Journal of Functional Foods 85 (2021) 104656

Contents lists available at ScienceDirect

Journal of Functional Foods

journal homepage: www.elsevier.com/locate/jff

The antihypertensive and antihypertrophic effect of lycopene is not affected by and is independent of age

Pedro Ferreira-Santos [a,c], Rosalía Carrón [a,b], M. José Montero [a,b], M. Ángeles Sevilla [a,b,*]

[a] Department of Physiology and Pharmacology, Faculty of Pharmacy, University of Salamanca, 37007 Salamanca, Spain
[b] Biomedical Research Institute of Salamanca (IBSAL), Hospital Virgen de la Vega, 37007 Salamanca, Spain
[c] Centre of Biological Engineering, University of Minho, 4710-057 Braga, Portugal

ARTICLE INFO

Keywords:
Lycopene
Aging
SHR
Cardiovascular
Oxidative stress

ABSTRACT

Hypertension of SHR appears at an early age and progressively increases. This study aimed to evaluate the effect of lycopene in SHR with mild hypertension (young rats) or with high level of hypertension (adult rats). Four-weeks treatment with 10 mg/kg/day of lycopene progressively decreased blood pressure in young (from 144 ± 2 to 119 ± 3 mmHg) and adult (from 177 ± 5 to 159 ± 7 mmHg) SHR. Heart and renal hypertrophy and fibrosis were increased in adult compared to young SHR and lycopene, regardless of the age of the rats, improved the injury in both organs. Although aging induced morphological and functional alterations in the aorta, the treatment was only effective in preventing the former. Lycopene helped to improve all the parameters linked to oxidative stress determined in this study. In conclusion, lycopene treatment improved the age-associated harmful changes in hypertension, cardiovascular and renal remodelling, and indicators of oxidant-antioxidant systems in both young and adult SHR.

1. Introduction

Aging is a progressive and continual natural process that results in decreased physiologic functions across all organ systems. These physiological changes result in development in chronic and inflammatory diseases that dramatically increase the risk of mortality (Buford, 2016). Hypertension associated with aging is recognized as major risk factor for the development of cardiovascular diseases (CVD) (World Health Organization, 2017). This pathological state is a multifactorial, complex disorder involving various organ systems (Silva et al., 2017). In general, the elevation of blood pressure leads to vascular dysfunction, increased arterial stiffness, cardiac and renal alterations as well as changes in the redox state (Montezano et al., 2015; Zhou et al., 2017). Hypertension is a global public health problem therefore, prevent or delay its development as well as find an effective treatment is very important. In this sense, antihypertensive therapy has a relevant role, resulting in reduction in the incidence of other associated CVD pathologies, such as stroke, myocardial infarction and heart failure, among others (Touyz et al., 2018). Recent studies have been reporting the action of new antihypertensive agents, demonstrating the effectiveness at the level of the cardiovascular system (Llorens-Cortes & Touyz, 2020; Sánchez-Rivera et al., 2016).

Non-pharmacological options play a central role in the treatment of hypertension. Several epidemiologic and prospective studies have provided convincing evidence that the Mediterranean diet (rich in fruits and vegetables, limiting the intake of red meats and using a healthy source of fat) results in a lower risk of developing CVD (Babio et al., 2014; Ferreira-Santos et al., 2018). In this regard, dietary intake of lycopene, a carotenoid compound found in large quantities in various fruits and vegetables included in the Mediterranean diet, has received considerable scientific interest due to its high antioxidant capacity (Anlar & Bacanli, 2020; Arain, Zhuang, Hassan, & Saeed, 2018; Müller, Caris-Veyrat, Lowe, & Böhm, 2016). Lycopene supplementation shown effectiveness for prevention or treatment of certain chronic pathologies, such as hypertension, cancer, diabetes, dyslipidemia, etc. (Ferreira-Santos et al., 2018; Friedman, 2013; Jiang, Guo, & Hai, 2016;

Abbreviations: ACh, acetylcholine; BW, body weight; CVD, cardiovascular diseases; GSH-PX, glutathione peroxidase; LVH, left ventricular hypertrophy; MDA, malondialehyde; NADPH, nicotinamide adenosine dinucleotide phosphate; $O_2^{\cdot-}$, superoxide anion; PE, phenylephrine; RLU, relative luminescence units; ROS, reactive oxygen species; SBP, systolic blood pressure; SEM, standard error of the mean; SHR, spontaneous hypertensive rats; SNP, sodium nitroprusside; SOD, superoxide dismutase; TBARS, thiobarbituric acid reactive substances.

* Corresponding author at: Department of Physiology and Pharmacology, Faculty of Pharmacy, University of Salamanca, 37007 Salamanca, Spain.
E-mail address: masevilla@usal.es (M.Á. Sevilla).

https://doi.org/10.1016/j.jff.2021.104656
Received 23 February 2021; Received in revised form 25 June 2021; Accepted 25 July 2021
Available online 1 August 2021

Roohbakhsh, Karimi, & Iranshahi, 2017; Thies, Mills, Moir, & Masson, 2017). Many studies have shown that lycopene exhibits cardiovascular effects in humans and animals and provides a protection against damage caused by reactive oxygen species (ROS), regulating redox status and antioxidant enzyme activity and improving the plasma lipid profile (Bose & Agrawal, 2007; Chen, Song, & Zhang, 2013; Ferreira-Santos et al., 2018; Mordente et al., 2011; Paran, Novack, Engelhard, & Hazan-Halevy, 2009).

Given the important role of oxidative stress in the pathogenesis of many clinical conditions and aging, antioxidant therapy could be interesting to improve the health of the population. In this way, our research group has been developing studies of drugs and dietary modifications (intake of antioxidants and bioactive peptides), for the treatment and prevention of CVD, such as hypertension, dyslipidemia and diabetes.

This research work was carried out in young and adult SHR rats in order to verify that the cardiovascular alterations caused by hypertension worsened with age. In this context, our objective was to study whether both the antihypertensive effect of lycopene and its benefits on cardiovascular disorders caused by high blood pressure were different depending on the severity of these damages caused by aging.

2. Materials and methods

2.1. Experimental protocol

All the experiments were performed according to the European Union guidelines for the ethical care and use of laboratory animals and the protocol was approved by the Bioethics Committee of University of Salamanca (Register N°: 006N°201400039292). Twenty-eight male SHR (Janvier Labs, Le Genest Saint Isle, France), half 7-weeks old and the other fourteen 21 weeks old, were housed in boxes of 3–4 rats and maintained at a temperature of 23 °C with 12 h light/dark cycles. Rats were fed on a solid standard diet (Global Diet 2014, Harlan Laboratories, Inc., Indianapolis, IN, USA) and tap water with *ad libitum* intake.

After two weeks of adaptation, baseline blood pressure measurements were performed. The systolic blood pressure (SBP) in conscious rats was measured by a CODA tail-cuff blood pressure system (Kent Scientific, Torrington, CT, USA), using the same methodology previously reported by us (Ferreira-Santos et al., 2018).

Thereafter, young SHR (with mild hypertension) and adult SHR (with high level of hypertension) were distributed in the following groups of seven animals each. Non-treated groups (SHR): **1)** young (9 weeks) and **2)** adult (21 weeks) rats received tap water.

Lycopene treated groups (SHR-LYC): **1)** young (9 weeks) and **2)** adult (21 weeks) rats received lycopene dissolved in drinking water.

Lycopene concentration was adjusted to daily water consumption to achieve a correct dosage of 10 mg/kg/day. This dose of lycopene was previously used in other studies of our group, showing effects on the cardiovascular system (Ferreira-Santos, Aparicio, Carrón, Montero, & Sevilla, 2020; Ferreira-Santos et al., 2018).

The BW and SBP were monitored weekly in all groups during the experimental period (4 weeks). After this time, animals were anaesthetized with sodium pentobarbital (60 mg/kg BW, i.p.). Blood samples were collected, centrifuged, and the plasma was stored at –80 °C until use. Heart, kidney, liver and thoracic aorta were immediately harvested, placed in chilled Krebs buffer (composition in mM: NaCl, 118; KCl, 4.7; $CaCl_2$, 2.5; KH_2PO_4, 1.2; $MgSO_4$, 1.2; $NaHCO_3$, 25 and glucose, 11, pH = 7.4) and appropriately processed for further studies.

2.2. Hypertrophy and fibrosis

After sacrifice, the heart, kidney and aorta were removed and placed immediately in Krebs solution, gassed with carbogen (5% CO_2, 95% O_2) and kept at 37 °C to remove excess blood. For the determination of left ventricular hypertrophy (LVH) index, the atria were removed from the heart and all the epicardial fat was scraped off, the right and left ventricles were separated, regarding the interventricular septum as an integral part of the left ventricle, and weighed. To determine the renal hypertrophy (RH) index, the left kidney was properly dried with filter paper to remove excess Krebs solution and all fat was removed. Afterwards the kidney was weighed. Both, ventricular and renal hypertrophy were calculated as an index using organ weight/BW ratio.

Sections of ventricle, kidney and a portion of the aorta were fixed in 10% formaldehyde and then processed for paraffin embedding. Slices of 5-μm were stained with Sirius-red collagen-specific stain or hematoxylin-eosin and examined under light microscopy. At least eight areas from each preparation were captured using a high-resolution digital camera with attached video camera (Olimpus BX50 and DP50, respectively, Tokyo, Japan).

Adobe Photoshop® CS3 software was used to quantified the collagen in histologic sections, and the percentage of cardiac and renal interstitial fibrosis was determined for each image as the ratio of the collagen surface area to the total area (5–7 fields for each section were measured).

Estimation of cardiac and renal collagen was also performed by measuring the content of hydroxyproline using a spectrophotometric method reported elsewhere (Sauzeau et al., 2006). Total collagen was calculated assuming that collagen contains 12.7% hydroxyproline.

Morphometric parameters of arteries were determined using aortic slices, the internal and external perimeters of the medial layer were measured using the ImageJ software, and internal and external radii (R_i and R_e respectively) calculated according to the formula: perimeter = $2\pi R$, where $2R_i$ is the internal diameter (L) and $R_e - R_i$ is the medial thickness (W_m). The medial cross-sectional area (CSA_m) was calculated as: $CSA_m = \pi(R_e^2 - R_i^2)$.

2.3. Vascular reactivity in aorta

Vascular reactivity was performed in isolated aorta rings (3 mm in length) using a methodology reported previously by our group (Ferreira-Santos et al., 2018). Rings were placed in organ bath with Krebs solution at 37 °C aerated with carbogen. The preparations were precontracted with phenylephrine (PE, 10^{-6} M) and at the steady maximal contraction, cumulative concentration–response curves were obtained for acetylcholine (ACh, 10^{-8}–10^{-4} M) or sodium nitroprusside (SNP, 10^{-8}–10^{-5} M).

Each curve was obtained in different rings, and responses to ACh and SNP were expressed as percentage of PE contraction.

2.4. Oxidative stress parameters

2.4.1. Detection of superoxide anion

The superoxide anion ($O_2^{\bullet -}$) produced by NADPH-oxidases is the main ROS in vessels. $O_2^{\bullet -}$ production was assessed by lucigenin-enhanced chemiluminescence assay in segments of thoracic aorta stimulated by nicotinamide adenosine dinucleotide phosphate (NADPH, 10^{-4} M) using a luminometer (LUMAT LB-9507, Berthold Technologies, Bad Wildbad, Germany). The results of $O_2^{\bullet -}$ production were expressed as relative luminescence units (RLU)/min/mg dry tissue.

2.4.2. Lipid peroxidation determination

The formation of products of lipid peroxidation was quantified in plasma using the thiobarbituric acid-reactive substances (TBARS) method, as reported previously by us (Ferreira-Santos et al., 2018) and results were expressed as malondialdehyde (MDA) concentration (nmol/mL).

2.4.3. Antioxidant enzymes

The activity of endogenous antioxidant enzymes (superoxide dismutase (SOD) and glutathione peroxidase (GSH-PX)) was assessed in liver homogenates previously centrifuged (1200 rpm for 30 min at 4 °C)

to remove crude fractions. The supernatants were used to carry out the determination of enzymatic activity by spectrophotometric methods using commercially available assay kits (Sigma-Aldrich, USA). Total proteins were measured by the Bradford method, and results of enzymatic activity were reported as U/mg protein.

2.5. Statistical analysis

Values are expressed as the mean ± standard error of the mean (SEM). GraphPad Prism® software (version 5.0; GraphPad Software, Inc., San Diego, CA, USA) was used for calculations, fitting and statistical analysis. Comparison of concentration–response curves of ACh and SNP were performed according to the extra sum of squares F-test principle. The level of statistical significance was determined by one-way analysis of variance (ANOVA) followed by Bonferroni's test for multiple comparisons and two-way ANOVA for blood pressure data. Significance was accepted at $P < 0.05$.

2.6. Chemical compounds

Lycopene (Redivivo®, 10% CWS/S-TG, DSM Nutritional Products, USA). Acetylcholine chloride (PubChem CID: 6060); phenylephrine hydrochloride (PubChem CID: 5284443); Sirius-red (PubChem CID: 5464587); sodium nitroprusside (PubChem CID: 11953895); thiobarbituric acid (PubChem CID: 2723628), trichloroacetic acid (PubChem CID: 6421), nicotinamide adenosine dinucleotide phosphate (PubChem CID: 5884) and lucigenin (PubChem CID: 65099) were purchased from Sigma-Aldrich (St. Louis, MO, USA). All other chemicals were analytical grade. Stock solutions were prepared in ultrapure water, stored at −20 °C and appropriate dilutions were made on the day of the experiments.

3. Results

3.1. Systolic blood pressure

Initially, young rats had a mild hypertension state with SBP values around 140 mmHg, while adult animals had SBP values above 170 mmHg, ie, established hypertension. A slight increase in these values throughout the study was observed in both groups of non-treated rats.

As shown in Fig. 1A, the treatment with lycopene progressively decreased SBP in young SHR (from 144 ± 2 mmHg to 119 ± 3 mmHg at the end of treatment, $P < 0.001$) and in adult SHR (from 177 ± 5 mmHg to 159 ± 7 mmHg at the end of treatment, $P < 0.01$).

At the end of treatment, BW was similar between treated and non-treated groups for young and adult SHR, respectively (Fig. 1B).

3.2. Hypertrophy and fibrosis

3.2.1. Heart

LVH index in adult SHR was shown to be significantly higher compared to young rats (2.71 ± 0.03 mg/g BW *vs.* 2.55 ± 0.01 mg/g BW, respectively, $P < 0.05$) (Fig. 2A).

According to SBP and LVH results, hypertension associated with aging leads to a significant increase in collagen deposition, quantified by interstitial fibrosis stained with Sirius red in histological slices (Fig. 2B and D) and the content of hydroxyproline present in the left ventricle homogenates (Fig. 2C). The lycopene supplement was effective in reducing hypertrophy and fibrosis.

3.2.2. Kidney

RH index in adult rats was shown to be significantly higher compared to young rats (3.31 ± 0.05 mg/g BW *vs.* 3.13 ± 0.06 mg/g BW, respectively, $P < 0.05$) (Fig. 3A). Also the collagen deposition was increased as can be seen in interstitial fibrosis quantified in the histological sections of the kidney as percentage (Fig. 3B and D) or in kidney homogenates as hydroxyproline content (Fig. 3C). The lycopene treatment showed a significant reduction of renal hypertrophy and was able to decrease the collagen deposition.

3.2.3. Aorta

According to the other factors studied, the morphology of the aorta was also altered with the age. In Table 1 and Fig. 4 it is possible to observe a significant increase in all parameters studied (L, W_m, CSA_m and W_m/L) in adult animals. The treatment with lycopene significantly prevented the morphometric alterations in the thoracic aorta, decreasing the W_m, CSA_m and W_m/L in relation to the respective young and adult SHR control group. Lycopene treatment increased the aorta lumen in young rats, but no changes were observed in adult rats.

3.3. Vascular reactivity

As shown in Fig. 5A and B, the aging promotes vascular dysfunction manifested in the reduction of endothelial-dependent vasorelaxation to ACh (E_{max}, 80.8 ± 1.6 % for young rats *vs.* 52.7 ± 1.6 % for adult rats, $P < 0.001$) and endothelium-independent relaxation induced by SNP (E_{max}, 94.5 ± 2.0 % for young rats *vs.* 82.6 ± 2.6 % for adult rats, $P < 0.01$) on aortic rings of SHR. Treatment with lycopene was not able to improve the impairment of relaxation responses caused by aging.

Moreover, the contractile response to PE was similar in the two

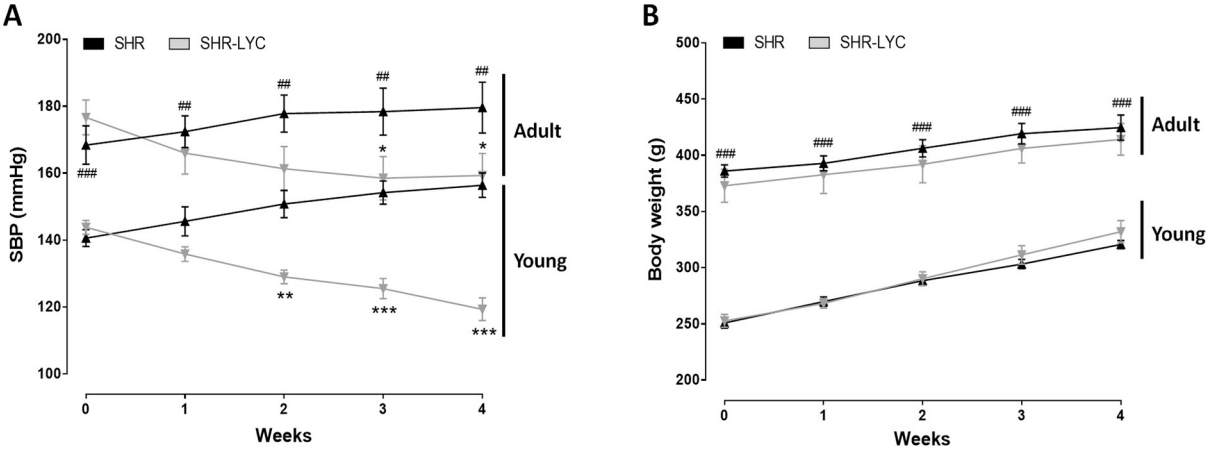

Fig. 1. Systolic blood pressure (SBP) (**A**), and body weight (**B**) in young and adult spontaneously hypertensive rats (SHR) and SHR treated with 10 mg/kg/day of lycopene (SHR-LYC). Values are expressed as mean ± SEM (n = 7). $^{\#\#}P < 0.01$ and $^{\#\#\#}P < 0.001$ *vs.* young SHR; *P < 0. 05, **P < 0. 01 and ***P < 0.001 *vs.* the corresponding SHR without treatment.

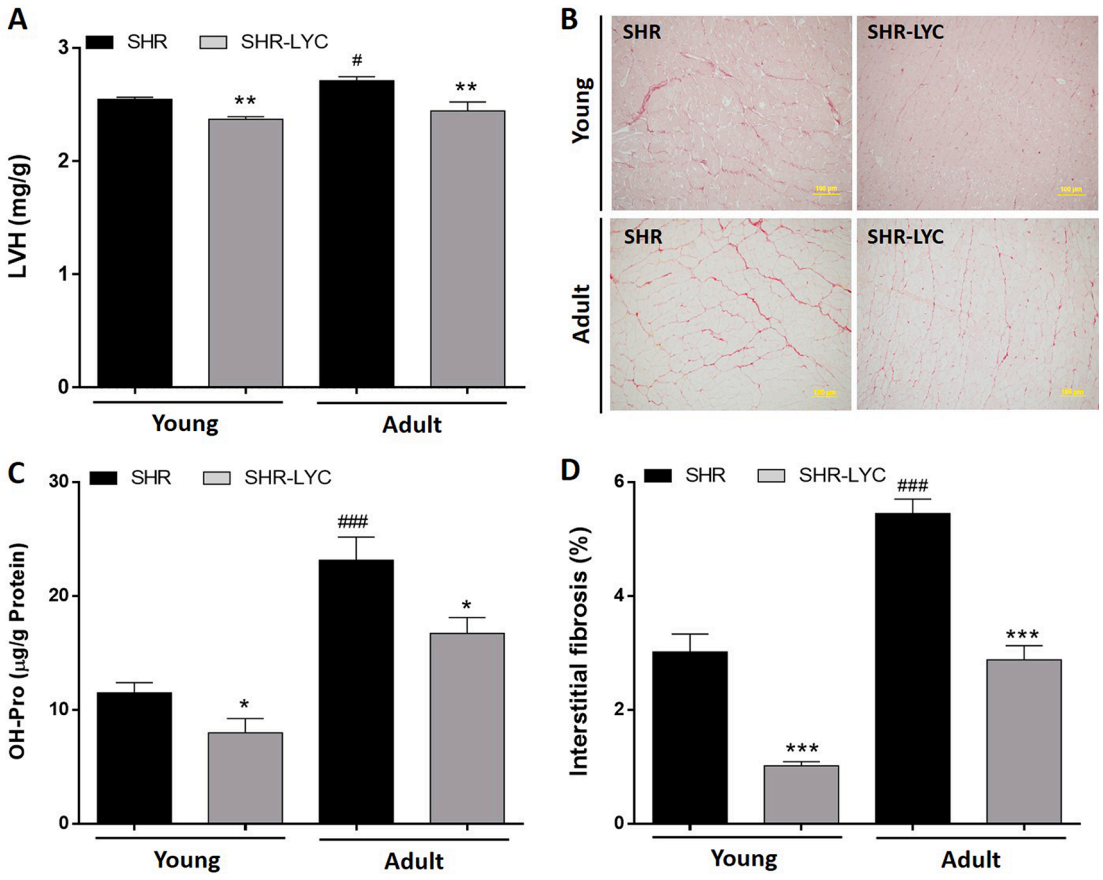

Fig. 2. Left ventricular hypertrophy (LVH) (**A**), left ventricle sections stained with Sirius-red (magnification x200) (**B**), cardiac hydroxyproline content (OH-Pro) (**C**), and cardiac interstitial fibrosis quantification (**D**) in young and adult spontaneously hypertensive rats (SHR) and SHR treated with 10 mg/kg/day of lycopene (SHR-LYC). Values are expressed as mean ± SEM (n = 7). $^{\#}P < 0.05$ and $^{\#\#\#}P < 0.001$ *vs.* young SHR; *P < 0. 05, **P < 0. 01 and ***P < 0.001 *vs.* the corresponding SHR without treatment.

groups (young and adult rats), and the intake of lycopene during the 4 weeks did not significantly modify any response (data not shown).

3.4. Parameters of oxidative stress

$O_2^{\bullet -}$ production stimulated by NADPH was higher in aortic rings of adult SHR control group compared to young animals (5723 ± 523 RLU/min/mg *vs.* 2543 ± 232 RLU/min/mg, respectively, P < 0.001). Chronic treatment with lycopene decreased $O_2^{\bullet -}$ production. This effect was more visible in adult animals, where ROS production was higher than in younger animals (Fig. 6A).

Fig. 6B shows that adult SHR rats had a significant elevation of MDA content compared to the young SHR group (4.6 ± 0.1 nmol/mL *vs.* 3.9 ± 0.2 nmol/mL, P < 0.05). Moreover, lycopene significantly attenuated the plasmatic MDA levels in both groups of animals (3.8 ± 0.1 nmol/mL for adult and 3.2 ± 0.1 nmol/mL for young rats).

3.5. Activity of antioxidant enzymes

The enzymatic activities of SOD and GSH-PX in the liver homogenates were determined. As shown in Table 2, untreated SHR animals have similar values of antioxidant enzymes activity. Treatment with lycopene in young and adult SHR rats increased significantly the activity of SOD and GSH-PX.

4. Discussion

This study examines, for the first time, the effect of the lycopene on the cardiac, vascular and renal alterations using the SHR model and comparing two states of the hypertension development associated to the aging.

Aging is a primary risk factor for many diseases and in particular for CVD and its derived morbidity and mortality (Izzo et al., 2021). Oxidative stress is involved in the development of many degenerative and chronic age-related disorders: CVD, chronic kidney disease, neurodegenerative diseases, cancer, etc. ROS play a physiological role in the cardiovascular system, controlling endothelial function, vascular tone, and cardiac function, and a pathophysiological role in inflammation, hypertrophy, proliferation, apoptosis, migration, fibrosis, angiogenesis, and rarefaction, all of which are important processes contributing to endothelial dysfunction and cardiovascular remodeling in hypertension and other CVD (Montezano & Touyz, 2012).

Among other complications, hypertension can cause serious damage to the heart, manifested by ventricular hypertrophy, enlargement of cardiomyocytes and collagen deposition. In addition, hypertensive process can cause kidney damage and endothelial dysfunction, directly linked to the production of ROS, leading to so-called oxidative stress (Silva et al., 2017; World Health Organization, 2017).

Different rat genetic models of hypertension have been used in genetic, (patho)physiological and pharmacological studies, but the most commonly studied is the SHR (Lerman, Kurtz, Touyz, Ellison, Chade, Crowley, & Coffman, 2019). In this strain the hypertension appears early and progressively increases over time. Ours results confirm that age in SHR was associated with elevation of SBP, young rats had blood pressure values coincident with mild hypertension while adults were clearly hypertensive, and they are in agreement with other studies (Berenyiova, Drobna, Cebova, Kristek, & Cacanyiova, 2018).

After a four-week of the lycopene supplemented diet, we observed an

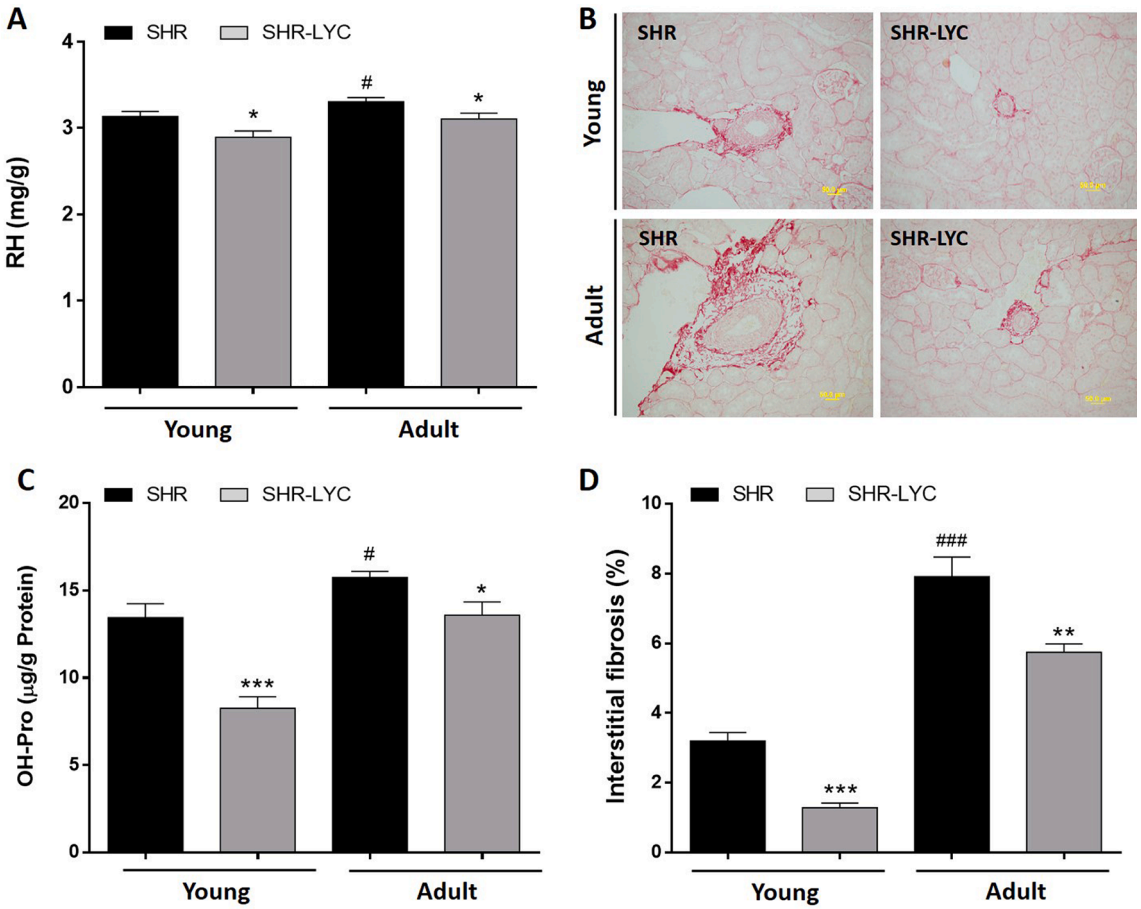

Fig. 3. Renal hypertrophy (RH) (**A**), kidney sections stained with Sirius-red (magnification x200) (**B**), renal hydroxyproline content (OH-Pro) (**C**), and renal interstitial fibrosis quantification (**D**) in young and adult spontaneously hypertensive rats (SHR) and SHR treated with 10 mg/kg/day of lycopene (SHR-LYC). Values are expressed as mean ± SEM (n = 7). $^{\#}P < 0.05$ and $^{\#\#\#}P < 0.001$ *vs.* young SHR, *P < 0. 05, **P < 0. 01 and ***P < 0.001 *vs.* the corresponding SHR without treatment.

Table 1
Effects of lycopene on thoracic aortic wall geometry.

	Young		Adult	
Parameters	SHR	SHR-LYC	SHR	SHR-LYC
L (µm)	1427.8 ± 7.6	1486.8 ± 9.7**	1587.5 ± 23.8###	1560.0 ± 18.4
W_m (µm)	134.0 ± 1.8	115.8 ± 2.2***	151.5 ± 1.0###	133.2 ± 3.5***
CSA_m (mm^2)	0.66 ± 0.01	0.58 ± 0.02**	0.83 ± 0.01###	0.71 ± 0.03**
W_m/L	0.09 ± 0.00	0.08 ± 0.00***	0.10 ± 0.00###	0.09 ± 0.00***

Internal diameter (L), medial wall thickness (W_m), and medial cross-sectional area (CSA_m). Values are expressed as mean ± SEM of 7 rats.
P < 0.001 *vs.* young SHR, ** P < 0.01 and *** P < 0.001 *vs.* the corresponding SHR without treatment.

effective reduction on SBP in both young and adult rats. This effect was more pronounced in young animals, reaching normal values of blood pressure. We have previously reported that lycopene plays an important role in regulating blood pressure in other animal models. On the one hand it showed antihypertensive action, but not hypotensive effects in Wistar rats infused with angiotensin II and on the other hand prevented the increase in blood pressure in the metabolic syndrome (Ferreira-Santos et al., 2020, 2018). Human studies also show the action of lycopene at the cardiovascular level, highlighting the advantages of its consumption and no toxic effects (Cheng et al., 2017; Ried & Fakler, 2011). All of these results could support the usefulness of lycopene in the prevention of hypertension.

Chronic hypertension and aging are typically associated with organs hypertrophy and remodeling, in addition to increasing the incidence of inflammatory processes. Our results of morphometric and histological measures show that cardiac, renal and vascular hypertrophy and fibrosis was higher in adult SHR and these parameters were decreased, in both young and adult SHR, after 4 weeks of treatment with lycopene. Wang and co-workers (Wang et al., 2014) indicated that lycopene improved the cardiac function and ventricular remodeling.

It is important to note that the oxidative stress has a negative effect in vascular structure and function. Moreover, several experimental studies revealed that the aorta presents vascular remodeling and endothelial dysfunction associated with the presence of hypertension and the impact of age in SHR (Berenyiova et al., 2018; Zhang et al., 2018). The present study confirms that ACh-dependent endothelial relaxation and SNP-independent endothelial relaxation decreased significantly in adult SHR compared to young rats. Moreover, this reduction in relaxing responses was accompanied by changes in the morphology of the arteries, visible in wall thickening or lumen increase of the older animals. Lycopene treatment showed effective action to prevent morphological changes, but failed to improve aortic vasodilation. These results are in line with previous findings obtained by our group with lycopene and astaxanthin, both carotenoids improved the cardiac, renal, and aortic hypertrophy and fibrosis, but had no effect on ACh-induced relaxations in aortic rings obtained from continuously perfused angiotensin II rats and SHR, respectively (Ferreira-Santos et al., 2018; Monroy-Ruiz,

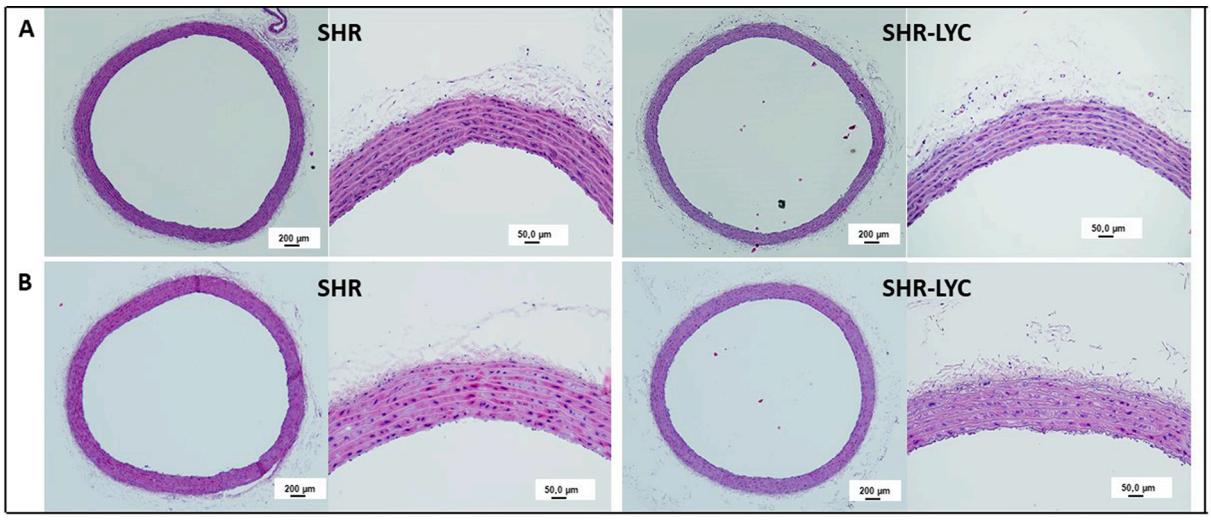

Fig. 4. Histological sections of the aorta stained with hematoxylin-eosin in young (**A**) and adult (**B**) spontaneously hypertensive rats (SHR) and SHR treated with 10 mg/kg/day of lycopene (SHR-LYC). Original magnification x40 and x200.

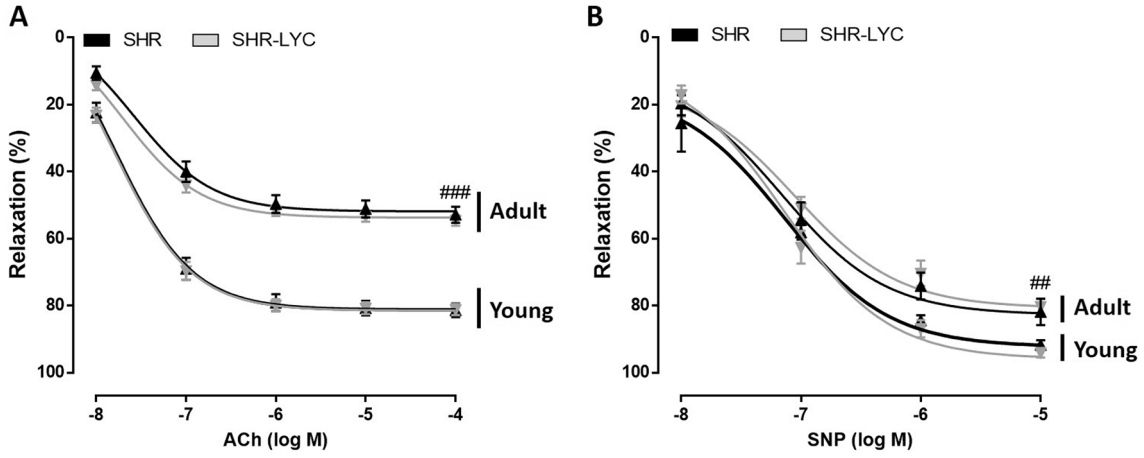

Fig. 5. Cumulative concentration–response curves to acetylcholine (ACh) (**A**) and sodium nitroprusside (SNP) (**B**) in pre-constricted aortic rings from young and adult spontaneously hypertensive rats (SHR) and SHR treated with 10 mg/kg/day of lycopene (SHR-LYC). Values are expressed as mean ± SEM (n = 7). $^{\#\#}P < 0.01$ and $^{\#\#\#}P < 0.001$ *vs*. young SHR.

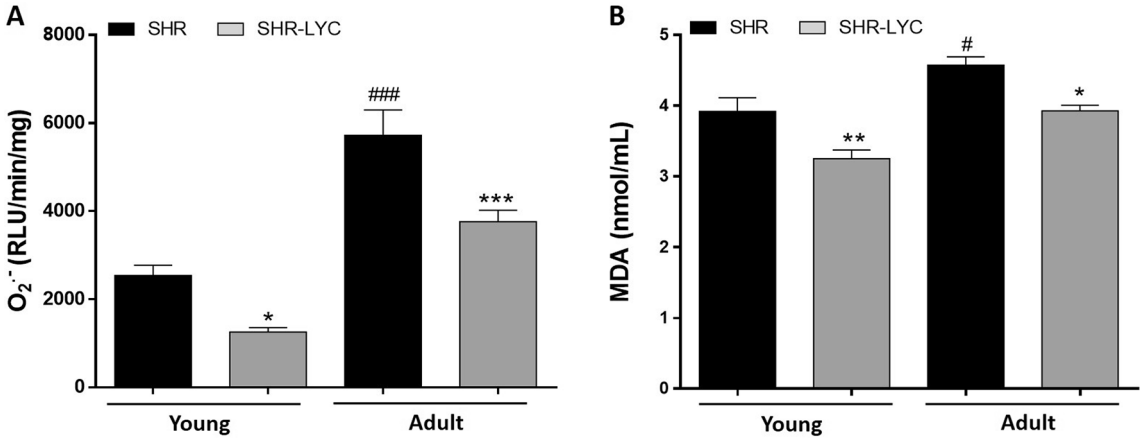

Fig. 6. Generation of vascular superoxide anion ($O_2^{\cdot -}$) stimulated by NADPH in aortic rings (**A**) and assessment of lipid peroxidation through the quantification of malondialdehyde (MDA) in plasma (**B**) from young and adult spontaneously hypertensive rats (SHR) and SHR treated with 10 mg/kg/day of lycopene (SHR-LYC). Values are expressed as mean ± SEM (n = 7). $^{\#}P < 0.05$, and $^{\#\#\#}P < 0.001$ *vs*. young SHR, *P < 0. 05, **P < 0. 01 and ***P < 0.001 *vs.* the corresponding SHR without treatment.

Table 2
Effects of lycopene on antioxidant enzymes activity on liver homogenates.

	Young		Adult	
Enzymes	SHR	SHR-LYC	SHR	SHR-LYC
SOD (U/mg prot)	94.93 ± 1.78	105.27 ± 2.35**	100.54 ± 2.63	108.70 ± 3.10*
GSH-PX (U/mg prot)	1.25 ± 0.14	1.62 ± 0.15*	1.49 ± 0.11	2.07 ± 0.17**

SOD, superoxide dismutase. GSH-PX, glutathione peroxidase. Values are expressed as mean ± SEM of 7 rats. * $P < 0.05$ and ** $P < 0.01$ *vs.* the corresponding SHR without treatment.

Sevilla, Carrón, & Montero, 2011).

The present study also reveals the beneficial effect of lycopene on oxidative stress on this model of hypertension. Oxidative stress occurs from the imbalance between ROS production and antioxidant defenses and several studies have proven that heart tolerance to oxidative stress decreases with age because of a reduction in the concentrations of the antioxidant enzymes, contributing to the development of cardiovascular alterations (Abete et al., 1999; Adwas, Elsayed, Azab, & Quwaydir, 2019). The current study shows that the age leads to an increase in oxidative stress biomarkers. There is an age-dependent significant increase of MDA plasma levels and $O_2^{\bullet -}$ production induced by NADPH. Although the adult SHR used in this study did not show differences respect to the young ones in markers of antioxidant defenses like SOD and GSH-PX, lycopene was able to significantly increase these endogenous defenses, in both groups.

A recent study also found that similar doses of lycopene increase antioxidant defenses as a result of the improvement in activity of catalase, SOD, and GSH-PX in animals with type 2 diabetes mellitus (Zheng, Yin, Lu, & Jiang, 2019). It has been reported that lycopene serves as a precursor for various oxidative cleavage products and metabolites that can interact with multiple transcription factors to overexpress antioxidant and cytoprotective enzymes (Saini, Rengasamy, Mahomoodally, & Keum, 2020).

In both young and adult rats treated with lycopene the $O_2^{\bullet -}$ production and plasma MDA levels were reduced compared to their controls. These results are in line with those obtained in a previous study where we demonstrated that lycopene had a beneficial effect in an experimental model of hypertension with a high component of oxidative stress, in which the levels of angiotensin II are continually elevated (Ferreira-Santos et al., 2018).

A study, performed in hypertensive patients, demonstrates the action of the diet supplemented with tomatoes (vegetable in which lycopene is the predominant carotenoid) changes the redox state, increases antioxidant enzymes and decreases peroxidation lipid (Bose & Agrawal, 2007).

In conclusion, hypertension causes cardiovascular complications such as endothelial dysfunction, remodeling or oxidative stress, which intensify with age and treatment with lycopene improved most of these alterations in a similar way in both young and adult animals.

Ethical statement

All authors who have signed the MS entitled "**The antihypertensive and antihypertrophic effect of lycopene is not affected by and is independent of age**" declare that all the experiments were performed according to the European Union guidelines for the ethical care and use of laboratory animals, and the protocol was approved by the Bioethics Committee of the University of Salamanca (Registry No.: 006N°201400039292).

CRediT authorship contribution statement

Pedro Ferreira-Santos: Validation, Investigation, Formal analysis, Visualization. **Rosalía Carrón:** Conceptualization, Resources, Writing - original draft, Visualization, Supervision. **M José Montero:** Conceptualization, Resources, Writing - original draft, Visualization, Supervision, Project administration, Funding acquisition. **M Ángeles Sevilla:** Conceptualization, Writing - original draft, Visualization, Supervision.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

This work was financed by the University of Salamanca (Spain).

Pedro Ferreira Santos was the recipient of a fellowship funded by a project Art.83/LOU of University of Salamanca.

Lycopene was kindly supplied by DSM NUTRITIONAL PRODUCTS.

References

Abete, P., Napoli, C., Santoro, G., Ferrara, N., Tritto, I., Chiariello, M., … Ambrosio, G. (1999). Age-related decrease in cardiac tolerance to oxidative stress. *Journal of Molecular and Cellular Cardiology, 31*(1), 227–236. https://doi.org/10.1006/jmcc.1998.0862.

Anlar, H. G., & Bacanli, M. (2020). Lycopene as an antioxidant in human health and diseases. In Pathology (pp. 247–254). Elsevier. http://dx.doi.10.1016/b978-0-12-815972-9.00024-x.

Arain, M. A., Zhuang, M., Hassan, F.-U., & Saeed, M. (2018). Lycopene: A natural antioxidant for prevention of heat-induced oxidative stress in poultry. *World's Poultry Science Journal, 74*(1), 89–100. https://doi.org/10.1017/S0043933917001040.

Babio, N., Toledo, E., Estruch, R., Ros, E., Martínez-González, M. A., Castañer, O., … Salas-Salvadó, J. (2014). Mediterranean diets and metabolic syndrome status in the PREDIMED randomized trial. *CMAJ.*

Berenyiova, A., Drobna, M., Cebova, M., Kristek, F., & Cacanyiova, S. (2018). Changes in the vasoactive effects of nitric oxide, hydrogen sulfide and the structure of the rat thoracic aorta: The role of age and essential hypertension. *Journal of Physiology and Pharmacology, 69*(4). https://doi.org/10.26402/jpp.2018.4.05.

Bose, K. S. C., & Agrawal, B. K. (2007). Effect of lycopene from tomatoes (cooked) on plasma antioxidant enzymes, lipid peroxidation rate and lipid profile in grade-I hypertension. *Annals of Nutrition & Metabolism, 51*(5), 477–481. https://doi.org/10.1159/000111170.

Buford, T. W. (2016, March 1). Hypertension and aging. Ageing Research Reviews. Elsevier Ireland Ltd. http://dx.doi.10.1016/j.arr.2016.01.007.

Chen, J., Song, Y., & Zhang, L. (2013). Effect of Lycopene Supplementation on Oxidative Stress: An Exploratory Systematic Review and Meta-Analysis of Randomized Controlled Trials. *Journal of Medicinal Food, 16*(5), 361–374. https://doi.org/10.1089/jmf.2012.2682.

Cheng, H. M., Koutsidis, G., Lodge, J. K., Ashor, A., Siervo, M., & Lara, J. (2017). Tomato and lycopene supplementation and cardiovascular risk factors: A systematic review and meta-analysis. *Atherosclerosis, 257*, 100–108. https://doi.org/10.1016/j.atherosclerosis.2017.01.009.

Adwas, A. A., Elsayed, A., Azab, A. E., & Quwaydir, F. A. (2019). Oxidative stress and antioxidant mechanisms in human body. *Journal of Applied Biotechnology & Bioengineering, 6*(1), 43–47. https://doi.org/10.15406/jabb.2019.06.00173.

Ferreira-Santos, P., Aparicio, R., Carrón, R., Montero, M. J., & Sevilla, M.Á. (2020). Lycopene-supplemented diet ameliorates metabolic syndrome induced by fructose in rats. *Journal of Functional Foods, 73*, Article 104098. https://doi.org/10.1016/j.jff.2020.104098.

Ferreira-Santos, P., Aparicio, R., Carrón, R., Sevilla, M.Á., Monroy-Ruiz, J., & Montero, M. J. (2018). Lycopene-supplemented diet ameliorates cardiovascular remodeling and oxidative stress in rats with hypertension induced by Angiotensin II. *Journal of Functional Foods, 47*, 279–287. https://doi.org/10.1016/J.JFF.2018.06.002.

Friedman, M. (2013). Anticarcinogenic, cardioprotective, and other health benefits of tomato compounds lycopene, α-tomatine, and tomatidine in pure form and in fresh and processed tomatoes. *Journal of Agricultural and Food Chemistry, 61*(40), 9534–9550. https://doi.org/10.1021/jf402654e.

Izzo, C., Vitillo, P., Di Pietro, P., Visco, V., Strianese, A., Virtuoso, N., … Vecchione, C. (2021). The role of oxidative stress in cardiovascular aging and cardiovascular diseases. *Life, 11*(1), 1–42. https://doi.org/10.3390/life11010060.

Jiang, W., Guo, M.-H., & Hai, X. (2016). Hepatoprotective and antioxidant effects of lycopene on non-alcoholic fatty liver disease in rat. *World Journal of Gastroenterology, 22*(46), 10180. https://doi.org/10.3748/wjg.v22.i46.10180.

Lerman, L. O., Kurtz, T. W., Touyz, R. M., Ellison, D. H., Chade, A. R., Crowley, S. D., … Coffman, T. M. (2019). Animal Models of Hypertension: A Scientific Statement From the American Heart Association. Hypertension (Dallas, Tex. : 1979) (Vol. 73). http://dx.doi.10.1161/HYP.0000000000000090.

Llorens-Cortes, C., & Touyz, R. M. (2020). Evolution of a New Class of Antihypertensive Drugs: Targeting the Brain Renin-Angiotensin System. *Hypertension, 75*(1), 6–15. https://doi.org/10.1161/HYPERTENSIONAHA.119.12675.

Monroy-Ruiz, J., Sevilla, M.Á., Carrón, R., & Montero, M. J. (2011). Astaxanthin-enriched-diet reduces blood pressure and improves cardiovascular parameters in spontaneously hypertensive rats. *Pharmacological Research, 63*(1), 44–50. https://doi.org/10.1016/j.phrs.2010.09.003.

Montezano, A. C., Dulak-Lis, M., Tsiropoulou, S., Harvey, A., Briones, A. M., & Touyz, R. M. (2015). Oxidative stress and human hypertension: Vascular mechanisms, biomarkers, and novel therapies. *The Canadian Journal of Cardiology, 31*(5), 631–641. https://doi.org/10.1016/j.cjca.2015.02.008.

Montezano, A. C., & Touyz, R. M. (2012). Molecular Mechanisms of Hypertension-Reactive Oxygen Species and Antioxidants: A Basic Science Update for the Clinician. *Canadian Journal of Cardiology, 28*(3), 288–295. https://doi.org/10.1016/j.cjca.2012.01.017.

Mordente, A., Guantario, B., Meucci, E., Silvestrini, A., Lombardi, E., Martorana, G., …, Bohm, V. (2011). Lycopene and Cardiovascular Diseases: An Update. *Current Medicinal Chemistry, 18*(8), 1146–1163. https://doi.org/10.2174/092986711795029717.

Müller, L., Caris-Veyrat, C., Lowe, G., & Böhm, V. (2016). Lycopene and Its Antioxidant Role in the Prevention of Cardiovascular Diseases—A Critical Review. *Critical Reviews in Food Science and Nutrition, 56*(11), 1868–1879. https://doi.org/10.1080/10408398.2013.801827.

Paran, E., Novack, V., Engelhard, Y. N., & Hazan-Halevy, I. (2009). The effects of natural antioxidants from tomato extract in treated but uncontrolled hypertensive patients. *Cardiovascular Drugs and Therapy, 23*(2), 145–151. https://doi.org/10.1007/s10557-008-6155-2.

Ried, K., & Fakler, P. (2011). Protective effect of lycopene on serum cholesterol and blood pressure: Meta-analyses of intervention trials. *Maturitas, 68*(4), 299–310. https://doi.org/10.1016/j.maturitas.2010.11.018.

Roohbakhsh, A., Karimi, G., & Iranshahi, M. (2017). Carotenoids in the treatment of diabetes mellitus and its complications: A mechanistic review. *Biomedicine and Pharmacotherapy, 91*, 31–42. https://doi.org/10.1016/j.biopha.2017.04.057.

Saini, R. K., Rengasamy, K. R. R., Mahomoodally, F. M., & Keum, Y. S. (2020). Protective effects of lycopene in cancer, cardiovascular, and neurodegenerative diseases: An update on epidemiological and mechanistic perspectives. *Pharmacological Research, 155*(October 2019), 104730. https://doi.org/10.1016/j.phrs.2020.104730.

Sánchez-Rivera, L., Santos, P. F., Miralles, B., Carrón, R., José Montero, M., & Recio, I. (2016). Peptide fragments from β-casein f(134–138), HLPLP, generated by the action of rat blood plasma peptidases show potent antihypertensive activity. *Food Research International, 88*, 348–353. https://doi.org/10.1016/J.FOODRES.2015.12.007.

Sauzeau, V., Sevilla, M. A., Rivas-Elena, J. V., De Álava, E., Montero, M. J., López-Novoa, J. M., & Bustelo, X. R. (2006). Vav3 proto-oncogene deficiency leads to sympathetic hyperactivity and cardiovascular dysfunction. *Nature Medicine, 12*(7), 841–845. https://doi.org/10.1038/nm1426.

Silva, S. D., Jara, Z. P., Peres, R., Lima, L. S., Scavone, C., Montezano, A. C., … Michelini, L. C. (2017). Temporal changes in cardiac oxidative stress, inflammation and remodeling induced by exercise in hypertension: Role for local angiotensin II reduction. *PLoS ONE, 12*(12), 1–19. https://doi.org/10.1371/journal.pone.0189535.

Thies, F., Mills, L. M., Moir, S., & Masson, L. F. (2017). Cardiovascular benefits of lycopene: Fantasy or reality? *Proceedings of the Nutrition Society, 76*(2), 122–129. https://doi.org/10.1017/S0029665116000744.

Touyz, R. M., Alves-Lopes, R., Rios, F. J., Camargo, L. L., Anagnostopoulou, A., Arner, A., & Montezano, A. C. (2018). Vascular smooth muscle contraction in hypertension. *Cardiovascular Research, 114*, 529–539. https://doi.org/10.1093/cvr/cvy023.

Wang, X., Lv, H., Gu, Y., Wang, X., Cao, H., Tang, Y., … Huang, C. (2014). Protective effect of lycopene on cardiac function and myocardial fibrosis after acute myocardial infarction in rats via the modulation of p38 and MMP-9. *Journal of Molecular Histology, 45*(1), 113–120. https://doi.org/10.1007/s10735-013-9535-2.

World Health Organization. (2017). World Health Statistics 2017: Monitoring Health for The SDGs. *World Health Organization*. https://doi.org/10.1017/CBO9781107415324.004.

Zhang, Y., Liao, J., Zhang, L., Li, S., Wu, Y., & Shi, L. (2018). BK Ca channel activity and vascular contractility alterations with hypertension and aging via β1 subunit promoter methylation in mesenteric arteries. *Hypertension Research, 41*(2), 96–103. https://doi.org/10.1038/hr.2017.96.

Zheng, Z., Yin, Y., Lu, R., & Jiang, Z. (2019). Lycopene Ameliorated Oxidative Stress and Inflammation in Type 2 Diabetic Rats. *Journal of Food Science, 84*(5), 1194–1200. https://doi.org/10.1111/1750-3841.14505.

Zhou, B., Bentham, J., Di Cesare, M., Bixby, H., Danaei, G., Cowan, M. J., … Zuñiga Cisneros, J. (2017). Worldwide trends in blood pressure from 1975 to 2015: A pooled analysis of 1479 population-based measurement studies with 19·1 million participants. *The Lancet, 389*(10064), 37–55. https://doi.org/10.1016/S0140-6736(16)31919-5.