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**GABRIELA MOREIRA SOARES**

# ALTERAÇÕES NO METABOLISMO LIPÍDICO HEPÁTICO EM RATOS OBESOS SUBMETIDOS À DERIVAÇÃO DUODENO-JEJUNAL

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

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GABRIELA MOREIRA SOARES

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

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CASCAVEL-PR
(janeiro/2015)

Dedico este trabalho às pessoas mais importantes da minha vida:

Meus pais: Fátima e Rodrigo

Meus irmãos: Manuela e Bruno

Minhas sobrinhas: Sophia e Antonella

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(Madre Teresa de Calcutá)

# RESUMO

A obesidade hipotalâmica (OH) é uma condição severa que não apresenta nenhuma terapia eficaz. Cirurgias bariátricas têm surgido como uma alternativa de tratamento, porém, os efeitos deste procedimento são controversos. No presente trabalho, investigamos os efeitos da derivação duodeno-jejunal (DDJ) sobre o perfil lipídico e sobre a expressão gênica de proteínas e fatores de transcrição envolvidos em vias do metabolismo lipídico hepático em ratos com OH. Durante os cinco primeiros dias de vida, ratos *Wistar* neonatos receberam uma injeção subcutânea de glutamato monossódico (MSG) [4 g/kg de peso corporal, grupo OH]. O grupo controle (CTL) recebeu solução salina [1,25 g/kg de peso corporal]. Aos 90 dias de idade, os ratos OH foram aleatoriamente submetidos à pseudo-cirurgia (PC) ou à DDJ, formando os grupos OH PC e OH DDJ, respectivamente. Seis meses após a DDJ, foram verificados, os parâmetros de obesidade, concentração de lipídios e expressão gênica e proteica no fígado. Ratos OH PC apresentaram obesidade, hiperinsulinemia, resistência à insulina, hipertrigliceridemia, concentrações elevadas de ácidos graxos livres (AGL) e do conteúdo de triglicerídeo (TG) hepático. Também, os animais OH PC tiveram aumento na quantidade de mRNA da acetil-CoA carboxilase (ACC), ácido graxo sintetase (FASN) e estearoil-CoA desaturase-1 (SCD-1) e da expressão proteica da ACC e FASN no fígado. A expressão gênica da carnitina palmitoil-transferase-1a (CPT-1a) e da proteína de transferência de triglicerídeos microssomal (MTTP) foram menores no fígado do grupo OH PC. A cirurgia de DDJ normalizou a concentração de insulina, AGL e TG séricos e o conteúdo de TG hepático, sem alterar a obesidade nesses animais. Além disso, a DDJ reduziu a expressão do mRNA da piruvato quinase hepática (LPK), ACC, SCD-1, acil-CoA oxidase (ACO) e da proteína de ligação do elemento responsivo à carboidratos (ChREBP). A expressão proteica

de ACC e FASN foi normalizada em animais OH DDJ. A DDJ reduz a lipogênese *de novo* e melhora o conteúdo de TG hepático em ratos OH DDJ, indicando que esta cirurgia é eficiente na resolução da doença hepática gordurosa não alcoólica (DHGNA) na OH.

**Palavras-chave:** Derivação duodeno-jejunal; obesidade hipotalâmica; lipogênese *de novo*; ratos MSG.

## ABSTRACT

Hypothalamic obesity (HyO) is a severe condition without any effective therapy. Bariatric operations appear as an alternative treatment, but the effects of this procedure are controversial. Here, we investigated the effects of duodenal-jejunal bypass (DJB) upon lipid profile and expression of main genes, protein and transcription factors involved in hepatic lipid metabolism pathways in HyO rats. During the first 5 days of life, male newborn *Wistar* rats received a subcutaneous injection of monosodium glutamate (MSG) [4 g/kg body weight (BW), HyO group]. Control (CTL) group received saline [1.25 g/kg BW]. At 90 days of age, HyO rats were randomly submitted to DJB or sham operations forming HyO DJB and HyO Sham group, respectively. Six months after DJB, obesity parameters, lipids levels, and expression of genes and protein in the liver were verified. HyO Sham rats displayed obesity, hyperinsulinemia, insulin resistance, hypertryglyceridemic and presented higher free fatty acids (FFA) levels and hepatic triglyceride (TG) content. Also, HyO Sham animals enhanced acetyl-CoA carboxylase (ACC), fatty acid synthase (FASN) and stearoyl-CoA desaturase-1 (SCD-1) mRNA levels and ACC and FASN protein in the liver. Carnitine palmitoyltransferase-1a (CPT-1a) and microsomal TG transfer protein (MTTP) were down-regulated in HyO Sham rats. DJB operation normalized serum insulin, TG and FFA levels and hepatic TG content, without changing obesity in these animals. In addition, DJB reduced mRNA levels of liver pyruvate kinase (LPK), ACC, SCD-1, acyl-CoA oxidase (ACO) and carbohydrate response elemento-binding protein (ChREBP). ACC and FASN protein expression were normalized in HyO DJB animals. DJB reduces *de-novo* lipogenesis and improves hepatic TG content in HyO DJB rats, indicating that this surgery is efficient in the resolution of nonalcoholic fatty liver disease (NAFLD) in HyO.

**Keywords:** Duodenal-jejunal bypass; Hypothalamic obesity; *de-novo* lipogenesis; MSG rats.

# SUMÁRIO

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# LISTA DE ABREVIATURAS

**ACC** – acetil-CoA carboxilase

**ACO** – acil-CoA oxidase

**AG** – ácidos graxos

**AGCL** – ácidos graxos de cadeia longa

**AGL** – ácidos graxos livres

**apoB** – apolipoproteína B

**ARC** – núcleo arqueado

**ATP** – adenosina trifosfato

**ChREBP** – proteína de ligação do elemento responsivo à carboidratos

**COL** – colesterol

**CPT** – carnitina palmitoil-transferase

**CPT-1a** – carnitina palmitoil-transferase-1a

**CPT-2** – carnitina palmitoil-transferase-2

**DDJ** – derivação duodeno-jejunal

**DGYR** – derivação gástrica em Y de *roux*

**DHGNA** – doença hepática gordurosa não alcóolica

**DM2** – diabetes *mellitus* tipo 2

**DMN** – núcleo dorsomedial

**EHNA** – esteato-hepatite

**EM** – eminência mediana

**FASN** – ácido graxo sintetase

**FxR** – *farnesoid X receptor*

**G1P** – glicose-1-fosfato

**G3P** – gliceraldeído-3-fosfato

**G6P** – glicose-6-fosfato

**GCK** – glicoquinase

**GH** – hormônio do crescimento

**GHRH** – hormônio liberador do hormônio do crescimento

**GLUT2** – transportador de glicose tipo 2

**GS** – gastrectomia *sleeve*

**HDL** – lipoproteínas de alta densidade

**IMC** – índice de massa corporal

**LDL** – lipoproteínas de baixa densidade

**LH** – hipotálamo lateral

**LPK** – piruvato quinase hepática

**LXR** – receptor X do fígado

**MSG** – glutamato monossódico

**MTTP** – proteína de transferência de triglicerídeos microssomal

**NADPH** – fosfato de dinucleotídeo de nicotinamida e adenina

**NPY** – neuropeptídeo Y

**PPAR-α** – receptor ativado pelo proliferador de peroxissoma-α

**PVN** – núcleos paraventriculares

**RI** – resistência a insulina

**SCD-1** – estearoil-CoA desaturase-1

**SM** – síndrome metabólica

**SNC** – sistema nervoso central

**SNP** – sistema nervoso parassimpático

**SREBP-1c** – proteína de ligação do elemento regulador de esterol-1c

**SUS** – Sistema Único de Saúde

**TCA** – ciclo do ácido tricarboxílico

**TG** – triglicerídeos

**TyG** – triglicerídeo-glicose

**VLDL** – lipoproteínas de densidade muito baixa

**VMH** – hipotálamo ventromedial

**WHO** – *World Health Organization*

# INTRODUÇÃO

A obesidade pode ser conceituada como uma doença metabólica onde o indivíduo apresenta um acúmulo excessivo de gordura corporal, generalizada ou localizada, que leva a um comprometimento da saúde do mesmo (LUZ; ENCARNAÇÃO, 2008). Segundo a *World Health Organization* (WHO), em 2008, mais de 200 milhões de homens e quase 300 milhões de mulheres estavam obesos e aproximadamente 1,5 bilhões de adultos apresentavam sobrepeso em todo o mundo (WHO, 2014). No Brasil, cerca de 39% da população apresentavam sobrepeso e obesidade nessa mesma época (TARASTCHUK et al., 2008). Já em 2013, 17,5% da população brasileira apresentavam obesidade e 50,8% estavam acima do peso (BRASIL, 2013). Dessa forma, estudos populacionais tem considerado a obesidade como um problema de Saúde Pública, visto que seu predomínio ocorre em países desenvolvidos e em desenvolvimento e atinge indivíduos em diversas faixas etárias (CARVALHO, 2008).

Fatores genéticos, metabólicos, endócrinos, neurais, ambientais entre outros, estão relacionados com a gênese da obesidade (MOLINATTI; LIMONE, 1992). A facilidade de transportes e o avanço da tecnologia provocaram uma redução na atividade física que, quando associados a um comportamento alimentar inadequado, auxiliam no desenvolvimento desta síndrome (TAUBES, 1998). O sistema nervoso central (SNC) desempenha um papel de extrema importância nos mecanismos que controlam o peso corporal através de regiões hipotalâmicas específicas (DOLNIKOFF et al., 1988). Lesões nestas áreas podem provocar alterações metabólicas e endócrinas levando a um tipo de obesidade denominada de obesidade hipotalâmica (HOCHBERG; HOCHBERG, 2010).

Pacientes com obesidade hipotalâmica apresentam deficiência na secreção de um ou mais hormônios hipofisários (KARAVITAKI et al., 2006; MULLER, 2008), desequilíbrio nos neurotransmissores orexígenos e anorexígenos hipotalâmicos (LEE; KORNER, 2009), acúmulo excessivo de gordura, hiperfagia, hiperleptinemia, redução do tônus simpático e aumento do parassimpático (HOCHBERG; HOCHBERG, 2010). A atividade desregulada do sistema nervoso parassimpático (SNP), na obesidade hipotalâmica, envolve um aumento na

estimulação vagal das células β, levando a uma hipersecreção de insulina (LUSTIG, 2008; LUSTIG, 2011).

Independente da sua causa, as consequências da obesidade podem levar a outras desordens no metabolismo incluindo o diabetes *mellitus* tipo 2 (DM2), dislipidemias, hipertensão arterial, doenças cardiovasculares e cerebrais, certos tipos de cânceres, doenças respiratórias e osteoarticulares (SOWERS, 1998; WALLEY; BLAKEMORE; FROGUEL, 2006). A associação entre a obesidade e essas alterações metabólicas se dá através de um fator comum denominado resistência à insulina (RI) (VASQUES et al., 2009) e o desenvolvimento de tais patologias concomitantemente caracteriza a síndrome metabólica (SM). Outra característica da SM é o acúmulo de gordura nos hepatócitos devido o estado obeso, que quando ocorre em excesso origina a doença hepática gordurosa não alcóolica (DHGNA). Segundo Ângulo (2002), a DHGNA pode estar presente em 74% dos adultos obesos e é considerada a principal causa de morbimortalidade relacionada a doenças hepáticas.

Para o tratamento de tais doenças, são propostas mudanças no estilo de vida relacionado à dieta saudável e prática de exercícios, porém, quando essas alterações não demonstram resultados satisfatórios outros meios de intervenção devem ser utilizados como o medicamentoso ou cirúrgico (YANG; PARK; SONG, 2002; RIBEIRO; OLIVEIRA; MELLO, 2007). Estudos demonstram que a cirurgia bariátrica pode diminuir a morbidade da obesidade em até 40% e reduzir os riscos de doenças associadas (SJÖSTRÖM et al., 2004). Com base no tipo de procedimento cirúrgico ela pode ser dividida em categorias, sendo classificada como: restritiva, disabsortiva ou mista. A derivação duodeno-jejunal (DDJ) é um tipo de cirurgia bariátrica disabsortiva, que tem por objetivo desviar parte do intestino proximal a fim de diminuir a absorção dos alimentos (RUNKEL et al., 2011).

A DDJ em ratos obesos pré-diabéticos reduz o conteúdo de gordura hepático (ARAUJO et al., 2012), a concentração de triglicerídeos (TG) sérico e hepático e o índice triglicerídeo-glicose (TyG), bem como diminui a esteatose hepática (EBERTZ et al., 2014). Em ratos diabéticos obesos a DDJ promoveu redução de TG sérico e hepático, bem como diminuição da expressão proteica de fatores de transcrição e enzimas reguladoras da lipogênese *de novo* (HAN et al., 2014). A DDJ em ratos diabéticos não obesos promoveu redução da concentração de TG, colesterol (COL) total e ácidos graxos livres (AGL) de jejum, oito semanas após a cirurgia, bem como diminuiu a esteatose hepática, balonização e inflamação dos hepatócitos (KASHIKARA et al., 2014), e na 12ª semana de pós-operatório reduziu TG e AGL (HU et al., 2013).

Existem poucos estudos na literatura demonstrando o efeito das cirurgias bariátricas na obesidade hipotalâmica e estes estudos são controversos, demonstrando que a cirurgia bariátrica pode ser benéfica na obesidade hipotalâmica (INGE et al., 2007; SCHULTES et al., 2009; BRETAULT et al.; 2013; GATTA et al., 2013) ou não (WEISMANN et al., 2013). Para mimetizar a obesidade hipotalâmica, o modelo de obesidade induzido por lesões químicas pela administração de glutamato monossódico (MSG) é amplamente utilizado. O MSG quando administrado no período neonatal provoca lesões hipotalâmicas em regiões específicas que levam ao desenvolvimento da obesidade (OLNEY, 1969) e co-morbidades associadas semelhantes em humanos. Nosso grupo de pesquisa recentemente publicou um artigo onde foi demonstrado o efeito da DDJ em ratos com obesidade hipotalâmica dois meses após o procedimento cirúrgico. Observou-se que a DDJ não alterou a adiposidade, porém, melhorou a insulinemia, a RI no fígado e a secreção de insulina. Com relação ao metabolismo lipídico foi demonstrado que a DDJ melhora a concentração de TG plasmáticos sem alterar o conteúdo hepático deste lipídio (BONFLEUR et al., 2014).

Considerando que existem poucos estudos que demonstram o efeito da DDJ sobre o perfil lipídico na obesidade e que os resultados apresentados até hoje são controversos; que o tempo após a cirurgia é um fator importante para avaliação das alterações no metabolismo lipídico; que demonstramos que a DDJ tem efeito sobre a obesidade hipotalâmica em ratos; propomos o presente trabalho para responder o seguinte questionamento: Quais os efeitos da DDJ, seis meses após o procedimento cirúrgico, sobre a homeostase lipídica em ratos com obesidade hipotalâmica?

# REVISÃO DE LITERATURA

***Obesidade e doenças associadas***

Desde a pré-história a obesidade esteve presente nos seres humanos, sendo vista como símbolo de beleza e fertilidade. No período neolítico, as "deusas" eram cultuadas por seus quadris, coxas e mamas volumosas. Entretanto, nesta mesma época, Hipócrates (médico greco-romano) já alertava em seus manuscritos para os perigos que a obesidade oferecia para a saúde, afirmando que indivíduos gordos apresentavam maior chance de morte súbita que indivíduos magros. Já Galeno, afirmava que a obesidade era consequência da falta de disciplina do indivíduo (CUNHA; NETO; JUNIOR, 2006).

Nas últimas décadas a obesidade alcançou proporções alarmantes, principalmente em países desenvolvidos e em desenvolvimento, atingindo indivíduos de todas as idades. O fato deste aumento ocorrer em todas as faixas etárias demonstra a importância da adoção de medidas de prevenção, controle e tratamento de doenças não transmissíveis. No Brasil, políticas de saúde que visam o combate à obesidade infantil, como o Programa Saúde na Escola, Programa Nacional de Alimentação Escolar, regulamentação dos alimentos comercializados nas cantinas escolares, entre outras medidas, tem demonstrado influência na saúde desde a infância até a vida adulta. O impacto de uma intervenção de promoção à saúde certamente poderá refletir nos gastos do Sistema Único de Saúde (SUS) em relação às enfermidades e mortes evitáveis, na melhoria da qualidade de vida da população e na compreensão de que manter a saúde é uma tarefa que exige um esforço em conjunto, mobilizando o indivíduo, a comunidade, o governo e as diversas áreas do conhecimento (REIS; VASCONCELOS; BARROS, 2011).

A obesidade é uma doença caracterizada pelo acúmulo anormal ou excessivo de gordura, proveniente de um desequilíbrio energético entre o consumo e o gasto de calorias. Quando a quantidade de energia gasta é menor do que a quantidade consumida o indivíduo passa a apresentar um quadro de balanço energético positivo, que é considerado um determinante imediato da obesidade visto que resulta em um ganho de peso. A obesidade pode ser estimada através da utilização do índice de massa corporal (IMC), onde o cálculo realizado se dá através da relação entre peso e estatura, expresso em $Kg/m^2$ sendo que um

resultado superior ou igual a 30 Kg/m² indica obesidade (WHO, 2014). Diversos fatores estão envolvidos com o desenvolvimento da obesidade, como fatores genéticos, ambientais, neurais etc. (MOLINATTI; LIMONE, 1992). Cerca de 80% dos indivíduos que possuem histórico de obesidade entre os pais tendem a apresentar esse mesmo quadro, podendo esse fato estar intimamente ligado aos hábitos de vida daquela família, além da possível relação genética (GIGANTE et al., 2004).

Alterações no estilo de vida e no ambiente também contribuem para o aumento do sobrepeso e obesidade. A progressão da demanda populacional urbana, a inserção da mulher no mercado de trabalho, o processo de industrialização dos alimentos, a diminuição do esforço físico e gasto energético no trabalho e na rotina diária caracterizam alterações de ordem demográfica, cultural, política e econômica, destacando então a importância das modificações de caráter social no ambiente (GIGANTE et al., 2004).

O SNC desempenha um papel de extrema importância nos mecanismos que controlam o peso corporal através de áreas hipotalâmicas, tais como hipotálamo ventromedial (VMH) e hipotálamo lateral (LH). Outras áreas atuam em conjunto para esta regulação, como eminência mediana (EM), núcleos paraventriculares (PVN), dorsomedial (DMN) e arqueado (ARC) (DOLNIKOFF et al., 1988). Lesões nestas áreas, causadas por leucemia, traumatismo craniano, craniofaringeoma, alterações vasculares, meningites, entre outras (SOUZA et al., 2001), podem provocar alterações no controle do peso corporal levando a um tipo de obesidade endógena denominada de obesidade hipotalâmica (INGE et al., 2007; SCHULTES et al., 2009; KRYSIAK et al., 2010). Como consequência dessa doença, ocorrem alterações no funcionamento do sistema nervoso autonômico e alterações de ordem metabólica levando aos quadros de hiperfagia e hiperinsulinemia. As consequências dessas lesões levam a prejuízos na homeostase energética visto que o indivíduo passa a apresentar um desequilíbrio na ação dos neurotransmissores orexígenos e anorexígenos caracterizando uma disfunção na sinalização pelo hipotálamo (LEE; KORNER, 2009).

Independente dos fatores responsáveis pelo seu desenvolvimento, às consequências do estado obeso podem levar a outras desordens no metabolismo como as dislipidemias, intolerância a glicose, DM2, estados inflamatórios, problemas cardiovasculares (SOWERS, 1998), entre outros, caracterizando a SM. Essas alterações encontradas na SM estão interligadas através de diferentes mecanismos, porém, apresentam um mediador fundamental, em comum, denominado de RI, caracterizado por uma resposta anormal dos tecidos periféricos à ação deste hormônio circulante (VASQUES et al., 2009). De forma direta ou indireta, a RI contribui para o aparecimento de três principais anormalidades no metabolismo

lipídico em pacientes diabéticos: hipertrigliceridemia, altas concentrações de lipoproteínas de baixa densidade (LDL) e baixas concentrações de lipoproteínas de alta densidade (HDL) (SBC, 2007). Em situações fisiológicas a gordura (TG) é armazenada no tecido adiposo, porém, com o desenvolvimento da obesidade, esse armazenamento se torna prejudicado ou insuficiente fazendo com que a gordura seja depositada em outros órgãos, como por exemplo, o fígado (GUILHERME et al., 2008). Quando esse depósito de gordura nos hepatócitos ocorre em excesso, caracteriza a DHGNA (ÂNGULO, 2002).

***Metabolismo lipídico hepático e doenças associadas***

O fígado é considerado um órgão chave no controle do metabolismo lipídico, atua direcionando as gorduras com base nas condições hormonais e metabólicas dos indivíduos fornecendo para os tecidos periféricos o substrato energético necessário (SPASSIANI; KUK, 2008). Dessa forma, as gorduras podem ser armazenadas (GIBBONS; ISLAM; PEASE, 2000), oxidadas para a produção de adenosina trifosfato (ATP) (NGUYEN et al., 2008) ou encaminhadas para os tecidos periféricos pelas lipoproteínas de densidade muito baixa (VLDL), sendo utilizadas pelo músculo esquelético e armazenadas pelo tecido adiposo (DURSTINE et al., 2002).

Após a digestão dos alimentos, os metabólitos chegam até o fígado através do sistema porta-hepático. A glicose é captada pelos hepatócitos através dos transportadores de glicose tipo 2 (GLUT2), e fosforilada pela enzima glicoquinase (GCK) gerando glicose-6-fosfato (G6P). A G6P pode ser convertida em glicose-1-fosfato (G1P) que é adicionada às cadeias de glicogênio através da enzima glicogênio sintase (RUI, 2014). Uma vez que a quantidade de armazenamento de glicogênio é alcançada, o excesso de glicose é redirecionado para síntese de ácidos graxos (AG), em um processo denominado de lipogênese *de novo* hepática.

Na lipogênese *de novo* a glicose entra no fluxo glicolítico gerando gliceraldeído-3-fosfato (G3P), fosfoenolpiruvato e piruvato. A piruvato quinase hepática (LPK) é considerada uma enzima glicolítica chave nesse processo (YAMASHITA et al., 2001). O piruvato é oxidado e gera acetil-CoA o qual entra no ciclo do ácido tricarboxílico (TCA) na mitocôndria gerando citrato. A alta concentração de ATP e fosfato de dinucleotídeo de nicotinamida e adenina (NADPH) no estado alimentado inibe a progressão do citrato no TCA, promovendo acúmulo intramitocondrial desse metabólito. O citrato é então transportado para o citoplasma onde é convertido a Acetil-CoA. A enzima acetil-CoA carboxilase (ACC) transforma acetil-CoA em malonil-CoA, que por sua vez é transformado em ácido palmítico, através da enzima ácido graxo sintetase (FASN). O ácido palmítico pode ser desaturado pela enzima estearoil-

CoA desaturase-1 (SCD-1), formando AG insaturados (FABBRINI; SULLIVAN; KLEIN, 2010; BECHMANN et al., 2012; KAWANO; COHEN, 2013). O ácido palmítico e os AG insaturados são esterificados com o G3P para formação de TG. Em condições fisiológicas normais os TG não são armazenados no fígado em grandes quantidades, são transportados para o tecido adiposo.

Os TG hepáticos provenientes da síntese *de novo* ou da dieta, são transportados ao tecido adiposo pelas VLDL. Essa partículas são ricas em uma apoproteína denominada de apolipoproteína B (apoB) (HUSSAIN; NIJSTAD; FRANCESCHINI, 2011). No retículo endoplasmático a proteína de transferência de triglicerídeos microssomal (MTTP) realiza a transferência de fosfolipídios, TG, COL livre e ésteres de COL para a apo B dando origem a partícula de VLDL (SPARKS; SPARKS, 2008). A MTTP pode ser regulada por hormônios e macronutrientes, ocorrendo em níveis transcricionais, pós-transcricionais e pós-translacionais (HUSSAIN; NIJSTAD; FRANCESCHINI, 2011). Por exemplo, a insulina reduz a transcrição do gene da MTTP em células hepáticas e consequentemente há uma menor secreção de VLDL visto que sua produção é dependente de MTTP (HAGAN et al., 1994).

O fígado utiliza ácidos graxos de cadeia longa (AGCL) para manutenção das quantidades de ATP necessário (MURTHY; PANDE, 1994). A beta-oxidação é a via de oxidação de AGCL mais importante no fígado, podendo ocorrer via mitocondrial ou peroxissomal (LIMA et al., 2005). Os mecanismos de oxidação em ambas as estruturas são semelhantes, diferindo apenas no tipo de AG que será oxidado pela mitocôndria (AG derivados dieta) ou pelo peroxissomo (conjunto diferente de AG e seus derivados). Na beta-oxidação peroxissômica a enzima acil-CoA oxidase (ACO) catalisa os primeiros e determinantes passos desse processo (WANDERS, 2004). Já na oxidação mitocondrial, para que os AG permeiem a membrana da mitocôndria se faz necessário à ativação de um complexo enzimático denominado de carnitina palmitoil-transferase (CPT). O complexo CPT é formado por duas proteínas (CPT-1a e CPT-2) e de maneira geral conduz os AGCL pelas membranas da mitocôndria a fim de serem encaminhados à beta-oxidação (LIRA et al., 2010). A proteína CPT-1a se localiza na membrana externa da mitocôndria e é uma proteína integral de membrana responsável pela conversão de acil-CoA em acil-carnitina. Já a CPT-2 é uma proteína periférica presente no lado interno da mitocôndria e realiza o processo inverso ao da CPT-1a (KERNER; HOPPEL, 2000). Dessa forma, os AGCL conseguem ser transferidos de forma efetiva para a matriz mitocondrial para sofrerem o processo de beta-oxidação. Durante a formação de AG através da ação da ACC ocorre à geração de um produto denominado de

malonil-CoA que pode inibir o sítio de ligação da CPT-1a impedindo a beta-oxidação (WITTERS et al.,1988)

Fatores de transcrição regulam a expressão dos genes envolvidos com o processo de lipogênese e beta-oxidação hepática . A proteína de ligação do elemento regulador de esterol-1c (SREBP-1c) é o principal regulador da lipogênese (SHIMANO, 2001; HORTON; GOLDSTEIN; BROWN, 2002). Estudos têm demonstrado que a insulina ativa o SREBP-1c que por sua vez estimula a transcrição dos genes lipogênicos (ACC, FASN e SCD-1) e estimula a lipogênese hepática (FORETZ et al., 1999; SHIMOMURA et al., 1999; AZZOUT-MARNICHE et al., 2000). Recentemente foi identificada a proteína de ligação do elemento responsivo a carboidratos (ChREBP) que responde a estímulos mediados pela glicose aumentando sua transcrição, independente da insulina, induzindo a expressão de genes glicolíticos (LPK, G6P e GCK) e genes lipogênicos (DENTIN et al., 2004; ISHII et al., 2004). O receptor X do fígado (LXR), ativa a transcrição direta dos genes lipogênicos via ChREBP, e indiretamente através da SREBP-1c (CHA; REPA, 2007). Outro membro da família de receptores X é o *farnesoid X receptor* (FXR), importante regulador na homeostase glicêmica e lipídica no fígado (FORMAN et al., 1995; LU et al., 2000; ZHANG; KAST-WOELBERN; EDWARDS, 2003). Sua ativação realiza a redução de TG por diversos mecanismos, sendo os principais: redução da expressão de SREBP-1c e LXR, com consequente redução da lipogênese e indução da beta-oxidação através do aumento da expressão do receptor ativado pelo proliferador de peroxissomas-α (PPAR-α) (MODICA; GADALETA; MOSCHETTA, 2010; TEODORO; ROLO; PALMEIRA, 2011).

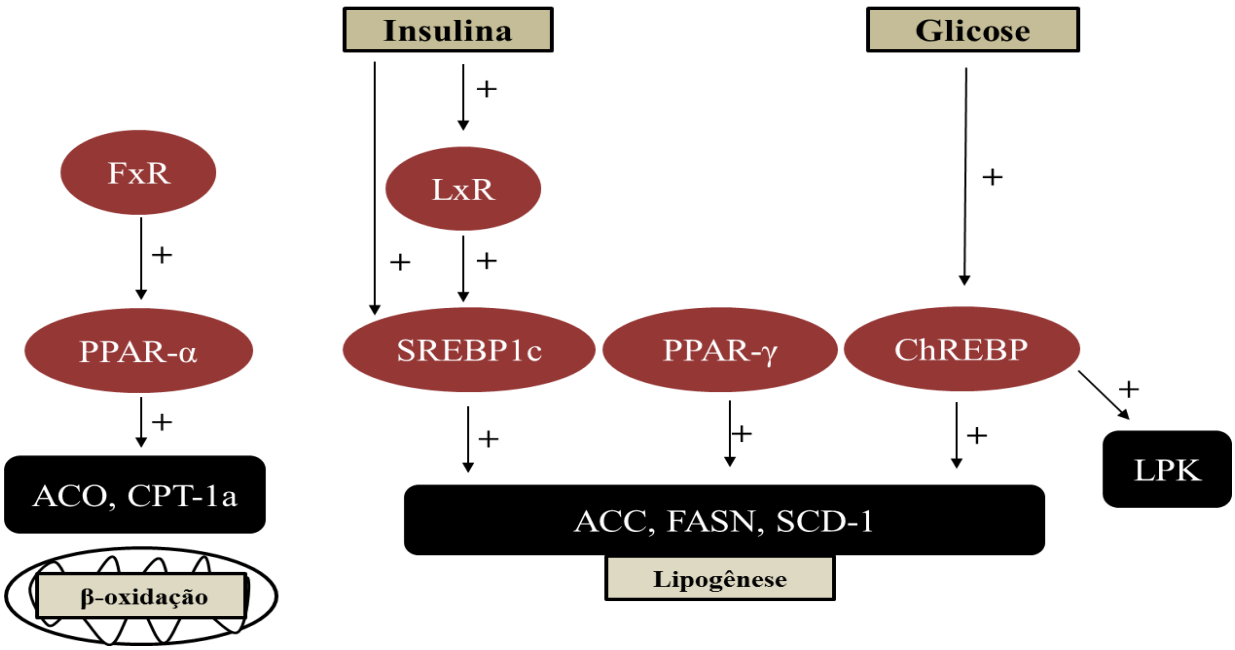

**Figura 1** - Fatores de transcrição envolvidos na via lipogênica e de beta-oxidação hepática (BERLANGA et al., 2014, modificado).

Alterações nos processos descritos acima, que levam a um desequilíbrio na captação e eliminação de TG nos hepatócitos, podem levar a um acúmulo de TG no citoplasma. Isso pode ocorrer devido as seguintes situações: 1) aumento da absorção de AG da circulação, que são provenientes de uma dieta altamente calórica ou da lipólise do tecido adiposo; 2) síntese *de novo* AG pela ativação da via lipogênica; 3) diminuição da beta-oxidação de AG e 4) diminuição da secreção hepática de VLDL (BERLANGA et al., 2014).

Esse excesso de gordura hepática em indivíduos que não apresentam histórico de consumo abusivo de álcool caracteriza a DHGNA (ÂNGULO, 2002). A DHGNA inclui desde uma simples esteatose hepática, onde o indivíduo apresenta acúmulo de gordura, até uma esteato-hepatite (EHNA), onde além do acúmulo citado anteriormente há a presença de inflamação e degeneração hepatocelular, muitas vezes podendo ser acompanhada do aparecimento de fibrose e podendo evoluir para a cirrose hepática e carcinoma hepatocelular (COHEN; HORTON; HOBBS, 2011).

A patogênese da DHGNA/EHNA é explicada pela hipótese de *two-hit* onde no primeiro *hit* há o acúmulo de gordura nos hepatócitos devido a alguns mecanismos que levam a maior importação ou síntese de AG quando comparado com a exportação ou degradação dos mesmos (ANSTEE; GOLDIN, 2006). O segundo *hit* é caracterizado pela presença de inflamação e fibrose que pode ocorrer no fígado sensibilizado pela esteatose por diversos estímulos que envolvem a ação de citocinas pró-inflamatórias, estresse oxidativo, e outros mecanismos relacionados com o excesso de lipídios nesse órgão (DAY; JAMES, 1998).

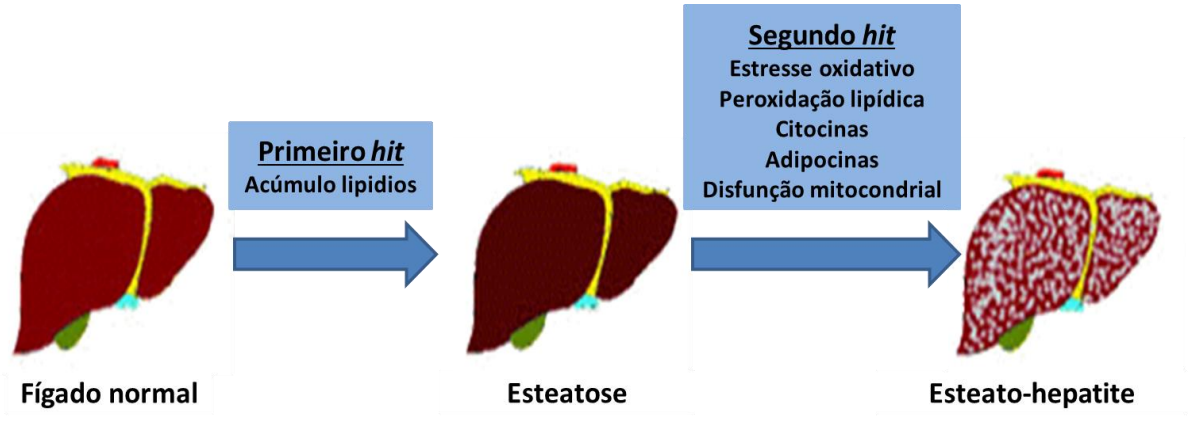


**Figura 2** – Hipótese de *two-hit* (TEVAR et al., 2010, modificado).

***Cirurgia bariátrica***

A fim de reverter o estado da obesidade e as síndromes associadas a ele, são utilizadas diferentes estratégias como dietas com restrição calórica, administração de medicamentos e cirurgias metabólicas e/ou bariátricas. Entre todas essas estratégias a utilização da cirurgia tem se mostrado mais efetiva (RUBINO; GAGNER, 2002; ZIMMET et al., 2011). Foi demonstrado por Adams et al. (2007) que as cirurgias bariátricas levam a uma melhora nas doenças associadas a obesidade, bem como diminuem em até 40% a morbidade em pacientes obesos a longo prazo (SJÖSTRÖM et al., 2004). A indicação da cirurgia se dá com base no IMC do indivíduo; se este for superior a 40 Kg/m² ou entre 35 e 40 Kg/m² com presença de outras disfunções associadas à obesidade que não conseguiram ser solucionadas pelos métodos convencionais (BUCHWALD; WILLIAMS, 2004).

As cirurgias bariátricas são classificadas, de acordo com o procedimento cirúrgico, em restritivas, disabsortivas e mistas (FANDIÑO et al., 2004). Os procedimentos restritivos estão relacionados com uma redução no tamanho do estômago e consequente limitação na quantidade de alimentos que podem ser consumidos. Entre estes procedimentos destaca-se a gastroplastia vertical, a banda gástrica ajustável laparoscópica e a gastrectomia vertical. Nos procedimentos disabsortivos, ocorre um desvio de um segmento do intestino delgado para que a absorção ocorra em menor proporção. Neste procedimento destacam-se as cirurgias de derivação biliopancreática com ou sem a bolsa duodenal. Nos procedimentos mistos ou híbridos, há a restrição do consumo alimentar juntamente com uma menor absorção devido ao desvio de pequena parte do intestino. A cirurgia mais conhecida desse grupo é a derivação gástrica em Y de *Roux* (DGYR) (RUNKEL et al., 2011). Outro procedimento de cirurgia bariátrica experimental que vêm se destacando é a DDJ. Nessa cirurgia não há alterações restritivas no estômago, somente alterações na absorção de nutrientes visto que ocorre uma exclusão do duodeno e de parte inicial do jejuno (RUBINO; MARESCAUX, 2004; RUBINO et al., 2006; KINDEL et al., 2010).

A DDJ promove melhora da homeostase glicêmica antes das alterações no peso corporal, em animais diabéticos (RUBINO; MARESCAUX, 2004; RUBINO et al., 2006; BREEN et al., 2012; HU et al., 2013; JUROWICH et al., 2013) e animais obesos pré-diabéticos (ARAUJO et al., 2012). Estudos também demonstram que a DDJ apresenta efeitos sobre o metabolismo lipídico. A DDJ normalizou o conteúdo de gordura hepático (ARAUJO et al., 2012; EBERTZ et al., 2014) e a concentração de TG sérico (EBERTZ et al., 2014) em ratos obesos pré-diabéticos, dois meses após o procedimento cirúrgico. Em ratos diabéticos não obesos, a DDJ promoveu redução da concentração de TG, COL total e AGL de jejum oito

semanas após a cirurgia (KASHIKARA et al., 2014), bem como, diminuição de TG e AGL na 12ª semana de pós-operatório (HU et al., 2013). Ratos diabéticos obesos no período de oito semanas após a cirurgia demonstraram melhora no perfil lipídico caracterizado pela diminuição de TG sérico e hepático, bem como redução da expressão proteica de fatores de transcrição e enzimas reguladoras da lipogênese (HAN et al., 2014). Recentemente, em um estudo com animais com obesidade hipotalâmcia, foi verificado que, dois meses após o procedimento de DDJ, ocorreu melhora no perfil lipídico com normalização de TG e COL total de jejum (BONFLEUR et al., 2014).

Com relação ao impacto da DDJ sobre a esteatose hepática, foi demonstrado recentente em ratos obesos pré-diabéticos, que a cirurgia levou a uma melhora nesse parâmetro, visto que com oito semanas de pós-operatório 30% dos animais não apresentavam mais o acúmulo de gordura (EBERTZ et al., 2014). Em outro estudo, analisando o mesmo período, foi verificado após a DDJ o desaparecimento total das lesões hepáticas, incluindo a esteatose, balonização hepatocelular e inflamação em animais diabéticos induzidos por modificação genética (KASHIKARA et al., 2014).

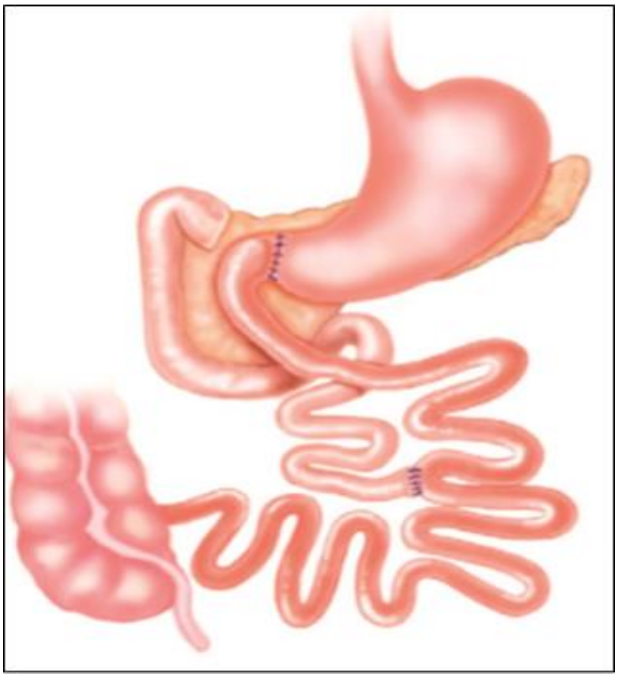

**Figura 3** – Cirurgia bariátrica de derivação duodeno-jejunal (RUBINO; MARESCAUX, 2004).

### *Cirurgia bariátrica e obesidade hipotalâmica*

O tratamento habitual da obesidade está relacionado com a restrição calórica e o aumento da atividade física, porém, essas medidas parecem ser ineficazes para o tratamento

da obesidade hipotalâmica (BRAY; GALLAGHER, 1975). A terapia medicamentosa também tem sido utilizada para esse fim, com ênfase no tratamento simpaticomimético e utilização de análogos da somatostatina, entretanto tem sugerido apenas resultados parciais e questionáveis (MOLLOY et al., 1998; HOCHBERG; HOCHBERG, 2010). A cirurgia bariátrica tem surgido como outra opção de tratatamento devido a sua eficácia na obesidade exógena, porém, na obesidade hipotalâmica seus resultados precisam ser mais bem investigados (GATTA et al., 2013) devido a escassez e controvérsia dos achados na literatura, como descrito abaixo.

Após cirurgia de banda gástrica ajustável laparoscópica, realizada em quatro indivíduos com obesidade hipotalâmica, foi observado redução da ingestão alimentar e diminuição lenta do IMC (MULLER et al., 2007). A utilização da cirurgia de DGYR associada com uma vagotomia troncular anterior foi eficaz na diminuição do peso corporal, melhora na concentração de TG sérico e diminuição da hiperfagia em um jovem do sexo masculino, apresentando uma manutenção dessa melhora até dois anos e meio após o procedimento (INGE et al., 2007). Outro paciente adulto do sexo masculino foi submetido à DGYR distal e dezoito meses após a cirurgia verificou-se a redução do IMC e remissão total do DM2 nesse indivíduo (SCHULTES et al., 2009).

Rottembourg et al. (2009), demonstraram o efeito da cirurgia em adolescentes portadores de obesidade hipotalâmica de ambos os sexos. Na adolescente do sexo feminino foi realizado o procedimento DGYR e após quatro anos pode-se verificar perda de peso e normalização do quadro de dislipidemia. No jovem do sexo masculino foi realizado o desvio biliopancreático com bolsa duodenal e observado uma diminuição no IMC dois anos após a cirurgia. Em um estudo de caso realizado com uma paciente do sexo feminino, foi observada redução do peso corporal aos nove meses após a realização da DGYR que se manteve estável até os dezenove meses de pós-operatório (PAGE-WILSON et al., 2012).

Outro estudo realizado com quatro indivíduos utilizou-se das técnicas de gastrectomia *sleeve* (GS) (2 pacientes) e DGYR (2 pacientes). Pode-se observar, trinta meses após o procedimento cirúrgico, redução do IMC nos pacientes submetidos à GS, bem como em um dos pacientes submetidos a DGYR na 48º semana de pós-operatório. O outro paciente que foi submetido a DGYR apresentou aumento no IMC que não foi contornado até sessenta e quatro meses após o procedimento (GATTA et al., 2013).

Weismann e colaboradores (2013) demonstraram o efeito da cirurgia bariátrica sobre o peso corporal em indivíduos portadores de obesidade hipotalâmica. Nesse estudo, 9 pacientes foram submetidos a três procedimentos cirúrgicos diferentes, sendo eles: banda gástrica

ajustável laparoscópica (6 pacientes); GS (2 pacientes) e DGYR (1 paciente). Dos 6 pacientes submetidos a banda gástrica, 3 foram operados novamente devido a ineficácia da cirurgia, sendo que 1 passou pelo procedimento de DGYR e outros 2 por GS. Os pacientes foram acompanhados por um período médio de três a cinco anos após as cirurgias e demonstrou-se que os indivíduos submetidos a banda gástrica e a GS não tiveram alteração do peso corporal. Em contrapartida, a DGYR levou a redução do peso corporal que foi mantida durante todo o período anteriormente citado. Desta forma, observa-se que existe na literatura controvérsias sobre a eficácia das cirurgias bariátricas em pacientes portadores de obesidade hipotalâmica.

***Modelo animal de obesidade hipotalâmica***

Para estudarmos os mecanismos fisiopatológicos associados com a obesidade hipotalâmica, roedores são submetidos ao tratamento neonatal com MSG, o qual promove lesões químicas em regiões específicas do hipotálamo como a EM (OLNEY, 1969) e ARC (NAGATA et al., 2006). Durante o período neonatal a barreira hematoencefálica ainda não se encontra totalmente desenvolvida, o que facilita o acesso da substância ao SNC (CESARETTI; KOBLMANN-JUNIOR, 2006).

A administração de MSG leva ao surgimento de distúrbios endócrinos e metabólicos levando ao desenvolvimento da obesidade. As lesões hipotalâmicas levam a redução da secreção do hormônio liberador do hormônio do crescimento (GHRH) (SASAKI; KAWAI; OHTA, 1994) e consequente redução da concentração de hormônio do crescimento (GH) circulante (MAITER et al., 1991). Consequentemente, ocorre redução do peso corporal, da massa muscular e da maioria dos órgãos (HAMAOKA; KUSUNOKI, 1986), bem como redução no comprimento e desenvolvimento impróprio do esqueleto nesses animais (IWASE et al., 2000). Apresentam ainda diminuída capacidade termogênica do tecido adiposo marrom (REMKE; WILSDORF; MÜLLER, 1988). Ratos obesos-MSG apresentam diminuição na concentração de neuropeptídio Y (NPY) em várias áreas hipotalâmicas (MORRIS et al., 1998) e em contrapartida alta concentração de leptina circulante (DAWSON et al., 1997), além de apresentar altas concentrações de corticosterona (RIBEIRO et al.., 1997; RIBEIRO et al., 2013).

A ineficiência na mobilização de gordura nesses animais contribui para a obesidade (SOUZA et al., 2001) que se desenvolve apresentando normo ou hipofagia (HIRATA et al., 1997). Além disso, são evidentes sintomas relacionados ao DM2, como RI (BALBO et al., 2007) e normo ou moderada glicemia (NAGATA et al., 2006). Pode-se observar também, alterações relacionadas ao metabolismo lipídico, onde há aumento da concentração de TG

(NARDELLI et al., 2010) e VLDL no plasma (OIDA et al., 1984). Camundongos MSG apresentam esteatose hepática, bem como alterações no fígado que se assemelham às encontradas em humanos com EHNA (SASAKI et al., 2009).

Praticamente todas as alterações acima citadas no modelo MSG são encontradas em pacientes com obesidade hipotalâmica, sendo assim, um bom modelo para se estudar essa síndrome.

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# SIX MONTHS OF DUODENO-JEJUNAL BYPASS REDUCES *DE NOVO* LIPOGENESIS AND AMELIORATES LIVER STEATOSIS IN HYPOTALAMIC OBESE RATS

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**Running title:** DJB improves liver steatosis in HyO rats

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**List of abbreviation:** ACC, acetyl-CoA carboxylase; ACO, acyl-CoA oxidase; AMPK-α, adenosine monophosphate activated protein kinase; AUC, area under curve; BA, bile acids; BW, body weight; CHOL, cholesterol; ChREBP, carbohydrate-responsive element-binding protein; CPT-1a, carnitine palmitoyl transferase-1a; CTL, control; DJB, duodeno-jejunal bypass; FA, fatty acids; FASN, fatty acid synthase; FXR, farnesoid X receptor; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; GLP-1, glucagon-like peptide 1; HOMA, homeostasis model assessment; HyO, hypothalamic obese; LPK, liver pyruvate kinase; MSG, monosodium glutamate; mTORC1, rapamycin complex; MTTP, microsomal triglyceride transfer protein; NAFLD, nonalcoholic fatty liver disease; NASH, nonalcoholic steatohepatitis; NEFAs, non-esterified free fatty acids; pACC, phosphorylated acetyl-CoA carboxylase; pAMPK-αThr, phosphorylated AMPK; PPAR, peroxisome proliferator-activated receptor; PTG/R5, glycogenic scaffolding protein; RIA, radioimmunoassay; SCD-1, stearoyl-CoA desaturase-1; SHAM, sham-operation; SREBP-1c, sterol regulatory element-binding protein; TG, triglyceride and VLDLs, very low-density lipoproteins.

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**Abstract**

*Background & Aims:* Here, we investigated the effects of duodeno-jejunal bypass (DJB) upon lipid profile and expression of genes and proteins, involved in the regulation of hepatic lipid metabolism, in hypothalamic obese (HyO) rats. *Methods:* During the first 5 days of life, male newborn *Wistar* rats received subcutaneous injections of monosodium glutamate [4 g/kg body weight (BW), HyO group] or saline (control, CTL group). At 90 days of life, HyO rats were randomly submitted to DJB (HyO DJB) or Sham-operations (HyO Sham group). Six months after DJB, adiposity, hepatic steatosis and lipid metabolism were verified. *Results:* HyO Sham rats were obese, hyperinsulinemic, insulin resistant and dyslipidemic. These rats had higher content of trygliceride (TG) in the liver with disorganization of the hepatocyte structures. These effects are associated with higher content of hepatic acetyl-CoA carboxylase (ACC), fatty acid synthase (FASN), and stearoyl-CoA desaturase-1 (SCD-1) mRNAs and protein in HyO rats. DJB surgery normalized insulinemia, insulin resistance, and TG and NEFA levels in the serum of HyO rats. TG content in the liver and the hepatic microscopic structures were also normalized in HyO DJB rats. The bariatric procedure decreased the expression of ACC and FASN proteins in the liver of HyO. *Conclusions:* The amelioration in hepatic steatosis, induced by DJB, was manifested as a late effect in HyO rats, and is in part associated with downregulation of the hepatic *de novo* lipogenesis processes, indicating that DJB protects against nonalcoholic fatty liver disease (NAFLD), in hypothalamic obesity.

**Keywords:** Bariatric surgery; *de novo* lipogenesis; monosodium glutamate; nonalcoholic fatty liver disease (NAFLD).

**Key points:**

- Hypothalamic obesity is characterized by morbid adiposity and several comorbidities, such as NAFLD; however life style modifications and traditional pharmacotherapies

are not effective against this syndrome. Also, bariatric surgeries demonstrated contradictory benefits in hypothalamic obese (HyO) patients.

- Here, HyO rats displayed liver steatosis associated with higher hepatic content of ACC, FASN and SCD-1 proteins.
- Our study revealed that only after six months of DJB procedure, HyO DJB rats decreased liver steatosis in part due to downregulation of the hepatic *de novo* lipogenesis. Remarkable, DJB protection against NAFLD is not accompanied by reductions in body adiposity in HyO rats.

**Introduction**

The hypothalamus plays an important role in the control of energy expenditure and body adiposity (1). Lesions in the hypothalamus may cause neuroendocrine and metabolic alterations, provoking hypothalamic obesity (2). Hypothalamic obese (HyO) patients show deficiency in the secretion of one or more pituitary hormones (3, 4), disruption in the orexigenic and anorexigenic signals in the hypothalamus (5), excessive fat accumulation, hyperleptinemia, reduction in the sympathetic and increase in the parasympathetic tones (2), hyperinsulinemia, insulin resistance (6, 7), dyslipidemias and nonalcoholic fatty liver disease (NAFLD) (8).

The NAFLD is characterized by higher hepatic lipids deposition (9) that can gradually progress to nonalcoholic steatohepatitis (NASH), cirrhosis and hepatocellular carcinoma (10). The accumulation of triglycerides (TG) in hepatocytes leading to hepatic steatosis occurs through increased fatty acids (FA) absorption from circulation, due to a high caloric diet and/or from adipose tissue lipolysis. Also, increases in the activation of the transcription factors and enzymes involved in *de novo* lipogenesis, or downregulation in the FA β-

oxidation, together or not, with reduced hepatic secretion of very low-density lipoproteins (VLDLs) are key mechanisms involved in NAFLD pathology (11).

Weight loss has been suggested as the best treatment against NAFLD and obesity. However, changes in feed behavior, physical exercise or the use of traditional anti-obesity pharmacotherapies are not effectiveness for hypothalamic obesity (2, 5). The bariatric operations can be an alternative against this syndrome, but the fewer studies with HyO patients, that was underwent to this surgical procedures, exhibit controversial results about its benefits (12-16).

Among the bariatric surgeries employed in HyO patients for weight loss, the restrictive procedures as sleeve gastrectomy, gastric bypass, and gastric banding are often used (12, 15, 16). However, the duodeno-jejunal bypass (DJB), a procedure that kept the volume of the stomach, but avoids the passage of food from the duodenum and part of the jejunum, was poorly investigated against this syndrome. DJB ameliorated glucose homeostasis and hepatic steatosis, independent on reductions in body weight in genetic and diet-induced diabetic obese rodents (17-21). However, these DJB benefits can be influenced by the type of obesity and the time after the surgery (22-25). Recently, using neonatal treatment with monosodium glutamate (MSG) in rats, that induce lesions in arcuate nuclei and median eminence of the hypothalamus (26), we demonstrated that HyO rats exhibited better peripheral actions of insulin, normalization of pancreatic islets morphofunction, but displayed persistent hepatic steatosis, after 2-months of DJB (27) (Cantelli submetido). Here, we aimed at verify whether the benefits of DJB, against hepatic lipid accumulation and lipogenesis regulation, manifest at a late stage in HyO rats.

## Methods

### *Hypothalamic Obesity Induction*

Hypothalamic obesity was obtained by daily subcutaneous injection of MSG [4 mg/g body weight (BW), HyO group], during 5 consecutive days, in male newborn *Wistar* rats, while control group (CTL) received saline (1.25 mg/g BW). From 21 days to 90 days of age, all rats were maintained at collective cages, under controlled lighting (light/dark cycle of 12 h) and temperature (22 ± 1°C) conditions, and had free access to standard diet (Algomix®, PR, BRA) and water.

*Duodeno-Jejunal Bypass and Sham Operations*

At 90 days of life, HyO rats were randomly submitted to DJB (HyO DJB group) or sham operations (HyO Sham group). Preoperative procedures were performed as previously described by Meguid et al (28). The DJB was performed as described by Rubino and Marescaux (17). Briefly, one gastroduodenal transection tied back duodenal stump was performed. After the jejunum was divided at a distance of 10 cm distal to the ligament of Treitz, and its distal end connected to the stomach (gastrojejunostomy). The proximal stump was reunited end-to-side, at a distance of 15 cm distal of the gastrojejunostomy, called jejunojejunostomia. For sham surgery a laparotomy was performed and subsequently the intestine was massaged to mimic the surgical and suture movements.

*Food intake and feces production*

After 6 months of DJB or sham operations, five rats of each experimental group were maintained, individually, in metabolic cages during 3 days for measurements of food consumption and excreted feces per 12 h. The results are expressed as the average of the three days of evaluation.

*Obesity parameters and Serum biochemical analysis*

Six months after operation, all rats had the glycemia measured using a glucose analyzer (FreeStyle® Lite, Illinois, USA), after 6 hours of fasting. Subsequently, all rodents were weighed and the nasoanal length was measured in all groups to obtain the Lee index [from the ratio of body weight $(g)^{1/3}$/ nasoanal length (cm) x 1000], which was used as a predictor of obesity in rodents. The rats were euthanized by decapitation and total blood was collected to obtain the serum that was used to measure total cholesterol (CHOL), TG and non-esterified free fatty acids (NEFA) using commercial kits according to the manufacturer's instructions (LaborClin®, Bioliquid, Pinhais, PR, BRA and Wako®, Germany, respectively). Insulinemia was also measured by radioimmunoassay (RIA). In addition, the retroperitoneal and perigonadal fat pads were removed and weighed.

*TyG index and HOMA-IR*

Insulin sensitivity was evaluated by the TyG index that was obtained from the ratio of TG and fasting glucose concentrations as the following formula: Ln [fasting TG (mg/dL) x fasting glucose (mg/dL)/2] (29, 30). Tissue insulin resistance was also evaluated by the homeostasis model assessment (HOMA), using the HOMA index of insulin resistance [(HOMA-IR) = fasting insulin (U/mL) x fasting glucose (mM)/22.5] (31).

*Lipids and glycogen content in the liver*

Fragments of liver from rats of all groups were collected and the lipids were extracted by the method of Folch (32). The extract was evaporated and diluted in isopropanol for the determination of TG and CHOL content in the liver, using commercial kits, as described above. Glycogen content in the liver fragments was measured as previously described by Ropelle et al. (33).

*Liver Histology*

Liver samples were fixed in 10% formalin per 24 h, dehydrated in alcohol, permeabilized with xylene to embedding in Paraplast® (Sigma-Aldrich Chemicals, St. Louis, MO, USA). Sections of 7 µm in thickness were stained with hematoxylin and eosin. To identify liver collagen fibers, the Mallory's trichrome staining was performed. For the descriptive analyses, 3 sections of each liver was analyzed by a blind researcher, using a light microscope (Olympus DP71; Olympus BX60; Olympus, Tokyo, Japan) with 400x magnification lens.

*Isolation of RNA and qPCR*

Liver samples were separated for RNA extraction which was performed using RNA minikit PuriLink® (Life Technologies, CA, USA). The mRNA quantification was done using the Fast System 7500 & 7500 Real-Time PCR System (Applied Biosystems, Carlsbad, CA, USA) and the amount of expression of each gene was normalized by the glyceraldehyde 3-phosphate dehydrogenase (GAPDH) gene. The absolute amount of gene expression was calculated by the use of standard curves ($10^8$-$10^3$ copies/DNA molecules in 2 µL), produced from the gene amplification products on 2% agarose gels. Primers for expression of the genes of interest were designed and purchased from Sigma-Aldrich® (MO, USA). The sequences used are shown in table 1.

*Protein expression*

A fragment of the liver was solubilized in extraction buffer (containing 10 mM EDTA, 100 mM tris, 10 mM sodium pyrophosphate, 100 mM sodium fluoride, 10 mM sodium orthovanadate, 2 mM phenylmethylsulfonyl fluoride and 0.1 mg/mL aprotinin) using a mechanical homogenizer (Marconi®, MA102/MINI, Piracicaba, SP, BRA). Then, the extracts

were centrifuged at 12,600 g at 4°C for 30 min and the supernatant was reserved. The protein quantification was measured by Bradford method using BSA for standard curve and the Bradford reagent (Bio-Agency Lab., São Paulo, SP, BRA). The samples (100 µg) were incubated at 100°C for 5 min with Laemmli sample buffer (0.1% bromophenol blue, 1 M sodium phosphate, 50% glycerol, 10% SDS) and separated by electrophoresis in a biphasic polyacrylamide gel (SDS-PAGE, 6.5 or 10%). Subsequently, samples were transferred to nitrocellulose membrane (BioRad®, CA, USA) and incubated with primary antibody for: adenosine monophosphate activated protein kinase (AMPK-α; cat. # 2532S, Cell Signaling Technology, Boston, MA, USA), phosphorylated AMPK (pAMPK-$\alpha^{Thr172}$; cat. # 2531S, Cell Signaling Technology, Boston, MA, USA), phosphorylated acetyl-CoA carboxylase ($pACC^{Ser79}$; cat. # 3661, Cell Signaling Technology, Boston, MA, USA), acetyl-CoA carboxylase (ACC; cat. # 3662S, Cell Signaling Technology, Boston, MA, USA), fatty acid synthase (FASN; cat. sc-20140, Santa Cruz Biotechnology Inc., Santa Cruz, CA, USA), stearoyl-CoA desaturase-1 (SCD-1; cat. ab19862, Abcam, Cambridge, UK), carnitine palmitoyl transferase-1a (CPT-1a; cat. sc-20669, Santa Cruz Biotechnology Inc., Santa Cruz, CA, USA) or microsomal triglyceride transfer protein (MTTP; cat. AV43618, Sigma-Aldrich, St. Louis, MO, USA). α-Tubulin was used as internal control (1:1000, cat T5168; Sigma-Aldrich Chemicals, St. Louis, MO, USA). The visualization of specific bands was performed by incubating the membranes with secondary anti-rabbit or anti-mouse antibodies (1:10.000; Cell Signaling Technology, Boston, MA, USA), followed the incubation with chemiluminescent reagents. The image was captured with the photodocumentador Chemi L-Pix Express (Loccus Biotecnologia®, Cotia, SP, BRA). The intensity of the bands was quantified by densitometry using the software to LabImage analysis 1D (Loccus Biotecnologia®, Cotia, SP, BRA).

*Statistical analysis*

Data are expressed as means ± SEM accompanied by the indicated number of independent experiments. For statistical analyses, the rat groups were compared using one-way analysis of variance (ANOVA) followed by the Tukey post-test ($P < 0.05$). Graphs were performed using GraphPad Prism version 5.00 for Windows (GraphPad Software®, San Diego, CA, USA).

**Results**

*Nutritional and Obesity Parameters*

Figure 1 shows weekly BW, registered before and after DJB procedure in HyO rats. HyO displayed lower BW than CTL rats, from the third week to the end of the experimental period ($P < 0.05$; Fig. 1A). This effect is also confirmed by a reduction in total BW in HyO group, in comparison with CTL, before and after the Sham surgery ($P < 0.0001$; Fig. 1B). DJB operation did not change BW in HyO DJB rats, when compared with HyO sham group (Fig. 1A and 1B).

At the end of the experimental period, HyO Sham rats presented lower food intake, excreted feces, and final BW than CTL rats ($P < 0.001$; Tab. 2). However, feed efficiency did not differ between HyO and CTL groups (Tab. 2). But, HyO rats displayed obesity with increases of 13% in Lee index, 130% in perigonadal, and 36% in retroperitoneal fat pad weights, compared with CTL rats ($P < 0.001$, $P < 0.001$, $P < 0.05$, respectively; Fig. 1 C and 1D). After 6 months of DJB operation, food consumption, excreted feces, and obesity, were similar between HyO DJB and HyO Sham groups (Tab. 2 and Fig 1C and 1D).

HyO rats displayed normoglycemia, but hyperinsulinemia, hypertrygliceridemia and higher NEFAs serum levels, when compared with CTL rats ($P < 0.05$, $P < 0.01$ and $P < 0.01$;

respectively; Tab. 3). The TyG and HOMA-IR indexes were higher in HyO Sham group, in comparison with CTL rats ($P < 0.001$ and $P < 0.01$; Tab. 3). After 6 months of DJB operation, HyO DJB, but not HyO Sham group, had normalized serum insulin, TG, and NEFA concentrations ($P < 0.01$, $P < 0.001$ and $P < 0.05$, respectively; Tab. 3). DJB operation also improved insulin action, since the TyG and HOMA-IR indexes were similar in HyO DJB rats and CTL group (Tab. 3).

*Liver morphology and content of lipids and glycogen*

Figure 2A shows that the macroscopic appearance of the liver from HyO Sham rats was uniformly pale, when compared to CTL. But, after 6 months of DJB operation, HyO DJB livers presented a partial reestablishment of the macroscopic appearance, as that observed in CTL rats (Fig. 2A). In addition, liver weight was lower in HyO Sham, when compared with CTL rats ($P < 0.0001$; Fig. 2D). DJB procedure did not alter liver weight in HyO DJB rats.

Figure 2B demonstrated that liver of HyO presented hepatocytes with several cytoplasmic lipid vacuoles, associated with displacement of nuclei toward the cell periphery. These histological characteristics indicate that liver of HyO Sham has steatosis, with a profile of the “microvesicular fat disease” (34), when compared with the CTL group. These microscopic features differ, significantly, from the CTL liver histology, which has the hepatocytes arranged in rows, delimited by connective tissue that contains sinusoidal capillaries (Fig. 2B). As expected, CTL hepatocytes displayed abundant and homogeneous cytoplasm, central nuclei and absence of steatosis. DJB surgery normalized hepatic steatosis, restoring the aspect of hepatocyte cytoplasm and nuclei localization in HyO DJB group similar to that observed for CTL (Fig. 2B). Liver fibrosis, which occurs in most types of chronic liver diseases, was not evidenced in HyO rats (Fig. 2C).

Hepatic steatosis was also confirmed in HyO rats by a higher TG content in the liver, but without alteration in the amount of hepatic CHOL, when compared with CTL rats ($P < 0.002$; Fig. 2E). Hepatic glycogen content was also higher in HyO Sham than that observed for CTL (Tab. 3). Six months after DJB operation, hepatic TG and glycogen content in HyO DJB rats were normalized (Fig. 2E and Tab. 3).

*Expression of Gene and Proteins Involved in de novo Lipogeneses and FA oxidation*

The hepatic expression of the lipogenic genes: ACC-1, SCD-1 and FASN were higher in HyO Sham group, when compared to CTL ($P < 0.01$, $P < 0.01$ and $P < 0.001$, respectively; Fig. 3A). However, the gene expression of CPT-1a, involved with β-oxidation process, and the MTTP, involved in apolipoprotein B assembly, were approximately reduced by 26% in HyO Sham liver, when compared with CTL ($P < 0.05$; Fig. 3A). In accordance with these data, the content of hepatic ACC, SCD-1 and FASN proteins were 73%, 34%, and 96% higher in HyO Sham than that observed for CTL rats ($P < 0.05$, $P < 0.04$ and $P < 0.001$, respectively; Fig. 4A, 4C and 4D). No modifications in the expression of pACC/ACC, CPT-1a and MTTP proteins were observed between the groups (Fig. 4B, 4E and 4F).

In addition, the mRNA levels of the transcription factor: peroxisome proliferator-activated receptor (PPAR)-γ was 50% lower in HyO Sham liver, than that observed for CTL ($P < 0.001$; Fig. 3B). However, no modifications in liver pyruvate kinase (LPK), acyl-CoA oxidase (ACO; Fig. 3A), carbohydrate-responsive element-binding protein (ChREBP), sterol regulatory element-binding protein (SREBP)-1c, farnesoid X receptor (FXR), and PPAR-α (Fig. 3B) mRNA expressions, were evidenced in the livers of HyO Sham and CTL groups (Fig. 3A and 3B). After 6 months, DJB operation decreased LPK, ACC-1, SCD-1and ChREBP gene expressions in HyO DJB liver to similar levels observed for CTL (Fig. 3). In agreement, the protein expression of ACC and FASN enzymes were reduced in the liver of

HyO DJB, in comparison with HyO Sham rats ($P < 0.02$ and $P < 0.0005$, respectively; Fig. 4A and 4C). Remarkable, the hepatic ACO and FXR mRNAs were lower in HyO DJB, than that observed for HyO Sham group ($P < 0.05$; Fig. 3B). As a final point, no modifications in pAMPK/AMPK protein expressions were observed between the groups (Fig. 4G).

**Discussion**

Hypothalamic obesity is a devastating condition that has no effective treatment at the present time (35). The bariatric surgery, frequently used to treat diet or genetic-induced obesity, shows no consistent benefits against hypothalamic obesity (2, 35) (14-16, 36). Here, for the first time, we demonstrated that after 6 months of DJB surgery, HyO rats showed normalized lipid profile in the plasma and liver, probably due to reduction in the expression of enzymes involved in *de novo* lipogenesis. Interetingly, this effect of DJB against liver steatosis in HyO rats was not due to reductions in adiposity or body weight.

In our study, HyO rats were hypertriglyceridemic, with increased NEFA serum levels, higher hepatic TG content and histological alterations in hepatocytes, consistent with hepatic steatosis. These characteristics are often finding in patients with hypopituitarism, hypothalamic obesity, craniopharyngioma, or those who underwent resection of a hypothalamic tumor (8, 37). These evidences demonstrated that the neonatal treatment with MSG can mimic, efficiently, the metabolic comorbidities, evidenced in human hypothalamic obesity.

Frequently, the majority of obese patients, that were submitted to bariatric surgeries, show NAFLD, which is linked to insulin resistance, type 2 diabetes, and dyslipidemias (38-41). The better way to treat NAFLD is to lose weight, through lifestyle modifications. However, when this is not successful, an alternative is bariatric surgery, a method for reaching substantial and sustained weight loss (42). In accordance, after 8-week of DJB

operation, genetic obese OLETF (*Otsuka Long-Evans Tokushima Fatty*) rats had improvement in hepatic steatosis, associated with weight loss, and increased glucagon-like peptide 1 (GLP-1) and bile acids (BA), in the serum (43). However, in western diet rats, after 1 or 2 months of DJB operation, decreased hepatic TG content, without alteration in body adiposity, was observed (19, 22). Similar effects of DJB were evidenced in high-fat diet type 2 diabetic rats, after 8-week of the surgery (23). Despite these benefits of DJB, upon hepatic steatosis in genetic and diet-induced obese rodents, the literature is limited about the effect of this malabsorption procedure in hypothalamic obesity (27). Recently, we demonstrated that HyO DJB rats, after 2 months of the surgery, already displayed hepatic steatosis, despite a reduction in serum TG levels (Cantelli et al., submitted). These data demonstrated that the improvement in liver steatosis manifests as a later effect of the DJB operation, in hypothalamic obesity. Hepatic FA metabolism is normalized only after 6 months of the DJB operation, indicating that the benefits of bariatric surgeries consist in a slow phenomenon.

In NAFLD, there is an upregulation in the hepatic lipogenic enzymes (44-46). In accordance, HyO Sham rats presented enhanced hepatic mRNA and protein levels of enzymes involved in *de novo* lipogenesis: ACC, FASN and SCD-1 (47-49) that, possibly, increased hepatic TG content and steatosis, in HyO rats. Among the transcriptions factors that regulate the expression of genes, involved in *de novo* lipogenesis (50, 51), only a down-regulation in PPAR-γ mRNA was evidenced in HyO liver. Therefore, additional studies are necessary to dissect which transcriptional signals may be involved in the regulation of lipogenic genes, in HyO Sham rats.

Furthermore, NAFLD may manifests due to reductions in the expression/activity of enzymes involved in β-oxidation or VLDL assembling (11). However, no modifications in protein content of CPT-1a and MTTP were observed in HyO liver, despite a reduction in their mRNAs. Possibly, post- transcriptional modifications may be responsible for these apparent

contradictory results. Since, enzymes involved in lipid metabolism are up and downregulated by several mechanisms (52, 53). In addition, the reduction in MTTP mRNA in HyO rats may be due to the hyperinsulinemia, observed in these rodents, since insulin decreased the transcription of MTTP gene in a dose- and time dependent manner (54).

DJB surgery lead to a reduction in the expression of LPK, ACC, and SCD-1 genes, and normalized the ACC and FASN protein contents in HyO DJB liver. Additionally, these rodents displayed a lower hepatic mRNA content of ChREBP, a well-recognized transcription factor that mediates the transcriptional effect of glucose upon LPK and lipogenic genes (55). Therefore, downregulation in LPK, ACC, and SCD-1 mRNAs induced by DJB operation in HyO rats, may be linked to reduction in ChREBP mRNA.

The FXR is a ligand-activated transcription factor, which activation can inhibit the expression of SREBP-1c, and its target lipogenic genes, preventing excessive FA synthesis (56). In addition, FXR activate PPAR-α, which regulates the transcription of the FA oxidation genes: CPT-1 and ACO (56, 57). Here, we observed that DJB surgery reduced hepatic FXR and ACO mRNAs in HyO DJB group. The mRNA for PPAR-α in liver of HyO DJB is lower than CTL rats. Possibly, the downregulation of FXR/PPAR-α decrease ACO transcription. This is a contradictory point, since such an effect would reduce peroxisome β-oxidation in HyO DJB rats. In addition, the FXR is a target for BA and regulates BA metabolism, via an autocrine mechanism (56). Increased serum levels of BA were evidenced in diet and genetic-obese diabetic rats, submitted to DJB surgery (23, 43). An improvement in glucose homeostasis and hepatic steatosis, after DJB, in diabetic rats were linked with enhanced gut secretion of GLP-1by BA stimulation (43). In this way, the reduction in the expression of hepatic FXR gene, in HyO DJB rats, may suggest that the mechanism of action, by which DJB operation exerts benefits in hypothalamic obesity, possibly differs from that evidenced in genetic and diet-induced obesity.

Other important feature, observed in this study, is that HyO rats were insulin resistant, but normoglycemic, exhibiting increased content of hepatic glycogen. Enhanced glycogen content was also evidenced in liver from high-fat and western diet obese rodents (19, 58). This effect may increase hepatic lipogenesis and development of NAFLD, Lu et al. (58) demonstrated that, in insulin resistant high-fat diet mice, the increased hepatic glycogen was linked to enhanced expression of gene and protein of the glycogenic scaffolding protein (PTG/R5). This protein regulates the mobilization and storage of glycogen, and also directs the glucose excess, that cannot be storage as glycogen, to FA synthesis, this mechanism involves the activation of the target of rapamycin complex 1 (mTORC1) that regulates downstream lipogenic genes. In accordance, deletion of PTG prevented hepatic glycogen accumulation and steatosis in high-fat diet mice (58).

Here, we tried to verify whether the better insulin action and decreased lipogenesis promoted by DJB in HyO rats may be associated with AMP-activated protein kinase (AMPK) activation. It is known that the AMPK inhibits lipogenesis, via phosphorylation of ACC, decreasing malonyl-CoA levels, which enhances CPT-1a action and FA oxidation (59). Furthermore, a report demonstrated that AMPK improved hepatic insulin sensitivity and inhibited lipogenesis by downregulation of mTORC1 pathway (60). However, no modifications in pAMPK/AMPK protein expressions were evidenced between HyO DJB and HyO Sham rats. Therefore, new studies are necessary to dissect how lipogenesis is downregulated in hypothalamic obesity by DJB surgery.

In summary, here we report the first evidence that DJB surgery has a late effect upon NAFLD in hypothalamic obesity, and the improvement in hepatic steatosis in HyO rats was not linked with weight loss or reductions in adipose tissue stores. The benefits of DJB against steatosis in hypothalamic obesity were associated in part by amelioration of insulin sensitivity and reduction in the expression of hepatic enzymes involved in *de novo* lipogenesis.

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**Authorship**

GMS, SLB and MLB: conception and experimental design; GMS, KRC and ACPAM: execution of experiments; SLB and ACFA: execution of bariatric procedure; GMS and HCLBS: execution of genic expression and data interpretation; MLB, RAR and ACB: intellectual contribution and provide materials and reagents. GMS, MLB, ACB and RAR: data interpretation and manuscript writing.

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**Table 1** – Primer sequences for real-time qPCR assays.

| Gene | Forward (5' – 3') | Reverse (5' – 3') |
|---|---|---|
| ACC-1 | AGGAAGATGGTGTCCCGCTCTG | GGGGAGATGTGCTGGGTCAT |
| ACO | CCCAAGACCCAAGAGTTCATTC | TCACGGATAGGGACAACAAAGG |
| ChREBP | GAAGACCCAAAGACCAAGATGC | TCTGACAACAAAGCAGGAGGTG |
| CPT-1a | CTCCTGAGCAGTTACCAATGC | GAACCTTGGCTGCGGTAAGAC |
| FASN | AGGTGCTAGAGGCCCTGCTA | GTGCACAGACACCTTCCCAT |
| FXR | GCAACTGCGTGATGGATATG | TTCGCTGTCCTCATTCACTG |
| LPK | GACCCGAAGTTCCAGACAAGG | ATGAGCCCGTCGTCAATGTAG |
| MTTP | CTTCTGCCTACACTGGCTACG | GTTCTCCTCTCCCTCATCTGG |
| PPAR-α | GTACGGTGTGTATGAAGCCATCTT | GCCGTACGCGATCAGCAT |
| PPAR-γ | GCCCTTTGGTGACTTTATGGAG | GCAGCAGGTTGTCTTGGATGT |
| SCD-1 | CAGTTCCTACACGACCACCACTA | GGACGGATGTCTTCTTCCAGAT |
| SREBP-1c | GGAGCCATGGATTGCACATT | AGGAAGGCTTCCAGAGAG |
| GAPDH | GGAGAAACCTGCCAAGTATGATG | AACCTGGTCCTCAGTGTAGCCCC |

ACC-1 – acetyl-CoA carboxylase, ACO – acyl-CoA oxidase, ChREBP – carbohydrate response element-binding protein, CPT-1a – carnitine palmitoyl transferase-1a, FASN – fatty acid synthase, FXR – farnesoid X receptor, LPK – liver pyruvate kinase, MTTP - microsomal triglyceride transfer protein, PPAR-α - peroxisome proliferator-activated receptor-α, PPAR-γ - peroxisome proliferator-activated receptor-γ, SCD-1 – stearoyl-CoA desaturase-1, SREBP-1c – sterol regulatory element binding protein-1c, GAPDH – glyceraldehyde 3-phosphate dehydrogenase.

**Table 2** – Final body weight, food intake and excreted feces in 12 hours of CTL, HyO Sham and HyO DJB rats.

| | CTL | HyO Sham | HyO DJB |
|---|---|---|---|
| Body weight (g) | 440 ± 7.8[a] | 337 ± 11.7[b] | 337 ± 10.5[b] |
| Food intake in 12 h (g) | 15.5 ± 0.7[a] | 9.8 ± 0.7[b] | 10.8 ± 0.5[b] |
| Food intake/BW (mg/g) | 32 ± 0.1 | 28 ± 0.2 | 31 ± 0.2 |
| Excreted feces in 12 h (g) | 6.3 ± 0.3[a] | 4.2 ± 0.3[b] | 4.1 ± 0.3[b] |

Data are mean ± SEM (n = 14 - 19 rats). Different letters over the bars indicate significant difference (One-way ANOVA followed by the Tukey post-test, $P < 0.05$).

**Table 3** – Fasting serum biochemical parameters, TyG index, HOMA-IR and liver glycogen content in CTL, HyO Sham and HyO DJB rats.

| | CTL | HyO Sham | HyO DJB |
|---|---|---|---|
| Glucose (mg/dL) | 65 ± 1.7 | 66 ± 2.5 | 66 ± 2.8 |
| Insulin (ng/mL) | 0.4 ± 0.1[a] | 1.0 ± 0.2[b] | 0.3 ± 0.1[a] |
| Triglyceride (mg/dL) | 155 ± 7.9[a] | 237 ± 16.7[b] | 140 ± 16.5[a] |
| Cholesterol (mg/dL) | 110 ± 4.3 | 122 ± 4.3 | 102 ± 5 |
| NEFA (mol/L) | 0.6 ± 0.02[a] | 0.9 ± 0.1[b] | 0.6 ± 0.1[a] |
| TyG index | 9.2 ± 0.1[a] | 9.6 ± 0.1[b] | 9 ± 0.1[a] |
| HOMA-IR | 1.5 ± 0.2[a] | 2.8 ± 0.5[b] | 1.6 ± 0.2[a] |
| Liver glycogen content (mg/100 mg liver) | 25 ± 1.6[a] | 38 ± 1 .8[b] | 31 ± 1.3[a] |

Data are mean ± SEM (n = 8 - 14 rats). Different letters over the bars indicate significant difference (One-way ANOVA followed by the Tukey post-test, $P < 0.05$).

**Figure legends**

Figure 1: *HyO rats not changed body weight (BW) and adiposity after 6 months of DJB surgery*. (A) Means ± SEM (n = 17 rats) of BW measured weekly, and (B) total BW, expressed as area under growth curve (AUC), registered before and after DJB or Sham surgeries in HyO rats. *HyO DJB and HyO Sham groups are different from CTL ($P < 0.05$). (D) Lee index and (E) retroperitoneal and perigonadal fat pad weights in CTL, HyO Sham and HyO DJB rats. Different letters over the bars indicate significant difference (One-way ANOVA followed by the Tukey post-test, $P < 0.05$).

Figure 2: *DJB surgery, after six months, ameliorates liver steatosis in HyO rats*. Macroscopic aspect of the liver (A), light microscopy of (B) hematoxylin and eosin and (C) Mallory's trichrome-stained liver sections from CTL, HyO Sham and HyO DJB rats. Means ± SEM (n= 5-19 rats) of the liver weight (D), and hepatic TG (E) and CHOL (F) contents in CTL, HyO Sham and HyO DJB rats. Arrows indicate microvesicular steatosis. Different letters over the bars indicate significant difference (One-way ANOVA followed by the Tukey post-test, $P < 0.05$).

Figure 3: *DJB surgery improves the expression of hepatic genes involved in de novo lipogenesis*. Means ± SEM (n= 4-7 rats) of the mRNA expressions for (A) LPK, ACC-1, FASN, SCD-1, ACO, CPT-1a, MTTP; and (B) ChREBP, SREBP-1c, PPAR-γ, PPAR-α and FXR in the liver of CTL, HyO Sham and HyO DJB rats. Different letters over the bars indicate significant difference (One-way ANOVA followed by the Tukey post-test, $P < 0.05$).

Figure 4: *DJB operation normalizes hepatic protein expression of de novo lipogenesis enzymes in HyO rats*. Means ± SEM (n= 6-10 rats) of the ACC (A), pACC/ACC (B) FASN

(C), SCD-1 (D), CPT-1a (E), MTTP (F) and pAMPK/AMPK (G) protein expressions in the liver of CTL, HyO Sham and HyO DJB rats. Different letters over the bars indicate significant difference (One-way ANOVA followed by the Tukey post-test, $P < 0.05$).

Figure 1

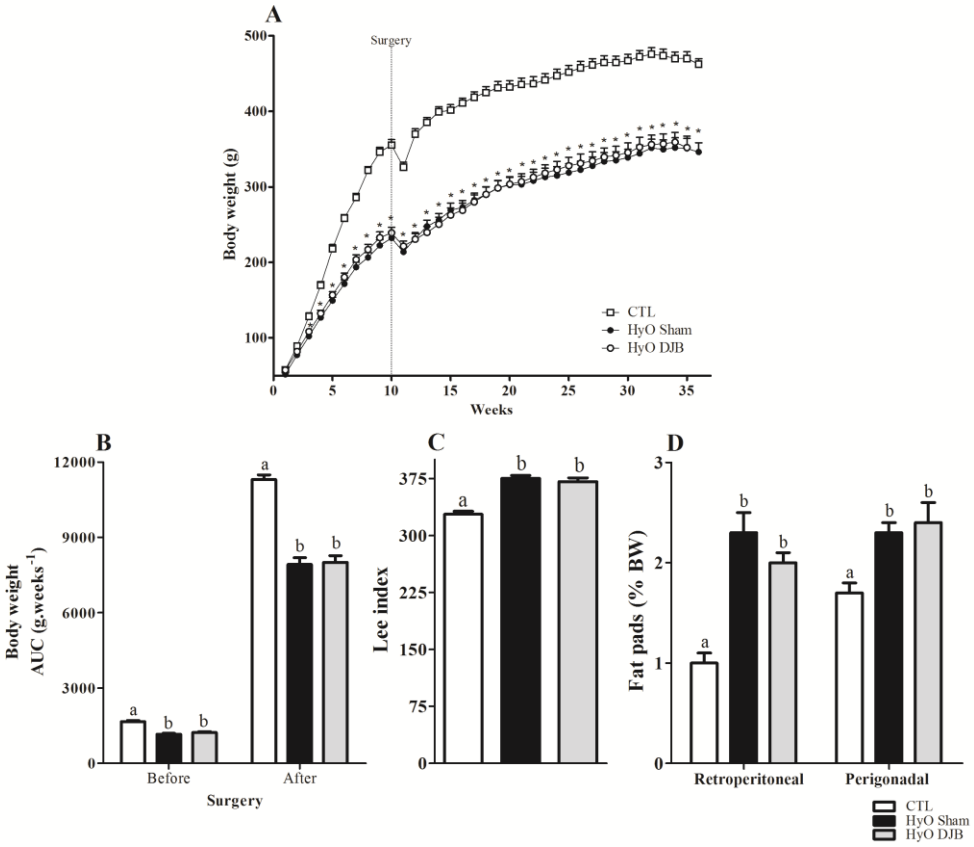
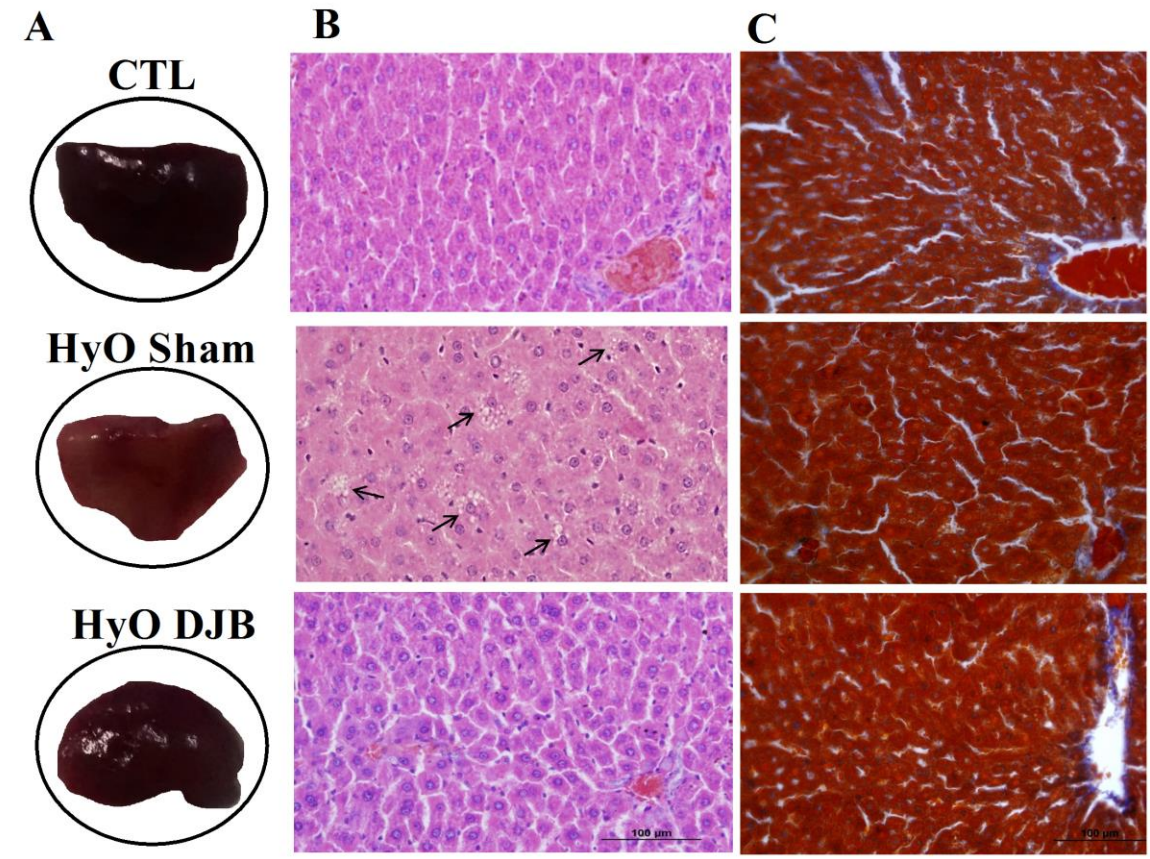

A
Surgery
Body weight (g)
Weeks
CTL
HyO Sham
HyO DJB
B
Body weight AUC (g.weeks-1)
Before
After
Surgery
C
Lee index
D
Fat pads (% BW)
Retroperitoneal
Perigonadal
CTL
HyO Sham
HyO DJB

Figura 2


A
B
C
CTL
HyO Sham
HyO DJB
100 µm
100 µm

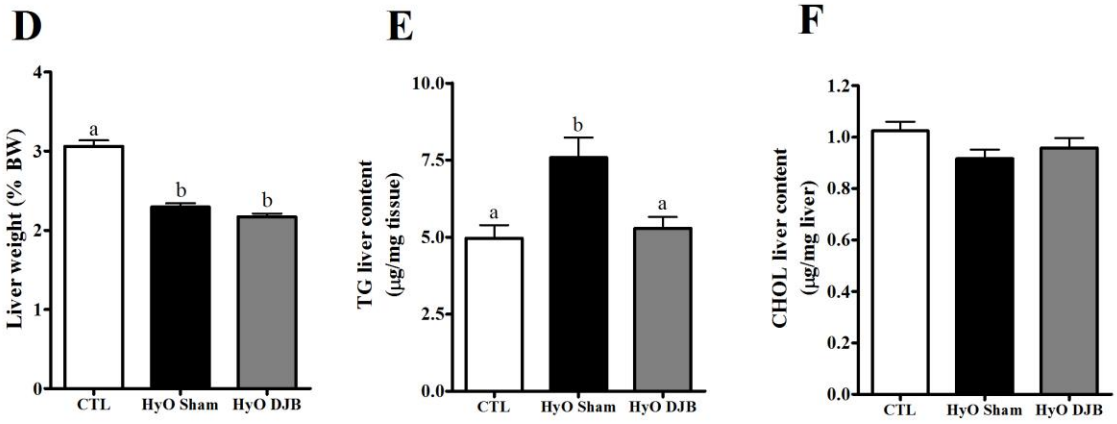

D
Liver weight (% BW)
a
b
b
CTL
HyO Sham
HyO DJB
E
TG liver content (µg/mg tissue)
a
b
a
CTL
HyO Sham
HyO DJB
F
CHOL liver content (µg/mg liver)
CTL
HyO Sham
HyO DJB

Figure 3

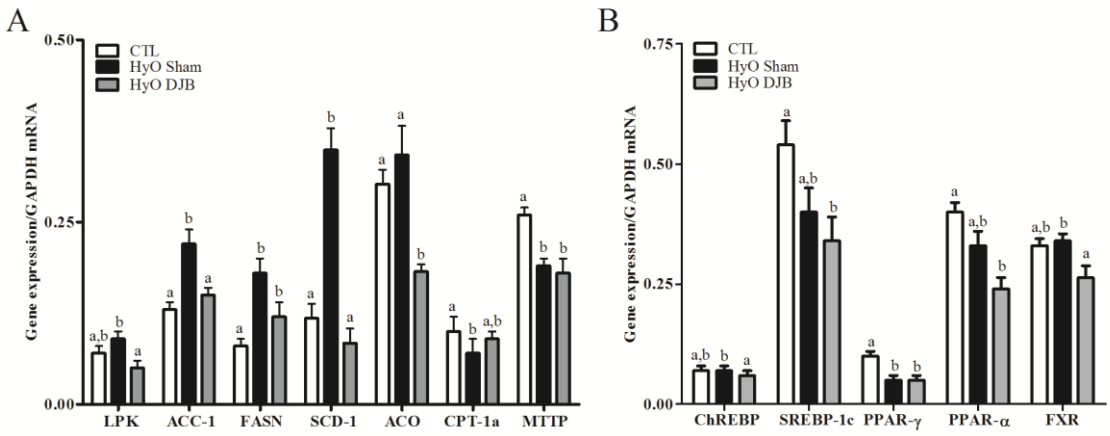
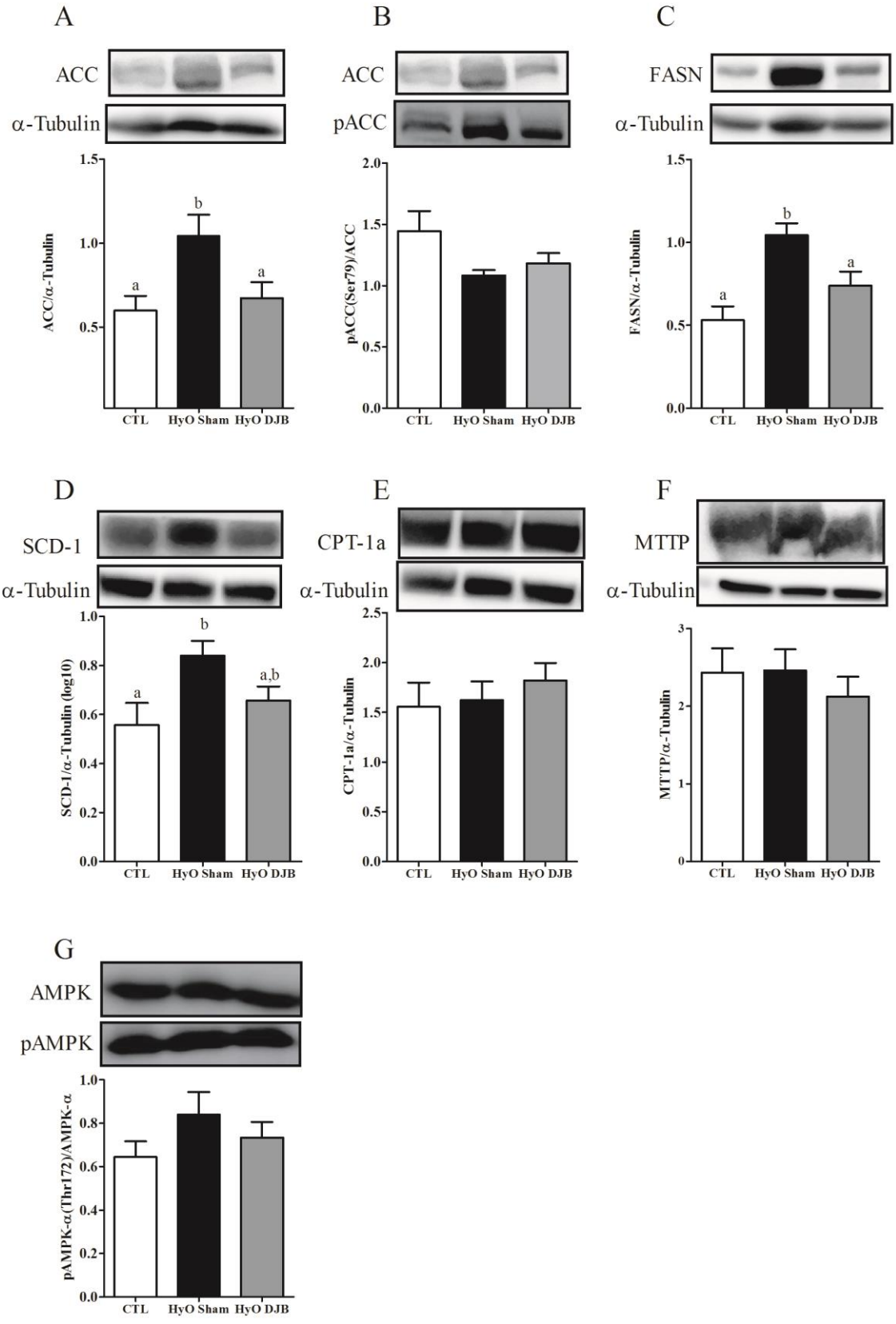

A
CTL
HyO Sham
HyO DJB
Gene expression/GAPDH mRNA
0.50
0.25
0.00
LPK
ACC-1
FASN
SCD-1
ACO
CPT-1a
MTTP
B
CTL
HyO Sham
HyO DJB
Gene expression/GAPDH mRNA
0.75
0.50
0.25
0.00
ChREBP
SREBP-1c
PPAR-γ
PPAR-α
FXR

Figura 4


A
ACC
α-Tubulin
ACC/α-Tubulin
a
b
a
CTL
HyO Sham
HyO DJB
B
ACC
pACC
pACC(Ser79)/ACC
C
FASN
α-Tubulin
FASN/α-Tubulin
D
SCD-1
α-Tubulin
SCD-1/α-Tubulin (log10)
a,b
E
CPT-1a
α-Tubulin
CPT-1a/α-Tubulin
F
MTTP
α-Tubulin
MTTP/α-Tubulin
G
AMPK
pAMPK
pAMPK-α(Thr172)/AMPK-α

# ANEXO A:

## Normas da revista científica

**Author Guidelines**

***Updated 22 February 2016***

***Liver International is published in an online-only format.***

**TYPES OF MANUSCRIPTS**

***Original Manuscripts:*** Liver International publishes both clinical and experimental research in all areas of normal and abnormal liver function and disease. Purely descriptive research or methodology papers will not be considered for publication. Basic science manuscripts will be considered for publication only if they have translational significance.

Manuscript length **should not exceed 5,000 words excluding figures and/or tables**.

Manuscripts are allowed **no more than 8 figures and/or tables with up to 6 individual panels each**.

**Additional supporting information can be submitted along with the original manuscript.** Authors preparing supporting information for publication should carefully read the guidelines at: https://authorservices.wiley.com/bauthor/suppinfo.asp.

Abstract:

- The abstract must not exceed **250** words.
- The title must not exceed **130** characters.
- Key points must be organized in a box with **4 bullet points** which highlight your paper's originality. Must not exceed **100 words**.

- The abstract **must be organized as follows:**
  - *Background & Aims*
  - *Methods*
  - *Results*
  - *Conclusions*

Do not use abbreviations, footnotes or references in the abstract. An electronic word count of the abstract must be included.3-5 key words at the end of the abstract must be provided.

The manuscript must be arranged as follows:

- Title page
- Abstract in the *Liver International* format
- Key points box
- Introduction
- Materials and methods (or Patients and methods)
- Results
- Discussion
- Acknowledgements
- References
- Tables
- Figure legends

As a rule, original manuscripts will be evaluated by two independent reviewers and by the Editors. The Editors reserve the right of early rejection without further external review if the manuscript is judged unlikely to be accepted. Manuscripts requiring extensive revision will be at a disadvantage for publication and will be rejected. Authors shall be responsible for the quality of language and style and are strongly advised against submitting a manuscript which is not written in grammatically correct English. The Editors reserve the right to reject poorly written manuscripts even if their scientific content is qualitatively suitable for publication. Manuscripts are submitted with the understanding that they are original contributions and do not contain data that have been published elsewhere or are under consideration by another journal. Meeting abstracts do not constitute prior publication. Revised manuscripts should be accompanied by a point-by-point reply to the critiques, specifying the changes made in the revised version, which should be highlighted.

***Rapid Communications***: will be considered for important and timely scientific contributions; authors should explain in their accompanying letter why they wish to submit their paper as a rapid communication. Rapid communications will undergo regular peer-review as original manuscripts but will be granted fast-track processing. Such papers **should not exceed 3,000 words**, including no more than **2 tables or figures and 20 references**. Additional supporting information can be submitted along with the original manuscript. Authors preparing supporting information for publication should carefully read the guidelines at: https://authorservices.wiley.com/bauthor/suppinfo.asp.

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- The recommended length is **5,000 words** with an upper limit of 6000 words excluding references, figures and/or tables.
- References should not exceed a maximum of 150.
- The inclusion of a reasonable number of tables and figures to summarize critical points is highly desirable.
- Review articles must be accompanied by a title page and an abstract.
- Reviews should include a **key points' box**, with a maximum of 5 bullet points that briefly summarizes the content of the review.

Review articles are reviewed by the Editors and are normally sent off for peer review. Invitation to write a review article does not imply automatic acceptance.

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***Debates in Hepatology:*** Case Reports or Original Articles eliciting a debate on patient management or relevant controversial topics meriting discussion are encouraged to be submitted in a rapid communication format for consideration of publication in this section. Upon acceptance, they will be accompanied by two short invited commentaries providing a PRO and a CON view on the subject.

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**ORGANIZATION OF THE MANUSCRIPT**

Submitted manuscripts must be typed **double-spaced** throughout, preferably using a "standard" font (we prefer **Times/Arial 12**). Tables and figures must be numbered. For mathematical symbols, Greek letters, and other special characters, use normal text. The references must be in accordance with *Liver International* reference style (**see References**). Approved nomenclature for gene and protein names and symbols should be used, including appropriate use of italics (all **gene symbols** and loci should be in **italics**) and capitalization as it applies for each organism's standard nomenclature format, in text, tables, and figures. Full **gene names** are generally **not in italics** and Greek symbols are not used. **Proteins** should **not be italicized**.

**Improperly prepared manuscripts will not be entered into the peer review process and will be sent back to the author for correction.**

**A letter of submission** must be uploaded with all manuscripts. The letter may be used to outline the strengths of the manuscript. All commercial relationships (i.e. consultancies, patent-licensing agreements) that might pose a conflict of interest in connection with the submitted manuscript must be included in the letter. **In case of possible conflicts of interest, the letter must include a detailed description of the nature of the conflict of interest, the full name of the entity with which there is a conflict, as well as address, telephone number, webpage address, a detailed financial disclosure, and any other important, relevant details.**

The **Title page** must contain:

a. A title of no more than 130 characters.
b. Names of the Authors including the first names of all the Authors in full.
c. Names of department(s) and institution(s) where the work was done.
d. Name, address, telephone and fax numbers, and electronic mail address of the corresponding Author.
e. Electronic word count for main body of manuscript.
f. Number of figures and tables.
g. List of abbreviations in the order of appearance.
h. Conflict of interest.
i. Financial support.
j. Trial registration number, if applicable (see below).

**Animal trials** – Manuscripts reporting experiments using animals must include a statement giving assurance that all animals received human care and that study protocols comply with the institution's guidelines. Statistical methods used should be outlined.

**Human trials** – Manuscripts reporting data from research conducted on humans must include a statement of assurance in the methods section of the manuscript reading that: (1) informed consent was obtained from each patient included in the study and (2) the study protocol conforms to the ethical guidelines of the 1975 Declaration of Helsinki as reflected in a priori approval by the institution's human research committee.

**Randomised controlled trials** – Any paper that is a randomised control trial should adhere to the guidelines that can be found at the following web-site: www.consort-statement.org. The checklist should be downloaded, completed and uploaded with your submission. **The trial**

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**References** – References must be in accordance with the *Liver International* reference style. References are ordered as they appear in the text and citation numbers for references are placed between "brackets" ("[ ]") in the text as well as in the reference list.
Authors should be listed surname first, followed by the initials of given names (e.g. Bolognesi M). **If there are more than six authors, the names of the first six authors followed by et al. should appear.** Titles of all cited articles are required. Titles of articles cited in reference list should be in upright, not italic text; the first word of the title is capitalized, the title written exactly as it appears in the work cited, ending with a full stop. Journal titles are abbreviated according to common usage, followed by Journal years, semicolon (;) before volume and colon (:) before full page range (see examples below).
All articles in the list of references should be cited in the text and, conversely, all references cited in the text must be included in the list. Personal communications and unpublished data should be cited directly in the text by the first Author, without being numbered.
**An example of how references should look within the text**: HVPG was measured by hepatic vein catheterization using a balloon catheter according to a procedure described elsewhere [14, 15] and used as an index of portal hypertension [16].
**An example of how the reference list should look:**
[14] Merkel C, Bolognesi M, Bellon S, Zuin R, Noventa F, Finucci G, et al. Prognostic usefulness of hepatic vein catheterization in patients with cirrhosis and esophageal varices. Gastroenterology 1992;102:973-979.
[15] Groszmann RJ, Wongcharatrawee S. The hepatic venous pressure gradient: anything worth doing should be done right. Hepatology 2004;39:280-282.
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**Tables** – Tables should be provided as Word files (*.doc), Excel (.xls) or Illustrator (*.eps) compatible files. **TIFF and JPG files are not acceptable for table submission**. Tables should contain a maximum of 10 columns. **Tables submitted in landscape orientation will not be accepted**. Tables should include a title, table legend, and if necessary footnotes.
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Two standard widths are used and **figures should fit in one (8.5 x 23.5 cm) or two (17.5 x 23.5 cm) columns** (see Figure and Table Guidelines).

Figure files should be provided in high resolution .eps format, minimum 800dpi (for graphs and charts) or .tiff format, minimum 300dpi (for photographs or a combination of images and text). Figures with multiple parts (A, B, C) should be provided as separate files. Panel lettering should be in Arial bold 16 pt, capitalized and no full stop (A) while lettering in figures (axes, conditions) should be in Arial 14 pt, lower case type with the first letter capitalized and no full stop. Do not copy and paste figure files into the manuscript word document.

Figures can be in grayscale or CMYK. All photomicrographs should have a scale on the photograph. Photographs of identifiable patients should be accompanied by written permission to publish from patient(s).

If you no longer have the original data to improve/recreate graphs, charts or combination figures to high resolution, please crop the graph portion in Microsoft PowerPoint and re-type any text in Arial/Times New Roman in minimum 14pts. This will ensure that at least the text is clear. Any lines in the figures must be at least 1.5 or 2pts thick. We will accept the revised .ppt file. For more information on file requirements, please refer to http: //authorservices.wiley.com/prep_illust.asp.

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**ACKNOWLEDGEMENTS**

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