UNIVERSIDADE ESTADUAL PAULISTA
"JÚLIO DE MESQUITA FILHO"
FACULDADE DE MEDICINA

Jéssica Leite Garcia

IMPACTO DA INGESTÃO DE CARBOIDRATOS SIMPLES E GORDURA SOBRE PARÂMETROS METABÓLICOS, INFLAMATÓRIOS E PRÓ-OXIDANTES NO PLASMA E NO TECIDO ADIPOSO INDEPENDENTE DE OBESIDADE

Dissertação apresentada ao Programa de Pós-Graduação em Patologia da Faculdade de Medicina, Universidade Estadual Paulista "Júlio de Mesquita Filho", Câmpus de Botucatu, para obtenção do título de Mestre(a) em Patologia.

Orientador(a): Prof(a). Dr(a). Camila Renata Corrêa
Coorientador(a): Prof(a). Dr(a). Igor Otávio Minatel

Botucatu
2018

JÉSSICA LEITE GARCIA

IMPACTO DA INGESTÃO DE CARBOIDRATOS SIMPLES E GORDURA SOBRE PARÂMETROS METABÓLICOS, INFLAMATÓRIOS E PRÓ-OXIDANTES NO PLASMA E NO TECIDO ADIPOSO INDEPENDENTE DE OBESIDADE

Dissertação apresentada ao Programa de Pós-Graduação em Patologia da Faculdade de Medicina, Universidade Estadual Paulista "Júlio de Mesquita Filho", Câmpus de Botucatu, para obtenção do título de Mestre(a) em Patologia.

Orientador (a): Prof(a).Dr(a). Camila Renata Corrêa
Coorientador(a):Prof(a).Dr(a). Igor Otávio Minatel

Botucatu
2018

FICHA CATALOGRÁFICA ELABORADA PELA SEÇÃO TÉC. AQUIS. TRATAMENTO DA INFORM.
DIVISÃO TÉCNICA DE BIBLIOTECA E DOCUMENTAÇÃO – CÂMPUS DE BOTUCATU – UNESP
BIBLIOTECÁRIA RESPONSÁVEL: ROSEMEIRE APARECIDA VICENTE-CRB 8/5651

Garcia, Jéssica Leite.
Impacto da ingestão de carboidratos simples e gordura sobre parâmetros metabólicos, inflamatórios e pró-oxidantes no plasma e no tecido adiposo independente de obesidade / Jéssica Leite Garcia. - Botucatu, 2018

Dissertação (mestrado) - Universidade Estadual Paulista "Júlio de Mesquita Filho", Faculdade de Medicina de Botucatu
Orientador: Camila Renata Corrêa
Coorientador: Igor Otávio Minatel
Capes: 40501000

1. Stress oxidativo. 2. Tecido adiposo. 3. Inflamação. 4. Obesidade. 5. Dietas.

Palavras-chave: Dieta; Estresse oxidativo; Inflamação; Tecido adiposo.

Dedicatória

Dedico aos meus pais, Reginaldo e Tânia e à minha irmã Ana Cláudia, os quais são minha base, exemplo, fonte de força e amor, que nunca falharam em me apoiar nessa jornada. Todas as minhas conquistas são, em verdade, nossas.
Não há palavras que possam expressar o quanto fizeram por mim e o quanto os amo.

Dedico ao Guilherme Guida de Oliveira por me mostrar que há muito mais que o esperado além da nossa zona de conforto, que posso realizar todos os meus objetivos e por caminhar ao meu lado.

Aos amigos pelo apoio, compreensão, disposição em ajudar e por tornar a cidade de Botucatu também um lar. Vocês estão em meu coração.

Agradecimentos

Primeiramente agradeço à Profª. Drª. Camila Renata Corrêa pela oportunidade que me concedeu, pela orientação sempre presente, paciente e disposta. Por sempre querer o melhor para os seus alunos e apoia-los. Obrigada por me receber de forma tão acolhedora em seu grupo e por ser um exemplo de ser humano.
Sou imensamente grata.

Agradeço ao co-orientador deste trabalho, Dr. Igor Otávio Minatel.

Ao Profº. Emérito Dr. Antônio Carlos Cicogna, por todos os ensinamentos e por instigar a todos ao seu redor a nunca parar de aprender.

À Profª. Drª. Denise Fecchio.

À Profª. Drª Ana Lúcia dos Anjos Ferreira.

Aos parceiros de pesquisa:

Fabiane Valentini Francisqueti, aluna de doutorado do Programa de Pós-graduação em Patologia, por sua boa vontade em compartilhar seu conhecimento no laboratório, pelo trabalho em equipe e por me guiar quando iniciei essa jornada

Profº Dr. Fernando Moreto

Artur Junio Togneri Ferron, aluno de doutorado do Programa de Pós-gradução em Fisiopatologia em Clínica Médica

E em especial, ao Dr. Klinsmann Carolo dos Santos e a mestranda Ana Paula Costa Rodrigues Ferraz do Programa de Pós-graduação em Patologia, pelos ensinamentos, pela amizade e por tudo o que me transmitiram.

À coordenadora do Programa de Pós-graduação em Patologia Profª. Drª Márcia Guimarães da Silva.

Aos funcionários do Programa de Pós-graduação em Patologia, especialmente à secretária Vânia do Amaral Soler, sempre gentil e prestativa.

A todos os funcionários da Unidade de Pesquisa Experimental.

A Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) pelo suporte concedido.

Epígrafe

“Eu levanto a minha voz, não para que eu possa gritar, mas para que aqueles sem voz possam ser ouvidos...não é possível prosperar quando metade das pessoas ficam para trás”

Malala Yousafzai

Resumo

Introdução: O padrão alimentar atual é conhecido como dieta ocidental composta por grandes quantidades de carboidratos simples e gordura. O consumo desse tipo de dieta é frequentemente associado às complicações metabólicas e comorbidades decorrentes da disfunção do tecido adiposo em condição de obesidade. Os adipócitos hipertrofiados liberam maior quantidade de adipocinas como a leptina e resistina e os macrófagos residentes respondem liberando citocinas pró-inflamatórias como Interleucina-6 (IL-6) e Fator de necrose tumoral alfa (TNF-α) e quimiocinas. O estresse oxidativo também é desencadeado na condição de hipertrofia, ampliando a resposta inflamatória. A literatura relata que determinados nutrientes podem levar à inflamação do tecido adiposo independente da obesidade. **Objetivo:** Avaliar o impacto de uma dieta rica em carboidratos simples e gordura sobre parâmetros metabólicos plasmáticos e parâmetros inflamatórios e pró-oxidantes no tecido adiposo independente de obesidade. **Materiais e métodos:** Esse estudo foi aprovado pela Comissão Ética no Uso de Animais da Faculdade de Medicina de Botucatu (1233/2017). Para a obtenção dos grupos experimentais foi utilizado um cut-off calculado através do índice de adiposidade dos animais obtendo-se dois grupos: Normocalórico (n=7) e Hipercalórico (n=8). O grupo Normocalórico recebeu ração padrão e água de beber e o grupo Hipercalórico recebeu ração rica em carboidratos simples e gordura com 25% de sacarose adicionados a água de beber pelo período experimental de 30 semanas. Foram avaliados o estado nutricional e a composição corporal (ingestão de ração, água e de calorias, eficiência alimentar, peso corporal, ganho de peso, índice de adiposidade e massa muscular) e realizadas análises plasmáticas bioquímicas (glicose, triglicérides, ácido úrico, proteínas totais, ureia, albumina e proteína e creatinina urinárias) e hormonais (insulina, adiponectina e leptina). No tecido adiposo foi avaliado inflamação (IL-6 e TNF-α) e estresse oxidativo (Malondialdeído e Carbonilação). Dados paramétricos estão apresentados em média e desvio padrão e não-paramétricos apresentados em mediana e intervalo interquartil e analisados por Teste *t* de Student e teste U de Mann-Whitney, respectivamente,

para $p<0,05$. **Resultados:** Os grupos apresentaram o consumo calórico (kcal/dia) estatisticamente iguais acompanhado de menor ingestão de ração (g) com maior ingestão de água (mL) pelo grupo H e, ainda, menor eficiência alimentar (g/kcal). Não houve diferença no peso inicial (g) dos grupos, já o peso final (g) e o ganho de peso (g) foram inferiores no grupo H. A gordura total (g) e o índice de adiposidade (%) foram iguais e o peso dos músculos Sóleo e Extensor Digitorum Longus (EDL) (g) foram menores no grupo H. A glicemia, triglicérides, ácido úrico e relação proteína/creatinina apresentaram-se elevados no grupo H, assim como a insulina e adiponectina, no entanto não houve diferença para a leptina e para o índice de resistência à insulina (HOMA-IR). No tecido adiposo, o grupo H apresentou menor a área dos adipócitos e maior número de adipócitos por campo e a expressão do receptor ativado por proliferados do peroxissoma gama (PPAR-γ) superior ao grupo N. As citocinas pró-inflamatórias (IL-6 e TNF-α) estavam aumentados no grupo H enquanto que os parâmetros de estresse oxidativo estavam diminuídos. **Conclusão:** A qualidade dietética independente do aumento de adiposidade é um fator de risco para o desenvolvimento da inflamação no tecido adiposo e complicações bioquímicas que podem resultar em futuras comorbidades.

Palavras-chave: dieta, estresse oxidativo, inflamação, tecido adiposo.

Abstract

Introduction: The current eating pattern is known as the Western diet made up of large amounts of simple carbohydrates and fat. The intake of this type of diet is often associated with metabolic complications and comorbidities due to the dysfunction of adipose tissue in obesity. Hypertrophied adipocytes release more adipokines such as leptin and resistin and resident macrophages respond by releasing proinflammatory cytokines such as Interleukin-6 (IL-6) and Tumor Necrosis Factor alpha (TNF-α) and chemokines. Oxidative stress is also triggered in the hypertrophic condition and contributes to increase the inflammatory response. The literature reports that certain nutrients can lead to adipose tissue inflammation independent of obesity. **Aim:** To evaluate the impact of simple carbohydrates and fat intake in metabolic, inflammatory and pro-oxidant parameters in plasma and adipose tissue independent of obesity. **Materials and methods:** This study was approved by the Animal Ethical Committee of Botucatu Medical School (1233/2017). To obtain the experimental groups, a cut-off based on the adiposity index of the animals was used, obtaining two groups: Normocaloric (n=7) and Hypercaloric (n=8). The Normocaloric group received standard chow and drinking water and the Hypercaloric group received chow rich in simple carbohydrates and fat and 25% of sucrose added to drinking water for the experimental period of 30 weeks. The nutritional status and body composition was evaluated (chow fed, water intake, caloric intake, dietary efficiency, body weight, weight gain, adiposity index and muscle mass) and performed biochemical plasma analyzes (glucose, triglycerides, uric acid, totals, urea, albumin and urinary protein and creatinine) and hormones (insulin, adiponectin and leptin). In the adipose tissue, inflammation (IL-6 and TNF- α) and oxidative stress (Malondialdehyde and Carbonylation) were evaluated. Parametric data are presented in mean and standard deviation and non-parametric data are presented in median and interquartile range and analyzed by Student's t test and Mann-Whitney U test, respectively, for $p < 0.05$. **Results:** The groups presented caloric intake (kcal / day) statistically equal. The H group presented lower feed intake (g), higher water intake (mL) and lower feed efficiency (g / kcal). There was no difference in the initial weight (g) of the groups, since the final weight (g) and

weight gain (g) were lower in group H. The total fat (g) and the adiposity index (%) were the equals and the weight of the soleus and digitorum longus (EDL) muscles (g) were lower in group H. Blood glucose, triglycerides, uric acid and protein/creatinine ratio were elevated in group H, as were insulin and adiponectin, but there was no difference for leptin and insulin resistance index (HOMA-IR). In adipose tissue, the H group had the lowest adipocyte area and the highest number of adipocytes per field and the proliferation-activated receptor expression of peroxisome gamma (PPAR-γ) higher than the N group. The proinflammatory cytokines (IL-6 and TNF-α) were increased in the H group whereas the oxidative stress parameters were decreased. **Conclusion:** Dietary quality independent of adiposity gain is a risk factor for the development of inflammation in adipose tissue and biochemical complications that may result in future comorbidities.

Key words: diet, oxidative stress, inflammation, adipose tissue.

Lista de Ilustrações

Lista de Tabelas

Lista de Abreviaturas e siglas

AGL	Ácidos graxos livres
C/EBPs	Proteínas ligadoras ao amplificador CCAAT
EO	Estresse oxidativo
ERNs	Espécies reativas de nitrogênio
EROs	Espécies reativas de oxigênio
HDL	Lipoproteína de alta densidade
HSL	Lipase hormônio sensível
IGF-1	Fator de crescimento semelhante à insulina tipo 1
IL-6	Interleucina-6
LDL	Lipoproteína de baixa densidade
LPL	Lipase lipoproteica
LPS	Lipopolissacarídeo
MCP-1	Proteína quimiotática de macrófagos 1
NF-kB	Fator nuclear kappa B
OMS	Organização Mundial da Saúde
PAI-1	Inibidor do ativador de plasminogênio tipo 1
PCR	Proteína C reativa
PPAR-γ	Receptor gama ativado por proliferados de peroxissomas
RBP-4	Proteína ligante de retinol 4
SM	Síndrome Metabólica
SNC	Sistema nervoso central
TAG	Triacilglicerol
TGF-β	Fator de transformação de crescimento beta
TNF-α	Fator de necrose tumoral alfa
TRL4	*Receptor Toll-like* 4

Sumário

CAPÍTULO I – Revisão de literatura

CAPÍTULO II – Artigo científico

CAPÍTULO III – Conclusão

Capítulo I

Revisão de Literatura

1 O Padrão dietético e implicações

A literatura relata que nas últimas décadas ocorreram mudanças drásticas nos hábitos alimentares, impactando na saúde da população (1, 2). Assim, a prevalência do déficit nutricional diminuiu e houve aumento de sobrepeso e obesidade (3). Segundo a Organização Mundial de Saúde (OMS), o número de mortes associado a hábitos alimentares não saudáveis acompanhados de inatividade física, atingem no mundo 1,6 milhões e 4,1 milhões, respectivamente (4). Ainda de acordo com a OMS, em 2016 mais de 1,9 bilhões de adultos apresentavam sobrepeso e mais de 650 milhões eram obesos (5). Até o mesmo ano no Brasil, 18,9% da população foi considerada obesa (6). Esses números elevados se devem em grande parte ao padrão alimentar consumido atualmente.

O padrão alimentar atual é representado pela Dieta Ocidental caracterizada por uma alta ingestão de carnes vermelhas, laticínios ricos em gordura e alimentos industrializados contendo grandes quantidades de sódio, ácidos graxos saturados e carboidratos simples (principalmente sacarose e frutose) (7). Esse tipo de dieta proporciona excesso calórico de macronutrientes (carboidratos, gorduras e proteínas), porém deficiente em micronutrientes (vitaminas e minerais) podendo acarretar diversos problemas a saúde (2).

O consumo exagerado desses alimentos está associado principalmente ao desenvolvimento de obesidade que, consequentemente, associa-se a hiperglicemia, hipertrigliceridemia, resistência à insulina, Diabetes Mellitus tipo II, doenças cardiovasculares, renais e hepáticas (8-10). Trabalhos científicos relatam que em condição de obesidade, o aumento do tecido adiposo está diretamente relacionado ao desenvolvimento dessas alterações uma vez que se torna disfuncionante (7, 11-14). Portanto, estudos apontam que o tecido adiposo como uma conexão entre hábito alimentar e desenvolvimento de complicações metabólicas e comorbidades.

2 O tecido adiposo

O tecido adiposo foi, por muito tempo, tradicionalmente visto como um órgão passivo de estocagem de energia, isolante térmico e proteção mecânica a traumas, porém, atualmente é considerado um órgão de função dinâmica devido a sua atividade metabólica e endócrina que contribui para a homeostase energética do organismo e está envolvida em processos fisiológicos e patológicos (15, 16). É composto por adipócitos e suas células precursoras, os pré-adipócitos, fibroblastos, macrófagos residentes, células do estroma vascular e tecido nervoso (17).

Os adipócitos são as células predominantes nesse tecido e são especializados em armazenar gordura na forma de triacilglicerol (TAG) em seu citoplasma. Desempenham atividades metabólicas de lipogênese e lipólise; sendo lipogênese o processo que resulta em biossíntese e armazenamento de TAG na gotícula intracitoplasmática quando a oferta energética é abundante e lipólise o processo que resulta na quebra de TAG armazenado e liberação de ácidos graxos livres (AGL), quando há déficit energético (18, 19).

Os adipócitos juntamente com as demais células do tecido adiposo desempenham atividade endócrina secretando uma grande variedade de substâncias bioativas, denominadas adipocinas (20), as quais participam de diversos processos fisiológicos. Na Tabela 1 estão representadas algumas adipocinas e suas funções no organismo.

Tabela 1. Adipocinas e suas principais funções ou efeitos no organismo.

Adipocinas	Funções ou efeitos	Referências
Adipsina	Promove secreção de insulina pelas células β	(15, 21)
Adiponectina	Sensibilidade à insulina, anti-inflamatória	(15, 22)
Apelina	Estimula entrada de glicose no tecido adiposo e músculo esquelético, regulação da ingestão alimentar	(23)
HSL	Metabolismo lipídico	(24)
IGF-1	Metabolismo lipídico	(25)
IL-6	Inflamação	(26,27)
Leptina	Regulação do apetite, inflamação	(15)
LPL	Metabolismo lipídico	(28)
MCP-1	Quimiotaxia de macrófagos	(29)
Omentina-1	Sensibilidade à insulina	(30)
PAI-1	Homeostase vascular	(31)
PCR	Inflamação	(32)
RBP-4	Resistência à insulina	(15)
Resistina	Resistência à insulina, inflamação	(33)
TGF-β	Migração e adesão celular, crescimento e diferenciação tecidual	(27, 32)
TNF-α	Inflamação, resistência à insulina	(26)
Visfatina	Resistência à insulina	(26, 31)

Algumas adipocinas produzidas pelo tecido adiposo. HSL: lipase hormônio sensível, IGF-1: fator de crescimento semelhante à insulina tipo 1, IL-6: interleucina-6, LPL: lipase lipoproteica, MCP-1: proteína quimiotática de macrófagos 1, PAI-1: inibidor do ativador de plasminogênio tipo 1, PCR: proteína C reativa, RBP-4: proteína ligante de retinol 4, TGF-β: fator de transformação de crescimento beta, TNF-α: fator de necrose tumoral alfa.

Dentre todas as adipocinas produzidas no tecido adiposo, a adiponectina é a mais abundantemente e age como um fator protetor por apresentar efeito anti-inflamatório e por aumentar a sensibilidade à insulina via supressão da produção de citocinas inflamatórias induzidas pelo TNF-α (14, 15).

Outra adipocina de papel muito significante é a leptina que atua via sistema nervoso central (SNC) reduzindo a ingestão alimentar, proporcionando saciedade e aumentando o gasto energético (26).

De forma geral, as adipocinas possuem ações variadas atuando na homeostase do tecido adiposo, porém em determinadas condições a secreção dessas substâncias pode ser alterada como em condição de disfunção do tecido adiposo (34).

3 Disfunção do tecido adiposo

O tecido adiposo não é capaz de exercer sua função de armazenamento de energia ilimitadamente. Em situação de aporte excessivo de nutrientes, há aumento de tamanho dos adipócitos (hipertrofia) e do número (hiperplasia) até o tecido atingir uma inflexibilidade para se expandir tornando-se disfuncionante (35, 36).

Os adipócitos hipertrofiados liberam maior quantidade de adipocinas como a leptina e resistina (37), enquanto os macrófagos residentes respondem a esse estímulo liberando citocinas pró-inflamatórias como IL-6 e TNF-α e quimiocinas como MCP-1 (38, 39). As quimiocinas agem recrutando monócitos circulantes que chegam ao tecido adiposo e se diferenciam em macrófagos (40). Os macrófagos possuem a capacidade de alterar seu perfil de secreção de citocinas de acordo com o estímulo que recebem, o perfil M1 é conhecido por uma atividade pró-inflamatória (IL-1, IL-2, IL-6, TNF-α) e o perfil M2, anti-inflamatória (IL-10, IL-4, IL-13) (41).

Nessa condição de disfunção do tecido adiposo, há o predomínio de macrófagos M1 que são os grandes responsáveis por ampliar e manter a

inflamação tecidual e pelos níveis elevados de IL-6 e TNF-α (38, 41, 42). Essas citocinas interferem no metabolismo lipídico induzindo a lipólise e levando a liberação de AGL, que são reconhecidos por receptores Toll-like 4 (TRL4), o qual é membro de uma família de receptores presentes em diferentes células e tecidos, que reconhecem lipopolissacarídeos (LPS), e ativam fatores transcricionais intracelulares responsáveis pela resposta imune (43, 44). Evidências mostram que ácidos graxos saturados possuem semelhanças ao LPS das bactérias e também podem ativar particularmente o TLR4 favorecendo a liberação de citocinas pró-inflamatórias (45, 46).

Quanto à leptina, seu efeito inflamatório se dá via indução da produção de citocinas pelos macrófagos (16), enquanto os níveis de adiponectina reduzem, pois, sua expressão é fortemente inibida pelo TNF-α (47) (Figura 1).

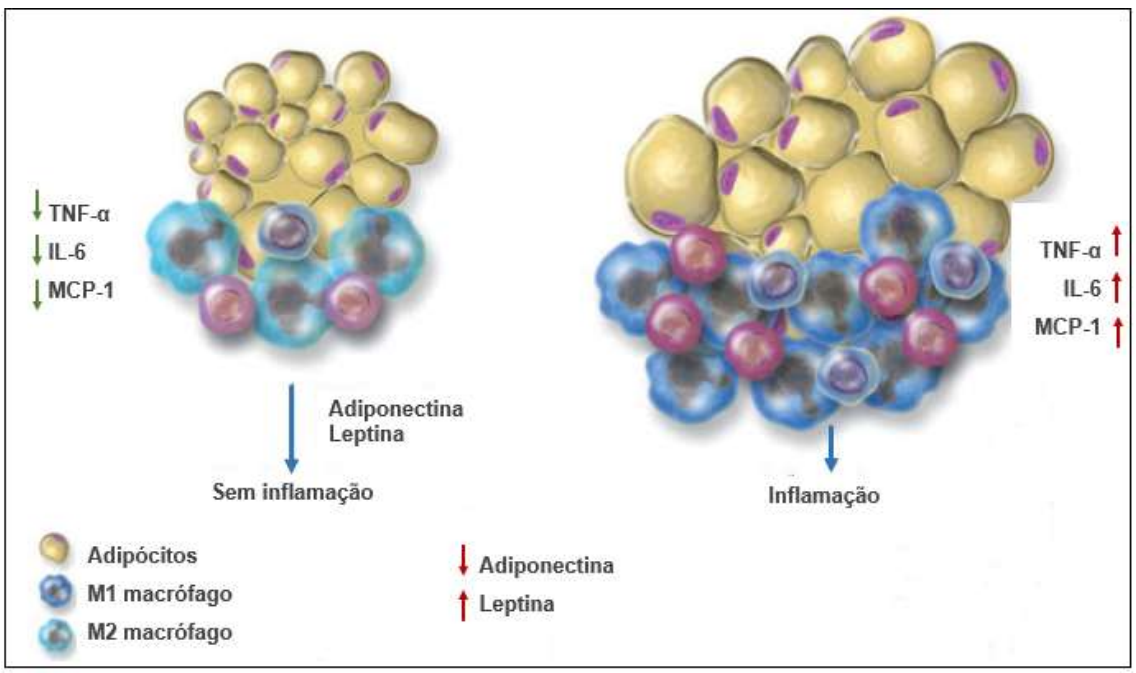

Figura 1. Adaptado de Vilahur, Ben-Aicha & Badimon (2017). Tecido adiposo saudável sem presença de inflamação: presença de macrófagos do perfil M2; tecido adiposo hipertrófico e inflamado: presença de macrófagos do perfil M1. Secreção alterada de adipocinas.

Outro fator que contribui para a inflamação nesse cenário é o estresse oxidativo (EO), classicamente definido como o desequilíbrio entre espécies

oxidantes e antioxidantes (48). Normalmente, as espécies oxidantes, denominadas espécies reativas de oxigênio (EROs) e de nitrogênio (ERNs) atuam em processos fisiológicos, no entanto, quando sua produção é exacerbada e supera a defesa antioxidante, apresentam efeitos nocivos (49, 51).

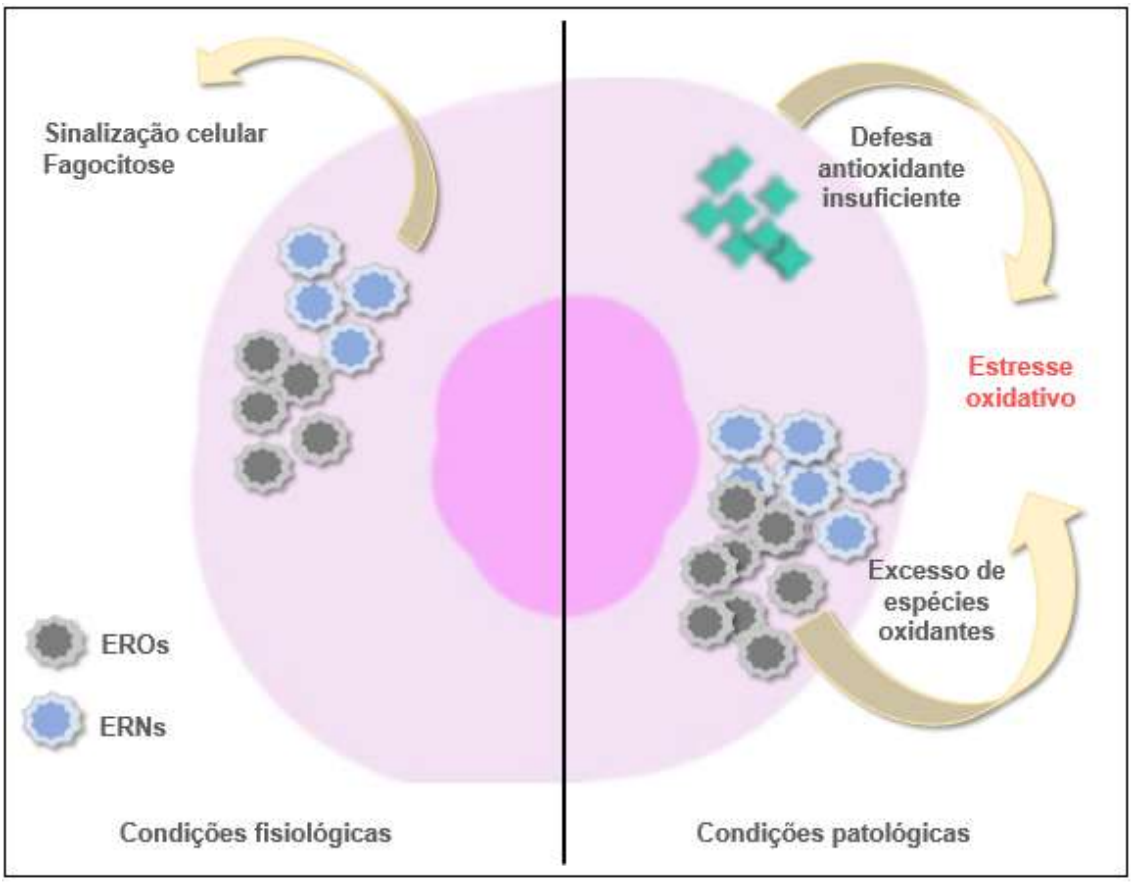

Figura 2. Desequilíbrio entre espécies oxidantes e antioxidantes. Condições fisiológicas atuando na fagocitose e sinalização celular em condições patológicas onde a produção é exacerbada. Espécies reativas de oxigênio (EROs) e de nitrogênio (ERNs).

O EO nessa condição pode ser desencadeado por diferentes mecanismos como, por exemplo, a hipóxia, uma vez que no tecido adiposo expandido pode ocorrer má oxigenação acarretando em áreas de necrose tecidual nas quais os macrófagos atuam na fagocitose dos restos celulares, um processo que libera espécies oxidantes em excesso (52, 53). Além desse mecanismo, o EO pode ser gerado pela intensa atividade do retículo endoplasmático e da mitocôndria

atuando como sinalizador capaz de estimular fatores de transcrição como o fator nuclear kappa B (NF-kB) que induz a produção de citocinas (52). Sendo assim, podemos observar que o EO e a inflamação são processos que se retroalimentam e esse quadro local pode se tornar sistêmico e chegar a outros órgãos através de vascularização do tecido adiposo (10).

Por todos esses mecanismos descritos acima, são decorrentes da hipertrofia dos adipócitos, com isso, é considerada mais prejudicial comparada à hiperplasia, pois o processo hiperplásico evitaria a hipertrofia juntamente com o estresse celular e a disfunção dela decorrente (36). O próprio processo hipertrófico acompanhando de disfunção reflete no processo de adipogênese, diferenciação de pré-adipócitos em adipócito maduro, um processo altamente controlado por fatores de transcrição, como o receptor gama ativado por proliferados de peroxissomas (PPAR-γ) e proteínas ligadoras ao amplificador CCAAT (C/EBPs) (54).

O PPAR-γ é um fator transcricional adipogênico altamente expresso no tecido adiposo que desempenha papel essencial na diferenciação dos adipócitos (18, 36, 53). Nesse estado de hipertrofia dos adipócitos, pode ocorrer uma inibição da adipogênese via regulação negativa do PPAR-γ por citocinas inflamatórias como o TNF-α (55), sendo esse mais um fator contribuidor para a disfunção do tecido.

4 Complicações metabólicas, comorbidades e qualidade dietética

As complicações metabólicas compreendem alterações como resistência à insulina, hiperglicemia e hipertrigliceridemia que podem resultar em comorbidades como diabetes, doenças renais, hepáticas e cardiovasculares (56). Esses fatores normalmente estão relacionados à obesidade decorrente do desequilíbrio alimentar por uma ingestão elevada de carboidratos simples (açúcares) e gordura.

A resistência à insulina é extremamente relevante no desenvolvimento

das comorbidades (57-59). Na obesidade, pode resultar do excesso de gordura que diminui a entrada da insulina aos seus receptores e, ainda, quando há disfunção do tecido adiposo por ação do TNF-α que interfere em diversos processos dependentes de insulina e leva a resistência impedindo a fosforilação dos receptores consequentemente, impedem sua função (26).

A desregulação das adipocinas é um fator importante. A adiponectina destaca-se por estar significantemente reduzida em indivíduos obesos, ocorrendo uma correlação negativa entre seus níveis circulantes e o grau de obesidade, sendo considerada um biomarcador para Síndrome Metabólica (SM) (29). Enquanto que a leptina é diretamente proporcional à quantidade de massa adiposa, no entanto, há resistência à sua ação no SNC (60), além do efeito pró-inflamatório já citado. As comorbidades cardíacas, renais e hepáticas podem ter diversas causas. Na obesidade, dentre os mecanismos propostos destaca-se a disseminação da inflamação e estresse oxidativo originados no tecido adiposo (61) e a lipotoxicidade (62).

A literatura aponta também que o consumo de uma dieta rica em gordura, está relacionado com elevados níveis de ácidos graxos circulantes, os quais ao serem reconhecidos pelos receptores TLR4 podem desencadear a inflamação no tecido adiposo independente de obesidade, pois estimula a produção de quimiocinas e citocinas pró-inflamatórias, atraindo monócitos que se diferenciam em macrófagos aumentando a produção de citocinas, principalmente, IL-6 e TNF-α (43, 44, 63, 64). Esse mecanismo de ativação tem sido considerado um fator de ligação entre inflamação e sobrecarga nutricional.

Outro mecanismo pelo qual a dieta pode levar a inflamação é através da modificação da flora intestinal (65-67). O consumo de uma dieta rica em carboidratos simples e gordura aumenta a população de bactérias gram negativas no intestino provocando aumento da permeabilidade das vilosidades intestinais e maior liberação de LPS pelos capilares endoteliais (68). O LPS liberado é reconhecido pelos receptores TRL4 que dá início a inflamação (65). Trabalhos recentes mostram que essa modificação da flora intestinal pela dieta está relacionada a inflamação no tecido adiposo (65-69). Sendo assim, a

disfunção no tecido adiposo poderia ser associada ao tipo de dieta ingerida.

Em contrapartida a esses efeitos maléficos associados à dieta, a literatura relata hábitos alimentares que apresentem efeitos positivos na saúde. Dietas ricas em vegetais, legumes, frutas, cereais, fibras, ácidos graxos monoinsaturados e peixes proporcionam aumento da lipoproteína de alta densidade (HDL) e diminuição da lipoproteína de baixa densidade (LDL), baixos níveis de glicemia, triglicérides e insulina e demonstram efeito antioxidante e anti-inflamatório (40, 70, 71). Esse tipo de dieta previne as complicações metabólicas e tem se mostrado importante no auxílio do tratamento de indivíduos com síndrome metabólica (72).

Dessa forma, um indivíduo pode ter impactos positivos ou negativos em sua saúde de acordo com o tipo de dieta consumida, tornando importante avaliar esses efeitos uma vez que a literatura pouco relata sobre esse assunto na ausência de obesidade. A qualidade dietética pode ser um fator primário desencadeador de alterações e estar relacionada a impactos na saúde através da disfunção do tecido adiposo mesmo na ausência de obesidade.

5 Objetivo

O objetivo desse trabalho foi avaliar o impacto de uma dieta rica em carboidratos simples e gordura sobre parâmetros metabólicos plasmáticos e parâmetros inflamatórios e pró-oxidantes no tecido adiposo independente de obesidade.

Referências bibliográficas

1. Urlacher SS, Kramer KL. Evidence for energetic tradeoffs between physical activity and childhood growth across the nutritional transition. Sci Rep. 2018;8(1):369.
2. Kearney J. Food consumption trends and drivers. Phil Trans R Soc. 2010;365:2793–807.
3. Popkin BM, Adair LS, Ng SW. Now and Then: The Global Nutrition Transition: The Pandemic of Obesity in Developing Countries. Nutr Rev. 2013;70(1):3–21.
4. World Health Organization. Noncommunicable diseases. Fact sheet N°355. 2017.
5. World Health Organization. WHO | Obesity and overweight. Fact sheet N°311. 2017.
6. Ministério da Saúde. Vigitel Brasil 2016. 2017. Available from: http://portalarquivos.saude.gov.br/images/pdf/2017/abril/17/Vigitel.pdf
7. Caliceti C, Calabria D, Roda A, Cicero A. Fructose Intake, Serum Uric Acid, and Cardiometabolic Disorders: A Critical Review. Nutrients. 2017;9(4):395.
8. Calder PC, Ahluwalia N, Brouns F, Buetler T, Clement K, Cunningham K, et al. Dietary factors and low-grade inflammation in relation to overweight and obesity. Br J Nutr. 2011;106(S3):S5–78.
9. Archundia Herrera MC, Subhan FB, Chan CB. Dietary Patterns and Cardiovascular Disease Risk in People with Type 2 Diabetes. Curr Obes Rep. 2017;1–9.
10. Moreno-Indias I, Tinahones FJ. Impaired adipose tissue expandability and lipogenic capacities as ones of the main causes of metabolic disorders. J Diabetes Res. 2015;2015.
11. Micha R, Peñalvo JL, Cudhea F, Imamura F, Rehm CD, Mozaffarian D. Association Between Dietary Factors and Mortality From Heart Disease, Stroke, and Type 2 Diabetes in the United States. Jama. 2017;317(9):912.
12. Yogarajah T, Bee Y-TG, Noordin R, Yin KB. Increased peroxisome proliferator-activated receptor γ expression levels in visceral adipose tissue, and serum CCL2 and interleukin-6 levels during visceral adipose tissue accumulation. Mol Med Rep. 2015;11(1):515–20.
13. Poulos SP, Dodson M V, Culver MF, Hausman GJ. The increasingly complex regulation of adipocyte differentiation. Exp Biol Med. 2016;241(5):449–56.
14. Vaiopoulos AG, Marinou K, Christodoulides C, Koutsilieris M. The role of adiponectin in human vascular physiology. Int J Cardiol. 2012;155(2):188–93.
15. Choi CHJ, Cohen P. Adipose crosstalk with other cell types in health and disease. Exp Cell Res. 2017;360(1):6–11.
16. Krysiak R, Handzlik-Orlik G, Okopien B. The role of adipokines in

connective tissue diseases. Eur J Nutr. 2012;51(5):513–28.
17. Fonseca-Alaniz MH, Takada J, Alonso-Vale MIC, Lima FB. O tecido adiposo como centro regulador do metabolismo. Arq Bras Endocrinol Metabol. 2006;50(2):216–29.
18. Baumgard LH, Hausman GJ, Sanz Fernandez M V. Insulin: Pancreatic secretion and adipocyte regulation. Domest Anim Endocrinol. 2016;54:76–84.
19. Proença ARG, Sertié RAL, Oliveira AC, Campaña AB, Caminhotto RO, Chimin P, et al. New concepts in white adipose tissue physiology. Brazilian J Med Biol Res. 2014;47(3):192–205.
20. Halberg N, Wernsted I, Scherer P. The adipocyte as an endcrine cell. Endocrinol Metab Clin North Am. 2009;37(3):1–15.
21. Lo JC, Ljubicic S, Leibiger B, Kern M, Leibiger IB, Moede T, et al. Adipsin is an adipokine that improves β cell function in diabetes. Cell. 2014;158(1):41–53.
22. Botulinum RI, Study R. HHS Public Access. 2014;4(1):139–48.
23. Hwangbo C, Wu J, Papangeli I, Adachi T, Sharma B, Park S, et al. Endothelial APLNR regulates tissue fatty acid uptake and is essential for apelin's glucose-lowering effects. Sci Transl Med. 2017;9(407).
24. Xia B, Cai GH, Yang H, Wang SP, Mitchell GA, Wu JW. Adipose Tissue Deficiency of Hormone- Sensitive Lipase Causes Fatty Liver in Mice. 2017;1–17.
25. Berryman DE, List EO. Growth Hormone's effect on adipose tissue: Quality versus quantity. Int J Mol Sci. 2017;18(8):1–28.
26. Andrade-Oliveira V, Câmara NOS, Moraes-Vieira PM. Adipokines as drug targets in diabetes and underlying disturbances. J Diabetes Res. 2015;2015.
27. Skurk T, Alberti-Huber C, Herder C, Hauner H. Relationship between adipocyte size and adipokine expression and secretion. J Clin Endocrinol Metab. 2007;92(3):1023–33.
28. Wang H, Eckel RH. Lipoprotein lipase: from gene to obesity. Am J Physiol Endocrinol Metab. 2009;297(1522–1555):E271–88.
29. Opatrilova R, Caprnda M, Kubatka P, Valentova V, Uramova S, Nosal V, et al. Adipokines in neurovascular diseases. Biomed Pharmacother. 2018;98:424–32.
30. Ouerghi N, Ben Fradj MK, Bezrati I, Feki M, Kaabachi N, Bouassida A. Effect of High-Intensity Interval Training on Plasma Omentin-1 Concentration in Overweight/Obese and Normal-Weight Youth. Obes Facts. 2017;10(4):323–31.
31. Vilahur G, Ben-Aicha S, Badimon L. New insights into the role of adipose tissue in thrombosis. Cardiovasc Res. 2017;113(9):1046–54.
32. Esteve Ràfols M. Tejido adiposo: Heterogeneidad celular y diversidad funcional. Endocrinol y Nutr. 2014;61(2):100–12.
33. Parreño Caparrós E, Illán Gómez F, Gonzálvez Ortega M, Orea Soler I, Pérez Paredes M, Lozano Almela ML, Arjonilla Sampedro E, Alcaráz Tafalla MParreño Caparrós E, Illán Gómez F, Gonzálvez Ortega M, Orea

Soler I, Pérez Paredes M, Lozano Almela ML, Arjonill ATM. Nutrición Hospitalaria. Nutr Hosp. 2017;34(6):1333–7.
34. Klöting N, Blüher M. Adipocyte dysfunction, inflammation and metabolic syndrome. Rev Endocr Metab Disord. 2014;15(4):277–87.
35. Goossens GH, Blaak EE. Adipose Tissue Dysfunction and Impaired Metabolic Health in Human Obesity: A Matter of Oxygen? Front Endocrinol. 2015;6:1–5.
36. Castro JP, Grune T, Speckmann B. The two faces of reactive oxygen species (ROS) in adipocyte function and dysfunction. Biol Chem. 2016;397(8):709–24.
37. Francisqueti FV, Nascimento AF do, Corrêa CR. Obesidade , inflamação e complicações metabólicas. 2015;40(1):81–9.
38. Castoldi A, De Souza CN, Saraiva Câmara NO, Moraes-Vieira PM. The macrophage switch in obesity development. Front Immunol. 2016;6:1–11.
39. Jayarathne S, Koboziev I, Park OH, Oldewage-Theron W, Shen CL, Moustaid-Moussa N. Anti-Inflammatory and Anti-Obesity Properties of Food Bioactive Components: Effects on Adipose Tissue. Prev Nutr Food Sci. 2017;22(4):251–62.
40. Paniagua JA. Nutrition, insulin resistance and dysfunctional adipose tissue determine the different components of metabolic syndrome. World J Diabetes. 2016;7(19):483.
41. Kraakman MJ, Murphy AJ, Jandeleit-Dahm K, Kammoun HL. Macrophage polarization in obesity and type 2 diabetes: Weighing down our understanding of macrophage function? Front Immunol. 2014;5:1–6.
42. Liu Y-C, Zou X-B, Chai Y-F, Yao Y-M. Macrophage Polarization in Inflammatory Diseases. Int J Biol Sci. 2014;10(5):520–9.
43. Necela BM, Su W, Thompson EA. Toll-like receptor 4 mediates cross-talk between peroxisome proliferator-activated receptor γ and nuclear factor-kB in macrophages. Immunology. 2008;125(3):344–58.
44. Garibotto G, Carta A, Picciotto D, Viazzi F, Verzola D. Toll-like receptor-4 signaling mediates inflammation and tissue injury in diabetic nephropathy. J Nephrol. 2017;30(6):719–27.
45. Poulain-Godefroy O, Le Bacquer O, Plancq P, Lecœur C, Pattou F, Frühbeck G, et al. Inflammatory role of toll-like receptors in human and murine adipose tissue. Mediators Inflamm. 2010;2010.
46. Huang S, Rutkowsky JM, Snodgrass RG, Ono-Moore KD, Schneider DA, Newman JW, et al. Saturated fatty acids activate TLR-mediated proinflammatory signaling pathways. J Lipid Res. 2012;53(9):2002–13.
47. Prado WL do, Lofrano MC, Oyama LM, Dâmaso AR. Obesidade e Adipocinas Inflamatórias : Implicações Práticas para a Prescrição de Exercício. 2009;15(5):378–83.
48. Luvizotto R de AM, Nascimento AF, Imaizumi E, Pierine DT, Conde SJ, Correa CR, et al. Lycopene supplementation modulates plasma concentrations and epididymal adipose tissue mRNA of leptin, resistin and IL-6 in diet-induced obese rats. Br J Nutr. 2013;110(10):1803–9.
49. Ner AA, Es VV. Oxidant mechanisms in childhood obesity : the link.

2011;(90):369–84.
50. Noeman SA, Hamooda HE, Baalash AA. Biochemical Study of Oxidative Stress Markers in the Liver, Kidney and Heart of High Fat Diet Induced Obesity in Rats. Diabetol Metab Syndr. 2011;3(1):17.
51. Francisqueti FV, Chiaverini LCT, Santos KC dos, Minatel IO, Ronchi CB, Ferron AJT, et al. The role of oxidative stress on the pathophysiology of metabolic syndrome. Rev Assoc Med Bras. 2017;63(1):85–91.
52. Rudich A, Kanety H, Bashan N. Adipose stress-sensing kinases: linking obesity to malfunction. Trends Endocrinol Metab. 2007;18(8):291–9.
53. Minihane AM, Vinoy S, Russell WR, Baka A, Roche HM, Tuohy KM, et al. Low-grade inflammation, diet composition and health: current research evidence and its translation. Br J Nutr. 2015;114(7):999–1012.
54. Feng S, Reuss L, Wang Y. Potential of natural products in the inhibition of adipogenesis through regulation of PPARγ expression and/or its transcriptional activity. Molecules. 2016;21(10).
55. Weisberg S, McCann D, Desai M, Rosenbaum M, Leibel R, Ferrante Jr A. Obesity is assciated with macrophage accumulation in adipose tissue. J Clin Invest. 2003;112(12):1796–808.
56. Adorni CS, Corrêa CR, Vileigas DF, de Campos DHS, Padovani CR, Minatel IO, et al. The influence of obesity by a diet high in saturated fats and carbohydrates balance in the manifestation of systemic complications and comorbidities. Nutrire. 2017;42(1):16.
57. Basciano H, Federico L, Adeli K. Fructose, insulin resistance, and metabolic dyslipidemia. Nutr Metab. 2005;2(1):5.
58. Hocking S, Samocha-Bonet D, Milner KL, Greenfield JR, Chisholm DJ. Adiposity and insulin resistance in humans: The role of the different tissue and cellular lipid depots. Endocr Rev. 2013;34(4):463–500.
59. Zhang DM, Jiao RQ, Kong LD. High dietary fructose: Direct or indirect dangerous factors disturbing tissue and organ functions. Nutrients. 2017;9(4).
60. Zhang Y, Chua S. Leptin Function and Regulation. Compr Physiol [Internet]. 2017;8:351–69.
61. Farhangi MA, Mesgari-Abbasi M, Hajiluian G, Nameni G, Shahabi P. Adipose Tissue Inflammation and Oxidative Stress: the Ameliorative Effects of Vitamin D. Inflammation. 2017;40(5):1688–97.
62. Butler TJ, Ashford D, Seymour A-M. Western diet increases cardiac ceramide content in healthy and hypertrophied hearts. Nutr Metab Cardiovasc Dis. 2017;1–8.
63. Sears DD, Kim JJ. TLR4 and insulin resistance. Gastroenterol Res Pract. 2010;2010.
64. Shah PK. Innate immune pathway links obesity to insulin resistance. Circ Res. 2007;100(11):1531–3.
65. Hersoug L-G, Møller P, Loft S. Role of microbiota-derived lipopolysaccharide in adipose tissue inflammation, adipocyte size and pyroptosis during obesity. Nutr Res Rev. 2018
66. Collins KH, Paul HA, Hart DA, Reimer RA, Smith IC, Rios JL, et al. A High-

Fat High-Sucrose Diet Rapidly Alters Muscle Integrity, Inflammation and Gut Microbiota in Male Rats. Sci Rep. 2016;6:1–10.
67. del Bas JM, Guirro M, Boqué N, Cereto A, Ras R, Crescenti A, et al. Alterations in gut microbiota associated with a cafeteria diet and the physiological consequences in the host. Int J Obes. 2017.
68. Liu D, Zhang Y, Liu Y, Hou L, Li S, Tian H, et al. Berberine Modulates Gut Microbiota and Reduces Insulin Resistance via the TLR4 Signaling Pathway. Exp Clin Endocrinol Diabetes. 2018;4.
69. Jordan BF, Gourgue F, Cani PD. Adipose Tissue Metabolism and Cancer Progression: Novel Insights from Gut Microbiota? Curr Pathobiol Rep. 2017;5(4):315–22.
70. Kant AK. Dietary patterns: biomarkers and chronic disease risk. Appl Physiol Nutr Metab. 2010;35(2):199–206.
71. Naja F, Nasreddine L, Itani L, Adra N, Sibai AM, Hwalla N. Association between dietary patterns and the risk of metabolic syndrome among Lebanese adults. Eur J Nutr. 2013;52(1):97–105.
72. Romagnolo DF, Selmin OI. Mediterranean Diet and Prevention of Chronic Diseases. Nutr Today. 2017;1.

Capítulo II

Artigo Científico

(Apresentado nas normas para submissão à revista Nutrients)

Dietary Quality Is a Risk Factor for Metabolic Complications

Jéssica Leite Garcia[1], Klinsmann Carolo dos Santos[1], Fabiane Valentini Francisqueti[1], Ana Paula da Costa Rodrigues Ferraz[1], Artur Junio Togneri Ferron[1], Igor Otávio Minatel[2], Camila Renata Corrêa[1,*]

[1] São Paulo State University (Unesp), Medical School, Botucatu 18618-687, Brazil; jessleitegarcia@gmail.com (J.L.G); klinsmanncarolo@gmail.com (K.C.S); fabiane_vf@yahoo.com.br (F.V.F); ferrazapcr@gmail.com (A.P.C.R.F.); artur.ferron@gmail.com (A.F.J.)

[2] São Paulo State University (Unesp), Insitute of Bioscience, Botucatu 18618-689, Brazil; igorminatel@gmail.com (I.O.M)

* Correspondence: ccorrea@fmb.unesp.br (C.R.C.), Tel.: +55-14-3880-1722

Abstract: Evidences indicate that dietary quality is directly related to positive or negative health consequences. The aim of this study was to evaluate if dietary quality is a risk factor for the development of metabolic complications independent of adiposity gain. An adiposity index (IA) cut-off was used and the groups Normocaloric (N) and Hypercarolic (H) were obtained, n=7 and n=8, respectively. The Hypercaloric diet was composed by high-sugar-fat chow and 25% sucrose added to drinking water. The experimental period was 30 weeks. Data are presented as means ± standard deviation or medians (interquatile range), and the differences among the groups were determined by Student's t test and Mann-Whitney U test, respectively. A p value <0.05 was considered as statistically significant. The groups presented caloric intake and adiposity index

statistically equal, however the H group showed higher glycemia, triglycerides, uric acid and protein/creatinine ratio compared to N group. Same were observed to the hormonal parameters insulin and adiponectin, whereas Homeostatic Model Assessment for Insulin (HOMA-IR) presented no statistical difference between the groups. In adipose tissue, proinflammatory citokines IL-6 (interleukin-6) and TNF-α (tumor necrosis factor alpha) levels were increased in H group vs. N group, as well as Peroxisome proliferator-activated receptor gamma (PPAR-γ) expression. Dietary quality, independent of adiposity gain, is a risk factor to the metabolic complications development.

Keywords: diet, simple carbohydrate, adipose tissue, metabolic complications.

1. Introduction

Evidences indicate that dietary quality is direct related to positive or negative health consequences (1-4). Studies have shown that a diet composed by low amounts of animal fat and simple sugar, rich in vegetables, fibers, mono and polyunsatured fatty acids and moderated in complex carbohydrates has benefit effects on body (1, 2, 5). These benefit effects include the increase of high lipoprotein density, triglycerides decrease, low levels of glucose and insulin, besides providing a possible anti-inflammatory and antioxidant effect (2, 6). However, this is not the reality presented in the last decades, where the dietary pattern is mostly composed of processed foods rich in simple carbohydrates (fructose and sucrose), saturated fatty acids, red meat and high-fat dairy (3, 7). Studies have associated this food habit to the development of obesity, which is resulting from adipose tissue expansion due to the excessive accumulation of

fatty acids and/or adipogenesis, a mechanism regulated by several factors, among them, the transcriptional factor PPAR-γ (8). Futhermore, it is known that individuals with adequate body weight but who consume an unbalanced diet rich in industrialized foods composed of simple sugars and fats are subject to the same risks for complications as obese individuals (6), such as cardiovascular disease, type II diabetes and some cancers (6, 9-11). These complications can be originated in the adipose tissue, whose main function is fat storage, but it is also considered an organ with endocrine function secreting a vast amount of substances called adipokines, which includes hormones and cytokines (12). When there is a great energy intake, the adipose tissue expands to a level of inflexibility leading to deregulation in adipokine secretion (13, 14). This hypertrophic state leads to a chronic low-grade inflammation by increasing the release of cytokines such as TNF-α and IL-6. This condition also triggers oxidative stress, mainly by the release of reactive oxygen species (ROS) by inflammatory cells (15). At this point, when inflammation and oxidative stress become established, adipose tissue becomes dysfunctional and it is unable to maintain the fat storage function, contributing to the development of metabolic complications and chronic diseases (13, 14, 16, 17). Therefore, since the literature reports the existence of diets considered to be pro-inflammatory (18, 19) and due the scarcity of studies that approach metabolic and nutritional aspects related to adipose tissue not associated with the development of obesity, the aim of this study was to evaluate if dietary quality is a risk factor for the development of metabolic complications independent of adiposity gain.

2. Materials and Methods

2.1. Animals and experimental protocol

This study was approved by the Animal Ethics Committee of Botucatu Medical School (1233/2017) and the experiments were performed in accordance with the National Institute of Health's Guide for the Care and Use of Laboratory Animals (20). 35 male Wistar rats, aged 21 days, were kept in individual cages at temperature (24±2ºC) and relative humidity (55±5%) controlled in 12 h light-dark cycle. The animals were distributed into two experimental groups, Normocaloric (N) and Hypercaloric (H) over 30 weeks. The diets and water were offered ad libitum Both diets were produced according to Francisqueti et al. (2017) (21). The Hypercaloric diet was composed by high-sugar-fat chow and 25% sucrose added to drinking water.

2.2. Cut-off

In the experimental studies, even at similar laboratory conditions, the homogeneity response is not ensured due to the individuality of each animal (22). Animals submitted to hypercaloric diets may present characteristics that are different from each other and similar to animals submitted to normocaloric diets, such as adiposity index. Aiming to study animals with similar adiposity index under different diets, a cut-off (22-24) with 95% confidence interval was established for the average adiposity level in N and H groups. The cut-off value corresponds to the mean point between the upper limit in group N and the lower limit in group H. Considering this, animals with adiposity index above the cut-off were excluded. This process made it possible to reclassify animals in group N with n = 7 and group H with n = 8 (Figure 1), which were evaluated in this study.

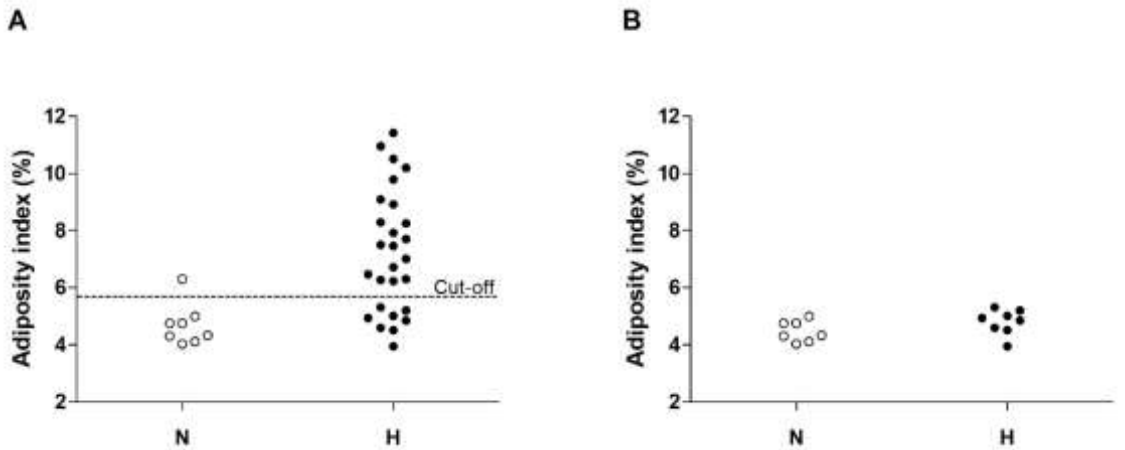

Figure 1. (**A**) Cut-off according to the adiposity index. Groups Normocaloric (N), n=7 and Hypercaloric (H), n=8. (**B**) Groups composition after cut-off.

2.3. Biological material and euthanasia

At the end of the 30ª week, preceding euthanasia, 24 hours urine samples were collected, and the total volume of diuresis was measured and subsequently all samples were centrifuged (3000 rpm at 4ºC per 10 minutes; Eppendorf® Centrifuge 5804-R, Hamburg, Germany), stored in Eppendorf® tubes at -80ºC. At the end of 30 weeks, the animals were fasted for 12 hours and then anesthetized with Xylazine (1 mg/kg, i.p.) and Ketamine (100 mg/kg, i.p.) (Syntec, Rhobifarma Indústria Farmacêutica Ltda., Hortolândia, São Paulo, Brazil) and euthanized by decaptation. The blood samples were collected in Falcon® tubes and centrifuged (3000 rpm at 4ºC per 10 minutes, Eppendorf® Centrifuge 5804-R, Hamburg, Germany) and stored at -80 ° C. The epididymal, retroperitoneal and visceral fat deposits were dissected and the epididymal deposits were stored at -80ºC in sterile cryotubes (Alfa Ltda-EPP, Ipiranga, São Paulo, Brazil).

2.4. Nutritional status and body composition

To evaluate the nutritional status, the food intake (chow and water), caloric intake and dietary efficiency were analyzed. Food and water intake were calculated daily from the individual leftovers of each animal. The caloric intake was calculated by the food intake multiplied by the energy value of each diet. In order to analyze the capacity of conversion of ingested energy in body weight, the dietary efficiency was calculated, dividing the total gain of body weight by the total energy ingested. Weight gain was calculated by the final body weight minus the initial body weight. After the experimental period, the deposits of retroperitoneal, visceral and epididymal fat were dissected and weighed and the adiposity index was calculated by summing the weight of the deposits divided by body weight and multiplied by 100, giving the result in percentage of fat (25). In addition, the soleus and Extensor Digitorum Longus (EDL) muscles were dissected right and left, and later weighed as a parameter of muscle mass.

2.5. Biochemical and hormonal analysis

To evaluate the possible complications associated to the diet, were analyzed the following parameters.

2.5.1. Biochemical

Plasma glucose, triglycerides, uric acid, total proteins, urea, serum albumin (BioClin, Quibasa Química Básica Ltda., Belo Horizonte, Minas Gerais, Brazil); protein and creatinin urinary (CELM®, Barueri, São Paulo, Brazil) were measured by colorimetric-enzymatic method in automatic enzymatic analyzer system (Chemistry Analyzer BS-200, MindrayMedical International Limited, Shenzhen, China). Protein and creatinin urinary were measured to obtain

protein/creatinin ratio (26).

2.5.2. Hormonal

The hormones insulin, adiponectin (EMD Millipore Corporation, Billerica, MA, USA) and leptin (Crystal Chem, Elk Grove Village, IL, USA) by the ELISA technique. As readings were performed on Spectra Max 190 microplate reader (Molecular Devices®, Sunnyvale, CA, USA). The HOMA-IR (27), which allows the evaluation of insulin resistance was also calculated, given by the formula: Insulin fasting (μUI / mL) x Glucose fasting (mmol / L) / 22.5.

2.6. Adipose tissue histology

The epididymal adipose tissue was fixed for 24 hours in 4% paraformaldehyde with 0.1M phosphate buffer (pH = 7.4). After 24 hours were placed in 70% alcohol until processing. The tissue was automatically processed in histotechnical apparatus (LEICA TP1020, LEICA Biosystems Inc., Richmond, Ilinois, USA) and then included in paraffin. Were obtained 5 μm sections (LEICA RM2155, LEICA Biosystems Inc., Richmond, Illinois, USA) and stained with hematoxylin-eosin (H&E). The histological slides were analyzed using a LEICA DM LS microscope coupled to an IBM PC compatible computer equipped with an image analysis program Image Pro-plus (Media Cybernetics, Silver Spring, Maryland, USA) in which images of the slides were captured and analyzed in 40x magnification. One slide was made per animal and 10 fields per slide were evaluated for the area and number of adipocytes. The area was obtained according to the method described by Osman et al. (2013) (28).

2.7. RNA extraction, cDNA synthesis and PCR real time

Frozen fragments of epididymal adipose tissue were homogenized in TRI-zol® (Invitrogen, Carlsbad, CA, USA) for the extraction of ribonucleic acid (RNA) according to the manufacturer's specifications. RNA was subjected to reverse transcription for conversion to complementary deoxyribonucleic acid (cDNA) (High Capacity cDNA Reverse Transcription Kit, Applied Biosystems, CA, USA). The cDNA was used in the real-time polymerase chain reaction (PCR) kit (Taq-Man® Gene Expression Assays, Applied Biosystems, Foster City CA, USA) to evaluate the expression of PPAR-γ.

2.8. Inflammation and oxidative stress parameters

The following analyzes were performed on epididymal adipose tissue. This was chosen because it reflected the behavior of visceral adipose tissue (29).

2.8.1. Inflammation

The tissue samples were homogenized in phosphate-buffered saline (PBS) at 1:10 (sample: buffer) and IL-6 and TNF-α cytokines were measured by ELISA (EMD Millipore Corporation, Billerica, MA, USA) and readings performed on Spectra Max 190 microplate reader (Molecular Devices®, Sunnyvale, CA, USA). Total proteins (BioClin, Quibasa Química Básica Ltda., Belo Horizonte, Minas Gerais, Brazil) were measured in the homogenates by a colorimetric-enzymatic method and analyzed in automatic enzymatic analyzer system (Chemistry Analyzer BS-200, MindrayMedical International Limited, Shenzhen, China), correction of the results expressed in pg / mg protein.

2.8.2. Oxidative stress

Oxidative stress was evaluated by the dosages of Malondialdehyde (MDA) and Carbonylation in homogenate of adipose tissue.

For the quantification of MDA, 250 μL of homogenate was used for 750 μL of trichloroacetic acid 10% for precipitation of proteins. Samples were centrifuged (3000 rpm; for 5 minutes; Eppendorf® Centrifuge 5804-R, Hamburg, Germany) and the supernatant removed. Thiobarbituric acid (TBA) was added to the supernatant in the ratio 1: 1 and the samples heated for 15 minutes at 100 ° C. MDA reacts with TBA in the ratio 1: 2 MDA-TBA, absorbed at 535 nm. After cooling, the reading at 535 nm was performed on Spectra Max 190 microplate reader (Molecular Devices®, Sunnyvale, CA, USA). The MDA concentration was obtained by the molar extinction coefficient ($1.56x10^5$ M^{-1} cm^{-1}) and the absorbance of the samples and the final result expressed in nmol / g protein (30).

Carbonylation was quantified by a method adapted from Mesquita et al. (2014). 100 μL of homogeneous to 100 μL 2,4-dinitrophenylhydrazine (DNPH) (10 mM in 2 M HCl) was used. The samples were incubated for 10 minutes at room temperature and then 50 μL of NaOH (6 M) were added and incubated again for 10 minutes at room temperature. The reading was performed at 450 nm on a Spectra Max 190 microplate reader (Molecular Devices®, Sunnyvale, CA, USA) and the result obtained from the absorbance of the samples and the molar extinction coefficient (22000 M^{-1} cm^{-1}) (31). The final results expressed in nmol/mg protein.

2.9 Statistical analysis

Parametric data are presented as means ± standard deviation and compared by Student's t test. Non-parametric data are presented as median

(interquatile range) and compared by Mann-Whitney U test. The software used was Sigma Plot version 12.0 for Windows (Systat Software Inc., San Jose, CA, Estados Unidos). A p value <0.05 was considered as statistically significant.

3. Results

3.1. Nutritional status and body composition

There was no difference in caloric intake (kcal/day) between the groups (Figure 2).

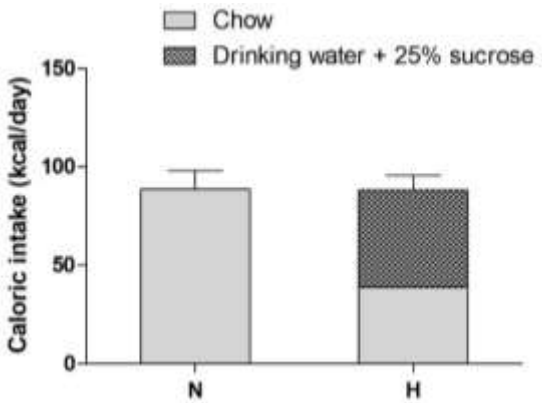

Figure 2. Caloric intake (kcal/dia). Normocaloric (N), n=7; Hypercaloric (H), n=8. Data presented as mean ± standard deviation. Student's t test, p=0,691.

Group H presented lower feed intake (g) accompanied by higher water intake (mL) and dietary efficiency (g / kcal) than group N (Table 1). Total protein and urea levels were lower in group H, but there was no difference between groups for albumin levels. The final body weight and weight gain were lower in the H group and there was no difference in the adiposity index between the groups, but when the weight of the skeletal muscles Soleus and EDL as a parameter of muscle mass was evaluated, the weights were lower in group H.

Table 1. Nutritional status and body composition.

Parameters	Normocaloric n=7	Hypercaloric n=8	*p* value
Chow fed (g/day)	24,0 (22,6 – 27,8)	8,52 (7,83 – 9,59)	p<0,001*
Water intake (mL/day)	36,0 ± 5,47	50,0 ± 5,98	p<0,001*
Dietary efficiency (g/kcal)	0,016 ± 0,00	0,012 ± 0,00	p<0,001*
Total protein (g/dL)	6,06 ±0,20	5,75 ± 0,19	p=0,011*
Albumin (g/dL)	2,52 ± 0,41	2,59 ± 0,40	p=0,735
Urea (mg/dL)	54,1 ± 14,5	40,6 ± 5,68	p=0,030*
Initial body weight (g)	183 ± 13,9	171 ± 9,38	p=0,084
Final body weight (g)	492 ± 54	405 ± 30	p=0,002*
Weight gain (g)	302 ± 53,0	237 ± 37,5	p=0,010*
Total fat (g)	21,6 ± 1,85	19,3 ± 2,5	p=0,095
Adiposity index (%)	4,46 ± 0,36	4,79 ± 0,43	p=0,142
Soleus weight (g)	0,21 ± 0,02	0,17 ± 0,02	p=0,035*
EDL weight (g)	0,18 ± 0,01	0,16 ± 0,00	p=0,001*

EDL: Extensor Digitorum Longus. Data are presented as means ± standard deviation or as median (interquatile range). *: statistical difference to $p<0,05$.

3.2. Biochemical and hormonal analysis

Group H had higher glycemia, triglycerides, uric acid, insulin and adiponectin. There was no statistical difference for leptin and HOMA-IR. The urinary protein-creatinine ratio was elevated in the H group (Table 2).

Table 2. Biochemical and hormonal parameters.

Parameters	Normocaloric n=7	Hypercaloric n=8	*p* value
Glucose (mg/dL)	86,6 ± 10,3	98,4 ± 5,5	p=0,001*
Triglycerides (mg/dL)	59,0 ± 14,9	75,7 ± 11,8	p=0,031*
Uric acid (mg/dL)	0,41 ± 0,07	0,57 ± 0,07	p=0,006*
Insulin (ng/mL)	0,64 ± 0,18	1,05 ± 0,30	p=0,043*
Adiponectin (ng/mL)	0,018 ± 0,003	0,029 ± 0,006	p=0,001*
Leptin (ng/mL)	0,55±0,44	1,29±0,70	p=0,072
HOMA-IR	6,70 ± 2,02	10,24 ± 3,44	p=0,103
Protein/creatinin ratio	0,0021 ± 0,0004	0,0025 ± 0,0002	p=0,023*

HOMA-IR: Homeostatic Model Assessment for Insulin Resistance. Data are presented as means ± standard deviation. *: statistical difference to $p<0,05$.

3.3. Adipose tissue histology

The histology of the epidydimal adipose tissue is presented in Figure 3. The H group presented smaller area and greater number of adipocytes per field of view.

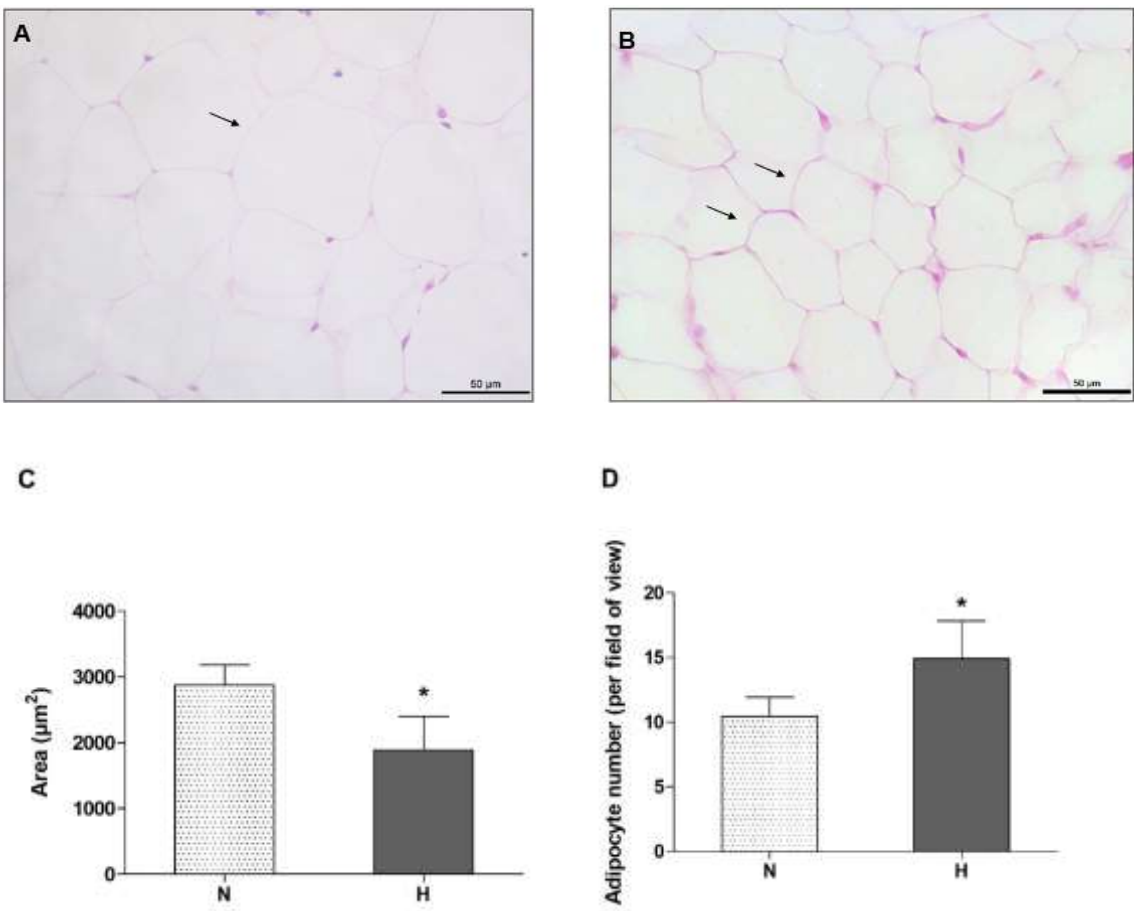

Figure 3. Adipose tissue histology stained with H&E in 40x magnification: (**A**) N group e (**B**) H group; arrow: adipocytes. (**C**) adipocytes area e (**D**) adipocytes number per field of view. Data are presented as means ± standard deviation. *: statistical difference to $p<0,05$.

3.4. Genic expression

The PPAR-γ expression was higher in group H (Figure 4).

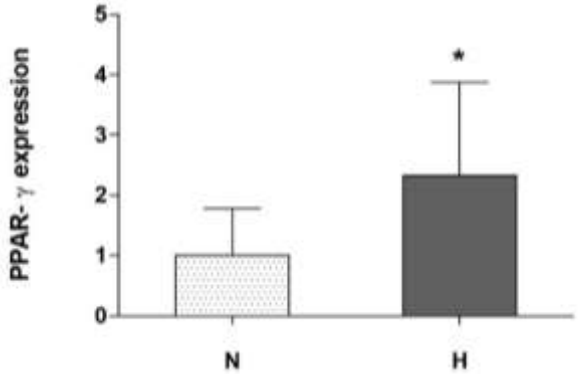

Figure 4. PPAR-γ: pproliferator-activated receptor gamma. PPAR-γ expression in epidydimal adipose tissue. Normocaloric (N), n=7; Hypercaloric (H), n=8. Data are presented as means ± standard deviation. *: statistical difference to p<0,05.

3.5. Inflammation and Oxidative stress

In adipose tissue, proinflammatory cytokines IL-6 and TNF-α levels were elevated in the H group and the oxidative stress parameters, MDA and Carbonylation levels were decreased (Table 3).

Table 3. Adipose tissue inflammation and oxidative stress.

Parameters	**Normocaloric n=7**	**Hypercaloric n=8**	***p* value**
TNF-α (pg/mg protein)	52,8 ± 17,4	283,44 ± 67,9	p<0,001*
IL-6 (pg/mg protein)	17,8 ± 6,86	100,8 ± 54,4	p<0,008*
MDA (nmol/g protein)	121,9 (102,0 – 181,8)	90,4 (72,6 – 97,9)	p=0,009*
Carbonylation (nmol/g de protein)	34,0 (29,1 – 39,0)	11,3 (10,7 – 13,7)	p<0,001*

TNF-α: tumor necrosis factor alpha; IL-6: interleukin-6; MDA: malondialdehyde. Data are presented as means ± standard deviation or median (interquatile range) *: statistical difference to p<0,05.

4. Discussion

The aim of this study was to evaluate if dietary quality is a risk factor for metabolic complications development independent of adiposity gain. It was observed that the animals submitted to the Hypercaloric diet ate less amount of feed in grams and more water. This fact did not interfere with the amount of calorie ingested (kcal / day), since the upper water intake in the H group, which contained 25% sucrose, provided energy values in kcal similar to the N group. Although caloric intake was similar between groups, macronutrient quality was different reflecting in the alteration of some body composition and biochemical parameters. The fact that the animals in the H group consumed less feed resulted in lower protein intake, lowering the levels of total proteins and urea, the final product of protein degradation, in relation to the N group. However, these alterations do not characterize a state of malnutrition (32), which can be confirmed by albumin levels that did not differ between groups, indicating that the synthesis of systemic proteins by the liver is preserved (33). On the other hand, the synthesis of muscle proteins is impaired and it is confirmed by the lower weight of the soleus and EDL muscles, body weight and food efficiency in group H.

Changes observed in group H for biochemical parameters such as glycemia, triglycerides, and uric acid are explained by the unbalanced intake of macronutrients. These changes are originated from the high consumption of simple carbohydrates (sucrose and fructose), and fat present in the diet. Sucrose, composed of glucose and fructose in the same proportion, has a hyperglycemic effect due to glucose, and it is not directly associated with fructose. Fructose is absorbed through the intestine through the portal vein and it is metabolized in

the liver, where it can enter different metabolic pathways to form energy substrates such as glucose, glycogen, lactate and fatty acids (34). In addition, unlike glucose, fructose breakdown is not regulated by the main glycolysis limiting step at the phosphofructokinase level, acting as a substrate for new hepatic lipogenesis and lipid production (34, 35). In addition, the Hypercaloric Diet of the present study also contains lard, which is composed of saturated and monounsaturated fatty acids (36) whose ingestion is associated with elevated plasma levels of triglycerides (37).

Another parameter elevated in the H group, due to the influence of fructose, is uric acid, a product originated from one of the degradation pathways of this sugar. Evidences suggest that hyperuricemia is directly related to the excessive intake of fructose (35). This is a risk factor for gout (38), and it is also a biomarker for cardiovascular and renal diseases (39, 40). Among the mechanisms associated with the development of these complications, it is proposed that uric acid at high concentrations behaves as a pro-oxidant, triggering oxidative stress and inflammation, and it may leads to damage of peripheral organs such as the kidneys (41). Our study showed that the animals of H group, in addition to hyperuricemia, presented changes in the protein/creatinin ratio, so there may be an association between both parameters. Adorni et al. (2017), also observed biochemical changes similar to those found in our study, even without significant difference in caloric intake (9). These data reinforce the idea that diet quality is a worrying factor, regardless of the caloric intake.

In this study, hormonal parameters were also evaluated, and insulin was observed to be significantly elevated in the H group. This data is in agreement with the literature that highlights that this hormone increases according to the type of diet, especially those rich in refined sugars and saturated fats (42). In

parallel, Pagliassotti et al. (2000) demonstrated higher values of insulin in rats fed with hyperlipid diet without altering body fat, strengthening the idea that, independent of obesity, diet may be a factor of metabolic complications (43). Although there is no significant difference for HOMA-IR between the groups, increased circulating glycemia and insulin indicate possibly an initial process of insulin resistance in target organs such as adipose tissue.

The hormones adiponectin and leptin were also evaluated in this study and they are the most studied hormones secreted by adipose tissue, having a great influence on the energy balance (44). Leptin showed no difference between groups, and this was expected, since both groups were not obese. However, adiponectin, a hormone directly related to food intake, energy homeostasis, protection against atherosclerosis, and increased insulin sensitivity (45) decreased chronic inflammation and hypertrophy (46), is increased in the hypercaloric group. This data suggest that this elevation may be an early anti-inflammatory response due to diet quality according to Westerink et al. (2014) (47). This hormone is also positively regulated in response to activation of PPAR-γ, a nuclear factor widely expressed in adipose tissue (48), which was also elevated in the animals belong to the H group. While adiponectin is inversely correlated with increased adipose mass, secretion of leptin is directly proportional. H group presented leptin levels statistically equal to the N group, an expected result, since there was no increase in adiposity in group H.

PPAR-γ is responsible for controlling the storage of fatty acids through the proliferation of adipocytes (8, 49). In the presente study, only the gene expression was evaluated in the adipose tissue of the animals, consequently it is not possible to affirm that its respective protein was transcribed and if it was an active factor in the adipogenesis. In addition to this data, the number and the area of the

adipocytes were determined, and the H group showed a smaller area and a larger number of these cells. These results, coupled with increased PPAR-γ gene expression, may indicate an increase in the number of adipose cells, since this nuclear factor is involved in the regulation of adipocyte maturation (adipogenesis) and absorption and storage of triglycerides and associated with adipose tissue hyperplasia (49). The adipogenesis has a beneficial action because it preserves the adipose tissue from the hypertrophy avoiding its dysfunction and the development of metabolic complications (50). In contrast, changes in PPAR-γ expression in adipose tissue may result in late obesity with greater fat accumulation due to the greater number of adipose tissue cells (51).

Adipose tissue is directly affected by dietary quality, the literature reports that some nutrients such as fatty acids can stimulate Toll like-4 receptors (TRL4) presented in adipocytes and macrophages residing in adipose tissue by increasing production of proinflammatory cytokines (52). In the present study, it was observed that dietary metabolites also influence the inflammation of adipose tissue via polarization of resident macrophages, which may be polarized from the anti-inflammatory profile (M2) to the inflammatory profile (M1) (53). In group H, an increase in proinflammatory cytokines, TNF-α and IL-6, could be associated with these mechanisms. According to Asterholm et al., (2014), the increase of pro-inflammatory cytokines in response to hypercaloric diets may be an adaptive local and acute pro-inflammatory response and can also be a predictor for systemic inflammation and future metabolic complications (54). Another stimulus for adipose tissue inflammation involving TRL4 is the gut microbiota modification. Recent studies shows that the gut microbiota is altered by high sugar fat diet intake leading to an increase in the permeability of the intestinal villi and greater release of LPS through the endothelial capillaries,

which is recognized by the TRL4 receptors in adipose tissue triggering inflammation (55-57). One of the limitations of the present study was not to evaluate the gut microbiota, then is not possible to attribute the observed inflammation to this mechanism.

The inflammation developed in adipose tissue increases the levels of ROS resulting in oxidative stress that consequently leads to the formation of metabolites such as MDA and protein carbonylation (58). Our data showed a decrease in MDA levels and carbonylation of proteins in adipose tissue in H group vs. to N group. This result was not expected considering that cytokine levels were increased in this tissue. In a study of animals with obesity induced by hypercaloric diet, Farhangi et al. (2017) did not observe a significant difference for MDA between control and obese animals, but found that obese animals had increased levels of the antioxidant enzyme catalase in adipose tissue, this increase may have been a response in combating reactive species by preventing lipid peroxidation and not altering MDA levels (59). In our study, antioxidant enzymes were not evaluated, a limiting factor to discuss the significant decrease in oxidative stress parameters in group H.

Although some alterations associated with adipose tissue dysfunction were not observe in animals of the H group, such as insulin resistance and adiponectin decrease, it was observed that, independently of the expansion of this tissue, there is an increase in proinflammatory cytokines and alteration of metabolic parameters. These data are relevant to emphasize that dietary quality - independent of adiposity gain - may be a risk factor for the development of inflammation in adipose tissue and some biochemical complications that may result in future comorbidities.

Acknowledgements: This work was supported by Fundação de Amparo à Pesquisa do Estado de São Paulo – FAPESP (2015/10626-0).

Author contributions: J.L.G., F.V.F. and A.P.C.R.F. conducted the experiment; J.L.G., K.C.S. and A.F.J. analyzed the data; J.L.G., K.C.S. I.O.M and C.R.C. wrote the paper.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Archundia Herrera, M.C.; Subhan, F.B.; Chan, C.B. Dietary Patterns and Cardiovascular Disease Risk in People with Type 2 Diabetes. *Curr. Obes. Rep*. **2017**, 6(4), 405-413.
2. Romagnolo, D.F.; Selmin, O.I.; Mediterranean Diet and Prevention of Chronic Diseases. *Nutr. Today* 2017, 52(5), 208-222.
3. Butler, T.J.; Ashford, D.; Seymour, A.M. Western diet increases cardiac ceramide content in healthy and hypertrophied hearts. *Nutr. Metab. Cardiovasc. Dis*. **2017**, 27(7), 991-998.
4. Frölich, S.; Lehmann, N.; Weyers, S.; Wahl, S.; Dragano, N.; Budde, T.; et al. Association of dietary patterns with five-year degree and progression of coronary artery calcification in the Heinz Nixdorf Recall study. *Nutr. Metab. Cardiovasc. Dis.* **2017**, 27(11), 999-1007.
5. Baratta, F.; Pastori, D.; Polimeni, L.; Bucci, T.; Ceci, F.; Calabrese, C.; et al. Adherence to Mediterranean Diet and Non-Alcoholic Fatty Liver Disease: Effect on Insulin Resistance. *Am. J. Gastroenterol*. **2017**, 112(12), 1832-1839.
6. Paniagua, J. A. Nutrition, insulin resistance and dysfunctional adipose tissue determine the different components of metabolic syndrome. *World J. Diabetes*. **2016**, 7(19), 483-514.
7. Naja, F.; Nasreddine, L.; Itani, L.; Adra, N.; Sibai, A. M.; Hwalla, N. Association between dietary patterns and the risk of metabolic syndrome among Lebanese adults. *Eur. J. Nutr.* **2013**, 52(1), 97–105.
8. Feng, S.; Reuss, L.; Wang, Y. Potential of natural products in the inhibition of adipogenesis through regulation of PPARγ expression and/or its transcriptional activity. *Molecules*. **2016**, 21(10), 1-19.
9. Adorni, C. S.; Corrêa, C. R.; Vileigas, D. F.; de Campos, D. H. S.; Padovani, C. R.; Minatel, I. O.; et al. The influence of obesity by a diet high in saturated fats and carbohydrates balance in the manifestation of systemic complications and

comorbidities. *Nutrire*. **2017**, 42(16), 1-6.
10. Micha, R.; Peñalvo, J. L.; Cudhea, F.; Imamura, F.; Rehm, C. D.; Mozaffarian, D. Association Between Dietary Factors and Mortality From Heart Disease, Stroke, and Type 2 Diabetes in the United States. *Jama*. **2017**, 317(9), 912.924.
11. Kant, A. K. Dietary patterns: biomarkers and chronic disease risk. *Appl. Physiol. Nutr. Metab*. **2010**, 35(2), 199–206.
12. Fonseca-Alaniz, M.; Takada, J.; Alonso-Vale, M. I. C.; Lima, F. B. O tecido adiposo como centro regulador do metabolismo. *Fisioter. e Pesqui*. **2006**, 50(2), 216–229.
13. Moreno-Indias, I.; Tinahones, F. J. Impaired adipose tissue expandability and lipogenic capacities as ones of the main causes of metabolic disorders. *J. Diabetes Res.* **2015**, 2015, 1-12.
14. Klöting, N.; Blüher, M. Adipocyte dysfunction, inflammation and metabolic syndrome. *Rev. Endocr. Metab. Disord*. **2014**, 15(4), 277–87.
15. Francisqueti, F. V.; Chiaverini, L. C.T.; Santos, K. C. dos, Minatel, I. O.; Ronchi, C. B.; Ferron, A. J. T.; et al. The role of oxidative stress on the pathophysiology of metabolic syndrome. *Rev. Assoc. Med. Bras*. **2017,** 63(1), 85–91.
16. Minihane, A. M.; Vinoy, S.; Russell, W. R.; Baka, A.; Roche, H. M.; Tuohy, K. M.; et al. Low-grade inflammation, diet composition and health: current research evidence and its translation. *Br. J. Nutr.* **2015**, 114(7), 999–1012.
17. Calder, P. C.; Ahluwalia, N.; Brouns, F.; Buetler, T.; Clement, K.; Cunningham, K.; et al. Dietary factors and low-grade inflammation in relation to overweight and obesity. *Br. J. Nutr.* **2011**, 106(S3), S5–78.
18. O'Connor, L.; Imamura, F.; Brage, S.; Griffin, S. J.; Wareham, N. J.; Forouhi, N. G. Intakes and sources of dietary sugars and their association with metabolic and inflammatory markers. *Clin. Nutr*. **2017**, 1–10.
19. Mazidi, M.; Kengne, A. P.; Mikhailidis, D. P.; Cicero, A. F.; Banach, M. Effects of selected dietary constituents on high-sensitivity C-reactive protein levels in U.S. adults. *Ann. Med*. **2017**, 50(1), 1–6.
20. Canadian Council on Animal Care. Guide to the Care and Use of Experimental Animals. *CCAC*. 1993, 1, 209.
21. Francisqueti, F. V.; Minatel, I. O.; Ferron, A. J. T.; Bazan, S. G. Z.; Silva, V. S.; Garcia, J. L.; et al. Effect of Gamma-Oryzanol as Therapeutic Agent to Prevent Cardiorenal Metabolic Syndrome in Animals Submitted to High Sugar-Fat Diet. *Nutrients*. **2017**, 9(12), 1299-1309.
22. Freire, P. P.; Alves, C. A. B.; Deus, A. F. de; Leopoldo, A. P. L.; Leopoldo, A. S.; Silva D. C. T. da; et al. Obesity does not Lead to Imbalance Between Myocardial Phospholamban Phosphorylation and Dephosphorylation. *Arq. Bras. Cardiol*. **2014**, 103(1), 41–50.
23. Oliveira-Junior, S. A.; Dal Pai-Silva, M.; Martinez, P. F.; Campos, D. H.S.; Lima-Leopoldo, A. P.; Leopoldo, A. S.; et al. Differential nutritional, endocrine, and cardiovascular effects in obesity-prone and obesity-resistant rats fed standard and hypercaloric diets. *Med. Sci. Monit*. **2010**, 16(7), 208-217.

24. Nascimento, A. F.; Sugizaki, M. M.; Leopoldo, A. S.; Lima-Leopoldo, A. P.; Nogueira, C. R.; Novelli, E. L. B.; et al. Misclassification probability as obese or lean in hypercaloric and normocaloric diet. *Biol. Res*. **2008**, 41(3), 253–259.
25. Dobrian, A. D.; Davies, M. J.; Schriver, S. D.; Lauterio, T. J.; Prewitt, R. L. Oxidative Stress in a Rat Model of Obesity-Induced Hypertension. *Hypertension*. **2001**, 37(2), 554–560.
26. Chen, J. Y.; Jian, D. Y.; Lien, C. C.; Lin, Y. T.; Ting, C. H.; Chen, L. K.; et al. Adipocytes play an etiological role in the podocytopathy of high-fat diet-fed rats. *J. Endocrinol*. **2016**, 231(2), 109–120.
27. Matthews, D. R.; Hosker, J. P.; Rudenski, A. S.; Naylor, B. A.; Treacher, D. F.; Turner, R. C. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia*. **1985**, 28(7):412–419.
28. Osman, O. S.; Selway, J. L.; Kępczyńska, M. A.; Stocker, C. J.; O'Dowd, J. F.; Cawthorne, M. A.; et al. A novel automated image analysis method for accurate adipocyte quantification. *Adipocyte*. **2013**, 2(3), 160–164.
29. Luvizotto, R. A. M.; Nascimento, A. F.; Imaizumi, E.; Pierine, D. T.; Conde, S. J.; Correa, C. R.; et al. Lycopene supplementation modulates plasma concentrations and epididymal adipose tissue mRNA of leptin, resistin and IL-6 in diet-induced obese rats. *Br. J. Nutr.* **2013**, 110(10), 1803–1809.
30. Samarghandian, S.; Farkhondeh, T.; Samini, F.; Borji, A. Protective Effects of Carvacrol against Oxidative Stress Induced by Chronic Stress in Rat 's Brain, Liver and Kidney. *Biochem. Research Inter*. **2016,** 2016, 1-7.
31. Mesquita, C. S.; Oliveira, R.; Bento, F.; Geraldo, D.; Rodrigues, J. V.; Marcos, J. C. Simplified 2,4-dinitrophenylhydrazine spectrophotometric assay for quantification of carbonyls in oxidized proteins. *Anal. Biochem*. **2014**, 458, 69–71.
32. Branco, A. C. S. C.; Diniz, M. F. F. M.; Almeida, R. N.; Santos, H. B.; Oliveira, K. M.; Ramalho, J. A.; Dantas, J. G. Parâmetros bioquímicos e hematológicos de ratos Wistar e camundongos Swiss do biotério professor Thomas George. *Ver. Bras. Ciências da Saúde*. **2011**, 15(2), 209–214.
33. Kuwahata, M.; Hasegawa, M.; Kobayashi, Y.; Wada, Y.; Kido, Y.; An oxidized/reduced state of plasma albumin reflects malnutrition due to an insufficiente diet in rats. *J. Clin. Biochem*. Nutr. **2017**, 60(1), 70–75.
34. Zhang, D. M.; Jiao, R. Q.; Kong, L. D. High dietary fructose: Direct or indirect dangerous factors disturbing tissue and organ functions. *Nutrients*. **2017**, 9(4), 335-355
35. Legeza, B.; Marcolongo, P.; Gamberucci, A.; Varga, V.; Bánhegyi, G.; Benedetti, A.; et al. Fructose, glucocorticoids and adipose tissue: Implications for the metabolic syndrome. *Nutrients.* **2017**, 9(5), 1–19.
36. Crescenzo, R.; Bianco, F.; Mazzoli, A.; Giacco, A.; Cancelliere, R.; di Fabio, G.; et al. Fat quality influences the obesogenic effect of high fat diets. *Nutrients*. **2015**, 7(11), 9475–9491.

37. Sociedade Brasileira de Cardiologia. I Diretriz Sobre o Consumo de Gorduras e Saúde Cardiovascular. *Arq. Bras. Cardiol.* **2013**, 101(1), 1- 40
38. Zamudio-Cuevas, Y.; Hernández-Díaz, C.; Pineda, C.; Reginato, A. M.; Cerna-Cortés, J. F.; Ventura-Ríos, L.; et al. Molecular basis of oxidative stress in gouty arthropathy. *Clin. Rheumatol*. **2015**, 34(10), 1667–1672.
39. Stack, A. G.; Hanley, A.; Casserly, L. F.; Cronin, C. J.; Abdalla, A. A.; Kiernan, T. J.; et al. Independent and conjoint associations of gout and hyperuricaemia with total and cardiovascular mortality. *Qjm*. **2013**,106(7), 647–658.
40. Caliceti, C.; Calabria, D.; Roda, A.; Cicero, A. Fructose Intake, Serum Uric Acid, and Cardiometabolic Disorders: A Critical Review. *Nutrients*. **2017**, 9(4), 1-15.
41. Nakagawa, T.; Tuttle, K. R.; Short, R. A.; Johnson, R. J. Hypothesis: fructose-induced hyperuricemia as a causal mechanism for the epidemic of the metabolic syndrome. *Nat. Clin. Pract. Nephrol.* **2005**, 1(2), 80–86.
42. DiNicolantonio, J.J.; Lucan, S. C.; O'Keefe, J. H. The Evidence for Saturated Fat and for Sugar Related to Coronary Heart Disease. *Prog. Cardiovasc. Dis*. **2016**, 58(5), 464–472.
43. Pagliassotti, M. J.; Gayles, E. C.; Podolin, D.A.; Wei, Y.; Morin, C. L. Developmental stage modifies diet-induced peripheral insulin resistance in rats. *Am. J. Physiol. Integr. Comp. Physiol.* **2000**, 278(1), 66-73.
44. Meriga, B.; Parim, B.; Chunduri, V. R.; Naik, R. R.; Nemani, H.; Suresh, P.; et al. Antiobesity potential of Piperonal: Promising modulation of body composition, lipid profiles and obesogenic marker expression in HFD-induced obese rats. *Nutr. Metab*. **2017**, 14(1), 1–14.
45. Hocking, S.; Samocha-Bonet, D.; Milner, K. L.; Greenfield, J. R.; Chisholm, D. J. Adiposity and insulin resistance in humans: The role of the different tissue and cellular lipid depots. *Endocr. Rev.* **2013**, 34(4), 463–500.
46. Bahceci, M.; Gokalp, D.; Bahceci, S.; Tuzcu, A.; Atmaca, S.; Arikan, S. The correlation between adiposity and adiponectin, TNF-alpha, IL-6 and high sensitivity CRP protein levels. *J. Endocrinol. Invest*. **2007**, 30(3), 210–4.
47. Westerink, J.; Hajer, G. R.; Kranendonk, M. E. G.; Schipper, H. S.; Monajemi, H.; Kalkhoven, E.; et al. An oral mixed fat load is followed by a modest anti-inflammatory adipocytokine response in overweight patients with metabolic syndrome. *Lipids.* **2014**, 49(3), 247–54.
48. Sharma, A. M.; Staels, B. Peroxisome proliferator-activated receptor γ and adipose tissue - Understanding obesity-related changes in regulation of lipid and glucose metabolism. *J. Clin. Endocrinol. Metab*. **2007**, 92(2), 386–95.
49. Choi, S-S.; Park, J.; Choi, J. H. Revisiting PPARγ as a target for the treatment of metabolic disorders. *BMB Rep.* **2014**, 47(11), 599–608.
50. Poulos, S. P.; Dodson, M. V.; Culver, M. F.; Hausman, G. J. The increasingly complex regulation of adipocyte differentiation. *Exp. Biol. Med*. **2016**, 241(5), 449–456.
51. Yogarajah, T.; Bee, Y-T, G.; Noordin, R.; Yin, K. B. Increased peroxisome

proliferator-activated receptor γ expression levels in visceral adipose tissue, and serum CCL2 and interleukin-6 levels during visceral adipose tissue accumulation. *Mol. Med. Rep*. **2015**, 11(1), 515–520.

52. Francisqueti, F. V.; Nascimento, A. F.; Minatel, I. O.; Dias, M. C.; Luvizotto, R. D. A. M.; Berchieri-Ronchi, C.; et al. Metabolic syndrome and inflammation in adipose tissue occur at different times in animals submitted to a high-sugar/fat diet. *J. Nutr. Sci*. **2017**, 6(41), 1-8.
53. Camell, C.; Goldberg, E.; Dixit, V. D. Regulation of Nlrp3 inflammasome by dietary metabolites. *Semin. Immunol*. **2015**, 27(5), 334–42.
54. Wernstedt-Asterholm I.; Tao, C.; Morley, T. S.; Wang, Q. A.; Delgado-Lopez, F.; Wang, Z. V.; et al. Adipocyte inflammation is essential for healthy adipose tissue expansion and remodeling. *Cell. Metab*. **2014**, 20(1), 103–18.
55. Hersoug, L-G.; Møller, P.; Loft, S. Role of microbiota-derived lipopolysaccharide in adipose tissue inflammation, adipocyte size and pyroptosis during obesity. *Nutr Res Rev*. **2018**, 1-11.
56. Collins, K. H.; Paul, H. A.; Hart, D. A.; Reimer, R. A.; Smith, I. C.; Rios, J. L.; et al. A High-Fat High-Sucrose Diet Rapidly Alters Muscle Integrity, Inflammation and Gut Microbiota in Male Rats. *Sci Rep*. **2016**, 6(October),1–1.
57. Liu, D.; Zhang, Y.; Liu, Y.; Hou, L.; Li, S.; Tian, H.; et al. Berberine Modulates Gut Microbiota and Reduces Insulin Resistance via the TLR4 Signaling Pathway. *Exp Clin Endocrinol Diabetes*. **2018**, 4, 1-8
58. Noeman, S. A.; Hamooda, H. E.; Baalash, A. A. Biochemical Study of Oxidative Stress Markers in the Liver, Kidney and Heart of High Fat Diet Induced Obesity in Rats. *Diabetol. Metab. Syndr.* **2011**, 3(1), 1-8.
59. Farhangi, M. A.; Mesgari-Abbasi, M.; Hajiluian, G.; Nameni, G.; Shahabi, P. Adipose Tissue Inflammation and Oxidative Stress: the Ameliorative Effects of Vitamin D. *Inflammation*. **2017**, 40(5), 1688–97.

Capítulo III
Conclusão

De acordo com os resultados desse trabalho, podemos concluir que a qualidade dietética independente do aumento de adiposidade é um fator de risco para o desenvolvimento da inflamação no tecido adiposo e complicações bioquímicas que podem resultar em futuras comorbidades.

Anexo

UNIVERSIDADE ESTADUAL PAULISTA
CAMPUS DE BOTUCATU
FACULDADE DE MEDICINA

unesp

FMB

Ética CEUA

Comissão de Ética no Uso de Animais

Criada através da Portaria DFM nº 611 de 13/12/2012

CERTIFICADO Nº 1233/2017-CEUA

Certificamos que a proposta intitulada **"Impacto da ingestão de carboidratos simples e gordura sobre parâmetros metabólicos, inflamatórios e pró-oxidantes no plasma e no tecido adiposo independente de obesidade"**, registrada com o nº 1233/2017, sob a responsabilidade de Jéssica Leite Garcia, Orientada pela Profa. Dra. Camila Renata Correa Camacho, Coorientada por Prof. Dr. Igor Otavio Minatel, com a colaboração de Fabiane Valentini Francisqueti – que envolve a produção, manutenção ou utilização de animais pertencentes ao filo Chordata, subfilo Vertebrata (exceto humanos), para fins de pesquisa científica (ou ensino) – encontra-se de acordo com os preceitos da Lei n. 11.794, de 8 de outubro de 2008, do Decreto n. 6.899, de 15 de julho de 2009, e com as normas editadas pelo Conselho Nacional de Controle de Experimentação Animal (CONCEA), e foi aprovado pela Comissão de Ética no Uso de Animais da Faculdade de Medicina de Botucatu, em reunião de 31 de agosto de 2017.

Finalidade ( ) Ensino (X) Pesquisa Científica	
Vigência da autorização	27/08/2018
Espécie/Linhagem/Raça	Ratos Wistar
Nº de animais	60
Peso/Idade	80 gramas/21 dias
Sexo	Macho
Origem	Biotério Central da UNESP - FMB

Prof. Dr. Guilherme Antônio Moreira de Barros
Presidente da CEUA

Alberto Santos Capelluppi
Secretário da CEUA

Distrito Rubião Junior, s/nº - Botucatu – S.P. CEP: 18.618-970 Fone: (14) 3880-1608/3880-1609 e-mail secretaria: ceua@fmb.unesp.br