ECRONICON OPEN ACCESS

EC CARDIOLOGY

Research article

# Modulation of Serum Adropin Levels by Isoproterenol-Induced Acute Myocardial Infarction in Normal and Obese Male Albino Rats

**Sherif Wagih Mansour[1]*, Ali Khalil Ali Asalah[2], Mai M Hasan[2] and Sara G Seada[3]**

[1]*Professor and Head of Medical Physiology, Faculty of Medicine, Zagazig University, Egypt*

[2]*Professor of Medical Physiology, Faculty of Medicine, Zagazig University, Egypt*

[3]*Assistant Lecturer of Medical Physiology, Faculty of Medicine, Zagazig University, Egypt*

***Corresponding Author:*** Sherif Wagih Mansour, Professor and head of Medical Physiology, Faculty of Medicine, Zagazig University, Egypt.

**Received:** September 24, 2019; **Published:** October 16, 2019

## Abstract

**Background:** Acute myocardial infarction (AMI) is a major cause of morbidity and mortality all over the world. Adropin, which is a peptide synthesized in the heart, is suggested to be released into the bloodstream after ischaemic cardiac injury.

**Aim:** To clarify the effect of the ISO-induced AMI in normal and obese rats on serum adropin level and to localize any potential association between its level and other cardiac enzyme markers, oxidative stress markers, pro-inflammatory and metabolic parameters.

**Material and Methods:** 85 adult male albino rats were used. 15 animals died and the remaining animals (70) were divided into 5 main groups: control group and four ISO-induced AMI groups where blood samples and hearts were taken at 1h, 2h, 6h and 24h. post AMI. Each group is subdivided into lean and obese subgroups. In all groups, serum adropin, cTn-I, CK-MB, CRP, TNF-α, oxidative stress markers (MDA, SOD, GSH and catalase), lipid profile, insulin and glucose levels were measured. HOMA-IR was calculated. ECG and blood pressure were recorded. Histo-pathological examination of the heart was done.

**Results:** The results of the present study showed that serum adropin levels was increased after AMI together with cTn-I, CK-MB, CRP, TNF-α, MDA, TC, TGs and LDL-C. Adropin showed positive correlation with abovementioned parameters in all studied groups. SOD, GSH, catalase and HDL-C were decreased after AMI with negative correlation with adropin in all groups. Histo-pathological examination revealed edema, inflammatory cell infiltration, congestion, haemorrhage and necrosis in ISO-induced AMI.

**Conclusions:** Serum adropin can be used as an alternative biomarker to other cardiac enzyme markers (cTn-I, CK-MB) for early diagnosis of AMI especially in cases of obesity.

***Keywords:*** *Oxidative Stress Markers; Adropin; Acute Myocardial Infarction*

## Introduction

Cardiovascular diseases are the first cause of morbidity and mortality all over the world [1] where AMI is the main cause of death and disability in middle-aged and elderly adults in both developing and developed countries [2]. AMI causes injury to the cardiac muscle tissue or even cause its death due to prolonged exposure to hypoxia and ischemia [3].

Many studies showed that overweight and obesity are associated with AMI [4]. Therefore, accurate, valid, precise, cost-effective, readily accessible cardiac markers are needed for better diagnosis and prognosis of AMI.

Adropin was firstly isolated in 2008 by Kumar and his team in mice. Adropin is encoded by the Enho gene which is expressed in the brain, liver sinusoids, hepatocytes [5,6] and cardiomyocytes in addition to endocardium and epicardium [3,5,7].

It was shown that adropin influences the regulation of metabolic pathways during prolonged ischemia and hypoxia in isoproterenol (ISO)-induced AMI rats [3], where in the ischemic region ATP synthesis is markedly reduced due to the lack of oxygen and the washout of metabolic products. So, the cardiac infarcted tissues is in need to maintain energy homeostasis, which could explain the increased adropin expression [8].

Also, some studies reported that adropin has a potential protective role for endothelium that is likely regulated via increased endothelial nitric oxide synthase (eNOS) expression where adropin influences gene expression of eNOS through vascular endothelial growth factor receptor2 (VEGFR2) -phosphatidylinositol 3-kinase-Akt and VEGFR2-extracellular signal regulated kinase (ERK) 1/2 pathways, leading to increase of the bioavailability of NO [5].

Another study reported a decrease in serum adropin level in human subjects with AMI compared to controls [10]. Also, a study found that serum adropin level is significantly lower in coronary artery disease patients than that in the control group [11].

Adropin also appears to play a role in the preservation of energy balance and insulin response [5]. Adropin levels have been observed to be relatively high in mice fed a diet with a high fat and low carbohydrate content, whereas adropin levels have been reported to be lower in mice fed a diet with low fat and high carbohydrate content [12]. Adropin has a role in metabolic homeostasis which is evidenced by improving glucose homeostasis, fatty liver and dyslipidemia with obesity using synthetic peptide or transgenic over expression [9]. Adropin knockout mice became obese, despite maintaining normal food intake and energy consumption [12].

Therefore, we decided to investigate the effect of the ISO-induced AMI in normal and high fat diet (HFD) -induced obese rats on serum adropin level and to define any potential association between its level and other cardiac enzyme markers, oxidative stress markers, pro-inflammatory and metabolic parameters.

## Materials and Methods

The experimental protocol was approved by animal handling standard ethics, physiology department and by local medical ethics committee in faculty of medicine of Zagazig university (Institutional Review Board, IRB (3338/29/1/2017).

This experimental prospective cohort study was carried out on a total number of 87 healthy adult male albino rats, weighing 150 - 200g. The animals were kept in steel wire cages (7 - 8/cage) in the animal house of the faculty of medicine, Zagazig University under hygienic conditions. Rats had free access to water, kept at room temperature and were maintained on a 12 h light/dark cycle.

After being acclimatized to the housing for one week, rats were randomly divided according to feeding protocol into two groups: a group fed normal chow (Lean group) which provided 3. 3 kcal/g of food and was composed of 5% fat, 18% proteins and 77% carbohydrates [13] and a group fed an HFD for 12 weeks which provided 4. 73 kcal/g of food and was composed of 35% carbohydrate, 20% protein, 45% fat [14] (HFD-obese group). BMI (weight/nose-anus length$^{2,}$ g/cm$^2$) was measured where rats with BMI > 0.68 g/cm$^2$ were considered to be obese [15].

Then rats were divided into two main groups:

- **Control group (Group A):** 14 rats which were subdivided into lean (1A) and obese (2A) subgroups, (7rats each).
- **ISO-induced AMI Groups**: 73 rats, 17 of which died after ISO injection to become 56: Rats were divided into 4 equal groups, 14 rats each (7 lean and 7 obese) -according to the time of taking samples-as following: Group B: 1 hour post induction of infarction. Group C: 2 hours post induction of infarction. Group D: 6 hours post induction of infarction. Group E: 24 hours post induction of infarction.

### Induction of AMI, ECG recording and measurement of arterial blood pressure

Rats were injected once subcutaneously (S.C.) with ISO in a dose 200 mg/Kg body weight, dissolved in 5 ml normal saline [3], where control rats were S.C. injected with the same volume of saline.

AMI was diagnosed during the experiment by recording electrocardiogram (ECG) using PowerLab4/20 (data acquisition system, AD Instruments Pty Ltd, Australia) where lead II was recorded. Blood pressure was measured using non-invasive blood pressure rat tail device (NIBP250). Rats were anesthetized with intraperitoneal (i.p.) injection of urethane (1.5 gm/kg) [16].

### Blood collection and heart isolation

At the end of the experiment and according to the timing of each group, blood samples were collected from the retro-orbital vascular plexus in dry clean and screw capped tubes and left for 30 minutes to be clotted. Then sera were separated by centrifugation at 3000 r.p.m for 15 minutes. The supernatant serum was stored and deeply frozen at -20°C until assayed. Hearts were excised, fixed in formalin, embedded in paraffin wax, stained with H&E and examined using light microscope.

### Biochemical analysis

- **Serum adropin levels:** Were measured using rat Adropin (AD) ELISA kits. SunRed, Biotechnology company, Shanghai, Ca 201-11-3361.
- **Serum cardiac troponin-I (cTn-I) levels:** Were measured using ELISA kits for for cTn-I:Bioassay technology laboratory, China Cat. No E0639Ra.
- **Serum CK-MB levels:** Were measured using kits for CK-MB level estimation: Pointe Scientific, Inc. USA.5449 Research Drive, Canton, MI 48188.
- **Serum CRP levels:** Were measured by using kits for CRP estimation: Monobind, Inc Lake Forest, Ca 92630, USA.
- **Serum TNF-α levels:** Were measured using kits for estimation of TNF-α: R and D systems, Inc., USA and Canada, Ca RTA00, SRTA00, PRTA00.
- **Serum malondialdehyde (MDA) levels:** Were measured by using kits for MDA level estimation: Sigma-Aldrich, USA, Ca.MAK085.
- **Serum superoxide dismutase enzyme activity (SOD):** Were measured by using kits for SOD level: Thermofisher, USA, colorimetric assay, Ca.EI ASODC.
- **Serum Reduced glutathione (GSH) level:** Were measured by using kits for GSH estimation, Elabscience, colorimetric assay, Ca E-BC-K030-S.
- **Serum Catalase activity:** Were measured by using kits for catalase estimation, Thermofisher, USA, colorimetric assay, Ca EIACATC.
- **Serum Total cholesterol (TC) level**: Were measured using TC kits**.** BioSource Europe S.A.-Rue de l'Industrie, 8-B-1400 Nivelles-Belgium.
- **Serum Triglycerides (TGs) level:** Were measured by using kits for estimation of serum TGs, Thermofisher, USA, Ca TR22421
- **Serum HDL-cholesterol (HDL-C) level**: Were measured by using kits for estimation of HDL-C. BioSource Europe S.A.-Rue de l'Industrie, 8-A-1340 Nivelles-Belgium.
- **Serum low density lipoprotein-cholesterol (LDL-C) levels:** Were measured according to the equation: LDL-C=TC-HDL-C-TGs/5 [17].
- **Serum insulin level:** Were measured by using kits for estimation of insulin levels. INS-EASIA, KAP1251 (BioSource Europe S.A. -Rue de l'Industrie, 4-A- 1300 Nivelles-Belgium)**.**

- **Serum glucose:** Were measured by using kits for estimation of glucose levels. INS-EASIA, KAP1251 (BioSource Europe S.A. -Rue de l'Industrie, 8-B- 1400 Nivelles-Belgium).
- **HOMA-IR:** Were measured was calculated according to the equation: Insulin (μU/ml) xGlucose (mg/dl)/405 [18].

## Statistical analysis

The data were expressed as mean ± SD for quantitative variables and statistically analyzed by using SPSS program (version26 for windows) (SPSS Inc. Chicago, IL, USA). One way Analysis of Variance (ANOVA) was used to compare means of the groups followed by LSD test to compare statistical differences between groups. The correlations between parameters were analyzed using Pearson, s correlation. P values < 0.05 were considered to be significant.

## Results

In this study, body weight (g), BMI (g/cm$^2$) and abdominal circumference (cm) were measured and they were significantly higher in obese (342 ± 41g, 0.8 ± 0.09 g/cm$^2$ and 24. 2 ± 1. 2 cm respectively) groups when compared to lean groups (232g ± 29, 0.53 ± 0.09 g/cm$^2$ and 17. 9 ± 0.7 cm respectively). Also ECG was recorded before and after ISO injection, where we found (in lean and obese groups) ST segment depression (0.128 ± 0.03 and 0.137 ± 0.03mV respectively), decreased R-wave amplitude (0.45 ± 0.1 and 0.31 ± 0.1 mV respectively), decreased duration of R-R interval (0.2 ± 0.01 and 0.19 ± 0.02 seconds respectively) and consequently increased heart rate (297 ± 22 and 325 ± 24 BPM respectively) after ISO injection when compared to those before injection [ (0.026 ± 0.007, 0.034 ± 0.01 mV), (0.58 ± 0.18, 0.5 ± 0.15 mV), (0.29 ± 0.02, 0.27 ± 0.02sec.), (208 ± 11, 224 ± 13 BPM) respectively]. Blood pressure was measured (mmHg) before and after ISO injection and it was significantly decreased (in lean and obese groups) after ISO injection (56 ± 8 and 68 ± 8 respectively) than that before injection (93 ± 15 and 124 ± 21 respectively). These findings indicate anterior myocardial infarction where lead II is considered a reciprocal lead (Figure 1).

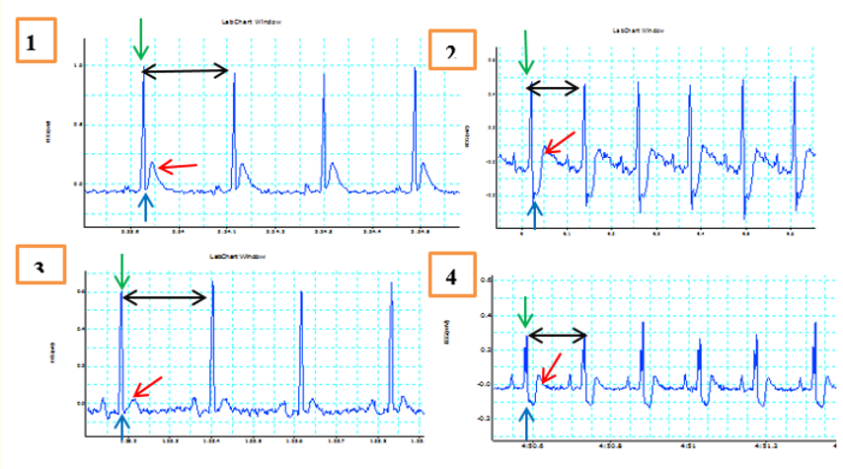


***Figure 1:*** *Shows ECG recording of lead II before and after ISO injection in lean and obese groups. [1] ECG of a rat from the lean group before injection. [2] ECG of a rat from the lean group after injection showing depressed ST segment (blue arrow), upright T-wave (red arrow), decreased R-wave amplitude (green arrow) and decreased duration of R-R interval (tachycardia) (black arrow). [3] ECG of an obese rat before injection. [4] ECG of an obese rat after injection showing depressed ST segment (blue arrow), upright T-wave (red arrow), decreased R-wave amplitude (green arrow) and decreased duration of R-R interval (tachycardia) (black arrow).*

**Serum adropin (pg/ml):** Our study showed that in lean groups, serum adropin levels in groups 1B, 1C and 1D were significantly ($p < 0.05$) elevated compared to that of group 1A, with a peak in group 1D. Regarding obese groups, it was found that serum adropin levels in groups 2B, 2C, 2D and 2E were significantly ($p < 0.05$) higher compared to that of group 2A with a peak in group 2C. All values of serum adropin levels in obese groups were significantly ($p < 0.05$) lower than their corresponding lean groups (Table 1, 2 and figure 2).

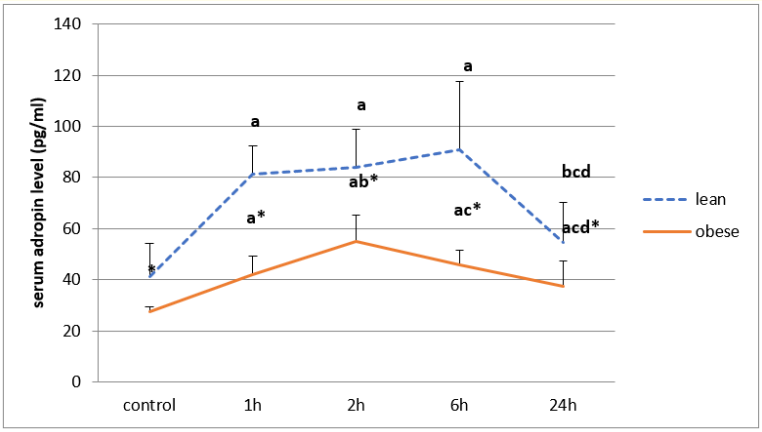


***Figure 2:*** *Illustrates serum adropin levels in all studied groups. a:vs group A, b: vs group B, c: vs group C, d: vs group D. * means significant difference between the obese group and its corresponding lean group.*

| | | 1A | 1B | 1C | 1D | 1E |
|---|---|---|---|---|---|---|
| | | Control (n = 7) | 1h. post AMI (n = 7) | 2h. post AMI (n = 7) | 6h. post AMI (n = 7) | 24h. post AMI (n = 7) |
| Adropin (pg/ml) | | 41.2 ± 13 | 81.4 ± 11[a] | 83.9 ± 15[a] | 90.7 ± 27[a] | 54.4 ± 16[bcd] |
| Metabolic paramerters | Glucose (mg/dl) | 77 ± 14 | 110 ± 17[a] | 119 ± 14[a] | 103 ± 6[ac] | 102 ± 21[ac] |
| | Insulin (µU/ml) | 17 ± 2 | 22.4 ± 2[a] | 25 ± 2[ab] | 23 ± 2[a] | 22.7 ± 1[a] |
| | HOMA-IR | 3.3 ± 0.6 | 6 ± 0.8[a] | 7.3 ± 1[ab] | 6 ± 0.5[ac] | 5.8 ± 1[ac] |
| | TC (mg/dl) | 80 ± 27 | 104 ± 13[a] | 105 ± 16[a] | 116 ± 22[a] | 106 ± 16[a] |
| | TGs (mg/dl) | 93 ± 27 | 143 ± 33[a] | 184 ± 62[a] | 181 ± 52[a] | 149 ± 42[a] |
| | HDL (mg/dl) | 46 ± 5 | 30.7 ± 4[a] | 33.9 ± 6[a] | 36.9 ± 7[a] | 30.9 ± 9[a] |
| | LDL (mg/dl) | 15 ± 5 | 44 ± 14[a] | 34 ± 10[a] | 43 ± 12[a] | 45 ± 12[a] |
| Cardiac markers | cTn-I (ng/dl) | 0.07 ± 0.02 | 0.13 ± 0.05 | 0.27 ± 0.09[ab] | 0.33 ± 0.03[ab] | 0.27 ± 0.09[ab] |
| | CK-MB (U/l) | 9.25 ± 3.6 | 12.7 ± 2.4 | 24.3 ± 5.4[ab] | 30.2 ± 5.3[abc] | 17.4 ± 5.3[acd] |
| Inflammatory markers | CRP (mg/l) | 0.07 ± 0.02 | 0.14 ± 0.04 | 0.15 ± 0.06 | 0.24 ± 0.08[a] | 0.5 ± 0.2[abcd] |
| | TNF-α (pg/ml) | 16.2 ± 6 | 37 ± 8[a] | 43.4 ± 14[a] | 54.6 ± 12[ab] | 46.8 ± 11[a] |
| Oxidative stress markers | MDA (mg/dl) | 11.06 ± 3.1 | 14.38 ± 3.1 | 15.2 ± 2.53[a] | 15.28 ± 3.02[a] | 19.51 ± 5.3[abcd] |
| | SOD (U/ml) | 211.7 ± 46 | 174.4 ± 51 | 128.4 ± 42[ab] | 125.1 ± 25[ab] | 111.3 ± 34[ab] |
| | GSH (mg/dl) | 5.54 ± 1.5 | 4.97 ± 1.55 | 4.46 ± 1.5 | 3.8 ± 0.9[a] | 2.54 ± 0.7[abc] |
| | Catalase (U/l) | 161.6 ± 21 | 145.2 ± 42 | 144.3 ± 25 | 132.8 ± 28 | 109.9 ± 33[abc] |

***Table 1:*** *Shows mean ± SD of metabolic parameters, cardiac markers, inflammatory markers and oxidative stress markers in all lean groups.*
*a:vs group A; b: vs group B; c: vs group C; d: vs group D.*

| | | 2A | 2B | 2C | 2D | 2E |
|---|---|---|---|---|---|---|
| | | Control (n = 7) | 1h. post AMI (n = 7) | 2h. post AMI (n = 7) | 6h. post AMI (n = 7) | 24h. post AMI (n = 7) |
| Adropin (pg/ml) | | 27.44 ± 2* | 42.07 ± 7.13a* | 55 ± 10.2 ab* | 45.7 ± 5.9 ac* | 37.3 ± 10.04acd* |
| Metabolic paramerters | Glucose (mg/dl) | 114 ± 34* | 204 ± 69a* | 211 ± 82a* | 202 ± 57a* | 191 ± 9a* |
| | Insulin (µU/ml) | 24 ± 4* | 31 ± 5a* | 33 ± 5a* | 31 ± 4a* | 30 ± 5a* |
| | HOMA-IR | 6.9 ± 2* | 15.8 ± 6a* | 17.4 ± 7a* | 15.4 ± 4a* | 14.5 ± 3a* |
| | TC (mg/dl) | 126 ± 29* | 161 ± 20a* | 151 ± 14a* | 153 ± 16a* | 152 ± 22a* |
| | TGs (mg/dl) | 173 ± 19* | 240 ± 39a* | 246 ± 22a* | 255 ± 51a* | 221 ± 57a* |
| | HDL (mg/dl) | 36 ± 4* | 23 ± 5a* | 25 ± 6a* | 26 ± 6a* | 22 ± 3a* |
| | LDL (mg/dl) | 55 ± 7* | 90 ± 11a* | 76 ± 11ab* | 76 ± 10ab* | 86 ± 18a* |
| Cardiac markers | cTn-I (ng/dl) | 0.06 ± 0.01 | 0.09 ± 0.03 | 0.19 ± 0.04ab | 0.38 ± 0.1abc | 0.28 ± 0.06abcd |
| | CK-MB (U/l) | 43 ± 6* | 59.3 ± 10* | 76.3 ± 12 ab* | 78.3 ± 22.5ab* | 55.7 ± 18 cd* |
| Inflammatory markers | CRP (mg/l) | 0.24 ± 0.04* | 0.28 ± 0.09* | 0.28 ± 0.04* | 0.38 ± 0.06abc* | 0.78 ± 0.07abcd* |
| | TNF-α (pg/ml) | 34.2 ± 10* | 49.8 ± 6a* | 59.2 ± 12a* | 70.4 ± 7abc* | 62 ± 10ab* |
| Oxidative stress markers | MDA (mg/dl) | 17.3 ± 3.8* | 20.7 ± 3* | 29.9 ± 5.4 ab* | 29 ± 5.2 ab* | 34.1 ± 5.9ab* |
| | SOD (U/ml) | 100.43 ± 32.5* | 95 ± 13.8* | 87.7 ± 8.9* | 70.57 ± 13ab* | 69.86 ± 8.7ab* |
| | GSH (mg/dl) | 4 ± 0.7* | 3.3 ± 1* | 2.7 ± 0.9 a* | 2.68 ± 0.8 a* | 1.59 ± 0.68abcd* |
| | Catalase (U/l) | 95 ± 20.6* | 93.2 ± 38* | 79.1 ± 31.8* | 71.7 ± 31.5* | 46 ± 23abc* |

***Table 2:*** *Shows mean ± SD of metabolic parameters, cardiac markers, inflammatory markers and oxidative stress markers in all obese groups.*

*a:vs group A; b: vs group B; c: vs group C; d: vs group D.*

**means significant difference between obese group and its corresponding lean group.*

**Regarding serum glucose (mg/dl), insulin (µU/ml) and HOMA-IR:** In lean and obese groups, it was found that their levels in all AMI groups (B, C, D, E) were significantly ($p < 0.05$) higher than that group (A). Moreover, these values were significantly ($p < 0.05$) higher in obese than lean groups (Table 1 and 2).

**Lipid profile:** Was also measured (mg/dl), where we found that in both lean and obese groups, serum TC, TGs and LDL-C were significantly ($p < 0.05$) higher, while HDL-C was significantly ($p < 0.05$) lower in all AMI groups compared to that of controls. Moreover, there were significant differences between obese and their corresponding lean groups (Table 1 and 2).

**Serum cTn-I (ng/dl):** It was found that in lean groups serum cTn-I levels in groups 1C, 1D and 1E were significantly ($p < 0.05$) higher compared to that of group 1A. Regarding obese groups, it was found that serum cTn-I levels in groups 2C, 2D and 2E were significantly ($p < 0.05$) higher compared to that of group 2A. No significant differences were found between obese and their corresponding lean groups (Table 1 and 2).

**Serum CK-MB (U/l):** In lean groups, serum CK-MB levels in groups 1C, 1D and 1E were significantly ($p < 0.05$) higher compared to that of group 1A. Regarding obese groups, it was found that serum CK-MB levels in groups 2C and 2D were significantly ($p < 0.05$) higher

compared to that of group 2A. Moreover, serum CK-MB levels in all obese groups were significantly ($p < 0.05$) higher than those of their corresponding lean groups (Table 1 and 2).

**Serum inflammatory markers:** In lean groups, serum CRP levels (mg/l) in groups 1D and 1E were significantly ($p < 0.05$) higher than that of group 1A. In obese groups, serum CRP levels in groups 2D and 2E were significantly ($p < 0.05$) higher compared to that of group 2A. Moreover, the mean values of serum CRP levels in all obese groups were significantly ($p < 0.05$) higher than those of their corresponding lean groups (Table 1 and 2).

Regarding serum TNF-α (pg/ml), it was found that in lean groups, serum levels of TNF-α in groups 1B, 1C, 1D and 1E were significantly ($p < 0.05$) higher than that of group1A. Also in obese groups, serum TNF-α levels in groups 2B, 2C, 2D and 2E were significantly ($p < 0.05$) higher than that of group 2A. Serum TNF-α levels in all obese groups were significantly ($p < 0.05$) higher than those of their corresponding lean groups (Table 1 and 2).

**Serum oxidative stress markers:** In lean groups, serum MDA levels (mg/dl) in groups 1C, 1D and 1E were significantly ($p < 0.05$) higher compared to that of group 1A. In obese groups, it was found that serum MDA levels in groups 2C, 2D and 2E were significantly ($p < 0.05$) higher compared to that of group 2A. Moreover, serum MDA levels in all obese groups were significantly ($p < 0.05$) higher than those of their corresponding lean groups (Table 1 and 2).

Regarding serum SOD activity (U/ml) in lean groups, it was found that in groups 1C, 1D and 1E, serum SOD activity was significantly ($p < 0.05$) lower compared to that of group 1A. Regarding obese groups, it was found that serum SOD activity in groups 2D and 2E was significantly ($p < 0.05$) lower compared to that of group 2A. Serum SOD activity in all obese groups were significantly ($p < 0.05$) lower than those of their corresponding lean groups (Table 1 and 2).

Regarding serum GSH levels (mg/dl) in lean groups it was found that in groups 1D and 1E, serum GSH levels were significantly (p 0.05) lower than that of group 1A. While in obese groups, it was found that serum GSH levels in groups 2C, 2D and 2E were significantly ($p < 0.05$) lower than that of group 2A. Moreover, serum GSH levels in all obese groups were significantly ($p < 0.05$) lower than those of their corresponding lean groups (Table 1 and 2).

Regarding catalase activity (U/l) in the lean groups, it was found that in group 1E, it was significantly ($p < 0.05$) lower compared to that of group 1A. While in obese groups, it was found that serum catalase activity in group 2E was significantly ($p < 0.05$) lower compared to that of group 2A. Moreover, it was found that serum catalase activity in all obese groups were significantly ($p < 0.05$) lower than those of their corresponding lean groups (Table 1 and 2).

There were significant positive correlations ($p < 0.05$) between serum adropin levels and each of cTn-I, CK-MB, CRP, TNF-α, MDA, glucose, insulin, HOMA-IR, TC, TGs and LDL-C, while there were significant negative correlations ($p < 0.05$) between serum adropin levels and each of SOD, catalase, GSH and HDL-C in both lean and obese groups (Table 3).

Histopathological changes were found in AMI groups (Figures 3 and 4).

## Discussion

AMI is one of the most common causes of death worldwide and it is defined as myocardial cell death due to prolonged ischemia. Rapid diagnosis of AMI using ECG and assessment of early cardiac biomarkers is mandatory to initiate effective treatment [19].

In this study, AMI was induced by ISO injection. Myocardial damage with ISO was explained by an imbalance between oxygen supply and demand of cardiomyocytes, which is related to myocardial hyperfunction due to increase both in chronotropism and inotropism as well as coronary hypotension [20].

| | Lean group (at 6h. post-AMI) | | HFD-induced obese group (at 2h. post-AMI) | |
|---|---|---|---|---|
| | r | p-value | r | p-value |
| TC | +0.868 | <0.05 | +0.932 | <0.01 |
| TGs | +0.872 | <0.05 | +0.845 | <0.05 |
| HDL | -0.963 | <0.01 | -0.938 | <0.01 |
| LDL | +0.846 | <0.05 | +0.892 | <0.01 |
| Glucose | +0.839 | <0.05 | +0.955 | <0.01 |
| Insulin | +0.899 | <0.01 | +0.946 | <0.01 |
| HOMA-IR | +0.869 | <0.05 | +0.911 | <0.01 |
| CRP | +0.861 | <0.05 | +0.772 | <0.05 |
| TNF-α | +0.769 | <0.05 | +0.993 | <0.001 |
| cTn-I | +0.906 | <0.01 | +0.851 | <0.05 |
| CK-MB | +0.779 | <0.05 | +0.923 | <0.01 |
| MDA | +0.968 | <0.001 | +0.934 | <0.01 |
| SOD | -0.777 | <0.05 | -0.935 | <0.01 |
| GSH | -0.911 | <0.01 | -0.976 | <0.001 |
| Catalase | -0.830 | <0.05 | -0.861 | <0.05 |

***Table 3:*** *Shows correlation between serum adropin levels (at its peak) and other parameters in both lean and HFD-induced obese groups.*

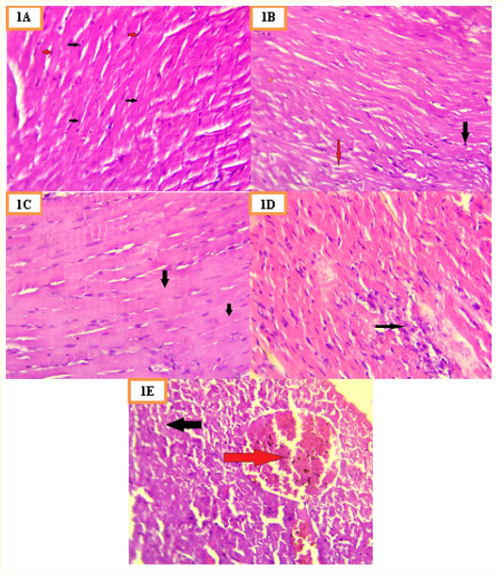


***Figure 3:*** *Photomicrographs of cardiac tissue from the lean group (HE x400). [1A] Group 1A: Normal architecture of cardiac wall with branching and anastomosing myofibers bounded with endomysium. Cardiomyocytes have central oval, euchromatic nuclei (black arrows) and surrounded with numerous blood capillaries flat nuclei of fibroblasts (red arrow). [1B] Group1B: Normal architecture of cardiac wall with branching and anastomosing myofibers with mild interstitial edema (red arrow) with few apoptotic nuclei (black arrow). [1C] Group1C: Myofibers with loss of myocardial striation (black arrows) [1D] Group1D: Mild inflammatory infiltrate (black arrow) [1E] Group1E: Moderate tissue necrosis (black arrow), moderate congested blood vessels (red arrow).*

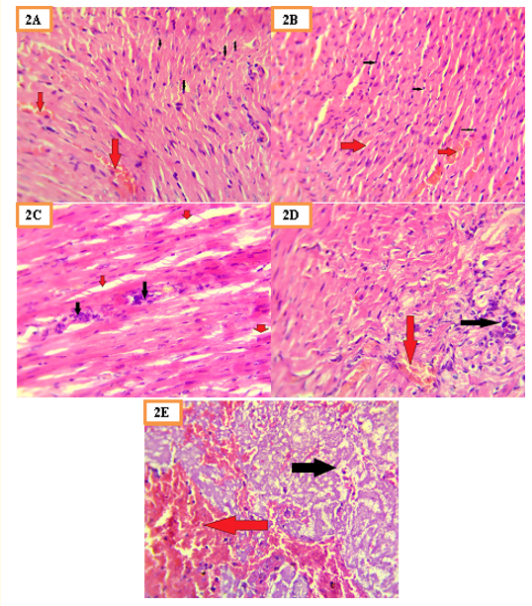


***Figure 4:*** *Photomicrographs of cardiac tissue from the obese group (HE x400). [2A] Group2A: pyknotic (apoptotic) nuclei (black arrow), congested blood vessels (red arrow), hemorrhage. [2B] Group2B: Marked pyknotic (apoptotic) nuclei (black arrows), severe congested blood vessels (red arrow). [2C] Group2C: marked interstitial edema (red arrows), mild inflammatory infiltrate (black arrows). [2D] Group2D: moderate interstitial infiltrate (black arrow), severe congested blood vessels (red arrow). [2E] Group2E: marked tissue necrosis (black arrow), severe congested blood vessels and haemorrhage (red arrow).*

It was found that adropin was increased significantly at 1 hour post-AMI induction in both lean and obese groups compared to that of controls and continued to increase to reach peak levels at 6 hours in the lean groups and 2 hours in the obese groups. This may be explained by the need of the infarcted tissues to maintain energy homeostasis, where adropin could influence the regulation of metabolic pathways during long periods of ischemia and hypoxia in AMI rats [8]. Accordingly, adropin could be increased to protect the ischemic region by maintaining glucose homeostasis and restoring myocardial glycogen stores, thus improving myocardial energy status to avoid more hypoxia, enlargement of the infarcted area and further impairment of cardiac function [8].

Our results were consistent with other studies [3,6] which reported significant increase in serum adropin levels as early as 30 minutes and within 2 hours post-infarction respectively compared to that of controls.

However, our results were not in line with those of another study [10] who found that serum adropin levels were significantly lower in patients with AMI compared to controls.

In the present study, serum adropin levels in all obese groups were found to be significantly lower than that of their corresponding lean. This finding is in agreement with those of a previous study [5] which reported decreased levels of adropin in obesity.

Regarding cTn-I, we found that-in both lean and obese groups-cTn-I levels began to increase significantly 2 hours post AMI induction. A previous study reported that cTnI began to increase significantly at 1 hour post-AMI induction [3]. A human study found that serum cTnI rose within 30 minutes post AMI [6].

Serum CK-MB-in both lean and obese groups-began to increase significantly at 2 hours post-AMI induction. Moreover, we found that serum levels of CK-MB were significantly higher in obese groups compared to that of the lean groups. Our results are in line with those of another study which found that serum levels of CK-MB were significantly elevated 2hours post-AMI [3]. In a human study, serum CK-MB was significantly elevated within 30 - 40 minutes after the onset of chest pain [6]. Also, our results came in consistency with those of another study which found that HFD-induced obesity in rabbits was associated with increased CK-MB activity [21].

When myocardial cells are damaged, the cardiac membrane becomes permeable resulting in leakage of enzymes [22]. ISO increases permeability of cardiac muscle cells leading to release of cytosolic enzymes such as CK-MB [23].

Regarding inflammatory markers, we found that CRP increased gradually to become significantly higher at 6 hours post-AMI induction, in both lean and obese groups, while TNF-α began to increase significantly at 1 hour post AMI induction in both lean and obese groups. These results are in consistency with those of another study which found that CRP was increased in acute coronary syndrome after 12 hours since CRP requires > 6 hours to be synthesized and secreted by hepatocytes [24].

TNF-α is expressed in cardiomyocytes only after AMI as myocardial tissue with ischemia and anoxia activates cardiomyocytes and local mononuclear macrophages, which causes the myocardium in the infarcted zone and infarction border zone to produce large amounts of TNF- α [25]. We found also that both serum CRP and TNF-α were significantly higher in the obese groups than that in the lean groups. Obesity predisposes to a pro-inflammatory state by increasing inflammatory mediators IL-6 and TNF-α [26]. The liver drains free fatty acids and the circulating triacylglycerol promoting release of cytokines such as IL-1β that induces IL-6 production by adipose tissue, which in turn triggers hepatocyte expression and release of CRP [27].

Regarding MDA, which is an indicator of lipid peroxidation, we found that its serum levels -in both lean and obese groups- became significantly higher than that of the controls at 2 hours post AMI induction.

On the other hand, we found that serum levels of SOD decreased to become significantly lower at 2 hours post AMI induction (in the lean group) and at 6hours post AMI induction (in the obese group).

Also, it was found that serum levels of GSH began to decrease significantly at 6 hours post AMI induction in the lean group and at 2 hours in the obese group.

Serum catalase activity became significantly lower at 24 hours post AMI induction than that of controls in both lean and obese groups.

It is worth noting that serum levels of MDA were significantly higher in the obese groups than that of their corresponding lean groups, while SOD, GSH and catalase were significantly lower in the obese groups than that of their corresponding lean groups.

Our results are in agreement with those of other investigators who reported that the levels of MDA were significantly increased and total antioxidant status was significantly decreased in AMI [28]. A study showed that in obese mice, serum levels of MDA were significantly higher, while serum levels of GSH and catalase were significantly lower than that of non-obese [29]. Moreover, it was observed that the mean MDA concentration was highest in AMI patients with previous hyperlipidemia. Thus AMI is associated with greater oxidative stress and diminished activity of antioxidant defense system to combat free radical [30].

Growing evidence indicates that the mitochondria of white adipose tissue, particularly of obese persons, are the main site of reactive oxygen species (ROS) generation, with augmented expression of NADPH oxidase and decreased expression of antioxidative enzymes [31]. The activation of antioxidant enzymes in overweight individuals may be to counteract the effect of oxidative stress generated by ROS. Also, it was found that in the early stages of obesity development there may be an initial elevation in antioxidant enzymes to counteract oxidative stress [32]. The depletion of the antioxidant activities in obese individuals may be attributed to the high production of ROS which may

destroy these antioxidant enzymes. Also, decreased activity of SOD may be attributed to excess production of ox-LDL which inhibits the expression of SOD [33].

During AMI, superoxide radicals modulate the activity of catalase resulting in reduced activity of this enzyme and accumulation of superoxide radicals, with consequent damage to the myocardium [34].

The mechanism of free radical formation in obesity is the increase of pro-inflammatory cytokines produced by adipocytes and pre-adipocytes such as TNF-α, IL-1 and IL-6 which are potent stimulators for the production of reactive oxygen and nitrogen species by macrophages and monocytes. TNF-α increases the interaction of electrons with oxygen to produce superoxide anions [35].

Regarding metabolic parameters, significant increases were found in serum levels of TC, TGs and LDL-C with significant decreases in HDL-C in AMI groups in both lean and obese groups. These results are in consistency with that of other investigators [36]. We also found that lipid profile in obese group was different from lean group, where TC, TGs and LDL-C were significantly higher in obese than that of lean groups, while HDL-C was significantly lower in obese group when compared to that of the lean group, which agreed with a previous study [37]. ISO-induced elevation in cholesterol levels could be due to increase in biosynthesis and decrease in its utilization [38]. Hypertriglyceridemia might be due to decrease in the activity of lipoprotein lipase accompanied by increase in the activity of hormone-sensitive lipase, resulting in the decreased uptake of TGs from the circulation [36].

We also found significant increase in serum glucose, insulin levels and insulin resistance (HOMA-IR) in AMI groups compared to that of controls and also in obese groups compared to their corresponding lean groups. These results were in line with those of Aydin., *et al.* Who found that glucose levels in patients with acute coronary syndrome were elevated compared to controls. The elevated glucose levels may be due to the effect of increased epinephrine secretion after AMI, causing glycogenolysis in the liver and releasing glucose into the blood [6].

In this study we found significant positive correlations between serum adropin and each of cTn-I, CK-MB, CRP, TNF-α, MDA, glucose, insulin, HOMA-IR, TC, TGs and LDL-C while there were significant negative correlations with SOD, catalase, GSH and HDL-C in both lean and obese groups at the peaks of serum adropin. These results came in line with another study that found a positive correlation between serm adropin and cTn-I [3].

Histopathological changes were found in all AMI groups such as interstitial edema, apoptotic cells, loss of striations, inflammatory cell infiltration, congestion, haemorrhage and tissue necrosis. These finding came in line with a previous study [3]. Myocardial edema may be the cause of decreased R wave amplitude [39]. It was reported that neutrophils exert potent cytotoxic effects through the release of proteolytic enzymes and the adhesion with Intercellular Adhesion Molecule (ICAM) -1 expressing cardiomyocytes. Monocyte Chemoattractant Protein (MCP) -1 is also markedly upregulated in the infarcted myocardium inducing recruitment of mononuclear cells in the injured areas. Monocyte-derived macrophages and mast cells may produce cytokines and growth factors necessary for fibroblast proliferation and neovascularization, leading to effective repair and scar formation [40].

## Conclusion

The present study revealed a significant increase of serum adropin levels following AMI. These levels were correlated with the changes in other cardiac enzyme markers, lipid profile, proinflammatory and oxidative stress markers. Thus, serum adropin can be used as an alternative biomarker to other cardiac enzyme markers (cTn-I, CK-MB) for early diagnosis of AMI especially in cases of obesity.

## Recommendations

Further studies are required to clarify the effect of adropin injection on cardiovascular system in absence and presence of different diseases such as AMI and heart failure and its possible mechanism/s of action under different conditions as obesity and diabetes mellitus.

## Bibliography

1. Vasan RS. "Biomarkers of cardiovascular disease: molecular basis and practical considerations". *Circulation* 113.19 (2006): 2335-2362.
2. Damiani G., *et al*. "Influence of socioeconomic factors on hospital readmissions for heart failure and acute myocardial infarction in patients 65 years and older: evidence from a systematic review". *Clinical Interventions in Aging* 10 (2015): 237-245.
3. Aydin S., *et al*. "A candidate diagnostic marker for myocardial infarction in conjunction with troponin-I". *Peptides* 58 (2014): 91-97.
4. Oliveira A., *et al*. "Self-reporting weight and height: misclassification effect on the risk estimates for acute myocardial infarction". *European Journal of Public Health* 19.5 (2009): 548-553.
5. Kumar KG., *et al*. "Identification of adropin as a secreted factor linking dietary macronutrient intake with energy homeostasis and lipid metabolism". *Cell Metabolism* 8.6 (2008): 468-481.
6. Aydin S., *et al*. "Adropin as a potential marker of enzyme-positive acute coronary syndrome". *Cardiovascular Africa Journal of Africa* 28.1 (2017): 40-47.
7. Aydin S., *et al*. "Expression of adropin in rat brain, cerebellum, kidneys, heart, liver, and pancreas in streptozotocin-induced diabetes". *Molecular and Cellular Biochemistry* 380.1-2 (2013): 73-81.
8. Grossman AN., *et al*. "Glucose-insulin-potassium revived: current status in acute coronary syndromes and the energy-depleted heart". *Circulation* 127.9 (2013): 1040-1048.
9. Lovren F., *et al*. "Adropin is a novel regulator of endothelial function". *Circulation* 122 (2010): S185-S192.
10. Yu HY., *et al*. "Serum adropin levels are decreased in patients with acute myocardial infarction". *Regulatory Peptides* 190-191(2014): 46-49.
11. Zhang C., *et al*. "Correlation of serum adropin level with coronary artery disease". *Zhonghua Yi Xue Za Zhi* 94.16 (2014):1255-1257.
12. Kumar KG., *et al*. "Adropin deficiency is associated with increased adiposity and insulin resistance". *Obesity* 20.7 (2012): 1394-1402.
13. Surwit R., *et al*. "Differential effects of fat and sucrose on the development of obesity and diabetes in C57BL/6J and A/J mice". *Metabolism* 44.5 (1995): 645-651.
14. Barbier de La Serre C., *et al*. "Propensity to high-fat diet-induced obesity in rats is associated with changesin the gut microbiota and gut inflammation". *The American Journal of Physiology-Gastrointestinal and Liver Physiology* 299.2 (2010): G440-G448.
15. Novelli ELB., *et al*. "Anthropometrical parameters and markers of obesity in rats". *Index of obesity in Rats, Laboratory Animals* 41.1 (2007): 111-119.
16. Zaafan MA., *et al*. "Protective effects of atorvastatin and quercetin on isoprenaline-induced myocardial infarction in rats". *Bulletin of Faculty of Pharmacy Cairo University* 51.1 (2013): 35-41.
17. Friedewald WT., *et al*. "Estimation of the concentration of low density lipoprotein cholesterol in plasma without use of the preparative ultracentrifuge". *Clinical Chemistry* 18.6 (1972): 499-502.
18. Sun G., *et al*. "Serum visfatin concentrations are positively correlated with serum triacylglycerols and downregulated by overfeeding in healthy young men". *The American Journal of Clinical Nutrition* 85.2 (2007): 399-404.

19. Mythili S and Malathi N. "Diagnostic markers of acute myocardial infarction (Review)". *Biomedical Reports* 3.6 (2015): 743-748.

20. Siddiqui MA., *et al*. "Isoprenaline: A tool for inducing myocardial infarction in experimental animal". *International Journal of Research and Reviews in Pharmacy and Applied Science* 6.1 (2016): 1318-1326.

21. Sibouakaz D., *et al*. "Biochemical and Ultrastructural Cardiac Changes Induced by High-Fat Diet in Female and Male Prepubertal Rabbits". *Analytical Cellular Pathology* (2018): 16.

22. Priscilla DH and Prince PSM. "Cardioprotective effect of gallic acid on cardiac troponin-T, cardiac marker enzymes, lipid peroxidation products and antioxidants in experimentally induced myocardial infarction in Wistar rats". *Chemico-Biological Interactions* 179.2-3 (2009): 118-124.

23. Marjomaki V., *et al*. "Isoproterenol-induced redistribution of endosomes in cardiac myocytes". *European Journal of Cell Biology* 65.1 (1994): 1-13.

24. Sano T., *et al*. "C-reactive protein and lesion morphology in patients with acute myocardial infarction". *Circulation* 108.3 (2003): 282-285.

25. Zeng M., *et al*. "Suppression of NF-kappa B reduces myocardial no-reflow". *PLOS One* (2012): e47306.

26. Ellulu MS., *et al*. "Obesity and inflammation: the linking mechanism and the complications". *Archives of Medical Science* 13.4 (2017): 851-863.

27. Brooks GC., *et al*. "Relation of C-reactive protein to abdominal adiposity". *The American Journal of Cardiology* 106.1 (2010): 56-61.

28. Khan HA., *et al*. "Serum markers of tissue damage and oxidative stress in patients with acute myocardial infarction". *Biomedical Research* 24.1 (2013): 15-20.

29. Susantiningsih T., *et al*. "The Effect of Treadmill Treatment on Oxidative Stress Markers and Endogenous Antioxidant Status in Obesity Mice". *Open Access Macedonian Journal of Medical Sciences* 6.10 (2018): 1803-1808.

30. Patil N., *et al*. "Antioxidant status in patients with acute myocardial infarction". *Indian Journal of Clinical Biochemistry* 22.1 (2007): 45-51.

31. Roberts CK., *et al*. "Oxidative stress and dysregulation of NAD(P)H oxidase and antioxidant enzymes in diet-induced metabolic syndrome". *Metabolism* 55.7 (2006): 928-934.

32. Woo J., *et al*. "The effects of de-training on blood adipokines and antioxidant enzyme in Korean overweight children". *European Journal of Pediatrics* 171.2 (2012): 235-243.

33. Codoñer-Franch P., *et al*. "Oxidant mechanisms in childhood obesity: the link between inflammation and oxidative stress". *Translational Research* 158.6 (2011): 369-384.

34. Saravanan G and Prakash J. "Effect of garlic (Allium sativum) on lipid peroxidation in experimental myocardial infarction in rats". *Journal of Ethnopharmacology* 94.1 (2004): 155-158.

35. Vincent HK., *et al*. "Obesity exacerbates oxidative stress levels after acute exercise". *Medicine and Science in Sports and Exercise* 36.5 (2004): 772-779.

36. Subashini R. "Pretreatment with Nelumbo Nucifera leaf extract ameliorates on lipids, lipoproteins, marker enzymes of lipid metabolism and ECG pattern against isoproterenol induced cardiotoxicity". *International Journal of Pharmacy and Pharmaceutical Sciences* 6.5 (2014): 459-464.

37. Lee JJ., *et al*. "Effects of Brassica juncea L. leaf extract on obesity and lipid profiles of rats fed a high-fat/high-cholesterol diet". *Nutrition Research and Practice* 12.4 (2018): 298-306.

38. Deepa PR and Varalakshmi P. "Beneficial cardio-renovascular effects of a low molecular weight heparin derivative on Adriamycin induced glycosaminoglycanuria and tissue lipid abnormalities". *Toxicology* 211.1-2 (2005): 77-85.

39. Derbali A., *et al*. "Cardioprotective effects of linseed oil against isoproterenol-induced myocardial infarction in Wistar rats: A bio-chemical and electrocardiographic study". *Journal of Physiology and Biochemistry* 71.2 (2015): 281-288.

40. Frangogiannis NG., *et al*. "The inflammatory response in myocardial infarction". *Cardiovascular Research* 53.1 (2002): 31-47.

**Volume 6 Issue 11 November 2019**