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Vanessa Azevedo de Jesuz

EFEITO DA SUPLEMENTAÇÃO DE LICOPENO E BETACAROTENO SOBRE O TECIDO MUSCULAR CARDÍACO EM RATAS WISTAR ALBINAS ALIMENTADOS COM DIETA HIPERLIPÍDICA

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Tese de doutorado apresentada ao Programa de Pós-Graduação em Alimentos e Nutrição, da Universidade Federal do Estado do Rio de Janeiro como requisito para obtenção do título de Doutor em Ciência de Alimentos.

Orientador: Dr. Anderson Junger Teodoro.
Coorientador: Dra. Vilma Blondet de Azeredo.

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BANCA EXAMINADORA

Prof. Dr. Anderson Junger Teodoro – PPGAN/UNIRIO

Profª. Drª. Vilma Blondet de Azeredo - UFF

Profª. Drª Leandro Miranda Alves - UFRJ

Prof. Dr. Juliana Furtado Dias - UNIRIO

Prof. Dr. Cassiano Felippe Gonçalves de Albuquerque - UNIRIO

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Dedico este trabalho a minha família e
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RESUMO

O padrão alimentar atual da população é de uma dieta rica em gorduras que estimulam a expressão de citocinas pró-inflamatórias, estresse oxidativo e dano ao tecido celular, levando a prejuízos nas células cardíacas e podendo acarretar doenças cardiovasculares. Os carotenóides são compostos bioativos com potente ação antioxidante e anti-inflamatória que têm sido investigados na prevenção e no tratamento de doenças cardiovasculares. Assim, o presente estudo teve como objetivo avaliar o efeito do consumo de carotenóides isolados e na matriz alimentar sobre a função e integridade do músculo esquelético cardíaco. Para tanto, foi realizado estudo experimental em duas etapas. Na primeira etapa foram avaliados os efeitos do molho de tomate e licopeno sobre biomarcadores de células cardíacas em ratos alimentados com dieta hiperlipídica. *Rattus norvegicus, wistar*, albinos, fêmeas e adultas foram divididos em cinco grupos: grupo controle, grupo hiperlipídico, grupo de molho de tomate, grupo licopeno 2 mg e grupo licopeno 4 mg. Ração e água foram oferecidas *ad libitum*, enquanto molho de tomate e licopeno (2 e 4 mg/dia) foram oferecidos diariamente por 60 dias. Na segunda etapa avaliou-se o efeito do betacaroteno e do suco de buriti nos parâmetros das células cardíacas de ratos submetidos à dieta hiperlipídica. *Rattus norvegicus, wistar*, albinos, fêmeas e adultas foram distribuídas em cinco grupos: grupo controle; grupo hiperlipídico; grupo suco de buriti; grupo betacaroteno 2mg e grupo betacaroteno 4mg. Ração e água foram oferecidas *ad libitum*, enquanto suco de buriti e betacaroteno (2 e 4 mg/dia) foram oferecidos diariamente por 60 dias. Após eutanásia dos animais foram coletados: sangue e tecido cardíaco e avaliados o índice cardiossomático, o perfil lipídico, os marcadores inflamatórios (IL-1β e TNF-α), ciclo celular e apoptose do tecido cardíaco (citometria de fluxo). Utilizou-se o *Software GraphPad inStat* e o *SPSS Statistical* para a realização da análise estatística, sendo aceito o nível de significância de 5%. A análise do ciclo celular de células cardíacas mostrou uma menor porcentagem de células na fase G0/G1 e um aumento na fase G2/M ocasionado pela dieta hiperlipídica. Tanto o licopeno quanto o molho de tomate reverteram o efeito negativo da dieta rica em gordura no ciclo celular e preveniram a morte celular cardíaca estimulada por dieta rica em gordura. A suplementação de molho de tomate e licopeno apresentou efeitos benéficos no metabolismo cardíaco e hepático. O suco de buriti e a suplementação de betacaroteno foram capazes de reduzir o efeito de uma dieta rica em gordura na IL-1. No entanto, a suplementação com suco de buriti e com betacaroteno isolado aumentam a morte celular, principalmente a suplementação com 4 mg de betacaroteno isolado, agravando os danos histológicos causados pela dieta hiperlipídica e demonstrando um possível efeito tóxico sobre essas células.

Palavras-chave: carotenóides; licopeno; betacaroteno; dieta hiperlipídica; inflamação; ciclo celular; apoptose.

ABSTRACT

The current dietary pattern of the population is a diet which is rich in fats that stimulate the expression of pro-inflammatory cytokines, oxidative stress and damage to cell tissue, leading to damage to cardiac cells and potentially causing cardiovascular disease. Carotenoids are bioactive compounds with a powerful antioxidant and anti-inflammatory action which have been investigated in the prevention and treatment of cardiovascular diseases. Thus, the present study aimed to evaluate the effect of the consumption of carotenoids both in isolation and in the food matrix on the function and integrity of the cardiac skeletal muscle. Therefore, an experimental study was carried out in two stages. In the first step, we evaluated the effects of tomato sauce and lycopene on cardiac cell biomarkers in rats fed a high-fat diet. *Rattus norvegicus*, *wistar*, albinos, females and adults were divided into five groups: control group, hyperlipidic group, tomato sauce group, 2 mg lycopene group and 4 mg lycopene group. Food and water were offered *ad libitum*, while tomato sauce and lycopene (2 and 4 mg / day) were offered daily for 60 days. In the second stage, we focused on the effect of beta-carotene and buriti juice on the parameters of cardiac cells of rats submitted to a high-fat diet. *Rattus norvegicus*, *wistar*, albinos, females and adults were divided into five groups: control group; hyperlipidic group; buriti juice group; 2mg beta-carotene group and 4mg beta-carotene group. Food and water were offered *ad libitum*, while buriti juice and beta-carotene (2 and 4 mg / day) were offered daily for 60 days. After euthanasia of the animals, blood and cardiac tissue were collected and the cardiosomatic index, lipidic profile, inflammatory markers (IL-1β and TNF-α), cell cycle and apoptosis of cardiac tissue (flow cytometry) were evaluated. The GraphPad inStat software and the SPSS Statistical software were used to perform the statistical analysis, with a significance level of 5% being accepted. The analysis of the cell cycle of cardiac cells showed a lower percentage of cells in the G0 / G1 phase and an increase in the G2 / M phase caused by the high-fat diet. Both lycopene and tomato sauce reversed the negative effect of a high-fat diet on the cell cycle and prevented cardiac cell death stimulated by a high-fat diet. Supplementation of tomato sauce and lycopene had beneficial effects on cardiac and hepatic metabolism. Buriti juice and beta-carotene supplementation were able to reduce the effect of a high-fat diet on IL-1. However, supplementation with buriti juice and beta-carotene in isolation increases cell death, especially supplementation with 4 mg of beta-carotene, aggravating the histological damage caused by the high-fat diet and demonstrating a possible toxic effect on these cells.

Keywords: carotenoids; lycopene; betacarotene; high-fat diet; inflammation; cell cycle; apoptosis.

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Effect of beta carotene and buriti juice (*Mauritia Flexuosa*) on hepatic and cardiac cell biomarkers in rats fed with high-fat diet

INTRODUÇÃO

Sabe-se que o padrão alimentar atual da população é de uma dieta rica em gorduras que pode desencadear o aumento do estresse oxidativo, estado inflamatório e danos em diversas biomoléculas, dentre elas o DNA, bem como favorecer o desenvolvimento de diversas doenças crônicas não transmissíveis como as doenças cardiovasculares (BRAGA *et al,* 2002; GERALDO *et al.,* 2008; OLIVEIRA & SCHOFFEN, 2010).

As doenças cardiovasculares são consideradas um problema de saúde pública e uma das principais causas de morte em todo o mundo. Dentre elas, destaca-se a aterosclerose, uma patologia assintomática caracterizada pelo estreitamento ou oclusão da artéria por um ateroma. Por conseguinte, cada vez mais estudos têm sido realizados a fim buscar estratégias preventivas e o uso de compostos bioativos presentes nos alimentos parece contribuir para a prevenção de doenças cardiovasculares por diversos mecanismos de ação, primordialmente, pelo seus potenciais antioxidantes e anti-inflamatórios (UPADHYAYA *et al,* 2007).

Diversas pesquisas evidenciam uma relação inversa entre o consumo de alimentos ricos em carotenóides e o risco de doenças induzidas pelo estresse oxidativo (ZERN & FERNANDEZ, 2005; SILVER *et al.,* 2011; LAU *et al.,* 2005; VENTURINI *et al.,* 2011). Os carotenoides são amplamente distribuídos na natureza, tipicamente vistos como pigmentos em frutas, flores e vegetais, e são responsáveis pela coloração amarela, laranja e vermelha (FRASER and BRAMLEY, 2004; RODRIGUEZ-AMAYA E KIMURA, 2004).

Atualmente, mais de 700 carotenóides foram isolados de produtos naturais, sendo β-caroteno, licopeno, luteína, α-caroteno, zeaxantina e β-criptoxantina são os carotenóides mais prevalentes no soro humano (GERSTER, 1997). Dentre os carotenoides mais amplamente estudados os que possuem maior atividade antioxidante é o licopeno e o β-caroteno (DIAS *et*

al., 2008; RUHL, 2013, YOUNG, 2001). Eles podem atuar na desativação de espécies reativas, evitando assim a iniciação de cadeias de oxidação em nível celular que conduz a danos ao ácido desoxiribonucléico (DNA) e peroxidação lipídica (SILVA *et al.*, 2001).

O tomate e seus derivados são as melhores fontes dietéticas de licopeno e, quanto mais avermelhado for o alimento, maior é sua concentração (BRAMLEY *et al.*, 2000). Dentre os alimentos com maior teor de betacaroteno, encontra-se o buriti (*Mauritia flexuosa*) que é uma fruta pertencente à família *Arecaceae*, encontrado na Floresta Amazônica brasileira (DELGADO, COUTURIER, & MEJIA, 2007).

Todavia, os estudos ainda não são conclusivos quanto a melhor forma de utilização dos carotenóides com o objetivo de prevenção ou benefícios sobre a saúde cardiovascular, se a partir da ingestão de suplementos isolados ou na matriz alimentar. Deste modo, cabe identificar possíveis meios dietéticos capazes de amenizar estes efeitos e atuarem na proteção do tecido muscular cardíaco. Neste aspecto, torna-se relevante estudar o efeito do consumo de carotenóides isolados e na matriz alimentar sobre a função e integridade do musculo esquelético cardíaco.

CAPÍTULO 1

REVISÃO BIBLIOGRÁFICA

Carotenóides e Saúde Cardiovascular

A Pesquisa de Orçamento Familiar realizada pelo IBGE nos anos de 2017-2018 (POF 2017-2018) evidenciou o alto consumo de produtos processados e prontos para consumo como pães, biscoitos recheados, sanduíches, salgados, pizzas, refrigerantes, sucos e cerveja pela população brasileira, em especial nas áreas urbanas. Na pesquisa, menos de 10% da população brasileira atingiu as recomendações de consumo de frutas, verduras e legumes.

Além disso, a ingestão inadequada de micronutrientes foi observada em todas as Grandes Regiões do País, evidenciando o padrão inadequado da alimentação da população brasileira que pode desencadear déficits de nutrientes e diversas doenças crônicas não transmissíveis, como a obesidade e doenças cardiovasculares (IBGE, 2018).

A ingestão de dietas hiperlipídicas, ricas principalmente em ácidos graxos saturados, pode estar associada ao desenvolvimento de estresse oxidativo, estado inflamatório e danos em diversas biomoléculas, dentre elas o DNA, bem como favorecer o desenvolvimento de diversas doenças crônicas (BRAGA *et al*, 2002; GERALDO *et al.*, 2008; OLIVEIRA & SCHOFFEN, 2010). Acredita-se que dietas ricas em gordura saturada podem estimular a síntese de citocinas pró-inflamatórias, aumento de espécies reativas de oxigênio e estresse oxidativo (FRANÇA *et al.*, 2013; GENTILE & PAGLIASSOTTI, 2008). Desta maneira, o consumo de uma dieta rica em gordura saturada poderia contribuir para o desenvolvimento de doenças cardiovasculares.

As doenças cardiovasculares hoje em dia são consideradas um problema de saúde pública e uma das principais causas de morte em todo o mundo. Estima-se que 17,7 milhões de pessoas morreram por doenças cardiovasculares em 2015, representando 31% de todas as mortes em nível global (WHO, 2017).

Diversos fatores podem contribuir de maneira negativa ou positiva na gênese das doenças cardiovasculares. Como fatores negativos podemos incluir alimentação rica em gorduras saturadas, trans e açúcar; obesidade; uso de cigarro e bebida alcóolica em excesso. Existem alguns fatores negativos que não são controlados como idade, sexo e genética (**Figura 1**). E existem ainda diversos fatores que podem contribuir positivamente para que a doença não ocorra, tais como alimentação rica em compostos bioativos, vitaminas e antioxidantes e a prática de atividade física (GARCÍA-FERNÁNDEZ, 2014).

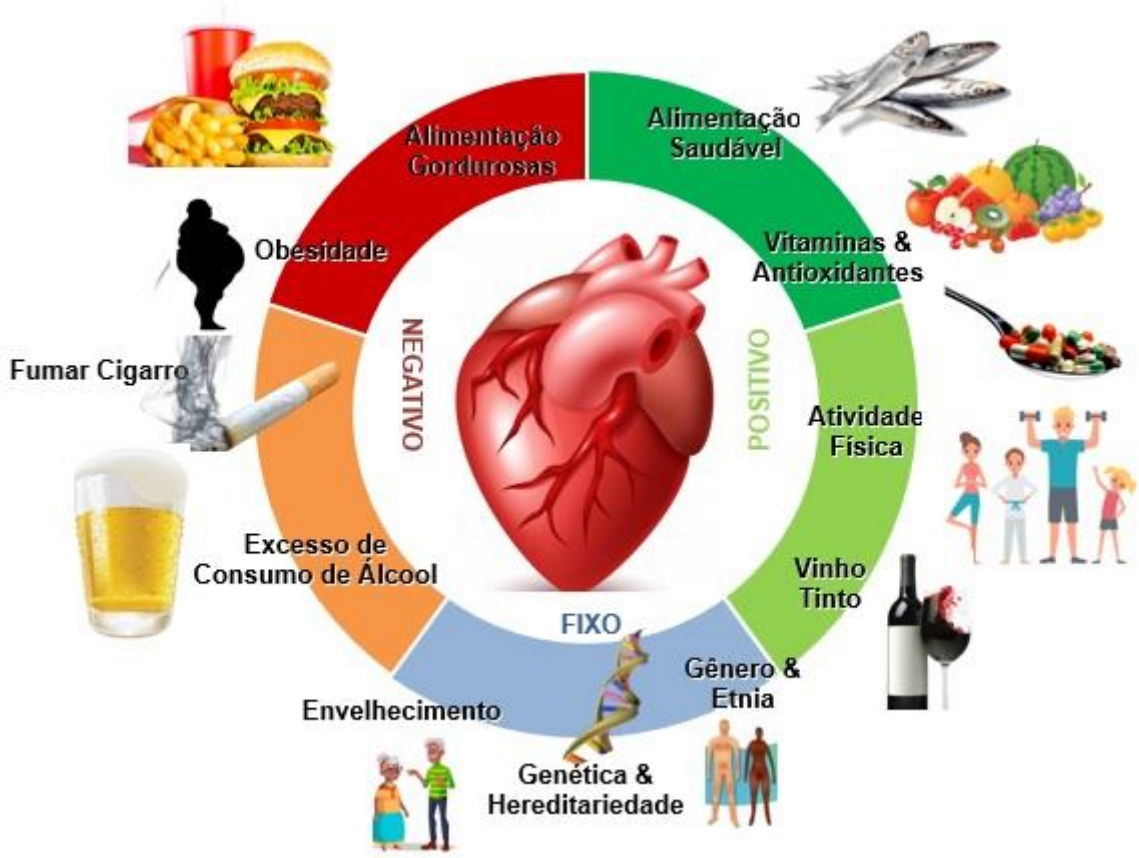

Figura 1. Fatores de risco e fatores preventivos para doenças cardiovasculares

Dentre as doenças cardiovasculares mais acometidas e principal responsável pela patogênese do infarto miocárdico, pelo acidente vascular cerebral e por doenças vasculares periféricas, encontra-se a aterosclerose que é uma doença caracterizada pelo estreitamento ou oclusão da artéria por um ateroma que, por sua vez, pode ser constituído por uma grande

variedade de elementos, tais como lipídios, monócitos, plaquetas, elementos fibrosos e cálcio (RAPOSO, 2010).

Diversos podem ser os agentes e condições fisiopatológicas a promover as lesões iniciais do processo aterosclerótico, como níveis elevados de LDL-c oxidado, níveis baixos de HDL-c, níveis elevados de colesterol total, hipertrigliceridemia, alterações nos valores de homocisteína e de PCR-us, hipertensão arterial, obesidade, diabetes mellitus tipo 2, bem como fatores do estilo de vida, tais como dieta aterogênica, sedentarismo, tabagismo, etilismo (LUZ & COIMBRA, 2004; RAPOSO, 2010; WONG, 2012; BARBALHO, 2015).

A aterosclerose em si é uma patologia assintomática e alterações precoces nas paredes dos vasos que levam a lesões ateroscleróticas podem ser encontradas mesmo em jovens saudáveis (VIOLA & SOEHNLEIN, 2015). Acredita-se que mediante um estado inflamatório e formação de espécies reativas de oxigênio (ERO), como superóxido e peróxido de hidrogênio, a LDL-colesterol pode ser oxidada e, a partir disso, produzir uma série de produtos de oxidação e gerar lesões nas células endoteliais (STEINBERG *et al*., 1989; HANSSON & HERMANSSON, 2011; ROCHA & LIBBY, 2009) (**Figura 2**).

Na gênese da aterosclerose, além da inflamação crônica da parede da artéria e consequente formação de placas, encontra-se também a ativação de diferentes células inatas do sistema imune que estão envolvidas diretamente na origem do depósito das substâncias constituintes destas placas. As lesões ateroscleróticas são, de fato, uma série de respostas celulares e moleculares altamente específicas e dinâmicas, essencialmente inflamatórias, por natureza (BARBALHO, 2015).

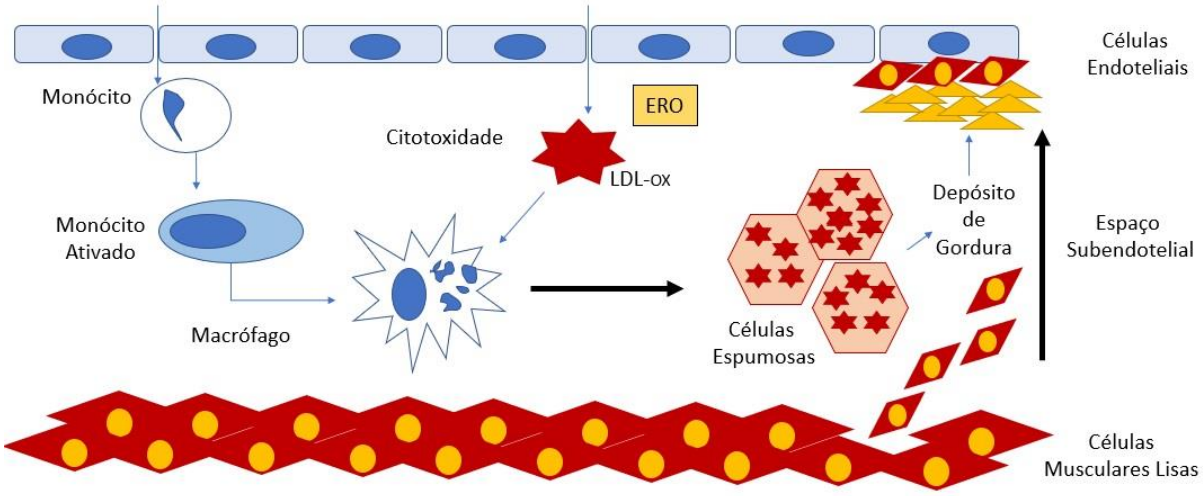

Figura 2. Conjunto de interações dos monócitos, macrófagos e no processo de formação da placa aterosclerótica. Internalização e modificação das LDL pelas espécies reativas de oxigénio (ROS), diapedese dos monócitos, internalização das LDL-ox e formação de células espumosas. Migração das células musculares lisas e formação de trombos.

É cada vez mais evidente que a inflamação é um mecanismo central envolvido em todo o ciclo de vida da aterosclerose (WONG, 2012). No entanto, a fisiopatologia da aterosclerose compreende várias etapas importantes, como aumento da permeabilidade endotelial, expressão de moléculas de adesão, imigração e adesão de monócitos e formação de células espumosas (PANDIT & PANDEY, 2016).

A inflamação na parede do vaso prossegue na forma de cascatas com seu início na ativação das células endoteliais, resultando na expressão de moléculas de adesão na superfície celular (moléculas de adesão intracelular), moléculas de adesão de células vasculares, selectinas, integrinas e outros, que tem como consequência a produção e liberação de citocinas pró-inflamatórias e quimiocinas (fator de necrose tumoral, interferons, proteína-1 quimioatraente de monócitos e outras). Essas cascatas podem ser intensificadas por outros fatores, tais como espécies reativas de oxigênio (ROS), hipóxia e fator de crescimento alterado (WONG, 2012).

O processo inflamatório também contribui para o aumento da secreção de citocinas pró-inflamatórias e com o consequente recrutamento de monócitos e infiltração de

macrófagos na parede arterial (BARBALHO, 2015). Mediante este processo, inicia-se a produção de moléculas de adesão para leucócitos/monócitos (como molécula de adesão intercelular (ICAM-1), molécula de adesão vascular (VCAM-1) e e-selectina), responsáveis pela adesão destas células no vaso. Uma vez aderidos, os monócitos podem migrar para a camada íntima da artéria, onde se diferenciam em macrófagos (HANSSON & LIBBY, 2006; AGRAWAL *et al.*, 2010). Após este evento, a LDL-colesterol oxidada passa a ser reconhecida por receptores *scavengers* localizados nos macrógafos, formando as células espumosas que, por sua vez, serão responsáveis pela formação do ateroma e pelo estímulo para a secreção de fatores de transcrição que modulam a expressão de moléculas pró-inflamatórias (BROWN & GOLDSTEIN, 1983; ROCHA & LIBBY, 2009; BARBALHO, 2015) (**Figura 2**).

A ativação da via NF-kB tem um papel central na inflamação, uma vez que é responsável pela regulação de genes que codificam citocinas pró-inflamatórias (como TNF-α, IL-1 e IL-6), moléculas de adesão, quimiocinas, fatores de crescimento, enzima cicloxigenase-2 (COX2) e enzima óxido nítrico sintase induzível (iNOS) (ROBBESYN *et al*., 2004; KUNDU & SURH, 2004; TEDGUI & MALLAT, 2006).

Não obstante, a resposta inflamatória envolvida na aterosclerose desencadeia não só a liberação de citocinas e quimiocinas, mas também de proteínas da fase aguda, principalmente proteína C reativa (PCR). Desta forma, inúmeros estudos evidenciam a associação entre níveis elevados de PCR e progressão da aterosclerose, provavelmente, em virtude da capacidade desta proteína em aumentar a expressão de MCP-1 e de moléculas de adesão que, por sua vez, favorecem a ligação dos monócitos às células endoteliais e contribuem para a formação de células espumosas (LIBBY *et al*., 2002; DEVARAJ *et al*., 2003).

A sobrevivência dos macrófagos e sua proliferação na placa, bem como a capacidade limitada dos leucócitos de deixar lesões ateroscleróticas, representam processos importantes que controlam criticamente o número de macrófagos dentro das lesões ateroscleróticas (VIOLA & SOEHNLEIN, 2015). A proliferação de músculo liso e a formação de matriz extracelular ao redor do depósito de gordura em desenvolvimento, levam a formação do ateroma ou mancha aterosclerótica. Essa placa tende a se desprender e desencadear tromboembolismo e outras manifestações clínicas (PANDIT & PANDEY, 2016).

Entende-se que o aumento do tecido adiposo visceral está diretamente associado à elevação dos níveis de PCR e aos componentes da síndrome da resistência insulínica, o que sugere que o tecido adiposo visceral poderia exercer papel fundamental no desenvolvimento da aterosclerose. Dessa forma, a síndrome da resistência insulínica, o aumento do IMC (obesidade, principalmente a visceral) e a aterosclerose estão intimamente relacionados e podem ser determinantes na resposta exacerbada dos eventos inflamatórios do endotélio vascular (BARBALHO, 2015).

Foi demonstrado aumento da produção de IL-1B em resposta às dietas hiperlipídidas em modelos de rato de aterosclerose. Foi sugerido que as células dentro da parede do vaso medeiam interações dependentes de IL-1B com células mieloides infiltrantes, que a atividade de IL-1B promove a cascata inflamatória por meio da supra regulação de citocinas, quimiocinas e moléculas de adesão em células endoteliais e que IL- 1B também pode estimular a proliferação de células musculares lisas e fibroblastos. Desta forma, destaca-se o papel relevante que a sinalização de IL-1 desempenha em várias etapas no início e progressão da aterosclerose (WONG, 2012).

Além disso, a importância das células imunes, particularmente das células T auxiliares e reguladoras, foi observada no desenvolvimento de ateroma (KHAN, 2015). O desenvolvimento da placa aterogênica envolve múltiplos sinais extras e intracelulares,

envolvendo células do sistema imune e da vasculatura (PANDIT & PANDEY, 2016). Evidências crescentes sugerindo que as vias do *Toll like receptor* (TLR) estão envolvidas na iniciação e progressão da placa da aterosclerose. A presença de TLRs nas placas ateroscleróticas e nos leucócitos infiltrantes fornece evidências do envolvimento da imunidade inata na aterogênese (WONG, 2012).

O LDL oxidado regula positivamente a expressão de TLR em macrófagos. A via TLR-NFkB é ativada nas lesões, transcrevendo genes relacionados à inflamação e proliferação celular, fundamentais para a aterogênese (PANDIT & PANDEY, 2016). O TLR4, em particular, é expresso mais fortemente em áreas de placa propensas a ruptura, e polimorfismos neste gene estão associados à suscetibilidade a eventos coronarianos e resposta ao tratamento com estatina no cenário de complicações ateroscleróticas. Dado o envolvimento potencial de TLRs na progressão da aterosclerose, eles se tornaram alvos terapêuticos significativos (WONG, 2012).

A inquietação com o aumento da incidência das doenças cardiovasculares é crescente e muitos estudos mostram as mais diferentes abordagens quanto à complexidade e aos aspectos etiopatogênicos. Todavia, é consenso que as modificações no estilo de vida são a forma mais eficaz de melhorar ou prevenir os fatores de risco. A modificação na alimentação e a prática regular de exercício físico modifica o perfil metabólico e inflamatório, levando a um quadro de equilíbrio metabólico (BARBALHO, 2015), o que seria de grande valia para a prevenção da aterosclerose ou mesmo para a redução do estado inflamatório de indivíduos com aterosclerose.

O papel dos antioxidantes na prevenção da aterosclerose é apoiado por várias linhas de evidência. Os efeitos benefícios dos ácidos graxos ômega-3 e sua relação com a capacidade antioxidante são amplamente relatados. Micronutrientes dietéticos, manganês, zinco, cobre e selênio podem atuar como cofatores para enzimas com ação antioxidante, cuja atividade pode

ser prejudicada em meio à deficiência desses nutrientes. Entretanto, há falta de consistência nos relatos dos benefícios dos suplementos antioxidantes (PANDIT & PANDEY, 2016).

Estudos anteriores relacionaram a alta ingestão de produtos à base de tomate ou licopeno com menor risco de doenças metabólicas, efeito protetor contra dieta hiperlipídica e diminuição da inflamação hepática (WANG *et al.*, 2008, KIM *et al.*, 2011; MELENDEZ-MARTINEZ *et al.*, 2013; FENNI *et al.*, 2017).

O fígado é um dos órgãos mais importantes para o metabolismo de carboidratos, proteínas e lipídeos, tendo como principal função digestiva a produção e secreção de bile, responsável pela emulsificação das gorduras dietéticas. Além disso, atua no armazenamento de vitaminas e minerais, na degradação e excreção de hormônios, na biotransformação e excreção de drogas e no auxílio à resposta imune. O tecido hepático é essencial não só para e homeostase do organismo, mas também para reações metabólicas vitais à saúde que podem ser desreguladas em processos de lesão hepática (SCHINONI, 2008).

A alta ingestão dietética de gordura, principalmente de ácidos graxos saturados, está associada ao aumento de peso corporal, acúmulo de gordura visceral, dislipidemias e resistência à insulina (MCLELLAN *et al.*, 2007; MACHADO *et al*., 2003; RODRIGUES *et al.*, 2003; CHARBONNEAU, 2007) além de afetar diretamente a integridade e função do tecido hepático, por promover um estado de lipotoxicidade no órgão associado a um elevado nível de estresse oxidativo e redução da defesa antioxidante do organismo (ALEGRÍA-EZQUERRA, 2008; JIAN-GAO & QIAO, 2009).

O alto consumo de gordura dietética induz ao aumento da produção de citocinas pró-inflamatórias, como a interleucina-6 (IL-6) e o fator de necrose tumoral-α (TNF-α). As citocinas enviam sinais de estímulo, modulação ou inibição para diferentes células do sistema imunológico como processo de defesa anti-inflamatória. O TNF-α é sintetizado principalmente por macrófagos e se apresenta em altas concentrações em situação de

inflamação. Já a IL-6 pode ser produzida por diversos tipos de células, como células B, T e monócitos, e consiste em um dos maiores mediadores de fase aguda da inflamação (GHANIM, et al., 2010).

A alta atividade energética do fígado gera consumo aumentado de oxigênio e elevada produção de radicais livres que promovem danos à membrana, às proteínas, aos lipídios e ao DNA celular (HALLIWELL & GUTTERIDGR, 2000; SIES *et al.*, 2005; JAESCHKE *et al.*, 2000), o que torna as células vulneráveis à apoptose e favorece a resposta inflamatória no tecido e no organismo de maneira geral (CAVE *et al.*, 2007; FRANÇA, 2013).

A ingestão de dietas ricas em gorduras, especialmente ácidos graxos saturados, afeta diretamente a integridade e a função do tecido hepático e pode ser agravada pelo consumo de álcool (ALEGRÍA-EZQUERRA, 2008). Esses hábitos podem estar associados ao desenvolvimento de estresse oxidativo, estado inflamatório e danos a várias biomoléculas, incluindo o DNA, resultando em rupturas simples ou duplas e também no aparecimento de mutações, favorecendo o aparecimento e progressão de várias doenças crônicas (GERALDO *et al*., 2008; OLIVEIRA & SCHOFFEN 2010; BUCCHIERI *et al*, 2002).

O consumo excessivo de gorduras gera um aumento do fluxo de lipídios para o fígado promovendo um estado de lipotoxicidade do órgão, associado a um alto nível de estresse oxidativo e redução da defesa antioxidante do organismo (JIAN-GAO & QIAO *et al*., 2009). Nos hepatócitos, o estresse oxidativo e a peroxidação lipídica promovem danos à membrana plasmática, tornando-a vulnerável à apoptose e favorecendo a resposta inflamatória nos tecidos e no corpo em geral, com aumento da secreção de TGF-B1 e ativação dos miofibroblastos. responsável pela formação de cicatrizes, onde lesões crônicas podem desencadear fibrose hepática (CAVE *et al*., 2007; FRANÇA *et al*., 2013).

Neste contexto, cada vez mais estudos têm sido realizados a fim buscar estratégias preventivas e o uso de compostos bioativos presentes nos alimentos parece contribuir para a

prevenção das mesmas por diversos mecanismos de ação, aos quais podem ser considerados seus potenciais antioxidantes e anti-inflamatórios (UPADHYAYA *et al*, 2007).

Os compostos antioxidantes são substâncias capazes de retardar ou inibir as taxas de oxidação, podendo ser produzidos endogenamente ou absorvidos através dos alimentos na dieta (BARREIROS *et al.,* 2006; HALLIWELL & GUTTERIDGE, 2007). Neste sentido, estudos evidenciam que a redução dos níveis de inflamação crônica e estresse oxidativo podem contribuir para a diminuição da morbidade e mortalidade cardiovascular. Além disso, alguns autores demonstraram relação inversa entre o consumo de alimentos ricos em carotenóides e o risco de doenças induzidas pelo estresse oxidativo (ZERN & FERNANDEZ, 2005; SILVER *et al.,* 2011; LAU *et al.*, 2005; VENTURINI *et al.,* 2011).

Os carotenoides são amplamente distribuídos na natureza, tipicamente vistos como pigmentos em frutas, flores e vegetais, e são responsáveis pela coloração amarela, laranja e vermelha. Como não são sintetizados por células animais, os carotenóides dependem da dieta como fonte (FRASER & BRAMLEY, 2004; RODRIGUEZ-AMAYA & KIMURA, 2004).

Em alimentos, os carotenóides são encontrados em estruturas de tetraterpenos formadas a partir de oito unidades de isoprenóides, sendo descrita com a fórmula geral C40H56On, no qual n varia de 0 a 6. Desta forma, uma grande variedade de estruturas pode ser encontrada, oriundas de diversos processos, como ciclização, hidrogenação, desidrogenação, migração de duplas ligações, encurtamento ou extensão da cadeia, reordenamento, isomerização, entre outras (CASTENMILLER & WEST, 1998; 20 RODRIGUES-AMAYA, 1997, RODRIGUES-AMAYA, 1999, RODRIGUES-AMAYA, 2003; FRASER & BRAMLEY, 2004).

De maneira geral, as formas conjugadas das duplas ligações dos compostos carotenóides existem na configuração *trans*. Os isômeros *cis* de alguns carotenóides podem

ser encontrados naturalmente em tecidos de plantas, especialmente algas (FENNEMA, 1996; CASTENMILLER & WEST, 1998; FRASER & BRAMLEY, 2004).

A estrutura molecular dos carotenóides é responsável por determinar suas funções e ações naturais (DUTTA et al., 2005). A geometria molecular global é essencial para o funcionamento dos carotenóides, pois permite que eles se encaixem nas estruturas celulares e subcelulares corretamente. Além disso, o sistema de dupla ligação conjugada de propriedades fotoquímicas e reatividade química também são determinantes para suas funções (BRITTON, 1995). Finalmente, as interações com outras moléculas próximas são cruciais para o funcionamento adequado dos carotenóides.

Dentre as inúmeras funções dos carotenóides, destaca-se a capacidade antioxidante e de eliminação relacionada à sua estrutura (WILLCOX *et al.*, 2008; RODRIGUES *et al.*, 2012). Sua estrutura de dupla ligação conjugada está diretamente associada à sua reatividade química com radicais livres, como os radicais peroxila, hidroxila e superóxido. Assim, estudos têm observado que os carotenóides podem atuar na prevenção ou redução do dano oxidativo ao DNA, lipídios e proteínas (PALOZZA, 1992; KRINSKY, 1993; RAO, 1998).

Atualmente, mais de 700 carotenóides foram isolados de produtos naturais, mas apenas alguns podem ser detectados em tecidos ou soro de animais (KRINSKY, 1993; CREWS, 2001). β-caroteno, licopeno, luteína, α-caroteno, zeaxantina e β-criptoxantina são os carotenóides mais prevalentes no soro humano (GERSTER, 1997), sua estrutura é ilustrada na (**Figura 3**).

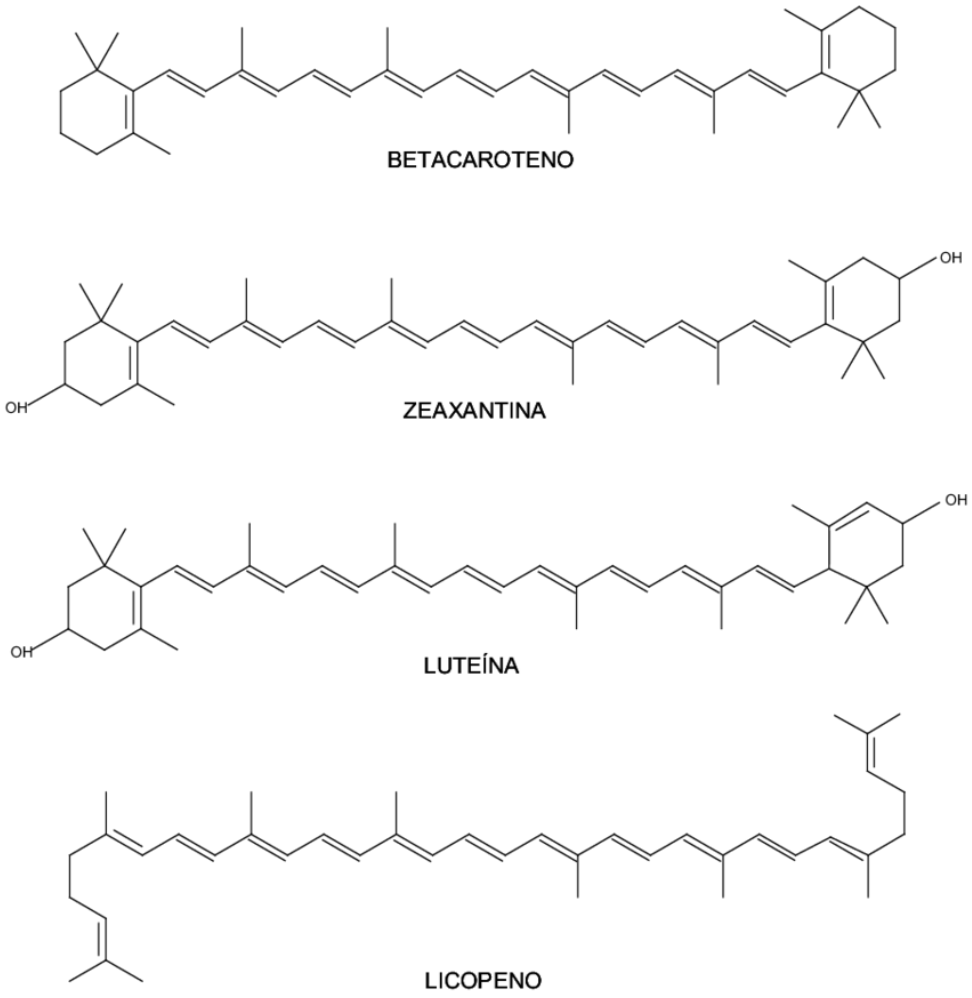

Figura 3. Estrutura química de carotenóides.

Dentre os carotenoides mais amplamente estudados os que possuem maior atividade antioxidante é o licopeno e o β-caroteno (DIAS *et al.*, 2008; RUHL, 2013, YOUNG, 2001). Eles podem atuar na desativação de espécies reativas, evitando assim a iniciação de cadeias de oxidação em nível celular que conduz a danos ao ácido desoxiribonucléico (DNA) e peroxidação lipídica (SILVA *et al.*, 2001). Os carotenóides eliminam radicais peroxil gerados no processo de oxidação lipídica e interrompe a sequência de reação que leva a danos nos compartimentos lipofílicos, como na LDL-colesterol oxidada, e ao início e progressão da aterosclerose (TAPIERO *et al.*, 2004).

Uma correlação inversa entre os carotenóides plasmáticos e IL-6, TNF-alfa e PCR foi demonstrada em estudos anteriores, apoiando a hipótese de que os carotenóides podem interferir de forma positiva em marcadores inflamatórios (HERPEN-BROEKANS, 2004; WALSTON, 2006; HOZAWA, 2007). Não obstante, um estudo demonstrou que baixas concentrações séricas de licopeno e betacaroteno podem aumentar o risco de doenças cardiovasculares (KARPPI *et al.*, 2011).

Em um estudo anterior, a combinação de compostos fenólicos e carotenóides levou a efeitos sinérgicos ao prevenir a oxidação da LDL-colesterol de forma mais eficaz do que a suplementação isolada de carotenóides (MILDE *et al.*, 2007). Desta forma, torna-se relevante o questionamento em relação a melhor forma de utilização dos carotenóides com o objetivo de prevenção ou benefícios sobre a saúde cardiovascular: através de suplementos isolados ou a partir da matriz alimentar.

Licopeno

O licopeno é um caroteno lipossolúvel, acíclico, com 40 carbonos (C40H56) e composto por onze duplas ligações conjugadas e duas ligações duplas não conjugadas. Como a maioria dos carotenóides, o licopeno não apresenta atividade pró-vitamina A devido à ausência dos anéis β-ionona na sua estrutura (TAPIERO et al., 2004). Ele pode ser encontrado no plasma e tecidos humanos com grande variação na sua distribuição e, de todos os carotenoides, ele é considerado um dos mais abundantes no corpo humano, sendo sua concentração sérica associada principalmente à ingestão alimentar (KHACHIK *et al.*, 2002).

O licopeno é um dos carotenóides com maior propriedade antioxidante (LIDEBJER *et al.*, 2007) e pode desempenhar relevante papel na regulação do metabolismo do colesterol

(VOUTILAINEN *et al.*, 2006). A potencial ação antiaterogênica do licopeno tem sido atribuída principalmente à sua capacidade antioxidante e, por isso, associada a prevenção da oxidação do LDL-colesterol (RAO, 2020). No entanto, existem evidências científica de que o licopeno também apresenta ação na regulação do metabolismo do colesterol pelo fato do licopeno compartilhar vias sintéticas iniciais semelhantes ao colesterol (HEBER *et al.*, 2002). Evidências crescentes evidenciam que a suplementação de licopeno é eficaz na redução LDL-colesterol, com ação semelhante ao de baixas doses de estatinas em pacientes com níveis ligeiramente elevado de colesterol (PALOZZA *et al.*, 2012).

Assim como alguns outros carotenóides, o licopeno foi inversamente associado à proteína C reativa (AKBARALY *et al.*, 2008). No entanto, existem resultados divergentes na literatura quanto a suplementação de curto prazo de licopeno dietético e seus efeitos sobre o colesterol. Mais estudos de intervenção de curto prazo são necessários para explorar mecanismos específicos através dos quais a suplementação de licopeno ou a ingestão de fontes de licopeno na dieta poderiam induzir alterações em outros marcadores bioquímicos relevantes que afetam o risco subsequente de doenças cardiovasculares (SESSO *et al.*, 2003).

O tomate e seus derivados são importantes fontes dietéticas de licopeno e, quanto mais avermelhado for o alimento, maior é sua concentração. A absorção do licopeno é maior quando o alimento passa pelo processo de cocção, pois o rompimento das paredes celulares facilita o contato deste com a mucosa intestinal (BRAMLEY *et al.*, 2000).

Estudos anteriores observaram correlação entre o consumo de pelo menos 7 porções / semana de produtos à base de tomate e a redução de 30% no risco de doenças cardiovasculares (SESSO, 2003). No entanto, alguns estudos científicos não mostraram associação significativa entre a concentração sérica de licopeno e a ingestão dietética de produtos à base de tomate com a redução do risco de DCV (SESSO *et al.*, 2005; CICCONE *et*

al., 2013). Em um estudo prospectivo (12 anos de acompanhamento) envolvendo 73.286 mulheres, não foi observada associação significativa entre alta ingestão de licopeno e risco de DCV, enquanto maior ingestão de alimentos ricos em α e β-caroteno foi associada a menor incidência de DCV (OSGANIAN *et al.*, 2003). Desta forma, ainda há divergência nos estudos quanto a correlação entre o licopeno dietético ou molho de tomate com os níveis plasmáticos de licopeno, levantando questões sobre o mecanismo biológico pelo qual o licopeno dietético pode estar direta ou indiretamente relacionado à DCV (SESSO *et al.*, 2003) (**Tabela 1**).

Tabela 1: Estudos da associação de licopeno com risco cardiovascular e aterosclerose

Ref.	**Fonte de licopeno**	**Tipo de estudo**	**Sexo**	**n**	**Desfecho**	**Principais achados**
KARPPI *et al*, 2013	Consumo de frutas e vegetais	Estudo de base populacional	Homens	840	Progressão precoce de aterosclerose	Altas concentrações séricas de carotenóides podem ser protetoras contra a aterosclerose precoce
ZOU *et al*, 2013	Suplementação de licopeno e luteína	Transversal, controle de caso	Homens, mulheres	144	Aterosclerose	Combinação de luteína e suplementação de licopeno foi mais eficaz do que luteína sozinha para proteção contra o desenvolvimento da espessura da íntima-média da artéria carótida em indivíduos chineses com aterosclerose subclínica
JACQUES *et al*, 2013	Fontes alimentares de licopeno e suplemento vitamínico	Análise de riscos proporcionais de Cox	Homens, mulheres	5,135	Risco de doença cardiovascular	A ingestão de licopeno foi inversamente associada à incidência de DCV e ao risco de DCV

WANG *et al*, 2008	Produtos à base de tomate e suplementação de licopeno	Coorte	Homens, mulheres	54	Risco de doença cardiovascular; atividade da LCAT no HDL-colesterol	A ingestão de licopeno leva a alterações no HDL, aumenta a atividade de LCAT e diminui a atividade de CETP, sugerindo suas propriedades antiaterogênicas
DANIELS *et al*, 2014	Ingestão de frutas e vegetais	Prospectivo, Ensaio clínico	Homens, mulheres	80	Risco de doença cardiovascular; atividade da LCAT no HDL-colesterol	O consumo de frutas e vegetais aumenta o licopeno no soro, HDL2 e HDL3, assim como as atividades de PON-1 e LCAT em HDL3
GAJENDRAGADKAR *et al*, 2014	Suplementação de licopeno	Prospectivo, Ensaio clínico	Homens, mulheres	72	Doença cardiovascular	A suplementação de licopeno melhora a função endotelial em pacientes com DCV na prevenção secundária ideal, mas não em voluntários saudáveis
ARRANZ *et al*, 2015	Suco de tomate com e sem azeite de oliva	ensaio aberto, controlado, randomizado e cruzado	Homens, mulheres	11	Lipemia pós-prandial	Todos os isômeros de licopeno aumentaram em indivíduos que consumiram suco de tomate com azeite; colesterol LDL e total diminuíram 6h após o consumo de suco de tomate com azeite, o que se correlacionou significativamente com um aumento de trans-licopeno e 5-cis-licopeno
BIDDLE *et al*, 2015	Suco de vegetais (com 29,4 mg de licopeno)	Transversal, controle de caso	Homens, mulheres	40	Infarto	Alimentação rica em produtos dietéticos ricos em licopeno nos níveis plasmáticos de licopeno; níveis séricos de PCR não diminuíram no grupo de

						intervenção como um todo, mas diminuíram significativamente no grupo do sexo feminino.
HAN *et al*, 2016	Consumo de frutas e vegetais	Análise de riscos proporcionais de Cox	Homens, mulheres	2499	Doenças cardiovasculares	Maior concentração de licopeno sérico tem uma associação significativa com a redução do risco de mortalidade entre os indivíduos com síndrome metabólica
LI *et al*, 2017	Suplementação de licopeno	Experimental	Ratos	60	Doenças cardiovasculares	O licopeno modula a resposta à inflamação nas DCV; suplementação de licopeno aliviou a lesão cardíaca por meio da modulação dos sistemas geradores de NO

Vale ressaltar ainda que alguns estudos levantaram o questionamento se de fato o consumo de produtos derivados do tomate, predominantemente tomate e molho de tomate, poderia ser correlacionado a melhora da saúde cardiovascular ou se o efeito positivo estaria enviesado por um padrão alimentar mediterrâneo, sugerido por ter maiores benefícios cardiovasculares (KARPPI *et al.*, 2013). O consumo de fontes alimentares à base de tomate junto com frutas, vegetais frescos e azeite de oliva são comuns em um padrão alimentar mediterrâneo e fornecem uma variedade de nutrientes com potenciais benefícios cardiovasculares (SESSO *et al.*, 2003).

Betacaroteno

O betacaroteno é um carotenóide com atividade de pró-vitamina A, que apresenta variedade de funções, incluindo ação antioxidante e aumento da comunicação intercelular pelas junções comunicantes em diversos tipos de tecidos do organismo (PAIVA, 1999; YEH *et al.*, 2003).

Estudos epidemiológicos vêm sugerindo que compostos antioxidantes, entre eles o β-caroteno, podem desempenhar importante papel no que diz respeito a causas primárias de diversas doenças, inclusive doenças cardiovasculares. Esse fato estaria associado à atividade antioxidante dos carotenoides que é consequência de sua estrutura singular. O β-caroteno pode reagir múltiplas vezes com radicais peroxila para formar moléculas estáveis e é capaz de inibir a modificação oxidativa do LDL-colesterol (LAVY, 1993; CHEN, *et al* 2002; SHAISH, *et al.*, 2006).

Em um estudo anterior, células endoteliais humanas foram expostas à concentração fisiológica de β-caroteno e a condições pró-oxidantes induzidas por TNF-α. Os resultados mostraram que a atividade antioxidante do β-caroteno foi contrária ao estresse oxidativo inflamatório, permitindo efeitos protetores contra DCV (TOMO *et al.*, 2012).

Estudo recente evidenciou o potencial do betacaroteno aumentar o efluxo de colesterol dos macrófagos da lesão endotelial para HDL-colesterol, regulando a resposta inflamatória aterosclerótica e, consequentemente, a inibição da aterogênese (BECHOR *et al.*, 2016) (**Tabela 2**). Street et al. demonstraram uma correlação significativamente inversa entre o risco de infarto do miocárdio subsequente e níveis mais baixos de β-caroteno (STREET, 1994). Portanto, baixo as concentrações de β-caroteno sérico foram associadas ao aumento do risco

de mortalidade por doenças cardíacas, confirmando os efeitos cardioprotetores dos carotenóides (KARPPI *et al.*, 2013).

Acredita-se ainda na capacidade dos carotenóides e mais provavelmente seus metabólitos de interagir com os receptores nucleares RAR/PPARs (RAR/receptores ativados por proliferadores de peroxissoma) para melhorar funções do sistema imunológico (RÜHL *et al*, 2007). Além disso, foi proposto que, especialmente os metabólitos mais polares, são capazes de interagir com NF-κB (fator nuclear kappa B) e Nrf-2 (fator nuclear 2 relacionado ao eritróide 2), o que resultaria em diminuição dos níveis de algumas citocinas, como TNF-α (LINNEWIEL *et al*, 2009; LINNEWIEL *et al*, 2014); ou promoção a translocação de Nrf-2 para o núcleo, estimulando a expressão de enzimas antioxidantes ativas tais como catalase (CAT) e superóxido dismutase (SOD), contribuindo para a redução dos marcadores de estresse oxidativo (RÜHL *et al*, 2007; LINNEWIEL *et al*, 2014) (**Figura 4 e 5**).

Outros resultados não apoiaram o papel benéfico da ingestão de β-caroteno (VIVEKANANTHAN *et al.,* 2003; BJELAKOVIC *et al.*, 2008). Em um estudo randomizado controlado com placebo, 22.071 homens aparentemente saudáveis foram convidados a tomar β-caroteno ou placebo por 12 anos; durante o período de acompanhamento, a suplementação de β-caroteno não influenciou o câncer, DCV ou incidência de morte (HENNEKENS, 1996).

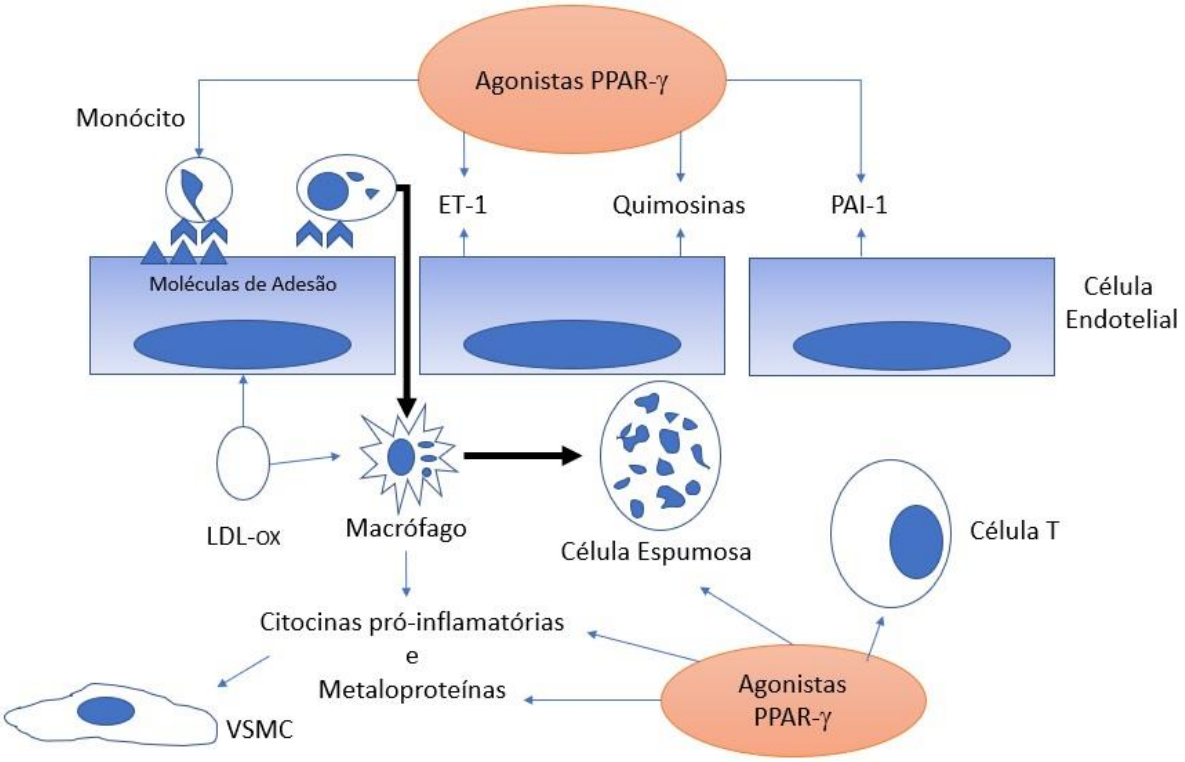

Figura 4. Possíveis ações de agonistas de PPAR-Ɣ na proteção da formação de placas ateroscleróticas.

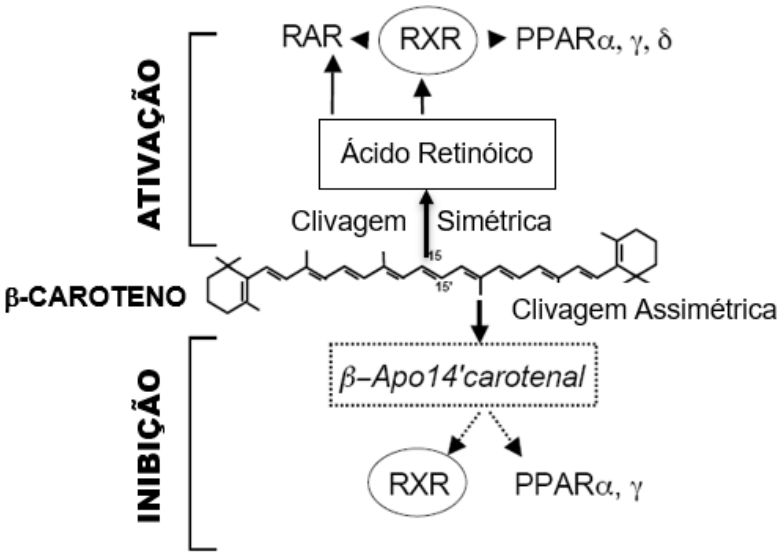

Figura 5. O metabolismo do β-caroteno pode gerar moléculas específicas com diferentes efeitos celulares. Simétrico a clivagem do β-caroteno finalmente produz duas moléculas de ácido retinóico (RA), um conhecido agonista para RAR e RXR, incluindo todos os PPAR. Alternativamente, β-caroteno também pode sofrer clivagem assimétrica produzindo apocarotenais, uma série específica de moléculas denominadas de acordo com o comprimento de seu lado correntes. Acredita-se que os apocarotenais sejam gerados sob condições de estresse oxidativo e/ou inflamação. Adaptado de ZIOUZENKOVAA & PLUTZKY, 2008.

Tabela 2: Estudos da associação de betacaroteno com risco cardiovascular e aterosclerose

Ref.	Fonte de betacaroteno	Tipo de estudo	Sexo	n	Desfecho	Principais achados
OSGANIAN *et al*, 2003	α-caroteno e β-caroteno	Prospectivo	Homens e mulheres	73286	Doenças cardio-Vasculares	Associação significativamente inversas entre ingestão de α-caroteno e β-caroteno e DCV
D'ODORICO *et al*, 2000	α-caroteno e β-caroteno	Prospectivo	Homens e mulheres	392	Prevalência e incidência de placas carótidas	α-caroteno e β-caroteno foram inversamente associados com a prevalência de aterosclerose na carótida
KRITCHEVSKY *et al*, 1998	Carotenóides com atividade de pró-vitamina A	Transversal	Homens e mulheres	12773	Prevalência de placas na carótida	Indivíduos com alto consumo de carotenóides tiveram prevalência menor de placas
MATOS et al, 2018	Vitamina A	Transversal	Homens e mulheres	90	Aterosclerose	Inflamação crônica decorrente da aterosclerose está relacionada à gravidade da doença
KARPPI *et al*, 2013	Consumo de frutas e vegetais	Estudo de base populacional	Homens	840	Progressão precoce de aterosclerose	Altas concentrações séricas de carotenóides podem ser protetoras contra a aterosclerose precoce
BECHOR et al, 2016	9-cis - Carotene	Experimental	Ratos	30	Efeito benéfico na inibição da aterosclerose	Aumento do efluxo de colesterol para HDL em macrófagos

Dentre os alimentos com maior teor de betacaroteno, encontra-se o buriti (*Mauritia flexuosa*) que é uma fruta pertencente à família *Arecaceae* e ao gênero *Mauritia*, encontrado na Floresta Amazônica do Brasil (DELGADO, COUTURIER, & MEJIA, 2007).

Diversas espécies de frutas exóticas da Amazônia brasileira têm sido estudadas pela sua produção de frutas com características sensoriais únicas e alta concentração de nutrientes e compostos bioativos, como o buriti (*Mauritia flexuosa*). Apesar da potencialidade da Amazônia brasileira, toda essa diversidade ainda tem sido pouco explorada ou aproveitada devido à falta de estudos e pesquisas relevantes a fim de correlacionar seu consumo com efeitos benéficos à saúde.

O buriti é o fruto de uma palmeira da família Arecaceae, encontrada nos estados do Pará, Amazonas, Amapá, Rondônia, Goiás, Bahia, Minas Gerais, Mato Grosso, Ceará, Maranhão (BONDAR, 1964, FERRI, 1980; PALLET *et al*., 2002). Sua frutificação em maior escala ocorre nos meses de dezembro a junho na maioria das regiões (SALAY *et al.*, 2005). Cada fruto pesa em média 50g, possui uma casca escamosa e dura de coloração vermelha escura e apresenta polpa macia de coloração amarela escura de característica oleaginosa (MARIATH *et al*., 1989) (**Figura 6**).

O buriti vem ganhando destaque em pesquisas sobre avaliação dos seus valores nutricionais, especialmente em relação à sua atividade antioxidante. Segundo ALBUQUERQUE *et al.* (2003), o óleo do buriti é basicamente composto de tocoferol, carotenóides e em maiores quantidades ácidos graxos de cadeia longa.

Figura 6. Buriti (*Mauritia flexuosa*)

Estudos recentes avaliaram a capacidade antioxidante e conteúdo fenólico de frutos de buriti, sendo observado elevada capacidade antioxidante na fruta, em razão do teor de β-caroteno presente nela, o que faz desse fruto a maior fonte já estudada desse pigmento (GODOY & RODRIGUEZ-AMAYA, 1994). Desta forma, ela pode apresentar um potencial terapêutico na prevenção de doenças cardiovasculares.

CAPÍTULO 2

Lycopene and Tomato Sauce Improve Hepatic and Cardiac Cell Biomarkers in Rats

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AUTORES: Vanessa Azevedo de Jesuz, Monique de Barros Elias Campos, Vanessa Rosse de Souza, Teresa Palmiciano Bede, Bianca Portugal Tavares de Moraes, Adriana Ribeiro Silva, Cassiano Felippe Gonçalves de Albuquerque, Vilma Blondet de Azeredo and Anderson Junger Teodoro

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Abstract

This study evaluated the effects of tomato sauce and lycopene on hepatic and cardiac cell biomarkers in rats fed a high-fat diet. Animals were split into five groups: control group, high-fat group (HG), high-fat tomato sauce group, high-fat lycopene 2 mg, and high-fat lycopene 4 mg. Food and water were offered ad libitum, whereas tomato sauce and lycopene (2 and 4 mg/day) were offered daily for 60 days. Body, heart, and liver weights, cardiosomatic and hepatosomatic indices, and serum parameters were also analyzed in rats. The animals' hearts and liver were processed, and cells were examined by flow cytometry. Results showed that the groups receiving tomato sauce and lycopene had lower glycemia. The serum concentration of high-density lipoprotein cholesterol, hepatic enzymes, and tumor necrosis factor-a did not change upon treatment. Tomato sauce and lycopene supplementation did not increase interleukin-1b in response to a high-fat diet. Cell cycle analysis of cardiac and liver cells showed a lower percentage of cells in the G0/G1 phase and an increase in the G2/M phase in HG. Both lycopene and tomato sauce reversed this effect. Both lycopene and tomato sauce reversed this effect and prevented high-fat diet-stimulated cardiac and liver cell death. Supplementation of tomato sauce and lycopene showed beneficial effects on cardiac and liver cell metabolism; therefore, it is suggested as a nutritional approach for the prevention and treatment of cardiovascular diseases and nonalcoholic hepatic steatosis.

Keywords: apoptosis; carotenoids; cell cycle; high-fat diet; inflammation.

Introduction

Obesity is a risk factor for various chronic diseases, and the metabolic defects of obesity and type 2 diabetes, characterized by fatty liver disease, insulin resistance, and dyslipidemia, lead to an increased risk of cardiovascular disease and cancer.1–3 Although diagnosed worldwide, it has variations in prevalence, reaching 20–30% in western countries.

In the United States, a country where 25% of the adult population is obese, the disease affects more than 60% of these individuals. It is estimated that 2–3% of the population has hepatic steatosis.3 The consumption of diets rich in saturated fats is linked to synthesis of proinflammatory cytokines, an increase in reactive oxygen species, development of oxidative stress, and damage to several biomolecules. It is also a predisposing factor in the development of a variety of chronic diseases, including obesity, cognitive dysfunction, diabetes, and cancer.4–9Thus, a high-fat diet has a central role in the development of oxidative events, as occurs in hepatic steatosis and atherosclerosis. Fatty liver is associated with several atherosclerotic risk factors such as hypertension, diabetes, and dyslipidemia.10,11

Bioactive compound supplements are a potential disease-preventing or health promoting treatment to be taken daily.12 Bioactive compounds are substances discovered from natural sources, which are capable of retarding or inhibiting oxidation rates and can be produced endogenously or absorbed through foods in the diet.13–15 Some authors have demonstrated an inverse relationship between the consumption of carotenoid rich foods and the risk of diseases induced by oxidative stress.6,16–18

Lycopene is a lipophilic non-provitamin A carotenoid, responsible for the red color in some fruits and vegetables, such as tomatoes. It has a capacity to protect against many diseases, mainly due to its antioxidative effects, lipid regulating enzyme activities, capacity to induce adipocyte differentiation, and improvement of the plasma lipid profile in rats fed with a high-

fat diet.12 Previous studies have linked the high intake of tomato products or lycopene with a lower risk of metabolic diseases, protective effects against high-fat diets, and decreased hepatic inflammation.19–22 However, there is no consensus in the literature regarding which form of lycopene (i.e., tomato products or isolated lycopene supplement) is more beneficial to these inflammatory diseases.

The aim of this study was to evaluate the effect of tomato sauce and isolated lycopene on changes related to cardiac and hepatic tissues in Wistar rats, such as glycemia, lipid profile, inflammatory mediators, hepatic, and cardiac cell cycle.

Materials and methods

Samples

Samples of Brazilian tomato sauce (ingredients: tomato [97%], sugar, salt, modified starch, vegetable oil, onion, parsley, marjoram, celery, thickener xanthan gum, aromatizing and potassium sorbate, and sodium benzoate) were obtained from a local market (Rio de Janeiro, RJ, Brazil).

Water-soluble lycopene 10% (containing sucrose, corn starch, fish gelatin, lycopene, corn oil, ascorbyl palmitate, and dl-alpha-tocopherol) was provided by Roche (Rio de Janeiro, RJ, Brazil). Samples of tomato sauce and lycopene were kept in a refrigerator (10 °C) and freezer (-18 °C), respectively, in appropriate packaging.

Appropriate solutions for each experimental group were prepared daily in the laboratory by dissolving the content in filtered water at 50 °C and adding 20% refined sugar to obtain a palatable solution.

Experimental model

Fifty female, adultWistar rats were individually housed and maintained in a 12-h light–12-h dark cycle at 22°C (–2°C). Animal maintenance was in accordance with the Animal Research: Reporting of In Vivo Experiments (ARRIVE) guidelines.23 Rat care and experimental protocols were approved by the Institution's Scientific, Academic, and Ethics Board.

Animals were divided into five groups (Fig. 1): control group (CG), which received a standard diet (based casein) ad libitum, consisting of protein (minimum) 12.95%, fat (minimum) 4.0%, and water; high-fat group (HG), which received a high-fat diet ad libitum, consisting of protein (minimum) 12.95%, fat (minimum) 20.0%, and water; tomato sauce group (TG), which received the high-fat diet ad libitum, plus a tomato sauce solution providing 2.0 mg of lycopene per day; 2.0 mg lycopene group (L2G), which received the high-fat diet ad libitum, plus a solution containing 2.0 mg all-trans lycopene (water soluble) 10% dissolved in water daily, and water; 4.0 mg lycopene group (L4G), which received the high-fat diet ad libitum, plus a solution containing 4.0 mg all-trans lycopene (water soluble) 10% dissolved in water daily, and water. The ingredients for the formulation of the control and high-fat rations used in the experiment are shown in the Supplementary Table S1.

After 60 days of the experiment, the vaginal smear procedure was performed on all animals to identify their phase in the estrous cycle and to establish that all were in the same physiological state without hormonal interference in the analysis. After the estrous cycle check, rats in the ''estrus'' phase were separated and trapped. Body weight was measured, and trapped animals were sacrificed. Serum and organs (heart and liver) were obtained, weighed, frozen, and kept at -70 °C until analysis.

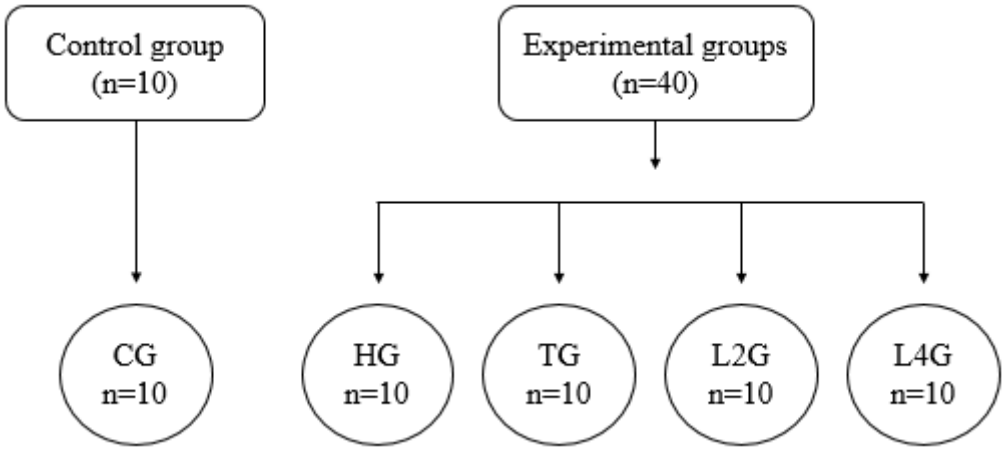


Figure 1. Dietary protocol. Experimental model: Control group (CG): standard diet plus water; Experimental groups: high-fat group (HG). high-fat diet plus water; group tomato sauce (TG). high-fat diet plus solution with tomato sauce and water; 2mg lycopene group (L2G). high-fat diet plus solution with 2.0 mg *all-trans* lycopene and water; 4mg lycopene group (L4G). high-fat diet plus solution with 4.0 mg *all-trans* lycopene and water. Diets and solutions were administered during a period of 60 days.

Experimentals procedures were conducted according to the study of Ribeiro et al.13 The hearts and livers were weighed to determine the relative weight of the organ denominated cardiosomatic index and hepatosomatic index, which is calculated according to the formula:

(Heart/liver weight (g) / body weight) x 100

Analytical methods

A glucometer was used to measure serum glucose concentration. Serum total cholesterol, high-density lipoprotein (HDL) cholesterol, triglycerides, aspartate aminotransferase (AST), alanine aminotransferase (ALT), interleukin (IL)-1b, and tumor necrosis factor (TNF)-a concentration were measured using the BioClin_ Commercial Kits and wavelengths specific to each biochemical indicator, using the colorimetric method with automated

spectrophotometer reading (BioClin BS-120 Chemistry Analyzer_). Low-density lipoprotein (LDL) cholesterol was calculated according to Friedwald's formula.24

Cell cycle

Animal heart muscle and liver tissues were processed, and cardiac and liver cell cycles and apoptosis were measured using flow cytometry. Cell extractions were performed through maceration of the tissue and addition of 0.5 mg/mL collagenase (Sigma_). After centrifugation, the cells were washed twice with phosphate-buffered saline and resuspended

in 500 lL of ice-cold Vindelov solution containing 0.1% Triton X-100, 0.1% citrate buffer, 0.1mg/mL RNase, and 50mg/mL propidium iodide (PI; Sigma Chemical Co., St. Louis, MO, USA). After 15min of incubation, the cell suspension was analyzed for DNA content by

flow cytometry using a FACSCalibur flow cytometer (Becton Dickinson, Mountain View, CA, USA). The relative proportions of cells with DNA content haploid subG1(<2n), diploid G0/G1 (2n), S phase (>2n and <4n), and G2/M phase (4n) were acquired and analyzed using CellQuest and WinMDI 2.9. Respectively, the percentage of cell population at a particular phase was estimated with FlowJo software following the acquisition of 30,000 events. To our knowledge, the cell dissociation procedure does not affect fluorescence under the experimental conditions used in this study. Nuclei of viable cells were gated according to the FL-2W· FL2-A relationship based on the study conducted by Guimara˜es et al.25

Apoptosis assay

Cells were resuspended in 400 lL of binding buffer containing 5 lL of Annexin V fluorescein isothiocyanate and 5 lL PI (Apoptosis Detection Kit II; BD Biosciences, BD Pharmingen, Mountain View, CA, USA) for 15 min at room temperature (25_C). Annexin V binding was evaluated by flow cytometry (FACScalibur; BD Biosciences) after acquisition of 30,000 events. The data were analyzed in CellQuest and FlowJo software.

Data analysis

The effects of tomato sauce and isolated lycopene supplementation were analyzed using one-way analysis of variance followed by Tukey's post-hoc test for the multiple mean comparison test. Results are expressed as mean – standard deviation, and the significance level was set at $P < .05$.

Results

At the end of the experiments, body weights were measured, and the final body weight was higher ($P < .05$) in the TG (277.7 – 11.48 g) and lower in the L2G (197.3 – 13.12 g) and L4G (188.9 – 15.02 g) groups compared with the control group (Table 1). Heart weight was higher ($P < .05$) in the TG (1.06 – 0.05 g) and lower in the L4G (0.72 – 0.11 g) groups compared with the control group. No differences were observed in liver weight or hepatosomatic and cardiosomatic index among the groups (Table 1). Images of rat hearts included in this study are displayed in Figure 2.

Table 1. Body and tissue weight in control and experimental groups

Parameters (g)	CG	HG	TG	L2G	L4G
Initial body weight	160.4 ± 4,83[a]	161.4 ± 6,23[a]	163.4 ± 5.68[a]	162.0 ± 5.34[a]	158.6 ± 5.94[a]
Final body weight	261.00 ± 31.04[a]	268.20 ± 17.68[a]	277.70 ± 11.48[a]	197.30 ± 13.12[b]	188.90 ± 15.02[b]
Heart weight	0.94 ± 0.21[a]	0.90 ± 0.07[a]	1.06 ± 0.05[b]	0.80 ± 0.16[a]	0.72 ± 0.11[c]
Liver weight	7.86 ± 1.52[a]	7.10 ± 0.57[a]	8.50 ± 1.01[a]	5.78 ± 0.55[b]	5.82 ± 0.37[b]
Cardiosomatic index	0.36 ± 0.09[a]	0.34 ± 0.01[a]	0.38 ± 0.06[a]	0.40 ± 0.06[a]	0.38 ± 0.04[a]
Hepatosomatic index	2.99 ± 0.23[a]	2.65 ± 0.25[a]	3.06 ± 0.43[a]	2.95 ± 0.43[a]	3.09 ± 0.17[a]

*Values are mean–SD. Different letters mean statistical difference between groups. Statistical significance was determined by ANOVA followed by Tukey's post hoc multiple mean comparison test. CG: control group; HG: high-fat group; TG: group tomato sauce; L2G: lycopene 2mg; L4G: lycopene 4mg.

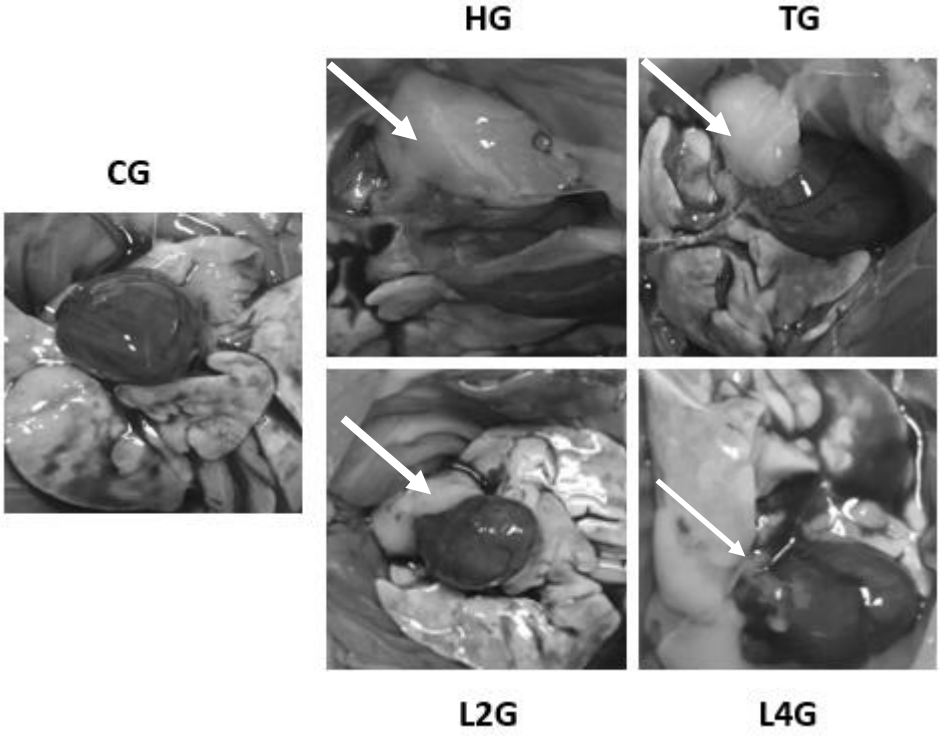


Figure 2. Heart extracted from sacrificed rats after dietary protocol. CG: control group; HG: high-fat group; TG: group tomato sauce; L2G: lycopene 2mg; L4G: lycopene 4mg. White arrows indicate accumulated fat in tissues.

Significant differences (P < .05) in glycemia, total cholesterol, and triglycerides were found among the groups analyzed (Table 2). Groups receiving tomato sauce (TG) and lycopene (L2G, and L4G) had lower glycemic values compared with the control. Furthermore, the group receiving a high-fat diet had higher triglyceride levels compared with the CG group; however, no differences were observed when compared with the L4G group. In addition, there were no differences in the concentrations of serum HDL cholesterol and liver enzymes (AST and ALT) among the groups (Table 2). High-fat diet induced an increase in LDL when compared with the control group. However, lycopene and tomato sauce treatment did not promote changes in the LDL concentration caused by the hyperlipidic diet.

Table 2. Serum parameters in rats fed with experimental diets

Parameters (mg/dL)	**CG**	**HG**	**TG**	**L2G**	**L4G**
Glycemia	107.00 ± 2.65[a]	101.67 ± 4.51[a.c]	86.20 ± 4.76[b]	88.60 ± 4.56[b]	88.60 ± 6.80[b]
Total cholesterol	46.00 ± 8.60[a.b]	45.00 ± 3.54[b]	60.00 ± 4.06[c]	56.80 ± 8.14[a.b.c]	53.80 ± 4.49 [a.b.c]
HDL cholesterol	21.60 ± 3.51[a]	22.40 ± 1.34 [a]	25.00 ± 2.55 [a]	24.20 ± 1.30 [a]	23.40 ± 1.34 [a]
LDL cholesterol	13,6 ±5,01	26,76 ± 2,50	23,8 ± 3,77	19,53 ± 4,80	21,8 ±4,01
Triglycerides	31.60 ± 8.17[a]	47.00 ± 7.94[b]	41.20 ± 7.66 [b]	40.00 ± 6.20[b]	33.25 ± 7.68[a]
AST	195.60 ± 52.03[a]	219.80 ± 33.39[a]	198.20 ± 52.77[a]	177.80 ± 41.81[a]	176.40 ± 26.60[a]
ALT	32.20 ± 6.42[a]	32.00 ± 5.39[a]	29.00 ± 4.80[a]	28.00 ± 5.43[a]	33.20 ± 5.81[a]

*Values are mean–SD. Different letters mean statistical difference between groups. Statistical significance was determined by ANOVA. followed by Tukey's post hoc multiple mean comparison test. CG: control group; HG: high-fat group; TG: group tomato sauce; L2G: lycopene 2mg; L4G: lycopene 4mg. AST - Aspartate aminotransferase. ALT - Alanine aminotransferase

HG showed an increase in IL-1b expression compared with the other groups. Tomato sauce and lycopene, however, did not show this effect and presented a similar profile. Importantly, TG showed similar results to CG. There were no variations in the levels of TNF-a among the groups (Fig. 3).

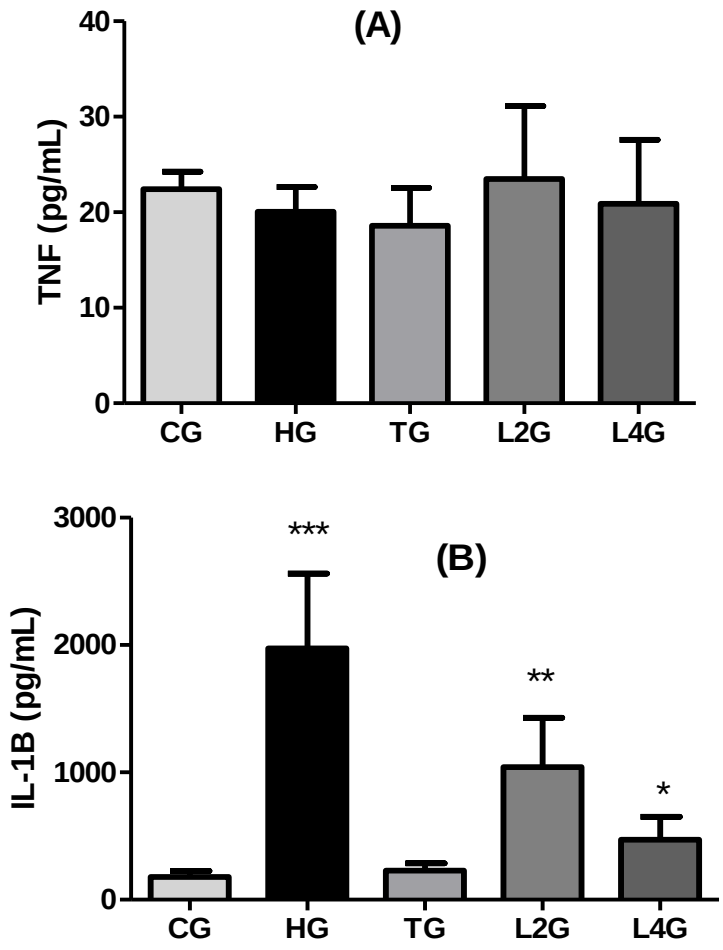


Figure 3. (A) TNF-α e (B) IL-1β from rats fed a high fat diet supplemented with lycopene and tomato sauce. CG: control group; HG: high-fat group; TG: group tomato sauce; L2G: lycopene 2mg; L4G: lycopene 4mg. *p<0.05; **p<0.01; p<0.001 ANOVA - Tukey test.

Cell cycle analysis of cardiac cells showed that the HG group presented a lower percentage of cells in the G0/G1 phase (47.98 – 6.28), compared with the other groups (Table 3). Furthermore, a high-fat diet increased the percentage of cells in the G2/M phase, which was

reversed by the action of both lycopene and tomato sauce ($P < .05$). In liver cells, high-fat diet decreased the number of cells in the G0/G1 phase and increased the percentage of cells in the G2/M phase. We hypothesized that the highest rate of cell death in that group promoted an increase in cellular proliferation to reduce cellular loss. Both tomato sauce and ycopene treated groups increased the number of cells in the G0/G1 phase and decreased numbers in the G2/M phase induced by the high-fat diet (Table 4).

Table 3. Effect of lycopene and tomato sauce on cell cycle progression of cardiac cells in rats fed high fat diet

Groups	Sub-G1	G_0/G_1	S	G_2/M
CG	2.35 ± 1.42^{a}	79.80 ± 4.34^{a}	8.90 ± 0.44^{a}	7.95 ± 1.50^{a}
HG	2.35 ± 1.42^{a}	71.39 ± 3.88^{b}	9.76 ± 0.85^{a}	16.03 ± 1.82^{b}
TG	2.35 ± 1.42^{a}	78.86 ± 7.14^{a}	8.53 ± 0.49^{a}	12.66 ± 1.62^{c}
L2G	2.35 ± 1.42^{a}	81.55 ± 7.88^{a}	9.06 ± 2.67^{a}	5.95 ± 2.03^{a}
L4G	2.35 ± 1.42^{a}	$80.17 \pm 10.35^{a.b}$	10.95 ± 1.73^{a}	8.87 ± 1.87^{a}

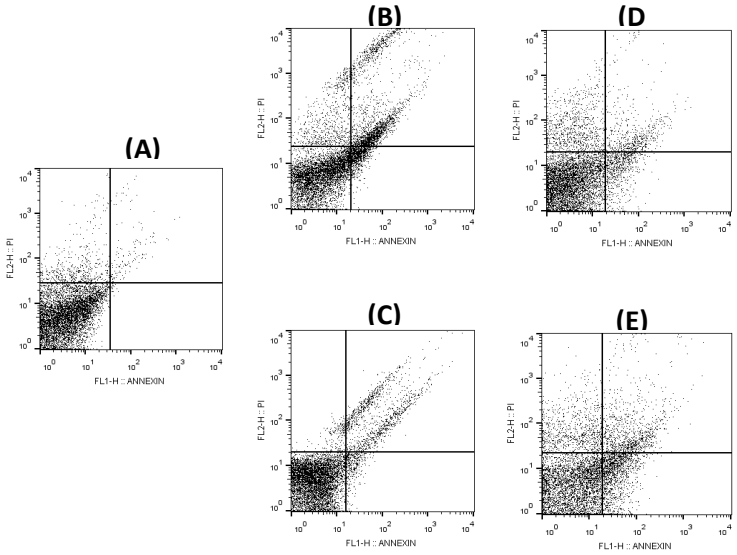
The results are expressed as the percentages of total cells. Values are mean–SD. Statistical significance was determined by ANOVA. followed by Tukey's post hoc multiple mean comparison test. Control group; HG: high-fat group; TG: group tomato sauce; L2G: lycopene 2mg; L4G: lycopene 4mg.

Table 4. Effect of lycopene and tomato sauce on cell cycle progression of hepatic cells in rats fed high fat diet

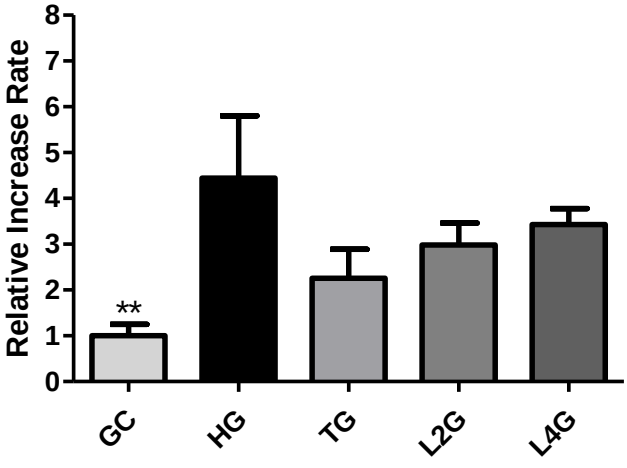
Groups	Sub G1	$G_0/G1$	S	G_2/M
CG	2.35 ± 1.42^{a}	63.49 ± 6.06^{a}	20.20 ± 8.47^{a}	14.41 ± 3.85^{a}
HG	2.35 ± 1.42^{a}	53.67 ± 5.79^{b}	28.18 ± 3.40^{b}	18.15 ± 3.08^{a}
TG	2.35 ± 1.42^{a}	67.50 ± 10.10^{c}	15.66 ± 6.10^{a}	16.84 ± 4.40^{a}
L2G	2.35 ± 1.42^{a}	$77.96 \pm 4.89^{c.d}$	10.57 ± 2.05^{a}	11.47 ± 2.85^{a}
L4G	2.35 ± 1.42^{a}	71.27 ± 7.39^{d}	13.81 ± 3.65^{a}	14.92 ± 3.93^{a}

The results are expressed as the percentages of total cells. Values are mean–SD. Statistical significance was determined by ANOVA. followed by Tukey's post hoc multiple mean comparison test. Control group; HG: high-fat group; TG: group tomato sauce; L2G: lycopene 2mg; L4G: lycopene 4mg.

Annexin V and PI biomarkers were used to assess apoptosis. An increase in apoptotic cells was present in liver and heart cells in every group receiving the high-fat diet compared with the CG group. High-fat diet promoted a significant decrease in the population of viable cells and a significant increase of 5.2 and 3.9 times, respectively, in cardiac and hepatic cells compared with controls (Figs. 4 and 5). High-fat diet supplemented with lycopene and tomato sauce reversed the increase in apoptosis caused by the hyperlipidic diet, exhibiting higher values when compared with the control group.

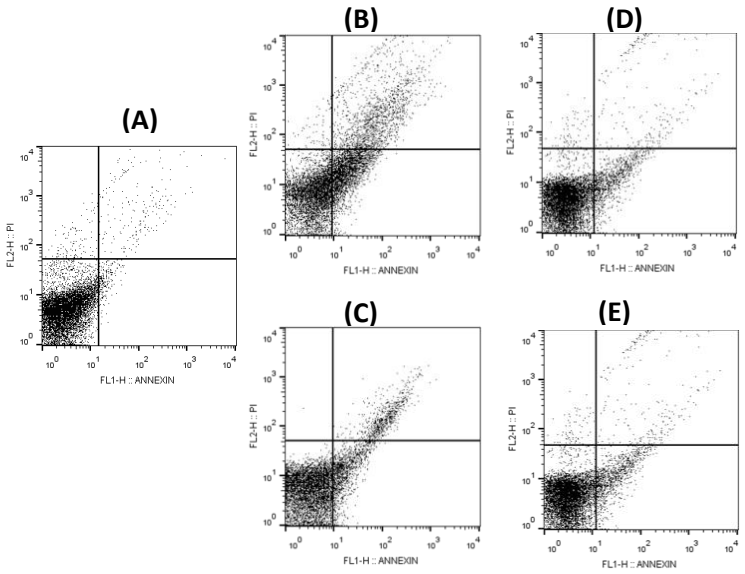


(a)



(b)

Figure 4. Monitoring of cell death of cardiac cells from rats fed a high fat diet supplemented with lycopene and tomato sauce. (a) Flow cytometry analysis of cardiac cells from rats fed a high fat diet supplemented with lycopene and tomato sauce. (b) Quantitative effects of lycopene and tomato sauce of of cell death of cardiac cells from rats fed a high fat diet. (A): control group (CG); (B): high-fat group (HG); (C): group tomato sauce (TG); (D): lycopene 2mg (L2G); (E): lycopene 4mg (L4G). The results are expressed as mean ± SD, with significant differences compared by 1-way ANOVA followed by Tukey's multiple comparison post hoc test. $*p < 0.05$. $**p < 0.01$.



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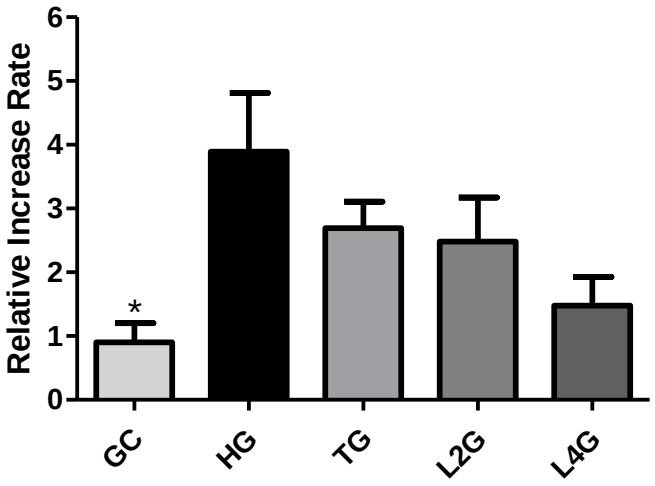

(b)

Figure 5. Monitoring of cell death of hepatic cells from rats fed a high fat diet supplemented with lycopene and tomato sauce. (a) Flow cytometry analysis of hepatic cells from rats fed a high fat diet supplemented with lycopene and tomato sauce. (b) Quantitative effects of lycopene and tomato sauce of of cell death of hepatic cells from rats fed a high fat diet. (A):

control group (CG); (B): high-fat group (HG); (C): group tomato sauce (TG); (D): lycopene 2mg (L2G); (E): lycopene 4mg (L4G). The results are expressed as mean ± SD, with significant differences compared by 1-way ANOVA followed by Tukey's multiple comparison post hoc test. $*p < 0.05$. $**p < 0.01$.

Discussion

Tomato sauce is a rich source of lycopene, which has potente antioxidant activity. Nevertheless, very little is known about different forms of lycopene supplementation in cardiovascular diseases and nonalcoholic hepatic steatosis.4,21,26–30

Previous study have shown that 762 individuals with hepatic steatosis (76.8%) had at least one atherosclerotic plaque, evidencing a higher prevalence of atherosclerotic plaques in patients with hepatic steatosis. The study observed a direct association between hepatic steatosis and carotid plaques independent of age and sex.15 For this reason, it is important to evaluate factors that may contribute to their prevention.

The present study provided information regarding the effects of lycopene and lycopene in food matrix supplementation on cardiac and liver metabolism, cell cycle, and apoptosis. Our outcomes agree with published results in which quercetin supplementation in rats was able to reduce weight gain and increase heart size even with ingestion of a high-fat diet when compared with the control.12 Herein, lycopene supplementation in rats reduced high-fat dietinduced weight gain and was cardioprotective.

Previous studies have shown that a tomato juice intervention in vivo did not affect glucose and lipid profiles.31,32 Furthermore, no changes in weight gain were observed in accordance with our results.

Obesity and dyslipidemia are considered risk factors of cardiovascular disease and nonalcoholic hepatic steatosis. Furthermore, it has been reported that a high-fat diet and high cholesterol levels can favor metabolic alterations.33 Our results revealed that a high-fat diet promoted an increase in triglycerides and cholesterol, and lycopene and tomato sauce supplementation improved the rat's lipid profile. Previous study observed that treatment with a lycopene mix and other bioactive compounds promoted an increase in HDL levels and reduced oxidative stress through prevention of LDL oxidation.18

Cellular mechanisms triggered by the consumption of a high-fat diet include apoptosis, necrosis, and autophagy.34–37 Those effects may be linked to the genesis of cardiovascular diseases and nonalcoholic hepatic steatosis. Therefore, it is vital to identify strategies that may contribute to their prevention and reduction.

Stress factors can induce IL-1b and TNF-a. Proinflammatory cytokine IL-1b is elevated in chronic inflammatory diseases, such as obesity, and can be associated with increased cell proliferation, cell cycle arrest, and increased apoptosis in different cell types.37,38 We showed that tomato sauce and lycopene supplementation reversed the increase in IL-1b levels induced by high-fat diet.

Numerous studies have investigated the effects of individual compounds on vital cellular parameters and apoptosis to determine the underlying mechanisms of action. However, few studies have investigated the influence of phytochemical combinations in this context.39,40

To determine the basic mechanisms by which carotenoids present in the food matrix may be more effective in preventing and treating diseases than individual compounds, this study compared two versions of lycopene, isolated and food matrix lycopenes, and their inhibitory effects on cell growth and apoptosis. Few studies have reported the mechanism by which fruits and vegetables could prevent or reduce inflammatory diseases, such as cardiovascular

diseases and nonalcoholic hepatic steatosis. Cardiac cell cycle analysis reported that a high-fat diet promoted a decrease in the G0/G1 phase and an increase in the percentage of cardiac cells in the G2/M phase, demonstrating compensation by cell proliferation for higher levels of cell death. Nevertheless, it is important to mention that the pronounced cell proliferation may be harmful to the organism.

Lycopene promoted an increase of cells in the G0/G1 phase and a decrease in the cell percentage in the G2/M phase in different cancer cell lines.4 Our study showed that both tomato sauce and lycopene supplementation increased the number of cardiac cells in the G0/G1 phase and a decrease in the number of cardiac cells in the G2/M compared with the high-fat diet. Tomato sauce reduced the effects caused by high-fat diet in the cardiac cells of the study animals. Lycopene increased the percentage of cardiac cells in the G0/G1 phase, perhaps lessening damage caused by the high-fat diet. Similar results were observed in the liver cell cycle, in which tomato sauce and lycopene supplementation increased and decreased the percentage of cells in the G0/G1 and G2/M phase, respectively. Consumption of tomato juice and pure lycopene regulates the cell cycle of HepG2 cells. Nonetheless, tomato juice did not promote apoptotic changes; only lycopene supplementation was able to induce apoptosis in HepG2 cells.40,41

Apoptosis is characterized by a series of distinct changes in cell morphology, loss of cell attachment, cytoplasmic contraction, DNA fragmentation, and other biochemical changes, including the activation of caspases through extrinsic and/or intrinsic mitochondrial pathways. Therefore, an inhibitory effect on cell proliferation is very desirable for a compound. It is known that changes in the cell growth process and cell cycle are main features of different pathologies.

This study showed a damaging effect of high-fat diet on cardiac cell apoptotic induction. However, tomato sauce and lycopene were not able to reduce high-fat diet induced apoptosis. Similar results have been observed in different cancer cell lines in which lycopene promoted na increase in apoptosis.4,41

We demonstrated that tomato sauce and lycopene supplementation have beneficial effects on cardiac and liver cell metabolism and may be considered as a nutritional approach for the prevention and treatment of cardiovascular diseases and nonalcoholic hepatic steatosis.

Acknowledgments

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Author Disclosure Statement

No competing financial interests exist.

Supplementary Material

Supplementary Table S1.

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Supplementary

Table S1. Ingredients used to formulate the control and high fat rations offered in the experiment (g/100g ration)

Ingredients	Control Ration	High Fat Ration	TS	L2	L4
Casein*	14.0	14.0	14.0	14.0	14.0
Starch	62.1	46.07	46.07	46.07	46.07
Soil oil	4.0	-	-	-	-
Lard	-	20.0	20.0	20.0	20.0
Cellulose	5.0	5.0	5.0	5.0	5.0
[1]Mix vitamins	1.0	1.0	1.0	1.0	1.0
[2]Mix minerals	3.5	3.5	3.5	3.5	3.5
B-colin	0.25	0.25	0.25	0.25	0.25
L-cystine	0.18	0.18	0.18	0.18	0.18
Sugar	10.0	10.0	10.0	10.0	10.0
Lycopene(mg)	0.00	0.00	2.0	2.0	4.0

Legend: (*)% casein protein = 92.5% protein / 100g casein; (1) Vitamin blend (mg / kg diet): retinol palmitate 2.4, cholecalciferol 0.025, benadione sodium bisulfite 0.8, biotin 0.22, cyanocobalamin 0.01, riboflavin 6.6, thiamine hydrochloride 6 , 6 and tocopherol acetate 100; (2) Mineral mix (g / kg diet): copper sulphate 0.1, ammonium molybdate 0.026, sodium iodate 0.0003, potassium chromate 0.028, zinc sulphate 0.091, calcium hydrogen phosphate 0.145, iron sulphate ammoniated 2,338, magnesium sulfate 3.37, manganese sulfate 1,125, sodium chloride 4, calcium carbonate 9.89 and potassium dihydrogen phosphate 14.75.

CAPÍTULO 3

EFFECT OF BETA CAROTENE AND BURITI JUICE (*MAURITIA FLEXUOSA*) ON HEPATIC AND CARDIAC CELL BIOMARKERS IN RATS FED WITH HIGH-FAT DIET

AUTORES: Vanessa Azevedo de Jesuz, Monique de Barros Elias Campos, Vanessa Rosse de Souza, Teresa Palmiciano Bede, Vilma Blondet de Azeredo and Anderson Junger Teodoro

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Abstract

Diets high in saturated fat stimulate the detection of proinflammatory cytokines and increased reactive oxygen species, oxidative stress, and cell tissue damage, like in hepatic and cardiac cells. Carotenoids are potent antioxidant and anti-inflammatory micronutrients that have been investigated in the prevention and treatment of various chronic conditions. This work evaluated the effect of beta carotene and buriti juice in liver and cardiac cells parameters of rats submitted to high-fat diet. Buriti (*Mauritia Flexuosa*) is a fruit from the Amazon that has a high content of beta-carotene in its composition. Experimental model was conducted with groups of adult females Rattus norvegicus: control (CG); high-fat (HG); buriti juice (BUG); beta carotene - 2mg (B2G) and beta carotene - 4mg (B4G). Animals supplemented with beta carotene and buriti juice and with a high-fat diet showed lower body weight gain. The hyperlipid group presented higher levels of IL-1B than the control group. However, both the buriti juice and the beta carotene supplementation were able to reduce the effect of a high-fat diet in IL-1B. The high fat diet altering the cell cycle of hepatic and cardiac cells. In addition, supplementation did not prevent excess dietary fat from reducing viable cell content and increasing the apoptosis process. Both supplements increased cell death and liver cell necrosis, especially the supplementation with 4 mg beta carotene isolated that aggravated the histological damage caused by the high-fat diet, demonstrating a possible toxic effect on these cells.

Keywords: Beta carotene; high-fat diet; liver; cardiac cell; buriti juice;

Introduction

Metabolic disorders and obesity related to excessive fat intake play an important role in the pathogenesis of many chronic diseases such as cancer, cardiovascular disease, diabetes, hypertension and neurodegenerative diseases. Previous investigations suggest dietary interventions may modulate the expression of some genes that may affect various disorders [1].

There has been a trend of nutritional transition present in many countries, which leads to a new dietary model based on a diet rich in saturated fats, sugars and refined foods, and poor in complex carbohydrates and fiber, also known as the Western diet [2]. Excessive fat consumption generates increased lipid flow to the liver, promoting a state of lipotoxicity in the organ, associated with a high level of oxidative stress and reduced antioxidant defense of the organism [3]. Diets high in saturated fat stimulate the detection of proinflammatory cytokines and increased reactive oxygen species, oxidative stress, and cell tissue damage, like in hepatic and cardiac cells [4,5].

Currently, it is known that cardiovascular diseases and cancer are the conditions with the highest mortality rates and, among the types of cancer, liver cancer is the one most associated with the mortality rate. In addition, consumption of a high-fat diet is associated with the development of liver steatosis, which in turn may lead to several atherosclerotic risk factors such as hypertension, diabetes, dyslipidemia, and insulin resistance [6, 7].

As the results of recent investigations show, dietary measures may modulate the expression of some genes, as well as the risk of diseases induced by oxidative stress [8,9,10,11]. These metabolic changes contribute to increased oxidative stress and inflammation in the body. Therefore, antioxidant and anti-inflammatory agents may play a role in preventing these disorders. Carotenoids are potent antioxidant and anti-inflammatory micronutrients that have

been investigated in the prevention and treatment of various chronic conditions, like hepatic steatosis and cardiovascular diseases [12].

Several studies observed a relation of beta carotene consumption and the risk of coronary disease, stroke, cardiovascular disorders, cancer, and mortality in general [13,14]. Beta carotene is a carotenoid present in vegetables and fruits of yellow-orange colour, with emphasis on carrot, pumpkin, papaya and mango, as well as in dark green leafy vegetables such as cabbage and spinache. The highest levels of this carotenoid, which presents considerable pro-vitamin A activity, have been found in fruits whose pulps have orange coloration [15]. Nevertheless, some studies have yielded conflicting results, by emphasizing the uncertainty regarding the protective role of antioxidant action of carotenoids on cardiovascular disease, or even, by underlining that increased intake of β-carotene does not allow cardioprotective effects, but conversely correlates with increased risk of cardiovascular events [16,17].

Buriti (*Mauritia Flexuosa*), a species of palm of Amazonian origin, has recently gained great interest in studies because it is the richest natural source of beta carotene known (152,000 µg RAE / 100g in oil) [18]. Animal studies have shown extremely high bioavailability, probably due to its oily composition. The effectiveness of buriti in treating and preventing diseases has caught the attention of researchers.

Therefore, it is relevant to study liver and cardiac cells after treatment with beta carotene and buriti. However, as most of this human data is still inconclusive, experimental animal models were used to better understand the mechanisms of action of these products. In this sense, the aim of this study is to evaluate the effects of carotenoids on liver and cardiac cell function and integrity in *in vivo* models (rats submitted to high-fat diet), through cell cycle and apoptosis analysis, as well as biochemical parameters.

Materials and methods

Experimental design and sampling

The study was conducted in the Laboratory of Experimental Nutrition at Department of Nutrition and Dietetics of Federal Fluminense University (LabNE-UFF). Samples of Brazilian buriti juice (ingredients: buriti fruit (97%), sugar and water) were obtained from Amazônia, Brazil. Water-soluble (WS) beta carotene 10% (containing sucrose, corn starch, fish gelatin, lycopene, corn oil, ascorbyl palmitate, and DL-alpha tocopherol) was provided by Roche (Rio de Janeiro, RJ, Brazil). Samples of buriti juice and beta carotene were kept in a refrigerator (10°C) and freezer (-18°C), respectively, in appropriate packaging. Appropriate solutions for each experimental group were prepared daily in the laboratory by dissolving the content in filtered water at 50°C and adding 20% refined sugar to obtain a palatable solution. Fifty female, adults Wistar rats were individually housed and maintained in a 12-h light–12-h dark cycle at 22ºC (± 2ºC). Animal maintenance was in accordance with the ARRIVE guidelines [19]. Rat care and experimental protocols were approved by the Institution's Scientific, Academic and Ethics Board.

Animals were divided into five groups: 1) control group (CG), which received a standard diet (based casein) *ad libitum*, consisting of protein (minimum) 12.95%, fat (minimum) 4.0%, and water; 2) high-fat group (HG), which received a high-fat diet *ad libitum*, consisting of protein (minimum) 12.95%, fat (minimum) 20.0%, and water; 3) buriti juice group (BUG), which received the high-fat diet *ad libitum*, plus a buriti juice solution providing 2.0 mg of beta carotene per day; 4) 2.0 mg beta carotene group (B2G), which received the high-fat diet *ad libitum*, plus a solution containing 2.0 mg all-trans beta carotene (water soluble) 10% dissolved in water daily, and water; and 5) 4.0 mg beta carotene group (B4G), which received the high-fat diet *ad libitum*, plus a solution containing 4.0 mg all-trans lycopene (water soluble) 10% dissolved in water daily, and water. The ingredients for the formulation of the

control and high-fat rations used in the experiment are shown in the Table 1 and the experimental model are illustrated in Figure 1.

TABLE 1. Ingredients used to formulate the control and high fat rations offered in the experiment (g/100g ration)

Ingredients	Control Ration	High Fat Ration	BUG	B2G	B4G
Casein*	14.0	14.0	14.0	14.0	14.0
Starch	62.1	46.07	46.07	46.07	46.07
Soil oil	4.0	-	-	-	-
Lard	-	20.0	20.0	20.0	20.0
Cellulose	5.0	5.0	5.0	5.0	5.0
[1]Mix vitamins	1.0	1.0	1.0	1.0	1.0
[2]Mix minerals	3.5	3.5	3.5	3.5	3.5
B-colin	0.25	0.25	0.25	0.25	0.25
L-cystine	0.18	0.18	0.18	0.18	0.18
Sugar	10.0	10.0	10.0	10.0	10.0
Beta carotene(mg)	0.00	0.00	2.0	2.0	4.0

Legend: (*)% casein protein = 92.5% protein / 100g casein; (1) Vitamin blend (mg / kg diet): retinol palmitate 2.4, cholecalciferol 0.025, benadione sodium bisulfite 0.8, biotin 0.22, cyanocobalamin 0.01, riboflavin 6.6, thiamine hydrochloride 6 , 6 and tocopherol acetate 100; (2) Mineral mix (g / kg diet): copper sulphate 0.1, ammonium molybdate 0.026, sodium iodate 0.0003, potassium chromate 0.028, zinc sulphate 0.091, calcium hydrogen phosphate 0.145, iron sulphate ammoniated 2,338, magnesium sulfate 3.37, manganese sulfate 1,125, sodium chloride 4, calcium carbonate 9.89 and potassium dihydrogen phosphate 14.75.

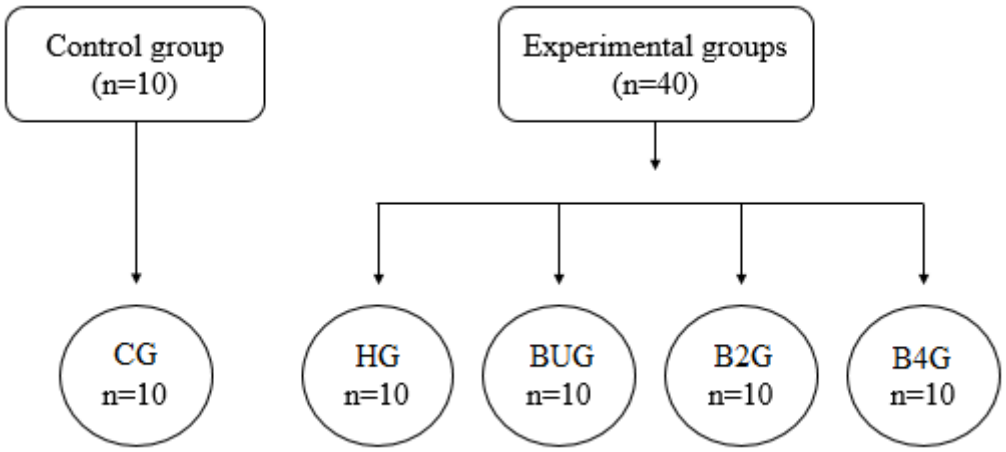

FIGURE 1. Dietary protocol. Experimental model: Control group (CG): standard diet plus water; Experimental groups: high-fat group (HG). high-fat diet plus water; group buriti juice (BUG). high-fat diet plus buriti juice and water; 2mg beta carotene group (B2G). high-fat diet plus solution with 2.0 mg *all-trans* beta carotene and water; 4mg beta carotene group (B4G). high-fat diet plus solution with 4.0 mg *all-trans* beta carotene and water. Diets and solutions were administered during a period of 60 days.

After 60 days of the experiment, the vaginal smear procedure was performed on all animals to identify their phase in the estrous cycle and to establish that all were in the same physiological state without hormonal interference in the analysis. After the estrous cycle check, rats in the "estrous" phase were separated and trapped. Body weight was measured and trapped animals were sacrificed. Serum and organs (heart and liver) were obtained, weighed, frozen and kept on -70°C until analysis. Experimental procedures were conducted according to the study of Ribeiro *et al.* (2018) [20].

The hepatic and cardiac tissue was carefully removed and weighed with a BioPrecisa® precision scale. Then, the relative weight of the organ, denominated hepatic/cardiac index, was calculated according to the equation: Hepatic/Cardiac index = [(Liver/ Heart Weight (g) ÷ Body Weight) X 100].

After weighing, four fragments of different hepatic lobes and cardiac tissue were sectioned with a surgical scalpel of each animal and immediately treated for cell cycle analysis and apoptosis assay [21].

Analytical Methods

A glucometer was used to measure serum glucose concentration. Serum total cholesterol, triglycerides, aspartate aminotransferase (AST), alanine aminotransferase (ALT), IL-1β, and TNF-α concentration were measured using BioClin® commercial kits and wavelengths specific to each biochemical indicator, using the colorimetric method with automated spectrophotometer reading (BioClin® BS-120 Chemistry Analyzer®).

Cell Cycle Analysis and Cell Viability

Flow cytometry analysis was performed to measure cell cycle and cell viability of tissue hepatic. The tissue was macerated and the extractions were made with the addition of 0.5 mg/ml collagenase (Sigma®). The cells were washed twice with phosphate buffered saline and resuspended in 500 μl of ice-cold Vindelov solution containing 0.1% Triton X-100, 0.1% citrate and 0.1 mg/mL of RNase and 50 mg/mL of propidium iodide (Sigma Chemical Co., St. Louis, MO) after centrifugation. Cells were incubated for 15 minutes and the suspension was analyzed for DNA content by flow cytometry using a FACS Calibur flow cytometer (Becton Dickinson. Mountain View, CA). After acquisition of 30.000 events, the relative proportions of cells with DNA content haploid subG1 (<2n), diploid G0/G1 (2n), S phase (>2n and <4n) and G2/M phase (4n) were acquired and analyzed using CellQuest and WinMDI 2.9. Considering the experimental conditions that were used in this study, or in any others we were aware of, the fluorescence was not affected by the cell dissociation process. According

Guimarães *et al*. (2017) [21], nuclei of viable cells were gated according to FL- 2W × FL2-A relation.

Apoptosis Assay

For the apoptosis assay, the cells were subjected to staining with Annexin V conjugated to FITC. The non-adherent cells were collected, and adherent cells were quickly washed with a calcium/magnesium-free buffered saline solution (BSS) and were detached with 0.125% trypsin/EDTA (Sigma Chemical Co., St. Louis, MO, USA) at room temperature. Subsequently, apoptotic and necrotic cells were stained with Annexin V FITC/Propidium Iodide (PI) (BD Pharmingen, Mountain View, CA, USA) according to the manufacturer's instructions, quantified with a flow cytometer 30.000 events (FACS Calibur, BD Bioscience, Mountain View, CA, USA), and the data were analyzed in CellQuest and FlowJo software.

Statistical Analysis

Data were analyzed using software package Graphpad Prism for Windows. Differences between the groups were analyzed using the Student's t-test, and the values were considered unpaired and parametric. For means of comparison between the groups, they were analyzed using one-way ANOVA followed by Tukey's post-hoc test for the multiple mean comparison test. Results are expressed as mean–standard deviation, and the significance level were set at $p < 0.05$.

Results

During the experiment the different dietary treatments did not influence ($p>0.05$) the intake of ration and water of animals which were similar among all groups. However, animals supplemented with beta carotene and buriti juice and with a high-fat diet (B2G, B4G and BUG) showed lower body weight gain ($p <0.05$) (Table 2). Although no difference was observed in the hepato-somatic and cardiac index of the animals.

TABLE 2. Body and tissue weight in control and experimental groups

GROUPS	CG	HG	B2G	B4G	BUG
Start weight (g)	207,60 ± 30,33	209,60 ± 17,15	258,00 ± 22,88	253,00 ± 26,54	250,40 ± 10,33
Final weight (g)	261,00 ± 31,04	268,20 ± 17,68	274,00 ± 30,13	262,60 ± 14,50	270,60 ± 13,76
Liver weight (g)	7,8g ± 1,15	7,1g ± 1,34[6]	7,5g ± 1,85	8,01 ± 0,11	8,25 ± 0,18
Heart weight (g)	0,94 ± 0,21	0,90 ± 0,07	1,04 ± 0,11	0,98 ± 0,08	0,98 ± 0,13
Glycemia	100,40 ± 9,24	97,60 ± 6,50	107,20 ± 7,09	107,60 ± 10,71	97,40 ± 9,53
Triglycerides	31,60 ± 8,17	41,25 ± 13,20	73,40 ± 38,71	43,40 ± 11,52	46,00 ± 12,33
Total cholesterol	46,00 ± 8,60	45,00 ± 3,54	78,40 ± 22,74	62,80 ± 9,31	65,40 ± 7,54
AST	195,60 ± 52,03	219,80 ± 33,39	162,80 ± 56,86	133,80 ± 28,45	111,60 ±27,52
ALT	32,20 ± 6,42	32,00 ± 5,39	32,80 ± 11,88	35,00 ± 4,53	22,00 ± 6,56
IL-1B	266,38 ± 133,17	1135,38 ± 730,58	571,97 ± 412,78	452,00 ± 208,48	531,60 ± 565,86
TNF-α	25,66 ± 7,87	20,05 ± 5,84	35,78 ± 22,24	39,56 ± 14,55	27,42 ± 9,14

*Values are mean–SD. Different letters mean statistical difference between groups. Statistical significance was determined by ANOVA followed by Tukey's post hoc multiple mean comparison test. CG: control group; HG: high-fat group; B2G: group betacarotene 2 mg; B4G group betacarotene 4 mg; BUG: group buriti juice. AST - Aspartate aminotransferase. ALT - Alanine aminotransferase

The group supplemented with 2mg beta carotene had higher cholesterol and triglyceride levels when compared to the other groups. The group that received only the high-fat diet had the lowest levels of cholesterol and triglycerides (Table 2). We observed that HG presented higher levels of AST (219,80 ± 33,39) when compared to the different groups CG (195,60 ± 52,03); B2G (162,80 ± 56,86); B4G (133,80 ± 28,45); BUG (111,60 ±27,52). We found no significant difference in relation to ALT between the groups.

The hyperlipid group presented higher levels of IL-1B than the control group. However, both the buriti juice and the beta carotene supplementation were able to reduce the effect of a high-fat diet in IL-1B. We did not observe statistical difference in glycemic and TNF analysis among the groups (Figure 3).

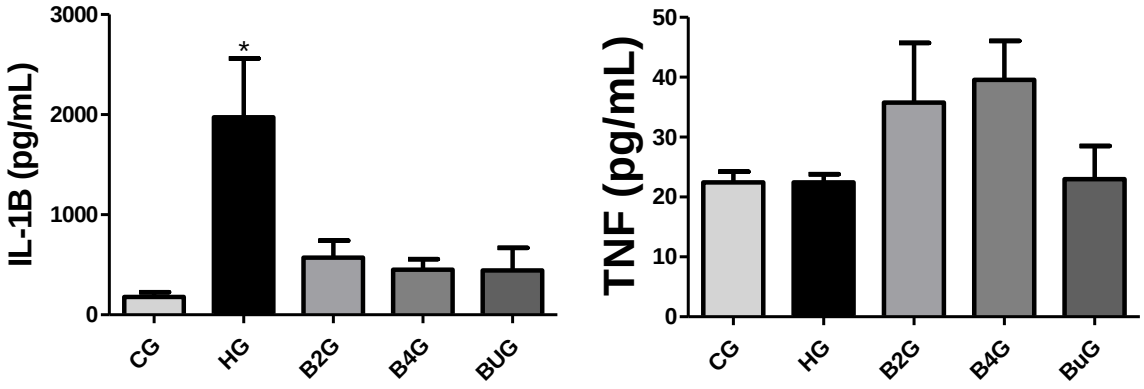

FIGURE 3. Serum inflammatory parameters of IL-1*b and* TNF-*a* from rats fed a high-fat diet supplemented with Beta Carotene and Buriti juice **P* <.05; ***P* <.01; *P* <. 001. ANOVA–Tukey's test. ANOVA, analyis of variance; TNF, tumor necrosis factor; IL, interleukin.

Our results of hepatic cell cycle are described below (Table 3). GBU presents the lowest percentage of cells in phase G0 / G1 (12.00 ± 2.95), when compared to the groups GC (63.49 ± 6.06), GH (53.67 ± 5.79), GB2 (49.60 ± 29.59) and GB4 (67.50 ± 10.10). In addition, GB2 reached a maximum value of 25.98 ± 16.54 in the G2 / M phase, 55% higher when compared to the CG.

TABLE 3. Effect of Beta carotene and Buriti Juice on Cell Cycle Progression of Hepatic Cells in Rats Fed with a High-Fat Diet.

Groups	G0/G1	S	G2/M
CG	63,49 ± 6,06 [a]	20,20 ± 8,47 [a]	14,41 ± 3,85[a]
HG	53,67 ± 5,79 [b]	28,18 ± 3,40[b]	18,25 ± 3,08[a]
BUG	12,00 ± 2,95[d]	4,13 ± 2,23[a,c]	9,21 ± 1,40[b]
B2G	49,60 ± 29,59	3,48 ± 0,43[c]	25,98 ± 16,54
B4G	67,50 ± 10,10	12,22 ± 4,95[a,c]	9,21 ± 2,76[b]

The results are expressed as mean – SD, with significant differences compared by one-way ANOVA followed by Tukey's multiple comparison post hoc test. *P < .05. *P < .01.

In relation to the cardiac cell cycle (Table 4), BUG and B4G decreased G_0-G_1 when compared to the control group, while B2G increased. BUG decreased the G_2-M phase, while B2G increased when compared to the control group (Figure 4).

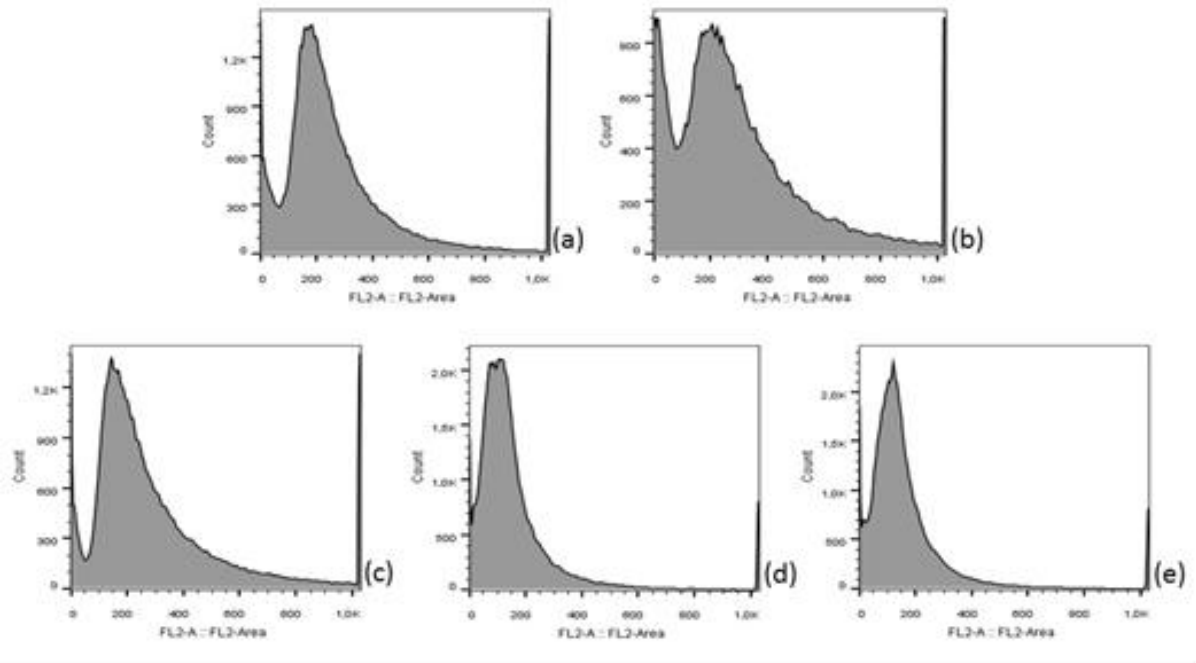

FIGURE 4. Flow cytometry analysis of cardiac cells from rats fed a high fat diet supplemented with Beta carotene and Buriti Juice. (a): control group (CG); (b): high-fat group (HG); (c): group buriti juice (BuG); (d): beta carotene 2mg (B2G); (e): beta carotene 4mg (B4G).

TABLE 4. Effect of Beta carotene and Buriti Juice on Cell Cycle Progression of Cardiac Cells in Rats Fed with a High-Fat Diet.

GROUPS	**G0/G1**	**S**	**G2/M**
CG	$56,2 \pm 4,34^{a,c}$	$6,3 \pm 0,44^{a,b}$	$7,9 \pm 1,50^{a}$
HG	$49,9 \pm 6,88^{a}$	$6,6 \pm 0,86^{a,b}$	$10,8 \pm 1,82^{a}$
BUG	$39,09 \pm 1,94^{b}$	$4,69 \pm 1,31^{a}$	$6,73 \pm 1,33^{b}$
B2G	$61,24 \pm 7,46^{c}$	$8,24 \pm 1,81^{b}$	$14,41 \pm 1,96^{c}$
B4G	$35,29 \pm 8,25^{b}$	$5,20 \pm 0,71^{a}$	$10,24 \pm 3,36^{a}$

The results are expressed as mean – SD, with significant differences compared by one-way ANOVA followed by Tukey's multiple comparison post hoc test. *P < .05.

The high-fat (HG) diet altered the liver cell cycle of the animals, leading to a smaller number ($p < 0.05$) of viable cells and a higher apoptotic cell rate (Table 5). The supplements did not reverse the effects of high fat diet on apoptosis of liver cells. Beta carotene supplementation, especially B4G, showed to be even more aggressive to the liver tissue.

In addition to the alterations already caused by the high-fat diet, beta carotene treatment caused an increase in hepatic cells in initial apoptosis in GH (22.98 ± 7.78) when compared to CG (2.18 ± 1.55). In the beta carotene-treated groups (GBU - 0.04 ± 0.01, GB2 - 0.26 ± 0.27 and GB4 0.75 ± 0.71a), a significant decrease in initial apoptosis was observed when compared to the control and hyperlipidic group (Figure 5).

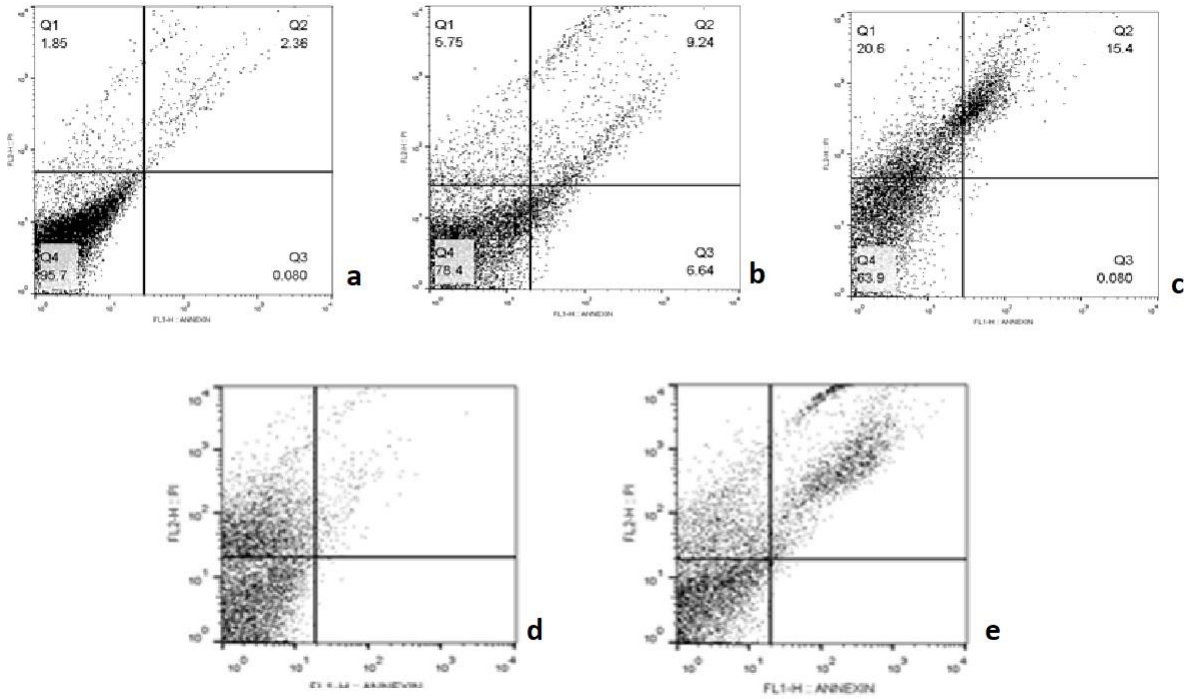

FIGURE 5. Flow cytometry analysis of cell death of hepatic cells from rats fed a high fat diet supplemented with beta carotene and buriti juice. (a): control group (CG); (b): high-fat group

(HG); (c): group buriti juice (BuG); (d): beta carotene 2mg (B2G); (e): beta carotene 4mg (B4G).

TABLE 5. Monitoring of cell death of hepatic cells from rats fed a high-fat diet supplemented with Betacarotene and Buriti Juice.

Groups	Viable cells (Annexin V- PI-	Initial apoptosis (Annexin V+ PI-)	Late apoptosis (Annexin V+ PI+)	Non-apoptotic cells (Annexin V- PI+)
CG	89,85 ± 3,96 [a]	2,18 ± 1,55 [a]	3,91 ± 1,90[a]	2,69 ± 1,67[a]
HG	66,58 ± 10,16 [b]	22,98 ± 7,78[b]	6,59 ± 1,80[a]	1,72 ± 0,43[a]
BUG	75,90 ± 12,18[c]	0,04 ± 0,01[c]	5,33 ± 5,82[a]	18,70 ± 6,52[b]
B2G	64,36 ± 12,11[b]	0,26 ± 0,27[c]	15,27 ± 9,22[b]	20,08 ± 6,44[b]
B4G	35,34 ± 12,59[d]	0,75 ± 0,71[a,c]	32,23 ± 20,15[b]	31,68 ± 9,54[b]

The results are expressed as mean – SD, with significant differences compared by one-way ANOVA followed by Tukey's multiple comparison post hoc test. *P < .05. *P < .01.

However, both groups receiving beta carotene supplementation (GB2 - 15.27 ± 9.22 and GB4 32.23 ± 20.15) showed a marked increase in the percentage of late apoptosis cells compared to the CG (3.91 ± 1 90), with no significant difference in GBU (5.33 ± 5.82) and GH (6.59 ± 1.80) in relation to the control group. GBU increased viable cells relative to GH, but also increased cell necrosis compared to the control and hyperlipidic group. The most significant result was GB4, which showed the highest programmed induction of death of these cells, reaching 32.23 ± 20.15 in late apoptosis and 31.68 ± 9.54 in necrosis when compared to the

control group (3, 91 ± 1.90; 2.69 ± 1.67) and GH (6.59 ± 1.80; 1.72 ± 0.43), respectively, demonstrating a possible toxic effect on these hepatic cells (Table 5).

Necrosis was increased 128% in HG when compared to CG and beta carotene supplementation was not enought to reduce this efecct of high-fat diet (Figure 6). Beta carotene increased dose-dependent apoptosis in cardiac cells. The results of this study suggest that the suplementation of 4mg beta carotene could resulte in a possible toxic effect on these cardiac cells (Table 6).

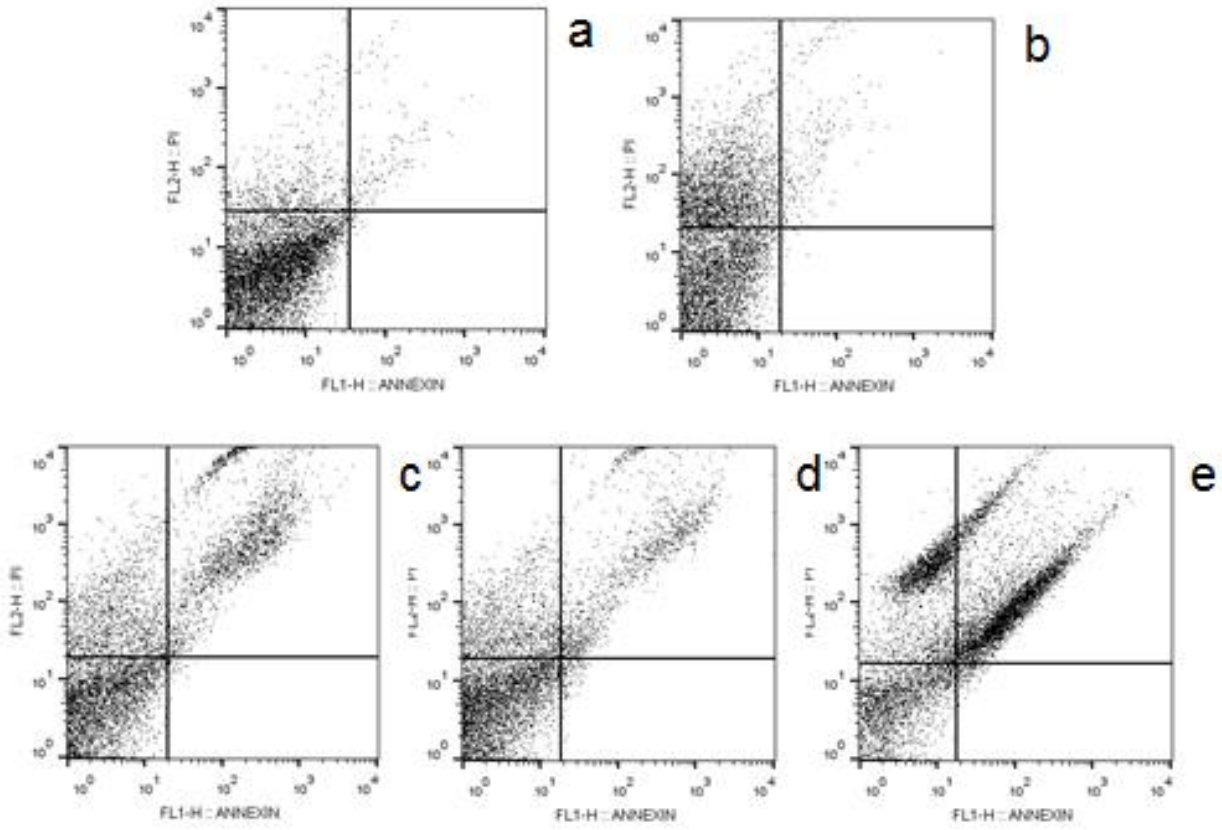

FIGURE 6. Flow cytometry analysis of cell death of cardiac cells from rats fed a high fat diet supplemented with beta carotene and buriti juice. (a): control group (CG); (b): high-fat group (HG); (c): group buriti juice (BuG); (d): beta carotene 2mg (B2G); (e): beta carotene 4mg (B4G).

TABLE 6. Monitoring of cell death of cardiac cells from rats fed a high-fat diet supplemented with Betacarotene and Buriti Juice.

Groups	Viable cells (Annexin V- PI-)	Initial apoptosis (Annexin V+ PI-)	Late apoptosis (Annexin V+ PI+)	Non-apoptotic cells (Annexin V- PI+)
CG	90,84 ± 3,43[a]	0,65 ± 0,34	3,89 ± 2,42[a]	4,76 ± 1,09[a]
HG	76,84 ± 12,65[a]	3,11 ± 4,01	7,98 ± 8,35[a]	10,86 ± 11,51[a]
BUG	69,73 ± 10,83[a]	0,78 ± 0,66	15,91 ± 6,41[a]	13,62 ± 8,81[a]
B2G	67,50 ± 9,81[a]	1,14 ± 0,30	20,21 ± 15,44[a]	11,16 ± 5,88[a]
B4G	37,38 ± 18,20[b]	1,58 ± 0,71	37,32 ± 16,24[b]	23,70 ± 9,43[b]

The results are expressed as mean – SD, with significant differences compared by one-way ANOVA followed by Tukey's multiple comparison post hoc test. *P < .05.

Discussion

The consumption of a high energy density, high-fat diet is thought to be one of the main factors for increased obesity and associated comorbidities. A study show that the key point is that when individuals are exposed, on a chronic basis, to a higher mean level of dietary fat, the otherwise incredibly robust negative feedback system that regulates body fat decreases [22]. More fat is stored and the individual moves towards obesity. High-fat intake combined with high levels of oxidative stress results in increased liver fat loading in the setting and reduced antioxidant levels resulting in lipotoxicity [23]. Obesity is correlate to metabolic syndrome, dyslipidemia, cardiac diseases, intolerance glucose and insulin resistance. Studies have shown the relationship between metabolic syndrome, especially obesity and

dyslipidemia, and the presence of nonalcoholic fatty liver disease (NAFLD) refers to a wide spectrum of liver damage [24]. Other studies showed the relation between liver disorders and cardiovascular diseases [25,26].

In addition, previous *in vitro* and *in vivo* studies showed that beta carotene is a powerful antioxidant capable of neutralizing intracellular free radicals involved in the development of chronic illnesses, including CVD, hepatic steatosis and cancer. Serum beta carotene has also been inversely associated with systemic markers of inflammation and insulin resistance [27,28].

Our outcomes agree with published results in which beta carotene supplementation in rats was able to reduce weight gain even with ingestion of a high-fat diet when compared with the control group. Our results revealed that a high-fat diet promoted an increase in triglycerides and IL-1B. Moreover, beta carotene 4mg and buriti juice supplementation improved the rat's lipid profile. Furthermore, the intervention with beta carotene supplementation *in vivo* did not affect glucose profiles. Stress factors can induce pro-inflammatory cytokine IL-1B, associated the chronic inflammatory diseases, such as obesity, and can interfere with increased cell proliferation, cell cycle and cell death in different cell types. We showed that beta carotene and buriti juice supplementation reversed the increase in IL-1B levels induced by high-fat diet.

Hozawa et al (2007) demonstrated an inverse relationship between serum levels of beta carotene and oxidative stress markers and inflammation associated with cardiovascular disease [29]. Our study showed a reduction of IL-1B after beta carotene and buriti juice supplementation, corroborating with the previous study.

It is suggested that beta carotene is an important factor in reducing the risk of cancer and cardiovascular diseases. Human cancer risks have been shown to be inversely correlated with

high blood levels of retinol or beta carotene and ingestion of foods containing retinol [30]. Thus, many studies investigate the action of bioactive compounds on interferences in cellular parameters and apoptosis of cells. Most studies are directed at isolated compounds and few are concerned with the synergy of compounds in the food matrix.

In this context, our study compared the results of isolated beta carotene supplementation and beta carotene in the cell matrix (in buriti juice). Moreover, their effects on cell growth and apoptosis were evaluated in the face of a scenario of tissue inflammation, due to the effect of a high-fat diet. Liver cell cycle analysis reported that a high-fat diet promoted a decrease in the G0/G1 phase and an increase in the percentage of hepatic cells in the G2/M phase, demonstrating compensation by cell proliferation for higher levels of cell death. Nevertheless, it is important to mention that the pronounced cell proliferation may be harmful to the organism. Our results of hepatic cell cycle demonstrate GBU presented the lowest percentage of cells in phase G0 / G1, when compared to the others groups. In addition, GB2 reached a maximum value in the G2 / M phase, 55% higher when compared to the GC.

Beta carotene supplementation did not prevent the change caused by the high-fat diet in the hepatic and cardiac cell cycle. In addition, the high-fat diet altered the liver cell cycle of the animals, leading to a smaller number of viable cells and a higher apoptotic cell rate, the supplements did not reverse the effects of high fat diet on apoptosis of liver cells. Beta carotene supplementation, especially B4G, showed to be even more aggressive to the liver tissue. In addition to the alterations already caused by the high-fat diet, beta carotene treatment caused an increase in hepatic cells in initial apoptosis in GH when compared to control group. In the beta carotene-treated groups, a significant decrease in initial apoptosis was observed when compared both to the control group and hyperlipidic group. However, both groups receiving beta carotene isolated supplementation showed a marked increase in the percentage of late apoptosis cells compared to the control group. Buriti juice was able to

increase viable cells relative to GH, but also increased cell necrosis compared to control and hyperlipidic group. The most significant result was GB4, which showed the highest programmed induction of death of these cells, in late apoptosis and in necrosis when compared to the control group and hyperlipidic group, respectively, demonstrating a possible toxic effect on these cells.

Mukherjee *et al* (2011) demonstrates that, even when administered at high doses during long periods of time, beta-carotene does not cause toxicity, whereas high doses of vitamin A and retinoids, as possible prophylactic agents for cancer prevention, can lead to acute hepatotoxicity and cause other adverse effects [31]. On the other hand, contrary to the beneficial effects of beta carotene, the literature presents cases of hypervitaminosis associated with megadoses, which resulted in hepatic impregnation, causing adverse effects such as injury, fibrosis, portal hypertension and hepatic disorders [32]. There were also reports of experimental and interventionist studies that suggested a possible antioxidant action, which contrasts with its already known antioxidant action.

This study showed a beta carotene supplementation caused a possible toxic effect on liver and cardiac cells, increasing the harmful effect of a high-fat diet.

Conclusions

The high fat diet caused damage to liver and cardiac tissue, altering the cell cycle. In addition, supplementation did not prevent excess dietary fat from reducing viable cell content and increasing the apoptosis process. Both supplements increased cell death and liver cell necrosis, especially the supplementation with 4 mg beta carotene isolated, demonstrating a possible toxic effect on these hepatic and cardiac cells.

Conflicts of Interest

The authors declare no conflicts of interest

Acknowledgment

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CONSIDERAÇÕES FINAIS

O presente estudo forneceu informações sobre os efeitos do licopeno e betacaroteno, na matriz alimentar e suplemento isolado, a função e integridade de células cardíacas do tecido estriado esquelético. Tanto a suplementação de licopeno quanto de betacaroteno isolados e na matriz celular foram capazes de reduzir o ganho de peso e reduzir a IL-1β induzidos pela dieta hiperlipídica em ratas.

O estudo observou que o licopeno isolado e o molho de tomate exerceram efeito protetor sobre células do tecido cardíaco através do aumento da apoptose e da parada de ciclo celular, processos estes alterados por uma dieta hiperlipídica.

No entanto, a suplementação de betacaroteno não evitou os efeitos nocivos causados pela dieta hiperlipídica no ciclo celular cardíaco. A suplementação com suco de buriti foi capaz de aumentar as células viáveis quando comparado ao grupo hiperlipídico, mas também aumentou a necrose celular. A suplementação de 4mg de betacaroteno isolado apresentou a maior indução programada de morte celular, na apoptose tardia e na necrose, demonstrando um possível efeito tóxico sobre essas células.

Desta maneira, demonstramos que a suplementação com molho de tomate e licopeno tem efeitos benéficos no metabolismo das células cardíacas e pode ser considerada uma abordagem nutricional para a prevenção e tratamento de doenças cardiovasculares. Em contrapartida, a suplementação de betacaroteno causou um possível efeito tóxico nas células cardíacas, sendo necessários mais estudos para avaliar os mecanismos moleculares associados ao betacaroteno e sua ação sobre as células cardíacas.

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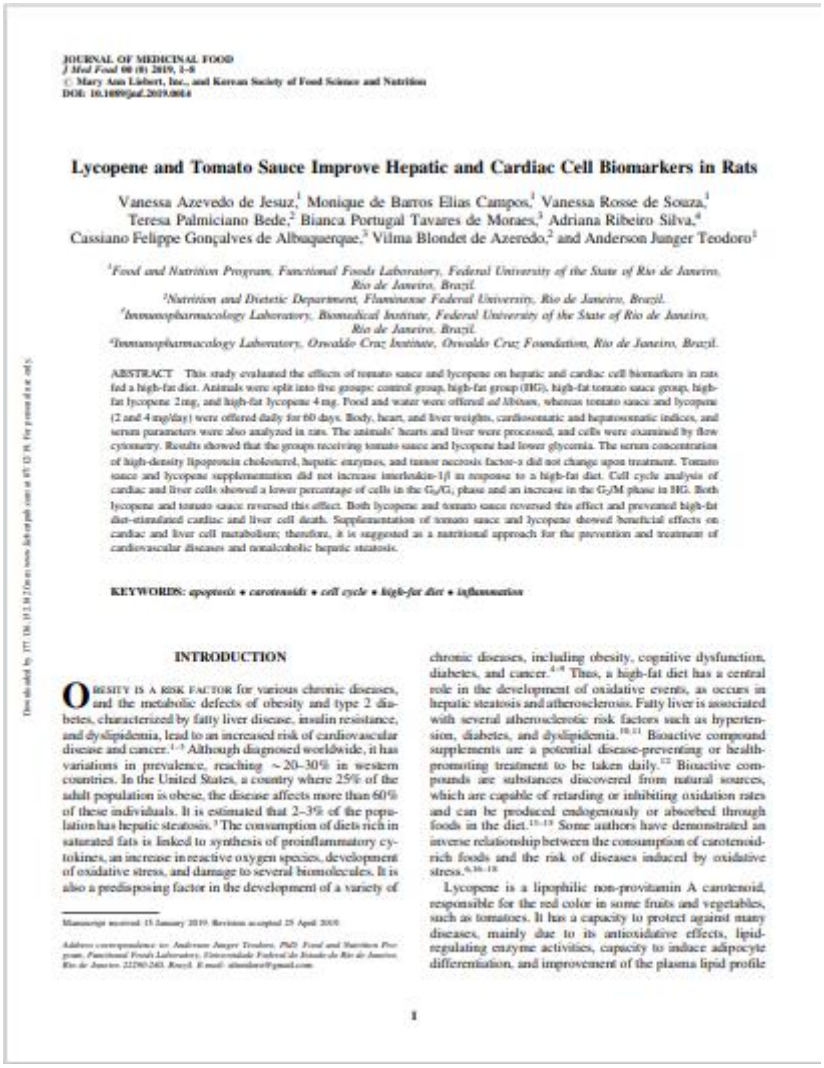
APÊNDICES

APÊNDICE A – Artigo publicado



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Lycopene and Tomato Sauce Improve Hepatic and Cardiac Cell Biomarkers in Rats

Vanessa Azevedo de Jesuz,[1] Monique de Barros Elias Campos,[1] Vanessa Rosse de Souza,[1] Teresa Palmiciano Bede,[2] Bianca Portugal Tavares de Moraes,[3] Adriana Ribeiro Silva,[4] Cassiano Felippe Gonçalves de Albuquerque,[3] Vilma Blondet de Azeredo,[2] and Anderson Junger Teodoro[1]

[1]*Food and Nutrition Program, Functional Foods Laboratory, Federal University of the State of Rio de Janeiro, Rio de Janeiro, Brazil.*
[2]*Nutrition and Dietetic Department, Fluminense Federal University, Rio de Janeiro, Brazil.*
[3]*Immunopharmacology Laboratory, Biomedical Institute, Federal University of the State of Rio de Janeiro, Rio de Janeiro, Brazil.*
[4]*Immunopharmacology Laboratory, Oswaldo Cruz Institute, Oswaldo Cruz Foundation, Rio de Janeiro, Brazil.*

ABSTRACT This study evaluated the effects of tomato sauce and lycopene on hepatic and cardiac cell biomarkers in rats fed a high-fat diet. Animals were split into five groups: control group, high-fat group (HG), high-fat tomato sauce group, high-fat lycopene 2 mg, and high-fat lycopene 4 mg. Food and water were offered *ad libitum*, whereas tomato sauce and lycopene (2 and 4 mg/day) were offered daily for 60 days. Body, heart, and liver weights, cardiosomatic and hepatosomatic indices, and serum parameters were also analyzed in rats. The animals' hearts and liver were processed, and cells were examined by flow cytometry. Results showed that the groups receiving tomato sauce and lycopene had lower glycemia. The serum concentration of high-density lipoprotein cholesterol, hepatic enzymes, and tumor necrosis factor-α did not change upon treatment. Tomato sauce and lycopene supplementation did not increase interleukin-1β in response to a high-fat diet. Cell cycle analysis of cardiac and liver cells showed a lower percentage of cells in the G_0/G_1 phase and an increase in the G_2/M phase in HG. Both lycopene and tomato sauce reversed this effect. Both lycopene and tomato sauce reversed this effect and prevented high-fat diet–stimulated cardiac and liver cell death. Supplementation of tomato sauce and lycopene showed beneficial effects on cardiac and liver cell metabolism; therefore, it is suggested as a nutritional approach for the prevention and treatment of cardiovascular diseases and nonalcoholic hepatic steatosis.

KEYWORDS: *apoptosis • carotenoids • cell cycle • high-fat diet • inflammation*

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INTRODUCTION

OBESITY IS A RISK FACTOR for various chronic diseases, and the metabolic defects of obesity and type 2 diabetes, characterized by fatty liver disease, insulin resistance, and dyslipidemia, lead to an increased risk of cardiovascular disease and cancer.[1–3] Although diagnosed worldwide, it has variations in prevalence, reaching ~20–30% in western countries. In the United States, a country where 25% of the adult population is obese, the disease affects more than 60% of these individuals. It is estimated that 2–3% of the population has hepatic steatosis.[3] The consumption of diets rich in saturated fats is linked to synthesis of proinflammatory cytokines, an increase in reactive oxygen species, development of oxidative stress, and damage to several biomolecules. It is also a predisposing factor in the development of a variety of chronic diseases, including obesity, cognitive dysfunction, diabetes, and cancer.[4–9] Thus, a high-fat diet has a central role in the development of oxidative events, as occurs in hepatic steatosis and atherosclerosis. Fatty liver is associated with several atherosclerotic risk factors such as hypertension, diabetes, and dyslipidemia.[10,11] Bioactive compound supplements are a potential disease-preventing or health-promoting treatment to be taken daily.[12] Bioactive compounds are substances discovered from natural sources, which are capable of retarding or inhibiting oxidation rates and can be produced endogenously or absorbed through foods in the diet.[13–15] Some authors have demonstrated an inverse relationship between the consumption of carotenoid-rich foods and the risk of diseases induced by oxidative stress.[6,16–18]

Lycopene is a lipophilic non-provitamin A carotenoid, responsible for the red color in some fruits and vegetables, such as tomatoes. It has a capacity to protect against many diseases, mainly due to its antioxidative effects, lipid-regulating enzyme activities, capacity to induce adipocyte differentiation, and improvement of the plasma lipid profile

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Address correspondence to: Anderson Junger Teodoro, PhD, Food and Nutrition Program, Functional Foods Laboratory, Universidade Federal do Estado do Rio de Janeiro, Rio de Janeiro, 22290-240, Brazil. E-mail: ajteodoro@gmail.com

APÊNDICE B – Artigo submetido

Food &
Function

Effect of betacarotene and buriti juice (Mauritia Flexuosa) on hepatic and cardiac cell biomarkers in rats fed with high-fat diet

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