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Original Paper

Tomato-Oleoresin Anti-Inflammatory Effect Recovers Obesity-Induced Cardiac Dysfunction by Modulating Myocardial Calcium Handling

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Key Words
Obesity • Inflammation • Cardiac dysfunction • Calcium kinetic • Papillary muscle • Lycopene

Abstract
Background/Aims: Considering the importance of inflammation on obesity-related disorders pathogenesis, including cardiac dysfunction, the interest in natural anti-inflammatory therapeutic strategies has emerged. The lycopene is a carotenoid presents in tomato and red fruits that displays anti-inflammatory properties. In this sense, we will evaluate the anti-inflammatory effect of tomato-oleoresin supplementation on obesity- related cardiac dysfunction by modulating myocardial calcium kinetic. ***Methods:*** Male Wistar rats were initially randomized into 2 experimental groups: (Control, n= 20) or high sugar- fat diet (HSF, n=20) for 20 weeks. At week 20th, once detected the cardiac dysfunction (cardiac remodeling, systolic and diastolic dysfunction) by echocardiography in HSF group, animals were randomly divided to begin the treatment with tomato-oleoresin, performing 4 groups: Control (n= 10); Control + tomato tomato-oleoresin supplementation (Control + Ly, n= 10); HSF (n= 10) or HSF + tomato tomato-oleoresin supplementation (HSF + Ly, n= 10). Tomato oleoresin was mixed with maize oil equivalent to 10mg lycopene/kg body weight (BW) per day and given orally, by gavage, every morning for a 10-week period. It was analyzed cardiac inflammatory parameters by the enzyme-linked immunosorbent assay (ELISA) and *in vivo* (echocardiography) and *in vitro* (studying isolated papillary muscles from the left ventricle) cardiac

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function. The groups were compared by Two-Way analysis of variance (ANOVA). ***Results:*** The HSF diet induced cardiac dysfunction (FS(%) C: 60.4±1.3; C+Ly: 60.9±1.3; HSF: 51.7±1.3; HSF+Ly: 59.4±1.4) and inflammation (TNF-α: C: 1.88±0.41; C+Ly: 1.93±1.01; HSF: 4.58±1.99; HSF+Ly: 2.03±0.55; IL-6: C: 0.58±0.16; C+Ly: 0.40±0.16; HSF: 2.00±0.45; HSF+Ly: 0.53±0.26; MCP-1: C: 0.31±0.08; C+Ly: 0.43±0.22; HSF: 1.54±0.32; HSF+Ly: 0.50±0.16). Tomato-oleoresin supplementation improved cardiac remodeling and dysfunction, cardiac inflammation and myocardial calcium kinetic. ***Conclusion:*** the anti-inflammatory effect of tomato-oleoresin supplementation treated the obesity-induced cardiac dysfunction by modulating myocardial calcium handling.

Introduction

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

It has been described that the inflammation also exerts an important role on both cardiac remodeling and dysfunction [8, 9]. Corroborating this information, increased tumoral necrosis factor- α (TNF- α) and interleukin- 6 (IL-6) plasma levels have already been reported in patients with chronic heart failure, suggesting a positive correlation between inflammation and disease severity [10, 11]. However, the mechanism by how inflammation leads to cardiac dysfunction in obesity condition needs to be clarified [12–15]. The adipose tissue produces, physiologically, several adipokines, such as leptin, adiponectin, proinflammatory cytokines- as IL-6 and TNF-α [16]- and monocyte chemoattractant protein -1 (MCP-1). Yet, in obesity condition occurs an increase in adipokines generation [17], initiating a local inflammatory process that later reaches other organs [18]. In the heart, pro-inflammatory cytokines seem to induce apoptosis and necrosis as well as hypertrophy in cardiomyocytes [19]. Interesting, a study showed an association between proinflammatory cytokines and myocardial contractile depression, especially by altering the calcium homeostasis and handling [20].

Considering the importance of inflammation on obesity-related disorders pathogenesis, including cardiac dysfunction, the interest in natural anti-inflammatory therapeutic strategies has emerged. Lycopene is a carotenoid present in tomato and red fruits that displays anti-inflammatory properties previously demonstrated by our research group in a study involving adipose tisse of obese animals [21]. The relation between lycopene and cardiovascular disease has been evaluated in clinical [22, 23] and experimental studies [24]. Our recent study [25] demonstrated that 10 weeks of tomato-oleoresin supplementation reduced cardiac oxidative stress and attenuated insulin resistance. As part of our previous published study, we investigated the lycopene anti-inflammatory effect to recovery myocardial contractile. We hypothesized that the antinflamatory propertie of lycopene recovery cardiac function by improving calcium handling.

Materials and Methods

Animals and Experimental Protocol

All the experiments and procedures were approved by the Animal Ethics Committee of Botucatu Medical School (1196/2016) and were performed in accordance with the National Institute of Health's Guide for the Care and Use of Laboratory Animals [26]. Male Wistar rats (±187 g) were kept in an environmental controlled room (22°C ± 3°C; 12 h light-dark cycle and relative humidity of 60 ± 5%) and initially randomly divided into 2 experimental groups: (Control, n= 20) or high sugar-fat diet (HSF, n=20)

for 20 weeks. The diets and water were provided *ad libitum*. The HSF diet contained soybean meal, sorghum, soybean peel, dextrin, sucrose, fructose, lard, vitamins, and minerals, plus 25% of sucrose in drinking water. Control diet contained soybean meal, sorghum, soybean peel, dextrin, soy oil, vitamins, and minerals. The nutrients and nutritional composition of each diet was described in our previous studies [27, 28] (Supplementary Table 1 – for all supplementary material see www.cellphysiolbiochem.com).

At week 20th of this study, once detected the cardiac dysfunction by echocardiogram in the HSF group (Supplementary Table 2), the animals were randomly divided to begin the treatment with tomato-oleoresin, performing four groups: Control (n= 10); Control + tomato tomato-oleoresin supplementation (Control + Ly, n= 10); HSF (n= 10) or HSF + tomato tomato-oleoresin supplementation (HSF + Ly, n= 10). Tomato oleoresin was mixed with maize oil equivalent to 10mg lycopene/kg body weight (BW) per day and given orally, by gavage, every morning for a 10-week period [21, 29]. In order to avoid differences in the energy provided, all the groups received the same maize oil volume (approximately 2 mL/kg BW per day). At the end of the 30-week period, after 8 h of fasting the animals were anesthetized (thiopental 120 mg/kg/i.p.) and euthanized by decapitation after the absence of foot reflex to obtain blood and heart.

Lycopene preparation

Tomato-oleoresin (Lyc-O-Mato 6% dewaxed; LycoRed Natural Products Industries) was mixed with maize oil and stored at 4°C in the dark until to be used, as described previously [30]. The tomato-oleoresin maize oil mixture was stirred for 20min in a water-bath at 54°C before being fed to the animals. Each milliliter of the solution contained 5mg of total lycopene. Stability of lycopene was monitored at 450nm, and confirmed by diode-array spectra, as described previously [31].

Inflammatory parameters

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

Echocardiographic study

All the animals were evaluated *in vivo* by transthoracic echocardiography, using a Vivid S6 system equipped with multifrequency ultrasonic transducer 5.0 to 11.5 MHz (General Electric Medical Systems, Tirat Carmel, Israel). All exams were performed by the same examiner and obtained according to the leading-edge method recommended by the American Society of Echocardiography [32, 33]. Rats were lightly anesthetized by intramuscular injection with a mixture of ketamine (50 mg/kg) and xylazine (1 mg/kg). After shaving their chest, the animals were placed in left decubitus position. To implement structural measures of the heart, the images were obtained in one-dimensional mode (M-mode) guided by the images in two-dimensional mode with the transducer in the parasternal position, minor axis. Left ventricular (LV) evaluation was performed by positioning the cursor M-mode just below the mitral valve plane at the level of the papillary muscles. The images of the aorta and left atrium were obtained by positioning the M-mode course to plan the level of the aortic valve. The following cardiac structures were measured: The relative thickness of the LV (RWT) was calculated by dividing LVPWD multiplied by two by LVDD. Left ventricular mass (LVM) was calculated using the formula [(LVDD +LVPWD IVSDT) 3 − (LVDD) 3] × 1.04 where 1.04 is the specific density of the myocardium. MVE index (LVMI) was calculated by normalizing to body weight Estimated LV mass. The LV systolic function was assessed by the following parameters: percentage of endocardial shortening (Δ% endo) [(LVDD − LVSD)/LVDD] × 100; midwall fractional shortening (% Δ meso) {[(LVDD + ½ + ½ LVPWD IVSDT) − (LVSD + ½ + ½ IVSST LVPWS)]/(LVDD + ½ + ½ LVPWD IVSDT)} × 100. The LV diastolic function was evaluated by the following indices: transmitral flow early peak velocity (E wave); transmitral flow late peak velocity (A wave); ratio between the E and A waves (E/A); isovolumetric relaxation time in absolute values (IRT). The joint assessment of diastolic and systolic LV function was performed by myocardial performance index also known as Tei index (sum of isovolumetric contraction and IRT time, divided by the left ventricular ejection time).

Post-mortem morphological analysis

After euthanasia, the rats were submitted to thoracotomy, and the hearts, ventricles and tibia were separated, dissected, weighed and measured. Cardiac remodeling at the macroscopic level, which identifies the presence or absence of cardiac hypertrophy, was determined by analyzing the following parameters: heart weight (HW), left ventricle (LV) weights, HW and LV/tibia length ratios.

Myocardial function by isolated papillary muscle study

Myocardial function was evaluated by studying isolated papillary muscles from the LV. This procedure has been used by several authors [4, 5, 34]. This preparation permitted the measurement of the capacity of cardiac muscle to shorten and develop forces independent of influences that can modify *in vivo* mechanical performance of the myocardium, such as the heart rate, preload, and afterload. After euthanasia, the hearts were quickly removed and placed in oxygenated Krebs-Henseleit solution at 28°C. The LV papillary muscles were dissected, mounted between two spring clips, placed vertically in a chamber containing Krebs-Henseleit solution (118.5 mM NaCl; 4.69 mM KCl; 2.5 mM $CaCl_2$; 1.16 mM MgSO4; 1.18 mM KH_2PO4; 5.50 mM GL, and 24.88 mM $NaHCO_3$) and maintained at 28°C with a thermostatic water circulator. The bathing solution was bubbled with 95% oxygen and 5% carbon dioxide, with a pH of 7.4. The lower spring clip was attached to a 120T-20B-force transducer (Kyowa, Tokyo, Japan) by a thin steel wire (1/15,000 inch), which passed through the mercury seal at the bottom of the chamber. The upper spring clip was connected with a thin steel wire to a rigid lever arm, above which a micrometer stop was mounted for adjusting the muscle length. The muscle preparation was placed between two parallel platinum electrodes (Grass E8, GRASS Technologies, An Astro-Med, Inc. Product Group, West Warwick, RI, USA) and stimulated at a frequency of 0.2 Hz (12 pulses/min) with 5 ms square-wave pulses. Voltage was set to a value 10% greater than the minimum required to produce a maximal mechanical response. Conventional mechanical parameters at *Lmax* were calculated from isometric contraction: maximum developed tension normalized per cross-sectional area (DT [g/mm^2]), resting tension normalized per cross-sectional area (RT [g/mm^2]), positive (+dT/dt [g/mm^2/s]) and negative (-dT/dt [g/mm^2/s]) tension derivative normalized per cross-sectional area of papillary muscle (CSA). To determine the mechanism by which obesity induces negative inotropic effects on contractile function, the papillary muscles were evaluated under the baseline condition of 2.5mM Ca^{2+} and after inotropic and lusitropic maneuvers: increases in extracellular Ca^{2+} concentration (to test their effects on myofilament machinery) and post-rest contraction (PRC), mainly related to sarcoplasmic reticulum (SR) storage and release capacity [5, 34]. Inotropic responses were recorded 5 min after the addition of each dose of extracellular Ca^{2+} (0.5, 1.0, 1.5, 2.0, 2.5, 3.0 and 3.5 mM) to the bathing solution. PRC was studied at an extracellular Ca^{2+} concentration of 0.5 mM, where the stimulus was paused for 10, 30, and 60 s before restarting the stimulation.

Statistical Analysis

Results are expressed as mean ± standard deviation (SD). Differences among the groups were determined by using Two-Way analysis of variance (ANOVA) for independent samples. A repeated-measures Two-Way ANOVA was used to evaluate the positive and negative inotropic effects on myocardial function. Once detected the differences ($p < 0.05$), the Tukey post- hoc test for multiple comparisons were carried out. All the statistical analyses were performed using SigmaStat for Windows (Version 3.5).

Results

As previously reported [25], both groups that received the HSF diet (HSF an HSF + Ly) presented higher daily caloric intake, final body weight, adiposity index, glucose levels and systolic blood pressure compared to the respective controls (control and control + Ly), with no effect to tomato oleoresin supplementation on these parameters. HOMA-IR was higher in the HSF group compared to control group, however, the tomato oleoresin attenuated insulin resistance in the HSF + Ly group compared to HSF (Supplementary Fig. 1).

The echocardiographic parameters are presented in the Table 1. The HSF group presented cardiac remodeling (increased estimated LV mass and relative wall thickness (RWT)) and deterioration of both systolic (decreased fraction shortening (FS), endocar-

Table 1. Echocardiographic evaluation. Data are expressed in mean ± standard deviation (n= 8 animals/group). LV: left ventricle; RWT: Relative wall thickness; FS: Fraction shortening; ES: Endocardial shortening; E/A waves: diastolic mitral inflow velocity ratio; IRT: Isovolumetric relaxation time. TEI: Myocardial Performance Index; Lateral E: mitral tissue Doppler velocity lateral; Septal E mitral tissue Doppler velocity septal; Average E: mitral tissue Doppler velocity (medium of lateral and septal velocities); E´/A: diastolic mitral inflow doppler velocity ratio. Comparison by Two-way ANOVA with Tukey post-hoc ($p < 0.05$). *HSF vs Control; # HSF vs HSF + Ly; $HSF + Ly vs Control + Ly

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

Table 2. Cardiac post-mortem analyses. Data are expressed in mean ± standard deviation (n= 8 animals/group). LV: left ventricle; LVW: left ventricle weight; HW: heart weight. Comparison by Two-way ANOVA with Tukey post-hoc ($p < 0.05$). *HSF vs Control; # HSF vs HSF + Ly; $HSF + Ly vs Control + Ly

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

dial shortening (ES) and cardiac output) and diastolic function (decreased E average) compared to control group. The tomato-oleoresin supplementation in HSF + Ly group was effective to atenuate cardiac remodeling (reduced estimated LV mass and RWT) and to recover systolic-diastolic function, visualized by increased fraction shortening (FS) and E'/A, respectively, compared to HSF.

Table 2 shows the *post- mortem* cardiac morphological parameters. It is possible to verify that the HSF group developed pathological cardiac remodeling process (higher heart weight, LV weight, HW/tibia and LV/tibia) after 30 weeks of experimental protocol compared to control group. On the other hand, the treatment with tomato-oleoresin was effective to attenuate the cardiac remodeling process in the HSF + Ly compared to HSF group.

The analysis of myocardial papillary muscle function at baseline condition with 2.5 mM Ca^{2+} concentration is presented in the Fig. 1. Fig. 1A showed that there was no difference for cross sectional area (CSA) of papillary muscle among the groups as soon as there was no difference for positive tension derivative normalized per cross-sectional area of the papillary muscle (+dT/dt, g/mm2/s) (Fig. 1C). In Fig. 1B is possible to note a functional impairment in the maximum developed tension (DT) in the HSF group compared to control group while the tomato-oleoresin supplementation was effective to recovery the DT capacity in the HSF + Ly group compared to HSF group. In Fig. 1D it notes a reduced negative tension derivative normalized per cross-sectional area of the papillary muscle (-dT/dt, g/mm2 /s) in the HSF group compared to control group with no effect of tomato oleoresin supplementation.

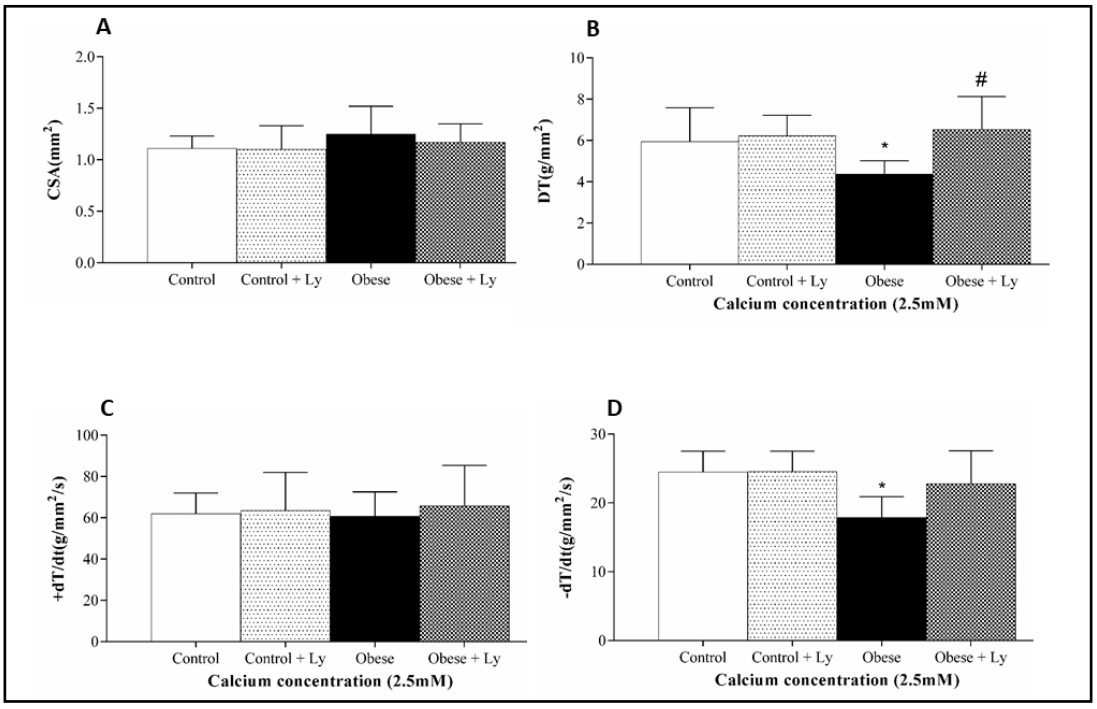

Fig. 1. Basal papillary muscles response. Baseline calcium concentration (2.5mM). A- CSA, cross-sectional area; B- Maximum developed tension normalized per cross-sectional area [DT, g/mm2]; C- positive [+dT/dt, g/mm2 /s]; D- negative [-dT/dt, g/mm2 /s] tension derivative normalized per cross-sectional area of the papillary muscle. Data are expressed in mean ± standard deviation (n= 8 animals/group). Comparison by repeated-measures two-way ANOVA with Tukey post-hoc ($p<0.05$): *HSF vs Control; #HSF vs HSF + Ly.

Fig. 2 shows the post-rest contractile response of papillary muscle. Fig. 2A shows that the HSF presented a functional impairment in the DT (60s) compared to control group and the tomato-oleoresin supplementation was effective to recovery this condition in HSF + Ly compared to HSF group. Fig. 2B shows that there was no difference for positive tension derivative normalized per cross-sectional area of the papillary muscle (+dT/dt, g/mm2/s) among the groups. Fig. 1C shows that the HSF presented a functional impairment in the -dT/dt (30 and 60s) compared to control group and the tomato-oleoresin supplementation was effective to recovery this condition in HSF + Ly compared to HSF group.

Fig. 3 shows the contractile response of papillary muscle in different concentrations of extracellular calcium (from 0.5 to 3.5 mM). Fig. 3A shows that the HSF presented a functional impairment in the DT (2.5mM) compared to control group and the tomato-oleoresin supplementation was effective to recovery this condition in HSF + Ly compared to HSF group. Fig. 3B shows that there was no difference for positive tension derivative normalized per cross-sectional area of the papillary muscle (+dT/dt, g/mm2/s) among the groups. Fig. 3C shows that the HSF presented a functional impairment in the -dT/dt (2.5, 3.0 and 3.5mM) compared to control group and the tomato-oleoresin supplementation was effective to recovery this condition in 3.0 and 3.5mM in HSF + Ly compared to HSF group.

Fig. 4 shows the cardiac inflammatory parameters. It notes that the HSF group presented higher TNF-α levels (Fig. 4A), MCP-1 levels (Fig. 4B) and IL-6 levels (Fig. 1C) compared to control group, representing an inflammatory condition. In opposition, the treatment with tomato-oleoresin was effective to attenuate cardiac inflammation in the HSF + Ly group compared to the HSF group.

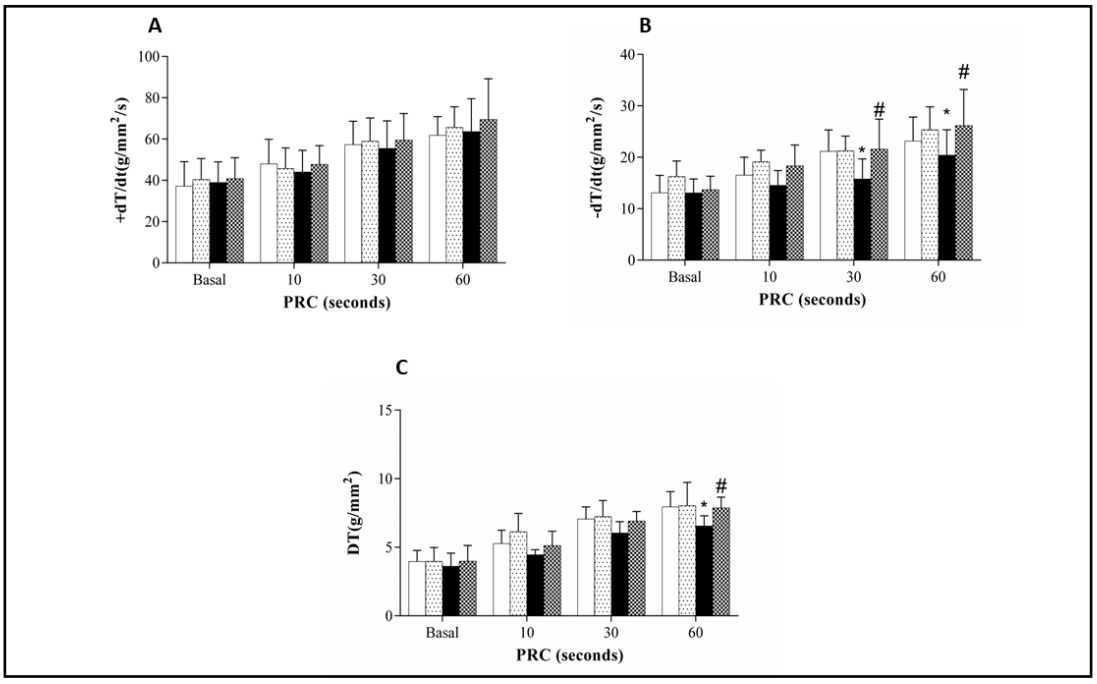

Fig. 2. Effect of post-rest contraction in papillary muscles. Baseline calcium concentration (0.5mM) is presented as 100%. A- Maximum developed tension normalized per cross-sectional area [DT, g/mm2]; B- positive [+dT/dt, g/mm2 /s]; C- negative [-dT/dt, g/mm2 /s] tension derivative normalized per cross-sectional area of the papillary muscle. Data are expressed in mean ± standard deviation (n= 8 animals/group). Comparison by repeated-measures two-way ANOVA with Tukey post-hoc ($p<0.05$): *HSF vs Control; #HSF vs HSF + Ly.

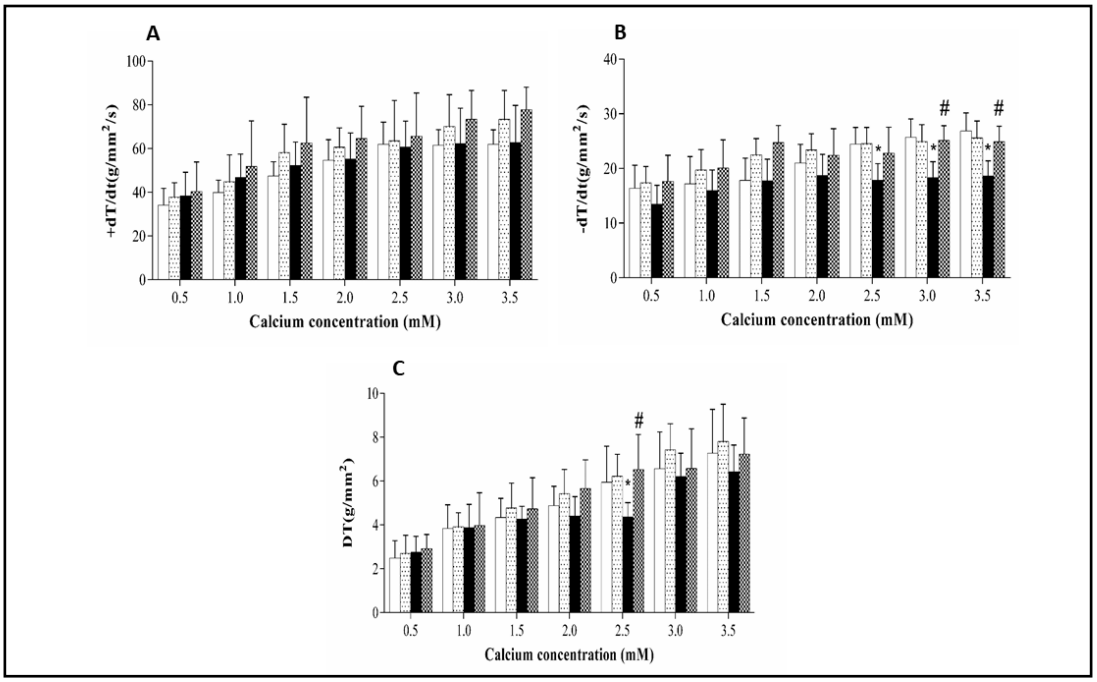

Fig. 3. Effects of increasing extracellular calcium concentration in papillary muscles. A- Maximum developed tension normalized per cross-sectional area [DT, g/mm2]; B- positive [+dT/dt, g/mm2 /s]; C- negative [-dT/dt, g/mm2 /s] tension derivative normalized per cross-sectional area of the papillary muscle. Data are expressed in mean ± standard deviation (n= 8 animals/group). Comparison by repeated-measures two-way ANOVA with Tukey post-hoc ($p<0.05$): *HSF vs Control; #HSF vs HSF + Ly.

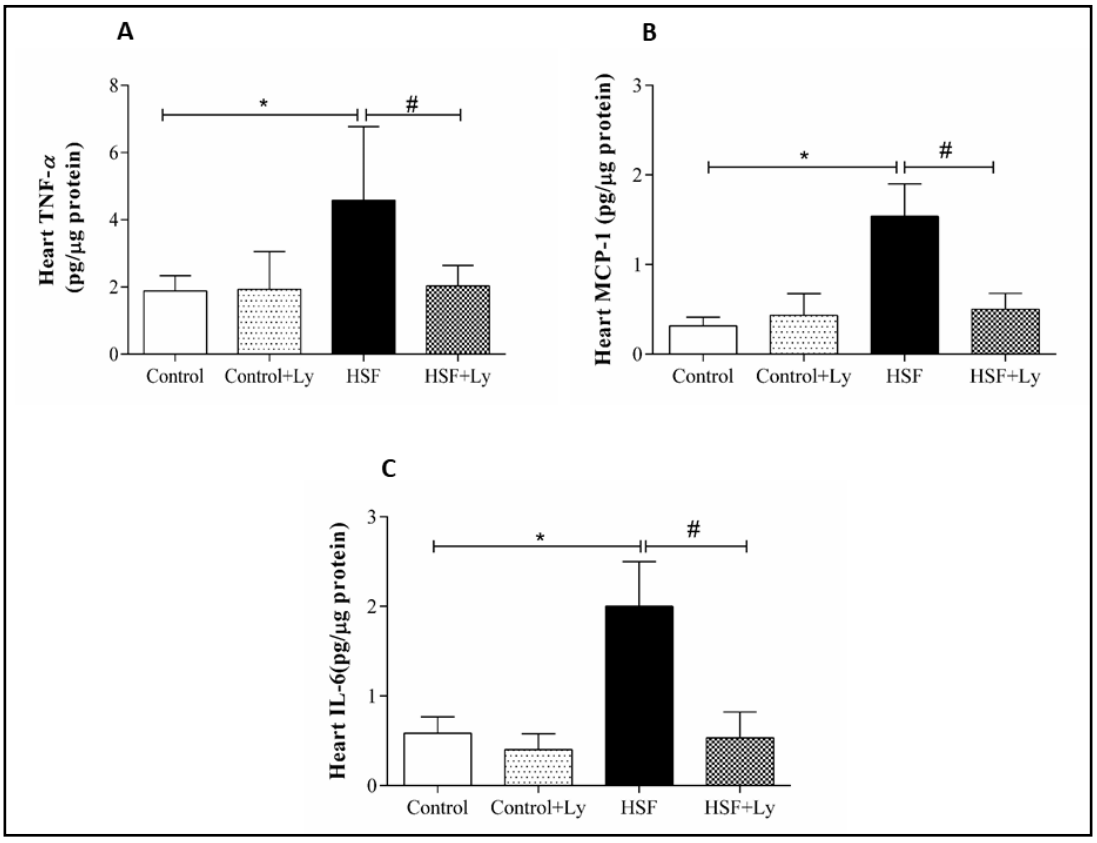

Fig. 4. Cardiac inflammatory parameters. A- tumoral necrosis factor – alpha (TNF-α, pg/µg protein); B- monocyte chemo attractant protein- 1 (MCP-1, pg/µg protein); C- Interleukin- 6 (IL-6, pg/µg protein). Data are expressed in mean ± standard deviation (n= 8 animals/group). Comparison by two-way ANOVA with Tukey post-hoc ($p<0.05$): *HSF vs Control; #HSF vs HSF + Ly.

Discussion

In our previous study [25], we demonstrated a positive effect of tomato oleoresin to improve insulin resistance and to reduce cardiac oxidative stress. Although some researchers have investigated the clinical and experimental effects of lycopene and tomato-oleoresin on several diseases, such as non-alcoholic fatty liver disease (NAFDL) [35], renal disease [29], adipose tissue inflammation [21, 31], cancer [36, 37] and cardiovascular diseases [23–25, 38–40], no studies have evaluated the lycopene anti-inflammatory effect to recover myocardial contractility by modulating calcium handling.

Cardiac disease in obesity has multifactorial causes that include metabolic dysregulation- as insulin resistance and hypertension- as risk factors able to lead to adverse cardiac remodeling, characterized by hypertrophy, systolic and diastolic function impairment [41]. The echocardiographic analysis showed cardiac remodeling and systolic and diastolic function impairment in the HSF group after 30 weeks. In addition, the *post-mortem* morphological analysis in the HSF group support the finding that obesity induces cardiac hypertrophy, visualized by increased total heart, left ventricle and their respective normalized weight [6]. Considering that tomato-oleoresin supplementation had no effect on obesity and systolic blood pressure (as previously reported [25]), it would be expected that the HSF + Ly animals displayed the same cardiac deterioration than the HSF group. However, the HSF + Ly group presented recovery of cardiac remodeling and function.

Several mechanisms try to explain cardiac dysfunction and remodeling induced by obesity. Recently, it was reported the role of inflammation on cardiac remodeling and dys-

function [8, 9]. Studies reveal that, although inflammation following tissue damage is an essential physiological reaction in the healing process, an excessive inflammatory response is associated with left ventricular (LV) hypertrophy as well as progression of cardiac diseases [8]. Pro-inflammatory cytokines, such as TNF-α and IL-6, seem to induce cardiomyocytes apoptosis and necrosis as soon as cells hypertrophy [19]. In addition, proinflammatory cytokines, seem to exert an important role in the myocardial contractile depression by altering calcium homeostasis and handling, characterized by lower systolic Ca^{2+} influx and Ca^{2+} recapture by SERCA2 resulting in reduced myofilament Ca^{2+} responsiveness [20]. Moreover, functional studies using isolated papillary muscle showed that obesity is able to impair the contractile regulation pathway due the slower recapture of calcium via SERCA2, and lower Ca influx by L-type Ca^{2+} channel activity impairment [1, 3, 5, 7].

Post-rest contraction (PRC) findings and extracellular calcium concentration changes suggest that obesity promotes regulatory Ca^{2+} channel dysfunction. Post-rest contraction allows us to study aspects of sarcoplasmic reticulum (SR) participation in the cardiac muscle contraction-relaxation cycle. In this study, the PRC induced a significant reduced response in -dT/dt and DT in the myocardium of the HSF animals. These data are consistent with a previous study that showed lower contractile response in obese Zucker after 60 weeks of cessation of stimulus [42]. As -dT/dt is influenced by the rate of calcium ions uptake into SR, the minor Ca^{2+} re-sequestration demonstrated by -dT/dt in obese rats suggests depressed SERCA2 activity. The elevation of extracellular Ca^{2+} alters the contraction and relaxation phases due increased cytoplasmic Ca^{2+} concentration availability, which interfer in the operation of the L-type channel, Sodium-calcium exchange NCX and SR function. The lower response to extracellular Ca^{2+} elevation in the HSF animals can be related to a reduction of Ca^{2+} influx across L-type channels and/or changes in SR function, as verified with post-rest contraction. These results confirm previous studies that verified cardiac dysfunction and depressed responsiveness to extracellular Ca^{2+} elevation in myocytes and papillary muscles of obese rats [4, 7, 43].

Obesity is a low-grade chronic inflammation condition, which the pro- inflammatory cytokines produced by adipose tissue can reach other organs, as the heart [44]. Thus, our findings suggested that the post- rest contraction and the extracellular calcium maneuver leaded to negative outcomes in developed force (DT) and in relaxation capacity (-dT/dt) in the HSF group compared to the control group, establishing a relation between increased myocardial pro- inflammatory concentration cytokines and calcium kinetic change [20]. In opposition, the anti-inflammatory tomato-oleoresin effect attenuated the contractile cardiac impairment in the HSF + Ly group restoring (DT) and (-dT/dt) capacity.

Lycopene is a symmetrical molecule formed from eight C5 isoprenoid units joined head-to-tail, except at the center, where thereis a tail-to-tail link that reverses the order. Several research groups have suggested that cis-isomers of lycopene are better absorbed than the all-transform because of the shorter length of the cis-isomer, the greater solubility of cis-isomers in mixed micelles, and/or as a result of the lower tendency of cisisomers to aggregate [45]. However, at present, the exact functions and relative activities of these different isomers are unknown [46]. Several mechanisms have been proposed to explain the cardiovascular protective effects of lycopene, including the reduction of cholesterol levels and inflammatory response, the reduction of oxidation of biomolecules, the improvement of intercellular communication and the stimulation of apoptosis and finally, the antiangiogenic effects. It is important to remember that all these factors play a fundamental role in the development of atherosclerosis and CVD [45]. Although we have observed a benefic effect of lycopene on cardiac dysfunction, remodeling and calcium kinetic, it must be emphasized that there are other nutrients present in tomato oleoresin (γ-tocopherol, α-tocopherol, β-carotene, phytofluene, and phytene) that may contribute to explain the results of the present study. Although we have observed a benefic effect of lycopene on cardiac dysfunction, remodeling and calcium kinetic, it must be emphasized that there are other nutrients present in tomato oleoresin (γ-tocopherol, α-tocopherol, β-carotene, phytofluene, and phytene) [47] that may contribute to explain the results of the present study.

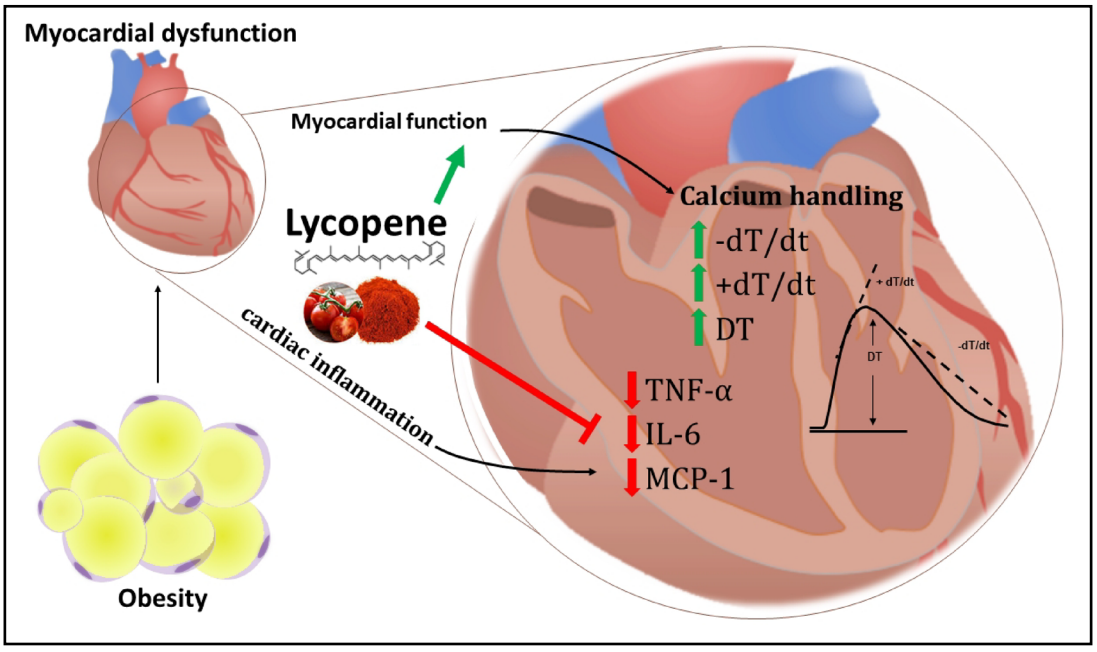

Fig. 5. Anti-inflammatory effects of tomato oleoresin in cardiac function recovery. The obesity promotes increase of inflammatory markers (TNF-α, MCP-1 and IL-6). This inflammatory condition modifies the myocardial calcium handling, leading to an impairment in the Maximum developed tension [DT]; positive [+dT/dt]; negative [-dT/dt] tension derivative. The anti-inflammatory proprieties of Tomato oleoresin were able to recovery the cardiac function.

Conclusion

In summary, this study found that the HSF diet was able to induce obesity, cardiac dysfunction by calcium kinetic impairment due inflammation and the tomato-oleoresin was able to attenuate this condition. So, it is possible to conclude that the anti- inflammatory effect of the tomato-oleoresin supplementation treated obesity-induced cardiac dysfunction and remodeling by modulating the myocardial calcium handling (Fig. 5).

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Author Contributions

AJTF, FM, CRC and ALAF contributed to conception and design of the study; FVFF, CCVAS, SGZB, DHSC, JLG, LG, IOM and AJTF performed the experiments and analyzed the data; AJTF, FVFF and ALAF wrote the manuscript; all the authors contributed to manuscript revision, read and approved the submitted version.

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Statement of Ethics

All the experiments and procedures were approved by the Animal Ethics Committee of Botucatu Medical School (1196/2016).

Disclosure Statement

The authors declare that they have no competing interests.

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