

# Regular Aerobic Exercise and Vitamin D3 Supplementation-Reduced Anthropometric Measures and the Hydrogen Peroxide-Induced Expression of TNF- $\alpha$ in Rats

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Received May 11, 2018

Accepted October 7, 2018

Published online March 31, 2019



## Please cite this article

as follows: Mohseni Zadeh N, Najafipour H, Azarbayjani MA, Matin Homaei H, Keshtkar AA. Regular aerobic exercise and vitamin d3 supplementation-reduced anthropometric measures and the hydrogen peroxide-induced expression of TNF- $\alpha$  in rats. Int J Basic Sci Med. 2019;4(1):38-42. doi:10.15171/ijbsm.2019.08.

## Abstract

**Introduction:** Inflammation, oxidative stress (OS), and obesity are documented to play key roles in the pathogenesis of cardiovascular diseases (CVDs). Accordingly, tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) and hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>), as a main innate immunity pro-inflammatory cytokine and a main free radical, respectively, are the main risk factors for CVDs. The present study aimed to evaluate the effects of OS, regular aerobic exercise (RAE), and vitamin D3 (VD3) on the expression of TNF- $\alpha$  in the myocardial cells in a rat model.

**Methods:** In this experimental study, 48 male Wistar rats were divided into 8 groups (6 in each group) including healthy controls, sham (injected with dimethyl sulfoxide [DMSO] + saline), H<sub>2</sub>O<sub>2</sub> (either 1 or 2 mmol/kg), H<sub>2</sub>O<sub>2</sub> (1 mmol/kg) + VD3, H<sub>2</sub>O<sub>2</sub> (2 mmol/kg) + VD3, H<sub>2</sub>O<sub>2</sub> (1 mmol/kg) + RAE, and H<sub>2</sub>O<sub>2</sub> (2 mmol/kg) + RAE. TNF- $\alpha$  level of myocardial cells was evaluated after 8 weeks using the ELISA technique.

**Results:** The results of the study demonstrated that exposure to 2 mmol/kg of H<sub>2</sub>O<sub>2</sub> significantly increased TNF- $\alpha$  level of myocardial cells compared to the rats which were exposed to one mmol/kg H<sub>2</sub>O<sub>2</sub> ( $P=0.039$ ). Furthermore, RAE ( $P=0.040$ ), and the combination of RAE+VD3 ( $P=0.049$ ) significantly reduced the expression of myocardial TNF- $\alpha$ .

**Conclusion:** In general, VD3 and RAE were found to suppress TNF- $\alpha$  expression induced by H<sub>2</sub>O<sub>2</sub> in the rat myocardium. Therefore, they can be considered as potential therapeutic interventions for reducing OS-induced inflammation in the damaged myocardial cells.

**Keywords:** Inflammation, Tumor necrosis factor- $\alpha$ , Vitamin D3

## Introduction

Reactive oxygen species (ROS) can be considered as the risk factors for inducing several cardiovascular diseases (CVDs) pathogenesis.<sup>1</sup> Additionally, oxidative stress (OS), which is associated with several chronic inflammation conditions in the human tissues, is an imbalance between the ROS production and detoxification systems.<sup>2</sup> Furthermore, OS is involved in the pathogenesis of CVDs by the up-regulation of pro-inflammatory

molecules.<sup>2,3</sup> Thus, the factors which regulate the inflammatory effects of the ROS can be potential therapeutic agents for managing CVDs. In addition, ROS are produced in several conditions such as infections, stress, exercise, and exposition to the toxins.<sup>4,5</sup>

The pathogenesis of CVDs is multifactorial. In the one hand, the role of chronic inflammation is postulated as a contributing factor in CVDs. Tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ) is an important

pro-inflammatory cytokine which plays key roles in the increased expression of several down-stream pro-inflammatory molecules such as adhesion molecules, free radicals, ROS, and some other pro-inflammatory cytokines.<sup>6</sup> Based on the reports, TNF- $\alpha$  is involved in the pathogenesis of CVDs.<sup>7</sup> On the other hand, regular aerobic exercise (RAE) and safe dietary are believed to play crucial roles in cardiovascular health.<sup>8</sup> Additionally, vitamin D3 (VD3) is essential nutrition which is not only regarded as an important immunoregulatory factor, but also it is a key factor for bone growth.<sup>9,10</sup> Furthermore, VD3 deficiency is associated with CVDs.<sup>10</sup> Thus, the current study mainly sought to investigate the effects of RAE and VD3 on the expression of TNF- $\alpha$  in the heart muscles of the rats.

## Materials and Methods

### Animals

This study was performed on 48 male Wistar rats with 200 $\pm$ 20 g weight and 8-10 weeks of age at the Physiology Research Center, Kerman University of Medical Sciences. The animals were kept in standard conditions (i.e., the ambient temperature of 22 $\pm$ 2°C, free access to food and water, and 12 hours of a dark and light cycle). Then, they were divided into 8 experimental groups (6 rats in each group), the details of which are presented in Table 1.

### Treatments

H<sub>2</sub>O<sub>2</sub> (either 2 mmol/kg or 1 mmol/kg) was intraperitoneally injected three times weekly.<sup>11-13</sup> Further, VD3 was injected daily in 0.5  $\mu$ g/kg dose.<sup>14</sup> Normal saline and dimethyl sulfoxide were used as the solvents for VD3.

### Regular Aerobic Exercise

RAE was daily performed for eight weeks. During the first week, the rats were trained with a speed of 8 m/min and a slope of 10 degrees for 30 minutes on a treadmill, followed by a speed of 12 m/min and at the same slope and time on a treadmill in the second week. Furthermore, during the third and fourth weeks, rats were trained with a speed

of 16 m/min at the same slope for 45 minutes and with a speed of 20 m/min at the same slope for 45 minutes, respectively. For the fifth to eighth weeks, the rats were trained at a speed of 20 m/min at an angle of ten degrees for 60 minutes every day.<sup>15</sup>

### Evaluation of the Lee Index

The Lee index is a well fast and accurate marker for determining the obesity, as well as estimating the body composition in the rats. Accordingly, this index is calculated by dividing the cubicle root of the weight (grams) by the nasoanal length in millimeters.<sup>15</sup> In the current study, the Lee index was computed using the following formula:

Lee index = cube root of body weight (g)/nose-to-anus length (cm)<sup>16</sup>

### Calculation of the Running Speed

The running speed was calculated using the following protocol. First, 10 minutes of warm-up with 10-15 m/min took place, then, the test was started by running the rats at a speed of 15 meters per minute (for 2 minutes), followed by increasing the treadmill speed every 2 minutes as 0.3 m/s equivalent to 1.8 m/min so that the animals were unable to run.<sup>17</sup> The anthropometric indicators and running speed test were evaluated before and after the interventions. Moreover, 24 hours after the last running and after 12 hours of hunger, the animals were decapitated in an ethical and standard condition in order to avoid the production of intracellular H<sub>2</sub>O<sub>2</sub>.<sup>18</sup> Next, the heart tissues were immediately isolated and stored at -70°C for further investigations.

### Assessment of TNF- $\alpha$ Level of the Heart Tissue

The heart tissue was homogenized using a homogenizing buffer containing NaCl, 50 mM Tris-HCl, and 12  $\mu$ M leupeptin. Then, the homogenized samples were centrifuged at 4°C for 20 minutes in 3000 g and the supernatant was employed for measuring the TNF- $\alpha$  level. TNF- $\alpha$  level of the heart tissue was investigated by a commercial ELISA kit (Bioassay Technology Com, China).

### Statistical Analysis

For statistical analysis, the SPSS software, version 24 was used. Accordingly, the normal distributions of the data were checked using the Kolmogorov-Simonov test and the homogeneity of variance was analyzed utilizing the Levene test. Additionally, repeated measure ANOVA was applied to calculate the running speed and anthropometric indicators. Then, the TNF- $\alpha$  level of the heart tissue was compared between the groups using three-way analysis of variance and, Bonferroni follow-up test was utilized in the case of significant differences. The  $P \leq 0.05$  was considered statistically significant.

**Table 1.** The Intervention Protocol for Experimental Groups

Group Number	Experimental Groups	Treatments
1	Control	Non-treatment
2	Vehicle	IP of DMSO + saline (d)
3	H <sub>2</sub> O <sub>2</sub>	IP of 1 mmol/kg H <sub>2</sub> O <sub>2</sub> (tiw)
4	2H <sub>2</sub> O <sub>2</sub>	IP of 2 mmol/kg H <sub>2</sub> O <sub>2</sub> (tiw)
5	H <sub>2</sub> O <sub>2</sub> + VD3	IP of 1 mmol/kg H <sub>2</sub> O <sub>2</sub> (tiw)+0.5 $\mu$ g/Kg VD3 (d)
6	2H <sub>2</sub> O <sub>2</sub> 2 + VD3	IP of 2 mmol/kg H <sub>2</sub> O <sub>2</sub> (tiw)+0.5 $\mu$ g/Kg VD3 (d)
7	H <sub>2</sub> O <sub>2</sub> + RAE	IP of 1 mmol/kg H <sub>2</sub> O <sub>2</sub> (tiw)+RAE (d)
8	2H <sub>2</sub> O <sub>2</sub> + RAE	IP of 1 mmol/kg H <sub>2</sub> O <sub>2</sub> (tiw)+RAE (d)

**Results**

The results showed that RAE and vitamin D3 (VD3) significantly increased the Lee index ( $P=0.001$ ) and running speed ( $P=0.001$ ) in the rats under the treatment of  $H_2O_2$ . Data analysis using the Bonferroni follow-up test revealed that RAE led to a significant increase in Lee index during 8 weeks ( $P=0.001$ ).

In addition, the  $H_2O_2$  administration led to an increase in TNF- $\alpha$  level of the heart tissue of the rats. There was a significant difference in cardiac TNF- $\alpha$  in the rats either exposed to 2 mmol/kg or 1 mmol/kg of  $H_2O_2$  ( $P=0.039$ ). Additionally, 2 mmol/kg of  $H_2O_2$  significantly enhanced the Lee index ( $P=0.001$ ) while it decreased the running speed ( $P=0.047$ ) compared to 1 mmol/kg of  $H_2O_2$ .

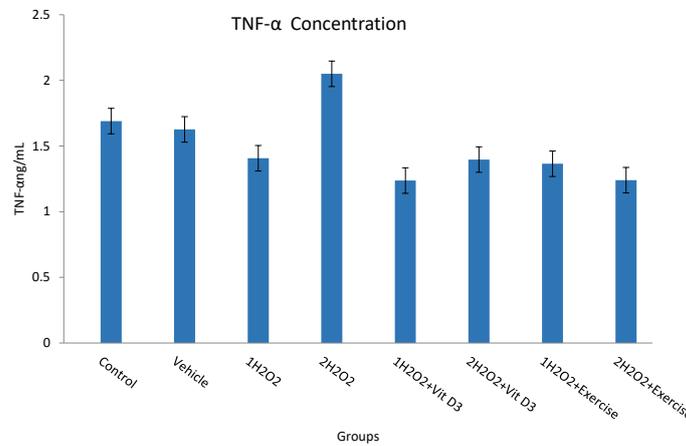
Further, VD3 and RAE administration decreased the expression of TNF- $\alpha$  ( $P=0.040$ ) and the Lee index ( $P=0.003$ ) whereas the administration of  $H_2O_2$  with

RAE increased this index ( $P=0.011$ ). Furthermore, the statistical analysis confirmed that the administration of VD3 and RAE down-regulated TNF- $\alpha$  ( $P=0.049$ , Figure 1) and decreased the Lee index ( $P=0.023$ , Figure 2) while it increased the running speed ( $P=0.018$ , Figure 3) in 2 mmol/kg of  $H_2O_2$  pre-administrated rats.

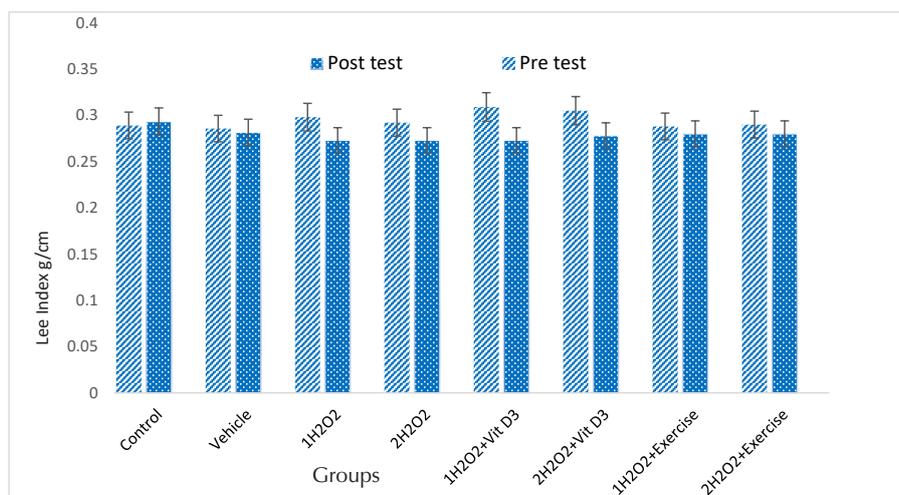
**Discussion**

It is reported that  $H_2O_2$  is a marker of OS and has a significant positive association with obesity and heart tissue inflammation.<sup>3,19</sup> Thus, the present study was designed to administrate  $H_2O_2$  to make a model of OS in the heart tissue. To the best of our knowledge, the current study, as the first investigation, used a combination of VD3 and RAE in order to evaluate their effects on the expression of TNF- $\alpha$  in the heart tissue.

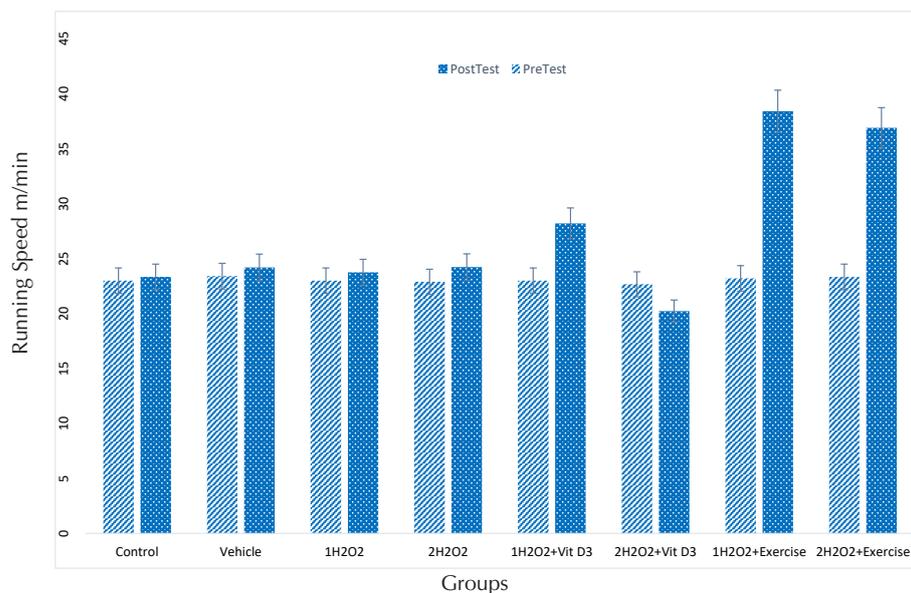
Based on the results, TNF- $\alpha$  level of the heart tissue



**Figure 1.** The Effects of Vitamin D3 and Regular Exercise on the Expression of TNF- $\alpha$  in the Heart Tissue of the Rat Oxidative Stress Animal Model. As shown, 2mmol/kg of  $H_2O_2$  significantly increase the expression of TNF- $\alpha$  ( $P=0.039$ ) while vitamin D3/regular exercise ( $P=0.049$ ) reduces its expression. Note. 2  $H_2O_2$ : 2mmol/kg of  $H_2O_2$ ; 1  $H_2O_2$ : 1mmol/kg of  $H_2O_2$ .



**Figure 2.** The Effects of Vitamin D3 and Regular Exercise on the Lee Index of the Rat Oxidative Stress Animal Model. Figure 2 illustrates that vitamin D3 decreases Lee index in both 2 mmol/kg of  $H_2O_2$  ( $P=0.023$ ) and 1 mmol/kg of  $H_2O_2$  (1  $H_2O_2$ ,  $P=0.001$ ) groups. Note. 2  $H_2O_2$ : 2 mmol/kg of  $H_2O_2$ ; 1  $H_2O_2$ : 1 mmol/kg of  $H_2O_2$ .



**Figure 3.** The Effects of Vitamin D3 and Regular Exercise on the Running Speed of the Rat Oxidative Stress Animal Model. As displayed, vitamin D3 and regular exercise increase the running speed in 2 mmol/kg of H<sub>2</sub>O<sub>2</sub> group ( $P=0.018$ ). Note. 2 H<sub>2</sub>O<sub>2</sub>: 2 mmol/kg of H<sub>2</sub>O<sub>2</sub>; 1 H<sub>2</sub>O<sub>2</sub>: 1 mmol/kg of H<sub>2</sub>O<sub>2</sub>.

significantly increased in the rats which were exposed to 2 mmol/kg of H<sub>2</sub>O<sub>2</sub> compared to those which were treated with 1 mmol/kg of H<sub>2</sub>O<sub>2</sub>. Moreover, the exposition to 2 mmol/kg of H<sub>2</sub>O<sub>2</sub> significantly increased the Lee index whereas it decreased the running speed. Therefore, the results confirmed the induction of OS in animals that were administrated 2 mmol/kg of H<sub>2</sub>O<sub>2</sub>. Additionally, the results approved the induction of a pro-inflammatory state in the heart of the rats which were exposed to H<sub>2</sub>O<sub>2</sub>-induced OS.<sup>1,20-22</sup> These are in line with the results of previous investigations regarding the impacts of OS in inducing the expression of pro-inflammatory molecules such as TNF- $\alpha$ .<sup>1,23</sup> Interestingly, VD3 and RAE administration decreased the TNF- $\alpha$  level of the heart tissue and Lee index while increasing the running speed in both rat groups exposed to 2 mmol/kg of H<sub>2</sub>O<sub>2</sub> and 1 mmol/kg of H<sub>2</sub>O<sub>2</sub>. Based on the results, it seems that VD3 and RAE modulate OS effects in the cardiovascular system. In addition, both VD3 and RAE are found to have immunomodulatory effects on the myocardium which may lead to increased cardiovascular functions and decreased inflammation by down-regulating the TNF- $\alpha$ . Immunoregulatory effects of VD3 were previously documented by several studies.<sup>9,20</sup> The present study further showed the critical effects of VD3 supplementation on the OS and the local expression of TNF- $\alpha$  in the heart. Further, VD3 administration ameliorated the Lee index, and RAE significantly increased the running speed in either 2 mmol/kg of H<sub>2</sub>O<sub>2</sub> or 1 mmol/kg of H<sub>2</sub>O<sub>2</sub> exposed rats. Accordingly, VD3 may assist in divulging the OS by manipulating the cell metabolism while RAE improved physical performance including the running speed. Thus, since OS is an important risk factor for inducing or stimulating the CVDs,<sup>21</sup> VD3 and RAE

can apparently be considered as complementary factors in managing the patients with CVDs. Similarly, previous studies revealed that RAE can modulate OS ramifications in various cells including the modulations of proteasome activation in the heart,<sup>13</sup> tyrosinase phosphorylation in the sperms,<sup>12</sup> prostaglandins production in the vessels,<sup>22</sup> as well as hypertension.<sup>11</sup> The results of the current study further demonstrated that RAE can improve physical performance and running speed while VD3 can modulate the Lee index, as a physiological and biochemical marker. However, based on the findings of previous studies, VD3 can modulate pro-inflammatory conditions by inhibiting several pro-inflammatory signaling molecules such as NF- $\kappa$ B (i.e., nuclear factor kappa-light-chain-enhancer of the activated B cells), which is the main factor for the expression of pro-inflammatory molecules,<sup>23</sup> by increasing the expression of NF- $\kappa$ B inhibitor (IkB).<sup>25</sup> Finally, studies have revealed that RAE is the main factor for inducing OS inhibitory pathways.<sup>13,25</sup>

### Conclusion

In general, RAE and VD3 can synergistically act to modulate the side effects of OS in both physical and molecular levels in the heart tissue.

### Ethical Approval

The Ethical Committee of Kerman University of Medical Sciences approved the protocol of the current study (Code: IR.KMU.REC.1396.1562).

### Competing Interests

Authors have no conflict of interest to declare.

## Acknowledgment

This project was supported by Kerman University of Medical Sciences and was derived from a Ph.D. thesis.

## References

- Husain K, Hernandez W, Ansari RA, Ferder L. Inflammation, oxidative stress and renin angiotensin system in atherosclerosis. *World J Biol Chem.* 2015;6(3):209-217. doi:10.4331/wjbc.v6.i3.209
- Rani V, Deep G, Singh RK, Palle K, Yadav UC. Oxidative stress and metabolic disorders: Pathogenesis and therapeutic strategies. *Life Sci.* 2016;148:183-193. doi:10.1016/j.lfs.2016.02.002
- Yang Y, Lv J, Jiang S, et al. The emerging role of Toll-like receptor 4 in myocardial inflammation. *Cell Death Dis.* 2016;7:e2234. doi:10.1038/cddis.2016.140
- Poljsak B, Suput D, Milisav I. Achieving the balance between ROS and antioxidants: when to use the synthetic antioxidants. *Oxid Med Cell Longev.* 2013;2013:956792. doi:10.1155/2013/956792
- Gorlach A, Dimova EY, Petry A, et al. Reactive oxygen species, nutrition, hypoxia and diseases: Problems solved? *Redox Biol.* 2015;6:372-385. doi:10.1016/j.redox.2015.08.016
- Shirazi LF, Bissett J, Romeo F, Mehta JL. Role of inflammation in heart failure. *Curr Atheroscler Rep.* 2017;19(6):27. doi:10.1007/s11883-017-0660-3
- Sepehri Z, Masoumi M, Ebrahimi N, et al. Atorvastatin, losartan and captopril lead to upregulation of TGF-beta, and downregulation of IL-6 in coronary artery disease and hypertension. *PLoS One.* 2016;11(12):e0168312. doi:10.1371/journal.pone.0168312
- Huang CJ, McAllister MJ, Slusher AL, Webb HE, Mock JT, Acevedo EO. Obesity-related oxidative stress: the impact of physical activity and diet manipulation. *Sports Med Open.* 2015;1(1):32. doi:10.1186/s40798-015-0031-y
- Arababadi MK, Nosratabadi R, Asadikaram G. Vitamin D and toll like receptors. *Life Sci.* 2018;203:105-111. doi:10.1016/j.lfs.2018.03.040
- Norman PE, Powell JT. Vitamin D and cardiovascular disease. *Circ Res.* 2014;114(2):379-393. doi:10.1161/circresaha.113.301241
- Makino A, Skelton MM, Zou AP, Cowley AW Jr. Increased renal medullary H<sub>2</sub>O<sub>2</sub> leads to hypertension. *Hypertension.* 2003;42(1):25-30. doi:10.1161/01.hyp.0000074903.96928.91
- Li SF, Liu HX, Zhang YB, Yan YC, Li YP. The protective effects of alpha-ketoacids against oxidative stress on rat spermatozoa in vitro. *Asian J Androl.* 2010;12(2):247-256. doi:10.1038/aja.2009.78
- Radak Z, Sasvari M, Nyakas C, Pucsok J, Nakamoto H, Goto S. Exercise preconditioning against hydrogen peroxide-induced oxidative damage in proteins of rat myocardium. *Arch Biochem Biophys.* 2000;376(2):248-251. doi:10.1006/abbi.2000.1719
- Halder SK, Sharan C, Al-Hendy A. 1,25-dihydroxyvitamin D3 treatment shrinks uterine leiomyoma tumors in the Eker rat model. *Biol Reprod.* 2012;86(4):116. doi:10.1095/biolreprod.111.098145
- Husain K, Hazelrigg SR. Oxidative injury due to chronic nitric oxide synthase inhibition in rat: effect of regular exercise on the heart. *Biochim Biophys Acta.* 2002;1587(1):75-82.
- Bernardis LL. Prediction of carcass fat, water and lean body mass from Lee's "nutritive ratio" in rats with hypothalamic obesity. *Experientia.* 1970;26(7):789-790.
- Hoydal MA, Wisloff U, Kemi OJ, Ellingsen O. Running speed and maximal oxygen uptake in rats and mice: practical implications for exercise training. *Eur J Cardiovasc Prev Rehabil.* 2007;14(6):753-760. doi:10.1097/HJR.0b013e3281eacef1
- Plant DR, Gregorevic P, Warmington SA, Williams DA, Lynch GS. Endurance training adaptations modulate the redox-force relationship of rat isolated slow-twitch skeletal muscles. *Clin Exp Pharmacol Physiol.* 2003;30(1-2):77-81.
- Granger DN, Kviety PR. Reperfusion injury and reactive oxygen species: The evolution of a concept. *Redox Biol.* 2015;6:524-551. doi:10.1016/j.redox.2015.08.020
- Portou MJ, Baker D, Abraham D, Tsui J. The innate immune system, toll-like receptors and dermal wound healing: A review. *Vascul Pharmacol.* 2015;71:31-36. doi:10.1016/j.vph.2015.02.007
- Bagul PK, Deepthi N, Sultana R, Banerjee SK. Resveratrol ameliorates cardiac oxidative stress in diabetes through deacetylation of NFkB-p65 and histone 3. *J Nutr Biochem.* 2015;26(11):1298-1307. doi:10.1016/j.jnutbio.2015.06.006
- Puri N, Zhang F, Monu SR, et al. Antioxidants condition pleiotropic vascular responses to exogenous H<sub>2</sub>O<sub>2</sub>: role of modulation of vascular TP receptors and the heme oxygenase system. *Antioxid Redox Signal.* 2013;18(5):471-480. doi:10.1089/ars.2012.4587
- Al-Rasheed NM, Al-Rasheed NM, Bassiouni YA, et al. Vitamin D attenuates pro-inflammatory TNF-alpha cytokine expression by inhibiting NF-small ka, CyrillicB/p65 signaling in hypertrophied rat hearts. *J Physiol Biochem.* 2015;71(2):289-299. doi:10.1007/s13105-015-0412-1
- Roy A, Srivastava M, Saqib U, et al. Potential therapeutic targets for inflammation in toll-like receptor 4 (TLR4)-mediated signaling pathways. *Int Immunopharmacol.* 2016;40:79-89. doi:10.1016/j.intimp.2016.08.026
- Sallam N, Laher I. Exercise Modulates Oxidative Stress and Inflammation in Aging and Cardiovascular Diseases. *Oxid Med Cell Longev.* 2016;2016:7239639. doi:10.1155/2016/7239639