# Differences between α-linolenic and linoleic acid supplementation on the redox status and cardiodynamic parameters of male and female *Wistar albino* rats

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**Abstract:** The aim of present study was to investigate the difference between α-linolenic acid (ALA, omega-3) and linoleic acid (LA, n-6) on the redox status and cardiac function of the isolated rat heart. ALA or LA were administered for 6 weeks by gavage to all animals, which were randomly divided into 4 groups: male rats treated with a linoleic acid (M-LA), dose of 7.3 mg/kg/day; female rats treated with a linoleic acid (F-LA), dose of 7.3 mg/kg/day; male rats treated with an α-linolenic acid (M-ALA), dose of 165 mg/kg/day; female rats treated with α-linolenic acid (F-ALA), dose of 165 mg/kg/day. Using the Langendorff technique, markers of heart function were evaluated: the maximum and minimum rates of pressure development in the left ventricle (LV; dp/dt max, dp/dt min), systolic and diastolic left ventricle pressure (SLVP, DLVP, respectively), heart rate (HR) and coronary flow (CF). We measured the concentrations of prooxidative markers: nitrites ($NO_2^-$), superoxide anion radicals ($O_2^-$) and hydrogen peroxide ($H_2O_2$), as well as the index of lipid peroxidation (TBARS) in the plasma and effluent. In the lysate, we measured the concentrations of reduced glutathione (GSH), catalase (CAT) and superoxide dismutase (SOD). ALA more negatively influenced the isolated rat heart, especially in females. In contrast, the administration of LA was linked to more prominent oxidative stress, while the application of ALA was associated with improved activity of the antioxidative defense system (with better values in males).

**Key words:** α-linolenic acid; linoleic acid; redox status; cardiac contractility; rat

## INTRODUCTION

Polyunsaturated fatty acids (PUFAs) are fatty acids (FAs) that contain more than one double bond in their carbon backbone [1]. Polyunsaturated FAs can be classified into different groups according to their chemical structure; essential FAs are all omega-3 and omega-6 methylene-interrupted fatty acids. Among omega-6 FA, linoleic acid (LA; 18:2) is the most common PUFA, whereas α-linolenic acid (ALA; 18:3), a *cis*-omega-3 PUFA, is the most prevalent omega-3 FA in the diet [2]. Alpha-linolenic acid (ALA), or *all-cis*-9,12,15-octadecatrienoic acid, is an 18-carbon omega-3 essential FA and is the precursor of both eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA). Linoleic acid (LA), or *all-cis*-9,12-octa-decadienoic acid, is an 18-carbon omega-6 essential FA used in the biosynthesis of arachidonic acid (AA) and, thus, some prostaglandins, leukotrienes (LTA, LTB, LTC), and thromboxanes (TXA) [3].

Almost forty years ago, it was first hypothesized that an increased dietary intake of omega-3 and omega-6 PUFAs from fish fat and plant oil could exert protective effects against several pathologies, including cardiovascular [4,5], neurodegenerative [6], neuropsychiatric [6] and inflammatory diseases [7], as well as against some cancer types [8]. Since then, a large number of preclinical studies have clarified molecular mechanisms underlying the preventive and therapeutic

effects of omega-3 PUFAs against atherosclerosis and cardiac arrhythmia [4-8], and cardiologists and other physicians have begun to prescribe them routinely [9].

There has been an enormous discontinuity in the intake of LA during the last century as the availability of vegetable oils containing LA has increased 20-fold, yet at the same time, the generally accepted benefit of LA in slowing the development of atherosclerosis has come into question [9-10]. While some studies showed that the highest category of LA intake corresponded to a 15% lower risk of coronary heart disease, others have observed no benefit of LA supplementation on coronary disease [9-12].

Since LA and ALA, as well as other PUFAs, are highly oxidizable molecules, there is concern about their use at high concentrations for the prevention of chronic diseases that have oxidative stress as an underlying mechanism [13]. Some *in vitro* and *in vivo* studies have demonstrated the protective effects of ALA and LA against oxidative stress and lipid peroxidation. However, only a few, conflicting studies have been conducted showing the antioxidant potential of these essential FAs [14-16]. Controversy still exists over the precise mechanisms by which essential fatty acids could modulate cardiovascular function.

Omega-3 PUFAs have been shown to upregulate the antioxidant system in various cells, counteracting oxidative stress as antiinflammatory and cardioprotective FAs, while the omega-6 PUFAs were shown to be proinflammatory [7,9,17]. The intake of omega 3, omega 6 and *trans*-FAs may influence testicular function and compromise cardiovascular function in males [17]. Data have suggested that the omega-3 PUFA status is inversely associated with type 2 diabetes in women but not in men, suggesting a sex-dependent effect of PUFAs [18]. Therefore, LA and ALA likely differentially modulate the redox status and cardiac function in different genders.

Using an *ex vivo* model of the isolated rat heart and biochemical analyses, we investigated the difference between the effects of ALA omega-3 and LA omega-6, on the redox status and cardiac function of rat heart. Additionally, this study also examined the controversy over the use of these FAs as preventive/curative tools for cardiovascular disease and pays special attention to the role of gender in this.

## MATERIALS AND METHODS

### Ethics statement

All research procedures were carried out in accordance with the European Directive for the Welfare of Laboratory Animals, No. 86/609/EEC and the principles of good laboratory practice (GLP). The protocol of the current study was approved by the Ethics Committee for experimental animal well-being of the Faculty of Medical Sciences at the University of Kragujevac, Serbia.

### Animals and experimental design

This study was carried out using 48 adult male and female *Wistar albino* rats (24 weeks old; b.w. 550±50 g). Animals were housed under controlled environmental conditions, at 25°C and a 12-h light/dark cycle with *ad libitum* access to food and tap water. For 6 weeks, the FA ALA or LA were administered by gavage to all animals [19], which were randomly divided into 4 groups as follows: (i) male rats treated with linoleic acid (M-LA) at a dose of 7.3 mg/kg/day [20]; (ii) female rats treated with linoleic acid (F-LA) at a dose of 7.3 mg/kg/day; (iii) male rats treated with α-linolenic acid (M-ALA) at a dose of 165 mg/kg/day [21]; (iv) female rats treated with α-linolenic acid (F-ALA) at a dose of 165 mg/kg/day.

### Isolated heart perfusion

On the 43rd day, after 6 weeks of treatment with the different FA, all animals were euthanized by cervical dislocation without the use of an anesthetic in order to prevent any drug interactions. The chest was then opened via midline thoracotomy. The hearts were immediately removed, immersed in cold saline and mounted on a stainless-steel cannula on the Langendorff perfusion apparatus that provided retrograde perfusion under gradually increasing coronary perfusion pressure (CPP from 40 to 120 cmH$_2$O). Krebs-Henseleit buffer was used for retrograde perfusion (in mmol/L: NaCl 118, KCl 4.7, CaCl$_2$x2H$_2$O 2.5, MgSO$_4$x7H$_2$O 1.7, NaHCO$_3$ 25, KH$_2$PO$_4$ 1.2, glucose 11 and pyruvate 2). The buffer was equilibrated with 95% O$_2$ and 5% CO$_2$, pH 7.4 at 37°C. Following initiation of the heart perfusion, the preparations were stabilized

within 30 min with a basal coronary perfusion pressure of 70 $cmH_2O$. Following the stabilization period, the perfusion pressure was reduced to 50 and then to 40 $cmH_2O$; the pressure was then gradually increased to 60, 80, 100 and 120 $cmH_2O$ to establish coronary autoregulation. Testing started immediately after the control experiment to avoid unwanted, time-dependent consequences. With a sensor placed in the left ventricle, software was used to measure the cardiodynamic markers at each perfusion pressure (40, 60, 80, 100 and 120 $cmH_2O$). Markers of heart function included maximum and minimum rates of pressure development in the LV (dp/dt max, dp/dt min), systolic and diastolic left ventricle pressure (SLVP, DLVP, respectively), heart rate (HR) and coronary flow (CF). We evaluated all of the mentioned parameters during hypoxic (40 $cmH_2O$), normoxic (60-80 $cmH_2O$) and hyperoxic conditions (100-120 $cmH_2O$) according to coronary perfusion pressure (CPP).

### Biochemical analyses

Blood samples were collected after 42 days of PUFA treatment and as the rats were killed by exsanguination. Samples of the coronary venous effluent were collected after stabilization of the coronary flow and for each perfusion pressure.

### Preparation of plasma and erythrocyte lysate

Blood was collected into vacutainer tubes with anticoagulant (sodium citrate) and centrifuged for 10 min at 2000 x g to separate the plasma. The obtained plasma was used for determination of prooxidative markers: nitrites (the amount of NO released), superoxide anion radicals ($O_2^-$), and hydrogen peroxide ($H_2O_2$), for the indirect quantification of the index of lipid peroxidation with reactive thiobarbituric substances (TBARS), which were measured by spectrophotometric quantification in the plasma. These parameters were also measured in the effluent samples. The remaining packed red blood cells (RBC) were washed three times with normal saline to remove the buffy coat. Hemolysis was performed by pipetting out 1 mL of washed RBC suspension in ice cold distilled water. RBC ghosts were sedimented in a high-speed refrigerated centrifuge at 10000 x g for 40 min. The cell

content was separated carefully and used for reduced glutathione (GSH), catalase (CAT) and superoxide dismutase (SOD) estimation. For hemoglobin (Hb) estimation we used the lysate and an equal volume of Drabkin's reagent, which were mixed and left for 3 min to determine the hemoglobin concentration. Absorbance was read at 540 nm against a reagent blank. Hemolysates containing about 50 g Hb/L and CAT, SOD and GSH were calculated as $U/Hbx10^3$ according to McCord and Fridovich [22].

### Determination of prooxidative parameters: superoxide anion radicals ($O_2^-$), nitrites ($NO_2^-$), hydrogen peroxide ($H_2O_2$), and the index of lipid peroxidation measured as TBARS

The concentration of the $O_2^-$ radical was measured using the nitro blue tetrazolium (NBT) reagent in TRIS buffer (assay mixture) with coronary venous perfusate or plasma. The measurement was performed at 530 nm. The Krebs-Henseleit solvent served as the blank for effluent samples, and distilled water was the blank for plasma samples [23].

Nitric oxide (NO) rapidly decomposes into stable nitrite and nitrate metabolites. Nitrites can therefore be used as an index of NO production via a spectrophotometric method that uses the Griess reagent. Briefly, 0.5 mL of the perfusate or plasma was precipitated with 200 μL of 30% sulfosalicylic acid, mixed for 30 min and centrifuged at 30000 x g. Equal volumes of the supernatant and Griess reagent were mixed and stabilized for 10 min in the dark and the sample was measured spectrophotometrically at 543 nm. Nitrite concentrations were determined using sodium nitrite as the standard [24].

Hydrogen peroxide ($H_2O_2$) measurement was based on the oxidation of phenol red by $H_2O_2$ in a reaction catalyzed by horseradish peroxidase (HRP). A total of 200 μL of perfusate or plasma was precipitated with 800 mL of freshly prepared phenol red solution, followed by the addition of 10 μL of (1:20) HRP (made *ex tempore*). For the blank, Krebs-Henseleit solution or distilled water was used. The concentration of $H_2O_2$ was measured at 610 nm [25].

The index of lipid peroxidation was determined indirectly by measuring the products of the reaction

with thiobarbituric acid (TBA) or reactive substances (RS). Briefly, 1% TBA in 0.05 M NaOH was incubated with coronary venous perfusate or plasma at 100ºC for 15 min and then spectrophotometrically measured at 530 nm. The Krebs-Henseleit solvent or distilled water served as the blanks [26].

### Determination of antioxidant parameters

In the RBC lysate, we determined the concentrations of nonenzymatic antioxidants, such as reduced glutathione (GSH), and the activities of the antioxidant enzymes, catalase (CAT) and superoxide dismutase (SOD).

CAT activity was determined according to Beutler [27]. The lysates were diluted with distilled water (1:7 v/v) and treated with chloroform-ethanol (0.6:1 v/v) to remove Hb. Fifty μL of CAT buffer, 100 μL of sample and 1 mL of 10 mM $H_2O_2$ were mixed. Detection was performed at 360 nm.

SOD activity was determined by the McCord method [22]. The lysate (100 μL) and 1 mL of carbonate buffer were mixed together and 100 μL of epinephrine was added. Detection was performed at 470 nm.

The concentration of reduced glutathione (GSH) was determined based on GSH oxidation with 5,5'-dithiobis(2-nitrobenzoic acid), using the method by Beutler [28].

### Drugs

α-Linolenic acid (product number L2376) and linoleic acid (product number L1376), both of ≥99% purity, were purchased from Sigma-Aldrich Chemie GmbH, Eschenstr. 5, Taufkirchen, Germany.

### Statistics

We used traditional parameters of descriptive statistics: average value±standard deviation (SD), and minimal and maximal values. Normality of the parameter distribution was evaluated with the Kolmogorov-Smirnov test. Dependent variables were compared using an analysis of variance (ANOVA) for repeated measurements (*post hoc* Bonferroni test). For independent groups, we used ANOVA in one direction (*post hoc* Tukey test). Statistical significance was based on $p<0.05$. Complete statistical evaluation was performed with SPSS Statistics 22 (SPSS, Chicago, IL).

## RESULTS

### Differences between chronic treatments with LA and ALA on the cardiodynamic parameters of isolated hearts of male and female rats

The values of dp/dt min (at all CPPs) and SLVP (60 cm CPP) after chronic administration of LA were significantly lower in male than in female rats (Fig. 1A-D). The values of CF were higher in the male group when compared with the female group under hypoxic (40-60 cm CPP) and normoxic conditions (80 cm CPP) (Fig. 1F). There were no differences in HR between the groups (Fig. 1E). Females treated with ALA had lower values of dp/dt max than males only under hypoxic conditions (Fig. 1A). In addition, CF and HR were also lower at all CPPs (Fig. 1 E and F).

In males, the values of SLVP (40, 60 cm CPP) and DLVP (at all CPPs) were lower in the ALA group as compared to the LA group (Fig. 1C and D). In contrast, ALA enhanced the HR and CF only in hypoxic conditions as compared with LA (Fig. 1E and F). In females, the values of all examined cardiodynamic parameters (except HR) were lower in the ALA group when compared to the LA group at all CPPs (Fig. 1A-D). CF was also lower under hyperoxic conditions (100 and 120 cm CPP) (Fig. 1E and F).

### Differences between chronic treatments with LA and ALA on the biomarkers of oxidative stress in isolated heart (male and female rats)

Male rats treated with LA had higher values of $H_2O_2$ and $NO_2$ (in hypoxic conditions) when compared to female rats (Fig. 2 B and D). In contrast, under normoxic and hyperoxic conditions, the females had higher values of $O_2^-$ and TBARS (Fig. 2A and C). Male rats treated with ALA showed a larger release of all examined biomarkers of oxidative stress (Fig. 2A-D).

Male groups treated with ALA had higher values of measured cardiac oxidative markers as compared to the LA groups (Fig. 2A-D). The values of $NO_2^-$ were higher only in hypoxic conditions (Fig. 2D), and of
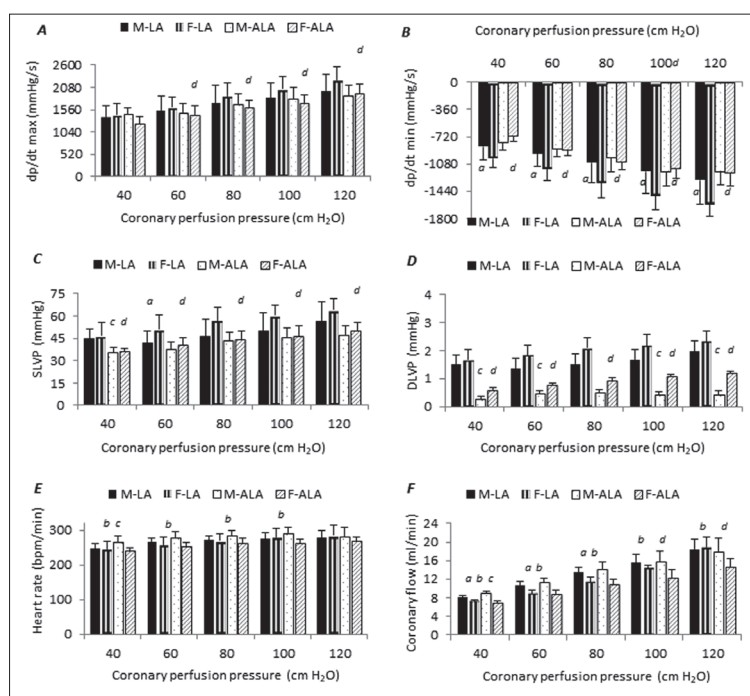
**Fig.1**. The effects of chronic administration of LA and ALA on the cardio-dynamic parameters of isolated rat heart. **A** – The maximum rate of pressure development in the left ventricle (dp/dt max); **B** – the minimum rate of pressure development in the left ventricle (dp/dt mix); **C** – SLVP; **D** – DLVP; **E** – HR; **F** – CF. All values are expressed as the means±standard deviation. Statistically significant differences (p<0.05) between the groups at the same coronary perfusion pressure are marked as follows: *a* – differences between the sexes treated with LA, *b* – differences between the sexes treated with ALA, *c* – differences between male rats treated with LA or ALA, *d* – differences between female rats treated with LA or ALA.
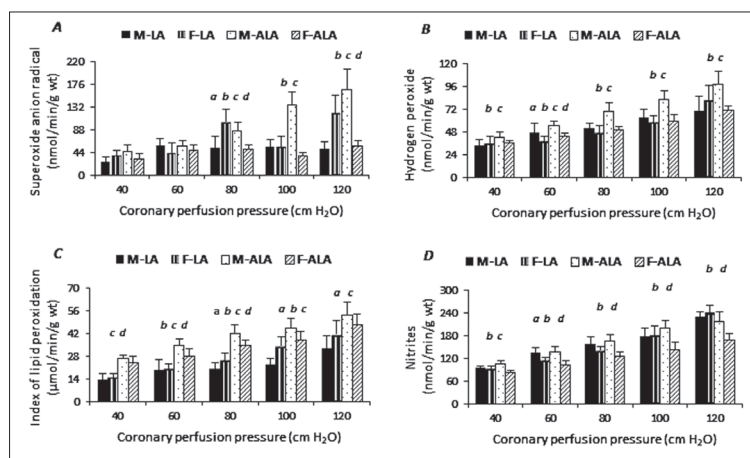


**Fig. 2.** The effects of the chronic administration of LA and ALA on the bio-markers of oxidative stress in isolated rat heart. **A** – $O_2^-$ concentration; **B** – $H_2O_2$ concentration; **C** – the index of lipid peroxidation, measured as TBARS; **D** – the concentration of nitrites ($NO_2^-$).

$O_2^-$ in normoxic and hyperoxic conditions (Fig. 2A). Female groups treated with ALA exhibited a stronger release of $H_2O_2$ (normoxic conditions) and TBARS (hypoxic and normoxic conditions) (Fig. 2B and C), and a lower release of $O_2^-$ and $NO_2^-$ (normoxic and hyperoxic conditions) in comparison to the LA groups (Fig. 2A and D).

**Differences between chronic treatments with LA and ALA on the biomarkers of oxidative stress in blood samples of male and female rats**

Males treated with LA showed higher release of $O_2^-$ and $NO_2^-$ (Fig. 3A and D). There were no statistically significant differences in the production of $H_2O_2$ and TBARS between the sexes (Fig. 3B and C). Males treated with ALA also had higher values of $O_2^-$ (Fig. 3A). There were no statistically significant differences in the production of other examined biomarkers between the sexes (Fig. 3B-D).

The male ALA group had lower values of TBARS and $NO_2^-$ when compared to the LA group (Fig. 3C and D). There were no statistically significant differences in the production of $O_2^-$ and $H_2O_2$ between the compared groups (Fig. 3A and B). In females, there were no statistically significant differences in the production of all of the examined biomarkers between groups (Fig. 3A-D).

**Differences between chronic treatments with LA and ALA on the levels of antioxidant markers in blood samples of male and female rats**

Males treated with LA had higher activities of SOD and CAT (Fig. 4A and B), while the concentration of GSH was lower (Fig. 4C). In contrast, males treated with ALA had a higher activity of SOD and concentration of GSH (Fig. 4A and C), whereas CAT activity was unchanged in both sexes (Fig. 4B).
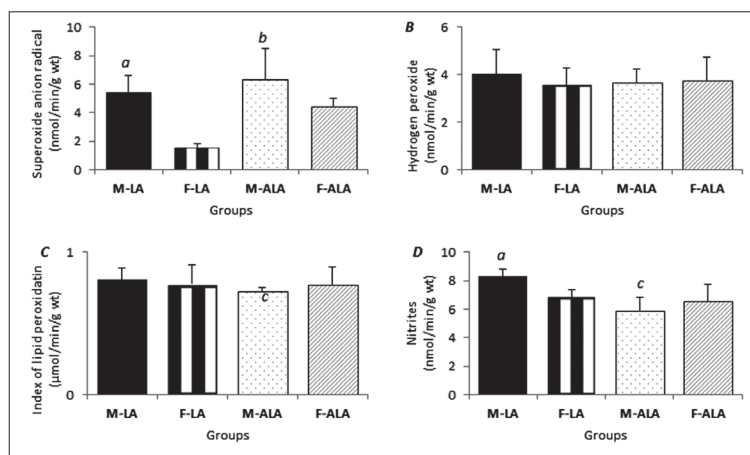
**Fig. 3.** The effects of the chronic administration of LA and ALA on the levels of prooxidant markers in blood samples. **A** – $O_2^-$ concentration; **B** – $H_2O_2$ concentration; **C** – the index of lipid peroxidation, measured as TBARS; **D** – the concentration of nitrites $NO_2^-$.
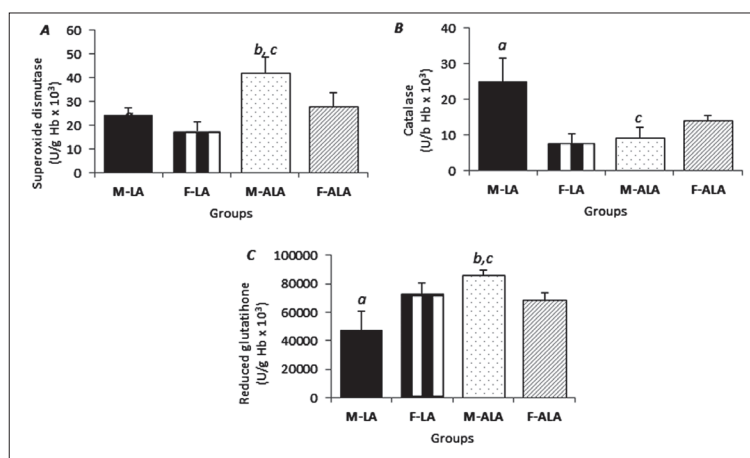


**Fig. 4.** The effects of the chronic administration of LA and ALA on the levels of antioxidant markers in blood samples, **A** – SOD; **B** – CAT; **C** – GSH.

The ALA group had higher values of SOD activity and GSH concentrations (Fig. 4A and C) and lower values of CAT activity when compared with the LA group (Fig. 4B). In females, there were no statistically significant differences in antioxidant markers between the compared groups (Fig. 4A-C).

## DISCUSSION

The aim of the current study was to investigate the differences between the two FAs, α-linolenic acid (ALA, omega-3) and linoleic acid (LA, omega-6), on the redox status and cardiac function of rat heart. Additionally, this study aimed to evaluate the potential gender-related differences in response to ALA and LA treatment.

The two major types of long-chain PUFAs, omega-3 and omega-6, were shown to have antagonistic effects [1-7]. While omega-3 PUFAs emerged as anti-inflammatory and cardioprotective, omega-6 PUFAs were shown to be proinflammatory. To test these assumptions and the differences between these FAs on cardiac contractility, we evaluated the changes in cardiac function after 6 weeks of FA treatment using the following markers of heart function: maximum and minimum rates of pressure development in the LV (dp/dt max, dp/dt min), SLVP, DLVP, HR and CF. Myocardial contractile dysfunction, a major manifestation of heart failure, can be revealed through the mechanical defects of left ventricular pressure development (+dp/dt max) and the rate of relaxation, as measured by LV negative (-dp/dt min).

The contractility parameters were lower in the F-ALA group, while the same parameters were not affected in male groups. The other important indicators used to assess LV (SLVP and DLVP) were higher in the F-LA group (compared with the F-ALA). HR had the most drastic decrease in the M-ALA group. Interestingly, CF was lower in male animals treated with LA in contrast to female rats. When comparing the effects of LA between the male and female rats, lusitropic force and cardiac perfusion was lower in the female group. When comparing the effects of ALA between male and female rats, the only differences were observed in HR and CF.

A likely explanation for the cardiac effects of PUFA is that application of FA has profound effects on calcium management. PUFA can also produce a dose-dependent reduction in sodium and calcium currents by inhibiting the ryanodine receptor (RyR). These findings support the hypothesis that a lower omega-3 index may also be a marker of an increased propensity for the hypertensive rat heart towards malignant arrhythmias [29]. Other studies have pre-

sented similar results [28]. In accordance with their results, our findings suggest that these two FAs induce changes in cardiac function of isolated rat heart and act in opposite manners between genders. Generally, compared with ALA, LA significantly affected cardiac function and strongly disturbed heart muscle function, especially in female animals. It is probable that the duration of FA treatment is one of the crucial factors in the development of cardiac dysfunction and consequently of heart hypertrophy.

LA intake may compromise the omega-3 PUFA status of the tissue since its conversion to omega-6 LC-PUFA shares a common enzymatic pathway with the n-3 family [30]. The ALA diet led to higher levels of omega-3 LC-PUFAs, including DHA in the brain and heart [30]. One investigator described the different distribution of omega-6 and omega-3 PUFAs throughout the whole rat body, and 18:2 omega-6 was the most concentrated in the heart (13 wt%); this may be one of the reasons for the diverse effects of LA and ALA on heart function in our study [31].

One more mechanism whereby LA and ALA can modulate cardiac function was demonstrated by Berecki et al [31]. They stated that membrane incorporation of PUFAs reduces the response to norepinephrine. In particular, incorporated PUFAs blunted the increase in the sarcoplasmic reticulum (SR) calcium content produced by norepinephrine and therefore attenuated the increase in transient calcium produced by norepinephrine.

To determine the mechanism by which these FAs act on cardiac function *in vitro*, we evaluated the effects of LA and ALA on the prooxidative markers and parameters of systemic redox balance in the heart. In our study, female animals treated with LA exhibited a higher release of $O_2^-$, while the male groups treated with ALA had higher values of $H_2O_2$. On the other hand, TBARS levels were significantly changed in almost all groups, with a higher release of both TBARS and NO observed in all female groups after treatment with either LA or ALA. It important to note that in the M-ALA group, we observed a higher rate of coronary flow and all the prooxidative parameters measured from the effluent had a positive correlation with coronary flow. Most likely, FA-induced cardiac dysfunction is a consequence of myocardial disorders

but not of coronary circulation pathologies or endothelial dysfunction.

In the third part of this study we decided to exclude the effects of coronary flow rate by measuring all prooxidative and antioxidant markers from rat blood. We found higher values of $O_2^-$ after treatment with both FAs in the male group, and higher levels of TBARS and nitrites in the M-LA group. These results are in accordance with the results of other relevant studies. Di Nunzio et al. [16] investigated the effect of LA and ALA on oxidative stress and confirmed the assumption that LA strongly induces lipid peroxidation. It is known that omega-3 PUFAs are important components of cell membranes that affect their function, and an omega-3 deficiency is deleterious to health. They are, however, prone to lipid peroxidation due to their many double bonds [4-10]. The metabolic reactions within the omega-3 and omega-6 PUFA families take place in the cell endoplasmic reticulum, apart from the last reaction of β-oxidation, which takes place in peroxisomes, thereby requiring translocation of adequate substrates into this cell compartment. PUFA residues of membrane phospholipids are very sensitive to oxidation and the action of reactive oxygen species (ROS).

Omega-3 PUFAs are known to have antiinflammatory effects. Excess production of NO is associated with inflammation [4,5]. NO is synthesized from L-arginine by NO synthase (NOS) with NADPH and oxygen as cosubstrates [32]. Inducible NOS (iNOS) is the key enzyme that produces large amounts of NO from macrophages stimulated by the bacterial endotoxin lipopolysaccharide (LPS) and by proinflammatory cytokines such as interferon-γ (IFN-γ) and tumor necrosis factor (TNF) [33]. In the present study, NO was significantly higher after LA supplementation, but the elevated levels of this parameter were decreased after ALA treatment.

Furthermore, SOD activity and GSH were significantly higher in the ALA group, in contrast to CAT activity. Generally, all antioxidant markers were significantly higher in ALA male and female groups, while LA induced higher levels of antioxidant parameters in the male group. Although different omega-3 acids share common chemical characteristics, a previous work showed that EPA and DHA modulate the

activity of antioxidant enzymes in different manners and they have different impacts on cell oxidative status. Popovic et al. [34] suggested that CAT activity and nitrite concentrations in liver were significantly decreased after the EPA/DHA supplementation. On the other hand, sex-related differences in this investigation indicate that the cardiodepressive effects of LA were more obvious in female rats during hypoxic conditions. Generally, males had higher values of cardiac and systemic oxidative parameters which was accompanied by a compensatory higher mobility of antioxidative protection.

Differences in the effects of LA and ALA on male and female animals may be related to the characteristics of male and female organisms. First, a higher content of body fat is characterized by a favorable fatty acid composition [35]. Second, the intake of omega-3 PUFAs is positively related to testicular volume, while the intake of omega-6 PUFAs is inversely related to testicular volume.

Finally, both fatty acids achieved their effects in hypoxic and hyperoxic conditions, suggesting that the level of oxygenation can also be an important factor in the response of the heart to the applied PUFAs. Thus, it seems that during normoxic conditions the heart is more resistant to their supplementation. ALA more negatively influenced the isolated rat heart in females. The administration of LA was connected to more prominent oxidative stress, while the application of ALA was also associated with improved activity of the antioxidative defense system (displaying higher values in males).

## CONCLUSION

The findings of present study may help to elucidate the difference between the effect of ALA and LA on the heart and on local and systemic oxidative stress. The obtained results demonstrate that these PUFAs had a cardiodepressive impact on the heart, especially ALA. ALA apparently possesses antioxidative properties. Moreover, the effects of both FA were gender-specific in terms of exhibiting more negative effects in females. Lastly, ALA can be considered a beneficial supplement in all conditions and pathophysiological processes characterized by increased ROS production.

**Author contributions:** KR and VJ designed the study, IS, DR, JJ and TN collected and analyzed all data, IS and VZ performed statistical analyses. All authors approved final version of manuscript.

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