# ROLE OF NITRIC OXIDE IN CARDIAC PERFORMANCE DURING EXPERIMENTAL ISCHEMIC CARDIAC ARREST AND RE-PERFUSION

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**ABSTRACT:**

***Background:*** *Re-perfusion strategies are the current standard therapy for acute myocardial infarction, despite the spectrum of re-perfusion-associated pathologies that may contribute to irreversible myocardial injury.*

***Aim of the work:*** *The aim of present study is to clarify the alterations in intrinsic cardiac functions in response to cardiac ischemic arrest followed by re-perfusion in isolated hearts perfused with nitric oxide (NO) donor, L-arginine, or NO inhibitor, Nω-Nitro-L-arginine methyl ester hydrochloride (L-NAME), to shed light on the possible role of NO in re-perfusion process.*

***Materials and Methods:*** *Cardiac activities of hearts isolated from Adult albino rats of both sexes were studied on Langendorff preparation under basal conditions and during 30 min re-perfusion following 30 min of total global ischemia. Rats were randomly allocated into three groups; control and L-arginine or L-NAME infused heart groups. Both L-arginine and L-NAME were infused over 20 min during the baseline activity before induction of total global ischemia. Thereafter, cardiac tissue levels of malondialdhyde, catalase and nitrite were assessed.*

***Results:*** *Compared to the control, both L arginine and L-NAME infused hearts showed increased basal chronotropy and myocardial flow rate. Significantly depressed basal inotropic state was only observed in L-arginine group. The three studied groups demonstrated significant deterioration in the inotropic activity and compromised myocardial flow rate during the whole period of reperfusion. L-arginine infused hearts demonstrated depressed inotropy and chronotropy, weak systolic and diastolic functions with compromised myocardial flow at early 5 min of reperfusion, yet with significantly higher myocardial flow rate % recovery by the end of reperfusion (82.7% ±3.01 in L-arginine vs. 56.4% ± 2.32 in control and 62.6% ±2.17 in L-NAME). The chronotropic activity was maintained in both the control and L-NAME infused hearts. Cardiac tissue NO showed the highest level in L-arginine group and the lowest level in L-NAME one. Both catalase and MDA were insignificantly changed among the three studied groups.*

***Conclusion:*** *Reducing NO availability by L-NAME revealed mild impact on the ischemia re-perfusion induced contractile dysfunction. Excess NO worsens cardiac performance at early re-perfusion. However, it may have potentially protective effect by acquiring higher the myocardial flow rate during the reperfusion.*

**Keywords:** Cardiac arrest, ischemia/re-perfusion, L-arginine, L-NAME, nitric oxide.

## INTRODUCTION:

Coronary heart disease is a growing problem in the whole world with the most common mode of cardiovascular deaths is ischemic heart disease[1&2]. Myocardial ischemia occurs when there is insufficient blood supply to the myocardium, which is commonly caused by atherosclerotic coronary artery disease; or during the course of coronary artery bypass graft surgery, when the aorta is cross-clamped and coronary flow is interrupted[3]. Furthermore,

myocardial ischemia occurs during the course of cardiac transplantation, as the donor's heart suffers from total global ischemia despite the use of hypothermia and cardioplegia[4].

Whatever the cause of myocardial ischemia, the most effective therapy for reducing myocardial damage, preserving ventricular function and preventing the onset of heart failure is the timely re-perfusion[5]. As re-perfusion is mandatory to salvage ischemic myocardium from infarction, however re-perfusion process itself also, contributes to irreversible myocardial injury, a phenomenon which has been termed myocardial re-perfusion injury[6&7].

The nitric oxide (NO), also known as endothelium derived relaxing factor, was first discovered in the late1700s, and was considered to be a toxic gas with environmental hazards[8]. The importance of NO in the field of biology and medicine was not fully appreciated until the 1980s when series of studies were conducted by several independent groups[8]. Nowadays, it is well known that normal production of NO plays a prominent role in controlling blood pressure via the regulation of vascular tone and also prevents cardiovascular disease, including atherosclerosis, stroke, and hypertension[10].

NO is generated in mammals, including humans, by members of family of enzymes, generally named nitric oxide synthase (NOS), using amino acid L-arginine as a precursor and molecular oxygen[11]. It was found that up to ≈70% of systemic NO is accomplished by endothelial nitric oxide synthase (eNOS), one of 3 members of the NOS family (other enzymes: neuronal nitric oxide; synthase (nNOS) and inducible nitric oxide synthase; (iNOS)[12].

Previous studies have suggested that decreased release of endothelial NO may be involved in cardiac ischemia re-perfusion injury, though it's exact role is not clear. Therefore, the present study was carried out to clarify the alterations in intrinsic cardiac functions in response to cardiac ischemic arrest followed by re-perfusion in isolated hearts perfused with NO donor, L-arginine, or NO inhibitor, Nω-Nitro-L-arginine methyl ester hydrochloride (L-NAME), to shed light on the possible role of NO in such conditions.

## MATERIALS AND METHODS:

### Animals:

The present study was performed on 36adult albino rats of both sexes, weighing 150–220 grams. Rats were purchased from the Research Institute of Ophthalmology (Giza, Egypt), and maintained in the Physiology Department Animal House under standard conditions of boarding. Rats were given regular diet composed of bread, milk and vegetables, with free access to water. All rats received care in accordance with the National Health Guidelines and the study protocol was approved by the Research Ethical Committee of the Faculty of Medicine, Ain Shams University.

### Experimental Protocol:

Rats were randomly allocated into the following groups (Figure 1):

1. **Control group**: Hearts of theses rats were subjected to 20 min of perfusion followed by 30 min of total global ischemia, then re-perfusion for another 30 min (n=12).
2. **L-arginine group**: Hearts of theses rats were infused with L-arginine during the 20 min of perfusion, and then followed by the same protocol of ischemia/re-perfusion exactly as control group (n=12).
3. **L-NAME group**: Hearts of theses rats were infused with L-NAME during the 20 min of perfusion, and then followed by ischemia/re-perfusion (n=12).

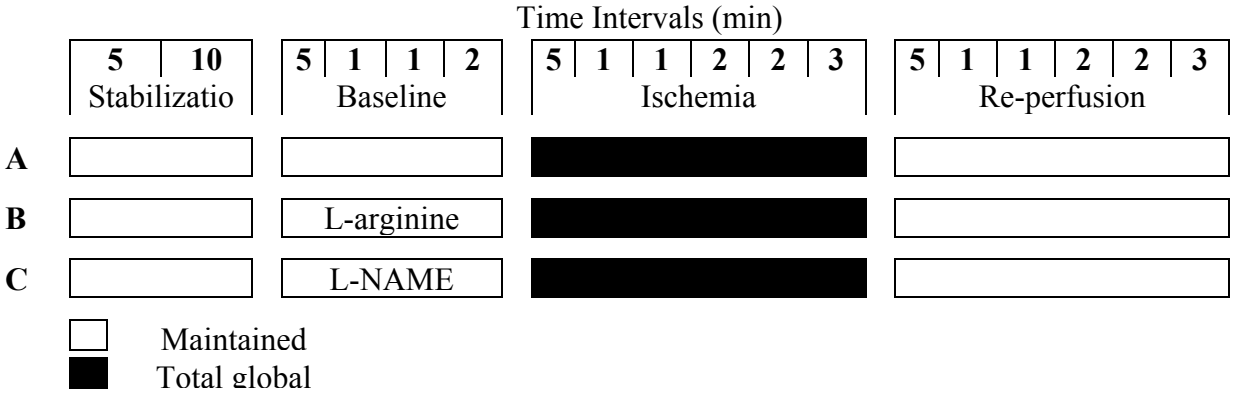


Figure (1):Protocol of ischemia/re-perfusion in isolated hearts from control (A), L-arginine (B) and L-NAME (C) rat groups.

**Experimental Procedure:**

On the day of experiments, overnight fasted rats, with free access to water, were weighed and injected intraperitoneally with heparin (5000 IU/Kg, Nile Company, Egypt). One hour later, rats were anaesthetized with intraperitoneal injection of Na thiopental (40 mg/kg, Sandoz, Austria).

***Perfusion of the isolated heart:***

A midline abdominal incision was made; the heart was excised and immediately placed in ice-cold Krebs–Henseleit bicarbonate buffer solution where it rapidly stops beating. The hearts were perfused according to ***Langendorff technique*** that was modified by ***Ayobe and Taraz***[13] with retrograde perfusion under constant pressure (=55 mmHg) without recirculation, using Krebs–Henseleit bicarbonate buffer (pH 7.4). The perfusion fluid was equilibrated with gas mixture (oxygen 95% and carbon dioxide 5%) and the temperature adjusted at 37°C.

Tension developed by the heart was measured by isometric force transducer (K-30 UGO BASILE S.R.L, model 7004-F, Italy), which is connected through an analog-digital converter and amplifier (the IWX-214 hardware) (UGO BASILE S.R.L, model 17304, Italy) to a computer software (Lab Scribe 2 ver.2.232) to be visualized on a computer screen (Dell Computer, Inc.).

***L-arginine / L-NAME infusion:***

The infusion was done through a catheter tube (PE-50; Clay Adams, New Jersey, USA), connected to an opening just above the aortic cannula using a Sage-355 infusion pump (Orion Research Mfg., Cambridge, Massachusetts, USA) at a rate of 1ml/min, over 20 minutes just before exposure to total global ischemia. Both L-arginine and L-NAME were supplied as powder (Sigma Chemical Co., St. Louis, Missouri, USA), dissolved in Krebs Henseleit bicarbonate buffer and infused at a dose of 100 μM[14] and 50 μM[15] respectively.

***Ischemia/re-perfusion (I/R) technique:***

At the end of 20 minutes baseline activity, total global ischemia was induced by stopping of the perfusing fluid to the heart by a clamp for 30 minutes; afterwards the heart was re-perfused for an additional 30 minutes to record the post-ischemic cardiac activities.

**Measurements:** Recordings of intrinsic cardiac activities were visualized on a computer screen, where the data could be printed or stored on disk for off-line analysis. Basal cardiac activities (20 minutes after stabilization) as well as 5, 15&30minutes of re-perfusion were recorded. In each record, heart rate (HR), peak developed tension (PT), time to peak tension (TPT) and half relaxation time

(1/2RT) were determined. Simultaneously the myocardial flow rate (MFR) was assessed by collecting the fluid for 3 minutes. The peak tension and myocardial flow rate both were calculated per 100 mg left ventricular (LV) mass and contraction time (CT) was calculated as the sum of TPT and 1/2RT.

***Cardiac weights:***

After perfusion, the heart was washed with normal saline and dried by filter paper. Both atria were separated, right ventricular wall was peeled evenly and the remaining were the left ventricle plus the septum. The left ventricle (LV) was weighed on a five-digit precision balance (Mettler, AE163), then divided into two pieces, wrapped separately in parafilm and stored at −80°C for later determination of cardiac tissue malondialdehyde (MDA), catalase (CAT) and nitrite levels.

***Assessment of cardiac tissue MDA, CAT& nitrite levels:***

Preparation of tissue homogenates:

On the day of preparation, one piece of the stored LV was allowed to thaw, blotted with filter paper, subdivided into two halves and weighed. Both halves were homogenized separately in 10 ml cold buffer (50 mM potassium phosphate, pH 7.5, and 1 mM EDTA) per gram LV tissue using tissue homogenizer (IKA–WERK, Ultra–Turrax, West Germany), and centrifuged at 4000 rpm for 15 minutes. The supernatant were stored in aliquots at −80°C for subsequent estimation of MDA&CAT levels.

The other stored piece of LV was homogenized in 10 ml cold buffer (100 mM potassium phosphate, pH 7.0, containing 2 mM EDTA) per gram LV tissue, and centrifuged at 4000 rpm for 15 minutes. The supernatant was also stored in aliquots at −80°C for subsequent estimation of nitrite level.

Biochemical analysis:

MDA, CAT and nitrite levels were measured in cardiac tissue homogenates by enzymatic colorimetric technique described by Satoh[16],Aebi[17] and Montgomery and Dymock (1961) respectively; using kits supplied by Bio-diagnostic, Egypt.

**Statistical analysis:**

Results were expressed as mean ± SEM. Percentage (%) recovery of intrinsic cardiac activities was calculated in all the studied groups, relative to their initial values. Statistical significance was determined by one-way analysis of variance (ANOVA) for differences between the means of different groups; further analysis was carried out using the Least Significance Difference (LSD) a multiple-range test to find intergroupal differences. Simple Student's "t" test for paired data was performed to detect significance from baseline value in the same group. Correlation between variables was evaluated using Pearson's correlation coefficient which is calculated by linear regression analysis using the Least Square Method. All statistical data, statistical significances, correlations and lines of regression were analyzed using Statistical Package for Social Science (SPSS) software (SPSS Inc., Chicago, Illinois, USA), version 14.0. For all statistical analysis, a probability of $P \leq 0.05$ is considered statistically significant.

## RESULTS:

**Intrinsic cardiac activities:** (Figure 2)

***a) Chronotropic activity***

Both L-arginine and L-NAME infused isolated hearts showed significant baseline tachycardia compared to their matching controls. After ischemia/re-perfusion (I/R), no change could be detected in HR of control rats compared to their baseline value. However, it was reduced in L-arginine infused hearts at all recorded times of re-

perfusion, though, it reaches the level of significance at 5 & 30 min of re-perfusion. On the other hand, L-NAME infused hearts showed significant increase in HR at 5 min of re-perfusion. On comparing post-ischemic responses, at the end of re-perfusion, HR was significantly higher in both L-arginine and L-NAME infused isolated hearts compared to controls. It was even significantly higher in L-NAME infused group at 5 min of re-perfusion compared to both controls and L-arginine infused hearts. The values of % recovery was highest for L-NAME infused hearts followed by L-arginine infused hearts, though it didn't reach the level of significance (Table 1).

***b) Inotropic activity***

Baseline PT/LV was significantly reduced in L-arginine infused hearts compared to controls. There was significant reduction in contractile activity through the re-perfusion period, following total global ischemia, in all the studied isolated hearts compared to their corresponding baseline value. On comparing post-ischemic responses, at the end of re-perfusion, the PT/LV was insignificantly different among the three studied groups (Table 2).

The baseline TPT was almost the same in all the studied groups. Following exposure to I/R, significant prolongation of TPT was observed in control hearts at 30 min of re-perfusion and 5 min in L-arginine infused hearts. Upon comparing post-ischemic responses, TPT was significantly shorter in L-arginine infused isolated hearts compared to both control and L-NAME groups, which was reflected on the value of % recovery (Table 3).

Similarly, no difference could be detected in the baseline pre-ischemia 1/2RT between the three studied groups. Also, the values of 1/2RT appeared to be stable even after I/R, except for the significant prolongation in the L-arginine infused hearts at 5 min of re-perfusion compared to their baseline value. On comparing post-ischemic responses, at the end of reperfusion 1/2RT was significantly shorter in both L-arginine and L-NAME infused hearts compared to controls. The % recovery showed the significantly lower value in L-NAME infused hearts compared to controls (Table 4).

Once more, no difference could be detected in the baseline pre-ischemia CT between the three studied groups. After I/R, significant prolongation of CT was detected in control hearts at 30 min and L-arginine infused hearts at 5 min of re-perfusion compared to their baseline value. On comparing post-ischemic responses, CT was significantly shorter in L-arginine infused hearts at 30 min of re-perfusion compared only to controls. L-arginine infused hearts also have the significantly lower % recovery compared to controls (Table 5).

***c) Myocardial flow rate***

Baseline MFR/LV was significantly higher in both L-arginine and L-NAME infused isolated hearts compared to controls. Following I/R, MFR/LV was significantly reduced at all times of re-perfusion in all the studied groups. Notably, despite the reduction in MFR/LV after I/R, it was still significantly higher in L-arginine infused hearts at all times of re-perfusion compared to controls and L-NAME infused hearts only at 30 min of re-perfusion. . The value of % recovery was significantly higher in L-arginine infused hearts compared to both L-NAME infused and control hearts (Table 6).

**Cardiac tissue MDA, CAT & nitrite levels:**

Nitric oxide level in cardiac tissue (represented by its end products nitrite) was significantly higher in L-arginine infused hearts compared to both L-NAME infused and control hearts. Meanwhile, it was significantly lower in L-NAME infused hearts than controls. The levels of MDA and CAT were statistically indifferent among the three studied groups (Table 7).

Table (1): Heart rate (HR, bpm) baseline values, post-ischemic responses and % of recovery of perfused hearts isolated from control, L-arginine, and L-NAME rat groups.

| HR | Baseline | Post-ischemic responses | | | % of |
|---|---|---|---|---|---|
| (bpm) | values | 5 min | 15 min | 30 min | Recovery |
| Control (11) | 158 ±13.30 | 170 ±16.40 | 148 ±16.90 | 138 ±16.10 | 86.0 ±7.23 |
| L-arginine (12) | 209[a] ±14.30 | 175[*] ±12.70 | 198[a] ±11.20 | 186[*a] ±13.90 | 90.0 ±4.73 |
| L-NAME (12) | 198[a] ±8.45 | 226[*ab] ±9.71 | 198[a] ±7.03 | 189[a] ±4.69 | 97.0 ±2.69 |

Values are represented as mean ± SEM.
The number of observations is given in parentheses.
*: Significance of differences from baseline pre-ischemia value of the same group calculated by Student's t-test for paired data at P≤0.05.
a: Significance of differences from control group calculated by LSD at P≤0.05.
b: Significance of differences from L-arginine group calculated by LSD at P≤0.05.

Table (2): Peak developed tension/left ventricle (PT/LV, g/100mg) baseline values, post-ischemic responses and % of recovery of perfused hearts isolated from control, L-arginine, and L-NAME rat groups.

| PT/LV | Baseline | Post-ischemic responses | | | % of |
|---|---|---|---|---|---|
| (g/100mg) | values | 5 min | 15 min | 30 min | Recovery |
| Control (11) | 1.32 ±0.11 | 0.95[*] ±0.08 | 0.98[*] ±0.09 | 0.86[*] ±0.08 | 64.8 ±1.73 |
| L-arginine (12) | 0.87[a] ±0.07 | 0.67[*a] ±0.07 | 0.61[*a] ±0.07 | 0.66[*] ±0.07 | 72.8 ±4.92 |
| L-NAME (12) | 1.09 ±.068 | 0.81[*] ±.055 | 0.72[*a] ±.061 | 0.67[*] ±.064 | 60.4[b] ±2.81 |

Values are represented as mean ± SEM.
The number of observations is given in parentheses.
*: Significance of differences from baseline pre-ischemia value of the same group calculated by Student's t-test for paired data at P≤0.05.
a: Significance of differences from control group calculated by LSD at P≤0.05.
b: Significance of differences from L-arginine group calculated by LSD at P≤0.05.

Table (3): Time to peak tension (TPT, msec) baseline values, post-ischemic responses and % of recovery of perfused hearts isolated from control, L-arginine, and L-NAME rat groups.

| TPT | Baseline | Post-ischemic responses | | | % of |
|---|---|---|---|---|---|
| (msec) | values | 5 min | 15 min | 30 min | Recovery |
| Control (11) | 90.90 ±6.53 | 90.90 ±4.04 | 96.40 ±6.57 | 105.00[*] ±9.08 | 115.0 ±6.10 |
| L-arginine (12) | 80.40 ±3.11 | 93.80[*] ±5.54 | 85.00 ±1.95 | 80.80[a] ±3.47 | 101.0[a] ±2.87 |
| L-NAME (12) | 91.30 ±2.55 | 96.30 ±3.99 | 97.10 ±3.56 | 100.00[b] ±5.27 | 110.0 ±4.79 |

Values are represented as mean ± SEM.
The number of observations is given in parentheses.
*: Significance of differences from baseline pre-ischemia value of the same group calculated by Student's t-test for paired data at P≤0.05.
a: Significance of differences from control group calculated by LSD at P≤0.05.
b: Significance of differences from L-arginine group calculated by LSD at P≤0.05.

Table (4): Half-relaxation time (1/2RT, msec) baseline values, post-ischemic responses and % of recovery of perfused hearts isolated from control, L-arginine, and L-NAME rat groups.

| 1/2RT | Baseline | Post-ischemic responses | | | % of |
|---|---|---|---|---|---|
| (msec) | values | 5 min | 15 min | 30 min | Recovery |
| Control (11) | 60.50 ±5.81 | 63.60 ±5.14 | 66.40 ±5.99 | 71.40 ±6.26 | 121.0 ±8.10 |
| L-arginine (12) | 55.00 ±4.52 | 72.10* ±7.96 | 55.40 ±2.98 | 55.80a ±2.94 | 106.0 ±6.38 |
| L-NAME (12) | 58.30 ±4.41 | 55.00b ±3.07 | 54.60 ±3.76 | 58.30a ±2.91 | 102.0a ±3.38 |

Values are represented as mean ± SEM.
The number of observations is given in parentheses.
*: Significance of differences from baseline pre-ischemia value of the same group calculated by Student's t-test for paired data at P≤0.05.
a: Significance of differences from control group calculated by LSD at P≤0.05.
b: Significance of differences from L-arginine group calculated by LSD at P≤0.05.

Table (5): Contraction time (CT, msec) baseline values, post-ischemic responses and % of recovery of perfused hearts isolated from control, L-arginine, and L-NAME rat groups.

| CT | Baseline | Post-ischemic responses | | | % of |
|---|---|---|---|---|---|
| (msec) | values | 5 min | 15 min | 30 min | Recovery |
| Control (11) | 151.00 ±11.70 | 155.00 ±8.75 | 163.00 ±11.9 | 176.00* ±14.40 | 118.0 ±6.27 |
| L-arginine (12) | 135.00 ±7.08 | 166.00* ±12.80 | 140.00 ±3.87 | 137.00a ±5.75 | 102.0a ±3.20 |
| L-NAME (12) | 150.00 ±6.81 | 151.00 ±6.69 | 152.00 ±6.19 | 159.00 ±7.76 | 106.0 ±3.06 |

Values are represented as mean ± SEM.
The number of observations is given in parentheses.
*: Significance of differences from baseline pre-ischemia value of the same group calculated by Student's t-test for paired data at P≤0.05.
a: Significance of differences from control group calculated by LSD at P≤0.05.

Table (6): Myocardial flow rate/left ventricle (MFR/LV, ml/min/100mg) baseline values, post-ischemic responses and % of recovery of perfused hearts isolated from control, L-arginine, and L-NAME rat groups.

| MFR/LV | Baseline | Post-ischemic responses | | | % of |
|---|---|---|---|---|---|
| (ml/min/100mg) | values | 5 min | 15 min | 30 min | Recovery |
| Control (12) | 1.51 ±0.08 | 1.31* ±0.08 | 1.05* ±0.1 | 0.86* ±0.07 | 56.4 ±2.32 |
| L-arginine (12) | 1.81a ±0.11 | 1.63*a ±0.14 | 1.47*a ±0.11 | 1.51*a ±0.14 | 82.7a ±3.01 |
| L-NAME (12) | 1.84a ±.058 | 1.58* ±.051 | 1.29* ±.051 | 1.14*ab ±.036 | 62.6b ±2.17 |

Values are represented as mean ± SEM.
The number of observations is given in parentheses.
*: Significance of differences from baseline pre-ischemia value of the same group calculated by Student's t-test for paired data at P≤0.05.
a: Significance of differences from control group calculated by LSD at P≤0.05.
b: Significance of differences from L-arginine group calculated by LSD at P≤0.05.

Table (7): Myocardial tissue levels of: malondialdehyde (MDA), catalase (CAT) and nitrite (NO) in the control, L-arginine, and L-NAME rat groups.

| | MDA | CAT | NO |
|---|---|---|---|
| | (nmol/g) | (u/g) | (µmol/g) |
| Control | (10) | (10) | (7) |
| | 20.69 ±2.86 | 6.92 ±1.01 | 11.54 ±1.80 |
| L-arginine | (9) | (9) | (9) |
| | 27.38 ±3.37 | 5.44 ±0.69 | 17.93[a] ±0.95 |
| L-NAME | (11) | (10) | (9) |
| | 28.03 ±7.77 | 5.42 ±1.43 | 7.55[ab] ±0.68 |

Values are represented as mean ± SEM.
The number of observations is given in parentheses.
a: Significance of differences from control group calculated by LSD at P≤0.05.
b: Significance of differences from L-arginine group calculated by LSD at P≤0.05.

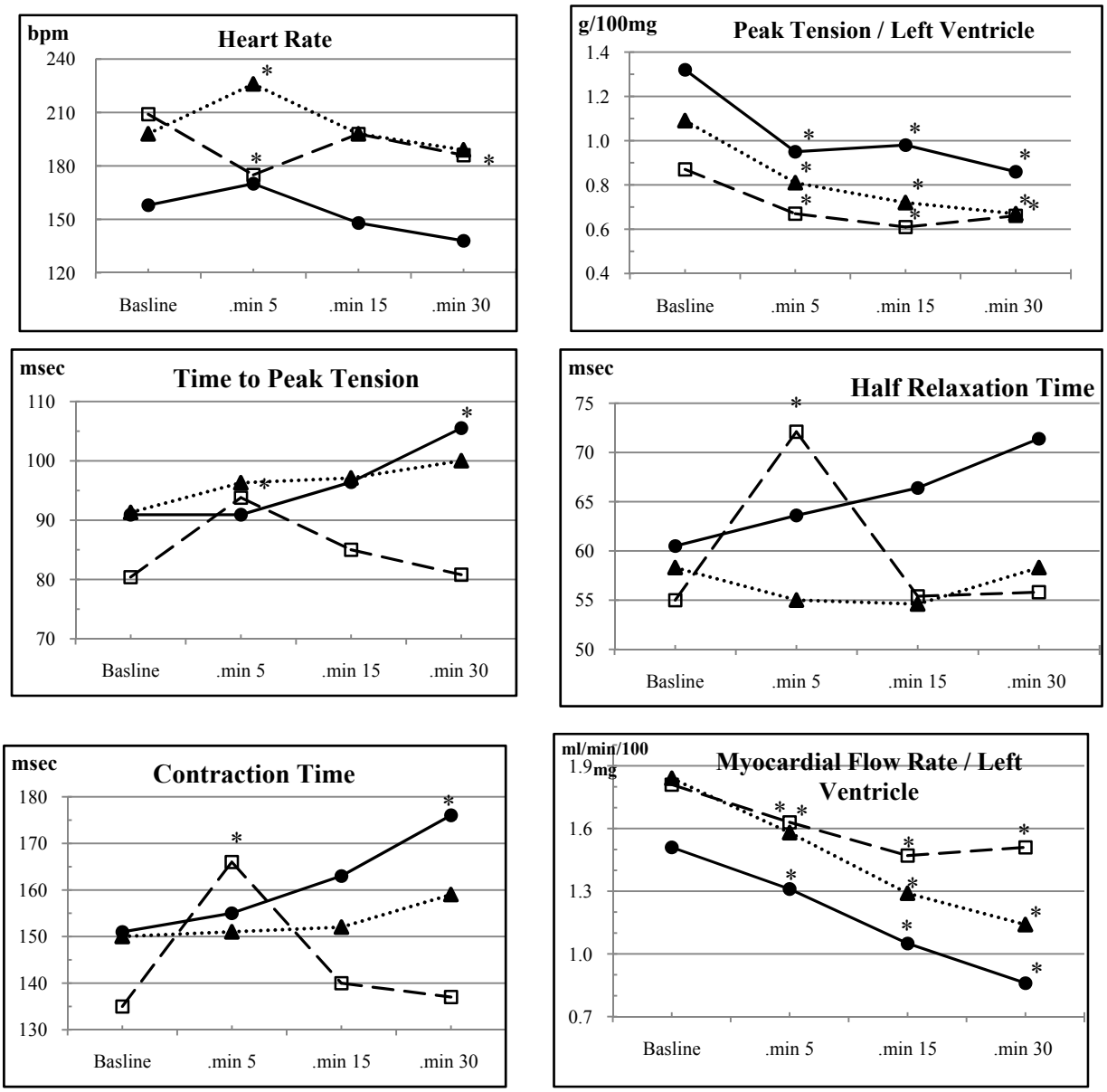


Figure (2): Intrinsic cardiac activities of hearts isolated from control( —●—), L-arginine ( —□—) and L-NAME (··▲··) rat groups.

**Correlation studies:**

As illustrated in figure (3), the percentage recovery of MFR/LV was found to be positively correlated with the MDA and NO levels.

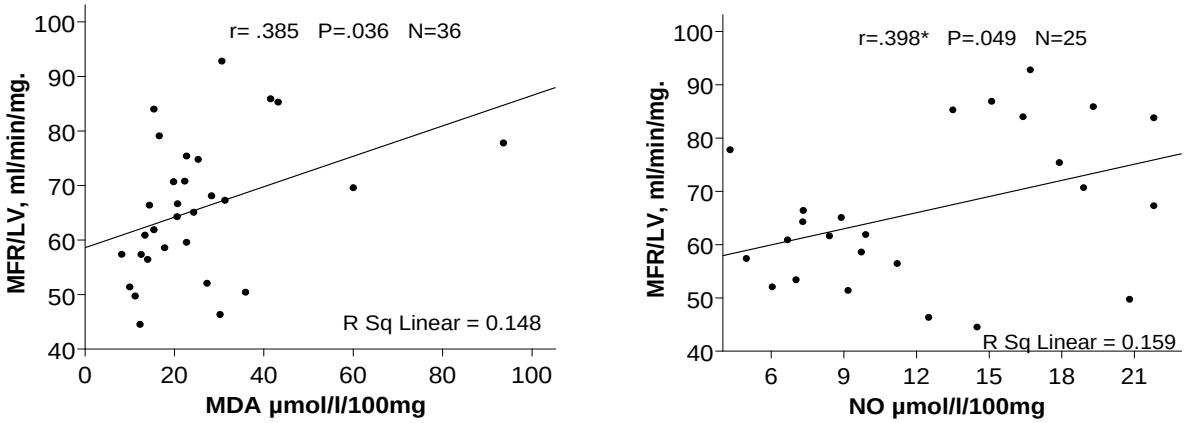


Figure (3): Graphs showing relationships between the percentage recovery of myocardial flow rate/left ventricle (MFR/LV) and myocardial tissue levels of malondialdehyde (MDA) and nitric oxide (NO) following total global ischemia in all the studied groups.

**DISCUSSION:**

Results of the present study demonstrated the changes in intrinsic cardiac activities in response to ischemic arrest for 30 min and then re-perfusion for 30 min in the presence of altered NO availability i.e. excess NO level (with the use of L-arginine as NO donor) and with reduced NO (with the use of L-NAME as non specific inhibitor for NO synthesis to reduce the endogenous NO).

**A) Chronotropic activity:**

The obtained results showed unaltered chronotropic activity of control hearts after I/R as manifested by the maintained heart rate that reached 86% at the end of 30 min re-perfusion. This was in agreement with the previously published studies by *Behmenburg et al.*[18] *& Hu et al.*[19], who reported that 30 minutes of global ischemia (no-flow) is not sufficient to induce serious sinoatrial (SA) node damage.

The present study revealed also that altering the level of NO during baseline period induced a positive chronotropic effect. Cardiac tissue NO level was found to be significantly higher in L-arginine (NO donor) infused hearts and significantly lower in L-NAME infused heart, however in both situations, a positive chronotropic effect was observed under basal condition. These conflicting observations would add and point to the complexity and diversity of the role of NO in controlling cardiac chronotropy.

*Wang*[20], has reported that the myocardial chronotropy is affected by NO in a biphasic manner as high doses of sodium nitroprusside increases heart rate, where as lower doses have a negative chronotropic effect. Also, *Fellet*[21] and his colleagues have reported that the diversity of intracellular pathways active ted by NO and their differing sensitivities to different levels of NO might account for some aspects of its reported specific but opposite effects[21]. Moreover, Kazanski et al.[22] showed that NO can modulate the current performance of stretch activated channels. They demonstrated that NO donors led to activation of mechanically gated channels in

unstretched ventricular myocytes, while in stretched cells with active channels, NO led to its inactivation and inhibition.

The observed positive chronotropic effect with L-arginine infusion under basal condition is in accordance with *Musialek et al.*[23] who have reported that low doses of NO donor increases the beating rate in isolated atria from guinea pig and in SA node cells from rabbit by activation of the hyperpolarization inward current. Also, the study performed by Fellet et al.[24] reported that NO donor may directly stimulate pacemaker activity of SA node. Moreover, *Wang*[20] had reported that the exogenous NO increases the heart rate both in vivo and in vitro, and attributed this positive chronotropic effect to the increment of the inward current of SA node via the NO–cGMP dependent pathway. Furthermore in 2017, *Salihi and his coworkers*[25] reported that NO exerts a tonic, direct positive chronotropic influence on the denervated human hearts.

On the other hand, isolated hearts infused with L-NAME in pre-ischemic period, also revealed a clearly positive chronotropic effect with the heart rate being statistically indifferent from the L-arginine group under basal condition. This finding is in accordance with reports that adopt negative chronotropic effect of NO[26&27]. Many reports have shown that inhibition of NOS induces an increase in the chronotropic response to β-agonist at cellular myocardial tissue and whole animal[28, 29&30].

In 2006, the in vivo study of *Fellet and his coworkers*[31] revealed that the down regulation of NOS activity, after autonomic blockade, led to decrease in the levels of cGMP and increased rate of $O_2$ uptake and ATP production. They added that lower levels of cGMP and higher levels of ATP, both would stimulate molecular mechanisms leading to a higher rate of impulse generation by the SA node and they suggested that the decreased level of ATP would be responsible for decreased rate of sinus node activity. Moreover, in 2008 *Fellet et al*[21]. suggested that there is a direct negative chronotropic effect of NO on the pacemaker activity. Also, *Navarro*[32] reported a negative chronotropic effect of endogenous NO, according to, two observations first: the increase in heart rate after L-NAME administration, and second: to the association of reduced HR with increased mitochondrial nitric oxide synthase (mtNOS) activities.

Upon exposure to 30 min of global ischemia and 30 min of re-perfusion, HR was about 90% recovery and 97% recovery at 30 min re-perfusion in L-arginine and L-NAME groups respectively. It worthy to note, that at 5 min of early re-perfusion, the heart rate of L-NAME infused hearts was the highest compared to both control and L-arginine groups and as well as to its basal value. While, HR at 5 min re-perfusion in L-arginine group, was significantly lower than its basal value. These observations would highlight the negative chronotropic effect of NO upon exposure to re-perfusion after global ischemia.

It should be noted that the redox state of the cardiac tissue cells wasn't significantly altered by the end of re-perfusion in the three studied groups, being slightly elevated MDA and slightly lowered CAT levels in both L-arginine and L-NAME groups compared the control. Therefore, it could be concluded that, the insignificant change in the heart rate % recovery in the control, L-arginine and L-NAME groups upon exposure to re-perfusion can be attributed to the maintained redox balance demonstrated in this model. This assumption is supported by the study done by *Ke et al.*[33] who reported that the arrhythmia induced by myocardial I/R injury is due to the associated increased reactive oxygen species (ROS) generation.

**B) Inotropic activity:**

In this I/R model, myocardial inotropy was severely deteriorated during the whole period of re-perfusion in the three studied groups, reaching by the end of 30 min re-perfusion about 64.8%, 72.8%, and 60.4% in the control, L-arginine and L-NAME infused hearts respectively. This is in agreement with numerous studies that clarify the deleterious effects of I/R injury that induces myocardial contractile dysfunction[34, 35 &36].

Several pathways have been proposed in I/R injury including cytosolic and mitochondrial $Ca^{2+}$ overload, release of ROS, acute inflammatory response, and impaired metabolism[37&38]. Outcomes subsequent to I/R occur in a time–dependent fashion[39], beginning with oxidative stress, inflammation, intracellular $Ca^{2+}$ overload, and rapidly proceeding to irreversible cell death by apoptosis and necrosis[40].

Mitochondria play a critical role in the pathogenesis of myocardial ischemia re-perfusion injury. They are the major contributors of ROS as well as the major target for ROS-caused damage. Its dysfunction, leads to diminished energy production, loss of myocyte contractility, altered electrical properties and eventual cardiomyocyte cell death[41&42].

It should be noted that, under basal condition, in the pre-ischemic period, L-arginine infused hearts clearly demonstrated the negative inotropic effect of NO, as the PT/LVwas significantly depressed compared to controls. This is in agreement with previous reports which demonstrated negative inotropic effects of NO[43,44,&45]. In the same manner, *Iwai-Kanai et al.*[46] observed an inverse relation between contraction in isolated cardiomyocytes and NO activity. Further evidences provided by *Heusch et al.*[47] *and Soski´c et al.*[48] that have been reported that an excessive NO formation is thought to contribute to myocardial contractile dysfunction.

Actually, physiological levels of NO could play a role in modulating cardiac function, by a direct effects via cGMP dependent pathway and linked to the phosphorylation of ion channels ($Ca^{2+}$and $Na^{+}$channels) or indirect effects mediated by an inhibition of phosphodiesterases that increase the levels of cAMP and the subsequent activation of the L-type $Ca^{2+}$ channel which results in positive inotropy[49].

Previous reports have described the bimodal effect of NO under basal state, with a positive inotropic effect at low amounts of NO exposure but a negative one at higher amounts. This bimodal effect of NO on cardiac inotropy was observed with exogenous or endogenous NO[50]. It is worthy to be noted that, this depressed inotropy of L-arginine infused hearts, under basal condition extended to and was still evident at early re-perfusion at 5 min (proposed period of still high NO level) and by the recovery at 30 min of re-perfusion, PT/LV was indifferent from the other studied groups.

The role of NO in myocardial re-perfusion injury remains under argument, though a slight increase in NO may be cardio protective, and a large increase seems to be detrimental because of the formation peroxynitrite[51]. The deleterious effects of NO have been reported to be linked to acceleration of apoptosis[52], inhibition of mitochondrial respiration and ROS production[53]. Therefore, reducing NO production and subsequently decreasing the levels of nitro-oxidative stress could protect the heart from I/R injury and promote recovery of myocardial contractility[34].

Surprisingly, PT/LV of L-NAME infused hearts, that had the lowest level of NO, was statistically indifferent from the controls whether under basal condition, early re-perfusion (5 min) or by its end. This inotropic state –being more or less similar to the control without any improvement–

would reflect that NO deficiency didn't not play an interfering mechanistic role in the re-perfusion induced contractile dysfunction in such I/R model.

Concerning time relations, the basal CT was statistically indifferent among the three studied groups, yet the all the cardiac times, TPT, 1/2RT and CT, were enhanced under basal conditions with increased NO availability in L-arginine infused group, though it did not reach level of significance. These results are in agreement with *Smith et al*.[54] who reported that administration of NO donor enhanced the relaxation with little change in the maximum rate of tension development. It was also reported that NO can modulate basal contractile function by inducing an early onset of isometric relaxation (relaxation hastening effect) even without affecting the rate of isometric tension development[55].

Upon exposure to I/R, control rats showed prolonged CT that was associated with prolonged TPT, late at 30 min re-perfusion. This would point to late impairment of cardiac systolic function. On the other hand, with L-arginine infusion, the prolongation in CT was observed early at 5 min of re-perfusion and was associated with prolongation of both the TPT and the 1/2RT, pointing to early impairment of both the systolic and diastolic cardiac functions with increased NO availability. It is worthy to note that the increased availability of NO, as early as 5 min of re-perfusion, in L-arginine infused hearts, resulted in marked cardiac depression and worsen the contractility. Increased NO induces negative inotropy, weak systolic and impaired diastolic function together with negative chronotropy. This would reflect the deleterious effects of NO on cardiac performance during early re-perfusion period.

This proposed concept goes in line with the results observed in LNAME group. As the decrease in NO availability in LNAME infused hearts, ameliorates the cardiac dysfunction at 5 min re-perfusion observed with L-arginine infusion with even better chronotropic and lusitropic activities and the cardiac performance become more or less nearer to the control response. This proposal is supported by the study done by *Paulus and Shah*[55], they reported that excessive production of NO or peroxynitrite may cause diastolic dysfunction which usually be accompanied by systolic dysfunction.

In 2007, *Yellon and Hausenloy*[56] stated that impaired cardiac contractility during I/R can be attributed to calcium paradox. They added that there is intracellular and mitochondrial $Ca^{+2}$ overload that induces hyper contracture of the cardiomyocytes, which accompanied by opening of mitochondrial permeability transitional pore (mPTP). It was found that NO has a dose dependent and paradoxical effects on mPTP; high concentration of NO opened mPTP, while its low concentration prevents its opening[57]. Thus, this could be the explanatory mechanism for the observed cardiac dysfunction observed with increased NO availability at early re-perfusion.

**C) Myocardial flow rate:**

The second cardinal feature of the re-perfusion syndrome demonstrated in the present model of I/R, beside the deteriorated inotropy, is the compromised MFR/LV during the whole period of 30 min re-perfusion which was also clearly evident in the three studied groups, despite of the alteration of NO level. This compromised coronary flow upon re-perfusion could be attributed to the impaired vasoactivity of coronary vessels which is in agreement with previously published reports[58,59&35].

*Deng et al.*[60] reported that after 15 min of ischemia, there was concomitant evidence of injury in large coronary conduit as well as coronary micro-vasculature with a defect in coronary vasodilatory response to acetylcholine that required about 90 min to return to baseline. Moreover, *Tsao and*

*Lefer*[61] demonstrated the impaired vaso-relaxtion to endothelial dependent vasodilator at 20 min of re-perfusion after 30 min of total global ischemia. The detective vasoreactivity could be accounted for the intense release of vasoconstrictive substances, as well as, the extra-vascular coronary micro- vascular compression by the edema of surrounding myocardium with primary physical destruction of capillary endothelium[62].

*Park and Lucchesi*[63] reported that the extent of re-perfusion injury was directly related to the duration of the ischemic insult and the restoration of the coronary flow; if the re-perfusion initiated within 20 min after the onset of ischemia, the myocardial damage would be reversible but with longer ischemic insult, the recovery would be incomplete with irreversible damage. The extent of re-perfusion injury also depends on state of cardiac performance under basal conditions before exposure to the ischemic insult.

In this studied model, we increased availability of NO with the use of L-arginine and the decreased level, with the use of non specific NOS inhibitor L-NAME, try to clarify how the manipulation in vascular reactivity under basal condition could change or alter the response or extend of re-perfusion injury.

Interestingly, under basal conditions the MFR/LV was significantly higher in both L-arginine and L-NAME infused groups compared to control one. The increase in basal MFR/LV with increased availability of NO in L-arginine group is in agreement with many studies demonstrating that basal endogenous NO is an important vasodilator and thus increases coronary flow[64]. In 2014, *Morita et al.*[65] reported that the enhancement in NO synthesis with increasing availability of L-arginine concentration induces vascular vasodilatation by relaxing the vascular smooth cells via decreasing intracellular $Ca^{2+}$ through NO-sGC–cGMP dependent pathway[66&67]. In addition, NO produces vasodilatations through cGMP dependent hyperpolarization of vascular smooth muscles via the opening of selective $K^{+}$ channels[68].

On the other hand, L-NAME infused isolated hearts also, demonstrated an increase in MFR/LV under basal condition. The apparent contradictory observation would point to other different regulatory mechanism(s) interplay in controlling MFR under basal condition rather than NO availability. In agreement with this, numerous studies have demonstrated little or no effect of NOS inhibition on baseline coronary flow[69&70]. Also, *Reffelmann et al.*[71] have reported that vasoactivity is achieved by the appropriate balance of molecules that cause vasodilatation (NO, Prostacyclin, Adenosine) and those that cause vasoconstriction (Endothelin, Angiotensin II, Thromboxan A2).Actually, the coronary flow has many determinants including; perfusion pressure, extra-vascular compression, auto-regulation, metabolic regulation, endothelium mediated regulation and neuro-hormonal regulation[72,73&74].

It is worthy to note that the beating heart rate in this group (i.e. L-NAME infused hearts) was significantly higher than the control. Therefore, it could be proposed that the observed tachycardia was responsible for such observed increase in MFR/LV in this group. In support of this assumption was the report of *Colin et al.*[75] who stated the increase in heart rate increases the myocardial oxygen consumption per min that results in a proportionate increase in coronary blood flow. *Nishikawa and Ogawa*[76] reported that NO did not regulate vascular resistance when myocardial flow was increased by rapid pacing and the release of NO is not an important factor for pacing induced arteriolar dilation. Also, they stated that the myogenic and metabolic mechanisms rather than the NO production

are responsible for the increase in coronary flow with such tachycardia. They added that there are other metabolic substances like Adenosine, lactate, phosphate, $CO_2$, potassium and prostaglandins may be implicated in such increase in coronary flow with increased heart rate.

It should be noted that despite the increase in myocardial flow rate under basal condition in both L-arginine and L-NAME infused hearts, yet upon exposure to 30 min of global ischemia and re-perfusion, both groups, similar to the control one, demonstrating progressive deterioration in the MFR/LV to reach 56.4%, 82.7%, 62.6% at 30 min re-perfusion in control, L-arginine and L-NAME respectively. With increased NO availability in L-arginine group, the myocardial flow was higher than the control one –not only under basal– but also all through the period of re-perfusion, whereas with decreased NO availability in L-NAME infused hearts MFR/LV still higher than the control and reach level of significance at 30 min of re-perfusion. But it was significantly less than that with L-arginine group.

The correlation studies in such I/R model revealed a significant positive correlation between this MFR/LV at 30 min of re-perfusion recovery with the NO and MDA levels.It should be noted that this significant positive correlation between the MFR/LV at 30 min of re-perfusion recovery and the MDA levels, the indicative marker of oxidative stress, would reflect the other good image of ROS on coronary vascular pathway reactivity rather than its typically injurious effect. This point of view is in agreement with the study done by *Burgoyne et al.*[77]; they stated that the amount and type of ROS are important signaling molecules for normal coronary vascular functioning in the healthy heart. Moreover, $H_2O_2$was found to be an activator for $K^+$ channels in coronary myocytes and thus a good mediator for coronary vasodilation[78].

**Conclusion:**

From the aforementioned data, it could be summarized that exposure of the control hearts to 30 min of re-perfusion after 30 min of no flow ischemia (I/R) revealed deteriorated inotropic state and compromised myocardial flow throughout the whole period of re-perfusion, together with systolic dysfunction and still preserved diastolic function at the end of re-perfusion.

On blocking the endogenously released NO by L-NAME before exposure to ischemia, resulted in increased chronotropy, improved coronary blood flow together with maintained inotropy under basal state, this would reflect the depressant effect of endogenously released NO on the HR and MFR. Yet upon exposure to I/R, the % recovery of the chronotropy, inotropy, systolic function and the MFR were indifferent from their matching control hearts. Only improvement in the % recovery of the diastolic function was observed, reflecting the deleterious effect of the endogenously released NO on the diastolic function.

On the other hand, increasing NO availability by infusing its substrate L-arginine before exposure to 30 min ischemia, revealed increased cardiac chronotropy and MFR together with depressed inotropy under basal condition. Upon exposure to 30 min of re-perfusion, there were enhancement of the systolic function and improvement of myocardial flow % recovery together with insignificant mild improvement in cardiac contractility compared to the control hearts.These observation might reflect the potentially protective effect of increased NO availability on the myocardial flow and systolic function at the end of 30 min of re-perfusion. However, it is worthy to note that excess NO availability worsen the cardiac performance at early re-perfusion period.

Therefore, it could be conclude that; reducing NO availability does not have great influence in the I/R induced contractile dysfunction. Although, increasing NO availability might have potential protective effect by improving the myocardial flow and enhancing the systolic function at the end of 30 min of re-perfusion. However, it should be noted that excess NO worsen the cardiac performance at early 5 minre-perfusion period.The findings of the present work revealed that the ischemic re-perfusion cardiac injury is multi-factorial and the mechanistic role played by NO still needs further research.

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## دراسة تأثير أكسيد النيتريك علي الأداء الوظيفي لعضلة القلب أثناء تعرضها للتوقف والإقفار التجريبي ثم إعادة التروية

**المقدمة:** إعادة التروية هي العلاج القياسي لاحتشاء عضلة القلب الحاد ، بالرغم من طيف المضاعفات المرتبطة بإعادة التروية والتي قد تساهم في اعتلال عضلة القلب.

**الهدف من الدراسة :** يهدف هذا البحث لدراسة التغيرات التي تطرأ على أداء عضلة القلب المعزول واستجابتها عند تعرضها للإقفار الدموي ثم إعادة التروية عندزيادة أكسيد النيتريك باستخدام L-arginine او عند نقص أكسيد النيتريك باستخدام L-NAME .

**الطرق والمواد المستخدمة:** تم تنفيد هذه الدراسة علي القلوب المعزولة من فئران التجارب البيضاء من الجنسين على جهاز لانجندورف بعد تعرضها للاقفار لمدة ٣٠ دقيقة ثم إعادة التروية لمدة ٣٠ دقيقة .وقسمت الى ثلاث مجموعات ، المجموعة الضابطة ومجموعة تعطي مادة L-arginine ومجموعة تعطي مادة L-NAME لمدة ٢٠ دقيقة قبل تعرضهما للإقفار ثم إعادة التروية ، وتم اجراء قياس الوظائف الاساسية للقلب قبل الإقفار و بعد إعادة التروية وقياس مستوي الأكسدة مالونديالدهيد ومضاد الأكسدة الكاتلاز ومستوي النترات في القلب .

**النتائج:** بالمقارنة مع المجموعة الضابطة لوحظ وجود زيادة غير ذات دلالة إحصائية في ضربات القلب الأساسية ومعدل سريان المحلول المغذي للقلب في المجموعتين مقارنة مع قيم الأساس للمجموعة الضابطة.

فيما يخص القوة المتولدة نتيجة الانقباض فقد لوحظ تتدهور ذات دلالة إحصائية من بدء اعادة التروية بعد الاقفار مقارنة بمعدلاتها الأساسية في جميع المجموعات. وكذلك تدهور في معدل الاساس في مجموعة L-arginine مقارنة باساس المجموعة الضابطة.

وبدراسة معدل سريان المحلول المغذي للقلب فان هذا المعدل تساوى مع معدل الأساسي في كل المجموعات في كامل فترة اعادة التروية.

المجموعة التي اعطيت مادة L-arginine اظهرت انخفاض في النبض وتدهور في القوة المتولدة نتيجة الانقباض والانبساط مع زيادة ذات دلالة إحصائية في معدل سريان المحلول عند نهاية اعادة التروية بلغت ( 3.0± 82.7% مقارنة مع 2.32± 56.4% في المجموعة الضابطة و 2.17± 62.6% في مجموعة L-NAME)

قياس مستوي النترات في القلب كان الاعلى في مجموعة L-arginine والادني في مجموعة L-NAME.

قياس مستوي الأكسدة ومضاد الأكسدة اظهرت تغيرات غير ذات دلالة احصائية بين المجموعات الثلاثة.

**الخلاصة:** اظهرت هذه الدراسة ان نقص أكسيد النيتريك ادي الي تأثير خفيف علي القوة المتولدة نتيجة الانقباض بعد الاقفار واعادة التروية اما زيادة أكسيد النيتريك ادت الى تدهور في اداء عضلة القلب عند بداية اعادة التروية و قد يكون لها تأثير وقائي محتمل من خلال الحصول على أعلى معدل سريان المحلول عند نهاية اعادة التروية.