneia obstrutiva do sono, Síndrome de Pickwick, infecções pulmonares e asma (28). Existem diversas manifestações psicossociais e psiquiátricas relacionadas à obesidade como discriminação social, isolamento com afastamento das atividades sociais, depressão e transtornos alimentares (bulimia e distúrbio do comer compulsivo) (29). Também podem ser observadas alterações da coagulação (hipercoagulabilidade e hipofibrinólise devido ao aumento da apolipoproteína B e do inibidor do ativador do plasminogênio/PAI-1 e fibrinogênio) (30). Existe evidência de que os pacientes obesos, ou mesmo com sobrepeso, apresentam um risco aumentado de mortalidade com relação aos que apresentam IMC menor que 25 kg/m², com diminuição da expectativa de vida, principalmente quando são portadores de obesidade mórbida. É justamente o avanço do conhecimento médico sobre o aumento da morbimortalidade que enfatiza a necessidade de intervenção médica no tratamento da obesidade (4, 31).

A associação de fatores de risco cardiovasculares com obesidade abdominal está bem estabelecida. Embora ainda seja necessária uma classificação universal, a obesidade abdominal é considerada componente importante da Síndrome Metabólica (SM) (32, 33). A SM é definida como um transtorno complexo representado pela coexistência, em graus variados, de um conjunto de fatores de risco cardiovasculares que incluem a obesidade visceral (centrípeta), dislipidemia, alteração na homeostase glicêmica (DM2, intolerância à glicose ou resistência insulínica) e hipertensão arterial sistêmica. Outros achados incluem estado pró-inflamatório, microalbuminúria e hipercoagulabilidade (34). A obesidade, resistência insulínica e DM2 têm sido caracterizados como um estado inflamatório crônico que está associado com concentrações anormais de citocinas, reagentes de fase aguda e vários biomarcadores de vias de sinalização inflamatórias (35). Em 1988, Reaven introduziu o termo síndrome X e identificou a resistência à insulina, definida como a menor captação da glicose pelos tecidos periféricos, como o substrato fisiopatológico comum da síndrome (36). Em 1999, a OMS estabeleceu o termo unificado Síndrome Metabólica, pois os estudos não identificaram a presença de resistência à insulina como único fator causal de todos os componentes da síndrome (37). Mutações e polimorfismos nos genes associados com a resistência à insulina, anormalidades nos adipócitos, hipertensão e alterações lipídicas ocupam também papel importante na etiopatogenia da síndrome (38). Existem critérios diferentes para

o diagnóstico de SM, podendo-se destacar os critérios da Federação Internacional de Diabetes (IDF), o NCEP-ATPIII (*National Cholesterol Education Program Adult Treatment Panel III*) e os critérios da Organização Mundial da Saúde (39). Os critérios da IDF reconheceram a obesidade central como fator importante e determinante da SM, destacando a associação entre circunferência abdominal, doenças cardiovasculares e outros componentes da SM. Desse modo, a obesidade visceral foi colocada na posição central e, nesta definição, como componente essencial da SM (40).

1.2 Tecido Adiposo como Órgão Endócrino

O tecido adiposo está localizado em diversos sítios anatômicos (depósitos múltiplos), que coletivamente foram chamados de *órgão adiposo* (41). É composto por dois compartimentos principais: subcutâneo e visceral. Porém, existem ainda diversos outros depósitos especializados em tecidos linfoides, adipócitos mamários e células-mãe da medula óssea, dentre outros (42). O tecido adiposo apresenta funções como armazenamento e liberação de energia e também atua como órgão endócrino, secretando diversas substâncias fundamentais na homeostasia do organismo (43). A capacidade de armazenamento energético é virtualmente ilimitada. Resulta do aumento das reservas de cada adipócito (favorecimento da lipogênese relativamente à lipólise) e da replicação e diferenciação de pré-adipócitos. A ausência de limite representa vantagem adaptativa em curto prazo, e desvantagem em longo prazo, traduzida em disfunção endócrino-metabólica (44). Existe uma grande especialização fisiológica e heterogeneidade de células adiposas no organismo. O tecido adiposo é composto por dois tipos citológicos funcionalmente distintos: o tecido adiposo branco (TAB) e o tecido adiposo marrom (TAM) (45). O TAB, localizado nas regiões subcutânea e visceral, armazena energia na forma de triglicerídeos e participa da regulação do balanço energético mediante processos de lipogênese e lipólise. É composto anatomicamente por adipócitos, células do sistema imune, tecido conjuntivo, nervoso e vascular. É considerado um importante órgão endócrino metabolicamente ativo (43, 46). O TAB visceral é composto principalmente pelos tecidos adiposos mesentéricos e omentais, os quais são drenados, através da circulação portal, para o fígado (42). O TAM é altamente ativo no metabolismo oxidativo e dissipação de

energia química através da proteína desacopladora 1 (UCP1). As células marrons utilizam mais UCP1 mitocondrial para realizar os processos químicos da respiração e dissipar energia química sob a forma de calor, constituindo um fator de proteção contra hipotermia, obesidade e diabetes. O efeito termogênico, obtido através da ativação do TAM, leva a aumento do gasto energético, redução da adiposidade, redução dos níveis de glicose e de lipídios plasmáticos, contribuindo para uma melhor homeostase. Além disto, os adipócitos marrons também secretam fatores que atuam localmente e sistemicamente para influenciar o metabolismo energético (47).

Mais recentemente, também ficou demonstrado que o TAB contém células que podem expressar altos níveis de UCP1 e adquirir uma aparência multilocular sob estímulo prolongado do frio ou outras vias que elevam o nível intracelular do AMP cíclico (cAMP). O clássico TAM, exemplificado pelos depósitos interescapulares dos roedores, é derivado de uma linhagem celular myf-5 de modo semelhante à da musculatura. As células semelhantes às do tecido marrom (*brown-like cells*) localizadas nos depósitos do tecido adiposo branco (linhagem não-myf-5) são denominadas células bege, que constituem um tipo citológico de tecido adiposo recém-descoberto (48). Estudos ainda são necessários para responder questões como presença e quantidade de células beges precursoras nos vários depósitos de TAB, e se e como o *pool* dessas células contribuem de fato para o *status* metabólico dos humanos. Acredita-se que, em estado basal, as células beges representam um alto percentual de adipócitos em depósitos subcutâneos em comparação com os depósitos viscerais (49). Quando os animais são expostos ao frio ou recebem um estímulo β-adrenérgico crônico, os adipócitos beges pré-existentes passam por uma diferenciação e irão se assemelhar morfologicamente e histoquimicamente às células marrons. Por outro lado, nos depósitos mais resistentes ao *browning*, como os depósitos de tecido branco abdominal, as células bege precursoras têm inicialmente que sofrer proliferação antes que a conversão em tecido marrom ocorra (49).

Com o desenvolvimento de técnicas moleculares, têm sido identificadas várias substâncias não peptídicas e peptídeos bioativos expressos e secretadas pelo tecido adiposo, que influenciam não apenas a função adipocitária, mas também interferem em várias vias metabólicas por meio da circulação sanguínea. Portanto, o tecido adiposo é um órgão endócrino dinâmico que secreta mediadores inflamatórios e imunomediadores denominados

em conjunto como adipocinas (50). As adipocinas são altamente diversificadas em termos de estrutura proteica e função fisiológica, atuando como fatores anti-inflamatórios e pró-inflamatórios (51). Exercem um importante papel na regulação do consumo alimentar e da sensibilidade insulínica e, em alguns casos, atuam diretamente na resistência insulínica do tecido muscular esquelético, fígado e tecido adiposo (51, 52). Elas incluem as citocinas clássicas, fatores de crescimento, proteínas envolvidas na regulação da pressão arterial, homeostase vascular, metabolismo lipídico, glicídico e angiogênese. A adiponectina e leptina são as adipocinas mais abundantes sintetizadas pelo tecido adiposo (53). A desregulação na secreção destas adipocinas, a lipotoxicidade pelo excesso de ácidos graxos livres e as diferenças na localização predominante do tecido adiposo (visceral ou subcutâneo) são fatores importantes associados à resistência insulínica, aumento do DM2 e das doenças cardiovasculares (54). Várias destas moléculas secretadas pelo tecido adiposo podem agir como sinais endócrinos, parácrinos e autócrinos na regulação da homeostase energética, incluindo a autorregulação do crescimento e desenvolvimento do adipócito, angiogênese (fator de crescimento endotelial vascular/VEGF), homeostase glicêmica (adiponectina, leptina e TNF-alfa), reatividade vascular (inibidor do ativador de plasminogênio 1/PAI-1) e na regulação da pressão sanguínea (angiotensinogênio) (43, 55).

Além da importância como órgão endócrino, o tecido adiposo também é considerado um órgão imunológico. A obesidade é uma adiposopatia (*sick fat*) com efeitos endócrinos e imunes adversos que podem levar a doenças metabólicas (56). Dentre as manifestações da adiposopatia, podemos citar: alterações na adipogênese (proliferação e diferenciação dos adipócitos), adiposidade visceral, crescimento de tecido adiposo mesmo em áreas sem adequado suprimento vascular e deposição ectópica de gordura (56). Desse modo, podemos agrupar as adipocinas, de acordo com a sua principal função, em adipocinas com função imunológica, cardiovascular, metabólica e endócrina. Dentro do primeiro grupo incluem-se a IL-6, o TNF-alfa, e os fatores do complemento B, C3 e D (adipisina). Estas moléculas têm funções bem definidas nos estados inflamatórios (57). Nos grupos das adipocinas com função predominantemente cardiovascular, destacam-se as moléculas do sistema renina-angiotensina e o inibidor do plasminogênio (PAI-1) (55). As moléculas com função metabólica desempenham um papel na homeostasia energética. As adipocinas envolvidas nesses processos são os ácidos graxos livres (AGL), a adiponectina, a resistina, o *agouti related*

peptide (AGRP) e a visfatina (ação insulinomimética) (58). Além das citadas anteriormente, existem outras com diferentes funções, como: interleucina 1 (IL-1), *CC- chemokine ligand 2* (CCL2), *visceral adipose-tissue-derived serine protease inhibitor* (VASPIN), proteína ligante de retinol 4 (RBP4), apelina (importante fator angiogênico), resistina e omentina, dentre outras (59). A omentina é uma nova adipocina expressa preferencialmente no tecido adiposo visceral que tem sido alvo de interesse devido seus efeitos favoráveis na inflamação, homeostase glicêmica e proteção cardiovascular. Está negativamente associada com a resistência insulínica e a obesidade, além de variáveis como IMC, leptina e circunferência abdominal. Em contrapartida, a omentina se relaciona positivamente com a adiponectina e HDL. Semelhantemente à adiponectina, a perda de peso induzida aumenta os níveis circulantes de omentina e melhora a sensibilidade insulínica (60). Além das adipocinas, o TAB expressa numerosos receptores responsivos a sinais aferentes do sistema nervoso central e do sistema endócrino. Dentre esses vários receptores, evidenciam-se os de insulina, glucagon, peptídeo semelhante ao glucagon 1 (GLP-1), leptina, IL-6 e TNF-alfa (53, 61).

Por outro lado, as células musculares têm sido identificadas como células com alta capacidade de secreção e também representam uma fonte importante de moléculas, com efeitos locais ou à distância (62). As miocinas são, portanto, citocinas ou outros peptídeos que são produzidos, expressados e liberados pelas fibras musculares podendo ser autócrinos, parácrinos ou endócrinos. A inter-relação entre adipocinas e miocinas representa um balanço necessário à homeostase metabólica (63). No caso da obesidade, o tecido adiposo secreta adipocinas, as quais contribuem para estabelecer um processo inflamatório crônico, que desencadeará processos patológicos como a aterosclerose e resistência insulínica (64). A musculatura esquelética é capaz de produzir miocinas, que conferem alguns dos benefícios do exercício físico (65). As miocinas podem regular os efeitos prejudiciais das adipocinas pró-inflamatórias. Mesmo curtos períodos de inatividade física são associados com alterações metabólicas, incluindo diminuição da sensibilidade insulínica, atenuação do metabolismo lipídico pós-prandial, perda de massa muscular e acúmulo de tecido adiposo visceral. As miocinas identificadas incluem: miostatina, *leukemia inhibitory factor* (LIF), interleucina-4 (IL-4), interleucina-6 (IL-6), interleucina-7 (IL-7), interleucina-15 (IL-15), *brain-derived neurotrophic factor* (BDNF), *insulin-like growth factor 1* (IGF-1), *fibroblast growth factor* (FGF-2), *follistatin-related protein 1* (FSTL-1), irisina, dentre outras (66).

1.3 Marcadores Inflamatórios

A composição do tecido adiposo pode variar de acordo com sua localização anatômica e peso corporal. Nos obesos, o tecido adiposo é caracterizado pela hipertrofia e hiperplasia de adipócitos, infiltração de macrófagos, ativação de células endoteliais e fibrose (67). Com a hipertrofia dos adipócitos, ocorre compressão dos vasos sanguíneos no TAB, hipóxia local e apoptose de alguns adipócitos, desencadeando a cascata da resposta inflamatória e também o processo de angiogênese. Portanto, a condição de hipóxia poderia ter um papel central como estímulo à quimiotaxia de macrófagos e indução da expressão de genes pró-inflamatórios (54). O conceito de obesidade como processo inflamatório crônico se sustenta no fato de que o nível circulante de muitas citocinas e proteínas de fase aguda associadas à inflamação apresenta-se elevado em obesos, há expressão de genes inflamatórios pelos adipócitos e existe infiltração de macrófagos no tecido adiposo correlacionada com o crescimento deste tecido. (68). Os adipócitos secretam várias citocinas e proteínas de fase aguda que, direta ou indiretamente, elevam a produção e circulação de fatores relacionados à inflamação (68, 69). A origem dos marcadores inflamatórios na obesidade, possivelmente, é a partir da secreção pelos próprios adipócitos, a partir da secreção do TAB que estimularia a produção de marcadores pelo fígado e outros órgãos, por órgãos não adiposos, principalmente fígado e células imunes, ou uma combinação destas três situações anteriores (67). O desenvolvimento de um processo inflamatório no tecido adiposo em excesso pode ser o evento primário na gênese das alterações metabólicas e vasculares e pode ser a condição que associa a obesidade com resistência insulínica, DM2, hiperlipidemia, aterosclerose e outros componentes da SM (35, 69).

O TNF-alfa é uma citocina pró-inflamatória com ação autócrina, parácrina e endócrina. Foi inicialmente identificada como um polipeptídeo produzido por macrófagos durante infecções e neoplasias, situações estas que contribuem para o desenvolvimento e indução de caquexia. Seus níveis circulantes estão elevados em pacientes obesos e reduzem após a perda de peso (70). O TNF-alfa apresenta grande diversidade de atividades biológicas, as quais incluem: respostas imunológicas, reações inflamatórias e neovascularização. Pode inibir a proliferação de células tumorais e promover apoptose celular. Atua no adipócito, desempenhando papel

regulador no acúmulo de gordura corporal, pela inibição da lipogênese, com diminuição da expressão da LPL, do GLUT-4 e da acetil-CoA sintetase, bem como com aumento da lipólise. Apesar do TNF-alfa ser pouco expresso no TAB, sua expressão está modificada no TAB de obesos, onde apresenta superexpressão (71). Sua expressão é maior no tecido adiposo visceral do que no subcutâneo e sua produção principal é dada pelos macrófagos e, com menor participação, pelos adipócitos (72). Estudos mostram que seus níveis plasmáticos possuem correlação positiva com a obesidade e a resistência insulínica. A neutralização de receptores de TNF-alfa ou deleção gênica deles parece melhorar a sensibilidade insulínica (67). Além de prejudicar a sinalização insulínica, o TNF-alfa influencia a expressão gênica. No TAB, ele inibe a expressão de genes envolvidos na captação e armazenamento de ácidos graxos livres e glicose (diminui GLUT-4, diminui captação de glicose); suprime genes de fatores de transcrição envolvidos na lipogênese (aumenta a lipólise, diminui a lípase lipoproteica (LPL), diminui lipogênese); diminui a expressão da adiponectina e aumenta a IL-6 (73). Está envolvido no processo de inflamação, pois desempenha um papel principal na cascata das citocinas e estimula a síntese de outras citocinas, como IL-6, e proteínas de fase aguda associadas ao processo inflamatório. Assim como a IL-6, o TNF-alfa é mediador central da resposta de fase aguda, pois também determina a produção e a elevação das concentrações plasmáticas estimuladas pelo fígado de fibrinogênio, proteína amiloide sérica A (SAA), inibidor do ativador de plasminogênio-1 (PAI-1) e, em especial, da proteína C reativa (PCR) (74). Estudos têm demonstrado correlações significativas entre o TNF-alfa e os componentes da SM: triacilglicerol, HDL e pressão arterial sistólica além das correlações entre TNF-alfa e IMC, sensibilidade insulínica e PAI-1. Visto que o TNF-alfa está correlacionado com os componentes da SM, pode predizer risco para doenças cardiovasculares (30).

A IL-6 é uma citocina pró-inflamatória produzida por várias células (fibroblastos, células endoteliais, monócitos, adipócitos, macrófagos, etc.). Desempenha importante papel no metabolismo lipídico por aumentar a lipólise, com inibição da LPL e aumento da liberação de ácidos graxos livres e glicerol. Importante papel também no metabolismo dos carboidratos pela supressão da expressão de receptores e sinalizadores de insulina (redução da expressão do substrato do receptor de insulina-1 (IRS-1) e GLUT-4 nos tecidos muscular e hepático) (67). Portanto, está relacionada com a obesidade, desenvolvimento de hiperinsulinemia e resistência insulínica (75). Valores séricos de IL-6 foram associados com hipertensão arterial

e com a circunferência da cintura, indicando que pessoas com obesidade central possuem maior risco de desenvolver SM, lesões ateroscleróticas e eventos cardiovasculares (74). A IL-6 desempenha funções nos mecanismos imunes celulares e humorais relacionados à inflamação. É mediadora central da resposta de fase aguda e a principal citocina pró-coagulante, pois determina a produção e elevação das concentrações plasmáticas das proteínas hepáticas de fase aguda como o fibrinogênio, SAA e, em especial, da PCR (73). Parece ter também propriedades anti-inflamatórias desde que haja diminuição do TNF-alfa e do Interferon γ (IFN-γ). A IL-6 pode agir de forma distinta, dependendo da sua concentração orgânica, tanto nos tecidos periféricos quanto no sistema nervoso central, influenciando o peso corporal, a homeostase energética e a sensibilidade insulínica (65).

A PCR é uma proteína de fase aguda, sintetizada predominantemente pelo fígado e com sua produção estimulada e regulada pelos níveis circulantes de IL-6, IL-1 e TNF-alfa. Seus níveis também podem sofrer alterações durante processos inflamatórios crônicos e níveis elevados no plasma são considerados como preditores independentes de doença coronariana (76, 77). A quantidade de PCR circulante é proporcional ao Índice de Massa Corporal (IMC), obesidade visceral e circunferência abdominal e inversamente proporcional à adiponectina. Níveis elevados deste marcador inflamatório têm sido associados à obesidade, risco de DM2 e doenças cardiovasculares (35, 78). A PCR não é apenas um marcador de atividade inflamatória. Participa diretamente do processo de aterogênese, modula a função endotelial, induz a expressão de várias moléculas (*intercellular adhesion molecule*/ICAM-1, *vascular cell adhesion molecule*/VCAM-1, *monocyte chemoattractant protein-1*/MCP-1 e selectinas), regula a produção de óxido nítrico no endotélio e coordena a produção e secreção de várias citocinas, aumentando a atividade pró-inflamatória de diversas adipocinas (79)

1.4 Sistema Renina-Angiotensina (SRA)

O SRA é um dos mais importantes sistemas envolvidos na regulação da pressão arterial e homeostasia cardiovascular, estando intimamente associado com a patogênese das doenças cardiovasculares (80). Consiste primariamente em uma cascata enzimática coordenada, na

qual a renina (enzima sintetizada e armazenada nas células justaglomerulares dos rins) age sobre o angiotensinogênio (AGT), que é uma globulina e, este é convertido para angiotensina (Ang) I com propriedades vasoconstrictoras brandas e, subsequentemente, para Ang II pela ação da enzima conversora de angiotensina (ECA). A Ang II é um potente vasoconstrictor e, assim como o AGT, tem várias funções metabólicas (81). Há evidências de que o SRA é um sistema hormonal complexo. Não é apenas circulante, apresenta também expressão e secreção tecidual e possui diferentes peptídeos com diversas atividades biológicas (82). As angiotensinas são formadas no plasma e em outros tecidos a partir de precursores e substratos localmente expressos ou provenientes da circulação, como por exemplo, no cérebro, rim, coração e vasos, exercendo importantes funções nestes órgãos (83).

O tecido adiposo representa também um local de produção de componentes do SRA e existe interação dos componentes teciduais deste sistema com componentes circulantes (83). A hipertrofia dos adipócitos aumenta a secreção de componentes do SRA podendo gerar várias alterações cardiovasculares e metabólicas (84, 85). O SRA do tecido adiposo, regulado por fatores hormonais e nutricionais, é influenciado pelo grau de obesidade. A ativação do SRA em obesos pode determinar efeitos deletérios locais e sistêmicos e pode contribuir para o quadro de hipertensão arterial sistêmica e outras comorbidades associadas à obesidade (55). Desse modo, a expressão de genes deste sistema e seus produtos em muitos tecidos, faz com seja considerado um sistema endócrino. As funções autócrina e parácrina são importantes na regulação tecidual local e atuam em sintonia com os efeitos na circulação (função endócrina) e, sob diferentes condições, podem influenciar a resposta farmacológica dos inibidores do SRA. Existe evidência de que a ECA tecidual pode ser o local primário de ação dos inibidores da ECA (IECA). Consequentemente, a duração da ação de um IECA pode depender mais da duração da inibição tecidual do que da meia-vida plasmática da droga (86). Há evidências também de que o SRA contribui para a regulação do peso corporal por sua ação em diversos tecidos (87, 88). Portanto, o SRA, além de sua importante função na regulação da função cardiovascular e balanço hidroeletrolítico, também é reconhecido por seus potenciais papéis em vários aspectos da SM, podendo ter atribuições como fator causal de várias comorbidades associadas à obesidade (87).

Os níveis de mRNA AGT são 60% maiores no tecido adiposo do que no fígado, que é

considerado a principal fonte de AGT. O AGT foi descrito no tecido adiposo visceral em maior quantidade do que no tecido adiposo subcutâneo e há correlação positiva entre AGT expresso no tecido adiposo visceral e o IMC e pressão arterial, corroborando com estudos que mostram que o AGT pode ser determinante na distribuição do tecido adiposo e pode estar envolvido na SM (55, 89).

A Ang I é catabolizada pela ECA em um octapeptídeo com relevantes ações biológicas, a Ang II, que age através de seus receptores específicos AT1 e AT2, os quais são glicoproteínas com 30% de similaridade em sua sequência estrutural e agem como mediadores da atividade da Ang II sobre o sistema cardiovascular e outros órgãos. Na maioria dos mamíferos, incluindo os humanos, já foi demonstrada a presença dos receptores AT1 no córtex e medula da suprarrenal, nos túbulos proximais e glomérulos renais, nos músculos cardíacos, na musculatura dos vasos sanguíneos e no cérebro. Os receptores AT2 também estão presentes em muitos tecidos, incluindo a glândula suprarrenal, coração e cérebro. Muitas das funções nestes locais ainda não foram elucidadas, mas pressupõe importantes papéis fisiológicos (90). A Ang II também pode ser formada por outras vias independentes da renina, pela ação de catepsinas e quimases. É um dos mais potentes vasoconstritores conhecidos, sendo considerada o principal componente biologicamente ativo do SRA (91). Dentre as principais ações da Ang II via AT1, podemos considerar a indução da vasoconstrição (preferencialmente renal, cerebral e coronariana); a retenção renal de sódio (via liberação de aldosterona); retenção renal de água (via liberação de vasopressina); supressão da renina (por *feedback* negativo); hipertrofia de músculo liso e cardiomiócitos; estimulação de fibrose no miocárdio e vascular; efeito inotrópico positivo (contração de cardiomiócitos); ativação do sistema nervoso simpático e estimulação da formação de espécies reativas de oxigênio (90). A Ang II está altamente correlacionada com a disfunção endotelial presente nos pacientes hipertensos, diabéticos, obesos e com SM (92). O receptor AT2, na maioria das vezes, exerce efeitos opostos às ações mediadas pelo receptor AT1. Os principais efeitos de sua ativação são o antiproliferativo (inibição do crescimento celular), vasodilatação e proteção contra isquemia cardíaca (87, 90).

No quadro de SM foi descrito aumento da atividade da renina plasmática, maior nível plasmático de angiotensinogênio e aldosterona e maior atividade da ECA (88). Além da

contribuição do SRA, principalmente via Ang II, na gênese da SM, interações em diferentes níveis desse sistema com a insulina são implicadas como fator fundamental para o desenvolvimento da DM2 (88). A Ang II, via receptor AT_1, e a aldosterona podem modular as ações metabólicas da insulina, induzindo à resistência insulínica, hiperglicemia e elevação dos níveis de lipoproteínas de densidade muito baixa (VLDL) e triglicérides (87). A Ang II também contribui com o estresse oxidativo, inflamação e apoptose das células β-pancreáticas (88). Por outro lado, a hiperglicemia e a hiperinsulinemia ativam o SRA, que pode assim induzir ao quadro de hipertensão arterial, disfunção cardiovascular e renal (80). Dessa forma, substâncias produzidas pelo tecido adiposo podem ativar o SRA, contribuindo com as alterações cardiovasculares e renais associadas à obesidade e à síndrome metabólica (92). Medicações utilizadas para o tratamento da hipertensão arterial, que reduzem a síntese ou ação da Ang II, também tem ações terapêuticas na prevenção da resistência insulínica e controle do DM2 (92).

O SRA possui outros peptídeos com atividades biológicas diversas. A Ang-(1-7) é um heptapeptídeo formado primariamente a partir da Ang II pela ação da ECA2 e prolil-carboxipeptidase (PCP) e a partir da Ang I através da ação da ECA2, da prolil-endopeptidase (PEP), e endopeptidase neutra (NEP) (93). A Ang-(1-7), agindo via receptor Mas, geralmente promove ações antagônicas às produzidas pela Ang II (94). Trabalhos experimentais mostram a importância da Ang-(1-7) sobre o perfil lipídico e glicídico e sobre o perfil inflamatório no tecido adiposo. Altos níveis plasmáticos de Ang-(1-7) cronicamente atenuam o perfil pró-inflamatório do tecido adiposo, protegendo contra o estresse metabólico induzido por dieta hiperlipídica (95, 96). O aumento dos níveis plasmáticos da Ang-(1-7) também gera um incremento no perfil glicídico e lipídico demonstrado por melhora na tolerância à glicose e sensibilidade insulínica bem como decréscimo na dosagem de triglicérides e colesterol (96).

1.5 Tratamento Cirúrgico da Obesidade

As cirurgias bariátricas são um conjunto de técnicas cirúrgicas, com respaldo científico, com ou sem uso de órteses, destinadas à promoção de redução ponderal e ao tratamento de doenças que estão associadas e/ou que são agravadas pela obesidade (97). A cirurgia bariátrica

apresentou fases distintas em sua evolução. A fase pioneira ocorreu quando se observou que procedimentos cirúrgicos poderiam ser realizados com a finalidade exclusiva de induzir a perda de peso. Posteriormente, houve um grande avanço com a realização das cirurgias bariátricas por videolaparoscopia. Na última década, tem sido estudado os efeitos metabólicos associados a estas cirurgias (98). O termo cirurgia bariátrica deriva da palavra grega *baros* que significa peso e inclui os procedimentos cirúrgicos realizados com a finalidade de levar à perda substancial de peso. Originalmente, o objetivo primordial destes procedimentos era alcançar esta perda de peso significativa e sustentada. Na realidade, a perda de peso é somente uma das consequências da cirurgia. A cirurgia bariátrica está associada com outros benefícios à saúde, incluindo a melhora ou mesmo a normalização da hiperglicemia, dislipidemia, pressão arterial, apneia obstrutiva do sono e melhora da qualidade de vida, com redução de riscos cardiovasculares e diminuição da mortalidade (99). Em virtude destes amplos benefícios da perda de peso e às evidências crescentes de que alguns procedimentos levam às mudanças metabólicas que não podem ser completamente explicadas exclusivamente por esta perda de peso, a denominação cirurgia bariátrico-metabólica tem sido considerada como um nome mais apropriado (100).

O tratamento da obesidade é complexo e considerado um problema de saúde pública (101). A cirurgia bariátrica é o método que, consistentemente, resulta em perda considerável do excesso de peso, de maneira sustentada, com manutenção dessa perda em longo prazo e com baixos índices de complicações peri e pós-operatórias e com morbimortalidade relacionada à cirurgia considerada aceitável (102, 103). Quando comparada com o tratamento clínico, a cirurgia resulta em perda de peso mais significativa em longo prazo, com melhora na qualidade de vida e controle de comorbidades em pacientes com obesidade grau III. Esta perda de peso é similar quando comparado às cirurgias realizadas por laparoscopia ou por via aberta, mas ocorrem menos complicações graves, a perda sanguínea é menor e a recuperação é mais rápida quando o procedimento é realizado por laparoscopia, embora a conversão para cirurgia aberta seja necessária algumas vezes (104). As indicações cirúrgicas, independentemente das técnicas utilizadas, de acordo com o Consenso Bariátrico da Sociedade Brasileira de Cirurgia Bariátrica (SBCB) incluem: a) IMC > 40 kg/m^2, independentemente da presença de comorbidades. b) IMC entre 35 e 40 kg/m^2 na presença de comorbidades. c) IMC entre 30 e 35 kg/m^2 na presença de comorbidades nas quais fique

demonstrada a intratabilidade clínica (97). Define-se comorbidade como estado patológico causado, agravado ou cujo tratamento/controle é dificultado pela presença do excesso de peso ou que apresente cura/controle com a perda ponderal (97). As técnicas cirúrgicas aprovadas no Brasil são: *bypass* gástrico (gastroplastia com reconstrução do intestinal em *Y de Roux),* banda gástrica ajustável, gastrectomia vertical e *Duodenal Switch* (97).

No Brasil, a partir de 1999, o Ministério da Saúde reconheceu a necessidade do tratamento cirúrgico dos obesos mórbidos e incluiu a gastroplastia entre os procedimentos cobertos pelo Sistema Único de Saúde. Na linha de cuidado do sobrepeso e obesidade na Rede de Atenção às Pessoas com Doenças Crônicas, a Portaria do Ministério da Saúde nº 424/GM/MS, de 19 de março de 2013, definiu que os pacientes com indicação para o tratamento cirúrgico da obesidade são aqueles com obesidade grau III e obesidade grau II com comorbidades (diabetes, hipertensão arterial, apneia do sono, artropatias, hérnia de disco, etc.) e que tenham seu quadro clínico agravado pela obesidade, obesidade estável há pelo menos cinco anos e que não responderam a tratamento conservador (dietas, psicoterapia, atividades física, etc.), devendo estar com acompanhamento especializado durante, pelo menos, dois anos. Em 2011, a Federação Internacional de Diabetes (IDF), considerando que o aumento da prevalência da obesidade e DM2 se tornou o maior problema de saúde pública no mundo, por meio de um grupo de endocrinologistas, cirurgiões e peritos em saúde pública definiu as recomendações das indicações da cirurgia e outras intervenções gastrointestinais no tratamento e prevenção do DM2 (105). Foi definido que a cirurgia bariátrica é uma terapêutica apropriada para pacientes com DM2 e obesidade que não conseguem alcançar os objetivos terapêuticos com o tratamento clínico, especialmente quando existem comorbidades importantes. A cirurgia deve ser considerada nos pacientes com DM2 e IMC $\geq$ 35 kg/m^2 ou uma alternativa terapêutica em pacientes com IMC entre 30 e 35 kg/m^2 quando o DM2 não puder ser controlado adequadamente mesmo com uma terapêutica clínica otimizada, especialmente na presença de outros fatores importantes de risco cardiovascular (105). A técnica a ser utilizada deve levar em consideração os riscos e benefícios bem como as particularidades de cada paciente (106).

A cirurgia bariátrica tem história relativamente recente e determinou impacto na história da cirurgia. Durante os últimos cinquenta anos, aproximadamente, várias técnicas cirúrgicas foram propostas e experimentadas, utilizando-se diferentes conceitos fisiopatológicos (mal

absorção, restrição de consumo e combinação de mal absorção e restrição). Todas as técnicas podem ser realizadas por cirurgia aberta ou por via laparoscópica (99, 107). Cada modificação ocorreu em resposta às deficiências, complicações e dificuldades surgidas no manejo do paciente ao longo do período pós-operatório. (107). Além destes, ainda existem procedimentos experimentais em desenvolvimento, como por exemplo, a estimulação gástrica por marcapasso gástrico, com finalidade de induzir gastroparesia e perda de peso por estimulação elétrica do antro. Neste procedimento, um eletrodo é posicionado na parede gástrica entre a incisura angularis e a junção esofagogástrica, ao longo da pequena curvatura gástrica, conectado por um fio condutor a um marcapasso posicionado no subcutâneo, programado para emitir estímulo bipolar para o estômago, com consequente saciedade e perda de peso induzidas por estímulos vagais eferentes (108).

Em 1954, Kremen, Linner e Nelson realizaram o primeiro *bypass* jejunoileal, que originalmente foi o primeiro procedimento mal absortivo realizado com o objetivo primário de induzir perda de peso. A cirurgia consistia em uma jejunoileostomia terminoterminal e uma ileocecostomia. Como a cirurgia desencadeou várias complicações, como diarreia, desequilíbrio hidroeletrolítico e insuficiência hepática, nesta década pouco foi produzido, indicando desinteresse dos cirurgiões por este tema (109). A década seguinte trouxe publicações como a de Payne, De Wind e Scott, em 1963, realizando *bypass* jejuno-cólico. Complicações como distúrbios hidroeletrolíticos, diarreia incontrolável e insuficiência hepática, tornaram estes procedimentos proibitivos (110). O uso de estratégias restritivas iniciou em 1966 com Manson e Ito e, rapidamente, suplantou os procedimentos que se caracterizavam apenas por mal absorção. Em seu procedimento inicial, seccionaram o estômago de maneira horizontal, ainda sem o uso de grampeadores mecânicos, e realizaram uma gastrojejunostomia em alça (111). A década de 70 se caracterizou por intensa atividade na cirurgia bariátrica. Enquanto o *bypass* jejunoileal declinava, outros procedimentos mal absortivos e restritivos emergiam. Na Itália, em 1979, Scopinaro criou uma derivação biliopancreática, realizando uma gastrectomia parcial associada à reconstrução do trânsito em *Y de Roux* com alça alimentar de 250 cm e alça comum de 50 cm (112). Anos mais tarde, Hess e Marceau modificaram a derivação biliopancreática realizando uma gastrectomia vertical associada à preservação do piloro e anastomose ileal ao duodeno, conhecida como *Duodenal Switch* (113, 114).

Em 1971, Mason e Printen realizaram, pela primeira vez, um procedimento puramente restritivo dividindo o estômago horizontalmente da pequena para a grande curvatura gástrica, com o trânsito das secreções sendo feito por conduto na grande curvatura. Embora fosse uma técnica mais fisiológica, com maior rapidez de execução e menos complicações do que o *bypass* gástrico, a dificuldade na manutenção da perda de peso fez este procedimento declinar (115). As décadas de 80 e 90 foram marcadas por muitas publicações descrevendo variantes da derivação gástrica em *Y de Roux* (*bypass* gástrico) (107). Em 1989, Fobi introduziu a gastroplastia vertical com anel de silicone restritivo proximal, criando um reservatório gástrico drenado por uma gastrojejunostomia e, em 1998, Fobi e Lee apresentaram uma nova variação da técnica adicionando gastrostomia para nutrição, se necessário, e anel de silicone radiopaco no estômago excluído para facilitar o acesso percutâneo e eventual estudo radiológico ou endoscópico futuro (116, 117).

Em 1991, Capella apresentou técnica semelhante à anterior, onde era realizada uma gastroplastia vertical e um *bypass* gástrico, mas com um reservatório gástrico menor (10 cm^3) ao longo da pequena curvatura com extremidade inferior livre e móvel. A anastomose era realizada na porção terminal livre da gastroplastia com alça jejunal em *Y de Roux* ascendida em posição transmesocólica e retrogástrica (118). Nos anos 90, a banda gástrica ajustável ganhou notoriedade com o uso da laparoscopia por sua maior facilidade de execução e menor tempo cirúrgico. Apesar de muito utilizada em alguns serviços, não apresenta os mesmos resultados em longo prazo com relação à perda de peso quando comparada ao *bypass* gástrico em *Y de Roux* (119, 120). No Brasil, Arthur B. Garrido Júnior foi o pioneiro a introduzir a técnica que chamou *Fobi-Capella*, utilizando recursos das técnicas destes dois autores. Publicou, em 2002, o resultado de mais de mil cirurgias realizadas por sua equipe, com mortalidade de 1% e resultados muito satisfatórios quanto à perda de peso em longo prazo bem como a melhora de comorbidades como distúrbios respiratórios, hipertensão arterial e DM2 (121). A NIH (*National Institutes of Health – Consensus Development Panel*), realizado em 1991, referendou o *bypass* gástrico em *Y de Roux* como a técnica padrão-ouro (122). A cirurgia de Capella é a técnica mais utilizada no Brasil, podendo ser realizada pelo método convencional ou através de cirurgia videolaparoscópica (97).

Vários fatores devem ser levados em consideração na escolha da melhor técnica de acordo

com as comorbidades e o risco cirúrgico do paciente (99). As novas perspectivas estão em torno de novos procedimentos, como a gastrectomia vertical (*sleeve gastrectomy*), e a indicação da cirurgia bariátrica em pacientes selecionados com IMC inferior a 35 kg/m^2 (105, 123). Com o avanço nas técnicas de biologia molecular, sabe-se hoje do papel hormonal da cirurgia bariátrica e, com isso, as pesquisas evoluem para o aspecto metabólico da cirurgia (124). Estas descobertas, no nível celular, podem contribuir no desenvolvimento de possíveis mecanismos de perda de peso e controle de comorbidades além da tradicional explicação da redução do consumo alimentar e mal absorção (99).

1.5.1 Gastrectomia Vertical (*Sleeve Gastrectomy*)

A gastrectomia vertical é uma técnica de cirurgia bariátrica reconhecida pela Sociedade Brasileira de Cirurgia Bariátrica e Metabólica e realizada, internacionalmente, de maneira crescente (97, 125). Foi originalmente realizada como componente restritivo do procedimento *Duodenal Switch*, onde a redução da capacidade gástrica iniciaria uma perda de peso em curto prazo enquanto o componente disabsortivo (derivação biliopancreática) manteria a perda de peso em longo prazo. Era, então, indicada como primeira etapa de tratamento para paciente com obesidade extrema e risco cirúrgico elevado (126). Envolve a criação de um estômago com lúmen reduzido ao longo da pequena curvatura gástrica através da ressecção gástrica ao longo da grande curvatura desde o fundo até o antro gástrico. Esta ressecção leva a formação de um tubo gástrico em continuidade com o esôfago superiormente e piloro e duodeno inferiormente. Através desta técnica, a capacidade gástrica é reduzida em 80% ou mais, as alças intestinais permanecem intactas e o fundo gástrico é removido (127). Mantém íntegra a ordem das estruturas do tubo digestivo, fator primordial para o desempenho de suas funções, mantendo a sequência estômago, piloro, duodeno, jejuno e íleo. Com a ressecção do fundo gástrico, é retirado o principal local de produção de grelina (128, 129). O nome desta operação permanece controverso. Em 1993, Marceau *et al.* foram os primeiros a descreverem-na dentro do contexto da *Duodenal Switch* e designaram-na como gastrectomia parietal (114). Em 1998, Hess denominou-a de gastrectomia vertical (113). Na *American Society for*

Metabolic and Bariatric Surgery é usado o termo *sleeve gastrectomy* (130). A Sociedade Brasileira de Cirurgia Bariátrica e Metabólica recomenda o termo gastrectomia vertical (97).

Muitos trabalhos recentes demonstram que este procedimento promove uma perda de peso acentuada, melhora do perfil metabólico dos pacientes obesos e eficácia da técnica semelhante à técnica mais comumente realizada (*bypass* gástrico em *Y de Roux*), podendo ser recomendada como procedimento único e definitivo para o tratamento da obesidade e suas comorbidades (124, 125, 131-133). Também tem efeito benéfico no controle ou cura do DM2 em pacientes com obesidade grau I sem levar a uma perda de peso indesejável (134). Embora seja considerada um procedimento restritivo, trabalhos experimentais e em humanos demonstram os efeitos metabólicos da gastrectomia vertical na diminuição da resistência insulínica e também melhora no perfil lipídico (135, 136). A gastrectomia vertical, que era indicada como uma primeira etapa de tratamento cirúrgico, tem sido proposta como procedimento primário e definitivo no tratamento da obesidade devido aos resultados satisfatórios no perfil glicídico e lipídico, tanto a curto como em longo prazo (137). Embora a gastrectomia vertical seja tipicamente referida como um procedimento restritivo, evidências sugerem que a redução do volume gástrico, isoladamente, provavelmente, não acarretaria resultados tão eficazes. Considera-se provável a participação de mudanças hormonais, envolvendo grelina, *glucagon-like peptide-1* (GLP1), *peptide YY* (PYY) e outros, nos efeitos benéficos desta técnica (124, 138).

1.5.2 Omentectomia

O omento maior é a maior prega peritoneal e pende inferiormente sob a forma de avental a partir da grande curvatura gástrica. É constituído por tecido adiposo e, em meio a este tecido, também existem tecidos (*milky spots*) ricos em macrófagos, linfócitos e células hematopoiéticas. Isto faz com que o omento seja um tecido de composição única no organismo com propriedades de neovascularização, hemostasia, desbridamento e defesa (139). Trabalhos experimentais demonstraram que o omento é composto principalmente por

tecido adiposo (93% da área omental) e pequenas ilhas de tecido linfoide. Após sua ativação, o componente não adiposo (*milky spots*) se expande quinze a vinte vezes, podendo aumentar de 7% até 76% da área omental. Juntamente com esse incremento do tecido não adiposo, ocorre também o aumento da expressão de fatores de crescimento e de angiogênese (*vascular endothelial growth factor*/VEGF), fatores quimiotáticos (*stromal cell-derived factor 1*/SDF-1α) e de células progenitoras (*C-X-C chemokine receptor type 4*/CXCR-4 e WT-1 gene), associados à promoção da cicatrização e regeneração de tecidos lesados (140, 141). Estas importantes propriedades do omento com relação à imunologia, neovascularização, hemostasia e regeneração, faz com que seja um órgão capaz de bloquear processos inflamatórios intraperitoneais e impedir quadros de peritonites difusas, bem como acelerar a hemostasia e regeneração de espaços mortos e cicatrização de tecidos lesados (142, 143).

Por outro lado, o omento constitui uma parte do tecido adiposo visceral que, quando em excesso, produz citocinas pró-inflamatórias, levando a um processo inflamatório crônico correlacionado com resistência insulínica, intolerância à glicose, dislipidemia, hipertensão e estado pró-trombótico (32, 33). Por este motivo, ressecções do grande omento têm sido propostas como componente de cirurgias bariátricas, no intuito de melhorar o perfil metabólico e maximizar a perda de peso nos pacientes obesos. A omentectomia consiste na dissecção e ressecção completa de todo omento maior ao longo da grande curvatura gástrica até o duodeno bem como de sua inserção no cólon transverso até o baço (144). Esta ressecção implicaria na retirada de uma quantidade significativa de gordura visceral (fonte de *plasminogen activator inhibitor-1*/PAI-1 e resistina, por exemplo), implicada no desenvolvimento da resistência insulínica e de fenômenos tromboembólicos (145). A inclusão da omentectomia como parte da técnica de cirurgia bariátrica permanece controversa e questionável quanto aos efeitos benéficos sobre o metabolismo e o incremento da perda de peso nos obesos (146). Estudos demonstram melhora na homeostase glicêmica e no perfil lipídico quando se associa a omentectomia à cirurgia bariátrica (145, 147). Outros autores demonstraram que a redução do tecido adiposo visceral com a realização da omentectomia não é uma abordagem útil para a melhora da sensibilidade insulínica e redução dos fatores de risco cardiometabólico associados com obesidade ou DM2 (148-150).

2 OBJETIVOS

2.1 Objetivo geral

- Avaliar o perfil glicídico e lipídico bem como a expressão de marcadores moleculares do perfil inflamatório e do sistema renina-angiotensina no tecido adiposo branco de ratos tratados com dieta padrão ou hiperlipídica e submetidos à gastrectomia vertical com ou sem omentectomia.

2.2 Objetivos específicos

- Comparar o peso corporal, consumo alimentar, consumo energético e adiposidade corporal entre os grupos estudados;
- Analisar os efeitos da gastrectomia vertical com ou sem omentectomia no perfil glicídico, por meio dos testes de sensibilidade insulínica e tolerância à glicose, e dosagens plasmáticas de glicose, peptídeo-C e insulina;
- Analisar os efeitos da gastrectomia vertical com ou sem omentectomia no perfil lipídico, por meio das dosagens séricas do colesterol total, HDL e triglicerídeos;
- Analisar os efeitos da gastrectomia vertical com ou sem omentectomia na expressão gênica de marcadores pró-inflamatórios (TNF-alfa e IL-6) e de componentes do sistema renina-angiotensina (enzima conversora de angiotensina, enzima conversora de angiotensina II e angiotensinogênio) no tecido adiposo periepididimal.

3 METODOLOGIA

3.1 Animais

Para realização dos experimentos foram utilizados ratos machos da linhagem Wistar, com oito semanas de idade, procedentes do Instituto de Fisiologia e Farmacologia da Universidade Federal de Minas Gerais – Belo Horizonte/MG (151-153). Os animais foram randomizados em seis grupos compostos por dez ratos/cada, mantidos em gaiolas individuais, em ambiente com ciclos de luminosidade de 12 horas (7 horas às 19 horas), com temperatura de 22 ± 2,0 ºC e acesso à alimentação e água *ad libitum*. As etapas de tratamento, testes de tolerância à glicose, sensibilidade insulínica e sacrifício foram realizadas na Sala de Experimentação Animal do Biotério das Faculdades Integradas Pitágoras – Montes Claros/MG e as demais etapas foram conduzidas no Laboratório de Pesquisa em Saúde do Hospital Universitário Clemente de Faria – Montes Claros/MG.

3.2 Dieta, Consumo Alimentar e Jejum

Todos os animais com oito semanas, desmamados, receberam as respectivas dietas por oito semanas. Os grupos experimentais G1, G2 e G3 receberam a dieta padrão (STD) e água *ad libitum*. Os grupos G4, G5 e G6 receberam dieta experimental hiperlipídica (HFD) e água *ad libitum*. Para a manutenção regular dos animais foi utilizada dieta padrão, que apresentava em sua composição 50,30% de carboidratos, 41,90% de proteínas e 7,80% de lipídios, com um total de 2,18 kcal por grama de dieta (Purina - Labina®, USA). A dieta hiperlipídica, utilizada para induzir obesidade, era composta de 24,55% de carboidratos, 14,47% de proteínas e 60,98% de lipídios, apresentando um total de 5,28 kcal por grama de dieta. Todos os componentes da dieta hiperlipídica foram obtidos da Rhoster® LTDA (São Paulo, Brasil) (154, 155). Após este período de oito semanas, os animais foram submetidos a tratamento cirúrgico (Tabela 1). No pós-operatório, os grupos continuaram com suas respectivas dietas

por mais quatro semanas: G1 a G3 receberam dieta padrão e G4 a G6 receberam dieta hiperlipídica (156). O consumo alimentar (g/peso corporal) dos animais foi mensurado três vezes por semana, em dias pré-estabelecidos, no mesmo horário, calculando-se em cada gaiola a diferença dos pesos das rações ofertada e consumida, fazendo-se o cálculo aritmético da estimativa diária do consumo de ração em gramas. O cálculo do consumo energético (kcal/g de peso corporal) foi feito pela multiplicação da dieta ingerida (g/peso corporal) por seu respectivo valor energético (kcal). A mensuração do consumo alimentar e energético foi realizada em todos os animais dos seis grupos durante todo o experimento. Foi oferecido somente água nas doze horas que antecederam a realização dos testes de tolerância à glicose, procedimento cirúrgico e sacrifício e nas primeiras vinte quatro horas após a cirurgia.

Status do Grupo	**Identificação dos Grupos**
Dieta Padrão (STD) / Laparotomia (L)	G1 (n = 10)
Dieta Padrão (STD) / Gastrectomia vertical (GV)	G2 (n = 10)
Dieta Padrão (STD) / Gastrectomia vertical + Omentectomia (GVO)	G3 (n = 10)
Dieta Hiperlipídica (HFD) / Laparotomia (L)	G4 (n = 10)
Dieta Hiperlipídica (HFD) / Gastrectomia vertical (GV)	G5 (n = 10)
Dieta Hiperlipídica (HFD) / Gastrectomia vertical + Omentectomia (GVO)	G6 (n = 10)

TABELA 1 – Grupos de animais conforme tipo de dieta e tratamento cirúrgico

3.3 Curva de Peso Corporal e Peso do Tecido Adiposo

O peso corporal foi avaliado durante todo o experimento e também imediatamente antes da cirurgia e antes do sacrifício (final da quarta semana de pós-operatório). Os animais foram pesados três vezes por semana, em dias pré-estabelecidos, tão logo se iniciava o período claro, com balança apropriada, de precisão, e a anotação do peso do animal feita em gramas. Para avaliação da adiposidade pós-operatória, após o sacrifício foram retirados e pesados os tecidos

adiposos brancos periepididimal, retroperitoneal e mesentérico e também o tecido adiposo marrom interescapular, os quais foram imediatamente congelados em nitrogênio líquido e armazenados em gelo seco (- 80° C) para análises posteriores. O peso do tecido adiposo foi corrigido pelo peso corporal.

3.4 Experimentos *in* vivo: Teste de Sensibilidade Insulínica e Teste de Tolerância à Glicose

Uma semana antes da cirurgia e, posteriormente, antes do sacrifício foi realizado o teste de sensibilidade insulínica (TSI), através de coletas de sangue na cauda do animal, para determinação dos níveis de glicose a qual foi avaliada nos tempos 0, 15, 30 e 60 minutos após injeção intraperitoneal de insulina (0.75 U/kg BW; Sigma, St. Louis, MO, USA) (96). Três dias após o TSI, com jejum prévio de doze horas, foi realizado o teste de tolerância à glicose (TTG) com a dosagem dos níveis de glicose nos tempos 0, 15, 30, 60 e 120 minutos após a injeção intraperitoneal de glicose (2 g/Kg BW), usando Accu-Check (Roche Diagnostics Corp. Indianapolis, USA) (96).

3.5 Anestesia, Antibioticoprofilaxia e Procedimentos Cirúrgicos

No tempo determinado (8ª semana de tratamento com as respectivas dietas), os ratos foram submetidos a tratamento cirúrgico após jejum de 12 horas. A anestesia foi feita com Cetamina 10% (60 mg/kg) associada à Xilazina 2% (8 mg/kg), via intraperitoneal, conforme o Protocolo Anestésico do Comitê de Ética em Experimentação Animal da Universidade Federal de Minas Gerais (157).

Os animais foram mantidos em ventilação espontânea durante os procedimentos, e o plano anestésico foi controlado, a cada 10 minutos, mediante avaliação dos reflexos auricular e interdigital, que deveriam estar abolidos. Em caso de necessidade, era administrado mais ¼ a ½ da dose inicial dos anestésicos utilizados (157). A antibioticoprofilaxia foi realizada com Ceftriaxona na dose de 100 mg/kg de peso, por via intramuscular, logo após o início da

anestesia. Foram adotados os princípios de assepsia e antissepsia de Halsted, utilizando instrumental cirúrgico estéril (158). Os animais anestesiados foram submetidos à tricotomia da região abdominal e posicionados em posição supina com suas extremidades e a cauda devidamente imobilizadas. A antissepsia da região abdominal foi feita com clorohexidine em veículo aquoso e colocado campos cirúrgicos sanitizados e fenestrados em região abdominal dos animais. Foi realizada incisão longitudinal mediana da parede, iniciando no epigástrio, de aproximadamente 5,0 cm de extensão, utilizando lâmina de bisturi descartável número quinze, com adequada exposição do estômago e omento. Nos grupos G1 e G4 foi feita apenas a manipulação das vísceras (incluindo o estômago) e posterior rafia da parede abdominal (laparotomia não terapêutica / simulada). Nos grupos G2 e G5 foi feita a gastrectomia vertical (*sleeve gastrectomy*) cujo plano de secção originava-se na parte distal do antro, a cerca de 1,5 a 2,0 mm do piloro, e estendia-se até o ângulo de Hiss. A síntese do tubo gástrico foi realizada com chuleio contínuo usando fio de poligalactina 5-0 (125). A ressecção do estômago foi de 80%, incluindo a remoção total do fundo gástrico. Nos grupos G3 e G6, além da gastrectomia descrita anteriormente, também foi realizada a omentectomia, com ressecção total do omento maior (147). Após as ressecções e revisão final, terminado o ato cirúrgico, os animais foram hidratados com 1,0 mL de solução cristalóide (soro fisiológico 0,9%) para cada 300g de peso, na temperatura de 36 ºC, via peritoneal. A síntese da parede abdominal foi realizada em dois planos. Inicialmente, com sutura contínua em monobloco do peritôneo parietal, da camada muscular e aponeurose com fio de poligalactina 4-0 pré-montado em agulha cilíndrica. E, por fim, a síntese da pele com sutura contínua com fio de poligalactina 4-0 pré-montado em agulha cilíndrica. Os animais foram mantidos aquecidos e em observação até a recuperação completa da anestesia, quando, então, foram encaminhados para suas respectivas gaiolas de origem, sob as mesmas condições ambientais pré-operatórias. Antes da recuperação anestésica, os animais receberam analgesia com 0,5 mL da solução de 500 mg de dipirona diluídos em 01 mL de água (159). A ingestão de água *ad libitum* foi liberada logo após a recuperação anestésica e o consumo alimentar, com a mesma composição calórica do pré-operatório (STD ou HFD), *ad libitum*, foi liberado 24 horas após o procedimento. Durante as primeiras 72 horas de pós-operatório foram mantidos com 01 grama de dipirona diluído em 100 mL de água (*ad libitum*). Os animais foram avaliados e acompanhados até o final da 4ª semana de pós-operatório.

3.6 Eutanásia

No final da 20ª semana de vida foram realizados os procedimentos de jejum, avaliação do peso corporal e, em seguida, efetuada a eutanásia pela técnica de decapitação, conforme orientações da Sociedade Brasileira de Ciência de Animais de Laboratório / Colégio Brasileiro de Experimentação Animal – SBCAL – COBEA (160). Após o sacrifício foram coletados sangue para testes laboratoriais e retirados os seguintes órgãos e tecidos: fígado, tecido adiposo branco (retroperitoneal, mesentérico e periepididimal) e tecido adiposo marrom (interescapular). Posteriormente, os tecidos foram congelados em – 80 ºC.

3.7 Coletas de Sangue e Testes Laboratoriais

A coleta de sangue ocorreu após jejum de 12 horas e imediatamente após o sacrifício. As amostras tiveram o soro e plasma separados por centrifugação e armazenados a uma temperatura de – 20 °C para dosagens séricas de colesterol total, lipoproteína de alta densidade (HDL), triglicerídeos, alanino aminotransferase (ALT), aspartato aminotransferase (AST), glicose, insulina, peptídeo-C, proteína C reativa (PCR) e ferritina. Os testes laboratoriais foram feitos utilizando kits enzimáticos de espectrofotometria específicos (DSA BioELISA, USA).

3.8 Histologia

Após o sacrifício, amostras do tecido adiposo periepididimal foram separadas, fixadas em solução de formaldeído a 10% e incluídas em parafina. Foram feitos cortes de 7 µm de espessura, em micrótomo específico, com posterior montagem em lâminas de vidro previamente preparadas as quais foram coradas por hematoxicilina e eosina (HE) e avaliadas

por microscopia de luz convencional (Olympus BX50 microscope (Tokyo, Japan)). Imagens de áreas de tecido adiposo (com lentes objetivas X40) foram capturadas com Evolution LC color light camera (Media Cybernetics, Rockville, MD, USA).

3.9 Retrotranscrição e Análise da Expressão de RNA por Reação em Cadeia da Polimerase Quantitativo em Tempo Real (qRT-PCR)

O RNA total do tecido adiposo foi obtido através do reagente Trizol (Invitrogen Corp., San Diego, CA, USA), tratado posteriormente com DNAse e transcrição reversa com *moloney murine leukemia vírus* (MMLV) *reverse transcriptase* (Invitrogen Corp.), usando primers hexâmeros aleatórios. O RNA total foi utilizado para a síntese de cDNA num volume final de reação de 30 µL utilizando-se 1.000 ng de RNA total, 0,2 µg de hexadeoxinucleotídeos, tampão para RT (concentrações finais: Tris-HCl 45 mM pH 8,3; KCl 68 mM), 5 µl de $MgCl_2$ 50 mM, DTT 15 mM, dNTPs 1,8 mM e 150 UI de transcriptase reversa. O cDNA foi sintetizado em termociclador durante um período de 60 minutos de incubação a 37º C. A reação foi finalizada pelo aquecimento a 90 ºC por 5 min. As reações de PCR quantitativo foram feitas utilizando primers específicos para o cDNA dos genes de interesse: TNF-alfa, IL-6, ECA, ECA2 e AGT. A confecção e análise dos pares de bases foram executadas através do programa BLASTN, sintetizados pela empresa Invitrogen, aliquotados na concentração de 200 µM e armazenados em freezer a – 20 ºC. O cDNA obtido na etapa de RT (2 µl) foi usado como fita molde para a amplificação por PCR. As reações de PCR quantitativo dos genes de interesse tiveram um volume final de 20 µl e foram feitas em duplicatas, utilizando 10 µl do *Master Mix do SYBR Green reagent* (Applied Biosystem, Grand Island, NY, EUA), 0,25 µl de cada primer na concentração de 1 nM e 8,5 µl de água nuclease free e 1 µl de cDNA e foram realizadas no equipamento QuantStudio™ 6 Flex Real-Time PCR System equipment (Applied Biosystems, EUA). A expressão gênica foi normalizada usando a Beta-actina endógena (FW: 5'-TGA CAG GAT ACA GAA GGA GA-3'; RV: 5'-TAG AGC CAC CAA TCC ACA CA-3'). Os genes de interesse e seus respectivos primers foram: TNF-α (FW: 5'-ATG GGC TCC CTC TCA TCA GT-3'; RV: 5'-GCT TGG TGG TTT GCT ACG AC-3'),

IL-6 (FW: 5’-GTC AAC TCC ATC TGC CCT TCA-3’; RV: 5’-GAA GGC AAC TGG CTG GAA GT-3’), AGT (FW: 5’-CCT AAC TGA CCC GAG CTG TAG-3’; RV: 5’-TGT GGA CTT GCT TCT GTG TGT-3’), ACE (FW: 5’-ATT GCA GCC GGG CAA CTT-3; RV: 5’-TCC TCC GTG ATG TTG GTG TC-3’) and ACE2 (FW: 5’-GCC CAA AAG ATG AAC GAG GC-3’; RV: 5’-CGC TTG ATG GTC GCA TTC TG-3’). O método CT comparativo relativo de Livak e Schmittgen foi aplicado para comparar os níveis de expressão de genes entre grupos usando a equação $2^{(-\text{Delta Delta C(T)})}$ (161).

3.10 Análises Estatísticas

Diferenças entre dois grupos foram avaliadas pelo teste t de Student. Diferenças entre mais de 2 grupos foram analisadas por análise de variância - ANOVA seguido do pós-teste de Bonferroni. O nível de significância previamente estabelecido foi $p < 0,05$. Essas análises foram realizadas com o programa *GraphPad Prism* versão 6.0.

3.11 Aspectos Éticos

O presente estudo seguiu os preceitos nacionais e internacionais que regem o desenvolvimento de pesquisa em animais. Este estudo foi aprovado pelo Comitê de Ética em Experimentação e Bem-Estar Animal da UNIMONTES, Montes Claros – MG, pelo processo número 031/2014 (Anexo A).

4 PRODUTOS

Os produtos foram dois artigos científicos.

4.1 Produto 1: *Effects of sleeve gastrectomy on metabolic profile and adipose renin-angiotensin system expression in obese rats,* formatado segundo as normas para publicação do periódico Obesity Surgery.

4.2 Produto 2: *Effects of omentectomy in addition to sleeve gastrectomy on metabolic and inflammatory profile of obese rats*, formatado segundo as normas para publicação do periódico Journal of Surgical Research.

4.1 Produto 1

EFFECTS OF SLEEVE GASTRECTOMY ON METABOLIC PROFILE AND ADIPOSE RENIN-ANGIOTENSIN SYSTEM EXPRESSION IN OBESE RATS

Short title: EFFECTS OF SLEEVE GASTRECTOMY

Manuscript type: Original contribution

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ABSTRACT

Background: Sleeve gastrectomy (SG) has been used as a multipurpose surgical procedure for the treatment of obesity. The present study assessed the effects of SG on the metabolic and inflammatory profile and renin-angiotensin system (RAS) in high-fat diet-induced obesity male rats. **Methods:** Male Wistar rats were treated with standard diet or high-fat diet and submitted to SG or sham surgery. The glycemic and lipid profile and the gene expression of inflammatory markers and RAS components in adipose tissue were evaluated. **Results:** The SG led to weight loss, decrease in adiposity ($p < 0.01$), increase in the brown adipose tissue weight ($p < 0.05$) and reduction in glucose plasma levels ($p < 0.05$), C-peptide ($p < 0.05$), insulin ($p < 0.001$) and total cholesterol ($p < 0.05$). In addition, SG led to a decrease in the expression of tumor necrosis factor-alpha (TNF-α) ($p < 0.01$), interleukin-6 (IL-6) ($p < 0.001$), angiotensinogen (AGT) ($p < 0.001$) and angiotensin convert enzyme (ACE) ($p < 0.05$) and increase in the expression of angiotensin convert enzyme 2 (ACE2) ($p < 0.05$) in white adipose tissue. **Conclusion:** This study evidenced the benefic effects of the SG in weight loss and improvement in metabolic parameters in this animal model, and RAS seems to have an important role.

Keywords: Obesity; Bariatric Surgery; Inflammation; Renin-Angiotensin System; Metabolism

INTRODUCTION

Obesity is considered a serious public health problem worldwide. It is characterized by an imbalance between the energy intake and expenditure resulting in the accumulation of corporal adipose tissue [1]. In adipose tissue, especially visceral, there is an increase in the expression of proinflammatory cytokines leading to the development of a chronic inflammatory state [2].

The comprehension of preventive and therapeutic strategies for the obesity remains inconclusive [1]. In this perspective, the renin-angiotensin system (RAS) may be considered a potential target for the treatment of obesity due to its metabolic properties recently described, especially in the adipose tissue [3, 4]. The RAS, is one of the most important systems involved in the pathogenesis of cardiovascular diseases, and consists primarily in a coordinated enzymatic cascade, in which the renin acts on the angiotensinogen (AGT) that is converted into angiotensin I (Ang I) and, subsequently, into angiotensin II (Ang II) by the angiotensin converting enzyme (ACE) [5]. The Ang II is a potent vasoconstrictor that under the action of the angiotensin converting enzyme 2 (ACE2), may originate the Ang-(1-7), which acting through the Mas receptor, promotes antagonistic actions to those produced by Ang II [6, 7].

Along with clinical approaches, surgical techniques that were originally developed for the treatment of severe obesity, constitute an important therapeutic approach for the treatment of diabetes mellitus type II (DM2) and other comorbidities in obese patients [8, 9]. Among those techniques, the sleeve gastrectomy (SG), which was initially indicated as the first stage in the treatment of patients with severe obesity or high surgical risk, has been considered a therapeutic option as a primary and definitive procedure in the treatment of obesity and DM2

due to its satisfactory results on the glycemic and lipid profile [10, 11].

As obesity progresses with increased endocrine activity, it becomes relevant the study of surgical treatment effects on the glycemic and lipid profiles and also in the gene expression of important components of the RAS and inflammatory markers.

MATERIALS AND METHODS

Animals and experimental diets

The experiment was conducted with forty male Wistar rats (eight weeks old), which were randomly divided into four groups (n=10/each) and fed with the respective experimental diets for eight weeks: standard diet (STD) and high-fat diet (HFD). The STD (Purina - Labina®, USA), used for the regular maintenance of the rats, is composed of 50.30% of carbohydrate, 41.90% of protein and 7.80% of fat, with a total of 2.18 kcal per 1 g of diet [12, 13]. The HFD is composed of 24.55% of carbohydrate, 14.47% of protein and 60.98% of fat, representing a total of 5.28 kcal per 1 g of diet [12, 13]. All of the high-fat diet components were purchased from Rhoster® LTDA (São Paulo, Brazil). After this period, the animals were submitted to surgical treatment: sham surgery (laparotomy) or sleeve gastrectomy. The experimental groups were: rats treated with standard diet and submitted only to laparotomy (STD+L), standard diet and sleeve gastrectomy (STD+SG), high-fat diet and laparotomy (HFD+L) and high-fat diet and sleeve gastrectomy (HFD+SG). After surgery, the animals continued to receive their respective diets for four additional weeks. The animals were individually housed in an environment with 12 hour light cycle (7 am to 7 pm), at a temperature of 22 ± 2.0 °C and had access to food and water *ad libitum*. This study was

approved by Ethics Committee of Experimentation and Animal Welfare of Unimontes, Montes Claros, Brazil, by the process number 031/2014.

Surgical procedures

The animals were anesthesized with ketamine (100 mg/kg) and xylazine (30 mg/kg) intraperitoneally under spontaneous ventilation. Antibiotic prophylaxis was performed by intramuscular administration of ceftriaxone (100 mg/kg), immediately after the onset of anesthesia. Median longitudinal incision was performed, starting at the epigastrium, with approximately 5.0 cm in length. The groups STD+L and HFD+L underwent sham surgery. The groups STD+SG and HFD+SG underwent SG with 80% resection of the stomach, including the complete removal of the gastric fundus and confection of a gastric tube from the distal part of the antrum (1,5 mm from the pylorus) to the Hiss angle using 5-0 polyglactin yarn.

Measurements of body weight, food intake, tissue collection and plasma parameters

Body weight (BW), food intake and energy intake (food intake in kcal) were measured three times a week both in the pre and postoperative period. One week before surgery and sacrifice insulin sensitivity tests (IST) were performed for determination of blood glucose levels, which were monitored at 0, 15, 30, and 60 min after intraperitoneal injection of insulin (0.75 U/kg BW; Sigma, St. Louis, MO, USA). The glucose tolerance test (GTT) was performed measuring the blood glucose levels at 0, 15, 30, 60, and 120 minutes after intraperitoneal injection of glucose (2 g /kg BW) following a fasting period of 12 hours, using

Accu-Check (Roche Diagnostics Corp. Indianapolis, USA) [14]. Overnight-fasted rats were sacrificed by decapitation with guillotine and blood samples were collected and centrifuged (3200 rpm for 10 min) when the plasma was separated for the determination of the total serum cholesterol, HDL cholesterol, triglycerides, glucose, insulin, C-peptide, ferritin and C-reactive protein (CRP) by enzymatic tests (DSA BioELISA, USA). Samples of adipose tissues were collected, weighted and immediately frozen in liquid nitrogen and stored in dry ice (- 80 °C) for further analysis.

Reverse transcription and quantitative real time-polymerase chain reaction (qRT-PCR)

Total RNA from the adipose tissue was prepared using TRIzol reagent (Invitrogen Corp., San Diego, CA, USA), treated with DNAse and reverse transcribed with MMLV (Invitrogen Corp.) using random hexamer primers. Levels of the interested genes were determined by qRT-PCR using SYBR Green reagent (Applied Biosystems, Grand Island, NY, USA) in QuantStudio™ 6 Flex Real-Time PCR System equipment (Applied Biosystems, USA). Gene expression was normalized to the endogenous Beta-actin (FW: 5'-TGA CAG GAT ACA GAA GGA GA-3'; RV: 5'-TAG AGC CAC CAA TCC ACA CA-3'). The genes of interest and respective primers were: TNF-α (FW: 5'-ATG GGC TCC CTC TCA TCA GT-3'; RV: 5'-GCT TGG TGG TTT GCT ACG AC-3'), IL-6 (FW: 5'-GTC AAC TCC ATC TGC CCT TCA-3'; RV: 5'-GAA GGC AAC TGG CTG GAA GT-3'), AGT (FW: 5'-CCT AAC TGA CCC GAG CTG TAG-3'; RV: 5'-TGT GGA CTT GCT TCT GTG TGT-3'), ACE (FW: 5'-ATT GCA GCC GGG CAA CTT-3; RV: 5'-TCC TCC GTG ATG TTG GTG TC-3') and ACE2 (FW: 5'-GCC CAA AAG ATG AAC GAG GC-3'; RV: 5'-CGC TTG ATG GTC GCA TTC TG-3').

Hematoxylin and eosin staining

Periepididymal adipose tissue samples were fixed in formaldehyde solution (10%), and embedded in paraffin. Sections of 5 mm were prepared for hematoxylin and eosin (HE) staining, and evaluated under a conventional light microscope (Olympus BX50 microscope (Tokyo, Japan). Images of fat tissue areas (X40 objective lenses) were captured with Evolution LC color light camera (Media Cybernetics, Rockville, MD, USA).

Statistical analysis

All data were transferred to GraphPad Prism software (Version 5.0®, San Diego, CA, USA) and analyzed with 95% ($P < 0.05$) confidence. Data are expressed as the mean ± SD. The statistical significance of differences in mean values among rats groups was assessed by one-way ANOVA followed by Bonferroni post-test.

RESULTS

During the preoperative period, the food intake (g/g BW) was similar between the groups treated with STD and HFD, but the energy intake (kcal/g BW) was higher for the HFD treated group, which led to the statistically significant increase in the body weight of the rats in this group (Figure 1). Four weeks after surgery, a significant reduction of the food intake, energy intake and body weight was observed in the groups submitted to STD+SG and HFD+SG. In these groups, a significant adiposity reduction in the white adipose tissues (periepididymal and retroperitoneal), related to the adipocyte area and diameter was observed

(Figure 2). Regarding the interscapular brown adipose tissue weight a significant increase was observed in the HFD+SG group (Figure 2).

For the glycemic profile, a significant increase in the insulin resistance and decrease in the glucose tolerance was observed in the HFD group pre surgery. The glycemic parameters, including measurements of IST and plasmatic measurements of glucose, C- peptide and insulin showed a significant improvement in the post surgery period in the HFD+SG group when compared to the HFD+L and STD+L groups (Figure 3 and 4). For the lipid profile, it was observed a decrease in the levels of total cholesterol in the HFD+SG group in comparison to the HFD+L and STD+L groups. Additionally, a decrease in the levels of triglycerides was observed in the group STD+SG in comparison to the HFD+L group. Regarding the HDL cholesterol levels, there was no difference between the groups submitted to surgery when compared to the respective control groups, which was also observed for the quantitative plasmatic measurements of CRP (Figure 4). The ferritin levels were significantly lower in the group HFD+SG group in comparison to the respective control group.

The mRNA expression of proinflammatory cytokines by qRT-PCR in periepididymal adipose tissue showed a statistically significant decrease in the levels of TNF-α and IL-6 in rats from the HFD+SG group in comparison to its respective sham group. The expression of components of the RAS by qRT-PCR in the same tissue showed a decrease in the mRNA levels of angiotensinogen and ACE and an increase in the mRNA levels of ACE2.

DISCUSSION

In the present study we evaluated the effects of SG on the glycemic, lipid and inflammatory profile as well as on the expression of renin-angiotensin system components in white adipose tissue of rats with obesity induced by high-fat diet.

There is evidence in the literature that this experimental model is suitable for the induction of obesity, accomplishment of SG and follow-up of body profile (loss of weight and adiposity) and biochemical and hormonal parameters [15, 16]. The SG performed in this study showed a reduction in food intake and a significant loss of weight in the postoperative period. This data is in accordance with the literature that demonstrates a loss of weight in humans and rodents after SG [15, 17]. However, a limitation in our study was the short period of follow-up devoted to the animals (four weeks only), preventing us to better assess the regain of weight after the SG. Nevertheless, authors have demonstrated with this same surgical technique, the maintenance of weight loss in the same rodent models for a period up to fifteen weeks of postoperative [15]. Studies with humans found the weight loss after SG as significant as the weight loss reported for patients submitted to Roux-en-Y gastric bypass after twelve months of postoperative follow-up [18]. Other authors showed the maintenance of weight loss even after a long period of follow-up, showing a significant improvement of the comorbidities [19]. Another limitation of our study regards in the fact that the sham surgery did not include a gastrostomy and closure. We only performed a laparotomy and closure of the abdominal wall. Authors describe that in the control group they performed the same surgical intervention, except the gastric excision. Instead of the resection, a slight pressure with blunt forceps along a vertical line between the esophageal sphincter and the pylorus was performed, resembling the stapling process [20, 21]. The SG consists in the

creation of a reduced stomach lumen with the excision of the gastric fundus, remaining the intestine itself intact. One of the factors that could be associated with weight loss could be a reduction in the levels of ghrelin, a hormone mainly produced by the A-cells in the fundus of the stomach. This correlation was demonstrated in humans, highlighting a more significant reduction in the levels of ghrelin and weight loss in the SG, with a follow-up of six months of postoperative period, not being observed with other technique as exemplified by adjustable gastric banding [22]. However, in genetically obese models of rodents (leptin-insensitive), it was observed that the weight loss after SG may be independent of the levels of ghrelin [23]. Furthermore, the authors demonstrated the normalization of the levels of ghrelin after SG in rodents with exogenous obesity, suggesting the existence of extragastric ghrelin production [24].

The brown adipose tissue (BAT) cells use more mitochondrial uncoupling protein 1 (UCP1) to perform the respiration chemical process and dissipate chemical energy as heat. The thermogenic effect, obtained by the BAT activation, leads to an increase in the energy expenditure, reduction of adiposity and in the glucose and plasma levels of lipids, thus contributing to the homeostasis improvement [25]. The evidence of a significant interscapular brown adipose tissue weight gain, in the group treated with high-fat diet and submitted to SG, corroborated with the literature that also shows an increment of the brown adipose tissue after SG [26]. In addition, a study performed with F-18-fluorodeoxyglucose positron emission tomography/computed tomography, before and one year after bariatric surgery showed a benefic impact of the surgery on the adipose tissue, including the brown adipose tissue regarding an improvement in the metabolic parameters in patients with metabolic syndrome [27]. Experimental studies show that the SG may mediate the effects with great physiological impacts, including changes of the intestinal microbiota, secretion of intestinal hormones, in

the levels and composition of bile acids and in the reduction of hepatic steatosis [21, 28]. Two receptors have been identified that respond to bile acids. The first is a G protein-coupled receptor found on the cell surface termed TGR5, and the second is a ligand-activated transcription factor farnesoid X receptor (FXR) [28]. FXR plays an important role in a wide range of gastrointestinal functions and experiments point to an important role of FXR as a molecular target for the potent effects of SG. Loss of function of the nuclear bile acid receptor (FXR) greatly diminishes the effects of SG [28, 29]

In this study, the GTT and IST performed in the preoperative period showed alterations in the glycemic profile induced by high-fat diet. In the postoperative, the plasma levels of glucose, C-peptide and insulin confirmed the improvement in the glycemic profile in the groups treated with high-fat diet and submitted to SG. This effect is widely documented in the literature, where authors have shown the surgical effect on glycemic profile with a significant reduction in the plasma levels of glucose and insulin and a normalization of the homeostatic model assessment for insulin resistance [30].

Regarding the lipid profile, a statistically significant reduction in the levels of total cholesterol was demonstrated, corroborating with studies that showed an improvement in the lipid profile after SG in both rodents and humans [31, 32], although the same was not observed with the dosage of HDL cholesterol and triglycerides.

Concerning the inflammatory parameters, in our study the plasmatic dosage of CRP did not show statistically significant difference among groups. Obesity evolves with increasing plasma concentrations of CRP, being considered an important risk factor for the development of cardiovascular diseases. A study performed in patients with severe obesity evidenced high concentrations of hs-CRP, which were significantly reduced after SG [33]. However, the ferritin dosage showed a statistically significant reduction in the high-fat diet

group submitted to SG. This result is in accordance with a study that showed decreased levels of ferritin in obese patients one year after SG [34].

The inflammatory markers are increased in obesity and described to be a link between obesity and its comorbidities, mainly due to their proinflammatory properties and consequent alterations in the cardiovascular function and insulin resistance state [35]. In this study, TNF-α and IL-6 were decreased in the periepididymal adipose tissue of rats submitted to SG, corroborating with a study that demonstrate the benefic effects of this surgical technique in the reduction of the inflammatory state associated with obesity [35].

As far as we know, this study is unique regarding the analyses of gene expression of the RAS in the periepididymal adipose tissue after SG, evidencing a significant decrease in the levels of AGT and ACE and an increase in the levels of ACE2. Studies demonstrate that the adipose tissue represents an important source of the RAS's components production and that there is an interaction between the secretions of the RAS's tissue components with the RAS's circulating components. The hypertrophy of the adipocytes increases the secretion of RAS's components, enabling the generation of several cardiovascular and metabolic changes [36, 37]. At the cellular level, RAS's components induce insulin resistance, contribute to the oxidative stress, inflammation and apoptosis in β-pancreatic cells [38]. On the other hand, experimental studies show the importance of the Ang-(1-7) on lipid and glycemic profile as well as on the inflammatory profile in the adipose tissue. High chronic levels of Ang-(1-7) are shown to attenuate the proinflammatory profile of the adipose tissue, protecting against metabolic stress induced by high-fat diet [39]. The increase in the plasma levels of Ang-(1-7) are also described to generate an increment in the glycemic and lipid profile evidenced by the improvement in the glucose tolerance and insulin sensitivity as well as a decrease in cholesterol and triglycerides levels [14].

CONCLUSIONS

In summary, the primary goal of the surgical treatment of obesity, in addition to weight loss, is the improvement of the metabolic profile. This study, showed the beneficial effects of the SG in the glycemic, lipid and inflammatory profile in a rat model. Furthermore, it was evidenced that this surgical technique altered the expression of components of the RAS in the white adipose tissue: reduced the expression levels of AGT and ACE and increased the expression of ACE2 in rats with obesity-induced by diet. Finally, in this experimental study, the sleeve gastrectomy presented satisfactory effects in the glycemic and lipid profile as well as in the gene expression of inflammatory and RAS markers.

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Conflict of interest

All authors declare no conflict of interest.

Statement Animals Rights

All applicable institutional and/or national guidelines for the care and use of animals were followed.

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LEGENDS OF FIGURES

Figure 1. Body weight, food intake and energy intake of rats fed with standard diet (STD) and high-fat diet (HFD) and submitted to sleeve gastrectomy (STD+SG and HFD+SG) or sham surgery (STD+L and HFD+L). **(A)** Body weight (g) preoperative. **(B)** Food intake (g/BW) preoperative. **(C)** Energy intake (Kcal/g BW) preoperative. **(D)** Body weight (g) postoperative. **(E)** Food intake (g/BW) postoperative. **(F)** Energy intake (Kcal/g BW) postoperative. **(G)** Pre and postoperative weight curves. *P < 0.05; **P < 0.01; ***P < 0.001 (t-tests, one-way ANOVA and Bonferroni post-test).

Figure 2. Effects on adipose tissues of rats fed with standard diet (STD) and high-fat diet (HFD) and submitted to sleeve gastrectomy (STD+SG and HFD+SG) or sham surgery (STD+L and HFD+L). **(A)** Periepididymal adipose tissue weight (g/BW). **(B)** Mesenteric adipose tissue weight (g/BW). **(C)** Retroperitoneal adipose tissue weight (g/BW). **(D)** Interscapular brown adipose tissue weight (g/BW). **(E)** Body adiposity/white adipose tissue weight (periepididymal, mesenteric and retroperitoneal) (g/BW). **(F)** Adipocyte area (μ/m^2). **(G)** Periepididymal adipose tissues. **G.1**: HFD+L; **G.2**: STD+L, **G.3**: HFD+SG; **G.4**: STD+SG **(H)** Hematoxylin and eosin (HE) staining of periepididymal adipose tissue samples. Inlet: HE staining. Scale bar: x40. **H.1**: STD+L; **H.2**: STD+SG; **H.3**: HFD+L; **H.4**: HFD+SG. *P < 0.05; **P < 0.01; ***P < 0.001 (one-way ANOVA and Bonferroni post-test).

Figure 3. Preoperative and pre-sacrifice insulin sensitivity tests (IST), glucose tolerance tests (GTT) and their respective areas under the curve (AUC) in rats fed with standard diet (STD) and high-fat diet (HFD) and submitted to sleeve gastrectomy (STD+SG and HFD+SG) or sham surgery (STD+L and HFD+L). **(A)** Preoperative IST (mg/dL). **A.1**: AUC of preoperative IST. **(B)** Preoperative GTT (mg/dL). **B.1**: AUC of preoperative GTT. **(C)** Pre-sacrifice IST (mg/dL). **C.1**: AUC of pre-sacrifice IST. **(D)** Pre-sacrifice GTT (mg/dL). **D.1**: AUC of pre-sacrifice GTT. *P < 0.05; **P < 0.01; ***P < 0.001 (one-way ANOVA and Bonferroni post-test).

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Figure 5. Analysis of mRNA expression of inflammatory-related targets by qRT-PCR in periepididymal adipose tissue of rats fed with standard diet (STD) and high-fat diet (HFD) and submitted to sleeve gastrectomy (STD+SG and HFD+SG) or sham surgery (STD+L and HFD+L). **(A)** mRNA expression of tumor necrosis factor - alpha (TNF- α) (Arbitrary Unit). **(B)** mRNA expression of interleukin-6 (IL-6) (Arbitrary Unit). **P < 0.01; ***P < 0.001 (one-way ANOVA and Bonferroni post-test).

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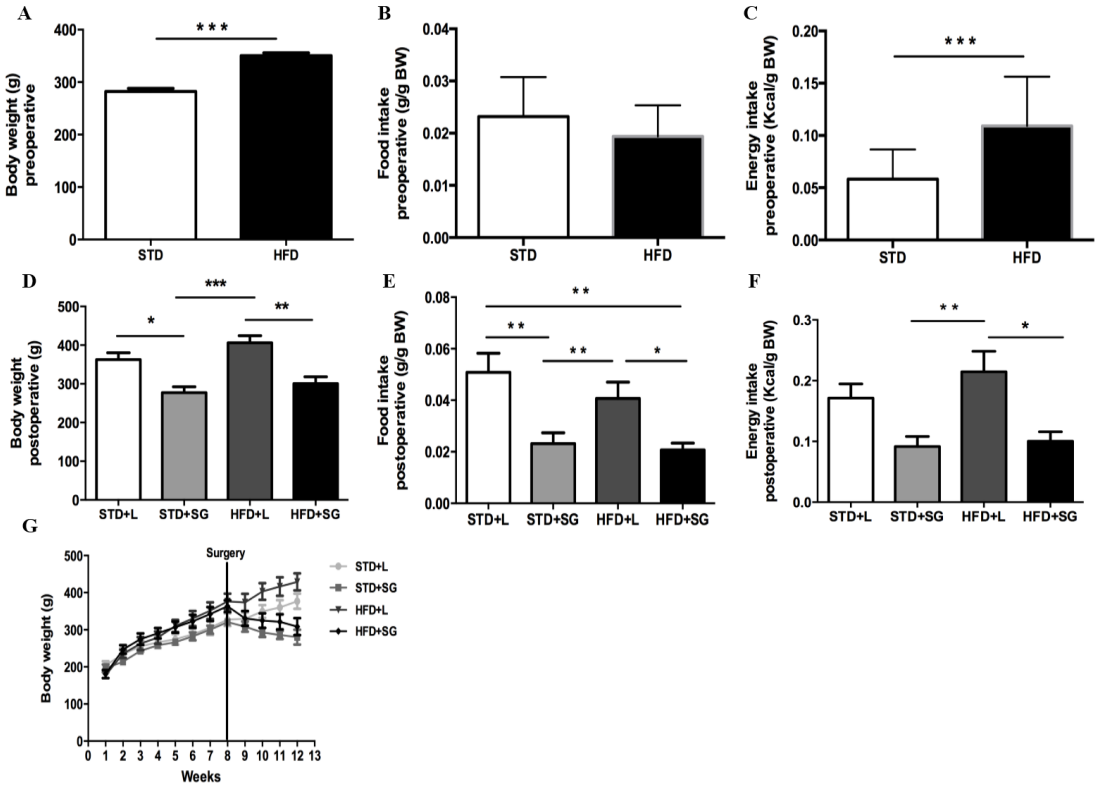
FIGURES



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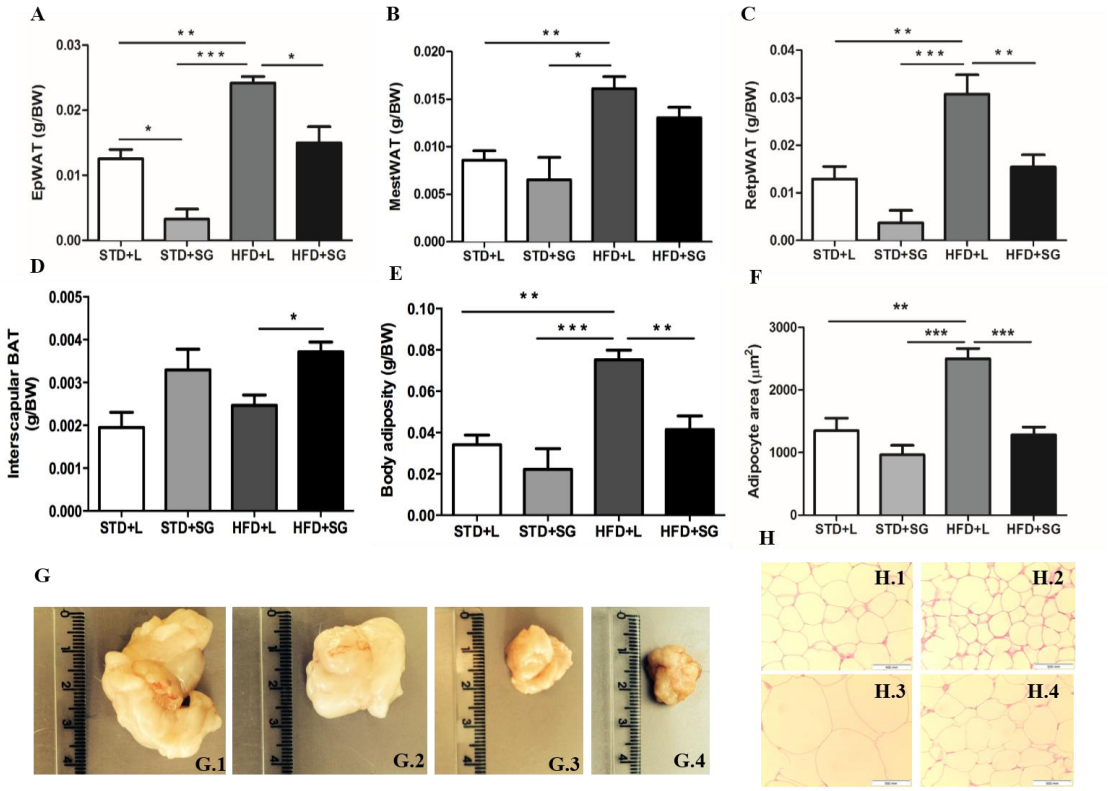

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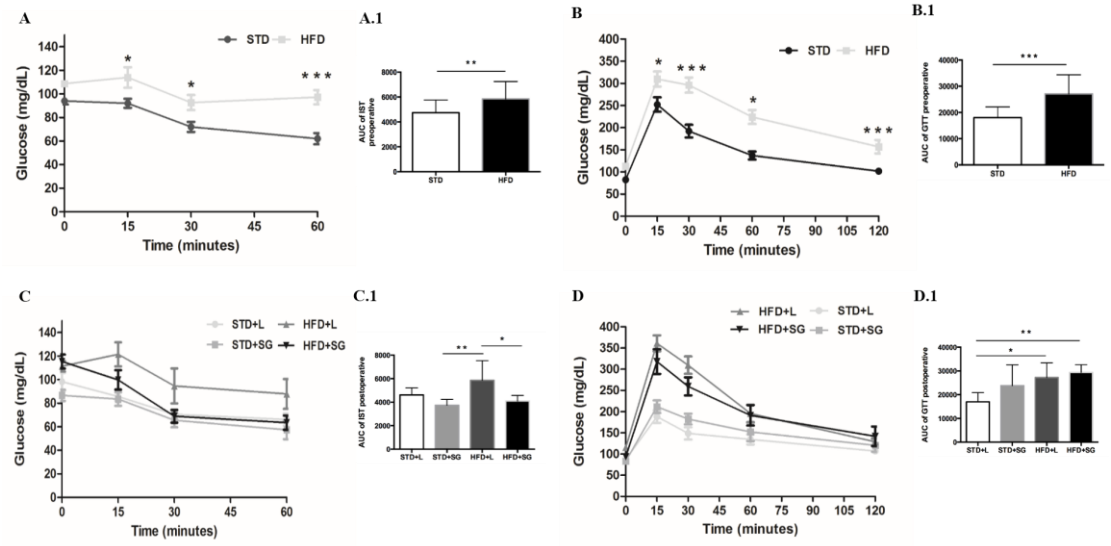

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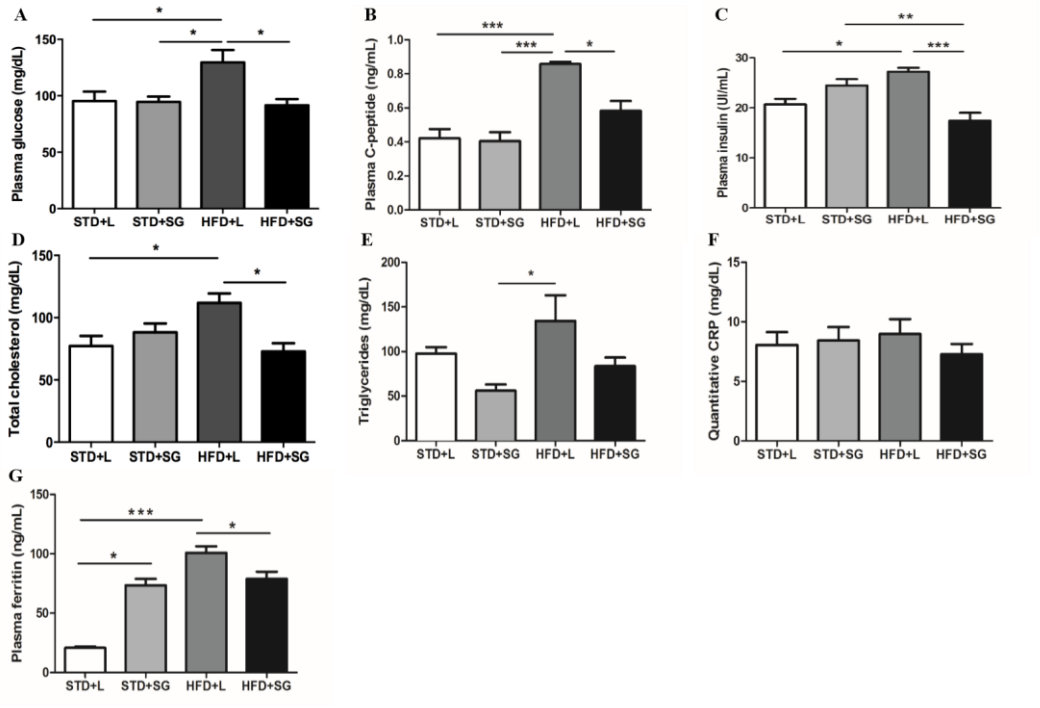

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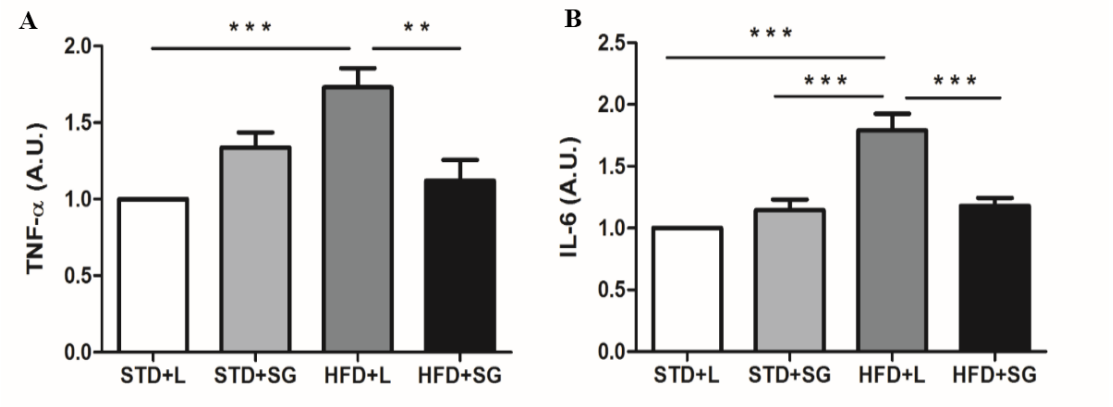

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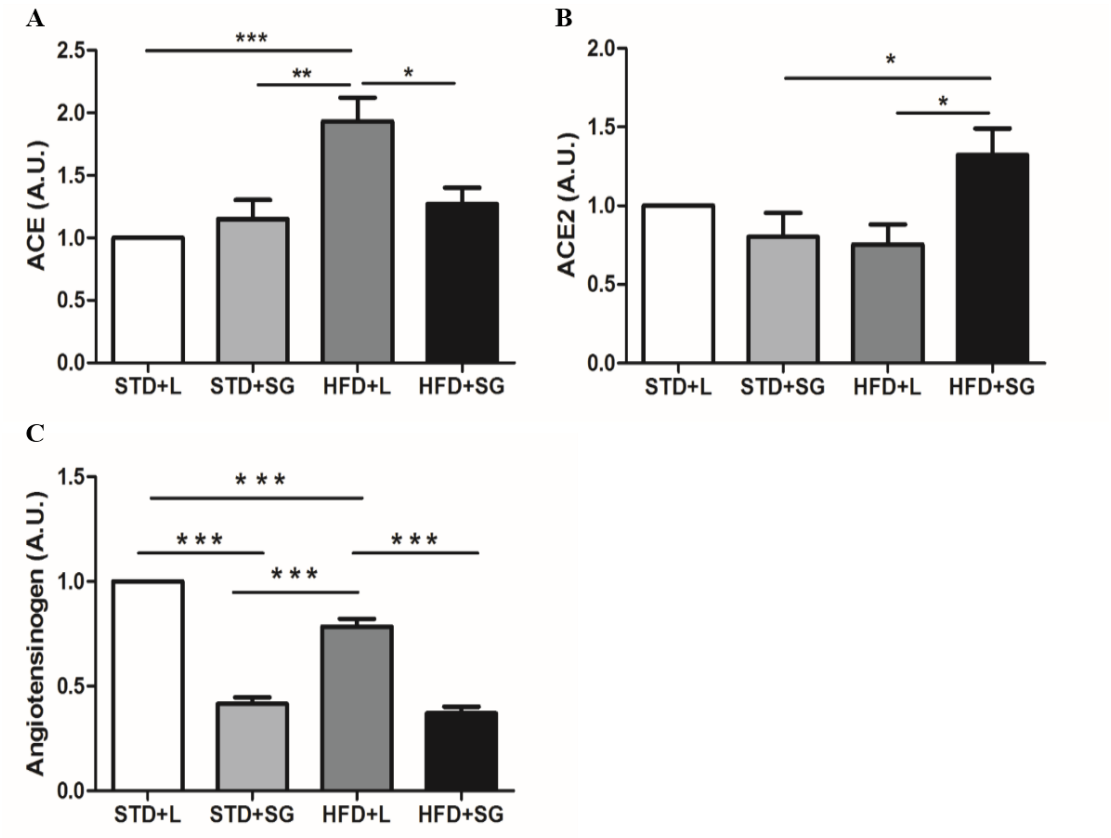

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4.2 Produto 2

EFFECTS OF OMENTECTOMY IN ADDITION TO SLEEVE GASTRECTOMY ON METABOLIC AND INFLAMMATORY PROFILE OF OBESE RATS

Short title: EFFECTS OF OMENTECTOMY ON OBESE RATS METABOLISM AND INFLAMMATION

Categorization of the manuscript: Gastrointestinal

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Author contributions

The authors Thaísa Soares Crespo, Deborah de Farias Lelis and Antônio Sérgio Barcala Jorge contributed to the design of the study, acquisition of data, or analysis and interpretation of data. The authors Thaísa Soares Crespo, João Marcus Oliveira Andrade and Alanna Fernandes Paraíso contributed drafting the article and revising it critically for important intellectual content and the authors Alfredo Maurício Batista de Paula, André Luiz Sena Guimarães and Sérgio Henrique Sousa Santos contributed to the final approval of the version to be submitted.

Abstract

Background: Visceral obesity has been considered a risk factor for metabolic and cardiovascular complications. In an attempt to reduce the visceral adipose tissue, the omentectomy has been proposed to be performed along with bariatric surgery. The goal of this study was to evaluate if the omentectomy associated with sleeve gastrectomy (SG) would be beneficial in the inflammatory and metabolic profile of rats treated with standard diet (STD) or high-fat diet (HFD). **Methods:** For this experiment male Wistar rats were randomly divided into six groups, and submitted to sham surgery (STD+L and HFD+L), SG alone (STD+SG and HFD+SG) or SG with omentectomy (STD+SGO and HFD+SGO). The animals were monitored until the fourth week of the postoperative period and the corporal, glycemic and lipid profiles and the tissue expression of inflammatory markers (TNF-alpha and IL-6) in the visceral adipose tissue were measured. **Results:** In rats with diet-induced obesity treated with SG with or without omentectomy there was a reduction in body weight

(HFD+SG: p<0.01 and HFD+SGO: p<0.05), adiposity (HFD+SG: p<0.001 and HFD+SGO: p<0.05), plasma levels of glucose (HFD+SG: p<0.01 and HFD+SGO: p<0.01), C-peptide (HFD+SG: p<0.01 and HFD+SGO: p<0.001), insulin (HFD+SG: p<0.05 and HFD+SGO: p<0.001), total cholesterol (HFD+SG: p<0.01 and HFD+SGO: p<0.01) and in the tissue expression of TNF-alpha (HFD+SG: p<0.001 and HFD+SGO: p<0.01), but with no statistically significant differences between the groups in which the omentectomy was performed or not. **Conclusion:** In this study we did not observe the additional benefic effects with the omentectomy associated to SG in the metabolic profile and in the tissue expression of inflammatory markers.

KEYWORDS: Obesity; bariatric surgery; sleeve gastrectomy; omentectomy; weight loss; inflammation mediators.

1. Introduction

The adipose tissue is a complex organ with endocrine and metabolic activities, from which several factors with local (autocrine and paracrine) and systemic (endocrine) functions are secreted (1). Obesity is associated with a chronic inflammatory process characterized by the abnormal production of proinflammatory cytokines, with the activation of different signaling pathways. Therefore, the excess of adipose tissue, especially the visceral compartment, is correlated with the development of insulin resistance, glucose intolerance, dyslipidemia, hypertension and prothrombotic state (2-4). For this reason, resections of the great omentum have been proposed during the performance of bariatric surgeries in order to improve the metabolic profile and to maximize the weight loss in obese patients (5).

However, the omentum possess healing factors (growth, angiogenesis, and chemotactic factors and progenitor cells), and when activated promotes healing and regeneration of injured tissues (6). These important properties of the omentum regarding its immunological functions and its roles in the process of vascularization, debridement, hemostasis, healing, and regeneration, allows it to be capable of blocking intraperitoneal inflammatory processes, adhering to it, and thus preventing the development of diffuse peritonitis (7-9).

The inclusion of omentectomy as a part of the bariatric surgery technique remains controversial and questionable. Authors show improvement in the glycemic homeostasis and in the lipid profile when the omentectomy is associated to the bariatric surgery (5, 10). However, other authors show that the reduction of the visceral adipose tissue, achieved by the omentectomy, it is not a useful approach to the improvement of the insulin sensitivity and reduction of the cardiometabolic risk factors associated with obesity or *diabetes mellitus* type II (DM2) (11, 12).

The goal of this study was to determine if the performance of omentectomy associated to sleeve gastrectomy (SG) provides additional beneficial effects on the glycemic and lipid profile and the expression of inflammatory markers in the white adipose tissue of Wistar rats treated with standard and high-fat diet.

2. Materials and methods

2.1 Animals and diets

Male Wistar rats aged eight weeks were used for this experiment, randomly divided into six groups (10 animals/group), maintained in individual cages and exposed to a light

cycle 12/12 h in a temperature of 22 ± 2 ºC. The rats were treated with standard diet (STD) or high-fat diet (HFD) for eight weeks and given *ad libitum* access to food and water. The STD (Purina - Labina®, USA) is composed of 50.30% of carbohydrate, 41.90% of protein and 7.80% of fat, with a total of 2.18 kcal per 1 g of diet (13). The HFD is composed of 24.55% of carbohydrate, 14.47% of protein and 60.98% of fat, presenting a total of 5.28 kcal per 1 g of diet (13). All of the high-fat diet components were purchased from Rhoster® LTDA (São Paulo, Brazil). After this period, the animals were submitted to surgical treatment as followed: simulated laparotomy (sham surgery) (L), sleeve gastrectomy (SG) and sleeve gastrectomy with omentectomy (SGO). Following the second day of the postoperative period, the animals received their respective diets for more four additional weeks. This study was approved by the Ethics committee of Experimentation and animal Welfare of UNIMONTES, Montes Claros, Brazil.

2.2 Surgical procedures

The animals were submitted to anesthesia (ketamine and xylazine) administered intraperitoneally following intramuscular application of ceftriaxone for antibiotic prophylaxis. In two groups, only the simulated lapatoromy was performed, with the manipulation of the stomach (STD+L and HFD+L); in another two groups, it was performed sleeve gastrectomy (STD+SG and HFD+SG) and lastly, in another two groups, in addition to the sleeve gastrectomy, it was included the omentectomy procedure (STD+SGO and HFD+SGO). The SG was performed with an 80% resection of the stomach, including complete removal of the gastric fundus, and confection of a new gastric tube, by manual suture, extending from the Hiss angle to the distal portion of the antrum (Figure 1.A, B, C and D). For the groups that

were submitted to the omentectomy, a complete dissection and resection of the whole greater omentum along the greater curvature of the stomach until the duodenum as well as the transverse colon until the spleen was performed (Figure 2.E and F).

2.3 Body weight, food intake and tissue collection

The body weight, food intake, (g/BW) and energy intake (kcal/g BW) were assessed three times a week during the pre and postoperative period. The animals were sacrificed by decaptation with guilhotine and blood samples were collected and centrifuged (3200 rpm for 10 minutes) for posterior plasma dosages. The following tissues were collected: white adipose tissue (periepididymal, retroperitoneal, mesenteric) and brown adipose tissue (interscapular), which were weighted and immediately frozen in liquid nitrogen and stored at – 80 ºC for posterior analysis.

2.4 In vivo experiments: Insulin sensitivity test and glucose tolerance test

One week before the surgery, and one week before sacrifice the insulin sensitivity test (IST) was performed with blood tail samples, collected from the animals for the determination of blood glucose levels, at the times 0, 15, 30 and 60 minutes after intraperitoneal injection of insulin (0.75 U/kg BW; Sigma, St. Louis, MO, USA) (14). The glucose tolerance test (GTT) allowed us to assess the levels of glucose at the times 0, 15, 30, 60 and 120 minutes after intraperitoneal injection of glucose (2 g/kg BW), following previous fasting period of 12 hours, using Accu-Check (Roche Diagnostics Corp. Indianapolis, USA) (14).

2.5 Laboratory tests

Plasma dosages of glucose, C-peptide, insulin, total cholesterol, high-density liproprotein (HDL) and triglycerides, were performed by using specific enzymatic Elisa kits (DSA BioELISA, USA).

2.6 Reverse transcription and quantitative real time-polymerase chain reaction (qRT-PCR)

Total RNA from the periepididymal adipose tissue was prepared using TRIzol reagent (Invitrogen Corp., San Diego, CA, USA), treated with DNAse and reverse transcribed with Moloney murine leukemia virus (Invitrogen Corp., San Diego, Calif) using random hexamer primers. Levels of the tumour necrosis factor (TNF-alpha) and interleukin (IL-6) were determined by qRT-PCR using SYBR Green reagent (Applied Biosystems, Grand Island, NY, USA) in QuantStudio™ 6 Flex Real-Time PCR System equipment (Applied Biosystems, USA) with the following primers: TNF-α FW: 5'-ATG GGC TCC CTC TCA TCA GT-3'; TNF-α RV: 5'-GCT TGG TGG TTT GCT ACG AC-3'; IL-6 FW: 5'-GTC AAC TCC ATC TGC CCT TCA-3'; IL-6 RV: 5'-GAA GGC AAC TGG CTG GAA GT-3'. Gene expression was normalized to the endogenous Beta-actin FW: 5'-TGA CAG GAT ACA GAA GGA GA-3' and RV: 5'-TAG AGC CAC CAA TCC ACA CA-3'. The relative comparative CT method of Livak and Schmittgen was applied to compare gene expression levels between groups using the equation $2^{(-\text{Delta Delta C(T)})}$ (15).

2.7 Statistical analysis

All data were transferred to GraphPad Prism® software (Version 6.0, San Diego, CA, USA) and analyzed with confidence of 95% ($P < 0.05$). Data are expressed as the mean ± SD. The differences between two groups were analyzed by the Student's T test. Statistically significant differences among more than two groups were analyzed by one-way ANOVA following Bonferroni post hoc test.

3. Results

In the preoperative period, the food intake (g/BW) was higher in the standard group but the energy intake (kcal/g BW) was higher in the group fed with high-fat diet, resulting in a statistically significant body weight increase in all groups fed with high-fat diet when compared to the groups fed with standard diets (Figure 2.A, B and C). In the postoperative period, an important decrease in the food intake, energy intake, body weight and adiposity (evaluated in the periepididymal, mesenteric and retroperitoneal adipose tissues) for the animals submitted to SG was observed, with no statistically significant difference between the groups with or without omentectomy (Figure 2. D, E, F, G, H, I and J). Concerning the brown adipose tissue (interscapular), it was observed a significant increase in the group HFD+SGO when compared to its respective control group (Figure 2. K).

Regarding the glycemic profile, the IST and GTT performed in the preoperative period showed significantly elevated glucose levels in the animals treated with high-fat diet (Figure 3.A and B). The same tests performed in the fourth week of the postoperative period, showed

the tendency of reduction in the glucose levels in the groups submitted to SG or SGO, but with no significant difference among the groups treated with the same diet (Figure 3.C and D). Similarly, the plasma glucose dosage (blood collected in the sacrifice) showed values significantly reduced in the groups HFD+SG and HFD+SGO (Figure 3. E). The C-peptide and insulin dosages showed reduced levels in the animals submitted to omentectomy, but with no significant difference when compared to the groups without omentectomy (Figure 3.F and G).

A significant reduction of the total cholesterol in the groups treated with high-fat diet and submitted to SG was observed, and similarly with or without omentectomy (Figure 4.A). In these same groups, there were no differences regarding the plasma levels of HDL and triglycerides, as showed in the figures 4.B and 4.C.

The gene expression of the inflammatory marker TNF-alpha was significantly lower in the groups treated with high-fat diet and submitted to SG when compared to the control group, but no significant difference was observed between the groups with or without omentectomy (Figure 5.A). On the other hand, the IL-6 expression was significantly lower for the animals in the group HFD+SG, while the same was not observed for the group HFD+SGO (Figure 5.B).

4. Discussion

In this study the effects of the omentectomy associated with SG on the weight loss, glycemic and lipid profile and gene expression of inflammatory markers in the white adipose

tissue of male Wistar rats treated with standard diet and obesity induced by high-fat diet were evaluated. There are evidences that this animal model is appropriate in the induction of obesity, performance of SG with hand-sewn or with suture staple technique and posterior evaluation of the corporal, biochemical and molecular profile (16-18). In the preoperative period, a significant increase in the body weight of the animals treated with high-fat diet was evidenced. On the other hand, in the postoperative period, a reduction in food intake (g/BW), energy intake (kcal/g BW), body weight and adiposity for the animals submitted to SG with or without omentectomy was observed, with a significant reduction in the adiposity in the group STD when compared to the group HFD. This weight loss after the SG procedure is observed even when the animals are maintained in the high-fat diet after the surgery, as performed in this study (19). In our study, the inclusion of omentectomy did not result in a statistically significant increase in weight loss when compared to the group submitted to SG only. Regarding the glycemic profile, both the animals in the groups STD and HFD showed a significant reduction in the levels of insulin and C-peptide after SG or SGO, wherein the animals in the group HFD it was also evidenced a significant decline in the glycemic levels but similarly in the groups with or without omentectomy. In the group HFD, the SG and SGO, in an equivalent manner reduced significantly the total cholesterol levels although this effect was not evidenced for the levels of HDL (no difference between groups) and triglycerides (significant reduction in the group STD+SG only). There are only a few studies in the literature dealing with the effects of the omentectomy associated to the bariatric surgery on the corporal and metabolic profile, remaining this subject still controversial and challenging. Thorne *et al.* showed the long-term benefic effects of the omentectomy associated to the adjustable gastric banding in the weight loss and improvement of the metabolic parameters in obese patients (5). CSendes *et al.* presented a prospective randomized study involving seventy

patients with obesity degree III, where the performance of gastric bypass with or without omentectomy and a follow up of two years. During this period, it was not evidenced differences regarding the body mass index (BMI), plasma levels of glucose, insulin, total cholesterol and triglycerides in the groups with or without omentectomy (20). Wu *et al.* performed laparoscopic SG with or without omentectomy in forty obese patients. In the group where the omentectomy was performed, it was also done a partial enterectomy with a resection ranging from 80 to 200 cm of the small bowel with the preservation of the first 100 cm of the jejunum and the last 200 cm of the ileum. Even in this complex technique, the follow up of up to twelve months showed that there were no significant differences in the body mass index loss or in the percentage of excess body weight loss and also there was no significant difference concerning the dosages of triglycerides, total cholesterol, HDL, fasting glucose and fasting insulin, concluding that in the period evaluated, the SG with or without omentectomy and partial enterectomy did not differ in its effects regarding weight loss. The authors in this study considered the SG alone as an effective and secure procedure and highlighted that the inclusion of omentectomy and partial enterectomy may present a higher risk for complications, such as leakage, stenosis, and bowel adhesion (21). However, Santoro *et al.*, in previous years, described an association between SG, omentectomy and jejunectomy, which he called *digestive adaptation*, with favorable results regarding the improvement of comorbidities, specially *diabetes mellitus*, attributing these results to a neuroendocrine postprandial increment, demonstrated by the reduction of ghrelin and resistin and an increase of glucagon-like peptide-1 and peptide YY, without the deleterious effects of mal-absorption (22). These same authors also showed the benefic effects of the entero-omentectomy alone on the glycemic profile of patients with DM2 and obesity degree I, and a follow up of three years (23). Heap *et al.* also presented favorable results regarding the improvement of comorbidities

associated to obesity performing a technique similar to the Santoro, which includes subtotal lateral gastrectomy, placement of gastric ring, partial resection of the jejunum and omentectomy (24). However, as this technique show four components it is difficult to define the real benefic role of the omentectomy.

An interesting evidence in our study was the significant weight increase of the interscapular brown adipose tissue (g/BW) observed in the HFD group submitted to SG with omentectomy. Bucerius *et al.* evaluated the bariatric surgery impact on the metabolic activity of different adipose tissues, showing an increment of the brown adipose tissue with an improvement of the metabolic parameters in morbidly obese patients (25). Schneck *et al.* showed that in high-fat diet induced obese mice a decrease in the white adipose tissue and an increment of the brown adipose tissue occurred after SG (26). As far as we know, there are no studies correlating the potential effects of the omentectomy associated to the bariatric surgery in the brown adipose tissue.

The adipose tissue is recognized as an endocrine organ by its expression and secretion of several factors with important endocrine functions (1). The expression of proinflammatory cytokines, among them tumor necrosis factor-alpha (TNF-alpha), has been described in the adipose tissue of different rodent models of obesity and diabetes, with an important role in the insulin resistance (27). Xia *et al.* performing total endoscopic resection of the omentum in rats, showed a decrease in the levels of TNF-alpha with improvement in the metabolic patterns (28). In the present study it was observed a decrease in expression of TNF-alpha in the periepididymal adipose tissue in the groups treated with HFD and submitted to SG when compared to the control group (sham surgery), but with no significant different among the

animals which have been submitted or not to omentectomy. Concerning the expression of IL-6 in this same tissue, it was observed a significant reduction only for the HFD group, in which the SG and omentectomy was performed. Herrera *et al.* performed a randomized study with twenty two patients that were submitted to a laparoscopic Roux-en-Y gastric bypass (LRYGB) with or without omentectomy. After a postoperative follow up of twelve months, it was evidenced comparative results in the patients with or without omentectomy regarding the glycemic and lipid profile and also concerning the inflammatory mediators. The surgical time was significantly more prolonged when the omentectomy was performed and a duodenal perforation occurred during this procedure (29). Sdralis *et al.* also reached similar conclusions performing SG alone or with omentectomy in thirty and one patients with obesity degree III. The follow up of one year showed a weight loss comparable in both groups, increase in the levels of adiponectin and HDL and a significant decrease in the levels of insulin, IL-6 and hs-CRP in both groups, but with no significant differences between them, and no significant modification in the levels of TNF-alpha (30).

5. Conclusion

In accordance with previous studies, our study concluded that the omentectomy associated with sleeve gastrectomy when compared to the SG alone, did not result in significant benefits for the glycemic and lipid profiles and in the attenuation of the expression of inflammatory markers (TNF-alpha and IL-6) in the white adipose tissue of rats treated with standard and high-fat diet in the period of experimentation. However, in the HFD group where the omentectomy was performed, there was a significant increase of the brown adipose tissue, and this correlation needs to be further investigated. As the omentectomy procedure

requires more surgical time, involves the potential risks for other intraoperative complications and implies in the resection of a structure with proven benefits, more studies are required to be conducted in order to obtain more consistent data proving that the reduction of the visceral adipose tissue with the resection of the great omentum associated to bariatric surgery improves the metabolic profile of obese patients with reduction of the cardiovascular risk and other comorbidities associated with obesity.

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Disclosure

The authors report no proprietary or commercial interest in any product mentioned or concept discussed in this article.

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Legends

Figure 1. Sleeve gastrectomy and omentectomy. **(A)** Exposition and repair of the stomach at the Hiss angle and distal antrum. **(B)** 70-80% resection of the stomach including the gastric fundus. **(C)** Formation of the gastric tube (sleeve gastrectomy performed). **(D)** Stomach resected. **(E)** Exposition of the greater omentum. **(F)** Omentectomy performed.

Figure 2. Food intake, energy intake, body weight and tissues weight of rats fed with standard diet (STD) and high-fat diet (HFD) and submitted to sham surgery (STD+L and HFD+L), sleeve gastrectomy (STD+SG and HFD+SG) and sleeve gastrectomy+omentectomy (STD+SGO and HFD+SGO). **(A)** Food intake (g/BW) preoperative. **(B)** Energy intake (Kcal/g BW) preoperative. **(C)** Body weight (g) preoperative. **(D)** Food intake (g/BW) postoperative. **(E)** Energy intake (Kcal/g BW) postoperative. **(F)** Body weight (g) postoperative. **(G)** Periepididymal adipose tissue weight (g/BW). **(H)** Mesenteric adipose tissue weight (g/BW). **(I)** Retroperitoneal adipose tissue weight (g/BW). **(J)** Body adiposity/white adipose tissue weight (periepididymal, mesenteric and retroperitoneal) (g/BW). **(K)** Interscapular brown adipose tissue weight (g/BW). (*P < 0.05; **P < 0.01; ***P < 0.001 (t-tests, one-way ANOVA and Bonferroni post-test).

Figure 3. Preoperative and pre-sacrifice insulin sensitivity tests (IST), glucose tolerance tests (GTT) and blood parameters of rats fed with standard diet (STD) and high-fat diet (HFD) and submitted to sham surgery (STD+L and HFD+L), sleeve gastrectomy (STD+SG and HFD+SG) and sleeve gastrectomy+omentectomy (STD+SGO and HFD+SGO). **(A)** Preoperative IST (mg/dL). **(B)** Preoperative GTT (mg/dL). **(C)** Pre-sacrifice IST (mg/dL). **(D)** Pre-sacrifice GTT (mg/dL). **(E)** Plasma glucose (mg/dL). **(F)** Plasma C-peptide (ng/mL). **(G)** Plasma insulin (UI/mL). *P < 0.05; **P < 0.01; ***P < 0.001 (one-way ANOVA and Bonferroni post-test).

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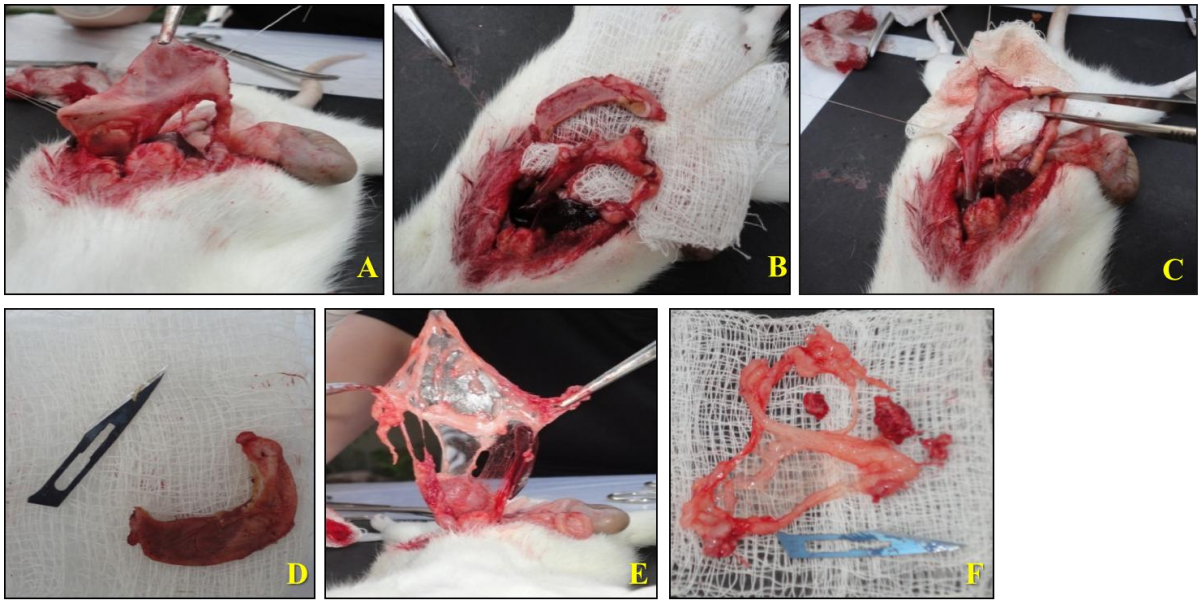

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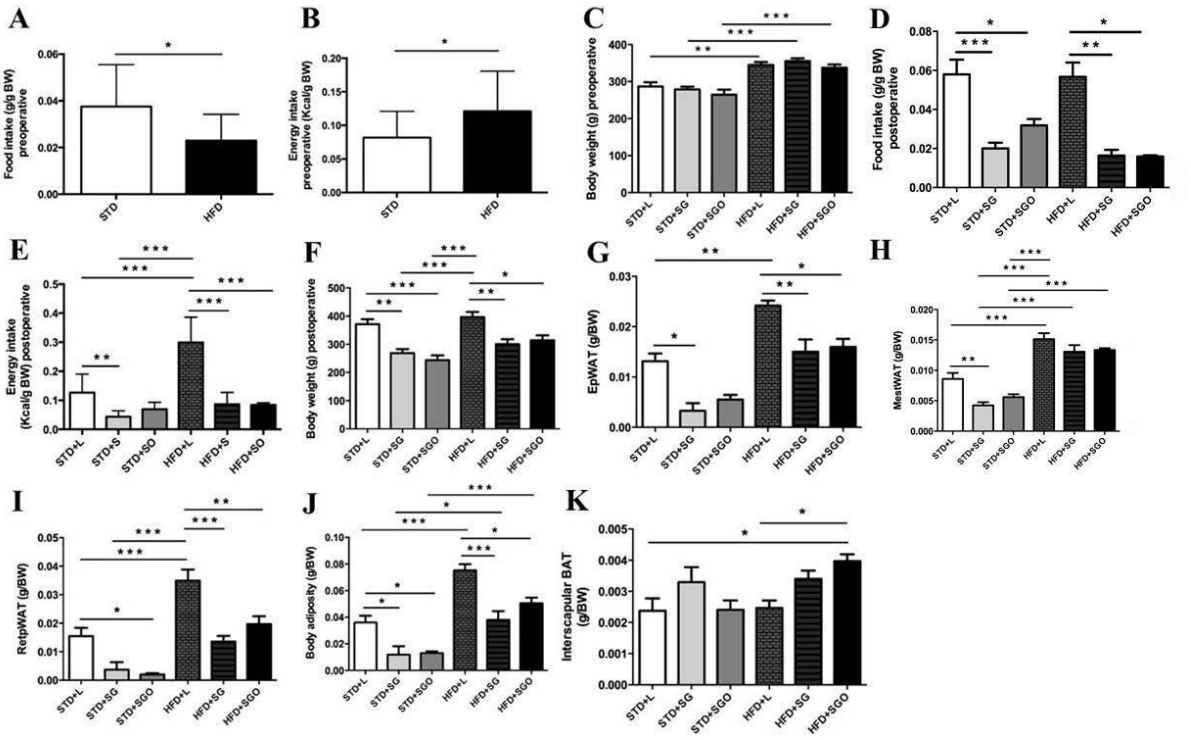

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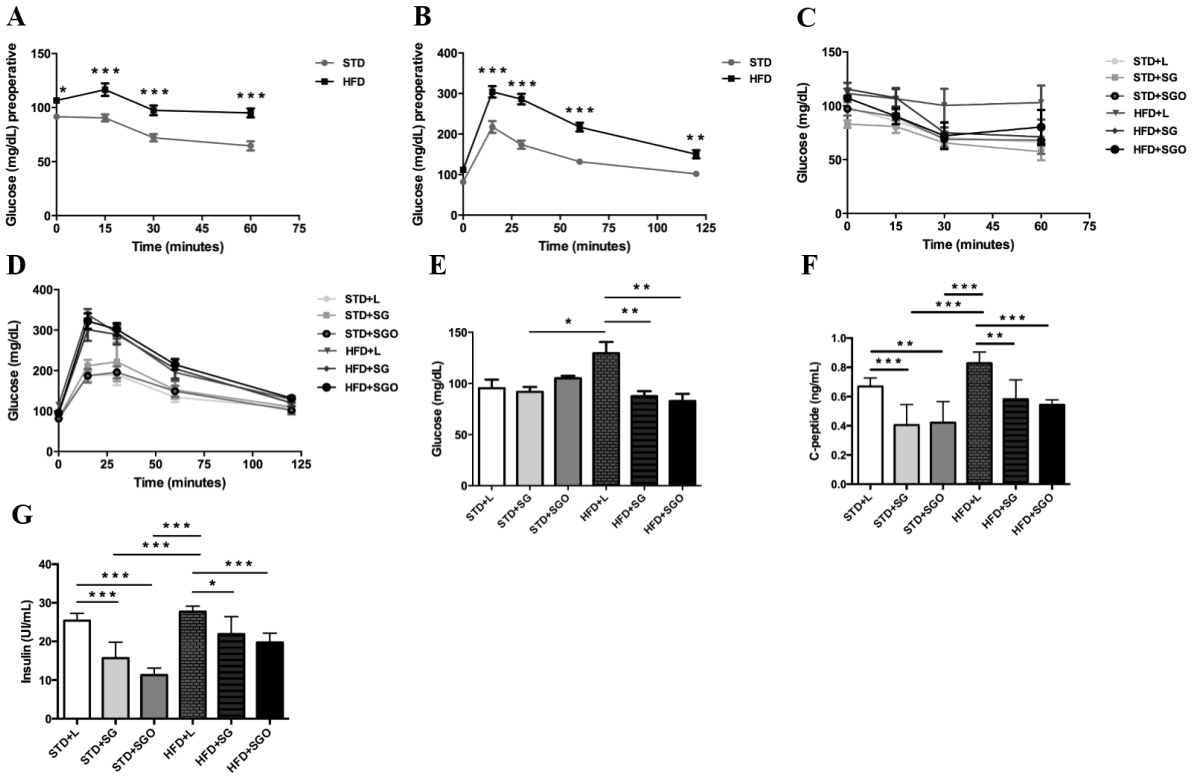

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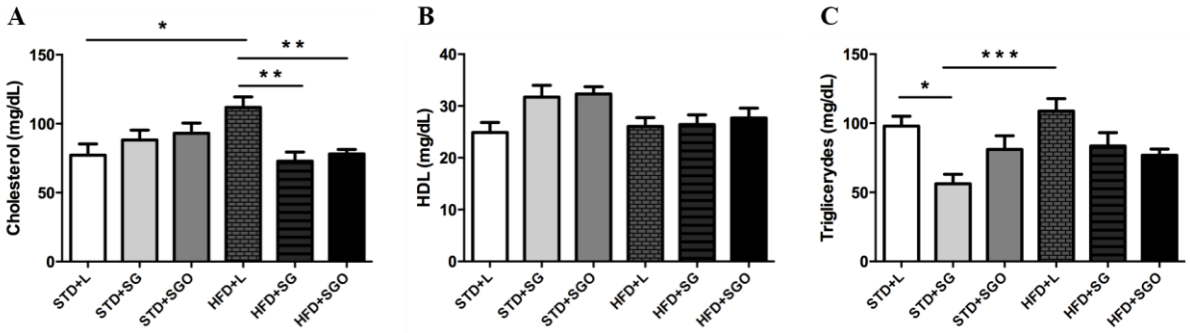

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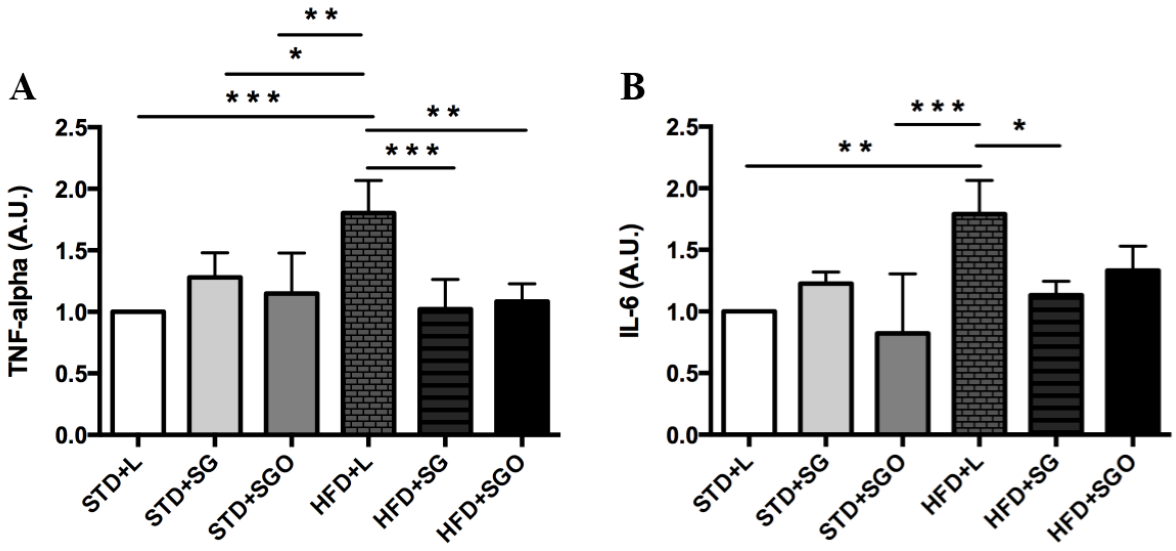

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COVER LETTER

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July 24, 2015

Dear Dr. D.W. McFadden and Dr. S.A. LeMaire

We are pleased to submit a new manuscript entitled “Effects of omentectomy in addition to sleeve gastrectomy on metabolic and inflammatory profile of obese rats” for consideration by the Journal of Surgical Research.

We confirm that this work is original and has not been published elsewhere nor is it currently under consideration for publication elsewhere.

In this paper, we report the effects of the inclusion of omentectomy in addition to sleeve gastrectomy on the lipid and glycemic metabolism as well as on the expression of important inflammatory markers in the white adipose tissue of rats. This is significant because the performance of omentectomy associated to different bariatric surgery techniques is still controversial regarding the benefic effects on the metabolism and weight loss in the obese. The paper should be of interest to readers in the areas of general surgery, bariatric surgery, gastroenterology, endocrinology and metabology.

Our study, in addition to evaluate the effect of the omentectomy as a part of bariatric surgery on the weight loss and the metabolic profile, also evaluates the gene expression of TNF-alpha and IL-6 in the white adipose tissue of male rats submitted to sleeve gastrectomy. In the

literature there are only a few studies concerning the effects of omentectomy on inflammation, which uses serum parameters of the inflammatory markers. As far as we know, there are no studies associating this procedure with the evaluation of inflammation through the analysis of the tissue expression of these important markers. Moreover, regarding the studies that evaluate the effects on the glycemic and lipid metabolism in obese patients, there are many controversies that make it clear the necessity of more studies that may define the actual value of the resection of the great omentum. If on the one hand we know that the omentum is part of the visceral adipose tissue, which in excess is associated to the secretion of proinflammatory cytokines and a higher cardiovascular risk, on the other hand, it is already well established that in the midst of the omental adipose tissue there are tissues rich in machrophages, lymphocytes and hematopoietic cells. This ensures the omentum as a tissue with unique composition, with neovascularization, hemostasis, debridement, healing and regeneration of injured tissues. Motivated by these controversies about this subject and its scarcity in the literature regarding the effects on the inflammation, we believe that is very relevant to develop an experimental study where the omentectomy is performed in association to the sleeve gastrectomy, which is a technique already known by its metabolic and bariatric effects.

In addition, we would like to indicate some potential reviewers for our paper, as follow: Mariane Bertagnolli (mariane.b@gmail.com), Sainte-Justine University Hospital Research Center; Katia de Angelis (prof.kangelis@uninove.br), Universidade de São Paulo (USP) / UniNove; Maria Cláudia Irigoyen (hipirigoyen@gmail.com), Universidade de São Paulo (USP); Adaliene Versiani Ferreira (adaliene@gmail.com), Universidade Federal de Minas Gerais (UFMG).

Please address all correspondence concerning this manuscript to me at sergiosousas@hotmail.com

Thank you for your consideration of this manuscript.

Sincerely,

Sérgio Henrique Sousa Santos

5 CONSIDERAÇÕES FINAIS

Como a prevalência da obesidade e suas alterações metabólicas, em especial, o *diabetes mellitus* tipo II (DM2) adquiriram proporções alarmantes, trabalhos que aprofundam o conhecimento desta patologia no nível celular e molecular contribuem para uma melhor compreensão dos mecanismos fisiopatológicos da doença, suas inter-relações e, consequentemente, apontam novas possibilidades terapêuticas. No caso da obesidade mórbida, a cirurgia bariátrica é considerada o método terapêutico mais eficaz, promovendo redução significativa do peso e das morbidades associadas. O *bypass* gástrico é a técnica mais empregada no mundo. No entanto, a gastrectomia vertical desponta como uma nova proposta cirúrgica para o tratamento da obesidade e doenças associadas, não como primeira etapa de tratamento para pacientes com obesidade extrema e de alto risco, mas como possibilidade de tratamento único e definitivo.

Neste trabalho, em ratos com obesidade induzida por dieta hiperlipídica, ficou demonstrado que a gastrectomia vertical é uma técnica cirúrgica com resultados satisfatórios quanto à perda de peso, incremento do perfil metabólico (glicídico e lipídico) e redução da expressão tecidual de marcadores inflamatórios e de componentes do sistema renina-angiotensina (SRA), os quais apresentam ações deletérias e contribuem para a gênese ou agravamento das comorbidades relacionadas à obesidade. Muitos destes efeitos benéficos no metabolismo não podem ser explicados somente em decorrência da perda de peso; portanto, a demonstração de que esta técnica interfere positivamente na expressão de marcadores inflamatórios e do SRA, contribui para que possa ser considerada uma cirurgia com efeitos bariátricos e metabólicos. Como é uma técnica cirúrgica segura, com maior facilidade de execução, não requer anastomoses intestinais e que preserva grande parte da fisiologia gastrointestinal, sustentamos a evidência de ser uma ferramenta terapêutica valiosa, com possibilidade de ampliação de suas indicações no tratamento da obesidade e DM2.

Com relação à controversa redução do tecido adiposo visceral através da inclusão da ressecção do omento maior durante a realização da cirurgia bariátrica, nosso trabalho não demonstrou benefício adicional quanto ao incremento da perda de peso e melhora dos

parâmetros metabólicos quando a omentectomia foi adicionada à gastrectomia vertical em ratos com obesidade induzida por dieta hiperlipídica. Nos animais tratados com dieta hiperlipídica e submetidos à gastrectomia vertical, o acréscimo da omentectomia esteve associado a maior quantidade de tecido adiposo marrom. Este fato merece novos estudos. Apesar de o omento ser parte importante do tecido adiposo visceral, o qual está associado com maior risco cardiovascular, mais estudos são necessários para que se estabeleça com propriedade a indicação da omentectomia nos pacientes obesos, já que esta estrutura possui propriedades imunológicas, hemostáticas e de regeneração tecidual.

Portanto, a gastrectomia vertical isolada pode ser considerada como terapêutica bariátrica e metabólica para obesidade e suas comorbidades, e, sob a luz da biologia molecular, ser compreendida além da perspectiva anatômica.

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ANEXOS

ANEXOS

ANEXO A - Parecer do Comitê de Ética em Experimentação e Bem-Estar Animal/UNIMONTES

UNIVERSIDADE ESTADUAL DE MONTES CLAROS
COMITÊ DE ÉTICA EM EXPERIMENTAÇÃO E BEM-ESTAR ANIMAL Unimontes

PARECER CONSUBSTANCIADO

Montes Claros, 21 de novembro de 2014.

Processo N. **031**
Título do Projeto: **Avaliação metabólica, inflamatória e molecular de ratos tratados com dieta hiperlipídica e normolipídica s submetidos a gastrectomia vertical com e sem omentectomia**
Orientador: **Prof. Sérgio Henrique Sousa Santos**

Histórico
A prevalência mundial da obesidade, o excesso de peso, a dislipidemia e as alterações metabólicas que os acompanham vêm se tornando alarmantes. Paralelamente, o *diabetes melitus* II, as doenças cardiovasculares e a hipertrigliceridemia representam um agravo expressivo que leva a um estado de doença de grande repercussão, ligada a vários fatores, com grande impacto na morbidade e mortalidade da população mundial. Recentemente, também foi demonstrada a associação causal entre obesidade e inflamação crônica subclínica e também a participação do Sistema Renina Angiotensina e Sirtuinas na regulação de processos metabólicos.
A descrição da Síndrome Metabólica, a gravidade de suas consequências deletérias e o aumento dramático de sua prevalência levaram os profissionais da saúde à considerá-la como um dos principais problemas de saúde pública do mundo. Existe, também, forte evidência de que uma ampla heterogeneidade nos riscos metabólicos e cardiovasculares, ainda não bem esclarecidos, estão relacionados principalmente ao local de acúmulo do excesso de tecido adiposo. O acúmulo do tecido adiposo visceral é um importante fator preditivo para distúrbios do metabolismo lipídico, glicídico ou aterogênico, enquanto que o acúmulo de tecido adiposo nas partes mais inferiores do corpo não está associado ao aumento destas alterações metabólicas. Diante disto, é de extrema importância contribuir para o tratamento clínico destes pacientes, estudando a expressão de diferentes marcadores inflamatórios e do Sistema Renina Angiotensina e Sirtuinas na obesidade bem como suas correlações com o metabolismo lipídico e glicídico. Evidências mostram que a modulação dos mediadores inflamatórios secretados pelo tecido adiposo em expansão pode ser uma ferramenta terapêutica importante para a prevenção de comorbidades associadas à obesidade.
No caso da obesidade mórbida, a cirurgia bariátrica é considerada o método terapêutico mais eficaz, promovendo redução significativa do peso e das morbidades relacionadas. A redução do peso corporal geralmente é acompanhada por uma diminuição ou até mesmo uma normalização dos parâmetros biológicos. Essas cirurgias têm sido citadas como alternativa de tratamento do *diabetes melitus* II. Entretanto, o retorno da glicemia ao normal e os níveis normais de triglicérides são observados logo nos primeiros dias após a cirurgia, sugerindo que a perda de peso não explica inteiramente por que a cirurgia melhora o diabetes. Efeitos relacionados aos entero-hormônios (GLP-1 e PYY), e suas ações sobre o Sistema Nervoso Central e o aparelho digestório aparecem como prováveis responsáveis pela melhora do controle do metabolismo da glicose.

Mérito
Os resultados do projeto permitirão conhecer os efeitos da gastrectomia vertical em modelos animais com Síndrome Metabólica induzida por dieta hiperlipídica bem como correlacionar o perfil metabólico, inflamatório, do Sistema Renina Angiotensina, Sirtuinas e principais entero-hormônios no pós-operatório, avaliando inclusive a relevância de se incluir a omentectomia como parte da técnica, já que o epíplon é parte importante do tecido adiposo visceral ainda com funções não completamente esclarecidas.
O projeto irá avaliar as inter-relações dos principais marcadores envolvidos na fisiopatologia da obesidade e nos estudos dos resultados da gastrectomia vertical com omentectomia como importante opção no tratamento da Síndrome Metabólica e suas doenças associadas. Assim, espera-se, no futuro, aprofundar o entendimento e ampliar a eficácia das terapêuticas medicamentosas bem como considerar a cirurgia bariátrica além da perspectiva anatômica e, sob a luz da biologia molecular, aumentar o foco na cirurgia metabólica.

Parecer
O Comitê de Ética em Experimentação e Bem-Estar Animal (CEEBEA) da UNIMONTES analisou o processo **nº 031** e entende que o mesmo encontra-se Aprovado.

Prof. Orlando Raphael Lopasso Júnior
Presidente da Comissão de Ética em Experimentação e Bem-Estar Animal da UNIMONTES

ANEXO B

OBESITY SURGERY

INSTRUCTIONS FOR AUTHORS

1. ABOUT OBSU

Obesity Surgery is published by Springer Science+Business Media LLC and is the official journal of the International Federation for the Surgery of Obesity and metabolic disorders (IFSO). Obesity Surgery publishes concise articles on Original Contributions, New Concepts, How I Do It, Review Articles, Brief Communications, Letters to the Editor and dedicated Video Submissions. Requirements are in accordance with the "Uniform Requirements for Manuscripts submitted to Biomedical Journals, "www.icmje.org. Articles that are accepted for publication are done so with the understanding that they, or their substantive contents, have not been and will not be submitted to any other publication.

2. ETHICAL RESPONSIBILITIES OF AUTHORS

This journal is committed to upholding the integrity of the scientific record. As a member of the Committee on Publication Ethics (COPE) the journal will follow the COPE guidelines on how to deal with potential acts of misconduct.

Authors should refrain from misrepresenting research results that could damage the trust in the journal and ultimately the entire scientific endeavor. Maintaining integrity of the research and its presentation can be achieved by following the rules of good scientific practice, which includes:

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• A single study is not split up into several parts to increase the quantity of submissions and submitted to various journals or to one journal over time (e.g. “salami-publishing”).

• No data have been fabricated or manipulated (including images) to support your conclusions

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Proper acknowledgements to other works must be given (this includes material that is closely copied (near verbatim), summarized and/or paraphrased), quotation marks are used for verbatim copying of material, and permissions are secured for material that is copyrighted.

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In addition:

• Changes of authorship or in the order of authors are not accepted after acceptance of a manuscript.

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- If the article has already been published online, depending on the nature and severity of the infraction, either an erratum will be placed with the article or in severe cases complete retraction of the article will occur. The reason must be given in the published erratum or retraction note.

- The author's institution may be informed

.

2a. DISCLOSURE OF POTENTIAL CONFLICT OF INTEREST

Authors must disclose all relationships or interests that could influence or bias the work. Although an author may not feel there are conflicts, disclosure of relationships and interests affords a more transparent process, leading to an accurate and objective assessment of the work. Awareness of real or perceived conflicts of interests is a perspective to which the readers are entitled and is not meant to imply that a financial relationship with an organization that sponsored the research or compensation for consultancy work is inappropriate. Examples of potential conflicts of interests that are directly or indirectly related to the research may include but are not limited to the following:

• Research grants from funding agencies (give the research funder and the grant number)
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• Financial support for attending symposia
• Financial support for educational programs
• Employment or consultation
• Support from a project sponsor
• Position on advisory board or board of directors or other type of management relationships
• Multiple affiliations
• Financial relationships, for example equity ownership or investment interest
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In addition, interests that go beyond financial interests and compensation (non-financial interests) that may be important to readers should be disclosed. These may include but are not limited to personal relationships or competing interests directly or indirectly tied to this research, or professional interests or personal beliefs that may influence your research.

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See below examples of disclosures:

Funding: This study was funded by X (grant number X).

Conflict of Interest: Author A has received research grants from Company A. Author B has received a speaker honorarium from Company X and owns stock in Company Y. Author C is a member of committee Z.

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2b. STATEMENT OF HUMAN AND ANIMAL RIGHTS

When reporting studies that involve human participants, authors should include a statement that the studies have been approved by the appropriate institutional and/or national research ethics committee and have been performed in accordance with the ethical standards as laid down in the 1964 Declaration of Helsinki and its later amendments or comparable ethical standards.

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The following statements should be included in the text before the References section:

i. Ethical Approval

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The welfare of animals used for research must be respected. When reporting experiments on animals, authors should indicate whether the institutional and/or national guidelines for the care and use of animals were followed.

For studies with animals, the following statement should be included:

"All applicable institutional and/or national guidelines for the care and use of animals were followed."

If articles do not contain studies with human participants or animals by any of the authors, Springer recommends including the following sentence:

"This article does not contain any studies with human participants or animals performed by any of the authors."

For retrospective studies, add the following sentence:
"For this type of study formal consent is not required."

ii. Informed Consent

All individuals have individual rights that are not to be infringed. Individual participants in studies e.g. have the right to decide what happens to the (identifiable) personal data gathered and to what they have said e.g. during a study or an interview as well as to any photograph that was taken. Hence it is important that all participants gave their informed consent in writing prior to inclusion in the study. Identifying details (names, dates of birth, identity numbers and other information) of the participants that were studied should not be published in written descriptions, photographs, and genetic profiles unless the information is essential for scientific purposes and the participant (or parent or guardian if the participant is incapable) has given written informed consent for publication. Complete anonymity is difficult to achieve in some cases, and informed consent should be obtained if there is any doubt. For example, masking the eye region in photographs of participants is inadequate protection of anonymity. If identifying characteristics are altered to protect anonymity, such as in genetic profiles, authors should provide assurance that alterations do not distort scientific meaning.
The following statement should be included:
Informed consent: "Informed consent was obtained from all individual participants included in the study."
If identifying information about participants is available in the article, the following statement should be included:
"Additional informed consent was obtained from all individual participants for whom identifying information is included in this article."

3. IMPORTANT SUBMISSION INFORMATION

3a. SYSTEM REQUIREMENTS

Authors will need the following items in order to use Editorial Manager:

- Internet access
- A current Adobe Acrobat browser plug-in
- Electronic files of all required documents for upload.

3b**. YOUR AUTHOR ACCOUNT**

Authors entering the journal's Editorial Manager site for the first time can create a new account at http://www.edmgr.com/obsu/ by clicking “Login” at the top of the screen, and “Register Now” at the next screen, and then following the online prompts in order to create your account and submit a manuscript.

NOTE: If you have previously logged into the system, you should always use your existing account for ALL subsequent submissions. If you have forgotten your Username or Password, you may use the “Send Username/Password” link at the OBSU Log In Page.

3c. **ONLINE SUBMISSION**

After you have logged into your account and entered your Submission Center, Editorial Manager will lead you through a step-by-step submission process. When submitting your manuscript through Editorial Manager, you will navigate through nine (9) submission steps.

The required documents for all online submissions include the main Manuscript document, and a Conflict of Interest (COI) form, which should be completed by each contributing author.

Note: Always keep copies of your word-processing, graphic, video and COI files. You may want to revise the manuscript text, images or forms after the review process and you will need the original files if your manuscript requires revisions.

Make sure that all required online fields are completed before attempting to submit; the system will not allow you to submit if any required fields are not completed. If you cannot finish your submission in one visit, you can save a draft and later re-enter the process at the same step by clicking on the “Incomplete Submissions” link in your Author Main Menu.

4. MANUSCRIPT PREPARATION

Please take note of the required terminology standards.

Mandatory

• Weight loss must be expressed as change in BMI or %total weight loss (%TWL)

Optional

• Weight loss can be expressed as % Excess Weight Loss (%EWL), with the calculation of ideal body weight as that equivalent to a BMI of 25 kg/m2 and/or % Excess BMI Lost (%EBMIL) with excess BMI > 25 kg/m2 as well as % total body weight loss.

• Data extending beyond 30 days must include lost to follow-up information in the Abstract and Results section, including all tables and figures, with the denominator provided as to how many patients were available at each time point and the number of patients actually seen.

4a. **MANUSCRIPT SECTIONS AND FILE ITEMS**

When you upload your manuscript documents to OBSU, the system will ask you to indicate the manuscript file "Item." Your manuscript should be submitted in various parts; for example, your "Manuscript" should be uploaded separately from the "Official Conflict of Interest Form." Images should be submitted separately, as should any electronic supplementary material (or "Other") and videos (either as supplementary videos or as dedicated video submissions).

Use the following format guidelines.

• Use a normal, plain font (e.g., 12-point Times Roman) for text.

• Double-space the text, and set page borders at one inch.

• Use italics for emphasis.

• Use the automatic page numbering function to number the pages.

• Do not use field functions.

• Use tab stops or other commands for indents; do not use the space bar for indents.

i. **Manuscript – Main Text (required)**

In the "Attach Files" step (final step) of your submission, the "Manuscript" file should include a Title Page, the Main Text (which should include a Conflict of Interest Disclosure Statement), References, and Figure Legends (if any). Tables may also be included at the end of this document, or submitted separately.

Title Page. This should include:

• The title of the article.

• The manuscript type.

• The complete names and academic degrees for each contributing author (first name, middle initial[s], surname, degree[s]).

• The departmental and institutional affiliations with complete email addresses for each contributing author. Include the city, state or province, and country where the work was performed.

• "Correspondence to" followed by the name and contact information for the corresponding author.

• A shortened title for use as a running head (not to exceed 30 characters in length, including spaces between words).

• At the bottom of the page, any detailed grant information, and acknowledgment of any grant support.

• Acknowledgments: Individuals, other than authors, who were of direct help in the reported work should be acknowledged by a brief statement. Each acknowledged person should give their written consent to be named in the manuscript.

Main Text. The main text document should be double-spaced and for most submissions include:

• Abstract (not required for Letters; optional for Brief Communications)

• Introduction/Purpose

• Materials and Methods

• Results

• Conclusion

• Conflict of Interest Disclosure Statement (see details below)

• References (see details below)

• If separate images or figures are provided, then a Figure Legend should be included in the main text document after the References.

Any Tables that you provide should be included at the end of the text.

Additional format requirements and details for specific manuscript types are included in the "Manuscript Types and Formats" section below.

Conflict of Interest Disclosure Statement (in Text). A Conflict of Interest Disclosure Statement is required to be included for each author within the manuscript text, and should be located just before the list of References. For each author, the statement must declare the potential conflict of interest, or "no conflict of interest." For additional details, refer to section 2a. above.

References

• Use Medline®/Pubmed® Style. Visit the following website for sample references: http://www.nlm.nih.gov/bsd/uniform_requirements.html.

• Type references double-spaced and list them in consecutive, numerical order as they appear in the text (not alphabetically).

• Identify reference citations in the text by numbers in square brackets (e.g., [1]). Once a reference is cited, all subsequent citations should be to the original number.

• Cite all references within the text or tables.

• Papers that have been accepted for publication or are in press may be listed in the References, but the Journal does not reference unpublished data and personal communications.

• If several references are available on the same subject, cite only the most recent and pertinent, giving preference to original articles over review articles or textbooks.

Journal Articles

Journal articles should be cited according to the Medline®/Pubmed® Journal Article Citation Format. An example follows:

Lee MJ, Fanelli F, Haage P, Hausegger K, Van Lienden KP. Patient safety in interventional radiology: a CIRSE IR checklist. Cardiovasc Intervent Radiol. 2012 Apr;35(2):244-6. Epub 2011 Oct 20. PMID: 22011783

Books and Other Published Material

For citation format examples of books, other monographs, other published material, and electronic material, visit http://www.nlm.nih.gov/bsd/uniform_requirements.html

Tables

• Use the table function (not spreadsheets) to make tables.

• Number all tables using Arabic numerals.

• Always cite tables in the text in consecutive, numerical order.

• For each table, supply a table heading. The table title should explain clearly and concisely the components of the table.

• Identify any previously published material by giving the original source in the form of a reference at the end of the table heading.

• Footnotes to tables should be indicated by superscript lower-case letters (or asterisks for significance values and other statistical data) and included beneath the table body.

• All tables should be supplied on a separate page at the end of the main document and have callouts in the text.

ii. Official Conflict of Interest Form(s) – (required)

Every contributing author must electronically complete the official ICMJE Conflict of Interest (COI) form, which is available by clicking the "Conflict of Interest Form" link in the "For visiting http://www.icmje.org/coi_disclosure.pdf. Depending on your internet browser settings, the uploaded form(s) may not appear as a readable part of the manuscript proofs for review.

During submission, please make sure that you upload your COI forms at the end of your submitted document list, so that reviewers do not have difficulties reviewing your submission.

Note: If you have trouble viewing the PDF form after you have downloaded it, make sure that you open and view the PDF directly from your "downloads" folder via Adobe Reader rather than by way of your online internet browser.

If any contributing author's COI form is incomplete or missing from the submission, the submission will be returned to the author for correction prior to review. Each author must complete the form even if no conflict of interest exists.

Details provided in the ICMJE COI forms must correspond with the required COI Disclosure Statement that the authors include in the manuscript text (see 4a.i. "Conflict of Interest Disclosure Statement (in Text)" above).

iii. Figures (optional)

Along with uploading main text document, you can also upload separate figure and graphic image documents. Common graphics files such as GIF, JPEG, EPS, TIFF and many others are supported. Do not upload figures as PDF files, or in PowerPoint; we also recommend that figures not be embedded in the main text of your article.

For vector graphics, the preferred format is EPS; for halftones, TIFF format is preferred. Very large figure files should be compressed as much as possible before uploading figures to the website. If the figures will be printed in black and white, do not refer to color in the captions. All figures are to be numbered using Arabic numerals. Figure parts should be denoted by lowercase letters. Figures should always be cited in text in consecutive numerical order. For each figure, include the figure legends at the end of the manuscript text. Make sure to identify all elements found in the figure in the caption.

Photographs of patients in which the subject is identifiable must either have the face masked out, or be accompanied by written permission from the individual in the photograph for publication.

Image Size

• Actual size of submitted image(s) should be as follows:

• Width: 39 mm, 84 mm, 129 mm or 174 mm wide.

• Height: No higher than 234 mm.

• The following open source image-conversion software is available in Mac and Windows format to assist you in standardizing your images:

o GraphicsMagick - www.graphicsmagick.org

o Image Magick - www.imagemagick.org

o Xn Convert - www.xnconvert.com

For detailed submission guidelines regarding Line Art, Halftone Art, Combination Art, Color Art, and other artwork details, click here for Artwork Instructions: http://www.springer.com/authors/manuscript+guidelines?SGWID=0-40162-12-331200-0

iv. Other (optional)

If you want to provide a file with your submission that does not fit any of the above file designations, you may submit it under "Other."

v. Multimedia (video)

We invite contributing authors to submit Supplementary Videos to a manuscript submission, as well as Dedicated Video Submissions. If any multimedia is submitted, it will be reviewed along with the submission, and if accepted will be published as-received from the author in the online version only. All standard instructions for manuscript and video submission should be followed (see "Videos" below).

Multimedia Articles may consist of:

• Information that cannot be printed: animations, video clips, sound recordings

• Information that is more convenient in electronic form: sequences, spectral data, etc.

• Large original data, e.g. additional tables, illustrations, etc.

• If supplying any multimedia, the text must make specific mention of the material as a citation, similar to that of figures and tables (e.g., "… as shown in Animation 3.")

Supplementary Videos

Upon submission of articles that include supplementary video, the author(s) will be required to submit the video according to the following specifications:

• To accommodate user downloads, keep to the recommended upper limit for the size of the different file types. Larger-sized files may require very long download times, and some users may experience problems during downloading or viewing for very large files.

• Video clips should not exceed one minute or 2MB. Anything exceeding 1 minute must be submitted in separate videos.

• Always use either .mp4 or .mov files.

• The content of these files must be identical to that reviewed and accepted by the editor in-chief.

• All narration should be in English supplementary videos related to the article via videos.springer.com.

Dedicated Video Submissions

For dedicated video submissions, author(s) will be required to submit an accompanying textual Abstract, and video according to the following specifications:

• Always use either .mp4 or .mov files.

• Additional details for dedicated Video Submissions can be found in the Table below.

4b. **MANUSCRIPT TYPES AND FORMATS**

The manuscript types for submission include Original Contributions, New Concepts, How I Do It, Review Articles, Brief Communications, Letters to the Editor, and Dedicated Video Submissions. You may submit your manuscript either as Type I, II, or III (detailed below).

i. Manuscript Type I

• Original Contribution: All papers involving clinical or basic science research.

• New Concept: All innovative technologies, devices, procedures or treatment protocols; should include a detailed description of the procedure and the results.

• How I Do It: A description of a technique or operative procedure of interest

ii. Manuscript Type II

• Review: A scholarly literature review of a current topic. May be solicited or unsolicited.
• Brief Communication: A short report that can present research, an innovated concept or procedure, or a small case series with important, but very straightforward results.
• Letter: A brief report of an opinion or unstructured comment on a published paper. The editors reserve the right to accept, reject or excerpt letters without changing the views expressed by the author(s).

iii. Manuscript Type III

• Video Submissions: Manuscripts submitted as dedicated video submissions must be accompanied by a textual Abstract that briefly describes the video. See section 4a.v. above, for specific video requirements.

Each of the above manuscript types requires a specific submission format. The specific format for each type can be found in the Table below. When required by the nature of the report, manuscripts that do not follow the specific formats below may be accepted; e.g., the listed page, word and figure/image limits may be used as a guideline rather than a rule. Please remain succinct in your wording.

Table: Manuscript Submission Formats* and Required Items**

FORMAT I	**#pp / #words**	**Main Text**	**Figures**	**COI Forms**
Original Contribution **New Concept** **How I Do It**	8pp / 2400	Title Page • Structured Abstract, includes subheadings (250 words) • Key Words •Introduction/Purpose • Materials/ Methods/ Results/ Conclusion • COI Disclosure Statement • References • Figure Legends (if any) • Tables (if any)	Up to 6	Official ICMJE Conflict of Interest forms must be completed by each contributing author (these are not viewable to reviewers) http://www.icmje.org/coi_disclosure.pdf
FORMAT II	**#pp / #words**	**Main Text**	**Figures**	**COI Forms**
Review Article	10pp / 3000	• Title Page • One-Paragraph Abstract (125 words) • Typically these are invited submissions; format varies based on topic. • COI Disclosure Statement • References	Up to 6	Must be completed by each contributing author (these are not viewable to reviewers) http://www.icmje.org/coi_disclosure.pdf

		• Figure Legends (if any) • Tables (if any)		
Brief Communication	5pp / 1500	• Title Page • Structured Abstract, includes subheadings (250 words) • Key Words •Introduction/Purpose • Materials/ Methods/ Results/ Conclusion • COI Disclosure Statement • References • Figure Legends (if any) • Tables (if any)	Up to 2	
Letter to Editor	4pp / 1200	• Title Page • No Abstract required • Unstructured • COI Disclosure Statement • Limited number of references	Up to 3	
FORMAT III	**#pp / #words**	**Main Text**	**Figures**	**COI Forms**
Dedicated Video	2pp / 500	• Textual Abstract includes Title, COI statement, Introduction, Materials/ Methods/ Results/ Conclusion/ COI Statement, References (if any) • Video(s) in .mp4 or .mov format only; not to exceed 10 minutes, with narration in English.	None	Must be completed by each contributing author (these are not viewable to reviewers) http://www.icmje.org/coi_disclosure.pdf

*All text, including references, must be double-spaced with one-inch wide margins, and pages numbered consecutively

** Title Page, References, COI Statement, Figures and Tables are not considered in Page/Word Count requirements.

4c. ADDITIONAL SUBMISSION DETAILS

i. Language Editing Services

If you would like your manuscript language edited by a scientific expert before submission or upon revision, Springer recommends using Edanz Group. Edanz provides scientific editing and related services that raise the quality of manuscripts to the standard necessary for ease of peer review. As the only international editing service centralized in China and Japan, Edanz understands the publication challenges faced by scientists whose first language is not English. For more information and a price quotation, contact: http://www.edanzediting.com/springer

ii. Special Characters

The Journal does not assume responsibility for errors in conversion of customized software, newly released software, and special characters. Indicate any special characters used in the file (e.g., Greek, math symbols) by using a symbol code (e.g., ‹ga› for Greek alpha), and defining these codes at the end of your paper.

iii. Abbreviations, Drug Names, Digits

Use the standard abbreviations and units listed in Scientific Style and Format: The CBE Manual for Authors, Editors, and Publishers, Sixth Edition (Reston, Va., Council of Biology Editors, 1994). The first time an uncommon abbreviation appears in the text, it should be preceded by the full name for which it stands. Generic names for drugs and chemicals should be used the first time the drug or chemical is mentioned in the text and, preferably, thereafter. If an author wishes, the trade name may be inserted in parentheses following the generic name the first time the generic name appears, and the manufacturer name and city should also be included. Express digits as numerals except when they are the first word in a sentence, and decimals should be written in North American format. Express units of measurement in the metric system whenever possible, and abbreviate them when used with numbers.

iv. Other Required Forms

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5. MANUSCRIPT SUBMISSION

5a. SUBMISSION STEPS

i. Submission Checklist

Please view a copy of the Submission Checklist below. We recommend that you have all items listed in the checklist complete and ready for upload before starting your online submission. During submission, please make sure that you upload your COI forms at the end

of your submitted document list. Submit all other documents first (main text, figures, tables, etc.).

ii. Review Your Submission

After uploading the files for your submission, the system will convert the files to PDF, and either open the PDF in a new window, or download it to your "downloads" folder for you to open.

Make sure to review the PDF of your submission before you confirm your submission. Once you have reviewed your PDF document for completeness, click "Submit" and all contributing authors will receive an emailed confirmation.

After the manuscript is submitted, the Editors will inspect the submission before assigning reviewers. If any part of the manuscript is not complete, the manuscript will be returned to your Submission Center, with an e-mail notification sent to the authors indicating a need for additional information and/or correction. Once a complete manuscript is correctly submitted, the OBSU editors will assign reviewers to your submission.

5b. KEEPING TRACK

After submission, you may monitor the progress of your submission through the review process. Only the submitting author can view the submission. In order to view your submission details and current status, you must enter the same User Name and Password that you originally used to submit your manuscript.

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If the manuscript is accepted pending minor revisions, or suggested for resubmission after major revisions, we emphasize the importance of authors providing their revisions as

promptly as possible, and providing a point-by-point reply to all reviewer comments. The annotated version of the revised manuscript should identify all changes and include each reviewer point in parentheses, e.g., "(Reviewer 1, Comment 2)."

6. AFTER ACCEPTANCE

If your article is accepted, you will receive a link to the special Springer web page with questions related to:

6a. AUTHOR PROOFS

After a submission is accepted and processed, the author will receive e-mailed notification from the Production Office, and a proof of the article is made available to the author. You should check the proof for typesetting errors, completeness, and accuracy of the text, tables and figures. Substantial changes in content, e.g., new results, corrected values, title and authorship, are not allowed without the approval of the Editor. Any such changes would require a written request and written approval/agreement from all contributing authors to the Editorial Office and to Production for their consideration.

The article will be published online after receipt of the corrected proofs. Online publication is the official first publication of the article, and citable with the DOI. After online publication, further changes can only be made in the form of an Erratum, which would be hyperlinked to the article. After release of the printed version, the paper can also be cited by issue and page numbers.

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Color figures may be used without charge for the electronic version of the journal that is published online via SpringerLink. However, color figures will appear in the print version of the Journal at the author's expense at $1,150 per article. You may provide your choice at the Springer web page.

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Can be ordered via the Springer web page.

7. SUPPORT AND ASSISTANCE

If you have questions or need assistance at any point during the submission and review process, contact the OBSU Managing Editor:

Attn: Deana Rodriguez
Managing Editor, OBSU Editorial Office
Phone: +001 (562) 961-9928
E-mail: obsu.rodriguez@gmail.com

SUBMISSION CHECKLIST

Authors: Make sure that all of the items below are ready and available for Step 6, “File Upload.”

TITLE PAGE REQUIRES:

[ ] Full Title
[ ] All Contributing Authors, Full Names/Degrees
[ ] All Author Email Addresses/Affiliations
[ ] "Correspond To" Information
[ ] Short Title for Running Head
[ ] Detailed Acknowledgments and Grant Information

MAIN MANUSCRIPT TEXT REQUIRES:

[ ] Text

[ ] Abstract (N/A for Letters to the Editor; optional for Brief Communications)

[ ] Required Ethical, COI, and Human/Animal Rights Statements

[ ] References in PubMed style

[ ] Tables (Optional)

[ ] Figure Legends (if providing figures)

FIGURES/IMAGES:

[ ] For vector graphics, the preferred format is EPS; for halftones, use TIFF format. MS Office files are also acceptable

[ ] Figure width should be 39 mm, 84 mm, 129 mm or 174 mm, and no higher than 234 mm

[ ] No identifying information about patients

[ ] Patient and/or publisher permissions, if needed

VIDEO/ELECTRONIC SUPPLEMENTARY MATERIAL:

[ ] Any Video or multimedia in either .MP4 or .MOV file format

[ ] Supplementary videos not to exceed 2 MB in size

[ ] Narration in English

REQUIRED OFFICIAL ICMJE CONFLICT OF INTEREST FORM(S):

[ ] One form completed by each author (ex: 5 authors = 5 forms)

REQUIRED FOR REVISIONS ONLY:

[ ] One copy of clean, revised text, tables and figures

[ ] One copy of annotated, revised text, tables and figures

ANEXO C

JOURNAL OF SURGICAL RESEARCH

A Journal of Surgical Basic Science, Clinical Investigation, Outcome Studies and Education Research Official Publication of the *Association for Academic Surgery*

DESCRIPTION

The Journal of Surgical Research: Clinical and Laboratory Investigation publishes original articles concerned with clinical and laboratory investigations relevant to surgical practice and teaching.

The journal emphasizes reports of clinical investigations or fundamental research bearing directly on surgical management that will be of general interest to a broad range of surgeons and surgical researchers. The articles presented need not have been the products of surgeons or of surgical laboratories.

The Journal of Surgical Research also features review articles and special articles relating to educational, research, or social issues of interest to the academic surgical community.

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GUIDE FOR AUTHORS

INTRODUCTION

The Journal of Surgical Research publishes original manuscripts dealing with clinical and laboratory investigations pertinent to the practice and teaching of surgery. Priority will be given to reports of clinical investigations or basic research bearing directly on surgical management, and of general interest to a wide range of surgeons and surgical investigators.

Manuscripts relating to surgical specialty interests will be judged onthe basis of general interest. Research need not have been done by surgeons or in surgical laboratories. The Journal publishes review articles and special articles relating to educational, research, or social issues pertinent to the academic surgical community. Such manuscripts should be designated as Research Review or Special Article in the cover letter, as well as on the title page. Preliminary reports of 1000 words or less which are accepted by the editorial board will be given priority for the earliest possible publication.

Submission of Manuscripts

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Page charges

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BEFORE YOU BEGIN

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for animal experiments http://ec.europa.eu/environment/chemicals/lab_animals/legislation_en.htm; Uniform Requirements for manuscripts submitted to Biomedical journals http://www.icmje.org. Authors should include a statement in the manuscript that informed consent was obtained for experimentation with human subjects. The privacy rights of human subjects must always be observed.

Conflict of interest

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Submission of an article implies that the work described has not been published previously (except in the form of an abstract or as part of a published lecture or academic thesis or as an electronic preprint, see http://www.elsevier.com/sharingpolicy), that it is not under consideration for publication elsewhere, that its publication is approved by all authors and tacitly or explicitly by the responsible authorities where the work was carried out, and that, if

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All authors should have made substantial contributions to all of the following: (1) the conception and design of the study, or acquisition of data, or analysis and interpretation of data, (2) drafting the article or revising it critically for important intellectual content, (3) final approval of the version to be submitted. Generally, the maximum number of expected authors for a clinical or basic science manuscript is 8.

Changes to authorship

This policy concerns the addition, deletion, or rearrangement of author names in the authorship of accepted manuscripts:

Before the accepted manuscript is published in an online issue: Requests to add or remove an author, or to rearrange the author names, must be sent to the Journal Manager from the corresponding author of the accepted manuscript and must include: (a) the reason the name should be added or removed, or the author names rearranged and (b) written confirmation (e-mail, fax, letter) from all authors that they agree with the addition, removal or rearrangement. In the case of addition or removal of authors, this includes confirmation from the author being added or removed. Requests that are not sent by the corresponding author will be forwarded by the Journal Manager to the corresponding author, who must follow the procedure as described above. Note that: (1) Journal Managers will inform the Journal Editors of any such requests and (2) publication of the accepted manuscript in an online issue is suspended until authorship has been agreed. After the accepted manuscript is published in an online issue: Any requests to add, delete, or rearrange author names in an article published in an online issue will follow the same policies as noted above and result in a corrigendum.

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Role of the funding source

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