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# ESTUDO DA DERIVAÇÃO DUODENOJEJUNAL SOBRE A ESTRUTURA DAS FIBRAS MUSCULARES E JUNÇÕES NEUROMUSCULARES DO MÚSCULO DIAFRAGMA DE RATOS OBESOS INDUZIDOS POR DIETA DE CAFETERIA

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Dissertação apresentada ao Programa De Pós-Graduação *Stricto Sensu* em Biociências e Saúde – Nível Mestrado, do Centro de Ciências Biológicas e da Saúde, da Universidade Estadual do Oeste do Paraná, como requisito parcial para a obtenção do título de Mestre em Biociências e Saúde.

Área de concentração: Biologia, processo saúde-doença e políticas de saúde

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FOLHA DE APROVAÇÃO

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Esta dissertação foi julgada adequada para a obtenção do título de Mestre em Biociências e Saúde e aprovada em sua forma final pelo Orientador e pela Banca Examinadora.

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CASCAVEL-PR
(Abril /2015)

# DEDICATÓRIA

*“Acreditastes em mim mais do que eu mesma,*
*e agora a minha vitória eu dedico a vocês”*

Ao meu noivo, Marcos Gausmann Koerich
À minha mãe, Inês Hart e
À minha irmã, Ana Flávia Hart Ulsenheimer

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# RESUMO GERAL

Na obesidade, a dinâmica do músculo diafragma pode ser prejudicada pelo excesso de tecido adiposo depositado no tórax e abdome, levando a alterações na mecânica respiratória. Uma técnica de cirurgia bariátrica conhecida como a derivação duodenojejunal (DDJ) tem sido investigada como estratégia de tratamento na obesidade e em suas comorbidades. Todavia, os efeitos desse procedimento sobre a musculatura esquelética ainda não foram observados. Assim, o presente estudo teve como objetivo investigar os efeitos da DDJ sobre as junções neuromusculares (JNMs) e nas fibras musculares do músculo diafragma de ratos obesos induzidos por dieta de cafeteria. Ratos *Wistar* machos foram separados em dois grupos: grupo controle (CTL) que recebeu dieta padrão e água, e grupo cafeteria (CAF) que recebeu dieta de cafeteria e refrigerante durante 10 semanas. Após este período, o grupo CAF foi distribuído em dois grupos: Grupo cafeteria submetido à falsa operação (CAF SHAM) e Grupo cafeteria submetido à DDJ (CAF DDJ). Após a cirurgia, ambos os grupos CAF continuaram a receber a dieta de cafeteria. Passadas oito semanas, os animais foram eutanasiados e amostras do músculo diafragma foram coletadas para análise das fibras musculares, quantificação de colágeno e avaliação morfométrica das JNMs. Os animais do grupo CAF SHAM apresentaram aumento do peso corporal, no índice de Lee e nas gorduras retroperitoneal e periepididial quando comparado ao grupo CTL e a cirurgia de DDJ não reverteu este parâmetro. A estrutura das fibras musculares e das JNMs foram semelhante entre os grupos CAF SHAM e CTL. No entanto, o grupo CAF SHAM apresentou alterações na ultraestrutura das fibras como miofibrilas frouxamente arranjadas e desorganização de linha Z no músculo diafragma. Além disso, o grupo CAF SHAM apresentou uma quantidade considerável de gotículas de lipídios e redução na porcentagem de colágeno quando comparado ao grupo CTL. A DDJ não afetou a estrutura e a ultraestrutura das fibras musculares ou das JNMs do músculo diafragma dos animais do grupo CAF DDJ. Dois meses após o procedimento, a DDJ não melhorou as alterações observadas no músculo diafragma de ratos obesos induzidos por dieta de cafeteria.



**PALAVRAS-CHAVE**: derivação duodenojejunal; morfometria; músculo diafragma; junção neuromuscular; dieta de cafeteria.

## GENERAL ABSTRACT

Concerning obesity, the diaphragm dynamics can be impaired due to the excess of fat deposited in thorax and abdomen, leading to changes in respiratory function. A technique of bariatric surgery known as duodenal-jejunal bypasses (DJB) has been investigated as a treatment strategy in obesity and its comorbidities. However, the effects of this procedure on skeletal muscles have not yet been observed. The present study aimed at investigating the DJB effects on the neuromuscular junctions (NMJs) and muscle fibers of diaphragm of obese rats induced by cafeteria diet. Male *Wistar* rats were divided into two groups: a control group (CTL) that received a standard diet and water, and Western Diet group (WD) that received a cafeteria diet and soft drink for 10 weeks. After this period, WD group was distributed into two groups: WD sham-operated rats (WD SHAM); and WD DJB-operated rats (WD DJB). Following surgery, both the WD groups continued to receive the cafeteria diet. After eight weeks, the animals were euthanized and samples of diaphragm muscle were collected to analyze its fibers, quantify its collagen and evaluate NMJs morphometric. WD SHAM rats displayed an increase in body weight, the Lee index and retroperitoneal and peri-epididymal fat pads compared to the CTL group and DJB surgery did not alter these parameters. The muscle fiber structure and NMJs were similar in the WD SHAM and CTL groups. However, the WD SHAM group showed alterations in the fiber ultrastructure, such as loosely arranged myofibrils and Z line disorganization in the diaphragm. In addition, WD SHAM animals presented a considerable amount of lipid droplets and a reduction in the percentage of collagen in diaphragm muscle compared to the CTL group. DJB did not affect the structure or ultrastructure of the muscle fibers or the NMJs in the diaphragm of the WD DJB animals. Two months after the procedure, DJB did not improve the alterations observed in the diaphragm of WD obese-rats.



**Keywords:** duodenal-jejunal bypass; morphometric; diaphragm muscle; neuromuscular junctions; cafeteria diet.

# SUMÁRIO

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## DISSERTAÇÃO



## ARTIGO CIENTÍFICO

# LISTA DE ABREVIATURAS

**CAF** - Grupo cafeteria submetido à dieta de cafeteria

**CAF DDJ** - Grupo cafeteria submetido à derivação duodenojejunal

**CAF FO** - Grupo cafeteria submetido à falsa operação

**CNA** - Comprimento nasoanal

**CTL** - Grupo controle

**CTP** - Carnitina palmitoil transferase

**DDJ** - Derivação duodenojejunal

**DJB** - *Duodenal-jejunal bypass*

**DGYR** - Derivação gástrica em *Y de Roux*

**FG** - Fibras glicolíticas de contração rápida

**FOG** - Fibras oxidativas-glicolíticas de contração rápida

**HE** - Hematoxilina-eosina

**IMC** - Índice de massa corporal

**JMNs** - Junções neuromusculares

**NMJs -** *Neuromuscular junctions*

**SBCBM** - Sociedade Brasileira de Cirurgia Bariátrica e Metabólica

**SO** - Fibras oxidativas de contração lenta

**SUS** - Sistema Único de Saúde

**OMS** - Organização Mundial da Saúde

**WD** - *Western diet*

**WD DJB** - *Western diet group submitted to duodenal-jejunal bypass*

**WD SHAM** - *Western diet group submitted to sham surgery*

# INTRODUÇÃO GERAL

A obesidade é uma doença crônica definida como acúmulo de tecido adiposo em um nível que compromete a saúde dos indivíduos (OMS, 1997). A capacidade de armazenar energia sob a forma de gordura é essencial para a manutenção das funções vitais. No entanto, tal capacidade tornou-se prejudicial com os padrões de vida atuais, devido ao excesso da oferta de alimentos calóricos e um crescente conforto da vida moderna (HALPERN, 1999). Desse modo, ocorre um balanço energético positivo, pois o valor calórico consumido é superior ao gasto (PEREIRA; FRANCISCHI; LANCHA JR, 2003).

Segundo a Organização Mundial da Saúde (OMS), cerca de 12% da população mundial é considerada obesa (ABESO, 2013). No Brasil, 50,8% dos brasileiros estão acima do peso e desses 17,5% são obesos (VIGITEL, 2013). Assim, a obesidade e o sobrepeso são considerados um problema de saúde pública, cuja obesidade está relacionada a várias comorbidades (OMS, 2000). Dentre as principais patologias normalmente associadas à obesidade, destacam-se os problemas respiratórios, caracterizados principalmente pela falta de ar, a apneia do sono e a síndrome da hipoventilação (PEREIRA; FRANCISCHI; LANCHA JR, 2003).

A origem de problemas respiratórios está relacionada principalmente com alterações na mecânica respiratória do indivíduo obeso. Tais alterações ocorrem devido ao acúmulo de tecido adiposo depositado na região torácica e abdominal, pois geram compressão mecânica sobre o músculo diafragma, pulmões e caixa torácica e levam à restrição da mecânica pulmonar. Assim, ocorre diminuição da complacência do sistema respiratório, aumento do trabalho da respiração e do consumo de oxigênio (DELGADO; LUNARDI, 2011).

Na obesidade, além do acúmulo de lipídios no tecido adiposo pode haver depósitos de lipídios em outros tecidos, como no músculo estriado esquelético (HERPEN; SCHRAUWEN-HINDERLING, 2008). Tais modificações podem ocorrer simultaneamente com alterações na estrutura das fibras musculares (MALENFANT *et al.,* 2001; BAYOL; SIMBI; STICKLAND, 2005; ALMEIDA *et al.,* 2008; SISHI *et al.,* 2010) e podem prejudicar o funcionamento muscular (CLEBIS; NATALI, 2001). Sugere-se que as possíveis alterações na estrutura das fibras musculares possam afetar o músculo diafragma e suas junções neuromusculares (JNMs), uma vez que ambos estão intimamente interligados. Assim, acredita-se que essas alterações podem levar a um comprometimento da força muscular respiratória: um achado que é observado em obesos mórbidos (CASTELLO *et al.,* 2007).

No tratamento da obesidade, várias abordagens podem ser utilizadas, entre elas a reeducação alimentar, a atividade física, o uso de medicamentos e as intervenções cirúrgicas (RAVELLI *et al.,* 2007). No entanto, para o controle e tratamento mais eficaz dessa doença, além de uma equipe de profissionais de saúde, é necessário que haja maneiras de motivar a população para a mudança de seus hábitos de vida. Segundo Reis, Vasconcelos e Barros (2011), ambientes que estimulem padrões saudáveis de alimentação, atividade física e ações que visam informar a importância de um estilo de vida saudável são importantes para apoiar e conscientizar os cidadãos. De acordo com Ravelli *et al*. (2007), a cirurgia bariátrica é considerada o melhor tratamento para a obesidade mórbida, devido à eficácia na perda de peso e à melhora das comorbidades associadas. No entanto, devem haver programas educativos multidisciplinares para o sucesso na redução de peso nos períodos pré e pós-operatório, pois são extremamente importantes para auxiliar os pacientes na mudança de novos hábitos.

Uma técnica cirúrgica experimental conhecida como derivação duodenojejunal (DDJ) vem sendo investigada como estratégia de tratamento para a obesidade e doenças associadas. Em modelos animais de diabetes e obesidade, a DDJ tem demonstrado melhorar a homeostase glicêmica (BREEN *et al.,* 2012; HU *et al.,* 2013; JUROWICH *et al.,* 2013), o perfil lipídico (HU *et al.,* 2013), a função renal (ZHIQING *et al.,* 2014), a doença hepática gordurosa (EBERTZ *et al.,* 2014) e a aterosclerose (CHEN *et al.,* 2014) sem promover alterações no peso corporal. Entretanto, esta é a primeira vez que o efeito desta cirurgia experimental está sendo analisado no que se refere à morfologia do músculo diafragma. Tendo em vista a

importância de tal músculo para a respiração, hipotetiza-se que a cirurgia de DDJ reverta às possíveis alterações morfológicas no músculo diafragma de ratos obesos induzidos pela dieta de cafeteria. Neste contexto, o presente estudo teve como objetivo investigar os efeitos da DDJ sobre as JNMs e nas fibras musculares do músculo diafragma de ratos obesos induzidos por dieta de cafeteria.

# REVISÃO GERAL DE LITERATURA

## Obesidade

A obesidade e o sobrepeso estão se tornando cada vez mais comuns entre as pessoas e a obesidade é considerada um dos maiores problemas de saúde pública (WHO, 2000). Atualmente, essa doença atinge proporções epidêmicas em todo o Planeta, com cerca de 2,8 milhões de pessoas que morrem a cada ano (OMS, 2013). O diagnóstico pode ser feito a partir do cálculo do índice de massa corporal (IMC), que verifica a relação entre peso corpóreo (kg) dividido pela estatura $(m)^2$. O valor superior ou igual a 30 $kg/m^2$ considera o indivíduo obeso, enquanto aquele superior a 40 $kg/m^2$ é classificado como obeso mórbido (OMS, 1997). Os custos dessa doença para os setores público e privado são cerca de 1,5 bilhões de reais por ano com internações hospitalares, consultas médicas e medicamentos. Desse valor, 600 milhões são enviados pelo governo via Sistema Único de Saúde (SUS) e representam 12% do orçamento gasto com todas as outras doenças (ANJOS, 2006).

A alta incidência da obesidade tem sido relacionada a vários fatores, incluindo hereditariedade, hábitos alimentares, redução de gasto energético, alterações hormonais e estilo de vida (RASSLAN *et al.,* 2009). Alguns fatores sociodemográficos como escolaridade, raça/cor, união conjugal, idade e renda também estão associados com o excesso de peso e a obesidade (RONSON *et al.,* 2005; VEDANA *et al.,* 2008; GIGANTE; MOURA; SARDINHA, 2009). Antigamente, a obesidade era associada apenas a países de alta renda, mas, atualmente, sua prevalência é maior em países com baixa e média renda (OMS, 2013).

Mudanças no orçamento familiar indicam incremento na aquisição de produtos industrializados e redução do consumo de alimentos *in natura,* devido à grande oferta dos produtos processados (TARDIDO; FALCÃO, 2006). Contudo, a

ideia de adesão à dieta ocidental, utilizada para justificar o aumento da incidência da obesidade, não explica a prevalência desta doença em mulheres obesas de baixa renda. Tais mulheres se alimentam basicamente de arroz, feijão, açúcares e gorduras; não ingerem produtos industrializados e enlatados e raramente consumem frutas e verduras (FERREIRA; MAGALHÃES, 2011). Desse modo, apesar das explicações sobre o surgimento e a manutenção da obesidade, ainda não se sabe claramente porque diferentes subgrupos populacionais são acometidos de forma distinta (MINAYO *et al.,* 2003).

A obesidade está associada a várias comorbidades, dentre as quais destacam-se as doenças cardiovasculares, hipertensão, diabetes *Mellitus* tipo 2, acidente vascular cerebral, vários tipos de câncer e cálculos biliares (STEIN; COLDITZ, 2004). Dentre as patologias mais comuns associadas à obesidade estão os problemas respiratórios, que incluem falta de ar, apneia do sono e síndrome da hipoventilação (PEREIRA; FRANCISCHI; LANCHA JR., 2003). As principais alterações observadas nestes quadros são: diminuição da complacência torácica, taquipneia, aumento do trabalho muscular respiratório, altos índices de hipoxemia e fadiga respiratória (DELGADO; LUNARDI, 2011).

O acúmulo de tecido adiposo na região abdominal, especialmente em torno do diafragma e da pleura e a hipertonia dos músculos do abdome levam ao comprometimento respiratório devido à redução do desempenho muscular e da expansão torácica (RASSLAN *et al.,* 2009). O excesso de tecido adiposo no tórax e abdome promove uma compressão sobre o músculo diafragma, pulmões e caixa torácica, cujas consequências são a restrição da mecânica pulmonar e a redução da complacência do sistema respiratório. Isso resulta em aumento do trabalho respiratório, do consumo de oxigênio e do custo energético da respiração (DELGADO; LUNARDI, 2011). Assim, a obesidade pode afetar o tórax e o diafragma e determinar modificações na função respiratória, mesmo que não ocorram alterações pulmonares (RASSLAN *et al.,* 2009).

Outras consequências do excesso de peso e da obesidade são as lesões músculo-esqueléticas, especialmente osteoartrite (WHO, 2013), desconfortos articulares (RASIA *et al.,* 2007), pés planos (ARRUDA; SIMÕES, 2006), alterações na postura corporal (ARRUDA; SIMÕES, 2006; GUIDETTI; 2010; SIQUEIRA; SILVA, 2011) e na morfologia do tecido muscular (MALENFANT *et al.,* 2001; BAYOL; SIMBI; STICKLAND, 2005; ALMEIDA *et al.,* 2008; SISHI *et al.,* 2010).

As complicações decorrentes da obesidade afetam diretamente a qualidade de vida das pessoas. Cada vez mais, o uso de modelos animais em estudos experimentais tem contribuído para o desenvolvimento de terapias para diversas patologias associadas à obesidade, dentre as quais destaca-se a cirurgia bariátrica.

**Modelo experimental de obesidade induzida por dieta**

Considerando-se que um dos fatores causais da obesidade humana é o consumo de alimentos ricos em gordura e com elevada densidade energética, certos modelos experimentais buscam simular esta condição por oferecerem um aporte maior de lipídios, carboidratos ou ambos. Assim, este modelo de indução de obesidade é o que mais se assemelha a obesidade em humanos (KRAUSS *et al.,* 1998; DEITEL, 2003; NASCIMENTO *et al.,* 2008; ABESO, 2009).

Existem vários tipos de dietas para indução da obesidade que se revelaram eficazes. As dietas hipercalóricas caracterizam-se por apresentar maior quantidade de carboidratos, enquanto que as dietas do tipo *high fat,* apresentam maiores porcentagens de lipídios *(DIEMEN; TRINDADE; TRINDADE, 2006; ROSINI et al., 2012).* Outro modelo de dieta experimental é a dieta de cafeteria, conhecida também como dieta ocidentalizada ou *fast-food* que consiste em uma variedade de alimentos altamente palatáveis predominantes na sociedade ocidental e associados com a atual pandemia de obesidade (SAMPEY *et al.,* 2011). Nesta dieta, alimentos como pão, queijo, doce, bolo, chocolate, massa e refrigerante podem ser oferecidos isoladamente ou em associação com a ração padrão (DIEMEN; TRINDADE; TRINDADE, 2008; SHAFAT *et al.,* 2009; GOULARTE; FERREIRA; SANVITTO, 2012).

Ratos alimentados com dieta de cafeteria são modelos experimentais amplamente utilizados para estudar a obesidade e desordens associadas (GOULARTE *et al.,* 2011), devido à grande semelhança com a gênese e as respostas metabólicas decorrentes da obesidade em humanos (ROSINI *et al.,* 2012). Esta dieta, produzida pela mistura de comidas consumida pelos humanos, induz a hiperfagia nos ratos, os quais ganham peso rapidamente e tornam-se obesos (SHAFAT *et al.,* 2009), além de desenvolverem disfunções associadas como hiperinsulinemia, hiperglicemia, intolerância à glicose e inflamação (SAMPEY *et al.,* 2011)

**Cirurgia bariátrica**

Atualmente, a cirurgia bariátrica é a ferramenta mais eficaz no controle e tratamento da obesidade mórbida. Dentre seus principais benefícios, destacam-se a perda e a manutenção de peso corporal em longo prazo, além da melhora das comorbidades como diabetes, hipertensão, colesterol elevado, incontinência urinária, dores de cabeça crônicas, doenças do fígado e artrites associadas (SBCBM, 2011). Além disso, a intervenção cirúrgica melhora outras doenças associadas à obesidade como a apneia obstrutiva do sono e a síndrome da hipoventilação (WEI; WU, 2012). O Brasil é o segundo país que mais realiza este tipo de cirurgia e o número de pacientes passou de 16 mil, em 2003, para 72 mil em 2012, ou seja, um aumento de 350% (SBCBM, 2011).

O SUS gasta uma quantia considerável em cirurgias bariátricas todos os anos. Em 2010, foram realizadas 4489 cirurgias pelo SUS e em 2013 o número de procedimentos cirúrgicos chegou a 6493 (BRASIL, 2014). Com a preocupação em proporcionar um tratamento mais humanizado e multidisciplinar, o Ministério da Saúde criou uma portaria que visa aos atendimentos com psicólogos, nutricionistas e até cirurgiões plásticos financiados pelo SUS para pacientes obesos (SBCBM, 2011). Com a melhora ou até mesmo a cura das doenças associadas à obesidade, há uma redução no uso de medicamentos, número de consultas aos profissionais de saúde e na quantidade de exames realizados pelos pacientes. Sendo assim, a cirurgia gera mais economia para os serviços de saúde. Estudos mostram que os custos da cirurgia são amortizados em menos de três anos, enquanto as pessoas obesas geram custos que aumentam em longo prazo (SÜSSENBACH, 2011).

A cirurgia bariátrica é indicada para pacientes portadores de obesidade mórbida com IMC > 40 $Kg/m^2$, há mais de cinco anos e com insucesso nos tratamentos anteriores, ou então, para pacientes com IMC entre 35 e 39,9 $kg/m^2$, associado à comorbidades (RAVELLI *et al.,* 2007). Em 2013, foi aprovada a Resolução 1.942 do Conselho Federal de Medicina, que visa à redução de 18 para 16 anos da idade mínima para realização da cirurgia bariátrica bem como ao aumento para 110 anos da idade máxima, que antes era de 65. Entretanto, esta escolha exige precauções especiais e o risco/benefício deve ser muito bem analisado (SBCBM, 2011).

Embora a cirurgia bariátrica promova vários benefícios, a intervenção cirúrgica pode ocasionar certas complicações como a deficiência nutricional em ferro, ácido fólico, vitamina B12 (MARCASON, 2004; PARKES, 2006; MECHANICK *et al.,* 2008), tiamina (vitamina B1) e vitaminas A, D, E e K, além de anormalidades eletrolíticas, com reduzidas concentrações de cálcio, magnésio, sódio e potássio (MECHANICK *et al.,* 2008). Além disso, complicações respiratórias pós-operatórias como embolia pulmonar, atelectasias e pneumonia são frequentes (DELGADO; LUNARDI, 2011).

Os procedimentos da cirurgia bariátrica são comumente divididos em três categorias: restritivo, disabsortivo ou mal-absortivo e misto. Os procedimentos restritivos incluem a banda gástrica, gastroplastia vertical com bandagem e gastrectomia vertical, os quais visam reduzir o volume gástrico. Os procedimentos disabsortivos ou mal-absortivos como a derivação jejunoileal, a DDJ e o desvio biliopancreático envolvem o desvio de uma ou mais porções do intestino para diminuir sua capacidade de absorção. Os procedimentos mistos, como a derivação gástrica em *Y de Roux* e derivação Bilio-Pancreática com *Duodenal Switch,* associam a restrição do estômago com o desvio de parte do intestino e levam a uma discreta má absorção (KARRA; YOUSSEIF; BATTERHAM, 2010; SBCBM, 2011).

A cirurgia de DDJ, foco deste estudo, foi introduzida como um procedimento que contribui para melhorar o diabetes sem promover a perda de peso em modelo animal de diabetes tipo 2 (RUBINO *et al.,* 2004). Esta técnica cirúrgica experimental consiste na exclusão do duodeno e do jejuno proximal do trânsito alimentar sem a restrição do volume gástrico. Tal procedimento tem comprovado melhorar a homeostase glicêmica (BREEN *et al.,* 2012; JUROWICH *et al*., 2013), o perfil lipídico (HU *et al.,* 2013), a função renal (ZHIQING *et al.*, 2014), além de atenuar a doença hepática gordurosa (EBERTZ *et al.*, 2014) e prevenir a aterosclerose (CHEN *et al.,* 2014) em modelo animal de diabetes adquirida ou obesidade, independente da perda de peso.

Um modelo de DDJ semelhante ao proposto por Rubino *et al. (*2004) foi descrito por Jurowich *et al*. (2013), caracterizado como um procedimento menos invasivo e com menores índices de morbidade e mortalidade, o qual foi adotado nesta pesquisa. Nesta cirurgia, é realizada uma transecção pós-pilórica no duodeno. Em seguida são feitos o fechamento do coto duodenal e a reconstrução da passagem intestinal através da gastrojejunostomia, união do piloro do estômago ao jejuno (Figura 1).

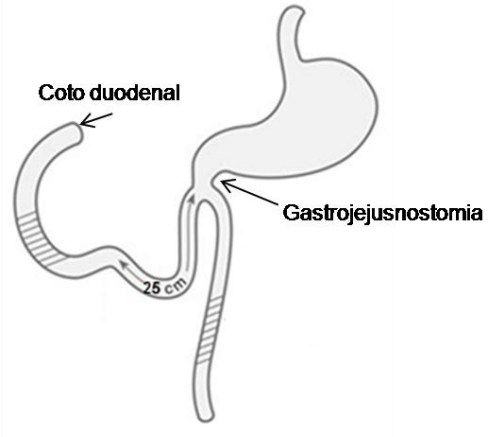


**Figura 1** - Representação da cirurgia de DDJ (Adaptado de JUROWICH *et al*., 2013).

Devido aos efeitos positivos que a cirurgia de DDJ tem mostrado em modelo animal de obesidade e diabetes, especulam-se quais seriam os efeitos desta modalidade cirúrgica sobre a morfologia do músculo estriado esquelético em animais obesos.

## Músculo estriado esquelético e JNMs

A musculatura esquelética pode ser extremamente afetada na obesidade, por isso é um importante alvo de investigação. Um dos principais músculos da respiração, o músculo diafragma, tem os movimentos limitados devido ao acúmulo de tecido adiposo, depositado principalmente na região abdominal (DELGADO; LUNARDI, 2011). Encontrado apenas nos mamíferos, este músculo está localizado entre as cavidades do tórax e do abdome e apresenta duas regiões: uma central tendínea e uma periférica muscular, na qual se distinguem, em cada antímero, as porções lombar, costal e esternal (LESSA *et al.,* 2012).

O músculo estriado esquelético é constituído por células longas, finas e multinucleadas, chamadas de fibras musculares (BADARO; SILVA; BECHE, 2007). Na musculatura esquelética, podem ser caracterizados três tipos de fibras musculares: fibras tipo I ou SO (fibras oxidativas de contração lenta); fibras tipo IIa ou FOG (fibras oxidativas-glicolíticas de contração rápida); fibras tipo IIb ou FG (fibras glicolíticas de contração rápida) (BROOKE; KAISER,1970; PETER *et al.,* 1972). Devido à importante função para a respiração, o músculo diafragma está em contínua atividade rítmica, por isso, suas fibras musculares precisam ser resistentes à fadiga. Assim, durante a respiração normal, são utilizadas principalmente as fibras lentas, enquanto as fibras rápidas são recrutadas especificamente quando a taxa de

respiração aumenta. O diafragma de humanos é constituído por 55% de fibras lentas (tipo I) enquanto as fibras rápidas representam 21% (tipo IIa) e 24% (tipo IIb) (POLLA *et al.,* 2004). Em ratos, as fibras lentas (tipo I) são cerca de 60%, enquanto as fibras rápidas (tipo IIa e IIb) representam 20% (PADYKULA; GAUTHIER, 1970).

No modelo animal de obesidade induzida por dieta de cafeteria, estudos mostram que a morfologia do músculo estriado esquelético é afetada e ocorre redução na área de secção transversal das fibras dos músculos gastrocnêmio (SISHI *et al.,* 2010), semitendíneo (BAYOL; SIMBI; STICKLAND, 2005) e sóleo (ALMEIDA, 2008), além de apoptose e atrofia muscular (SISHI *et al.,* 2010). Ademais, a oferta da dieta de cafeteria para ratas somente no período de gestação e também durante a lactação resultou na redução do número de núcleos e de fibras musculares do músculo semitendíneo nas proles (BAYOL; SIMBI; STICKLAND, 2005).

Algumas alterações também foram observadas na estrutura das fibras musculares em indivíduos obesos. Foi verificado aumento na área das fibras musculares (tipo IIb) (MALENFANT *et al.,* 2001) e maiores quantidades de lipídios intramusculares no músculo vasto lateral (GOODPASTER *et al.,* 2000; MALEFANT *et al.,* 2001). Os lipídios podem se acumular entre as fibras (intrafascicularmente) ou no citoplasma das fibras musculares (intramiocelularmente) (SILVESTRE, 2009). No interior dessas células, os lipídios estão sob a forma de gotículas lipídicas nas adjacências das mitocôndrias (BELMONTE; AOKI, 2005). Indivíduos magros apresentam cerca de 1,5% de gotículas de lipídios por fibra muscular, enquanto que em indivíduos obesos, esse valor chega a 3-4% (GOODPASTER *et al.,* 2000). De acordo com Malefant *et al.* (2001), as gotículas de lipídios estão localizadas na região central das fibras musculares em indivíduos obesos, o que sugere uma diminuição na sua utilização, pois os lipídios são oxidados essencialmente pelas mitocôndrias subsarcolémicas. Isto pode estar relacionado com a diminuição da capacidade oxidativa observada em indivíduos obesos.

Os lipídios são uma importante fonte de energia para o músculo esquelético. Para que ocorra a oxidação lipídica, os ácidos graxos presentes no sarcoplasma precisam atravessar as membranas mitocondriais através do complexo carnitina palmitoil transferase (CTP). Primeiro, os ácidos graxos são ativados, transformam-se em acil-CoA e, pela ação das enzimas CTP I e CTP II, atravessam as membranas mitocondriais e entram no processo de β-oxidação. Em seguida, ocorre a formação de acetil-CoA que é metabolizado no Ciclo de Krebs para a produção de ATP (CURI

*et al.,* 2003). Na obesidade, a diminuição na oxidação lipídica pode estar relacionada com a redução da atividade da enzima CPT I (KIM *et al.,* 2000), a qual pode ser explicada pelo excesso de malonil CoA, potente inibidor da CTP I. O excesso de malonil CoA ocorre devido à metabolização elevada da glicose, pela via glicolítica, que resulta em citrato; ao sair da mitocôndria, é transformado em acetil-CoA e posteriormente é convertido em malonil CoA (CURI *et al.,* 2003).

As alterações nas estruturas das fibras musculares relatadas anteriormente também podem afetar o tamanho das JNMs, uma vez que ambas estão intimamente interligadas. A JNM é um tipo de sinapse formada entre neurônios motores e fibras musculares esqueléticas (WU; XIONG; MEI, 2010). É considerada uma estrutura anatômica e funcionalmente diferenciada para a transmissão de um sinal do terminal nervoso para a fibra muscular (ENGEL, 2008).

As JNMs de todos os vertebrados têm basicamente a mesma estrutura. São formadas por um terminal pré-sináptico contendo um neurotransmissor: a acetilcolina; células de Schwann e seus prolongamentos citoplasmáticos que envolvem o terminal axônico, exceto na membrana pré-sináptica, cuja função é manter preso o terminal pré-sináptico; uma fenda contendo acetilcolinesterase e revestida por lâmina basal: a goteira sináptica primária; uma membrana pós-sináptica, correspondente a uma região especializada da membrana sarcoplasmática que contém receptores para acetilcolina e um sarcoplasma juncional, que suporta estrutural e metabolicamente a região pós-sináptica (Figura 2) (OGATA, 1988).

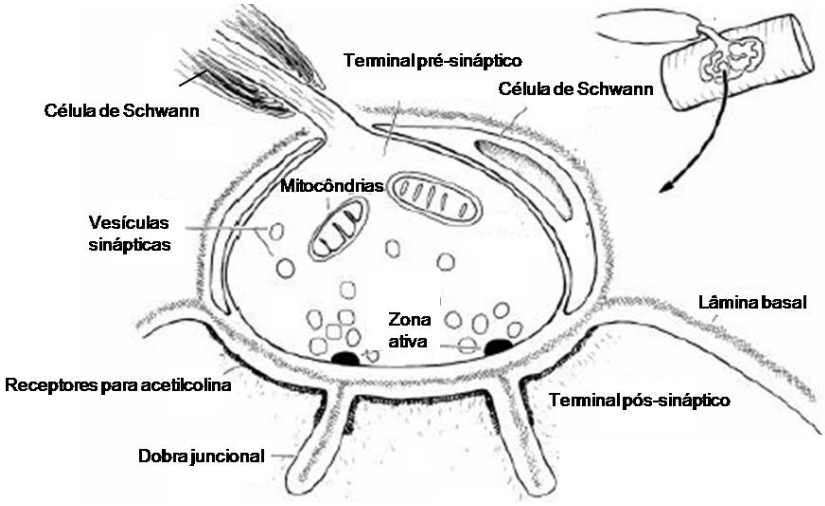


**Figura 2** - Estrutura de uma JNM com seus principais constituintes (Adaptado de HALL; SANES, 1993).

A forma e o tamanho do terminal axônico das JNMs variam de acordo com os diferentes tipos de fibras musculares. No diafragma de rato, as fibras tipo I apresentam poucas dobras juncionais e o terminal do axônio é pequeno e elíptico. As JNMs das fibras tipo IIb apresentam numerosas dobras juncionais e o terminal do axônio é longo e liso. Nas JNMs das fibras tipo IIa, as dobras juncionais são mais escassas do que nas fibras tipo I e o terminal do axônio é longo quando comparado às fibras tipo IIb (PADYKULA; GAUTHIER, 1970).

Para o nosso conhecimento, as informações na literatura que abordem os efeitos da obesidade sobre a morfologia do músculo diafragma e suas JNMs associadas são escassas. Portanto, há necessidade de mais estudos direcionados para essa área, a fim de que se ampliem os conhecimentos e proporcione-se uma melhor compreensão sobre as possíveis alterações funcionais que podem ocorrer, a partir de alterações estruturais na musculatura do diafragma, as quais podem estar associadas aos problemas respiratórios observados na obesidade.

**Efeito da cirurgia bariátrica sobre o músculo estriado esquelético**

Embora estudos recentes mostrem a eficácia dos procedimentos cirúrgicos no tratamento da obesidade e melhora das comorbidades associadas (KARRA; YOUSSEIF; BATTERHAM, 2010; SÜSSENBACH, 2011; ZEVE; NOVAIS; OLIVEIRA JÚNIOR, 2012), ainda há poucas informações em relação aos efeitos da cirurgia bariátrica sobre a musculatura estriada esquelética.

A cirurgia de derivação gástrica em *Y de Roux* (DGYR) é o procedimento mais realizado em indivíduos obesos para a redução do peso corporal (PORIES, 2008). Alguns estudos mostram que após a DGYR ocorre diminuição na espessura dos músculos quadríceps femoral, bíceps braquial e braquial (PEREIRA *et al.,* 2011; LYYTINEN *et al.,* 2013). A DGYR também promove redução na área de secção transversal das fibras no músculo quadríceps femoral (LYYTINEN *et al.,* 2013) e na quantidade de lipídios intramusculares no músculo vasto lateral, sem provocar alterações na capacidade oxidativa e glicolítica das fibras (GRAY *et al.,* 2003). A preservação de massa magra foi observada em indivíduos somente após redução de peso, induzida por banda gástrica (SERGI *et al.,* 2003).

Os poucos trabalhos encontrados na literatura que abordam os efeitos da cirurgia bariátrica sobre o músculo esquelético são referentes apenas a estudos realizados

em humanos. Há escassez de estudos realizados em animais de laboratório que visem esclarecer as alterações provocadas no músculo estriado esquelético após intervenções cirúrgicas. Até o momento, desconhecemos qualquer publicação na literatura que aborde os efeitos da cirurgia de DDJ sobre a musculatura esquelética. O conhecimento dessas possíveis alterações no músculo diafragma após procedimento bariátrico é importante para a compreensão de repercussões funcionais que podem ocorrer e que possam estar associadas às complicações respiratórias pós-operatórias.

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## ARTIGO CIENTÍFICO

# DUODENAL-JEJUNAL BYPASS DOES NOT AFFECT THE STRUCTURE OR ULTRASTRUCTURE OF THE MUSCLE FIBERS OR THE NEUROMUSCULAR JUNCTIONS IN THE DIAPHRAGM OF OBESE RATS

*(Artigo científico submetido à Revista Obesity Surgery)*

# DUODENAL-JEJUNAL BYPASS DOES NOT AFFECT THE STRUCTURE OR ULTRASTRUCTURE OF THE MUSCLE FIBERS OR THE NEUROMUSCULAR JUNCTIONS IN THE DIAPHRAGM OF OBESE RATS

**Manuscript type**: Original article

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**Short running head:** Effects of DJB on diaphragm of obese rats

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**Abstract**

*Purpose:* The present study investigates the effects of duodenal-jejunal bypass (DJB) on the structure and ultrastructure of the muscle fibers and neuromuscular junctions (NMJs) in the diaphragm of Western diet obese rats. *Methods:* Male *Wistar* rats were fed a standard rodent chow diet (CTL) or Western diet (WD) *ad libitum*. After 10 weeks, WD rats were submitted to a sham operation or DJB, forming the WD SHAM and WD DJB groups, respectively. After 8 weeks, the structure, ultrastructure and collagen content of the muscle fibers as well as the morphometry of the neuromuscular junctions (NMJs) were analyzed. *Results:* WD SHAM rats displayed an increase in body weight, the Lee index and retroperitoneal and peri-epididymal fat pads compared to the CTL group. DJB did not alter these parameters. The muscle fiber structure and NMJs were similar in the WD SHAM and CTL groups. However, the WD SHAM group showed alterations in the fiber ultrastructure, such as loosely arranged myofibrils and Z line disorganization in the diaphragm. In addition, WD SHAM animals presented a considerable amount of lipid droplets and a reduction in the percentage of collagen in diaphragm muscle compared to the CTL group. DJB did not affect the structure or ultrastructure of the muscle fibers or the NMJs in the diaphragm of the WD DJB animals. *Conclusions:* Two months after the procedure, DJB did not improve the alterations observed in the diaphragm of WD obese-rats.

**Keywords:** Duodenal-jejunal bypass, Diaphragm, Neuromuscular junction, Obesity.

## Introduction

Obesity is a public health problem associated with several diseases that directly affect quality of life [1]. Among the associated pathological conditions, those involving the respiratory system stand out - the most common being obstructive sleep apnea and

hypoventilation syndrome [2]. The occurrence of respiratory problems in obese people is mainly related to changes in respiratory function [3]. Because of its important function in breathing, the diaphragm must be in continuous rhythmic activity, which requires fatigue resistance from its muscle fibers [4]. However, excessive fat depositsin the chest and abdomen produces compression on the diaphragm, lungs and chest cavity, leading to decreased respiratory system compliance, increased work of breathing, oxygen consumption and respiratory energy expenditure [3]. As a result, the activity of the diaphragm increases in an attempt to maintain adequate alveolar ventilation [5].

Changes in the respiratory function of obese individuals may be related to alterations in the morphology of the diaphragm [5]. Studies conducted with genetically obese Zucker rats [5, 6] and rats with hypothalamic obesity [5] reported remodeling and alterations in muscle fiber size [5] muscle atrophy [5, 6] and fibrosis of the diaphragm [6]. Such alterations to muscle fibers may affect the neuromuscular junctions (NMJs) associated with this muscle, since these two structures are extensively interconnected. Furthermore, Zucker rats have been shown to display alterations to the diaphragm muscle action potential, as manifested by increased height, overshoot and area [7].

Recently, advances in the treatment of obesity have been achieved with the use of surgical interventions. Bariatric surgery is the most effective treatment for cases of morbid obesity, due to its effectiveness in inducing weight loss and improving comorbidities [8]. Duodenal-jejunal bypass (DJB) is a type of experimental malabsorptive bariatric surgery, which aims to divert some of the proximal intestine to decrease food absorption [9]. Studies have shown that in animal models of acquired diabetes or obesity, prior to producing weight loss, this procedure improves glucose homeostasis [10-12], the lipid profile [11] and renal function [13] attenuates fatty liver disease [14] and prevents

atherosclerosis [15]. However, there are no reports on the effects of this type of surgery on the morphology of the diaphragm in obese mice.

The model of animal obesity that most resembles human obesity is that induced by consuming a cafeteria diet [16]. In this diet, animals are offered highly palatable and caloric foods mimicking the westernized diet [17]. The cafeteria diet produces voluntary hyperphagia, rapid weight gain, increased fat mass and generates pre-diabetic parameters such as hyperglycemia and insulin intolerance [17, 18, 19]. Thus, our objective was to evaluate the effects of DJB on the structure of muscle fibers and NMJs of the diaphragm in cafeteria diet-induced obese rats.

## Methods

### *Animals*

All experimental procedures were approved by the Ethics Committee on Animal Experiments (CEUA) of the UNIOESTE, under Protocol N° 8709). At eight weeks of age, eighteen male Wistar rats (*Rattus norvegicus*) were randomly divided into two groups: a control group (CTL, n = 6) that received a standard diet and water *ad libitum*, and Western Diet group (WD; n = 12) that received a cafeteria diet and soda drinks *ad libitum*. After consuming the cafeteria diet for 10 weeks, the WD group was distributed into two groups: WD sham-operated rats (WD SHAM; n = 6); and WD DJB-operated rats (WD DJB; n = 6). For seven days before and seven days after surgery the WD SHAM and WD DJB groups were given a liquid cafeteria diet, while the CTL group received the standard liquid diet. Following surgery, both the WD groups continued to receive the cafeteria diet for eight weeks. The animals were kept in cages with standard lighting conditions (06:00 to 18:00) and a controlled temperature (22 ± 1 ° C) throughout the experimental period.

*Diets*

The CTL group received the standard rodent diet (Biobase, Brazil) composed of 3.8 kcal/g (70% carbohydrates, 20% protein and 10% fat) and water *ad libitum*. The WD groups received a cafeteria diet, according to the model described by Goularte *et al.* [20] with some modifications. This highly palatable and high calorie diet consisted of standard chow (Biobase, Brazil), Italian salami (Sadia, Brazil), mini bread rolls (Nutrella, Brazil), corn chips (Cheetos, Pepsico, Brazil), marshmallow (Fini, Brazil), mixed sausage (Sadia, Brazil), chocolate cake (Renata, Selmi, Brazil), corn-based cookies (Zadimel, Brazil), mortadella (Frimesa, Brazil), bacon snacks (Trophy, Helena, Brazil), chocolate wafer biscuits (Bauduco, Brazil) and 350 ml of degassed Coca-Cola (Coca-Cola, Brazil) and Guarana (Antarctica, Brazil) per day.

*The duodenal-jejunal bypass surgery (DJB) and the sham surgery*

The DJB and SHAM surgeries were performed after 10 weeks consuming the cafeteria diet. The perioperative procedures were performed as described by Meguid *et al.* [21] and the DJB surgery was performed as described by Jurowich *et al.* [12]. The animals were fasted for 12 to 16 hours prior to surgery and anesthetized with 1% isoflurane (Isoforine®, Cristália, SP, Brazil). Briefly, the DJB surgery consisted of a laparotomy followed by post-pyloric duodenal transection, closure of the duodenal stump and reconstruction of the intestinal transit through gastrojejunostomy (unionof the pyloricstomach to the jejunum). To demonstrate the correct execution of the surgery, a saline solution was injected into the pyloric region to test for any constriction or leakage of the liquid. For the sham operation, a midline incision was made into the anterior abdominal wall associated with the movement of the intestinal loops and stomach was performed and then sutured.

*Evaluating Obesity*

The body weight of the animals was measured from the 8th to 26th weeks of age. At the end of the trial period, the final body weight and nasal-anal length (NAL) were measured to obtain the Lee index - [weight corporal$^{1/3}$ (g)/nasal-anal length (cm)] X 1000, which is considered a parameter for assessing obesity. Then, the animals were desensitized in a $CO_2$ chamber and euthanized by decapitation (guillotine). The retroperitoneal and peri-epididymal fat was removed and weighed to assess the accumulation of fat.

*Collecting the diaphragm*

The animals were placed in a prone position and an incision was made in the midline immediately below the thorax, with the skin and muscle being subsequently folded back. The diaphragm was removed through an incision along its lumbar, costal and sternal portions.

*The histological study of the muscle fibers*

The samples of the diaphragm were fixed in Karnovsky's solution [22] and subsequently washed in phosphate buffered saline (PBS) to remove any excess fixative. The samples were embedded in paraplast (SIGMA, Missouri, USA) to facilitate the acquisition of cross sections of the muscle fibers. Subsequently, the muscle fragments were serially sectioned at 7μm using a microtome (R35, Leipzig, China). The obtained sections were placed on slides and placed in an oven at 60°C for 1 hour. After which the slides were subjected to deparaffinization, hydration and staining with either hematoxylin-eosin for morphological analysis of the muscle fibers [23] or Picrosirius Red, to reveal the collagen [24]. After staining, the slides were dehydrated, cleared and mounted with the aid of Permount (Fisher Scientific®, New Jersey, USA).

*The ultrastructural analysis of the muscle fibers*

Regarding the ultrastructure, samples of the diaphragm muscle were cut into longitudinal fragments (approximately one mm in width) and immersed in Karnovsky's fixative [22] for mounting. Subsequently, they were washed in 0.1M phosphate buffer, pH 7.3 (15 minutes) and post-fixed in 1% osmium tetroxide for two hours. The samples were then washed in distilled water, incubated in 0.5% uranyl acetate (2 hours), dried in acetone and soaked in a mixture of resin and 100% acetone (12 hours) to form blocks. The desired fields were selected using semi-thin sections (0.5 μm) and the ultrathin sections (90 nm thick) were obtained using an ultra-microtome (Ultracut UCT, Leica®, Germany). The ultrathin sections were stained with saturated uranyl acetate in 50% ethyl alcohol (20 minutes) and lead citrate (10 minutes).

*The morphological and morphometric study of the neuromuscular junctions*

For the analysis of the NMJs, the samples of diaphragm muscle were immersed in Karnovsky's fixative [22] at ambient temperature. They were then sectioned lengthwise into three or four slices using stainless steel blades. The obtained sections were washed in 0.1 M phosphate buffer, pH 7.4, for one minute and then subjected to nonspecific esterase reaction [25] to reveal the cholinesterase enzyme present in the synaptic cleft. The sections were then dehydrated, diaphanized, mounted on slides and covered with cover slips with the aid of Permount (Fisher Scientific®, New Jersey, USA).

*Analysis of the images*

The muscle fibers were examined under an Olympus microscope coupled to a Bx60® Olympus DP71 camera (Tokyo, Japan), with the aid of the DP Controller 3.2.1 276 program. Measurement of the muscle fiber area and the quantification of the number of

fibers and nuclei were carried out in five, randomly chosen, images (magnification 400X) per animal. Images of the NMJs were captured in the same microscope described above, with magnification of 200X. The area and larger and smaller diameter sof 50 NMJs were evaluated for each animal. To quantify the collagen, five random images of the samples per animal were captured (magnification 400X) using a Leica DMLB® coupled DFC 300 FX camera (Wetzlar, Germany) using QWinV3 software (Leica Microsystems, Wetzlar, Germany). All morphometric analyzes were performed using Image-Pro Plus 6.0® software (Media Cybernetics, Maryland, USA). In the ultrastructural analysis, the material was examined and photographed in a transmission electron microscope (CM100, Philips®, Netherlands), with 30 regions of muscle fibers being observed per group to quantify the structures in Table 3.

*Statistical analysis*

Data were initially analyzed using Shapiro-Wilk's normality test. For the analysis of body weight, one-way ANOVA was used together with Tukey's post-test. The Lee index, retroperitoneal and peri-epididymal fat weight, muscle fiber area and number, the number of peripheral and central nuclei, the percentage of collagen, the intrafascicular lipid quantification and the area and largest and smallest diameters of the NMJs were compared using the one-way ANOVA test followed by Bonferroni's post-test and Dunn's Newman-Keuls tests. The ultrastructural analysis, assuming a 1:1 ratio for each group, was evaluated using the $Chi^2$ test for K proportions, followed by the Marascuilo procedure. The resulting data were expressed as the mean ± standard deviation or percentage, according to the nature of the variable. In all tests, $P < 0.05$ was considered significant. Analyses were conducted with the aid of the Graph Pad Prism 5.0® (La Jolla, USA) statistical software.

## Results

*Body parameters*

Before surgery, the body weights of both groups submitted to the cafeteria diet were significantly higher compared to the CTL group ($P = 0.0001$). After the surgery date (10th week of the experimental protocol), the animals in the WD SHAM and WD DJB groups showed little weight loss during the first post-operative week, followed by rapid weight gain compared to the CTL group ($P = 0.0001$). At the end of the experiment, the body weight of the animals in the WD SHAM group was significantly higher compared to the CTL group ($P = 0.0001$), while there was no difference in body weight between the WD SHAM and WD DJB groups (Fig. 1A).

The WD SHAM group also presented increases of 4% in the Lee index ($P < 0.05$), 140% in the retroperitoneal fat ($P < 0.001$) and 85% in peri-epididymal fat ($P < 0.01$) compared to the CTL group. Moreover, regarding these parameters, the WD DJB group presented no significant difference when compared to the WD SHAM group (Figs. 1B, 1C and 1D).

*Morphological and morphometric analysis of muscle fibers and neuromuscular junctions*

The morphology of the diaphragm muscle fibers was found to be similar in appearancein the CTL, WD SHAM and WD DJB groups. The muscle fibers were arranged in fascicles surrounded by perimysium, with the presence of intrafascicular lipids. These fibers were polygonal or rounded in shape with different diameters, peripheral cores, with each fiber surrounded by endomysium.

Analysis of the muscle fiber morphometry showed no significant difference in the area or the number of muscle fibers between the WD SHAM and CTL groups. The animals in the WD DJB group also showed no difference in these variables compared to WD SHAM group (Fig. 2A and 2B). The amount of intrafascicular lipids was similar in the three

groups (Fig. 2C). In addition, there was no difference between the three groups in terms of the number of peripheral and central nuclei (Fig. 2D and 2E). Regarding the percentage of collagen, there was a decrease of 37% in the WD SHAM group compared to the CTL group ($P < 0.05$). However, there was no significant difference between the WD DJB and WD SHAM groups (Figs. 3A, 3B, 3C and 3D).

Regarding the ultrastructure of the muscle fibers, the diaphragm of animals form the CTL group had well-defined myofibrils and sarcomeres with organized band A, I and Z line. Peripheral nuclei and lipid droplets were also evident (Fig. 4A and 4G). In the WD SHAM group (Fig. 4B, 4J, $P < 0.001$) more regions were found with loosely arranged myofibrils and Z line disorganization, compared to the CTL group. Moreover, considerably more lipid droplets were found throughout the intermyofibrillar mitochondria in the WD SHAM group (Fig. 4H) compared to the CTL group. The WD DJB group (Fig. 4C and 4I) presented ultrastructural changes similar to those seen in the WD SHAM group, as there were no significant difference in relation to these changes between the WD DJB and WD SHAM groups (Fig. 4J). In the three studied groups, the presence of fragmented nuclei was observed in similar proportions (Fig. 4D, 4E, 4F and 4J).

The NMJs observed in the CTL, WD SHAM and WD DJB groups presented varied phenotypes, that is, with round, oval and elliptic shapes (Figs. 5A, 5B and 5C). In the morphometric analysis, there was no significant difference in the area and the largest and smallest endplate diameters between the WD SHAM and CTL groups. The WD DJB group also showed no significant difference in the size of the NMJs compared to WD SHAM group (Fig. 5D, 5E and 5F).

**Discussion**

In this study, we observed that, in the short-term, DJB failed to reverse the body parameters, the reduction of collagen and ultrastructural changes in the muscle fibers caused by obesity in cafeteria diet-induced obese rats. However, the DJB caused no changes in the morphology or morphometry in the muscle fibers of the diaphragm.

In this study, the cafeteria diet given to the animals induced obesity, since it led to body weight gain and increased stocks of retroperitoneal and peri-epididymal fat. The increase in the Lee index, analogous to the human body mass index, also confirmed the obesity of the animals. These data are consistent with those of other studies that reported increases in these body parameters in rats fed with the cafeteria diet [26, 27]. In order to investigate anypossible beneficial effects of DJB surgery, the animals submitted to the cafeteria diet continued to receive the same diet after the surgery. DJB surgery is a bariatric procedure capable of improving glucose homeostasis in animal models of acquired diabetes and diet-induced obesity [12, 14, 27, 28], regardless of body weight loss. In this study, there were no changes in body weight, the weight of retroperitoneal and peri-epididymal fator the Lee index eight weeks after the DJB. These results are in agreement with a previous study that also showed no reduction in these parameters in obese animals that continued to receive a cafeteria diet for eight weeks post-DJB [27]. In the study by Hu *et al.* [11], DJB was also unable to reduce the body weight of animals previously fed with a high-calorie diet and a standard diet for 12 weeks after surgery. According to Patel *et al.* [29], rats submitted to DJB showed no changes in the concentration of fasting ghrelin, a hormone involved in regulating food intake. Thus, it is suggested that animals did not lose weight after DJB because this surgical procedure does not provoke a change in food intake. Furthermore, one study reports [30] intestinal adaptation in obese rats after DJB, which suggests an increase in intestinal absorption.

Although the cafeteria diet led to increased stocks of retroperitoneal and peri-epididymal fat, the diaphragm muscle of animals in the WD SHAM group showed no increase in intrafascicular lipids compared to the CTL group. DJB surgery did not alter the amount of lipids in that muscle. Goodpaster *et al.* [31] reported an increase in the amount of intramuscular lipids in the *vastus lateralis* muscle of obese individuals and Gray *et al.* [32] noted a reduction in the amount of intramuscular lipids of obese people after weight loss induced by Roux-Y gastric bypass. The diaphragm may be less susceptible to such changes compared to the other skeletal muscles, possibly due to its constant activation during respiratory functions.

It is known that cafeteria diet-induced obesity tends to reduce the muscle fiber area of the hind limb muscles [33, 34]. However, the cafeteria diet and DJB surgery did not alter the structure of the diaphragm, as no changes were observed in the area, number of muscle fibers or the number of peripheral or central nuclei. Gosselin *et al.* [35] also noted that the size of the muscle fibers in the diaphragm was unaltered in young and senescent rats, even after physical training. Again, this suggests that the constant activity of the diaphragm muscles in maintaining the respiratory function becomes the resilient to the process of muscle atrophy.

Regarding the ultrastructure of the diaphragm, more regions were found to have foci of loosely arranged myofibrils and disorganized Z lines in the animals from the WD SHAM group compared to the CTL group and DJB did not reverse these changes. It is suggested that these changes in the myofibrils may jeopardize the functioning of the muscle [36], causing losses in the diaphragmatic dynamics. In addition, the presence of fragmented nuclei was observed in the three groups. Fragmentation of the nucleus is seen in the process of apoptosis, which occurs in cases of cellular renewal and defense against diseases [37].

To the best of our knowledge, this is the first study to investigate the association between cafeteria diet-induced obesity and the percentage of collagen in the diaphragm. A decrease in the percentage of collagen was observed in the WD SHAM group compared to the CTL group. This reduction in collagen may be related to its decreased synthesis and/or increased degradation. According to Hu *et al.* [11], moderately obese rats submitted to a high calorie diet have a high concentration of leptin. This hormone enhances the activity of the metalloproteinases MMP-2 [38] and the mRNA expression of MMP-9 [39], enzymes responsible for collagen degradation. As the percentage of collagen in the diaphragm muscle was similar in the WD DJB and WD SHAM groups, it is suggested that the continued use of the cafeteria diet and the maintenance of post-DJB body weight maintained the leptin concentration and activity of matrix metalloproteinases high, leading to increased degradation of collagen in relation to its synthesis.

Again, to the best of our knowledge, this is the first time the effect of the cafeteria diet and DJB on NMJ morphometry has been investigated. When compared to CTL group, the cafeteria diet did not alter the morphology of the NMJs in the WD SHAM group. Moreover, DJB surgery did not modify the size of these structures compared to the WD SHAM group. However, we found that the WD DJB group showed increases of 29% in the area, 28% in largest and 16% in the smallest diameter of the NMJs compared to the CTL group. This increase in the size of the NMJs may be related to a possible remodeling of the muscle fibers. Due to the lack of studies involving the characterization of these structures in obesity and after bariatric surgery, more studies are needed to clarify these findings.

In conclusion, obesity induced by the cafeteria diet caused ultrastructural changes in the muscle fibers of the diaphragm and reduced the percentage of collagen in that muscle. DJB surgery did not reduce body weight or the weight of the fat in obese animals and did not reverse the deleterious effects on the diaphragm.

**Conflict of interest**

The authors have no conflicts of interestto declare.

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**FIGURES LEGENDS**

**Figure 1: (A)** Changes in body weight after surgery in the CTL, WD SHAM and WD DJB animals. Values expressed as mean ± SD from 6-9 rats per group. *WD SHAM and WD DJB vs. CTL, P = 0.0001; #WD SHAM vs. CTL, P < 0.05 (one-way ANOVA test followed by Tukey's post-test). (**B**) Lee index and weights of the retroperitoneal (**C**) and peri-epididymal (**D**) fats. Values expressed as mean ± standard deviation obtained from 8-10 rats per group. The different letters refer to the significant differences between groups, p < 0.05 (one-way ANOVA followed by Bonferroni's post-test).

**Figure 2:** Morphometric analysis of the diaphragm muscle of the animals in the CTL, WD SHAM and WD DJB groups. (**A**) Muscle fiber area. (**B**) Number of muscle fibers. (**C**) Number of intrafascicular lipids. (**D**) Number of peripheral nuclei. (**E**) Number of central nuclei. Values expressed as mean ± standard deviation obtained from 5 rats per group. (Number of peripheral nuclei: Kruscal-Wallis Test followed by Dunn's post-test; Further analysis: one-way ANOVA test followed by the Newman-Keuls post-test).

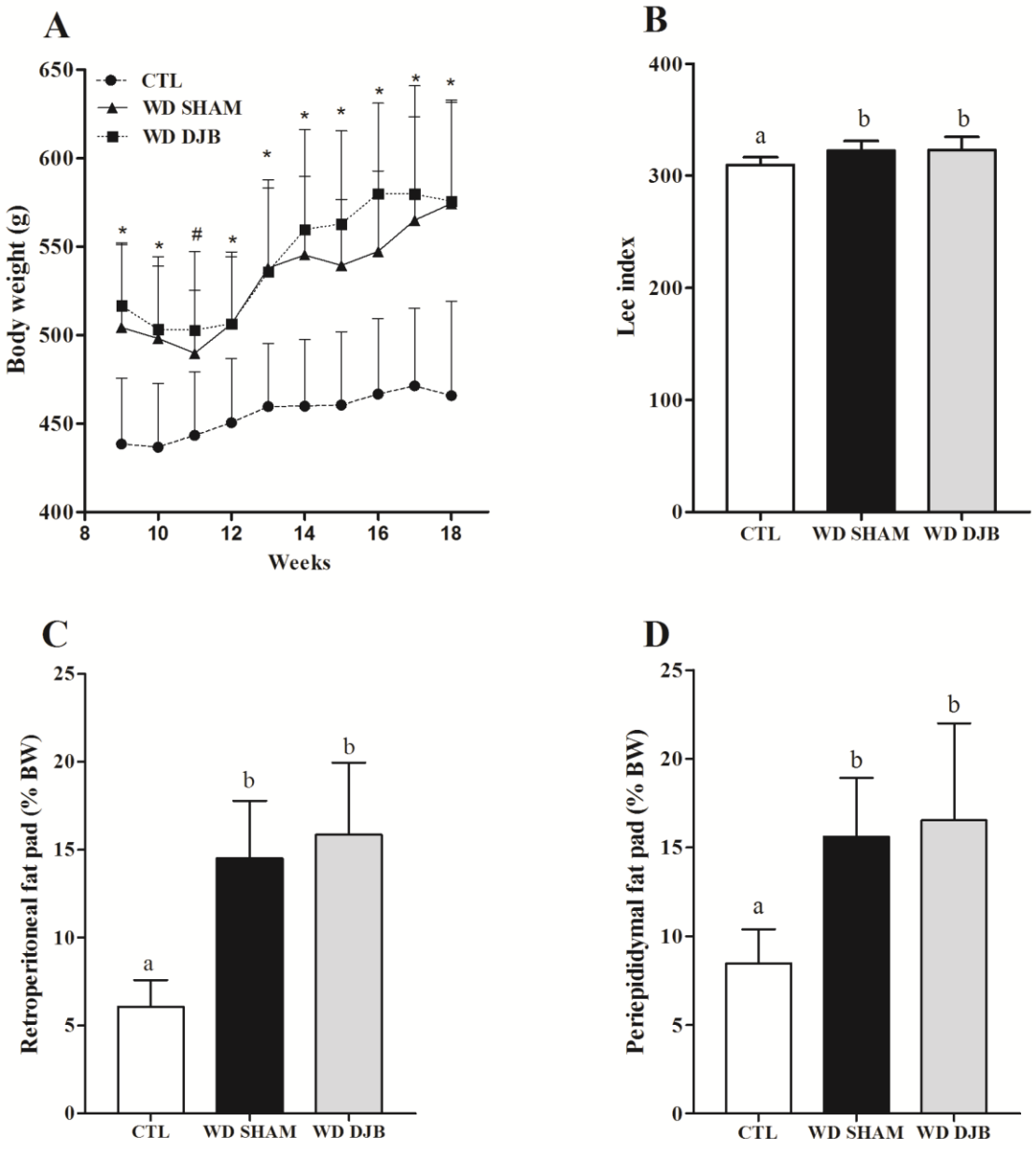
**Figure 3:** Photomicrographs of the collagen present in the muscle fibers of the diaphragm in animals from the CTL (**A**), WD SHAM (**B**) and WD DJB (**C**) groups. Cross section. Picrosirius red without polarized light. Bar = 20 µm. (**D**) Percentage of collagen in the diaphragm. Values expressed as mean ± standard deviation obtained from 5 rats per group. [a] represents a significant difference P < 0.05. (One-way ANOVA followed by Newman-Keuls post-test).

**Figure 4:** Transmission electron micrographs of the diaphragm muscle from rats. Longitudinal section. The right column corresponds to the CTL group; the central column

corresponds to the WD SHAM group; the left column corresponds to the WD DJB group. (**A**) Note the peripheral nucleus (short arrow), organized Z line (arrowhead), Band A (A) and Band I (I). Bar = 500 nm. (**B**, **C**) Note the disorganization of the Z line (arrowhead), foci of loosely arranged myofibrils (short arrow). Bar = 1 µm. (**D**, **E** and **F**) Note the presence of fragmented nuclei (short arrow) and lipid droplets (star). Bar = 2 µm. (**G**, **H** and **I**) lipid droplets are visible (star), and Z line disorganization (arrowhead). Bar = 1 µm. (**J**) Ultrastructural changes observed in the animals from the CTL, WD SHAM and WD DJB groups. Values expressed as percentages obtained from 2 to 3 rats per group. Different letters in the same line represent statistically significant differences, $P < 0.001$. ($\chi^2$ test followed by the Marascuilo monitoring test).

**Figure 5:** Photomicrographs of the NMJs from the diaphragm of rats in the CTL (**A**), WD SHAM (**B**) and WD DJB (**C**) groups. Longitudinal section. Nonspecific esterase. (**D**) NMJ area (**E**) largest NMJ diameter. (**F**) Smallest NMJ diameter. Bar = 100 µm. Values expressed as mean ± standard deviation obtained from 5 rats per group. (One-way ANOVA followed by the Newman-Keuls post-test).

**Figure 1**


A
CTL
WD SHAM
WD DJB
Body weight (g)
Weeks
B
Lee index
a
b
b
CTL
WD SHAM
WD DJB
C
Retroperitoneal fat pad (% BW)
a
b
b
CTL
WD SHAM
WD DJB
D
Periepididymal fat pad (% BW)
a
b
b
CTL
WD SHAM
WD DJB

# Figure 2

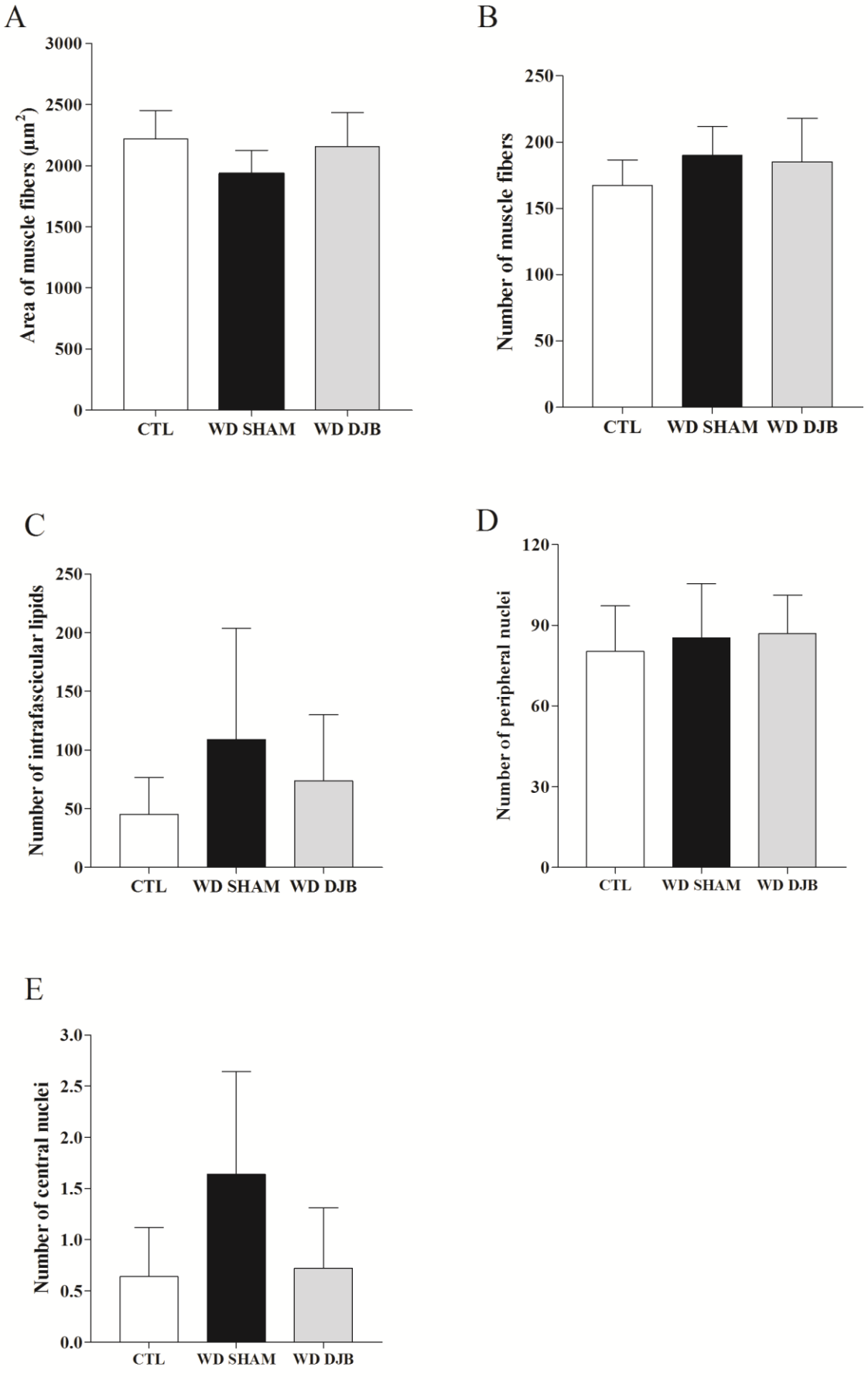
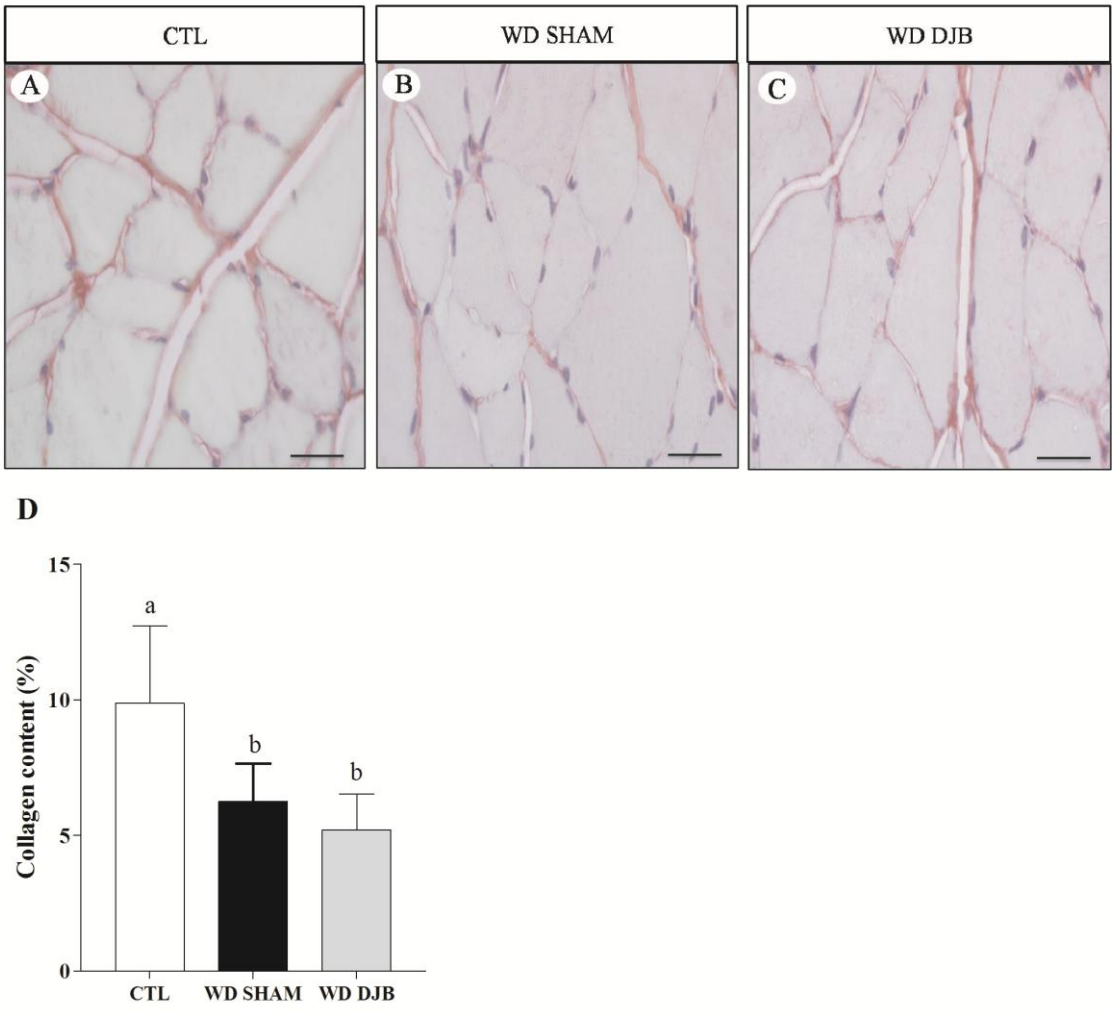
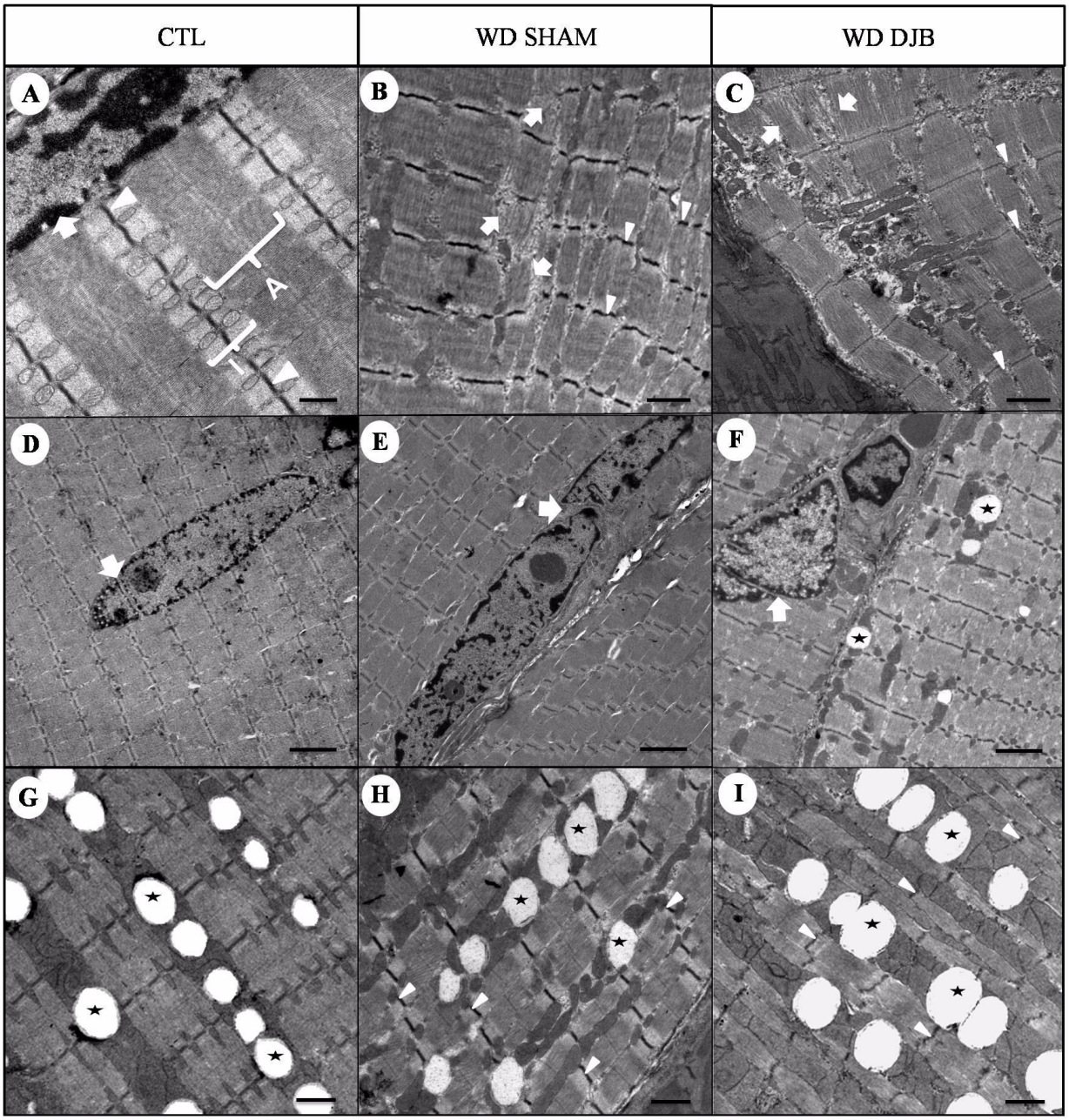

A
Area of muscle fibers (µm²)
3000
2500
2000
1500
1000
500
0
CTL
WD SHAM
WD DJB
B
Number of muscle fibers
250
200
150
100
50
0
CTL
WD SHAM
WD DJB
C
Number of intrafascicular lipids
250
200
150
100
50
0
CTL
WD SHAM
WD DJB
D
Number of peripheral nuclei
120
90
60
30
0
CTL
WD SHAM
WD DJB
E
Number of central nuclei
3.0
2.5
2.0
1.5
1.0
0.5
0.0
CTL
WD SHAM
WD DJB

**Figure 3**


CTL
WD SHAM
WD DJB
A
B
C
D
Collagen content (%)
15
10
5
0
a
b
b
CTL
WD SHAM
WD DJB

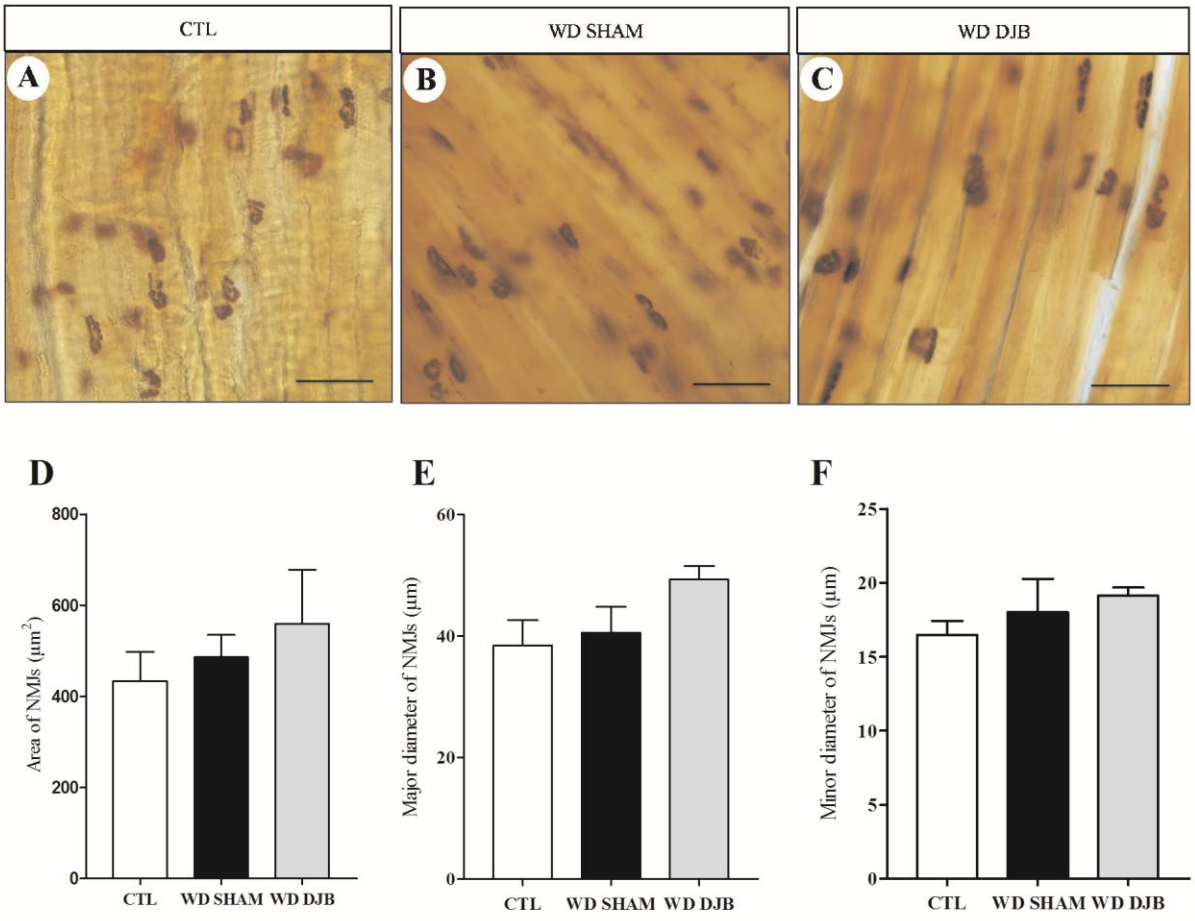
## Figure 4




J

| Observation | CTL | WD SHAM | WD DJB |
|---|---|---|---|
| Z-line disorganization (%) | 30[a] | 83[b] | 63[b] |
| Loosely arranged myofibrils (%) | 7[a] | 56[b] | 33[b] |
| Fragmented nuclei (%) | 10[a] | 16[a] | 16[a] |

Figure 5


CTL
WD SHAM
WD DJB
A
B
C
D
E
F
Area of NMJs (µm²)
800
600
400
200
0
CTL
WD SHAM
WD DJB
Major diameter of NMJs (µm)
60
40
20
0
CTL
WD SHAM
WD DJB
Minor diameter of NMJs (µm)
25
20
15
10
5
0
CTL
WD SHAM
WD DJB

**Anexo A**

unioeste
Universidade Estadual do Oeste do Paraná
*Pró-Reitoria de Pesquisa e Pós-Graduação*
*Comitê de Ética no Uso de Animais - CEUA*

## PARECER DE PROTOCOLO

A solicitação de prorrogação de prazo do protocolo intitulado "Regulação da secreção de insulina em ratos obesos submetidos à derivação duodeno-jejunal", sob vossa coordenação, foi avaliado pelo CEUA como **APROVADO.**

**ATENÇÃO!**

O Certificado Experimental deste Protocolo, somente será emitido após o encerramento das atividades previstas e após o encaminhamento do Relatório Final ao CEUA. Este Parecer **NÃO** tem valor como Certificado Experimental.

Cascavel, 09/04/2013

Profa. Dra. Luciana Oliveira do Fariña
Coordenadora do CEUA
Portaria nº 2861/2012-GRE

**Anexo B**

**Normas da Revista Científica**

**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:

- The manuscript has not been submitted to more than one journal for simultaneous consideration.
- The manuscript has not been published previously (partly or in full), unless the new work concerns an expansion of previous work (provide transparency on the re-use of material to avoid the hint of text-recycling (“self-plagiarism”)).
- 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
- No data, text, or theories by others are presented as if they were the authors own (“plagiarism”).
  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.
  Important note: the journal may use software to screen for plagiarism.

- Consent to submit has been received from all co-authors and responsible authorities at the institute/organization where the work has been carried out ***before*** the work is submitted.
- Authors whose names appear on the submission have contributed sufficiently to the scientific work and therefore share collective responsibility and accountability for the results.

In addition:

- Changes of authorship or in the order of authors are not accepted ***after*** acceptance of a manuscript.
- Requests to add or delete authors at revision stage or after publication is a serious matter, and may be considered only after receipt of written approval from all authors and detailed explanation about the role/deletion of the new/deleted author. The decision on accepting the change rests with the Editor-in-Chief of the journal.
- Upon request authors should be prepared to send relevant documentation or data in order to verify the validity of the results. This could be in the form of raw data, samples, records, etc.

If there is a suspicion of misconduct, the journal will carry out an investigation following the COPE guidelines. If, after investigation, the allegation seems to raise valid concerns, the accused author will be contacted and given an opportunity to address the issue. If misconduct has been proven, this may result in the Editor-in-Chief's implementation of the following measures, including, but not limited to:

- If the article is still under consideration, it may be rejected and returned to the author.
- 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)
- Honoraria for speaking at symposia
- 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
- Intellectual property rights (e.g. patents, copyrights and royalties from such rights)
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See below examples of disclosures:
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Conflict of Interest: The authors declare that they have no conflict of interest.

## 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.

If doubt exists whether the research was conducted in accordance with the 1964 Helsinki Declaration or comparable standards, the authors must explain the reasons for their approach, and demonstrate that the independent ethics committee or institutional review board explicitly approved the doubtful aspects of the study.

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

### i. Ethical Approval

"All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards."

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.

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"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."

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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.
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*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
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visiting http://www.icmje.org/coi_disclosure.pdf. The form(s) will not appear as part of the manuscript for review.

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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: ARTWORK INSTRUCTIONS

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Multimedia Articles may consist of:

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- 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.
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- All narration should be in English.

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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.
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- ***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 particular current topic. May be solicited or
- unsolicited.
- ***Brief Communication****:* A short report that can present research, an innovate concept or procedure, or a small case series with important, but very straightforward results.
- ***Letter****:* A very brief report of an opinion or an unstructured comment on a published
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### 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.

**Table: Manuscript Submission Formats and Required Items**

| FORMAT I * | #pp / #words | Main Text | Figures | COI Forms |
|---|---|---|---|---|
| **Original Contribution**<br><br>**New Concept**<br><br>**How I Do It** | 8pp / 2400 | • Title Page<br>• Structured Abstract, includes subheadings (250 words)<br>• Key Words<br>• Introduction/Purpose<br>• Materials/ Methods/ Results/ Conclusion<br>• COI Disclosure Statement<br>• References<br>• Figure Legends (if any)<br>• 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 | | COI Forms |
|---|---|---|---|---|
| **Review Article** | 10pp / 3000 | • Title Page<br>• 1-Paragraph Abstract (125 words)<br>• Typically these are invited submissions; format varies based on topic.<br>• COI Disclosure Statement<br>• References<br>• Figure Legends (if any)<br>• Tables (if any) | Up to 6 | Must be completed by each contributing author (these are not viewable to reviewers) http://www.icmje.org/coi_disclosure.pdf |
| **Brief Communication** | 5pp / 1500 | • Title Page<br>• 1-Paragraph Abstract (Optional; 125 words)<br>• Keywords<br>• Introduction /Methods /Results / Conclusion<br>• COI Disclosure Statement<br>• Limit references to ten (10)<br>• Figure Legends (if any)<br>• Tables (if any) | Up to 2 | |
| **Letter to Editor** | 4pp / 1200 | • Title Page<br>• No Abstract required<br>• Unstructured<br>• COI Disclosure Statement<br>• Limited number of references | Up to 3 | |

| Format III* | #pp / #words | Main Text | | COI Forms |
|---|---|---|---|---|
| **Dedicated Video** | 2pp / 500 | • Textual Abstract includes Title, COI statement, Introduction, Materials/ Methods/ Results/ Conclusion/ COI Statement, References (if any)<br>• 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 |

* References, COI Statement, Figures and Tables are not considered in Page Count. All text, including references, must be double-spaced with one-inch wide margins, and pages numbered consecutively.

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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.

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## 5. MANUSCRIPT SUBMISSION

### 5a. SUBMISSION STEPS

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Please view a copy of the SUBMISSION CHECKLIST here. We recommend that you have all items listed in the checklist complete and ready for upload before starting your online submission.

**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.

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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).”

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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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Managing Editor, OBSU Editorial Office
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E-mail: obsu.rodriguez@gmail.com

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## SUBMISSION CHECKLIST

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

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- ☐ 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 Conflict of Interest Statement (all authors must be included in this statement)
- ☐ 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:

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- ☐ One copy of annotated, revised text, tables and figures

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