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Suplementação com licopeno: Influência sobre o estado-redox e a modulação β-adrenérgica cardíaca em modelo experimental de obesidade induzida por dieta

Tese apresentada à Faculdade de Medicina, Universidade Estadual Paulista "Júlio de Mesquita Filho", Câmpus de Botucatu, para obtenção do título de Doutor em Fisiopatologia em Clínica Médica.

Orientadora: Profª. Adjunta Ana Lúcia dos Anjos Ferreira
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1. Coração - Doenças. 2. Obesidade. 3. Licopeno. 4. Antioxidantes.

Palavras-chave: Estado redox; Função cardíaca; Licopeno; Obesidade.

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Dedico este trabalho às pessoas mais importantes da minha vida,

Aos meus Pais, Lucinalva Togneri e ArturFerron (in memorian)

Representam minhas raízes, minha base; fé, força de vontade, trabalho e dignidade, ensinamentos que estarão sempre comigo e fazem parte do caráter. Amor eterno!

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"Mobilizar é convocar vontades para atuar na busca de um propósito comum, essa mobilização ocorre quando um grupo de pessoas, uma comunidade, uma sociedade decide e age com objetivo comum, buscando cotidianamente, os resultados desejados por todos".

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Sumário

Resumo

O desequilíbrio do sistema redox é uma das vias relacionadas à disfunção cardíaca associada à obesidade. O licopeno é considerado um dos melhores antioxidantes dentre os carotenoides. O objetivo deste estudo foi testar a capacidade antioxidante do licopeno na recuperação da função cardíaca, melhorando a resposta β-adrenérgica. 40 animais foram divididos aleatoriamente em 2 grupos experimentais para receber a dieta controle (Controle, n = 20) ou uma dieta rica em açúcar e gordura (HSF, n = 20) por 20 semanas. Uma vez que a disfunção cardíaca foi detectada pelo ecocardiograma no grupo HSF, os animais foram redivididos para iniciar o tratamento com licopeno ou veículo: Controle (n = 6); (Controle + Ly, n = 6); HSF (n = 6) e (HSF + Ly, n = 6). Licopeno (10 mg de licopeno / kg de peso corporal (PC) por dia) foi administrada por via gavage todas as manhãs durante um período de 10 semanas. A análise incluiu parâmetros bioquímicos nutricionais e plasmáticos, pressão arterial sistólica, parâmetros oxidativos nas análises plasmática, cardíaca e cardíaca in vivo e in vitro. A comparação entre os grupos foi realizada pela análise de variância two way (ANOVA). Resultados: A dieta HSF foi capaz de induzir obesidade, resistência à insulina, disfunção cardíaca e dano oxidativo. No entanto, a suplementação licopeno melhorou a resistência à insulina, a remodelação cardíaca e a disfunção, melhorando a resposta *β*-adrenérgica. É possível concluir que licopeno é capaz de reduzir o dano oxidativo cardíaco, melhorando a resposta β-adrenérgica do sistema, recuperando assim a função cardíaca.

Palavras Chave: Dieta hiperlipídica; obesidade; sistema *β*-adrenérgico; disfunção cardíaca; licopeno.

Abstract

The system redox imbalance is one of the pathways related to obesity-related cardiac dysfunction. Lycopene is considered one of the best antioxidants. The aim of this study was to test if the tomato-oleoresin would be able to recovery cardiac function by improving β-adrenergic response due its antioxidant effect. A total of 40 animals were randomly divided into two experimental groups to receive either the control diet (Control, $n = 20$) or a high sugar-fat diet (HSF, $n = 20$) for 20 weeks. Once cardiac dysfunction was detected by echocardiogram in the HSF group, animals were re- divided to begin the treatment with Tomato-oleoresin or vehicle, performing four groups: Control ($n = 6$); (Control + Ly, $n = 6$); HSF ($n = 6$) and (HSF + Ly, $n = 6$). Tomato oleoresin (10 mg lycopene/kg body weight (BW) per day) was given orally every morning for a 10-week period. The analysis included nutritional and plasma biochemical parameters, systolic blood pressure, oxidative parameters in plasma, heart, and cardiac analyses in vivo and in vitro. A comparison among the groups was performed by two-way analysis of variance (ANOVA). Results: The HSF diet was able to induce obesity, insulin-resistance, cardiac dysfunction, and oxidative damage. However, the tomato-oleoresin supplementation improved insulin-resistance, cardiac remodeling, and dysfunction by improving the β-adrenergic response. It is possible to conclude that tomato-oleoresin is able to reduce the oxidative damage by improving the system's β-adrenergic response, thus recovering cardiac function.

Keywords:high sugar-fat diet; obesity; β-adrenergic system; cardiac dysfunction; lycopene; tomato-oleoresin

Capítulo 1

Revisão de Literatura

1. Obesidade e desempenho cardíaco

Estudos clínicos mostram que o excesso de gordura acarreta diversas anormalidades cardíacas, entre elas, alterações morfológicas, hemodinâmicas e funcionais, que se correlacionam com a duração e intensidade da obesidade[1,2]. Diversos estudos experimentais têm demonstrado que a obesidade induzida por diferentes tipos de dietas (com alto teor de gordura e/ou altamente energéticas) resultam em disfunção miocárdica em roedores[3–7]. Alguns autores observaram prejuízo funcional em cardiomiócitos isolados, com depressão no pico de encurtamento e atraso no tempo de relaxamento, em modelos de obesidade induzidos por dietas ricas em gordura[3,5]. Estudos recentes tem revelado que ratos obesos alimentados com dieta rica em gordura, durante 15 semanas, apresentam disfunção miocárdica em condições basais e após manobras inotrópicas[6,7].

Embora seja evidente que uma variedade de alterações e/ou danos no desempenho cardíaco ocorram com a elevação do acúmulo de tecido adiposo, os mecanismos responsáveis por estas alterações não estão estabelecidos. Diversos fatores têm sido apontados como possíveis responsáveis por anormalidades cardíacas em modelos de obesidade[3,6], entre eles, o sistema beta (β)-adrenérgico, um importante mecanismo de regulação da contração e relaxamento do miocárdio[8,9]. O sistema β-adrenérgico é um dos principais mecanismos neuro-humorais de modulação da função cardíaca, atuando, tanto em condições fisiológicas quanto patológicas[8,10,11]. A via β-adrenérgica é constituída por adrenoreceptores β, proteínas de ligação ativadora e inibitória

(G_s e G_i), adenilato ciclase e adenosina monofosfato cíclico (AMPc)[12]. O tecido cardíaco é composto por três subtipos de β-adrenoreceptores, β_1, β_2 e β_3. Os receptores β_1 e β_2 são expressos na proporção de 75 a 80% e 15 a 18%, respectivamente[13], sendo que, ambos promovem efeitos inotrópicos, cronotrópicos e lusitrópicos positivos em resposta à um β-agonista[13,14]. Por outro lado, os receptores $\beta_{3,}$ (que representam 2 a 3% em condições normais) agem como mediadores do efeito inotrópico negativo[15]. Os receptores β_1 e β_2-adrenérgicos são acoplados à proteína G estimulatória (G_s), que acarreta ativação da adenilato ciclase e, posteriormente, aumento dos níveis de AMPc. O acúmulo de AMPc resulta em maior ativação da proteína quinase A (PKA) desencadeando alterações no ciclo Ca^{+2} intracelular, uma vez que, a PKA fosforila diversas proteínas essenciais para função cardíaca, incluindo os canais de cálcio do tipo L[16,17], fosfolambam[18], troponina I[19], e os receptores de rianodina[20,21] (Figura 1).

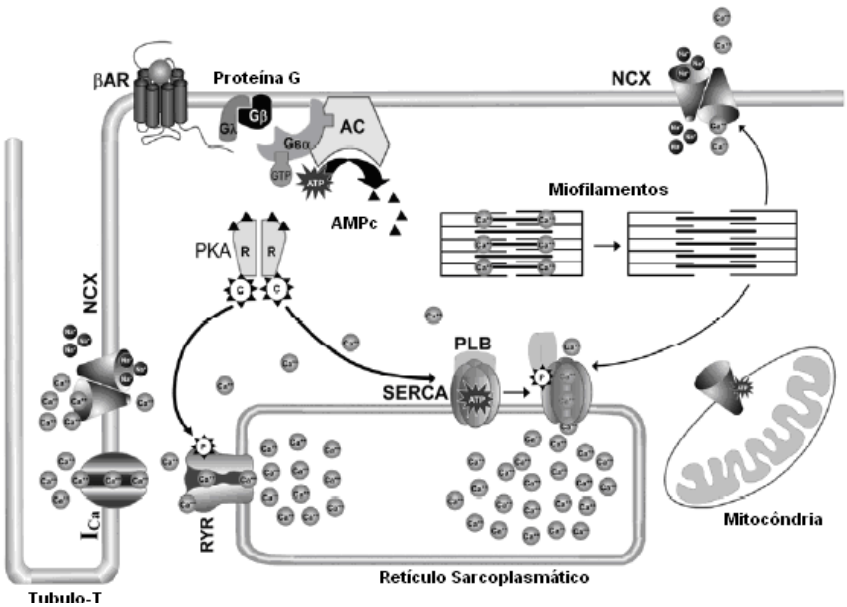

Figura 1. Diagrama esquemático dos efeitos gerais da ativação β-adrenérgica

em diferentes proteínas do ciclo de Ca^{+2} intracelular (modificado de Brum et al)[21].

A regulação da função cardíaca pelo sistema β-adrenérgico é mediada também pela atividade da proteína G, que possui a função de promover a ligação do receptor β à adenilato ciclase para que ocorra a formação de AMPc. A atividade da adenilato ciclase é modulada por duas proteínas G: G_s com capacidade de estimular e, G_i, capaz de inibir a ativação da adenilato ciclase[22]. A proteína G_s é formada pelas subunidades α, β e γ na forma inativa; a subunidade $G_s\alpha$ encontra-se acoplada à guanina difosfato (GDP). Após ação de um agonista sobre o receptor β, a $G_s\alpha$ substitui o GDP por guanina trifosfato (GTP), separa-se das subunidades β e γ, e, interage com a adenilato ciclase que, ao ser ativada, produz AMPc[12,23](Figura 2).

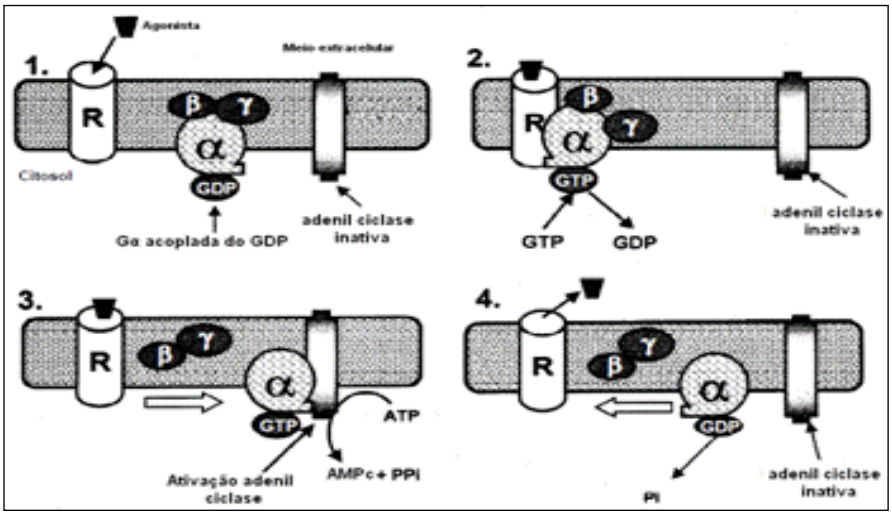

Figura 2. Representação esquemática do acoplamento entre R: receptores β-adrenérgicos e a proteína $G_s\alpha$; GTP, Guanina trifosfato; GDP, Guanina difosfato; Pi fósforo inorgânico; PPi difosfato inorgânico; AMPc, Adenosina monofosfato cíclico.(Modificado de Evora et al[24]).

Embora seja bem estabelecido que os receptores β-adrenérgicos e a

proteína G em cardiomiócitos desempenham funções importantes na regulação do desempenho cardíaco na obesidade, poucos estudos[25,26] abordam a relação entre a função cardíaca e a via β-adrenérgica. Esses autores mostram redução da resposta contrátil cardíaca à estimulação β-adrenérgica. Anormalidades cardíacas relacionadas à obesidade e inflamação subsidiam fibrose e apoptose celular e alguns biomarcadores indicam a presença de disfunção cardíaca[27].

Exposições crônicas a ROS estão associadas com estimulação adrenérgica sustentada podendo resultar em efeitos deletérios e contribuir para o desenvolvimento de arritmias cardíacas e insuficiência cardíaca[28]. O contato com o peróxido de hidrogênio (H_2O_2) aumenta a amplitude de transiente do cálcio, portanto, desempenhando papel modulador na contratilidade miocárdica[28].

No entanto, o estado regulatório destes biomarcadores na obesidade e sua relação direta com a presença de prejuízo cardíaco permanecem incertos. Embora dados recentes enfatizem a relação entre obesidade e desempenho cardíaco, há necessidade de esclarecimentos. Estudos têm demonstrado que as funções sistólica e diastólica do ventrículo esquerdo (VE) estão comprometidas em portadores de síndrome metabólica, mesmo que tenham fração de ejeção do VE (FEVE) normal[29]. No entanto, outros estudos têm revelado que a obesidade está apenas associada com remodelação concêntrica do VE sem mudança funcional[30].

2. Estado redox e obesidade

A alteração redox é o evento resultante do desequilíbrio de magnitude

entre a produção de espécies reativas (do oxigênio, ROS e nitrogênio, RNS) e o sistema de defesa antioxidante. Tal desequilíbrio tem como consequência a oxidação de importantes elementos biológicos (lipídeos, proteínas, carboidratos e DNA) o que leva a modificações químicas, geométricas e funcionais de tais elementos. A oxidação dos elementos resultam em produtos finais que, podem ser aferidos, refletindo indiretamente a magnitude do dano oxidativo[31]. Este desequilíbrio tem sido apontado como importante mecanismo de lesão de várias doenças como a obesidade[31].

O desenvolvimento de obesidade e de síndrome metabólica (SM) resulta da combinação de fatores genéticos e ambientais como a inadequação alimentar[32]. O processo fisiopatológico da SM é de origem multifatorial, podendo cursar de maneiras diferentes até o desfecho final que pode ser Diabetes *Mellitus* tipo 2 (DM-2) e/ou doenças cardiovasculares (DCV). Neste contexto, a SM parece ser desencadeada pelo aumento de adiposidade com subsequente desenvolvimento de resistência insulínica, aumento da pressão arterial e dislipidemia.

É bem conhecida a relação entre hiperadiposidade, inflamação e alteração do estado redox[33]. O tecido adiposo produz diversas adipocinas, dentre elas leptina, adiponectina, resistina, citocinas pró-inflamatórias, como interleucinas 1 (IL-1) e 6 (IL-6), e fator de necrose tumoral α (TNF-*α)* e o inibidor do ativador do plasminogênio tipo 1 (PAI-1), os quais são envolvidos em respostas pró-inflamatórias e pró-trombóticas[34]. Excluindo-se causas genéticas da obesidade, o balanço energético positivo representa um estímulo

para que os adipócitos estoquem energia em forma de triglicerídios. A partir do momento que existe hipertrofia dos adipócitos, inicia-se processo patológico resultante da disfunção endócrina do tecido adiposo[35], onde ele passa a produzir quantidades exacerbadas de algumas adipocinas, com desenvolvimento um estado pró-inflamatório [36]. Dessa maneira, a adipocina *monocyte chemoattractant protein -1* (MCP-1), em especial, atrai monócitos para o tecido adiposo, iniciando assim, um processo inflamatório local. A hipertrofia dos adipócitos pode ocorrer no tecido adiposo subcutâneo, epididimal e visceral, porém este último parece ter consequências patológicas caracterizadas por uma mudança funcional com maior produção de resistina e leptina, e menor de adiponectina[37]. Em particular, resistina e leptina parecem ser expressas na presença de inflamação[38], enquanto que a adiponectina parece bloquear a expressão de TNF-α[39].

O aumento da concentração plasmática de glicose e de ácidos graxos livres, frequente presente na obesidade, é outro fator que contribui para a elevação da produção de espécies reativas. Em desequilíbrio com a atividade antioxidante, o excesso de ROS e RNS se constitui em estímulo para o estresse inflamatório, que por sua vez também produz espécies reativas adicionais, estabelecendo um ciclo patológico, onde ambos eventos (redox e inflamatório) se retro-alimentam positivamente, caracterizando os estados pró-inflamatório e pró-oxidante [40].

A oxidação de lipídios gera vários produtos finais mensuráveis, entre eles o 4-hidroxinonenal (4-HNE) e o malondialdeído (MDA). O 4-HNE pode ser

formado após a oxidação, via espécies reativas, de ácidos graxos insaturados ω-6 tais como linolênico, linoleico e araquidônico[41]. É o biomarcador de peroxidação lipídica mais estudado e o elevado corpo de conhecimento é justificado pelas consequências patológicas atribuídas. O 4-HNE é aldeído altamente citotóxico que, se acumula no coração em resposta à lipoperoxidação. O aldeído forma adutos de proteína por meio de uma reação que permite a criação de ligações carbono – carbono e também de uma ligação carbono - enxofre (adutos de Michael)[42]. Foi demonstrado que, danos induzidos no DNA por adutos de aldeídos também inibem enzimas catalisadoras e detoxificadoras[43]. Outro fator que faz do 4-HNE uma molécula chave, é a inibição da contratilidade do músculo cardíaco[44].

O MDA é outro subproduto da lipoperoxidação enzimática e não enzimática de ácidos graxos poli-insaturados (PUFAs) como os ácidos araquidônico e docosahexaenoico após clivagem de suas ligações duplas e liberação de bis-aldeído- malondialdeído[45]. Concentrações elevadas de subprodutos da peroxidação lipídica tem sido associadas à presença de síndrome metabólica[46]. Recente estudo mostrou que a toxicidade resultante da presença de hiperglicemia, resistência insulínica e hipertrigliceridemia determinam a elevação nas concentrações de MDA em adultos com SM[47]. Além disso, prévios estudos mostraram que a estimulação β-adrenérgica aumenta a concentração de MDA em presença de noraepinefrina[48]; isoproterenol [28].

Concentrações elevadas de produtos finais da lipoxidação como MDA,

acroleína, glioxal e principalmente o 4-HNE, citados acima, podem promover carbonilação de proteínas[49]. O processo de oxidação de proteínas pode ocorrer diretamente via ROS/RNS ou indiretamente via produtos finais da lipoxidação. Em geral, a oxidação de proteínas confere modificações à estrutura geométrica proteica. O processo tóxico direto é caracterizado pela adição de radical nitrogenado enquanto que o indireto consiste na formação de grupamentos carbonila (aldeídos e cetonas) em determinados aminoácidos. A ação direta comumente modifica tirosina, arginina, lisina, prolina e treonina, enquanto que a ação indireta modifica cisteína, histidina e lisina presentes nas estruturas proteicas[50].

É difícil elucidar se a modificação ocorrida em determinada proteína foi proveniente de processo direto ou indireto, porém certos biomarcadores indicam a presença do processo (direto ou indireto) e sua magnitude. Como exemplo, 3-nitrotirosina (3-NT) é considerado biomarcador da oxidação direta de proteínas. Produção elevada de radical superóxido (O_2^-) e incapacidade de sua detoxificação enzimática facilitam sua combinação com o óxido nítrico (NO) resultando no radical peroxinitrito ($ONOO^-$), uma das espécies mais reativas[51]. O $ONOO^-$ é um potente agente de nitração de proteínas, agindo principalmente sobre resíduos de tirosina dando origem ao 3-NT ligado à proteína[52]. A presença dos íons metálicos Fe^{2+} e Cu^+ catalisam reações de nitração. Outra forma de oxidação de proteínas é a indireta. Neste processo, as proteínas sofrem ação de radicais lipídicos previamente oxidados por ROS e RNS. Os produtos finais da lipoxidação (4-HNE, malondialdeído e acroleína)

estão implicados neste processo oxidativo. A modificação proteica produzida corresponde ao acréscimo de grupamento carbonilas, principalmente em resíduos de cisteína, lisina e histidina[51,52]. A carbonilação de proteínas mensurada por método espectrofotométrico é direto e inespecífico, pois é identificada a presença de grupamentos cetona e aldeído na estrutura proteica. Porém, é amplamente utilizado e é considerado um dos melhores biomarcadores de dano oxidativo a proteínas[53].

Neste sentido as proteínas também podem ser alvos de glicação ou como conhecido atualmente da reação de Maillard, descrita como uma reação entre açúcares redutores e aminoácidos, que levam à formação de uma variedade de compostos chamados produtos de glicação (AGEs). Sua formação tem sido associada a importantes efeitos pró-oxidativos e pró-inflamatórios envolvidos nas alterações metabólicas inerentes a patogênese e à progressão de inúmeras doenças. Do ponto de vista químico, os AGEs são especialmente representados por proteínas covalentemente modificadas por processos oxidativos e não-oxidativos, envolvendo açúcares ou seus produtos de degradação. Adicionalmente, AGEs podem apresentar estrutura similar, desde que são originados de precursores comuns, como é o caso da Nε-carboximetilisina (CML), gerada a partir do glioxal (GO), o qual, por sua vez, pode ser formado por meio da oxidação dos açúcares. Portanto sendo a formação/concentração de CML um importante marcador no processo de aferição da glicação de proteínas.[54,55]

As consequências biológicas dos processos de glicação, oxidação,

carbonilação e nitração de proteínas vão desde a deformidade geométrica até a perda de funções fisiológicas no organismo. Exemplos são: a) aumento da contratilidade e hipertrofia cardíaca; b) deformação estrutural dos receptores β-adrenérgicos e a consequente incapacidade de ligação com seu agonista; e c) perda da capacidade de atuação do seu antagonista[56–58].

3. Sistema antioxidante na obesidade

Antioxidante corresponde a qualquer substância que em mínima concentração é capaz de impedir a oxidação de biomoléculas. Os antioxidantes podem ser classificados como endógenos e exógenos. Antioxidantes endógenos são constituídos pelo sistema glutationa, catalase, superóxido dismutase, produtos catabólicos (ácido úrico e bilirrubina) e proteínas circulantes como albumina e ceruloplasmina. Antioxidantes exógenos são constituídos por compostos naturais obtidos via alimentação, ou seja, não sintetizados pelo organismo. Ácido ascórbico, tocoferóis, carotenoides, compostos fenólicos e alguns aminoácidos são exemplos de antioxidantes exógenos[59].

Estudos clínicos e experimentais tem constatado a eficácia da suplementação com antioxidantes nas manifestações clínicas e laboratoriais da SM. Exemplo é a vitamina E que parece melhorar a ação da insulina e reduzir a peroxidação lipídica[60], enquanto que o ácido ascórbico parece estar associado com melhora do tônus vascular[61], e também com a diminuição nos níveis de MDA e aumento da capacidade antioxidante hidrofílica (FRAP) em indivíduos

hipertensos[62]. Humanos portadores de SM frequentemente consomem menos frutas e verduras, e consequentemente apresentam uma menor ingestão de antioxidantes e menores concentrações plasmáticas de ácido ascórbico e carotenoides[63]. Nestes indivíduos, a adequação alimentar com fibras alimentares provenientes da maior ingestão de frutas e verduras foi capaz de elevar as concentrações plasmáticas do β-caroteno[64]. A associação de ingestão de antioxidantes com o exercício físico também pode interferir no sistema antioxidante e nas manifestações da SM.

Estudo recente mostrou que a adequação alimentar (ingestão de frutas e verduras) associada à prática de exercício físico por 20 semanas resultou em maior condicionamento cardiorrespiratório em residentes da cidade de Botucatu, SP. Além disso, foi observada redução da adiposidade visceral (circunferência abdominal), redução da prevalência de SM e, principalmente, aumento significante das concentrações de glutationa e de capacidade antioxidante total (TAP) do plasma[65]. Portanto, é possível considerar que antioxidantes participam não só da detoxificação de espécies reativas, mas também estão intimamente relacionados à modulação de processos fisiopatológicos presentes na obesidade.

Para nosso conhecimento, este é o primeiro estudo a avaliar o efeito de carotenóides no sistema β-adrenérgico de sujeitos obesos. De fato, o papel de antioxidantes nesse sistema tem sido abordado em condições diferentes da obesidade. Ausência de efeito da suplementação com vitamina C nos níveis plasmáticos de neurotransmissores foi observada em portadores de hipertensão

arterial, situação em que o estado catecolaminérgico de noradrenalina está aumentado[62]. Por outro lado, a n-acetil cisteína administrada agudamente, alterou a resposta β-adrenérgica em estudo *in vitro* com cardiomiócitos estimulados com isoproterenol. Os autores mostraram que o antioxidante diminuiu a produção de ROS e também reduziu a atividade inotrópica positiva (com consequente atenuação da amplitude transiente de Ca^{2+} e piora da contratilidade celular)[28]. Examinando pacientes portadores de insuficiência cardíaca (caracterizada pelo aumento da atividade simpática), estudo prévio mostrou que a administração aguda de vitamina C foi eficaz em restaurar o controle vagal da frequência cardíaca e melhorar a sensibilidade do barorreflexo agudamente. Os autores sugerem a necessidade de trabalhos adicionais que avaliem a administração crônica de antioxidantes[66].

4. Licopeno

O licopeno é um carotenóide sem atividade pró-vitamina A encontrado no tomate e nas frutas vermelhas. Em decorrência de seu grande número de duplas ligações conjugadas (figura 3), o licopeno é considerado um dos melhores antioxidantes dentre os carotenóides. Outro atributo do licopeno se refere à sua altíssima capacidade em se ligar ao oxigênio *singlet*[67] apresentando capacidade antioxidante 2 vezes maior que o β-caroteno e 10 vezes maior que o α-tocoferol[68]. Além do oxigênio *singlet* e o radical peroxil[69], observa-se forte interação entre o licopeno e outras ROS como o H_2O_2[70].

A proteção do licopeno contra a oxidação de bases de DNA foi

recentemente demonstrada por nosso grupo em cardiomiócitos de ratos Wistar submetidos à cardiotoxicidade e suplementados com licopeno (5mg/kg/d/7semanas) na forma licopeno-oleoresina[71]. Usando modelo experimental de obesidade, observamos que a suplementação de licopeno (10mg/kg/6semanas) foi associada à menor concentração plasmática e menor nível de expressão gênica de leptina, resistina e IL-6 no tecido adiposo epididimal de ratos[72] e ao aumento da expressão gênica de adiponectina no plasma e no tecido adiposo epididimal além de aumento da expressão gênica de SIRT1 e FoxO1[73]. Como SIRT1 e Fox1 estão envolvidos na regulação da transcrição da adiponectina e tal adipocina é considerada protetora (contra aterogênese, contra a diminuição da sensibilidade à insulina e possui função anti-inflamatória), o aumento de sua expressão pela suplementação com licopeno pode sugerir um papel importante na obesidade e suas co-morbidades.

Para o nosso conhecimento, não existem estudos que tenham avaliado o efeito da suplementação com licopeno na oxidação de proteínas associada à obesidade. Por outro lado, foi observado benefício da suplementação com licopeno (25 mg/dia/5 semanas) nos níveis plasmáticos de proteína carbonilada após exercício físico intenso (na 72ª h) de portadores de isquemia miocárdica, doença frequentemente associada à obesidade [74].

A suplementação com licopeno (60 mg/dia de licopeno/3 meses) foi associada à redução significante das concentrações de LDL plasmático em homens (30 a 35 anos). O estudo ainda mostrou que a adição de licopeno à cultura de macrófagos resultou em diminuição de uma co-enzima importante

na síntese do colesterol [macrophage 3-hydroxy-3-methyl glutaryl coenzyme A (HMGCoA) reductase][75]. A suplementação com licopeno na forma de extrato de tomate (15 mg licopeno/dia/8 semanas) resultou em melhora da pressão sistólica e diastólica e da oxidação da LDL (induzida por $CuSO_4^-$) em portadores (30 a 70 anos) de hipertensão arterial sistêmica grau I[76]. Examinando ratos adultos Sprague Dawley hiperglicêmicos, estudo prévio mostrou que suplementação de licopeno (doses de 10, 30, 60 e 90 mg/kg) resultou em efeito dose-dependente na diminuição da concentração de glicose, aumento de insulina, diminuição na peroxidação lipídica e aumento de enzimas antioxidantes[77]. Os resultados sugerem que a suplementação com licopeno pode contribuir com a atenuação do desequilíbrio no estado redox nesse modelo. Ratos adultos Sprague Dawley hiperlipidêmicos suplementados com tomate em pó, pasta e ketchup (10 ou 20 mg de licopeno/kg de dieta) mostraram melhora em todos os parâmetros lipídicos. Além disso, este estudo demonstrou que a menor dose de licopeno (10mg/kg dieta) do tomate em pasta atingiu o melhor índice aterogênico e uma significante elevação da lipoproteína de alta densidade do colesterol (HDL)[78]. Em modelo de isquemia e reperfusão no coração de ratos adultos Wistar foi observado que o licopeno diminuiu os danos causados pela peroxidação lipídica, aumentou a concentração de enzimas antioxidantes e melhorou parâmetros hemodinâmicos, suprimindo o desequilíbrio no estado redox e reduzindo injúria do miocárdio[79]. Em camundongos hipercolesterolêmicos foi observado que o suco concentrado do tomate adicionado à dieta (20g de licopeno/100g da dieta) preveniu a

aterosclerose, protegendo os lipídeos plasmáticos da oxidação[80]. Recente estudo clínico não mostrou efeitos benéficos da suplementação com licopeno (30mg/dia por 4 semanas) sobre marcadores inflamatórios em portadores de obesidade grave (IMC 37.5 ± 2.5 kg/m^2)[81].

O nível plasmático de licopeno também tem sido associado a doença cardíaca isquêmica e dislipidemia. Importante estudo multicêntrico europeu realizado com homens (≤ 70 anos) mostrou associação inversa entre risco de infarto agudo do miocárdio (IAM) e concentrações plasmáticas de licopeno. Este estudo ainda mostrou que o licopeno foi o único carotenóide a apresentar associação independente para baixo risco de IAM [82.] Outros autores identificaram diminuição na concentração plasmática de licopeno em pacientes (homens e mulheres com idade média 55 anos) portadores de dislipidemia (colesterol total ≥ 240mg/dL e de triglicérides ≥ 250mg/dL)[83].

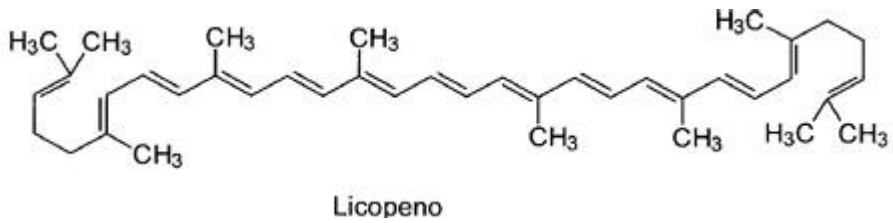

Figura 3. Fórmula estrutural do licopeno.

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Justificativa e hipótese

A indução de obesidade por dieta hiperlipídica e hiperenergética, contendo 45% das gorduras proveniente de banha de porco e 78% de carboidrato (53,5% na ração + 25% de sacarose na água de beber) está associada a maior deposição de gordura no tecido adiposo (hipertrofia de adipócitos) e disfunção cardíaca. Portanto, esta via de indução de obesidade resulta em várias alterações fisiopatológicas características da obesidade, como a presença de desequilíbrio no estado redox.

O desequilíbrio do estado redox é um dos mecanismos associados à obesidade onde há elevada produção e liberação de ROS/RNS e atividade antioxidante ineficiente. Exposições crônicas a ROS estão associadas com estimulação adrenérgica sustentada podendo resultar em efeitos deletérios e contribuir para o desenvolvimento de insuficiência cardíaca. Tendo em vista a associação entre obesidade, desequilíbrio do estado redox e disfunção cardíaca (via sistema β-adrenérgico), o presente estudo tem como hipótese que, a suplementação com licopeno em animais obesos resulta em maior proteção antioxidante com atenuação do estado pró-oxidante, e consequente restabelecimento da função miocárdica via regulação positiva da modulação β-adrenérgica cardíaca. A presença de antioxidantes, em especial o licopeno, orquestram processos metabólicos do organismo, atenuando a gravidade de fatores de risco de co-morbidades associadas à obesidade e restabelecendo a funcionalidade de órgãos vitais.

Capítulo 2

Artigo Científico

Tomato-oleoresin supplement atenuates obesity-induced cardiac dysfunction and inflammation

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Short title: Diet, obesity and Lycopene

Abstract

Introduction: Considering the importance of the inflammation in the pathogenesis of obesity and its cardiac disorders. The lycopene, carotenoid present in tomato has anti-inflammatory properties previously demonstrated by our research group. **Aim**: To evaluate the effect of tomato-oleoresin on cardiac inflammatory parameters and the impact on the cardiac calcium handling in diet- induced obesity in rats. **Materials and Methods:** 2 experimental groups (Control, n 20) and high sugar- fat diet (HSF, n=20) for 20 weeks. Was detected the cardiac dysfunction by echocardiogram in HSF group, animals were randomly divided to begin the treatment with Tomato-oleoresin, perfoming four groups: Control (n 10); (Control+Ly, n 10); HSF (n 10) and (HSF+Ly, n 10). Tomato oleoresin 10mg lycopene/kg body weight (BW) per day and given orally every morning for a 10-week period. It was analyzed nutritional parameters, biochemical parameters, systolic blood pressure, plasma and cardiac inflammatory parameters and cardiac function analyses in vivo and in vitro. Groups compare by using two-way analysis of variance (ANOVA). **Results**: HSF diet induced obesity, insulin-resistance, cardiac dysfunction and cardiac inflammation. However, the Tomato-oleoresin supplementation improved the insulin-resistance, cardiac remodeling and dysfunction by treatment cardiac inflammation. **Conclusion:** It is possible to conclude that tomato-oleoresin is able to modulate cardiac inflammatory parameters attenuating the cardiac dysfunction.

Key words: Obesity; inflammation, cardiac disfunction; Tomato-oleoresin.

1. Introduction

Several researches have been demonstrated the effect of obesity on cardiac dysfunction[1–5]. Some authors observed function impairment in isolated cardiomyocyte, with decrease in the peak shortening and also delay relaxation in diet-induced obesity model[1,3]. *In vitro* studies already reported cardiac dysfunction related to calcium handling in both basal condition and after maneuver in calcium concentration[4–7]. In front of this, calcium kinetic change seems to be one cause for the cardiac abnormalities in obese models[1,2]. However, the exact mechanism responsible for these changes is not well established.

Recently, the literature reports the role of inflammation on cardiac remodeling and dysfunction[8,9]. However, how the inflammation is able to lead to cardiac dysfunction needs to be clarify[10–13]. The adipose tissue produces many adipokines, among them leptin, adiponectin, proinflammatory cytokines- as interleukin 6 IL-6 and tumor necrosis factor α (TNF-*α)*[14]- and *monocyte chemoattractant protein -1* (MCP-1). However, in obesity condition occurs an increase in the production of this adipokines[15], initiating a local inflammatory process that later is able to reache other organs[16]. In the heart, pro-inflammatory cytokines, such as TNF-α and IL-6, seems to cause cardiomyocytes apoptosis and necrosis as well as cells hypertrophy[17]. Increased levels of TNF-a and IL-6 have been described in patients with chronic heart failure, showing a positive correlation between inflammation with disease severity[18,19].

Considering the importance of the inflammation in the pathogenesis of obesity and its cardiac disorders, therapeutic strategies using anti-inflammatory have been tested. The lycopene, carotenoid present in tomato and red fruits has anti-inflammatory properties previously demonstrated by our research group[20]. The relation between lycopene and cardiovascular disease has been evaluated in clinic[21,22] and experimental studies[23]. Although obesity and inflammation are able to lead to cardiac dysfunction, no studies investigated the anti-inflammatory effect of lycopene and consequently the cardiac modulation. The

aim of this study was to test if the tomato-oleoresin supplementation would be able to treat cardiac dysfunction and remodeling related to the high sugar fat diet consumption by modulating cardiac inflammation

2. Materials and Methods

2.1.Animals and Experimental Protocol

All the experiments and procedures were approved by the Animal Ethics Committee of Botucatu Medical School (1196/2016) and were performed in accordance with the National Institute of Health's Guide for the Care and Use of Laboratory Animals. Male Wistar rats (±187 g) were kept in an environmental controlled room (22 °C ± 3 °C; 12 h light-dark cycle and relative humidity of 60 ± 5%) and initially randomly divided into 2 experimental groups (Control, n 20) and high sugar- fat diet (HSF, n=20) for 20 weeks. HSF groups also received water + sucrose (25%). The diets and water were provided *ad libitum*. The HSF diet contained soybean meal, sorghum, soybean peel, dextrin, sucrose, fructose, lard, vitamins, and minerals, plus 25% sucrose in drinking water; Control diet contained soybean meal, sorghum, soybean peel, dextrin, soy oil, vitamins, and minerals. The nutrients and nutritional composition of each diet was described in our previous studies[24,25].

At week 20 of this study, when was detected the cardiac dysfunction by echocardiogram in HSF group, animals were randomly divided to begin the treatment with tomato-oleoresin, perfoming four groups: Control (n 10); Control supplemented with lycopene-rich tomato oleoresin (Control+Ly, n

10); HSF (n 10) and HSF supplemented with lycopene-rich tomato oleoresin (HSF+Ly, n 10). Tomato oleoresin was mixed with maize oil equivalent to 10mg lycopene/kg body weight (BW) per day and given orally every morning for a 10-week period[20,26]. To avoid differences in the energy provided, all groups received the same maize oil volume (approximately 2 ml/kg BW per day).

Feed consumption (FC) was measured daily and body weight (BW) was assessed weekly. Caloric intake (CI) for the control group was calculated according to the following formula: caloric intake (kcal/day) = feed consumption (g) × dietary energy (3.59 kcal/g). For the animals that received the HFS diet, the energy intake was calculated according to the formula: volume consumed (mL) × 0.25 (equivalent to 25% fructose) × 4 (calories per gram of carbohydrate) + caloric values offered by feeding (feed consumption (g) × dietary energy (4.35 kcal/g). Feed efficiency (FE) is the ability to convert caloric intake to BW and it was determined as follows: FE(%) = BW gain (g)/total caloric intake (kcal) × 100[24,25]. The adiposity index was calculated using the following formula: adiposity index = (total body fat (BF)/final body weight) x 100. BF was measured from the sum of the individual fat pad weights: BF = epididymal fat + retroperitoneal fat + visceral fat.

2.2. Lycopene preparation

Tomato oleoresin (Lyc-O-Mato 6% dewaxed; LycoRed Natural Products Industries) was mixed with maize oil and stored at 4ºC in the dark until used as described previously[27]. The tomato oleoresin–maize oil mixture

was stirred for 20 min in a water-bath at 54ºC before being fed to the animals. Each millilitre of the solution contained 5mg of total lycopene. Stability of lycopene was monitored at 450nm, and confirmed by diode-array spectra, as described previously[28].

2.3.Plasma Measurements

After 12-h fasting, blood was collected from the tail and the plasma was used for biochemical analysis. Plasma glucose was determined by using a glucometer (Accu-Chek Performa; Roche Diagnostics, Indianapolis, IN, USA). Insulin level was measured by enzyme-linked immunosorbent assay (ELISA) method using commercial kits (Millipore)[24].

2.4.Homeostatic Model Assessment Index (HOMA-IR)

The homeostatic model of insulin resistance (HOMA-IR) was used as an insulin resistance index, and it was calculated using the following formula: HOMA-IR = [fasting glucose (mmol/L) × fasting insulin (μU/mL)]/22.5[29].

2.5.Systolic Blood Pressure (SBP)

SBP evaluation was assessed in conscious rats by the non-invasive tail-cuff method with a NarcoBioSystems® Electro-Sphygmomanometer (International Biomedical, Austin, TX, USA). The animals were warmed in a wooden box (50 × 40 cm) between 38–40 °C with heat generated by two incandescent lamps for 4–5 min to cause arterial vasodilation in the tail and were then transferred to an iron cylindrical support that was specially designed

to allow total exposure of the animal's tail. After this procedure, a cuff with a pneumatic pulse sensor was attached to the tail of each animal. The cuff was inflated to 200 mmHg pressure and subsequently deflated. The blood pressure values were recorded on a Gould RS 3200 polygraph (Gould Instrumental Valley View, Ohio, USA). The average of two pressure readings was recorded for each animal.

2.6. Inflammatory parameters

Cardiac tissue (±150mg) was homogenized (ULTRA-TURRAX® T25 basic IKA® Werke Staufen/Germany) in 1.0 mL of Phosphate Buffered Saline (PBS) pH 7.4 cold solution, and centrifuged at 800g at 4°C for 10 min. The supernatant (100uL) was used to analysis. Tumoral necrosis factor- alpha (TNF-α), interleukin-6 (IL-6) and monocyte chemo attractant protein- 1 (MCP-1) levels were measured using the enzyme-linked immunosorbent assay (ELISA) method using commercial kits from R&D System, Minneapolis, USA. The supernatant (100uL) was used to analysis and the results were corrected by the protein amount.

2.7.Echocardiographic study

All the animals were evaluated *in vivo* by transthoracic echocardiography, using a Vivid S6 system equipped with multifrequency ultrasonic transducer 5.0 to 11.5 MHz (General Electric Medical Systems, Tirat Carmel, Israel). All exams were performed by the same examiner and obtained according to the

leading-edge method recommended by the American Society of Echocardiography. Rats were lightly anesthetized by intramuscular injection with a mixture of ketamine (50 mg/kg) and xylazine (1 mg/kg). After shaving their chest, the animals were placed in left decubitus position. To implement structural measures of the heart, the images were obtained in one-dimensional mode (M-mode) guided by the images in two-dimensional mode with the transducer in the parasternal position, minor axis. Left ventricular (LV) evaluation was performed by positioning the cursor M-mode just below the mitral valve plane at the level of the papillary muscles. The images of the aorta and left atrium were obtained by positioning the M-mode course to plan the level of the aortic valve. The following cardiac structures were measured: diameter of the aorta (DA) and left atrium (LA). The relative thickness of the LV (RWT) was calculated by dividing LVPWD multiplied by two by LVDD. Left ventricular mass (LVM) was calculated using the formula [(LVDD +LVPWD IVSDT) 3 − (LVDD) 3] × 1.04 where 1.04 is the specific density of the myocardium. MVE index (LVMI) was calculated by normalizing to body weight Estimated LV mass. The LV systolic function was assessed by the following parameters: percentage of endocardial shortening (Δ% endo) [(LVDD − LVSD)/LVDD] × 100; midwall fractional shortening (% Δ meso) {[(LVDD + ½ + ½ LVPWD IVSDT) − (LVSD + ½ + ½ IVSST LVPWS)]/(LVDD + ½ + ½ LVPWD IVSDT)} × 100. The LV diastolic function was evaluated by the following indices: peak velocity of early diastolic filling (E wave); peak velocity of late diastolic filling (A wave); ratio

between the E and A waves (E/A); isovolumetric relaxation time in absolute values (IRT). The joint assessment of diastolic and systolic LV function was performed by myocardial performance index also known as Tei index (sum of isovolumetric contraction and IRT time, divided by the left ventricular ejection time). The study was supplemented by evaluation by tissue Doppler early diastolic (E'), and late (A') of the mitral annulus (arithmetic average travel speeds of lateral and septal walls), and the ratio by the waves A and E'(E'/A).

2.8.Post-mortem morphological analysis

Rats were euthanised by thoracotomy, and the hearts, ventricles and tibia were separated, dissected, weighed and measured. Cardiac remodelling at the macroscopic level, which identifies the presence or absence of cardiac hypertrophy, was determined by analysing the following parameters: heart weight (HW), left ventricle (LV) weights, HW and LV/tibia length ratios.

2.9.Myocardial function by isolated papillary muscle study

Myocardial function was evaluated by studying isolated papillary muscles from the LV. This procedure has been utilised by various authors[4,5,30]. Conventional mechanical parameters at *Lmax* were calculated from isometric contraction: maximum developed tension normalised per cross-sectional area (DT [g/mm^2]), resting tension normalised per cross-sectional area (RT [g/mm^2]), positive (+d*T*/d*t* [g/mm^2/s]) and negative (-d*T*/d*t* [g/mm^2/s]) tension derivative normalised per cross-sectional area of papillary muscle (CSA). To

determine the mechanism by which obesity induces negative inotropic effects on contractile function, the papillary muscles were evaluated under the baseline condition of 2.5mM Ca^{2+} and after inotropic and lusitropic maneuvers: increases in extracellular Ca^{2+} concentration (to test their effects on myofilament machinery) and post-rest contraction (PRC), mainly related to SR storage and release capacity[5,30]. Inotropic responses were recorded 5 min after the addition of each dose of extracellular Ca^{2+} (0.5, 1.0, 1.5, 2.0, 2.5, 3.0 and 3.5 mM) to the bathing solution. PRC was studied at an extracellular Ca^{2+} concentration of 0.5 mM, where the stimulus was paused for 10, 30, and 60 s before restarting the stimulation.

2.10. Statistical Analysis

Results are expressed as mean ± standard deviation (SD). Differences among the groups were determined by using two-way analysis of variance (ANOVA) for independent samples. A repeated-measures two-way ANOVA was utilized to evaluate the positive and negative inotropic effects on myocardial function. When significant dif- ferences were found ($P < 0.05$), Tukey post hoc test for multiple comparisons were carried out. All statistical analyses were performed using SigmaStat for Windows (Version 3.5).

3. Results

The table 1 shows the nutritional, metabolic, and cardiovascular analysis. Both HSF groups exhibited a higher final body weight, adiposity index, weight gain, feed efficiency and SBP than their respective control

groups. Additionally, tomato-oleoresin prevented higher levels of insulin and HOMA-IR in HSF+Ly compared to HSF group.

Table 1. Effect of lycopene supplementation on nutritional, metabolic and blood pressure alterations induced by high sugar-fat diet

	Groups				Effect		
	Control	Control + Ly	HSF	HSF + Ly	diet	Ly	interaction
Initial BW(g/day)	203 ± 24	211 ± 14	221 ± 12	205 ± 18	0.41	0.55	0.12
Final BW(g/day)	485 ± 28	454 ± 52	556* ± 80	552$^{\$}$ ± 38	<0.001	0.43	0.54
Adiposity index (%)	3.44 ± 0.47	4.06 ± 0.61	10.3* ± 1.2	9.71$^{\$}$ ± 0.77	<0.001	0.979	0.079
Food intake(g/day)	22.1 ± 8.5	24.7 ± 3.0	11.7* ± 1.2	13.1$^{\$}$ ± 0.8	<0.001	0.305	0.728
Water intake(ml/day)	31.8 ± 12.5	34.8 ± 3.6	47.1* ± 5.5	41.7 ± 3.0	0.001	0.670	0.165
Cal. intake(kcal/day)	79.1 ± 30	90 ± 11	102* ± 8	102 ± 6	0.016	0.470	0.432
Weight gain(g)	131 ± 29	117 ± 32.8	174* ± 40	188$^{\$}$ ± 64	0.004	0.446	0.999
Feed eff. (%)	0.75 ± 0.29	0.63 ± 0.17	1.17* ± 0.08	0.85$^{\$}$ ± 0.14	<0.001	0.009	0.205
Glucose	77.2 ± 4.8	75.2 ± 6.7	102* ± 21	92.2$^{\$}$ ± 10.1	<0.001	0.245	0.428
Insulin	0.89 ± 0.16	1.32 ± 0.12	5.29* ± 0.67	1.42$^{\#}$ ± 0.19	<0.001	<0.001	<0.001
HOMA-IR	6.5 ± 1.8	9.5 ± 1.3	52.2* ± 31.7	12.6$^{\#}$ ± 1.3	<0.001	<0.001	<0.001
SBP (mmHg)	120 ± 6	117 ± 7	143* ± 24	138$^{\$}$ ± 15	0.001	0.524	0.877

Data are expressed in mean ± standard deviation (n, 6 animals/group); groups, Control; Control+Ly, Control + Lycopene; HSF, high sugar-fat; HSF+Ly, high sugar-fat + Lycopene; Diets, Control [carbohydrate, 60%; fat, 4%] or High sugar-fat diet [carbohydrate, 78%; fat, 14.6%] for 30 wks; Supplementation, Lycopene 10 mg / (Kg body wt / day) or corn oil orally for 10 wks; Two-way ANOVA with Tukey post-hoc was used to compare differences among groups ($p < 0.05$), *HSF *vs* Control; $^{\#}$ HSF *vs* HSF+Ly; $^{\$}$HSF+Ly *vs* Control+Ly; BW, body weight(g/day); Cal. Intake, Caloric intake (Kcal/day); Feed. Eff., Feed efficiency (%); HOMA-IR, Homeostatic Model Assessment Index; SBP, Systolic blood pressure (mmHg).

The cardiac parameters are presented in the table 2. The diet promoted an increase of estimated mass, RWT and deterioration of systolic function, visualized by the decrease in the FS, ES cardiac output. Regarding the diastolic function, the diet promoted average E decrease.

Although the tomato-oleoresin did not change any variables in the control group, it leaded to a decrease of estimated LV mas, RWT and prevented the deterioration of systolic and diastolic function in HSF + Ly group.

Table 2. Effect of lycopene supplementation in vivo cardiac alterations induced by high sugar-fat diet

	Groups				Effect		
	Control	Control + Ly	HSF	HSF + Ly	Diet	Ly	interaction
Est. LV mass (g)	1.76 ± 0.04	1.63 ± 0.04	1.94* ± 0.04	1.72$^{\#}$ ± 0.04	0.004	<0.001	0.324
LA/DA	1.25 ± 0.03	1.26 ± 0.03	1.29 ± 0.03	1.26 ± 0.03	0.371	0.602	0.345
RWT	0.46 ± 0.01	0.44 ± 0.01	0.52* ± 0.01	0.46$^{\#}$ ± 0.01	0.002	0.002	0.142
Cardiac output	87.3 ± 4.5	81.5 ± 4.5	69.9* ± 4.530	78.4 ± 4.7	0.032	0.769	0.132
FS (%)	60.4 ± 1.3	60.9 ± 1.3	51.7* ± 1.3	59.4$^{\#}$ ± 1.4	<0.001	0.005	0.015
ES (%)	29.1 ± 1.5	29.3 ± 1.5	22.2* ± 1.5	28.2 ± 1.6	0.016	0.054	0.075
E/A waves	1.50 ± 0.09	1.58 ± 0.09	1.63 ± 0.09	1.63 ± 0.10	0.363	0.712	0.654
IRT (ms)	26.1 ± 0.9	23.4 ± 0.9	24.8 ± 0.9	20.8$^{\$}$ ± 1.0	0.066	0.002	0.551
TEI index	0.34 ± 0.03	0.26 ± 0.03	0.29 ± 0.034	0.31 ± 0.03	0.861	0.378	0.155
Lateral E(cm/s)	5.09 ± 0.21	5.21 ± 0.21	4.89 ± 0.21	5.15 ± 0.22	0.56	0.379	0.738
Septal E cm/s)	6.03 ± 0.18	6.28 ± 0.18	5.22* ± 0.18	5.62 ± 0.19	<0.001	0.094	0.69
Average E	5.56 ± 0.15	5.74 ± 0.15	5.05* ± 0.15	5.38 ± 0.16	0.009	0.102	0.633
E'/A	1.26 ± 0.08	1.37 ± 0.08	1.10 ± 0.08	1.38$^{\#}$ ± 0.08	0.323	0.024	0.318

Data are expressed in mean ± standard deviation (n, 6 animals/group); groups, Control; Control+Ly, Control + Lycopene; HSF, high sugar-fat; HSF+Ly, high sugar-fat + Lycopene; Diets, Control [carbohydrate, 60%; fat, 4%] or High sugar-fat diet [carbohydrate, 78%; fat, 14.6%] for 30 wks; Supplementation, Lycopene 10 mg / (Kg body wt / day) or corn oil orally for 10 wks; Two-way ANOVA with Tukey post-hoc was used to compare differences among groups ($p < 0.05$), *HSF *vs* Control; $^{\#}$ HSF *vs* HSF+Ly; $^{\$}$HSF+Ly *vs* Control+Ly; LV, left ventricular; Est. LV. Mass, Estimated mass; LMV, Left ventricular mass; DA, diameter; aorta and LA, left atrium; RWT, Relative wall thickness; FS, Fraction shortening; ES, Endocardial shortening; IRT, Isovolumetric relaxation time.

Table 3 shows the cardiac morphological parameters. It is possible to verify the HSF influence on cardiac remodeling process after 30 weeks of experimental protocol. On the other hand, the treatment with tomato-oleoresin was effective to attenuate the cardiac remodeling process in the HSF+Ly.

Table 3. Effect of lycopene supplementation cardiac post-morten alterations induced by high sugar-fat diet

	Groups				Effect		
	Control	Control + Ly	HSF	HSF + Ly	Diet	Ly	Interaction
Heart weight (g)	1.21 ± 0.06	1.20 ± 0.12	1.61* ± 0.34	1.25$^{\#}$ ± 0.03	0.008	0.028	0.03
LV weight (g)	0.57 ± 0.02	0.58 ± 0.04	0.72* ± 0.03	0.61 ± 0.04	<0.001	0.003	0.002
Tibia Length (cm)	4.36 ± 0.5	4.43 ± 0.2	4.37 ± 0.8	4.32$^{\#}$ ± 0.5	0.329	0.818	0.256
HW/Tibia (g/cm)	0.27 ± 0.01	0.27 ± 0.02	0.37* ± 0.08	0.29$^{\#}$ ± 0.01	0.007	0.031	0.053
LVW/Tibia (g/cm)	0.13 ± 0.02	0.13 ± 0.01	0.16* ± 0.01	0.14$^{\#}$ ± 0.01	<0.001	0.005	0.011

Data are expressed in mean ± standard deviation (n, 6 animals/group); groups, Control; Control+Ly, Control + Lycopene; HSF, high sugar-fat; HSF+Ly, high sugar-fat + Lycopene; Diets, Control [carbohydrate, 60%; fat, 4%] or High sugar-fat diet [carbohydrate, 78%; fat, 14.6%] for 30 wks; Supplementation, Lycopene 10 mg / (Kg body wt / day) or corn oil orally for 10 wks; Two-way ANOVA with Tukey post-hoc was used to compare differences among groups ($p < 0.05$), *HSF *vs* Control; $^{\#}$ HSF *vs* HSF+Ly; LV, Left ventricle; LVW, Left ventricle weight; HW, Heart weight.

The analysis of myocardial papillary muscle function at baseline condition with 2.5 mM Ca^{2+} concentration is showed in the Table 3. HSF leaded to functional impairment in the maximum developed tension (DT) compared to control group. Tomato-oleoresin supplementation was effective to recovery the DT capacity compared to HSF group.

Table 4. Baseline data from isolated muscle preparation

	Groups				Effect		
	Control	Control + Ly	HSF	HSF + Ly	diet	Ly	interaction
DT(g/mm²)	5.96 ± 1.25	6.29 ± 1.65	4.41* ± 1.11	6.05$^{\#}$ ± 1.19	0.066	0.046	0.173
RT(g/mm²)	0.65 ± 0.11	0.61 ± 0.11	0.63 ± 0.08	0.57 ± 0.11	0.512	0.202	0.844
+d*T*/d*t* (g/mm²/s)	61.9 ± 10.1	63.5 ± 18.4	60.8 ± 11.7	65.5 ± 19.7	0.934	0.573	0.773
-d*T*/d*t*(g/mm²/s)	16.8 ± 2.4	17.5 ± 2.9	15.5 ± 3.3	16.1 ± 2.9	0.193	0.569	0.933
CSA (mm²)	1.11 ± 0.12	1.10 ± 0.23	1.25 ± 0.27	1.17 ± 0.3	0.181	0.801	0.912

Data are expressed in mean ± standard deviation (n, 6 animals/group); groups, Control; Control+Ly, Control + Lycopene; HSF, high sugar-fat; HSF+Ly, high sugar-fat + Lycopene; Diets, Control [carbohydrate, 60%; fat, 4%] or High sugar-fat diet [carbohydrate, 78%; fat, 14.6%] for 30 wks; Supplementation, Lycopene 10 mg / (Kg body wt / day) or corn oil orally for 10 wks; Two-way ANOVA with Tukey post-hoc was used to compare differences among groups ($p < 0.05$), *HSF *vs* Control; $^{\#}$ HSF *vs* HSF+Ly; Baseline condition, 2.5 mM (Ca^{2+}). DT, Maximum developed tension normalized per cross-sectional area of the

papillary muscle; RT, Resting tension normalized per cross-sectional area of the papillary muscle; peak of the positive, +d*T*/ d*t* and negative, -d*T*/d*t* tension derivatives normalized per cross-sectional area of the papillary muscle; CSA, cross-sectional area.

The figure 1 shows the response of PRC contraction stimulation on the papillary muscle function. The HSF leaded to functional impairment in the D T(30s) and -d*T*/d*t* (30 and 60s) compared to control group. Tomato-oleoresin supplementation was effective to recovery the DT (30s), -d*T*/d*t* (30 and 60s) capacity in the HSF+Ly compared to HSF.

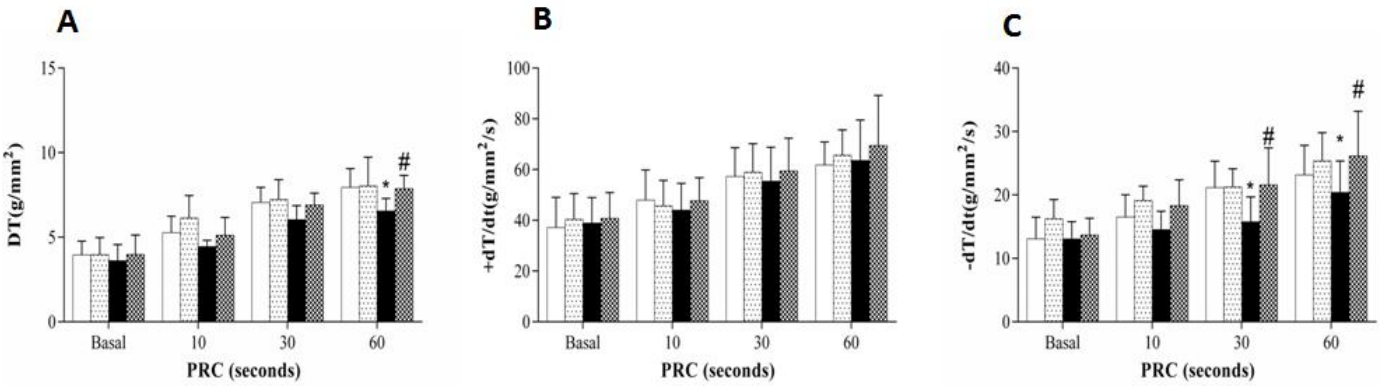

Figure 1. Effects of post-rest contraction in papillary muscles, data are expressed in mean ± standard deviation (n, 6 animals/group); groups: Control; Control+Ly, Control + Lycopene; HSF, high sugar-fat; HSF+Ly, high sugar-fat + Lycopene; Diets: Control [carbohydrate, 60%; fat, 4%] or High sugar-fat diet [carbohydrate, 78%; fat, 14.6%] for 30 wks; Supplementation: Lycopene 10 mg / (Kg body wt / day) or corn oil orally for 10 wks; Control (white bars); control+Ly (dotted bars); HSF(black bars); HSF+Ly(cross-hatched bars). Baseline calcium concentration (0.5mM) is presented as 100%; A, Maximum developed tension normalised per cross-sectional area [DT, g/mm2], B, positive [+d*T*/d*t*, g/mm2 /s] and C, negative [-d*T*/d*t*, g/mm2 /s] tension derivative normalized per cross-sectional area of the papillary muscle; repeated-measures two-way ANOVA with Tukey post-hoc. was used to compare differences among groups ($p < 0.05$): *HSF *vs* Control; #HSF *vs* HSF+Ly.

After basal condition, the papillary muscles were submitted to inotropic and lusitropic maneuvers. Figure 2 A–C shows that the extracellular Ca^{2+} concentration increase from 0.5 to 3.5 mmol/L, results in an inotropic effect in myocytes of all groups. The HSF diet leaded to functional impairment in the DT(2.5mM) and -d*T*/d*t*(3.0 and 3.5mM) compared to control group. Tomato-

oleoresin supplementation was effective to recovery the DT(2.5mM) and -d*T*/d*t*(3.0 and 3.5mM) capacity in HSF+Ly group.

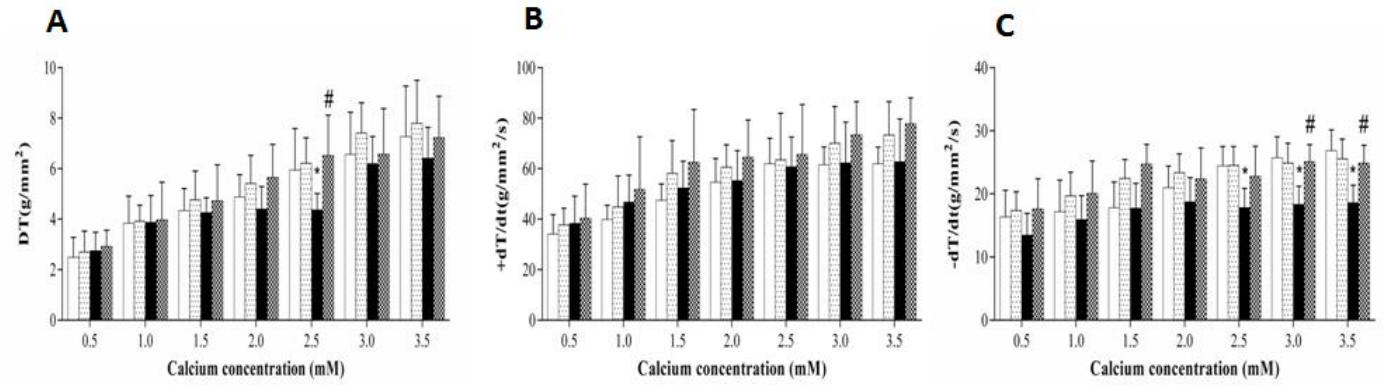

Figure 2. Effects of increasing extracellular calcium concentration in papillary muscles, data are expressed in mean ± standard deviation (n, 6 animals/group); groups: Control; Control+Ly, Control + Lycopene; HSF, high sugar-fat; HSF+Ly, high sugar-fat + Lycopene; Diets: Control [carbohydrate, 60%; fat, 4%] or High sugar-fat diet [carbohydrate, 78%; fat, 14.6%] for 30 wks; Supplementation: Lycopene 10 mg / (Kg body wt / day) or corn oil orally for 10 wks; Control (white bars); control+Ly (dotted bars); HSF(black bars); HSF+Ly(cross-hatched bars). A, Maximum developed tension normalised per cross-sectional area [DT, g/mm2], B, positive [+d*T*/d*t*, g/mm2 /s] and C, negative [-d*T*/d*t*, g/mm2 /s] tension derivative normalized per cross-sectional area of the papillary muscle; repeated-measures two-way ANOVA with Tukey post-hoc. was used to compare differences among groups ($p < 0.05$): *HSF *vs* Control; $^{\#}$HSF *vs* HSF+Ly.

The figure 3 shows the inflammatory parameters in cardiac tissue. It is possible to verify that the HSF influenced the cardiac levels of TNF-α, MCP-1 and IL-6. However, the treatment with tomato-oleoresin was effective to attenuate the cardiac inflammatory parameters in the HSF+Ly.

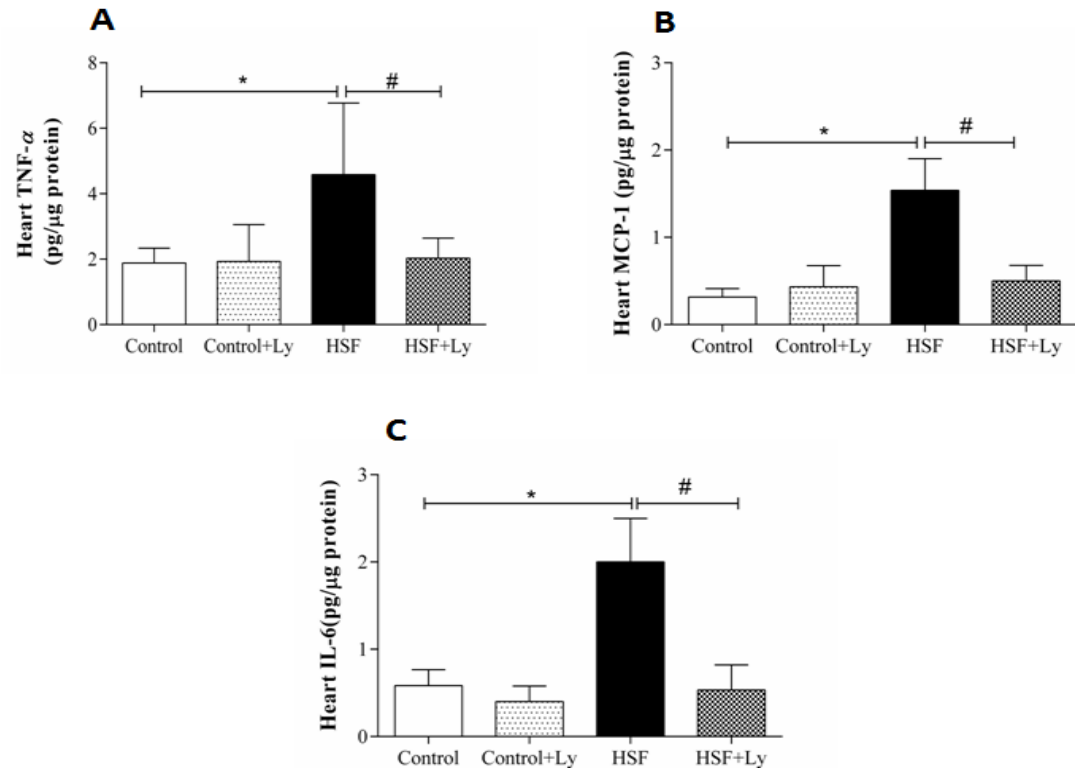

Figure 3. Effect of lycopene supplementation on cardiac inflammatory parameters induced by high sugar-fat diet, data are expressed in mean ± standard deviation (n, 6 animals/group); groups: Control; Control+Ly, Control + Lycopene; HSF, high sugar-fat; HSF+Ly, high sugar-fat + Lycopene; Diets: Control [carbohydrate, 60%; fat, 4%] or High sugar-fat diet [carbohydrate, 78%; fat, 14.6%] for 30 wks; Supplementation: Lycopene 10 mg / (Kg body wt / day) or corn oil orally for 10 wks; Control (white bars); control+Ly (dotted bars); HSF(black bars); HSF+Ly(cross-hatched bars); A, tumoral necrosis factor – alpha (TNF-α; pg/µg protein); B, monocyte chemo attractant protein- 1 (MCP-1; pg/µg protein); C, Interleukin- 6 (IL-6; pg/µg protein).

4. Discussion

According to the literature, the combination of large amounts of simple carbohydrate and saturated fat is part of modern eating habits and is one of the main causes of the development of obesity, comorbidities, cardiac dysfunction[24,25,31,32]. The aim of this study was to test if the tomato-oleoresin supplementation would be able to treat cardiac dysfunction and remodeling related to the high sugar fat diet consumption by modulating cardiac

inflammation.

The results show a better feed efficiency in both HSF groups which can explain the development of obesity, characterized by the higher values of body weight e adiposity index in these groups compared to the respective controls. These finding confirm that the western diet used in this study was efficient to promote obesity in the experimental period of 30 weeks, corroborating also the literature regarding the effect of high sugar fat diets to develop obesity[4,5,24,25].

Several diseases and metabolic disorders, such as insulin resistance, dyslipidemia, and hypertension, known as metabolic syndrome, are linked to obesity[33], all of them presented in the HSF animals. On the other hand, HSF+ Ly group presented an improvement of insulin resistance, represented by reduction in HOMA- IR. High plasma levels of glucose can lead to oxidative stress via glucose auto-oxidation, increasing formation of advanced glycation end products which can damage various organs[34]. Studies show the beneficial effects of lycopene in diabetes have been consistently related to its antioxidant potential[35]. Another explanation for this amelioration is the anti-inflammatory effect of tomato-oleoresin. Insulin resistance and type 2 diabetes are closely related with inflammation and studies already showed that tomato-oleoresin ameliorates the inflammation which may be involved in the beneficial effect on glucose metabolism[36].

Cardiac disease in obesity is multifactorial and includes as risk factors metabolic dysregulation, such as insulin resistance, hypertension, and dyslipidemia leading to adverse cardiac remodeling that may be characterized

by hypertrophy, and impaired ventricular systolic and diastolic function[37]. Echocardiographic analysis showed cardiac remodeling and impairment in ventricular systolic and diastolic function in HSF group after 30 weeks. In addition, the morphological analysis *post-death* in the current study support the finding that obesity induced cardiac hypertrophy visualized by increased total heart, left ventricle and the respective normalized weight[6].

Several mechanisms have been postulated to identify obesity-induced cardiac dysfunction, among them intracellular handling calcium. Functional studies using isolated papillary muscle have showed that obesity is able to lead impairment in this contractile regulation pathway[1,3,38,39]. Our study demonstrated that the post- rest contraction and extracellular calcium elevation leaded to negative responses in the systolic (DT) and diastolic (-d*T*/d*t*) response. PRC findings and extracellular calcium concentration changes suggest that obesity promotes regulatory Ca^{2+} channel dysfunction. Post-rest contraction allows us to study aspects of RS participation in the contraction-relaxation cycle of the cardiac muscle. In this study, the PRC induced a significantly diminished response in -d*T*/d*t* and DT in HSF myocardium. These data are consistent with a previous study that showed lower contractile response in obese Zuckers after 60s of cessation of stimulus[40]. As -d*T*/d*t* is influenced by the rate of uptake of calcium ions into the SR32, the minor Ca^{2+} resequestration demonstrated by -dT/dt in obese rats suggests depressed SERCA2 activity. The elevation of extracellular Ca^{2+} alters the contraction and relaxation phases due to increases in available cytoplasmic Ca^{2+} concentration,

by interfering with the operation of the L-type channel, NCX, and SR function. The lower response to extracellular Ca^{2+} elevation in HSF rats can be related to a reduction of Ca^{2+} influx across L-type channels and/or changes in the SR function, as verified with post-rest contraction. These results are in line with previous studies that verified cardiac dysfunction and depressed responsiveness to extracellular Ca^{2+} elevation in myocytes and papillary muscles of obese rats[4,38,41]. However, another study found a high response to extracellular Ca^{2+} increase in rats provided with a high-fat diet.

Recently, the literature reports the role of inflammation on cardiac remodeling and dysfunction[8,9]. Studies reveal that, although inflammation following tissue damage is an essential physiological reaction in the healing process, an excessive inflammatory response is associated with left ventricular (LV) hypertrophy as well as progression of myocardial diseases[8]. Pro-inflammatory cytokines such as TNF-α and IL-6 appear to cause cardiomyocytes apoptosis and necrosis as well as cells hypertrophy[17]. In addition, proinflammatory cytokines seem to exert a main role in the myocardial contractile depression, especially TNF-α and IL-6, by alteration of calcium homeostasis and handling[42]. Our results revealed that HSF group presented heart inflammation while the HSF+Ly group showed a reduction of this inflammatory state. The antinflammatory effect from tomato-oleoresin could explain the cardiac protection in HSF+Ly group even in the presence of cardiac risk factors, as obesity and hypertension.

In summary, this study found that HSF diet was able to induce obesity and cardiac dysfunction and that tomato-oleoresin was able to attenuate this condition. So, it is possible to conclude that tomato-oleoresin is able to modulate cardiac inflammatory parameters attenuating the cardiac dysfunction.

Conflict of Interest

The authors declare no conflict of interest.

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Material suplementar

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Article

Association between Cardiac Remodeling and Metabolic Alteration in an Experimental Model of Obesity Induced by Western Diet

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Abstract: The high consumption of fat and sugar contributes to the development of obesity and co-morbidities, such as dyslipidemia, hypertension, and cardiovascular disease. The aim of this study was to evaluate the association between dyslipidemia and cardiac dysfunction induced by western diet consumption. Wistar rats were randomly divided into two experimental groups and fed *ad libitum* for 20 weeks with a control diet (Control, n = 12) or a high-sugar and high-fat diet (HSF, n = 12). The HSF group also received water + sucrose (25%). Evaluations included feed and caloric intake; body weight; plasma glucose; insulin; uric acid; HOMA-IR; lipid profile: [total cholesterol (T-chol), high-density lipoprotein (HDL), non-HDL Chol, triglycerides (TG)]; systolic blood pressure, and Doppler echocardiographic. Compared to the control group, animals that consumed the HSF diet presented higher weight gain, caloric intake, feed efficiency, insulin, HOMA-IR, and glucose levels, and lipid profile impairment (higher TG, T-chol, non-HDL chol and lower HDL). HSF diet was also associated with atrial-ventricular structural impairment and systolic-diastolic dysfunction. Positive correlation was also found among the following parameters: insulin versus estimated LV mass (r = 0.90, p = 0.001); non-HDL versus deceleration time (r = 0.46, p = 0.02); TG versus deceleration time (r = 0.50, p = 0.01). In summary, our results suggest cardiac remodeling lead by western diet is associated with metabolic parameters.

Keywords: dyslipidemia; obesity; cardiac remodeling

1. Introduction

Obese individuals are typically predisposed to an increased heart rate and stroke volume, which may lead to ischemic cardiomyopathy, compensatory left ventricular remodeling, non-ischemic dilated cardiomyopathy, cardiac fibrosis, and apoptosis [1]. Furthermore, obesity is associated with metabolic syndrome (MS), a clustering of risk factors that includes visceral obesity, dyslipidemia, hyperglycemia, and hypertension. Among these factors, dyslipidemia is the major risk factor for cardiovascular disease (CVD). Although obesity influences blood lipid and lipoprotein levels, dietary factors are also able to influence these parameters [2].

Western diets (WD), which are rich in fat and carbohydrates may be responsible for the obesity epidemic, especially in industrialized countries [1]. WD are characterized by the consumption of high caloric-dense foods, and unbalanced proportions of fat (saturated vs unsaturated) and carbohydrates (high glycemic vs low glycemic), eating habits associated with an increased CVD risk [3]. Sonestedt et al. observed that individuals with low sucrose intake had lower triglycerides and higher HDL concentrations compared to those with high sucrose consumption [2]. Moreover, it has been already confirmed that the consumption of high carbohydrate foods and beverages increases the risk of MS [4].

There are several spontaneous (genetic) rodent models for MS that present cardiovascular disorders, including Zucker diabetic fatty rats [5,6], Goto-Kakizaki rats [7], and spontaneously hypertensive rats [8]. Although these models provide important information regarding the pathogenesis and the treatment of some aspects of MS, they do not reflect the diet-induced human metabolic syndrome [9]. Therefore, appropriate animal models mimicking human metabolic syndrome and related CVD are necessary to investigate the causes and progression of this disease and potential pharmacological interventions [10,11].

Experimental studies regarding cardiac function and diet-induced obesity present divergent results. Rats fed hypercaloric diets for 8 to 14 weeks developed obesity [12–14], but not cardiac disfunction, as assessed by echocardiogram [13,14]. However, Panchal et al. [15] showed ventricular dilation, increased systolic volume and increased estimated left ventricular mass in rats fed with high-carbohydrate and high-fat diet for both 8 and 16 weeks. Moreover, other researchers found diastolic dysfunction in isolated heart and papillary muscles [16], reduction in diastolic compliance [17] and impaired mechanical function of ventricular myocytes [18] from Ob rabbits [12,18] and rats [19] fed high-fat diets for 12 weeks. In this way, this study was designed to verify the hypothesis that our proposed diet model is able to induce cardiac remodeling. So, the aim of this study was to evaluate the effect of a Western diet on cardiac remodeling and its association with metabolic parameters.

2. Materials and Methods

2.1. Animals and Experimental Protocol

All the experimental protocol was approved by the Ethics Committee on the Use of Animals (CEUA) from Botucatu Medical School, São Paulo State University (UNESP), under number 1196/2016. Male Wistar rats (5–6 weeks old, weighing 209 ± 18g, n = 24) were obtained from the Animal Center of Botucatu Medical School, São Paulo State University, UNESP (Botucatu, SP, Brazil) and randomly divided into 2 experimental groups to receive chow diet (control group, n = 12) or high-sugar high-fat

diet (HSF, n = 12) for 20 weeks. Animals were individually housed in temperature-controlled and 12-hour light-dark conditions. Diets were designed in our laboratory and previously published by our research group [20]. The control diet contained soybean meal, sorghum, soybean hulls, dextrin, soy oil, vitamins and minerals. The HSF diet was composed of soybean meal, sorghum, soybean hulls, dextrin, sucrose, fructose, lard, vitamins and minerals plus 25% sucrose in drinking water. Nutritional composition of both diets is presented in Table 1, without considering drinking water.

Table 1. Nutritional composition of the diets.

	Diet	
Ingredients	**Control**	**HSF**
Soybean meal (g/kg)	335	340
Sorghum (g/kg)	278	80
Soy hulls (g/kg)	188.5	116.7
Dextrin (g/kg)	146.5	20
Sucrose (g/kg)	-	80
Fructose (g/kg)	-	180
Soy oil (g/kg)	14	-
Lard (g/kg)	-	154.3
Minerals (g/kg)	25	25
Salt (g/kg)	4	8
Components		
Protein (%)	20	16
Carbohydrate (%)	60	70
Fat (%)	4	14.6
% Energy from protein	22.85	13.45
% Energy from carbohydrate	66.78	58.69
% Energy from fat	10.37	27.8
Energy (kcal/g)	3.59	4.35

HSF diet had 25% of sucrose in drinking water.

2.2. Nutricional Profile

Feed consumption (FC) was measured daily and body weight (BW) was assessed weekly. Caloric intake (CI) for the control group was calculated according to the following formula: caloric intake (kcal/day) = feed consumption (g) × dietary energy (3.59 kcal/g). For the animals that received the HFS diet, the energy intake was calculated according to the formula: volume consumed (mL) × 0.25 (equivalent to 25% fructose) × 4 (calories per gram of carbohydrate) + caloric values offered by feeding (feed consumption (g) × dietary energy (4.35 kcal/g). Feed efficiency (FE) is the ability to convert caloric intake to BW and it was determined as follows: FE(%) = BW gain (g)/total caloric intake (kcal) × 100 [16].

2.3. Plasma Measurements

After 12-h fasting, blood was collected from the tail and the plasma was used for biochemical analysis. Plasma glucose was determined by using a glucometer (Accu-Chek Performa; Roche Diagnostics, Indianapolis, IN, USA). Insulin level was measured by enzyme-linked immunosorbent assay (ELISA) method using

commercial kits (Millipore). Triglycerides, total cholesterol, high-density lipoprotein (HDL), urea, and creatinine were measured by an automatic enzymatic analyzer system (Biochemical analyzer BS-200, Mindray, China). Non-HDL cholesterol fraction (VLDL + IDL + LDL) which is considered an estimation of the total atherogenic particles in plasma, was calculated by the formula: (non-HDL Chol = total Cholesterol-HDL) [21].

2.4. Homeostatic Model Assessment Index (HOMA-IR)

The homeostatic model of insulin resistance (HOMA-IR) was used as an insulin resistance index, and it was calculated using the following formula: HOMA-IR = [fasting glucose (mmol/L) × fasting insulin (μU/mL)]/22.5 [16].

2.5. Systolic Blood Pressure (SBP)

SBP evaluation was assessed in conscious rats by the non-invasive tail-cuff method with a NarcoBioSystems® Electro-Sphygmomanometer (International Biomedical, Austin, TX, USA). The animals were warmed in a wooden box (50 × 40 cm) between 38–40 °C with heat generated by two incandescent lamps for 4–5 min to cause arterial vasodilation in the tail and were then transferred to an iron cylindrical support that was specially designed to allow total exposure of the animal's tail [22]. After this procedure, a cuff with a pneumatic pulse sensor was attached to the tail of each animal. The cuff was inflated to 200 mmHg pressure and subsequently deflated. The blood pressure values were recorded on a Gould RS 3200 polygraph (Gould Instrumental Valley View, Ohio, USA). The average of two pressure readings was recorded for each animal.

2.6. Echocardiographic Analysis

At 20th week, all the animals were evaluated in vivo by transthoracic echocardiography, using a Vivid S6 system equipped with multifrequency ultrasonic transducer 5.0 to 11.5 MHz (General Electric Medical Systems, Tirat Carmel, Israel). All exams were performed by the same examiner and obtained according to the leading-edge method recommended by the American Society of Echocardiography. Rats were lightly anesthetized by intramuscular injection with a mixture of ketamine (50 mg/kg) and xylazine (1 mg/kg). After shaving their chest, the animals were placed in left decubitus position. To implement structural measures of the heart, the images were obtained in one-dimensional mode (M-mode) guided by the images in two-dimensional mode with the transducer in the parasternal position, minor axis. Left ventricular (LV) evaluation was performed by positioning the cursor M-mode just below the mitral valve plane at the level of the papillary muscles. The images of the aorta and left atrium were obtained by positioning the M-mode course to plan the level of the aortic valve. The following cardiac structures were measured: diastolic diameter (LVDD) and systolic (LVSD) LV; diastolic thickness posterior wall of the left ventricle (LVPWD); diameter of the aorta (DA) and left atrium (LA). The relative thickness of the LV (ERVE) was calculated by dividing LVPWD multiplied by two by LVDD. Left ventricular mass (LVM) was calculated using the formula [(LVDD +LVPWD IVSDT) 3 − (LVDD) 3] × 1.04 where 1.04 is the specific density of the myocardium. MVE index (LVMI) was calculated by normalizing to body weight Estimated LV mass. The LV systolic function was assessed by the following

parameters: percentage of endocardial shortening (Δ% endo) [(LVDD − LVSD)/LVDD] × 100; midwall fractional shortening (% Δ meso) {[(LVDD + ½ + ½ LVPWD IVSDT) − (LVSD + ½ + ½ IVSST LVPWS)]/(LVDD + ½ + ½ LVPWD IVSDT)} × 100; shortening velocity rear wall (LVPW), which is the maximum tangent of the stroke movement of the rear wall. The LV diastolic function was evaluated by the following indices: peak velocity of early diastolic filling (E wave); peak velocity of late diastolic filling (A wave); ratio between the E and A waves (E/A); deceleration time of E wave (DTE); isovolumetric relaxation time in absolute values (IRT) and normalized for heart rate (TRIVn = IRT/R-R0,5). The joint assessment of diastolic and systolic LV function was performed by myocardial performance index also known as Tei index (sum of isovolumetric contraction and IRT time, divided by the left ventricular ejection time). The study was supplemented by evaluation by tissue Doppler systolic displacement (S'), early diastolic (E'), and late (A') of the mitral annulus (arithmetic average travel speeds of lateral and septal walls), and the ratio by the waves E and E'(E/E') [23,24].

2.7. Statistical Analysis

Data are presented as mean ± standard deviation (SD) or median (interquartile range). Differences between the groups were determined by using Students-t test for independent samples. Pearson correlation was used for analytical statistic for association between cardiac parameters and metabolic variables. All statistical analyses were performed using SigmaStat for Windows (Version 3.5). P value < 0.05 was considered as statistically significant.

3. Results

After 20 weeks of treatment simulating a western diet, the HSF group showed higher weight gain than the control group. Even with a lower feed consumption, the HSF group showed higher caloric intake that resulted in higher feed efficiency (Figure 1).

In addition, the western diet induced changes in glucose metabolism homeostasis, characterized by higher glucose, insulin and HOMA-IR compared to the control group (Table 1).

The western diet was also associated with systolic and diastolic cardiac dysfunction, and remodeling at 20th week (Table 2), and changes in plasma lipid profile-higher TG, TC, non-HDL, and lower HDL (Figure 2).

Table 2. Effect of high-sugar and high-fat (HSF) diet on plasma metabolic parameters.

	Groups	
Variables	Control (*n* = 12)	HSF (*n* = 12)
Glucose (mg/dL)	83.4 ± 6.3	97.9 ± 8.5 *
Insulin (mg/dL)	2.5 ± 1.2	5.2 ± 1.3 *
HOMA-IR	21.3 ± 9.6	50.7 ± 11.2 *

Data presented as means ± SD. Control and high-sugar high-fat (HSF) groups; *n*: animals numbers; HOMA-IR: homeostatic model assessment index; * $p < 0.05$ versus C; Student's *t*-test for independent samples.

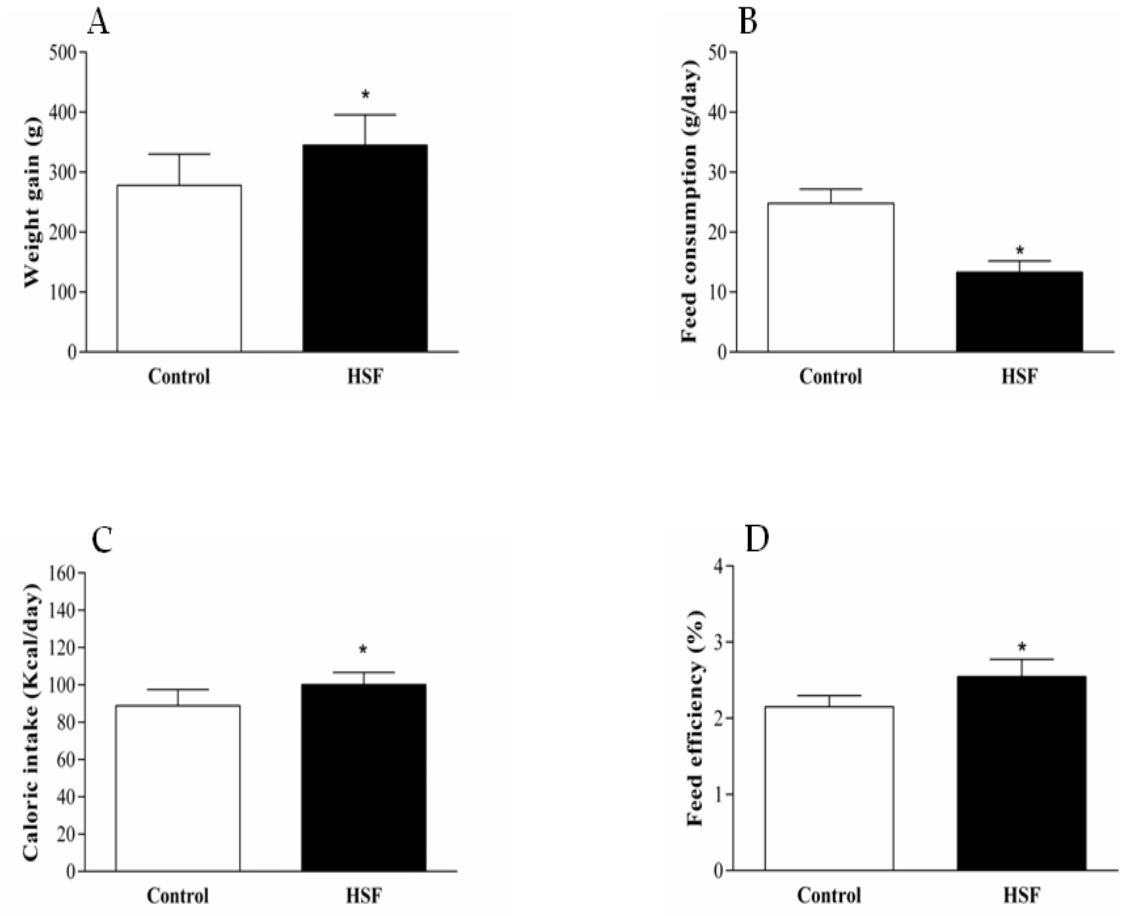

Figure 1. Nutritional profile of the groups. **A**—weight gain (g); **B**—feed consumption (g/day); **C**—caloric intake(kcal/day); **D**—feed efficiency (%). HSF—high-sugar high-fat group. * indicates $p < 0.05$.

The cardiac variables and systolic blood pressure are presented in Table 3. The diet promoted an increase in systolic blood pressure, cardiac remodeling, and deterioration of cardiac function, visualized by echocardiogram analysis.

Table 3. Effect of HSF diet on hemodynamic and cardiac remodeling.

Variables	Groups	
	Control (*n* = 12)	**HSF (*n* = 12)**
LVDD, mm	7.50 ± 0.40	6.53 ± 0.49 *
LVSD, mm	2.68 ± 0.34	3.31 ± 0.44 *
LVPWD, mm	1.54 ± 0.11	1.97 ± 0.11 *
Aorta diameter, mm	3.79 ± 0.24	4.01 ± 0.19 *
Left Atrium	4.73 ± 0.20	6.17 ± 0.41 *
Estimated LV mass, g	1.56 ± 0.32	2.03 ± 0.23 *
Relative wall thickness	0.45 ± 0.03	0.58 ± 0.06 *
Systolic volume, mL	23.5 ± 2.8	26.6 ± 5.8
Shortening Δ% endo	58.2 ± 3.3	52.5 ± 55.3 *

Shortening Δ% meso	25.6 ± 2.1	25.3 ± 2.7
Ejection fraction, %	0.92 ± 0.01	0.89 ± 0.03 *
Deceleration time, MS	44.1 ± 7.8	53.4 ± 9.4 *
Ew, m/s	78.9 ± 8.4	77.9 ± 6.6
Aw, m/s	48.7 ± 11.6	45.9 ± 14.1
E/A, m/s	1.67 ± 0.27	1.85 ± 0.64
IRT	22.9 ± 3.1	28.1 ± 4.8 *
Systolic blood pressure, mmHg	126 ± 5	136 ± 5 *

Data presented as means ± SD. Control and high-sugar high-fat (HSF) groups; n: animals numbers; LV:Left ventricular; LVDD:Left ventricular diastolic diameter; LVSD:Left ventricular systolic diameter; LVPWD:diastolic posterior wall thickness; Aw: A-wave mitral inflow velocity; Ew: E-wave mitral inflow deceleration time; IRT: Isovolumetric relaxion time; * $p < 0.05$ versus Control; Student's *t*-test for independent samples.

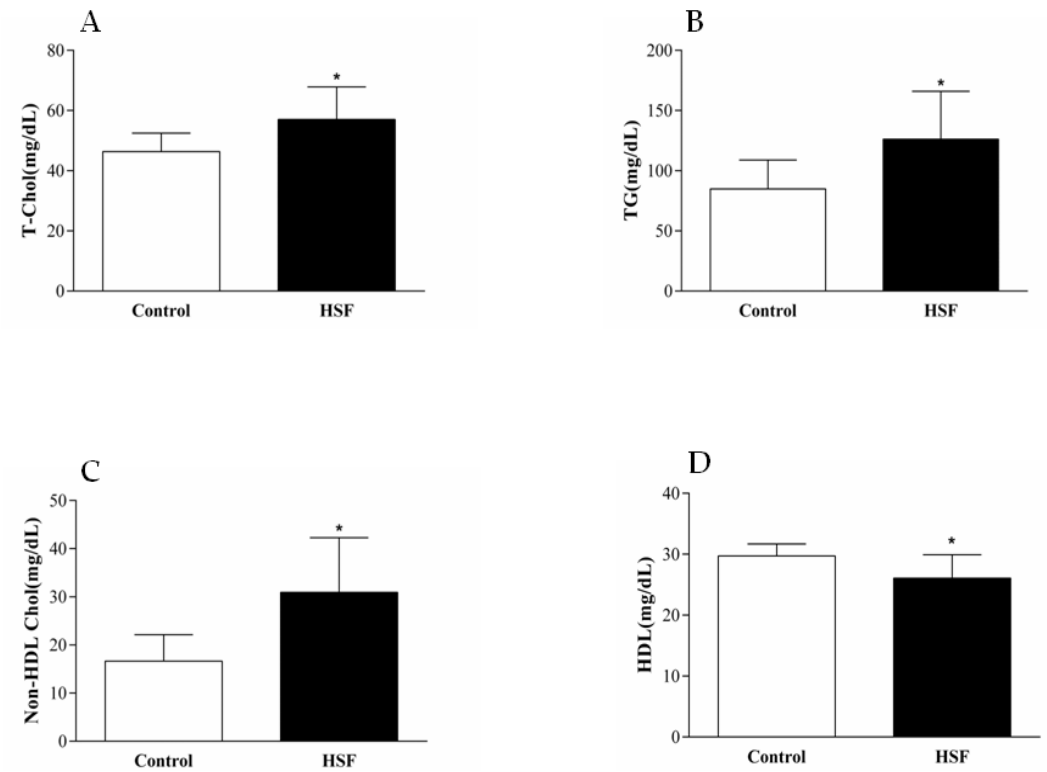

Figure 2. Effect of HSF diet on lipid profile. **A**—T-Chol: Total Cholesterol (mg/dL); **B**—TG: Triglycerides (mg/dL); **C**—Non-HDL Chol:(mg/dL); **D**—HDL: High-density lipoprotein (mg/dL). Control group; HSF-high-sugar high-fat group. * indicates $p < 0.05$.

Moreover, these cardiac changes were related to the altered lipid profile (Figure 3). Ejection fraction was inversely correlated with non-HDL cholesterol (r = 0.35; $p = 0.09$) and directly correlated with HDL cholesterol (r = 0.39, $p = 0.06$), although marginally significant. Positive correlation was also found among the following

parameters: insulin vs. estimated LV mass (r = 0.90, p = 0.001); non-HDL cholesterol vs. deceleration time (r = 0.46, p = 0.02); TG vs. deceleration time (r = 0.50, p = 0.01).

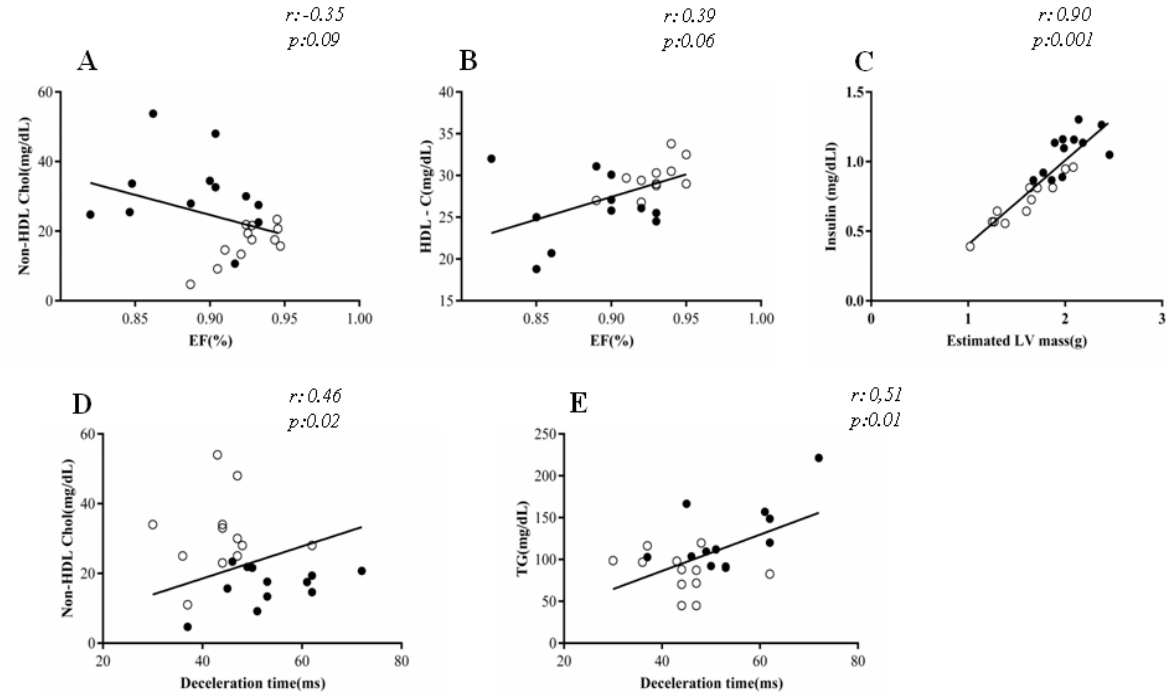

Figure 3. Correlation between echocardiographic and biochemical parameters. EF: Ejection Fraction; **A**—Non-HDL Chol:(mg/dL)/EF; **B**—TG: HDL: High-density lipoprotein(mg/dL)/EF; **C**—Insulin:(mg/dL)/estimated LV mass; **D**—Non-HDL Chol:(mg/dL)/Decelaration time and **E**—Triglycerides (mg/dL)/Decelaration time. Control group (○), HSF group (●); Pearson regression was used to examine the associations between variables.

4. Discussion

There is a lack of studies in the literature regarding cardiac function injuries and remodeling in obesity/MS models induced by western diets. The present study showed that the western diet model used was able to induce obesity, MS, and cardiac dysfunction and remodeling in the HSF group, even with a lower feed intake than that observed in control group. Since western diet combines high levels of fat and sugar, resulting in tasty but caloric food, the results can be explained by the better feed efficiency in the HSF group. This diet is the closest equivalent to the human ultra-processed food diet (western diet), and provided the animals with varied nutrients, high energy, and palatability, thereby mirroring the key obesogenic features of the human diet [25].

The development of obesity, characterized in this study by the significant difference in body weight, has occurred in the sixth week of experimental treatment and remained for more 14 weeks, with the HSF group presenting higher values compared to the control group. This result shows that the western diet used was efficient to promote obesity in the experimental period of 20 weeks. According to the literature, western diet/ hypercaloric diets are associated with higher body weight in

rodents [12,18,26,27]. Besides, the HSF animals in this study also presented many disorders similar to human obesity-related comorbidities, such as hypertension, dyslipidemia hypertriglyceridemia, glucose intolerance, insulin resistance, and hyperinsulinemia. Diets with high sugar and fat are extensively used to induce obesity and MS [28–30], hyperinsulinaemia, hyperglycemia and hepatic steatosis [15], dyslipidemia [31], and elevated blood pressure[32–36].

The cardiac morphological analysis in the current study revealed that the western diet leaded to hypertrophy, as characterized by increased left atrium, aorta diameter, left ventricular diastolic and systolic diameter, estimated mass of left ventricle and relative wall thickness. Moreover, it was also observed cardiac dysfunction, with decreased ejection fraction, shortening Δ% endo, and increased deceleration time. All these results show the robustness of the diet to induced cardiac disorders, since most of the experimental studies relating cardiac function and diet- induced obesity present divergent results. Some studies using echocardiogram analyses did not find cardiac dysfunction in the obesity model [12,13], whereas, other authors only demonstrated mild changes [15,37,38].

It is known that overweight and obesity can directly and indirectly modulate the heart, either by promoting an increased hemodynamic overload and neurohumoral activation, or by the secretion of proinflammatory adipokines [15,22,39–42]. This initial process of cardiac remodeling may be considered as the first step in the sequence of adaptive responses from heart to the stress leaded by a large number of physiological and pathological conditions, as changes in the volume and pressure loads and/or metabolic changes [43–45]. Rider et al. proposed that cardiac remodeling is an adaptive characteristic of obesity [46]. Thus, obesity-induced changes in cardiac structure may be elicited directly by obesity-induced increases in cardiac loading conditions (preload and afterload) or indirectly by obesity-induced cardiometabolic abnormalities such as dyslipidaemia and insulin resistance/diabetes [47,48]. The literature reports that the insulin resistance induced by obesity with associated hyperinsulinemia could promote cardiac remodeling via the growth-promoting properties of insulin or by attenuating the anti-apoptotic signaling of the phosphatidylinositol 3′-kinase (PI3K)-Akt (protein kinase B [PKB]) pathway elicited by insulin receptor activation [46,48]. Considering the strong correlation between insulin and left ventricular estimated mass found in this study, probably this pathway was activated in the animals. Clinical studies involving diabetes type 2, congestive heart failure, and obesity had correlated echocardiographic findings with insulin and lipid profile [49–52]. However, our model of diet-induced obesity showed an obesity-associated cardiomiopathy and brings new insights related to this condition and metabolic changes sought to elucidate whether this condition is correlated with metabolic changes.

There are recommendations to maintenance of target values of LDL cholesterol in order to protect against LV remodeling [53]. High-density lipoprotein (HDL) is one of the major lipoproteins in the blood. Studies have demonstrated that plasma HDL levels are inversely correlated with the incidence of coronary heart disease [54]. Post-infarct ejection fraction is lower in patients with low HDL cholesterol levels [55–57]. In addition, HDL cholesterol anti-inflammatory properties have been extensively discussed [58]. In vitro and in vivo studies suggest that the expression of Apo A1, the primary protein component of HDL cholesterol, is related with lower expressions of

cell adhesion molecules ICAM-1 and VCAM-1 [59,60] and lower expression of NF-kB and TNF-α. Inflammatory cell infiltration and heart tissue inflammation have been implicated on pathophysiology of DCVs [61]. Although HDL cholesterol anti-inflammatory properties on cardiac remain under discussion [62], this is a possible mechanistic pathway for the explanation of the relationship between higher HDL cholesterol levels and an efficient systolic function.

5. Conclusions

In summary, this paper brings important findings, in a Wistar rats experimental model, following the consumption of a western diet that promoted cardiac remodeling. The diet employed in the current study was able to induce cardiac disorders related to metabolic parameters. However, more studies to investigate the causal mechanisms are necessary, which will allow the development of a new therapy target for clinical practice.

Author Contributions: A.J.T.F., F.V.F., J.L.G. and F.M. conducted the experiment; A.J.T.F., F.V.F., I.O.M., S.G.Z.B., K.A.H.K., C.R.C., F.M., C.C.V.d.A.S. and A.L.F. analyzed the data; and A.J.T.F., F.V.F., I.O.M., F.M and A.L.F. wrote the paper.

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Protective effect of tomato-oleoresin supplementation on oxidative injury recoveries cardiac function by improving β-adrenergic response in a diet-obesity induced model

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Abstract:
Introduction: The system redox imbalance is one of the pathways related to obesity-related cardiac dysfunction. The lycopene is considered one of the best antioxidants. **Aim**: To test if the tomato-oleoresin would be able to recovery the cardiac function by improving *β*-adrenergic response due its antioxidant effect. **Materials and Methods:** 40 animals were randomly divided into two experimental groups to receive control diet (Control, n= 20) or high sugar- fat diet (HSF, n=20) for 20 weeks. Once detected the cardiac dysfunction by echocardiogram in HSF group, animals were re- divided to begin the treatment with Tomato-oleoresin or vehicle, performing four groups: Control (n=6); (Control + Ly, n=6); HSF (n=6) and (HSF + Ly, n=6). Tomato oleoresin (10mg lycopene/kg body weight (BW) per day) was given orally every morning for a 10-weeks period. The analysis included nutritional and plasma biochemical parameters, systolic blood pressure, oxidative parameters in plasma and heart and cardiac analyses *in vivo* and *in vitro*. Comparison among the groups were performed by Two-way analysis of variance (ANOVA). **Results**: The HSF diet was able to induce obesity, insulin-resistance, cardiac dysfunction and oxidative damage. However, the Tomato-oleoresin supplementation improved the insulin-resistance, cardiac remodeling and dysfunction by improving the *β*-adrenergic response. **Conclusion:** It is possible to conclude that conclude that tomato-oleoresin is able to reduce the oxidative damage improving the system *β*-adrenergic response recovering the cardiac function.

Keywords: high sugar-fat diet; obesity; *β*-adrenergic system; cardiac dysfunction; Lycopene; Tomato-oleoresin

1.Introduction

Clinical studies show that the excessive body fat leads to many cardiac abnormalities, among them, morphologic and functional changes[1], [2]. Animal studies have demonstrated myocardial dysfunction in obese rodents fed with hipercaloric diets[3]–[7]. Although is evident that many cardiac changes and/or impairment in performance occur due the adipose tissue accumulation[3], [8], the responsible mechanisms by these changes are not clarified. The system redox unbalance, characterized by high production of reactive species and inefficient antioxidant activity, is one of the pathways associated with the obesity- related cardiac dysfunction[9].

The β-adrenergic system is one of the most important mechanism responsible by myocardial contraction and relaxation[10]–[12]. However, chronic expositions to reactive species are associated with sustained adrenergic stimulation, resulting in arrhythmias and heart failure[13]. Considering the redox system role in the obesity and cardiac disorders pathogenesis, the use of antioxidants as therapeutic strategies have been tested[14], [15]

The lycopene is a carotenoid present in tomato and red fruits and considered a potent antioxidant[16]–[18]. The tomatoes and tomato product consumption is one of the Mediterranean diet characteristics, which is associated with health benefits[19]. However, there is a lack of studies regarding the lycopene dose ingestion in countries with Mediterranean diet. Moreover, the few studies which brings information about de lycopene consumption have a big variability[20] among the results(for example: in Italy the average intake is 7.4mg per day while in Spain is 1.6mg per day[19]). The lycopene effect on cardiovascular disease has been evaluated in clinic[21], [22] and experimental studies[23]. Although obesity and oxidative stress are able to lead to cardiac dysfunction, no studies evaluated the cardiac modulation by lycopene due the antioxidant effect. So, this study aimed to test if the tomato-oleoresin would be able to recovery the cardiac function by improving β-adrenergic response due its antioxidant effect.

2.Materials and Methods

2.1.Animals and Experimental Protocol

In the present study, male Wistar rats (±187g) were initially divided into two experimental groups to receive control diet (Control, n= 20) or high sugar- fat diet (HSF, n= 20) for 20 weeks. The diets and water were provided *ad libitum*. The diets composition has been described in our previous studies[24], [25]. All the animals were housed in an environmental controlled room (22°C ± 3°C, 12h light-dark cycle and relative humidity of 60 ± 5%). All of the experiments were performed in accordance with the National Institute of Health's Guide for the Care and Use of Laboratory Animals and the procedures were approved by the Animal Ethics Committee of Botucatu Medical School (1196/2016).

At the week 20th of this study, the cardiac dysfunction was detected by echocardiogram in the HSF group. Thus, the animals were casually divided to begin the treatment with tomato-oleoresin or vehicle, performing four groups: Control (n= 6); Control supplemented with lycopene- tomato oleoresin (Control+Ly, n= 6); HSF (n= 6) and HSF supplemented with lycopene- tomato oleoresin (HSF+Ly, n= 6). Tomato oleoresin was mixed with corn oil correspondent to 10mg lycopene/kg of

body weight (BW) per day and given orally every day, in the morning, for a 10 weeks period[26], [27]. To avoid differences in the energy provided, all the groups received the same corn oil amount (about 2 ml/kg BW per day). The supplementation time and dose were chosen based in previous studies from our research group and others from the literature[26]–[28]

2.2.Tomato- oleoresin preparation

The tomato- oleoresin (Lyc-O-Mato 6% dewaxed; LycoRed Natural Products Industries) was mixed with corn oil and kept in the dark, at 4ºC, until the moment to be used[29]. The tomato oleoresin–corn oil mixture stayed for 20min in a water-bath at 54ºC before the animals receive. The total amount of lycopene in each solution was 5mg/ml. Lycopene stability was confirmed by diode-array spectra at 450nm, as previously described [30].

2.3.Nutritional evaluation

Nutritional evaluation included: feed consumption (FC)- daily consumed amount in grams of chow feed; final body weight (BW); caloric intake (CI), calculating according to the following formula for the control group: caloric intake (kcal/day)= feed consumption (g) × dietary energy (3.59 kcal/g). For the HSF group, the caloric intake was calculated as following: water volume consumed (mL) × 0.25 (equivalent to 25% fructose) × 4 (calories per gram of carbohydrate) + caloric intake providing by the chow (feed consumption (g) × dietary energy (4.35 kcal/g).

Feed efficiency (FE) is defined as the ability to convert the caloric intake to body weight. It was calculated according to the formula: FE (%) = BW gain (g)/total caloric intake (kcal) × 100[24], [25]. The adiposity index, considered an obesity marker, was calculated as follow: adiposity index = (total body fat (BF)/final body weight) x 100. BF was evaluated considering the sum of the individual fat pad weights: BF = epididymal fat + retroperitoneal fat + visceral fat.

2.4.Metabolic and hormonal analysis

The plasma used for the biochemical analysis was collected after 12h fasting. The glucose levels were evaluated by a glucometer (Accu-Chek Performa; Roche Diagnostics, Indianapolis, IN, USA). The insulin levels were analyzed by ELISA assay with commercial kits (Millipore)[24]. The HOMA- IR (homeostatic model of insulin resistance), considered an insulin resistance index, was calculated by the following formula: HOMA-IR = [fasting glucose (mmol/L) × fasting insulin (μU/mL)]/22.5[15].

2.5.Systolic Blood Pressure (SBP)

SBP was evaluated by a non-invasive tail-cuff method with a NarcoBioSystems® Electro-Sphygmomanometer (International Biomedical, Austin, TX, USA) with the conscious rats. For this, the animals were heat during 4–5min in a wooden box (50 × 40 cm), with two incandescent lamps and temperature between 38–40°C, to induce arterial vasodilation in the tail. Then, the rats were transferred to an iron cylindrical support specially made to allow the total exposure of the animal's tail[31]. After this procedure, a cuff with a pneumatic pulse sensor was attached to the tail and inflated to 200 mmHg pressure and successively deflated. The blood pressure values were

domented on a Gould RS 3200 polygraph (Gould Instrumental Valley View, Ohio, USA). The final SBP of each animal considered the average of three pressure readings.

2.6.Lycopene Bioavailability Evaluation

The presence of lycopene was determined in plasma and cardiac tissue homogenate. To extract the carotenoids, samples were incubated with internal standard (equinenone), chloroform/methanol $CHCl_3/CH_3OH$ (3mL, 2:1, *v*/*v*) and 500mL of saline 8.5 g/L. Then the samples were centrifuged at 2000× *g* for 10 min and the supernatant was collected and hexane was added. The chloroform and hexane layers were evaporated under nitrogen and the residue was resuspended in 150mL of ethanol and sonicated for 30 s. 50μL of this aliquot was injected into the HPLC. The HPLC system was a Waters Alliance 2695 (Waters, Wilmington, MA, USA) and consisted of pump and chromatography bound to a 2996 programmable photodiode array detector, a C30 carotenoid column (5 μm, 150 x 4.6 mm, YMC-Yamamura Chemical Research, Wilmington, NC, USA), and Empower software (Empower 3, chromatographic data software Milford, MA, USA). The HPLC system programmable photodiode array detector was set at 450 nm for carotenoids. The mobile phase consisted of ethanol/methanol/methyl-tert-butyl ether/water (83:15:2, *v*/*v*/*v*, 15g/L with ammonium acetate in water, solvent A) and methanol/methyl-tert-butyl ether/water (8:90:2, *v*/*v*/*v*, 10 g/L with ammonium acetate in water, solvent B). The gradient procedure, at a flow rate of 1 mL/min (16 °C), was as follows: (1) 100% solvent A was used for 2 min followed by a 6 min linear gradient to 70% solvent A; (2) a 3 min hold followed by a 10 min linear gradient to 45% solvent A; (3) a 2 min hold, then a 10 min linear gradient to 5% solvent A; (4) a 4 min hold, then a 2 min linear gradient back to 100% solvent A. For the quantification of the chromatograms, a comparison was made between the area ratio of the substance and area of the internal standard obtained in the analysis[32]

2.7.Cardiac malondialdehyde (MDA) levels

MDA is the main lipid peroxidation marker. It is considered an oxidative stress index[33] and associated with cardiovascular diseases[34]. Thus, MDA levels were used to evaluate the cardiac lipid oxidation as follow:

Cardiac tissue (±150mg) was homogenized (ULTRA-TURRAX® T25 basic IKA® Werke Staufen/Germany) with 1.0mL of cold phosphate buffered saline (PBS) pH 7.4 , and centrifuged at 800g at 4°C for 10min. 100μL from the supernatant was mixed with 700μL of 1% orthophosphoric acid and 200μL of thiobarbituric acid (42mM). After this, the samples were kept at 100°C for 60min in a water bath, and immediately cooled on ice. In a 2mL tube, 200 μL was mixed with 200μL sodium hydroxide/methanol (1:12 *v*/*v*). After vortex, the samples were centrifuged for 3min at 13,000*g*. 200μL from the supernatant was transferred to a glass vial and 50μL was injected into the column. The HPLC used was a Shimadzu LC-10AD system (Kyoto, Japan) with a C18 Luna column (5μm, 150 × 4.60mm, Phenomenex Inc., Torrance, CA, USA), and a Shimadzu RF-535 fluorescence detector (excitation 525nm, emission 551nm), and 0.5mL/min phosphate buffer flow (KH2PO4 1mM, pH 6.8)[27]. The MDA levels considered the peak area determination in the chromatograms relative to the standard curve of known concentrations.

2.8.Circulating advanced oxidation protein products

Advanced oxidation protein products (AOPPs) are oxidized plasma proteins, result from the exposure to oxidation products and transported by albumin in the circulation[35]. The literature reports that high AOPP circulating levels contribute to cardiac diseases[36].

AOPP determination was based on spectrophotometric detection according to Kalousova et al[37]. Plasma samples (200μL) were diluted 1:5 with PBS. It was also used 200μL of chloramin T (0-100μmol/L) for calibration curve and the blank was only PBS (200μL). All the samples were put on a microtiter plate and mixed with 10μL of KI 1.16M and 20μL of acetic acid. The absorbance was measured immediately at 340nm (spectrophotometer Multiskan Ascent, Labsystems, Finland). The final AOPP concentration is expressed in chloramine units (μmol/L).

2.9.Circulating carboxymethyl lysine

Advanced glycation end products (AGEs) are a group of several molecules generated by both non-enzymatic glycation and protein, lipids and nucleic acids oxidation, able to modify tissue function and mechanical properties[38]. *In vivo*, CML is the main AGE associated with cardiac pathologies[39]. The plasmatic carboxymethyl lysine (CML) levels were evaluated by using ELISA commercial kit (OxiSelect™ CML, Cell Biolabs Inc.) following the manufacturer's instructions.

2.10.Echocardiographic study

The analyze was performed in the live animals by transthoracic echocardiography, with a Vivid S6 system equipped with multifrequency ultrasonic transducer 5.0 to 11.5MHz (General Electric Medical Systems, Tirat Carmel, Israel). The animals were lightly anesthetized by intraperitoneal injection with a mixture of ketamine (50 mg/kg) and xylazine (1 mg/kg), put in left decubitus position and only one examiner made all the exams. The heart image structural measures were obtained in one-dimensional mode (M-mode) guided by the images in two-dimensional mode with the transducer in the parasternal position, minor axis. Left ventricular (LV) evaluation was performed with the cursor M-mode just below the mitral valve plane at the level of the papillary muscles. The aorta and left atrium images were obtained by positioning the M-mode course to plan the aortic valve level.

The following cardiac structures were evaluated: diastolic diameter (LVDD); systolic (LVSD) LV; left ventricle diastolic thickness posterior wall (LVPWD); aorta diameter (AD); left atrium (LA). The LV diastolic function was assessed by the transmitral flow early peak velocity (E). The LV systolic function was evaluated by ejection fraction and posterior wall shortening velocity (PWSV). The joint assessment of diastolic and systolic LV function was performed by Tei index (sum of isovolumetric contraction and IRT time, divided by the left ventricular ejection time). The study was complemented by tissue Doppler evaluation, considering early diastolic (E'), and late (A') of the mitral annulus (arithmetic average travel speeds of lateral and septal walls), and the ratio by the waves E and E'(E/E').

2.11.Myocardial function by isolated papillary muscle study

Besides echocardiographic analysis, the myocardial function was also assessed by LV isolated papillary muscles. This procedure has been used by several authors[6],

[7], [40]. Conventional mechanical parameters at *Lmax* were calculated from isometric contraction: maximum developed tension normalized per cross-sectional area (DT [g/mm^2]), resting tension normalized per cross-sectional area (RT [g/mm^2]), positive (+dT/dt [g/mm^2/s]) and negative (-dT/dt [g/mm^2/s]) tension derivative normalized per cross-sectional area of papillary muscle (CSA).

2.12.β-adrenergic system study

β-adrenergic receptors (βAR) are important to regulate the cardiac function in both normal and pathologic conditions[10]. The receptors activity was assessed by the dose-response relationship between the isoproterenol and conventional mechanical parameters of papillary muscle at *Lmax*. After baseline values determination, the isoproterenol was added to the vat in the presence of 1.0 mM [Ca^{2+}] to increase progressively the concentrations for 10^{-8}, 10^{-7} and 10^{-6} mol/L.

The stabilization of contractile response occurs nearly 3- 5min after adding each isoproterenol dose. Data were sampled and expressed as the stimulation mean percent (%)[31]. At the end of the study, length (mm), weight (mg), and CSA (mm^2)[41] were measured for papillary muscle characterization. The CSA was calculated from papillary muscle length and weight, assuming uniformity and a specific gravity of 1.0. The muscle length at *Lmax* was measured with a cathetometer (Gartner Scientific Corporation, Chicago, IL, USA), and the muscle between the two clips was blotted dry and weighed.

2.13.Statistical Analysis

The results are expressed in mean ± standard deviation (SD). Two-way analysis of variance (ANOVA) for independent samples was used to determine the differences among the groups. In order to evaluate the positive and negative inotropic effects on myocardial function, it was used a repeated-measures two-way ANOVA. Once detected significant differences ($p< 0.05$), the Tukey post hoc test for multiple comparisons were carried out. All the statistical analyses were performed using SigmaStat for Windows (Version 3.5).

3.Results

The lycopene bioavailability is presented in the Table 1. It is possible to verify the presence of lycopene in both groups, which were supplemented (Control+Ly and HSF+Ly).

Table 1. Lycopene Bioavailability.

	Groups			
	Control	**Control + Ly**	**HSF**	**HSF + Ly**
Plasma(μg/mL)	ND	3.61 ± 0.68	ND	3.59 ± 2.31
Heart(μg/g of tissue)	ND	4.83 ± 2.37	ND	2.41 ± 0.36

Data are expressed in mean ± standard deviation (n= 4 animals/group).ND: Not detectable.

The HSF group presented increased caloric intake (kcal/d), final body weight (g), adiposity index, glucose levels, HOMA-IR and systolic blood pressure values

compared to the control group. The HSF + Ly showed the same changes observed in HSF group when compared to control + Ly, except for HOMA-IR. Tomato- oleoresin suppressed the insulin resistance in HSF + Ly compared to HSF (figure 1). No effect was observed of tomato- oleoresin on the other parameters.

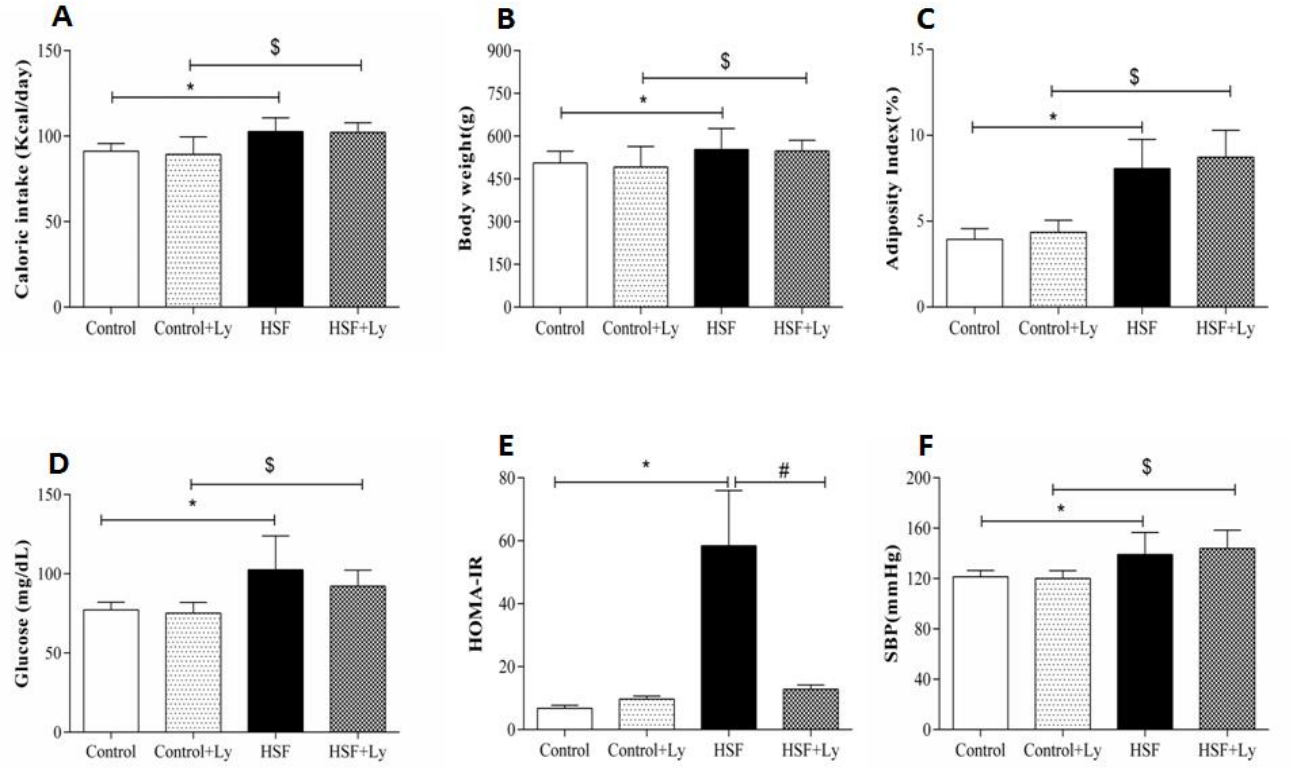

Figure 1. Nutritional and cardio- metabolic parameters. A- caloric intake (kcal/day); B- adiposity index (%); C- final body weight (g); D- glucose (mg/dL); E- HOMA-IR; F- systolic blood pressure (mmHg). Data are expressed in mean ± standard deviation (n= 6 animals/group). Comparison by Two-way ANOVA with Tukey post-hoc ($p < 0.05$): *HSF *vs* Control; $^{\#}$HSF *vs* HSF+Ly; $^{\$}$HSF+Ly *vs* Control+Ly.

The HSF group presented cardiac remodeling (increased LVDS, LVPWD and reduced LVDD), and deterioration of both systolic (decreased ejection fraction, Tei-a and Tei-b) and diastolic (increased E/E′ and decreased Tei-a and Tei-b) functions compared to control group. Regarding the tomato-oleoresin supplementation effect, HSF + Ly group showed improvement in some remodeling, systolic and diastolic parameters compared to HSF (Table 2).

Table 2. Echocardiographic study.

	Groups				Effect		
	Control	Control + Ly	HSF	HSF + Ly	Diet	Ly	interaction
LVDD (mm)	7.15 ± 0.11	7.02 ± 0.11	6.70 ± 0.12*	6.92 ± 0.11	0.019	0.665	0.123
LVDS (mm)	2.83 ± 0.10	2.74 ± 0.10	3.17 ± 0.11*	2.91 ± 0.10	0.016	0.098	0.417
LVPWD (mm)	1.63 ± 0.04	1.53 ± 0.04	1.73 ± 0.04*	1.62 ± 0.04$^{\#}$	0.031	0.014	0.932
AD (mm)	3.91 ± 0.06	3.86 ± 0.06	3.89 ± 0.07	3.88 ± 0.06	0.999	0.682	0.740
LA (mm)	4.86 ± 0.11	4.85 ± 0.11	5.02 ± 0.11	4.88 ± 0.11	0.388	0.483	0.536
HR (bpm)	254 ± 14	265 ± 14	262 ± 15	262 ± 14	0.871	0.716	0.713
E (cm/s)	73.6 ± 2.1	73.2 ± 2.18	76.1 ± 2.1	75.1 ± 2.3	0.351	0.742	0.895
PWSV (cm/s)	58.6 ± 1.3	61.1 ± 1.3	56.1 ± 1.3	59.8 ± 1.4	0.181	0.028	0.622
Dec. time (ms)	47.2 ± 1.3	42.1 ± 1.3	50.6 ± 1.3	42.7 ± 1.4	0.128	<0.001	0.322
Tei-a (ms)	116.1 ± 2.5	116.8 ± 2.5	99.1 ± 2.5*	111.7 ± 2.6$^{\#}$	<0.001	0.012	0.024
Tei-b (ms)	86.6 ± 2.9	92.6 ± 2.9	77.7 ± 2.9*	85.5 ± 3.1$^{\#}$	0.012	0.028	0.761
EF (%)	0.93 ± 0.008	0.93 ± 0.008	0.88 ± 0.008*	0.93 ± 0.008$^{\#}$	<0.001	0.006	0.008
E/E'	13.3 ± 0.4	12.7 ± 0.4	15.3 ± 0.4*	13.9 ± 0.50$^{\#}$	0.002	0.049	0.439

Data are expressed in mean ± standard deviation (n= 6 animals/group). Comparison by Two-way ANOVA with Tukey post-hoc ($p < 0.05$): *HSF *vs* Control; $^{\#}$HSF *vs* HSF+Ly; $^{\$}$ HSF+Ly *vs* Control+Ly. LVDD, left ventricular diastolic diameter; LVSD, left ventricular systolic diameter; LVPWD, diastolic thickness posterior wall of the left ventricle; AD, aorta diameter; LA, left atrium diameter during ventricular systole; HR, heart rate; E, E-wave peak transmitral early diastolic inflow velocity; PWSV, posterior wall shortening velocity; Dec. time, deceleration time; Transmitral flow, Tei-a and Tei-b; EF, ejection fraction; E/E'.

The myocardial papillary muscle study at baseline condition with 2.5 mM Ca^{2+} is presented in the Table 2. HSF group showed functional impairment in the maximum developed tension (DT) compared to control group. Tomato-oleoresin supplementation was effective to recovery the DT capacity in HSF + Ly group compared to HSF (table 3).

Table 3. Isolated papillary muscle at baseline condition (2.5mM Ca^{2+}).

	Groups				Effect		
	Control	Control + Ly	HSF	HSF + Ly	diet	Ly	interaction
DT(g/mm²)	5.96 ± 1.25	6.29 ± 1.65	4.41 ± 1.11*	6.05 ± 1.19$^{\#}$	0.066	0.046	0.173
RT(g/mm²)	0.65 ± 0.11	0.61 ± 0.11	0.63 ± 0.08	0.57 ± 0.11	0.512	0.202	0.844
+d*T*/d*t*(g/mm²/s)	61.9 ± 10.1	63.5 ± 18.4	60.8 ± 11.7	65.5 ± 19.7	0.934	0.573	0.773
-d*T*/d*t*(g/mm²/s)	16.8 ± 2.4	17.5 ± 2.9	15.5 ± 3.3	16.1 ± 2.9	0.193	0.569	0.933
CSA(mm²)	1.11 ± 0.12	1.10 ± 0.23	1.25 ± 0.27	1.17 ± 0.3	0.181	0.801	0.912

Data are expressed in mean ± standard deviation (n= 6 animals/group). Comparison by Two-way ANOVA with Tukey post-hoc ($p < 0.05$): *HSF *vs* Control; $^{\#}$HSF *vs* HSF+Ly; $^{\$}$ HSF+Ly *vs* Control+Ly. DT, Maximum developed tension normalized per cross-sectional area of the papillary muscle; RT, Resting tension normalized per cross-sectional area of the papillary muscle; peak of the positive, +dT/ dt and negative, -dT/dt tension derivatives normalized per cross-sectional area of the papillary muscle; CSA, cross-sectional area.

The figure 2 shows the β-adrenergic stimulation on the papillary muscle function. The isoproterenol stimulation demonstrated that the HSF group presented functional impairment in DT (10^{-6} M) and -dT/dt (10^{-7} and 10^{-6} M) compared to control group.

Tomato- oleoresin supplementation was effective to recovery the -dT/dt (10^{-7} and 10^{-6} M) capacity in HSF+Ly group compared to HSF.

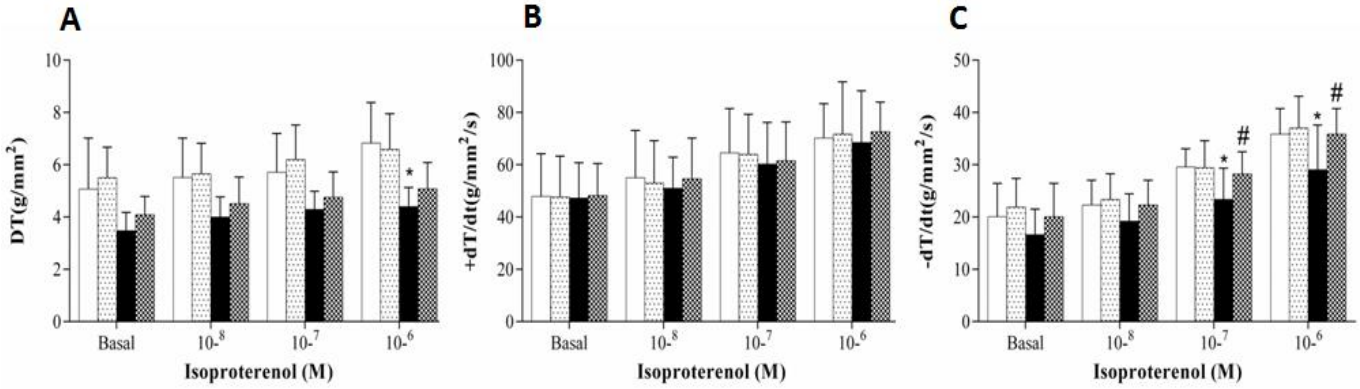

Figure 2. β- adrenergic stimulation in papillary muscles**.** Data are expressed in mean ± standard deviation (n= 6 animals/group). Baseline calcium concentration (1.0 mM) is presented as 100%. A, Maximum developed tension normalized per cross-sectional area [DT, g/mm2]. B, positive [+dT/dt, g/mm2 /s] and C, negative [-dT/dt, g/mm2 /s] tension derivative normalized per cross-sectional area of the papillary muscle. Two-way ANOVA repeated-measures with Tukey post-hoc was used to compare the groups ($p < 0.05$); *HSF *vs* Control; #HSF *vs* HSF+Ly.

The figure 3 shows the oxidative stress parameters in plasma and cardiac tissue. HSF group presented increased in all the parameters compared to control group. In opposition, it is possible to note a positive effect of the tomato-oleoresin on HSF+Ly group reducing plasma CML and AOPP and cardiac MDA levels compared to HSF.

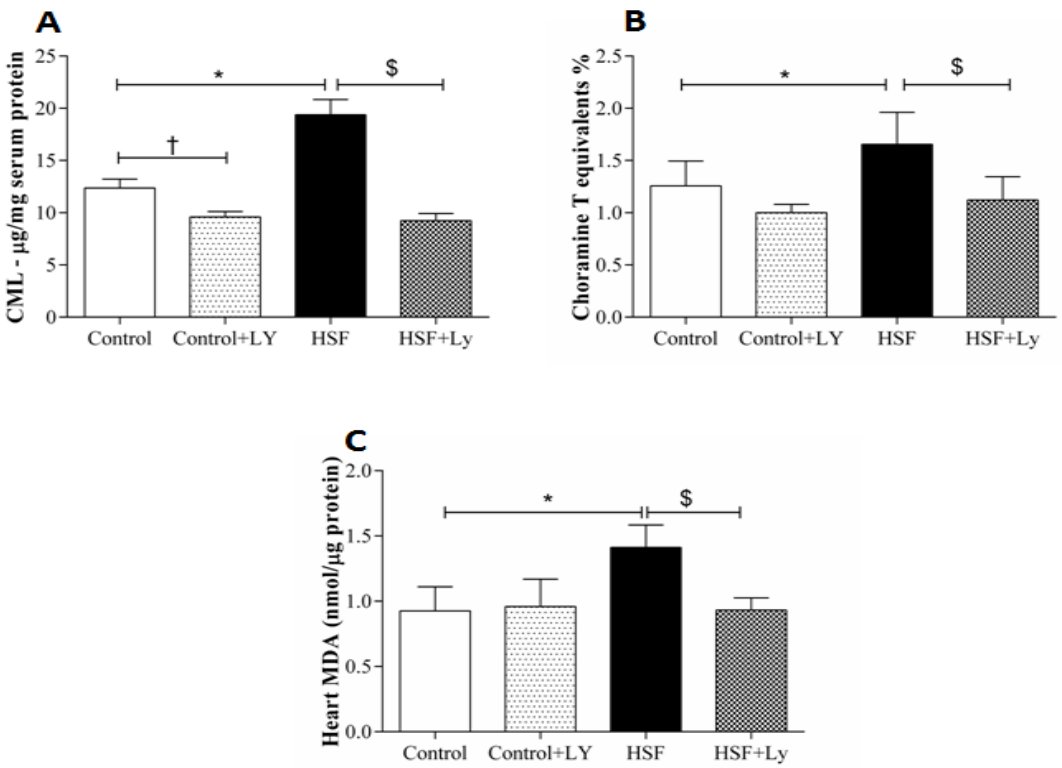

Figure 3. Plasma and cardiac tissue redox state parameters. A- Carboxymethyl lysine (CML- pg/mg protein); B- Cholaramine T equivalents %; C- Malondyhaldeide (MDA-nmol/μg protein). Data are expressed in mean ± standard deviation (n= 6 animals/group). Comparison by Two-way ANOVA with Tukey post-hoc ($p < 0.05$), *HSF *vs* Control; $HSF *vs* HSF+Ly.

4.Discussion

This study aimed to test if the tomato-oleoresin would be able to recovery the cardiac function by improving β-adrenergic response due its antioxidant effect. The results show that the HSF groups presented obesity, characterized by the higher values of body weight and adiposity index and also metabolic syndrome, with insulin resistance, dyslipidemia, and hypertension, all diseases usually associated with obesity[42]. These finding confirm that the diet model used in this study was efficient to lead obesity and related disorders, corroborating the literature [6], [7], [24], [25]. Regarding the lycopene effect on obesity and related disorders, it was observed a positive action only on insulin resistance in the HSF + Ly group, represented by the reduction in HOMA- IR. The literature attributes the tomato-oleoresin benefic effects on diabetes to the lycopene antioxidant potential[43]. Another explanation for this amelioration is the anti-inflammatory effect of tomato-oleoresin. Since insulin resistance and type 2 diabetes are conditions closely related with inflammation and studies already showed that tomato-oleoresin ameliorates the inflammation, this property may explain the beneficial effect on glucose metabolism[44].

The obesity is also associated with cardiac abnormalities, among them morphological, hemodynamic and functional alterations[1], [2], [24]. Considering the lycopene absence effect on obesity and hypertension in the HSF + Ly group, should both HSF groups present cardiac damage. However, the echocardiographic analysis showed cardiac remodeling and impairment in ventricular systolic and diastolic function only in HSF group after 30 weeks. In opposition, the HSF group supplemented with tomato- oleoresin showed a cardiac remodeling and function recovery.

Several mechanisms try to explain the obesity-induced cardiac dysfunction, among them, the β-adrenergic system responsiveness. The myocardial β-adrenergic mechanism is the main responsible by regulating the cardiac performance, especially by intracellular Ca^{2+} handling[10], [11]. Although functional studies using isolated papillary muscle have showed that obesity is able to lead to impairment in cardiac contractile[3], [5], [7], a small number of studies have evaluated the β-adrenergic response in high sugar-fat diet obesity-induced experimental models[31], [45]–[49]. Our results demonstrated that the isoproterenol stimulation leaded to negative responses in both systolic (DT) and diastolic ($-dT/dt$) response in the HSF group while the HSF + Ly group showed an improvement in the β-adrenergic response. However, it is still unclear how the high sugar-fat diet obesity-induced leads to a reduction in the β-adrenergic response.

One hypothesis for the β-adrenergic response impairment is the chronic exposition to reactive oxygen species (ROS) promoted by obesity[9], [50]. The literature reports that the direct contact with ROS exerts the same action of

isoproterenol on β-adrenergic response, increasing the calcium transient amplitude, therefore, exerting a modulator role in the myocardial contractility[13]. However, this continues exposition to ROS may result in deleterious effects and contribute the development of cardiac arrhythmias and failure[9]. Considering the lycopene antioxidant effect, the amelioration in the β-adrenergic responsiveness of the HSF+ Ly group can be attributed to this carotenoid property[14].

Another hypothesis is that the redox system unbalance in obesity condition may leads damage to lipid and protein, generating biomarkers as MDA, CML and AOPP, which were evaluated in this stud[35], [51]–[53]. These oxidative products can damage directly the cardiac tissue by altering its geometry and functionality or indirectly by carbonylation of proteins involved in the myocardial contractility regulatory response, as the β-adrenergic pathway[13], [54], [55]. While the HSF group presented higher levels of MDA, CML and AOPP and cardiac function deterioration, the tomato-oleoresin antioxidant effect is confirmed by the reduced levels of these markers and cardiac function recovery in the HSF + Ly group.

In summary, this study found that the HSF diet induced obesity- related cardiac dysfunction and the tomato-oleoresin was able to attenuate this condition. Therefore, it is possible to conclude that tomato-oleoresin is able to reduce the oxidative damage improving the system β-adrenergic response recovering the cardiac function.

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

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