

# Nandrolone Plus Moderate Exercise Increases the Susceptibility to Lethal Arrhythmias

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**Background:** Until now, no experimental study has directly assessed the arrhythmogenesis of chronic consumption of anabolic androgenic steroids along with moderate-intensity endurance exercise.

**Objectives:** We evaluated the influence of integration of anabolic androgenic steroids along with moderate-intensity endurance exercise on susceptibility to lethal ventricular arrhythmias in rat.

**Materials and Methods:** The animal groups were as follows: control group (CTL); exercise group (EX) which were under 6 weeks of treadmill exercise; nandrolone group (Nan) which received 5 mg/kg of nandrolone decanoate twice a week; vehicle group (Arach) which received Arachis oil (solvent of nandrolone); trained vehicle group (Arach + Ex); and trained nandrolone group (Nan + Ex). One day after ending of the intervention period, arrhythmia was induced by intravenous infusion of aconitine and ventricular arrhythmias were recorded. Then malondialdehyde (MDA) and glutathione peroxidase (GPX) of heart tissue were measured.

**Results:** Nandrolone, exercise, and their combination were associated with heart hypertrophy. Exercise could prevent the incremental effect of nandrolone on MDA/GPX ratio. Chronic administration of nandrolone with moderate-intensity endurance exercise had no significant effect on blood pressure, heart rate, and basal electrocardiographic parameters. Combination of nandrolone and exercise significantly increased the incidence of ventricular fibrillation (VF) and reduced the VF latency ( $P < 0.05$ ).

**Conclusions:** The findings suggest that chronic coadministration of nandrolone with moderate-intensity endurance exercise facilitates the VF occurrence in rat. Complementary studies are needed to elucidate the involved mechanisms of this abnormality.

**Keywords:** Nandrolone Decanoate; Aerobic Exercise; Ventricular Fibrillation

## 1. Background

Despite some beneficial effects of anabolic androgenic steroids (AAS) compounds in improvement of physical performance, chronic using of high doses of these drugs is associated with numerous adverse effects on the cardiovascular system including hypertension, myocardial infarction, dysrhythmias, hypertrophic cardiomyopathy, and cardiac remodeling (1-4).

Regular courses of exercise are the only practical method that can protect the heart against coronary artery diseases. Only one course of exercise can protect heart against ischemia/reperfusion injury (5). In addition, regular courses of aerobic exercise such as running or swimming can protect heart against the ischemia/reperfusion injury in animal models and can lead to diminution of infarcted area (6, 7). Regarding the cardiovascular adverse effects of AASs, usefulness of exercise training with consumption of high amounts of these drugs is controversial and questionable. Consumption of high doses of anabolic steroids might increase the possibility of cardiac arrhythmias. In animals with cardiac ischemia, acute injection of nandrolone

increases the possibility of cardiac arrhythmias (8). It is also reported that high and chronic consumption of nandrolone leads to ventricular repolarization disturbances (9). In addition, there are numerous evidence regarding occurrence of sudden death among athletics using anabolic steroids, especially nandrolone (10-12). However, to our knowledge, thus far no investigation has been directly performed to study the effect of chronic nandrolone consumption along with moderate exercise on the development of lethal cardiac arrhythmia.

## 2. Objectives

Based on the significance of this issue, we assessed the effects of chronic administration of nandrolone along with moderate-intensity treadmill exercise on susceptibility to lethal ventricular arrhythmias in rat.

## 3. Materials and Methods

Aconitine and sodium thiopental were purchased from

Sigma (England) and Sandoz (Austria), respectively. Nandrolone decanoate and Arachis oil, purchased respectively from Gedeon Richter (Hungary) and Henry Lamotte (Germany), were dedicated by Iran Hormone Company. Animal procedures were performed in accordance with the guidelines for the care and use of laboratory animals (Ethic Committee permission Kerman University of Medical Sciences, Kerman, Iran) and were performed on 48 male Wistar rats weighing 250 to 300 g. The animals were housed in a temperature-controlled room with free access to rat regular diet and water. Before the start of the experiment, all rats were run-tested on a motorized rodent treadmill (3 × 5 minutes, with 15 minutes breaks) for three successive days to habituate them to the training environment and to eliminate rats unwilling to run (13). Then they were randomly assigned to one of the following six experimental groups: 1) sedentary group (CTL group); 2) trained group with aerobic exercise by treadmill training (Ex group); 3) sedentary vehicle-treated (Arach group) (vehicle = 0.2 mL/kg Arachis oil as solvent of nandrolone decanoate twice a week, intramuscularly, for 6 weeks; 4) sedentary nandrolone-treated (Nan group), which received 5 mg/kg of nandrolone decanoate twice a week, intramuscularly, for 6 weeks (14); 5) trained vehicle-treated (Arach + Ex group) which received Arachis oil and swimming training; and 6) trained nandrolone-treated (Nan + Ex group) which received nandrolone decanoate and swimming training.

### 3.1. Exercise Training Protocol

Rats in the exercise groups were trained on a rodent treadmill (at 0° slope) five days a week, for 6 weeks. The treadmill speed was 16 m/min during the first week, increased by 4 m/min weekly over the next three weeks, and was kept at 28 m/min (~70 - 75% maximal aerobic capacity (VO<sub>2</sub> max)) for the remaining training sessions (13, 15). This protocol is considered as a moderate-intensity endurance training (15).

### 3.2. Blood Pressure and Electrocardiogram Recording and Arrhythmia Induction

Hemodynamic and electrocardiogram (ECG) recording and arrhythmia induction were performed as described previously (16). A day after the completion of the training session, the animals were anesthetized with intraperitoneal sodium thiopental (50 mg/kg). The right common carotid artery was cannulated and connected to a pressure transducer and a PowerLab system continuously recorded the heart rate (HR) and arterial blood pressure (BP). The trachea was cannulated and animals were artificially ventilated with room air at 50 strokes per minute during arrhythmia induction. Basal ECG (limb lead II) and BP were recorded following recovery time from surgery (15 minutes). Exclusion criteria were the presence of cardiac arrhythmia or a sustained drop in mean arterial BP to < 70 mm Hg during the stabilization period. For ar-

rhythmia induction, aconitine was infused through an angiocatheter (G-24) in the tail vein with a syringe pump at a velocity of 0.1 mL/min (15 µg/mL of saline) for ten minutes. The BP and ECG were simultaneously recorded during the infusion and for another five minutes after the infusion period ended (16).

### 3.3. Measured and Calculated Parameters

The mean arterial pressure (MAP) was estimated using the following formula:  $MAP = Pd + (Ps - Pd)/3$ , where Pd and Ps are respectively the diastolic and systolic arterial pressures. The PR interval (the earliest P-wave onset to the earliest onset of the QRS complex onset) and QT interval (the earliest Q or R-wave onset through the end of T wave) of basal ECG were determined by a mean of two minutes of ECG-recorded strip. In order to obviate the dependence of QT interval on HR, corrected QT interval (QTc) was measured using Bazett's formula normalized as:

$$QTc - B = QT / (RR/f)^{1/2}$$

Where RR is R-R interval and f = 150 msec (16-18).

The premature ventricular contraction (PVC), ventricular tachycardia (VT), and ventricular fibrillation (VF) were counted during the 15 minutes of the experiment, and the latency and duration of PVC, VT, and VF was measured in seconds. According to the Lambeth conventions, ventricular arrhythmias were defined as follows:

Ventricular premature beat (VPB; equal to PVC) is defined as a ventricular electrical complex (complete electrical event: QRS, RS, QRST, or RST) that is different in shape (voltage and/or duration, i.e. height and/or width) from the preceding (non-VPB) ventricular complex, and is premature in relation to the preceding ventricular complex. VT is a run of four or more consecutive ventricular premature beats. VF is defined as a sequence of a minimum of four consecutive ventricular complexes without intervening diastolic pauses in which the intrinsic shape, the peak-peak interval, and the height vary, and the variation between each is non-progressive (19).

The threshold dose of aconitine required for producing different ventricular arrhythmias (e.g. PVC, VT, or VF) was determined according to the following formula (16):

$$\text{Threshold } (\mu\text{g/kg}) \text{ for arrhythmia} = 15 \mu\text{g/mL} \times 0.1 \text{ mL/min} \times \text{time required for arrhythmia (min)} / \text{body weight (BW) (kg)} = 1.5 \mu\text{g/min} \times \text{time (min)} / \text{BW (kg)}$$

In addition, The severity of arrhythmias in the different groups was presented quantitatively by a previous scoring system (18) defined as follows: 0, < 10 PVCs; 1, ≥ 10 PVCs; 2, one to five episodes of VT; 3, > 5 episodes of VT or 1 episode of VF; 4, two to five episodes of VF; 5, > 5 episodes of VF; 6, VT or VF or both with duration > 300 sec.

Finally, the rats were killed and their hearts were removed, rinsed with cold saline, and weighed. The total heart weight (HW, left, and right ventricle) was normalized by total BW of the animal (mg/g). This ratio was considered as an index of cardiac hypertrophy (4, 20). A piece of heart apex dissected, weighed, and homogenized in 5 mL of 0.1% MTris-HCl buffer (pH 7.4) in ice-cold condition.

After centrifuging, the clear supernatant solution was taken for the biochemical analysis. Total proteins were measured by using the Lowry et al. method (21). Malondialdehyde (MDA) levels, as an index of lipid peroxidation, was estimated by concentration of thiobarbituric acid reactive substances (TBARS) in heart tissue (22). Glutathione peroxidase (GPX) of heart tissues was determined using relative Randox assay kits (according to the manufacturer's protocol) (23).

### 3.4. Statistical Analysis

The data were presented as mean  $\pm$  standard error of the mean. The normal distribution of quantitative data was confirmed by Kolmogorov-Smirnov test. Comparison of all parameters except arrhythmia scores among different groups were performed using one-way analysis of variance (ANOVA) and Tukey post-hoc test. Arrhythmia scores in the animal groups were compared using nonparametric Kruskal-Wallis test. The data was analyzed by use of SPSS 17 (SPSS Inc., Chicago, Illinois, the United States). P value  $< 0.05$  was considered as statistically significant.

## 4. Results

### 4.1. Ventricular Hypertrophy Index

Ventricular hypertrophy index showed significant increase in Nan group ( $P < 0.01$  versus CTL and  $P < 0.05$  versus Arach groups), Ex group ( $P < 0.01$  versus CTL and Arach groups) and in Nan + Ex group ( $P < 0.01$  versus CTL group) (Figure 1).

### 4.2. Redox Indices

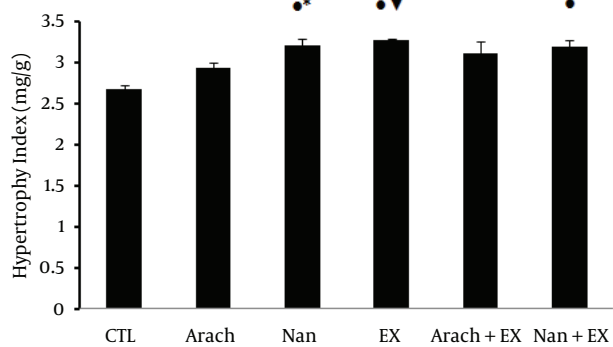
Six weeks of nandrolone consumption and moderate intensity exercise with or without nandrolone was associated with non-significant increase in MDA of cardiac tissue. In addition nandrolone-treated group showed in-

significant decrease in GPX of heart, so that the ratio of MDA/GPX only was significant in Nan group compared to CTL group ( $P < 0.05$ ) (Table 1).

### 4.3. Blood Pressure and Heart Rate and Basal Electrocardiographic Parameters

A sample strips of arterial BP and lead II of ECG from a rat of the Nan + Ex group, which were simultaneously recorded, are shown in Figure 2 A. Moderate-intensity endurance exercise or nandrolone alone or their combination didn't have significant effect on BP, HR, and RR, PR, J, QRS complex, and QTc intervals of electrocardiogram (Table 2).

**Figure 1.** Mean of Heart Hypertrophy Index in Different Experimental Groups at the End of 6<sup>th</sup> Week



Values are presented as mean  $\pm$  SEM. N = 7 - 9. CTL, control; Arach, animal group which received vehicle (Arachis oil); Nan, animal group which received 5 mg/kg/day of nandrolone decanoate twice a week; Ex, animal group which trained with prolonged moderate intensity exercise; Arach + Ex, animals group which received Arachis oil and exercise training; Nan + Ex, animals group which received nandrolone decanoate and exercise training; \*,  $P < 0.05$  compared with Arach group; •,  $P < 0.01$  compared with CTL group; ▼,  $P < 0.01$  compared with Arach group.

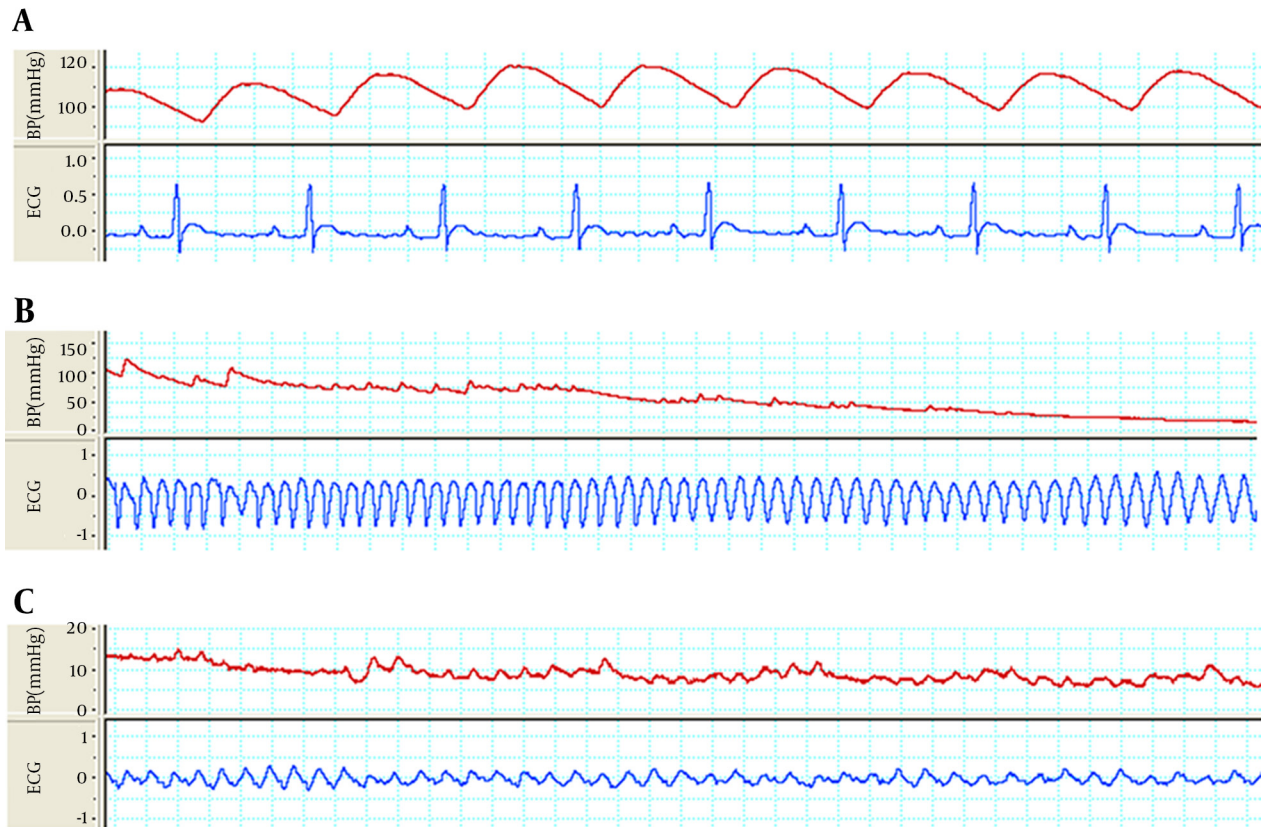
**Table 1.** Glutathione Peroxidase and Malondialdehyde of Heart Tissue in Different Animal Groups<sup>a</sup>

Group <sup>b</sup>	n	MDA, $\mu\text{mol}/\text{mg Protein}$	GPX, Unit/mg Protein	MDA/GPX
CTL	7	1.26 $\pm$ 0.267	15.98 $\pm$ 4.89	0.079 $\pm$ 0.019
Arach	7	1.305 $\pm$ 0.26	12.42 $\pm$ 1.69	0.105 $\pm$ 0.018
Nan	7	1.96 $\pm$ 0.27	12.02 $\pm$ 1.45	0.163 $\pm$ 0.019*
EX	9	2.08 $\pm$ 0.25	23.41 $\pm$ 3.51	0.089 $\pm$ 0.013
Arach + EX	9	2.16 $\pm$ 0.34	15.82 $\pm$ 1.64	0.136 $\pm$ 0.020
Nan + EX	8	1.8 $\pm$ 0.27	17.45 $\pm$ 2.46	0.103 $\pm$ 0.017
<b>P value</b>	-	0.056	0.131	0.046

<sup>a</sup> Abbreviations: GPX, glutathione peroxidase; MDA, malondialdehyde; and NA, non-applicable.

<sup>b</sup> CTL, control; Arach, animal group which received vehicle (Arachis oil); Nan, animal group which received 5 mg/kg/day of nandrolone decanoate twice a week; Ex, animal group which trained with prolonged moderate intensity exercise; Arach + Ex, animals group which received Arachis oil and exercise training; Nan + Ex, animals group which received nandrolone decanoate and exercise training.

**Figure 2.** The Strips of Arterial Blood Pressure and Lead II of ECG That Simultaneously was Recorded From an Animal From Nan+ Ex Group in Different Condition



A, Basal normal sinus rhythm (NSR) and blood pressure of an animal from Nan+ Ex group. B, Sustained ventricular tachycardia (VT) with regular morphology and rate along with blood pressure drop in same animal six minutes after onset of aconitine injection. C, Sustained ventricular fibrillation (VF) along with severe blood pressure drops in same animal 11 minutes after onset of aconitine injection.

**Table 2.** Mean Arterial Blood Pressure, Heart Rate, and parameters of Electrocardiogram in All Animal Groups <sup>a</sup>

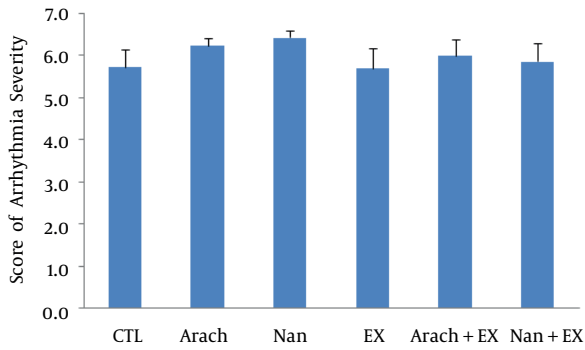
Group <sup>b</sup>	n	MAP, mmHg	HR, Beat/min	Electrocardiographic Parameters				
				RR Interval, msec	PR Interval, msec	QRS Interval, msec	JT Interval, msec	QTc-n Interval, msec
CTL	7	117 ± 2.7	406 ± 14	149 ± 5	44.7 ± 1	14.9 ± 0.9	40 ± 4.2	55 ± 4.4
Arach	8	116 ± 5	375 ± 36	153 ± 4	43.7 ± 1	16 ± 1	38 ± 2.9	55 ± 3.5
Nan	8	106 ± 8	382 ± 15	159 ± 7	46.5 ± 1.5	15.4 ± 0.8	38 ± 2.2	52 ± 2.9
EX	9	110 ± 5	400 ± 16	148 ± 7	44.5 ± 1	14.2 ± 0.7	40 ± 2.3	55 ± 2.4
Arach + EX	9	101 ± 9	376 ± 11	160 ± 4	44.5 ± 1.2	16.9 ± 1	42 ± 4.6	57 ± 3.7
Nan + EX	8	104 ± 8	395 ± 10	153 ± 4	42.5 ± 1.7	15 ± 0.5	43 ± 3.3	57 ± 3.4
P value	-	0.271	0.482	0.513	0.381	0.375	0.346	0.829

<sup>a</sup> Abbreviations: MAP, mean arterial pressure; HR, heart rate; NA, non-applicable.

<sup>b</sup> CTL, control; Arach, animal group which received vehicle (Arachis oil); Nan, animal group which received 5 mg/kg/day of nandrolone decanoate twice a week; Ex, animal group which trained with prolonged moderate intensity exercise; Arach + Ex, animals group which received Arachis oil and exercise training; Nan + Ex, animals group which received nandrolone decanoate and exercise training.

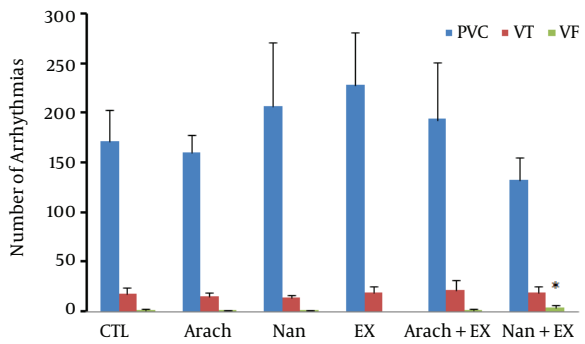


**Figure 3.** Scores of Arrhythmia Severity in Each Experimental Group



Values are presented as mean ± SEM. N= 7 - 9. CTL, control; Arach, animal group which received vehicle (Arachis oil); Nan, animal group which received 5 mg/kg/day of nandrolone decanoate twice a week; Ex, animal group which trained with prolonged moderate intensity exercise; Arach + Ex, animals group which received Arachis oil and exercise training; Nan + Ex, animals group which received nandrolone decanoate and exercise training.

**Figure 4.** The Number of Ventricular Arrhythmias in Animal Groups

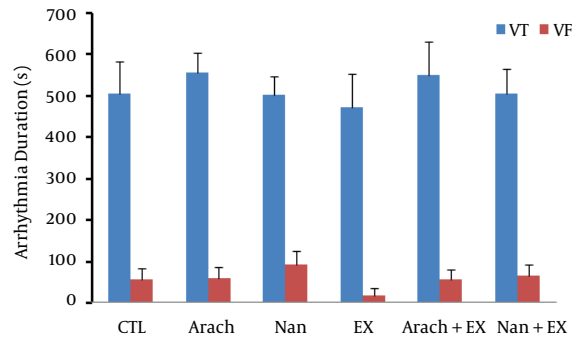


Values are presented as mean ± SEM. N= 7 - 9. CTL, control; Arach, animal group which received vehicle (Arachis oil); Nan, animal group which received 5 mg/kg/day of nandrolone decanoate twice a week; Ex, animal group which trained with prolonged moderate intensity exercise; Arach + Ex, animals group which received Arachis oil and exercise training; Nan + Ex, animals group which received nandrolone decanoate and exercise training. \*, P < 0.05 compared with Ex group.

#### 4.4. Susceptibility to Ventricular Arrhythmias

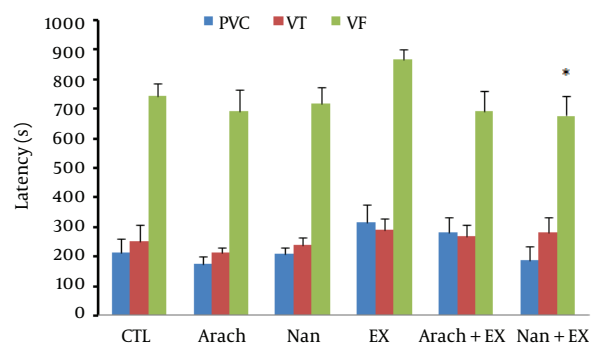
Chronic administration of nandrolone with moderate-intensity endurance exercise did not have significant effect on severity of ventricular arrhythmias (Figure 3) as well as the incidence of PVCs and VT, but increased the incidence of VF when compared with Ex group (P < 0.05) (Figure 4). The duration of VF showed increase in nandrolone group and decrease in Ex group, but did not reach to significant level (Figure 5). The latency of PVC and VT had no significant difference among groups, but latency of VF in Nan + Ex group had a significant reduction compared with Ex group (P < 0.05) (Figure 6). Sample strips of arterial BP and lead II of ECG during VT and VF from a rat of the Nan + Ex group that simultaneously were recorded are shown in Figure 2.

**Figure 5.** The Duration of Ventricular Tachycardia and Ventricular Fibrillation Arrhythmias in the Different Groups



Values are presented as mean ± SEM. N= 7 - 9. CTL, control; Arach, animal group which received vehicle (Arachis oil); Nan, animal group which received 5 mg/kg/day of nandrolone decanoate twice a week; Ex, animal group which trained with prolonged moderate intensity exercise; Arach + Ex, animals group which received Arachis oil and exercise training; Nan + Ex, animals group which received nandrolone decanoate and exercise training.

**Figure 6.** The Latency Periods for Induction of Ventricular Arrhythmias



Values are presented as mean ± SEM. N= 7 - 9. CTL, control; Arach, animal group which received vehicle (Arachis oil); Nan, animal group which received 5 mg/kg/day of nandrolone decanoate twice a week; Ex, animal group which trained with prolonged moderate intensity exercise; Arach + Ex, animals group which received Arachis oil and exercise training; Nan + Ex, animals group which received nandrolone decanoate and exercise training. \*, P < 0.05 compared with Ex group.

#### 5. Discussion

In the present study, the effects of chronic administration of nandrolone decanoate with moderate-intensity endurance exercise on the occurrence of lethal ventricular arrhythmias were investigated in rat. Nandrolone alone significantly increased the ratio of MDA/GPX of cardiac tissue, but moderate-intensity endurance treadmill exercise prevented this adverse effect of nandrolone. In addition, moderate exercise and nandrolone, alone or in combination, were associated with significant increase of the heart hypertrophy index. Nandrolone decanoate alone or along with moderate-intensity endurance exercise did not have significant effects on BP, HR, and basal ECG parameters. Combination of moderate-intensity exercise and nandrolone significantly increased the inci-

dence of VF, the most lethal cardiac arrhythmia, and significantly reduced the VF latency.

In agreement with results of present study, Franquni et al. (24) showed that chronic administration of nandrolone can lead to significant increase in HW to BW ratio. They showed that activity of angiotensin-converting enzyme during consumption of nandrolone increases and this can lead to increase of angiotensin II release, which is a factor for hypertrophic induction. Some previous animal studies also observed the significant increase of hypertrophy index following exercise training (25-27).

Consistent with our findings, other studies have shown that chronic administration of nandrolone along with exercise is associated with significant increase in heart hypertrophy index (25, 28-30). They showed that renin-angiotensin system activity has increased, which can justify the hypertrophic induction during exercise conditions with consumption of nandrolone. Overall, increase in heart collagen (20) changes the expression of myocardial enzymes (31) and increasing angiotensin-converting enzyme and rennin-angiotensin system activity (20, 24) may be involved in heart hypertrophy following to nandrolone consumption and exercise training.

The MDA is produced from lipid peroxidation, which can be caused by an increase in oxidants. Its production rate is proportional to the breakdown of unsaturated fatty acids. The GPX is an antioxidant enzyme that plays a role in removing free radicals and reducing their damage. In agreement with the findings of present study, others showed that endurance exercise has no significant effect on MDA of cardiac tissue in male rats (23, 32-36). In addition, another study reported that chronic administration of 10 mg/kg of nandrolone for 14 weeks did not have significant effect on GPX level in rats (37). Other studies on male rats showed that endurance exercise causes insignificant increase of GPX activity (35, 36, 38). Similar to findings of the present study, others also reported that exercise and nandrolone, when combined, have no significant effect on cardiac GPX (13, 39). On the other hand, some experiments showed that the treadmill exercise for eight weeks at a rate of 20 m/min significantly increased GPX activity in the male rat (40, 41) and nandrolone administration for 10 weeks at a dose of 10 mg/kg per week non-significantly decreases the GPX; however, nandrolone along with exercise had no effect on the heart GPX (13, 39). In the present study, MDA/GPX in nandrolone group, made up of increased MDA and decreased GPX, in this group was significantly higher than that in the control group; however, moderate-intensity endurance exercise prevented this adverse effect. Therefore, it can be assumed that chronic administration nandrolone induces redox imbalance in favor to oxidants agents and moderate-intensity endurance exercise helps to prevent the destructive effects of nandrolone by balancing redox system.

Regarding the effects of exercise and chronic administration of nandrolone on BP, consistent with the present

study, previous animal studies have reported ineffectiveness of endurance treadmill exercise (42, 43) and chronic administration of nandrolone on BP (44). It was also reported that chronic administration of nandrolone and swimming exercise, alone or in combination, have no effect on BP (29, 45). However, previous studies showed that in hypertensive mice, endurance exercise could significantly reduce BP (46, 47). A part of this useful effect of exercise on reduction of BP in hypertensive animals has been attributed to increased plasma-level of atrial natriuretic peptide (ANP) (a vasodilator and reducer of peripheral vascular resistance), reduction of Angiotensin II (a potent vasoconstrictor) and reduction of sympathetic nerve activity during swimming activity (46, 47). The difference between these two studies and ours could be due to examining hypertensive animals in those two studies, while animals were normotensive in our study, and it is evident that treatment protocols such as drugs and exercise can be more effective upon hypertensive animals compared with normotensive ones. Tseng et al. indicated that chronic seven-week administration of 20-mg/kg nandrolone to hypertensive animals can significantly increase BP (48). Of course, in contrast to our study, BP was measured in conscious mode, animals had hypertension, and nandrolone dose in Tseng et al. study was twice as high as our study, all of which justify the difference of their findings with ours.

Regarding the effect of chronic administration of nandrolone and exercise on HR, most of similar studies reported lack of significant effect of exercise (46, 47, 49) or chronic administration of nandrolone (48, 50) on HR of rats. Soares et al. (29) reported insignificant effect of exercise and nandrolone, alone or in combination, on HR of rats. In a human study, Maior et al. (51) also reported the same case as above. Some studies demonstrated contrary findings in which chronic administration of 20-mg/kg nandrolone for ten weeks led to significant reduction of HR (4, 24). In other studies, it was reported that swimming exercise for ten weeks resulted in significant decrease in HR (26, 52). It seems that type, intensity and length of exercise as well as amount of injected nandrolone are the reasons of discrepancy among these findings.

Similar to our results, Phillis et al. (53) demonstrated that chronic administration of 15-mg/kg nandrolone for 17 days had no effect on QTc, QRS, and PR intervals of ECG in male rats. In a human study, Bruder-Nascimento et al. (45) indicated that chronic administration of nandrolone has no effect on QRS. Maior et al. (51) reported that chronic administration of nandrolone results in significant increase of QTc interval in human. The reason of difference between findings of this study and ours is probably the kind of study, i.e., animal vs. human. Achar et al. (54) in a human study showed that chronic administration of nandrolone for five years leads to increase of lethal ventricular arrhythmias such as VF. In another study, Phillis et al. (53) reported that acute administration of nandro-

lone with a value of 10 to 160  $\mu\text{g}/\text{kg}/\text{minute}$  during 15 minutes before operation led to significant increase of VF duration while it had no effect on scores of arrhythmias in rat. In the present study, administration of nandrolone and exercise separately were associated with an insignificant increase and decrease in VF duration, respectively. The reason for significance of this parameter in the study of Phillis et al. (53) might be acute intravenous (IV) injection of nandrolone, while injections were intramuscular (IM) in our study that led to much slower absorption of drug and lower serum level peak. Williams and Franklin (55), reported that exercises such as running and walking reduce the risk of ventricular arrhythmias in young males. Other clinical studies on human (mean age,  $\geq 40$  years) showed that long-term and intense exercises result in increased risk of ventricular arrhythmias (56, 57). They also reported that stimulation of sympathetic system during exercise leads to increase of ventricular automaticity followed by increase the depolarization of Purkinje fibers, which could be a mechanism for VF induction by exercise. The reason of difference of findings in this study and ours could be the kind of study and exercise (i.e., human study and intense treadmill exercise vs. animal study and moderate-intensity endurance exercise).

We showed that moderate-intensity endurance exercise alone caused insignificant decline of VF duration, but exercise along with nandrolone led to significant increase of VF risk as the most dangerous type of ventricular arrhythmia. Because exercise with nandrolone has no significant effect upon ECG parameters, the reason of increase of VF risk in this case cannot probably be changes of basic ECG parameters. The reasons of decline in VF latency and increase in VF incidence in animals undergoing nandrolone plus exercise training might be remodeling of cardiac tissue including structural myocardial modifications, structural abnormality of myofibrils (1, 58), and obstruction of coronary arteries. The latter might originate from increased platelet aggregation due to increase of capacity of thromboxane A<sub>2</sub> receptor (59) as well as vascular changes including atherosclerosis, vascular stenosis, and coronary vasospasm during chronic administration of nandrolone and stimulation of sympathetic system during exercise, which leads to rise of ventricular automaticity and increase of phase IV of depolarization of Purkinje fibers (60-62). These conditions may lead to local and widespread ischemia of cardiac tissue; in stress or during administration of arrhythmogenic agents, electrical homogeneity of heart is disrupted more, which provides the condition of re-entry phenomenon and facilitates VF. In addition, it was reported that steroid hormones, chemically related to nandrolone, can acutely inhibit the reuptake of catecholamines into extraneuronal tissues, which could in turn increase catecholamine concentrations at receptor sites. Although normally responsible for reuptake of noradrenaline, the neuronal catecholamine transporter has been shown to be responsible for non-exocytotic release of noradrenaline from sympathetic

nerve terminals during ischemia. Increased release of noradrenaline has been implicated in ischemia-induced arrhythmia (63-65). Nandrolone has been shown to cause the release of intracellular calcium in rat primary myotubes, in a manner independent of intracellular androgen receptors but dependent on inositol triphosphate and the extracellular signal-regulated kinase pathway (66). This may be another potential mechanism to explain the increased susceptibility to ventricular arrhythmia in presence of nandrolone and exercise.

It should be noted that because of the limitations of working with animals including ECG recording in a state of consciousness and exercise, the arrhythmia induction performed in anesthetized condition in the present study. The results of this study suggest that chronic administration of nandrolone with long-term moderate-intensity treadmill exercises in male rats can cause significant increase in VF incidence and decrease in VF latency as the most dangerous ventricular arrhythmia. In the present study, this effect was appeared independent of changes of redox system and without vivid changes of ECG parameters. Clarification of involved mechanisms demands complementary studies.

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## Authors' Contributions

Hamideh Ghorbani Baravati and Zeinab Kordestani contributed in the preparation, treatment, and animals training, and helped in experimental procedure and sampling. Siyavash Joukar developed the original idea and the protocol, supervised the study, helped in experimental procedure, data analysis, and wrote the manuscript. Hossein Fathpour contributed as design consultant.

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