Effect of Short Term Caloric Restriction on Ischemic Reperfused Hearts in Adult Rats Subjected to Stress

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Abstract: Caloric restriction extends life span and decrease tissue susceptibility to stress –induced injury so it was intriguing to investigate a possible cardioprotective effect of short term caloric restriction during stress on ischemic reperfusion injury of the heart. This study was conducted on 32 adult albino rats which were assigned to 3 groups; control group C (n=10), Stress group S (n= 11) subjected to immobilization stress, and caloric restriction/stress group CR/S (n= 11) comprised of rats subjected to 35% caloric restriction and subjected to immobilization stress. The study was conducted for one month; obtained results revealed that S rats had significant elevation in ST segment , significant prolongation in half relaxation time (HRT) and significant decrease in plasma adiponectin level as well as cardiac tissue nitrate content. CR/S rats exhibited significant decrease in final body weight, BMI, absolute liver and heart weights compared to C and S groups as well as significant elevation of ST segment compared to C group. Ischemic reperfusion study of CR/S rat hearts revealed better ischemic tolerance compared to S rats as evidenced by the significant elevation of peak developed tension (PT/100mg LV)at 10 and 20 minutes of reperfusion, significant shortening of time to peak tension (TPT) at 20,30 minutes of reperfusion and HRT at 10, 20, 30 minutes of reperfusion as well as significant increase of myocardial flow rate (MFR/100 mg LV) at 20, 30 minutes of reperfusion. Biochemical analysis revealed significant elevation of tissue nitrate and plasma adiponectin in CR/S compared to S rats. Histopathological examination of the hearts of S rats showed large areas of leucocytic infiltration, marked vacuolation, undergoing apoptosis with small deeply stained nuclei and widely dilated and engorged blood vessels indicating injury of myocardium. On the other hand hearts of CR/S rats revealed apparently normal cardiac muscle fibers with small area of leucocytic infiltration.
In conclusion, short term caloric restriction improved tolerance of the heart to global ischemic reperfusion injury in stress-subjected rats.
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Key words: caloric restriction, ischemic reperfusion injury, cardioprotection ,immobilization stress, tissue nitrate, adiponectin.

Introduction

Stress is defined as an adaptive physiological response to disruption in homeostatic mechanisms *(Zhao et al., 2007)*. Moderate stress load can provoke protection, though overload may contribute to heart disease, high cholesterol, and high blood pressure *(Zhao et al., 2007)*.

An important relationship between stress and the heart has been for centuries the subject of debate *(Khanna et al., 2006)*.The effect of stress on rhythm of heart, myocardial blood flow, and hypertension has been extensively investigated *(Sawai et al., 2007)*. The response of the cardiovascular system to stress has been attributed to increased catecholamine secretion and enhanced platelet aggregation and when exaggerated, these bad effects can contribute to the pathogenesis of ischemic heart disease *(Hjemdahl et al., 1991)*.Many epidemiological studies have linked chronic stressors such as social isolation, depression, and self-reported stress to increased morbidity and mortality from ischemic heart disease *(Ketterer ,1993*; *Tennant et al., 1994 and Krantz et al., 1996*).

Caloric restriction has been widely investigated to increase life span in several species ranging from yeast to mammals *(Nisoli et al., 2005)*, also it was reported to ameliorate cardiac effects of ischemic reperfusion in middle aged and aged rats *(Abete et al., 2002 and Long et al., 2002*).. Caloric restriction causes mild stress similar to the preconditioning, in which a mild stress enhances the tolerance of the organ to severe stress (Stein *et al.*, 2004).

The present study was planned to find out if short term caloric restriction in adult rats subjected to stress could alleviate cardiac ischemic reperfusion injury.

Materials and Methods

Experimental animals:

This study was carried out on 32 adult male albino rats weighing 160-200 gm at the start of the experiment. Rats were purchased from Ophthalmic Diseases Research Institute (Giza) and housed 3/cage in plastic cages and maintained in Physiology Department animal house, Faculty of Medicine, Ain Shams University under standard conditions of boarding, at room temperature. Regular meals were introduced daily at 8 a.m. Control rats were fed *ad libitum* water and the standard rat chow diet (AIN-93 M diet formulated for adult rodents) prepared according to the National Research Council (NRC) 1978 and Reeves *et al.,* (1993).This formula was analyzed in the National Nutrition Institute (NNI) according to Official Methods of Analysis of Aoac International 2003 and was found to provide 418.98 C/100 g diet.

Experimental protocol:

Rats included in the present study were 32 rats. All animals received standard rat chow ad libitum in the first 3 weeks to calculate average daily food intake, on the fourth week; rats were allocated into the following 3 groups:

Control group (n= 10): C rats fed ad libitum standard chow diet.

Stress group (n= 11): S rats fed ad libitum the standard chow diet and subjected to immobilization stress for 4 weeks.

Caloric restriction/stress group (n= 11): CR/S rats subjected to 35% caloric restriction and immobilization stress for 4 weeks.

Immobilization stress: rats were subjected to immobilization stress in the prone position at room temperature (20-24^{O}C), 1 hour/day, 6 days/week for 4 weeks in tight animal restraining cages (Curtin Mathesson Scientific, regular size) according to *Scheuer and Mifflin, (1998).*

***Caloric restriction regimen*:** 35% caloric restriction was carried out by serving CR/S rats 65% of the average daily ad libitum food intake calculated before onset of the study according to *Shinmura et al. (2005).*

Experimental procedures:

At the end of the experimental period, all rats were fasted overnight, weighed and injected intraperitoneally (i.p) with 1000 IU heparin sodium (Nile CO), half an hour later, the rats were anaesthetized with intraperitoneal thiopental sodium (40 mg/Kg). Height (from the tip of the nose to the anus) was measured to the anus to calculate body mass index (BMI) according to the following equation BMI= Body weight (Kg)/ length (m^2) *(Guyton & Hall 2006)*and ECG was recorded for each rat, a midline abdominal incision was made, then the abdominal aorta was exposed and blood samples were collected in plastic tubes, centrifuged at 4000 r.p.m. for 15 minutes for separation of plasma and were stored at – 80^{o}C for biochemical study after one week.

ECG recording:

Needle electrodes were placed beneath the skin of the 4 limbs of the animal near the paws, and connected through an ECG coupler to a 2 channel oscillograph (Cardimax FX 121, Fukuda Denshi Co, LTD). The electrocardiographic tracing was recorded from lead II with paper speed of 25 mm/sec, heart rate (HR), P-R interval, QRS duration, QT interval, Q wave voltage, R wave voltage and ST segment deviation were measured. The heart rate was calculated using the following equation:

$$HR = \frac{7500}{\text{Distance in mm between 6 successive peaks of R waves}}$$

Biochemical measurements:

a- Plasma adiponectin byAvibion Human adiponectin (Acrp30) ELISA Kit, according to the method described by *Kissebah et al. (2000).*

b-Tissue nitrate, according to the coloremetric method described by *Bories and Bories, (1995).*

Heart Perfusion:

Thoracic cavity was opened, the heart was excised and immediately placed in ice cold -modified Krebs-Henseleit bicarbonate buffer solution for fast cardioplegia, and the aorta was cannulated and a retrograde perfusion with Krebs-Henseleit bicarbonate buffer (pH: 7.4), gassed with 95% O_2 and 5% CO_2 as previously described by Langendorff technique modified by *Ayobe and Tarazi,(1983).* The tension developed by the heart was measured by a light weight (0-50 g.) range D1-isometric force transducer which is connected through a strain gauge coupler FC 117 to a two channel oscillograph (Washington MD2-Bioscience). One gram weight was attached to the heart apex and was left to hang freely. After 15 minutes stabilization, the baseline record was taken. Total global ischemia was induced by stopping delivery of the perfusion fluid for 30 minutes; afterwards the hearts were reperfused again for an additional 30 minutes.

Measurements:

Records of basal heart activities as well as responses to 30 minutes of reperfusion after 30 minutes of global ischemia were analyzed to calculate heart rate (HR, beats/min.), peak developed tension (PT, g/100 mg LV), time to peak tension (TPT, msec.), time to half relaxation (HRT, msec.), and myocardial flow rate (MFR, ml/100 mg/min.), were determined at 1,10,20,30 minutes of reperfusion.

Determination of cardiac weights:

Hearts were plotted by filter paper and weighed in 5 Digit-Melter balance (AK 163). Weights of the whole hearts and left ventricle and were expressed as absolute values in (mg), as well as relative values; absolute weight/ body weight ratio (mg/gm).

Histopathological examinations:

The hearts were kept in 10% formaline for histopathological examinations, dehydrated, cleared in zylol and embedded in parablast. Paraffin sections were cut serially at 6 μm thickness and stained by Hematoxylin and Eosin (Hx & E) as described by *Drury and Wallington, (1980)*.

Statistical Analysis (Armitage and Berry, 1987):

All statistical data and significance tests were performed by using SPSS (Statistical Program for Social Science) statistical package (SPSS Inc) version 8.0.1. Statistical significance was determined by one-way ANOVA (analysis of variance) for differences between means of different groups; further analysis was made by LSD (least significance difference) to find intergroupal differences; paired t test was performed to detect significance from baseline value in the same group. A probability of P< 0.05 was considered statistically significant.All results were expressed as mean ±SEM.

Results

ECG changes:

As shown in Table(1) and Fig.(1), CR/S rats exhibited significant *(P<0.05)* decrease in heart rate compared to stress rats. ST segment showed significant *(P<0.05)* elevations in S and CR/S- rats compared to control rats.

Changes in Body weight, body mass index (BMI), heart weight, heart weight/body weight ratio, left ventricular weight & left ventricular /body weight ratio:

As shown in table (2), CR/S rats demonstrated significant *(P<0.05)* decrease in final body weight, body mass index (BMI) and absolute weights of the heart and left ventricle compared to C and S rats. However, relative weights of the whole heart as well as the left ventricle were not significantly changed.

Changes in cardiac tissue nitrate:

As shown in table (3), S rats showed significant *(P<0.05)* decrease in cardiac tissue nitrate compared to C rats, while CR/S rats showed significant *(P<0.05)* increase in tissue nitrate compared to S rats.

Changes in plasma Adiponectin level:

As shown in table (3), S rats showed significant *(P<0.05)* decrease in plasma adiponectin level compared to C, while CR /S rats showed significant *(P<0.05)* increase compared to S rats.

Isolated Perfused Hearts:

I-Chronotropic activity:

Table (4) and Figure (2) revealed that baseline values of heart rate (HR) were comparable among the 3 studied groups. C rats exhibited no significant change in HR in the reperfusion period compared to basal value. S rats showed significant *(P<0.05)* slowing of HR at 1 minute of reperfusion compared to basal value. In CR /S rats significant *(P<0.05)* slowing of HR at 1 minute and significant *(P<0.05)* acceleration at 10 minutes of reperfusion were observed when compared to basal value. HR of CR/S rats was significantly *(P<0.05)* higher than S rats at 10 and 30 minutes of reperfusion.

II-Inotropic activity:

A-Peak developed tension (PT/100mg LV):

Table (5) and Figure (2) revealed that baseline values of PT/100mg LV were comparable among the 3 studied groups. C rats showed no significant change in PT/100mg LV in the reperfusion period compared to basal value. S rats exhibited significant *(P<0.05)* decrease in PT/100 mg LV at 10, 20 and 30 minutes of reperfusion compared to basal value, while CR /S rats exhibited significant *(P<0.05)* decrease in PT/100mg LV only at 20 and 30 minutes of reperfusion compared to basal value and maintained at significantly *(P<0.05)* higher values of PT/100mg LV at 1 minute compared to C group and at 10 and 20 minutes of reperfusion compared to S group.

B-Time to peak tension (TPT):

Table (6) and Figure (2) revealed that baseline values of TPT were comparable among the 3 studied groups. C rats showed no significant change in TPT in the reperfusion period compared to basal value. Both S & CR/S rat hearts exhibited significantly *(P<0.05)* prolonged TPT after 1 minute

of reperfusion compared to basal values. However, TPT of CR/S rat hearts was significantly *(P<0.05)* shorter than S rats at 20 and 30 minutes of reperfusion.

C-Half relaxation time (HRT):

Table (7) and Figure(2) revealed that baseline values of HRT were comparable among the 3 studied groups. C rats showed no significant change in HRT in the reperfusion period compared to basal value. HRT of S rat hearts was significantly*(P<0.05)* more prolonged at 1 and 30 minutes of reperfusion compared to their basal value and at 20 minutes compared to C rats. CR/S rat hearts exhibited significant *(P<0.05)* prolongation in HRT only at 1 minute of reperfusion compared to basal value and significantly *(P<0.05)* shorter HRT at 10, 20 and 30 minutes of reperfusion compared to S rats.

D-Myocardial flow rate (MFR/100mg LV /min.):

Table (8) revealed that baseline values of MFR/ 100 mg LV/min. were comparable among the 3 studied groups. Isolated hearts of C, S, and CR/S rats showed significant *(P<0.05)* decreases in MFR/LV at 10, 20 and 30 minutes of reperfusion compared to their basal values. However, CR/S rats maintained significantly *(P<0.05)* higher MFR/100 mg LV/min. values at 20 and 30 minutes of reperfusion compared to S rats.

Histopathological examination:

C rats hearts had normal cardiac myocytes (Figure, 3). Meanwhile, hearts of S rats had large areas of leucocytic infiltration marked vacuolation, undergoing apoptosis with small deeply stained nuclei and widely dilated and engorged blood vessels indicating injury of myocardium (Figure 4). On the other hand hearts of CR/S rats revealed only apparent decrease in diameter of cardiac muscle fibers, with small area of leucocytic infiltration (Figure 5).

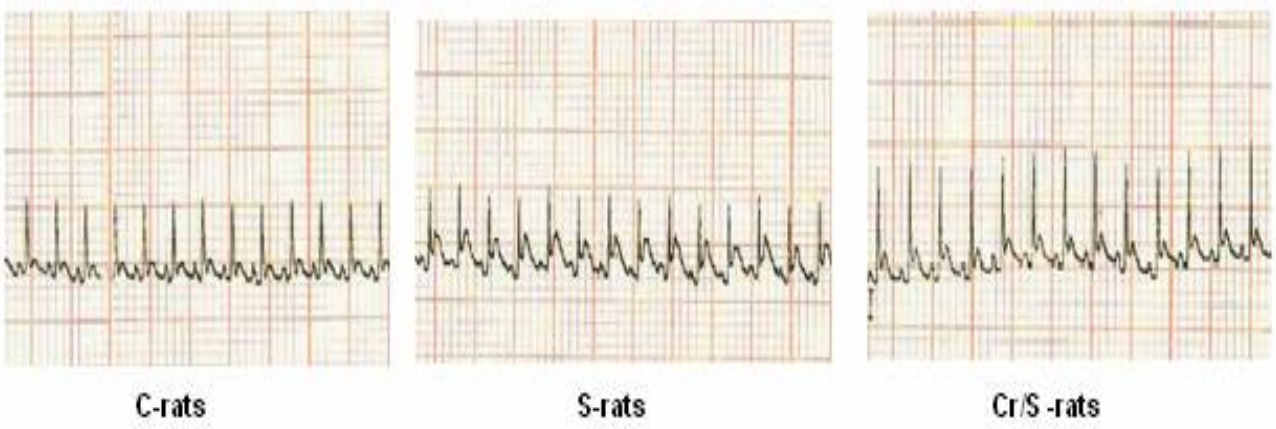

Figure (1): ECG records (lead II) of control (C), stress(S) and caloric restriction/ stress rats (CR/S) rats showing significant bradycardia in CR/S rats and significant ST segment elevation in S & CR/S rats.

Table (1): Changes in ECG parameters; heart rate (HR, beats/min), P-R interval (msec.), QRS wave (msec.), QT (msec.), Q wave (μv), R wave (μv), and ST segment elevation (μv) in control (C), stress(S) and caloric restriction /stress rats (CR/S) rats (M±SEM).

Groups	HR (beat/min.)	PR (msec.)	QRS (msec.)	QT (msec.)	Q (μv)	R (μv)	ST (μv)
C (10)	404 ± 23	44 ± 2.7	23 ± 1.5	70 ± 4.5	60 ± 6.7	640 ± 47.7	65 ± 7.6
S (11)	438 ± 14	47.3 ± 3.0	21.8 ± 1.2	63.6 ± 4.5	54.5 ± 4.5	681 ± 37.7	119 ±15.7*
CR/S (11)	384± 15**	43.6 ± 2.1	21.8± 1.2	65.5 ± 2.8	47.5 ± 2.8	727 ± 46.9	105±10.6*
P	NS	NS	NS	NS	NS	NS	< 0.05

*: Significance by LSD at P< 0.05 from control group.
**: Significance by LSD at P< 0.05 from stress group
P: Significance by one way ANOVA among the three studied groups
NS: not significant. In parenthesis is the number of rats

Table (2): Changes in body weight (BW, g), body mass index (BMI, Kg/m^2), heart weight (HW, g), heart weight/ body weight (HW/BW, mg/g), left ventricular weight (LV, g), left ventricular/ body weight (LV/BW, mg/g) in control (C), stress(S) and caloric restriction stress rats (CR/S) rats(M±SEM).

Groups	BW (gm)	BMI (Kg/m2)	HW (gm)	HW/BW (mg/gm)	LV (gm)	LV/BW (mg/gm)
C (10)	216 ± 4.8	5.1 ± 0.05	0.75 ± 0.02	3.5 ± 0.1	0.40 ± 0.01	1.85 ± 0.06
S (11)	209.8 ± 2.5	5.2 ± 0.2	0.75 ± 0.02	3.6 ± 0.08	0.39 ± 0.01	1.87 ± 0.06
CR/S (11)	162.3 ± 6.0*, **	4.1 ± 0.1*, **	0.60 ± 0.009*,**	3.7 ± 0.13	0.31 ± 0.01*,**	1.97 ± 0.09
P	< 0.001	< 0.001	< 0.001	NS	< 0.001	NS

*: Significance by LSD at P< 0.05 from control group.
**: Significance by LSD at P< 0.05 from stress group
P: Significance by one way ANOVA among the three studied groups
NS: not significant. In parenthesis is the number of rats

Table (3). Changes in tissue nitrate level (umollgm) and plasma adiponectin (ng/ml)in control (C), stress(S) and caloric restriction/ stress rats (CR/S) rats (M±SEM).

	Tissue nitrate (umollgm)	**Plasma adiponectin** (ng/ml)
C (10)	0.42±.037	2.7±0.12
S (11)	0.21±.019*	2.2±0.09*
CR/S(11)	0.40±.026**	2.9±0.16**
P	<0.001	<0.05

*: Significance by LSD at P< 0.05 from control group.
**: Significance by LSD at P< 0.05 from stress group
P: Significance by one way ANOVA among the three studied groups
NS: not significant. In parenthesis is the number of rats

Table (4): Preischemic (basal) and postischemic heart rate (HR, beats/min.) of hearts isolated from control (C), stress(S) and caloric restriction/ stress rats (CR/S) rats(M±SEM).

	Basal	**Postischemic reperfusion responses**			
		1 min.	10 min.	20 min.	30 min.
C (10)	164 ± 8.9	160 ± 12.8	187 ± 13.2	170 ± 15.7	170 ± 15.3
S (11)	155 ± 10.1	130 ± 12.8***	172 ± 13.7	160 ± 14.7	136 ± 14.3
CR /S (11)	163 ± 11.2	139 ± 12.8***	219 ±13.4**,***	190 ± 10.1	186 ± 14.4**
P	NS	N.S.	< 0.05	N.S.	NS

*: Significance by LSD at P<0.05 from the control group.
**: Significance by LSD at P<0.05 from the stress group.
***: Significance at 1, 10, 20, 30 minutes of reperfusion relative to baseline values
P: Significance by one way ANOVA among the three studied groups
NS: not significant In parenthesis is the number of rats

Table (5): Preischemic (basal) and postischemic values of peak developed tension (PT ,g/100mg LV) of the hearts isolated from control (C), stress(S) and caloric restriction/ stress rats (CR/S) rats(M±SEM).

	Basal	Postischemic reperfusion responses			
		1 min.	10 min.	20 min.	30 min.
C (10)	3.29 ± 0.16	3.1± 0.14	3.2 ± 0.17	3.2 ± 0.15	3.2 ± 0.16
S (11)	3.40 ± 0.10	3.3 ± 0.09	3.1 ± 0.09***	3.0 ± 0.09***	3.01 ± 0.11***
CR/S (11)	3.64 ± 0.13	3.5± 0.13*	3.6 ± 0.15**	3.5 ± 0.14**,***	3.4 ± 0.13***
P	NS	<0.05	NS	< 0.05	NS

*: Significance by LSD at P< 0.05 from the control group.
**: Significance by LSD at P< 0.05 from the stress group.
***: Significance at 1, 10, 20, 30 minutes of reperfusion relative to baseline values
P: Significance by one way ANOVA among the three studied groups
NS: not significant In parenthesis is the number of rats

Table (6): Preischemic (basal) and postischemic time to peak tension (TPT, msec.) of the hearts isolated from control (C), stress(S) and caloric restriction/ stress rats (CR/S) rats(M±SEM).

	Basal	Postischemic reperfusion responses			
		1 min.	10 min.	20 min.	30 min.
C(10)	158 ± 13.2	152 ± 7.9	138 ± 8.3	133 ± 7.9	148 ± 5.5
S (11)	135 ± 12.5	168 ± 10.7***	145 ± 9.3	156 ± 11.7	166 ± 13.7
CR/S (11)	141 ± 5.9	177 ± 14.6***	124 ± 7.7	126 ± 9.0**	127 ± 9.2**
P	NS	N.S.	NS	NS	< 0.05

*: Significance by LSD at P< 0.05 from the control group.
**: Significance by LSD at P< 0.05 from the stress group.
***: Significance at 1, 10, 20, 30 minutes of reperfusion relative to baseline values
P: Significance by one way ANOVA among the three studied groups
NS: not significant In parenthesis is the number of rats

Table (7): Preischemic (basal) and postischemic half relaxation time (HRT, msec.) of hearts isolated from control (C), stress(S) and caloric restriction/ stress rats (CR/S) rats(M±SEM).

	Basal	Postischemic reperfusion responses			
		1 min.	10 min.	20 min.	30 min.
C(10)	40 ± 3.7	42 ± 6.8	40 ± 5.4	36 ± 4.3	38 ± 4.9
S (11)	35.5 ± 5.2	52 ± 6.7***	51 ± 7.1	55 ± 8.0*	51 ± 6.7***
CR/S (11)	36.4 ± 5.1	55 ± 5.9***	24 ± 4.3**	25 ± 3.9**	31 ± 3.8**
P	NS	N.S.	< 0.05	< 0.05	< 0.05

*: Significance by LSD at P< 0.05 from the control group.
**: Significance by LSD at P< 0.05 from the stress group.
***: Significance at 1, 10, 20, 30 minutes of reperfusion relative to baseline values
P:Significance by one way ANOVA among the three studied groups
NS : not significant In parenthesis is the number of rats

Table (8): Preischemic (basal) and postischemic myocardial flow rate (MFR, ml/100mg LV/min.)of hearts isolated from control (C), stress(S) and caloric restriction stress rats (CR/S) rats(M±SEM).

	Basal	Postischemic reperfusion responses			
		1 min.	10 min.	20 min.	30 min.
C (10)	2.42 ± 0.09	2.18 ± 0.13	2.13 ± 0.15***	1.95 ± 0.15***	1.8± 0.11***
S (11)	2.32± 0.14	2.07 ± 0.21	1.8 ± 0.19***	1.5 ± 0.16***	1.47 ± 0.14***
CR/S(11)	2.40 ± 0.12	2.28 ± 0.16	2.07 ± 0.11***	1.98 ± 0.12**,***	1.88 ± 0.10**,***
P	NS	N.S.	NS	NS	<0.05

*: Significance by LSD at P< 0.05 from the control group.
**: Significance by LSD at P< 0.05 from the stress group.
***: Significance at 1, 10, 20, 30 minutes of reperfusion relative to baseline values
P:Significance by one way ANOVA among the three studied groups
NS : not significant In parenthesis is the number of rats

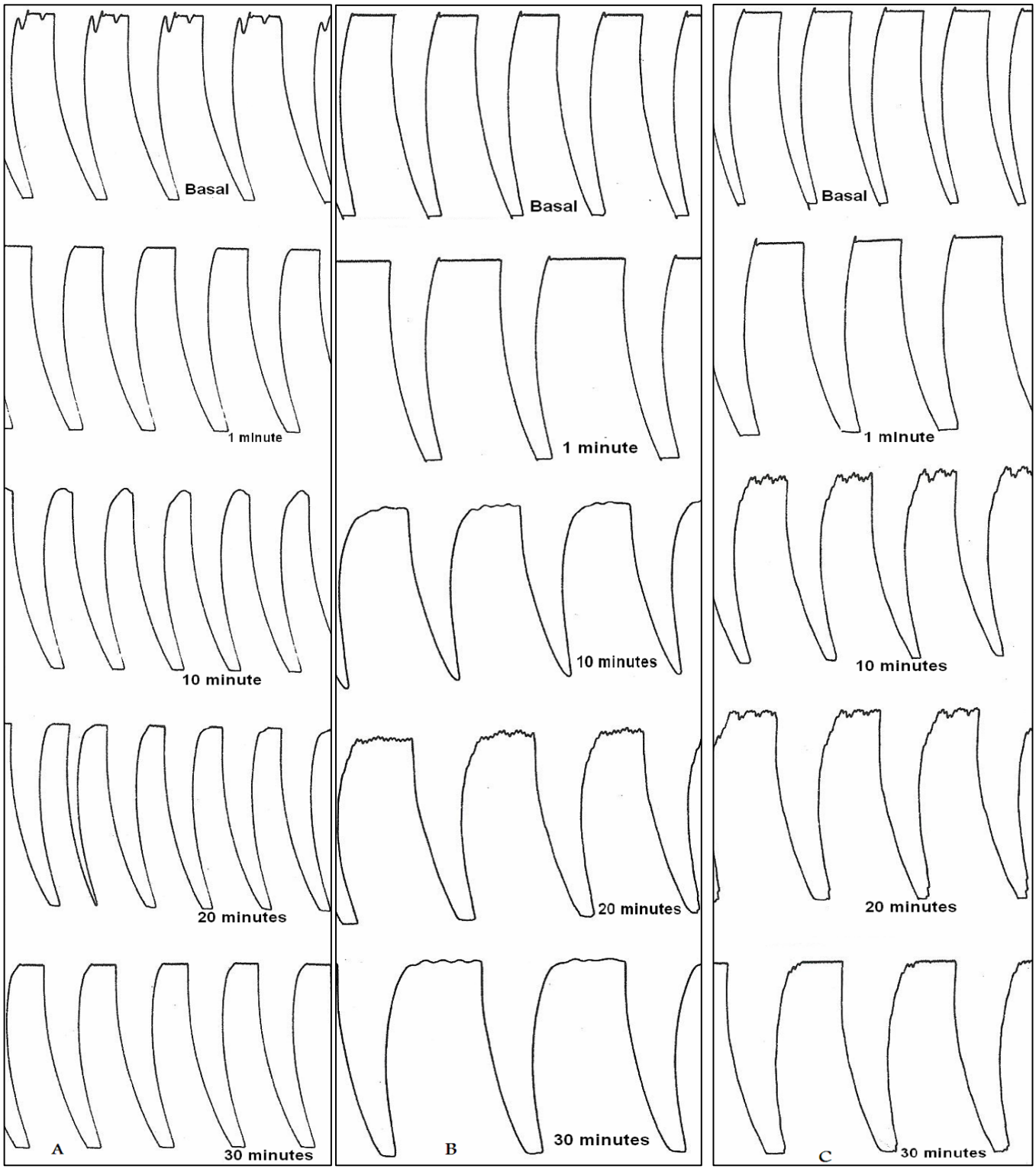

Figure (2);In vitro recording of isolated perfused hearts of C group(panel A),S group (panel B) and CR/S group (panel C) showing basal activity as well as after global ischemia and at1,10,20 ,30 minutes after reperfusion.

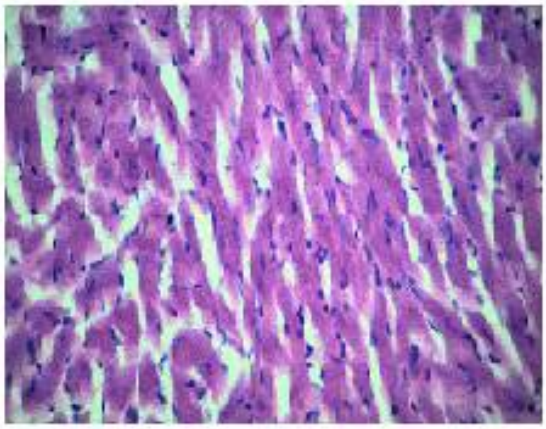

Figure (3). H&E stained histological heart sections of C rats revealing normal cardiac muscle fiber (magnification: 250x).

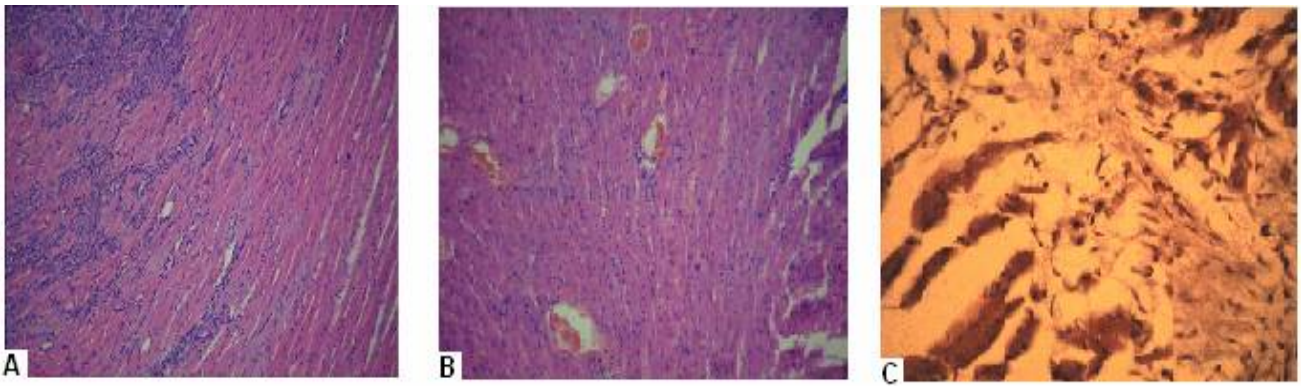

Figure (4). H&E stained histological heart sections of S rats revealing large areas of leukocyte infiltration(A), marked vacuolation ,undergoing apoptosis with small deeply stained nuclei and widely dilated and engorged blood vessels(B& C), indicating severe injury of myocardium (magnification: A & B250x & C- 640x).

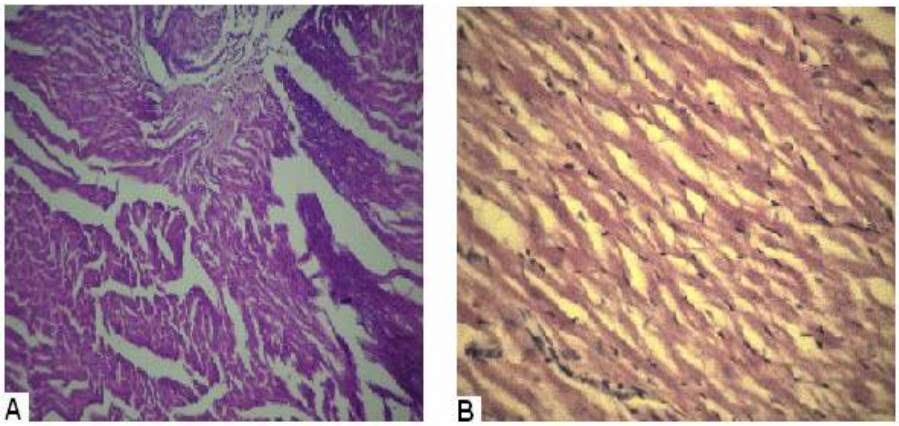

Figure (5): H&E stained histological heart sections of CR/S rats revealing an almost normal cardiac muscle fiber with small area of leukocyte infiltration (magnification: A 250x & B- 640x).

4. Discussion

Exposure to stress (.e.g. pain, inflammation and emotional stress) is inevitable in the course of life of any individual. Chronic stress in humans had been correlated with increased risk for ischemic heart disease *(Scheuer et al., 1998)* and those with ischemic heart disease are at higher risk to morbidity and mortality on exposure to stress *(Schwartz et al., 2010)*, so it was of value to look for a cardioprotective approach for stressed patients who might have a concurrent ischemic cardiac insult. Immobilization stress employed in the present study was estimated to be of mild intensity due to lack of significant change in heart rate, absolute and relative cardiac weights of S rats compared to unstressed controls. S rat hearts showed ischemic changes as evidenced by the significant elevation of ST segment. Moreover, myocardial flow rate at 30 minutes of reperfusion in S rats showed the lowest value compared to basal value indicating an exaggerated vasoconstrictor response of their coronary blood vessels to reperfusion injury. The significant decrease in cardiac tissue nitrate in S rats to 50% of control value might have contributed to these ischemic changes. It could be suggested that the physiological decrease in NO synthesis by coronary vascular endothelium in response to decreased shear stress *(Lam et al., 2006)* might have contributed to coronary vasoconstriction and decreased coronary blood flow during reperfusion period and that this decrease in NO production was more aggravated in S rat hearts leading to substantial decrease in myocardial flow rate even when reperfusion of the heart was resumed. Persistence of ST segment elevation in CR/S rats despite the significant bradycardia indicates that stress induced –cardiac risk was not completely abolished by short term caloric restriction. The significant bradycardia observed in CR/S compared to S rats disappeared during the *in vitro* study of isolated hearts of CR/S rats indicating that the underlying mechanism was either neural or humoral. Caloric restriction was reported to improve sympathetic /parasympathetic nervous system balance in favor of parasympathetic nervous system which would enhance cardioprotection *(de Jong et al., 2010).*

Isolated perfused heart study revealed that ischemic reperfusion induced evident decline of cardiac performance in S and CR/S rats compared to basal recordings which was consistent with the findings of *Flaherty and Weisfeldt, (1988)* and *Valen et al. (1993).* CR/S rat hearts showed significant increase in heart rate after 10 minutes of reperfusion compared to S rats which might reflect increased Na^+ and Ca^{++} ion influx in pace maker tissue leading to enhanced automaticity and inotropy which might have contributed to maintenance of PT/100mg LV in the first 10 minutes of reperfusion. The coincident significant shortening of HRT in CR/S compared to S rats reflects better ability of cardiac myocytes to decrease intracellular Ca^{++} concentration by Ca^{++} ATP_{ase} and Na^+ /Ca^{++} exchanger which might be due to better energy handling by cardiac myocytes. This improvement was shown to persist till 30 minutes of reperfusion indicating that caloric restriction had triggered adaptive mechanisms possibly involving coronary blood vessels as evidenced by the less deterioration of MFR/100mg LV compared to S rats leading eventually to alleviation of ischemic reperfusion injury and better cardiaoprotection.

Various mechanisms were proposed to explain ischemic reperfusion injury like increased cytosolic Ca^{++} due to Ca^{++} release from mitochondria, sarcoplasmic reticulum and nuclear organelles *(Boys et al., 2010)*, altered function of Na^+/ Ca^{++} exchanger *(Toth et al., 2009)*, endothelial injury and endogenous endothelin-1 release*(Han et al., 1995)*, burst of reactive oxygen species and mitogen activated protein kinase (MAPK)- mediated Na^+/H^+ exchanger phosphorylation and reactivation *(Garciarena et al., 2011).* Exposure to stress was reported to aggravate ischemic reperfusion injury by various mediators like immediate early genes (IEG), calcium leakage via ryanodine receptor 2, and catecholamine induced increase in free radicals *(Uevama et al., 2003 and Wittsteinet al,. 2005).*

Histopathological examination showed that hearts of S rats had inflammatory changes in the form of leukocyte infiltration, vascular congestion, apoptosis and myocyte injury which were in agreement with the findings of *Zhao et al. (2007)*.A possible role of inflammatory mediators and lymphokines in increasing susceptibility of S rat hearts to ischemic reperfusion injury cannot be excluded. *Cao et al. (2003)* found that coexistence of IL-2 during anoxia aggravates the effect of reoxygenation on the cell contraction and calcium homeostasis in the isolated rat ventricular myocytes, in which the mitochondrial lipid peroxidation induced by IL-2 was involved. *Lochner et al. (2009)* suggested that injury by necrosis and apoptosis share activation of p38MAPK as a common signal transduction pathway of ischemic reperfusion injury. Restoration of almost normal cardiac structure and amelioration of leukocyte infiltration by short term caloric restriction and consequently reduction of oxidative stress and apoptosis provide plausible explanation for improvement of systolic and diastolic functions after global ischemia and reperfusion in CR/S rats, a finding previously reported by *Sinclair (2005).*

The restoration of normal levels of plasma adiponecctin and cardiac nitrate levels with caloric restriction during stress came in accordance with the study of Zhu *et al.* (2004) and could be implicated in cardioprotection. Adiponectin- a circulating adipocyte –derived hormone was found to exert cardioprotection via its insulin sensitizing effect as well as antiatherogenic properties *(Han et al., 2007)*. It was reported that adiponectin improved recovery of left ventricular function after ischemia/reperfusion and limited infarct size in mice via increasing the phosphorylated form of AMP-activated protein kinase and acetyl-CoA carboxylase *(Shimura et al., 2007)* in addition to its ability to suppress inflammation ,apoptosis and oxidative stress (*Kondo et al., 2010)*. In a recent study by *Wang et al. (2010)*, biologically active adiponectin was found to be secreted by cardiac myocytes to exert protection against ischemic reperfusion injury by autocrine and paracrine fashion on adiponectin receptors (APN_1). We may suggest that short term caloric restriction adopted in the present study might have enhanced adiponectin expression in cardiac myocytes by a mechanism that needs to be resolved.

Absence of significant changes in absolute and relative cardiac weights as well as heart rate in S rats reflects unchanged arterial blood pressure. This was in accordance with the findings of *Puzserova et al. (2010)* who reported that 4 weeks mild stress had no effect on arterial blood pressure, heart rate and relative left ventricular mass due to elevated NO production by vascular endothelium in an important way of adaptation for prevention of normotensive rats from development of stress induced-hypertention. However, in our study, cardiac tissue nitrate level in S rats was found to be 50% decreased than control values which indicates considerable decrease in NO synthesis *(Lundberg et al.,2008)*. It could be suggested that although short term mild stress might have triggered an adaptational increase in vascular NO production, yet it exerted an opposite unfavorable effect on cardiac NO synthesis leaving the heart more susceptible to ischemic reperfusion injury. The underlying mechanism of stress-induced cardiac NO deficiency in S rats is difficult to speculate but it could be due to stress –induced oxidative stress which was reported by *Nishio et al. (2007)* to occur as early as 7 days of social isolation stress in mice. Oxidative state results in uncoupling of endothelial nitric oxide synthase (eNOS) resulting in production of superoxide by the eNOS monomer whereas the dimer, in the absence of oxidative stress produces mainly NO *(Landmesser et al., 2003)*. It was of interest to observe that absolute weights of the whole heart and left ventricle were significantly decreased in CR/S rats compared not only to S but also to C rats which might indicate considerable decrease in arterial blood pressure which together with the significant decrease in heart rate *in vivo* would decrease myocardial oxygen consumption. The observed increase in cardiac tissue nitrate level in CR/S than S rats could be expected to improve coronary blood flow in these rat hearts although the ST segment deviation was not completely abolished. Thus, it was not unexpected to observe that CR/S rat hearts could maintain a higher PT/100mgLV, MFR/100mgLV and shorter HRT during the reperfusion period compared to their stressed counterparts. NO homeostasis in the heart is determined by the cross talk between neuronal/endothelial nitric oxide (NO) synthase (n/eNOS) ,inducible nitric oxide synthase (iNOS) as well as the non enzymatic NO production by nitrite and nitrate which may end by either beneficial or toxic effects *(Lundberg et al.,2008 and Darra et al.,2010)*. However, it is generally accepted that increased nitric oxide production exerts vasculoprotective and cardioprotective effects *(Darra et al., 2010)*. Short term caloric restriction in stressed rats returned cardiac nitrate level to normal values which contributed to better tolerance to ischemic/reperfusion injury as recently confirmed by *Shinmura (2011)*. Little information is known about myocardial expression of silent information regulator 1 ($SIRT_1$) but several studies have indicated that $SIRT_1$ activates NOS and induces eNOS protein in endothelial cells *(Mattagajasingh et al., 2007 and Ota et al., 2007)*. *Mattagojasingh et al. (2007)* also reported that $SIRT_1$ and eNOS co-localize in endothelial cells and that $SIRT_1$ deacetylates eNOS, and thus increases endothelial NO. $SIRT_1$ is distributed in all mammalian tissues and evidence suggests that $SIRT_1$ regulates energy metabolism, endocrinal signals and some stress responses *(Bordone and Guarente, 2005)*. Animals and humans subjected to caloric restriction have high levels of $SIRT_1$ protein in brain, kidneys, muscles and liver *(Cohen et al., 2004)*, thus increasing the resistance of cells to apoptosis. Moreover, *Alcendor et al. (2007)* reported overexpression of $SIRT_1$ in starved animals which prevented apoptosis in cardiac myocytes.

In conclusion, this study demonstrated that short term mild stress decreased cardiac tissue nitrate with increased cardiac vulnerability to ischemic reperfusion injury. Nutritional approach by short term mild caloric restriction improve cardiac structural and functional abnormalities induced by stress. Improved cardiac tolerance to ischemic reperfusion injury with caloric restriction could be helpful in improving the outcome of ischemic cardiac injury in stressed patients.

Abbreviations: CR (caloric restriction), LV (left ventricle), MAPK (mitogen activated protein kinase), IEG (immediate early genes), (APN_1) adiponectin receptors, NO (nitric oxide). eNOS (endothelial nitric oxide synthase).$SIRT_1$ (silent information regulator 1).

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