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Tomato (*Lycopersicon esculentum*) or lycopene supplementation attenuates ventricular remodeling after myocardial infarction through different mechanistic pathways

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Abstract

The objective of this study was to evaluate the influence of tomato or lycopene supplementation on cardiac remodeling after myocardial infarction (MI). Male Wistar rats were assigned to four groups: the sham group (animals that underwent simulated surgery) that received a standard chow (S; $n=18$), the infarcted group that received a standard chow (MI; $n=13$), the infarcted group supplemented with lycopene (1 mg of lycopene/kg body weight/day) (MIL; $n=16$) and the infarcted group supplemented with tomato (MIT; $n=16$). After 3 months, morphological, functional and biochemical analyses were performed. The groups MIL and MIT showed decreased interstitial fibrosis induced by infarction. Tomato supplementation attenuated the hypertrophy induced by MI. In addition, tomato and lycopene improved diastolic dysfunction evaluated by echocardiographic and isolated heart studies, respectively. The MI group showed higher levels of cardiac TNF-α compared to the MIL and MIT groups. Decreased nuclear factor E2-related factor 2 was measured in the MIL group. Lipid hydroperoxide levels were higher in the infarcted groups; however, the MIT group had a lower concentration than did the MI group [S=223±20.8, MI=298±19.5, MIL=277±26.6, MIT=261±28.8 (nmol/g); $n=8$; $P<.001$]. We also examined left ventricle miRNA expression; when compared to the S group, the MIL group uniquely down-regulated the expression of eight miRNAs. No miRNA was found to be up-regulated uniquely in the MIT and MIL groups. In conclusion, tomato or lycopene supplementation attenuated the cardiac remodeling process and improved diastolic function after MI. However, the effect of lycopene and tomato supplementation occurred through different mechanistic pathways.

Keywords: Myocardial infarction; Remodeling; microRNA; Epigenetics; Nutrition

1. Introduction

Cardiovascular diseases are the major cause of death worldwide. Among them, acute coronary syndromes, including myocardial infarction (MI), have the highest mortality rate [1]. Heart failure is a common complication after MI and cardiac remodeling has a prominent role in this scenario [2–5].

Defined as genetic, morphological and biochemical changes in response to the determined injury, cardiac remodeling results in progressive ventricular dysfunction and death. Several factors are associated with cardiac remodeling after MI, and among them, we can highlight the increase in oxidative stress and the inflammatory process [6–8].

In order to attenuate cardiac remodeling and reduce mortality after MI, strategies have been used such as administration of converting

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enzyme inhibitors, angiotensin II, beta-blockers and blockers of aldosterone [6]. However, because of the socioeconomic impact and high mortality rates despite treatment, it is important to identify other factors that modulate the ventricular remodeling process. One of these factors is inclusion of foods in the diet having antioxidant properties, such as tomato (*Lycopersicon esculentum*), and one of its constituents, lycopene.

Lycopene is the main fat-soluble pigment responsible for the red color of ripe tomatoes. *In vitro* and *in vivo* studies have shown that lycopene acts as a potent antioxidant [9,10]. It is the most efficient scavenger of singlet oxygen and peroxyl radicals among the carotenoids, and it modulates the production of antioxidant enzymes, such as superoxide dismutase (SOD) and catalase (CAT), mainly due to their actions on nuclear factor E2-related factor 2 (Nrf2) [11,12]. Moreover, lycopene also modulates the inflammatory process through the NF-κB pathway. Indeed, Hung et al. [13] showed in a study with human endothelial cells that lycopene administration reduced the activation of NF-κB and the expression of intercellular adhesion molecule 1 (ICAM-1) by reducing TNF-α.

In addition to its action on inflammation and oxidative stress, lycopene and tomato could influence epigenetic pathways. In a previous study, we showed the effects of tomato supplementation on the expression of miRNAs in the normal rat heart [14]. Considering that inflammation, oxidative stress and expression of miRNAs modulate the remodeling process, tomato or lycopene supplementation might be an attractive treatment after coronary occlusion. Therefore, the purpose of this study was to evaluate the influence of tomato or lycopene supplementation on cardiac remodeling after MI.

2. Materials and methods

This research protocol was approved by the Animal Ethics Committee of Botucatu Medical School, and it was performed in accordance with the National Institute of Health's Guide for the Care and Use of Laboratory Animals.

Male Wistar rats, weighing 200–250 g, underwent experimental MI, according to the method described previously [15,16] or a simulated surgery (without coronary artery occlusion).

After 7 days, the first echocardiographic study was performed to ensure that the variables were similar between infarcted groups before beginning treatment. After the echocardiogram, the animals were assigned to four groups: the sham group (the animals underwent simulated surgery) that received a standard chow (S; n=18), the infarcted group that received a standard chow (MI; n=13), the infarcted group that received a standard chow supplemented with lycopene [1 mg of lycopene/kg body weight (BW)/day] (MIL; n=16) and the infarcted group that received a standard chow supplemented with tomato (MIT; n=16). Tomato supplementation was equivalent to 1 mg of lycopene/kg BW/day [17,18]. Lycopene and tomato were diluted in corn oil to be added to the chow (0.5 ml of corn oil/kg BW/day). The S and MI groups had the same amount of corn oil added to the chow, so there was no difference regarding macronutrients supplied to each group.

We selected only animals with an infarct size greater than 30% as assessed by histological analysis, because we considered that animals with small infarct size do not undergo cardiac remodeling [19]. The rats were housed in individual cages, in a temperature-controlled room (24 °C) with a 12-h light/dark cycle. Water was supplied *ad libitum*. The dietary intake was recorded daily. The rats were observed for 3 months, after which morphological, functional and biochemical analyses were performed.

2.1. Chow preparation

Fresh tomatoes were cooked for 5 min at 80 °C and placed in tap water. The tomato skin was removed manually and they were triturated and dried at 65 °C for 48 h. The lycopene content of the tomato powder was analyzed by high-performance liquid chromatography according to the method described by Riso and Porrini [20] and by Yeum et al. [21]. The lycopene concentration was 5.9 μg/mg of tomato powder. For each kilogram of standard chow, 4.2 g of tomato powder was added to supply the equivalent of 1 mg of lycopene/kg BW/day. This amount is equivalent to 700 mg of tomato/kg/day in humans. For this calculation, we used the following formula described by Reagan-Shaw et al. [22].

$$\text{Humanequivalentdoses} = \text{Animaldose}(\text{mg/kg})^{*}(\text{animalKm/humanKm}).$$

The Km factor, BW (kg) divided by BSA (m^2), is used to convert the mg/kg dose used in a study to an mg/m^2 dose (human Km of 60 kg: 37 and rat Km of 400 g: 7.7). Considering that our tomato had 95% of water, the result was 700 mg/kg/day [14].

2.2. Echocardiographic analysis

After 3 months, all the rats were weighed and evaluated by a transthoracic echocardiographic exam (General Electric Medical Systems, Vivid S6, Tirat Carmel, Israel). The following structural variables were measured: left atrium diameter, left ventricle (LV) diameter, diastolic and systolic dimensions, and LV diastolic posterior wall thickness. Systolic function was assessed based on the fractional area change (FAC), endocardial fractional shortening (FS) and posterior wall shortening velocity (PWSV).

Table 2
Isolated heart data

Variable	S group (n=8)	MI group (n=5)	MIL group (n=6)	MIT group (n=5)	P value
+dp/dt max (mm Hg/s)	2766±419	1200±326[a]	1479±339[a]	1042±246[a]	<.001
−dp/dt max (mm Hg/s)	2078±258	850±205[a]	1188±304[a,b]	750±177[a,c]	<.001
DP (mm Hg)	51.3±6.68	49.5±10.5	52.5±10.8	39.6±5.59	.054

+dp/dt max: maximum rate of ventricular pressure rise; −dp/dt max: decreased maximum rate of ventricular pressure rise; DP: developed pressure. Data are expressed as the mean±S.D. One-way ANOVA/Tukey.

[a] ≠ S group;
[b] ≠ MI group.
[c] ≠ MIL group.

Table 1
Morphological and functional data evaluated by echocardiography

Variable	S group (n=18)	MI group (n=13)	MIL group (n=16)	MIT group (n=15)	P value
BW (g)	432 (417–441)	445 (440–462)	459 (442–479)[a]	460 (418-477)	.039
HR (bpm)	261±29.7	271±25.2	276±37.2	261±25.5	.431
LA/BW (mm/kg)	12.2 (11.6–13.0)	14.5 (13.7–17.6)[a]	13.9 (12.5-17.6)[a]	14.2 (12.6-17.6)[a]	<.001
LVDD/BW (mm/kg)	18.3±1.33	22.5±1.99[a]	20.8±2.87[a]	22.1±2.46[a]	<.001
LVSD/BW (mm/kg)	9.39 (8.37–9.91)	17.2 (13.9–19.8)[a]	16.3 (15.2-17.9)[a]	16.5 (15.7-18.8)[a]	<.001
PWT (mm)	1.31 (1.29–1.37)	1.63 (1.47–1.71)[a]	1.64 (1.49-1.71)[a]	1.57 (1.50-1.71)[a]	<.001
SA (cm^2)	12.6 (11.0–15.7)	63.5 (58.6–80.6)[a]	55.4 (51.8-67.4)[a]	60.1 (52.4-73.1)[a]	<.001
DA (cm^2)	42.9 (40.4–49.8)	86.6 (73.4–106)[a]	77.7 (71.3-89.1)[a]	84.1 (73.7-91.6)[a]	<.001
FS (%)	49.2 (46.8–52.7)	20.4 (18.7–31.1)[a]	22.9 (20.2-24.2)[a]	21.8 (17.8-23.0)[a]	<.001
PWSV (mm/s)	36.9±3.97	24.9±6.96[a]	23.3±5.47[a]	22.9±7.30[a]	<.001
FAC	70.7±5.79	24.8±5.99[a]	26.6±9.51[a]	28.4±9.37[a]	<.001
IRT/$RR^{0.5}$ (ms)	53.9±5.61	64.5±12.3[a]	67.9±11.0[a]	60.4±11.8	.002
E/A	1.54 (1.46–1.71)	1.53 (1.23–1.79)	1.60 (1.30–3.14)	1.66 (1.30–3.52)	.807
EDT (ms)	47.8±8.35	47.6±9.61	50.7±8.50	47.8±8.35	.862

BW: body weight; HR: heart rate; LA: left atrium; LVDD: LV end-diastolic dimension; LVSD: LV end-systolic dimension; PWT: LV posterior wall thickness; SA: systolic area; DA: diastolic area; FS: endocardial fractional shortening; PWSV: posterior wall shortening velocity; FAC: fractional area change; IRT/$RR^{0.5}$: isovolumetric relaxation time adjusted by heart rate; E/A: peak velocity of early ventricular filling/peak velocity of transmitral flow during atrial contraction; EDT: E wave deceleration time. One-way ANOVA/Tukey. Data are expressed as the mean±S.D. or as the median (lower quartile–upper quartile).

[a] ≠ with S group.

Table 3
Morphometrical analysis

Variable	S group (n=17)	MI group (n=11)	MIL group (n=10)	MIT group (n=8)	P value
LV (g)	0.89 (0.79–1.06)	1.0 (0.91–1.27)	0.96 (0.91–1.03)	0.97 (0.89–1.09)	.592
RV (g)	0.23 (0.21–0.30)	0.33 (0.29–0.38)	0.28 (0.23–0.32)	0.23 (0.13–0.28)	.083
LV/BW	2.05 (1.87–2.34)	2.42 (2.00–2.82)	2.15 (1.98–2.25)	2.20 (1.87–2.34)	.691
RV/BW	0.59 (0.49–0.68)	0.73 (0.67–0.86)	0.63 (0.50–0.70)	0.59 (0.49–0.68) [b]	.050
CSA [a] (μm^2)	455±357	474±465	398±359	305±302 [b]	.008

RV: right ventricle; BW: body weight; LV: left ventricle; CSA: myocyte cross-sectional area. Data are expressed as the mean±S.D. or as the median (lower quartile–upper quartile). One-way ANOVA/Tukey.
[a] CSA analysis performed in six animals per group.
[b] ≠ MI group.

The E/A ratio, the isovolumetric relaxation time and the isovolumetric relaxation time corrected by the heart rate ($IRT/RR^{0.5}$) were used as indices of LV diastolic function [23].

2.3. In vitro left ventricular function analysis

After echocardiography analysis, five to eight animals in each group received sodium pentobarbital (50 mg/kg) and heparin (1000 UI) intraperitoneally, and underwent an isolated heart study by the Langendorff technique, following a previously described method [24]. The hearts that were used for the isolated heart study were not used for any other analysis, only for infarct size measurement, because retrograde perfusion can interfere with further biochemical analysis.

2.4. Morphometric analysis

On completion of the echocardiographic study, the right and left ventricles (including the interventricular septum) were dissected, separated and weighed. The myocyte cross-sectional area (CSA) was determined as previously described [25]. The lengths of the infarcted and the viable muscle for both the endocardial and epicardial circumferences were determined by planimetry. Infarct size was calculated by dividing the endocardial and epicardial circumferences of the infarcted area by the total epicardial and endocardial ventricular circumferences. The measurements were performed on ventricular sections (5–6 mm from the apex) under the assumption that the left mid-ventricular slice showed a close linear relationship with the sum of the area measurements from all of the heart sections [26].

2.5. Myocardial lipid hydroperoxide and antioxidant enzyme analysis

LV samples (100 mg) were homogenized in 5 ml of 0.1 M cold sodium phosphate buffer, pH 7.4, containing 1 mM ethylenediaminetetraacetic acid (EDTA). Tissue homogenates were prepared and total protein concentration, glutathione peroxidase (GSH-Px), SOD and CAT were assessed as previously specified [27]. All reagents were from Sigma (St. Louis, MO, USA).

2.6. Western blot analysis for Nrf-2, NF-κB, TNF-α, and type I and III collagen

Samples of LV were homogenized in RIPA buffer and diluted in Laemmli buffer, to detect type I collagen (rabbit polyclonal IgG, sc8784R; Santa Cruz Biotechnology, Inc., Dallas, TX, USA), type III collagen (mouse monoclonal IgG1, ab6310; Abcam, Inc.,

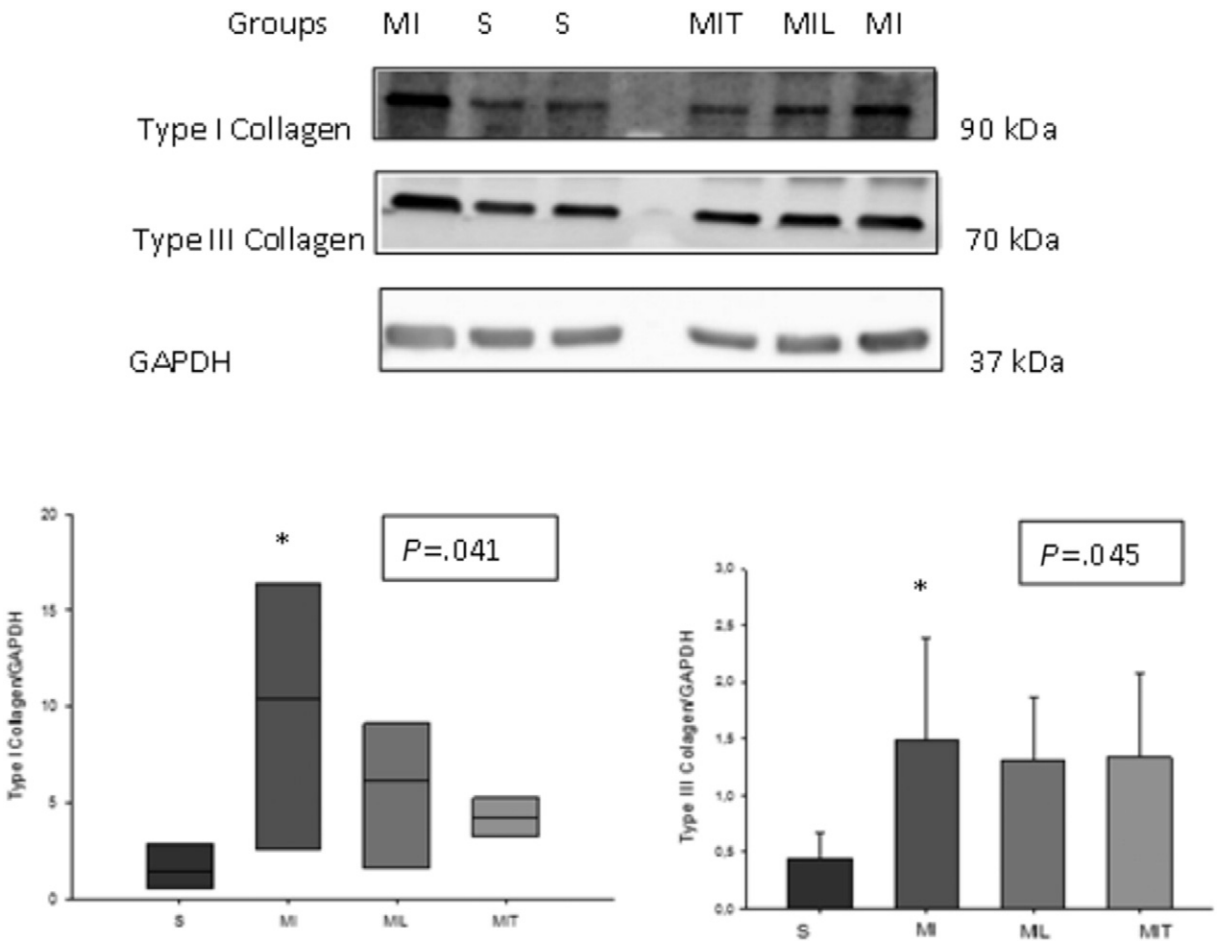

Fig. 1. Left ventricle collagen type I and III expression. (representative blots) *: ≠ S group. Groups: S (n=7), MI (n=7), MIL (n=7), MIT (n=7).

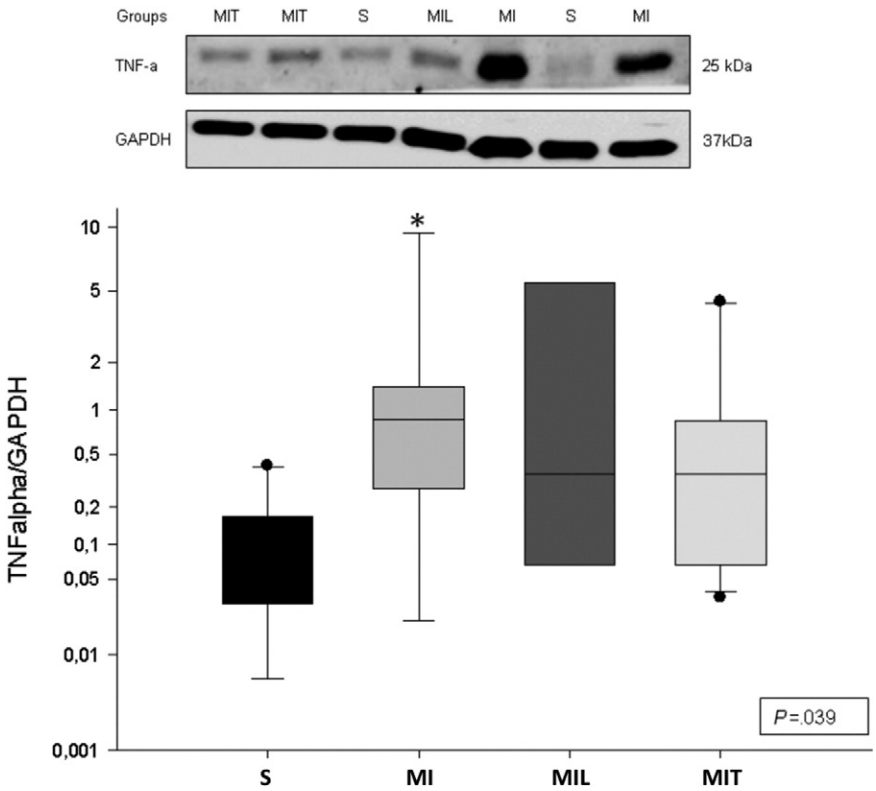

Fig. 2. Left ventricle TNF-α expression (representative blots). *: ≠ S group. Groups: S (n=11), MI (n=9), MIL (n=7), MIT (n=11).

Cambridge, UK), TNF-α [rabbit monoclonal IgG (Rodent Specific), 119,485; Cell Signaling Technology, Danvers, MA, USA), and total (mouse monoclonal IgG, sc8008) and phosphorylated NF-κB (pNF-κB; rabbit monoclonal IgG, sc3302; (Santa Cruz Biotechnology, Inc., Europe). Nuclear protein extraction from the LV was performed with the NE-PER Nuclear Extraction Reagents kit (Pierce Biotechnology, Rockford, IL, USA). Nuclear extracts were used to detect Nrf-2 (C-20, rabbit polyclonal IgG, sc722; Santa Cruz Biotechnology, Inc.). Secondary antibodies were used according to the manufacturer's recommendations, and GAPDH [GAPDH (6C5), mouse monoclonal IgG1, sc32233, Santa Cruz Biotechnology, Inc.] was used for normalization [27].

2.7. Left ventricular miRNA expression

Left ventricular miRNA expression was analyzed following a previously described method [14]. Six animals in each group were used for miRNA expression analyses. RNA was extracted from LV samples with the Recover All Total Nucleic Acid Isolation Kit (Ambion/Life Technologies, Carlsbad, CA, USA). The RNA quality and concentration were determined with a NanoDrop 8000 (Thermo Scientific, Waltham, MA, USA). Global miRNA expression analysis was determined using TaqMan Array Rodent MicroRNA Cards (A+B card sets v3.0; Life Technologies), according to the manufacturer's instructions. The miRNA data were generated following the MIQE guidelines [28]. The molecular targets (mRNAs) were derived from validated databases: miRwalk (http://zmf.umm.uni-heidelberg.de/apps/zmf/mirwalk2/miRpub.html) and filtered using the IDI tool (Integrated Database Interactions) (http://dcv.uhnres.utoronto.ca/iid/) in order to select mRNAs with interaction with cardiac tissue [29].

2.8. Statistical analysis

Data are expressed as the mean±S.D. or as the median (lower quartile–upper quartile). Comparisons among groups were performed using one-way analysis of variance (ANOVA) complemented with Tukey's test when variables had normal distribution or Kruskal–Wallis with Dunn posttest, when variables had nonnormal distribution. For mortality comparison, we used chi-square test. Data analysis was performed with SigmaStat for Windows v2.03 (SPSS Inc., Chicago, IL, USA). The significance level was 5%.

3. Results

There were no differences among the infarcted groups in the initial echocardiogram (data not shown).

There were no differences in global infarct sizes among the groups [MI (n=13): 44.8±9.4%; MIL (n=16): 38.1±6.1%; MIT (n=15): 41.6 ±7.7%; P=.070). In addition, there were no differences in infarct sizes considering each specific analysis.

Table 4
Left ventricle inflammation and oxidative stress

Variable	S group (n=8)	MI group (n=8)	MIL group (n=8)	MIT group (n=8)	P value
NF-κB (arbitrary units)	0.30 (0.12–1.04)	1.01 (0.87–4.35)	0.92 (0.28–2.16)	0.54 (0.34–2.03)	.369
pNF-κB (arbitrary units)	0.65 (0.37–0.91)	2.14 (1.37–3.73)	2.61 (0.75–3.78)	0.95 (0.70–3.80)	.107
NF-κB/pNF-κB (arbitrary units)	0.82±0.62	0.78±0.45	0.61±0.37	0.91±0.78	.763
LH (nmol/g)	223±20.8	298±19.5[a]	277±26.6[a]	261±28.8[a,b]	<.001
CAT (μmol/g)	69.3±8.92	40.8±9.65[a]	72.1±13.3[b]	86.8±18.2[a,b,c]	<.001
SOD (nmol/g)	16.0±2.81	24.5±3.24[a]	14.4±3.71[b]	12.8±1.01[b]	<.001
GSH-Px (nmol/g)	39.5±5.61	22.5±4.04[a]	31.7±4.88[a]	43.4±5.82[b,c]	<.001

Data are expressed as the mean±S.D. or as the median (lower quartile–upper quartile). pNF-κB: phosphorylated NF-κB; LH: lipid hydroperoxide; CAT: catalase; SOD: superoxide dismutase; GSH-Px: glutathione peroxidase. Data are expressed as the mean±S.D. One-way ANOVA/Tukey.

[a] ≠ S group;
[b] ≠ MI group;
[c] ≠ MIL group.

There was no difference in mean daily dietary intake (g) among the groups [S (n=18): 21.6 (21.2–22.1); MI (n=13): 21.6 (21.2–22); MIL (n=16): 21.2 (21.2–22.4); MIT (n=14): 21.7 (21.2–23.5) (P=.751)]. Nevertheless, BW was higher in the MIL compared to the S group (P=.039).

Regarding the mortality rate, there was no difference in mortality among groups (S=1, MI=3, MIL=3, MIT=2; P=.771).

Morphological and functional echocardiographic data are presented in Table 1. There was an increase in left heart chambers and in LV wall thickness induced by the coronary occlusion. The infarction caused a decrease in FS, FAC and PWSV and an increase in the IRT/$RR^{0.5}$ indicating systolic and diastolic dysfunction. However, the IRT/$RR^{0.5}$ was not different between the S and MIT groups.

Isolated heart analysis data are presented in Table 2. The MI group had lower values of both the maximum rate of ventricular pressure rise (+dp/dt max) and the maximum rate of ventricular pressure decrease (−dp/dt max). However, the MIL group had higher values of −dp/dt max compared to the MI and MIT groups.

Morphometric data are presented in Table 3. The MIT group had lower values of RV weight corrected by BW compared to the MI group. In addition, the CSA in the MIT group was lower than the MI group. Regarding collagen content, the MI group had a higher expression of type I and type III collagen than the other three groups (Fig. 1).

With regard to the inflammatory process, the MI group showed higher values of LV expression of TNF-α compared to the MIL and MIT groups (Fig. 2). However, there was no alteration in the expression of NF-κB, pNF-κB and NF-κB/pNF-κB ratio among the groups (Table 4).

The LV antioxidant enzyme activity and LH concentration are shown in Table 4. The oxidative stress marker, LH, was higher in the MI, MIL and MIT groups than in the S group; however, the MIT group had lower concentrations of LH than the MI group. Regarding CAT activity, the MI group showed lower activity compared to the S group. In addition, the MIL group showed higher CAT activity compared to the MI group, and the MIT group showed higher activity compared to the other three groups. In the MI group, SOD was higher when compared to the S group. However, animals that underwent MI and received diets supplemented with tomato or lycopene showed lower SOD activity than the MI group. The MI and MIL groups showed lower GSH-Px activity than the S group, and the MIT group had higher enzyme activity compared to the MI and MIL groups. Considering Nrf-2 expression, only the MIL group showed a significant reduction when compared to the S group (Fig. 3).

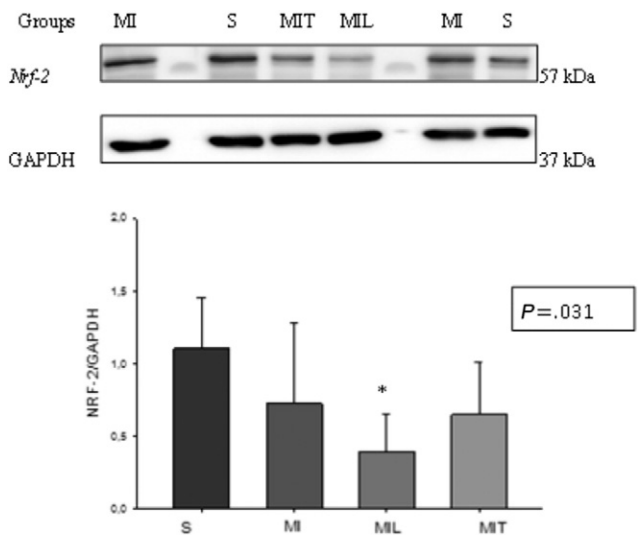

Fig. 3. Left ventricle *NRf-2* expression (representative blots). * ≠ S group. Groups: S (n=7), MI (n=7), MIL (n=7), MIT (n=7).

Observing LV miRNA expression, deregulated miRNAs are shown in detail in the Venn diagram for each group, specifying decreased expression (down-regulated, Fig. 4A) and increased expression (up-regulated, Fig. 5).

The changes of all miRNA expression compared to the S group are presented in Table 5. However, we were interested in miRNAs uniquely expressed in each group. When compared to the S group, the MIL group uniquely decreased the expression of eight miRNAs: hsa-miR-29c-3p, hsa-miR-194-5p, hsa-miR-503-5p, hsa-miR-20a-5p, hsa-miR-30a-5p, hsa-miR-192-5p, hsa-miR-30e-5p and hsa-miR-126-3p (Fig. 4A). These miRNAs regulate specific mRNA targets, and the results are presented in Fig. 4B. The MI group receiving tomato supplementation did not uniquely decrease any miRNA. Bioinformatics analysis showed that these miRNAs in the MIL group regulate the expression of the following mRNAs: IGF1R, SIRT1, IFRD1, LIMCH1, HDGF, CCND2 and HNRNPH1. We identified one miRNA with unique increased expression in the MI group: miR-214-3p (Fig. 5).

4. Discussion

The objective of this study was to evaluate the influence of tomato or lycopene supplementation on cardiac remodeling after acute MI. Our data showed that both tomato and lycopene supplementation attenuated the cardiac-remodeling process and improved diastolic dysfunction after MI. However, the effect of lycopene and tomato supplementation occurred through different mechanistic pathways, including cardiac inflammation, oxidative stress and expression of miRNAs.

It is important to note that in our study, tomato supplementation attenuated the increase in IRT/$RR^{0.5}$; this was observed in the MI and MIL groups and assessed by echocardiography. In addition, tomato supplementation reduced the CSA and RV/BW in infarcted animals. The reduction of hypertrophy could explain the diastolic improvement, reduction in pulmonary hypertension and RV hypertrophy observed in the MIT group. This confirms our previous study, in which we showed that tomato supplementation reduced CSA and improved diastolic function in the normal rat heart [14]. On the other hand, the lycopene supplementation attenuated the reduction of −dp/dt max evaluated by the isolated heart study. These differences observed between *in vivo* and *in vitro* studies could be related to neurohumoral factors that influence cardiac performance in studies *in vivo*. Importantly, tomato and lycopene supplementation attenuated the increase in both type I and type III collagen content in LV, and collagen tissue is an important modulator of diastolic function [6]. Thus, our data suggested that both tomato and lycopene supplementation attenuated cardiac remodeling, and improved diastolic dysfunction induced by MI. In agreement with our results, Wang et al. [30], in a study of infarcted rats that received saline or lycopene, found that treatment with lycopene 40 mg/kg/day for 28 days increased the ejection fraction, associated with decreased collagen fraction in the peri-infarct zone.

There are several mechanisms involved in the cardiac-remodeling process; among them, we can highlight inflammation. The cytokine TNF-α stimulates production of other inflammatory cytokines, promotes neutrophil activation, and increases endothelium thrombogenic capacity [31], and NF-κB is the main factor that stimulates the synthesis of TNF-α. Considering the effects of lycopene on inflammation, Hung et al [13]. showed in a study with human endothelial cells that lycopene administration reduced the activation of NF-κB and the expression of ICAM-1. In another study, He et al. [32] assessed the effects of lycopene on cardiac function and molecular mechanisms after MI in mice and observed improvement in cardiac function and remodeling with lycopene supplementation. The inhibition of inflammatory response by NF-κB and TNF-α suppression could be involved. In our work, lycopene or tomato supplementation attenuated the increased myocardial levels of TNF-α observed in the MI group.

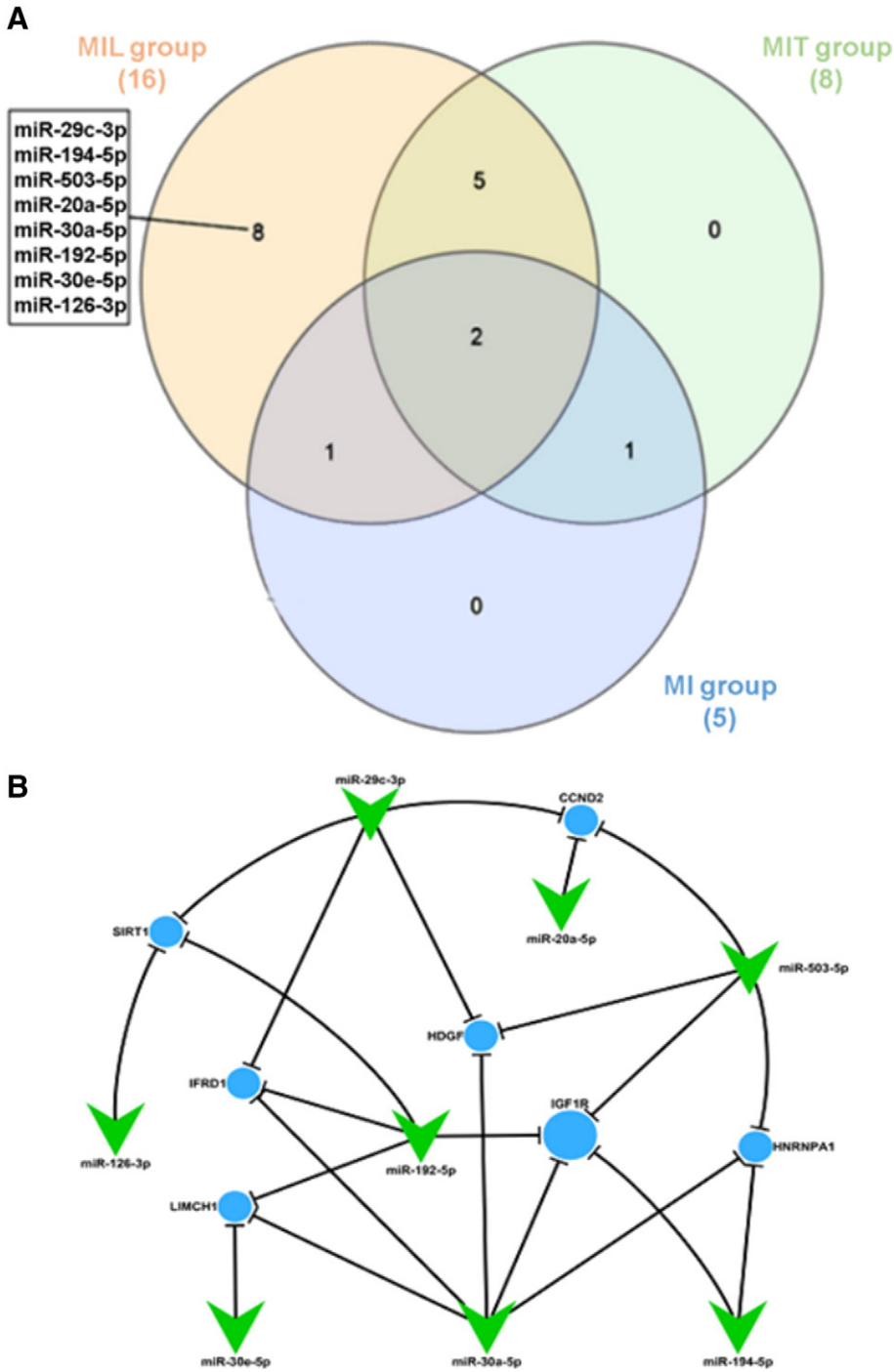

Fig. 4. (A) Venn diagram: down-regulated miRNAs expression of infarcted groups compared with S group. (B) MIL group: down-regulated miRNAs expression and specific targets. $P<.05$ ≠ S group.

However, the supplementation did not influence the total and phosphorylated NF-κB expression. Therefore, our results suggest that tomatoes or lycopene supplementation can reduce the inflammatory process after MI, independently of NF-κB. These discrepancies observed in lycopene supplementation among different studies could be explained, at least in part, due to different doses and time of lycopene supplementation.

Another critical mechanism involved in the cardiac-remodeling process is oxidative stress. It is important to emphasize that Nrf-2 is one of the most important regulators of antioxidant enzymes production. The main effect of lycopene and tomato supplementation is the reduction of oxidative stress, and besides being a potent scavenger of singlet oxygen and peroxyl radicals, lycopene modulates the production of antioxidant enzymes through the Nrf-2 pathway. Indeed, Tong et al. [10] investigated the effects of acute lycopene administration on oxidative stress in both *in vitro* and *in vivo* ischemia–reperfusion injury models. The authors showed that lycopene treatment before reperfusion reduced cardiomyocyte death, ROS production and JNK phosphorylation in the cardiac tissue. Similarly, Ferreira et al. [33] observed a reduction in DNA oxidative

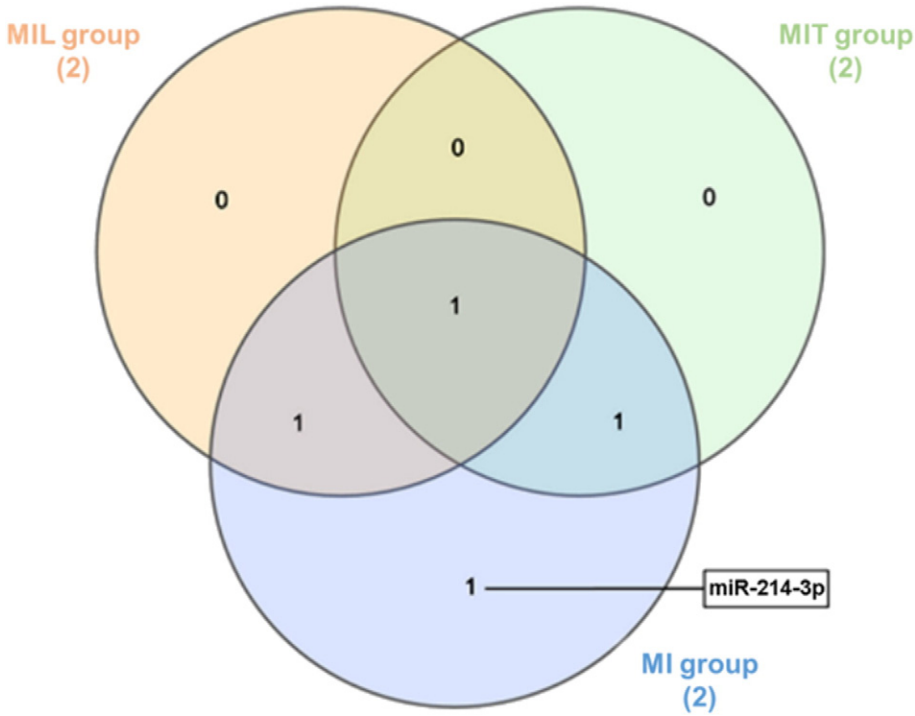

Fig. 5. Venn diagram: up-regulated miRNAs expression of infarcted groups compared with S group.

damage after lycopene supplementation in a model of acute cardiotoxicity induced by doxorubicin. In the present study, the MIT group showed lower cardiac levels of LH compared to the MI group. Thus, tomato supplementation attenuated oxidative stress in our study. This attenuation could be explained, in part, by alterations in antioxidant enzyme activity. In addition, Ojha et al. [34] evaluated the cardioprotective potential of lycopene against isoproterenol-induced MI. They showed that concurrent administration of lycopene and isoproterenol for 30 days inhibited lipid peroxidation and malondialdehyde formation in the heart. Interestingly, in our study, although the group supplemented with lycopene presented the same alterations in antioxidant enzymes in comparison with tomato, the supplementation did not change LH concentration.

Despite the changes in oxidative stress, found mainly in the group supplemented with tomato, we observed a decrease in myocardial expression of Nrf-2 in the MIL group, suggesting that this transcriptional factor did not influence the effects of lycopene and tomato supplementation in oxidative stress in this model. We observed the same features in a previous study, with tomato supplementation in the normal rat heart [14]. However, we cannot exclude Nrf-2 participation during the follow-up period.

We also evaluated the expression of miRNAs in infarcted rats supplemented with tomato or lycopene and observed that these substances induced different expression of miRNAs in the heart. MicroRNAs are small, noncoding RNA molecules that act as posttranscriptional regulators of gene expression. Several studies have already shown the involvement of miRNAs in certain cardiovascular diseases, and we have shown that tomato supplementation up-regulated the expression of miR-107 and miR-486 and down-regulated the expression of miR-350 and miR-872 in the normal rat heart [14].

In this study, to identify differences between lycopene or tomato supplementation, we were interested in miRNAs which were uniquely expressed in each group. Thus, regarding the two groups in which the diet was supplemented with tomato or lycopene, only the MIL group uniquely down-regulated eight miRNAs. Bioinformatics analysis showed that IGF-1R and SIRT-1 mRNAs are regulated by these miRNAs [35], and that cultures of human cardiac fibroblasts treated with IGF-1 display a selective up-regulation in elastin gene expression and a consequent increase in elastic fiber production through the cross-activation of the IGF-1R. The protein SIRT1 is implicated in the regulation of cardiomyocyte growth and survival under stress conditions [36] and SIRT-1 depletion aggravates ischemia/reperfusion-induced oxidative injury [37]. Thus, in our study, IGF-1R and SIRT-1 mRNA could have their expression increased, and this could explain some of our results.

Finally, lycopene makes up 90% of the total carotenoids and other phytochemicals present in tomatoes. However, the participation of other carotenoids in the effects from tomato supplementation cannot be ruled out. Moreover, the synergy among phytochemicals indicates that tomato may be more important than lycopene in reducing

Table 5
Up-regulated and down-regulated miRNA expression among groups compared with S group

	Down-regulated miRNAs	Up-regulated miRNAs
MI group	hsa-miR-208a-3p	hsa-miR-224-5p, hsa-miR-214-3p, hsa-miR-511-5p, hsa-miR-31-5p
MIL group	hsa-miR-208a-3p, hsa-miR-29c-3p, hsa-miR-194-5p, hsa-miR-503-5p, hsa-miR-20a-5p, hsa-miR-30a-5p, hsa-miR-30d-5p, hsa-miR192-5p, hsa-miR-106a-5p, hsa-miR-200c-3p, hsa-miR-30e-5p, hsa-miR-126-3p, hsa-miR-126-5p, hsa-miR-190a-5p	hsa-miR-224-5p, hsa-miR-511-5p
MIT group	hsa-miR-208a-3p, hsa-miR-30d-5p, hsa-miR-106a-5p, hsa-miR-200c-3p, hsa-miR-126-5p, hsa-miR-190a-5p	hsa-miR-224-5p, hsa-miR-31-5p

oxidative stress. Therefore, we suggest that it would be more helpful to prioritize the selection of food and eating habits in general, rather than the consumption of individual nutrients or specific dietary factors [38]. In addition, it is interesting to highlight that 700 of tomato/kg/day in humans is a relatively small portion, which could be easily introduced in the diet.

We also have to consider that serum or cardiac levels of lycopene were not measured in this study. Thus, we cannot exclude that differences in lycopene bioavailability between rats supplemented with tomato or lycopene could have influenced our results.

In conclusion, tomato or lycopene supplementation attenuated the cardiac remodeling process and improved diastolic function after MI. However, the effect of lycopene and tomato supplementation occurred through different mechanistic pathways, including cardiac inflammation, oxidative stress and the expression of miRNAs. In addition, our data suggest that we must prioritize the selection of foods instead of single nutrients.

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The funding source had no involvement in study design, in the collection, analysis and interpretation of data, in the writing of the report, and in the decision to submit the article for publication.

Conflicts of interest

None.

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