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Cornelian cherry consumption increases the L-arginine/ADMA ratio, lowers ADMA and SDMA levels in the plasma, and enhances the aorta glutathione level in rabbits fed a high-cholesterol diet

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ABSTRACT

In our previous publication we showed that the oral administration of cornelian cherry fruits prevented feed-induced atherosclerosis.

In this study we have examined the effect of cornelian cherry lyophilisate on proteins responsible for regulation of NO synthesis: asymmetric and symmetric dimethylarginine (ADMA, SDMA) and L-arginine in plasma, and dimethylarginine dimethylaminohydrolase (DDAH) in the liver. We have also assessed the systemic and local redox status in the blood and the aorta, and the thickness of the thoracic aorta.

We have shown that 60-days of administering lyophilisate to rabbits fed 1% cholesterol increased the L-arginine and L-arginine/ADMA ratio, decreased ADMA and SDMA, increased DDAH activity, and had a positive impact on the redox state in the aorta but not in the blood, measured as decreased MDA, and increased glutathione, GPx, and SOD. Moreover, lyophilisate significantly decreased intima thickness and the intima/media ratio in the thoracic aorta.

Concluding, the L-arginine-ADMA-DDAH pathway may contribute to the beneficial effects of the cornelian cherry in feed-induced atherosclerosis.

1. Introduction

Cornelian cherry is a native or cultivated plant in Europe and southwest Asia. Its fruits have been used for years in both cuisine and traditional medicine of many countries. Before the World War II, cornelian cherry was cultivated widely in Poland and used for home production of liqueurs, jams, and soups. Cornelian cherries have been also used in folk medicine to relive many conditions, especially gastrointestinal and metabolic disorders, diarrhea, fever, and common cold. Unfortunately, the plant has nowadays lost its popularity in Europe and is rarely consumed.

The network of metabolic pathways involved in the development of atherosclerosis and cardiovascular diseases includes several mechanisms that impact one another. Functional food may modulate many of these pathways. Attempts to elucidate these interactions help us understand how diet may prevent cardiovascular diseases. In our previous publications we have shown that cornelian cherry fruits contained large amounts of iridoids – mainly loganic acid – and anthocyanins, and proven their preventive effect on diet-induced dyslipidemia and atherosclerosis in rabbits. A pronounced, enhanced liver expression of the peroxisome proliferator-activated receptor alpha (PPARα) was shown, suggesting its role in the mechanism responsible for the positive effect of cornelian cherry on lipids. We have also shown its

Abbreviations: ADMA, asymmetric dimethylarginine; DDAH, dimethylarginine dimethylaminohydrolase; MDA, malondialdehyde; ELISA, enzyme-linked immunosorbent assay; eNOS, endothelial nitric oxide synthase; HPLC, high-performance liquid chromatography; PPARα, peroxisome proliferator-activated receptor alpha; PRMTs, protein arginine methyltransferases; RONS, reactive oxygen and nitrogen species; SDMA, symmetric dimethylarginine; TNFα, tumor necrosis factor α.

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anti-inflammatory effect (Kucharska, Szumny, Sokół-Łętowska, Piórecki, & Klymenko, 2015; Sozański et al., 2014; Sozański et al., 2016).

Studies completed over a couple of last decades have demonstrated an important role of the ADMA-L-arginine-DDAH pathway in the development of vascular dysfunction – an early pathology found in the development of atherosclerosis and subsequent cardiovascular diseases (Sibal, Agarwal, Home, & Böger, 2010). Asymmetric dimethylarginine (ADMA) and symmetric dimethylarginine (SDMA) inhibit endothelial NO synthesis, their levels are correlated with atherosclerosis, and the compounds are postulated as markers of cardiovascular events and mortality, especially in high-risk populations (Böger, Maas, Schulze, & Schwedhelm, 2009; Gore et al., 2013). Both ADMA and SDMA levels are regulated throughout their synthesis by the protein arginine methyltransferases (PRMTs). ADMA level in blood also depends on its metabolism in the liver by dimethylarginine dimethylaminohydrolase (DDAH), while SDMA is directly excreted by the kidneys. Unlike ADMA and SDMA, the L-arginine and L-arginine/ADMA ratio is positively correlated with NO synthesis and inversely correlated with the cardiovascular risk (Notsu, Yano, Shibata, Nagai, & Nabika, 2015).

Moreover, ADMA, SDMA, and L-arginine interact with the redox balance on many levels, e.g. by regulation of one another's synthesis, and under influence of transformations of reactive oxygen and nitrogen species (RONS) (Daiber et al., 2013). RONS have harmful effect on many biological tissues, and their contribution to the development of atherosclerosis and cardiovascular diseases has been established (Kang & Kang, 2013; Karbach, Wenzel, Waisman, Munzel, & Daiber, 2014).

Because the increased ADMA level and particularly the increased L-arginine/ADMA ratio are regarded independent risk factors for progression of atherosclerosis and cardiovascular diseases (Böger et al., 2009; Notsu et al., 2015), and considering their interaction with redox factors, their modulation by diet or natural supplements may be relevant. To the best of our knowledge, there are no studies that had investigated the effects of anthocyanins, iridoids, or anthocyanin- and iridoid-rich fruits on L-arginine and its derivatives, ADMA and SDMA. Results of our previous studies on the effect of cornelian cherry on induced atherosclerosis raise questions about its possible impact on factors affecting NO bioavailability.

The aim of this investigation was to assess the impact of oral administration of the cornelian cherry fruit lyophilisate on plasma levels of L-arginine and its derivatives (ADMA, SDMA), DDAH activity in the liver, systemic and local redox state in blood and in the aorta, as well as intima thickness and intima/media ratio in the thoracic aorta in cholesterol-fed rabbits.

Results of our study suggest beneficial effects of cornelian cherry consumption via mechanisms involving improvement of blood L-arginine and L-arginine/ADMA balance and via reduced oxidative stress in the aorta, thus reducing risk of CVDs.

2. Materials and methods

2.1. Chemicals

Simvastatin was kindly provided by Gedeon Richter, Poland, Sp z.o.o. (formerly GZF Polfa, Poland). Cholesterol was purchased from POCH, S.A., Poland.

2.2. Plant materials and sample preparation

Cornelian cherry (*Cornus mas* L.) fruits were obtained from the Bolestraszyce Arboretum and the Institute of Physiography, Poland. The plant material was authenticated by Prof. Jakub Dolatowski. A voucher specimen (BDPA 3 967) was deposited at the Herbarium of Arboretum and the Institute of Physiography in Bolestraszyce, Poland. The lyophilisate was prepared in the Department of Fruit, Vegetable and Cereals Technology at the Wrocław University of Environmental and Life Science, as described previously (Sozański et al., 2014). Ripe fruits were washed, frozen, and stored frozen at −20 °C until processed into lyophilisate. After freezing and removal of pits, the still-frozen samples were freeze-dried (Alpha 1-4 LSC, Christ, Osterode am Harz, Germany) for 24 h. Approximately 20 g of cornelian cherry lyophilisate was obtained from 100 g of ripe fruit. After freeze-drying, samples were ground into homogeneous powder using a laboratory mill (IKA 11A; BIOSAN, Vilnius, Lituania).

The qualitative and quantitative composition of cornelian cherry lyophilisate is presented in Table 1 (Sozański et al., 2014).

2.3. The in vivo study

This study was approved by the Local Ethical Committee on Animal Research at the Institute of Immunology and Experimental Therapy, Polish Academy of Sciences in Wrocław, and was conducted in accordance with the NIH Guide for the Care and Use of Laboratory Animals.

2.4. Animals and treatment

Animals, the schedule of administration of investigated substances, and the procedure of feeding were described in detail in our previous publication (Sozański et al., 2014). Briefly, 40 New Zealand rabbits, age between 8 and 12 months, were used in the experiment. Animals had unrestricted access to water and were given the same daily portion of laboratory feed (40 g/kg b.w.). After three weeks of adaptation rabbits were randomly divided into 4 groups of 10 animals each. Animals in group P were given a standard feed, the group CHOL received the same feed + 1% cholesterol. The CHOL + CM and CHOL + SIM groups were fed with the same feed + 1% cholesterol, and for 60 consecutive days were administered the cornelian cherry lyophilisate at the dose of 100 mg/kg. b.w., and simvastatin at the dose of 5 mg/kg b.w., respectively. On days 0 and 60 blood samples were collected from the marginal vein of the ear or the saphenous vein from each animal. On day 60 the animals were sacrificed with terminal anesthesia containing thiopenthal (250 mg/kg, administered intravenously).

2.5. Measurements of plasma levels of L-arginine, ADMA, and SDMA

L-arginine, ADMA, and SDMA concentrations were assessed simultaneously using the high-performance liquid chromatography (HPLC) with fluorescence detection. Details of the method were described previously in our earlier experiment (Trocha et al., 2014) and based on previous studies (Böger et al., 1998; Parker, Huang, & Tesfamariam, 2003).

Table 1
Characterization of selected constituents from cornelian cherry (*Cornus mas* L.) lyophilizate using HPLC-DAD (λ_{max}) and negative and positive ions in UPLC-ESI/MS (MS and MS/MS) (Sozański et al., 2014).

Compound	Mean ± SD* (mg/100 g)
Loganic acid	820.4 ± 68.0
Delphinidin 3-*O*-galactoside	2.5 ± 0.8
Cyanidin 3-*O*-galactoside	123.5 ± 19.7
Cyanidin 3-*O*-robinobioside	15.1 ± 5.1
Pelargonidin 3-*O*-galactoside	87.9 ± 19.9
Pelargonidin 3-*O*-robinobioside	6.7 ± 2.6
Cornuside	99.1 ± 16.1

* Standard deviation.

2.6. DDAH activity

The DDAH activity in liver homogenates was measured using the colorimetric method (spectrophotometer MARCEL S350 PRO, Marcel, sp. z. o. o., Poland). The method is based on the L-citrulline production rate and was described in detail in our previous study (Trocha et al., 2014). The DDAH activity was presented as μm of L-citrulline/gram of protein/minute at 37 °C.

2.7. Biomarkers associated with the redox state in the blood and aorta

Spectrophotometrical methods were used for the assessment of all redox parameters in blood and the aorta. The aortic malondialdehyde (MDA) level was determined using the BIOXYTECH-MDA-586 kit (OxisResearch, USA) and expressed as μM/g of tissue. The glutathione (GSH) concentration in the aorta was examined using the BIOXYTECH GSH-400 kit (OxisResearch, USA) and expressed as mM/mg of tissue. Superoxide dismutase (SOD) activity was determined using the Ransod kit (Randox Laboratories, UK) and expressed as U/mg of protein in the aorta and as UI/g hemoglobin in whole blood. Glutathione peroxidase (GPx) activity was measured using the GPx-340 kit (Randox Laboratories, UK) and expressed as U/mg of protein in the aorta, and as UI/g hemoglobin in whole heparinised blood.

2.8. Histopathological assessment of the thoracic aorta

Tissue sections of the thoracic aorta were washed with ice-cold sterile physiological saline and then fixed in the 7% buffered formalin solution. 4 μm thick slices were dehydrated, embedded in paraffin, and stained with hematoxylin and eosin. Samples were blindly examined under a light microscope by an experienced pathomorphologist. Intima thickness, and the intima/media ratio were estimated according to earlier studies by Brant et al. (2014) and El-Sheakh, Ghoneim, Suddek, and Ammar (2015).

2.9. Statistical analysis

Results were expressed as mean ± standard deviation (mean ± SD). The normality of all continuous variables was verified by the Shapiro-Wilk test. Statistical comparisons of data were performed using the ANOVA, followed by a post-hoc LSD test. Values of $P < 0.05$ were considered significant.

3. Results

We examined the effects of oral administration of cornelian cherry fruit lyophilisate on plasma levels of L-arginine, ADMA, and SDMA, DDAH activity in the liver, the redox status in blood and the aorta, and intima thickness and intima/media ratio in the thoracic aorta.

The cholesterol-rich diet induced an increase in ADMA and SDMA levels, a decrease in L-arginine, and a decrease in the L-arginine/ADMA ratio. Consumption of cornelian cherries reversed these changes. The CHOL + CM group, compared to the CHOL group, showed a significant decrease in ADMA and SDMA levels, an increase of L-arginine levels, and a pronounced increase in the L-arginine/ADMA ratio (by 63%) (Fig. 1).

Similar but less pronounced effects on the L-arginine-ADMA-SDMA pathway were observed in the group receiving simvastatin.

Cholesterol feeding only slightly decreased the activity of DDAH in the liver, compared to the control group. The addition of cornelian cherry significantly increased DDAH activity to the level higher than in all other groups. No significant effect on DDAH was observed for simvastatin (Fig. 2).

We also demonstrated the significant impact of cornelian cherry on lipid peroxidation and redox parameters in the aorta

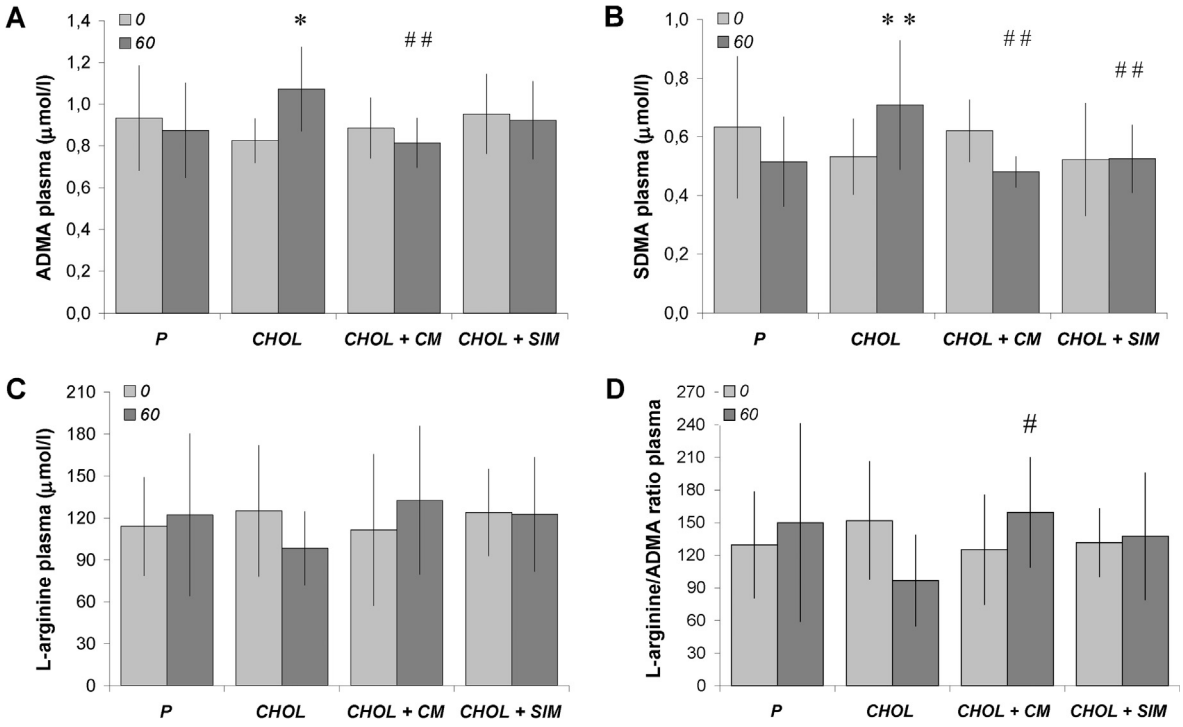

Fig. 1. ADMA (A), SDMA (B), l-arginine (C), and l-arginine/ADMA ratio (D) in plasma on day 0 and day 60 of the experiment. P, standard feed and vehicle-treated rabbits, CHOL, cholesterol-treated rabbits, CHOL + CM, rabbits treated with cholesterol + Cornelian cherry 100 mg/kg body weight, CHOL + SIM, rabbits treated with cholesterol + simvastatin 5 mg/kg body weight. Values presented as the mean ± SD. Specific comparisons: * $p < 0.05$, ** $p < 0.01$ vs. P, # $p < 0.05$, ## $p < 0.01$ vs. CHOL.

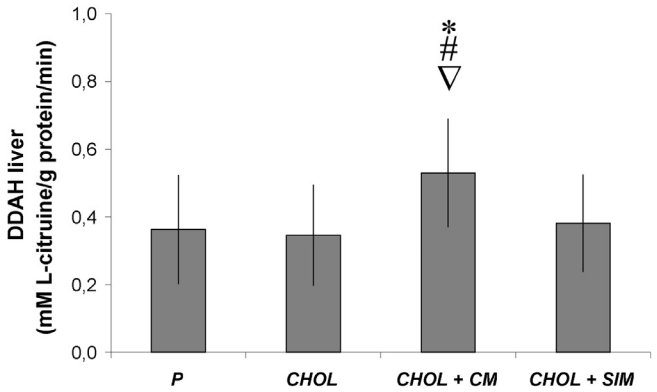

Fig. 2. Liver DDAH activity on day 60 of the experiment. P, standard feed and vehicle-treated rabbits, CHOL, cholesterol-treated rabbits, CHOL + CM, rabbits treated with cholesterol + Cornelian cherry 100 mg/kg body weight, CHOL + SIM, rabbits treated with cholesterol + simvastatin 5 mg/kg body weight. Values presented as the mean ± SD. Specific comparisons: * $p < 0.05$ vs. P, # $p < 0.05$ vs. CHOL, Δ $p < 0.05$ vs. CHOL + SIM.

(Fig. 3). The cholesterol-rich diet increased the MDA level. Cornelian cherry completely reversed these changes. The MDA level in the CHOL + CM group was lower than in the CHOL group, and comparable to the control group. Simvastatin did not, however, change the MDA level compared to the cholesterol-fed group.

Cholesterol feeding caused a significant depletion of glutathione and was completely reversed by cornelian cherry. The glutathione level in the CHOL + CM group was higher than in all other groups.

Gpx activity in the cholesterol-fed group was over 2 times higher than in the control group. Cornelian cherry partially reversed GPx activity to a level lower than in the CHOL group, but still higher than in the control group. Simvastatin significantly increased GPx activity to levels higher than in other groups. SOD activity in the cholesterol-fed group was moderately increased compared to the control group. Cornelian cherry significantly decreased SOD activity, reducing it to the level lower than in both the control and the cholesterol-fed group. Simvastatin increased SOD activity compared to all other groups.

No significant differences were observed when analyzing blood SOD activities in serum among study groups. Blood GPx activity was decreased in the CHOL group compared to the control group, and the effect was not reversed by either cornelian cherry or simvastatine treatment (Fig. 4).

Feeding with high-cholesterol diet caused a significant thickening of the intima layer and increased the intima/media ratio. Administration of cornelian cherry significantly diminished those changes. Intima thickness and the intima/media ratio in the CHOL + CM group were lower compared to the CHOL group. Simvastatin completely prevented thickening of the intima layer. Intima thickness and the intima/media ratio in the CHOL + SIM group were similar to values measured in the P group, fed with a standard diet. Thoracic aorta histomorphometric measures and representative photos of the aortic sections are presented in Fig. 5.

4. Discussion and conclusions

The most important finding made in our study is that oral administration of cornelian cherry had a positive impact on plasma levels L-arginine and its derivatives (ADMA, SDMA), as well as on DDAH activity in the liver in rabbits fed with the high-cholesterol diet. This suggests the possible impact of cornelian cherry consumption on the NO synthesis and the endothelial function. Key results are the following: oral administration of cornelian cherry caused: (1) a significant increase in the L-arginine/ADMA ratio and a decrease in ADMA and SDMA levels, (2) an enhanced activity of DDAH – the key enzyme responsible for ADMA metabo-

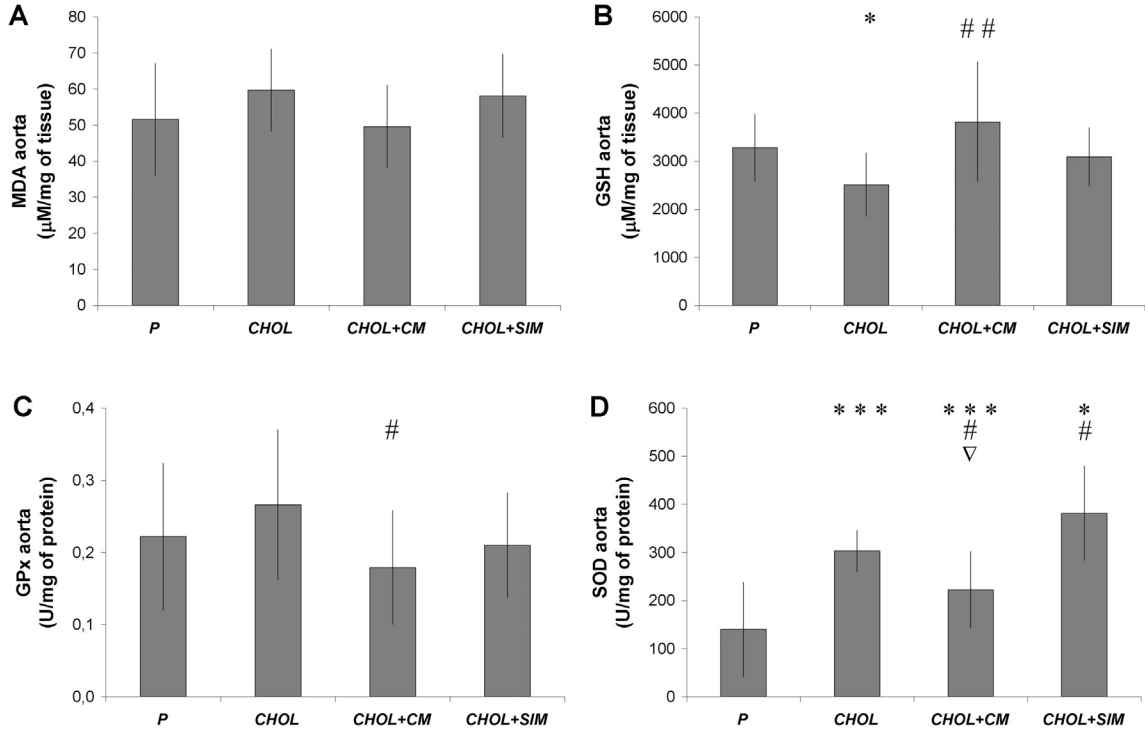

Fig. 3. Malondialdehyde concentrations (A), glutathione concentrations (B), GPx activities (C), and SOD activities (D) in the aorta. P, standard feed and vehicle-treated rabbits, CHOL, cholesterol-treated rabbits, CHOL + CM, rabbits treated with cholesterol + Cornelian cherry 100 mg/kg body weight, CHOL + SIM, rabbits treated with cholesterol + simvastatin 5 mg/kg body weight. Values presented as the mean ± SD. Specific comparisons: * $p < 0.05$, *** $p < 0.001$ vs. P, # $p < 0.05$, ## $p < 0.01$ vs. CHOL, Δ $p < 0.05$ vs. CHOL + SIM.

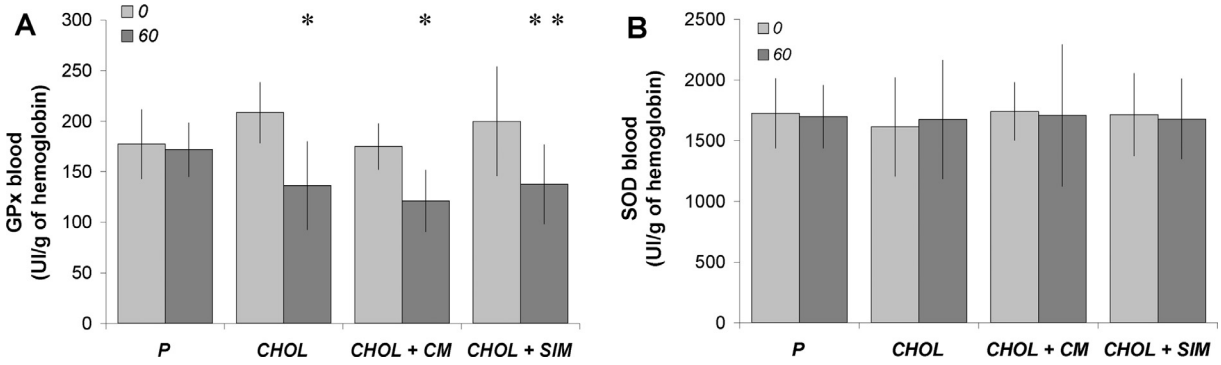

Fig. 4. Blood GPx (A) and SOD (B) activities. P, standard feed and vehicle-treated rabbits, CHOL, cholesterol-treated rabbits, CHOL + CM, rabbits treated with cholesterol + Cornelian cherry 100 mg/kg body weight, CHOL + SIM, rabbits treated with cholesterol + simvastatin 5 mg/kg body weight. Values presented as the mean ± SD. Specific comparisons: * $p < 0.05$, ** $p < 0.01$ vs. P.

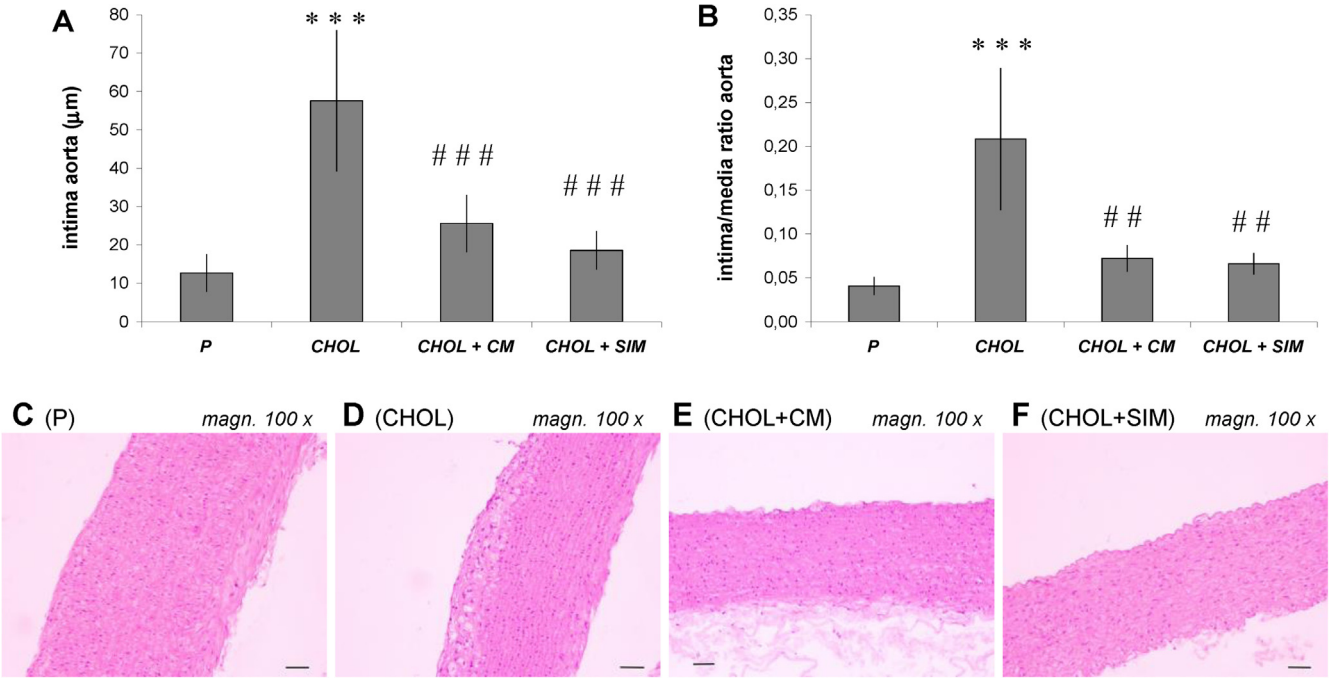

Fig. 5. Intima thickness (A), and the intima/media ratio (B) in the thoracic aorta. Values presented as mean ± SD. Specific comparisons: *** $p < 0.001$ vs. P; ## $p < 0.01$, ### $p < 0.001$ vs. CHOL. Representative hematoxylin-eosin stained cross-sections of thoracic aorta segments, in the (C) P group – rabbit No. 5, (D) CHOL group – rabbit No. 14, (E) CHOL + CM group – rabbit No. 19, and (F) CHOL + SIM group – rabbit No. 35.

lism, (3) a diminished local lipid peroxidation and oxidative stress in the aorta (4) no changes in systemic redox balance measured by blood antioxidant enzymes, and (5) a decreased intima thickness and the intima/media ratio in the thoracic aorta.

Changes in the L-arginine-ADMA-DDAH pathway caused by the administration of iridoid- and anthocyanin-rich cornelian cherry may provide a new insight into the cardiovascular benefits of the plant. The observed influence of cornelian cherry on the L-arginine-ADMA-DDAH pathway suggests a possible modulation of factors affecting NO synthesis in subjects fed with the high-cholesterol diet. In the available literature we have not encountered any previous reports describing effects of cornelian cherry, anthocyanins, or iridoids, on L-arginine and its derivatives, ADMA and SDMA. ADMA is considered a potent key competitive inhibitor of NO synthases, involved in the development of atherosclerosis and cardiovascular diseases (Böger et al., 2009). SDMA does not directly impact NO synthases, but may down-regulate its activity, acting as a competitor to L-arginine transport via the cell membrane mediated by the cationic amino acid transporter (CAT) y+ system (Bode-Böger et al., 2006). Both ADMA and SDMA down-regulate the NO synthesis in response to harmful factors, and their increased level is observed in several diseases, i.e. dyslipidemias, atherosclerosis, and cardiovascular diseases. A decreased NO bioavailability leads to subsequent arterial dysfunction and other pathological consequences, including increased platelet aggregation, leukocyte and endothelial cell interaction, inflammatory cell migration, and increased vascular permeability (Alpoim, Sousa, Mota, Rios, & Dusse, 2015). Both ADMA and SDMA are regarded predictors of cardiovascular and all-cause mortality in patients with cardiovascular risk factors (Gore et al., 2013). Unlike ADMA and SDMA, L-arginine is a key substrate for NO synthesis and it is inversely associated with endothelial dysfunction and cardiovascular diseases, especially in subjects with increased ADMA levels (Böger, 2014). In our experiment administration of anthocyanin- and iridoid-rich cornelian cherry slightly increased the plasma L-arginine level. The observation that consumption of fruit may increase the L-arginine level is important, because although food protein contain some considerable amounts of L-arginine – a normal, daily western diet contains approximately 5 g of L-arginine – majority of it is metabolized in the liver in the urea

cycle, and only small amount of dietary L-arginine contributes to the regulation of vascular NO synthesis (Böger, 2014; Lüneburg et al., 2011). Nevertheless, although plasma L-arginine deficiencies are very rare, results of previous studies demonstrate that L-arginine supplementation enhances the bioavailability of NO, improves vascular ability to relax, and prevents atherosclerosis in both animals and humans (Böger et al., 1997; Napoli et al., 2006; Brinkmann et al., 2015). Likely, the beneficial effect of L-arginine supplementation depends on the ADMA level and is observed primarily in subjects with increased ADMA (Böger, 2014).

We also found a significant increase in the L-arginine/ADMA ratio in cornelian cherry-fed animals. The L-arginine/ADMA ratio is a potent indicator of NO bioavailability (Bode-Böger, Böger, Kienke, Junker, & Frolich, 1996). A low L-arginine/ADMA ratio is also independently associated with the intima/media ratio in carotid arteries (Notsu et al., 2015). Some authors suggested its role as an indicator of the early stages of atherosclerosis and subsequent cardiovascular diseases (Notsu et al., 2015; Brinkmann et al., 2015). We found that positive impact of administration of cornelian cherry on L-arginine and its methylated derivatives i.e. ADMA and SDMA, was associated with decreased intima thickness and reduced intima/media ratio in the thoracic aorta. This finding proves a possible impact of dietary cornelian cherry on early atherosclerosis through the L-arginine-ADMA pathway. Moreover, histomorphometric results confirm that changes in both L-arginine-ADMA pathway and redox system observed in our experiment occurred during atherogenesis, and also confirm that the high-cholesterol diet used in our study induced atherosclerosis.

The exact mechanism leading to increased levels of ADMA and SDMA in atherosclerosis is not fully understood. Oxidative stress may inactivate the DDAH enzyme through increased levels of ox-LDL, or of chemokines like TNFα (Ito et al., 1999). DDAH is the key enzyme responsible for ADMA metabolism into citrulline and dimethylamine in the liver. Unlike ADMA, that is eliminated by the liver, SDMA is eliminated directly by renal excretion (Bode-Böger et al., 2006). There is no evidence that changes in SDMA elimination may be correlated with DDAH activity. In our study a considerable increase of DDAH activity in the CHOL + CM group compared to both the control and the cholesterol-fed CHOL groups was found. Comparing DDAH activity with the control and the CHOL groups we found only a slight decrease in the latter one. The pronounced increase of DDAH activity in the CHOL + CM group, much higher compared to all other groups, especially the control group – was surprising. We can hypothesize that cornelian cherry may affect DDAH beyond changes in lipid levels caused by cholesterol feeding.

Another mechanism in ADMA elevation may be related to stress or enhanced native or ox-LDL up-regulation of PRMT type 1, the key enzyme responsible for ADMA synthesis (Böger et al., 2000; Osanai et al., 2003). This may suggest a need for further studies of cornelian cherry effect on PRMT expression.

We also made an attempt to compare the effect of cornelian cherry on systemic and local redox parameters in blood and the aorta, a target organ in atherogenesis. Antioxidant enzymes in blood reflect the general redox status of the organisms investigated organism. Previous studies have demonstrated that consumption of plants possessing some known antioxidant properties may affect redox parameters in blood (Bravo et al., 2014), although currently there are no guidelines indicating their concomitant measurement with levels of lipids in patients with cardiovascular risk factors, or patients suffering from cardiovascular diseases. In our study we did not find any differences between the CHOL + CM and CHOL + SIM supplemented groups versus the cholesterol-fed CHOL group. We also did not notice changes in activity of SOD in the high-cholesterol diet compared to animals fed with a regular diet, as was seen to be slightly decreased in other studies (Casamassima, Chiosi, et al., 2016; Casamassima, Palazzo, et al., 2016).

In contrast, we found differences in the redox status in the thoracic aorta of examined rabbits. Arteries are the main target organs in the development of atherosclerosis. Our results demonstrated that cornelian cherry supplementation may reduce lipid peroxidation and oxidative stress in arteries. Besides we found a pronounced increase in SOD activities in simvastatine-treated rabbits. The observation was surprising because of positive effects on other redox parameters and the lack of any significant effect on MDA.

NO inhibitors – ADMA and SDMA, as well as DDAH changes described above, depend on the redox state. Increased ADMA leads to enhanced production of O_2^-, which – like other RONS – leads to decreased L-arginine and increased ADMA levels (Daiber et al., 2013). Oxidative stress, through different redox switches, causes endothelial dysfunction, mediated by direct endothelial nitric oxide synthase (eNOS) uncoupling, and indirectly mediated by increased ADMA and L-arginine depletion, as well as other mechanisms (Daiber et al., 2013; Karbach et al., 2014). Moreover, oxidative stress enhances expression of inflammatory related genes engaged in atherogenesis (Aboonabi & Singh, 2015). Diet-induced dyslipidemia leads to the development of RONS that contribute to the development of arterial dysfunction, LDL oxidation, and inflammation with subsequent atherogenesis. All these described pathomechanisms may interact with each other and lead to a vicious circle. A diet affecting NO synthesis may break that vicious circle and diminish endothelial dysfunction and atherogenesis.

Finally, some of limitations of this study should be noted. We did not measure non-enzymatic plasma redox parameters, such as MDA or GSH. We also did not measure inflammatory blood parameters, e.g. C-reactive protein, a possible indicator of inflammation and plaque vulnerability in atherogenesis. It would be valuable to expand our subsequent surveys with the measurement of inflammatory parameters as well as redox blood parameters, and to compare them with corresponding parameters in target tissues and L-arginine-ADMA-DDAH levels.

Our study has demonstrated that the beneficial effect of the cornelian cherry on feed-induced atherosclerosis may involve the L-arginine-ADMA-DDAH pathway. Additionally, we have proven the antioxidant effect of cornelian cherry on the aorta, and pointed to possible differences in the response of systemic and vascular redox balance to both high cholesterol feed and dietary intervention with cornelian cherry fruit. Results of our study suggest that iridoid- and anthocyanin-rich fruits may modulate NO synthesis and prevent early stages of atherosclerosis, caused by its depletion.

Conflicts of interest

None.

Competing interests statement

None.

List of contributions

Tomasz Sozański

1. The main conception and design of the study
2. Participation in the acquisition of laboratory data
3. Literature search
4. Analysis and interpretation of the data collected
5. Primary contribution in drafting the article
6. Final approval and guarantor of the article

Alicja Z. Kucharska

1. Preparation of cornelian cherry lyophilisate, acquisition of laboratory data
2. Drafting the method section concerning plant materials and sample preparation
3. Critical revision of the article and submission approval

Dorota Szumny

1. Acquisition of laboratory data
2. Critical revision of article and submission approval

Jan Magdalan

1. Literature search
2. Analysis and interpretation of the data collected
3. Drafting the article
4. Critical revision of article and submission approval

Merwid-Ląd Anna

1. Literature search
2. Analysis and interpretation of the data collected
3. Critical revision of article and submission approval

Nowak Beata

1. Literature search
2. Analysis and interpretation of the data collected
3. Critical revision of article and submission approval

Piórecki Narcyz

1. Providing the cornelian cherry fruits
2. Critical revision of article and submission approval

Dzimira Stanisław

1. Histopathological evaluations, histomorphometric measures of the intima thickness, and the intima/media ratio
2. Critical revision of manuscript and its approval to be submitted

Anna Jodkowska

1. Acquisition of laboratory data
2. Critical revision of article and submission approval

Adam Szeląg

1. Literature search
2. Analysis and interpretation of the data collected
3. Critical revision of article and submission approval

Trocha Małgorzata

1. Literature search
2. Analysis and interpretation of the data collected
3. Critical revision of article and submission approval

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