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Centro de Ciências Biológicas e da Saúde
Programa de Pós-Graduação em Ciências da Saúde
Doutorado

EFEITOS DE DIETA HIPERPROTEICA SOBRE A COMPOSIÇÃO CORPORAL E O METABOLISMO DE RATOS WISTAR ADULTOS OBESOS

ROSÂNGELA MARIA LOPES DE SOUSA

SÃO LUÍS – MA
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Tese apresentada ao Programa de Pós-Graduação em Ciências da Saúde da Universidade Federal do Maranhão, como requisito para obtenção do título de Doutora em Ciências da Saúde.

Orientador: Prof. Dr. José Albuquerque de Figueiredo Neto

Co-orientador: Prof. Dr. Antonio Marcus de Andrade Paes

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"O mundo pode dizer que não, mas a tese na qual defendo, é que pelos nossos sonhos somos movidos. E a nossa felicidade é o resultado da nossa vontade de realização".

Francisco S. Lourenço

Aos meus pais, **Francisca e Raimundo Nonato**,
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SUMÁRIO

LISTA DE SIGLAS E ABREVIATURAS

5-HT2c	Receptor de serotonina do tipo 2c
AMPA	Receptor glutamatérgico do tipo alfa-amino-3-hidroxi-metil-5-4-isoxazolpropiónico
ANVISA	Agência Nacional de Vigilância Sanitária
FDA	*Food and Drug Administration*
FTO	Gene ligado a obesidade e massa gorda
GABA	Ácido gama aminobutírico
GIP	Polipeptídeo inibitório gástrico
GLP-1	Peptídeo semelhante a glucagon 1
GLUT-2	Transportador de glicose tipo 2
HDL	Lipoproteína de alta densidade
HP	Dieta hiperproteica
ICQ	Índice cintura/quadril
IMC	Índice de massa corpórea
LDL	Lipoproteína de baixa densidade
MC4R	Receptor 4 de melanocortina
NHANES	Pesquisa Nacional de Saúde e Exame Nutricional
RI	Resistência à insulina
TG	Triglicerídeos
VIGITEL	Vigilância de Fatores de Risco e Proteção para Doenças Crônicas por Inquérito Telefônico
VLDL	Lipoproteína de densidade muito baixa

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RESUMO

Introdução: O consumo de açúcares adicionados foi considerado uma preocupação mundial em saúde pública por sua associação implícita com a epidemia de obesidade, diabetes mellitus tipo 2 (DM2) e síndrome metabólica (SM). Neste contexto, a manipulação dietética é um fator primário no controle e prevenção da obesidade e suas comorbidades. Muitas das abordagens priorizaram intervenções com baixo teor de gordura e / ou com baixo teor de carboidratos. Por outro lado, estudos atuais sugeriram dietas ricas em proteínas para promover uma maior perda de peso e resultados metabólicos. **Objetivo**: Investigar os efeitos de uma dieta rica em proteínas contra disfunções metabólicas induzidas por sacarose em ratos desde o desmame até a idade adulta, bem como para comparar os benefícios metabólicos entre maior ingestão de proteína e retirada do excesso de açúcar. **Material e Métodos**: Ratos Wistar machos desmamados foram distribuídos aleatoriamente em dois grupos: ratos controle, alimentados com uma ração normal e ratos obesos (HS/HS), alimentados com uma ração elevada de sacarose, por um período de 20 semanas. Posteriormente, os animais HS/HS foram distribuídos aleatoriamente em três novos grupos: ratos mantidos na dieta HS; ratos inicialmente submetidos à dieta HS e, em seguida, substituídos por comida normal; e aqueles substituídos por dieta rica em proteínas (HS/HP). Todos os grupos foram acompanhados por uma intervenção nutricional adicional de 12 semanas. Foram avaliadas nos grupos: peso corporal, consumo de energia, desenvolvimento da obesidade, perfil glicêmico/lipídico, tolerância à glicose, resistência à insulina, morfometria do tecido adiposo, músculos esqueleto e fígado, atividade lipolítica, histologia do fígado e perfil bioquímico da função hepática. **Resultados**: A exposição pós-desmame a dieta HS conduz ao fenótipo da síndrome metabólica na fase adulta, caracterizada aqui pela obesidade central, intolerância à glicose, dislipidemia e resistência à insulina. Além disso, a dieta HP foi efetivo na reversão dos danos pelo consumo de açúcar, marcados pela perda de peso e redução da obesidade com diminuição de massa de gordura. A substituição de HS pela comida padrão também resultou em diminuição da acumulação de tecido adiposo, embora em menor grau do que a ocasionada pela dieta HP. Notoriamente, a dieta HP restabeleceu a capacidade de resposta do tecido adiposo ao agonista adrenérgico, tornando o tecido suscetível à lipólise. A retirada de sacarose atenua os efeitos deletérios sobre o eixo glicose-insulina. No entanto, a sua substituição pela dieta HP promoveu os mesmos efeitos, mas melhorou a tolerância à glicose e a sensibilidade à insulina. Finalmente, a exposição ao HS levou a esteatose hepática sem inflamação, com a consequente elevação da atividade ALT. No entanto, ambas as intervenções nutricionais foram capazes de restaurar essas alterações. **Conclusão**: Nosso conjunto de dados mostra que a dieta HP sempre reestabeleceu os resultados metabólicos induzidos por sacarose. Por outro lado, a simples retirada do consumo elevado de sacarose também melhora os danos metabólicos, embora menos pronunciada. Assim, ambas as intervenções nutricionais se mostram mutuamente eficientes, potencialmente ajudando a tratar pacientes com síndrome metabólica.

Palavras-chave: Dieta rica em proteínas; Dieta rica em sacarose; Retirada de sacarose; Síndrome metabólica.

ABSTRACT

Introduction: The consumption of added sugars has been considered a global public health concern because of its implicit association with the epidemic of obesity, type 2 diabetes mellitus (DM2) and metabolic syndrome (MS). In this context, dietary manipulation is a primary factor in the control and prevention of obesity and its comorbidities. Many of the approaches prioritized low-fat and/or low-carbohydrate interventions. On the other hand, current studies have suggested high protein diets to promote greater weight loss and metabolic outcomes. **Objective**: To investigate the effects of a high-protein diet against sucrose-induced metabolic dysfunction in rats from weaning to adulthood, as well as to compare the metabolic benefits between increased protein intake and excess sugar removal. **Material and Methods**: Weaned male Wistar rats were randomly divided into two groups: control rats, fed a normal chow and obese rats (HS/HS), fed a high sucrose chow, for a period of 20 weeks. Subsequently, the HS/HS animals were randomly distributed into three new groups: rats kept on the HS diet; rats initially submitted to the HS diet and then replaced by normal food; and those replaced by a high protein diet (HS/HP). All groups were followed by an additional 12-week nutritional intervention. The following groups were evaluated: body weight, energy consumption, development of obesity, glycemic/lipid profile, glucose tolerance, insulin resistance, adipose tissue morphometry, skeletal muscle and liver, lipolytic activity, liver histology and biochemical profile of function liver. **Results:** Post-weaning exposure to the HS diet leads to the phenotype of metabolic syndrome in adulthood, characterized here by central obesity, glucose intolerance, dyslipidemia and insulin resistance. In addition, the HP diet was effective in reversing the damage caused by sugar consumption, marked by weight loss and obesity reduction with decreased fat mass. Replacing HS with the standard food also resulted in decreased adipose tissue accumulation, although to a lesser degree than that brought about by the HP diet. Notably, the HP diet restored the responsiveness of adipose tissue to the adrenergic agonist, making the tissue susceptible to lipolysis. Sucrose withdrawal attenuates the deleterious effects on the glucose-insulin axis. However, its replacement by the HP diet promoted the same effects, but improved glucose tolerance and insulin sensitivity. Finally, exposure to HS led to liver steatosis without inflammation, with a consequent elevation of ALT activity. However, both nutritional interventions were able to restore these changes. **Conclusion:** Our dataset shows that the HP diet has always re-established sucrose-induced metabolic outcomes. On the other hand, simply withdrawing high sucrose consumption also improves metabolic damage, although less pronounced. Thus, both nutritional interventions are mutually effective, potentially helping to treat patients with metabolic syndrome.

Keywords: High protein diet; High sucrose diet; Sucrose removal; Metabolic syndrome.

1 INTRODUÇÃO

A obesidade uma doença multifatorial que está associada ao desenvolvimento de diabetes mellitus tipo 2, doenças cardiovasculares, alguns tipos de câncer, assim como diversas condições patológicas (GUH et al., 2009). É definida como um peso desproporcional para a altura com presença de inflamação sistêmica leve ou crônica (SELLAYAH; CAGAMPANG; COX, 2014) e advém de um desequilíbrio entre a ingestão calórica e gasto de energia e da adoção de um estilo de vida sedentário. Entretanto o status socioeconômico, ambiental e interações genótipo-fenótipo têm que ser levados em consideração no que se refere a gênese da obesidade (SELLAYAH; CAGAMPANG; COX, 2014).

É considerada uma epidemia do século XXI, representando um grande risco para saúde global tendo em vista seu rápido crescimento (COLLABORATION, 2016a; GONZÁLEZ-MUNIESA et al., 2017). Em 2016, de acordo com a Organização Mundial de Saúde, mais de 1,9 bilhões de adultos com mais de 18 anos possuíam excesso de peso. Destes, mais de 650 milhões eram obesos. E considerando o gênero, a prevalência estimada desta clínica com idade igual ou superior a 18 anos foi de 11% nos homens adultos e 15% entre mulheres (WHO, 2017).

A obesidade pode ser caracterizada de acordo com os locais de acúmulo de depósitos adiposos, podendo ser subcutânea, quando o excesso de gordura subcutânea é encontrado nas áreas do quadril e da coxa (forma do corpo tipo pera ou obesidade ginoide), que é mais comum em mulheres; ou visceral, quando o acúmulo adiposo se concentra na região abdominal (forma de corpo tipo maçã ou obesidade androide), sendo mais comum em homens e mais perigosa em termos de saúde, particularmente aumentando o risco de doenças cardiovasculares (GONZÁLEZ-MUNIESA et al., 2017).

Ao longo dos anos, diferentes abordagens e tratamentos ao indivíduo obeso foram desenvolvidos e prescritos, visando o controle das alterações metabólicas relacionadas à morbidade. Estratégias como educação e controle da dieta, programas de atividade física, farmacoterapia e cirurgia bariátrica são as com maior destaque e, independente da intervenção escolhida, é consenso que devam ser iniciadas de forma mais precoce possível (GONZÁLEZ-MUNIESA et al., 2017).

Dietas para o manejo do peso corporal têm sido rigorosamente testadas em ensaios clínicos randomizados nos últimos 20 anos (BRAY et al., 2016). No entanto, a adesão para o

programa da dieta é um grande desafio (JENSEN et al., 2014). Para manutenção de perda de peso em longo prazo, além da aderência à dieta, a composição dos macronutrientes também possui um papel relevante (ABETE et al., 2010).

Em função da prevalência de sobrepeso e obesidade, especialmente nos países ocidentais (MOKDAD et al., 1999), as Dietas Hiperproteicas (HP) são muitas vezes consideradas alternativas eficientes e bem sucedidas para perda de peso em longo prazo (ASHLEY et al., 2001). De fato, as dietas HP atualmente são conhecidas pelos seus efeitos anti-obesogênicos em ratos (PETERS; HARPER, 1985; BENSAÏd et al., 2002; MOURAO et al., 2007) e humanos (JOHNSTON; TJONN; SWAN, 2004; WESTERTERP-PLANTENGA et al., 2004) especialmente quando 50% da densidade energética da dieta é constituída por proteínas (EISENSTEIN; HARPER 1991; MATTHEWS; CAMPBELL, 1992; GARLICK; MCNURLAN; PATLAK, 1999; MORENS et al., 2001).

Uma maior ingestão proteica associada ao consumo de alimentos com baixo índice glicêmico e lipídico podem trazer diversos benefícios como a perda de peso e recuperação de alterações metabólicas relacionadas a um fenótipo obesogênico, enquanto que uma elevação do consumo de carboidratos antagonizam todos estes efeitos (LARSEN et al., 2010; BRAY et al., 2016).

Comumente dietas com alto teor proteico possuem composição centesimal relativa de aproximadamente 20% de proteínas, 50% de carboidratos e 30% de gorduras e têm demonstrado efeitos benéficos perante alterações metabólicas (KRIEGER et al., 2006). A utilização de dieta com elevado teor de proteína em ratos obesos demonstrou melhorar os níveis séricos de insulina, leptina, lipídeos aterogênicos (especialmente de triglicerídeos), assim como atuar na redução de peso e no quadro de intolerância à glicose, etapa inicial de um quadro de Resistência Insulínica (RI) (LACROIX et al., 2004). Estudos em camundongos mostraram que a dieta HP também atua na prevenção e reversão da esteatose e inflamação hepática (GARCIA-CARABALLO et al., 2013). Resultado este, corroborado por Li et al. (2010) em um estudo com indivíduos obesos e com sobrepeso que consumiram dieta HP com densidade energética semelhante pelo período de um ano.

Dados na literatura demonstram que a ingestão de proteínas aumenta a termogênese pós-prandial e auxiliam na conservação de massa magra (DUE et al., 2004; NOAKES et al., 2005; WESTERTERP-PLANTENGA et al., 2006), assim como promovem redução do armazenamento de gordura, sugerindo que este nutriente seja componente chave para induzir

um balanço energético negativo e promover a perda de peso (VELDHORST et al., 2008). Ademais, é referido que todas as melhorias metabólicas observadas estão relacionadas ao aumento do conteúdo proteico e não à redução da composição de carboidratos (SOENEN et al., 2012).

Mesmo com a evidência da redução de peso corporal e melhora de respostas metabólicas relacionadas ao consumo de proteínas, o quantitativo de estudos avaliando as consequências fisiológicas e funcionais de uma dieta HP por períodos prolongados ainda são escassos na literatura científica tanto em animais quanto humanos. Neste contexto, nosso estudo se propõe a avaliar efeitos positivos de uma dieta hiperproteica sobre parâmetros metabólicos e histológicos em ratos com obesidade induzida por dieta rica em sacarose. Este estudo serve como base para o melhor entendimento dos efeitos dos macronutrientes sobre o metabolismo e planejamento futuro de dietas mais eficazes para o controle de morbidades metabólicas.

A obesidade é uma doença crônica, multifatorial e de difícil controle. Várias pesquisas, em todo o mundo, têm sido desenvolvidas com o intuito de propor medidas eficazes no tratamento e controle da obesidade. No entanto, observa-se grande dificuldade na adesão ao tratamento, principalmente dietético. Sendo este de considerável relevância, e em contrapartida ainda é bastante discutível sobre a composição dietética ideal que proporcione a perda de peso, redução de tecido adiposo nocivo e de risco para o desenvolvimento de agravos para a saúde como síndrome metabólica, diabetes mellitus do tipo II, doenças cardiovasculares e posteriormente a manutenção da massa corporal total.

A dieta hiperproteica é uma das estratégias dietéticas que tem sido estudada, devido ao fato da proteína apresentar características importantes que auxiliam no controle do balanço energético e da perda de massa corporal, além de ter mostrado resultados importantes na redução da hiperlipemia e glicemia. Portanto, a dieta hiperproteica poderia ser uma alternativa no controle da obesidade e suas comorbidades. Porém, devido às observações sobre a redução das gorduras viscerais com o consumo de dietas hiperproteicas ainda serem escassas e que as mesmas tem grande associação com o desfecho de agravos à saúde é que estudar os efeitos da dieta hiperproteica no controle da obesidade tornam-se necessários mais estudos controlados.

Assim, é de interesse avaliar efeito da composição corporal, redução de gorduras viscerais e alterações metabólicas em ratos que consumiram dieta com 34,4% de proteína e comparar com estudos na literatura.

Esta tese apresenta-se no *Formato de Artigo*, cuja organização está contida nas seções *Introdução* (que problematiza, apresenta e justifica esta pesquisa, além de fixar as hipóteses de estudo), *Referencial Teórico* (que fundamenta as pesquisas até aqui desenvolvidas, bem como a lacuna de conhecimento a ser preenchida), *Objetivos* (*Geral* e *Específicos*), *Resultados*, onde é inserido o artigo oriundo desta pesquisa, apresentado no Capítulo I, com título *"Long-term high-protein diet reverts weight gain and attenuates metabolic dysfunction in high-sucrose-fed adult rats"*, que foi submetido no periódico *Nutrition & Metabolism*, uma revista A2, na área de Medicina I, com fator de impacto 2.974, após isto, são apresentados as *Considerações Finais*, contendo principais resultados e alcance dos objetivos e ainda limitações, e finalmente a *Conclusão*, onde registra-se o alcance desta pesquisa.

2 REFERENCIAL TEÓRICO

2.1 Obesidade: definição e epidemiologia

A obesidade é uma doença crônica e multifatorial caracterizada pelo acúmulo excessivo de gordura corporal, resultante do desequilíbrio entre o ganho e o gasto de energia corporal e pode ser decorrente tanto do consumo calórico excessivo quanto da redução do gasto energético. A obesidade é considerada um dos problemas de saúde públicos mais desafiadores no século XXI (DI ANGELANTONIO et al., 2016) e constitui um reconhecido fator de risco para muitas outras doenças debilitantes e de alto custo social, como diabetes mellitus tipo 2, hipertensão arterial, acidentes vasculares cerebrais, cardiopatias, dislipidemias e alguns tipos de câncer (DOBBINS; DECORBY; CHOI, 2013; WILLIAMS; FRANKLIN 2015; COLLABORATION, 2016b).

Diversos são os métodos diagnósticos utilizados para confirmação de obesidade, sendo os parâmetros antropométricos os mais utilizados, principalmente por serem de baixo custo, não invasivos, universalmente aplicáveis e com boa aceitação pela população (DESPRÉS, 2012). Dentre os indicadores antropométricos mais utilizados está o Índice de Massa Corpórea (IMC), que é claculado a partir da razão da massa corporal (em quilogramas) pelo quadrado da estatura (em metros) e padroniza que valores de IMC iguais ou maiores a 30 kg/m2 são característicos de obesidade (QUADRO 1).

Quadro 1. Classificação de peso pelo Índice de Massa Corpórea (IMC).

CLASSIFICAÇÃO	IMC (Kg/m^2)
Peso Baixo	< 18,5
Peso Normal	18,5 - 24,9
Sobrepeso	25 - 29,9
Obesidade leve (grau I)	30 – 34,9
Obesidade moderada (grau II)	35 – 39,9
Obesidade mórbida (grau III)	≥ 40
Superobesidade	≥ 50

Fonte: WHO (2017).

Embora existam métodos mais precisos para medir a quantidade e a localização do tecido adiposo no corpo, o IMC ainda é o método mais comumente usado devido sua simplicidade, entretanto ele não constitui um bom índice para diferenciar a adiposidade mais nociva (como a gordura intra-abdominal da linha da cintura) da gordura potencialmente menos nociva em outras áreas do corpo (FRANCO; MORAIS; COMINETTI, 2016). Assim, o Índice Cintura/Quadril (ICQ) que relaciona à medida em centímetros da cintura e quadril se mostra mais eficiente em predizer níveis adiposos e estabelece valores de corte para riscos cardiovasculares abaixo de 0,85 para mulheres e abaixo de 0,9 para homens (CERHAN et al., 2014).

O excesso de peso e obesidade tem aumentado em proporções epidêmicas, com uma prevalência mundial estimada em mais de um bilhão de indivíduos afetados. Segundo a Organização Mundial de Saúde (WHO, 2017), a obesidade quase triplicou desde 1975. Em 2016 mais de 1,9 bilhão de adultos maiores de 18 anos tinham excesso de peso sendo que destes, mais de 650 milhões de adultos eram obesos (13%) e 1,35 bilhões com sobrepeso (39%). A maioria da população mundial vive em países onde o excesso de peso e a obesidade mata mais pessoas do que o baixo peso (WHO, 2017).

Estima-se que em 2030, 57,8% (3,3 bilhões de pessoas) da população adulta do mundo terá um IMC superior a 25 kg /m2 (FINKELSTEIN et al., 2012). A obesidade também aumentou em vários países desenvolvidos nas últimas duas décadas e hoje estima-se que 53,1% da população adulta europeia esteja com excesso de peso e obesidade (MARQUES et al., 2017) e nos Estados Unidos, a prevalência de obesidade propriamente dita é de 37,9% (FLEGAL et al., 2016).

No Brasil a realidade não é diferente, visto que dados da Vigilância de Fatores de Risco e Proteção para Doenças Crônicas por Inquérito Telefônico (VIGITEL) coletados em 2016 nas 27 capitais brasileiras apontam que a frequência de adultos com excesso de peso foi de 53,8%, sendo maior em homens (57,7%) do que entre mulheres (50,5%), tendo maior frequência na cidade Rio Branco (60,6%) e menor em Palmas (47,7%). A frequência de adultos obesos variou entre 14,5% em Florianópolis e 23,8% em Rio Branco. São Luís, a capital do estado do Maranhão, foi uma das cidades com menor frequência de excesso de peso (50,9%) e obesidade (12,5%) entre homens adultos (FACINA, 2014).

A obesidade é considerada em grande parte evitável e causada principalmente por mudanças recentes no chamado ambiente obesogênico, como aumento no consumo de

alimentos processados, açúcares simples, aquisição de calorias excessivas e ações automatizadas com tecnologias que reduzem ou substituem a atividade física gerando menos gasto calórico (DOBBINS et al., 2013; DI ANGELANTONIO et al., 2016).

O que se torna complexo para estudar fatores de risco para a obesidade são as diferenças notáveis na forma como há acumulo de gordura, o que é observado com a variação na configuração do corpo e na região de acumulação de gordura corporal em qualquer nível de adiposidade e a heterogeneidade nos fenótipos da obesidade faz com que o que foi descrito no passado como singular termo ”obesidade”, seja um desafio, uma vez que esta condição não pode ser considerada como uma entidade homogênea (GONZÁLEZ-MUNIESA et al., 2017).

A este respeito, o desenvolvimento de tecnologias, incluindo dispositivos para identificar melhor o fenótipo de indivíduos com obesidade, avaliação da composição corporal e depósitos de gordura (como a rotulação de isótopos para determinar presença de gordura marrom) tem sido um grande avanço e assim tornar-se possível identificar grandes subgrupos de indivíduos com obesidade com características comuns que possivelmente compartilhe fatores etiológicos comuns (GONZÁLEZ-MUNIESA et al., 2017). Compreender as causas da obesidade é um fator importante para a sua prevenção e tratamento. Estudos recentes sobre esse agravo vêm contribuindo para uma série de conhecimentos científicos referentes aos diversos mecanismos pelos quais se ganha peso (BRAY; POPKIN, 2014; SAEED et al., 2015; DEHGHAN et al., 2017).

2.2 Mecanismos fisiopatológicos da obesidade

A obesidade pode ser determinada pela interação de fatores genéticos, endócrinos, ambientais, psicossociais, econômicos e comportamentais que atuam modulando a ingestão alimentar e o gasto energético, afetando a deposição de gordura e a instalação da morbidade (BHUPATHIRAJU; HU, 2016).

A variabilidade do peso corporal está relacionada com alteração na expressão de diversos genes, entretanto já são conhecidos genes específicos, que quando alterados são responsáveis por formas monogênicas da patologia (SAEED et al., 2015). Por exemplo, mutações gênicas no Receptor 4 de Melanocortina (MC4R) são responsáveis por até 5% dos casos de obesidade extrema de início precoce e são a causas genéticas mais prevalentes, juntamente com as variantes do gene ligado a Massa Gorda e Obesidade Associada (FTO),

demonstrando de forma clara o envolvimento de genes específicos na gênese da obesidade (GONZÁLEZ-MUNIESA et al., 2017). No entanto, a genética por si só não é capaz de explicar o aumento rápido da prevalência mundial de obesidade, ratificando que a obesidade resulta da interação de genes de susceptibilidade com fatores de estilo de vida diversificados (FLIER, 2004).

Os fatores biológicos e ambientais contribuem para desequilíbrio entre a ingestão e gasto de energia favorecendo o desenvolvimento da obesidade. A análise de ingestão alimentar realizado pela Pesquisa Nacional de Saúde e Exame Nutricional (NHANES) confirmou essa tendência (KANT; GRAUBARD, 2018). As escolhas entre as refeições e lanches que produzem mais energia bem como tipo e qualidade de nutrientes, especialmente lipídeos saturados versus não saturados, a fonte de gorduras, carboidratos e proteínas, além de atitudes em comer fora de casa e o aumento do tamanho das porções, têm sido associados à obesidade durante as últimas décadas (BES-RASTROLLO et al., 2010).

Os fatores comportamentais que são moldados por todos os fatores relatados anteriormente, como fatores genéticos, pela maior disponibilidade e fácil acesso à alimentos, fatores socioeconômicos e culturais e sedentarismo, levando em conjunto para o aumento dos valores médios de IMC em países de alta renda (VANDEVIJVERE et al., 2015), assim como nos países de renda baixa e média (GONZÁLEZ-MUNIESA et al., 2017).

A obesidade tem fundamentação biológica no aumento do tecido adiposo, um órgão endócrino, complexo e dinâmico, em que células vasculares do estroma tecidual sofrem alterações numéricas e/ou funcionais, com importantes funções fisiológicas e metabólicas como proteção mecânica, isolamento térmico e secreção de proteínas envolvidas na inflamação, na regulação do apetite e no controle energético (ALGIRE et al., 2013; CHOE et al., 2016). A formação do tecido adiposo se inicia durante a vida intrauterina, originária do mesoderma. Existem dois tipos distintos de tecido adiposo: o tecido adiposo branco, que tem como função armazenamento de energia, e o tecido adiposo marrom, que participa da termogênese (ALGIRE et al., 2013).

O tecido adiposo marrom se desenvolve antes do nascimento, sua cor distinta é atribuída à sua alta densidade mitocondrial, fundamental para a geração de calor e oxidação dos ácidos graxos (CANNON; KUMAR, 2009; ALGIRE et al., 2013). Ele acaba sendo um dos principais responsáveis pelos processos de termorregulação e dissipação do excesso de calorias, favorecendo desse modo a manutenção do equilíbrio calórico e do peso corporal

(BIRSOY; FESTUCCIA; LAPLANTE, 2013; TRAYHURN 2013). É um tecido observado em roedores infantis e adultos, entretanto, propôs-se inicialmente que esse tecido nos seres humanos estaria limitado aos recém-nascidos e que seria gradualmente substituído por tecido adiposo branco com envelhecimento (ALGIRE et al., 2013). De forma mais recente, foi demonstrado por meio de tomografia por emissão de pósitrons/tomografia computadorizada que o tecido adiposo marrom é viável e funcional mesmo em adultos humanos (VIRTANEN et al., 2009).

A formação do tecido adiposo branco em seres humanos tem início no segundo trimestre de gestação e após o nascimento se acumula em depósitos viscerais e subcutâneos (POISSONNET; BURDI; GARN, 1984). Em roedores, esse tecido desenvolve-se principalmente após o nascimento, apresentando-se primeiro nos depósitos perigonadais e subcutâneos, e apenas mais tarde no depósito omental (GESTA et al., 2006). Anatomicamente, o tecido adiposo branco compreende dois depósitos principais, tecido adiposo branco subcutâneo e tecido adiposo branco visceral. O tecido adiposo branco subcutâneo, que se encontra por baixo da pele, e o tecido adiposo branco visceral, que está concentrado na cavidade abdominal, o qual é ainda subdividido em mesentérico, omental, retroperitoneal. Na obesidade, no entanto o excesso de tecido adiposo branco visceral está intimamente ligado às complicações metabólicas, tais como RI, diabetes tipo 2 e doenças cardiovasculares (GESTA; TSENG; KAHN, 2007).

O tecido adiposo branco apresenta funções mais abrangentes que o tecido marrom por ser especializado em armazenar energia, fornecendo substrato para outros tecidos, como os músculos, durante períodos de jejum ou de necessidade de aumento da demanda energética (CHOE et al., 2016). Os adipócitos brancos são células secretórias, capazes de liberar diferentes moléculas de lipídeos e proteínas adicionados a ácidos graxos mobilizados na lipólise. A lipotoxidade assim como o acúmulo de lipídeos ectópicos em órgãos como fígado e músculos, decorre da grande quantidade de ácidos graxos liberados pelo tecido adiposo (RUTKOWSKI et al., 2015).

Nos adultos, a expansão do tecido adiposo branco é regulada por fatores ambientais e genéticos e ocorre por hipertrofia (aumento do volume celular) e hiperplasia (aumento do número e diferenciação de adipócitos) (SPIEGELMAN; FLIER, 2001). O potencial de gerar novas células de gordura persiste mesmo na fase adulta (FONSECA-ALANIZ et al., 2006). Com o aumento do tecido adiposo, ocorre armazenamento excessivo de lipídeos, afetando sua

função primária e gerando inflamação tecidual, que por sua vez, induz a desregulação de citocinas, hormônios e produtos derivados do tecido adiposo, diminuição da remoção de gorduras e progressão das doenças associadas à obesidade (JUNG; CHOI, 2014).

Dietas podem induzir aumento dos depósitos de gordura por um processo complexo, impulsionado ou acompanhado por mudanças gênicas, levando a uma maior adipogênese, diferenciação do tecido adiposo, armazenamento de gordura e disfunção mitocondrial (CHANSEAUME et al., 2006). O número de células adiposas pode aumentar quando ratos são alimentados com dieta rica em carboidratos (ASHLEY et al., 2001) ou lipídeos (AMIN; KAMEL; ELTAWAB, 2011).

Durante a ingestão de alimentos, a oxidação do açúcar como fonte de energia primária, faz com que os mecanismos de oxidação da gordura sejam suprimidos e, consequentemente aumentem o armazenamento de lipídeos no tecido adiposo (SARIS et al., 2003). O armazenamento assim como a liberação de lipídeos pelo tecido adiposo branco, está fisiologicamente sob controle hormonal, pois a insulina promove o armazenamento de triglicerídeos (TG) durante a fase pós-prandial em um processo conhecido como lipogênese, e as catecolaminas desencadeiam a degradação de TG em glicerol e ácidos graxos no processo conhecido como lipólise, que fornece substratos energéticos para outros órgãos, incluindo músculo esquelético e hepático, durante o jejum (YU; GINSBERG, 2005). Assim, o tecido adiposo e a insulina desempenham um papel fundamental no armazenamento de gordura, pois inibem diretamente a lipólise, estimulam a lipogênese e aumentam a captura de ácidos graxos pelos adipócitos (KAHN; FLIER, 2000).

A gordura visceral representa um poderoso fator de risco de resistência à insulina (HUH et al., 2014). Os adipócitos do abdômen são resistentes à ação antilipolítica da insulina e, com o aumento da lipólise, ácidos graxos livres são liberados na veia porta, expondo o fígado altos níveis de lipídeos. Esse aumento de gordura, por consequência, acaba transformando tecidos que originalmente são insulino-sensíveis em tecidos insulinorresistentes (FOSTER; PAGLIASSOTTI, 2012) (FIGURA 1).

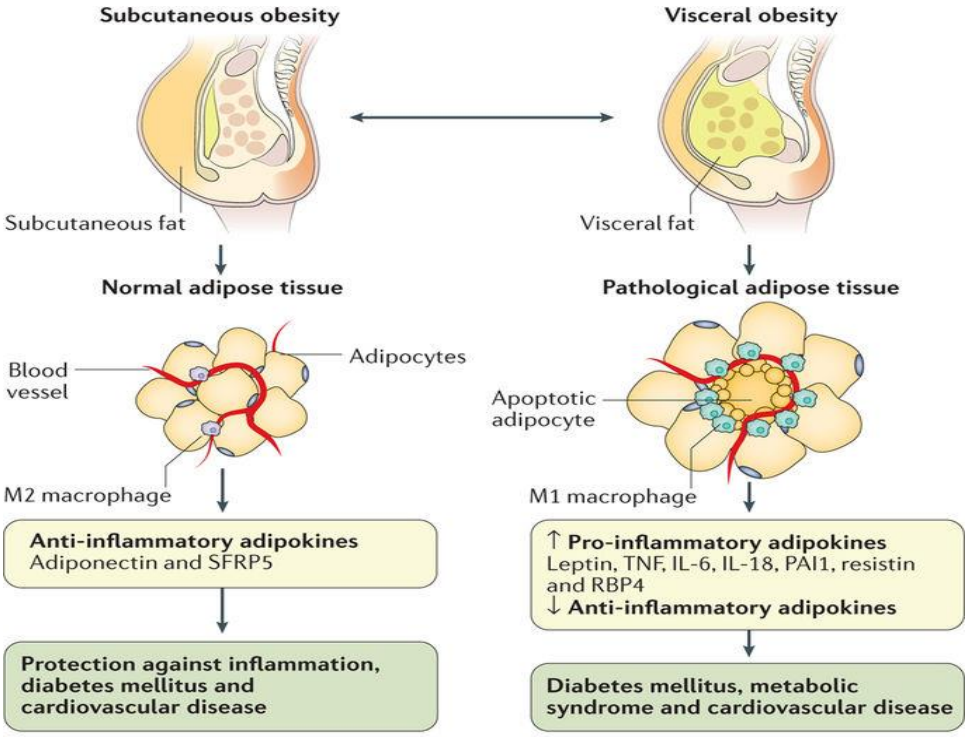

Figura 1. Alterações patológicas no tecido adiposo.
Fonte: Adaptado de González-Muniesa et al. (2017).

No estudo de Hiuge-Shimizu et al. (2012) em larga escala, revelou que o valor absoluto da área de gordura visceral correlacionava-se com os riscos cardiovasculares associados à obesidade, mas os riscos cardiovasculares não aumentaram com o aumento da gordura subcutânea. Consequentemente, os tecidos adiposos na obesidade gordurosa subcutânea podem funcionar normalmente com a liberação esperada de adipocinas anti-inflamatórias, enquanto que os tecidos adiposos na obesidade gordurosa visceral liberam uma quantidade aumentada de adipocinas pró-inflamatórias e suprimem a secreção de dipocitocinas anti-inflamatórias, criando assim de baixo grau inflamação, o que contribui para o comprometimento metabólico e cardiovascular sistêmico que está associado a distúrbios relacionados à obesidade (OUCHI et al., 2011; HEREDIA; GOMEZ-MARTINEZ; MARCOS, 2012; GONZÁLEZ-MUNIESA et al., 2017).

Na obesidade, ocorre também um aumento na produção e liberação de adipocinas, estresse oxidativo e hipóxia, resultando em um ambiente pró-inflamatório, que pode evoluir para inflamação sistémica de baixa intensidade sem aumento proporcional do débito cardíaco e do fluxo sanguíneo tecidual e adicionalmente instaurar resistência à insulina (TRAYHURN, 2013).

2.3 Ações e efeitos metabólicos de dietas ricas em carboidratos

A composição dos alimentos é um fator chave para a resposta fisiológica que será obtida após seu metabolismo. Apesar de haver evidências de que as concentrações sanguíneas de glicose e, consequentemente, de insulina podem favorecer o ganho de peso (BRAND-MILLER et al., 2002), no que diz respeito à ampla variedade de açúcares de adição, não se pode considerar que apenas a resposta insulínica tenha efeito sobre o acúmulo de gordura proveniente do alto consumo destas substâncias. Um exemplo disto é o fato da frutose apresentar índice glicêmico baixo e mesmo assim contribuir para o aumento do tecido adiposo (TAPPY; LÊ, 2010).

A Associação Americana de Cardiologia recomenda que o consumo de açúcar adicionado ao alimento não ultrapasse 100 e 150 calorias/dia para homens e mulheres, respectivamente, o que corresponde a aproximadamente 5% do consumo diário, o que por sinal não é atendido na maioria dos padrões alimentares globais. Na Inglaterra, o aumento do consumo de açúcar aumentou 1.500% entre os séculos XVIII e XIX, e até a virada do século XX, os açúcares (sacarose, glicose e frutose) se tornaram um dos principais constituintes da nossa dieta (LICHT, 2005). Bray, Nielsen e Popkin (2004) demonstram que o aumento da ingestão de açúcares de adição, especialmente a frutose livre e o xarope de milho rico em frutose (que possuem frutose em concentrações elevadas) ao longo dos últimos anos está diretamente correlacionado com o aumento da prevalência de obesidade na população.

Muitos estudos abordam o efeito das dietas enriquecidas com frutose ou sacarose, indicando que estas dietas levam a vários efeitos metabólicos e cardiovasculares adversos, como: dislipidemia, acúmulo de gordura ectópica, intolerância à glicose, hiperinsulinemia, resistência à insulina, hipertensão, aumento de peso corporal e estresse oxidativo (BASCIANO; FEDERICO; ADELI, 2005; BIZEAU; PAGLIASSOTTI, 2005; BOTEZELLI et al., 2012; PINTO et al., 2016).

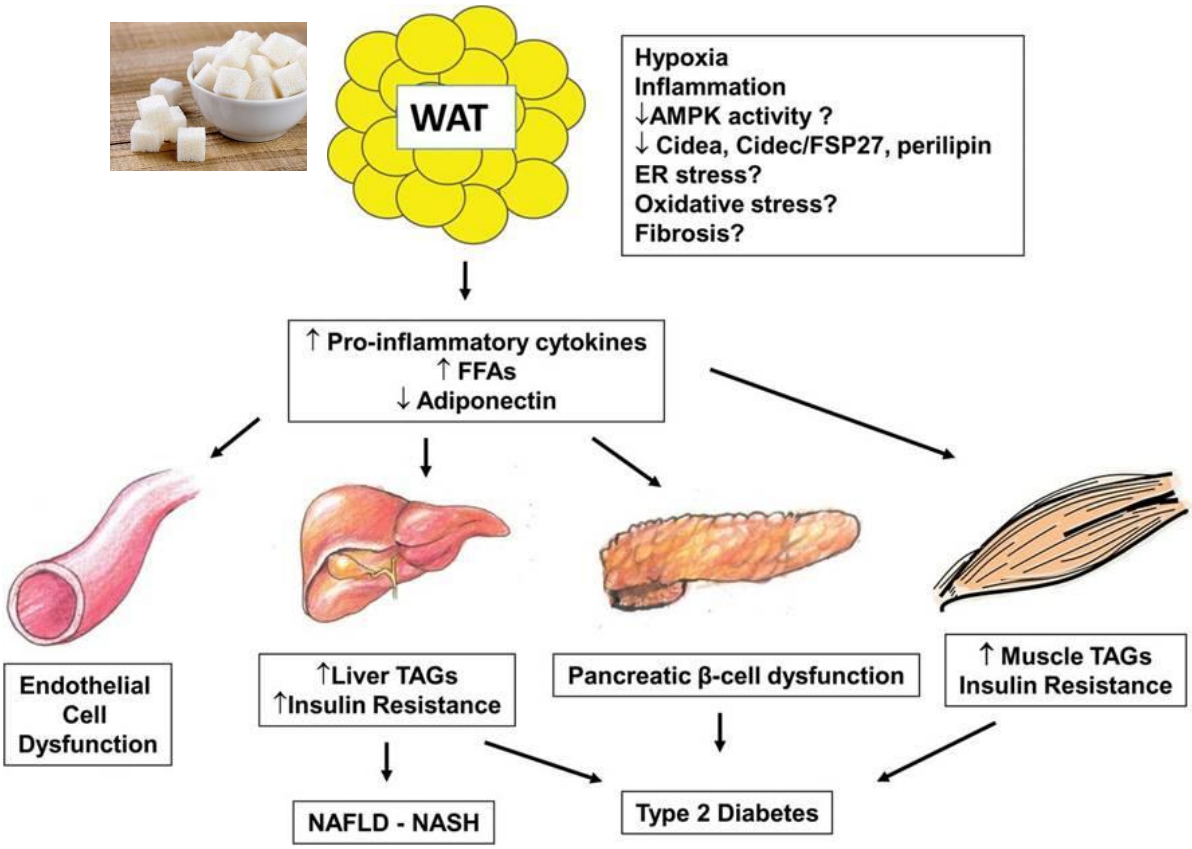

Figura 2. Efeito de açúcar simples nos tecidos e órgãos nobres.
Fonte: Adaptado de Abete et al. (2010).

Consumo de carboidrato simples promove alterções acentuadas no tecido adiposo branco e geram consequencias e disfunções para tecidos e órgãos nobres especificamente tecido hepático, músculo, pâncreas e endótélio (ABETE et al., 2010).

Diversos estudos demonstram um relação direta entre o consumo elevado de açúcar ou alimentos com elevado índice glicêmico e o acúmulo de tecido adiposo, contribuindo para o ganho de peso e obesidade (LUDWIG; FRIEDMAN, 2014). Naderali, Fatani e Williams (2004) demonstraram que ratos Wistar machos alimentados com dieta rica em sacarose manufaturada a partir da adição de 33% de leite condensado e 7% de açúcar refinado para cada quilo de ração, apresentaram um aumento relevante de ganho de peso em relação ao grupo controle em um período de 15 semanas (LIMA et al., 2008), utilizando um padrão dietético bem semelhante, observaram aumento de peso após 4 semanas de acompanhamento e ao final da 19ª semana esta diferença já ultrapassava 28% em relação ao controle.

Aumento de peso e de adiposidade visceral também foi observado por Pawlak et al. (2004) após exposição de ratos a uma dieta hiperglicídica (45% de carboidratos) por um período de 32 semanas, e Kawasaki et al. (2005) por após 42 semanas de exposição a uma

dieta rica em sacarose. Ensaio controlado aleatório em crianças e adultos com duração de 6 meses a 2 anos mostraram que a redução da ingestão de açúcares, diminui o peso corpóreo (BRAY; POPKIN, 2014).

Este ganho de massa corporal está relacionado principalmente ao aumento de depósitos adiposos e alterações da homeostase de parâmetros bioquímicos, tornando mais suscetível um perfil lipêmico nas espécies estudadas. Botezelli et al. (2012) submeteram ratos a uma dieta enriquecida em 60% com frutose por 60 dias e apresentaram aumento dos depósitos adiposos retroperitoneal, mesentérico e subcutâneo, tolerância à glicose, hiperinsulinemia, dislipidemia e aumento dos níveis de gordura hepática. Já Pinto et al. (2016) demonstraram a eficiência de uma dieta constituída de 25% de sacarose em induzir disglicemia em estado de jejum e alimentado, intolerância à glicose, hipertrigliceridemia e resistência à insulina mesmo em um período de acompanhamento relativamente curto (3 meses).

Estas alterações ocorrem especialmente pelos desfechos metabólicos associados a estes açúcares. A sacarose absorvida pelos enterócitos é imediatamente hidrolisada pela enzima sacarase em glicose e frutose, que mesmo possuindo estruturas isoméricas possuem destinos bioquímicos distintos. A glicose estimula a secreção de insulina pelas células β das ilhotas pancreáticas, que por sua vez estimula a captação de glicose para os tecidos sensíveis à insulina (músculo, tecido adiposo e fígado) e os processos anabólicos de glicogênese, lipogênese e proteogênese, garantindo desta forma o restabelecimento da homeostase glicêmica e de outros substratos energéticos. Entretanto, quando mantida em altas concentrações, desencadeia desfechos metabólicos deletérios como hiperinsulinemia, glicotoxicidade e RI (TAPPY; LÊ, 2010).

A frutose, por sua vez, é rapidamente captada para o fígado (via GLUT-2) e, de forma independente da insulina, estimula fatores lipogênicos e a síntese de novo de lipídeos de forma mais acentuada que a glicose, levando ao acúmulo de triglicerídeos hepáticos e exportação de Lipoproteína de Densidade Muito Baixa (VLDL), e consequentemente esteatose e dislipidemia. O excesso de frutose também está relacionado à sobrecarga mitocondrial e estresse oxidativo (TAPPY; LÊ, 2010). É importante frisar que isoladamente estes monossacarídeos já levam a todos estes desfechos metabólicos, no entanto quando ingeridos em conjunto (na sacarose, por exemplo) estes efeitos deletérios são potencializados (KOLDERUP; SVIHUS, 2015).

De forma importante, a frutose também contribui para o ganho de peso ao interferir negativamente sobre a liberação da insulina, leptina e grelina, hormônios que afetam diretamente o centro de saciedade no sistema nervoso central, resultando em uma ingestão aumentada de alimentos, que serão armazenados na forma de gordura (TEFF et al., 2004). A frutose de adição também torna os alimentos mais palatáveis e isso pode contribuir para uma maior ingestão (JOHNSON et al., 2007).

A relação entre o índice glicêmico dos alimentos na elevação dos níveis insulínicos vem sendo debatida há muitos anos. Oettle, Emmett e Heaton (1987) avaliaram a resposta insulínica para alimentos compostos por grãos refinados e alta quantidade de açúcar (barrinha coberta com chocolate, refrigerante de cola) e alimentos integrais (uva passa com amendoim, banana e amendoim) com quantidades energéticas e de gordura similares. Os resultados mostraram que os alimentos adoçados artificialmente contendo grãos refinados aumentaram os níveis de glicose e insulina quando comparados aos integrais. Esta maior resposta insulinêmica é responsável por uma maior conversão de glicose em ácidos graxos e lipogênese estimulada pela secreção de insulina (WILLIAMS; FRANKLIN, 2015), acarretando em acúmulo de tecido adiposo e contribuindo para o ganho de peso e obesidade (LUDWIG; FRIEDMAN, 2014).

Para Lomba et al. (2009) uma dieta rica em sacarose aumenta a adiposidade, promove um perfil lipídico aterogênico, marcado pelo aumento dos níveis de colesterol total, redução da Lipoproteína de Alta Densidade (HDL) e aumento da Lipoproteína de Baixa Densidade (LDL), além de definir regiões focais e crescentes de armazenamento de gordura no parênquima hepático. Embora a literatura evidencie esteatose em modelos animais, é menos frequente nesses modelos a esteato-hepatite, caracterizada por presença de balonização hepatocelular e infiltrado inflamatório (ANSTEE; GOLDIN, 2006). Isto se dá provavelmente pela estimulação da síntese de novo de lipídeos e redução da oxidação de ácidos graxos (TAPPY; LÊ, 2010). Sobre a relação entre o consumo de açúcares e seus efeitos a curto prazo são definidos os mecanismos de sinalização dos sistemas regulatórios para alimentação onde é sabido que tais substâncias estimulam a saciedade e promovem a reduzem da ingestão de alimentos e, que essa resposta não deve ser atribuída unicamente, ao seu efeito sobre a glicemia (ANDERSON; WOODEND, 2003).

Para Jankiewicz et al. (2015) a ingestão de dietas ricas em sacarose está associado ao consumo de menos grânulos e bebidas, menos lipídeos e proteínas; nesses casos, há um maior

armazenamento de tecido adiposo retroperitoneal. Tais dietas demonstram alterações de maiores níveis de glicemia, triglicerídeos, adiponectina, leptina, a redução dos níveis de HDL e insulina, porém é capaz de promover também a redução do colesterol total.

Mesmo com todos impactos negativos relacionados ao consumo de açúcares, ainda se observa uma expansão em seu consumo e a utilização de medidas de controle a obesidade associadas a este consumo merecem ser pesquisadas com maior destaque. Neste contexto, dietas com maior teor de proteínas podem ter destaque.

2.4 Ações e efeitos metabólicos da dieta hiperproteica

Em meados do século XX, Waterlow (1985) afirmou que uma dieta hiperproteica requer um processo de adaptação metabólica, sem efeitos benéficos e ou adversos cumulativos conhecidos. Tal processo é definido como uma resposta intencional a uma nova condição circunstancial e alimentar na qual foi submetido o organismo.

Em outras regiões parenquimais de órgãos anexos e de fundamental importância no metabolismo das proteínas, as variações no teor dessas substâncias na dieta hiperproteica, mesmo que em reduzido consumo de açúcares, pode interferir nos processos específicos bioquímicos e de adaptação molecular das áreas esplênicas de diferentes órgãos digestórios, em particular o fígado, com alterações do metabolismo celular (JEAN et al., 2001).

Mesmo com poucos estudos, especialmente avaliações de longo prazo, diversos são os benefícios atribuídos ao aumento do consumo de proteínas em detrimento às gorduras e carboidratos, principalmente no que se refere à perda de peso relacionada à maior saciedade conferida pelo substrato, assim como aumento de termogênese (CANNON; NEDERGAARD, 2004).

A saciedade promovida pela dieta hiperproteica é maior do que outros macronutrientes, considerando a mesma quantidade de calorias ingeridas. As proteínas têm menor tendência a promover o armazenamento de gordura, o que sugere que tais elementos sejam componentes chave para induzir um balanço energético negativo e promover a perda de peso (VELDHORST et al., 2008). O efeito da saciedade é multifatorial e influenciado por muitos componentes, incluindo, mas não se limitando, ao sistema endócrino, o sistema cognitivo e neural e o sistema gastrointestinal, visto que dietas hiperproteicas promovem aumento da energia desprendida, aumentam os níveis de hormônios anorexígenos e alteram a

gliconeogênese. A hierarquia da eficiência de saciedade induzida pelos macronutrientes é semelhante à observada para a termogênese induzida pela dieta (VELDHORST et al., 2008), com maior saciedade específica sensorial de proteína em comparação com os carboidratos e gorduras (BENSAÏd et al., 2002).

Iglay et al. (2007) relataram uma maior sensação de saciedade após o consumo de uma refeição isocalórica com 68% de proteína em comparação a uma refeição com 10% de proteína. No estudo conduzido por Belobrajdic, McIntosh e Owens (2004), observou-se que uma dieta hiperproteica reduziu o armazenamento de gordura visceral e subcutânea na carcaça dos ratos em até 26% quando comparado a uma dieta hipoproteica. Esta redução de gordura corporal pode ser parcialmente explicada pela diminuição equivalente a 19% do consumo energético. Este fato assemelha-se a estudos anteriores realizados com ratos, exibindo a proteína como sendo o macronutriente que mais induz saciedade (JEAN et al., 2001; BENSAÏd et al., 2002; BELOBRAJDIC; MCINTOSH; OWENS, 2004).

A ingestão proteica auxilia também transição de massa adiposa branca para massa magra. Estudo experimental sobre os efeitos da ingestão de elevadas concentrações de proteína na deposição adiposa em animais acompanhados com dietas ricas em gordura ou em sacarose não demonstraram alterações no consumo cumulativo de energia e peso corporal. Entretanto, as dietas ricas em proteínas reduziram em 20% o ganho de adiposidade induzida por dieta rica em sacarose e gordura. Os pesquisadores sugerem que a alta ingestão proteica reduz a capacidade hepática para a lipogênese, assim como a expressão de genes relacionados a síntese de ácidos graxos. Em conjunto, esses resultados sugerem que dietas podem reduzir a adiposidade pela inibição da lipogênese e estimular a cetogênese no fígado (CHAUMONTET et al., 2015).

Dietas hiperproteicas estão cada vez mais relacionadas à perda de peso, fornecendo benefícios na saciedade e redução da massa gordurosa. Alguns dos mecanismos potenciais que contam para a perda de peso associada a tais dietas, envolve uma secreção aumentada de incretinas como o peptídeo semelhante a glucagon 1 (GLP-1), polipeptídeo inibitório gástrico (GIP) e redução da secreção de grelina, assim como o aumento do processo de gliconeogênese (RIETMAN et al., 2014). Adicionalmente, a ingestão de proteínas aumenta a termogênese pós-prandial e auxiliam na conservação de massa magra (LEJEUNE et al., 2006), assim como promovem redução do armazenamento de gordura, sugerindo que este nutriente seja um

componente chave para induzir um balanço energético negativo e promover a perda de peso (VELDHORST et al., 2008).

O consumo de proteína afeta diferentemente outros tecidos e sistemas fisiológicos. No tecido ósseo, por exemplo, um estudo avaliando a densidade mineral óssea total da pelve e coluna vertebral em ratos albinos adultos da linhagem Wistar submetidos à dieta hiperproteica demonstrou uma redução significante da largura do córtex do fêmur e dos níveis de cálcio ósseo. Efeito semelhante ocorreu com o nível de osteocalcina. Igualmente, houve redução do cálcio. Os efeitos da alta ingestão de proteínas e possíveis mudanças no tecido ósseo estão associados a uma maior propensão à perda óssea (SILVA et al., 2014). No entanto, Jesudason e Clifton (2011) relataram efeitos benéficos de uma dieta hiperproteica, considerando o aumento da densidade mineral óssea. No que concerne a funcionalidade do sistema cardiovascular, dietas hiperproteicas em ratos adultos se mostraram capazes de preservas as funções aórticas, ventriculares e reduzir estresse oxidativo nas artérias mesentéricas (BEDARIDA et al., 2014).

Experimento com ratos machos da linhagem Wistar, submetidos a dietas hiperproteica não demonstraram alterações histopatológicas no fígado e dos rins, assim como no equilíbrio de cálcio; enquanto que, animais submetidos a uma dieta de proteína normal apresentaram esteatose hepática em grandes áreas do parênquima do órgão, assim como alterações nas concentrações de cálcio e síntese de hormônios relacionados aos mecanismos de captação mineral (LACROIX et al., 2004). Portanto, uma dieta rica em proteína, por longos períodos promove redução acentuada no tecido adiposo branco, especificamente hepático e, nas concentrações basais de triglicerídeos, glicose, leptina e insulina, sugerindo que tal conduta, experimental não apresenta efeitos deletérios orgânicos e pode prevenir a síndrome metabólica.

Do mesmo modo, ao escolher consumir alimentos ricos em proteínas deve-se considerar a alta ingestão de aminoácidos de cadeia ramificada em combinação com uma dieta ocidental para o risco de promoção e desenvolvimento de doenças metabólicas, além de disponibilizar uma carga ácida significativa para os rins. No contexto final, uma carga baixa de energia, excesso de proteína convertida em glicose (via gliconeogênese) e/ou corpos cetonônicos acabarão contribuindo para um balanço energético positivo não desejável (PESTA; SAMUEL, 2014).

2.5 Tratamento da obesidade

Como primeira linha de atuação, a mudança dos hábitos alimentares, a prática de exercício físico regular e a modificação comportamental, constituem as opções não farmacológicas do tratamento da obesidade (GONZÁLEZ-MUNIESA et al., 2017). Vários estudos mostram que uma ingestão calórica entre 500 a 1000 kcal/dia inferiores ao necessário para manter o peso corrente resulta, em média, numa perda de 0,5 kg/semana. Apesar de restrições calóricas mais severas poderem aumentar a velocidade de perda de peso, em longo prazo, culminam no insucesso da terapêutica pela dificuldade em se conseguir manter a redução de peso. A combinação da prática de exercício físico com a modificação dietética não acelera a perda de peso em curto-prazo, porém, o exercício físico é o componente mais preponderante na manutenção da redução de peso em longo prazo (LESER; YANOVSKI; YANOVSKI, 2002).

Outro auxiliar na terapêutica não farmacológica da obesidade é a terapia comportamental, ensinando e permitindo aos adultos obesos desenvolverem comportamentos adaptativos a um novo estilo de vida, tais como saber o que comer, quando comer, possuir um estilo de vida mais ativo e optar pela prática exercício físico de forma regular, de modo a atingir uma perda de peso favorável, mas também mantê-la. O tratamento não farmacológico deve ser tentado durante pelo menos 6 meses, recorrendo-se então à farmacoterapia caso a redução de peso obtida não seja satisfatória (CANNON; KUMAR, 2009).

A partir da década de 90, após a obesidade ser considerada doença, a comunidade cientifica e médica vem aprofundando pesquisas e métodos de combate a obesidade, incidindo primordialmente na prevenção por meio de reeducação alimentar e alterações de um ambiente obesogênico (NIKOLAOU; HANKEY; LEAN, 2015). Segundo o Consenso Latino-Americano de Obesidade, a intervenção farmacoterapêutica torna-se necessária quando mudanças no estilo de vida não produzem os efeitos desejados e, ou comorbidades podem colocar em risco a vida do paciente obeso. Consequentemente, a terapêutica farmacológica foi proposta como um adjuvante à dieta e às mudanças no estilo de vida, de forma a melhorar a manutenção da perda de peso (KHERA et al., 2016).

A farmacoterapia é aprovada para inclusão no tratamento de pacientes com obesidade de risco moderado ou de alto risco (um IMC $\geq$ 30 kg/m2 com ou sem efeitos metabólicos associados ou IMC $\geq$ a 27 kg/m2 se houver comorbidades, desde que associadas

como adjuvante para dieta, exercício físico e a modificação comportamental) (JENSEN et al., 2014). Uma perda de peso gradativa entre 5- 10%, que pode ser obtida com o uso responsável de medicações antiobesidade, pode reduzir de forma significativa os riscos de aquisição de doenças correlacionadas a obesidade (YUMUK et al., 2015).

Cinco estratégias farmacêuticas são atualmente aprovadas pelo Food and Drug Administration (FDA) para o tratamento de obesidade: orlistat, lorcaserina, liraglutida, fentermina/topiramato e bupropiona/naltrexona. Estes medicamentos em mono ou politerapia demonstram eficácia para perda de peso e melhoram marcadores cardiometabólicos (FUJIOKA, 2015; BRAY et al., 2016).

O Orlistat é a única droga disponível sem receita médica que desde setembro de 2013 é considerada segura para controle de peso crônico pelo FDA e Agência Européia de Medicamentos (KIM et al., 2014). Ele atua via inibição da lipase pancreática e intestinal, reduzindo assim a absorção de gordura e produzindo um déficit energético significante, levando a perda de peso. A maior parte dos efeitos indesejáveis causados por este fármaco são gastrointestinais e relacionados ao seu mecanismo de ação, dentre estes, o mais frequente é diarreia, em função da gordura não digerida no intestino (SRIVASTAVA; APOVIAN, 2018). Apesar do seu status aprovado, o orlistat teve uma série de problemas de segurança, incluindo hepatotoxicidade, nefrotoxicidade, pancreatite e cálculos renais (WEIR et al., 2011).

A lorcaserina é um agonista do receptor de serotonina do tipo 2c (5-HT2c) que atua no cérebro para reduzir a ingestão de alimentos. Há indicações de seu uso oral para períodos prolongados. O medicamento normalmente é bem tolerado, mas com efeitos adversos leves, como dor de cabeça, tonturas, náuseas, xerostomia e constipação podem surgir (SRIVASTAVA; APOVIAN, 2018). A liraglutida é um análogo do GLP que promove redução da velocidade de passagem dos nutrientes pelo intestino, permitindo maior saciedade e consequentemente redução da ingestão de alimentos. Os efeitos colaterais mais observados são náuseas e vômitos, porém pancreatite aguda e doença da vesícula biliar também já foram relatadas (JONES; BLOOM, 2015). O tratamento está associado a uma redução de 13% nos principais eventos cardiovasculares e uma redução de 15% na mortalidade (DAVIES et al., 2015).

A fentermina é um fármaco agonista noradrenérgico que atua como um supressor de apetite e tem como principais efeitos colaterais tonturas, xerostomia, insônia, constipação, irritabilidade e efeitos cardioestimulatórios (KIORTSIS, 2013; SMITH et al., 2013). Pode ser

utilizada em combinação com o topiramato, um antiepiléptico que atua inibindo receptores glutamatérgicos do tipo Alfa-Amino-3-Hidroxi-Metil-5-4-Isoxazolpropiónico (AMPA) e aumenta a condutância ao Ácido Gama Aminobutírico (GABA). Quando utilizados em associação o potencial anorexígeno é potencializado e consequentemente a perda de peso, de forma mais intensa que qualquer um dos outros medicamentos aprovados. Porém, estes apresentam efeitos adversos tais como parestesias e disgeusias, e de forma mais rara acidose metabólica e glaucoma (SRIVASTAVA; APOVIAN, 2018).

A combinação de naltrexona e bupropiona aumenta a saciedade e diminuem o apetite, inibindo a recaptação de dopamina e noradrenalina, bloqueando o receptor μ-opioide e ativando pro-opiomelanocortina (SOLAS et al., 2016). Efeitos colaterais como náuseas, constipação e cefaleias podem surgir. Não recomendado para pacientes que usam opióides, antidepressivos inibidores da enzima monoamina oxidase e com histórico de convulsões. A literatura não apresenta certeza sobre a segurança cardiovascular deste tratamento (KHERA et al., 2016).

Outra terapia farmacológica alvo de muita discussão entre as instituições reguladoras é o do anorexígeno anfetamínico sibutramina que em 2002 foi retirado mercado europeu e em 2010 do mercado americano (BRAY et al., 2016). No Brasil, sua venda é permitida de forma controlada. A sibutramina atua como um inibidor da recaptação da serotonina e noradrenalina, promovendo efeitos anorexígenos (ARAUJO; MARTEL, 2012). Ensaios clínicos mostraram que a substância promove expressiva perda de peso de cerca de 4 kg em 12 meses quando comparados a placebos. No entanto, aumentos de pressão arterial sistólica e diastólica e de frequência cardíaca também foram relatados nestes pacientes (RUCKER et al., 2007). Desta forma, é contraindicada para pacientes com doença cardiovascular, acidente vascular cerebral, arritmia ou hipertensão não controlada (SRIVASTAVA; APOVIAN, 2018).

Independente da medicação selecionada, durante seu uso é necessário um estilo de vida adequado para a eficaz perda de peso. O prescritor e o paciente devem estar familiarizados com as drogas e seus potenciais efeitos colaterais. Se os pacientes não perderem 4 a 5% do peso corporal após 3 meses, o medicamento deve ser interrompido e um novo plano de tratamento deve ser implementado. Nenhum medicamento é eficaz em todos os pacientes, assim como nem todos os pacientes são apropriados para todas as medicações (SOLAS et al., 2016).

As plantas são fontes naturais de moléculas úteis para o tratamento da obesidade e o

desenvolvimento de princípios ativos como alvo o intestino, tecido adiposo e sistema nervoso (JUNG; LIM; KIM, 2014). Adicionalmente, sob prescrição médica e orientação farmacêutica, medicamentos fitoterápicos podem ser uma alternativa no tratamento da obesidade com melhoria do perfil de risco cardiovascular e menor incidência de diabetes, segundo vários estudos (LIU et al., 2017) Compostos bioativos para o tratamento da obesidade são classificados de acordo com seu mecanismo de ação, tais como a restrição de apetite, sensação de saciedade aumentada ou diminuída de acordo com o poder calorífero; ativação de lipólise, oxidação de gordura e termogênese; redução da degradação e assimilação de energia que produzem macronutrientes ou declínio na biossíntese da gordura endógena; e processos diversos envolvendo adipogênese, inflamação e alterações associadas à obesidade (SOLAS et al., 2016; LIU et al., 2017).

A Garcinia cambolia é um medicamento fitoterápico registrado pela Agência Nacional de Vigilância Sanitária (ANVISA) com indicação terapêutica especifica para este fim (CARVALHO et al., 2008). Estudos clínicos e experimentais mostraram que a espécie vegetal *Syzygium cumini* também possui um potencial terapêutico para as alterações metabólicas decorrentes da obesidade. O uso do extrato hidroalcoólico das folhas de *Syzygium cumini* por 30 dias melhorou a glicemia em jejum, restabeleceu os níveis de triglicerídeos e perda de peso em animais obesos induzidos com L-Glutamato Monossódico (SANCHES et al., 2016).

3 OBJETIVOS

3.1 Objetivo geral

Avaliar os efeitos da dieta hiperproteica sobre o metabolismo geral e composição corporal de ratos adultos com obesidade e demais alterações metabólicas induzidas por dieta rica em sacarose.

3.2 Objetivos específicos

– Avaliar o desenvolvimento de obesidade e outras alterações metabólicas induzidas pela exposição precoce e sustentada à dieta rica em sacarose em ratos adultos;

– Avaliar as consequências metabólicas da substituição da dieta rica em sacarose pela dieta controle ou dieta rica em proteínas;

– Analisar histopatologicamente tecidos chaves dos grupos experimentais para averiguar o reflexo tecidual das alterações bioquímicas e morfométricas.

4 RESULTADOS

4.1 Capítulo I – Long-term high-protein diet reverts weight gain and attenuates metabolic dysfunction in high-sucrose-fed adult rats

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Capítulo 1 – Long-term high-protein diet reverts weight gain and attenuates metabolic dysfunction in high-sucrose-fed adult rats

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Abstract Background

The objective of this study is to investigate the effects of a high-protein diet against metabolic dysfunctions sucrose-induced in rats from weaning to adulthood, as well as to compare the metabolic benefits between higher protein intake and withdrawal of excess sugar.

Methods

Weaned male Wistar rats were randomized into two groups: control rats, fed a standard chow or obese rats (HS/HS), fed a high-sucrose chow, for a 20-weeks period. Afterwards, HS/HS animals were randomized into 3 new groups: rats maintained on HS diet; rats initially submitted to HS diet and then replaced by standard chow; and those replaced by high-protein diet (HS/HP). All groups were followed up for an additional 12-week nutritional intervention. Were assessed in groups: the body weight, energy intake, obesity development, glicemic/lipid profile, glucose tolerance, insulin resistance, morphometry of adipose tissue, liver and skeletal muscles, lipolitic activity, liver histology and biochemical profile of hepatic function.

Results

The post-weaning exposure to HS diet leads to metabolic syndrome phenotype at adulthood, herein characterized by central obesity, glucose intolerance, dyslipidaemia and insulin resistance. Furthermore, the HPD was effective in reversing the sugar intake damages, marked by weight loss and reduction of obesity and adipose fat pads. The replacement of HS by standard chow also resulted in decreased adipose accumulation, even though in a lesser extent than that caused by HPD. Notoriously, the HPD reestablished the responsiveness of adipose tissue to adrenergic agonist, making the tissue susceptible to lipolysis. The sucrose

withdrawal attenuates the deleterious effects on glucose-insulin axis. However, its replacement by HPD promoted the same effects but further improved glucose tolerance and insulin sensitivity. Finally, the exposure to HS led to hepatic steatosis without inflammation, with consequent rising of ALT activity. However, both nutritional interventions were able to restore such alterations.

Conclusions

Our set of data show that HPD consistently defeated against metabolic outcomes high-sucrose-induced. On the other hand, simple withdrawal from high-sucrose consumption also ameliorate the metabolic damages, even though less pronounced. Thus, both nutritional interventions show themselves to be mutually efficient, potentially helping to treat patients with metabolic syndrome.

Keywords: High-protein diet; High-sucrose diet; Withdrawal of sucrose; Metabolic syndrome.

Background

Consumption of added sugars has been considered a worldwide public health concern by its implied association with the epidemic of obesity, type 2 diabetes mellitus (T2DM) and metabolic syndrome (MetS) [1]. Although this is still an issue of intense debate [2, 3], huge evidence from basic [4] and clinical [5] studies consistently support such concern. Additionally, it has been demonstrated the ability of sugar-based western diet to genetically modify the activity of thrifty genes on Pacific people, which was not historically prone to obesity [6]. This scenario becomes still worse in face of the fact that sucrose is one of the few

components of our diet for which no upper limits are suggested in the majority of dietary guidelines around the globe [1], although it has been recommended a total sugar intake of 10% at maximum [7].

Sucrose intake dually promotes glucose-elicited insulin release toward its own uptake by insulin-sensitive tissues, as well as insulin-independent fructose uptake by hepatocytes and adipocytes [8]. Inside the latter, fructose is converted into fatty acids through *de novo* lipogenesis (DNL) that favours triacylglycerol (TAG) synthesis, very-low- density lipoprotein (VLDL) secretion and adipocyte hypertrophy [9, 10]. Sustained DNL activation in both territories intensifies lipid accumulation which also impairs insulin signalling within a vicious cycle towards non-alcoholic fatty liver disease (NAFLD) onset [11] and adipose tissue dysfunction [10]. Experimentally, we have shown that 60-day exposure of weaning rats to a high-sucrose but isocaloric diet (HSD) augmented body mass in association with increased fat accumulation in both subcutaneous and visceral depots. These animals also presented hyperglycaemia, hypertriglyceridemia, glucose intolerance and impaired insulin sensitivity [12].

Dietary manipulation is a primary factor in controlling and preventing obesity and its comorbidities. Much of the approaches have prioritized low-fat and/or low- carbohydrate interventions [13]. On the other hand, current studies have suggested high- protein diets to promote greater weight loss and metabolic outcomes in both human beings [14, 15] and rodents [16, 17]. The main rationale behind such advantage is that dietary proteins induce the release of anorexigenic and orexigenic hormones, which ultimately modulate neuronal pathways involved in the regulation of appetite and satiety, as well as energy expenditure [17-19]. Contrariwise, some studies have shown high-protein intake effects are only temporary and subjects (human beings and rodents) regain body weight upon mid- to long-term

interventions, suggesting a metabolic adaptation to high-protein regimens [20-22]. Such controversial outcomes put high-protein strategy into checkmate, deserving additional studies to support the development of differential dietary interventions to prevent or treat patients with obesity associated or not to MetS.

Therefore, in the present study we took advantage of our previously described model of MetS induced by HSD in rats [12] to assess the hypothesis that a 12-week high- protein nutritional intervention is more effective than mere excess sucrose withdrawal on the management of body weight and MetS-associated comorbidities. Our data provide a body of evidence that only long-term high protein diet (HPD) intake promotes sustained body weight management, as well as reestablishment of white adipose tissue (WAT) function, although excess sucrose withdrawal had equally improved glycemic control and dyslipidemia on those animals.

Methods

Experimental design

Weaned male Wistar rats (postnatal day 21) provided by the animal facility house of the Federal University of Maranhão were randomized into two groups: control rats (CT/CT; n = 7), fed a standard chow (Nuvital®, Nuvilab, Brazil) composed by 55.4% total carbohydrate (10% sucrose), 21% protein, 5.2% total lipids, totaling 3.52 kcal/g; or high-sucrose diet rats (HS/HS, n = 18), fed a high-sucrose chow composed by 65% total carbohydrate (25% sucrose), 12.3% protein, 4.3% total lipids, totaling 3.48 kcal/g, as previously described [12], for a 20-weeks period. Afterwards, HS/HS animals were randomized into 3 new groups: rats maintained on HSD (HS/HS; n=6); rats submitted to HSD replacement by standard chow (HS/CT; n=6); and those with HSD replaced by HPD (HS/HP; n=6). HPD was composed by

49% total carbohydrate (no sucrose), 34.3% protein, 2.2% total lipids, totaling 3.53 kcal/g. All groups were followed up for an additional 12- week nutritional intervention, totaling a 32-week follow-up, during which they were maintained under controlled conditions (21 ± 2 °C; 60% humidity and 12h light/dark cycle) with water and chow *ad libitum*.

Throughout observational period, body weight and energy intake were assessed twice a week. In parallel, Lee index ((body weight $(g)^{1/3}$ / naso-anal length (cm)) / 1000) was monthly calculated for assessment of obesity development [23]. During the 20^{th} week of observational period, 8-hour fasted rats had their tail-tip cut out and venous blood collected for fasting glucose measurement through glucometer and subsequent operation of the intraperitoneal glucose tolerance test. Collected blood samples were further processed for serum separation and measurement of total cholesterol (TC) and triglycerides (TAG) levels prior to 12-week nutritional intervention.

During the last week of nutritional intervention, animals were submitted to intraperitoneal glucose and insulin tolerance tests. By the completion of the 32-week follow-up, 8-hour fasted animals were anesthetized (40:10 mg/kg ketamine:xylazine solution) for blood and tissue collection upon laparotomy. White (retroperitoneal, periepididymal and mesenteric) and brown (interscapular) adipose tissue fat pads, liver and posterior skeletal muscles (gastrocnemius and soleus) were weighed for morphometric assessment and expressed as tissue mass (g) per 100 g body weight. Periepididymal adipose tissue samples were further used for *ex vivo* lipolysis assay. Liver lobes were processed for histological analysis and serum samples used for determination of biochemical and hormonal profile.

All procedures were performed in accordance with the rules of Brazilian Council for the Control of Animal Experimentation (CONCEA) and approved by the Ethical Committee on Animal Use and Welfare (CEUA) of the Federal University of Maranhão.

Assessment of glucose-insulin axis function

For intraperitoneal glucose tolerance test (*ip*GTT), animals were submitted to 8- hours fasting prior to administration of glucose 2 g/kg. Tail vein blood drops were collected immediately before (time 0) and 15, 30, 60 and 120 min after glucose load for glycemia measurement through glucometer (Accu check Active®, Roche Diagnostic, Germany). Similar procedure was carried out for intraperitoneal insulin tolerance test (*ip*ITT), excepting animals were fed and received 1 UI/kg insulin (Humulin 70/30®, Lilly, USA). Glucose disappearance rate (kITT) was derived from ITT curve and calculated as 0.693/ $t_{1/2}$[24]. The insulin resistance was inferred from TyG Index calculation [ln (fasting glucose (mg/dL) □ fasting triglyceride (mg/dL)) / 2] [25].

Assessment of serum biochemical profile

Serum samples were obtained upon spontaneous coagulation and centrifugation (3500 rpm; 10 min; 4 ºC) and used for colorimetric measurement of TAG, TC, aminotransferases enzymes (AST and ALT), γ-glutamyl transferase, alkaline phosphatase, albumin, total protein and urea (Labtest, Brazil) levels according to manufacturer's instructions. Insulin levels were assessed by immunoenzymatic method according to manufacturer's instructions (Sigma-Aldrich, USA).

Ex vivo lipolysis assay

To assess the lipolytic activity on WAT, periepididymal samples (~100 mg) were fragmented and incubated with Krebs buffer (120 mM NaCl, 15 mM $NaHCO_3$, 4.8 mM KCl, 1.2 mM $MgSO_4$, 1.2 mM KH_2PO_4, 2.4 mM $CaCl_2$, 1 % BSA and 0.1% glucose after pH adjustment to 7.4) for 1h at 37 °C in the absence or presence of 20 µM isoproterenol (Sigma-

Aldrich, USA). At the end of incubation, samples were cooled out to stop the reaction. Glycerol release (µg of glycerol/mg of tissue/h) was determined by colorimetric assay using a triglyceride assay kit according to manufacturer's instructions (Labtest, Brazil) [26].

Histological analysis

Samples from hepatic tissue were fixed in 10% phosphate-buffered formalin solution and they were analyzed by light microscopy either after hematoxilin-eosin (HE) or Masson's Trichrome staining. NAFLD activity score (NAS) [27] was applied for semi- quantitative analysis of the three definer criteria of non-alcoholic steatohepatitis: steatosis (0-3), ballooning (0-3), and lobular inflammation (0-2).

Statistical analysis

Statistical analysis was conducted using GraphPad Prism 7.0 software (GraphPad Software Inc., USA). Data were expressed as mean ± SEM and submitted to normality test (Kolmogorov-Smirnov) followed by parametrical analysis through unpaired *t* test (one-tailed) or one-way ANOVA (post test Newman-Keuls) for a significance level of 5% ($p < 0.05$).

Results

Post-weaning exposure to high-sucrose diet leads to metabolic syndrome phenotype at adulthood

Male rats exposed to HSD from weaning up to adulthood (24-weeks-old) evolved over time to a metabolic syndrome phenotype. As showed in Figure 1A, HS/HS rats presented higher body weight than CT/CT from 4th post-weaning week (260.0 ± 2.8 g *vs.* 248.6 ± 4.2 g, $p = 0.038$), reaching a 10% higher body weight at 20th post-weaning week (495.0 ± 4.1 g *vs.*

447.8 ± 9.4 g, $p < 0.0001$). A proportional increase was observed when analysed the area under the body weight curve (Figure 1B). Assessment of body mass through Lee Index calculation showed HS/HS rats were obese, as compared to CT/CT (331.0 ± 1.2 *vs.* 320.7 ± 1.7 $g^{1/3}$ cm^{-1}, $p < 0.0001$, Figure 1C). Interestingly, HS/HS rats developed an obese phenotype in spite of having a decreased energy intake, which was statistically lower from the 5th post-weaning week (Figure 1D-E).

Long-term HSD consumption negatively impacted on glucose and lipid homeostasis. HS/HS rats did not present fasting hyperglycaemia (Figure 2A), but had impaired glucose tolerance in comparison to CT/CT (Figures 2B-C), $p < 0.05$. Serum TC levels were slightly augmented in HS/HS (74.1 ± 2.2 mg/dL) when compared to CT/CT (64.7 ± 4.6 mg/dL, Figure 2D), $p < 0.05$; whereas serum TAG levels were 2-fold higher in HS/HS than CT/CT rats (139.1 ± 9.1 *vs*. 69.1 ± 4.1 mg/dL, Figure 2E), $p < 0.0001$. HS/HS rats also presented insulin resistance, since TyG Index values for this group (8.8 ± 0.05) were significantly higher than those obtained for CT/CT (8.1 ± 0.09, Figure 2F), $p < 0.0001$. Overall, this set of data consistently demonstrate that early introduction of dietary sugars leads to metabolic syndrome at adulthood, herein characterized by presence of the following metabolic outcomes: obesity, glucose intolerance, dyslipidaemia and insulin resistance.

High-protein diet reverts the weight gain induced by high-sucrose diet consumption

We next assessed the effects of a 12-week nutritional intervention with HPD on HSD-fed rats. HS/HP rats lose weight from 495.0 □ 4.1 g to 475.0 □ 6.3 g ($p < 0.0001$), no longer differing from CT/CT (481.5 □ 10.4 g; Figure 3A). On the other hand, HS/HS and HS/CT rats maintained similar upward trend of weight gain (Figure 3A). The effect of HPD on weight loss is better seen when analysed the area under body weight curve (Figure 3B), as well as the

Lee Index (Figure 3C) measured at the end of the nutritional intervention period. Such decreases are, at least in part, related to the lower energy intake of HS/HP rats, as compared to all the other groups (Figure 3D), $p < 0.0001$. Additionally, HPD consistently decreased white and brown adipose tissue depots in HS/HP rats, as compared to HS/HS (Table 1). Replacing HSD by standard chow also resulted in decreased adipose tissue accumulation, even though in a lesser extent than that caused by HPD (Table 1). HPD had no effect on liver weight, but increased skeletal muscle mass in comparison with the other two HSD-fed groups (Table 1).

To further characterize HPD effects on adiposity, we measured basal and isoproterenol-evoked *ex vivo* lipolysis in periepididymal fat pads from all groups. Data in Figure 4 show that all groups had similar lipolytic activity at basal conditions, but that long-term exposure to HSD abolished the physiological lipolytic response to sympathetic stimulus. HS/HS group was incapable of rising glycerol release under isoproterenol presence, as compared to the 4-fold increase observed in CT/CT group (6.74 ± 0.82 *vs.*24.81 ± 6.32 μg/mg/h, $p < 0.01$). On the other hand, HS/HP group showed a completely restored adipose tissue responsiveness to isoproterenol (9.83 ± 0.65 *vs.* 33.61 ± 6.01 μg/mg/h, $p < 0.001$), an effect not observed in HS/CT group, which had HSD simply replaced by standard chow (Figure 4). This set of data emphasizes the deleterious effects of high-sucrose consumption on sympathetic-evoked lipolysis and the counter modulatory effect promoted by high-protein intake instead of the simple excess sucrose withdrawal.

High-protein diet repairs glucose-insulin axis disruption induced by high-sucrose consumption

Exposure to HPD, as well as HSD withdrawal attenuated the disruption of glucose homeostasis verified at 20th week on HSD diet. As shown in Figure 5A, HS/HS rats presented

fasting dysglycaemia in comparison to CT/CT group (95.8 ± 2.8 *vs.* 85.9 ±2.2 mg/dL, $p < 0.05$), which was equally attenuated by either HPD introduction or HSD withdrawal, reaching levels not different from both HS/HS and CT/CT groups. When glucose levels were measured in fed state, only HS/HP group presented levels significantly lower than CT/CT group (86.6 □ 1.6 *vs.* 96.8 □ 3.2 mg/dL, $p < 0.05$; Figure 5B). In accordance to the latter, glucose levels at 15-min peak on *ip*GTT from HS/HP group were brought back to CT/CT-like levels ($p < 0.01$), an outcome not showed by HS/CT group (Figure 5C). Analysis of glycaemia decay during ITT (kITT) showed HS/HS rats presented impaired peripheral insulin sensitivity, which was restored by both nutritional interventions (Figure 5D). Surprisingly, fasting serum insulin levels did not differ among groups (Figure 5E).

Dyslipidaemia induced by long-term exposure to HSD was completely normalized upon 12-week nutritional intervention period. Fasting serum TC levels were reduced in both HS/HP and HS/CT groups (72.4 ± 8.1 and 69.9 ± 6.7 mg/dL, respectively), as compared to HS/HS group (101.9 ± 4.1 mg/dL, $p < 0.01$), which was 2-fold higher than CT/CT (53.7 ± 4.6 mg/dL, $p < 0.001$; Figure 5F). Similarly, serum TAG levels were 2.5- fold higher in HS/HS group (135.6 ± 17.0 mg/dL, $p < 0.001$) than in CT/CT (53.0 ± 3.8 mg/dL), but were strongly reduced to 61.6 ± 4.8 mg/dL and 77.6 ± 12.2 mg/dL in HS/HP and HS/CT, respectively ($p < 0.01$; Figure 5G). Notwithstanding, TyG Index values (Figure 5G) indicated the maintenance of hepatic insulin resistance in HS/HS group (8.74 ± 0.06), an outcome attenuated by HSD replacement for standard chow (HS/CT, 8.08 ± 0.14; $p < 0.001$), but completely reverted in HSD-fed rats (HS/HP, 7.94 ± 0.09; $p < 0.0001$), making the latter not different from CT/CT (7.64 ± 0.06). According to these data, excess sucrose withdrawal importantly attenuates the deleterious effects of long-term sucrose exposure on glucose-insulin axis. However, its replacement by HPD promoted the same effects but further improved peripheral insulin

sensitivity and glucose tolerance in the first 15 minutes after glucose load.

High-sucrose diet withdrawal restores hepatic lipids homeostasis

Microscopic analysis of H&E-stained liver slices according to the NAFLD activity score (NAS) showed that long-term exposure to HSD led to hepatic steatosis (Figure 6), with consequent rising of serum ALT activity, but no other marker of liver function (Table 2). However, both nutritional interventions were able to restore such alterations. Surprisingly, no signal of neither hepatocyte ballooning nor inflammatory infiltration were observed. These data reinforce the direct effects of sucrose consumption on NAFLD onset.

Discussion

Body weight management should be reached by simple appliance of energy balance equation, which leads to either weight loss if energy intake is less than energy expenditure, or weight gain if it occurs vice-versa. However, since meals with identical energy content may have unlimited combinations of dietary fats, carbohydrates, proteins or even alcohol; dietary manipulation has proven to be a challenging task for health professionals and billion people worldwide who are overweight or obese [13]. Most of the weight-loss diet literature has focused on the duel between low-fat and low-carbohydrate approaches, whereas high-protein interventions have currently attracted increasing attention [14, 15]. To our knowledge, the effects of long-term high-protein intake versus excess added sugar withdrawal on body weight loss and MetS-associated comorbidities have never been assessed. Consequently, we analysed two different groups of rats initially fed an HSD for 20-weeks, to then have their chow replaced by an isocaloric HPD or standard diet for additional 12-weeks to comparatively evaluate their metabolic responses.

Our results showed that post-weaning exposure to excess sucrose led HS/HS rats to progressively increase their body weight achieving an obese status, as assessed by Lee Index calculation, in accordance with previous studies from us [12] and others [28]. Intriguingly, such weight gain occurred in spite of the fact HS/HS rats had a total energy intake significantly lower than CT/CT ones. Sucrose-derived monosaccharides, glucose and fructose, have been shown to induce malonyl-CoA expression and consequent suppression of signalling pathways activated by orexigenic neuropeptides on hypothalamic nuclei responsible for appetite control [29]. There is additional evidence that high-sucrose but not high-fat induces oxytocin-mediated mechanisms which supress certain types of feeding reward, as a kind of protection against overeating carbohydrates [30]. Despite huge literature supporting hyperphagia induced by sucrose [31-34], it has been shown that over time this hyperphagia declines and total energy intake returns to a level that resembles control [35, 36]. Thus, though we did not aim to assess feeding behaviour and reward in our current study, this set of data certainly laid the groundwork for future studies on the effect of long-term exposure to excess sucrose on appetite control.

Besides obese, HS/HS rats were dyslipidemic, glucose intolerant and insulin resistant, gathering enough risk factors to be diagnosed as suffering from MetS [37]. These alterations are primarily ascribed to long-term consumption of sucrose-derived fructose. Fructose undergoes high uptake rate in an insulin-independent manner by the liver, where it is readily metabolized into TAG, not passing by the multiple control points that regulate the conversion of glucose to fat through DNL [38]. Nevertheless, glucose also plays an important role, since upregulation of hepatic DNL has been shown to be exacerbated upon glucose and fructose co-ingestion, like as in excess sucrose feeding [39]. Long-term DNL upregulation intensifies ectopic lipid accumulation that locally impairs insulin signalling [11]. Elevated serum levels

of TAG and TC found in HS/HS rats are also directly associated to the impairment of hepatic insulin signalling, which closely regulates the assembly and secretion of VLDL particles [40]. The assumption of hepatic insulin resistance in HS/HS rats was further supported by the calculation of TyG Index, a surrogate method to assess insulin sensitivity [25].

Once obesity and MetS were characterized in HS/HS rats, as compared to CT/CT ones, we next tested our hypothesis. HS/HS group was split up in three different groups (HS/HS, HS/CT and HS/HP, as made explicit in Methods Section) and followed up for an additional 12-week nutritional intervention period. HPD-fed rats were the only to consistently lose weight, achieving body weight and Lee Index values slightly below those from CT/CT rats. In accordance, HS/HP rats had the lowest energy intake among all groups, which was assessed in the last week of nutritional intervention. Previous studies in rodents have confirmed the capacity of diets containing up to 60% of energy as protein to reduce energy intake and body weight [17, 21, 41]. In both rats [19] and mice [17], high- protein effects on satiety have been ascribed to the action of gastrointestinal peptides, such as cholecystokinin and glucagon-like peptide 1, on the nucleus of solitary tract, while the possible role of HPD low palatability has been ruled out by different studies [17, 19, 42, 43].

Noteworthy, our HPD-fed rats did not regain body weight, as did in other studies with identical nutritional intervention period [17, 20, 44, 45]. This finding may be related to the fact that our HPD contained nearly 35% of energy as protein, whereas those used in the aforementioned studies had a minimum of 48%. To our knowledge, no study has compared long-term effects of diets containing different high-protein levels on satiety and body weight management. Nevertheless, we speculate that long-term very high intake of dietary proteins would activate feedback mechanisms to restore carbohydrate-driven rewarding feeding behaviour, in an opposite way to that proposed for high-sucrose intake [30]. On the other

hand, HS/CT rats did neither reduce body weight nor energy intake, but rather kept an upward trend of weight gain in parallel with HS/HS ones. Although contradictory, it has been shown that sometimes high-fat diet-induced body weight gain is defended completely when dietary palatability is reduced, for instance by giving standard chow back, whereas others give rise to additional body weight that is only partially defended [46]. There is poor literature on the mechanisms involved in body weight defence upon withdrawal from long-term high-sucrose consumption, however the involvement of hedonic systems has been proposed as the most likely candidate for such effect [46, 47].

Long-term exposure to HPD, as well as withdrawal from HSD promoted very similar metabolic outcomes upon 12-week nutritional intervention. HS/HP and HS/CT rats showed reduced fasting serum levels of glucose, TAG and TC, which are directly correlated with the improvement of peripheral insulin sensitivity, as inferred from *k*ITT and TyG Index values. Accordingly, lowering sucrose intake also resulted in return of hepatic steatosis and serum ALT activity back to control patterns. These outcomes are mainly ascribed to the counter-modulatory effect of the lower sucrose-derived fructose content in the strandard chow, which is indeed absent in HPD, allowing downregulation of DNL- related genes, reestablishment of hepatic insulin sensitivity and reduced assembly and secretion of VLDL particles [38, 40, 48]. On the other hand, only HPD-fed rats presented significantly lower serum glucose levels at 15-min *ip*GTT peak and fed state as well. This augmented glucose handling capacity is directly associated to the increased skeletal muscle mass found only in HS/HP group, as previously demonstrated elsewhere [16, 48].

Last but not least, long-term HPD intake completely reverted WAT accumulation, whereas it was only partially reduced upon withdrawal from HSD, in agreement with previously reported studies [43, 48]. These results can be explained to some extent by the

reduction in caloric intake on HS/HP rats. However, the subsequent huge regression of all measured fat pads could also arise from an increased thermogenic response to a high-protein intake. The long-term effect of HPDs on total energy expenditure and metabolic rate is still a matter of intense debate [17, 44, 45, 48]. However, there is consistent evidence for the stimulating role of dietary high-protein consumption on the WAT responsiveness to the lipolytic action of sympathetic agonists [49], which is importantly impaired in diet-induced obese rats [50, 51]. Moreover, it has been suggested that vagus nerve afferences carry information relative to the quantity of protein ingested to hypothalamic sites responsible for food intake control, which may alter peripheral sympathetic nervous system activity [52]. Thus, given the complete rescue of WAT sensitivity to isoproterenol observed in HS/HP, but not HS/CT rats, these data consistently support high-protein intake as an important strategy for adiposity decrease and weight loss in the context of MetS.

Conclusions

Although we have prioritized functional instead of molecular approaches in this study, our data importantly show that 12-week intake of an isocaloric moderately high- protein diet consistently defeated high-sucrose-induced central adiposity and obesity in addition to the attenuation of other important metabolic outcomes, such as improvement of glucolipid homeostasis associated to increased insulin sensitivity and reversal of hepatic steatosis. On the other hand, simple withdrawal from high-sucrose consumption also promoted the abovementioned metabolic outcomes with no impact on body weight. Thus, both nutritional interventions show themselves to be mutually efficient, potentially helping health professionals to make better decisions on how to treat patients with MetS associated or not to overweight/obesity.

Abbreviations

CT	Control diet
DNL	*De novo* lipogenesis
HP	High-protein diet
HS	High-sucrose diet
***ip*GTT**	Intraperitoneal glucose tolerance test
***ip*ITT**	Intraperitoneal insulin tolerance test
MetS	Metabolic syndrome
NAFLD	Non-alcoholic fatty liver disease
NAS	NAFLD activity score
T2DM	Type 2 diabetes mellitus
TAG	Triacylglicerol
TC	Total cholesterol
WAT	White adipose tissue

Declarations

Ethics approval and consent to participate

All procedures were performed in accordance with the rules of Brazilian Council for the Control of Animal Experimentation (CONCEA) and approved by the Ethical Committee on Animal Use and Welfare (CEUA) of the Federal University of Maranhão.

Consent for publication

Not applicable

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

RMLS, NLXR, BASP and AMAP conceived and designed the experiments. RMLS, NLXR, BASP, JRS, MUS, CFFC and LMF contributed to acquisition of data. RMLS, NLXR, BASP and AMAP analysed and interpreted the data. LMF and JAFN contributed to the discussion of the data. RMLS, BASP and AMAP contributed to drafting the article. All authors have revised the manuscript critically for important intellectual content and approved the final version to be published. RMLS, BASP, JAFN and AMAP are responsible for the integrity of the work as a whole.

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Figure legends

Figure 1. Morphometric parameters before nutritional intervention. A, Body weight (g); B, area under curve (AUC) of body weight; C, Lee index ($g^{1/3}$ cm^{-1} × 1000); D, energy intake (Kcal/100g/day); and E, AUC of energy intake were assessed in rats fed a standard chow (CT/CT, n = 7) and high-sucrose diet (HS/HS, n = 18) during twenty weeks from weaning (postnatal day 21). Points and bars represent mean ± SEM, compared by unpaired t-test (one-tailed). [a] represents $p < 0.05$ when compared to CT/CT.

Figure 2. Glycemic/lipid profile and insulin resistance before nutritional intervention. A, blood glucose levels (mg/dL) in fasting state; B, blood glucose levels (mg/dL) during glucose tolerance test (GTT); C, AUC of GTT; D, serum total cholesterol levels (mg/dL); E, serum triglycerides levels (mg/dL); F, TyG index assessed assessed in rats fed a standard chow (CT/CT, n = 7) and high-sucrose diet (HS/HS, n = 18) during twenty weeks from weaning (postnatal day 21). Points and bars represent mean ± SEM, compared by unpaired t-test (one-tailed). [ns] represents $p > 0.05$ and [a] represents $p < 0.05$ when compared to CT/CT.

Figure 3. Morphometric parameters after nutritional intervention. A, Body weight (g); B, area under curve (AUC) of body weight; C, energy intake (Kcal/100g/day); and D, Lee index ($g^{1/3}$ cm^{-1} × 1000) assessed in rats fed a standard chow (CT/CT, n = 7), high-sucrose diet (HS/HS, n = 6), initially fed a HS diet and then replaced by standard chow (HS/CT, n=6) and fed a HS diet then replaced by high-protein diet (HS/HP, n =6). The nutritional intervention started after 20 weeks post weaning and lasted for 12 weeks. Points and bars represent mean ± SEM, compared by one-way ANOVA (Newman Keuls). [a] represents $p <$

0.05 when compared to CT/CT, [b] represents $p < 0.05$ when compared to HS/HS and [c] represents $p < 0.05$ when compared to HS/CT.

Figure 4. ***Ex vivo*** **lipolytic activity.** Glycerol release rate (μg/mg of tissue/h) from periepididymal fat pads under basal or isoproterenol (Iso; 20 μM) stimulation were assessed in rats fed a standard chow (CT/CT, n = 7), high-sucrose diet (HS/HS, n = 6), initially fed a HS diet and then replaced by standard chow (HS/CT, n =6) and fed a HS diet then replaced by high-protein diet (HS/HP, n =6). The nutritional intervention started after 20 weeks post weaning and lasted for 12 weeks. Bars represent mean ± SEM, compared by one-way ANOVA (Newman Keuls). [*] represents $p < 0.05$, [**] represents $p < 0.01$, [***] represents $p < 0.001$.

Figure 5. Glycemic/lipid profile and insulin resistance after nutritional intervention. A-B, blood glucose levels (mg/dL) in fasting and fed states; C, blood glucose levels (mg/dL) during glucose tolerance test (GTT); D, blood glucose disappearance rate (kITT) after insulin tolerance test (ITT); E, serum insulin levels (μLU/mL); F, serum total cholesterol levels (mg/dL); G, serum triglycerides levels (mg/dL); and H, TyG index assessed in rats fed a standard chow (CT/CT, n = 7), high-sucrose diet (HS/HS, n = 6), initially fed a HS diet and then replaced by standard chow (HS/CT, n =6) and fed a HS diet then replaced by high-protein diet (HS/HP, n =6). The nutritional intervention started after 20 weeks post weaning and lasted for 12 weeks. Values represent mean ± SEM, compared by one-way ANOVA (Newman Keuls). [a] represents $p < 0.05$ when compared to CT/CT, [b] represents $p < 0.05$ when compared to HS/HS and [c] represents $p < 0.05$ when compared to HS/CT.

Figure 6. Histological analysis of livers. Sections of liver samples stained with H&E for visualization of hepatocytes morphologies and score for steatosis, ballooning and inflammation assessed in rats fed a standard chow (CT/CT, n = 7), high-sucrose diet (HS/HS, n = 6), initially fed a HS diet and then replaced by standard chow (HS/CT, n =6) and fed a HS diet then replaced by high-protein diet (HS/HP, n =6). The nutritional intervention started after 20 weeks post weaning and lasted for 12 weeks. Values represent mean ± SEM, compared by one-way ANOVA (Newman Keuls). [a] represents $p < 0.05$ when compared to CT/CT, [b] represents $p < 0.05$ when compared to HS/HS and [c] represents $p <$ 0.05 when compared to HS/CT.

Tables legends

Table 1. Tissues morphometry after nutritional intervention. Relative weight (g/100 g of body weight) of retroperitoneal, periepididymal and mesenteric fat pads, interscapular brown adipose tissue, liver and posterior skeletal muscles (gastrocnemius and soleus) assessed in rats fed a standard chow (CT/CT, n = 7), high-sucrose diet (HS/HS, n = 6), initially fed a HS diet and then replaced by standard chow (HS/CT, n =6) and fed a HS diet then replaced by high-protein diet (HS/HP, n =6). The nutritional intervention started after 20 weeks post weaning and lasted for 12 weeks. Values represent mean ± SEM, compared by one-way ANOVA (Newman Keuls). [a] represents $p < 0.05$ when compared to CT/CT, [b] represents $p < 0.05$ when compared to HS/HS and [c] represents $p < 0.05$ when compared to HS/CT.

Table 2. Biochemical profile of hepatic function after nutritional intervention. Serum levels of aminotransferases enzymes (AST and ALT, U/L), γ-glutamyl transferase (γ-GT, U/L), alkaline phosphatase (U/L), albumin (g/dL), total protein (g/dL) and urea (mg/dL) assessed in rats fed a standard chow (CT/CT, n = 7), high-sucrose diet (HS/HS, n = 6), initially fed a HS diet and then replaced by standard chow (HS/CT, n =6) and fed a HS diet then replaced by high-protein diet (HS/HP, n =6). The nutritional intervention started after 20 weeks post weaning and lasted for 12 weeks. Values represent mean ± SEM, compared by one-way ANOVA (Newman Keuls). [a] represents $p < 0.05$ when compared to CT/CT, [b] represents $p < 0.05$ when compared to HS/HS and [c] represents $p < 0.05$ when compared to HS/CT.

Figures

Figure 1. Morphometric parameters before nutritional intervention.

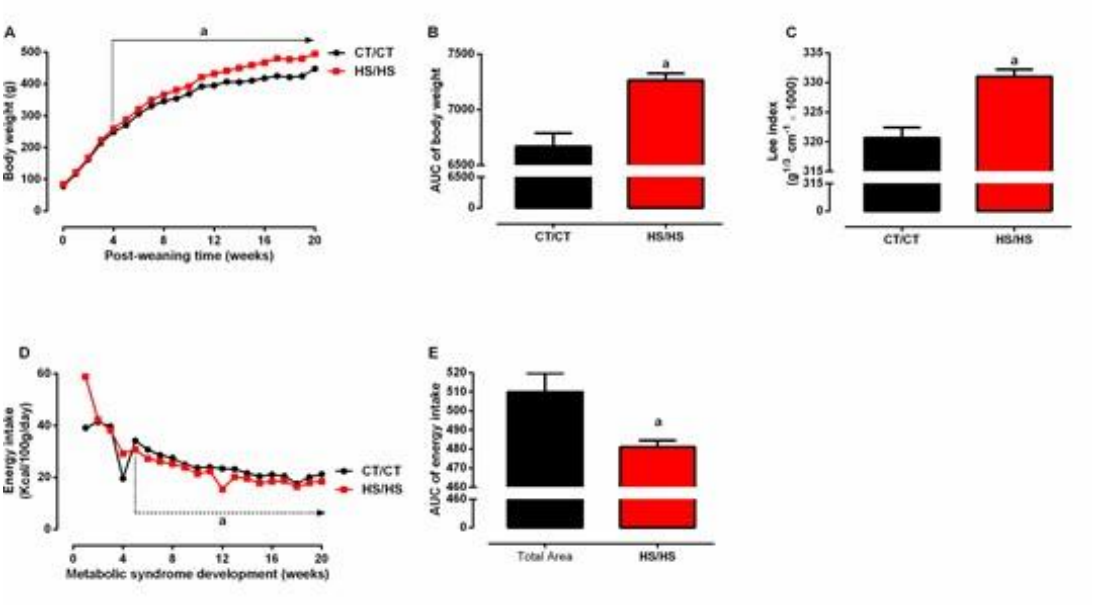


Figure 2. Glycemic/lipid profile and insulin resistance before nutritional intervention.

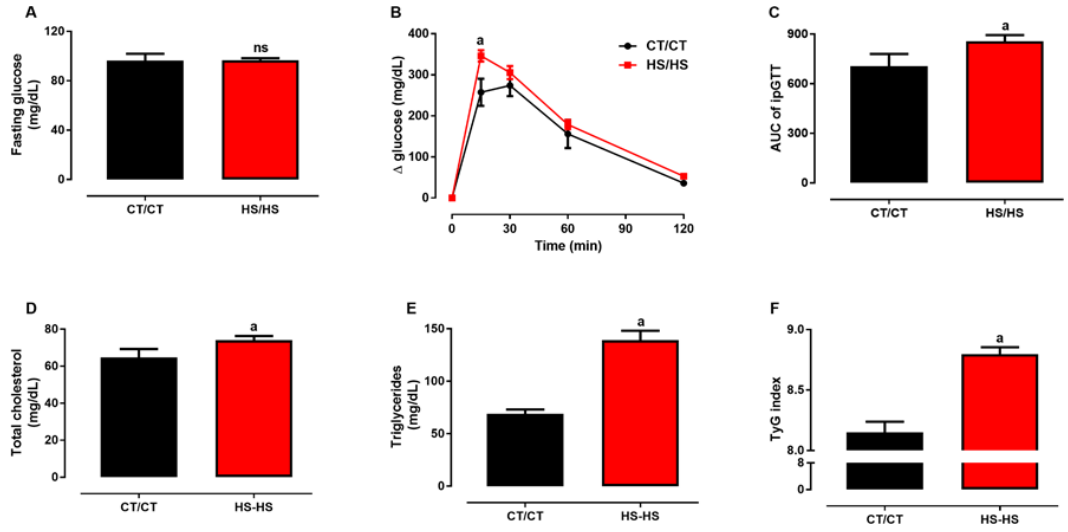


Figure 3. Morphometric parameters after nutritional intervention.

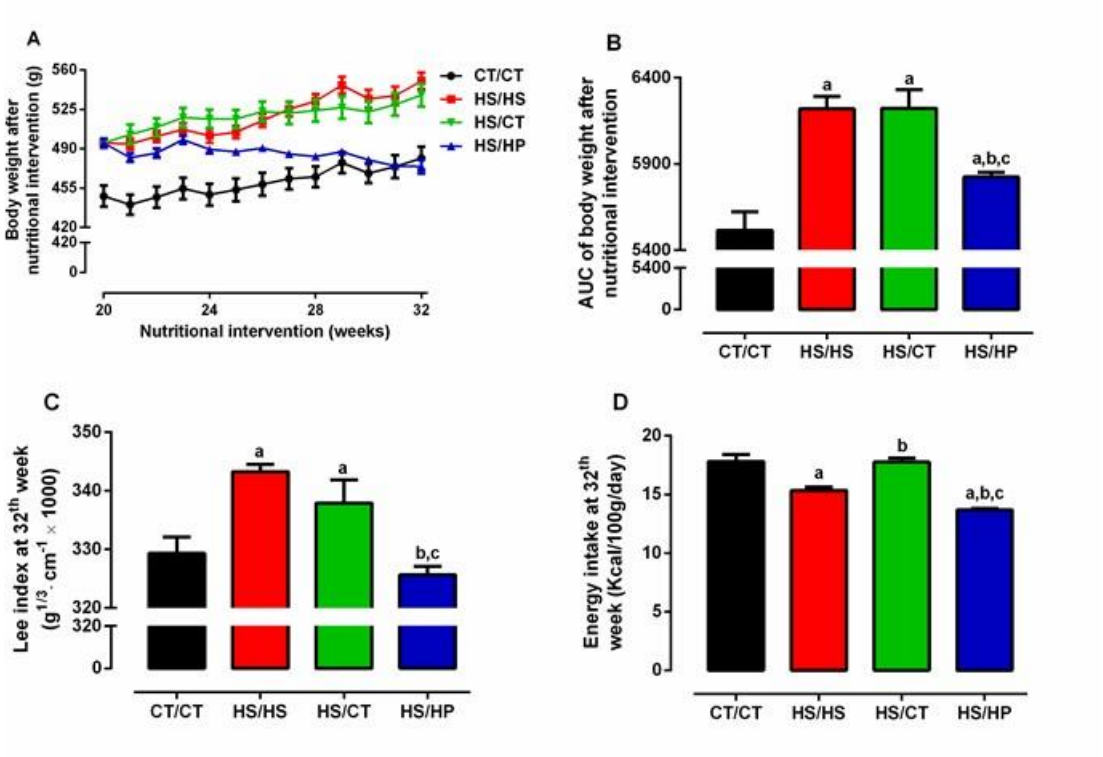


Figure 4. ***Ex vivo*** **lipolytic activity.**

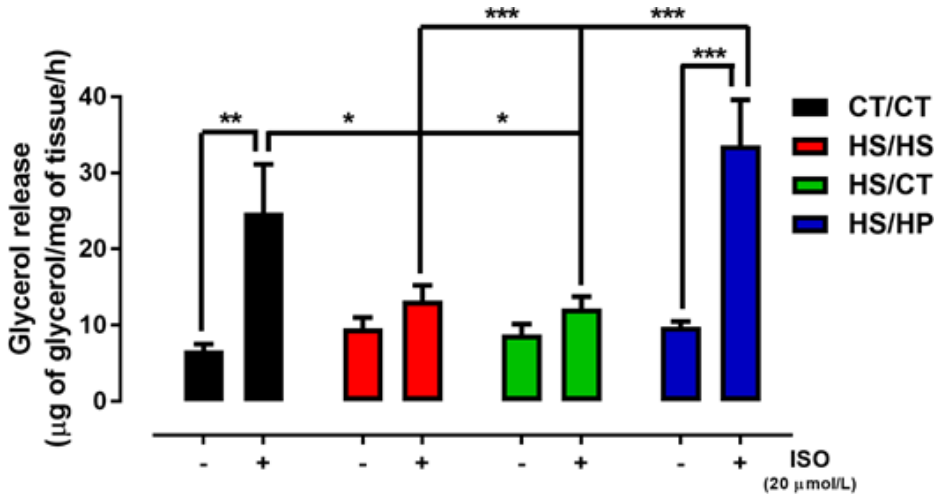


Figure 5. Glycemic/lipid profile and insulin resistance after nutritional intervention.

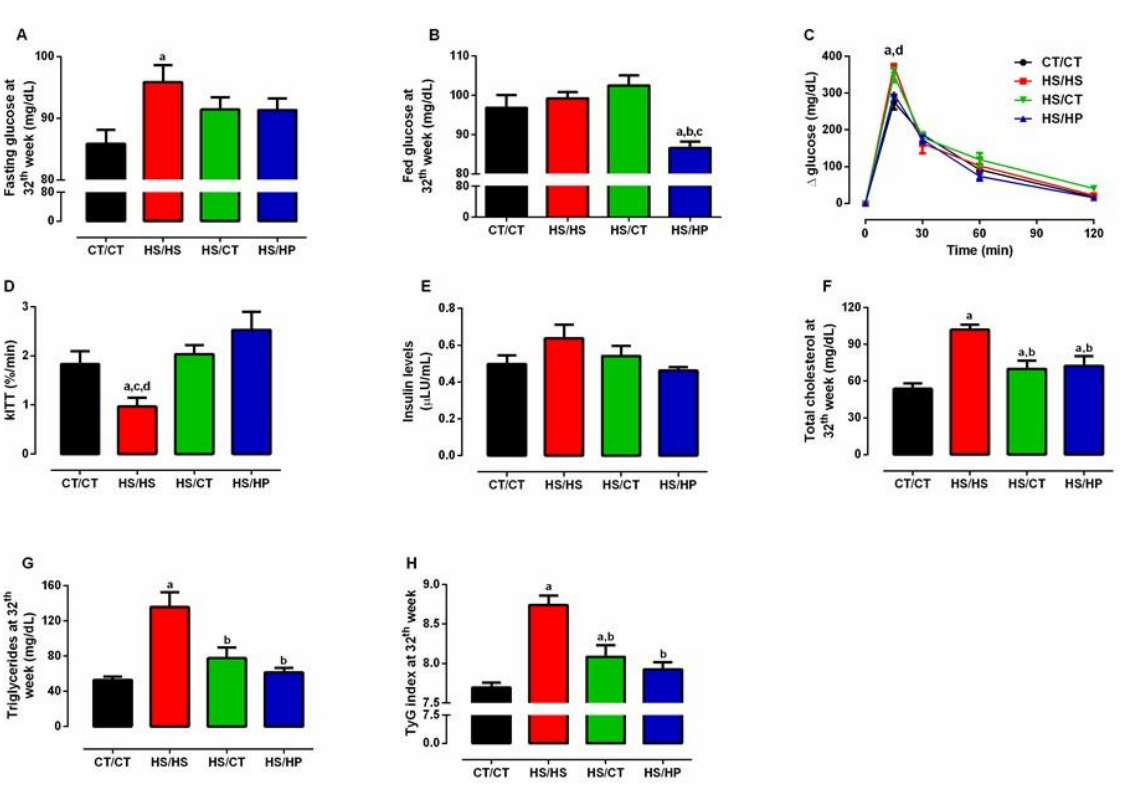
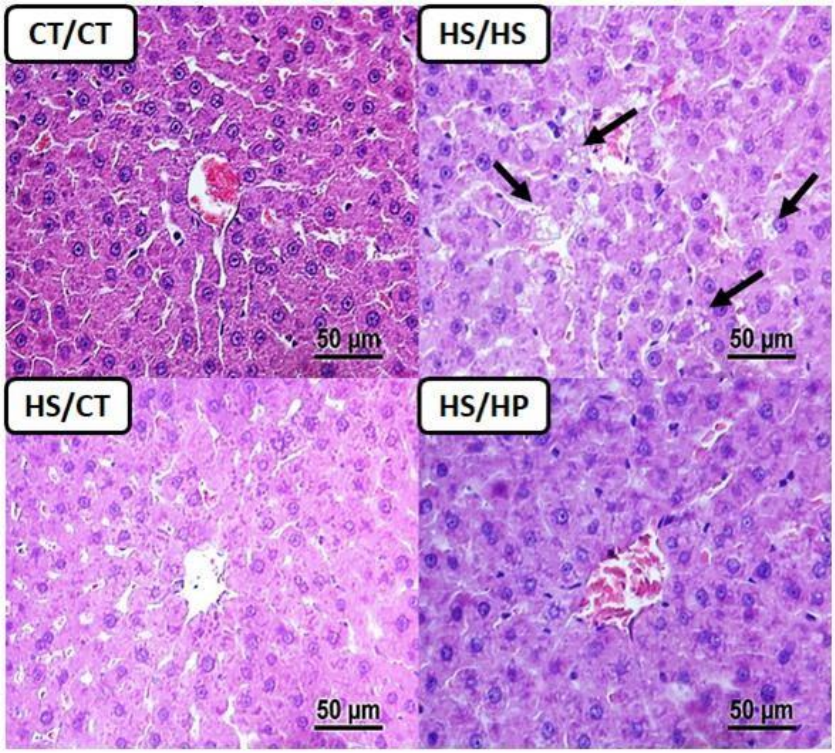


Figure 6. Histological analysis of livers.



	CT/CT	HS/HS	HS/CT	HS/HP
Steatosis	0.0 ± 0.0	0.67 ± 0.2 [a,c,d]	0.2 ±0.2	0.0 ± 0.0
Ballooning	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Inflammation	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Total	0.0 ± 0.0	0.67 ± 0.2 [a,c,d]	0.2 ±0.2	0.0 ± 0.0

Tables

Table 1. Tissues morphometry after nutritional intervention.

Tissues (g/100g BW)	CT/CT	HS/HS	HS/CT	HS/HP
Retroperitoneal fat	1.46 ± 0.13	3.09± 0.1 [a]	2.07 ± 0.19 [a,b]	1.19 ± 0.09 [b,c]
Periepididymal fat	1.28 ± 0.06	3.23 ± 0.25 [a]	2.19± 0.17 [a,b]	1.58 ± 0.12 [b,c]
Mesenteric fat	1.48 ± 0.16	2.88 ± 0.32 [a]	2.08 ± 0.19 [a,b]	1.29 ± 0.12 [b,c]
Brown adipose tissue	0.12 ± 0.01	0.21 ± 0.01 [a]	0.16 ± 0.01 [a,b]	0.1 ± 0.01 [b,c]
Liver	2.50 ± 0.06	2.55 ± 0.03	2.37 ± 0.06	2.60 ± 0.10
Skeletal muscles	1.17 ± 0.03	1.07 ± 0.02	1.09 ± 0.04	1.25 ± 0.03 [b,c]

Table 2. Biochemical profile of hepatic function after nutritional intervention.

Biochemical profile of hepatic function	CT/CT	HS/HS	HS/CT	HS/HP
AST (U/L)	27.34 ± 3.58	35.99 ± 3.66	29.04 ± 2.24	30.95 ± 4.59
ALT (U/L)	18.03 ± 0.81	30.94 ± 4.88 [a,c,d]	16.83 ± 1.41	17.69 ± 1.19
γ-GT (U/L)	21.52 ± 0.52	19.62 ± 1.04	21.75 ± 0.3	22.96 ± 0.28
Alkaline phosphatase (U/L)	26.93 ± 1.77	31.48 ± 3.42	34.59 ± 2.76	32.84 ± 2.47
Albumin (g/dL)	2.43 ± 0.06	2.56 ± 0.09	2.46 ± 0.08	2.35 ± 0.09
Total protein (g/dL)	2.54 ± 0.05	2.67 ± 0.05	2.59 ± 0.02	2.68 ± 0.04
Urea (mg/dL)	48.34 ± 2.58	44.83 ± 3.45	51.42 ± 0.85	51.41 ± 2.21

5 CONSIDERAÇÕES FINAIS

A obesidade é uma doença crônica, multifatorial e de difícil controle. Várias pesquisas, em todo o mundo, têm sido desenvolvidas com o intuito de propor medidas eficazes no tratamento e controle da obesidade. No entanto, observa-se grande dificuldade na adesão ao tratamento, principalmente dietético. Mesmo de considerável relevância, ainda é muito discutível e pouco consensual uma composição dietética ideal que proporcione a perda de peso, redução de tecido adiposo nocivo e redução de riscos para o desenvolvimento dos distúrbios metabólicos associados à síndrome metabólica, diabetes mellitus do tipo II e doenças cardiovasculares.

Neste contexto, uma dieta constituída por um teor mais elevado de proteínas surge como uma instigante estratégia dietética, visto que apresenta características importantes para o controle do balanço energético e da perda de massa corporal, além de ter mostrado resultados importantes na redução de dislipidemias e hiperglicemia. No entanto, mesmo com os efeitos positivos apontados, ainda são escassos na literatura científica estudos que apontem de forma mais ampla os benefícios relacionados a esta intervenção dietética em animais com obesidade induzida por consumo de açúcares de adição, principalmente em longo prazo.

Em conjunto, os dados aqui apresentados neste estudo reforçam os efeitos deletérios relacionados ao consumo precoce e sustentado de açúcares de adição, acarretando em obesidade com aumento dos depósitos adiposos brancos viscerais e não-viscerais, hiperglicemia, intolerância à glicose, dislipidemias e resistência à insulina. Além de diminuição da atividade lipolítica do tecido adiposo branco visceral e desenvolvimento de esteatose hepática sem inflamação.

Adicionalmente, demonstramos que a alteração do padrão alimentar obesogênico para um padrão normal não foi capaz de reverter todos os efeitos deletérios induzidos, reforçando a hipótese de que as alterações mediadas pelo consumo de carboidratos são persistentes e talvez até irreversíveis e que intervenções dietéticas realizadas sem alteração de outras variáveis fisiológicas (sedentarismo, por exemplo) não são suficientes para mitigar os danos instalados.

Por outro lado, demonstramos de forma satisfatória o sucesso da dieta hiperproteica em controlar/reverter todas as alterações metabólicas, atuando de forma importante na redução de peso corporal e obesidade, redução de massa adiposa e aumento de massa magra,

aumento da sensibilidade do tecido adiposo branco a processos de lipólise, normalização da glicemia, estado de intolerância e do perfil lipídico sérico e, por fim, redução do acúmulo de gordura ectópica no fígado.

6 CONCLUSÕES

Embora tenhamos priorizado abordagens funcionais em vez de moleculares neste estudo, nossos dados mostram de maneira importante que a ingestão de 12 semanas de uma dieta isocalórica moderadamente rica em proteínas derrotou consistentemente a adiposidade central induzida por muita sacarose e a obesidade, além da atenuação de outros fatores metabólicos importantes desfechos, como melhora da homeostase dos glicolipídios associada ao aumento da sensibilidade à insulina e reversão da esteatose hepática. Por outro lado, a simples retirada do consumo de alto teor de sacarose também promoveu os resultados metabólicos mencionados acima, sem impacto no peso corporal. Assim, ambas as intervenções nutricionais mostram-se mutuamente eficazes, podendo auxiliar os profissionais de saúde a tomarem melhores decisões sobre como tratar pacientes com SM associada ou não ao sobrepeso / obesidade.

Concluímos com sucesso que a dieta hiperproteica apresentou um potencial anti-obesogênico relevante e sugerimos este padrão nutricional como uma alternativa para a reversão de um perfil obesogênico severo induzido pelo consumo de sacarose, perfil este em expansão epidemiológica em nível global. Esperamos também que este estudo sirva como base para o planejamento futuro de terapias nutricionais mais eficientes para o controle destas morbidades endócrinas.

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ANEXOS

ANEXO A. DECISÃO DA CEUA – UNICEUMA SOBRE PROTOCOLO SUBMETIDO.

CEUMA – UNIVERSIDADE
Reitoria
Gerências de Graduação e Pós-Graduação
Comissão de Ética no Uso de Animais – CEUA UNICEUMA

DECISÃO DA CEUA – UNICEUMA SOBRE PROTOCOLO SUBMETIDO

DATA DO RECEBIMENTO: 03/07/2017

Nº DO PROTOCOLO: 177/17

Nº DO PARECER: 24/17

DATA DO PARECER: 11/12/2017

TÍTULO DO PROJETO/AULA: EFEITOS METABÓLICOS E DE COMPOSIÇÃO CORPORAL DA DIETA HIPERPROTEICA EM RATOS WISTAR ADULTOS OBESOS: Análise para considerações da aplicabilidade no homem.

CARACTERÍSTICAS DA AMOSTRA: 32 ratos machos, adultos, com 21 dias de idade pesando aproximadamente 70g.

PESQUISADOR/PROFESSOR RESPONSÁVEL: Rosângela Maria Lopes de Sousa

COLABORADORES: Adrielle Zagmignan, Ângela Tâmara Lemos Souza, Alexsandro Ferreira dos Santos, Antônio Marcus de Andrade Paes, Bruno Araújo Serra Pinto, Jonas Rodrigues Sanches, Lucas Martins França, Bismarck Ascar Sauaia

DECISÃO: (X) APROVADO ( ) PENDENTE ( ) EXCLUÍDO ( ) NÃO APROVADO

A CEUA-UNICEUMA, em sua função de examinar previamente os procedimentos de ensino e pesquisa a serem realizados na Instituição, para determinar sua compatibilidade com a legislação aplicável (Lei. 11794 e Resoluções do Conselho Nacional de Controle de Experimentação Animal – CONCEA). Reuniu-se no dia 11/12/2017, para apreciar a análise do relator da proposta de protocolo nº **177/17**, tendo chegado por votação da maioria dos membros presentes, as seguintes considerações:

Considerações: O projeto tem como objetivo avaliar os efeitos metabólicos e de composição corporal da dieta hiperproteica em ratos wistar adultos obesos. A indução da obesidade nos ratos será por meio de alimentação com uma ração rica em sacarose (HS) e um grupo controle (n=7) que receberá uma ração padrão, ambos os grupos serão tratados e acompanhados por 19 semanas. Na 20ª semana o grupo que recebeu a ração rica em sacarose será dividido randomicamente em 3 novos grupos: um grupo continuará recebendo alimentação rica em sacarose (HS-HS; n=6); um segundo grupo receberá uma alimentação hiperproteica (HS-HP; n=6) e o terceiro grupo receberá uma alimentação padrão (HS-CTR; n=6). Os animais serão acompanhados por mais 32 semanas. Serão coletadas o peso dos animais (2 vezes por semana), e mensalmente serão coletados alíquotas de sangue periférico para avaliação lipídica, glicêmica, hormonal e ao final de 32 semanas serão realizadas histológicas e expressão gênica.

Conclusão: Aprovado

Com base nos dados fornecidos pelo proponente, a Comissão, autoriza o protocolo supracitado, devendo o presente documento ser apresentado a Coordenação do Biotério, para agendamento do início dos procedimentos.

* Cópia do protocolo segue anexa.

São Luís 11/12/2017

Lídio Gonçalves Lima Neto
Coordenador CEUA-UNICEUMA

Prof. Dr. Lídio Gonçalves Lima Neto
Coord. Comissão de Ética no Uso de Animais - CEUA
Universidade Ceuma

ANEXO B. ARTIGO PUBLICADO NO PERIÓDICO A2, MEDICINA I, NUTRITION & METABOLISM.

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Nutrition & Metabolism

RESEARCH Open Access

Long-term high-protein diet intake reverts weight gain and attenuates metabolic dysfunction on high-sucrose-fed adult rats

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Abstract

Background: Consumption of added sugars has been considered a worldwide public health concern by its association with metabolic syndrome and its comorbidities. Meanwhile, current studies have suggested high-protein diets to promote weight loss and improved metabolic outcomes. Thus, this study aimed to investigate the effects of long-term high-protein diet (HPD, 34.3% protein) intake on high-sucrose-fed rats.

Methods: Weaned male Wistar rats were randomized into two groups: rats fed a standard chow (CT/CT, 10% sucrose) or rats fed a high-sucrose diet (HSD, 25% sucrose) for a 20-week observational period. Subsequently, HS/HS animals were randomized into 3 new groups: rats maintained on HSD diet (HS/HS); rats submitted to HSD replacement by standard chow (HS/CT); and those with HSD replaced by HPD (HS/HP). All groups were followed up for 12 weeks during which we investigated the effects of HPD on body weight, energy intake, obesity development, glicemic/lipid profile, glucose tolerance, insulin resistance, tissue weight (adipose tissue, liver and skeletal muscles), lipolytic activity, liver lipoperoxidation and histology, as well as serum markers of hepatic function.

Results: Post-weaning exposure to HSD led to metabolic syndrome phenotype at adulthood, herein characterized by central obesity, glucose intolerance, dyslipidaemia and insulin resistance. Only HPD feeding was able to revert weight gain and adipose tissue accumulation, as well as restore adipose tissue lipolytic response to sympathetic stimulus. On the other hand, either HPD or withdrawal from HSD promoted very similar metabolic outcomes upon 12-week nutritional intervention. HS/HP and HS/CT rats showed reduced fasting serum levels of glucose, triacylglycerol and total cholesterol, which were correlated with the improvement of peripheral insulin sensitivity, as inferred from *k*ITT and TyG Index values. Both nutritional interventions restored liver morphofunctional patterns, but only HPD restored lipid peroxidation.

(Continued on next page)

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(Continued from previous page)
Conclusions: Our data showed that 12-week intake of an isocaloric moderately high-protein diet consistently restored high-sucrose-induced central adiposity and obesity in addition to the attenuation of other important metabolic outcomes, such as improvement of glucolipid homeostasis associated to increased insulin sensitivity and reversal of hepatic steatosis. On the other hand, simple withdrawal from high-sucrose consumption also promoted the abovementioned metabolic outcomes with no impact on body weight.

Keywords: High-protein diet, High-sucrose diet, Withdrawal of sucrose, Metabolic syndrome

Background

Consumption of added sugars has been considered a worldwide public health concern by its implied association with the epidemic of obesity, type 2 diabetes mellitus (T2DM) and metabolic syndrome (MetS) [1]. Although this is still an issue of intense debate [2, 3], evidence from basic [4] and clinical [5] studies consistently support such concern. Additionally, it has been demonstrated the ability of sugar-based western diet to genetically modify the activity of thrifty genes on Pacific people, which were not historically prone to obesity [6]. This scenario becomes still worse in face of the fact that sucrose is one of the few components of our diet for which no upper limits are suggested in the majority of dietary guidelines around the globe [1], although it has been recommended a total sugar intake of 10% at maximum [7].

Sucrose intake dually promotes glucose-elicited insulin release toward its own uptake by insulin-sensitive tissues, as well as insulin-independent fructose uptake by hepatocytes and adipocytes [8]. Inside the latter, fructose is converted into fatty acids through de novo lipogenesis (DNL) that favours triacylglycerol (TAG) synthesis, very-low-density lipoprotein (VLDL) secretion and adipocyte hypertrophy [9, 10]. Sustained DNL activation in both territories intensifies lipid accumulation which also impairs insulin signalling within a vicious cycle towards non-alcoholic fatty liver disease (NAFLD) onset [11] and adipose tissue dysfunction [10]. Experimentally, we have shown that 60-day exposure of weaning rats to a high-sucrose but isocaloric diet (HSD) augmented body mass in association with increased fat accumulation in both subcutaneous and visceral depots. These animals also presented hyperglycaemia, hypertriglyceridemia, glucose intolerance and impaired insulin sensitivity [12].

Dietary manipulation is a primary factor in controlling and preventing obesity and its comorbidities. Much of the approaches have prioritized low-fat and/or low-carbohydrate interventions [13]. On the other hand, current studies have suggested high-protein diets to promote greater weight-loss and metabolic outcomes in both human beings [14, 15] and rodents [16, 17]. The main rationale behind such advantage is that dietary proteins induce the release of anorexigenic and orexigenic hormones, which ultimately modulate neuronal pathways involved in the regulation of appetite and satiety, as well as energy expenditure [17–19]. Contrariwise, some studies have shown high-protein intake effects are only temporary and subjects (human beings and rodents) regain body weight upon mid- to long-term interventions, suggesting a metabolic adaptation to high-protein regimens [20–22]. Such controversial outcomes put high-protein strategy into checkmate, deserving additional studies to support the development of differential dietary interventions to prevent or treat patients with obesity associated or not to MetS.

Therefore, in the present study we took advantage of our previously described model of MetS induced by HSD in rats [12] to assess the hypothesis that a 12-week high-protein nutritional intervention is more effective than mere excess sucrose withdrawal on the management of body weight and MetS-associated comorbidities. Our data provide a body of evidence that only long-term high protein diet (HPD) intake promotes sustained body weight management, as well as reestablishment of white adipose tissue (WAT) function, although excess sucrose withdrawal had equally improved glycemic control and dyslipidemia on those animals.

Methods

Experimental design

Weaned male Wistar-Hannover rats (postnatal day 21) provided by the animal facility house of the Federal University of Maranhão were randomized into two groups: control rats (CT/CT; $n = 7$), fed a standard chow (Nuvital®, Nuvilab, Brazil) composed by 55.4% total carbohydrate (10% sucrose), 21% protein, 5.2% total lipids, totalling 3.52 kcal/g; or high-sucrose diet rats (HS/HS, $n = 18$), fed a high-sucrose chow composed by 65% total carbohydrate (25% sucrose), 12.3% protein, 4.3% total lipids, totalling 3.48 kcal/g, as previously described [12], for a 20-week period. Afterwards, HS/HS animals were randomized into 3 new groups: rats maintained on HSD (HS/HS; $n = 6$); rats submitted to HSD replacement by standard chow (HS/CT; $n = 6$); and those with HSD replaced by HPD (HS/HP; $n = 6$). HPD was composed by 49% total carbohydrate (no sucrose), 34.3% protein, 2.2% total lipids, totalling 3.53 kcal/g. Micronutrient composition

of each diet is described in Additional file 1: Table S1. All groups were followed up for an additional 12-week nutritional intervention, totalling a 32-week follow-up, during which they were maintained under controlled conditions (21 ± 2 °C; 60% humidity and 12 h light/dark cycle) with water and chow ad libitum.

Throughout observational period, body weight and energy intake were assessed twice a week. In parallel, Lee index ((body weight (g)$^{1/3}$ ÷ naso-anal length (cm)) × 1000) was monthly calculated for assessment of obesity development [23]. During the 20th week of observational period, 8-h fasted rats had their tail-tip cut out and venous blood collected for fasting glucose measurement through glucometer and subsequent operation of the intraperitoneal glucose tolerance test. Collected blood samples were further processed for serum separation and measurement of total cholesterol (TC) and triglycerides (TAG) levels prior to 12-week nutritional intervention.

During the last week of nutritional intervention, animals were submitted to intraperitoneal glucose and insulin tolerance tests. By the completion of the 32-week follow-up, 8-h fasted animals were anesthetized (40:10 mg/kg ketamine:xylazine solution) for blood and tissue collection upon laparotomy. White (retroperitoneal, periepididymal and mesenteric) and brown (interscapular) adipose tissue fat pads, liver and posterior skeletal muscles (gastrocnemius and soleus) were weighed for morphometric assessment and expressed as tissue mass (g) per 100 g body weight. Periepididymal adipose tissue samples were further used for ex vivo lipolysis assay. Liver lobes were processed for histological analysis and serum samples used for determination of biochemical and hormonal profile.

All procedures were performed in accordance with the rules of Brazilian Council for the Control of Animal Experimentation (CONCEA) and approved by the Ethical Committee on Animal Use and Welfare (CEUA) of CEUMA University under the protocol number 177/17.

Assessment of glucose-insulin axis function

For intraperitoneal glucose tolerance test (*ip*GTT), animals were submitted to 8-h fasting prior to administration of glucose 2 g/kg. Tail vein blood drops were collected immediately before (time 0) and 15, 30, 60 and 120 min after glucose load for glycemia measurement through glucometer (Accu check Active®, Roche Diagnostic, Germany). Similar procedure was carried out for intraperitoneal insulin tolerance test (*ip*ITT), excepting animals were fed and received 1 UI/kg insulin (Humulin 70/30®, Lilly, USA). Glucose disappearance rate (kITT) was derived from ITT curve and calculated as 0.693/ $t_{1/2}$ [24]. The insulin resistance was inferred from TyG Index calculation [ln (fasting glucose (mg/dL) × fasting triglyceride (mg/dL)) / 2] [25].

Assessment of serum biochemical profile

Serum samples were obtained upon spontaneous coagulation and centrifugation (3500 rpm; 10 min; 4 °C) and used for colorimetric measurement of TAG, TC, aminotransferases enzymes (AST and ALT), γ-glutamyl transferase, alkaline phosphatase, albumin, total protein and urea (Labtest, Brazil) levels according to manufacturer's instructions. Insulin levels were assessed by immunoenzymatic method according to manufacturer's instructions (Sigma-Aldrich, USA).

Ex vivo lipolysis assay

To assess the lipolytic activity on WAT, periepididymal samples (~ 100 mg) were fragmented and incubated with Krebs buffer (120 mM NaCl, 15 mM $NaHCO_3$, 4.8 mM KCl, 1.2 mM $MgSO_4$, 1.2 mM KH_2PO_4, 2.4 mM $CaCl_2$, 1% BSA and 0.1% glucose after pH adjustment to 7.4) for 1 h at 37 °C in the absence or presence of 20 μM isoproterenol (Sigma-Aldrich, USA). At the end of incubation, samples were cooled out to stop the reaction. Glycerol release (μg of glycerol/mg of tissue/h) was determined by colorimetric assay using a triglyceride assay kit according to manufacturer's instructions (Labtest, Brazil) [26].

Assessment of hepatic oxidative damage

To assess hepatic oxidative damage, we measured tissue levels of malondialdehyde (MDA), as previously described [27, 28]. Briefly, liver samples were homogenized in 20 mM phosphate buffer solution containing 140 mM KCl (pH 7.4; 1:10 *w*/*v*) and centrifuged (750 ×g; 10 min; 4 °C) to separate the supernatant. To avoid MDA formation during sample processing, 0.01 vol% butylated hydroxytoluene (BHT, Sigma-Aldrich, USA) and 1 mM EDTA (Sigma-Aldrich, USA) were added to the buffer. Then, 150 μL of supernatant was added to 300 μL of cold 10% trichloroacetic acid (Sigma-Aldrich, USA) and 0.67% thiobarbituric acid (Merck, Germany) in 7.1% sodium sulfate and incubated in a boiling water bath for 25 min. The mixture was cooled and the pink chromogen was isolated by addiction of buthanol (2:1 *v*/v) followed by centrifugation (3000 ×g; 10 min; 4 °C). The resulting pink chromogen TBARS were determined spectrophotometrically at 535 nm. Calibration curve was performed using 1,1,3,3-tetramethoxypropane (Sigma-Aldrich, USA) as standard. TBARS levels were expressed as μM of MDA/mg protein.

Histological analysis

Samples from hepatic tissue were fixed in 10% phosphate-buffered formalin solution and they were

analysed by light microscopy either after hematoxilin-eosin (HE) or Masson's Trichrome staining. NAFLD activity score (NAS) [29] was applied for semi-quantitative analysis of the three definer criteria of non-alcoholic steatohepatitis: steatosis (0–3), ballooning (0–3), and lobular inflammation (0–2).

Statistical analysis

The sample size was calculated by using the free software G* Power 3.1 (Heinrich-Heine University Düsseldorf, Düsseldorf (NRW), Germany) [30] and on the basis of the effects of HSD on body weight gain, as well as plasma parameters from previous studies [12, 31, 32]. In accordance, a sample size of six animals per group was found to provide the appropriate power ($1 - \beta = 0{\cdot}8$) to identify significant differences ($\alpha = 0{\cdot}05$) in the variables analysed. Statistical analysis was conducted using GraphPad Prism 7.0 software (GraphPad Software Inc., USA). Data were expressed as mean ± SEM and submitted to normality test (Kolmogorov-Smirnov) followed by parametrical analysis through unpaired *t* test (one-tailed) or one-way ANOVA (post-test Newman-Keuls) for a significance level of 5% ($p < 0.05$).

Results

Post-weaning exposure to high-sucrose diet leads to metabolic syndrome phenotype at adulthood

Male rats exposed to HSD from weaning up to adulthood (24-weeks-old) evolved over time to a metabolic syndrome phenotype. As showed in Fig. 1a, HS/HS rats presented higher body weight than CT/CT from 4th post-weaning week (260.0 ± 2.8 g vs. 248.6 ± 4.2 g, $p = 0.038$), reaching a 10% higher body weight at 20th post-weaning week (495.0 ± 4.1 g vs. 447.8 ± 9.4 g, $p < 0.0001$). A proportional increase was observed when analysed the area under the body weight curve (Fig. 1b). Assessment of body mass through Lee Index calculation showed HS/HS rats were obese, as compared to CT/CT (331.0 ± 1.2 vs. 320.7 ± 1.7 $g^{1/3} \times cm^{-1}$, $p < 0.0001$, Fig. 1c). Interestingly, HS/HS rats developed an obese phenotype in spite of having a decreased energy intake, which was statistically lower CT/CT rats from the 5th post-weaning week (Fig. 1d, e).

Long-term HSD consumption negatively impacted on glucose and lipid homeostasis. HS/HS rats did not present fasting hyperglycaemia (Fig. 2a), but had impaired glucose tolerance in comparison to CT/CT (Figs. 2b, c), $p < 0.05$. Serum TC levels were slightly augmented in HS/HS (74.1 ± 2.2 mg/dL) when compared to CT/CT (64.7 ± 4.6 mg/dL, Fig. 2d), $p < 0.05$; whereas serum TAG levels were 2-fold higher in HS/HS than CT/CT rats (139.1 ± 9.1 vs. 69.1 ± 4.1 mg/dL, Fig. 2e), $p < 0.0001$. HS/HS rats also presented insulin resistance, since TyG Index values for this group (8.8 ± 0.05) were significantly higher than those obtained for CT/CT (8.1 ± 0.09, Fig. 2f), $p < 0.0001$. Overall, this set of data consistently demonstrate that early introduction of dietary sugars leads to metabolic syndrome at adulthood, herein

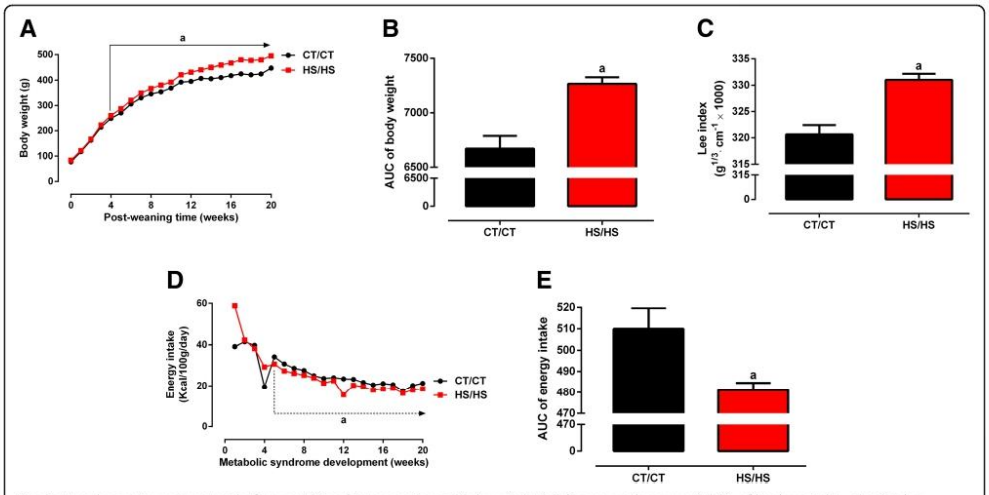


Fig. 1 Morphometric parameters before nutritional intervention. **a** Body weight (g); **b** area under curve (AUC) of body weight; **c** Lee index ($g^{1/3}$ $cm^{-1} \times 1000$); **d** energy intake (Kcal/100 g/day); and **e** AUC of energy intake were assessed in rats fed a standard chow (CT/CT, $n = 7$) and high-sucrose diet (HS/HS, $n = 18$) during 20 weeks from weaning (postnatal day 21). Points and bars represent mean ± SEM, compared by unpaired t-test (one-tailed). [a] represents $p < 0.05$ when compared to CT/CT

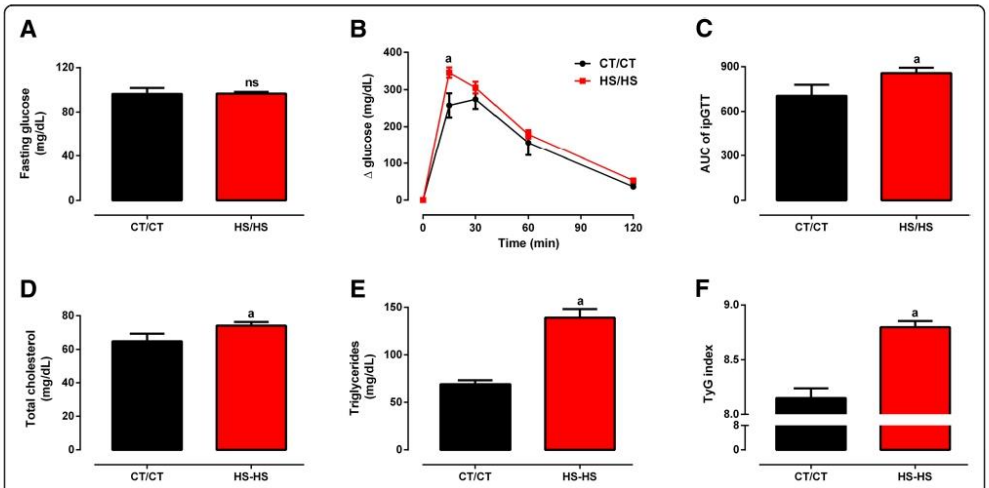


Fig. 2 Glycemic/lipid profile and insulin resistance before nutritional intervention. **a**, blood glucose levels (mg/dL) in fasting state; **b**, blood glucose levels (mg/dL) during glucose tolerance test (GTT); **c**, AUC of GTT; **d**, serum total cholesterol levels (mg/dL); **e**, serum triglycerides levels (mg/dL); **f**, TyG index assessed in rats fed a standard chow (CT/CT, $n = 7$) and high-sucrose diet (HS/HS, $n = 18$) during 20 weeks from weaning (postnatal day 21). Points and bars represent mean ± SEM, compared by unpaired t-test (one-tailed). ^{ns} represents $p > 0.05$ and ^{a} represents $p < 0.05$ when compared to CT/CT

characterized by presence of the following metabolic outcomes: obesity, glucose intolerance, dyslipidaemia and insulin resistance.

High-protein diet reverts the weight gain induced by high-sucrose diet consumption

We next assessed the effects of a 12-week nutritional intervention with HPD on HSD-fed rats. HS/HP rats lose weight from 495.0 ± 4.1 g to 475.0 ± 6.3 g ($p < 0.0001$), no longer differing from CT/CT (481.5 ± 10.4 g; Fig. 3a). On the other hand, HS/HS and HS/CT rats maintained similar upward trend of weight gain (Fig. 3a). The effect of HPD on weight loss is better seen when analysed the area under body weight curve (Fig. 3b), as well as the Lee Index (Fig. 3c) measured at the end of the nutritional intervention period. Such decreases are, at least in part, related to the lower energy intake of HS/HP rats throughout the nutritional intervention, as compared to all the other groups (Fig. 3d, e), $p < 0.001$. On the other hand, HS/CT rats increased their energy intake back to CT/CT levels. Additionally, HPD consistently decreased white and brown adipose tissue depots in HS/HP rats, as compared to HS/HS (Table 1). Replacing HSD by standard chow also resulted in decreased adipose tissue accumulation, even though in a lesser extent than that caused by HPD (Table 1). HPD had no effect on liver weight, but increased skeletal muscle mass in comparison with the other two HSD-fed groups (Table 1).

To further characterize HPD effects on adiposity, we measured basal and isoproterenol-evoked ex vivo lipolysis in periepididymal fat pads from all groups. Data in Fig. 4 show that all groups had similar lipolytic activity at basal conditions, but that long-term exposure to HSD abolished the physiological lipolytic response to sympathetic stimulus. HS/HS group was incapable of rising glycerol release under isoproterenol presence, as compared to the 4-fold increase observed in CT/CT group (6.74 ± 0.82 vs. 24.81 ± 6.32 μg/mg/h, $p < 0.01$). On the other hand, HS/HP group showed a completely restored adipose tissue responsiveness to isoproterenol (9.83 ± 0.65 vs. 33.61 ± 6.01 μg/mg/h, $p < 0.001$), an effect not observed in HS/CT group, which had HSD simply replaced by standard chow (Fig. 4). This set of data emphasizes the deleterious effects of high-sucrose consumption on sympathetic-evoked lipolysis and the counter modulatory effect promoted by high-protein intake instead of the simple excess sucrose withdrawal.

High-protein diet repairs glucose-insulin axis disruption induced by high-sucrose consumption

Exposure to HPD, as well as HSD withdrawal attenuated the disruption of glucose homeostasis verified at 20th week on HSD diet. As shown in Fig. 5a, HS/HS rats presented fasting dysglycaemia in comparison to CT/CT group (95.8 ± 2.8 vs. 85.9 ± 2.2 mg/dL, $p < 0.05$), which was equally attenuated by either HPD introduction or

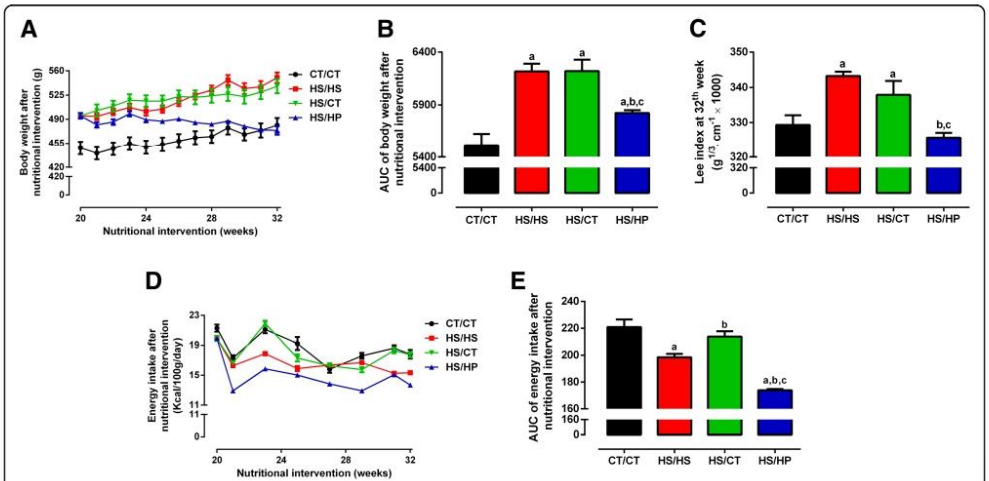


Fig. 3 Morphometric parameters after nutritional intervention. **a**, Body weight (g); **b**, area under curve (AUC) of body weight; **c**, Lee index ($g^{1/3}$ $cm^{-1} \times 1000$); **d**, energy intake (Kcal/100 g/day) and **e**, area under curve (AUC) of energy intake assessed in rats fed a standard chow (CT/CT, $n = 7$), high-sucrose diet (HS/HS, $n = 6$), initially fed a HS diet and then replaced by standard chow (HS/CT, $n = 6$) and fed a HS diet then replaced by high-protein diet (HS/HP, $n = 6$). The nutritional intervention started after 20 weeks post weaning and lasted for 12 weeks. Points and bars represent mean ± SEM, compared by one-way ANOVA (Newman Keuls). [a] represents $p < 0.05$ when compared to CT/CT, [b] represents $p < 0.05$ when compared to HS/HS and [c] represents $p < 0.05$ when compared to HS/CT

HSD withdrawal, reaching levels not different from both HS/HS and CT/CT groups. When glucose levels were measured in fed state, only HS/HP group presented levels significantly lower than CT/CT group (86.6 ± 1.6 vs. 96.8 ± 3.2 mg/dL, $p < 0.05$; Fig. 5b). In accordance to the latter, glucose levels at 15-min peak on *ip*GTT from HS/HP group were brought back to CT/CT-like levels ($p < 0.01$), an outcome not showed by HS/CT group (Fig. 5c). Analysis of glycaemia decay during ITT (kITT) showed HS/HS rats presented impaired peripheral insulin sensitivity, which was restored by both nutritional interventions (Fig. 5d). Surprisingly, fasting serum insulin levels did not differ among groups (Fig. 5e).

Dyslipidaemia induced by long-term exposure to HSD was completely normalized upon 12-week nutritional intervention period. Fasting serum TC levels were reduced in both HS/HP and HS/CT groups (72.4 ± 8.1 and 69.9 ± 6.7 mg/dL, respectively), as compared to HS/HS group (101.9 ± 4.1 mg/dL, $p < 0.01$), which was 2-fold higher than CT/CT (53.7 ± 4.6 mg/dL, $p < 0.001$; Fig. 5f). Similarly, serum TAG levels were 2.5-fold higher in HS/HS group (135.6 ± 17.0 mg/dL, $p < 0.001$) than in CT/CT (53.0 ± 3.8 mg/dL), but were strongly reduced to

Table 1 Tissues morphometry after nutritional intervention

Tissues (g/100 g BW)	CT/CT	HS/HS	HS/CT	HS/HP
Retroperitoneal fat	1.46 ± 0.13	3.09 ± 0.1[a]	2.07 ± 0.19[a,b]	1.19 ± 0.09[b,c]
Periepididymal fat	1.28 ± 0.06	3.23 ± 0.25[a]	2.19 ± 0.17[a,b]	1.58 ± 0.12[b,c]
Mesenteric fat	1.48 ± 0.16	2.88 ± 0.32[a]	2.08 ± 0.19[a,b]	1.29 ± 0.12[b,c]
Brown adipose tissue	0.12 ± 0.01	0.21 ± 0.01[a]	0.16 ± 0.01[a,b]	0.1 ± 0.01[b,c]
Liver	2.50 ± 0.06	2.55 ± 0.03	2.37 ± 0.06	2.60 ± 0.10
Skeletal muscles	1.17 ± 0.03	1.07 ± 0.02	1.09 ± 0.04	1.25 ± 0.03[b,c]

CT/CT rats fed a standard chow ($n = 7$), *HS/HS* rats fed a high-sucrose diet ($n = 6$), *HS/CT* rats initially fed HSD and then replaced by standard chow ($n = 6$), and *HS/HP* rats fed a HS diet and then replaced by high-protein diet ($n = 6$). Values represent mean ± SEM, compared by one-way ANOVA (Newman Keuls)
[a]represents $p < 0.05$ when compared to CT/CT
[b]represents $p < 0.05$ when compared to HS/HS
[c]represents $p < 0.05$ when compared to HS/CT

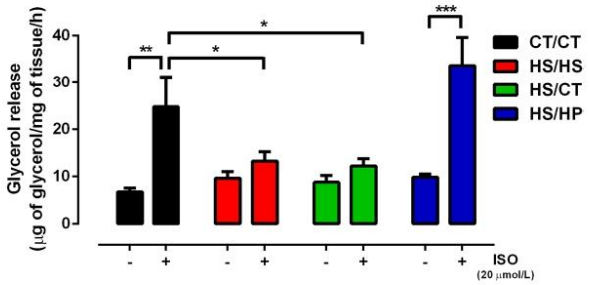


Fig. 4 Ex vivo lipolytic activity after nutritional intervention. Glycerol release rate (µg/mg of tissue/h) from periepididymal fat pads under basal or isoproterenol (Iso; 20 µM) stimulation were assessed in rats fed a standard chow (CT/CT, $n = 7$), high-sucrose diet (HS/HS, $n = 6$), initially fed a HS diet and then replaced by standard chow (HS/CT, $n = 6$) and fed a HS diet then replaced by high-protein diet (HS/HP, $n = 6$). The nutritional intervention started after 20 weeks post weaning and lasted for 12 weeks. Bars represent mean ± SEM, compared by one-way ANOVA (Newman Keuls). * represents $p < 0.05$, ** represents $p < 0.01$, *** represents $p < 0.001$

61.6 ± 4.8 mg/dL and 77.6 ± 12.2 mg/dL in HS/HP and HS/CT, respectively ($p < 0.01$; Fig. 5g). Notwithstanding, TyG Index values (Fig. 5g) indicated the maintenance of hepatic insulin resistance in HS/HS group (8.74 ± 0.06), an outcome attenuated by HSD replacement for standard chow (HS/CT, 8.08 ± 0.14; $p < 0.001$), but completely reverted in HSD-fed rats (HS/HP, 7.94 ± 0.09; $p < 0.0001$), making the latter not different from CT/CT (7.64 ± 0.06). According to these data, excess sucrose withdrawal importantly attenuates the deleterious effects of long-term sucrose exposure on glucose-insulin axis. However, its replacement by HPD promoted the same effects but further improved peripheral insulin sensitivity and glucose tolerance in the first 15 min after glucose load.

High-protein diet regresses hepatic lipid accumulation and peroxidation

Microscopic analysis of H&E-stained liver slices according to the NAFLD activity score (NAS) showed that long-term exposure to HSD led to hepatic steatosis, whereas neither hepatocyte ballooning nor inflammatory infiltration were observed (Fig. 6). Because of ectopic lipid accumulation, HS/HS rats also presented increased serum ALT activity (Table 2) and 2.5 higher levels of MDA in liver (Fig. 7). Both nutritional interventions led to regression of steatosis, as well as ALT activity to CT/CT-like levels. On the other hand, only HS/HP rats presented attenuated oxidative stress, since MDA levels were not statistically different from neither HS/HS nor CT/CT rats (Fig. 7). These data reinforce the direct effects of sucrose consumption on NAFLD onset. Furthermore, they support the persistence of oxidative damage even after steatosis regression, suggesting that HPD intake seems to be more beneficial than mere high-sucrose withdrawal.

Discussion

Body weight management should be reached by simple appliance of energy balance equation, which leads to either weight loss if energy intake is less than energy expenditure, or weight gain if it occurs vice-versa. However, since meals with identical energy content may have unlimited combinations of dietary fats, carbohydrates, proteins or even alcohol; dietary manipulation has proven to be a challenging task for health professionals and billion people worldwide who are overweight or obese [13]. Most of the weight-loss diet literature has focused on the duel between low-fat and low-carbohydrate approaches, whereas high-protein interventions have currently attracted increasing attention [14, 15]. To our knowledge, the effects of long-term high-protein intake versus excess added sugar withdrawal on body weight loss and MetS-associated comorbidities have never been assessed. Consequently, we analysed two different groups of rats initially fed an HSD for 20-weeks, to then have their chow replaced by an isocaloric HPD or standard diet for additional 12-weeks to comparatively evaluate their metabolic responses.

Our results showed that post-weaning exposure to excess sucrose led HS/HS rats to progressively increase their body weight achieving an obese status, as assessed by Lee Index calculation, in accordance with previous studies from us [12] and others [33]. Intriguingly, such weight gain occurred in spite of the fact that HS/HS rats had a total energy intake significantly lower than CT/CT ones. Sucrose-derived monosaccharides, glucose and fructose, have been shown to induce malonyl-CoA expression and consequent suppression of signalling

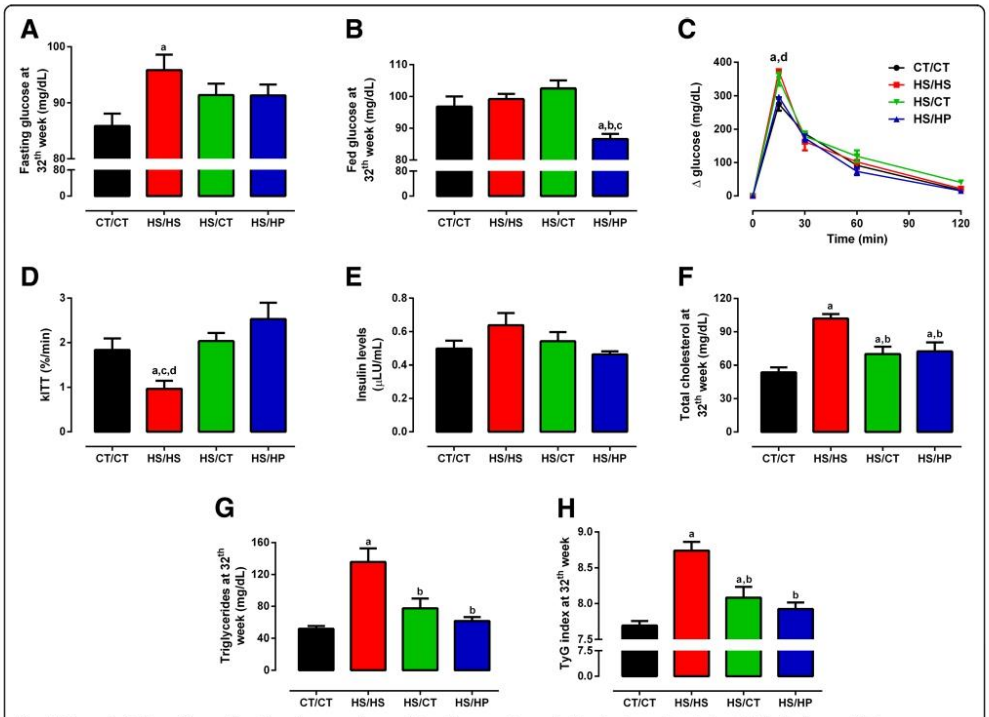
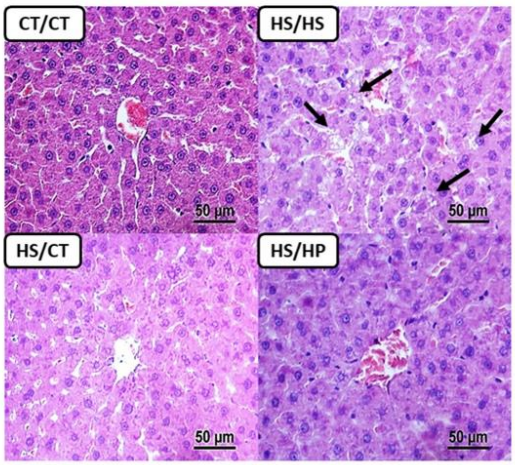


Fig. 5 Glycemic/lipid profile and insulin resistance after nutritional intervention. **a**-**b**, blood glucose levels (mg/dL) in fasting and fed states; **c**, blood glucose levels (mg/dL) during glucose tolerance test (GTT); **d**, blood glucose disappearance rate (kITT) after insulin tolerance test (ITT); **e**, serum insulin levels (μLU/mL); **f**, serum total cholesterol levels (mg/dL); **g**, serum triglycerides levels (mg/dL); and **h**, TyG index assessed in rats fed a standard chow (CT/CT, $n = 7$), high-sucrose diet (HS/HS, $n = 6$), initially fed a HS diet and then replaced by standard chow (HS/CT, $n = 6$) and fed a HS diet then replaced by high-protein diet (HS/HP, $n = 6$). The nutritional intervention started after 20 weeks post weaning and lasted for 12 weeks. Values represent mean ± SEM, compared by one-way ANOVA (Newman Keuls). [a] represents $p < 0.05$ when compared to CT/CT, [b] represents $p < 0.05$ when compared to HS/HS, [c] represents $p < 0.05$ when compared to HS/CT and [d] represents $p < 0.05$ when compared to HS/HP

pathways activated by orexigenic neuropeptides on hypothalamic nuclei responsible for appetite control [34]. There is additional evidence that high-sucrose but not high-fat induces oxytocin-mediated mechanisms which supress certain types of feeding reward, as a kind of protection against overeating carbohydrates [35]. Despite huge literature supporting hyperphagia induced by sucrose [36–39], it has been shown that over time this hyperphagia declines and total energy intake returns to a level that resembles control [40, 41]. Thus, though we did not aim to assess feeding behaviour and reward in our current study, this set of data certainly laid the groundwork for future studies on the effect of long-term exposure to excess sucrose on appetite control.

Besides obese, HS/HS rats were dyslipidemic, glucose intolerant and insulin resistant, gathering enough risk factors to be diagnosed as suffering from MetS [42]. On the other hand, HS/HS rats only showed dysglycaemia around 28 week on HSD diet (data not shown), suggesting the Wistar-Hannover strain to be someway resistant to diet-induced hyperglycaemia. Even after 32-weeks of HSD consumption, HS/HS rats showed only mild steatosis, reinforcing the possible resistance of Wistar-Hannover rats to some of the HSD-induced metabolic outcomes. To our knowledge, this specific rat strain has not yet been assessed for its obesity proneness, although regular Wistar rats have shown greater resistance to diet-induced obesity than other rat strains, such as Wistar Kyoto and Sprague-Dawley [43–45].

Despite the abovementioned issues, the alterations herein described are primarily attributed to long-term consumption of sucrose-derived fructose. Fructose undergoes high uptake rate in an insulin-independent manner by the liver, where it is readily metabolized into



	CT/CT	HS/HS	HS/CT	HS/HP
Steatosis	0.0 ± 0.0	0.67 ± 0.2 [a,c,d]	0.2 ±0.2	0.0 ± 0.0
Ballooning	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Inflammation	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
Total	0.0 ± 0.0	0.67 ± 0.2 [a,c,d]	0.2 ±0.2	0.0 ± 0.0

Fig. 6 Histological analysis of livers after nutritional intervention. Sections of liver samples stained with H&E for visualization of hepatocytes morphologies and score for steatosis, ballooning and inflammation assessed in rats fed a standard chow (CT/CT, $n = 7$), high-sucrose diet (HS/HS, $n = 6$), initially fed a HS diet and then replaced by standard chow (HS/CT, $n = 6$) and fed a HS diet then replaced by high-protein diet (HS/HP, $n = 6$). The nutritional intervention started after 20 weeks post weaning and lasted for 12 weeks. Values represent mean ± SEM, compared by one-way ANOVA (Newman Keuls). [a] represents $p < 0.05$ when compared to CT/CT, [c] represents $p < 0.05$ when compared to HS/CT and [d] represents $p < 0.05$ when compared to HS/HP

TAG, not passing by the multiple control points that regulate the conversion of glucose to fat through DNL [46]. Nevertheless, glucose also plays an important role, since upregulation of hepatic DNL has been shown to be exacerbated upon glucose and fructose co-ingestion, like as in excess sucrose feeding [47]. Long-term DNL upregulation intensifies ectopic lipid accumulation that locally impairs insulin signalling [11]. Elevated serum levels of TAG and TC found in HS/HS rats are also directly associated to the impairment of hepatic insulin signalling, which closely regulates the assembly and secretion of VLDL particles [48]. The assumption of

Table 2 Biochemical profile of hepatic function after nutritional intervention

Biochemical profile of hepatic function	CT/CT	HS/HS	HS/CT	HS/HP
AST (U/L)	27.34 ± 3.58	35.99 ± 3.66	29.04 ± 2.24	30.95 ± 4.59
ALT (U/L)	18.03 ± 0.81	30.94 ± 4.88[a,c,d]	16.83 ± 1.41	17.69 ± 1.19
γ-GT (U/L)	21.52 ± 0.52	19.62 ± 1.04	21.75 ± 0.3	22.96 ± 0.28
Alkaline phosphatase (U/L)	26.93 ± 1.77	31.48 ± 3.42	34.59 ± 2.76	32.84 ± 2.47
Albumin (g/dL)	2.43 ± 0.06	2.56 ± 0.09	2.46 ± 0.08	2.35 ± 0.09
Total protein (g/dL)	2.54 ± 0.05	2.67 ± 0.05	2.59 ± 0.02	2.68 ± 0.04
Urea (mg/dL)	48.34 ± 2.58	44.83 ± 3.45	51.42 ± 0.85	51.41 ± 2.21

CT/CT rats fed a standard chow ($n = 7$), *HS/HS* rats fed a high-sucrose diet ($n = 6$), *HS/CT* rats initially fed HSD and then replaced by standard chow ($n = 6$); and *HS/HP* rats fed a HS diet and then replaced by high-protein diet ($n = 6$). Values represent mean ± SEM, compared by one-way ANOVA (Newman Keuls)
[a]represents $p < 0.05$ when compared to CT/CT
[c]represents $p < 0.05$ when compared to HS/CT
[d]represents $p < 0.05$ when compared to HS/HP

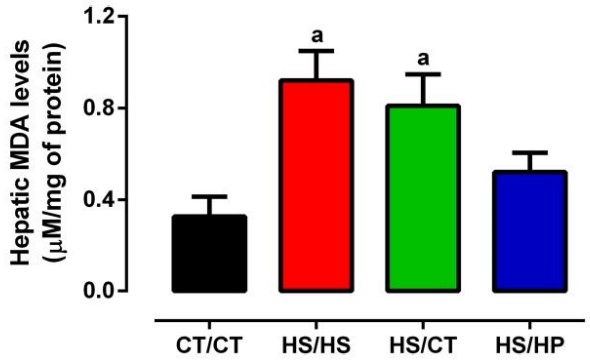


Fig. 7 Hepatic lipoperoxidation after nutritional intervention. Spectrophotometric quantification of malondialdehyde (MDA) in homogenated samples of livers from rats fed a standard chow (CT/CT, $n = 7$), high-sucrose diet (HS/HS, $n = 6$), initially fed a HS diet and then replaced by standard chow (HS/CT, $n = 6$) and fed a HS diet then replaced by high-protein diet (HS/HP, $n = 6$). The nutritional intervention started after 20 weeks post weaning and lasted for 12 weeks. Values represent mean ± SEM, compared by one-way ANOVA (Newman Keuls). [a] represents $p < 0.05$ when compared to CT/CT

hepatic insulin resistance in HS/HS rats was further supported by the calculation of TyG Index, a surrogate method to assess insulin sensitivity [25].

Once obesity and MetS were characterized in HS/HS rats, as compared to CT/CT ones, we next tested our hypothesis. HS/HS group was split up in three different groups (HS/HS, HS/CT and HS/HP, as made explicit in Methods Section) and followed up for an additional 12-week nutritional intervention period. HPD-fed rats were the only to consistently lose weight, achieving body weight and Lee Index values slightly below those from CT/CT rats. In accordance, HS/HP rats had the lowest energy intake among all groups, which was assessed throughout the nutritional intervention. Previous studies in rodents have confirmed the capacity of diets containing up to 60% of energy as protein to reduce energy intake and body weight [17, 21, 49]. In both rats [19] and mice [17], high-protein effects on satiety have been ascribed to the action of gastrointestinal peptides, such as cholecystokinin and glucagon-like peptide 1, on the nucleus of solitary tract, while the possible role of HPD low palatability has been ruled out by different studies [17, 19, 50, 51].

Noteworthy, our HPD-fed rats did not regain body weight, as did in other studies with identical nutritional intervention period [17, 20, 52, 53]. This finding may be related to the fact that our HPD contained nearly 35% of energy as protein, whereas those used in the aforementioned studies had a minimum of 48%. To our knowledge, no study has compared long-term effects of diets containing different high-protein levels on satiety and body weight management. Nevertheless, we speculate that long-term very high intake of dietary proteins would activate feedback mechanisms to restore carbohydrate-driven rewarding feeding behaviour, in an opposite way to that proposed for high-sucrose intake [35]. On the other hand, HS/CT rats did not reduce body weight nor energy intake, but rather kept an upward trend of weight gain in parallel with HS/HS ones. Although contradictory, it has been shown that sometimes high-fat diet-induced body weight gain is defended completely when dietary palatability is reduced, for instance by giving standard chow back, whereas others give rise to additional body weight that is only partially defended [54]. There is poor literature on the mechanisms involved in body weight defence upon withdrawal from long-term high-sucrose consumption, however the involvement of hedonic systems has been proposed as the most likely candidate for such effect [54, 55].

Long-term exposure to HPD, as well as withdrawal from HSD promoted very similar metabolic outcomes upon 12-week nutritional intervention. HS/HP and HS/CT rats showed reduced fasting serum levels of glucose, TAG and TC, which are directly correlated with the improvement of peripheral insulin sensitivity, as inferred from *k*ITT and TyG Index values. Accordingly, lowering sucrose intake also resulted in return of hepatic steatosis and serum ALT activity back to control patterns. These outcomes are mainly ascribed to the counter-modulatory effect of the lower sucrose-derived fructose content in the standard chow, which is indeed absent in HPD, allowing downregulation of DNL-related genes, reestablishment of hepatic insulin sensitivity and reduced assembly and secretion of VLDL particles [46, 48]. Besides, long-term HPD intake per se has been shown also to downregulate

lipogenic genes expression in both liver and white adipose tissue from HSD fed rats [56]. On the other hand, only HPD-fed rats presented significantly lower serum glucose levels at 15-min *ip*GTT peak and fed state as well. This augmented glucose handling capacity might be directly associated to the increased skeletal muscle mass found only in HS/HP group, as previously demonstrated elsewhere [16, 56]. Surprisingly, hepatic lipid peroxidation was attenuated only in HS/HP rats, supporting previous report on the protective action of long-term HPD intake against oxidative stress damage in liver [57].

Last but not least, long-term HPD intake completely reverted WAT accumulation, whereas it was only partially reduced upon withdrawal from HSD, in agreement with previously reported studies [51, 56]. These results can be explained to some extent by the reduction in caloric intake on HS/HP rats. However, the subsequent huge regression of all measured fat pads could also arise from an increased thermogenic response to a high-protein intake. The long-term effect of HPDs on total energy expenditure and metabolic rate is still a matter of intense debate [17, 52, 53, 56]. However, there is consistent evidence for the stimulating role of dietary high-protein consumption on the WAT responsiveness to the lipolytic action of sympathetic agonists [58], which is importantly impaired in diet-induced obese rats [59, 60]. Moreover, it has been suggested that vagus nerve afferences carry information relative to the quantity of protein ingested to hypothalamic sites responsible for food intake control, which may alter peripheral sympathetic nervous system activity [61]. Thus, given the complete rescue of WAT sensitivity to isoproterenol observed in HS/HP, but not HS/CT rats, these data consistently support high-protein intake as an important strategy for adiposity decrease and weight loss in the context of MetS.

Conclusions

Although we have prioritized functional instead of molecular approaches in this study, our data importantly show that 12-week intake of an isocaloric moderately high-protein diet consistently restored high-sucrose-induced central adiposity and obesity in addition to the attenuation of other important metabolic outcomes, such as improvement of glucolipid homeostasis associated to increased insulin sensitivity and reversal of hepatic steatosis. On the other hand, simple withdrawal from high-sucrose consumption also promoted the abovementioned metabolic outcomes with no impact on body weight. Thus, both nutritional interventions show themselves to be mutually efficient, potentially helping health professionals to make better decisions on how to treat patients with MetS associated or not to overweight/obesity.

Additional file

Additional file 1: Table S1. Micronutrient composition of diets. (PDF 79 kb)

Abbreviations

CT: Control diet; DNL: De novo lipogenesis; HP: High-protein diet; HS: High-sucrose diet; *ip*GTT: Intraperitoneal glucose tolerance test; *ip*ITT: Intraperitoneal insulin tolerance test; MetS: Metabolic syndrome; NAFLD: Non-alcoholic fatty liver disease; NAS: NAFLD activity score; T2DM: Type 2 diabetes mellitus; TAG: Triacylglicerol; TC: Total cholesterol; WAT: White adipose tissue

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

RMLS, NLXR, BASP and AMAP conceived and designed the experiments. RMLS, NLXR, BASP, JRS, MUS, CFFC and LMF contributed to acquisition of data. RMLS, NLXR, BASP and AMAP analysed and interpreted the data. LMF and JAFN contributed to the discussion of the data. BASP and AMAP drafted the article. All authors have critically revised the manuscript for intellectual content and approved its final version. RMLS, BASP, JAFN and AMAP are responsible for the integrity of the work as a whole.

Ethics approval

All procedures were performed in accordance with the rules of Brazilian Council for the Control of Animal Experimentation (CONCEA) and approved by the Ethical Committee on Animal Use and Welfare (CEUA) of CEUMA University under the protocol number 177/17.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Publisher's Note

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