UNIVERSIDADE ESTADUAL PAULISTA
"JÚLIO DE MESQUITA FILHO"
INSTITUTO DE BIOCIÊNCIAS DE BOTUCATU

MARIANA PIRANI ROCHA MACHADO

PARÂMETROS GASTRINTESTINAIS E METABÓLICOS NA OBESIDADE: EFEITOS TRANSGERACIONAIS E DE PLICATURA GÁSTRICA EM ROEDORES

Orientadora: Profa. Dra. Madileine F. Américo

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Tese apresentada ao Programa de Pós-graduação em Farmacologia e Biotecnologia do Instituto de Biociências, Campus de Botucatu, como exigência parcial para obtenção do título de Doutora em Farmacologia e Biotecnologia.

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1. Cirurgia bariátrica. 2. Dieta hiperlipídica. 3. Inflamação. 4. Motilidade gastrointestinal.

Palavras-chave: Cirurgia bariátrica; Contratilidade; Dieta hiperlipídica; Inflamação; Motilidade.

Dedicatória

Dedico este trabalho aos meus alunos e a toda minha família, em especial ao meu filho Davi, que ainda irá iniciar a sua jornada estudantil. Este trabalho é uma demonstração de que tudo que almejamos na vida conseguimos por meio de muito esforço e dedicação. O conhecimento transforma vidas!

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RESUMO

A obesidade paterna pode acarretar alterações na função gastrointestinal (GI) e tal efeito pode se manifestar também na prole masculina por questões epigenéticas. Além disso, tratamentos cirúrgicos da obesidade como a Plicatura Gástrica (PG) podem causar alterações no trânsito GI e em níveis hormonais, apesar de reduzirem o peso e quantidade de gordura corporal. Diante do exposto, empregar dois modelos de obesidade experimentais *in vivo* e a técnica biomagnética BAC, pode contribuir para a caracterização da motilidade gastrintestinal e de um tratamento cirúrgico reversível em obesos. Assim, os objetivos desse trabalho foram: 1) Avaliar comparativamente a função gastrointestinal e a morfologia em modelo experimental de obesidade paterna induzido por glutamato monossódico e duas gerações subsequentes de descendentes machos; 2) Verificar as funções metabólicas e gastrointestinais em modelo experimental de obesidade induzida por dieta hiperlipídica (High-Fat Diet - HFD) submetido à cirurgia de plicatura gástrica. Ratos obesos induzidos por MSG (F1) apresentaram diminuição na frequência e amplitude das contrações gástricas, enquanto ratos obesos da geração F2 mostraram MGET acelerado e MCAT e MSITT lentificados. Os níveis de glicose e leptina estavam aumentados em F1 e F2. Os níveis de insulina diminuíram nas gerações F1, F2 e F3. A morfometria duodenal mostrou-se alterada nas três gerações sugerindo que a obesidade pode ter transmissão transgeracional paterna. Em suma, foram observados distúrbios na função gastrointestinal de três gerações, ressaltando a importância de se considerar um ciclo paterno de obesidade para entender a extensão dos distúrbios da doença sobre a fisiologia gastrointestinal. Na segunda parte deste estudo, observou-se que a plicatura gástrica (PG) não foi capaz de reverter o esvaziamento gástrico lentificado presente em obesos e até piorou a variabilidade em torno da frequência média, sugerindo uma disritmia no período analisado após o procedimento. A PG possivelmente desencadeou remodelamento nas camadas mucosas e musculares das mucosas, tornando-se mais espessas e apresentando infiltrados inflamatório. Todavia, houve uma diminuição significativa dos níveis de leptina e do peso corporal, principalmente nos grupos obesos. Este estudo analisou as consequências para o trato GI do desenvolvimento de novas estratégias cirúrgicas para o tratamento, bem como da contribuição transgeracional na obesidade. Desta forma, os modelos de obesidade aqui adotados foram eficazes e ressaltaram a importante contribuição da função gastrointestinal sobre a manutenção do peso corporal e na saúde da prole. Nesse contexto, alterações na motilidade nem sempre são totalmente compreendidas ou consideradas relevantes durante a condução de estudos em obesos.

Palavras-chave: Dieta hiperlipídica, cirurgia bariátrica, contratilidade, inflamação, motilidade.

ABSTRACT

Paternal obesity can lead to changes in gastrointestinal (GI) function and this effect can also be manifested in male offspring due to epigenetic reasons. In addition, surgical treatments for obesity such as Gastric Plication (PG) can cause changes in GI transit and hormone levels, despite reducing weight and the amount of body fat. In view of the above, employing two experimental obesity models *in vivo* and the BAC biomagnetic technique can contribute to the characterization of gastrointestinal motility and a reversible surgical treatment in obese individuals. Thus, the objectives of this work were: 1) Comparatively evaluate gastrointestinal function and morphology in an experimental model of paternal obesity induced by monosodium glutamate and two subsequent generations of male offspring; 2) To verify the metabolic and gastrointestinal functions in an experimental model of obesity induced by a high-fat diet (High-Fat Diet - HFD) submitted to gastric plication surgery. MSG-induced obese rats (F1) showed decreased frequency and amplitude of gastric contractions, while F2-generation obese rats showed accelerated Mean Gastric Emptying Time (MGET) and slowed Mean Cecum Arrival Time (MCAT) and Mean Small Intestine Transit Time (MSITT). Glucose and leptin levels were increased in F1 and F2. Insulin levels decreased in the F1, F2 and F3 generations. Duodenal morphometry was altered in the three generations, suggesting that obesity may have transgenerational paternal transmission. In summary, disturbances in gastrointestinal function over three generations were observed, underscoring the importance of considering a paternal cycle of obesity to understand the extent of disease disturbances on gastrointestinal physiology. In the second part of this study, it was observed that gastric plication (GP) was not able to reverse the slowed gastric emptying present in obese and even worsened the variability around the mean frequency, suggesting a dysrhythmia in the period analyzed after the procedure. GP possibly triggered remodeling in the mucosal and muscular layers of the mucosa, becoming thicker and presenting inflammatory infiltrates. However, there was a significant decrease in leptin levels and body weight, especially in the obese groups. This study analyzed the consequences for the GI tract of the development of new surgical strategies for treatment, as well as the transgenerational contribution to obesity. Thus, the obesity models adopted here were effective and highlighted the important contribution of the gastrointestinal function on the maintenance of body weight and on the health of the offspring. In this context, changes in motility are not always fully understood or considered relevant when conducting studies in obese subjects.

Key words: bariatric surgery, contractility, high-fat diet, inflammation, motility.

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LISTA DE ABREVIATURAS E SIGLAS

BAC - Biosusceptometria de Corrente Alternada

CB – Cirurgia Bariátrica

CCK - Colecistocinina

cpm – Ciclos por Minuto

DIO – Obesidade Induzida por Dieta

EG – Esvaziamento Gástrico

GI – Gastrintestinal

HFD – High Fat Diet / Dieta hiperlipídica

ICC – Células Intersticiais de Cajal

IMC - Índice de Massa Corporal

MCAT - Mean Cecum Arrival Time / Tempo Médio de Chegada ao Ceco

MGET - Mean Gastric Emptying Time / Tempo Médio de Esvaziamento Gástrico

MSITT - Mean Small Intestine Transit Time/Tempo Médio de Trânsito de Intestino Delgado

MSG – Monosodium Glutamate / Glutamato Monossódico

PG – Plicatura Gástrica

PYY - Peptídeo YY

SNE – Sistema Nervoso Entérico

TGI – Trato Gastrointestinal

SUMÁRIO

Introdução

INTRODUÇÃO

1. Fisiologia do trato gastrointestinal

O trato gastrointestinal (TGI) inclui a cavidade oral, faringe, esôfago, estômago, intestino delgado e intestino grosso (REED & WICKHAM, 2009). As paredes do TGI são compostas essencialmente por mucosa (epitélio, lâmina própria e mucosa muscular), submucosa, muscular própria (músculo circular interno e músculo longitudinal externo) e serosa (TREUTING, ARENDS, DINTZIS, 2018). Os músculos lisos responsáveis pelos movimentos do TGI estão dispostos em uma camada circular interna e uma longitudinal externa. A contração da camada circular diminui o diâmetro do lúmen favorecendo digestão e absorção, enquanto a longitudinal encurta o segmento e contribui para a propulsão de força aboral do conteúdo (KUMRAL & ZFASS, 2018).

A regulação da função motora gastrointestinal é um processo complexo que envolve colaboração e comunicação de vários tipos de células, como neurônios entéricos, células intersticiais de Cajal (ICC), células musculares lisas, e ainda, alguns hormônios (AL-SHBOUL, 2013; KITAZAWA & KAIYA, 2019). As ICC são encontradas no TGI agindo como células marcapasso de motilidade intestinal e podem estar associadas a distúrbios de motilidade (SANDERS, WARD & KOH, 2014; WEI, PARSONS & HUIZINGA, 2017). O plexo mioentérico e ICCs atuam sobre as contrações fásicas, alternando entre o relaxamento distal e a contração proximal dos músculos (CAMILLERI et al., 2018; SANDERS, WARD & KOH, 2014; KAJI et al., 2018).

Uma série de funções essenciais são desempenhadas pelo TGI, incluindo digestão, absorção, secreção, peristaltismo, movimentos de mistura e segmentação, excreção e defesa. Muitas dessas funções são controladas por neurônios do sistema nervoso entérico (SNE), com modulação do sistema nervoso simpático e parassimpático, além de neurônios sensoriais (FURNESS et al., 2013; UESAKA et al., 2016). O SNE é composto

pelos plexos submucoso e mioentérico encontrados, respectivamente, entre as camadas submucosa e circular e entre as camadas de músculo liso circular e longitudinal ao longo da parede do intestino. É caracterizado por uma rede altamente conservada, mas complexa, de neurônios e células gliais, responsável por coordenar os processos digestivos e a homeostase gastrointestinal (LIAO, 2009; BREDENOORD; SMOUT; TACK, 2016; HOLLAND et al., 2021).

A motilidade gastrointestinal (GI) é um componente-chave da saúde digestiva e é um processo complexo, governado por muitos fatores fisiológicos (KITAZAWA & KAIYA, 2019). Métricas fisiologicamente relevantes, como frequência, amplitude e índice de motilidade, podem ser testadas *in vivo* e *in vitro* independentemente para quantificar as contrações do músculo liso (DU et al., 2019). Apesar da dificuldade de avaliação da contratilidade do músculo liso gastrointestinal *in vivo* pela dimensão do órgão e natureza relativamente frágil do tecido, é de suma importância para compreensão da função muscular (KITAZAWA & KAIYA, 2019). Existem estudos de contratilidade realizados a partir de preparações *in vitro* de alças isoladas de segmentos intestinais ou tiras das camadas musculares (KADOWAKI; WADE; GERSHON, 1996), e métodos *ex vivo* (LENTLE et al., 2012). Porém, é difícil extrapolar alterações na contratilidade observadas *in vitro* diretamente para condições in vivo, já que se deve considerar uma série de influências como nervos entéricos, presença de ICCs e moduladores endócrinos e imunológicos do organismo (KHAN; COLLINS, 2006).

Quaisquer alterações no funcionamento do TGI comprometem a farmacocinética de medicamentos e a absorção de nutrientes, tornando assim relevantes os estudos que avaliem a motilidade do TGI por meio de técnicas seguras e eficazes (MONTORO-HUGUET, BELLOC & DOMÍNGUEZ-CAJAL, 2021). O trânsito GI pode ser quantificado em modelos experimentais por meio de análise de substâncias incorporadas

à refeição teste como o deslocamento de carvão vegetal, corantes e substâncias não-absorvíveis (PADMANABHAN et al., 2013). Estudos de distribuição de refeições em modelos animais permitem realizar uma avaliação cuidadosa do trânsito do TGI (deslocamento de conteúdo ao longo dos segmentos), sendo útil para identificar vários distúrbios de motilidade (PINTO et al., 2021). Essas avaliações têm um papel importante, pois representam uma visão global das funções GI, podendo ser utilizadas para quantificação do esvaziamento gástrico e do trânsito intestinal (QUINI et al., 2012). Nesse sentido, o esvaziamento gástrico (EG) é um dos processos fisiológicos de deslocamento com controle mais complexo e que tem por finalidade a transferência do conteúdo alimentar do estômago para o duodeno (CAMILLERI, 2019).

Existem vários métodos para quantificar o EG em animais e humanos, mas é importante que estes sejam padronizados para estudos *in vivo* em modelos de doenças e tratamento por drogas, devendo atender ao máximo os preceitos éticos dos 3R. Para avaliação do EG, classicamente é realizado pela comparação do conteúdo residual de corante gástrico, como por exemplo pelo uso de compostos radioativos (cintilografia) ou vermelho de fenol, porém estes métodos requerem excisão do estômago do animal experimental (JORDI, VERRY & LUZ, 2014). A cintilografia tem sido considerada padrão-ouro para avaliar o EG tanto na pesquisa quanto na prática clínica (LEE et al., 2019). Todavia, devido à exposição à radiação ionizante, outros métodos validados foram desenvolvidos para roedores, sendo não letais e/ou que não utilizam radiação ionizante como por exemplo o teste de absorção de paracetamol e testes respiratórios com acetato de carbono 13 (CREEDON et al., 2013; MÜLLER, CANFORA & BLAAK, 2018). No entanto, ambos são indiretos e limitados pelo fato do metabolismo e da cinética de absorção entre os grupos experimentais.

Apesar de amplamente utilizadas, as técnicas supracitadas contrariam alguns preceitos éticos da experimentação animal devido ao uso de radiação, grande número de morte de animais, dentre outros (SANGNES et al., 2020). Por outro lado, a Biosusceptometria de Corrente Alternada (BAC) é uma técnica capaz de registrar a variação de fluxo magnético obtida a partir de material magnético ingerido e/ou fixado à parede do TGI do animal (QUINI, 2015; PINTO et al., 2021). A BAC permite a detecção e monitoramento de rastreadores magnéticos em sistemas biológicos, permitindo analisar a contratilidade gástrica, o esvaziamento e o trânsito gastrointestinal em humanos, cães e roedores (AMÉRICO et al., 2009; AMÉRICO et al., 2010; QUINI et al., 2012; GAMA et al., 2020; PINTO et al., 2021). Com essa técnica biomagnética é possível repetir análises em um mesmo animal, constituindo uma alternativa interessante para o estudo das propriedades do TGI devido à sua segurança e eficácia.

2. Modelos experimentais de obesidade e o TGI

O TGI é a interface chave entre nutrientes ingeridos e o corpo, desempenhando um papel principalmente na regulação da homeostase energética. Isto acontece a partir de sinais derivados do intestino, que transmitem informações sobre a entrada de nutrientes para o cérebro, ocasionando mudanças no comportamento alimentar e no gasto energético (MONTEIRO & BATTERHAM, 2017). Desta forma, o TGI tem sido considerado como iniciador dos eventos que contribuem para a inflamação sistêmica associada à obesidade, já que é uma região exposta a nutrientes excessivos e prejudiciais ingeridos, como o excesso de carboidratos e gordura que levam ao aumento da adiposidade (MAGNUSON et al., 2015; OZCAN et al., 2017).

A pesquisa sobre obesidade abrange modelos animais que apresentam vantagens e deficiências distintas (KLEINERT et al., 2018). Desta forma, para selecionar o melhor

modelo, deve-se considerar que cada espécie e gênero respondede forma diferente ou nem mesmo responde à obesidade induzida, seja pela dieta ou pelouso de substâncias químicas como o glutamato monossódico (MSG) (DE MOURA E DIAS et al., 2021).

Os modelos animais mais comuns de obesidade podem ser induzidos por dieta, modificação genética e por administração de MSG (LIANG et al., 2021). O momento da exposição dos animais a essas dietas e o tipo de dieta são cruciais, influenciando claramente os resultados. Em estudos para verificar influência da obesidade paterna, os efeitos são observados na geração F1 (transmissão intergeracional) e nas subsequentes (transmissão transgeracional) (DIMOFSKI et al., 2021).

A obesidade induzida por MSG pode desencadear efeitos tóxicos em vários sistemas biológicos na geração induzida (F1) (UTI et al., 2020; AFIFI et al., 2011), de modo que uma estratégia adotada é estudar sua prole. Nessa segunda geração, a toxicidade encontra-se diminuída, mas ainda há consequências no que se refere ao metabolismo e ao peso corporal destes. A obesidade induzida por MSG é considerada de início precoce, resultante de lesões no núcleo arqueado no hipotálamo de ratos neonatos causando danos ao centro de saciedade (YULYANINGSIH et al., 2017; LIANG et al., 2021). Estudos recentes buscam explorar os efeitos epigenéticos relacionados à obesidade em células espermáticas que podem causar doenças na prole (MCPHERSON et al., 2014; BELLASTELLA et al., 2019; HADDAD, ESMAIL & KHAZALI, 2021). Estado nutricional e a exposição ambiental (incluindo poluentes e produtos químicos) dos pais durante a infância e/ou o período periconcepcional têm consequências transgeracionais significativas (DIMOFSKI et al., 2021). No estudo de Zhou e colaboradores demonstraram efeitos intergeracionais da obesidade paterna na função cognitiva da prole por meio de modificações epigenéticas nos espermatozoides. Filhos de pais obesos pré-

concepcionais são propensos a desenvolver obesidade e/ou disfunção metabólica independente do peso da mãe (CAMPBELL & MCPHERSON, 2019; SHARP & LAWLOR, 2019).

Modelos animais de obesidade induzida por dieta (DIO) podem reproduzir o sobrepeso humano e a obesidade, e existem muitos protocolos usados para levar ao excesso de deposição de gordura (BORTOLIN et al., 2018). O modelo atual mais utilizado é induzido por dieta rica em calorias provenientes de lipídios/gorduras (conhecida como High Fat Diet – HFD), culminando em obesidade de início tardio causado pela ingestão excessiva de calorias (LIANG et al., 2021). Pais roedores alimentados com HFD podem transmitir disfunção das células β pancreáticas e intolerância à glicose para sua prole F1 fêmea (NG et al., 2010), e uma resposta alterada à insulina na prole de machos F1 (GRANDJEAN et al., 2016). Além disso, a exposição paterna à HFD pode levar a um risco aumentado de doença renal crônica (CHOWDHURY et al., 2016) e ao desenvolvimento de um fenômeno semelhante à síndrome metabólica (MASUYAMA et al., 2016).

Por sua vez, a obesidade pode alterar padrões da motilidade gastrointestinal (JACOBSON et al., 2006; NILSSON et al., 2003; FRIEDENBERG et al., 2008). Por exemplo, a taxa de EG é conhecida por ter influência na sensação de plenitude e saciedade e, portanto, tem sido sugerida estar associada à obesidade. Alguns estudos mostraram que o EG está aumentado em pacientes humanos obesos (WRIGHT et al., 1983; TOSETTI et al., 1996) e em modelo de obesidade experimental (LI, MA &WANG, 2011; LI, 2013; WAN, YIN, CHEN, 2019), e em outros estudos o EG apresentou-se reduzido na clínica (MADDOX et al., 1989), e em animais (VAZQUEZ et al., 2006; LI, MA &WANG, 2011). Em um estudo foi examinado o efeito do MSG sobre o EG e motilidade duodenal,

através de ressonância magnética rápida, como resultado foi observado que o MSG acelerou o EG facilitando a motilidade duodenal (NAKAYAMA & TERAMOTO, 2016). O TGI desempenha um papel fundamental na obesidade através de suas contribuições quanto à saciedade, produção de incretinas (GLP-1 - peptídeo-1 semelhanteao glucagon) e hormônios intestinais (como grelina, colecistocinina - CCK, e peptídeoYY) que influenciam o apetite. Estes fatores, por sua vez, podem afetar a glicemia pós-prandial, a absorção de nutrientes e por último determinam o balanço energético positivoque pode resultar em obesidade, além de alterações nos ácidos biliares, no microbioma e em produtos metabólicos da digestão de nutrientes (ácidos graxos de cadeia curta) que modificam fatores metabólicos associados à obesidade (ADAMSKA et al., 2014; CAMILLERI et al., 2017).

Vários hormônios medeiam a resposta do corpo à gordura na dieta. A CCK, o Peptídeo YY e a leptina inibem o apetite e induzem atraso no esvaziamento gástrico, enquanto a grelina é um estimulador do apetite e causa esvaziamento gástrico mais rápido (HELLSTRÖM et al., 2006; CAMILLERI, 2009). Em alguns animais induzidos por DIO apresentaram esvaziamento gástrico mais lento, sendo associado à redução dos níveis de grelina e aumento dos níveis de colecistocinina e leptina. Li e colaboradores (2011) sugeriram uma possível compensação com aumento da saciedade e diminuição da ingestão de alimentos para equilibrar o ganho de peso. Outro estudo com machos e dieta rica em gordura durante quatorze dias, demonstrou esvaziamento gástrico acelerado, mas sem alterações nos níveis de hormônios leptina, CCK, PYY e grelina (MUSHREF & SRINIVASAN, 2013). Enquanto Boyd e colaboradores defenderam que a dieta rica em gordura pode ter induzido mudanças na sensibilidade a esses hormônios ao invés de mudanças em suas taxas de secreção. Vázquez e colaboradores estudaram a associação entre o esvaziamento gástrico mais rápido em obesos e os níveis de leptina, PYY e grelina

e encontraram níveis mais elevados de leptina (possível resistência à leptina) e menor redução pós-prandial de grelina com níveis semelhantes de PYY em ambos os grupos.

Os impactos da DIO materna na adiposidade e no metabolismo da prole já estão bem estabelecidos, enquanto a extensão de qualquer contribuição de pais obesos não é clara (MORRIS, 2009; SOUBRY 2018; NG et al., 2010). Nesse sentido, é importante avaliar como a função do TGI pode ser afetada pela obesidade paterna transgeracional. Estudos em animais têm relatado que a obesidade induzida por HFD, bem como várias exposições ambientais pré-concepcionais afetam o desenvolvimento de células germinativas masculinas. Desta forma, epimutações adquiridas neste estágio pré-concepcional de desenvolvimento podem influenciar a saúde futura da prole (SOUBRY 2018).

3. Tratamento da obesidade experimental: protocolos cirúrgicos

As adaptações fisiológicas à perda de peso favorecem a recuperação do peso, devido a distúrbios nos níveis circulantes de hormônios relacionados ao apetite e homeostase energética, além de alterações no apetite e no metabolismo de nutrientes (ANASTASIOU, KARFOPOULOU, YANNAKOULIA, 2015). No tratamento da obesidade, para a manutenção da perda de peso efetiva e controle dessas adaptações fisiológicas, na maioria das vezes apenas modificações de dieta e exercício físico não são sustentáveis a longo prazo. Logo, medicamentos para perda de peso e procedimentos cirúrgicos comoa cirurgia bariátrica (CB) por alterarem a fisiologia da regulação do peso corporal são a melhor chance de sucesso a longo prazo (GREENWAY 2015).

A era da CB se desenvolveu na década de 1950, quando inicialmente se observou perda de peso após ressecções do intestino delgado (ANGRISANI et al., 2017). O refinamento das técnicas cirúrgicas, principalmente das minimamente invasivas, levou a

um aumento dos procedimentos cirúrgicos bariátricos na década de 2000. A banda gástrica, gastrectomia vertical, bypass gástrico em Y de Roux (RYGB) e derivação biliopancreática (DBP), com ou sem duodenal switch (DS), são os procedimentos bariátricos mais comumente realizados atualmente na prática clínica (Wu et al., 2019). Essas operações têm sido tradicionalmente categorizadas como procedimentos restritivos (banda e manga), disabsortivos (BPD, DS) ou procedimentos combinados restritivos e disabsortivos (RYGB) (LE ROUX & HENEGHAN, 2018). O bypass gástrico Roux-en-Y envolve a criação de uma pequena bolsa gástrica conectada ao intestino delgado para impedir boa parte da absorção de nutrientes (BUETER et al., 2012; MITCHELL & GUPTA, 2020).

A gastrectomia vertical ou sleeve gástrico (SG), consiste na remoção cirúrgica de 80% do estômago ao longo da grande curvatura, incluindo fundo, corpo e antro, com preservação do piloro, deixando um reservatório gástrico estreito. Isso restringe a distensão e promove a saciedade precoce, levando a tamanhos de porções drasticamente reduzidos (SHI et al., 2010). Comparada a RYGB, as cirurgias de banda gástrica e SG podem ter um risco maior de reganho de peso no acompanhamento a longo prazo, porém alcançam melhorias significativas no controle glicêmico, que se correlacionam diretamente com a quantidade de peso perdido (BUCHWALD, ESTOK, FAHRBACH et al., 2009; PATROKAKOS et al., 2009).

Estima-se que a obesidade seja responsável por 16,5% de todos os gastos médicos por ano para o sistema de saúde, sendo que possivelmente hoje as CB representam economia à saúde (WEINER et al., 2013; SCHROEDER, HARRISON, MCGRAW, 2016). Apesar das CB serem ainda o tratamento mais eficaz na obesidade, convém lembrar que estas induzem muitas vezes processo inflamatório, grandes mudanças na anatomia e na função do sistema digestivo, podendo gerar, consequentemente, numerosas

complicações, tanto gastrointestinais quanto nutricionais (BAPTISTA & WASSEF,2013; PICHE et al., 2015). Para isto, novos métodos cirúrgicos menos invasivos têm sido desenvolvidos para promover tratamento eficaz (RAMOS, 2010; MÜLLER et al., 2017). Recentemente, por exemplo, tem sido utilizada a plicatura da grande curvatura gástrica ou plicatura gástrica (PG) para redução do volume gástrico, sendo consideradamenos invasiva e menos dispendiosa do que outros procedimentos cirúrgicos bariátricos(EL SOUEIDY et al., 2021). A PG utiliza suturas que invaginam a grande curvatura, evitando grampeamento ou ressecção gástrica, e visa reduzir a morbidade (FRIED, 2012), permitindo a restrição mecânica à ingestão de alimentos (MÜLLER et al., 2017). A PG é uma técnica de CB baseada nos princípios anatômicos da videolaparoscopia gastrectomia vertical e pode produzir efeitos semelhantes na perda de peso, na regulação da ingestão e o metabolismo de carboidratos. Além disso, alguns estudos analisaram alterações seguindo a PG por certo tempo e relataram melhorias na homeostase da glicose juntamente com diminuições na hemoglobina glicosilada, insulina e grelina (BRADNOVA et al., 2014; CABRERA et al., 2018).

Metabolicamente, de forma geral, em todos os tipos de cirurgias bariátricas são encontradas quedas significativas na insulina, hemoglobina glicosilada (HbA1c), índice de massa corporal, triglicérides, colesterol e pressão arterial após cirurgia bariátrica (JI et al., 2021). E ainda, sabe-se que a obesidade é uma doença complexa associada ao aumento de vários marcadores inflamatórios, o que induz o quadro de inflamações crônicas de baixo grau da doença (KHANNA et al., 2022).

O estado inflamatório em indivíduos obesos pode ser medido pela quantificação de vários marcadores inflamatórios, como citocinas pró-inflamatórias (fator de necrose tumoral-α e interleucina-6), número de leucócitos e várias adipocinas (LAUTENBACH et al., 2021). No entanto, os estudos sobre as concentrações de citocinas/marcadores

inflamatórios após a cirurgia bariátrica têm sido inconsistentes. Enquanto alguns estudos observaram reduções nos níveis desses marcadores inflamatórios após cirurgia metabólica, outros não relataram tal declínio (BIOBAKU et al., 2020).

Finalmente, uma vez que vários estudos observaram um componente inflamatório na dualidade obesidade e estômago pós plicatura gástrica, as alterações nos parâmetros gastrointestinais e histológicos nestas condições devem ser analisadas com atenção. E mais, uma maior compreensão da fisiologia da perda/recuperação do peso aliadas aos mecanismos de regulação gastrointestinal através de procedimentos cirúrgicos podem compor um importante pilar para o desenvolvimento de estratégias para apoiar indivíduos com sobrepeso e obesos em seus esforços para alcançar e manter a perda de peso.

Os efeitos da obesidade transgeracional e de procedimentos cirúrgicos novos como a plicatura gástrica sobre a função GI não foram elucidados em termos motores e morfológicos, e ainda precisam ser adequadamente caracterizados. A obesidade paterna acarreta alterações na função gastrointestinal e tal efeito pode se manifestar também na prole masculina. A PG pode causar alterações no trânsito do TGI e em níveis hormonais, enquanto reduz o peso e quantidade de gordura corporal. Diante do exposto, o estudo de modelos de obesidade experimentais *in vivo*, empregando a técnica biomagnética BAC, podem trazer muitas contribuições para a caracterização da motilidade gastrintestinal e tratamentos em obesos. Assim, os objetivos desse trabalho foram:

1) *Avaliar comparativamente a função gastrointestinal e a morfologia em modelo experimental de obesidade paterna induzido por glutamato e duas gerações subsequentes de descendentes machos (Capítulo 1).*
2) *Verificar as funções metabólicas e gastrointestinais em modelo experimental de obesidade induzida por dieta hiperlipídica (HFD) submetido à cirurgia de plicatura gástrica (Capítulo 2).*

Capítulo 1

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Paternal obesity and its transgenerational effects on gastrointestinal function in male rat offspring

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Abstract

The interplay between obesity and gastrointestinal (GI) motility is contradictory, and the transgenerational influence on this parameter is unknown. We aimed to evaluate the GI function in a model of paternal obesity and two subsequent generations of their male offspring. Newborn male rats were treated with monosodium glutamate (MSG) and composed the F1 generation, while control rats (CONT) received saline. At 90 days, male F1 were mated with non-obese females to obtain male offspring (F2), which later mated with non-obese females for obtaining male offspring of F3 generation. Lee Index analysis was adopted to set up the obesity groups. Alternating current biosusceptometry (ACB) technique was employed to calculate GI transit parameters: mean gastric emptying time (MGET), mean cecum arrival time (MCAT), mean small intestinal transit time (MSITT), and gastric frequency and amplitude of contractions. Glucose, insulin, and leptin levels and duodenal morphometry were measured. F1 obese rats showed a decrease in the frequency and amplitude of gastric contractions, while obese rats from the F2 generation showed accelerated MGET and delayed MCAT and MSITT. Glucose and leptin levels were increased in F1 and F2 generations. Insulin levels decreased in F1, F2, and F3 generations. Duodenal morphometry was altered in all three generations. Obesity may have paternal transgenerational transmission, and it provoked disturbances in the gastrointestinal function of three generations.

Key words: Epigenetics; Gastric emptying; Intestinal transit; Monosodium glutamate

Introduction

Obesity is a multifactorial disease and has become a global pandemic. Over 650 million obese people (1,2) face a complex interplay between biological, epigenetic, psychosocial, environmental, and industrial factors (2,3).

Both non-genetic effects and genetic inheritance as well as epigenetic dysregulation can contribute to the predisposition to obesity (4). In this context, parental health or exposures can affect the lifetime health outcomes of offspring (4,5). Since maternal effects have been the focus of the transgenerational transmission of metabolic disorders (5–9), the role of paternal obesity in offspring metabolic programming has been investigated but still not fully elucidated (8,10).

Experimental models might contribute towards understanding the pathophysiological mechanisms and consequences concerning transgenerational obesity (6,9). When administered to neonatal rats, monosodium glutamate (MSG) is recognized to cause obesity and clinical manifestations of metabolic syndrome (6,11). MSG provokes hypothalamic lesions resulting in several endocrine disturbances, stunted growth, and obesity (7,12). MSG-induced maternal obesity results in spontaneously obese animals in the second generation (6).

Obesity is often associated with complications that affect cardiovascular, endocrine, and gastrointestinal (GI) systems (2,13,14). GI motility, in particular, plays a very critical role in the digestion and absorption of nutrients (15), and also participates in the control of appetite and satiety (13). However, the relationship between obesity and GI motility is conflicting, and transgenerational effects have not been evaluated properly (10,16). We hypothesized that obesity can alter parenteral GI function and

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such effect can also manifest in the male offspring. The aim of this study was to evaluate the GI function in a model of paternal obesity and in two subsequent generations of their male offspring.

Material and Methods

Experimental protocol

All experiment procedures were approved by the Ethics Committee on Animal Research of the Federal University of Mato Grosso (protocol number 23108. 705702/13-9), following the 3Rs principle (Replacement, Reduction, and Refinement) of animal research. Wistar rats had free access to water and standard diet (Labina Presence®, USA) and were kept in individual cages under controlled conditions of temperature (22 ± 1°C), humidity (50 ± 10%), and a 12-h light/dark cycle.

For obesity induction, male neonatal Wistar rats received 4 mg/g body weight subcutaneous (sc) injections of MSG (Sigma-Aldrich, USA) on days 2, 4, 6, 8, and 10 of postnatal life. Control Wistar rats received subcutaneous injections of saline solution (0.9% NaCl) on the same days (6,9). Both groups were weaned at 21 days of age and maintained in controlled conditions.

The Lee index was applied to all the male rats at 90 days of life. This index is defined as the cube root of body weight (g) $\times$ 10 / naso-anal length (cm) and values >0.300 are classified as obese (6,9). All obese animals included in the MSG group were defined as F1 generation.

Obese rats of F1 generation were mated with non-obese adult female rats (90 days of age) and upon pregnancy detection (sperm-positive confirmation), pregnant rats were housed in individual cages. At 90 days of life, the male offspring from F1 were classified using the Lee index and established the F2 generation. Subsequently, the F2 generation rats, at 90 days of life, were mated with non-obese adults female rats (90 days of age), and their male offspring were also classified using the Lee index establishing the F3 generation.

The experimental groups were categorized as: Control group (CONT, n=14), MSG-induced obese rats (F1, n=14), Obese offspring (F2, n=14), and Grand offspring (F3, n=14). After assessing the Lee index, the animals from each generation had gastrointestinal motility, metabolic, and histological parameters evaluated and compared with the non-obese control group. All the variables were analyzed at the same age in all animals.

Gastrointestinal motility

GI motility was evaluated by the alternating current biosusceptometry (ACB) technique. ACB uses non-invasive and radiation-free sensors to record variations on the biomagnetic field generated by an ingested magnetic material. The intensity of the signal is proportional to the amount of magnetic material and depends on the distance to the sensor (17). To measure the GI parameters (contractility and transit), all the animals fasted for 12 h. From then on, they were fed with a magnetic meal (1.6 g commercial chow plus 0.4 g ferrite powder) before the measurements. Ferrite ($MgZnFe_2O_3$, Imag, Brazil) is an inert ferromagnetic material that is not absorbed by the GI tract and is used as a magnetic marker. The ACB sensor was used to monitor the magnetic signals on the abdominal surface at 15 min intervals for at least 6 h (18).

For gastric contractility records, all the animals fasted for 12 h. Afterward, they ingested the magnetic meal described above and were anesthetized with 100 mg/kg ketamine (Cetamin®, Syntec, Brazil) plus 2.5 mg/kg acepromazine (Acepran®, Vetnil, Brazil), intraperitoneally. The animals were laid in a supine position and the ACB sensor was placed on the animal's abdominal surface in the corresponding anatomic region of the stomach. Magnetic signals were recorded continuously for 30 min at a sampling rate of 20 Hz, using a multichannel recorder (MP100 System; BIOPAC, USA) (17).

Magnetic data analysis

To quantify the GI transit, the statistical moment was applied (19). The temporal average determined by magnetic intensity curves normalized by the area under the curve was employed to quantify the following parameters: mean gastric emptying time (MGET), which was defined as the time t (min) when a mean amount of magnetic meal was emptied from the stomach; mean cecum arrival time (MCAT), defined as the time t (min) when an increase occurred in the mean amount of magnetic meal that arrived in the cecum; and mean small intestinal transit time (MSITT), which was quantified by the difference between MCAT and MGET (18).

To quantify the gastric contractility parameters, all magnetic signals were analyzed in MatLab (Mathworks, Inc., USA) by visual inspection and thereafter using bi-directional Butterworth band-pass filters by Fast Fourier Transform (FFT). The highest frequency peak for each FFT was defined as the gastric dominant frequency and the smallest, as the signal noise. The amplitude of contraction (A) was calculated by the relationship between the power of gastric peak (P) and power of noise peak (P') and is expressed in decibels (dB) as follows: $A = 10 \log_{10} (P/P')$ (20).

Serum and tissue sample collection

At the end of the GI motility evaluation, the animals were sacrificed at 120 days of life by anesthetic overdose through an intraperitoneal injection of ketamine (240 mg/kg, Cetamin®, Syntec) plus xylazine hydrochloride (45 mg/kg, Dopaser®, HertapeCalier, Brazil), followed by decapitation. Blood serum was sampled and stored under –80°C refrigeration until further assays.

Histological preparations and measurements

Duodenum tissue samples were carefully collected and fixed with Metacarn (60% methanol, 30% chloroform, and 10% glacial acetic acid), dehydrated with serial alcohol, diaphanized in xylol, and embedded in paraffin. Histological cuts were performed with Micron HM 355S Automatic Microtomer (Thermo Scientific, Germany) with 4-μm thickness. The sections were stained with hematoxylin-eosin (H&E) and the images were captured on an optical microscope (Zeiss, Germany) coupled to a high-resolution camera (AxioCam ERc5s, Zeiss). Fifteen images per animal were randomly selected for morphometric analysis of the thickness of circular muscle and longitudinal muscle layers, villus height, and crypt depth using ImageJ software (NIH, USA). The villus height:crypt depth ratio was calculated (20).

Glucose, insulin, and leptin serum quantifications

Glucose levels were measured immediately after serum collection by Glucose Liquiform enzymatic kit (Labtest, Brazil). ELISA immunoassays were used for leptin (Rat Leptin kit, Invitrogen, USA) and insulin (Rat Insulin kit, Elabscience Biotechnology Co. Ltd., USA) serum concentration analyses according to manufacturers' instructions.

Statistical analysis

Kolmogorov-Smirnov test was employed to assess the normality of data. The data are reported as means ± SD with a limit of statistical significance up to 5% ($P<0.05$). Analysis of variance (ANOVA) followed by Tukey's *post hoc* test was performed for the multiple comparisons.

Results

Table 1 shows the frequency of obesity, Lee index, and body weight for all groups studied. From the MSG-induced obese rats (F1), 73.3% of offspring in the F2 generation, and 7.0% of grand offspring in the F3 generation were obese, according to the parameters evaluated. Considering these results, only obese animals from F1 and F2 generations, and non-obese animals from F3 generation continued in the study because they were the large majority in each group. Furthermore, body weight was significantly higher for F1 and F2 groups (obese generations) compared with CONT group, whereas body weight of F3 generation (non-obese) was not different from the CONT group.

Figure 1 shows the glucose, insulin, and leptin levels for F1, F2, and F3 generations. Glucose levels were higher for the obese groups, MSG-induced obese rats (F1) and F2 generation offspring compared with CONT and F3 ($P<0.001$). In addition, F2 showed a slightly lower glucose level compared with F1 ($P<0.05$). No differences were found between F3 generation and CONT groups. In F1 obese and F2 obese groups, insulin levels were lower compared to CONT ($P<0.05$). Insulin levels for F3 were

Table 1. Frequency of obesity, Lee index, and body weight of Control (CONT), monosodium glutamate-induced obese rats (F1), obese offspring (F2), and grand offspring (F3) groups.

	CONT	F1	F2	F3
Obesity in the generations	0% (n=14)	100% (n=14)	77.8% (14/18)	7.0% (1/15)
Obesity in the experimental groups included in analysis	0% (n=14)	100% (n=14)	100% (n=14)	0% (n=14)
Lee index	<0.300	0.313 ± 0.010	0.325 ± 0.020	<0.300
Body weight (g)	296.1 ± 23.1	341.8 ± 20.7*	336.2 ± 60.8*	329.8 ± 26.4

Only the 14 obese animals from F2 and the 14 non-obese animals in F3 were included in the analysis. Data are reported as means ± SD. *$P<0.05$ compared with CONT (ANOVA followed by Tukey's *post hoc*).

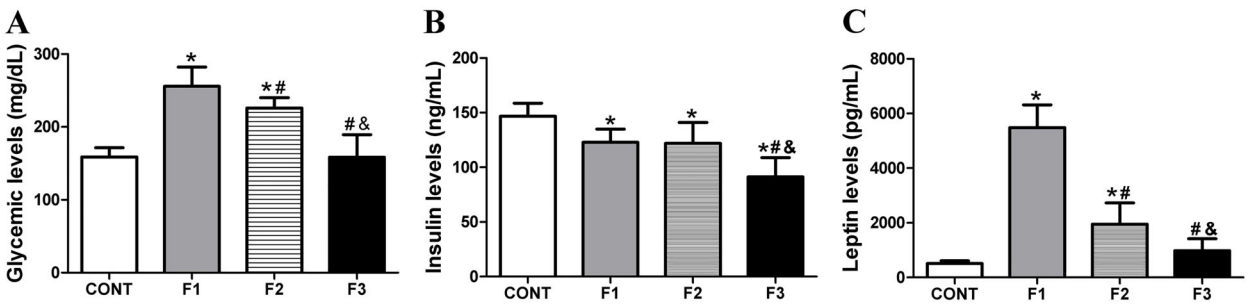

Figure 1. Glucose (**A**), insulin (**B**), and leptin (**C**) levels for Control group (CONT), monosodium glutamate (MSG)-induced obese rats (F1), obese offspring (F2), and grand offspring (F3). Data are reported as means ± SD (n=14 per group). *$P<0.05$ compared with CONT, #$P<0.05$ compared with F1, and &$P<0.05$ compared with F2 (ANOVA followed by Tukey's *post hoc*).

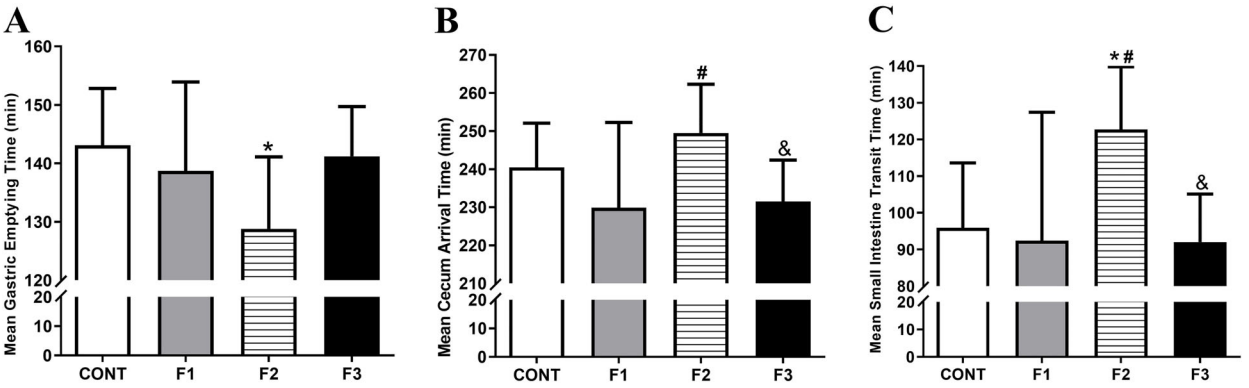

Figure 2. Gastrointestinal transit parameters evaluated for the Control group (CONT), monosodium glutamate (MSG)-induced obese rats (F1), obese offspring (F2), and grand offspring (F3). Mean gastric emptying time (MGET, **A**), mean cecum arrival time (**B**); mean small intestine transit time (**C**). Data are reported as means ± SD (n=14 per group). *$P<0.05$ compared with CONT, $^{\#}P<0.05$ compared with F1, and $^{\&}P<0.05$ compared with F2 (ANOVA followed by Tukey's *post hoc*).

significantly lower compared with CONT ($P<0.01$), F1 ($P<0.05$), and F2 ($P<0.01$) generations. Leptin levels were increased in the F1 and F2 obese groups compared to CONT ($P<0.001$ and $P<0.01$, respectively). The F1 group presented higher leptin levels than the F2 generation ($P<0.001$). Rats in the F3 generation had no difference in leptin levels compared to the CONT group.

The GI transit parameters evaluated in all groups are shown in Figure 2. The F2 obese group had accelerated gastric emptying compared with the CONT group ($P<0.05$). No difference was found among F1, F2, and F3 groups for gastric emptying time. Cecum arrival in F2 was delayed compared with F1 ($P<0.05$) and F3 groups ($P<0.05$), although it was not different from the CONT group. The small intestine transit time in the F2 group was delayed compared with CONT, F1, and F3 groups ($P<0.05$).

Gastric contractility parameters evaluated in all groups are shown in Figure 3. In the F1 group, the frequency of gastric contractions was reduced compared with CONT, F2, and F3 groups ($P<0.01$). No significant difference was found between the F2 and F3 generations compared to the CONT group. The amplitude of gastric contractions was similar between the CONT and F3 groups (41.30 ± 4.78 dB and 43.10 ± 8.10 dB), and both were significantly higher compared with the F1 generation (33.65 ± 3.43 dB; $P<0.05$ and $P<0.01$, respectively).

Representative H&E-stained duodenum tissue and the histological and morphometric analyses are presented in Figure 4. The thickness of the circular muscle layer for the F1 and F3 groups was thinner compared with the CONT group ($P<0.01$), and in F1 it was significantly thinner than F2 ($P<0.01$). No significant difference was observed in the F2 group compared with the CONT group. In the longitudinal muscle layer, the thickness was even thinner in F1, F2, and F3 groups compared with CONT ($P<0.01$). All the generations presented both lower crypt depth and

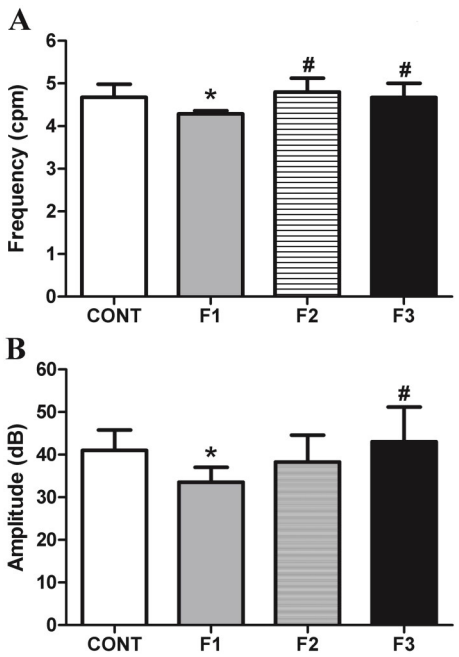

Figure 3. Gastric contractility parameters calculated for the Control group (CONT), monosodium glutamate (MSG)-induced obese rats (F1), obese offspring (F2), and grand offspring (F3). Frequency in contractions per min (cpm) (**A**) and amplitude (**B**) of gastric contractions. Data are reported as means ± SD (n=14 per group). *$P<0.05$ compared with CONT, $^{\#}P<0.05$ compared with F1 (ANOVA followed by Tukey's *post hoc*).

villus height compared with CONT ($P<0.001$). The F2 group presented greater villus height compared with the F1 and F3 groups ($P<0.01$). In this regard, villus height to crypt depth ratios in F1 and F3 groups were smaller compared with CONT ($P<0.01$). The F2 generation presented a higher villus height to crypt depth ratio

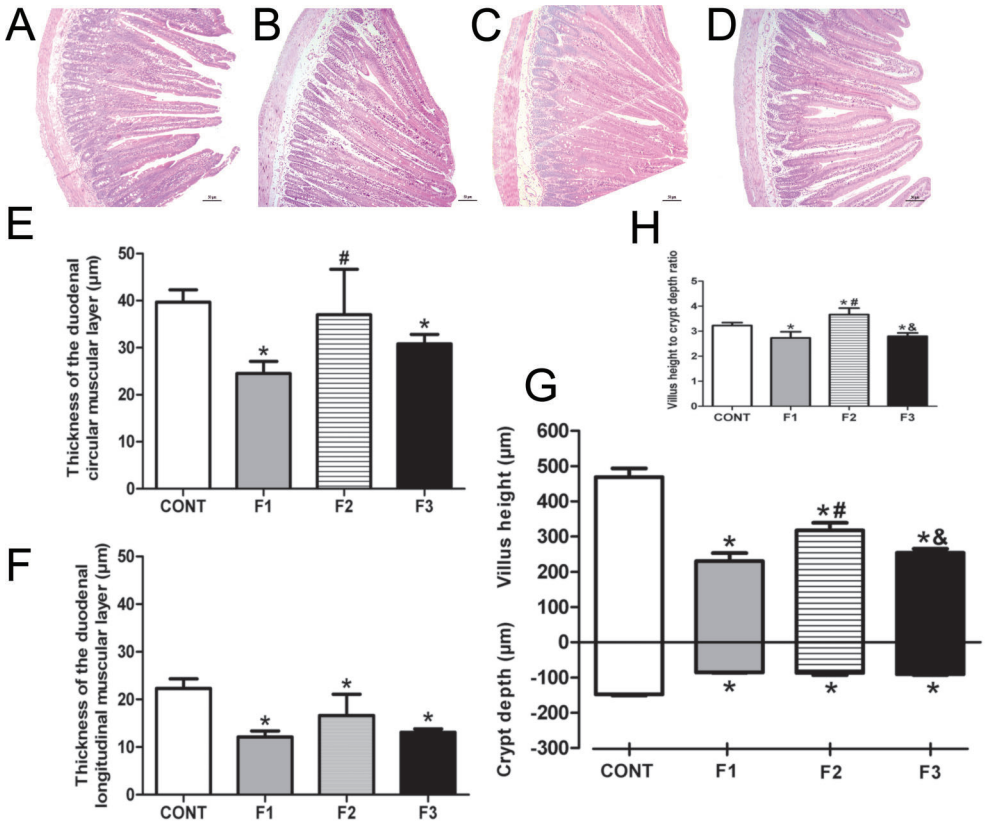

Figure 4. Histological photomicrographs of H&E staining of the duodenum (**A**–**D**) of Control (CONT), monosodium glutamate (MSG)-induced obese rats (F1), obese offspring (F2), and grand offspring (F3) (× 10 objective, scale bar, 50 µm). Quantification of the thickness of the duodenal circular muscle layer (**E**) and longitudinal muscle layer (**F**), crypt depth, villus height (**G**), and villus height to crypt depth ratio (**H**). Data are reported as means ± SD (n=14 per group). *$P < 0.05$ compared with CONT, $^{\#}P < 0.05$ compared with F1, and $^{\&}P < 0.05$ compared with F2 (ANOVA followed by Tukey's *post hoc*).

compared with all groups ($P < 0.01$ compared to CONT and $P < 0.001$ compared to F1 and F3).

Discussion

In this study, we demonstrated for the first time that MSG-induced obesity led to paternally derived, trans-generational obesity, and gastrointestinal alterations in male rats. The MSG-induced obese rats (F1) and their obese offspring generation (F2) showed disorders in GI motility with increased glucose and leptin levels. No functional changes or obesity phenotype was observed in the grand offspring generation (F3). All generations had changes in the muscular layers and duodenal mucosa, strengthening the extent of the disturbances driven by obesity.

Body weight was significantly higher for the F1 and F2 generations (obese rats) compared to the control group, whereas the F3 generations, characterized as non-obese, presented body weight that was not different from the control. The percentage of obesity was 100.0, 77.8, and 7.0% for the F1, F2, and F3 generations, respectively. This pattern indicated that MSG-induced obesity leads to paternally derived obesity in the subsequent generation (F2), with minor effects in the grand offspring generation (F3). Studies have shown that neonatal administration of MSG sets a precedent for the development of obesity due to the hypothalamic lesion resulting in a Lee Index with values higher than 0.300, typical physical characteristics, and delay in growth (12).

There are important interactions among adipose tissue, gut hormones, central nervous system, and GI tract in the development and maintenance of obesity (14,21). The investigation of the relationship between obesity and GI motility remains inconsistent and scarce, despite that it could represent an opportunity for novel therapeutic approaches for the treatment of obesity or other GI diseases (13,14).

Gastric emptying plays an important role in the intestinal exposure to nutrients and satiety (22,23), and it is closely related to glycemic control since it acts as a major determinant of postprandial glycemic regulation (24). In our study, both the F1 and F2 generations had

changes in gastric motility and, in parallel, their glycemic levels were higher compared to the CONT and F3 groups. Our group has already demonstrated the relationship between these variables in mildly diabetic rats (25). The severity of gastric dysmotility and gastric repercussions seems to be inversely proportional to glycemic control (26).

Small bowel transit regulates nutrient absorption and seems to be related to the development of obesity (23). In our study, the F2 generation had a delayed MCAT and MSITT compared to all groups, which reinforced that some programmed effects can be transmitted to subsequent generations (27). As the absorption of nutrients from the small intestine is based on the efficiency of both digestive and epithelial transport mechanisms and the area of the intestinal mucosa, it is supposed that a delay in the intestinal transit may increase absorption and weight gain (15,23). Other studies have shown that small intestinal transit (13,21) and intestinal myoelectrical activity (13) were not affected by obesity induced by a high-fat diet (HFD).

Such conflicting findings regarding GI motility reported in obesity may be justified by different methodologies, analyses of the experimental data, and different species and/or strains used as animal models (13,28). Although several methods are available to evaluate GI motility, most of them are invasive or restricted by ethical issues (29). ACB is a magnetic technique validated to evaluate GI motor functions in rodents repeatedly (17,18,20). It is non-invasive and allows recording the GI motility physiologically in real time, without interfering with the system itself.

Despite motility changes, the gut is a target for histologic changes that need to be evaluated to handle the impact of the disease on different segments. In our study, the thickness of the longitudinal muscle layer in the duodenum was reduced in all generations; however, the circular muscle was reduced only in the F1 generation. Another study also observed a decrease of the small intestine wall thickness in MSG-induced obese rats, but subsequent generations were not evaluated (30).

Muscular layers offer different contributions to the intestinal motor patterns: the circular layer acts on the mixing motions of the luminal content, whereas the propulsion activity is triggered by the longitudinal layer (31). Based on these findings, we assume that thinner muscular layers could have slowed the intestinal transit in the F2 group. In addition, disorders on the myenteric plexus may also contribute to the damage on GI transit, since it is located between the longitudinal and circular layers of the muscular layer (32).

In duodenal mucosa, the height of the villi and the depth of the crypt as well as the villus:crypt ratio of the F1 and F3 generations were reduced. In the F2 group, there was a higher villus:crypt ratio compared with CONT, despite the height of the villi and depth of crypt being reduced. The small intestine absorptive function also depends on the villus distribution, and enterocyte and crypt integrity (33). Hence, while such a function may be compromised in F1 and F3, the greater villus height in F2 may have been an adaptive morphometric characteristic of this spontaneous obese generation to increase the intestinal absorptive area. The ileum of the MSG-induced obese mice had lower expression levels of the junction proteins increasing the intestinal permeability, suggesting that the damage of the intestinal barrier induces systemic inflammation (34).

Peptides secreted by adipocytes can modulate GI motility by acting on the central and enteric nervous system (14). Leptin is produced by adipose tissue and exerts its primary effect on the hypothalamic nuclei to regulate food intake and body weight (35). In addition, leptin seems to influence gastric emptying and small intestine motility (21,35). Obese rats from F1 and F2 generations had higher leptin levels compared to CONT and F3 groups. Specifically, in the F1 generation, leptin concentrations were higher compared to F2. There was no difference between leptin levels from F3 and CONT groups. Thus, we believe that this may be one of the factors involved in the changes in GI motility that was observed in obese generations.

In our study, a reduction in the serum insulin concentrations in the F1, F2, and F3 generations compared to CONT was observed. Insulin secretion dysfunction seems to be a more permanent change across generations (7) and could be a characteristic inherited from an obese father. In female offspring from a father submitted to the HFD with normal adiposity, β-cell alterations with impaired insulin secretion and glucose tolerance have been observed, showing the intergenerational transmission of the metabolic sequel from father to offspring (36).

It is important to investigate the paternal implications of the onset of obesity and other metabolic disorders in offspring. MSG has shown toxic effects on several systems (11,37), and offspring from MSG-induced obese rats can be used as a model for obesity studies since it impacts on offspring metabolism and body weight and can be compared to other models (6,37).

In humans, overweight parents are considered as a risk factor for offspring overweight/obesity (38). Children from obese parents have an increased risk for obesity, reaching 80% when both parents are obese, 40% when only one parent is obese, and 10% when none is obese (39). In experimental studies, 80% of offspring rats from obese females were also obese (6). In our study, the F2 generation also showed a similar obese percentage in males, corroborating the strong paternal role in the transmission of obesity as pointed out in human studies (40). Thus, our data showed that the father can onset the intergenerational transmission of obesity induced or inherited, and this condition can also affect the gastrointestinal function. Furthermore, our study can contribute to new approaches in the field of developmental programming by

considering the influence of paternal obesity and its transgenerational effects on offspring health.

In conclusion, MSG-induced obese rats and their obese offspring generations had disorders in the gastrointestinal function associated with increased levels of glucose and leptin parallel to the reduction of insulin levels. From the third generation, GI variables were similar to control animals, despite morphometry being altered in all three generations. Our data added new insights about the physiology of obesity in rats and contributed to understanding the extent of the disturbances in gastrointestinal physiology provoked by a paternal cycle of obesity.

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Capítulo 2

GASTRIC PLICATION SURGERY CHANGES GASTROINTESTINAL AND METABOLIC PARAMETERS IN AN OBESITY-INDUCED HIGH FAT DIET MODEL

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ABSTRACT

Background: Obesity has grown worldwide, and treatment includes less invasive procedure as the gastric plication (GP) surgery. However, effects of surgical interventions in gastrointestinal (GI) motor parameters are underestimated.

Objective: Our study aiming to verify metabolic and gastrointestinal functions in experimental obesity model submitted to a gastric plication surgery.

Setting: Brazil.

Methods: Obesity was induced in male Wistar rats adopting a high-fat diet (HFD) for about 12 weeks. Four experimental groups were set up: Control sham (n=7); Control GP (n = 10), Obese sham (n = 6), and Obese GP (n = 10). Over thirty days were analyzed nutritional and murinometric parameters, Oral Glucose Tolerance Test, gastric motility (Mean Gastric Emptying Time, gastric frequency, amplitude of contractions and half-bandwidth), adiposity index, total fat weight, leptin, and lipoproteins levels. Data were analyzed by ANOVA followed by Tukey ($p<0.05$).

Results/Discussion: Obese groups showed a high index of adiposity and a relative weight of the total fat above of controls. In relation to leptin levels, this parameter increased in obese animals, being improved after GP surgery. The mean gastric emptying time was longer in obese groups, and GP was no able to modify this parameter. Although no changes in gastric frequency and amplitude contractions, the half-bandwidth of gastric contractions increased in obese GP group. There was an increasing in the thickness of mucosa and muscularis mucosa layers, in addition to the presence of inflammatory infiltrates mainly in mucosal layers after GP. GP decreased the energy intake, in obese groups, confirmed by the greater food efficiency and significantly decreased the body weight in all experimental groups submitted to the surgery.

Conclusion: Gastric plication decreased the leptin levels and the body weight significantly, mainly in obese groups. GP did not reverse slower gastric emptying observed in obese and even worsened variability around the mean gastric frequency. Our results suggesting a dysrhythmia after procedure that could be associated to inflammatory infiltrates. GP procedure possibly triggered remodeling in the mucosa and muscular of the mucosa layers which reinforcing the importance of evaluate gastrointestinal motility to upgrade the treatment of obesity.

Keywords: bariatric surgery, gastrointestinal tract, obesity, western diet.

1. INTRODUCTION

Obesity is a serious, costly condition and it increases the risks for multiple contracting diseases (DE LORENZO et al., 2019; HECKER et al., 2022). Obesity may be viewed as a multifactorial pathology and chronic low-grade inflammatory disease (De Lorenzo et al., 2019)). Clinically it is characterized as a nutritional and metabolic disorder that culminates in abnormal or excessive body fat (adiposity) that may impair health and reflects in an increase in body weight (WHARTON et al., 2020). The prevalence of obesity and its complications have reached epidemic proportions globally (ASSADI et al., 2022).

Bariatric surgery modalities are still the most effective and sustainable treatment for obesity (STOICA et al., 2021) and novel methods less invasive have been developed to avoid the potential problems of injuring or drying out the gastric wall (MÜLLER *et al.*, 2017). Recently, large gastric curvature plication or also called gastric plication (GP) has been used to reduce gastric volume. This technique uses sutures that invade the great curvature, avoiding stapling or gastric resection, and reducing the morbidity (EL SOUEIDY et al., 2021). GP reduce stomach capacity and allow mechanical restriction on food intake leading to weight loss (EL SOUEIDY et al., 2021), and improving plasma glucose levels (STOICA et al., 2021).

However, bariatric interventions induce underestimated complications in the anatomy and function of the gastrointestinal tract (GI) that are poorly understood (MESUREUR & ARVANITAKIS, 2017). GI has essential functions such as digestion, nutrient absorption, excretion, hormone production, drug absorption and immunological protection (KIELA & GHISHAN, 2016). Currently, the GI tract has been considered as

starter of systemic inflammation associated with obesity, since is the first interface between the body and the diet (KHOSHBIN & CAMILLERI, 2020).

Animal models are employed to study disorders in gastrointestinal motility after surgical intervention for obesity treatment (AL-SAFFAR et al., 2019). Several methods are available to assess the motor parameters, but generally these methods are invasive and incompatible with ethical principles due to the use of radiation, number of animal deaths, among others (DALLAGNOL et al., 2020). Alternating Current Biosusceptometry (ACB) is a validated biomagnetic alternative for the study of the properties of GI in clinical, physiological, and pharmaceutical research (GAMA et al., 2020; PINTO et al., 2021).

After consumption of a high-fat diet (HFD), in rats and human classified as obese, showed a slower gastric emptying (GE) rate and a disturbed hormonal response to fat that resulting in delayed satiety (MARTINEZ-GURYN et al., 2019). Other studies have shown a slower GE rate in parallel with a reduction in plasma ghrelin and increasing in leptin levels (LI, MA & WANG, 2011). The HFD exposure is associated with dyslipidemia, which involves higher triglycerides, total cholesterol, and LDL concentrations, as well as a reduction in HDL-cholesterol levels (MORAES-SOUZA et al., 2021). And yet, gastric dysmotility appears to be predominantly dependent on epigenetic modifications and can be associated to inflammatory infiltrates (KAYA, 2021).

Our hypothesis is that PG can accelerate the rate of gastric emptying, increase the frequency and/or amplitude of gastric contractions and contribute to the reduction of body weight and adiposity index. In addition, it improved feed efficiency and energy consumption, decreasing leptin, lipid (except HDL-c) and glycemic levels. Considering new surgical strategies for the treatment of obesity, the aim of this study was to evaluate GI motility and morphometric parameters in the obese rats underwent gastric plication or sham surgery.

2. MATERIAL AND METHODS

2.1. Animals

This study was approved by the Animal Research Ethics Committee (registered number: 1273 / 2018) and carried out in accordance with specific resolutions of Bioethics in Experiments with Animals. Thirty-two male *Wistar* rats obtained and kept in the Central Vivarium of UNESP (FMB), from Universidade Estadual Paulista, UNESP, Botucatu Campus.

The animals were kept in a light / dark cycle 12/12 hours, with a temperature of 22 ± 3ºC and controlled humidity between 60 ± 5% and separated in collective cages (maximum of 4 animals), receiving water and feed supply *ad libitum* in the whole experiment. The timeline of the experiment is summarized in Figure 1.

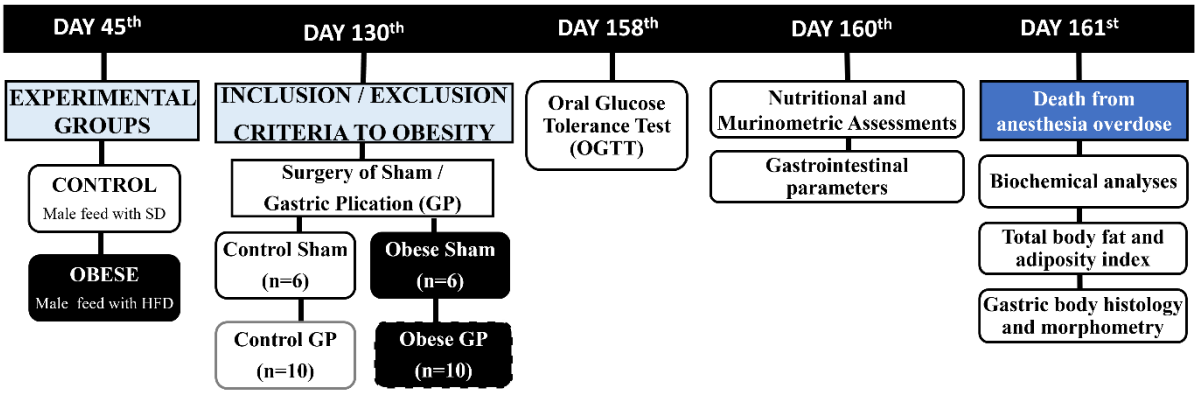

Figure 1. Timeline of the experiment. Control (SD: standard diet), Obese (HFD: High-Fat Diet), Control sham and Obese sham (performed a small midline laparotomy and closed immediately), and Control GP and Obese GP (performed the procedure of gastric plication surgery).

2.3. Obesity induction

Obesity was induced by adopting the High-fat diet (HFD) for about12 weeks, starting at 45 days of age. This procedure was performed according to the protocol proposed by Faradina et al., 2021. The nutrients and nutritional composition of each diet can be described as: HFD - contained soybean meal, sorghum, soybean peel, dextrin, sucrose, fructose, lard, vitamins, and minerals. While, standard Diet (SD) contained soybean meal, sorghum, soybean peel, dextrin, soy oil, vitamins, and minerals. Considering that the energy (kcal/g) from control diet was 3.59 and HFD diet 4.35 kcal/g. The diets used in this study were developed at the Laboratory for Experimental Research Unit (UNIPEX) - FMB / Unesp according to the components and nutritional values adopted by Francisqueti et al., 2017 (Table 1).

The body weight (BW) was evaluated from 98th to 128th day of life once a week, to evaluate the difference of body weight mean of the animals in thirty days. The animals of the HFD groups (Obese and Obese + GP) had a BW greater than the control group, presenting mean of BW(g) + SD (≥ 23.0 g), and were designated as obese rats after this measurement over thirty days, according to the method previously described (CAI et al., 2016).

Table 1. Diet composition and nutritional values.

Components	**Control**	**HFD**
Soybean meal (g/kg)	335	340
Sorghum (g/kg)	278	80
Soy hulls (g/kg)	188	116
Dextrin (g/kg)	146	20
Sucrose (g/kg)	-	80
Fructose (g/kg)	-	180
Soybean oil (g/kg)	14	-
Lard (g/kg)	-	154
Minerals (g/kg)	25	25
Salt (g/kg)	4	8
Nutritional values		
Protein (% of ingredients)	20.0	18.0
Carbohydrate (% of ingredients)	60.0	53.5
Fat (% of ingredients)	4.00	16.5
% of unsaturated	69.0	47.0
% of saturated	31.0	53.0
% Energy from protein	22.9	16.6
% Energy from carbohydrate	66.8	49.2
% Energy from fat	10.4	34.2
Energy (kcal/g)	3.59	4.35

HFD: High-Fat Diet.

2.4. Gastric Plication Surgery

Surgical procedures (sham or GP) were realized at the 135th life's day. Animals were fasted from 12h the evening prior to surgery, with ad libitum access to water. General anesthesia was induced using ketamine 80mg/kg (Cetamin®, Syntec, Brazil) and Xylazine 10mg/kg (Xilazin®, Syntec, Brazil) administered intraperitoneally.

In GP group (n = 10 for each group), a midline laparotomy 4 cm in length expose the gastric greater curvature to be dissected. Polyethylene cannulas and continuous sutures were applied via invagination in a caudocranial direction using 4/0 polypropylene from the antrum to the fundus.

Sham surgery consisted of the same midline laparotomy closed with sutures (n = n = 6 for each group). Closure of the abdominal wall in all animals were performed on musculoaponeurotic tissue with 3/0 polypropylene continuous sutures and on skin with rapid absorption 3/0 polyglycolic acid continuous sutures.

Post-surgical care included clinical supervision by a professional, in addition to analgesia and dressings, individual accommodation at the time of recovery to avoid fighting and preventing animal disturbance at that time, as well as preventing infections. Furthermore, the animals were transferred daily to clean cages, avoiding contamination of the surgical wound.

2.5. Experimental evaluations

2.5.1. Nutritional and murinometric assessments

The body weight gain and the average food consumption (g) were evaluated throughout the experiment until the 30th day to calculate the feed efficiency (%) and the energy intake (kcal/day), respectively. Then, these nutritional indices were evaluated according to De Oliveira and collaborators (2019), as following:

1. Body weight gain (g) **=** (average weight at day 30 – average weight at day 0); reproducing the average weight gaining during the 30 days of the experiment.

2. Feed efficiency (%) **=** [average body weight gain (g) × 100) / energy intake (kcal/day)].

3. Energy intake (kcal/day) **=** [average food consumption (g) × metabolizable energy of the diet (kcal)].

2.5.2. Oral Glucose Tolerance Test (OGTT)

The oral glucose tolerance test (OGTT) was performed in day 28 after surgery. In which, after prolonged fasting (± 12 hours), a drop of blood from the tail was collected after punctured for glycemic determination by conventional glucometer (time 0 – basal glycemia). Then, the rats received an administration of glucose solution (200 g/L) via gavage at a dose of 2.0 g/kg body weight. Blood glucose was determined again at intervals30, 60 and 120 minutes after administration of the glucose solution (MACHADO et al., 2020). The glycemic response during TOTG was assessed by the total estimate obtainedin the area under the curve (ASC) (TAI, 1994).

2.5.3. Alternating Current Biosusceptometry (ACB) and gastrointestinal parameters

On day 160th of experiment and thirty days after surgery, the gastrointestinal parameters (recordings of gastric contractility and gastric emptying time) were evaluated by the Alternating Current Biosusceptometry (ACB) technique in all groups.

ACB is a biomagnetic technique consisting of a magnetic sensor, a lock-in amplifier, a power amplifier, an analog / digital board (Biopac MP150 System, Inc.) and a computer for data storage and processing (AMÉRICO et al., 2010; QUINI et al., 2012). When the ACB sensor is approached to a magnetic sample, in this case the magnetic meal ingested, a value corresponding to the magnetic intensity is obtained. This value, in turn, depends on the distance between the sensor and the magnetic material and on the amount of magnetic material. The mechanical contraction of the stomach moves away thematerial

from the sensor positioned on the abdominal surface, reducing the recorded magnetic intensity; while the relaxation of the stomach brings the magnetic meal closer to the sensor, increasing the magnetic intensity (AMÉRICO et al., 2010). In terms of gastrointestinal transit, the displacement of the magnetic meal along the GI tract segments reduces, over time, the signal intensity recorded at the beginning in the gastric region. It allows to characterize this displacement in different conditions and without anesthesia or death of animals (QUINI et al., 2012).

For the preparation of the magnetic meal, the commercial feed (Nuvilab®, Brasil) was incorporated plus ferrite [composed of 2.4 g of feed and 0.6 g of powdered ferrite (Fe_2MnO_4)], modeled in pellet remaining inert at any pH and not being absorbed by the GI tract. After 12 hours of fasting, it was initialized the gastrointestinal transit measures, established through two points in the animal's abdominal region, one corresponding to the projection of the stomach, and the other to the cecum (Pinto et al., 2021).

Past one hour of the beginning of MGET registration, the animals were anesthetized (isoflurane anesthesia (dose of induction: 3.0% in 100% oxygen) initiating contractility records. Five minutes after anesthesia, with the ACB sensor positioned on its abdominal surface, the magnetic signals were registered continuously for 20-minutes at a sampling rate of 20 Hz, by using a multichannel recorder (MP100 System; BIOPAC) (maintenance dose of isoflurane for maintenance, 2.0%). To quantify gastric contractility parameters, the data were analyzed in MatLab® and, subsequently, the Fast Fourier Transform was applied. The frequencies were expressed in cycles per minute (cpm). The amplitude of contraction was determined by the ratio between the intensity of intestinal peak (P) and noise peak intensity (P′) and expressed in decibels (dB) as follows: $A = 10 \log10 (P/P')$ (CHEN et al., 1995; GAMA et al., 2020). After that, was quantified the half bandwidth (mHz) that is defined as the bandwidth at 1/2 peak height and quantified using the two points on the curve where the intensity was half the maximum intensity (y-axis). At these two points, the corresponding frequencies (x-axis) indicate the morphology of spectral distribution. Such an analysis allows for the observing of narrow spectral profiles due to the single peak and wide spectrum represented by several peaks resulting from recordings (MARQUES et al., 2013).

After this period, the gastric emptying measurements are returned (without anesthesia and in completely awake animals), with the points being monitored at 15-minute intervals for another 4,5 hours. From these data, a curve of variation of the intensity of the magnetic signal over time is constructed at each analyzed point, thus

generating a temporal distribution of the magnetic meal eaten. The statistical moment was calculated and called Mean Gastric Emptying Time (MGET) (CALABRESI, 2015; DALLAGNOL et al., 2020).

Finally, after carrying out all the evaluations, at next day all experimental groups were killed by anesthetic overdose consisting of 220 mg/kg ketamine (Cetamin®, Syntec, Brazil) plus 45 mg/kg xylazine chlorhydrate solution (Xilazin®, Syntec, Brazil) administered intraperitoneally (TAVORE et al., 2021). The animals were decapitated to collect the total body fat and the stomach for further analysis. In addition, blood was collected in a tube without anticoagulant, centrifuged at 3500 rpm for 10 minutes to collect the serum supernatant, which was separated and stored at -80ºC.

2.5.4. Total Body Fat and Adiposity Index

The total body fat was measured as the sum of the following individual fat pad dissected and weighed: epididymal fat + retroperitoneal fat + visceral fat. Adiposity Index (%) was calculated by (total body fat/final body weight × 100) for each animal per group. Adiposity index was used because the degree of fat tends to increase gradually (DAVID, ADEDEJI, FASANMADE, 2019), mainly the visceral fat with the development of obesity in rodents (GRYCEL *et al.*, 2019).

2.5.6. Serum Biochemical Analysis

The determinations of total cholesterol (mg/dL), triglycerides (mg/dL), high density lipoproteins – HDL (mg / dL), were performed through enzymatic methodology (YOUNG, 2001). Non- HDL cholesterol was calculated by subtracting HDL-C from CT, considering that this fraction can provide a better estimate of the concentration of atherogenic particles (XAVIER et al., 2013). The leptin hormone was quantified by ELISA immunoassays, following the procedures determined by the specific kit manufacturer (kit Rat Leptin®, Invitrogen - EUA).

2.5.7. Histological preparation and gastric morphometry analysis

For histological preparation, tissue samples from the body of stomach samples were carefully collected and fixed with Metacarn (60% methanol, 30% chloroform and 10% glacial acetic acid), dehydrated with serial alcohol, diaphanized in xylol and embedded in paraffin. Histological cuts were performed with Micron HM 355S Automatic Microtomer (Thermo Scientific, Germany) with 4 micrometers (μm) thickness. The slides were incubated at 60ºC to fix the cut and then washed in xylene to

remove excess paraffin and hydrated with decreasing concentrations of alcohol (from absolute to 70% alcohol). The sections were stained with hematoxylin-eosin (H&E), dehydrated with increasing concentrations of alcohol (from 70% alcohol to absolute), washed with xylol and mounted on a coverslip with Canada Balsam (VETEC QUÍMICA, Rio de Janeiro, Brazil).

After that, the images were captured on an optical microscope using 10x objective lens (Zeiss, Germany) coupled to a high-resolution camera (AxioCam ERc5s, Zeiss, Germany), connected to a computer with an image capture program (TCapture). Fifteen images per animal were randomly select for morphometric analysis using the ImageJ software (NIH, USA) to measure the gastric mucosa and muscularis mucosa thickness. Specimens were analyzed under light microscopy using a scoring system that includes the graded assessment of neutrophil infiltration of all experimental groups (Table 2), following the methodology of Shahin, Abdelkader & Safar (2018).

Table 2. Histopathological scoring criteria.

Pathological state	Score	
Leukocyte infiltration	0	Absent
	1	2-10/HPF
	2	11-20/HPF
	3	21-30/HPF
	4	>31/HPF

HPF, high power field

2.6. Statistical analysis

Statistics were performed using Prism software (V6, GraphPad, La Jolla, CA). The test of Kolmogorov-Smirnov was utilized to assess normality of data. The results were expressed as mean ± standard deviation (SD) with limit of statistical significance up to 5% ($p<0.05$). Analysis of Variance (ANOVA) followed by the Tukey's post-hoc test, was performed to determine group differences. And was performed the Dunn's multiple comparison test on the data of histological assessments.

3. RESULTS

Nutritional and murinometric parameters

Figure 2 represents nutritional and murinometric parameters. In 2A, an increase in body weight gain was observed in obese sham and GP surgery significantly decreased body weight gain in obese GP ($p<0.05$), and the same pattern was shown by the control GP group compared to its control ($p<0.05$).

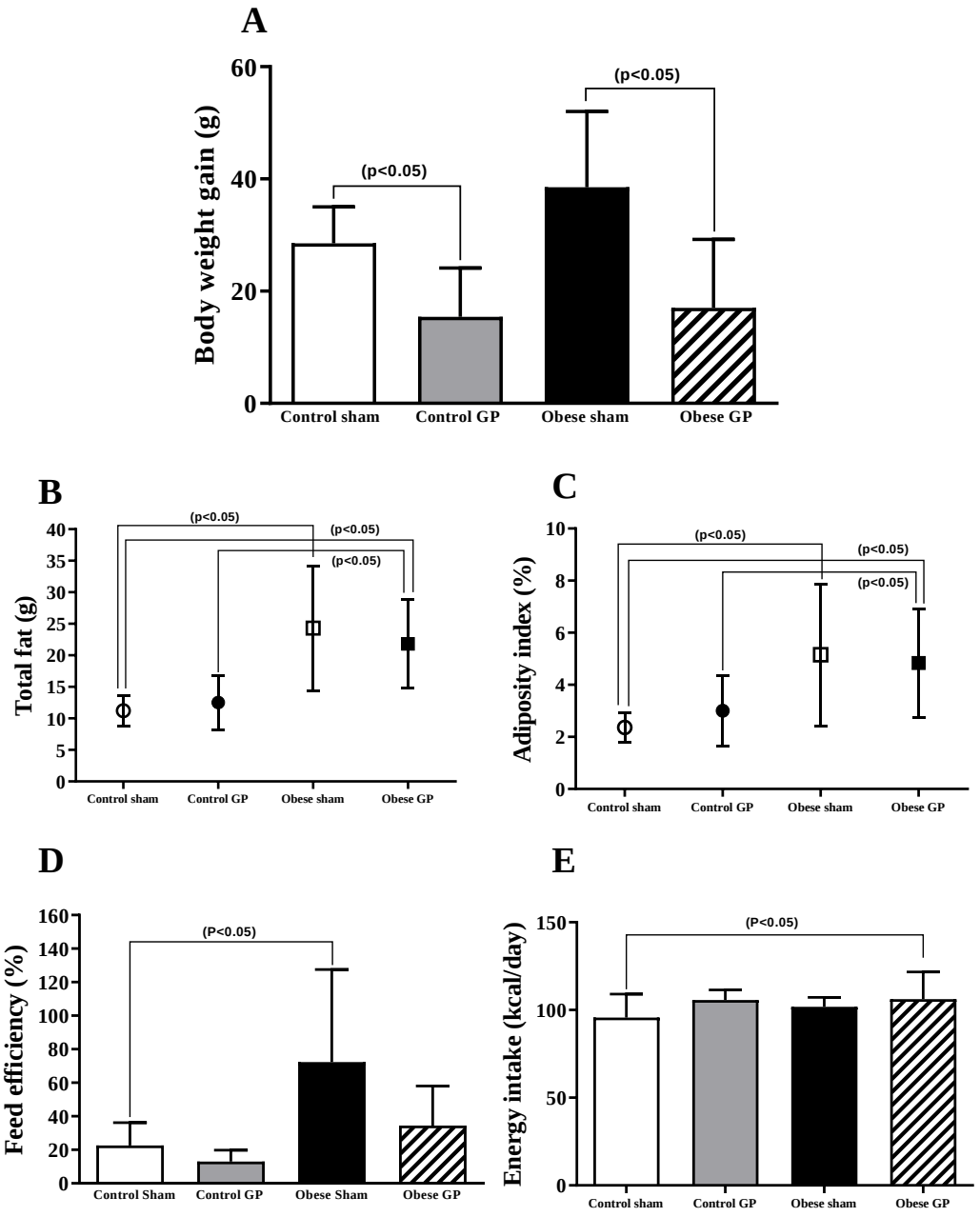

Figure 2. Nutritional and murinometric parameters: [A] Body weight gain (g) over thirty days, [B] Total fat (g) and [C] Adiposity index (%) measured at the final of experiment, [D] Feed efficiency (%) and [E] Energy intake (kcal/day) were determined by the mean of thirty days. Experimental groups: Control sham (n=6); Control GP (n = 10), Obese sham (n = 6), and Obese GP (n = 10). Data analyzed by ANOVA followed by Tukey and expressed as mean ± SD.

In 2B, high values of total fat were observed in obese sham e GP compared to control sham; also, these values were higher in Obese GP than Control GP. In 2C, it is possible to notice that adiposity index was higher in obese and in obese GP compared to both control groups. Figure 2D, it is noticed difference in the feed efficiency (%) between the groups Obese sham and Control sham ($p<0.001$). In 2E was showed a higher energy intake (kcal/day) in Obese GP compared to control sham ($p<0.05$).

Gastrointestinal parameters

In Figure 3A, the Gastric Emptying Time increased in obese groups compared to control sham ($p<0.05$) and in obese GP compared to control GP ($p<0.001$). No differences related to the frequency (cpm) and amplitude (dB) were found. In addition, half-bandwidth (cpm) was higher in obese GP group compared to obese sham ($p<0.05$) and control GP ($p<0.05$) indicating variability around the mean frequency.

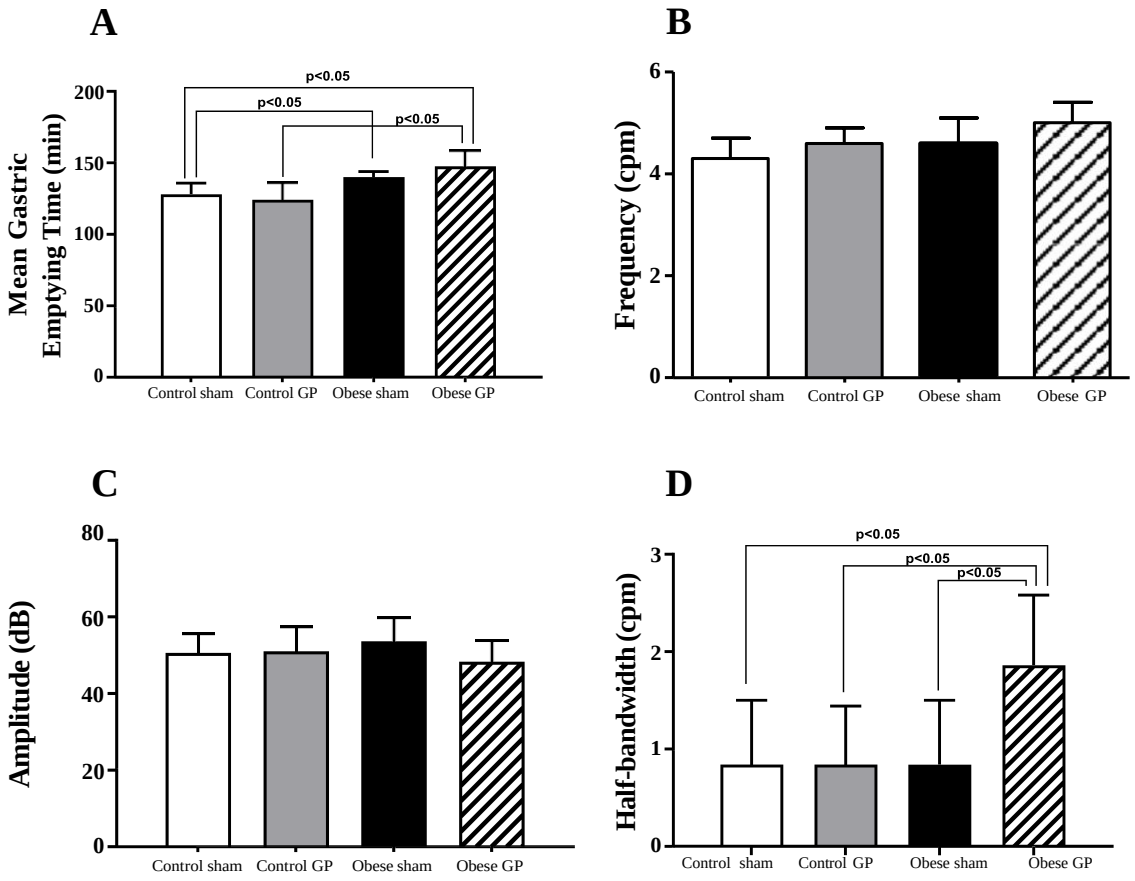

Figure 3. Gastrointestinal parameters were determined by: [A] Mean Gastric Emptying Time (MGET), [B] Frequency of gastric contractility, [C] Amplitude (dB), [D] Half-bandwidth (cpm). All experimental groups were analyzed thirty days post-surgery: Control sham (n=6); Control GP (n = 10), Obese sham (n = 6), and Obese GP (n = 10). Data analyzed by ANOVA followed by Tukey test and expressed as mean ± SD.

Biochemical Analyses

Obese sham and Obese GP showed high basal glucose levels on OGTT compared to controlgroup ($p<0.05$ and $p<0.001$, respectively) (Table 2). At this same time, Obese GP also presented higher glucose levels compared to Control GP ($p<0.001$). At 30 minutes, the obese GP group continue with higher glucose levels compared to Control GP ($p<0.05$). No difference was found at 60 minutes among the groups. At the last time point, 120 minutes, the group Obese GP presented yet higher levels of glucose than Control GP ($p<0.001$).

Table 2. Mean of blood glucose levels (mg/dL) obtained by Oral Glucose Tolerance Test (OGTT) performed thirty days post-surgery: Control sham (n=6); Control GP (n = 10), Obese sham (n = 6), and Obese GP (n = 10).

EXPERIMENTAL GROUPS	Blood glucose level (mg/dL)			
	Basal	***30min***	***60min***	***120 min***
Control sham	78.8 ±6.8	105.3±9.3	95.8±11.4	96.1±9.8
Control GP	84.9±7.5	108.4±10.4	83.9±10.3	86.6±4.9
Obese sham	107.8±12.0*	125.3±14.5	124.7±14.2	111.3±5.3
Obese GP	106.9±11.7$^{*\#}$	127.4±21.5$^{\#}$	115.3±10.2	104.3±11.4$^{\#}$
p value	*p<0.05 vs Control sham; #p<0.05 vs Control GP	#p<0.05 vs Control GP	NS	#p<0.05 vs Control GP

Data analyzed by ANOVA followed by Tukey test and expressed as mean ± SD, NS = non-significant.

Table 3 shows lipid profile of all experimental groups. Obese sham and obese GP presented higher levels of serum total cholesterol compared to control sham ($p<0.01$and $p<0.001$ respectively). Also, obese GP was significantly higher than control GP ($p<0.001$). All groups presented lower levels of high-density lipoprotein (HDL-C) compared to control sham ($p<0.001$). Non-HDL cholesterol, in obese sham group and obese GP presented higher levels compared to control sham ($p<0.001$), and these levels in obese GP was significantly higher than control GP ($p<0.001$). There was no difference among triglycerides (TG) levels in groups.

Table 3. Biochemical analyses determined the lipid profile of experimental groups:Control sham (n=6); Control GP (n = 10), Obese sham (n = 6), and Obese GP (n = 10).

VARIABLES (mg/dL)	Control sham	Control GP	Obese sham	Obese GP	p value
Total cholesterol	67.6±10.6	70.1±13.0	82.2±34.9*	100.3±13.3*#	*p<0.05 vs Control sham; #p<0.05 vs Control GP
Triglycerides	63.0±21.9	55.8±10.9	52.2±25.5	70.4±20.0	NS
HDL-C	47.0±3.5	31.3±4.7*	26.5±8.6*	28.6±4.1*	*p<0.05 vs Control sham
Non-HDL-C	21.4±7.2	36.4±11.9	54.2±26.8*	76.1±17.1*#	*p<0.05 vs Control sham

Data analyzed by ANOVA followed by Tukey test and expressed as mean ± SD, NS = non-significant.

In figure 4 is possible to notice that leptin levels were increased in obese sham compared to control sham group ($p<0.001$). In addition, obese GP presented reduced levels compared to the obese sham ($p<0.05$).

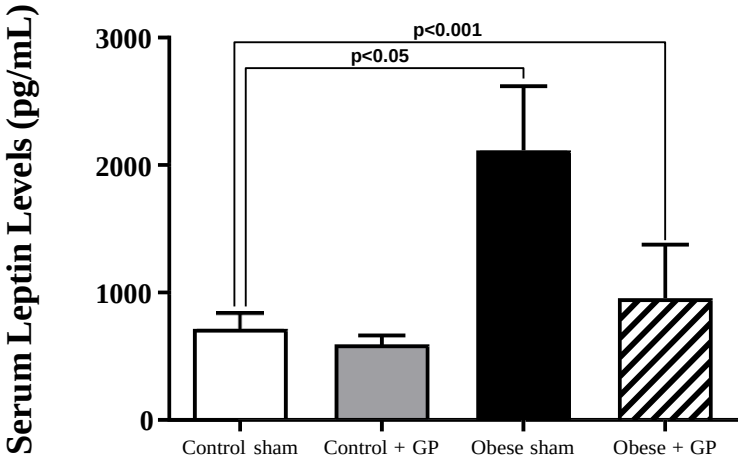

Figure 4. Serum leptin levels of all experimental groups analyzed thirty days post-surgery: Control sham (n=6); Control GP (n = 10), Obese sham (n = 6), and Obese GP (n = 10). Data analyzed by ANOVA followed by Tukey and expressed as mean ± SD.

Gastric body histology, morphometry, and pathological score

Histological photomicrographs [A] and gastric morphometry [B] are presented in Figure 5. Both GP groups had an increase in thickness of mucosa and muscularis mucosa layers compared to sham groups. The gastric mucosa layer on GP groups were thicker in

Control GP and Obese GP compared to control sham and obese sham respectively ($p<0.001$). The thickness of muscularis mucosa layer in obese sham and obese GP were thinner compared to control sham ($p<0.001$) and control GP ($p<0.001$). It was not possible to observe the thickness of the submucosa layer in the groups submitted the surgery, which may be related to gastric tissue remodeling after GP surgery.

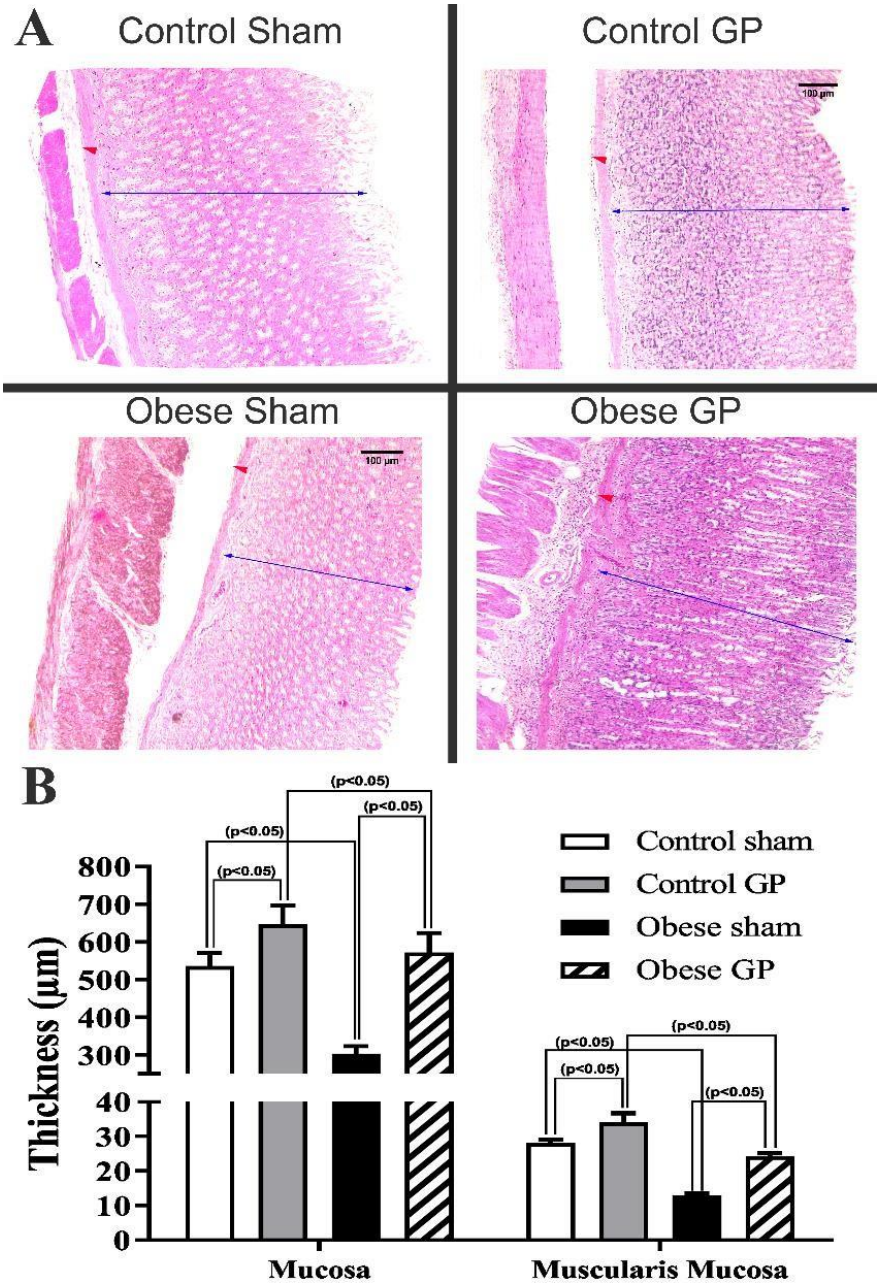

Figure 5. Histological photomicrographs [A] from gastric body using HE stain (×10 objective) (red arrow: muscularis mucosa layer / blue arrow: mucosa layer), and [B] Morphometrical quantification of gastric mucosa and muscularis mucosa. All experimental groups were analyzed thirty days post-surgery: Control sham (n=6); Control GP (n = 10), Obese sham (n = 6), and Obese GP (n = 10). Data analyzed by ANOVA followed by Tukey test and expressed as mean ± SD: $^{*}p<0.05$ compared to Control sham, $^{\#}p<0.05$ compared to Control GP, $^{\&}p<0.05$ compared to Obese sham. The morphometrical quantification was analyzed by Non-parametric Kruskal-Wallisone-way ANOVA followed by Dunn's multiple comparisons test).

Pathological state of the gastric mucosa layer (Figure 6) revealed that PG surgery was able to induce leukocyte infiltration, an inflammation state, evidenced by comparing control sham to control GP ($p<0.01$) and obese GP ($p<0.001$). The same pattern occurred observing obese GP, that presented a higher pathological score compared to obese sham ($p<0.05$).

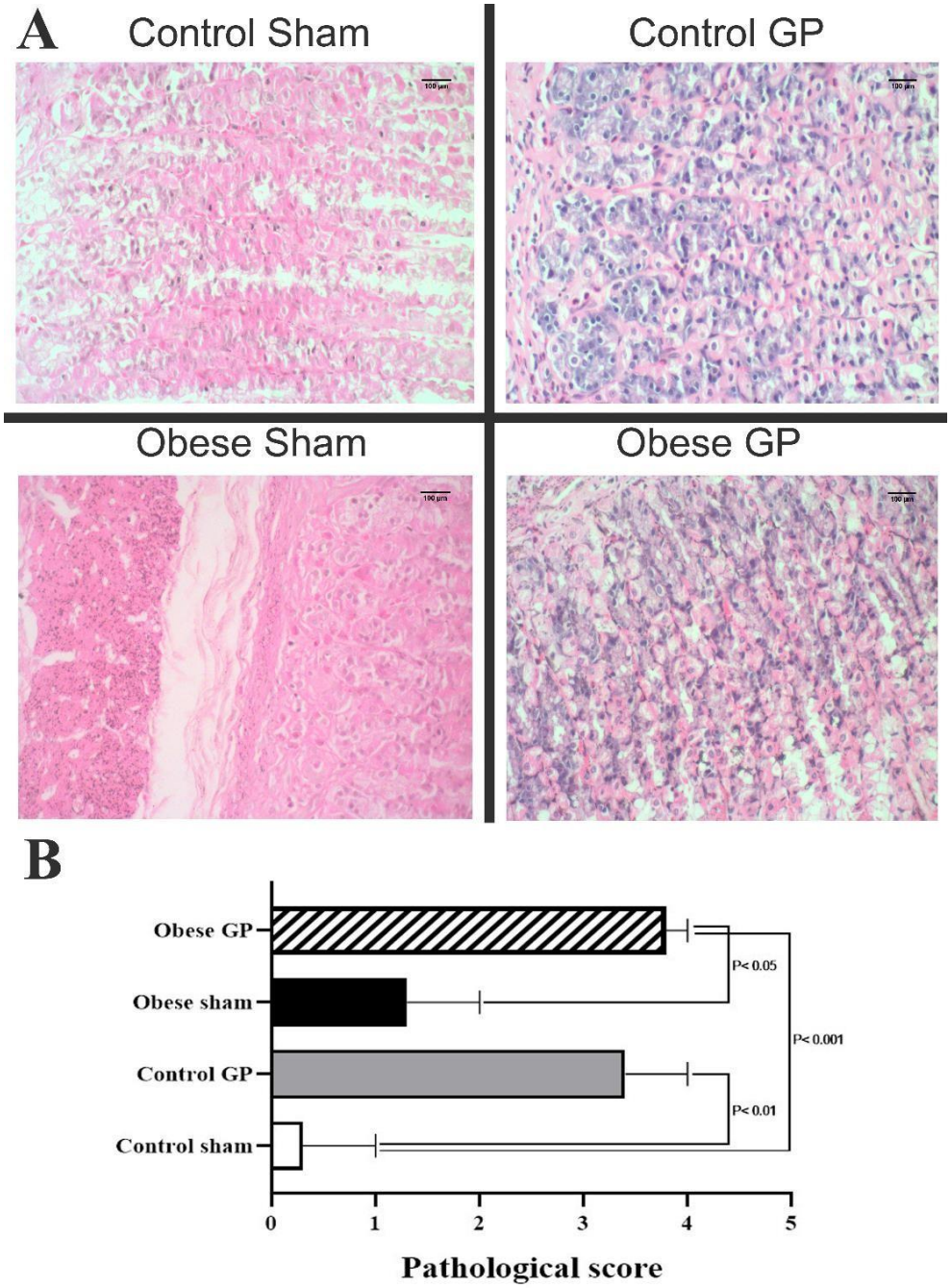

Figure 6. Histological assessment of gastric tissue using H&E stain (x40) of Control Sham (A), Control GP (B), Obese sham (C), and Obese GP (D) and the leukocytes infiltration score (E) showing multiple infiltrates in mucosa layer of gastric body. The pathological score was analyzed by Non-parametric Kruskal-Wallis one-way ANOVA followedby Dunn's multiple comparisons test.

4. DISCUSSION

To better understand and elucidate the pathophysiology of obesity, studies using animal models have adequately mimicked all aspects of human disease (KANASAKI & KOYA, 2011; SULEIMAN, MOHAMED & BAKAR, 2019). Diet Induced Obesity (DIO) rodent models are frequently used to get better knowledge about the pathways implied in the development of metabolic syndrome and obesity (VARLAMOV, 2017). Currently, diets high in both carbohydrates and fat, are the most used models to resemble overconsumption of modern, highly palatable, energy-dense processed foods, exacerbated by a sedentary lifestyle (LUDWIG et al., 2021). Our model was based on previous studies as Faradina (2021), that used a high-fat diet-induced obesity and successfully the animals of the HFD groups had a body weight greater than the control group.

Bariatric surgery is currently considered to efficiently produce long-term body weight loss, improve comorbidities, and improve quality of life in morbidly obese patients (STOICA et al., 2021). There are three mechanisms proposed for weight loss after GP mainly decreased capacity (restriction), decreased receptive relaxation (no fundus), and hormonal (decreased ghrelin and leptin levels). It has been suggested that weight loss is accompanied by changes of the adipokine levels (HOSSEINZADEH-ATTAR et al., 2013). However, there are contradictory data regarding serum levels of ghrelin, leptin, and adiponectin after bariatric surgery (STOICA at al., 2021).

Although GP has the advantage of potential reversibility, GP surgery impact in physiological functions of the GI tract (TALEBPOUR et al., 2015; JI et al., 2014). In GP, the gastric volume is reduced by application of the greater curvature and occurs a rapid delivery of partially digested nutrients to the distal bowel which up-regulates the secretions of gut hormones such as glucagon-like peptide-1 (GLP-1). GLP-1 enhanced glucose-dependent insulin secretion and activated other mechanisms related to glucose tolerance (TALEBPOUR et al., 2015). Additionally, bariatric surgery also has the potential to cause a variety of nutritional and metabolic complications (LUPOLI et al., 2017).

Studies suggests that bariatric surgery successfully decreases body weight and excess fat mass through targeting both energy intake and energy expenditure (EVERS et al., 2017; HAO et al., 2016). In our study, the GP surgery was able to significantly decrease the body weight gain of obese animals over thirty days, as well as influenced the long-term energy intake, as showing a decreased mean, confirmed by the greater food

efficiency related to a high-density calorie diet consumed by obese groups. Furthermore, different types of bariatric surgery may trigger differential energy intake dynamics resulting in variable weight loss outcomes (SHAH & SHIN, 2020). The body weight gain was significantly higher in obese sham compared to control sham as expected, resulted by the consumption of HFD adopted at this study, based on energy from lipids.

In experimental studies in rats, several parameters, such as body weight, body mass index, total body fat, fat pad mass, adiposity index, the Lee index, magnetic resonance imaging, have commonly been used to demonstrate increased adiposity and investigate the mechanisms underlying obesity (LEOPOLDO et al., 2016). Several studies have been used the adiposity index as an indicator of fat accumulation (DOBRIAN et al., 2000; BOUSTANY et al., 2004; SINITSKAYA et al., 2007). In our study, the relative weight of total fat and adiposity index were very different between the obeseand control groups. Although, there was no difference between obese sham and obese GP, probably for the short time of observation after surgery. Hu et al., 2019, verify inhibitof fat accumulation 28 days after the surgery; however, they employed other types of bariatric surgery as sleeve gastrectomy (SG), and Roux-en-Y gastric bypass (RYGB). Ourtime limitation it can be corrected by new studies that follow up GP surgery for a longertime to check reduction of body weight, adiposity index and total fat, as well as observe its effects on gastrointestinal motility.

Biochemical lipid parameters were measured, and obese animals presented higher lipid levels compared to control groups, while HDL-cholesterol levels were lower. This evidenced worsening the lipid profile of obesity and showed that thirty days of GP surgery was not enough to improve this profile, which is mainly driven by the effects of insulin resistance and pro-inflammatory adipokines (VEKIC et al., 2019). In a study of Mushi, Joshi & Rane (2014), they have used a High Fat and High Sugar diet and developed an experimental model of hyperlipidemia and insulin resistance. Their resultscorroborate ours demonstrating a significant increase in blood glucose, total and low- density lipoprotein cholesterol and triglycerides, with a decrease in high density lipoprotein cholesterol. Hyperlipidemia has been ranked as one of the greatest risk factorscontributing to the prevalence and severity of coronary heart diseases (EL-TANTAWY & TEMRAZ).

The consumption of high fat diet also leads to insulin resistance (IR) because the saturated fatty acids interfere with the action of insulin. In thisway, increases the chances of developing Diabetes *mellitus* and its associated complications such as diabetic nephropathy, retinopathy, neuropathy, gastroparesis, oxidative stress (PARK, KIM, KANG, 2011; STORLIEN et al., 1991).

The impaired glucose tolerance represents an abnormality in the regulation of glucose in the post-overload state, diagnosed by OGTT, since the glycemic uptake capacity of peripheral tissues [skeletal muscle and tissue adipose] (DEFRONZO & TRIPATHY, 2009). Analyzing the OGTT performed, it was possible to observe a worsening of glucose tolerance in obese groups, and in obese GP, the surgery improves this condition at basal time, at 30 and 120 minutes, compared to control GP, yet presenting a glycemic return. In clinical iseasy to observe improvement in the control of blood glucose after bariatric surgery (GREENHILL, 2019; SHAH & LAFERRÈRE, 2017) or after gastric plication (CABRERA et al., 2018). In experimental studies, GP surgery also shows improvement in glucose control and even remission in type 2 Diabetes *mellitus* (CHUMAKOVA-ORIN et al., 2021). Also,decreased the levels of hormones regulators of the energy balance and body adiposity asleptin (IZQUIERDO et al., 2019).

Regarding to leptin levels, obese sham showed higherlevels of leptin compared to the control; whereas obese GP presented significantly decrease in this level compared to the obese sham. Leptin regulates food intake and energy expenditure over the long term, to maintain weight (RAMOS-LOBO & DONATO, 2017). Decreasing levels of leptin were observed independently of the type of surgery (TERRA et al., 2013). In our data, obese GP group presented a significant decreasing in leptin levels showing the effectiveness on weight loss.

Leptin, a peptide hormonemainly produced by white adipose tissue, regulates energy homeostasis by stimulating energy expenditure and inhibiting food intake (MONTSERRAT-DE LA PAZ et al, 2021), observed in our study. Dietary food components, such as phenols, peptides, and vitamins, can decrease inflammation and improve leptin sensitivity. On the other hand, saturated fatty acids may worsen chronic inflammation increasing the risk for pathological complications (LEE et al., 2013). In our data, leptin levels improved after GP surgery in obese group. Furthermore, GP was able to significantly decrease the weight of all animals over thirty days, and in obese the GP surgery increased the energy intake and was able to regulate the feed efficiency of them. Leptin resistance is involved in the pathogenesis of diet-induced obesity (DIO), which is the main cause of obesity in humans. High fat diet consumption triggers central and peripheral leptin resistance and, in this sense, hyperleptinemia due to the expansion of adipose tissue, which is a key player in the development of leptin resistance (CRUJEIRAS et al., 2015).

The GP surgery improved GE time in obese since it presented MGET slower than its control. Gastric emptying (GE) is one important parameter of gastric motility

observed. It is a complex and improved physiological process of control, adapted to the individual's digestive and absorptive conditions (HIRATA et al., 2007). GE plays an important role in the intestinal exposure of nutrients and satiety, and it is closely related to glycemic control (MARATHE et al., 2013; MIHAI et al., 2018). A complex bidirectional relationship exists between gastric emptying and glycaemia, such that the rate of gastric emptying is partially modulated by acute changes in blood glucose concentrations (PHILLIPS et al., 2015). It was observed that the obese groups had higher basal glycemic levels after fasting on OGTT, directly influencing the EG, since it was slower than controls and possibly both hyperglycemia and slow EG leading to satiety combined with high feed efficiency.

The data found about GE has been generally contradictory in different clinical and experimental studies on obesity. In experiments using obese model is common to find data presenting a slower gastric emptying time (SUZUKI et al., 2005; LI, MA & WANG, 2011), as our result when compared to control sham. Although there are studies showing accelerated gastric emptying with diet-induced obese (DIO) (WAN, YIN & CHEN, 2019; LI, 2013). In addition to delayed gastric emptying, impaired fundic accommodation, antral hypomotility, and gastric dysrhythmias can occur in patients with gastroparesis caused by diabetes, gastric surgery, or idiopathic causes (FRIEDENBERG & PARKMAN, 2006). Highlighting the importance of the evaluation of parameters as frequency, amplitude, and half-bandwidth of gastric contractions since obesity may cause changes in all these parameters.

The frequency and amplitude of gastric contractions are important to understand the existence of dysrhythmias that were characterized by increases in the slow wave frequency, amplitude, and wave morphology common on obesity (CHENG, 2015). In obese GP group, there were differences in sign morphology compared to other groups. The average frequency can be the same between the groups, but it is important to remember that the gastric pacemaker frequency may present instability. Aiming to quantify instantaneous frequency instability the half-bandwidth was adopted, being a strong marker of higher frequency oscillations over time (MARQUES et al., 2013). It was observed that obese GP rats have a higher half-bandwidth than all groups, related to a possibly dysrhythmia.

Our histopathological analysis revealed that PG induced leukocyte infiltration mainly in the mucosa layer, these data probably also affected the motility parameters. Kaji and collaborators analyzed a mouse model of postoperative gastrointestinal

dysmotility, and propagation of electrical pacemaker activity were disrupted, possibly mediated by nitric oxide. Damaged ICC networks leading to disordered intestinal rhythmicity in regions of the gut (SUZUKI et al., 2004; MISCHOPOULOU et al., 2022). Despite, no studies were found relating the direct influence of surgery on the gastric mucosa, not allowing for correlations with our finding of inflammatory infiltrates after the PG procedure.

All four major layers of gastric wall was comprised in our data after GP in control and obese groups. The constitutive properties and thickness of the different layers of the gastric wall differ significantly between different regions of the stomach, corresponding to their respective physiological functions (BRANDSTAETER et al., 2019). There are few studies correlating motility with morphometry. We observed in GP groups both layers (muscular and mucosa) thicker compared to the sham groups. Possibly due to a remodeling of the mucosal layer and muscularis mucosa layer after surgery. This data corroborates to a study of bariatric surgery in obese rats and individuals operated on Roux-en-Y gastric bypass and SG displaying a new gastric mucosa phenotype with hyperplasia (RIBEIRO-PARENTI et al., 2021). The gastric mucosa barrier increased can prevent drug penetration and subsequent absorption (ENSIGN, CONE, HANES, 2012), consequently interfering in GE and glycemic levels as observed in our data. In addition to increasing of the thickness of the mucosa and muscularis mucosa layer, considerable gastric tissue remodeling was found after the PG procedure. Chronic inflammation is well documented in obesity, increased inflammation in obese individuals accentuates the comorbidities observed in this condition (BIOBAKU et al., 2020). Furthermore, it can lead to insulin resistance, β-cell dysfunction, and ultimately type 2 diabetes (ROHM et al., 2022).

There are reports from simple short-term complications in bariatric surgery or even death (HERRON & BLOOMBERG, 2006; MA & MADURA, 2015). In long term studies, there are notable improvements in insulin resistance and inflammatory cytokines/markers (O'BRIEN et al., 2019; BIOBAKU et al., 2020; COURCOULAS et al., 2014). In this way, long term studies are necessary to confirm a resolution of this local inflammation and to evaluate the concentrations of TNFα, IL-6, MCP-1, and other inflammatory cytokines/markers following bariatric surgery (BIOBAKU et al., 2020, LAUTENBACH et al., 2021).

Our study brings the relevant contribution of the GI, which is not fully understood in obesity, as a stage of the effects provoked by new and less invasive surgical strategies for the treatment of obesity. In summary, gastric plication decreased the leptin levels and

the body weight significantly, mainly in obese groups. GP did not reverse slower gastric emptying observed in obese and even worsened variability around the mean gastric frequency. Our results suggesting a dysrhythmia after procedure that could be associated to inflammatory infiltrates. GP procedure possibly triggered remodeling in the mucosa and muscular of the mucosa layers which reinforcing the importance of evaluate gastrointestinal motility to upgrade the treatment of obesity.

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CONCLUSÕES

A partir do modelo de obesidade paterna foram observadas diferenças no trânsito gastrintestinal, na contratilidade duodenal e na morfologia do TGI associado ao aumento dos níveis de glicose e leptina e redução da insulina em pais e filhos. Por outro lado, as variáveis gastrointestinais dos netos foram semelhantes a registradas nos animais controle, apesar da morfometria apresentar-se alterada nas três gerações. Nossos dados trouxeram novas perspectivas sobre a importância de se considerar um ciclo paterno de obesidade para entender a extensão dos distúrbios de obesidade na fisiologia gastrointestinal.

A plicatura gástrica avaliada após 30 dias mostrou-se eficaz na perda de peso tanto de animais controle quanto de obesos, bem como foi capaz de diminuir os níveis de leptina em obesos. Em relação a motilidade gastrointestinal, observou-se que os obesos com plicatura gástrica presentaram esvaziamento gástrico mais lento o que influenciou diretamente nos níveis glicêmicos. Nota-se que a glicemia elevada possivelmente conduziu à saciedade e a alta eficiência alimentar nos obesos.

Os modelos de obesidade adotados foram eficazes e ressaltaram a importante contribuição da função gastrointestinal sobre a manutenção do peso corporal e na saúde da prole. Nesse contexto, alterações na motilidade nem sempre são totalmente compreendidas ou consideradas relevantes durante a condução de estudos em obesos. Assim, este estudo apresentou uma temática em potencial para o desenvolvimento de novas estratégias cirúrgicas para o tratamento, bem como contribuição transgeracional na obesidade.

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