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IALA MILENE BERTASSO

EFEITO DA INTERVENÇÃO CIRÚRGICA BARIÁTRICA SOBRE O METABOLISMO LIPÍDICO HEPÁTICO MATERNO DE RATAS OBESAS E SUA REPERCUSSÃO SOBRE A PROLE

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“Daria tudo o que sei, pela metade do que ignoro”

René Descartes

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RESUMO GERAL

No presente estudo, foram avaliados, em ratas obesas submetidas à derivação gástrica em Y de Roux (DGYR), o perfil lipídico e a expressão gênica e proteica de enzimas envolvidas no metabolismo lipídico hepático e sua repercussão nos machos da prole adulta. Ratas *Wistar* obesas pela dieta de cafeteria (CAF) foram submetidas à falsa operação (CAF FO) ou à DGYR (CAF DGYR). Após cinco semanas, iniciaram o período de acasalamento. Os machos da prole (F1) obtida foram denominados segundo o procedimento realizado em suas mães, em CAF FO-F1 e CAF DGYR-F1, os quais receberam dieta padrão do desmame aos 120 dias de vida. O perfil lipídico e vias metabólicas lipídicas hepáticas foram verificados nas mães e nos filhotes. Mães submetidas à DGYR apresentaram redução do peso corporal, acúmulo de gordura e dislipidemia. Todavia, a DGYR materna promoveu acúmulo de gordura hepática (esteatose grau 3), um mecanismo provavelmente decorrente da redução da lipogênese hepática e aumento da β-oxidação nas ratas do grupo CAF DGYR. Os descendentes CAF DGYR-F1 apresentaram, aos 120 dias de vida, redução do peso corporal, do acúmulo de gordura, da concentração sérica de triglicerídeos (TG) e conteúdo de colesterol hepático (COL). A quantidade de mRNA da acetil-CoA carboxilase (ACC) e da estearoil-CoA desaturase-1 (SCD-1) foi menor, ao passo que a quantidade do mRNA da carnitina palmitoil-transferase-1 (CPT-1) foi maior nos animais do grupo CAF DGYR-F1, comparados aos animais do grupo CAF FO-F1. A expressão proteica da ACC e da proteína de transferência de triglicerídeos microssomal (MTTP) foi maior, enquanto que a expressão da enzima ácido graxo sintetase (FASN) e da ACC fosforilada (pACC) foi menor nos animais do grupo CAF DGYR-F1, quando comparados aos animais do grupo CAF FO-F1. A DGYR em ratas CAF melhorou a obesidade após prenhez e lactação. Entretanto, estes animais apresentaram danos hepáticos severos, os quais não repercutiram nos machos da prole adulta, apesar das alterações em vias metabólicas lipídicas.

Palavras-chave: Obesidade; Esteatose hepática; Programação metabólica; Derivação gástrica em Y de *Roux*.

GENERAL ABSTRACT

In the present study, the lipid profile and the gene and protein expression of enzymes involved in hepatic lipid metabolism and its repercussion in males of adult offspring were evaluated in obese rats submitted to *Roux* - en - Y gastric bypass (RYGB). Wistar rats obese by the cafeteria diet (CAF) were submitted to false operation (CAF SHAM) or to RYGB (CAF RYGB). Five weeks later, they began the mating season. The offspring males (F1) obtained were named according to the procedure performed in their mothers, in CAF SHAM-F1 and CAF RYGB-F1, who received a standard weaning diet at 120 days of age. Lipid profile and hepatic lipid metabolic pathways were verified in the mothers and pups. Mothers submitted to the RYGB presented reduction of body weight, fat pad and dyslipidemia. However, maternal RYGB promoted accumulation of hepatic fat (grade 3 steatosis), a mechanism probably due to the reduction of hepatic lipogenesis and increased β-oxidation in the rats of the CAF RYGB group. CAF RYGB-F1 offspring presented a reduction in body weight, accumulation of fat, serum triglycerides (TG) and hepatic cholesterol (CHOL) at 120 days. The amount of acetyl-CoA carboxylase (ACC) and stearoyl-CoA desaturase-1 (SCD-1) mRNA was lower, whereas the amount of carnitine palmitoyl transferase-1 (CPT-1) mRNA was higher in animals from the CAF RYGB-F1 group, compared to the animals from the CAF SHAM-F1 group. The protein expression of ACC and the microsomal triglyceride transfer protein (MTTP) was higher, whereas the expression of fatty acid synthetase (FASN) and phosphorylated ACC (pACC) was lower in CAF RYGB-F1 group, when compared to the animals of the CAF SHAM-F1 group. RYGB in CAF rats improved obesity after pregnancy and lactation. However, these animals had severe liver damage, which did not affect males of adult offspring, despite changes in lipid metabolic pathways.

Key words: Obesity; Hepatic steatosis; Metabolic programming; Roux-en-Y gastric bypass.

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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
ALT – Alanina aminotransferase
Apo – Apoproteína
AST – Aspartato aminotransferase
ATP – Adenosina trifosfato
ChREBP – proteína de ligação ao elemento de resposta aos carboidratos
COL – Colesterol
CPT-1a – Carnitina palmitoil-transferase 1
CTP-2 – Carnitina palmitoil-transferase 2
DAG – Diacilgliceróis
DBP – Derivação biliopancreática
DGYR – Derivação gástrica em Y de Roux
DHGNA – Doença hepática gordurosa não alcoólica
Dieta de CAF – Dieta de cafeteria
DM-2 – Diabetes Mellitus tipo 2
DNA – Ácido desoxirribonucleico
EH – Esteatose hepatica
EHNA – Esteato hepatite não alcoólica
FASN – Complexo enzimático de ácido graxo sintetase
FXR – Farnesoid X receptor
GCK – Glicoquinase
G1P – Glicose-1-fosfato
G3P – Gliceraldeído-3-fosfato
G6P – Glicose-6-fosfatase
GLUT2 – Transportador de glicose tipo 2
HDL – Lipoproteína de densidade alta
IDL – Lipoproteína de densidade intermediária
IMC – Índice de massa corporal
LDL – Lipoproteína de densidade baixa
LDN – Lipogênse *de novo*
LPA – Ácido lisofosfatídico
LPK – Piruvato quinase
LPL – Lipoproteína lipase
LXR – Receptor X do fígado
miRNA – microRNA
MTTP – Proteína de transferência de triglicerídeos microssomal
PL – Fosfolipídeos
PPAR-α - receptor ativado pelo proliferador de peroxissoma-α
QM – Quilomícron
RI – Resistência à insulina
SCD-1 – estearoil-CoA desaturase-1
SM – Síndrome metabólica
SREBP-1c – proteína de ligação do elemento regulador de esterol-1c
TCA – Ciclo do ácido tricarboxílico
TG – Triglicerídeo
VLDL - lipoproteínas de densidade muito baixa

INTRODUÇÃO GERAL

Alterações no contexto histórico, econômico, científico e social na sociedade refletiram em mudanças no estilo de vida e hábitos da população, envolvendo o consumo alimentar e a prática de atividades físicas. O âmbito alimentar foi marcado pelo fenômeno de transição nutricional, em que o consumo de fibras e nutrientes passa a ser substituído por alimentos de preparo rápido, altamente energéticos e de menor custo, fato intimamente relacionado ao avanço da industrialização. Eventos cotidianos envolvendo o gasto calórico vêm sendo substituídos por novas tecnologias amplamente distribuídas, seja no ambiente de trabalho ou no lar. Dessa forma, o aumento do consumo de alimentos cada vez mais calóricos, aliado à inatividade física, contribui para a pandemia da obesidade (BLEIL, 1998; MONTEIRO; CONDE; POPKIN, 2002; KRZYSZTOSZEK et al., 2015).

Evidenciada por sua etiologia multifatorial, a obesidade é caracterizada pelo desequilíbrio entre a ingestão e o gasto energético, resultando no armazenamento do excesso de energia em forma de gordura (HILL; COMMERFORD, 1996). Essa doença assumiu caráter epidêmico, abrangendo países desenvolvidos e em desenvolvimento, como o Brasil, onde 18% de sua população adulta está obesa (BRASIL, 2014). Além disso, atua como importante fator de risco para doenças cardiovasculares, desordens metabólicas, incluindo a intolerância à glicose, diabetes melittus tipo 2 (DM-2), hipertensão arterial, dislipidemia, hipercolesterolemia, além de certos tipos de câncer, doenças respiratórias e osteoarticulares (SOWERS, 1998; ROSIEK et al., 2015).

Em mulheres, a obesidade está intimamente associada ao aumento na prevalência de diversos riscos obstétricos, como diabetes gestacional, hipertensão, pré-eclâmpsia e mortalidade neonatal. Adicionalmente, estudos sugerem que a obesidade materna também está associada a danos importantes nos descendentes. O indivíduo em formação é sensível às condições ambientais que modulam as vias do desenvolvimento fetal e as consequências da nutrição gestacional podem modificar sua homeostase energética ao longo da vida

(NAEYE, 1990; CNATTINGIUS et al., 1998; KING, 2006). Dessa forma, o estado nutricional materno representa um “desafio” ambiental ao indivíduo em formação, como forma de preparo para a vida fora do útero (DELAGE; DASHWOOD, 2008; LILLYCROP, 2011). O mecanismo desta “memória pré-natal” ocorre devido à grande plasticidade gênica do organismo em desenvolvimento. Tal característica permite modificações do perfil epigenético, como a superexpressão ou silenciamento de genes relacionados à manutenção da homeostase energética. Com isso, as diferenças entre o que está sendo previsto e o ambiente externo real, predispõem o organismo a “padrões nutricionais não ideais”, durante o período de formação e crescimento, com o possível desenvolvimento de diversas doenças metabólicas ao longo da vida (LILLYCROP, 2011; CHAUDHARY et al., 2012).

A associação entre a obesidade e as alterações no metabolismo materno pode ocorrer por meio do fator comum denominado resistência à insulina (RI), um dos componentes da síndrome metabólica (SM) (VASQUES et al., 2009). Uma das características da SM é o acúmulo de lipídeos nos hepatócitos, que, em excesso, origina o espectro de doenças, denominado doença hepática gordurosa não alcóolica (DHGNA) (ÂNGULO, 2002).

A primeira manifestação relacionada à DHGNA é a esteatose hepática (EH), a qual é caracterizada pelo acúmulo de lipídeos, excedendo 5% da massa hepática. Essa doença é influenciada pela dieta e perfil lipídico materno alterado, ao se observar características de EH em descendentes de ratas tratadas com dieta hiperlipídica (SONG et al., 2012; SOUZA et al., 2012).

Objetivando reverter o estado de obesidade e síndromes associadas, diferentes estratégias vêm sendo utilizadas, como administração de fármacos e dietas com restrição calórica. No entanto, vários pacientes não respondem às medidas clínicas ou terapêuticas e recorrem às operações bariátricas (COUTINHO, 1999; ZEVE; NOVAIS; JÚNIOR, 2012). Os resultados dos procedimentos bariátricos têm sido superiores às medidas tradicionais na redução de peso em pessoas obesas, podendo reduzir a morbidade em até 40% e, consequentemente, o aparecimento de doenças associadas à obesidade (SJOSTROM et al., 2004; ADAMS; GRESS; SMITH, 2007; HAFEEZ; AHMED, 2013). Dentre os procedimentos realizados, a operação bariátrica de derivação gástrica em Y de *Roux* (DGYR), um procedimento misto por reduzir o volume do

estômago associado ao desvio de uma porção do intestino, é considerada uma das mais efetivas e sustentáveis para perda de peso em obesos mórbidos (MACDONALD et al., 1997; KARRA; YOUSSEIF; BATTERHAM, 2010). Entretanto, poucos estudos demonstram se a perda de peso e a melhora metabólica após a operação pode prevenir os efeitos deletérios da obesidade materna sobre parâmetros metabólicos dos descendentes.

Sabe-se que crianças nascidas após a operação bariátrica materna de derivação biliopancreática (DBP) exibem menor prevalência de obesidade severa, maior sensibilidade à insulina e melhora no perfil lipídico, quando comparadas a irmãos que nasceram antes do procedimento cirúrgico materno (KRAL et al., 2006; SMITH et al., 2009).

O conhecimento relacionado aos fatores indutores da obesidade, suas consequências metabólicas, endócrinas, bem como suas respostas às operações bariátricas tem sido ampliado em grande parte, devido à utilização de modelos animais experimentais (SCLAFANI; SPRINGER, 1976; MEGUID et al., 2004; KENNEDY et al., 2010). Apesar dos diversos modelos animais experimentais para o estudo da obesidade e suas comorbidades, o mais semelhante à obesidade humana é o modelo induzido pela dieta de cafeteria (CAF) (WEST; YORK, 1998; PRADA et al., 2005). Neste modelo, os animais têm acesso livre a alimentos e a líquidos hipercalóricos selecionados. A dieta de cafeteria promove hiperfagia voluntária, resultando em ganho rápido de peso, maior teor de gordura abdominal e parâmetros pré-diabéticos, além de disfunções neuronais, relacionadas à alimentação desregulada, comuns em algumas formas humanas de obesidade (SAMPEY et al., 2011).

Existem poucos estudos demonstrando o efeito do procedimento bariátrico de DGYR sobre o perfil lipídico da prole de ratas obesas pela dieta CAF e os resultados apresentados até hoje são controversos. Considerando que o tempo após a cirurgia é um fator importante para avaliação das alterações no metabolismo lipídico, propomos o presente trabalho para responder ao seguinte questionamento: o procedimento bariátrico de DGYR é capaz de reverter os efeitos da obesidade materna induzida por dieta CAF, sobre o perfil lipídico hepático dos descendentes machos?

REVISÃO GERAL DE LITERATURA

Obesidade

A obesidade é caracterizada pelo acúmulo excessivo de gordura corporal, devido ao desequilíbrio energético entre o consumo alimentar e o gasto de energia (WHO, 2016). Quando a energia consumida excede o gasto calórico, há um estado de balanço energético positivo, sendo o excesso de energia armazenado na forma de gordura no tecido adiposo (HILL; COMMERFORD, 1996).

Para diagnóstico de sobrepeso e obesidade em adultos, o método mais utilizado é o Índice de Massa Corporal (IMC), definido como o peso em quilogramas, dividido pelo quadrado da estatura em metros (kg/m²). É um índice simples, onde indivíduos com IMC entre 25 a 30 kg/m² são classificados com sobrepeso e indivíduos com IMC acima de 30 kg/m² são classificados como obesos (WHO, 2016). Usando esse parâmetro, a Organização Mundial da Saúde (WHO) mostrou que, em 2014, 39% da população adulta mundial apresentava sobrepeso e 13% estavam obesos. Entretanto, em 2013, 56,9% dos brasileiros adultos estavam com sobrepeso e 20,8% obesos (IBGE, 2013).

A obesidade, que era vista como um mal quase restrito à população adulta e idosa, vem crescendo entre os mais jovens. No Brasil, o excesso de peso atinge 33,5% das crianças de 5 a 9 anos, e a obesidade 6,6% e 11,8% do total de meninos e meninas, respectivamente (WHO, 2016). Acredita-se que esses índices não estejam relacionados apenas à má alimentação e ao sedentarismo dos jovens, mas também ao “ciclo vicioso” sustentado pela obesidade, já que filhos de pais obesos têm 80 a 90% de probabilidade de serem adultos obesos (SHANKAR et al., 2008). Neste contexto, assume caráter preocupante o aumento na prevalência da obesidade em mulheres em idade reprodutiva, bem como, o aumento do ganho de peso durante a gestação. No Brasil, em 2014, 4%, 14% e 18% das mulheres das faixas etárias de 18 a 24, 25 a 34 e 35 a 44 anos, respectivamente, estavam obesas (BRASIL, 2014) e 25% das gestantes adultas apresentavam sobrepeso e 5,5% obesidade (NUCCI et al., 2001).

As funções reprodutivas femininas são negativamente influenciadas pelo estado obeso, o qual reduz a implantação e o desenvolvimento fetal, a taxa de gravidez, induzindo ciclos anovolutórios, alterações menstruais, síndrome do ovário policístico, além de estar associado à morte materna e fetal, pré-eclâmpsia, diabetes gestacional e anormalidades congênitas (DOUCHI et al., 2002; BRANNIAN; FURMAN; DIGGINS, 2005; CATALANO; EHRENB, 2006; LEUNG et al., 2008; GAILLARD et al., 2013; SCOTT-PILLAI et al., 2013). O acréscimo de 20% sobre o peso médio, leva ao aumento de 10% nas taxas de mortalidade para mulheres, sendo apresentadas em suas formas moderadas (IMC entre 28 e 35) e, ainda, nas formas mais graves (IMC>35) (MOKDAD et al., 2003).

O fato da pandemia da obesidade abranger 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, têm 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 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 esforço em conjunto, mobilizando o indivíduo, a comunidade, o governo e as diversas áreas do conhecimento (REIS; VASCONCELOS; BARROS, 2011).

Além de impor ao indivíduo forte estigma social, a obesidade é fator preponderante ao desenvolvimento de inúmeras doenças, como o DM-2, hipertensão arterial sistêmica, cardiomiopatia hipertrófica, dislipidemia, aterosclerose, doenças respiratórias além de estar relacionada com alterações metabólicas dos descendentes (HUBERT et al., 1983; KOPELMAN, 2000, PATTI, 2013).

Programação metabólica

A gestante obesa apresenta resposta inflamatória exagerada, com acúmulo de macrófagos e intensa produção de mediadores pró-inflamatórios

(CHALLIER et al., 2008). Alterações induzidas pelo perfil inflamatório materno se estendem à placenta, sugerindo que a obesidade expõe o feto em desenvolvimento a um ambiente inflamatório, através do qual, fenótipos programados podem manifestar-se na vida adulta (PUDER; MUNSCH, 2010; GUPTA et al., 2011; JUONALA et al., 2011). Desta forma, a nutrição exerce grande influência sobre o desenvolvimento dos organismos, especialmente em períodos críticos do desenvolvimento, incluindo a gestação, lactação e os primeiros meses de vida (SILVEIRA et al., 2007). A característica do ambiente nutricional nestas “janelas críticas” é o principal fator determinante para o crescimento do indivíduo (MCMILLEN; ROBINSON, 2005; ALFARADHI; OZANNE, 2011; HOFFMAN, 2014).

Levando em consideração o estado nutricional materno e sua forte relação com o desenvolvimento, Hales e Barker (2001) propuseram a teoria do “fenótipo econômico”, sugerindo que o desenvolvimento do feto fosse sensível ao ambiente nutricional gestacional e lactacional. Portanto, o metabolismo de um indivíduo apresenta grande capacidade de se adequar a diversos fatores ambientais, aumentando sua capacidade de sobrevivência a condições adversas (SCHEINER, 1993). A programação fetal tem inicialmente caráter adaptativo e protetor. Em períodos onde os fetos de mamíferos vivem em ambiente gestacional adverso, alterações permanentes em seu metabolismo e estrutura ocorrem em resposta à carência nutricional. Nesta fase de adaptação ocorre a priorização da estrutura de alguns órgãos, principalmente o sistema nervoso central (SNC), possibilitando assim proporcionar a sobrevivência do feto (GOTTILIEB; CRUZ; BODANESE, 2008). Considerando que essas alterações tenham sido permanentes, esse organismo em desenvolvimento poderá responder de maneira não adequada a variações na ingestão alimentar quando adulto. Nesse contexto, se esse indivíduo for exposto à maior disponibilidade de nutrientes na vida adulta, seu organismo pode apresentar maior predisposição ao acúmulo de energia, apresentando hiperfagia e aumentado conteúdo de tecido adiposo, favorecendo a instalação de doenças metabólicas (BARKER, 2007).

Em humanos, estudos demonstram que a exposição à hiperglicemia durante a gestação provoca a expansão do tecido adiposo nos filhos, predispondo ao risco de desenvolvimento de obesidade e DM-2 quando adultos, comparados a

irmãos nascidos antes do desenvolvimento da doença nas mães (DABELEA et al., 2000). Em um estudo de coorte, conduzido no Reino Unido, mais de 900 mulheres e seus bebês foram acompanhados desde a gestação até os seis anos após o nascimento. Os autores encontraram associação direta do ganho de peso excessivo materno na gestação com maior adiposidade das crianças ao nascimento e entre 4 e 6 anos (CROZIER et al., 2010). Assim, a obesidade materna pode “alimentar um ciclo vicioso”, aumentando o risco para o desenvolvimento desta doença na primeira e segunda geração, acelerando a epidemia da obesidade (PATTI, 2013).

Estudos utilizando modelos animais demonstraram que proles adultas de ratas obesas e hiperglicêmicas possuíam seis vezes mais chances de apresentarem RI e aumento de 20% na gordura corporal, comprimento naso-anal (CNA) e pressão arterial mais elevada e intolerância à glicose. Essas alterações foram detectadas já no início da vida e se mantiveram a longo prazo (BUCKLEY; JAQUIERY; HARDING, 2005).

Pesquisas ainda apontam diferenças intersexuais na resposta metabólica e epigenética da obesidade pré-concepcional e gestacional. Em estudo realizado com camundongos sobre os efeitos da obesidade materna induzida por dieta rica em gordura concluiu-se que machos e fêmeas da prole desenvolveram hipercolesterolemia e hiperinsulinemia, sendo que os descendentes masculinos apresentaram maiores consequências. Os resultados desse estudo fornecem evidências adicionais dos efeitos prejudiciais da obesidade materna sobre o colesterol e sensibilidade à insulina dos descendentes, que se manifestaram de modo diferente entre os sexos (DAHLHOFF et al., 2014).

As alterações derivadas das condições não ideais, as quais o feto é submetido em casos de obesidade materna, são denominadas modificações epigenéticas (MARMO et al., 1994). O conceito de epigenética foi introduzido por Waddington em 1939, e, mais tarde, relacionado a alterações na expressão gênica hereditária, onde não há alterações na sequência do DNA (ácido desoxirribonucléico), mantendo-se a integridade do genoma (WEBER et al., 2007; ESTELLER, 2008). A metilação do DNA e modificações das histonas são dois mecanismos importantes para a epigenética, que atuam na regulação gênica, desenvolvimento e carcinogênese (JONES et al., 2002). Os microRNAs (miRNAs)

também podem desempenhar papel importante no controle da metilação do DNA e modificações das histonas. Estes fazem parte do grupo dos pequenos RNAs não codificantes de proteínas e atuam na regulação pós-transcricional da expressão gênica (WILFRED; WANG; NELSON, 2008). A expressão de diversos miRNAs tem sido associada à regulação do crescimento, diferenciação e morte celular e, principalmente, ao metabolismo energético, incluindo a homeostase da glicose e metabolismo lipídico hepático (LAGOS-QUINTANA et al., 2002; BARTEL, 2004).

Metabolismo lipídico hepático

O fígado é considerado um órgão essencial no controle do metabolismo lipídico e glicêmico, atuando no direcionamento das gorduras com base nas condições hormonais e metabólicas dos indivíduos e fornecendo para os tecidos periféricos o substrato energético necessário (SPASSIANI; KUK, 2008). Os lipídeos podem ser armazenados, oxidados para a produção de adenosina trifosfato (ATP) ou encaminhados para os tecidos periféricos pelas lipoproteínas de densidade muito baixa (VLDL), sendo utilizados pelo músculo esquelético e armazenados pelo tecido adiposo (DURSTINE et al., 2002; NGUYEN et al., 2008).

Os metabólitos provindos da digestão alimentar são transportados até o fígado, por meio do sistema porta-hepático. A glicose é transportada, principalmente pelo transportador de glicose tipo 2 (GLUT2), para o interior do citoplasma do hepatócito, onde é fosforilada pela enzima glicoquinase (GCK) à glicose-6-fosfato, a qual é convertida em glicose-1-fosfato (G1P) pela enzima fosfoglicomutase. Por ação das enzimas UDP-glicose pirofosforilase e glicogênio sintase, a G1P pode ser adicionada às cadeias de glicogênio. Quando a capacidade de armazenamento de glicogênio hepático é saturada, a glicose é redirecionada à síntese de ácidos graxos (AG), processo denominado lipogênese *de novo* hepática (LDN) (Fig. 1; RUI, 2014). Em condições fisiológicas normais, os triglicerídeos (TG) não são armazenados no fígado em grandes quantidades, sendo transportados principalmente ao tecido adiposo e muscular via lipoproteína de densidade muito baixa (VLDL) (KOEPPEN; STANTON, 2009).

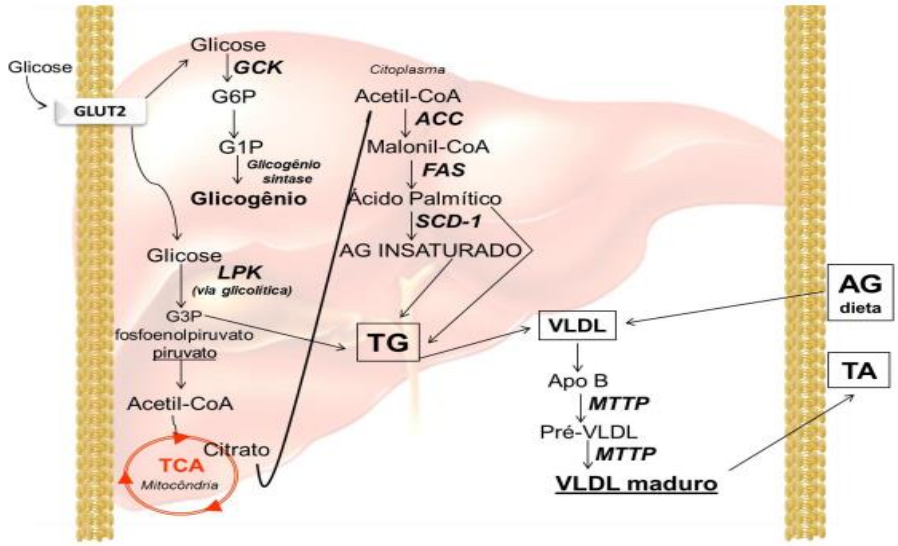

Figura 01: Lipogênese *de novo* e formação das lipoproteínas de densidade muito baixa (VLDL). Transportador de glicose tipo 2 (GLUT2); Glicoquinase (GCK); Piruvato quinase hepática (LPK); Acetil-CoA carboxilase (ACC); Ácido graxo sintetase (FASN); Estearoil-CoA desaturase-1 (SCD-1); Triglicerídeos (TG); Ácidos graxos (AG); Proteína de transferência de triglicerídeos microssomal (MTTP); Ciclo do ácido tricarboxílico (TCA) e tecido adiposo (TA), segundo Dentin; Girard; Postic, 2005.

Na LDN, a glicose é convertida à gliceraldeído-3-fosfato (G3P), fosfoenolpiruvato e piruvato por meio da enzima piruvato quinase hepática (LPK). A oxidação do piruvato produzido gera acetil-CoA, o qual é conduzido à mitocôndria para se integrar ao ciclo do ácido tricarboxílico (TCA). A elevada concentração de ATP e fosfato de dinucleotídeo de nicotinamida e adenina (NADPH), decorrente do estado alimentado, inibe a progressão do citrato no TCA, induzindo ao acúmulo intramitocondrial deste metabólito. Ocorre, então, o transporte de citrato ao citoplasma do hepatócito, sendo convertido à acetil-CoA (YAMASHITA et al., 2001). A enzima acetil-CoA carboxilase (ACC) catalisa a transformação de acetil-CoA à malonil-CoA, o qual é transformado em ácido palmítico, por adições repetidas de grupos acetil pelo complexo enzimático de ácido graxo sintetase (FASN). O ácido palmítico pode ser dessaturado pela enzima estearoil-CoA desaturase-1 (SCD-1), formando AG monoinsaturados. A enzima glicerol-3-fosfato aciltransferase catalisa a esterificação do G3P com o AG monoinsaturado recém-sintetizado, gerando ácido lisofosfatídico (LPA). O LPA é substrato para a enzima acilglicerol fosfato aciltransferase catalisar a formação de ácido fosfatídico (PA). Os PAs são processados com diacilgliceróis (DAG) pela enzima lipina e, em seguida, pela formação de triglicerídeo (TG) por ação da

enzima acil-CoA: diacilglicerol aciltransferase (BECHMANN et al., 2012; KAWANO; COHEN, 2013).

Os TGs também podem ser provenientes diretamente da dieta, sem a necessidade de glicose para sua formação. Nesse caso, para serem absorvidos pela mucosa intestinal, primariamente, recebem a secreção da vesícula biliar, formando micelas, para melhor ação das lipases. Após ação dessas enzimas, os TGs são convertidos em monoglicerídeos, diglicerídeos, ácidos graxos livres (AGL) e glicerol, que são ressintetizados a TG no interior dos enterócitos. Os TGs podem ser direcionados à circulação sanguínea, formando os AGL ou agrupados em fosfolipídeos (PL), colesterol (COL) e apoproteínas (apo) específicas (principalmente apoB48, apoCII e apoCIII), formando a lipoproteína quilomícrom (QM). Devido ao seu tamanho, os QM movem-se da mucosa intestinal para o sistema linfático e para a corrente sanguínea, onde são transportados para os músculos e tecido adiposo (NELSON; COX, 2002).

A apoCII presente nos QM atua como ativador da enzima lipoproteína lipase (LPL), também presente nos capilares do tecido adiposo e muscular. A função desta enzima é hidrolisar o TG a glicerol e AGL, para que possam sofrer influxo para o interior celular. Uma vez no citoplasma da célula adiposa ou muscular, os AGL são reesterificados e armazenados na forma de TG ou oxidados para a formação de ATP, respectivamente. À medida que vai sendo transportado pelos tecidos, o QM tem sua quantidade de TG reduzida e passa a ser denominado remanescente de QM. Estes são depurados pelo fígado pelo processo de endocitose mediada pelo receptor de apoE. Ao entrar no fígado por essa via, os TGs podem ser oxidados para produção de ATP ou corpos cetônicos quando o organismo necessita de energia. Entretanto, quando a dieta fornece maior aporte de AGL que o necessário, eles são convertidos em TG e empacotados como VLDL (KOEPPEN; STANTON, 2009).

Tanto os TGs hepáticos provenientes da LDN quanto os oriundos da dieta são transportados ao tecido adiposo pelas VLDLs. A síntese dessa lipoproteína tem início no retículo endoplasmático do hepatócito através da proteína de transferência de triglicerídeos microssomal (MTTP). Essa proteína realiza a transferência de PL, TG, COL livre e ésteres de COL para a apoB durante o processo de translação, dando origem a uma molécula de pré-VLDL.

Posteriormente, essa molécula se une a MTTP e forma a VLDL madura, a qual é secretada pelo fígado (MCGARRY; FOSTER, 1980; TOMKIN; OWENS, 2015). Dessa forma, o TG é transportado via VLDL do fígado ao tecido adiposo, onde é hidrolisado pela LPL dos capilares (NELSON; COX, 2002). A MTTP pode ser regulada por hormônios e macronutrientes, em níveis transcricionais, pós-trascricionais e pós-translacionais. Por exemplo, a insulina reduz a transcrição do gene de MTTP em células hepáticas e consequentemente diminui a secreção de VLDL, visto que sua produção é dependente de MTTP (HAGAN et al., 1994; HUSSAIN; NIJSTAD; FRANCESCHINI, 2011).

À medida que vão sendo hidrolisadas pela LPL, as VLDLs têm seu tamanho reduzido e se transformam em lipoproteínas de densidade intermediária (IDL). Grande parte das IDLs são rapidamente captadas pelo fígado através de receptores específicos. O restante continua circulando e transformando-se em LDL, as quais contêm pouco TG, mas são ricas em ésteres de COL e preservam apenas a apoB-100 (GINSBERG; TUCK, 2001; KOEPPEN; STANTON, 2009). Entretanto, os tecidos extra-hepáticos não são capazes de degradar o COL e necessitam de alternativas para descartá-lo. Para isso, o fígado, intestino delgado e macrófagos sintetizam a HDL, através da secreção de apoA1. O fornecimento de PL e COL livre dos QM e VLDLs à apo A1 desencadeia a formação da pré-β-HDL, a qual recebe o COL liberado pelas células, principalmente pelos macrófagos. Na medida em que vai passando pelos tecidos, essa molécula vai se tornando uma HDL rica em ésteres de COL. A enzima lecitina colesterol acetiltransferase transforma os PL em éster de COL que acabam formando um núcleo na molécula, o que confere seu formato redondo e a torna uma HDL madura, necessitando, assim, transportar o COL ao fígado. Esse processo é conhecido como transporte reverso de COL, onde a HDL madura recebe TG do QM e transfere ésteres de COL ao remanescente de QM, VLDL e LDL por intermédio da proteína de transferência de éster de COL e pela proteína de transferência de PL, e os transfere ao fígado para posterior reutilização ou excreção na forma de sais biliares. Essa transferência ocorre por ação da enzima lipase hepática e transportadores do tipo SRB (STEIN; STEIN, 1999; RUI, 2014).

Quanto à função catabólica hepática, em estado de jejum, os TGs armazenados no tecido adiposo necessitam ser mobilizados para fornecimento

energético. Concentrações altas de catecolaminas, glucagon e baixas de insulina, estimulam a fosforilação da lipase sensível a hormônio (LHS), que hidrolisa os TG dos adipócitos liberando AGL e glicerol. No sangue, os AGL são transportados pela albumina até os tecidos, entre eles o fígado, onde são encaminhados para as vias β-oxidativas (KOEPPEN; STANTON, 2009). No hepatócito, AG de cadeia longa (AGCL) são oxidados via β-oxidação para manutenção das quantidades de ATP necessário, o que pode ocorrer na mitocôndria ou no peroxissomo (MURTHY; PANDE, 1994; LIMA et al., 2005). Os mecanismos envolvidos na oxidação são semelhantes em ambas estruturas, exceto no tipo de AG utilizado pela mitocôndria (AG derivados da dieta) ou pelo peroxissomo (conjunto diferente de AG e seus derivados). Na β-oxidação peroxissômica, a enzima acil-CoA oxidase (ACO) catalisa os primeiros e determinantes passos desse processo (WANDERS, 2004). Já na β-oxidação mitocondrial, os AGLs são ativados na membrana mitocondrial externa por esterificação com a coenzima A, formando tio-ésteres Acil-Coa graxos. Estes interagem com a enzima carnitina palmitoil-transferase 1a (CPT-1a), localizada na membrana externa da mitocôndria e responsável por sua conversão em ésteres graxos do tipo Acil-carnitina. Estes últimos são então conduzidos através da membrana mitocondrial interna até a matriz mitocondrial, onde o grupo Acil-Coa graxo interage com uma proteína periférica presente no lado interno da mitocôndria, a carnitina palmitoil-transferase 2 (CPT-2), formando novamente acil-CoA (KERNER; HOPPEL, 2000; LIRA, 2010). Após, o produto da CPT-2 passa por ciclos de β-oxidação, onde NADH e FADH também são formados, os quais transferem seus elétrons para a cadeia respiratória. Como esses elétrons migram até o citocromo-C-oxidase e os prótons são expulsos a partir da matriz mitocondrial para o espaço intermembranar, cria-se um gradiente eletroquímico, cuja energia é utilizada pelo sistema ATP sintase para a geração de ATP, que é expulso pela adenina nucleotídeo translocase em troca de adenosina difosfato citosólicos (PESSAYRE et al., 2001; NELSON; COX, 2002).

Os fatores de transcrição regulam a expressão dos genes envolvidos com o processo de β-oxidação hepática e lipogênese (Fig. 2) (HORTON; GOLDSTEIN; BROWN, 2002; KOEPPEN; STANTON, 2009). As proteínas de ligação do elemento regulador de esterol (SREBP) são fatores de transcrição que

regulam a expressão de genes envolvidos na síntese de COL (SREBP-2), AG (SREBP-1c) ou ambos (SREBP-1b). Estudos têm demonstrado que a insulina ativa a SREBP-1c que, por sua vez, estimula a transcrição dos genes lipogênicos (ACC, FASN e SCD-1) e, por consequência, a lipogênese hepática (FORETZ et al., 1999; SHIMOMURA et al., 1999; AZZOUT-MARNICHE et al., 2000).

Outro fator de transcrição é a proteína de ligação do elemento responsivo a carboidratos (ChREBP) induzido pela glicose, independente da insulina, estimula 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 β-oxidação através do aumento da expressão do receptor ativado pelo proliferador de peroxissomos-α (PPAR-α) (MODICA; GADALETA; MOSCHETTA, 2010; TEODORO; ROLO; PALMEIRA, 2011), o principal regulador da β-oxidação dos AGCL nas mitocôndrias e nos peroxissomos. No período de jejum, sua expressão é aumentada estimulando a oxidação dos AG (HUANG et al., 2012).

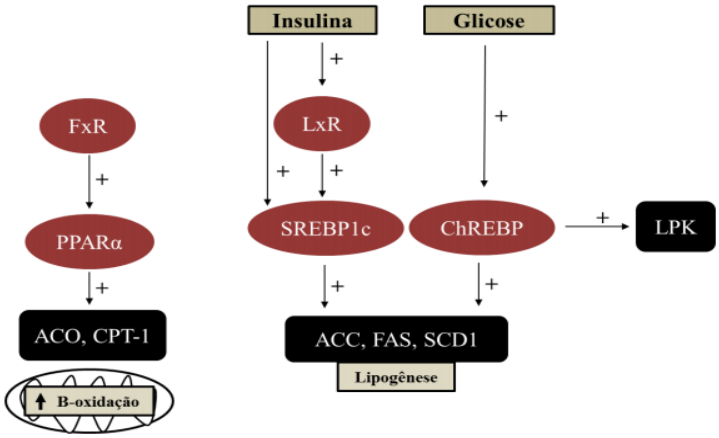

Figura 2: Fatores de transcrição envolvidos com o metabolismo lipídico. *farnesoid X receptor* (FXR); Receptor ativado pelo proliferador de peroxissoma-α (PPAR-α); Acil-CoA oxidase (ACO); Carnitina palmitoil-transferase-1 (CPT-1); Receptores X do fígado (LXR);

Proteína de ligação do elemento regulador de esterol-1c (SREBP-1c); Acetil-CoA carboxilase (ACC); Ácido graxo sintetase (FAS); Estearoil-CoA desaturase-1 (SCD-1); Proteína de ligação do elemento responsivo à carboidratos (ChREBP); Piruvato quinase hepática (LPK), segundo Berlanga et al., 2014.

As alterações nos processos descritos acima, que levam ao desequilíbrio na captação e eliminação de TG nos hepatócitos, podem levar ao acúmulo de TG no citoplasma. Isso pode ocorrer devido às 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 β-oxidação de AG, e 4) diminuição da secreção hepática de VLDL (ÂNGULO, 2002; MCCULLOUGH, 2006; ZÁMBÓ et al., 2013; BERLANGA et al., 2014). Esse acúmulo de TG no hepatócito pode resultar no desenvolvimento da doença hepática gordurosa não alcoólica (DHGNA).

Doença hepática gordurosa não alcoólica (DHGNA)

A DHGNA ou NAFLD, do inglês *Nonalcoholic Fatty Liver Disease*, é caracterizada pela deposição de lipídeos nos hepatócitos, cujo quadro patológico se assemelha ao da lesão hepática induzida pelo álcool, porém ocorre em indivíduos sem histórico de consumo abusivo da droga (DIEHL et al., 1999; ÂNGULO, 2002; MARCHESINI et al., 2003).

A DHGNA inclui um espectro de doenças hepáticas crônicas variando de simples esteatose hepática (EH), onde o fígado apresenta apenas acúmulo de lipídeos, até esteato-hepatite não alcoólica (EHNA). Nesse caso, além do acúmulo de lipídeos tem presença de inflamação e degeneração hepatocelular, podendo estar acompanhada do aparecimento de fibrose e até mesmo evoluir para a cirrose hepática e carcinoma hepatocelular (CHALASANI et al., 2012; WILLEBRORDS et al., 2015). A DHGNA é a doença hepática mais comum no mundo ocidental e afeta todos os grupos raciais e étnicos, sem predileção por sexo ou idade, e é a causa de elevação assintomática de aminotransferases em 45 a 90% dos casos. A prevalência dessa doença aumenta significativamente em obesos, podendo chegar de 50 a 75% (CARVALHEIRA; ZECCHIN; SAAD, 2002).

Estima-se que 1 bilhão de pessoas atualmente sofrem de alguma forma de DHGNA (LOOMBA; SANYAL, 2013).

O mecanismo molecular subjacente à progressão da DHGNA não é completamente compreendido, sua patogênese tem sido interpretada pela hipótese de “duplo-*hit*”. O “primeiro *hit*” inclui o acúmulo de lipídeos no fígado, seguido pelo “segundo *hit*”, onde mediadores pró-inflamatórios induzem a inflamação, lesão hepatocelular e fibrose (DAY; JAMES, 1998).

A característica do “primeiro *hit*” da DHGNA é o acúmulo de TG no citoplasma dos hepatócitos em decorrência do desequilíbrio entre a entrada e a saída de lipídeos. Ocorre o aumento na absorção hepática de AGLs derivados da circulação, devido ao aumento da lipólise do tecido adiposo e/ou a partir da dieta em forma de QM. Ocorre também o aumento na concentração de glicose e insulina em resposta à ingestão de carboidratos, que promovem a LDN. Esses eventos são acompanhados pela diminuição da oxidação mitocondrial de AGL e diminuição na secreção hepática de TG na forma de VLDL. Ou seja, em pacientes com DHGNA o aumento na aquisição de AG por captação e LDN, não são compensados pela oxidação de AGL e produção de VLDL (ANSTEE; GOLDIN, 2006; BERLANGA et al., 2014).

O “segundo *hit*” é geralmente atribuído ao estresse oxidativo, que causa peroxidação lipídica na membrana do hepatócito, e também a produção de citocinas que são, em parte, responsáveis pela progressão de EH para EHNA e cirrose. Toxinas bacterianas, hiperprodução de citocinas, especialmente o fator de necrose tumoral- α (TNF-α), alteração dos estoques de ATP e da atividade do citocromo P450 parecem ser gatilhos importantes para a progressão da doença e fibrogênese (DAY; JAMES, 1998; ÂNGULO, 2002; SASS; CHANG; CHOPRA, 2005).

O metabolismo lipídico hepático dos descendentes pode ser influenciado pela dieta materna. Em modelos animais, a obesidade materna e/ou ingestão de uma dieta rica em gordura durante a gravidez pode programar a adiposidade, RI, dislipidemias, hipertensão e EH na prole (GREGERSEN et al., 2005; KHAN et al., 2005; MCCURDY et al., 2009; ASHINO et al., 2012; SONG et al., 2012; SOUZA et al., 2012). Em humanos, estudos mostram efeitos semelhantes, onde a obesidade materna pré-gravidez é associada ao aumento da

obesidade, disfunção cardiovascular, RI e EH na infância, adolescência e vida adulta do indivíduo (MINGRONE et al., 2008; PIRKOLA et al., 2010).

Sabe-se que o excesso de lipídeos é armazenado no tecido adiposo branco, em condições homeostáticas, porém o desenvolvimento deste tecido só ocorre após o terceiro trimestre gestacional (SYMONDS et al., 2003). Portanto, acredita-se que a obesidade materna, que expõe o fígado fetal ao excesso de TG, adipocinas e outros fatores, provoca alterações na expressão de genes que regulam positivamente a lipogênese e negativamente a lipólise, o que contribui para a acumulação hepática de lipídeos e para o estado inflamatório (SEGOVIA et al., 2014).

McCurdy e colaboradores (2009) demonstraram os efeitos da programação em resposta à dieta materna hiperlipídica em macacos. Curiosamente, nem todas as mães que receberam a dieta desenvolveram obesidade e RI. No entanto, quando analisada durante o terceiro trimestre de prenhez, machos e fêmeas da prole de mães alimentadas com dieta hiperlipídica demonstrou sinais de EHNA, tais como a inflamação hepática, acúmulo de TG e ativação prematura do gene gliconeogênico. Altas concentrações de TG também foram observadas aos 30 e 180 dias de vida desses animais, além da duplicação na porcentagem de gordura corporal. Coletivamente, esses resultados sugerem que um feto em desenvolvimento é altamente vulnerável ao excesso de lipídeos, independente da obesidade e/ou diabetes materna, onde a exposição a esta pode aumentar o risco de DHGNA nos descendentes (MCCURDY et al., 2009).

Resultados semelhantes foram reproduzidos em ratos alimentados com dietas ricas em gordura durante a gestação e a lactação. A prole apresentou ganho de peso, aumento dos depósitos de gordura, elevação das concentrações séricas de insulina, TG e citocinas pró-inflamatórias (PARK et al., 2010; ASHINO et al., 2012). Em proles de ratas alimentadas com dieta hiperlipídica durante a gestação, lactação ou em ambas, foi observada EH. A expressão de SREBP-1c foi maior entre os filhotes cujas mães receberam dieta durante a lactação e durante a lactação e gestação, indicando estimulação da transcrição de genes lipogênicos e síntese dos AG (GREGÓRIO et al., 2010). Além disso, a maioria dos estudos que investigam os efeitos da programação da obesidade materna observam “deficiências acentuadas” na prole masculina, porém o motivo para

essas diferenças intersexuais não é bem compreendido. Para o tratamento da DHGNA, é indicada a perda de peso, incluindo tratamento medicamentoso, acompanhamento nutricional e prática de exercícios físicos. Porém, em muitos casos de obesidade mórbida, essas medidas não apresentam a eficácia esperada e o indivíduo acaba recorrendo aos procedimentos operatórios (JEFFERY et al., 2000).

Operação bariátrica

Os procedimentos bariátricos têm emergido como estratégia efetiva no tratamento do obeso mórbido, causando perda de peso durável e, além disso, melhoram a glicemia, dislipidemia, previnem a hipertensão arterial e o DM-2 (BRINCKERHOFF et al., 2013; KASHYAP et al., 2013; SCHAUER et al., 2014). Os mecanismos de ação da operação bariátrica são complexos e envolvem múltiplos sinais neuroendócrinos que exercem efeitos no sistema nervoso central, assim como em órgãos periféricos, constituindo um amplo campo para estudos (FRÜHBECK, 2015).

A intervenção cirúrgica no tratamento da obesidade teve seus primeiros estudos na década de 50, quando as primeiras técnicas consistiam em criar grande efeito disabsortivo por meio da exclusão de grande parte do intestino delgado (HYDOCK, 2005; MANCINI, 2006).

Tradicionalmente, as operações bariátricas são classificadas em restritivas, disabsortivas e mistas. Nas restritivas, o único órgão modificado é o estômago, visando provocar a redução da cavidade gástrica, assim, o paciente terá a sensação de saciedade com menor quantidade de alimento. As mais comuns são: gastroplastia vertical com bandagem, balão intragástrico e bandagem gástrica ajustável por vídeo (DAMASO; TOCK, 2005). Já os procedimentos disabsortivos (derivação duodenal-jejunal e derivação jejuno-ileal) envolvem alterações no trato intestinal que provocam a diminuição da área de absorção de nutrientes (TACK; DELOOSE, 2014). Nas operações bariátricas mistas, além do estômago, o intestino do paciente também é alterado, somando-se os efeitos de ambos os procedimentos. As técnicas mistas mais conhecidas são a DGYR e a derivação biliopancreática (DBP) (BALTASAR et al., 1996).

A DGYR caracteriza-se como procedimento misto por reduzir o volume do estômago associado ao desvio de uma porção do intestino, e tornou-se padrão ouro dentre as operações bariátricas, sendo o procedimento mais comumente realizado para perda de peso (KARRA; YOUSSEIF; BATTERHAM, 2010). Entre outros efeitos, esse procedimento melhora a hiperglicemia e frequentemente causa remissão do DM-2 em pacientes com obesidade mórbida, levando à melhora na sensibilidade à insulina e na função da célula β-pancreática (ANDERWALD et al., 2012).

Quanto ao efeito do procedimento bariátrico sobre o perfil lipídico, Holdstock e colaboradores (2005) verificaram que entre seis e doze meses após o procedimento de DGYR em pacientes com obesidade mórbida, houve redução das concentrações plasmáticas de insulina, TG, COL total e lipoproteína de densidade baixa (LDL). Um ano após o procedimento houve aumento da lipoproteína de densidade alta (HDL). Após a realização do procedimento bariátrico de DGYR em humanos que apresentavam obesidade hipotalâmica, verificou-se melhora na homeostase glicêmica e lipídica desses pacientes, bem como perda de peso e diminuição da hiperfagia (HOLDSTOCK et al., 2005; TENGHOLM; GYLFE, 2009).

Hady e colaboradores (2012) avaliaram o efeito da gastrectomia vertical em *sleeve* (GVS) em pacientes obesos, sete dias, um, três e seis meses após o procedimento cirúrgico. Verificou-se redução da massa corporal um mês após a cirurgia e melhora na concentração de grelina, insulina e glicose sete dias após o procedimento cirúrgico. Em relação ao perfil lipídico, observaram que a concentração de TG reduziu um mês após a gastrectomia, mantendo-se reduzida aos três e seis meses. O COL total e LDL apresentaram concentrações reduzidas aos três meses pós-cirurgia.

Em 2013, Buzga e colaboradores utilizando-se da GVS observaram melhora no peso corporal, na homeostase da glicose e na concentração de TG em mulheres obesas seis meses após o procedimento cirúrgico. Não houve diferenças na concentração da LDL. Foram demonstrados em ratos obesos, 50 dias após a realização dessa mesma operação, redução na concentração plasmática de TG, COL e fosfolipídios de jejum (STEFATER; WILSON-PÉREZ; CHAMBERS, 2012; BUZGA et al., 2013).

Um estudo sobre o impacto do procedimento bariátrico na DHGNA/EHNA foi realizado por Rabl e Campos (2012). Foram analisadas quatro técnicas operatórias distintas, sendo elas: DGYR (doze estudos); banda gástrica ajustável (dois estudos); derivação biliopancreática/desvio biliopancreático com duodenal *Switch* (dois estudos) e gastroplastia vertical (quatro estudos), totalizando vinte estudos realizados entre 1990 e 2011. Para cada um dos estudos, foi relatada a quantidade de meses após a operação onde ocorreu a segunda biópsia e os resultados da mesma que buscavam avaliar três critérios: esteatose, inflamação e fibrose. O resultado da histologia para EH verificado na segunda biópsia hepática demonstrou melhora significativa nesse parâmetro, em que dezenove estudos, do total de vinte, mostraram que os pacientes após a operação bariátrica apresentaram redução no acúmulo de lipídeos hepáticos. Também verificou-se uma melhora no estado inflamatório em quinze estudos e melhora no quadro de fibrose em treze estudos. Sendo assim, pode-se observar, por meio de estudos histológicos, que houve melhora no fígado da maioria dos pacientes portadores da DHGNA e EHNA, após a operação bariátrica (RABL; CAMPOS, 2012).

Um fator importante na determinação da progressão dos danos hepáticos nesses pacientes está intimamente relacionado com a acelerada perda de peso que ocorre após os procedimentos cirúrgicos. Essa situação leva ao aumento excessivo da lipólise e consequentemente ao acúmulo de AGCL no fígado, podendo acarretar piora precoce e transitória nesses pacientes (VERNA; BERK, 2008). Entretanto, poucos estudos têm demonstrado se a perda de peso e a melhora metabólica pós-operatória pode prevenir os efeitos deletérios da obesidade sobre parâmetros metabólicos dos filhos. Estudos demonstram que crianças nascidas após a operação bariátrica materna de DBP exibem menor prevalência de obesidade severa, maior sensibilidade à insulina e melhora no perfil lipídico, quando comparadas com os irmãos que nasceram antes do procedimento cirúrgico materno (KRAL et al., 2006; SMITH et al., 2009). Guénard e colaboradores (2013) verificaram superexpressão e metilação gênica diferencial em cinco vias imunes e inflamatórias em crianças nascidas após o procedimento bariátrico materno de DBP em relação aos irmãos nascidos antes do procedimento cirúrgico nas mães.

Em animais, um estudo utilizando ratas obesas pela dieta hiperlipídica submetidas à GVS, verificou que a prole apresentou redução do peso e do tamanho em relação à prole de mães controle. Quando a dieta hiperlipídica foi mantida até a puberdade, verificou-se que os animais nascidos de mães submetidas à GVS apresentaram propensão ao desenvolvimento de intolerância à glicose e aumento da adiposidade comparada aos animais de mães controles (GRAYSON et al., 2013).

Modelo animal para indução da obesidade

Para compreender os mecanismos envolvidos na fisiopatologia da obesidade, são utilizados diferentes modelos animais experimentais. Estes modelos podem ser de origem genética, como os camundongos ob/ob e db/db (BRAY; YORK, 1979; GAO et al., 2013), ou podem ser induzidos, como, por exemplo, pela ingestão de dieta CAF (SAMPEY et al., 2011), dietas hiperlipídicas, injeções neonatais de glutamato monossódico (MSG), entre outros (NAGATA et al., 2006).

Dentre os modelos de dietas experimentais para roedores, utilizados em laboratórios, a dieta CAF, ou dieta ocidentalizada, é o modelo que mais assemelha-se a grande variedade de alimentos relacionados à pandemia da obesidade na sociedade ocidental. Neste modelo, os animais têm acesso livre aos alimentos e líquidos hipercalóricos selecionados. A dieta CAF promove hiperfagia voluntária, resultando em rápido ganho de peso, gordura abdominal e parâmetros pré-diabéticos, além de disfunções neuronais duradouras, relacionadas à alimentação desregulada, comuns em algumas formas humanas de obesidade (SAMPEY et al., 2011).

Estudos de Sagae e colaboradores (2012) demonstraram que a obesidade iniciada precocemente afeta o número de oócitos e folículos pré-antrais e a espessura da camada folicular em ratas obesas pela dieta CAF. O pico pré-ovulatório de progesterona e hormônio luteinizante também é afetado, porém o pico de estradiol e prolactina não são alterados pela obesidade. A receptividade sexual em ratas, não é comprometida pela obesidade e a hiperinsulinemia pode estar associada às alterações na ovulação desses animais (SAGAE et al., 2012). Dessa forma, a obesidade materna induzida por dieta pode ter influência

permanente na expressão gênica dos descendentes, por interagir com mecanismos epigenéticos que alteram a conformação da cromatina e a acessibilidade dos fatores de transcrição (GALLOU-KABANI; JUNIEN, 2005).

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ARTIGO CIENTÍFICO

EFEITO DA INTERVENÇÃO CIRÚRGICA BARIÁTRICA SOBRE O METABOLISMO LIPÍDICO HEPÁTICO MATERNO E SUA REPERCUSSÃO SOBRE A PROLE

Effect of bariatric surgery intervention on maternal hepatic lipid metabolism and its effect on the offspring

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ABSTRACT

Aims: We evaluated in obese female rats undergoing *Roux*-in-Y gastric bypass (RYBG), the lipid profile and the gene and protein expression of enzymes involved in hepatic lipid metabolism and its repercussion in male adult offspring.
Main methods: Wistar female rats rendered obese on a cafeteria diet (CAF) were undergone to sham operation (CAF SHAM) or RYBG (CAF RYGB). After five weeks, they began the mating period. The F1 male offspring obtained was named in CAF SHAM-F1 and CAF RYGB-F1, who received standard diet.
Key findings: Maternal RYGB reduced weight, fat accumulation and improved serum lipid profile, however it was observed grade 3 steatosis. This event may be related with the reduction of *de novo* lipogenesis pathway and increased β-oxidation of the liver of the CAF RYGB group. At 120 days of life, the group CAF RYGB-F1 presented body weight and fat accumulation reduction, lower serum concentration of triglycerides and hepatic content of cholesterol. The amount of mRNA of acetyl-CoA carboxylase (ACC) and estearoil-CoA desaturase-1 was lower, while the gene expression of carnitine palmitoyl-transferase-1 was higher in the liver of animals of group CAF RYGB-F1 compared to the group CAF SHAM-F1. The ACC and transfer protein microssomal triglycerides were more expressed, while the enzyme fatty acid synthase and ACC phosphorylated were less expressed in the CAF RYGB, compared to the group CAF SHAM-F1.
Significance: The RYGB in CAF rats "improved obesity" after lactation. However, these animals presented liver damage, which did not affect the offspring, despite alterations in metabolic lipidic pathways.

Keywords: Obesity; Hepatic steatosis; Metabolic programming; *Roux*-en-Y gastric bypass.

1. Introduction

The consumption of CAF diet by rodents reproduces the central elements of obesity in humans. Thus, the CAF diet promotes voluntarily hyperphagia, rapid weight gain, accumulation of fat in the adipose tissue, hyperglycemia, hyperinsulinemia, dyslipidemia and insulin resistance (IR), reproducing the profile of the metabolic syndrome observed in human obese subjects [1].

Obesity predisposes to excessive accumulation of lipids in the hepatocyte, culminating with liver diseases not related to alcoholism, the non-alcoholic fatty liver disease (NAFLD) [2]. The first sign of NAFLD is hepatic triglyceride accumulation (steatosis) [3], which may be caused by increased absorption of fatty acids (AG) in circulation; increase in *de novo* lipogenesis (DNL); reduction of β-oxidation and/or

reduction of very low density lipoproteins assembly and secretion of the liver (VLDLs) [4, 5].

Epidemiological studies show that the environment in critical periods, including pregnancy, lactation and the first months of life, exert a strong influence on the development of the individuals, being associated with the installation of diseases throughout life, in particular obesity and its comorbidities [6]. This phenomenon is defined as a metabolic programming, molecularly explained by epigenetics [7]. In this way, the maternal obesity can "induce a vicious cycle" that culminates with the increase in the epidemic of this disease [8].

The *Roux*-en-Y gastric bypass (RYGB) is one of the surgical bariatric techniques most used and promotes rapid weight loss associated with improvement in glycemic profile, lipid and IR [9]. That way, can be one of the alternatives are most effective in interrupting the vicious cycle caused by obesity. However, few studies have demonstrated the effects of the RYGB about pregnancy and health of children [10-12]. Thus, the objective of this work was to evaluate in obese rats by CAF diet submitted to RYGB, the lipid profile and the gene and protein expression of enzymes involved in hepatic lipid metabolism and its impact on adult males of the offspring.

2. Materials and methods

2.1. Animal model

Experimental procedures are agreement with the Ethical Principles for Animal Research established by the National Council for Control of Animal Experimentation (CONCEA) and approved by the Institutional Committee for Ethics in Animal Experimentation (CEUA/UNIOESTE). Thirty-two female *Wistar* rats (21 days of age) were induced to obesity through the offering of the cafeteria diet (CAF) associated with the soft drink degassed (modified to GOULARTE et al., 2012) [13]. During the entire experimental period the rats were kept under light conditions (07h-19h) and temperature (23 ± 2 ºC) controlled. After 15 weeks of supply of CAF diet, the rats were randomly subjected to RYGB (CAF RYGB, n=18) or sham operation (CAF SHAM, n=14). Five weeks after the operation, the rats of both groups started the breeding season during the

evening period, at the ratio of two females to one male. After verified pregnancy through monitoring of the estrous cycle, the rats were placed individually in boxes, and during the pregnancy and lactation period, continued to receive the CAF diet. The birth of the offspring was considered postnatal day 0 and weaning occurred at 30 days of life. One week after weaning the pups, the dams were euthanized. The first generation (F1) of the male offspring obtained was named according to the treatment of mothers in CAF SHAM-F1 (n=28) and CAF RYGB-F1 (n=14), from seven litters of rats CAF SHAM and five litters of rats CAF RYGB, respectively. The puppies received standard diet for rodents (BioBase, Águas Frias, SC, BRA) of weaning to euthanasia, which occurred at 120 days of age.

2.2. Roux-en-Y gastric bypass and sham operation

Preoperative procedures were performed as described by Meguid et al., (2004) [14]. The rats were deprived of food for 12 hours, and were anesthetized with isoflurane (Isoforine®, Cristália, Brazil, SP, BRA). The RYGB was performed according to Hao et al., (2013) [15]. In the Sham group, a midline incision in the anterior abdominal wall was made; the stomach, duodenum and intestines were massaged, and the incision was closed.
For the first two postoperative days (PO) was administered a daily dose of saline (20 mL-0,9%) and sodium dipyrone (50 mg/kg, Medley, Germany). The rats of both groups received water *ad libitum*, but were fasted until the third PO day. From the 4th to the 10th PO day were offered CAF liquid diet and 350 ml of soft drink, after this period all the rats returned to solid diet.

2.3. Assessment of Obesity

For euthanasia, the dams and the offspring were fasted for 8 hours, with free access to water. A blood sample was collected from the tail vein for glucose measurement using a glucose analyzer (Tech Free®, SD Biosensor, Korea). The final body weight (BW) and nasoanal length were measured in all groups to obtain the Lee Index [from the ratio of body weight (g) 1/3/Nasoanal length (cm) x 1000] [16], which was used as a predictor of obesity in rodents. The rats were euthanized by decapitation and total blood samples were

collected to obtain the serum. In addition, the retroperitoneal and perigonadal fat pads were collected and weighed, as well as the liver.

2.4. Serum biochemical analysis

The whole blood was centrifuged at 4.000 revolutions per minute (rpm) for 15 minutes. From serum collected was performed the quantification of cholesterol (CHOL), triglycerides (TG), high density lipoprotein (HDL), aspartate aminotransferase (AST) and alanine aminotransferase (ALT) using 4,600 VITROS *chemistry system* equipment (Ortho Clinical Diagnostics, USA). The concentration of serum insulin was measured by enzyme-linked immunosorbent assay (RIA) (Sigma-Aldrich Chemicals, St Louis, MO, USA).

2.5. TyG index

The TG and glucose (TyG) index (Ln[fasting TG (mg/dL) × fasting glycemia (mg/dL) / 2]) was calculated and used for insulin resistance evaluation in all groups of rats, since it is a highly sensitive and specific method for assessing insulin sensitivity during the fasting state [17].

2.6. Lipid content in the liver

The mothers's and offspring's liver was weighed and approximately 500 mg of the left lobe were removed for determination of the total lipids extracted by the method of Folch [18]. The extract was evaporated and then diluted in isopropanol. The TG and CHOL liver content were measured through commercial kit, according to manufacturer's instructions (Laborclin®, Bioliquid, Pinhais, PR, BR and Wako®, Germany, respectively).

2.7. Liver histopathology

A liver sample was removed from the left lobe of each animal, in transverse direction to the board. The material was fixed in 4% paraformaldehyde during 24 hours, dehydrated

in alcohol ascending concentrations and after diaphanization in xylene was embedded in histologically paraplast (Sigma Co, Saint Louis, MO). Slices of three microns in thickness were prepared for staining with hematoxylin and eosin (H&E). The liver histopathology was examined and graded according to the magnitude of steatosis, based on Brunt's classification with modifications for rodent models. Briefly, steatosis was graded (0-3), as follows: 0, none to 5% of hepatocytes affected; 1, >5% to 30% affected; 2, >30% to 60% affected; and 3, >60% affected [19].

2.8. Hepatic mRNA expression

Approximately 30 mg of the lobe side of liver was collected and stored in 150 µl of RNAlater® solution. The liver RNA was isolated using commercial kit (Promega, Madison, USA) and the reverse transcription of mRNA was performed using the Superscript II kit (Invitrogen, Carlsbad, CA, USA). The transcripts were detected using the 7500 Real-Time PCR system with the SYBR Green PCR Master Mix (Applied Biosystems, Carlsbad, CA, USA). The Primer sequences used for gene expression in the ACC, FASN, SCD-1, CPT-1 and MTTP were designed and purchased from the manufacturer (Sigma-Aldrich Chemicals, St Louis, MO, USA) are shown in table 1. The amount of expression of each gene was normalized by the internal control glyceraldehyde-3-phosphate dehydrogenase (GAPDH) gene.

Table 1

Primer sequences for Real-time PCR assays.

Gene	***Forward* (5'–3')**	***Reverse* (5'–3')**
ACC	AGGAAGATGGTGTCCCGCTCTG	GGGGAGATGTGCTGGGTCAT
FASN	AGGTGCTAGAGGCCCTGCTA	GTGCACAGACACCTTCCCAT
SCD-1	CAGTTCCTACACGACCACCACTA	GGACGGATGTCTTCTTCCAGAT
CPT-1	CTCCTGAGCAGTTACCAATGC	GAACCTTGGCTGCGGTAAGAC
MTTP	CTTCTGCCTACACTGGCTACG	GTTCTCCTCTCCCTCATCTGG
GAPDH	GGAGAAACCTGCCAAGTATGATG	AACCTGGTCCTCAGTGTAGCCCC

ACC - Acetyl-CoA carboxylase; FASN - Fatty acid synthase; SCD-1 - Estearoil-CoA desaturase-1, MTTP - Microsomal triglyceride transfer protein; CPT-1a - Carnitine palmitoyltransferase 1a; GAPDH - Glyceraldehyde 3-phosphate dehydrogenase.

2.9. Hepatic protein expression

For protein expression determination, a fragment of liver was solubilized in homogenization buffer at 4ºC (containing:100 mM tris pH 7.5, 10 mM sodium pyrophosphate, 100 mM sodium fluoride, 10 mM EDTA, 10 mM sodium vanadate, 2 mM phenylmethylsulfonyl fluoride and 1% Triton-X 100) using a Polytron MA 102 generator (model MA 102/Mini; Piracicaba, SP, Brazil). After the addition of 10% Triton, the homogenate was centrifuged at 12.000 rpm, at 4ºC for 30 minutes to remove insoluble material. Protein concentration was measured by the Bradford dye method, using BSA to form a standard curve and Bradford reagent (Bio-Agency Lab., São Paulo, SP, BRA). For SDS gel electrophoresis and Western blot analysis, 80 µg of samples were homogenized with loading buffer containing beta-mercaptoethanol. After heating at 97ºC for 5 min, the proteins were separated by electrophoresis and afterwards transferred to nitrocellulose membranes that were subsequently blotted with polyclonal antibodies to Acetyl-CoA carboxylase (ACC, Cell Signaling Technology, Boston, MA, USA), phosphoric acid-acetyl-CoA carboxylase (pACC, Cell Signaling Technology, Boston, MA, USA), fatty acid synthase (FASN, Santa Cruz Biotechnology Inc., USA), estearoil-CoA desaturase-1 (SCD-1, Abcam, Cambridge, UK), carnitine palmitoyl-transferase-1 (CPT-1), transfer protein triglycerides microssomal (MTTP). α-Tubulin was used as an internal control (1:1,000, Sigma-Aldrich Chemicals, St Louis, MO, USA). Visualization of specific protein bands was performed by incubating the membranes with goat anti-rabbit secondary antibody (1:10,000; cat.7074, Cell Signaling Tecnology, Boston, MA, USA), followed by revelation in the system of image capture L-Pix Chemi Express (Loccus Biotechnology®, Cotia, SP, Brazil). The band intensities were quantified by optical densitometry using the LabImage 1D software of image analysis (Loccus Biotechnology®, Cotia, SP, BR).

2.10. Statistical analyzes

Results are presented as means ± SEM. For statistical analyses, the groups were compared using Student's T Test ($P < 0.05$) and graphs were performed using *GraphPad Prism Software*© version 6.00 for *Windows* (San Diego, CA, USA).

3. Results

3.1. Maternal results

3.1.1. Bodily and serum parameters

The RYGB significantly decreased body weight, Lee index and retroperitoneal and perigonadal fat pad in CAF RYGB female rats when compared with the CAF SHAM rats, without influencing the nasoanal length (Tab. 2). Although the glucose and HDL concentrations were similar between groups, the TG and CHOL serum concentrations were lower in CAF RYGB animals group regarding to CAF SHAM group (Tab. 3). The TyG index, used as a parameter for the evaluation of IR, was 25% lower in CAF RYGB group, when compared to CAF SHAM group.

Table 2

Maternal body parameters after pregnancy and one week after lactation in CAF SHAM and CAF RYGB rats.

	CAF SHAM	CAF RYGB
Body Weight (g)	321.0 ± 14.0	249.0 ± 12.5**
Nasoanal lenght (cm)	21.0 ± 0.5	21.0 ± 0.3
Lee index	330.0 ± 9.3	299.0 ± 2.3**
Retroperitoneal fat pad (g/100 g BW)	2.5 ± 0.2	1.0 ± 0.3**
Perigonadal fat pad (g/100 g BW)	5.0 ± 0.3	1.5 ± 0.3****

Data are means ± SEM (n= 13–16 rats). ** P < 0.01 and **** P < 0.0001 (Student's T Test).

Table 3

Maternal fasting serum parameters after pregnancy and one week after lactation in CAF SHAM and CAF RYGB rats.

	CAF SHAM	CAF RYGB
Glucose (mg/dL)	93.0 ± 4.50	91.0 ± 7.50
TG (mg/dL)	166.0 ± 40.00	62.0 ± 5.60*
CHOL (mg/dL)	109.0 ± 4.50	72.0 ± 6.20***
HDL (mg/dL)	55.0 ± 1.10	51.0 ± 5.40
TyG index	4.0 ± 0.08	3.0 ± 0.07*

Data are means ± SEM (n= 13–16 rats). * P < 0.05 and *** P < 0.001 (Student's T Test).

3.1.2. Liver's parameters

The serum ALT concentration was 52% higher in CAF RYGB group than CAF SHAM group, and the AST concentration was similar in both groups (Fig. 1A). The RYGB surgical intervention increased liver weight (77%), as well as, the liver total lipids content (113%) and TG (108%) in group CAF RYGB, when compared to CAF SHAM group, without influencing the liver CHOL content (Fig. 1C, F). Macroscopically, it is possible to observe that CAF RYGB rats liver has yellowish color (characteristic of liver steatosis) (Fig. 1H), when compared to CAF SHAM rats liver with a brown-reddish color (Fig. 1G). As for the histomorphological aspect, the liver of CAF SHAM rats presented hepatocytes arranged in rows, delimited by connective tissue, containing sinusoids capillaries. They have a typical morphology, abundant cytoplasm, homogeneous aspect, nucleus in central position in the cytoplasm and absence of steatosis (Fig. 1I). However, the CAF RYGB rats hepatocytes were distended by a single and large vacuoles, and with nuclear displacement to the cell periphery (Fig. 1J). As shown in Table 4, the liver of rats of CAF SHAM group, after pregnancy and lactation did not steatosis, however, the CAF RYGB animals group presented grade 3 (80%) and grade 2 (20%) macrovesicular steatosis.

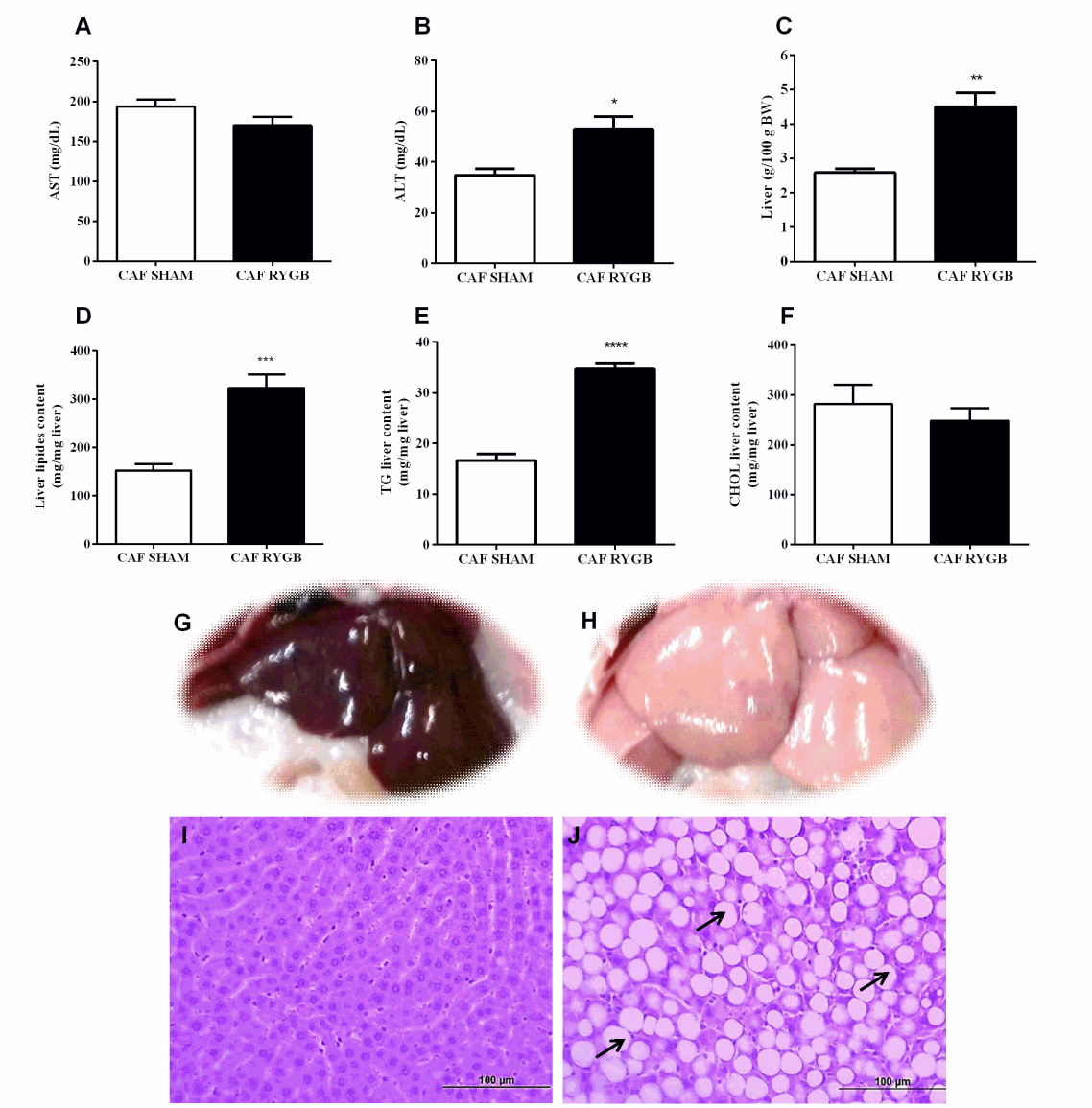

Fig. 1. (A) AST, (B) ALT, (C) liver weight, (D) Total lipids content, (E) TG and (F) CHOL hepatic content of CAF SHAM (n=13) e CAF RYGB (n=17) rats, after pregnancy and lactation. Macroscopic aspect and representative photomicrograph of liver sections stained in H&E on CAF SHAM (G and I) and CAF RYGB (H and J) group. The arrows indicate macrovesicular liver steatosis after pregnancy and lactation (n=5-7). The results were expressed as mean ± SEM. * $P < 0,05$; *** $P < 0,001$ and **** $P < 0,0001$. Student's T test.

Table 4

Degree of steatosis in the liver of CAF SHAM and CAF RYGB rats one week after lactation.

Groups	Degree 0	Degree 1	Degree 2	Degree 3
CAF SHAM	100%	0%	0%	0%
CAF RYGB	0%	0%	20%	80%

N=5-7.

3.1.3. Expression of genes and enzymes involved in liver lipid metabolism

To investigate possible factors related to hepatic steatosis development in CAF RYGB rats, we evaluated the gene and protein expression of enzymes involved in DNL, β-oxidation and VLDLs assembly in liver. The ACC (Fig. 2A, B) and SCD-1 (Fig. 2F, G) gene and protein expression, both related to the DNL are significantly reduced in CAF RYGB female rats, when compared to CAF SHAM rats. The same was observed in relation to the pACC protein expression (Fig. 2C). Also in relation to DNL markers, as can be observed in figure 2D that FASN gene expression was similar in two groups. However, the FASN protein expression was lower in CAF RYGB liver rats then CAF SHAM rats (Fig. 2E). The CPT-1 mRNA amount, an enzyme involved in mitochondrial β-oxidation, was lower in the liver of CAF RYGB animals in relation to CAF SHAM group (Fig. 2H), however the protein expression content was higher (Fig. 2I). In figure 2J it is observed that MTTP gene expression, related to VLDLs assembly, was similar in the two groups evaluated, but the protein expression was lower in CAF RYGB rats regarding to CAF SHAM group (Fig. 2K).

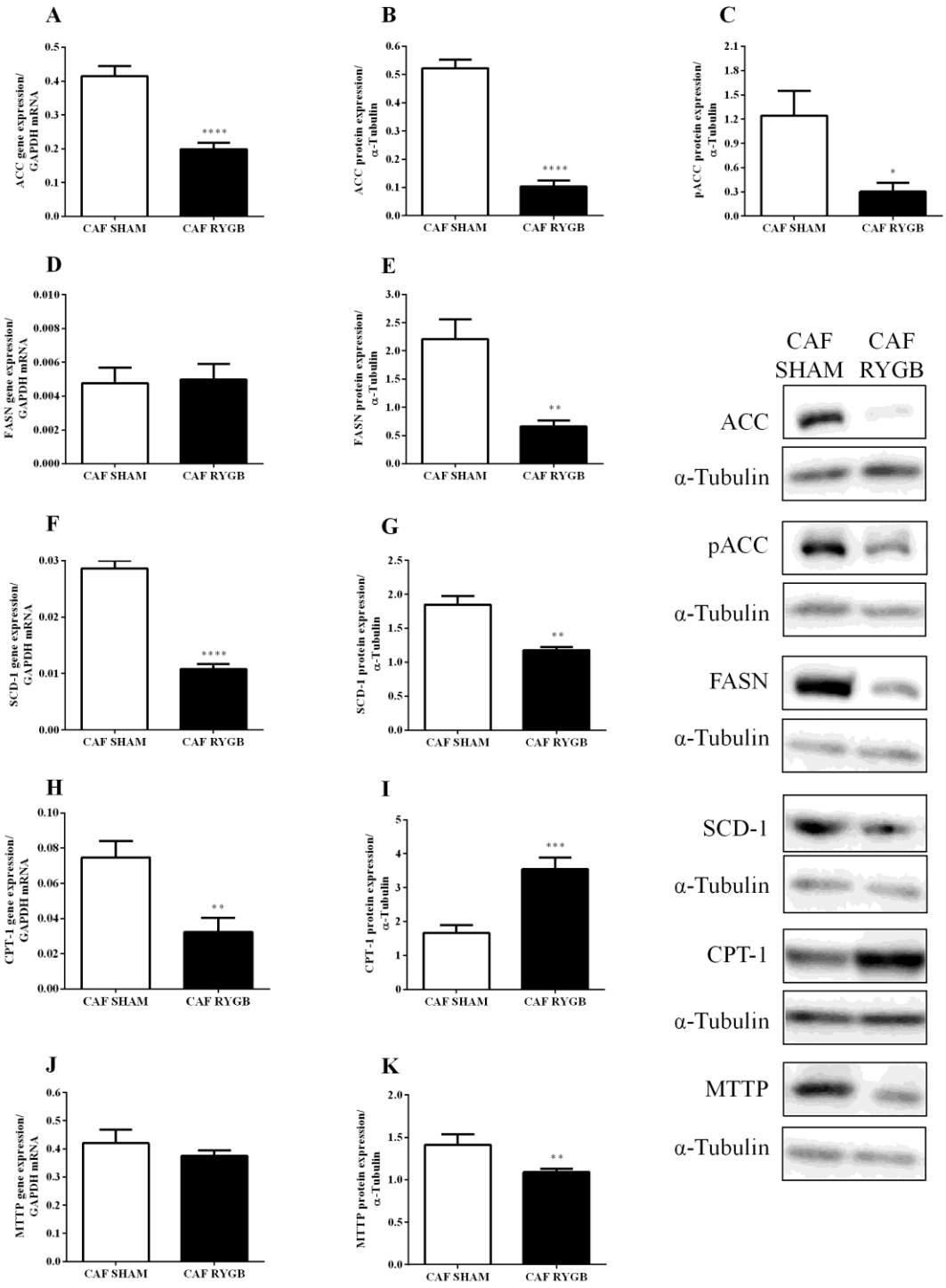

Fig. 2. (A) ACC gene and (B) protein expression; (C) pACC protein expression; (D) FASN gene and (E) protein expression; (F) SCD-1 gene and (G) protein expression; (H) CPT-1 gene and (I) protein expression; (J) MTTP gene and (K) protein expression of animals in CAF SHAM and CAF RYGB groups after pregnancy and one week after lactation (n=5). The results were expressed as mean ± SEM. * P < 0,05; ** P < 0,01; *** P < 0,001 e **** P < 0,0001. Student's T test.

3.2. Offspring results

3.2.1. Bodily and serum parameters

At 120 days of age, body weight, the nasoanal lenght, Lee index, and retroperitoneal and perigonadal fat pad were lower in the mothers's offspring who received cafeteria diet and were submitted to surgical intervention (CAF RYGB-F1) when compared to mothers's offspring who only received cafeteria diet (CAF SHAM-F1; tab. 5). The glucose, CHOL, and HDL serum concentrations were similar in both groups studied. However, triglyceridemia and TyG index were lower in CAF RYGB-F1 animals then CAF SHAM-F1 animals (tab. 6).

Table 5

Body parameters in CAF SHAM-F1 and CAF RYGB-F1 rats of 120 days of age.

	CAF SHAM-F1	CAF RYGB-F1
Body Weight (g)	384.0 ± 7.50	318.5 ± 12.0****
Nasoanal lenght (cm)	22.5 ± 0.13	21.5 ± 0.21***
Lee index	323.5 ± 1.60	316.0 ± 1.40**
Retroperitoneal fat pad (g/100 g BW)	3.5 ± 0.24	1.4 ± 0.18****
Perigonadal fat pad (g/100 g BW)	3.8 ± 0.3	2.1 ± 0.20***

Data are means ± SEM (n= 14–28 rats). ** P < 0.05; *** P < 0.001 and *** P < 0.0001 (Student's T Test).

Table 6

Fasting serum parameters in CAF SHAM-F1 and CAF RYGB-F1 rats with 120 days of age.

	CAF SHAM-F1	CAF RYGB-F1
Glucose (mg/dL)	98.1 ± 2.00	96.0 ± 2.40
TG (mg/dL)	127.0 ± 8.70	81.0 ± 8.40**
CHOL (mg/dL)	93.0 ± 2.80	89.0 ± 2.00
HDL (mg/dL)	60.0 ± 1.60	63.0 ± 1.50
TyG index	3.8 ± 0.04	3.5 ± 0.06**

Data are means ± SEM (n= 14–28 rats). ** P < 0.01 (Student's T Test).

3.2.2. Liver's parameters

It can be observed in Figure 3A, B, C and D that AST and ALT serum concentrations, liver weight and hepatic TG content, respectively, were similar in CAF RYGB-F1 and CAF SHAM-F1 groups. However, the CHOL liver content was lower in CAF RYGB-F group in relation to CAF SHAM-F1 group (Fig.3E). The histomorphological liver aspect the animals CAF RYGB-F1 group (Fig. 3F) was similar to CAF SHAM-F1 group (Fig. 3G).

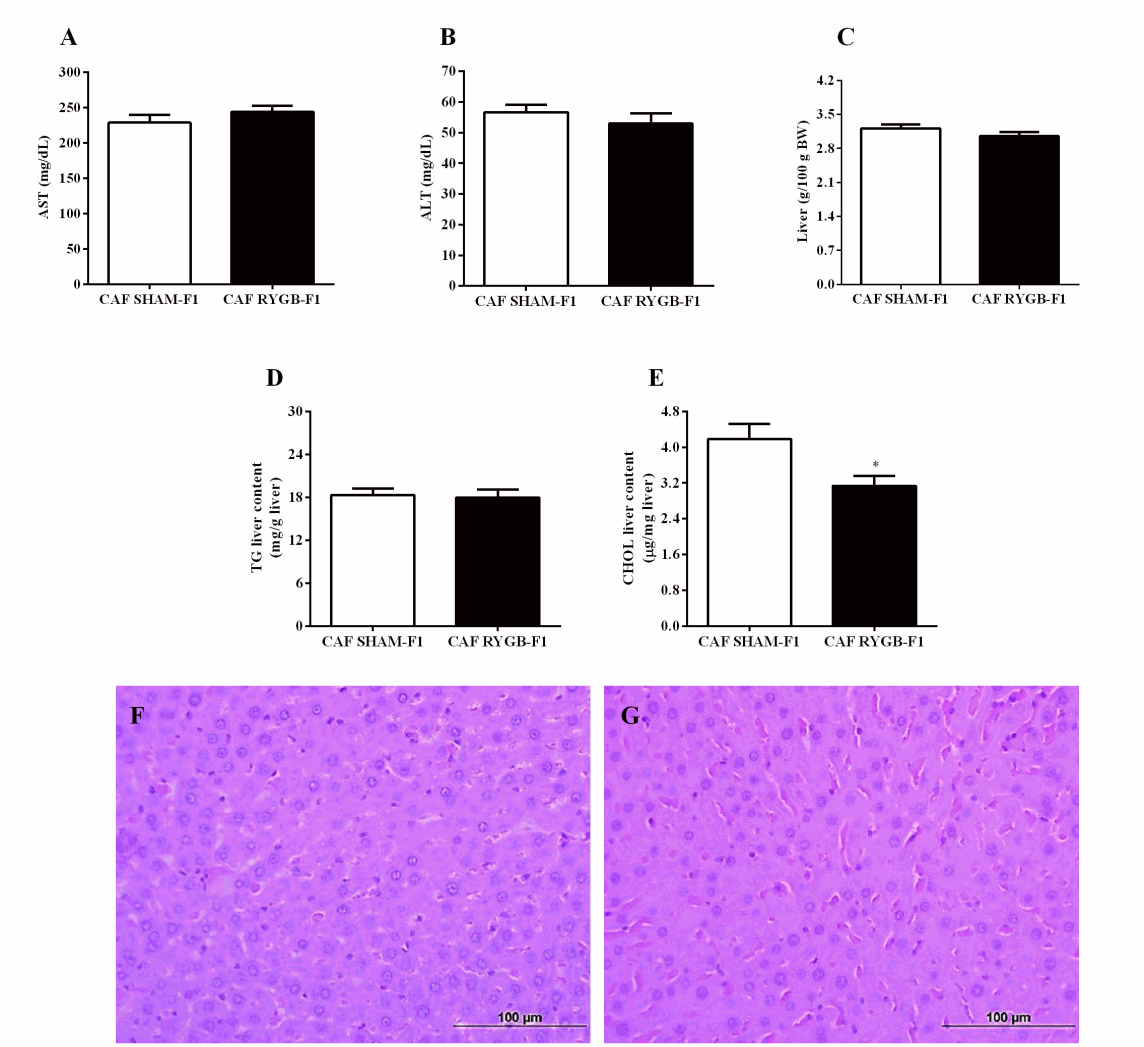

Fig. 3. (A) AST, (B) ALT, (C) liver weight, (D) TG and (E) CHOL hepatic content of CAF SHAM-F1 (n=28) and CAF RYGB-F1 (n=14) with 120 days of age. (F) Macroscopic aspect and representative photomicrograph of liver sections stained in H&E on CAF SHAM-F1 (G) and CAF RYGB-F1 group (H) (n=7). The results were expressed as mean ± SEM. * $P < 0,05$. Student's T test.

3.2.3. Expression of genes and enzymes involved in liver lipid metabolism

The ACC (Fig. 4A) and SCD-1 (Fig. 4F) gene expression were lower in livers of CAF RYGB-F1 animals group, when compared to CAF SHAM-F1 animals group, but FASN gene expression was not altered (Fig. 4D). The ACC protein expression (Fig. 4B) was higher, while the FASN (Fig. 4E) and pACC (Fig. 4C) expression was lower in CAF RYGB-F1 group then CAF SHAM-F1 group, however SCD-1 enzyme expression (Fig. 4G) was similar between the groups. The CPT-1 gene expression was higher in CAF RYGB-F1 animals in relation to CAF SHAM-F1 animals (Fig. 4H), however the protein expression content was similar in both groups (Fig. 4I). In figure 4J it is observed that MTTP gene expression was similar in two groups evaluated, but protein expression content was higher in CAF RYGB-F1 animals in relation to CAF SHAM-F1 animals (fig. 4K).

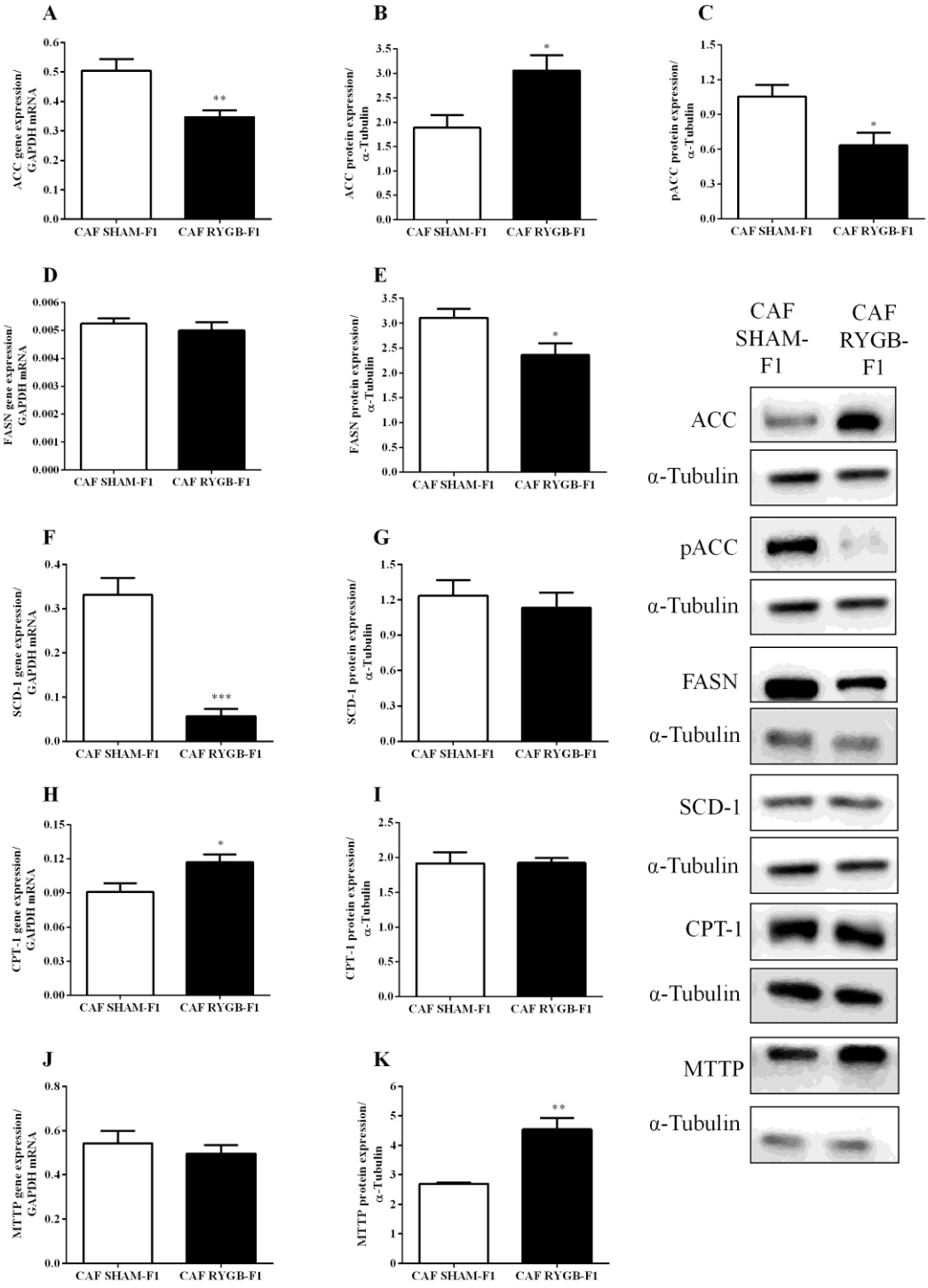

Fig. 4. (A) ACC gene and (B) protein expression; (C) pACC protein expression; (D) FASN gene and (E) protein expression; (F) SCD-1 gene and (G) protein expression; (H) CPT-1 gene and (I) protein expression; (J) MTTP gene and (K) protein expression of CAF SHAM-F1 and CAF RYGB-F1 males rats (n=5) with 120 days of age. The results were expressed as mean ± SEM. * P < 0,05; ** P < 0,01 e *** P < 0,001. Student's T test.

4. Discussion

Obesity in women is associated with an increase in prevalence of many obstetric risks such as gestational diabetes, hypertension, preeclampsia and neonatal mortality [20, 21], in addition promote changes in descendants growth and metabolism [22, 6]. Bariatric procedures stand out as the most effective treatment for morbid obesity, providing improved and/or resolution of associated comorbidities [23, 24]. However, the influences of these procedures on offspring metabolism and development of diseases in long term are little studied and are still controversial [25, 26].

The RYGB promotes body weight and fat pad reduction, both in humans and experimental models [27]. However, the glycemic homeostasis improvement may occur before weight loss after RYGB [28-30]. In the present study we observed that female rats submitted to RYGB presented after pregnancy and lactation, body weight and fat pad reduction and insulin tolerance improvement, verified by TyG index (Tab. 2), even with CAF diet continuous offer. Literature data show that RYGB is associated with serum lipid profile improvement in humans [31] and animals [32]. Corroborating these findings, we observed in this study, serum TG and CHOL concentrations reduction, without changes in HDL concentration (Tab. 3). These findings may be related to lipids emulsification and absorption reduction, fostered by changes in viscosity within the intestine lumen and time in contact with the enterocytes reduced, caused by proximal intestine deviation [31, 33].

In spite of all beneficial effects reported above, we observed that CAF RYGB rats showed ALT serum concentrations, liver weight and total hepatic lipids and TG content increase (Fig. 1B-E). Associated with these results, we also observed 80% occurrence of macrovesicular degree 3 hepatic steatosis in CAF RYGB rats (Fig. 1J; Tab. 4). This is the first study to demonstrate that obese female rats undergoing RYGB have liver changes after pregnancy and lactation, suggesting misfit in hepatic lipid metabolism. In this way, the gene and protein expression of enzymes related to DNL, β-oxidation and VLDLs assembly in liver were evaluated. Obese rats submitted to RYGB showed, after pregnancy and lactation, ACC and SCD-1 gene expression reduction (Fig. 2A, F) as well as ACC, pACC, FASN and SCD1 protein expression (Fig. 2B, C, E and G), suggesting DNL reduction. In addition, TG accumulation in hepatocytes may be due to hepatic fatty acids uptake increase from diet through the chylomicrons and free fatty acid concentration

increase generated by adipose tissue lipolysis [34]. Studies demonstrate lipolysis peripheral increased due to weight loss provided by the RYGB in tolerant glucose obese rats [35] and obese patients [36, 37]. Thus, it is speculated that iatrogenic changes in essential nutrients absorption and metabolism, associated with physiological changes related to pregnancy and lactation, induce excessive adipose tissue lipolysis, which lipids to liver supply increases, reducing the DLN and increasing hepatic TG stocks.

The TG accumulation in the liver may also be a consequence of changes in enzymes involved with β-oxidation and VLDLs assembly [3]. In this study, it was also observed that CPT-1 gene expression, a key enzyme in mitochondrial β-oxidation [38], was lower in rats submitted to RYGB (Fig. 2H), but its protein expression was higher (Fig. 2I). There were not differences in MTTP gene expression among the groups studied (Fig. 2J), however the protein expression was lower (Fig. 2K) in rats submitted to RYGB. The MTTP is the enzyme responsible for transfer of TG to B apolipoprotein (apoB), resulting in VLDL particles, which hepatic TG exported to adipose tissue [39]. Thus, associated with DNL reduction, we suggest that the likely amount of excessive lipids caused by peripheral lipolysis, leads to β-oxidation increase, providing more power to the fetus. In addition, the decrease in MTTP expression may be related to VLDLs assembly and/or secretion reduction, causing the containment of TG in hepatocytes. The difference between CPT-1 and MTTP gene and protein expression, suggests a post-transcriptional mechanism of protein expression control, which can be involved different microRNAs types, as miR-33a/b, by acting in processing after genes transcripcional involved in lipid metabolism [40].

This study showed that obese rats submitted to RYGB demonstred, after pregnancy and lactation, improvement in bodily and serum parameters, however severe liver damage was observed. Thus, considering that maternal metabolic status affects the offspring, the influence of these maternal effects on offspring males at 120 days of life was investigated. Adult CAF DGYR-F1 rats presented body parameters reduced (body weight, nasoanal length, Lee index and retroperitoneal and perigonadal fat pad, Tab. 5) when compared to the CAF SHAM-F1 group. Similar data were observed in pups of rats submitted to vertical gastrectomy and ileal interposition [25, 26]. Cummings et al., (2013) reported that these changes were independent of body weight loss, since meternal ileal interposition did not alter this parameter [25]. Children born to mothers undergoing biliopancreatic operation have low birth weight [41, 42]. The reduction of the essential nutrients absorption such as iron, vitamin B12 and folic acid after the RYGB, can lead to fetal complications such as

premature birth, low birth weight, neonatal hypocalcemia, rickets and fetal mental retardation [43]. In addition, it has been demonstrated that NFLD in obese mothers is associated with their descendants' body weight reduction [44]. In this way, more studies are needed to investigate the responsible mechanisms for these offspring changes.

Similar to that observed in female rats, the CAF RYGB-F1 offspring did not showed normoglycemia and serum TG concentration and IR reduction (Tab. 6). These results may be due to epigenetic mechanisms acting in pregnancy and lactation period. Still in relation to repercussions of the maternal RYGB on the offspring, it was observed that despite of the rats CAF RYGB presenting severe hepatic steatosis after lactation, the liver morphological aspect in offspring CAF RYGB-F1 showed absence of fat accumulation in the hepatocytes (Fig. 3G). The AST and ALT serum concentrations (Fig. 3A, B), liver weight (Fig. 3C) and TG hepatic content (Fig. 3D) were similar between the groups. However, the CHOL liver content was lower in CAF RYGB-F1 group compared to CAF SHAM-F1 group (Fig.3E). The amount of ACC and SCD1 mRNA was higher in offspring liver from obese female rats undergoing RYGB, while the amount of FASN mRNA was similar between the two groups. However, the ACC protein expression was higher, pACC and FASN reduced and the SCD1 was not altered in CAF RYGB-F1 group than CAF SHAM-F1 group. The CPT-1 gene expression was higher in CAF RYGB-F1 group when compared to CAF SHAM-F1 group, while the CPT-1 protein expression was not altered (Fig. 4H, I). On the other hand, the MTTP mRNA was similar between the two groups studied while its protein expression was higher in CAF RYGB-F1 group compared to CAF SHAM-F1 group (Fig. 4J, K). Thus, the maternal RYGB did not alter the β-oxidation in the offspring, but can be promoting VLDLs assembly and secretion increase. These data together show that offspring from obese rats submitted to RYGB, present post-transcriptional changes in enzymes involved in liver lipid metabolism, which may have been programed during pregnancy and lactation period.

5. Conclusions

In summary, it was demonstrated for the first time that obese rats submitted to RYGB, feature after pregnancy and lactation, normalization of bodily parameters and serum lipid profile. However, showed severe hepatic damage related to lipid

metabolism. The male descendants of CAF RYGB rats presented body weight and fat pad reduction in adulthood, as well as, the serum TG concentration, without developing liver steatosis. However, enzymes involved in hepatic lipid metabolism pathways, showed post-transcriptional changes. Thus, these results suggest the need for additional and more comprehensive studies, including the identification of epigenetic factors and/or other factors involved in passing the effects of bariatric operation for their offspring.

Conflict of interest statement

The authors report no conflicts of interest.

Author's contributions

Maria Lúcia Bonfleur: experimental design; Iala Milene Bertasso, Ana Claudia Paiva Alegre-Maller and Carla Bruna Pietrobon: implementation of experiments; Gabriela Moreira Soares: aid for implementation of quantitative PCR in real time technique; Allan Cezar Faria Araújo: implementation of surgical procedures; Antonio Carlos Boschero: intellectual contribution and supply of materials and reagents; Maria Lúcia Bonfleur, Sandra Lucinei Balbo and Rosane Aparecida Ribeiro: interpretation of data and drafting of the writing of this work.

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ANEXO A

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PARECER DE PROTOCOLO

O protocolo intitulado "Homeostase glicêmica e lipídica da prole de ratos obesos submetidos à derivação gástrica em Y de Roux", sob vossa coordenação, foi avaliado pelo CEUA como **APROVADO** para execução.

ATENÇÃO!

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

Cascavel, 13/02/2015

Profa. Dra. Luciana Oliveira de Fariña
Coordenadora do CEUA
Portaria nº 2729/2014 - GRE

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Life Sciences

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