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ANDRESA JESICA ZAMONER

EFEITO DA VAGOTOMIA SUBDIAFRAGMÁTICA E SELETIVA SOBRE A MORFOFISIOLOGIA DO TECIDO ADIPOSO DE CAMUNDONGOS OBESOS

CASCAVEL - PR
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ORIENTADORA: Prof.ª Dr.ª Maria Lúcia Bonfleur

COORIENTADORA: Prof.ª Dr.ª Sandra Lucinei Balbo

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EFEITO DA VAGOTOMIA SUBDIAFRAGMÁTICA E SELETIVA SOBRE A MORFOFISIOLOGIA DO TECIDO ADIPOSO DE CAMUNDONGOS OBESOS

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.

Orientador: Prof. Dr. (a)_____
Prof. Dra. Maria Lúcia - UNIOESTE

Prof. Dr. (a)_____
Prof. Dra. Dionízia Xavier Scomparim - UEPG

Prof. Dr. (a)_____
Prof. Dra. Elaine Manoela Porto Amorim - UNIOESTE

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RESUMO

ZAMONER, A. J. **Efeito da vagotomia subdiafragmática e seletiva sobre a morfofisiologia do tecido adiposo de camundongos obesos.** Dissertação (Mestrado). Programa de Pós-Graduação em Biociências e Saúde, Centro de Ciências Biológicas e da Saúde, *campus* Cascavel, Unioeste, 2023.

A obesidade tem assumido proporções alarmantes em todo o mundo, sendo considerada um importante problema econômico e de saúde global. Dentre os agravos à saúde ocasionados pela obesidade estão o diabetes mellitus tipo 2, a doença hepática gordurosa, os problemas cardiovasculares e vários tipos de câncer. Ainda que diversos fatores possam estar envolvidos no desenvolvimento da obesidade, evidências indicam que uma disfunção no sistema nervoso autônomo (SNA) contribui para o acúmulo de tecido adiposo e para a manutenção dessa síndrome. O sistema nervoso parassimpático, por meio do nervo vago, inerva órgãos viscerais abdominais, estando envolvido em muitas funções necessárias para a homeostase metabólica. A hiperinsulinemia é uma anormalidade frequente no estabelecimento e desenvolvimento da obesidade. Evidências experimentais sugerem que uma disfunção do SNA, com aumento da atividade parassimpática e redução da atividade simpática, pode levar ao aumento da secreção de insulina e da massa das células β, o que contribui para o acúmulo de gordura corporal e a manutenção da obesidade. Estudos demonstraram que a vagotomia subdiafragmática bilateral normaliza a concentração plasmática de insulina e a tolerância à glicose, além de diminuir o acúmulo de gordura em pacientes obesos mórbidos. Com base nessas evidências, investigamos a participação do SNP, por meio da vagotomia subdiafragmática e seletiva no metabolismo energético, secreção de insulina e consumo alimentar de camundongos obesos. Para tanto, aproximadamente 52 camundongos machos da linhagem C57BL6 com 40 dias de vida foram divididos em dois grupos: o grupo controle (CTL) recebeu uma dieta padrão para animais de laboratório e o grupo obeso (OB) recebeu dieta hiperlipídica (HFD). Após oito semanas de dieta HFD e indução da obesidade, os procedimentos cirúrgicos foram realizados formando os grupos: 1) Grupo OB-Falso operado (OB-Sham); 2) Grupo OB-Vagotomia Seletiva (OB-VagS) e 3) Grupo OB-Vagotomia Subdiafragmática Total (OB- Vag). Após oito semanas dos procedimentos cirúrgicos, os animais OB-VagT apresentaram redução do peso corporal e massa magra, juntamente com a diminuição dos estoques do tecido adiposo marrom (TAM). A vagotomia total não modificou os níveis de glicose e de insulina plasmáticos, mas normalizou os níveis de colesteróis totais. Já a vagotomia seletiva não foi eficaz na perda de peso dos animais, no entanto, alterou consumo alimentar diurno e aumentou o número de adipócitos e de inclusões lipídicas por campo no TAM, apresentando semelhanças ao encontrado no grupo OB-VagT.

Palavras-Chaves: Obesidade. Nervo vago. Vagotomia subdiafragmática e seletiva. Tecido adiposo

ABSTRACT

ZAMONER, A. J. **Effect of subdiaphragmatic and selective vagotomy on the morphophysiology of adipose tissue in obese mice.** Thesis (Master's degree). Graduate Program in Biosciences and Health, *campus* Cascavel, Unioeste, 2023.

Obesity has taken on alarming proportions all over the world, being considered an important economic and global health problem. Among the health problems caused by obesity are type 2 diabetes mellitus, fatty liver disease, cardiovascular problems and various types of cancer. Although several factors may be involved in the development of obesity, evidence indicates that a dysfunction in the autonomic nervous system (ANS) contributes to the accumulation of adipose tissue and to the maintenance of this syndrome. The parasympathetic nervous system, via the vagus nerve, innervates abdominal visceral organs and is involved in many necessary functions for metabolic homeostasis. Hyperinsulinemia is a frequent abnormality in the establishment and development of obesity. Experimental evidence suggests that an ANS dysfunction, with increased parasympathetic activity and reduced sympathetic activity, may lead to increased insulin secretion and β-cell mass, which contributes to body fat accumulation and maintenance of obesity. Studies have shown that bilateral subdiaphragmatic vagotomy normalizes plasma insulin concentration and glucose tolerance, in addition to decreasing fat accumulation in morbidly obese patients. Based on this evidence, we investigated the participation of the PSNS, through subdiaphragmatic and selective vagotomy, in energy metabolism, insulin secretion and food intake in obese mice. For this purpose, approximately 52 male mice of the C57BL6 lineage, 40 days old, were divided into two groups: the control group (CTL) received a standard diet for laboratory animals and the obese group (OB) received a high-fat diet (HFD). After eight weeks of HFD diet and obesity induction, surgical procedures were performed, forming the following groups: 1) OB-Sham-operated Group (OB-Sham); 2) OB-Selective Vagotomy Group (OB-VagS) and 3) OB-Total Subdiaphragmatic Vagotomy Group (OB-VagT). After eight weeks of the surgical procedures, the OB-VagT animals showed a reduction in body weight and lean mass, along with a decrease in brown adipose tissue (BAT) stores. Total vagotomy did not change glucose and plasma insulin levels, but normalized total cholesterol levels. Selective vagotomy was not effective in the weight loss of the animals, however, it altered daytime food intake and increased the number of adipocytes and lipid inclusions per field in the BAT, showing similarities to that found in the OB-VagT group.

Key words: Obesity. Vagus nerve. Subdiaphragmatic and selective vagotomy. Adipose tissue.

LISTA DE ILUSTRAÇÕES

LISTA DE ABREVIATURAS

CCK	Colestocinina
IMC	Índice de Massa Corporal
NV	Nervo Vago
NTS	Núcleo do Trato Solitário
NPY	Neuropeptídeo Y
OMS	Organização Mundial da Saúde
SAT	Tecido Adiposo Subcutâneo
SNA	Sistema Nervoso Autônomo
SNC	Sistema Nervoso Central
SNS	Sistema Nervoso Simpático
SNP	Sistema Nervoso Parassimpático
SNP	Sistema Nervoso Periférico
TAB	Tecido Adiposo Branco
TAM	Tecido Adiposo Marrom
UCP1	Proteína Desaclopadora 1
VAT	Tecido Adiposo Visceral

SUMÁRIO

1 INTRODUÇÃO

A incidência da obesidade vem crescendo em países desenvolvidos e em desenvolvimento, assumindo proporções alarmantes (BLÜHER, 2019). A elevação de sua prevalência torna a obesidade um importante problema econômico e de saúde global (FERRETTI; MARIANI, 2017). A falta de opções efetivas para a redução de peso sustentadas a longo prazo amplifica o tamanho desse problema. Indivíduos submetidos com sucesso a programas comportamentais e dietéticos para perda de peso, eventualmente, readquirem grande parte do peso perdido (SCHWARTZ *et al*., 2017).

O aumento global da obesidade tem sérios efeitos sobre a saúde (SWINBURN *et al*., 2011), haja vsita que o acúmulo excessivo de peso eleva o risco de se desenvolverem doenças como diabetes mellitus, problemas cardiovasculares, alguns tipos de cânceres entre outras enfermidades (CHOOI; DING; MAGKOS, 2019). Em todo o mundo, aproximadamente 44% dos casos de diabetes no mundo e 23% de doenças cardíacas isquêmicas são atribuídos ao sobrepeso e à obesidade. Em partes, isso ocorre devido à resistência à insulina induzida pela obesidade e ao fato de que o tecido adiposo em excesso não atua apenas como reservatório de energia, mas também como órgão endócrino, capaz de secretar citocinas, hormônios e proteínas que comprometem a funcionalidade das células em todo o corpo (GÓMEZ-HERNÁNDEZ *et al*., 2016). A obesidade também está associada a uma inflamação sistêmica crônica de baixo grau, por causa de concentrações mais elevadas de citocinas pró-infamatórias circulantes e ácidos graxos, o que contribui para o desenvolvimento de disfunções metabólicas (HENRIQUES; H. BEDARD; LUIZ BATISTA JÚNIOR, 2019).

A obesidade pode ser causada por fatores genéticos, metabólicos, endócrinos, ambientais, neurais e outros (WALLEY; BLAKEMORE; FROGUEL, 2006). O sistema nervoso central (SNC) desempenha papel de extrema importância nos mecanismos que controlam o peso corporal por meio de regiões hipotalâmicas específicas (VELLOSO; SCHWARTZ, 2011). O hipotálamo está envolvido na manutenção e no monitoramento da homeostase energética, tanto na saúde quanto em estados de doença, como na obesidade. O sistema nervoso autonômico (SNA), pincipalmente por

meio do nervo vago (X nervo craniano), faz a interface entre os sinais periféricos e o SNC. O nervo vago é o responsável pela inervação parassimpática para os órgãos viscerais abdominais, como estômago, intestino, fígado e pâncreas e, dessa forma, está envolvido em muitas funções necessárias para homeostase metabólica, incluindo produção hepática de glicose, secreção pancreática endócrina e exócrina e monitoramento do estado metabólico. Além das fibras eferentes, foi demonstrado que o nervo vago de acordo com a espécie animal contém aproximadamente 70 - 80% de fibras sensoriais (BROWNING; VERHEIJDEN; BOECKXSTAENS, 2017), as quais, dentro do trato gastrointestinal, podem ativar o nervo vago por estimulação mecânica do lúmen, por distensão e por estímulos químicos. Essa ampla distribuição fornece um substrato anatômico para o fornecimento em tempo real de informações sobre o estado metabólico do corpo para o SNC, bem como para modulação do metabolismo (MASI; VALDÉS-FERRER; STEINBERG, 2017).

O nervo vago também está envolvido no controle da secreção de insulina. As ilhotas pancreáticas são inervadas por terminações parassimpáticas derivadas do nervo vago, cujas fibras pré-ganglionares originam no núcleo motor dorsal do vago (LUITEIN *et al*., 1986) e estão sob controle do hipotálamo. As fibras pós-ganglionares são encontradas na periferia e próximas a todos os tipos celulares da ilhota (LOVE; SZEBENI, 1999; VAN DER ZEE *et al*, 1992), liberando acetilcolina (ACh), peptídeo inibitório vasoativo, peptídeo liberador da gastrina, óxido nítrico, polipeptídeo pituitário ativador da 4 adenilato ciclase etc. (AHRÉN, 2000).

A hiperinsulinemia é uma anormalidade frequente no estabelecimento e no desenvolvimento da obesidade. Evidências experimentais sugerem que uma disfunção do SNA, com aumento da atividade parassimpática e redução da atividade simpática, pode levar ao aumento da secreção de insulina e da massa das células β, o que contribui para o acúmulo de gordura corporal e manutenção da obesidade (BRAY AND YORK, 1979; BALBO *et al.,* 2007; SCOMPARIN *et al.*, 2009; CALEGARI *at al.*, 2011; BARELLA *at al.*, 2015). Também foi proposto que a inervação parassimpática do tecido adiposo branco (TAB) pode modular a captação de glicose mediada pela insulina, bem como o metabolismo dos ácidos graxos livres de forma anabólica, provocando, assim, o acúmulo de gordura nesse tecido (KREIER *et al*., 2002).

Estudos demonstraram que a vagotomia subdiafragmática bilateral normaliza a concentração plasmática de insulina e a tolerância à glicose, além de diminuir o

acúmulo de gordura em pacientes obesos mórbidos (KRAL, 1980) e em roedores com obesidade hipotalâmica (BALBO *et al*., 2007), genética (ROHNER-JEANRENAUD *et al*., 1983) e obesidade induzida por dieta de cafeteria (BALBO *et al*., 2017), reforçando que o desbalanço autonômico contribui para a instalação e manutenção da obesidade.

Outro tipo de vagotomia (a do ramo celíaco ou seletiva) em animais magros reduz a resposta secretória da insulina à glicose, carbacol (análogo a ACh) (SILVA *et al*., 2009) e ao aminoácido arginina (SILVA *et al*., 2012). Em ratos obesos Sprague-Dawley, a vagotomia celíaca, associada à cirurgia bariátrica de derivação gástrica em Y de Roux (RYGB), não foi eficiente para a perda de peso e para a supressão da ingestão alimentar em comparação com ratos RYGB com o nervo vago preservado. Nesse mesmo estudo, a vagotomia celíaca em ratos obesos foi eficaz em reduzir o ganho de peso e o consumo alimentar em comparação com os ratos obesos falso-operados (ZHENG *et al*., 2015). Outro estudo demonstrou que a vagotomia seletiva em ratos não obesos com diabetes tipo 2 piora o controle da glicose após a cirurgia bariátrica de transposição ileal (CHEN *et al.,* 2018). Dessa forma, a participação do nervo vago na instalação da obesidade tem sido amplamente discutida na literatura, mas ainda há dados contraditórios. Além disso, até o presente momento, não encontramos nada na literatura que mostre o efeito da vagotomia seletiva sobre os diferentes estoques de gordura corporal.

Considerando os aspectos supracitados, a pergunta norteadora deste trabalho é: *Quais são os efeitos da vagotomia subdiafragmática e seletiva realizada após a instalação da obesidade nos parâmetros biométricos e estoques de gordura em camundongos machos? Qual delas será mais efetiva para redução do peso corporal e acúmulo de gordura nos camundongos obesos*? Nossa hipótese é que os dois tipos de procedimentos cirúrgicos alterarão a secreção de insulina e o consumo alimentar, promovendo a perda de peso e, consequentemente, reduzindo o acúmulo de gordura corporal.

2 OBJETIVOS

2.1 Objetivo geral

Avaliar o efeito da vagotomia subdiafragmática e da vagotomia seletiva após a instalação da obesidade, sobre parâmetros corporais e morfofisiologia do tecido adiposo de camundongos obesos pela dieta hiperlipídica.

2.3 Objetivos específicos

Avaliar e comparar os efeitos da vagotomia subdiafragmática e da vagotomia seletiva sobre:

(a) Os parâmetros corporais e metabólicos nos camundongos obesos;
(b) A morfologia dos adipócitos dos diferentes estoques do tecido adiposo branco (TAB), bem como do tecido adiposo marrom (TAM) interescapular;
(c) A expressão de proteínas envolvidas com o processo de termogênese no TAM.

3 REVISÃO DE LITERATURA

3.1 Perfil epidemiológico da obesidade

A prevalência de excesso de peso e de obesidade vem aumentando em ritmo acelerado em diversos países (fig.1) (BLÜHER, 2019; FERREIRA; SZWARCWALD; DAMACENA, 2019). De acordo com a Organização Mundial da Saúde (OMS), em 2016, cerca de 1,9 bilhão de adultos no mundo estavam acima do peso (39% da população) e mais de 650 milhões (13% da população) eram obesos (OMS, 2020). No Brasil, o percentual de pessoas obesas em idade adulta no país passou de 12,2%, entre 2002 e 2003, para 26,8%, em 2019. Nesse mesmo período, a proporção da população adulta com excesso de peso passou de 43,3% para 61,7%, representando quase dois terços dos brasileiros (IBGE, 2020).

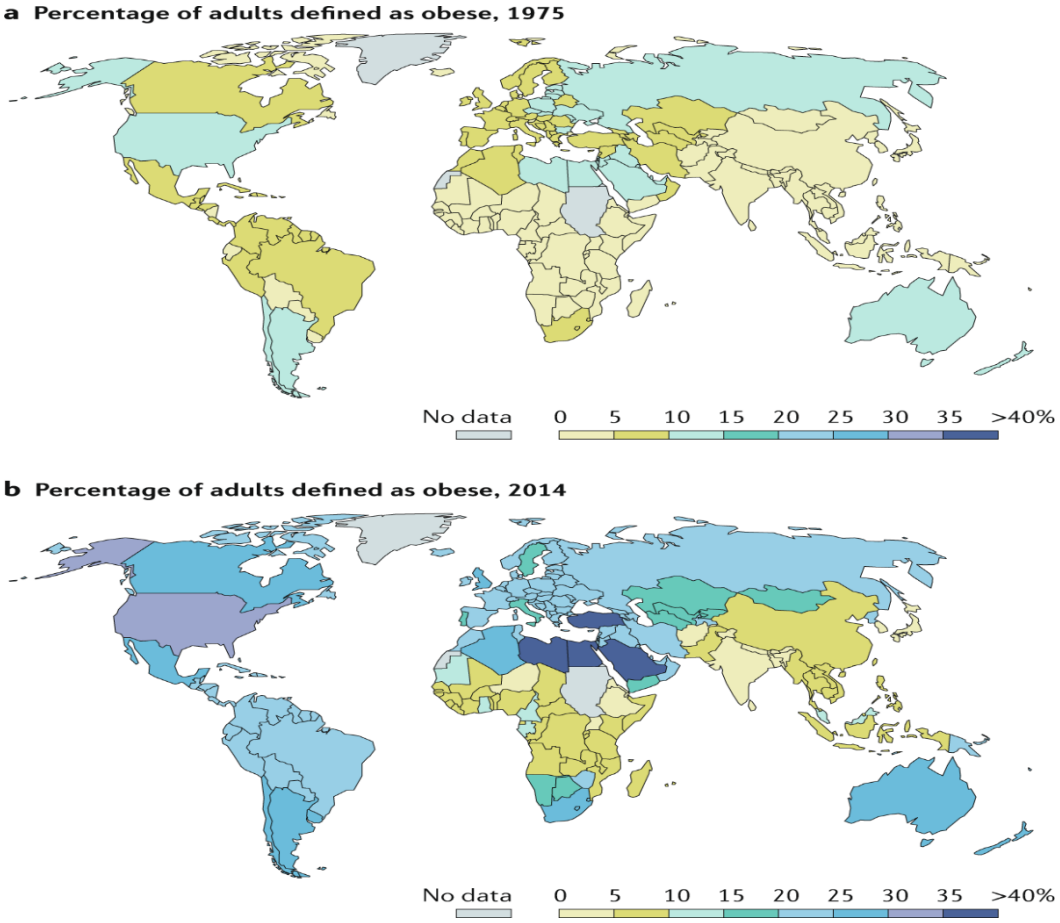

Figura 1. Porcentagem de adultos definidos como obesos por país em 1975 (parte a) e 2014 (parte b). Nota-se que o número de adultos com obesidade aumentou consideravelmente entre 1975 e 2014. Fonte: Blüher (2019).

A obesidade é definida como acúmulo anormal ou excessivo de gordura que pode prejudicar a saúde e, por isso, é caracterizada como uma doença crônica (POZZA; ISIDORI, 2018). Existem diferentes maneiras de mensuração da obesidade, no entanto, um índice de massa corporal (IMC) elevado, que representa o peso corporal (em quilogramas) em função da altura do corpo (em metros) ao quadrado, é amplamente utilizada como uma medida equivalente da gordura corporal e indicativa de obesidade. Estudos realizados em diferentes populações limitaram como um IMC normal em adultos para 25 kg/m, sendo que a obesidade é definida por um IMC > 30 kg/m. Ainda, o grau de obesidade pode ser subdividido em classe 1 (IMC de 30 a <34,9), classe 2 (IMC de 35 a <39,9) e classe 3 (IMC de> 40) (SCHWARTZ *et al.*, 2017).

Os valores de referência de IMC utilizados atualmente são calculados com base em estudos de morbidade e de mortalidade de população caucasiana. Alguns pacientes obesos não apresentem anormalidades metabólicas esperadas, embora

seja observado excesso substancial de gordura corporal, evidenciando que, enquanto a obesidade aumenta o risco de complicações, nem todo indivíduo obeso as desenvolverá. Ainda que o IMC seja o método amplamente utilizado para classificar e avaliar a obesidade e seus riscos em estudos epidemiológicos, não é capaz de determinar a composição do tecido magro em relação ao tecido adiposo e, por conseguinte, tem conduzido a interpretações errôneas (POZZA; ISIDORI, 2018).

3.2 Aspectos morfofisiológicos da obesidade

Uma alimentação não saudável e baixa atividade física estão entre os principais fatores de risco para a obesidade (FERREIRA; SZWARCWALD; DAMACENA, 2019). Esses aspectos, relacionados ou não a fatores genéticos, explicariam o excesso de gordura corporal presente em grandes proporções na população mundial. Sabe-se que dietas ricas em gordura são conhecidas por ocasionar um balanço energético positivo e acúmulo de massa adiposa (COELHO *et al*., 2011). Gordura, carboidratos e proteínas são os principais macronutrientes fontes de energia presentes na alimentação de humanos. Nesse contexto, tem sido demonstrado que a qualidade, ao invés da quantidade, é um fator de maior relevância nas desordens nutricionais. Dentre os macronutrientes, a gordura contém maior quantidade de energia por grama, fornecendo ácidos graxos essenciais, auxiliando na absorção de vitaminas solúveis em gordura e outros nutrientes vitais para o corpo. No entanto, existem mecanismos que relacionam o consumo de ácidos graxos saturados com o surgimento de inflamação sistêmica de baixo grau (crônica), resistência à insulina, síndrome metabólica e doenças cardiovasculares (RUIZ-NÚÑEZ; DIJCK-BROUWER; MUSKIET, 2016).

A progressão do estado magro para a obesidade traz consigo uma alteração fenotípica no tecido adiposo e o surgimento da inflamação crônica de baixo grau. Isso é marcado por aumento na concentração de ácidos graxos livres circulantes, fatores pró-inflamatórios solúveis e infiltração de células imunes em locais de inflamação. A obesidade também costuma apresentar um perfil lipídico específico, que inclui aumento da concentração de triglicerídeos e das partículas pequenas e densas de lipoproteínas de baixa densidade (LDL) e redução de lipoproteínas de alta densidade (HDL). O estado inflamatório crônico, aliado à dislipidemia de baixo grau, leva a disfunção vascular, a formação de aterosclerose e a fibrinólise prejudicada, os quais,

associados, aumentam o risco de acidente vascular cerebral e tromboembolismo venoso (FRUH, 2017).

A inflamação é uma resposta de defesa do organismo contra estímulos prejudiciais, por exemplo, a invasão por patógenos e danos celulares (CHOE *et al*., 2016). A inflamação crônica de baixo grau do tecido adiposo é marcada pela infiltração de macrófagos e outras populações de células imunes no tecido adiposo, junto a uma mudança para subtipos pró-inflamatórios de leucócitos (BURHANS *et al*., 2019). A expressão da citocina inflamatória TNFα está aumentada no tecido adiposo obeso. Outras citocinas pró-inflamatórias, como IL-1β, IL-6 e MCP-1, também são reguladas positivamente (CHOE *et al.,* 2016).

Em modelos de roedores e humanos, a inflamação do tecido adiposo está frequentemente ligada ao excesso de massa gorda e à resistência à insulina (BURHANS *et al.,* 2019). A resistência à insulina ocorre em diversos tecidos, incluindo o fígado, os músculos e os tecidos adiposos. O fígado ajuda manter as concentrações de glicose em jejum por meio da gliconeogênese e da glicogenólise mas quando esse órgão é resistente à insulina, a supressão da produção hepática de glicose é prejudicada e, portanto, a gliconeogênese e a glicogenólise permanecem em níveis elevados, apesar dos níveis normais ou elevados de glicose circulante. Para compensar a resistência à insulina nesses tecidos, as células β pancreáticas produzem mais insulina. Há, contudo, um limite de quanto pode ser produzido; à medida que esse patamar é alcançado, as células β falham. O diabetes melitos tipo 2 ocorre quando a secreção de insulina não se equipara a uma determinada concentração de glicose (LEE; LEE, 2014).

Além da inflamação periférica, a inflamação do sistema nervoso central (neuroinflamação), comprometendo hipotálamo e outras regiões do cérebro, também foi associada ao comprometimento cognitivo no contexto da obesidade. Diversas ligações entre alterações periféricas e cerebrais, compreendendo componentes inflamatórios, metabólicos e neurais, foram descritas em condições ligadas à obesidade. O sistema nervoso e o cérebro são responsáveis pelo ajuste do comportamento alimentar, da ingestão e do gasto energético. O nervo vago (o décimo nervo craniano) contém fibras que carregam sinais sensoriais ascendentes para o cérebro e sinais motores descendentes para órgãos viscerais, estando estritamente envolvido nesses processos regulatórios (CHANG; CHAVAN; PAVLOV, 2019).

3.3 Anatomia funcional do nervo vago

O nervo vago (NV) é o décimo nervo craniano; origina-se no tronco encefálico, passa pelo pescoço e pelo tórax até chegar ao abdome (BREIT *et al*., 2018). Considerado o nervo mais longo do corpo humano, o NV inerva a maioria dos órgãos, principalmente no tratogastrointestinal, constituindo-se um componente chave para o SNA. Trata-se de um nervo misto composto por 80% de fibras aferentes que transmitem sensações viscerais, somáticas e gustativas e 20% de fibras eferentes que levam a liberação de acetilcolina (ACh) na junção sináptica com músculos lisos, fibras nervosas intrínsecas ou células secretoras (BONAZ; SINNIGER; PELLISSIER, 2016).

3.4 Inervação vagal aferente

Fisiologicamente, o NV faz parte do sistema parassimpático e regula a frequência cardíaca, respiração e função do trato digestivo. Embora contenha fibras motoras e sensoriais, o NV é a principal via aferente da cavidade abdominal para o cérebro (LIU; FORSYTHE, 2021). As fibras aferentes superam amplamente as fibras eferentes dentro do NV. Essas fibras sensoriais e motoras ramificam-se ao nível do forame jugular, ao passo que as radículas aferentes mais espessas terminam no núcleo do trato solitário (NTS). Quando o NV deixa o crânio, os gânglios superiores (jugular) e inferior (nodoso) contêm os corpos celulares aferentes. Uma vez que todas as fibras aferentes vagais se originam desses gânglios e todas as fibras eferentes vagais se originam do cérebro posterior, o tronco cervical do NV, desse modo, é composto por fibras que inervam todos os órgãos do corpo (DE LARTIGUE, 2016).

Nesse sentido, as fibras nervosas aferentes vagais no trato gastrointestinal estão situadas em posição estratégica para captar informações como volume e composição do conteúdo luminal, sendo possível detectar diretamente toque mecânico, distensão e alongamento de diversos pontos. Além disso, são capazes de detectar de forma indireta a presença e a concentração de todos os três macronutrientes por meio da mediação de peptídeos e transmissores excretados por células endoteliais especializadas (BERTHOUD, 2008).

A distensão gástrica ativa os mecanorreceptores aferentes vagais de maneira dose-dependente, controlando o tamanho das refeições por meio da sinalização de volume ou carga existente. Em contrapartida, os aferentes vagais quimiossensíveis são ativados mediante resposta ao pH luminal, osmolalidade e estimulação química. Os terminais aferentes estão localizados próximos às células enteroendócrinas da mucosa, reagindo aos mediadores liberados. Atualmente, mais de 30 neuro-hormônios gastrointestinais foram identificados, sendo que muitos desses exercem papéis importantes na digestão, na absorção e na sinalização da saciedade (BROWNING; VERHEIJDEN; BOECKXSTAENS, 2017).

3.5 Inervação vagal eferente

As fibras eferentes parassimpáticas pré-ganglionares originam-se no núcleo motor dorsal do vago, formando sinapses com fibras eferentes parassimpáticas pós-ganglionares nos gânglios intrapancreáticos (MOULLÉ, 2021). Em humanos, essas fibras inervam o trato digestivo do esôfago à flexura esplênica, enquanto o cólon esquerdo e o reto são inervados pelo núcleo parassimpático sacral (BONAZ; SINNIGER; PELLISSIER, 2016).

O vago é o principal nervo que retransmite sinais parassimpáticos, cumprindo um papel importante na regulação da homeostase metabólica, por meio de mecanismos gastrointestinais, pancreáticos e hepáticos (STARUP-LINDE *et al*., 2016). Os corpos celulares eferentes vagais encontram-se agrupadas juntamente com as fibras aferentes, no entanto, são responsáveis por fornecer informações do cérebro para a periferia. Neurônios eferentes vagais inervam órgãos abdominais ao longo do canal alimentar, do esôfago anterior ao cólon transverso. Ainda que os neurônios vagais eferentes liberem exclusivamente acetilcolina, eles ativam duas populações diferentes de neurônios pós-ganglionares que podem atuar inibindo ou estimulando neurônios funcionais do órgão para controlar a digestão e a absorção dos alimentos (DE LARTIGUE, 2016). Diante doo envolvimento do NV na comunicação bidirecional entre a periferia metabólica e o SNC, pode-se acreditar que esse desenvolva um papel crítico no peso corporal e no equilíbrio energético.

3.6 O papel do nervo vago na obesidade

Considerando que um dos fatores envolvidos com a etiologia da obesidade é o desequilíbrio do SNA, com aumento do Sistema Nervoso Parassimpático (SNP) e redução do SNS, vários estudos têm buscado elucidar a participação do NV nas vias metabólicas envolvidas com o gasto energético e, portanto, com a obesidade.

O peso corporal é regulado por um complexo sistema homeostático, cujos principais componentes são a regulação do apetite, a saciedade e a modulação do gasto e do armazenamento de energia no tecido adiposo (GUARINO *et al*., 2017). O SNC desempenha um papel de extrema importância nos mecanismos que controlam o peso corporal por meio de regiões hipotalâmicas específicas (DOLNIKOFF *et al.,* 1988). O NV é o responsável pela inervação parassimpática para os órgãos viscerais abdominais, como estômago, intestino, fígado e pâncreas. Dessa forma, está envolvido em muitas funções necessárias para homeostase metabólica, incluindo a produção hepática de glicose, a secreção pancreática endócrina e exócrina e o monitoramento do estado metabólico (BROWNING; VERHEIJDEN; BOECKXSTAENS, 2017).

Nesse sentido, uma das importantes respostas regulatórias vagais acontece na sinalização pancreática, sendo a inervação vagal responsável pelo controle da secreção de insulina. As ilhotas pancreáticas são inervadas por terminações parassimpáticas derivadas do NV, cujas fibras pré-ganglionares originam-se no núcleo motor dorsal do vago (LUITEIN *et al*., 1986) e estão sob controle do hipotálamo. As fibras pós-ganglionares são encontradas na periferia e próximas a todos os tipos celulares da ilhota (LOVE; SZEBENI, 1999; VAN DER ZEE *et al*, 1992), liberando a acetilcolina (ACh),o peptídeo inibitório vasoativo, o peptídeo liberador da gastrina, o óxido nítrico e o polipeptídeo pituitário ativador da 4 adenilato ciclase (AHRÉN, 2000).

A obesidade é caracterizada por um desbalanço do SNA. Em humanos obesos e em modelos animais, ocorre aumento da atividade parassimpática e uma redução da atividade simpática, o que potencializa a secreção de insulina e o aumento da massa das células β por meio do NV, contribuindo para o acúmulo de gordura corporal e para a manutenção da obesidade (BRAY AND YORK, 1979; BALBO *et al*., 2007; SCOMPARIN *et al*., 2009; CALEGARI *et al*., 2011; BARELLA *et al*., 2015). Ainda que o mecanismo de ação do SNP sobre a regulação da massa pancreática endócrina não

esteja totalmente estabelecido, evidências científicas sugerem que, na obesidade, o aumento da ação vagal pode levar a um ciclo vicioso, que intensifica o armazenamento de gordura e interrompe a homeostase de energia corporal (LUBACZEUSKI *et al.,* 2015).

Ao compreender mais sobre os aspectos funcionais dos nervos vagais e a disponibilidade de métodos mais seletivos para manuseá-los, eles seguem sendo um alvo importante para a prevenção e/ou tratamento da obesidade. A seguir, detalhamos dois modelos de vagotomia e seus possíveis benefícios para a redução do acúmulo de peso corporal.

3.7 Vagotomia subdiafragmática total e seletiva

A vagotomia é um procedimento cirúrgico que envolve o corte ou a remoção de parte do NV. Ao estudar o papel desse nervo no organismo, o procedimento cirúrgico comumente utilizado é a vagotomia subdiafragmática total, na qual os troncos do vago anterior e posterior, assim como seus ramos, são dissecados (LIU; FORSYTHE, 2021). Na vagotomia denominada seletiva/celíaca, o ramo do nervo vago celíaco que segue para o pâncreas é seccionado (LAUSIER *et al*., 2010).

Sabe-se que o NV exerce inúmeras funções fisiológicas relacionadas com o consumo alimentar, com o metabolismo energético e com o controle da glicemia (PARDO *et al.*, 2008). Muitos estudos são conduzidos para avaliar o papel do NV e do SNP no desenvolvimento e instalação da obesidade. Pesquisas demonstraram que a vagotomia subdiafragmática bilateral normaliza a concentração plasmática de insulina e a tolerância à glicose, além de diminuir o acúmulo de gordura em pacientes obesos mórbidos (KRAL, 1980) e em roedores com obesidade hipotalâmica (BALBO *et al*., 2007), genética (ROHNER-JEANRENAUD *et al.*, 1983) e obesidade induzida por dieta de cafeteria (BALBO *et al.*, 2017), reforçando que o desbalanço autonômico contribui para a instalação e manutenção da obesidade.

A perda de peso após vagotomia troncular bilateral também foi relatada por Furness *et al.* (2001), em um estudo realizado em ratos submetidos a dieta a base de xarope de milho e óleos vegetais. A significante diminuição do peso corporal dos animais foi atribuída à perda de um sinal de alimentação transportado por neurônios aferentes vagais, ou a alterações humorais como aumento da produção de um

hormônio da saciedade. Sebaie *et al*. (2020), por sua vez, demonstraram que a vagotomia subdiafragmática realizada em ratos obesos diabéticos foi capaz de melhorar o perfil lipídico plasmático, de diminuir o peso da gordura e de reduzir a obesidade dos animais, além de atuar na diminuição da insulinemia de jejum e melhorar a tolerância à glicose.

Em outro estudo, a vagotomia foi associada à diminuição no desenvolvimento de diabetes tipo 2 em uma população de pacientes com doença gastrointestinal superior (STARUP-LINDE *et al.,* 2016). Isso pode ser explicado devido ao envolvimento do SNP na regulação da massa de células β em condições normais e patológicas. Neurônios hipotalâmicos especializados identificam variações locais na glicemia ou concentrações de ácidos graxos não esterificados o que, então, modificam suas taxas de disparo para o pâncreas. O SNA que inerva as ilhotas pancreáticas, por meio dos nervos parassimpáticos e simpático, liberam acetilcolina e catecolaminas, respectivamente. Enquanto a ativação dos receptores M_3 -muscarínicos de células β pela acetilcolina promove a liberação de insulina, a ativação do receptor adrenérgico pela epinefrina inibe a secreção de insulina. Dessa forma, a exposição a curto prazo ao excesso de nutrientes aumenta a liberação de insulina, intensificando as taxas de disparo parassimpático e diminuindo o simpático (MOULLÉ *et al.,* 2019).

A vagotomia do ramo celíaco/seletiva, realizada em animais magros, reduz a resposta secretória da insulina à glicose, ao carbacol (análogo a ACh) (SILVA *et al*., 2009) e ao aminoácido arginina (SILVA *et al*., 2012). Em ratos obesos Sprague-Dawley, a vagotomia celíaca associada à cirurgia bariátrica de derivação gástrica em Y de Roux (RYGB) não foi eficiente na perda de peso e na supressão da ingestão alimentar em comparação com ratos RYGB com o nervo vago preservado (ZHENG *et al*., 2015). Outro estudo demonstrou que a vagotomia seletiva em ratos não obesos com diabetes tipo 2 (Goto-Kakizaki) piorou o controle da glicose após a cirurgia bariátrica de transposição ileal (CHEN *et al*., 2018).

Os estudos supracitados demonstram que o NV participa da instalação da obesidade e que tema tem sido amplamente discutido na literatura, porém, os dados disponíveis até o presente momento ainda são contraditórios. Além disso, não encontramos pesquisas que mostrem o efeito da vagotomia seletiva sobre os diferentes estoques de gordura corporal.

3.8 Tecido adiposo

O tecido adiposo pode ser dividido em dois subtipos: (a) tecido adiposo branco (TAB), que armazena energia extra em forma de triglicerídeos; e (b) tecido adiposo marrom (TAM), que dissipa energia química em forma de calor (LUO; LIU, 2016). Os adipócitos, também chamados de células adiposas ou células de gordura, são o tipo celular predominante no tecido adiposo (LUO; LIO, 2016). Em mamíferos, esse se desenvolve em diversos locais, ocorrendo geralmente em áreas de tecido frouxo, como camadas subcutâneas entre o músculo e a derme. Entretanto, depósitos específicos desse tecido também se formam ao redor do coração, rins e outros órgãos internos (SETHI; VIDAL-PUIG, 2007).

Os adipócitos são formados por gotículas de lipídeos responsáveis por armazenar o excesso de calorias consumidas, como triglicerídeos, sem sofrer lipotoxicidade (MESSINA *et al*., 2017). Durante períodos de aumento de ingestão de alimentos e/ou de diminuição do gasto energético, o excesso de energia é depositado de forma eficiente no tecido adiposo na forma de triglicerídeos neutros. Esse processo é mediado por enzimas lipogênicas essenciais. No entanto, na ausência de alimentos e/ou de aumento de gasto energético, as reservas de lipídeos são liberadas para fornecer combustível para geração de energia (SETHI; VIDAL-PUIG, 2007). Essa habilidade única entre armazenar lipídeos sob demanda metabólica sistêmica une a biologia celular dos adipócitos e a fisiologia do tecido adiposo ao metabolismo do corpo inteiro (MESSINA *et al*., 2017).

O papel do tecido adiposo na homeostase metabólica está sendo fortemente valorizado nas últimas décadas, ao passo que se desenvolveu um entendimento mais profundo das suas funções biológicas essenciais (BURHANS *et al*., 2019). Acreditava-se que o tecido adiposo funcionava exclusivamente como um reservatório de energia e isolante térmico. Todavia, estudos demonstram se tratar de um órgão complexo que recebe inervações do SNC e exerce funções endócrinas e imunológicas fundamentais (BOOTH *et al*., 2016). Fatores bioativos são secretados pelo tecido adiposo, que circulam e retransmitem informações para outros órgãos metabolicamente ativos, como músculo, fígado, pâncreas e cérebro, modulando, assim, o metabolismo sistêmico (LUO; LIU, 2016).

3.9 Tecido adiposo branco

O TAB é composto principalmente por adipócitos, também chamados de células de gordura uniloculares, sustentados por um tecido conjuntivo frouxo ricamente vascularizado. O tamanho dos adipócitos varia em relação ao seu conteúdo lipídico. Em adipócitos maduros, uma grande gota lipídica preenche quase todo o volume da célula, sendo limitada apenas por uma monocamada lipídica reforçada com uma variedade de proteínas estruturais. A célula adiposa é composta por uma malha de colágeno que funciona para proteger a célula de ruptura mecânica. O tecido adiposo contém células-tronco com capacidade de se diferenciar não apenas em células de linhagem mesodérmica, como os adipócitos, mas também em células não mesodérmicas como neurônios e outros. Ademais, são encontrados no tecido adiposo determinadas células do sistema imune, como macrófagos e linfócitos, além de fibroblastos e células do endotélio vascular (WRONSKA; KMIEC, 2012).

O TAB é considerado o principal reservatório energético do organismo; trata-se de um órgão complexo que desempenha diversas funções em níveis celulares, teciduais e sistêmicos (GARCIA ROSA *et al*., 2019). Os adipócitos executam papel central na regulação da homeostase energética sistêmica, agindo como um depósito seguro no armazenamento de níveis excedentes de gordura (LONGO *et al.,* 2019).

Evolutivamente, armazenar energia em forma de triglicerídeos é vantajoso, no entanto, a presença de adipócitos especializados para esse fim é restrita aos vertebrados. Além de servir como depósito de energia, os adipócitos fornecem diversas funções fisiológicas adicionais. Animais em climas frios exibem aumento da adiposidade, o que os proporciona maior isolamento e termogênese. Ainda, os adipócitos também fornecem amortecimento mecânico e são numerosos em regiões anatômicas que recebem alto estresse mecânico, como a palma da mão, as nádegas e o calcanhar. Internamente, o tecido adiposo promove um maior amortecimento para órgãos como coração, glândulas suprarrenais, rins e ovários (GHABEN; SCHERER, 2019).

O tamanho e a renovação das células são os determinantes mais relevantes do metabolismo dos adipócitos brancos e da massa gorda, e as suas alterações estão associadas a condições patológicas (MORIGNY *et al*., 2021). Diante de um balanço energético positivo, os mecanismos dinâmicos reorganizam o tecido adiposo,

alterando sua morfologia (hiperplasia *versus* hipertrofia) (LONGO *et al*., 2019). Uma resposta hiperplásica protege contra o desenvolvimento de tecido adiposo desregulado e disfuncional. Já a obesidade hipertrófica é caracterizada por ser marcador de risco metabólico na obesidade, sendo ainda um fator de risco independente no desenvolvimento do diabetes tipo 2 (SMITH; KAHN, 2016).

Durante o excesso de nutrientes, quando a expansibilidade do tecido adiposo atinge seu limite, existe uma forte associação entre o tamanho dos adipócitos e a morte dessa célula. Em resposta à morte dos adipócitos, os macrófagos pró-inflamatórios cercam as células mortas e removem os detritos da área danificada. Durante esse processo, os macrófagos produzem agudamente citocinas inflamatórias, que, se não resolvidas, podem se tornar crônicas, ocasionando deficiência na sinalização da insulina dos adipócitos, inflamação adicional e disfunção do tecido adiposo (RICHARD *at al.,* 2020).

As funções metabólicas e endócrinas do tecido adiposo foram amplamente estudadas nas últimas décadas. Os adipócitos são capazes de produzir proteínas especificas em condições de volume normal. Em casos em que ocorre a hipertrofia do tecido, verificam-se alterações na síntese proteica, resultando na produção de proteínas inflamatórias (citocinas). Ao passo que grande parte das adipocinas - como o fator de necrose tumoral-α, IL-6 e PAI-1 - são pró-inflamatórias, a adiponectina, pelo contrário, é uma adipocina constituída de ações anti-inflamatórias, antidiabéticas, cardioprotetoras e antitumorais. Na obesidade, o tecido adiposo desregulado libera níveis elevados de adipocinas pró-inflamatórias e em contrapartida reduz níveis de adiponectina, estabelecendo um estado de inflamação crônica (DIVELLA *et al.,* 2016).

As adipocinas constituem uma classe de proteínas essenciais no equilíbrio entre apetite e saciedade, além de atuarem na regulação dos estoques de gordura corporal e gasto energético, na tolerância à glicose, na liberação e sensibilidade de insulina, no crescimento celular, na inflamação e na angiogênese. Dentro dessa ampla gama de sinais, os tecidos alvos do tecido adiposo são o cérebro, o fígado, o músculo, o coração, o pâncreas, o timo e o baço, porém, não se limita apenas a esses. No geral, a inflamação no tecido adiposo é apoiada para vincular a obesidade ao grupo das doenças metabólicas, servindo como uma espécie de gatilho para a hipóxia, que surge em decorrência da falta de acesso a vasculatura. A hipóxia desencadeia a inflamação induzida pela obesidade, a qual, vinculada aos níveis aumentados de adipocinas, se

torna central na perda da sensibilidade à insulina e no aumento dos níveis de glicose (BOOTH *et al*., 2016).

3.10 Tecido adiposo marrom

O TAM, ao contrário do TAB, está envolvido no gasto de energia; trata-se de um tecido termogênico que funciona para gerar calor em resposta à exposição ao frio (WHITE; DEWAL; STANFORD, 2019). O TAM é mais vascularizado do que o TAB, além de ser formado por uma trama especializada de adipócitos, cujo citoplasma contém várias inclusões lipídicas (multiloculares) e muitas mitocôndrias com a proteína desacopladora 1 (UCP1). Essa proteína localiza-se na membrana interna da mitocôndria e diminui o gradiente de prótons ao desacoplar a respiração celular e a síntese de ATP mitocondrial. Em resposta à exposição ao frio ou à ingestão de alimentos, a UCP1 é ativada, promovendo aumento na oxidação de glicose e ácidos graxos livres, respiração mitocondrial que leva à geração de calor (SIDOSSIS; KAJIMURA, 2015).

Erroneamente, no passado, acreditava-se que o TAM estava presente exclusivamente em humanos durante o período neonatal. Entretanto, atualmente, sabe-se que o TAM em adultos está localizado nas regiões cervicais, supraclavicular, paravertebral, mediastinal, para-aórtica e adrenal (GÓMEZ-HERNÁNDEZ *et al.,* 2016).

Em camundongos, o principal modelo animal utilizado para estudar a gordura marrom, o tamanho e a composição de cada depósito de TAM difere de acordo com a idade, sexo e linhagem. Grande parte dos repositórios de TAM estão concentrados em regiões dorsais posteriores do corpo do camundongo, abrangendo os interescapulares, subescapulares e cervicais. Os depósitos de gordura marrom são altamente irrigados por vasos sanguíneos, facilitando a troca de oxigênio, nutrientes e a dissipação de calor. Além de uma vasta vascularização, o TAM é também amplamente inervado, permitindo sua rápida estimulação pelo sistema nervoso simpático (SNS) (ELIA *et al*., 2015).

Em razão ao papel crítico desenvolvido pelo TAM no metabolismo energético do corpo, alterações na massa e na atividade do TAM podem ter alto impacto entre os distúrbios metabólicos, sobretudo, a obesidade e o diabetes tipo 2. Visto que a

atividade do TAM diminui em camundongos com obesidade induzida por dieta, a ativação do TAM poderia ser uma forma eficaz no controle do aumento do gasto energético (ZHANG *et al.,* 2018). Junto à sua necessidade de adaptação às mudanças dietéticas e às condições térmicas, o TAM é um tecido altamente plástico. Quando a termogênese é ativada, os depósitos do TAM são ampliados por meio de processos hipertróficos e hiperplásicos. (VILLARROYA *et al*., 2016).

Além de seu potencial terapêutico contra a obesidade, o TAM também exerce um papel notável no metabolismo da glicose, pois tem uma sensibilidade maior à insulina em comparação ao TAB, devido à captação de glicose no TAM, que pode ser aumentada em até cinco vezes após administração de insulina (ZHANG *et al*., 2018). No entanto, ainda não há evidências convincentes que demonstrem que o TAM seja um alvo farmacêutico viável para perda de peso corporal. Pesquisas ainda são necessárias para confirmar a relevância do tecido adiposo marrom e bege para o metabolismo energético em humanos (MARLATT; RAVUSSIN, 2017).

3.11 Modelos de obesidade animal e dieta hiperlipídica

A utilização de camundongos como modelo animal vem sendo amplamente utilizada pela comunidade acadêmica, de tal modo que, na atualidade, 60% de todas as pesquisas pré-clínicas realizadas em animais é conduzida em *Mus musculus*. Em parte, esse aumento pode ser decorrente das variadas ferramentas genéticas moleculares disponíveis para projetar mutações direcionadas ou não, troca de nucleotídeo único a rearranjo cromossômicos (KLEINERT *et al*., 2018).

Os camundongos são pequenos em seu tamanho, se reproduzem em grande quantidade, aproximadamente seis a 12 filhotes de acordo com sua respectiva linhagem, têm um ciclo reprodutivo consideravelmente curto, alcançando a sua maturidade dentro de quatro a oito semanas após o nascimento, tendo um período de gestação de somente três semanas. Todas essas características corroboram para a escolha destes animais, consolidando uma alternativa econômica para os pesquisadores (KLEINERT *et al.,* 2018).

Em roedores, a ingestão de dieta rica em gordura (HFD) normalmente induz a obesidade. Camundongos da linhagem C57Bl/6 são muito utilizados devido à sua facilidade de disponibilidade e tendência ao consumo excessivo de HFD, semelhante

ao comportamento humano, promovendo um fenótipo obeso. À medida que esses animais envelhecem, habitualmente apresentam características associadas à síndrome metabólica, que envolvem resistência à insulina, a intolerância à glicose, a hiperlipidemia, a hipertrigliceridemia e a hipertensão (GLASTRAS *et al.,* 2016).

Diversas pesquisas aplicam variados tipos de dietas com alto teor de gordura que variam entre 20 e 60% de energia total. Entre elas, podem ser citadas como fonte de componente de gordura os óleos derivados de plantas (milho, cártamo ou azeite) ou gorduras provindas de origem animal (por exemplo, sebo bovino e banha de porco). Dietas ricas em gordura têm sido frequentemente empregadas para induzir a obesidade em estudos experimentais, sendo eficazes na promoção da hiperglicemia, na resistência à insulina, na dislipidemia e no aumento de ácidos graxos livres no sangue (WONG *et al.,* 2016).

Em humanos, o aumento de tecido adiposo corporal pode estar relacionado não somente à quantidade diária de consumo e ao gasto de energia, mas também ao tipo de dieta, sobretudo aquelas ricas em gordura. Esse tipo de dieta, portanto, pode levar a várias alterações metabólicas, como a hiperfagia em humanos, a redução na secreção e ou a sensibilidade de leptina, a resistência à insulina e a obesidade (COELHO *et al*., 2011). Nessa perspectiva, a promoção de obesidade em roedores por meio de dietas hiperlipídicas almeja reproduzir o comportamento nutricional humano (DINIZ, B., *et al.,* 2008). Além disso, tem sido demostrado que as desordens originadas por uma alimentação rica em gordura em camundongos se assemelham às desordens metabólicas observadas em humanos (WHITE *et al.,* 2013).

4 ARTIGO CIENTÍFICO

INFLUENCE OF SUBDIAPHRAGMATIC AND SELECTIVE VAGOTOMY ON ADIPOSE TISSUE STOCKS IN OBESE MICE

Influence of subdiaphragmatic and selective vagotomy on adipose tissue stocks in obese mice

Andresa J. Zamoner [a], Sandra L. Balbo [a], Maria L. Bonfleur [a]

[a] Laboratório de Fisiologia Endócrina e Metabolismo (LAFEM), Centro de Ciências Biológicas e da Saúde, Universidade Estadual do Oeste do Paraná, Cascavel, Brazil

Correspondece to: Maria Lúcia Bonfleur

Universidade Estadual do Oeste do Paraná (UNIOESTE)

Laboratório de Fisiologia Endócrina e Metabolismo,

Cascavel, PR, Brazil CEP: 85819-110

E-mail: mlbonfleur@hotmail.com

Fone: +55 45 3220 3257

Abstract

Aims: Obesity has reached alarming proportions all over the world, being considered an important economic and global health problem. Evidence indicates a dysfunction in the autonomic nervous system (ANS), with increased parasympathetic activity and reduced sympathetic activity, leading to increased insulin secretion, which contributes to the accumulation of body fat and maintenance of obesity. Based on this evidence, we aimed to investigate the participation of the parasympatic, through subdiaphragmatic and selective vagotomy in biometric parameters and fat stores in obese male mice.

Main methods: 40-day-old male mice of the were fed a high-fat diet (HFD) to induce obesity. After eight weeks of HF diet, surgical procedures were performed, forming the groups: 1) OB-Sham-operated group (OB-Sham); 2) OB-Selective Vagotomy group (OB-VagS) and 3) OB-Total Subdiaphragmatic Vagotomy group (OB-VagT).

Key findings: Eight weeks after the surgical procedures, the OB-VagT animals showed a reduction in body weight and in lean body mass, along with a decrease in brown adipose tissue (BAT) stores. Total vagotomy did not change glucose and plasma insulin levels, but normalized total cholesterol levels. On the other hand, selective vagotomy was not effective in the weight loss of the animals, however, it altered daytime food consumption, and increased the number of adipocytes and lipid inclusions per field in the BAT, showing similarities to what was found in the OB-VagT group.

Significance: We suggest that the observed benefits of total and selective vagotomy are at least in part due to the likely activation of brown adipose tissue in these animals.

Keywords: Obesity, Vagus nerve, Subdiaphragmatic and selective vagotomy, Adipose tissue.

Introduction

The incidence of obesity has been growing in developed and developing countries, assuming alarming proportions (BLÜHER, 2019). Although several factors are involved in the development of obesity (TAUBES, 1998), the central nervous system (CNS), through the hypothalamus, is involved in the maintenance and monitoring of energy homeostasis via the autonomic nervous system (ANS) (VELLOSO; SCHWARTZ, 2011). It has been proposed that obese humans and animals exhibit ANS dysfunction, with high parasympathetic activity and decreased sympathetic tone (BRAY AND YORK, 1979; BALBO *et al.,* 2007; SCOMPARIN *et al*., 2009; CALEGARI *et al*., 2011; BARELLA *et al*., 2015).

Decreased sympathetic tone decreases lipolysis in white adipose tissue (WAT), which is specialized for energy storage, and decreases thermogenesis in brown adipose tissue (BAT), contributing to adiposity. The vagus nerve with sensory and motor fibers makes the interface between peripheral parasympathetic signals and the CNS. Although the WAT and the BAT do not present direct vagal innervation, studies show that the vagus indirectly influences the functioning of these tissues. In addition, increased parasympathetic activity stimulates insulin secretion, contributing to the accumulation of body fat and maintenance of obesity (BRAY AND YORK ,1979; BALBO *et al*., 2007; SCOMPARIN *et al*., 2009; CALEGARI *et al*., 2011; BARELLA *et al*., 2015).

Reinforcing that autonomic imbalance contributes to the onset and maintenance of obesity, studies show that bilateral subdiaphragmatic vagotomy normalizes plasma insulin concentration and glucose tolerance, in addition to decreasing fat accumulation in morbidly obese patients (KRAL, 1980) and in rodents with hypothalamic obesity (BALBO *et al*., 2007), genetic obesity (ROHNER-JEANRENAUD *et al.,* 1983) and cafeteria diet-induced obesity (BALBO *et al*., 2017).

Vagotomy of the celiac or selective branch, in lean animals, reduces the secretory response of insulin to glucose, carbachol (analogue to ACh) (SILVA *et al*., 2009) and to the amino acid arginine (SILVA *et al*., 2012). In rats with type 2 diabetes, the association of bariatric surgery of ileal interposition with vagotomy of the celiac branch, worsened glycemic control (CHEN *et al*., 2018). In this same study, in sham-operated diabetic animals, no change occurred after celiac vagotomy. Thus, the

participation of the vagus nerve in the onset of obesity has been widely discussed in the literature, but there are still contradictory data. Furthermore, until now, we have not found anything in the literature that shows the effect of selective vagotomy on different body fat stores. Therefore, our aim was to investigate the effects of subdiaphragmatic and selective vagotomy performed after the onset of obesity on biometric parameters and fat stores in male mice fed a high-fat diet.

Material and methods

Animals

All experimental protocols were approved by the Ethics Committee on Animal Use (CEUA) of the Western Paraná State University (UNIOESTE), Campus Cascavel, (approval number 22-20). 40-day-old C57BL/6 male mice were obtained from the central animal facility at UNIOESTE and kept in the sectoral animal facility at LAFEM at a temperature of 28 ± 2ºC, under a twelve-hour light/dark cycle. For obesity induction, all animals were fed a high-fat diet (HFD) for a period of 8 weeks. The diet was prepared from 50% ground standard chow; 31.2% lard; 14.8% casein and 4% soybean oil, containing 6.2 Kcal/g. The control group received a standard diet (Biobase Biotec, Águas Frias, SC. Brazil) and has 70% carbohydrates, 20% proteins and 10% fats, containing 3.8 Kcal/g. After 8 weeks on the diet, mice were randomly assigned to three groups: 1) sham-operated obese group (OB-Sham); 2) Obese group submitted to selective vagotomy (OB-VagS) and 3) Obese group submitted to total vagotomy (OB-VagT). After the surgical procedures, HFD continued to be offered for another 8 weeks.

Surgical procedures

For the surgical procedures, the animals were anesthetized with 1% isoflurane (Isoforine®, Cristália, Brazil). The animal was fixed in dorsal decubitus and an incision was made inferior to the sternum, in the upper half of the abdomen. For total vagotomy (subdiaphragmatic), the liver was removed and the two trunks of the vagus nerve and their respective branches were dissected. For selective surgery (celiac), the esophagus was located and then the anterior and posterior branches of the vagus, 1 cm below the diaphragm, were positioned and exposed for dissection. Sham-operated

animals (Sham) underwent the same procedures, but the vagus nerve was kept intact. At the end of the experimental period, to confirm the total vagotomy, the food retention in the stomach of all animals was evaluated by the ratio between stomach weight and body weight (BW), in accordance with previous studies (EDVELL; LINDSTRÖM, 1998; LAUSIER *et al*., 2010; LUBACZEUSKI *et al*., 2015).

Evolution of body weight and evaluation of food consumption

To determine the evolution of body weight, the animals were weekly weighed on a digital scale (KN WAAGEN, KN220/3, Brazil). The total weight gain was obtained through the final weight minus the initial weight in grams. Feed intake was measured in the eighth week after surgery, during daytime and nighttime. Consumption was obtained by the amount of feed offered minus the amount of feed left over, divided by the number of mice per box. Consumption was calculated in grams and in Kcal.

Assessment of obesity and collection of biological material

After 8 weeks of the surgical procedures, the final BW (g) and the nasoanal length (cm) were obtained to calculate the Lee index (cubic root of BW/nasoanal length x 1000) (BERNARDIS; PATTERSON, 1968). Then, capillary blood glucose was checked by the tail with the aid of a manual glucometer (Accu-Chek® Active, Roche, Brazil) and, subsequently, the mice were anesthetized with 9 mg/Kg of xylazine hydrochloride (Anasedan®, Vetbrands, Brazil) and 90 mg/Kg ketamine hydrochloride (Dopalen®, Vetbrands, Brazil). The whole blood was collected by cardiac puncture, and serum was obtained and stored at -20°C for measurement of insulin by radioimmunoassay (RIA) (Merck®, Germany). Total cholesterol and triglycerides were measured using standard commercial kits, according to the manufacturer's instructions (Bioclin®, Quibasa, Brazil). The different stocks of adipose tissue were removed, weighed and a fragment was stored for later histological analysis.

HOMA-IR and TyG index

Tissue sensitivity to insulin was assessed by the homeostasis assessment method (HOMA) previously validated (BONORA *et al*., 2000) and HOMA index of insulin resistance (HOMA-IR) = Fasting insulin (µU/ml) x Glucose fasting (mMol/l) / 22.5.

Carcass fat extraction by the Soxhlet method

For the extraction of fat from the animal carcasses, they were weighed (wet weight) and stored in a -20°C freezer. Subsequently, the carcasses were dehydrated in an oven at 65°C for 72 h, macerated and weighed (dry weight) before being sent for extraction. The total water content was calculated through the wet weight minus the dry weight. After dehydration, total carcass fat (subcutaneous adipose tissue) was extracted with petroleum ether (LabSynth, SP, Brazil) using a soxhlet extractor (MARQLABOR, EG-RE). The percentage of total carcass fat was obtained by calculating: % of total carcass fat = (Container Weight + Fat) - Container Weight X 100 / Dry Weight.

Histological analyzes of white adipose tissue

Retroperitoneal white adipose tissue fragments were fixed in Carson's formaldehyde and embedded in Paraplast® (Sigma-Aldrich Chemicals, St Louis, MO, USA). Sections were made at 5 µm and stained with hematoxylin-eosin. Images of six fields per section of each adipose tissue sample were captured with the 40x objective, and in each field, adipocytes were counted and the diameter was measured using Image J software (USA version 1.53a).

Histological processing of brown adipose tissue

After the removal and weighting, a fragment of brown adipose tissue (BAT) was removed and fixed in 4% paraformaldehyde for 24 hours, dehydrated in ascending alcohol concentrations and, after clearing in xylene, soaked in histological paraplast (Sigma- Aldrich Chemicals, St Louis, MO, USA). Sections of 5 µm were prepared for hematoxylin-eosin staining to verify the histological morphology. Five sections of each BAT sample were photographed using an optical microscope (Olympus DP71; Tokyo, Japan) with a 40x magnifying glass. Image J software was used for image analysis. The nuclei count per field was quantitatively performed and the area of the adipocytes was measured. The number and size of lipid inclusions were also verified.

Statistical analyzes

All data are presented as the mean ± standard error of the mean. Analysis of variance - one-way ANOVA, followed by Tukey's post-test, was used for statistical

differences. P < 0.05 was adopted as a significance criterion. Tests were performed using GraphPad Prism® version 8.00 for Windows.

Results

The high-fat diet was effective in inducing obesity, since the body weight of the OB animals was greater when compared to the CTL group, with this difference being significantly greater from the first week of the diet, intensifying in the following weeks (P<0, 0001). After one week of surgical procedures, the body weight of the OB-VagT animals was lower than that observed in the OB-Sham and OB-VagS groups until the eighth week after surgery (Fig. 1A, P<0.02). Reinforcing this result, we observed that the total body weight gain during eight weeks after the surgeries was lower in animals from the OB-VagT group (15.7 ± 1.6), when compared to the OB-Sham (21.6 ± 0.8) and OB-VagS (21.8 ± 1.8). Regarding food consumption, we observed that there were no differences between the groups at nighttime (Fig. 1c), however, during daytime, we observed that mice from the OB-VagS group showed an increase in food consumption (P<0.04) compared to the OB-Sham and OB-VagT groups (Fig. 1D).

As seen in Table 01, the animals in the OB-VagT group showed a reduction in plasma TG and COL concentration, compared to the OB-Sham mice (P<0.05). Fasting blood glucose, insulin, HOMA-IR and HOMA-beta indices were similar between the studied groups (Table. 0.1).

When analyzing the body composition of the animals, we observed that the total vagotomy promoted a reduction in the body weight of the OB-VagT animals (42.73 ± 1.3) compared to the OB-Sham (49.74 ± 0.7) and OB-VagS (48.43 ± 1.4) groups. In addition, total vagotomy promoted a reduction in carcass weight and in the percentage of lean body mass, compared to the OB-Sham group (Table 2, P<0.007 and P<0.02 respectively). Selective vagotomy did not influence these parameters. We also observed that the percentage of fat extracted from the carcass did not present a significant difference between the analyzed groups.

Next, we analyzed the different fat stores of the animals. As we can see in Figure 2A, all three groups showed fat deposition, but visually, the store of gonadal fat in the OB-VagT group is greater than in the OB-Sham and OB-VagS groups. When we measured the weight of the different fat stores, we verified that the OB-VagT animals had a higher weight of gonadal (Fig. 2B, P<0.004) and retroperitoneal (Fig. 2C,

P<0.0001) fat, compared to the OB-Sham and OB-VagS groups. Regarding interscapular brown adipose tissue (BAT), the OB-VagT group showed a decrease in weight, compared to the OB-Sham group (Fig.2D, P<0.02). Selective vagotomy did not alter fat weight, compared to OB-Sham animals (Fig. 2B, 3C and 3D).

In an attempt to better assess the effect of vagotomy on different fat stores, retroperitoneal fat and BAT morphometry was performed. In figure 3A, we observe that the retroperitoneal WAT adipocytes from all groups present the nucleus displaced to the periphery and a large fat droplet. When analyzing the diameter of the adipocytes, although the OB-VagS and OB-VagT groups presented higher values, no statistical differences were found when compared to the OB-Sham group (Fig. 3C). However, regarding the number of adipocytes per field, the OB-VagS and OB-VagT groups had a lower number than the OB-Sham group (Fig. 3B, P< 0.01).

When histologically analyzing the BAT (Fig. 4A), we observed that, in the OB-Sham animals, the adipose cells were expanded, with greater fat content and with a unilocular appearance. In the OB-VagS group, we observed that the adipocytes had unilocular and multilocular appearance, given that some cells had several small lipid inclusions, while others had larger inclusions. In the OB-VagT group, the BAT adipocytes showed characteristics of typical multilocular adipose tissue, since the adipose cells contained small droplets of lipids of different sizes. In the morphometric analysis, we observed that the number of adipocytes per field and the number of lipid inclusions per adipocyte was higher in the OB-VagS and OB-VagT groups, compared to the OB-Sham group (Fig. 4B and 5D, P<0.0002 and P<0.0004 respectively). The adipocyte area and the size of lipid inclusions was smaller in the OB-VagT group compared to the OB-VagS and OB-Sham groups (Fig. 4E and 4C, P<0.0001). The adipocyte area in the OB-VagS group was similar to the OB-Sham group, however, the size of the lipid inclusions was smaller compared to the OB Sham group (Fig. 4E, P<0.0002).

To better understand the effect of total and selective vagotomy on BAT, we analyzed the gene expression of iodothyronine deiodinase type 2 (DiO2), peroxisome proliferator-activated receptor coactivator 1α (Pgc-1α), transcriptional regulator of positive regulatory domain containing 16 (Prdm16), cell death-inducing DNA fragmentation factor alpha-like effector A (CIDEA), fibroblast growth factor 21 (Fgf21), beta-3 adrenergic receptor (Adrb3), Cytochrome C (Citc), mitochondrial uncoupling protein 1 (Ucp1), lipoprotein lipase (Lpl) and Leptin (Lep). We can observe that total

vagotomy promoted an increase in Dio2 expression, compared to the OB-Sham and OB-VagS groups (Fig. 5A). As for the expression of Pgc1α in the animals from OB-VagT and OB-VagS groups, the values were higher compared to the OB-Sham group (Fig. 5B). As for the evaluation of Prdm16 expression, the OB-VagT group showed higher values than the OB-Sham group (Fig.5C). Total vagotomy also modified Leptin expression, since it showed reduced values compared to the OB-VagS and OB-Sham groups (Fig.5J). Regarding Cidea, Fgf21, Adrb3, Citc, Ucp1 and Lpl, no change was observed between the three groups.

Discussion

In this study, we hypothesized that total and selective subdiaphragmatic vagotomy would be effective in promoting weight loss and reducing fat accumulation in obese animals, even while continuing a high-fat diet. Although we did not observe differences in the amount of fat in the OB-VagT animals, we noticed that total subdiaphragmatic vagotomy was effective in reducing weight gain and activating the brown adipose tissue that contributes to and attenuates the onset of obesity. Furthermore, for the first time we observed the effects of vagotomy of the celiac branch on fat stores. Even though the selective vagotomy did not change the weight of the WAT and the BAT, when morphologically analyzing this tissue, we noticed that its appearance, number of adipocytes and lipid inclusions were similar to the animals in the OB-VagT group.

Although the pathophysiological mechanisms of obesity are complex and not completely understood, several studies have shown that the development of obesity can be caused by imbalance in the ANS (BRAY AND YORK, 1979; STEARNS *et al*., 2012). In obese humans and in animal models, study has shown that there is an increase in parasympathetic activity and a reduction in sympathetic activity (BALBO et al., 2007; SCOMPARIN *et al*., 2009; CALEGARI *et al*., 2011; BARELLA *et al* 2015;). One of the main changes related to this imbalance is the increase in insulin secretion, potentiated by parasympathetic activity, and the reduction in lipolysis, caused by the decrease in sympathetic tone (AHRÉN, 2000). Together, hyperinsulinemia and decreased lipolysis in adipose tissue increase lipogenic activity, which contributes to

the accumulation of body fat and maintenance of obesity (AHRÉN, 2000; ERDMANN *et al*., 2008).

Parasympathetic changes result from greater activation of the vagus nerve. The vagus nerve interfaces between peripheral signals and the CNS, being responsible for parasympathetic innervation to abdominal visceral organs, thus, it is involved in many necessary functions for metabolic homeostasis, including hepatic glucose production, endocrine and exocrine pancreatic secretion, and monitoring of the metabolic status (BROWNING; VERHEIJDEN; BOECKXSTAENS, 2017). Complete section of the vagus nerve below the diaphragm (total or subdiaphragmatic vagotomy) has been shown to be effective in normalizing plasma insulin concentration and glucose tolerance, in addition to decreasing fat accumulation in morbidly obese patients (KRAL, 1980) and in rodents with hypothalamic obesity (BALBO *et al*., 2007), genetic obesity (ROHNER-JEANRENAUD *et al*., 1983) and cafeteria diet-induced obesity (BALBO *et al*., 2017). However, the effects of sectioning the celiac branch of the vagus nerve on obesity have yet to be explored. There are few studies with lean and diabetic animals and the results are still contradictory (SILVA *et al.,* 2009, 2012).

In the present study, we demonstrated that total vagotomy, performed after the onset of obesity, by offering a high-fat diet, was effective in promoting body weight reduction without affecting food intake and fat stores. Weight reduction was accompanied by a reduction of approximately 16% in lean body mass in animals from the OB-VagT group, compared to animals from the OB-Sham group. Unlike our results, in obese MSG rats, early performed total vagotomy was effective in reducing adiposity (BALBO *et al*., 2007). Also, Balbo (2016) showed that the total vagotomy, performed after the onset of obesity by the cafeteria diet, promoted a reduction in body weight and in the stores of gonadal fat.

A study showed that vagotomized rats have reduced IGF1 and growth hormone (GH), in addition to the down-regulation of the mRNA of the GHRH group in the arcuate nucleus of the hypothalamus, and of the mRNA of the receptors for GHRH and GHS-R in the pituitary gland, thus showing the importance of the vagus nerve in the regulation of GH (AL-MASSADI *et al*., 2011). Although we did not analyze the concentration of this hormone, possibly, in our study, the animals may have reduced

GH secretion as a result of vagotomy after the onset of obesity, leading to increased fat stores and reduced lean body mass.

Regarding celiac vagotomy, we observed that the OB-VagS animals showed increased food consumption during daytime, without changes in body weight and fat stores. The effects of vagal ablation on food intake still show contradictory results, given that time and surgical method are relevant aspects to be considered in the interpretation of results (BARELLA *et al.,* 2012; INOUE; BRAY, 1977). Studies show that the vagus nerve also influences dyslipidemias, since total vagotomy performed in MSG obese rats reduced triglyceride levels (KUCHLER *et al.,* 2021). Total vagotomy also normalized plasma triglyceride level, decreased cholesterol and increased plasma levels of high-density lipoprotein in obese diabetic and non-diabetic rats (SEBAEI; YOUSOF; ABDO, 2020). Corroborating our data, total vagotomy was effective in reducing plasma cholesterol. However, selective vagotomy did not alter these parameters.

The vagus nerve is also involved in controlling body glucose (FERNANDES *et al*., 2011; LAUTT *et al*., 2001) regulating insulin action by the pancreatic islets (GILON; HENQUIN, 2001). Vagal stimulation potentiates insulin secretion when glucose concentrations are high, influencing insulin release after food presentation. The increase in parasympathetic activity with elevation of circulating insulin levels, have been suggested in the development of obesity (BRAY; YORK, 1979; AHRÉN, 2000). Total vagotomy has been shown to be effective in restoring glucose tolerance in hypothalamic obesity (BALBO *et al*., 2007; LUBACZEUSKI *et al*., 2015).

However, in the present study, the benefits of vagotomy in the control of body glycemia and insulin levels in obese animals on a high-fat diet were not noticed. Indeed, it is not easy to explain the lack of decrease in fasting insulin levels in these animals, however, the stage of life in which the vagotomy was performed must be considered, since the blocking effect of hyperinsulinemia was effective in rats only when it was performed in the initial phase of life (BARELLA *et al*., 2015).

Differently from what was expected, the weight of the retroperitoneal and gonadal WAT stocks were higher in the animals from the OB-VagT group, compared to the OB-Sham group. Then we investigated possible morphological alterations in this tissue. When morphologically analyzed, the WAT of animals submitted to total

vagotomy showed a lower number of adipocytes per field. The ANS exerts direct control at the cellular and molecular levels in adiposity (MESSINA *et al.,* 2017), the sympathetic nervous system (SNS) has terminals in the WAT, playing an important role in homeostatic control (SHI; BOWERS; BARTNESS, 2004), however, there is rare evidence that points to the direct effect of the parasympathetic nervous system (PNS) on the metabolism of this tissue (GIORDANO *et al*., 2006). Regardless of these points of discussion, previous studies observed that the vagus nerve ablation (vagotomy) reduced WAT accumulation in obese rats submitted to cafeteria diet (Balbo *et al*., 2016). It is possible that the diet content influenced the observed results, either by reducing or increasing the effects of the vagus nerve ablation on the WAT content. Although the selective vagotomy did not influence the weight of the WAT, when morphologically analyzed, it also showed a lower number of adipocytes per field, similar to that observed in the OB-VagT group.

Another important adipose tissue in the regulation of energy metabolism is the BAT, due to its excellent thermogenic potential. The BAT is vastly vascularized, and its cytoplasm contains several lipid inclusions (multilocular) and many mitochondria with uncoupling protein 1 (UCP1). This protein is located in the inner mitochondrial membrane and decreases the proton gradient by uncoupling cellular respiration and mitochondrial ATP synthesis. In response to cold exposure or food ingestion, UCP1 is activated, promoting an increase in glucose and free fatty acid oxidation, mitochondrial respiration, which leads to heat generation (SIDOSSIS; KAJIMURA, 2015). This unique ability to generate heat through UCP1 makes this tissue an important therapeutic target for obesity and its complications (CARPENTIER *et al*., 2022).

Our results demonstrate an important influence of total vagotomy on the interscapular BAT stock, which showed weight reduction, and, when histologically analyzed, showed a higher number of adipocytes per field and number of lipid inclusions per adipocytes, in addition to a reduction in the size of adipocytes and lipid inclusions. Although the selective vagotomy did not change the BAT weight, in the histological analysis we observed an increase in the number of adipocytes per field and the number of lipid inclusions per adipocytes, compared to OB-Sham animals. In addition, we observed a reduction in the size of lipid inclusions compared to OB-Sham animals, but still significantly larger compared to OB-VagT animals. These results together point to an effect of vagotomies on the BAT.

BAT thermogenesis is triggered by the release of norepinephrine from its sympathetic nerve terminals, stimulating β3-adrenoceptors, which activate a cascade of intracellular events that result in the activation of uncoupling protein-1 (UCP-1) (BARTNESS; VAUGHAN; SONG, 2010). Thus, when the tissue is active, large amounts of lipids and glucose are burned (CANNON; NEDERGAARD, 2004).

Indeed, our results also showed an increase in the expression of Dio2 in the BAT in the animals from OB-VagT group. The Dio2 gene encodes type II iodothyronine deiodinase (D2), whose main function is to enzymatically convert tetraiodothyronine (T4) into triiodothyronine (T3) (ARROJO E DRIGO; BIANCO, 2011). In mice, triiodothyronine stimulates the sympathetic nervous system, leading to increased expression of UCP1 in BAT (LÓPEZ *et al.,* 2010). However, even though UCP1 expression was increased in animals from the OB-VagT group, the values were not statistically different in our study. PGC1α expression in BAT also showed increased levels in the OB-VagT and OB-VagS groups. PGC1α plays a central role in the regulation of cellular energy metabolism, being strongly induced by the exposure to cold, being associated with adaptive thermogenesis. Still, PGC1α stimulates mitochondrial biogenesis and promotes muscle tissue remodeling, and participates in the regulation of carbohydrate and lipid metabolism (LIANG; WARD, 2006). Protein expression (Prdm16) was also evaluated, which was higher in animals from the OB-VagT group. Prdm16 plays a key role in adipose biology, including several physiological processes, such as energy homeostasis, glucose and lipid metabolism, and body weight regulation (CHI; COHEN, 2016). Thus, our data suggest a possible increased BAT activity, since total vagotomy and, more discreetly, selective vagotomy, significantly increased genes involved in thermogenesis.

Furthermore, the gene expression of leptin in the BAT was evaluated, and showed decreased levels in the animals from OB-VagT group. Plasma leptin concentrations are highly correlated with adiposity, however, it is unclear how leptin production in each fat depot is coordinated. It was proposed that the specific differences of each adipose tissue depot, in the expression of the leptin gene, are correlated to the volume of the constituent adipocytes (ZHANG *et al*., 2002). In part, this would explain the decreased levels found in our study.

Conclusions

In summary, the effects of total vagotomy were seen in reducing body weight gain and in the possible activation of brown adipose tissue. However, total vagotomy increased WAT stores and decreased animal lean body mass. Furthermore, we observed that, although the effects of selective vagotomy were more discreet, they showed similarities to the total vagotomy group. Although glucose and insulin levels were not altered, total vagotomy provided a decrease in plasma cholesterol levels. It is possible that the observed benefits of total and selective vagotomy are, at least in part, due to the likely activation of brown adipose tissue, however, further studies are needed.

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Conflicts of interest statement

The authors report no conflicts of interest.

Ethical Standards

All experiments were approved by the UNIOESTE´s Committee on Ethics in Animal Experimentation.

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Table1. Fasting serum biochemical parameters analyzed 8 weeks after the operation procedures in OB-Sham, OB-VagS and OB-VagT mice.

	OB-Sham	OB-VagS	OB-VagT
TG (mg/dL)	98.4 ± 5.6 [a]	89.0 ± 5.5 [a b]	76.5 ± 7.3 [b]
CHOL (mg/dL)	253 ± 8.4[a]	207 ± 12[b]	200 ± 4.9[b]
Glucose (mg/dL)	144 ± 8.1	143 ± 8.9	120 ± 5.3
Insulin (µU/mL)	22.9 ± 0.9	25.8 ± 2.0	22.2 ± 1.1
HOMA-IR	7.7 ± 0.5	9.0 ± 1.3	6.6 ± 0.4
TyG index	8.8 ± 0.1[a]	8.7 ± 0.1[a,b]	8.5 ± 0.1[b]

Data are mean ± SEM (N=5-9). Different letters indicate significant difference (one-way ANOVA followed by the Tukey post-test, $P < 0.05$).

Table2. Carcass fat extraction, OB-Sham, OB-VagS and OB-VagT mice.

	OB-Sham	OB-VagS	OB-VagT
Final BW (g)	49.7 ± 0.7[a]	48.4 ± 1.4[a]	42.5 ± 1.4[b]
Carcass weight (g)	30.4 ± 0.5[a]	29.9 ± 1.1[a]	25.9 ± 1.3[b]
Water content (g)	13.7 ± 0.9	14.7 ± 1.0	15.2 ± 0.5
Lean mass (%)	22.4 ± 0.6[a]	21.9 ± 1.9[a,b]	18.8 ± 1.5[b]
Fat mass (%)	27.3 ± 0.9	29.5 ± 1.5	28.6 ± 1.7

Data are mean ± SEM (N=7-10). Different letters indicate significant difference (one-way ANOVA followed by the Tukey post-test, $P < 0.05$).

Figure Legends

Figure - 1 (A) Body weight of CTL, OB-Sham, OB-VagT, OB-VagS mice recorded over 16 weeks. * Represents statistical difference when compared to the corresponding value of the CTL group. # OB-VagT is different from OB-Sham and OB-VagS. (B) Food intake (g) Dark period and (C) Food intake (g) Light period of the groups OB-Sham (n= 10), OB-VagS (n=8) and OB-VagT (n=7). Intake data were evaluated for 24 hours, divided into day and night. Different letters over the bars represent significant differences. Date are means ± SEM. One-way ANOVA followed by the Tukey's posttest, P<0,05.

Figure - 2 (A) Images of animals at the time of euthanasia (B) weight of the white adipose tissue (WAT), (C) retroperitoneal and (D) brown adipose tissue (BAT) stocks between the OB-Sham (n=10), OB- VagS (n=8) and OB-VagT (n=7). Data expressed as mean ± SEM. Different letters on the bars represent statistical differences (P<0.05). One-way ANOVA followed by Tukey's post-test.

Figure - 3. Histological evaluation of retroperitoneal white adipose tissue stained with hematoxylin and eosin in the OB-sham (n=5), OB-VagS (n=7) and OB-VagT (n=7) groups. Images were obtained using conventional microscopy with same magnification of (40x) in 50 μm scale. Data expressed as mean + SEM, one-way ANOVA followed by Tukey's post test.

Figure - 4 Assessment of brown adipose tissue hypertrophy. Samples were stained with hematoxylin and eosin. Images were obtained using conventional microscopy at the same magnification (40x) at a scale of 50 μm. Data expressed as mean + SEM, one-way ANOVA followed by Tukey's post test.

Figure - 5 Gene expression (A) iodothyronine deiodinase type 2 (Dio2), (B) peroxisome proliferator-activated receptor coactivator 1α (Pgc-1α), (C) transcriptional regulator of positive regulatory domain containing 16 (Prdm16), (D) cell death-inducing DNA fragmentation factor alpha-like effector A (CIDEA), (E) fibroblast growth factor 21 (Fgf21), (F) beta-3 adrenergic receptor, (G) Cytochrome C (Citc), (H) mitochondrial

uncoupling protein 1, (I) lipoprotein lipase (Lpl), (J) and Leptin (Lep). Data expressed as mean + SEM, one-way ANOVA followed by Tukey's post test.

Figure 1

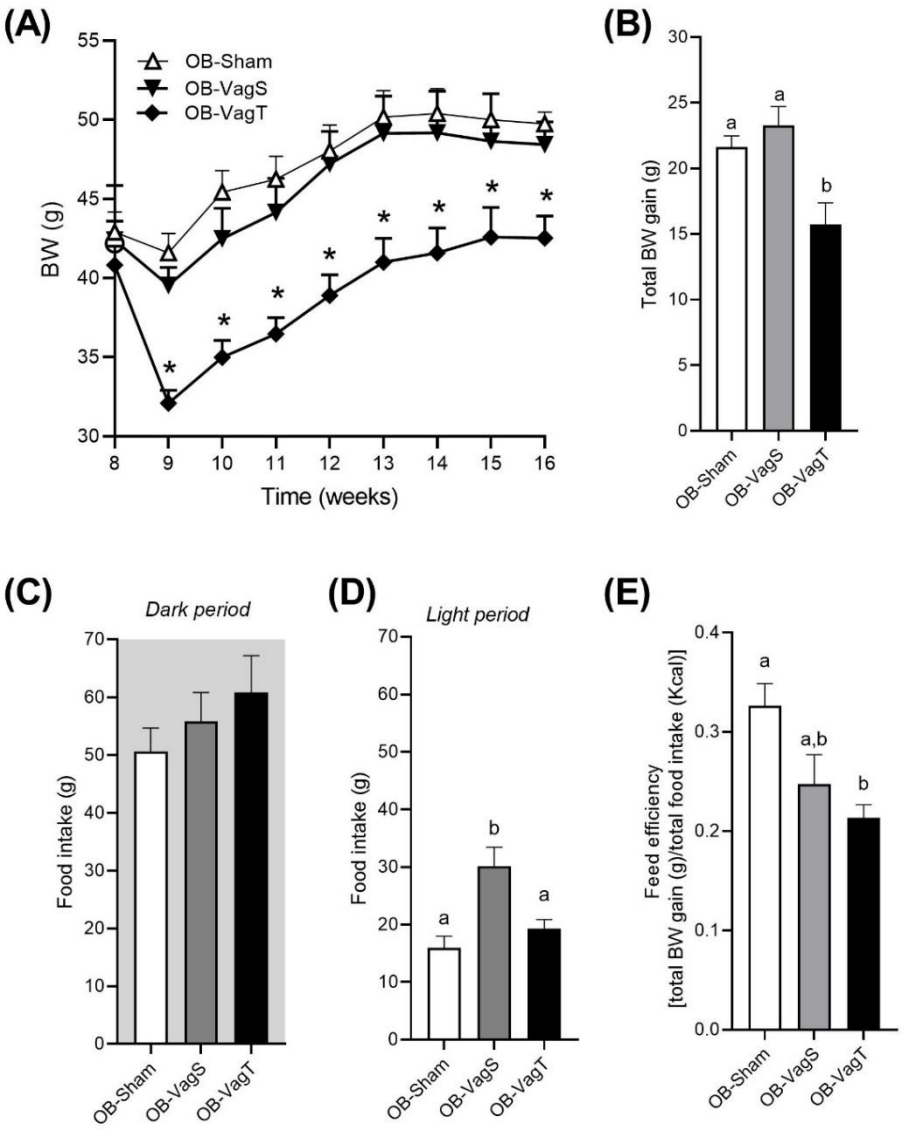

(A)
OB-Sham
OB-VagS
OB-VagT
BW (g)
Time (weeks)
(B)
Total BW gain (g)
OB-Sham
OB-VagS
OB-VagT
(C)
Dark period
Food intake (g)
(D)
Light period
Food intake (g)
(E)
Feed efficiency
[total BW gain (g)/total food intake (Kcal)]

Figure 2

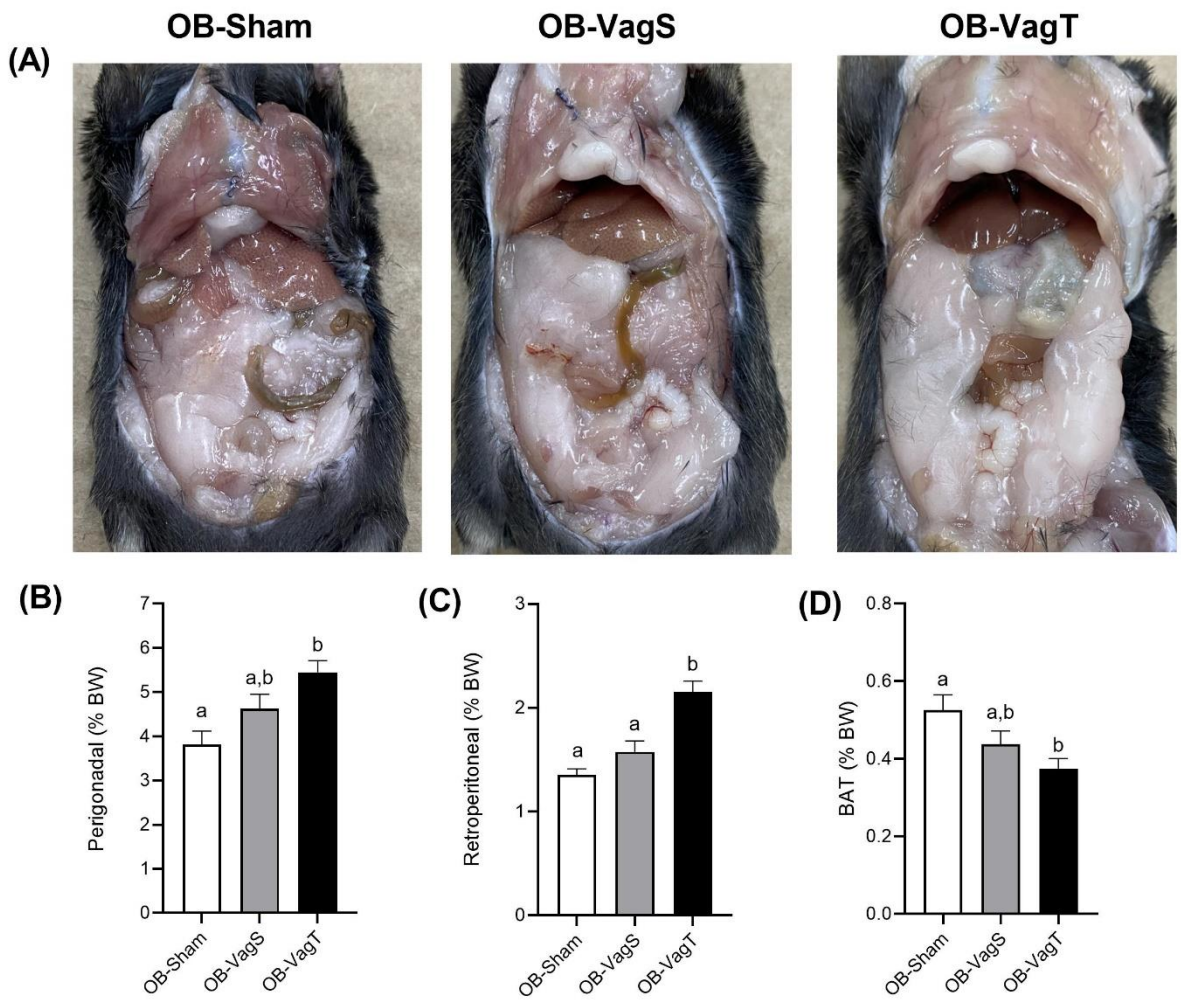

(A)
OB-Sham
OB-VagS
OB-VagT
(B)
Perigonadal (% BW)
0
1
2
3
4
5
6
7
a
a,b
b
OB-Sham
OB-VagS
OB-VagT
(C)
Retroperitoneal (% BW)
0
1
2
3
a
a
b
OB-Sham
OB-VagS
OB-VagT
(D)
BAT (% BW)
0.0
0.2
0.4
0.6
0.8
a
a,b
b
OB-Sham
OB-VagS
OB-VagT

Figure 3

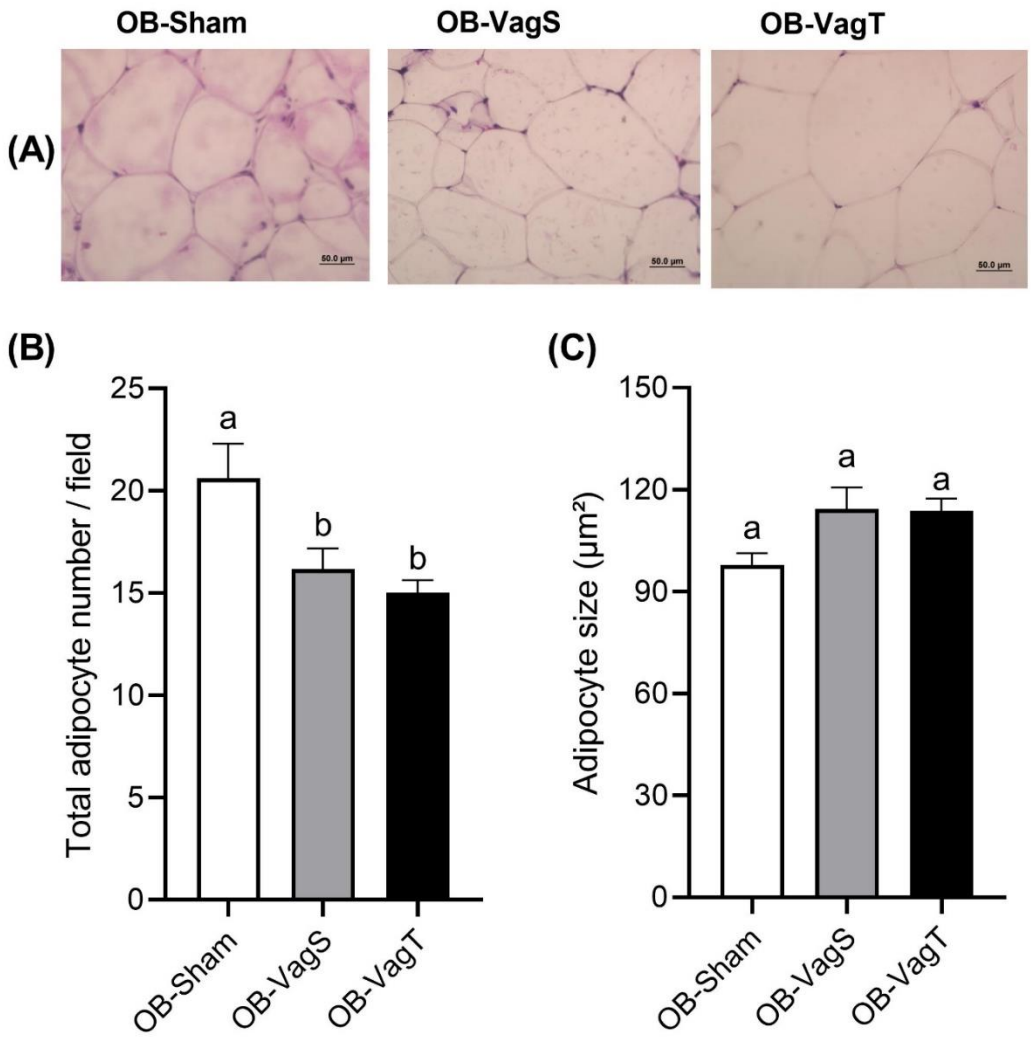

OB-Sham
OB-VagS
OB-VagT
(A)
50.0 µm
(B)
Total adipocyte number / field
25
20
15
10
5
0
a
b
b
OB-Sham
OB-VagS
OB-VagT
(C)
Adipocyte size (µm²)
150
120
90
60
30
0
a
a
a
OB-Sham
OB-VagS
OB-VagT

Figure 04

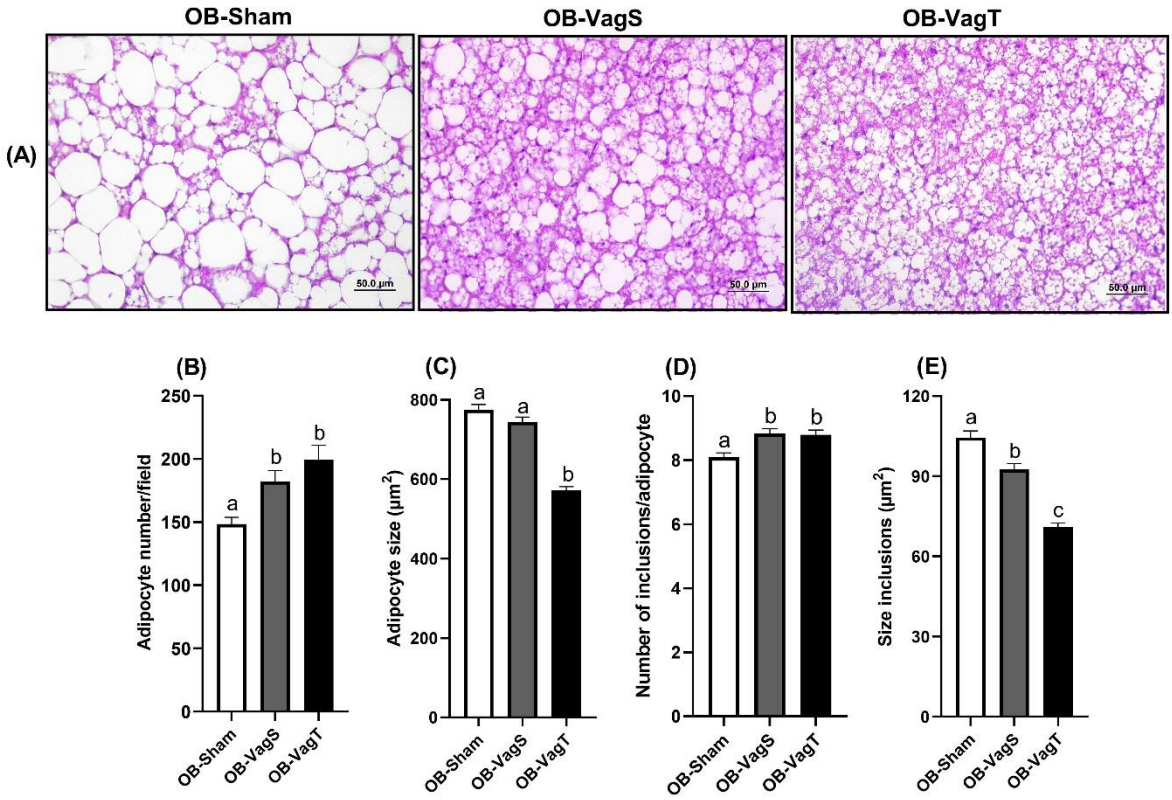
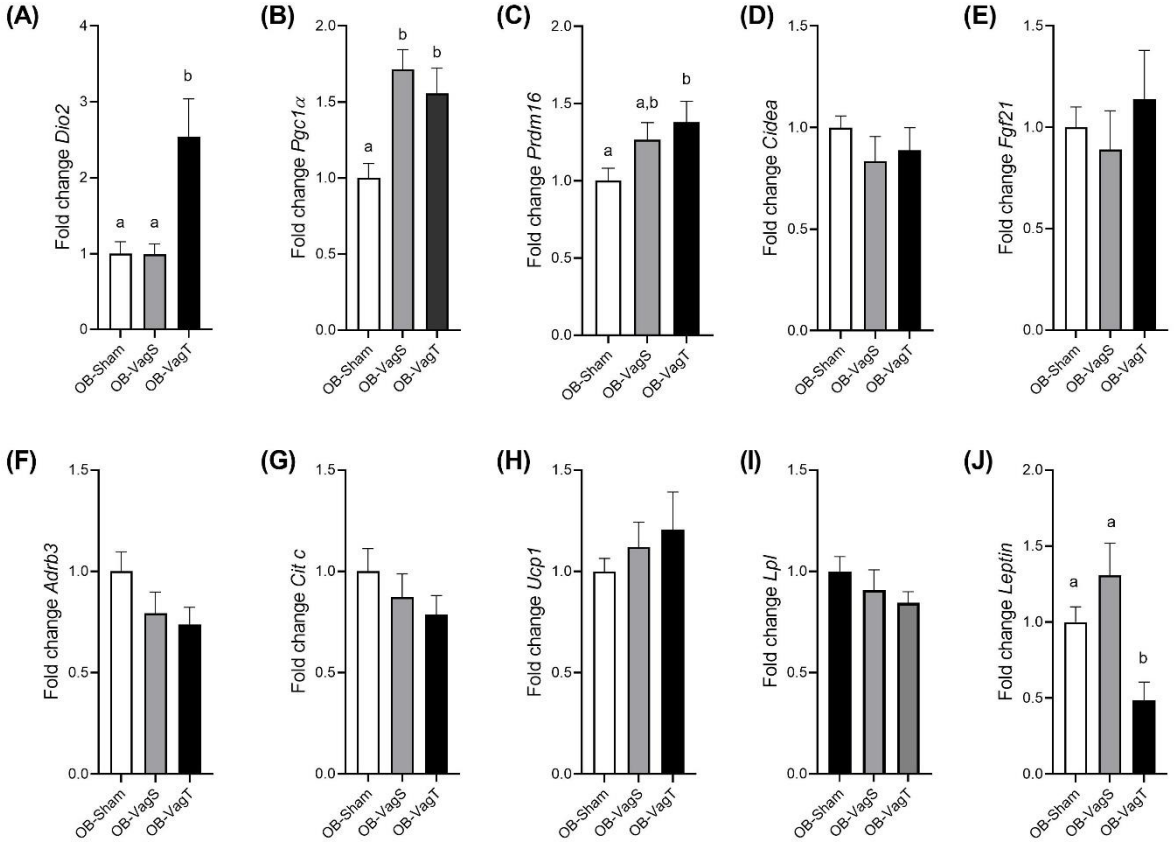

OB-Sham
OB-VagS
OB-VagT
(A)
50.0 µm
(B)
Adipocyte number/field
(C)
Adipocyte size (µm²)
(D)
Number of inclusions/adipocyte
(E)
Size inclusions (µm²)
a
b
c
OB-Sham
OB-VagS
OB-VagT

Figure 5


(A)
Fold change Dio2
a
a
b
(B)
Fold change Pgc1α
a
b
b
(C)
Fold change Prdm16
a
a,b
b
(D)
Fold change Cidea
(E)
Fold change Fgf21
(F)
Fold change Adrb3
(G)
Fold change Cit c
(H)
Fold change Ucp1
(I)
Fold change Lpl
(J)
Fold change Leptin
a
a
b
OB-Sham
OB-VagS
OB-VagT

CONSIDERAÇÕES FINAIS

Este estudo avaliou o efeito da vagotomia subdiafragmática e da vagotomia seletiva após a instalação da obesidade, sobre parâmetros corporais e morfofisiológicos do tecido adiposo de camundongos obesos submetidos à dieta hiperlipídica. Nossos resultados demonstraram que a vagotomia total reduziu peso corporal e a massa magra dos animais, juntamente com a diminuição dos estoques de tecido adiposo marrom. Os efeitos da vagotomia seletiva foram menos pronunciados, no entanto, alterou consumo alimentar diurno e aumentou o número de adipócitos e inclusões lipídicas por campo no tecido adiposo marrom, apresentando semelhanças ao encontrado no grupo vagotomia total. Diante disso, nossos dados colaboram para o esclarecimento e para o entendimento dos efeitos específicos da vagotomia total e seletiva sobre a adiposidade. No entanto, mais estudos são necessários para compreender os mecanismos envolvidos que resultaram na redução do peso corporal e da massa magra desses animais.

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ANEXOS

Anexo A - Parecer de Protocolo CEUA

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Pró-Reitoria de Pesquisa e Pós-Graduação
Comitê de Ética no Uso de Animais - CEUA

Autorização

O Protocolo nº **22-20** intitulado **"Avaliação dos parâmetros metabólicos em camundongos obesos por dieta hiperlipídica submetidos a vagotomia total subdiafragmática e a vagotomia celíaca"**, sob a responsabilidade de **Sandra Lucinei Balbo** que envolve a produção, manutenção ou utilização de animais pertencentes ao filo *Chordata*, subfilo *Vertebrata*, para fins de pesquisa científica encontra-se **Aprovado** para execução, está de acordo com as Normas editadas pelo Conselho Nacional de Controle de Experimentação Animal (CONCEA) e foi aprovado pelo Comitê de Ética no Uso de Animais (CEUA) do UNIOESTE em reunião de 05/03/2021. Essa Autorização não substitui o Certificado Experimental de realização ética da pesquisa, necessitando do encaminhamento do Relatório Final de execução do Projeto para sua emissão.

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Cascavel, 08/03/2021

Profa. Dra. Luciana Oliveira de Fariña
Coordenadora do CEUA
Portaria nº 3126/2018-GRE

Anexo B- Normas da Revista Científica

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[2] J. van der Geer, J.A.J. Hanraads, R.A. Lupton, 2018. The art of writing a scientific article. Heliyon. 19, e00205. https://doi.org/10.1016/j.heliyon.2018.e00205. Reference to a book:

[3] W. Strunk Jr., E.B. White, The Elements of Style, fourth ed., Longman, New York, 2000. Reference to a chapter in an edited book:

[4] G.R. Mettam, L.B. Adams, How to prepare an electronic version of your article, in: B.S. Jones, R.Z. Smith (Eds.), Introduction to the Electronic Age, E-Publishing Inc., New York, 2009, pp. 281–304. Reference to a website:

[5] Cancer Research UK, Cancer statistics reports for the UK. http://www.cancerresearchuk.org/ aboutcancer/statistics/cancerstatsreport/, 2003 (accessed 13 March 2003). Reference to a dataset: [dataset]

[6] M. Oguro, S. Imahiro, S. Saito, T. Nakashizuka, Mortality data for Japanese oak wilt disease and surrounding forest compositions, Mendeley Data, v1, 2015. https://doi.org/10.17632/ xwj98nb39r.1. Reference to software:

[7] E. Coon, M. Berndt, A. Jan, D. Svyatsky, A. Atchley, E. Kikinzon, D. Harp, G. Manzini, E. Shelef, K. Lipnikov, R. Garimella, C. Xu, D. Moulton, S. Karra, S. Painter, E. Jafarov, S. Molins, Advanced Terrestrial Simulator (ATS) v0.88 (Version 0.88), Zenodo, March 25, 2020. https://doi.org/10.5281/ zenodo.3727209.

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