



Research article

The role of fish oil in attenuating cardiac oxidative stress, inflammation and fibrosis in rat model of thyrotoxicosis

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ABSTRACT

Hyperthyroidism is associated with cardiovascular complications. Fish oil reduces risk of cardiovascular diseases. This study aims to evaluate the impact of fish oil on myocardial oxidative stress, inflammation and fibrosis in rat model of thyrotoxicosis. Rats were randomized into four groups; control rats, fish oil treated rats (FO, 100mg omega-3/100g body weight/day), hyperthyroid rats (Hyper, i.p levothyroxine 3 mg/kg/day), and hyperthyroid rats treated with fish oil (Hyper + FO) for 8 weeks. Changes in oxidants/antioxidants, inflammatory and fibrotic markers were measured. Thyrotoxicosis increased serum endothelin-1, thiobarbituric acid reactive substances (TBARS) and reduced activities of cardiac catalase and super oxide dismutase (SOD). Cardiac fibrosis paralleled with a decrease of matrix metalloproteinase -2 (MMP2) levels were observed in Hyper group. Use of FO increased activities of SOD and catalase, increased TBARS levels, and attenuated cardiac fibrosis by normalizing MMP-2 levels. Use of FO may attenuate cardiac oxidative stress and fibrosis in hyperthyroid states.

1. Introduction

Hyperthyroidism is a pathological condition defined by elevated serum levels of thyroid hormones [1]. Hyperthyroidism is associated with long-term cardiovascular complications including increased risk of angina, atrial fibrillation and heart failure [2]. In overt hyperthyroidism, risk of cardiovascular diseases (CVDs) increases up to 1.7 fold [3]. Cardiomyocytes are very sensitive and highly responsive to thyroid hormones due to a wide distribution of thyroid hormone receptors [4].

Oxidative stress, characterized by the imbalance between the production of free radical molecules and the ability of the body to neutralize them using anti-oxidant enzymes [5], is linked to CVD [6]. Excessive mitochondrial reactive oxygen species (ROS) generation promotes inflammation by activation of many redox and inflammatory signaling pathways such as the nuclear factor (NF)-kappa B. Both oxidative stress and inflammation mediate cardiac remodeling and fibrosis. Fibrosis

alters cardiac structure and function predisposing into CVDs [7]. The impact of hyperthyroidism on cardiac fibrosis is unclear.

In the myocardium, fatty acids are the most important source of energy. Recent studies focused on the utility of fish oil (FO) to prevent cardiovascular diseases development and progression. Fish oil is rich in omega-3 polyunsaturated fatty acids (ω -3 PUFA). The omega-3 fatty acids are known to be anti-inflammatory and anti-thrombotic affecting lipid mediators and various signaling pathways [8]. Previous studies showed that ω -3 PUFA can decrease serum triglycerides (TG) and lower the risk of arrhythmia, coronary artery disease (CAD) and heart failure (HF) [9, 10].

In human and animal models, the use of ω -3 PUFA reduce in-vivo oxidative stress by increasing antioxidant levels of superoxide dismutase (SOD) and glutathione (GSH) [11]. The ω -3 PUFA attenuate inflammation and prevent cardiac dysfunction associated with pressure volume overload [12]. In cardiac surgery model, the intake of high dose of ω -3 PUFA for 3 weeks decreased plasma and cardiac endothelin 1, peak C-reactive protein levels, inducible nitric oxides and risk

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Table 1. Characteristics of animal groups at 8 weeks.

	Control	FO	Hyper	Hyper + FO	P value
Body WT (g)	269.3 ± 7.64	290.6 ± 4.88#	245.7 ± 3.66*	263.7 ± 6.43\$	<0.0001
Heart WT (g)	0.953 ± 0.031	1.01 ± 0.029#	1.39 ± 0.046****\$	1.37 ± 0.042***\$	<0.0001
Heart WT/BWT (mg/g)	4.29 ± 0.135	4.43 ± 0.114#	6.09 ± 0.214****	6.00 ± 0.170****\$	<0.0001
Free T3 (pg/ml)	5.94 ± 0.649	5.92 ± 0.463	692.5 ± 101.4****	604.2 ± 67.6****	<0.0001
Free T4 (ng/dl)	9.79 ± 1.05	7.42 ± 0.697	472.1 ± 57.09****	460.3 ± 68.2****	<0.0001
Systolic Bp (mmHg)	120.6 ± 0.54	122.3 ± 0.38#	125.9 ± 0.80****\$	126.3 ± 0.47***\$	<0.0001
Diastolic Bp (mmHg)	68.8 ± 0.47	66.1 ± 0.46**	67.78 ± 1.29	66.64 ± 0.41*	0.0033
Heart Rate (beat/min)	411.5 ± 7.41	433.6 ± 13.44	489.1 ± 21.06*	435.1 ± 9.63	0.0271
Serum Triglyceride	104.9 ± 11.86	87.97 ± 10.95	117.0 ± 11.03	113.8 ± 13.85	0.2007
Serum HDL	14.03 ± 0.944	10.62 ± 0.498	17.96 ± 1.44\$	18.63 ± 1.68\$	<0.0001
Serum LDL	34.4 ± 2.93	20.88 ± 1.93*	37.35 ± 4.19\$	28.53 ± 4.27	0.0053
Serum Cholesterol	68.33 ± 3.29	49.09 ± 3.04**	72.23 ± 6.25\$	64.59 ± 5.07	0.0027

Data are presented as mean ± sem. FO: fish oil, Hyper: hyperthyroidism, WT: weight, BWT: body weight, Bp: blood pressure. Unit for serum lipids is mg/dl. Number is 10–15 reading each group. *p < 0.05, **<0.01, ***<0.001, <0.0001 vs. control, # vs. Hyper, \$ vs. FO.

of post-operative atrial fibrillation [13]. However, the impact of fish oil on factors affecting cardiac disease during hyperthyroidism is unclear.

The aim of this study was to evaluate the effect of fish oil on cardiac inflammation, oxidative stress, and fibrosis in rat model of thyrotoxicosis induced by levothyroxine.

2. Methodology

2.1. Animals and treatments

Adult male Wistar rats (200–250 gm) were housed in metal cages (5 rats per cage) under hygienic conditions and were kept at 24 °C and 12 h light/dark cycle with free access to adequate food and water. An ethical approval to perform the study was obtained by the Animal Care and Use Committee (ACUC) at Jordan University of Science and Technology (JUST). All animal procedures were performed in accordance with the ACUC regulations at JUST, which comply with the National Institutes of Health (NIH) guide for the care and use of laboratory animals (NIH Publications No. 8023, revised 1978).

Rats were randomized into four main groups (15 rats for each group):

1. Group 1 (control): Rats were infused with 1% intra-peritoneal (i.p) bovine serum albumin (BSA) buffer dissolved in 0.01 M NaOH, and were given corn oil (4 µL/g body weight) by oral gavage.
2. Group 2 (FO): Rats received Menhaden fish oil at a dose of 100mg omega-3/100g body weight/day by oral gavage and infused with i.p 1% BSA buffer dissolved in 0.01 M NaOH [14, 15].
3. Group 3 (Hyper): Rats were infused with i.p levothyroxine injection (T4, 3 mg/Kg/day, SIGMA-ALDRICH, Germany) freshly dissolved in 1% BSA buffer of 0.01 M NaOH, and were given corn oil (4 µL/g body weight) by oral gavage.
4. Group 4 (Hyper + FO): rats were infused with i.p levothyroxine, and given fish oil by oral gavage as described above.

2.2. Assessment of body weight and blood pressure

Body weight was read at baseline and then twice weekly for 8 weeks. Blood pressure was taken at the end of the study using the tail-cuff plethysmography method (computerized tail cuff plethysmography blood pressure system, IITC Life Science, Woodland Hills, CA, USA) as previously described [16]. Systolic blood pressure (SBP), diastolic blood pressure (DBP) and heart rate were recorded. Blood pressure for each rat was tested three times and data were averaged to produce a single valid value.

2.3. Blood collection and measurements of serum thyroxine (T4) and 3, 5, 3'-triiodothyronine (T3)

After 8 weeks, animals were killed by decapitation and fresh blood samples were collected. Serum samples were prepared by centrifugation at 5000 rpm for 10 min in serum tubes. Serum levels of thyroxine (T4) and 3, 5, 3'-triiodothyronine (T3) hormones were measured using Cobas e 411 analyzer (Roche Diagnostics GmbH, D-68298 Mannheim, Germany).

2.4. Analysis of serum and cardiac biomarkers

Following decapitation, hearts were removed, washed, and weighed. A small section from the right side of the heart was obtained from 5 rats in each group and was fixed in 10% formalin for histopathology. Tissue homogenizer (OMNI International, Tissue master 125, Kennesaw, USA) was used to homogenize residual heart tissues in cold phosphate buffer saline (PBS) containing protease inhibitor (Sigma Aldrich, MI, USA). Homogenized tissues were centrifuged at 15,000 rpm for 10 min at 4 °C. The lysates were collected in aliquots and stored at -80 °C until analysis. All procedures were carried out over crushed ice. Serum triglyceride and high density lipoprotein (HDL) levels were evaluated using colorimetric kinetic assay according to kit instructions (Biosystems S.A., Barcelona, Spain). Serum cholesterol levels were measured using colorimetric kinetic assay (Arcomex, Cholesterol kit, Amman, Jordan). Rat myeloperoxidase (MPO) ELISA kit (My BioSource Inc. CA, USA) was used for evaluation of MPO levels in cardiac homogenates. Cardiac and serum endothelin-1 protein levels were measured using sandwich ELISA (R&D systems, MA, USA). Total glutathione (GSH) levels were determined kinetically using the GSH assay kit (Sigma-Aldrich, MI, USA). Cardiac catalase enzyme activities were determined using specific kinetic kit (Cayman Chem., Ann Arbor, MI, USA). Superoxide dismutase (SOD) assay kit was used to examine cardiac activities of SOD (Sigma-Aldrich Corp., MI, USA). Thiobarbituric acid reactive substances (TBARS) and total nitrite levels were quantified using TBARS Assay kit and Griess reaction assay (R&D Systems, MA, USA); respectively. Measurements of cardiac MMP-2 and TGF-β1 protein levels were performed using sandwich ELISA kits (R&D systems, MA, USA). Plates were read at a wavelength specified for each kit using Epoch Biotek microplate reader (Bio-Tek, Winooski, VT, USA). All measurements were normalized to total protein concentrations (DC-Biorad, Hercules, CA, USA).

2.5. Masson's trichrome staining

Paraffin embedded sections (4µm thick) were deparaffinized by xylene and graded alcohol and washed with distilled water. Sections were then incubated with Bouin's solution overnight. Slides were washed

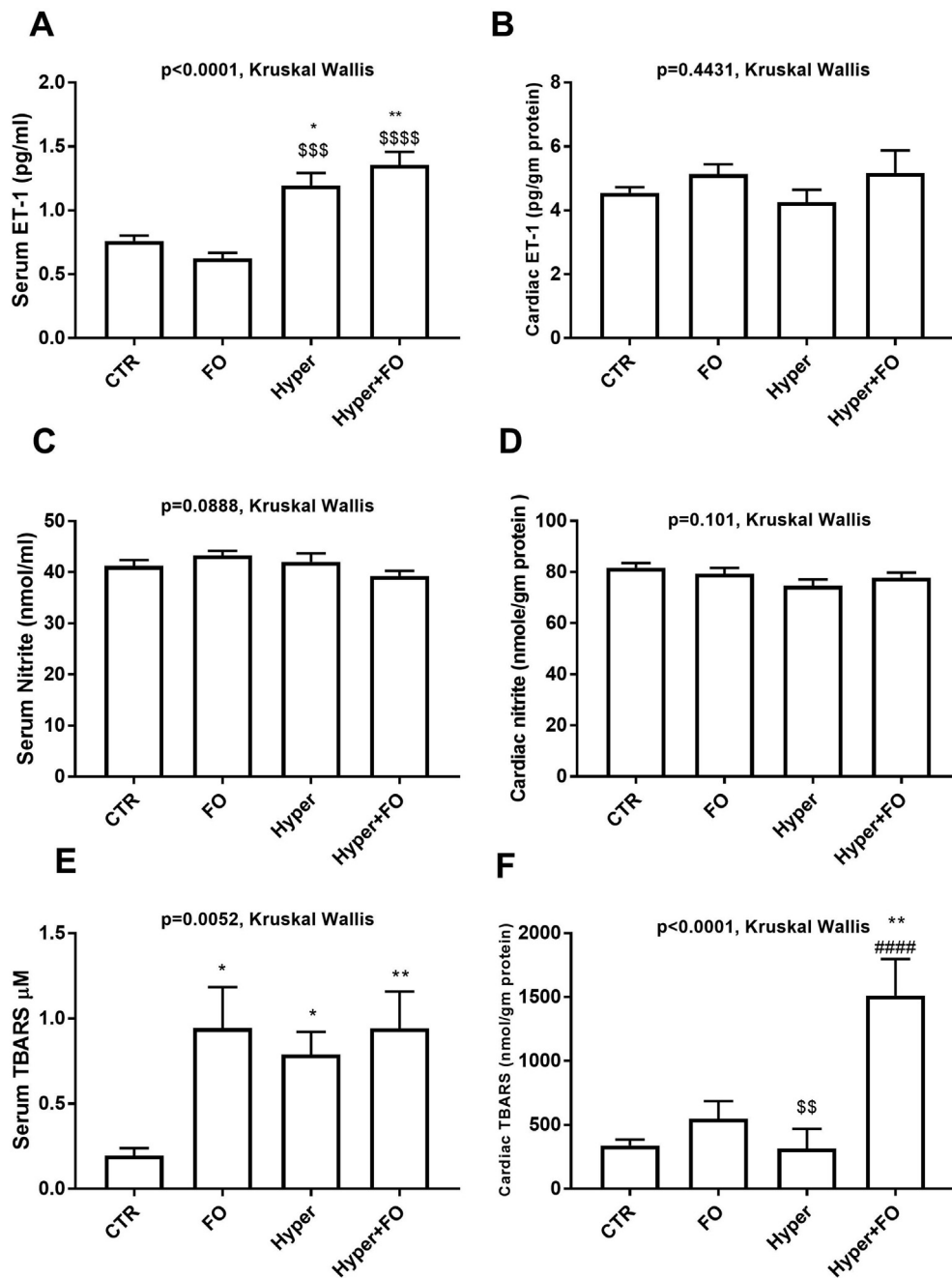


Figure 1. Levels of endothelin-1, nitrite and TBARS. Levels of serum and cardiac ET-1 (A–B), total nitrite (C–D), and TBARS (E–F) in the control (CTR); FO treated animals (FO); hyperthyroid animals (Hyper); and hyperthyroid animals treated with FO (Hyper + FO). * $p < 0.05$, ** $p < 0.01$, **** $p < 0.0001$ vs. Control; # $p < 0.05$, #### $p < 0.0001$ vs. Hyper; \$ $p < 0.05$, \$\$ $p < 0.01$ vs. FO.

with distilled water and stained with Masson's Trichrome according to manufacture instructions (Sigma-Aldrich Corp, St. Louis, MO, USA). Following gentle washing, slides were dehydrated by graded alcohols, cleared in xylene and cover-slipped. Sections were viewed at 100x magnification using high resolution light microscope (Nikon, Japan). Quantification of percentage area of fibrosis was performed by the NIH Image J software version 1.50i, USA. For each slide, 3 representative images were quantified and averaged to produce a single value.

2.6. Statistical analysis

Data are presented as mean \pm sem for continuous variables. Because molecular data were not normally distributed, Kruskal-Wallis test was

used to measure differences in serum and cardiac biomarkers. The Dunn's post-test was then used for pair wise comparisons. Statistical significance was considered at a $P < 0.05$. Graph Pad Prism 7 (La Jolla, CA, USA) was used for all analyses.

2.7. Theory/calculation

Previous studies have identified a key role for inflammation, oxidative stress and remodeling in the development and progression of CVDs. Hyperthyroidism is a secondary cause of CVDs, however, the mechanisms are not fully clear. Several experimental and clinical studies have documented the cardio-protective effects of fish oil in arrhythmia and coronary artery disease. However, the impact of fish oil on the substrates of

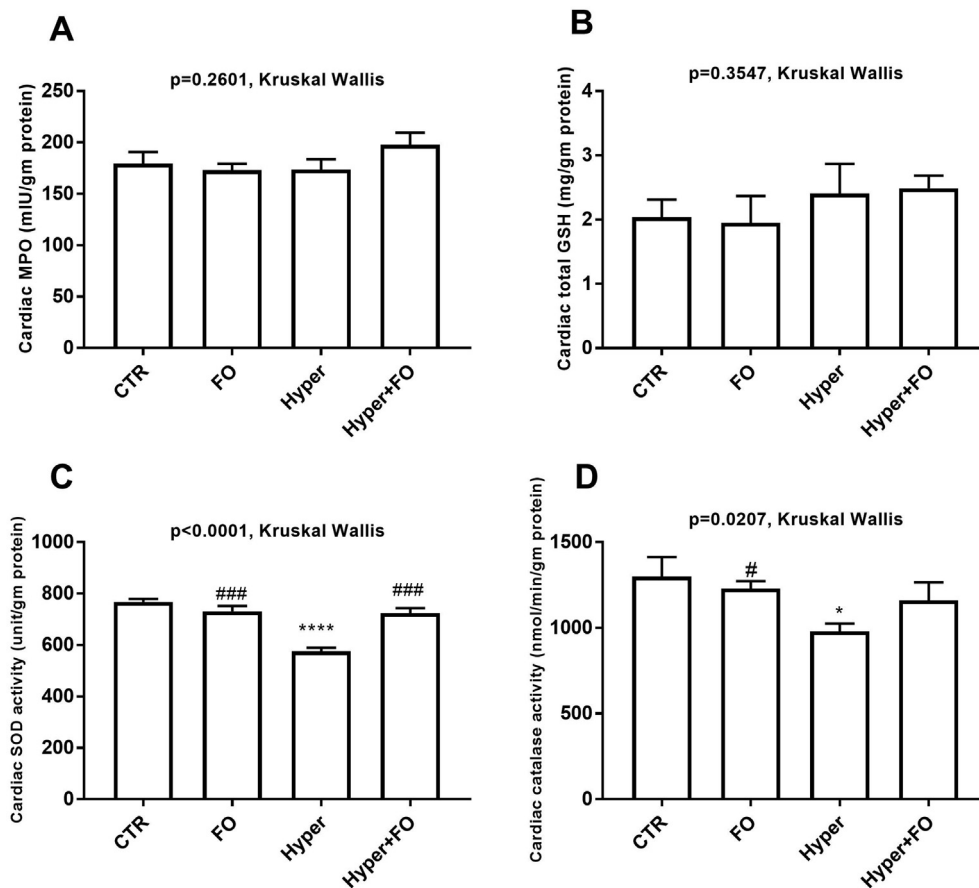


Figure 2. Levels of MPO, GSH and antioxidant activities of SOD and catalase. Cardiac contents of MPO (A), GSH (B), SOD (C), and catalase (D) in the control (CTR); FO treated animals (FO); hyperthyroid animals (Hyper); and hyperthyroid animals treated with FO (Hyper + FO). * $p < 0.05$, ** $p < 0.01$, **** $p < 0.0001$ vs. Control; # $p < 0.05$, ### $p < 0.01$ vs. Hyper; \$ $p < 0.05$, vs. FO.

CVDs development in hyperthyroidism is not clear. In this study, we evaluated the impact of hyperthyroid status on plasma and cardiac biomarkers of inflammation, oxidative stress and fibrosis and assessed the effects of fish oil on these substrates in thyrotoxicosis model induced by daily injection of levothyroxine. This study provides original findings regarding the mechanisms underlying development of cardiac disease in hyperthyroidism, and raises interesting questions for clinical and basic investigation related to use of fish oil to prevent the development or the progression of CVDs in hyperthyroidism.

3. Results

3.1. Characteristics of study groups

Thyrotoxicosis induced by daily administration of a high dose of levothyroxine for 8 weeks induced a hyperthyroid status as documented by the significant increase in thyroid hormones (T3 and T4) in both Hyper and Hyper + FO groups without differences between them (Table 1). This was coupled with a decrease in BWT that reached statistical significance in the Hyper group. Levothyroxine induced an increase in heart weight and in the ratio of heart WT/BWT in both Hyper and Hyper + FO groups ($p < 0.0001$, Table 1) without significant differences between them. Levothyroxine increased SBp and heart rate significantly in the Hyper group. Use of FO reduced heart rate but not SBp. Although no significant increase was found for DBp in hyper group, a slight but significant decrease was found for DBp in the FO and Hyper + FO groups (Table 1). Levothyroxine did not cause significant changes in serum lipids levels. However, use of FO reduced serum LDL and total

cholesterol levels which resulted in higher serum levels of LDL, HDL, and total cholesterol in Hyper group as compared to FO group (Table 1).

3.2. Biomarker levels of inflammation and oxidative stress

3.2.1. Endothelin-1 levels

Serum ET-1 was significantly increased in both Hyper and Hyper + FO groups as compared to control and use of FO did not change serum levels ($p < 0.0001$, Figure 1A). On the other hand, cardiac ET-1 levels were similar between groups (Figure 1B).

3.2.2. Serum and cardiac total nitrite and TBARS levels

There were no significant differences in both serum and cardiac total nitrite levels between all groups (Figure 1C, D).

On the other hand, serum TBARS levels were increased significantly in all groups relative to control (Figure 1D).

Interestingly, cardiac TBARS levels were elevated significantly in Hyper + FO group compared to both control and Hyper groups indicating that FO is promoting increased cardiac TBARS ($p < 0.0001$, Figure 1E).

3.2.3. Cardiac MPO and GSH levels

There was no significant difference in cardiac MPO or total GSH levels between all groups (Figure 2A, B).

3.2.4. Cardiac superoxide dismutase (SOD) and catalase activities

Relative to control, cardiac SOD and catalase activities were significantly decreased in the Hyper group and use of FO normalized their activities in the Hyper + Fo group (Figure 2C, D). Use of FO nor. Relative

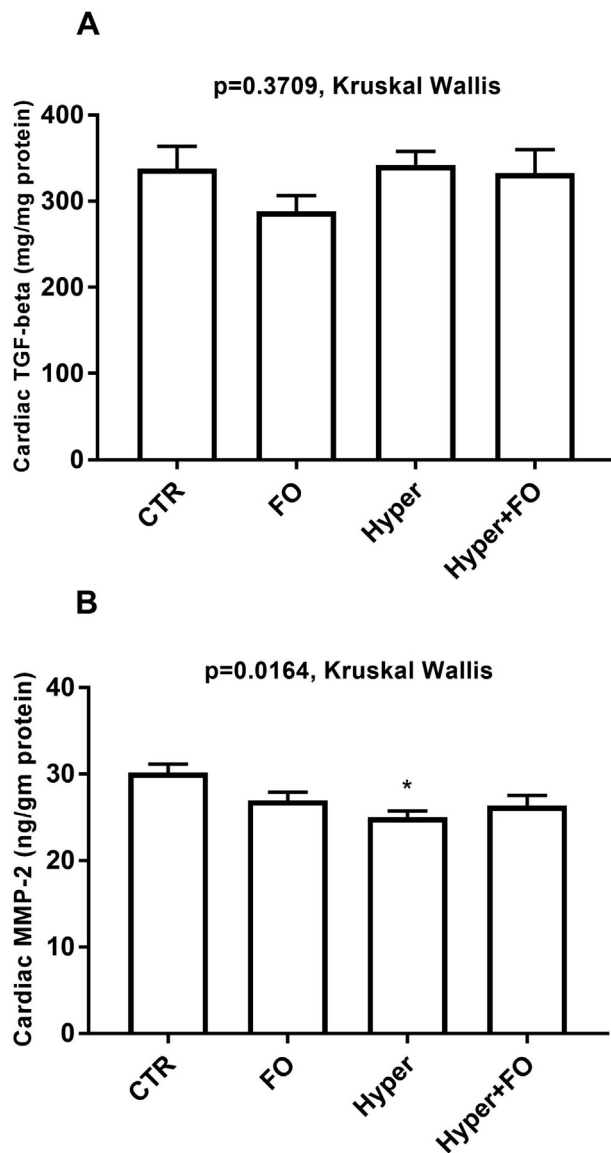


Figure 3. Levels of fibrotic factors. Cardiac contents of TGF-beta (A) and MMP-2 (B) in the control (CTR); FO treated animals (FO); hyperthyroid animals (Hyper); and hyperthyroid animals treated with FO (Hyper + FO). * $p < 0.05$ vs. Control.

to hyper group, use of FO increased SOD activities in Hyper + FO groups (Figure 2C).

3.3. Biomarker levels of cardiac fibrosis

3.3.1. Cardiac TGF- β 1 and MMP-2 levels

There were no significant differences in cardiac TGF- β 1 protein levels between all groups (Figure 3A). However, a significant decrease in cardiac MMP-2 levels was observed in Hyper group compared to CTR group. Use of FO normalized MMP-2 levels in the Hyper + FO group (Figure 3B).

3.3.2. Masson trichrome staining

Figure 4A–D represents cardiac sections stained with Masson's Trichrome staining to assess extent of interstitial fibrosis (blue). Relative to control, cardiac fibrosis was significantly elevated in Hyper group and was attenuated by fish oil supplementation. Figure 4E represents quantification of area of cardiac fibrosis among groups.

4. Discussion

In this study, we have evaluated the impact of thyrotoxicosis on cardiac oxidative stress, inflammation and remodeling and tested if the use of fish oil (FO) will affect these substrates. A dose of 100 mg omega-3/100g BWT was used. This dose has been shown to be cardio protective and associated with significant reduction in arachidonic acid concentrations and improvement of the ratio of omega 6 to omega 3 from 13:1 to 4:1 as we previously documented [17].

In the present study, a hyperthyroid status was induced by daily administration of a high dose of levothyroxine (3 mg/Kg) for 8 weeks as characterized by the significant increase in serum thyroid hormones in both Hyper and Hyper + FO groups. Use of FO had no significant effect on thyroid hormones levels. Patients with hyperthyroidism usually notice a loss in their body weights, which is not consistent with observations in animal models of hyperthyroidism. Previous studies have observed a reduction [18], an increase [19, 20] or no change in body weight in hyperthyroid animals [21].

Although an increase in BWT was observed over time, the BWT was significantly lower for the hyper group relative to control, and was normalized by the use of FO in the Hyper + FO group, suggesting that metabolic changes associated with prolonged hyperthyroidism promoted a reduction in body weight that could be optimized by the use of FO. Interestingly, despite the decrease in body weight, an increase in the heart to body weight ratio was observed in hyper thyroid rats regardless of FO treatment, indicating cardiac dilatation or remodeling that might occur in hyperthyroidism [22].

Hyperthyroidism is associated with elevated blood pressure and tachycardia [23]. Our hyperthyroid rats showed a significant increase in SBp without an effect of FO on SBp readings. As expected, an increase in HR was seen in hyperthyroid rats and use of FO attenuated this increase providing a potential heart rate slowing effect in hyperthyroid patients with tachycardia. We have suggested previously in a model of post-operative atrial fibrillation that use of FO may modulate heart rate through enhanced heart rate variability parameters associated with vagal tone [13]. Enhanced sympathetic activity or reduced vagal activity promotes tachycardia. Stimulation of vagal tone is accompanied by anti-inflammatory properties [24]. The ω 3 fatty acids have been found to inhibit pacemaker currents [25]. These mechanisms may contribute to heart rate slowing effect associated with FO intake [13].

In the present study, the induction of exogenous hyperthyroid status did not significantly change serum lipid levels relative to control. However, supplementation of FO for 8 weeks resulted in a decrease in LDL and total cholesterol, which is similar to our previous findings [17]. The relationship between serum levels of lipids and FO supplementation is controversial in the literature. Levels of LDL and total cholesterol were shown to be increased [26, 27], decreased [17, 28, 29], or not changed [30, 31]. Similarly, several studies reported the positive effect of FO on serum HDL [27, 31, 32], whereas others reported a negative effect [33, 34]. In our study, no differences in serum HDL levels were observed relative to control, but levels were higher in hyper groups relative to FO suggesting that thyroid hormones might affect HDL levels. Alterations in lipid levels have been observed in hyperthyroidism via different mechanisms [35].

Endothelin-1 (ET-1) is a potent vasoconstrictor and inflammatory factor that is produced by endothelial cells, fibroblasts and cardiac myocytes [36]. We have shown that patients with history of recent MI have increased plasma levels of ET-1 and increased risk of atherosclerotic cardiovascular events indicating a link to endothelial dysfunction [37, 38]. Furthermore, patients with hyperthyroidism have increased levels of plasma ET1 and this increase is associated with increased risk of atrial fibrillation [39, 40]. Interestingly, ET-1 levels return to normal values in the state of euthyroidism clearly indicating that thyroid hormones modulate ET-1 expression [41]. In our study, serum ET-1 was significantly increased in both Hyper and Hyper + FO groups with no significant effect of FO on their levels. On other hands, cardiac ET-1 levels were

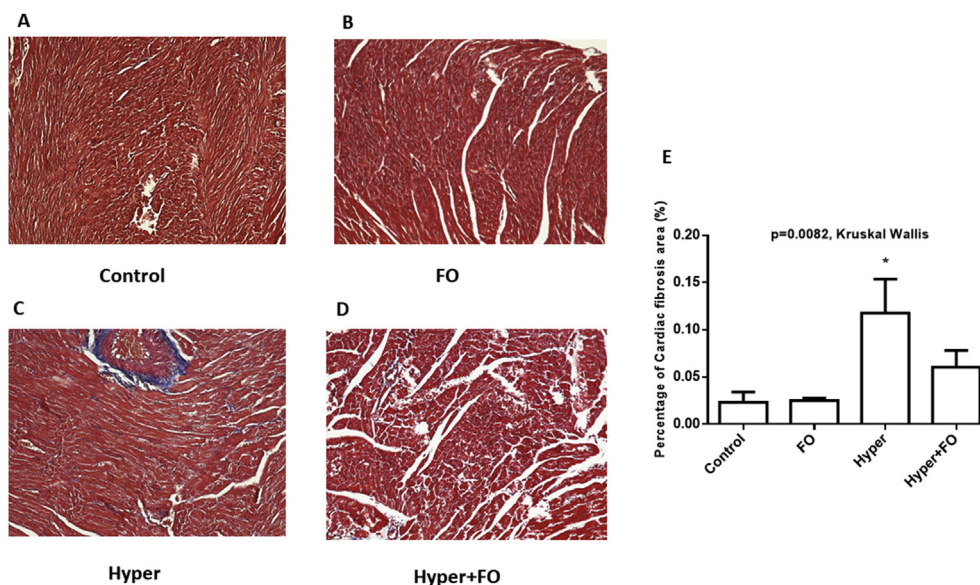


Figure 4. Cardiac fibrosis. Masson's Trichrome staining of right sided cardiac sections shows extent of fibrosis (blue), cardiac myocyte (red) in the control (CTR, A); FO treated animals (FO, B); hyperthyroid animals (Hyper, C); and hyperthyroid animals treated with FO (Hyper + FO, D). Sections were examined at 100× magnification. Figure 4E represent quantitative analysis of cardiac fibrosis. * $p < 0.05$ vs. Control.

similar among all groups, suggesting that thyroid hormones might mediate systemic ET-1 expression on a mechanism that is different from cardiac expression or independent of thyroid hormones. In addition, cardiac expression might need longer time to be changed as compared to circulating levels. Similar results were found for cardiac levels of MPO that were not affected by hyperthyroid status.

Oxidative stress and elevated ROS production have been linked to hyperthyroidism [42]. Production of ROS or reactive nitrogen species (RNS) such as superoxide anion, hydrogen peroxides and nitrites promote oxidative damage. The body neutralizes ROS and RNS by antioxidant enzymes and molecules such as SOD, catalase, and glutathione system to prevent oxidative damage [43]. The SOD converts superoxide to hydrogen peroxide (H_2O_2), which is a substrate for glutathione peroxidase and catalase enzymes. Catalase reduces H_2O_2 to water [44, 45]. In hyperthyroid hearts, reduced levels of SOD and catalase, and increased levels of nitrite, nitrate and TBARS (lipid peroxidation markers) have been reported [46, 47]. On the other hand, changes in levels of glutathione (GSH) in hyperthyroidism are still unclear.

Here, no changes in total nitrite or GSH levels have been found. However, cardiac activities of SOD and catalase were reduced in Hyper group and use of FO prevented the decrease in their activities, suggesting that FO supplementation may prevent oxidative stress partly by increasing antioxidant enzyme activities of SOD and catalase.

Thiobarbituric acid reactive substances (TBARS) are markers of lipid peroxidation [46]. Similar to our previous findings, serum TBARS were increased in FO treated animals as well as in hyperthyroid rats indicating that both factors increase peroxidation of lipids. Several studies reported increased TBARS after FO supplementation [48, 49, 50]. This might be due to increased number of unsaturated bonds incorporated into membrane phospholipids after FO supplementation. This finding suggests that although FO may improve defense mechanisms in the body, they may also increase lipid peroxides. On the other hand, the decrease in antioxidant enzyme activities of SOD and catalase in Hyper group might lead to elevated TBARS levels. Interestingly, cardiac TBARS levels were most elevated in Hyper + FO group compared to both control and Hyper groups. This suggests a significant interaction and synergism between FO and hyperthyroidism as changes in tissue levels of lipids require longer time than changes in plasma levels [17]. This finding may raise a concern

regarding the use of FO in patients. Future studies should evaluate the effect of FO on other ROSs such as H_2O_2 and superoxide anion.

Hyperthyroidism is associated with cardiac structural changes such as cardiac hypertrophy and fibrosis [51]. The transforming growth factor beta ($TGF-\beta 1$) is a primary fibrotic factor [52]. Many studies showed a trend of increased $TGF-\beta 1$ expression in rat models of cardiac diseases [53, 54]. In this study, cardiac $TGF-\beta 1$ levels were similar among all groups. However, Masson's Trichrome staining revealed an evidence of cardiac fibrosis in Hyper group relative control suggesting presence of other growth factors or pro fibrotic molecules that may mediate fibrosis in hyperthyroidism such as the platelet derived growth factors [55, 56]. Regional differences in $TGF-\beta 1$ expression among different cardiac chambers may also contribute [57]. The matrix metalloproteinases are proteolytic enzymes that are regulated by inflammatory signals to help the establishment of ECM homeostasis. Increased or decreased MMP-2 levels may promote fibrosis [51]. Patients with hypertension and cardiac hypertrophy were found to have reduced levels of MMP-1, MMP-2 and MMP-9 [58]. In this study, there was a significant decrease of cardiac MMP-2 levels in Hyper group. Interestingly, FO supplementation normalized MMP-2 levels and attenuated cardiac fibrosis. The reduction of MMP-2 in Hyper group may also contribute to the observed fibrosis in cardiac sections.

5. Conclusion

Thyrotoxicosis induced by daily injection of levothyroxine for 8 weeks was associated with elevated thyroid hormones, increased heart to body weight ratio, systolic BP and heart rate. Thyrotoxicosis was also associated with increased serum ET-1, TBARS and reduced activities of cardiac catalase and SOD. Cardiac fibrosis paralleled with a decrease of cardiac MMP-2 levels was seen in Hyper group. Use of FO reduced heart rate, increased antioxidant enzyme activities of SOD and catalase, increased TBARS levels and attenuated cardiac fibrosis by normalizing MMP-2 levels. Our study suggests that thyrotoxicosis may increase risk of cardiac diseases by increasing blood pressure, inflammation/oxidative stress and fibrosis. Use of FO may attenuate oxidative stress and fibrosis and reduce risk of cardiac disease in hyperthyroid states.

Declarations

Author contribution statement

Fadia Mayyas: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Ahmad Alsaheb: Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Karem Alzoubi: Conceived and designed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

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Competing interest statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

References

- D.S. Cooper, Hyperthyroidism, *Lancet* 362 (9382) (2003) 459–468.
- S. Ertek, A.F. Cicero, Hyperthyroidism and cardiovascular complications: a narrative review on the basis of pathophysiology, *Arch. Med. Sci.* 9 (5) (2013) 944–952.
- M. Dorr, H. Volzke, Cardiovascular morbidity and mortality in thyroid dysfunction, *Minerva Endocrinol.* 30 (4) (2005) 199–216.
- I. Stoykov, B. Zandieh-Doulabi, A.F. Moorman, V. Christoffels, W.M. Wiersinga, O. Bakker, Expression pattern and ontogenesis of thyroid hormone receptor isoforms in the mouse heart, *J. Endocrinol.* 189 (2) (2006) 231–245.
- R. Rodrigo, J. Gonzalez, F. Paoletto, The role of oxidative stress in the pathophysiology of hypertension, *Hypertens. Res.* 34 (4) (2011) 431–440.
- T.M. Paravicini, R.M. Touyz, Redox signaling in hypertension, *Cardiovasc. Res.* 71 (2) (2006) 247–258.
- D. Fan, A. Takawale, J. Lee, Z. Kassiri, Cardiac fibroblasts, fibrosis and extracellular matrix remodeling in heart disease, *Fibrogenesis Tissue Repair* 5 (1) (2012) 15.
- Y. Adkins, D.S. Kelley, Mechanisms underlying the cardioprotective effects of omega-3 polyunsaturated fatty acids, *J. Nutr. Biochem.* 21 (9) (2010) 781–792.
- A. Leaf, J.X. Kang, Y.F. Xiao, G.E. Billman, Clinical prevention of sudden cardiac death by n-3 polyunsaturated fatty acids and mechanism of prevention of arrhythmias by n-3 fish oils, *Circulation* 107 (21) (2003) 2646–2652.
- J.H. Rensson, D.R. Van Wagoner, Impact of dietary fatty acids on cardiac arrhythmogenesis, *Circ. Arrhythm. Electrophysiol.* 2 (4) (2009) 460–469.
- I. Romieu, R. Garcia-Esteban, J. Sunyer, C. Rios, M. Alcaraz-Zubeldia, S.R. Velasco, et al., The effect of supplementation with omega-3 polyunsaturated fatty acids on markers of oxidative stress in elderly exposed to PM(2.5), *Environ. Health Perspect.* 116 (9) (2008) 1237–1242.
- M.K. Duda, K.M. O'Shea, A. Tintinu, W. Xu, R.J. Khairallah, B.R. Barrows, et al., Fish oil, but not flaxseed oil, decreases inflammation and prevents pressure overload-induced cardiac dysfunction, *Cardiovasc. Res.* 81 (2) (2009) 319–327.
- F. Mayyas, S. Sakurai, R. Ram, J.H. Rensson, E.S. Hwang, L. Castel, et al., Dietary omega3 fatty acids modulate the substrate for post-operative atrial fibrillation in a canine cardiac surgery model, *Cardiovasc. Res.* 89 (4) (2011) 852–861.
- F. Mayyas, K.H. Alzoubi, Cardiac effects of cigarette tobacco smoking in rat model of diabetes, *Life Sci.* 211 (2018) 279–285.
- Z. Anna, S. Angela, B. Barbara, R. Jana, B. Tamara, V. Csilla, et al., Heart-protective effect of n-3 PUFA demonstrated in a rat model of diabetic cardiomyopathy, *Mol. Cell. Biochem.* 389 (1–2) (2014) 219–227.
- F. Mayyas, K.H. Alzoubi, Z. Al-Taleb, An evaluation of the effect of pentoxifylline on blood pressure and myocardial oxidative status following intake of western diet, *Clin. Exp. Hypertens.* 37 (8) (2015) 666–673.
- F. Mayyas, R. Jaradat, K.H. Alzoubi, Cardiac effects of fish oil in a rat model of streptozotocin-induced diabetes, *Nutr. Metab. Cardiovasc. Dis.* 28 (6) (2018) 592–599.
- A. Kumar, R.A. Sinha, M. Tiwari, R. Singh, T. Koji, N. Manhas, et al., Hyperthyroidism induces apoptosis in rat liver through activation of death receptor-mediated pathways, *J. Hepatol.* 46 (5) (2007) 888–898.
- K. Engels, H. Rakov, D. Zwanziger, G.S. Hones, M. Rehders, K. Brix, et al., Efficacy of protocols for induction of chronic hyperthyroidism in male and female mice, *Endocrine* 54 (1) (2016) 47–54.
- M.E. Asker, W.A. Hassan, A.M. El-Kashlan, Experimentally induced hyperthyroidism influences oxidant and antioxidant status and impairs male gonadal functions in adult rats, *Andrologia* 47 (6) (2015) 644–654.
- Y. Zhang, E.I. Dedkov, D. Teplitsky, N.Y. Weltman, C.J. Pol, V. Rajagopalan, et al., Both hypothyroidism and hyperthyroidism increase atrial fibrillation inducibility in rats, *Circ. Arrhythm. Electrophysiol.* 6 (5) (2013) 952–959.
- L. Liu, F. Yun, H. Zhao, S. Zhang, Z. Liu, X. Wang, et al., Atrial sympathetic remodeling in experimental hyperthyroidism and hypothyroidism rats, *Int. J. Cardiol.* 187 (2015) 148–150.
- G. Kaminski, K. Makowski, D. Michalkiewicz, J. Kowal, M. Ruchala, E. Szczepanek, et al., The influence of subclinical hyperthyroidism on blood pressure, heart rate variability, and prevalence of arrhythmias, *Thyroid* 22 (5) (2012) 454–460.
- Y. Zhang, Z.B. Popovic, S. Bibevski, I. Fakhr, D.A. Sica, D.R. Van Wagoner, et al., Chronic vagus nerve stimulation improves autonomic control and attenuates systemic inflammation and heart failure progression in a canine high-rate pacing model, *Circ. Heart Fail.* 2 (6) (2009) 692–699.
- P. Vreken, A. Van Lint, A. Bootsma, H. Overmars, R. Wanders, A. Van Gennip, Rapid stable isotope dilution analysis of very-long-chain fatty acids, pristanic acid and phytanic acid using gas chromatography–electron impact mass spectrometry, *J. Chromatogr. B Biomed. Sci. Appl.* 713 (2) (1998) 281–287.
- P.J. Nestel, Fish oil and cardiovascular disease: lipids and arterial function, *Am. J. Clin. Nutr.* 71 (1) (2000), 228S–31S.
- W.S. Harris, n-3 fatty acids and serum lipoproteins: human studies, *Am. J. Clin. Nutr.* 65 (5 Suppl) (1997), 1645S–54S.
- A. Asha, S. Mathew, P. Lakshmanan, Effect of PUFA from fish oil on lipid metabolism of heart in streptozotocin-induced diabetes in male albino rats, *J. Biomed. Sci.* (2012).
- I. Mahmud, A. Hossain, S. Hossain, A. Hannan, L. Ali, M. Hashimoto, Effects of Hilsa ilisa fish oil on the atherogenic lipid profile and glycaemic status of streptozotocin-treated type 1 diabetic rats, *Clin. Exp. Pharmacol. Physiol.* 31 (1–2) (2004) 76–81.
- S.E. Kasim, B. Stern, S. Khilnani, P. McIn, S. Baciorowski, K.-L.C. Jen, Effects of omega-3 fish oils on lipid metabolism, glycemic control, and blood pressure in type II diabetic patients, *J. Clin. Endocrinol. Metab.* 67 (1) (1988) 1–5.
- M. Petersen, H. Pedersen, A. Major-Pedersen, T. Jensen, P. Marckmann, Effect of fish oil versus corn oil supplementation on LDL and HDL subclasses in type 2 diabetic patients, *Diabetes Care* 25 (10) (2002) 1704–1708.
- M. Liu, R. Wallin, T. Saldeen, Effect of bread containing stable fish oil on plasma phospholipid fatty acids, triglycerides, HDL-cholesterol, and malondialdehyde in subjects with hyperlipidemia, *Nutr. Res.* 21 (11) (2001) 1403–1410.
- P.P. de Silva, P.J. Davis, S.K. Cheema, Hyperlipidaemic effect of fish oil in Bio F1B hamsters, *Br. J. Nutr.* 91 (3) (2004) 341–349.
- J.S. Parks, J.A. Martin, B.L. Sonbert, B.C. Bullock, Alteration of high density lipoprotein subfractions of nonhuman primates fed fish-oil diets. Selective lowering of HDL subfractions of intermediate size and density, *Arteriosclerosis* 7 (1) (1987) 71–79.
- M. Peppia, G. Betsi, G. Dimitriadis, Lipid abnormalities and cardiometabolic risk in patients with overt and subclinical thyroid disease, *J. Lipids* 2011 (2011) 575840.
- F. Mayyas, M. Niebauer, A. Zurick, J. Barnard, A.M. Gillinov, M.K. Chung, et al., Association of left atrial endothelin-1 with atrial rhythm, size, and fibrosis in patients with structural heart disease, *Circ. Arrhythm. Electrophysiol.* 3 (4) (2010) 369–379.
- F. Mayyas, D. Baydoun, R. Ibdah, K. Ibrahim, Atorvastatin reduces plasma inflammatory and oxidant biomarkers in patients with risk of atherosclerotic cardiovascular disease, *J. Cardiovasc. Pharmacol. Ther.* 23 (3) (2018) 216–225.
- F. Mayyas, M. Al-Jarrah, K. Ibrahim, D. Mfady, D.R. Van Wagoner, The significance of circulating endothelin-1 as a predictor of coronary artery disease status and clinical outcomes following coronary artery catheterization, *Cardiovasc. Pathol.* 24 (1) (2015) 19–25.
- F. Mayyas, N. Saadeh, K. Al-Muqbel, D.R. Van Wagoner, Plasma endothelin-1 levels are increased in atrial fibrillation patients with hyperthyroidism, *PLoS One* 13 (12) (2018), e0208206.
- J.M. Alegret, G. Aragones, R. Elosua, R. Beltran-Debon, A. Hernandez-Aguilera, C. Romero-Menor, et al., The relevance of the association between inflammation and atrial fibrillation, *Eur. J. Clin. Invest.* 43 (4) (2013) 324–331.
- C.H. Chu, J.K. Lee, H.M. Keng, M.J. Chuang, C.C. Lu, M.C. Wang, et al., Hyperthyroidism is associated with higher plasma endothelin-1 concentrations, *Exp. Biol. Med.* 231 (6) (2006) 1040–1043.
- K. Asayama, K. Dobashi, H. Hayashibe, Y. Megata, K. Kato, Lipid peroxidation and free radical scavengers in thyroid dysfunction in the rat: a possible mechanism of injury to heart and skeletal muscle in hyperthyroidism, *Endocrinology* 121 (6) (1987) 2112–2118.
- S. Matsushima, S. Kinugawa, T. Yokota, N. Inoue, Y. Ohta, S. Hamaguchi, et al., Increased myocardial NAD(P)H oxidase-derived superoxide causes the exacerbation of postinfarct heart failure in type 2 diabetes, *Am. J. Physiol. Heart Circ. Physiol.* 297 (1) (2009) H409–H416.
- M. Deponce, Glutathione catalysis and the reaction mechanisms of glutathione-dependent enzymes, *Biochim. Biophys. Acta* 1830 (5) (2013) 3217–3266.
- J. Nordberg, E.S. Arner, Reactive oxygen species, antioxidants, and the mammalian thioredoxin system, *Free Radic. Biol. Med.* 31 (11) (2001) 1287–1312.
- P. Venditti, S. Di Meo, Thyroid hormone-induced oxidative stress, *Cell. Mol. Life Sci.* 63 (4) (2006) 414–434.

- [47] R. Shinohara, T. Mano, A. Nagasaka, R. Hayashi, K. Uchimura, I. Nakano, et al., Lipid peroxidation levels in rat cardiac muscle are affected by age and thyroid status, *J. Endocrinol.* 164 (1) (2000) 97–102.
- [48] D. Harats, Y. Dabach, G. Hollander, M. Ben-Naim, R. Schwartz, E. Berry, et al., Fish oil ingestion in smokers and nonsmokers enhances peroxidation of plasma lipoproteins, *Atherosclerosis* 90 (2-3) (1991) 127–139.
- [49] M.J. Gonzalez, J.I. Gray, R.A. Schemmel, L. Dugan Jr., C.W. Welsch, Lipid peroxidation products are elevated in fish oil diets even in the presence of added antioxidants, *J. Nutr.* 122 (11) (1992) 2190.
- [50] M. Suzukawa, M. Abbey, P. Howe, P.J. Nestel, Effects of fish oil fatty acids on low density lipoprotein size, oxidizability, and uptake by macrophages, *J. Lipid Res.* 36 (3) (1995) 473–484.
- [51] P. Liu, M. Sun, S. Sader, Matrix metalloproteinases in cardiovascular disease, *Can. J. Cardiol.* 22 (Suppl B) (2006) 25B–30B.
- [52] P. Kong, P. Christia, N.G. Frangogiannis, The pathogenesis of cardiac fibrosis, *Cell. Mol. Life Sci.* 71 (4) (2014) 549–574.
- [53] U. Seeland, C. Haeuseler, R. Hinrichs, S. Rosenkranz, T. Pfitzner, K. Scharfetter-Kochanek, et al., Myocardial fibrosis in transforming growth factor-beta(1) (TGF-beta(1)) transgenic mice is associated with inhibition of interstitial collagenase, *Eur. J. Clin. Investig.* 32 (5) (2002) 295–303.
- [54] B. Wang, J. Hao, S.C. Jones, M.S. Yee, J.C. Roth, I.M. Dixon, Decreased Smad 7 expression contributes to cardiac fibrosis in the infarcted rat heart, *Am. J. Physiol. Heart Circ. Physiol.* 282 (5) (2002) H1685–H1696.
- [55] J. Andrae, R. Gallini, C. Betsholtz, Role of platelet-derived growth factors in physiology and medicine, *Genes Dev.* 22 (10) (2008) 1276–1312.
- [56] B. Burstein, E. Libby, A. Calderone, S. Nattel, Differential behaviors of atrial versus ventricular fibroblasts: a potential role for platelet-derived growth factor in atrial-ventricular remodeling differences, *Circulation* 117 (13) (2008) 1630–1641.
- [57] H. Nakajima, H.O. Nakajima, O. Salcher, A.S. Dittie, K. Dembowski, S. Jing, et al., Atrial but not ventricular fibrosis in mice expressing a mutant transforming growth factor-beta(1) transgene in the heart, *Circ. Res.* 86 (5) (2000) 571–579.
- [58] A. Zervoudaki, E. Economou, C. Stefanadis, C. Pitsavos, K. Tsioufis, C. Aggeli, et al., Plasma levels of active extracellular matrix metalloproteinases 2 and 9 in patients with essential hypertension before and after antihypertensive treatment, *J. Hum. Hypertens.* 17 (2) (2003) 119–124.