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A Pilot Study to Assess Adenosine 5'-triphosphate Metabolism in Red Blood Cells as a Drug Target for Potential Cardiovascular Protection

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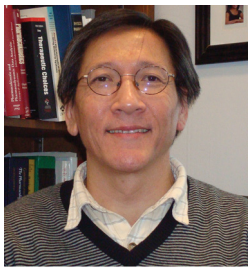

P.K.F. Yeung

Abstract: *Objective*: To study the effect of exercise preconditioning on adenosine 5'triphosphate (ATP) metabolism in red blood cells and cardiovascular protection against injury induced by isoproterenol *in vivo*.

Methods: Male Sprague Dawley rats (SDR) were each exercised on a treadmill for 15 minutes at 10 m/min and 10% grade (n = 7) (LowEx), or 14 m/min and 22% grade (n = 8) (VigEx). Two hours after the exercise, each rat received a single dose of isoproterenol (30 mg/kg) by subcutaneous (sc) injection. Two separate groups of SDR were used as control: One received no exercise (n = 10) (NoEx) and the other received no exercise and no isoproterenol (n = 11) (NoIso). Serial blood samples were collected over 5 hours for measurement of ATP and its catabolites by a validated HPLC. Hemodynamic recording was collected continuously for the duration of the experiment. Data were analysed using ANOVA and *t*-tests and difference considered significant at $p < 0.05$.

Results: Exercise pre-conditioning (both LowEx and VigEx) reduced mortality after isoproterenol from 50% to < 30% ($p > 0.05$). It attenuated the rebound in blood pressure significantly ($p < 0.05$ between NoEx vs VigEx), attenuated the increase of RBC adenosine 5'-monophosphate (AMP) concentrations induced by isoproterenol, and also decreased the breakdown of ATP to AMP in the RBC ($p < 0.05$ vs NoEx).

Conclusion: Exercise pre-conditioning decreased the blood pressure rebound and also breakdown of ATP in RBC after isoproterenol which may be exploited further as a drug target for cardiovascular protection and prevention.

Keywords: ATP, cardiovascular protection, exercise preconditioning, hemodynamic, RBC, target, toxicity, rats.

INTRODUCTION

The importance of adenosine and ATP in regulating many biological functions has long been recognized, especially for their effects on the cardiovascular system [1-5]. It is known that adenosine and ATP are key factors in the regulation of coronary blood flow [6], inhibition of platelet aggregation [7], protection of myocardium [8], neuro- and immunomodulation [4, 9-11], tissue necrosis modulation [2], ischemic preconditioning [5, 12-14], energy metabolism [3, 15], and perhaps other functions (e.g. pain mediation) as well which maintain cardiovascular homeostasis in the body. It has been shown that patients with effort angina and essential hypertension have altered adenosine metabolism compared to healthy individuals [16-18]. Plasma concentrations of adenosine have been shown to increase in patients with congestive heart failure (CHF) [19], which may represent a compensatory physiologic response to heart failure [20]. Adenosine is known also to interact with the renin angiotensin system (RAS) to maintain a homeostatic balance between blood pressure, vascular resistance and blood volume in hypertension and endothelial dysfunction [21-23]. Thus it has been postulated that adenosine and ATP may be used as sensitive biomarkers to quantify myocardial and endothelial ischemia [24-26], and for monitoring therapeutic effects of anti-ischemia drugs [27-29].

Exercise is known to have cardioprotective effect *via* a mechanism similar to that of ischemia preconditioning which affects energy metabolism in the body [30, 31]. Previous studies have shown that exercise preconditioning offered cardioprotective effect against injury induced by isoproterenol in rats [32, 33], and cardiotoxicity induced by doxorubicin [34]. We have recently shown that exercise improved cardiovascular hemodynamic profiles and increased red blood cell (RBC) concentrations of ATP in both normotensive Sprague Dawley rat (SDR) and spontaneously hypertensive rats (SHR) although the post exercise effects were more profound in the SHR [35, 36]. The increase of ATP concentrations in the RBC may be a key contributing factor for the acute effects of post exercise preconditioning and in the long term effects of program of regular exercise in cardiovascular protection. The current study investigates further the potential cardiovascular

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protective effect of exercise preconditioning against injury induced by isoproterenol using a previously described freely moving rat model *in vivo* [37]; and to determine the effect of exercise pre-conditioning on ATP metabolism in the RBC and its potential as a drug target for cardiovascular protection or prevention.

MATERIALS AND METHODS

Authentic standards of adenine nucleotides including ATP, adenosine 5'diphosphate (ADP) and adenosine 5' monophosphate (AMP) and other purine nucleotides were purchased from Sigma-Aldrich Chem Co. (St. Louis, MO, USA). Solvents were HPLC grade, and all other chemicals were reagent grade (Fisher Scientific, ON, Canada).

The protocol followed the Canadian Council on Animal Care guidelines and was approved by the Dalhousie University Committee on Laboratory Animals (UCLA 12-008). Male SDR weighing between 250 - 300 g with an indwelling carotid artery catheter were purchased directly from Charles River Laboratories (Wilmington, MA, USA). Each rat was acclimatized for at least 48 hours in the Carleton Animal Care Centre before experiment. The exercise test was performed on a research exercise treadmill (Columbus Instruments International Corporation, Columbus, Ohio, U.S.A.) (Fig. **1**). After 2 brief sessions of 3 – 5 minutes (min) each of training to acclimatize the rat with the treadmill on the day before the study, each rat was exercised on the treadmill for 15 min at 10 m/min and 10% grade (n = 7) (LowEx), or 15 min at 14 m/min and 22% grade (n = 8) (VigEx). Another group of rats without exercise was used as control (NoEx, n = 10) as described previously [35, 36]. Two hours after the exercise, each rat received a single dose of isoproterenol (30 mg/kg) by subcutaneous (sc) injection. A second control group of rats which received no exercise and no isoproterenol (only normal saline) (NoIso, n = 11) was used for further comparison. All the rats were housed in the same location and a similar number of blood samples (n = 10) was collected from each rat. Blood samples (0.3 mL each) were collected *via* the indwelling catheter using a "Stopping Solution" from each rat at 1, 1.05, 1.25, 2 (immediately before isoproterenol), 2.2, 2.5, 3, 4, 5 and 6 hr after the exercise for measurement of adenine nucleotide concentrations (i.e. ATP, ADP and AMP) in the RBC (Fig. **2**). Each blood sample withdrawn was replenished with the same volume of saline to avoid volume depletion. Hemodynamic recording was interrupted briefly during each blood sample collection, after which the catheter was flushed with heparin saline (10 IU/mL) to maintain patency and the quality of the tracing recorded. At the end of the experiment, the rat was euthanized by cardiac puncture under anaesthesia with isoflurane. The total length of the experiment was about 6 hrs from the start of the final exercise. Hemodynamic variables (SBP, DBP and HR) were continuously recorded (except interrupted briefly during each blood sample collection) *via* the intra-vascular catheter using a TruWave® disposable pressure transducer (Model PX601, Edwards Lifesciences Canada, Inc., Mississauga, ON, Canada) coupled to a Siemens hemodynamic monitor (Sirecust® 400) and chart recorder (Siredoc®) (Erlangen, FRG) as previously described [35, 36]. The hemodynamic data presented were averages of 10 – 15 seconds (sec) recording. The RBC samples collected were processed and lysed immediately using an ice cold 10% trichloroacetic acid. The lysate samples were stored at -80°C, and concentrations of ATP, ADP and AMP in the RBC determined by a validated HPLC assay [38]. Maximum concentrations of adenine nucleotides in the RBC (Cmax) were taken from the observed value, and area under the RBC concentration – time curve (AUC) was calculated using the trapezoid method (Prism 5, GraphPad Software Inc., La Jolla, CA, USA). Hemodynamic and biomarker variables were compared between groups using ANOVA followed by multiple comparison, student's unpaired and paired t-tests and considered significant when $p < 0.05$. In addition, correlation and regression analyses of the data from RBC concentration of the adenine nucleotides were assessed using Pearson Correlations (r) and linear regressions (β), respectively (Minitab® Inc., Release 17, State College, PA, USA), and differences between treatment groups considered significance at $p < 0.05$.

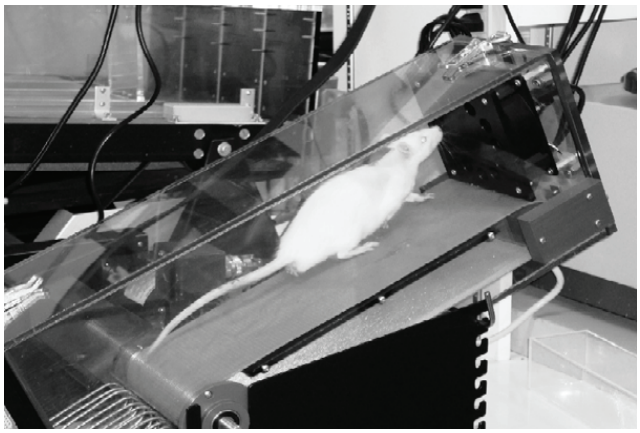

Fig. (1). Treadmill exercise rat model at a 22% incline.

RESULTS

Before Isoproterenol Injection

There were no significant differences in the baseline hemodynamic parameters (SBP, DBP and HR) measured at 1 hr after the final exercise between the four studied groups (Table **1**). The RBC concentrations of ATP were significantly higher ($p < 0.05$) in the LowEx group compared to VigEx group or the controls (NoEx and NoIso). There were no significant differences in the ADP or AMP concentrations between the study groups before isoproterenol injection (Fig. **3**).

After Isoproternol Injection

There was no fatality in the rats which did not receive isoproterenol (NoIso). On the other hand, 5 of the 10 rats died from the injury (50% mortality) within 4 hrs after the isoproterenol (30 mg/kg) injection without exercise pre-conditioning (NoEx) ($p < 0.05$). The mortality was reduced to < 30% in the exercise groups (2 of 7 and 2 of 8 died in

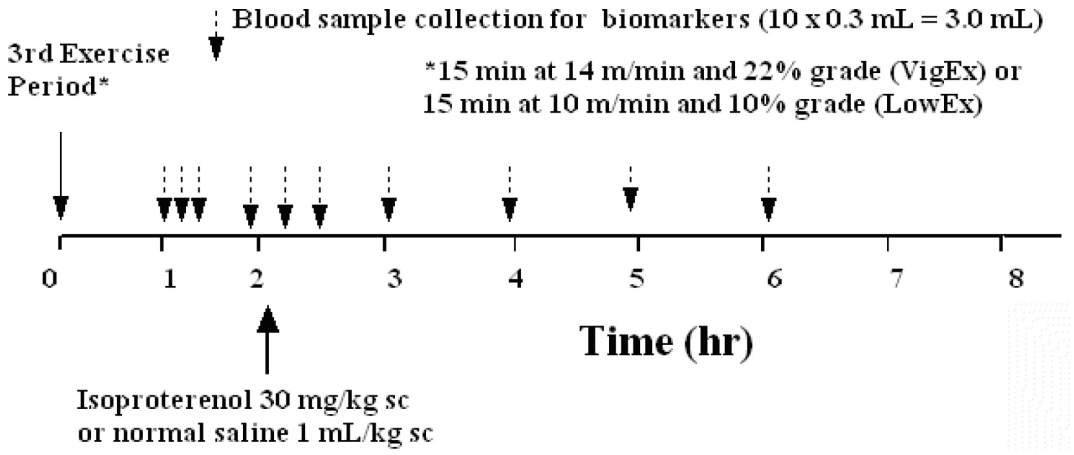

Fig. (2). Experimental design and blood sample collection scheme.

Table 1. Hemodynamic effect of exercise pre-conditioning before isoproterenol (Iso) injection in rats.

Biomarkers/Treatment	LowEx Group (n = 7)	VigEx Group (n = 8)	NoEx Group (n = 10)	NoIso Group (n = 11)
SBP (mmHg) 1 h after exercise	127 ± 7[a]	128 ± 11	127 ± 14	123 ± 11
DBP (mmHg) 1 h after exercise	96 ± 7	97 ± 13	104 ± 19	104 ± 11
HR (bpm) 1 h after exercise	382 ± 42	384 ± 38	396 ± 40	378 ± 48
Mean SBP (mmHg) before Iso injection	126 ± 10	127 ± 8	127 ± 14	123 ± 14
Mean DBP (mmHg) before Iso injection	95± 6	96 ± 10	101 ± 19	99 ± 12
Mean HR (bpm) before Iso injection	386 ± 39	386± 37	393 ± 26	377 ± 29

[a]Mean ± SD.

LowEx and VigEx, respectively). However due to the small number of rats in each group, the reduction in mortality from the exercise pre-conditioning was not statistically significant ($p > 0.05$). Immediately after isoproterenol injection (30mg/kg), SBP and DBP fell sharply in both exercise groups (LowEx and VigEx) and the NoEx group, in contrary, the heart rate (HR) increased in the study groups receiving isoproterenol ($p < 0.05$ vs before isoproterenol, paired t-test) (Table **2**). Both SBP and DBP rebounded shortly after (1 – 2 hr after isoproterenol) to near pre-isoproterenol levels in the three groups (Fig. **4**). However, the % rebound was significantly less in the VigEx than the NoEx group ($p < 0.05$). The % rebound was also blunted in the LowEx group, although the decrease did not reach statistical significance (Table **2**). HR remained elevated in all three isoproterenol groups until the end of the experiment (Fig. **4**).

There was a significant increase in the RBC concentrations of ATP in the rats without receiving isoproterenol and exercise (+0.93 ± 0.80 mM in NoIso group), and also in the rats receiving isoproterenol after an exercise preconditioning (+0.69 ± 0.60 mM and +0.50 ± 0.55 mM in LowEx and VigEx groups, respectively), but not in the rats without exercise (+ 0.52 ± 0.92 mM in NoEx group) (paired t-test, Table **3**). On the other hand, there was a significant increase in the ADP concentration in the rats receiving isoproterenol with or without exercise (LowEx, VigEx and NoEx groups), but not the rats in NoIso group (Table **3**). In contrast, the AMP concentration was significantly higher after isoproterenol injection by 0.28 ± 0.21 mM ($p < 0.05$ paired t-test) only in the NoEx group, but not in the exercise groups (LowEx and VigEx) or the control NoIso group (Table **3**). Similarly, the Cmax and AUC of AMP concentrations in the RBC were significantly greater in the NoEx group than the other groups ($p < 0.05$), particularly the VigEx group (Fig. **5**). When the ratios of the AUC of the adenine nucleotides in RBC were compared, the ratios of AMP/ATP and AMP/ADP were significantly higher in the NoEx group than all the other groups (Table **3**).

Correlation and regression analyses of the adenine nucleotide concentrations in RBC from each rat showed an inverse (-ve) relationships (both r and β) between ATP and ADP and also between ATP and AMP, but a positive (+ve) relationship between ADP and AMP in the NoEx group (Table **4**). A significant difference was found in the correlation (r) and regression coefficients (β) between the NoEx group and those from the NoIso group which did not receive isoproterenol, but there was no difference between

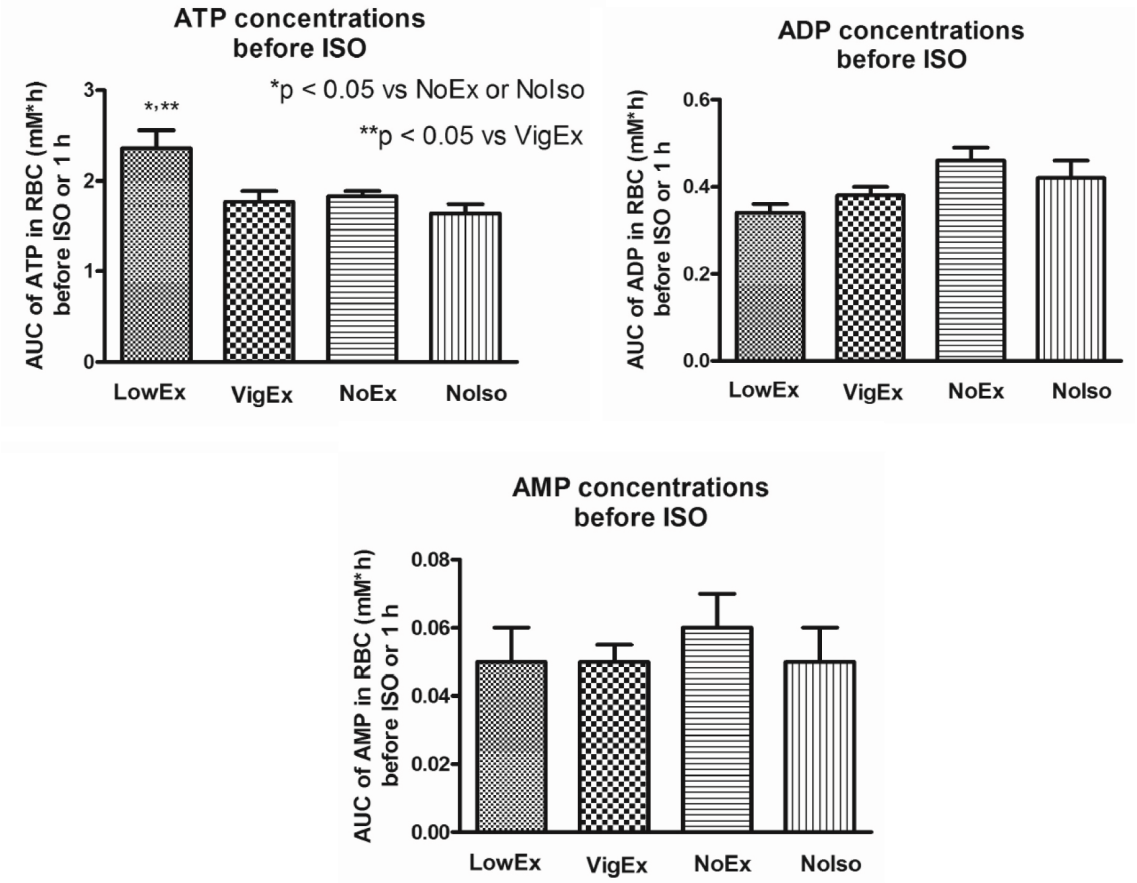

Fig. (3). Concentrations of adenine nucleotides in red blood cells (RBC) before isoproterenol injection (30 mg/kg) in rats. Each column represents mean ± SEM.

Table 2. Hemodynamic effects of exercise preconditioning after isoproterenol (Iso) injection in rats.

Biomarkers/Treatment	LowEx Group (n = 7)	VigEx Group (n = 8)	NoEx Group (n = 10)	NoIso Group (n = 11)
SBP (mmHg) immediately before Iso or 1 hr	125 ± 21[a]	127 ± 10	125 ± 13	129 ± 13
SBP (mmHg) 10 - 15 min after Iso	58 ± 23	68 ± 8	58 ± 16	NA[b]
Immediate fall of SBP (mmHg)	-67 ± 23**	-58 ± 13**	-64 ± 20**	NA
Rebound of SBP 1-2 hrs after Iso (mmHg)	+62 ± 41	+33 ± 19*	+69 ± 44	NA
Rebound of SBP (% of Pre-Iso)	+97 ± 34	+74 ± 16*	+103 ± 29	NA
DBP (mmHg) immediately before Iso or 1 hr	95 ± 9	92 ± 13	99 ± 20	99 ± 14
DBP (mmHg) 10 -15 min after Iso	35 ± 16	35 ± 24	32 ± 13	NA
Immediate fall of DBP (mmHg)	-60 ± 21**	-58 ± 18**	-60 ± 19**	NA
Rebound of DBP 1-2 hrs after Iso (mmHg)	+62 ± 40	+39 ± 25*	+76 ± 37	NA
Rebound of DBP (% of Pre-Iso)	+97 ± 42	+73 ± 31*	+110 ± 36	NA
HR (bpm) immediately before Iso or 1 hr	379 ± 38	368 ± 34	386 ± 33	372 ± 47
HR (bpm) 10 – 15 min after Iso	455 ± 45	458 ± 54	456 ± 50	NA
Immediate increase of HR (bpm)	+76 ± 65	+104 ± 42	+70 ± 53	NA
Increase of HR (bpm) at the end of experiment	+110 ± 65	+114 ± 44	+131 ± 49	NA

[a]Mean ± SD
[b]NA = Not applicable
*p < 0.05 vs NoEx Group
**p < 0.05 vs before isoproterenol (paired t-test)

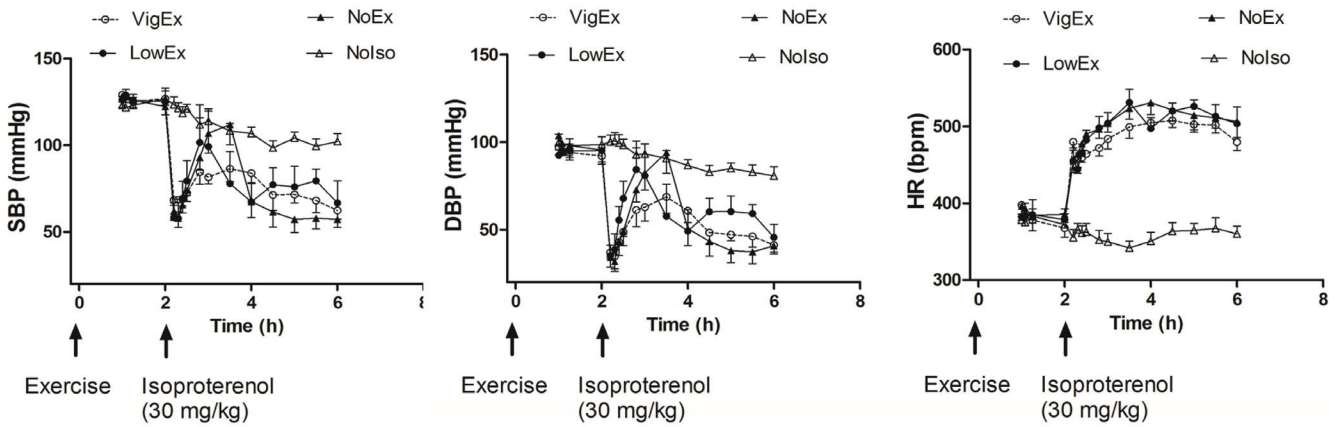

Fig. (4). Hemodynamic effects in response to isoproterenol injection (30 mg/kg) in rats with or without exercise preconditioning. Each point represents mean ± SEM. Systolic blood pressure SBP; diastolic blood pressure DBP; hear rate HR.

Table 3. Effects of exercise preconditioning on ATP metabolism in RBC after isoproterenol (Iso) injection in rats.

Biomarkers/Treatment	LowEx Group (n = 7)	VigEx Group (n = 8)	NoEx Group (n = 10)	NoIso Group (n = 11)
AUC ratio of AMP to ATP in RBC from T1 – T last	0.03 ± 0.02*	0.02 ± 0.02*	0.12 ± 0.12	0.03 ± 0.02*
AUC ratio of ADP to ATP in RBC from T1 – T last	0.22 ± 0.11	0.24 ± 0.10	0.34 ± 0.17	0.22 ± 0.08
AUC ratio of AMP to ADP in RBC from T1 – T last	0.12 ± 0.06*	0.11 ± 0.06*	0.25 ± 0.15	0.13 ± 0.07*
Max increase in [ATP] in RBC (mM)	0.69 ± 0.60**	0.50 ± 0.55**	0.52 ± 0.92	0.93 ± 0.80**
Max increase in [ADP] in RBC (mM)	0.24 ± 0.24**	0.21 ± 0.24**	0.41 ± 0.37**	0.04 ± 0.21*
Max increase in [AMP] in RBC (mM)	0.05 ± 0.07*	0.05 ± 0.12*	0.28 ± 0.21**	0.01 ± 0.02*

[a]Mean ± SD
[b]NA = Not applicable
*p < 0.05 vs NoEx Group
**p < 0.05 vs before isoproterenol injection (or baseline) by pair t-test

the NoIso group and those receiving isoproterenol after an exercise pre-conditioning (LowEx and VigEx groups) (Table **4**).

DISCUSSION

As reported in our previous communication, a modest level of exercise for 15 min at a treadmill speed of 10 m/min and 5% grade can induce a significant post exercise reduction in BP in rats. It also significantly increased RBC concentrations of ATP which may contribute to the post exercise BP effects [35, 36]. Our current study found similar results with significantly higher RBC ATP levels in the LowEx group more than one hour after the exercise compared to the other groups (Fig. **3**). Surprisingly, the rats in the VigEx group did not have increased RBC ATP levels. On the other hand, the ADP and AMP concentrations in RBC appeared to be lower in the exercise groups although the difference was not statistically significant (Fig. **3**). The results suggest that exercise may increase production of ATP from ADP and AMP in the RBC, and the effect was dependent and affected by the level or intensity of the exercise. An increase in ATP production in whole blood after exercise has previously been shown in healthy human volunteers suggesting that the exercise effect on ATP metabolism in the RBC is not species dependent [39]. However, it is not clear if the difference in ATP concentrations in the RBC between the two exercise groups (LowEx and VigEx) was a factor resulted in the significantly greater BP rebound effect seen in the LowEx group compared to the VigEx group (Table **2**). It is also interesting to note that both ADP and AMP concentrations in the RBC from the surviving rats in the LowEx group remained elevated at the end of the experiment (4 hr after isoproterenol) while the concentrations in the other groups (VigEx and NoEx) gradually returned to baseline levels (Fig. **6**). The difference never the less was not statistically significant ($p > 0.05$) because of the large variation of the data particularly in the LowEx group. The change was not apparent for the ATP concentrations in the RBC (Fig. **6**). Thus it remains to be tested if the ADP and AMP concentrations in LowEx group would return to baseline if the experiment was extended further. However, besides the greater rebound in blood pressure and the elevated AMP

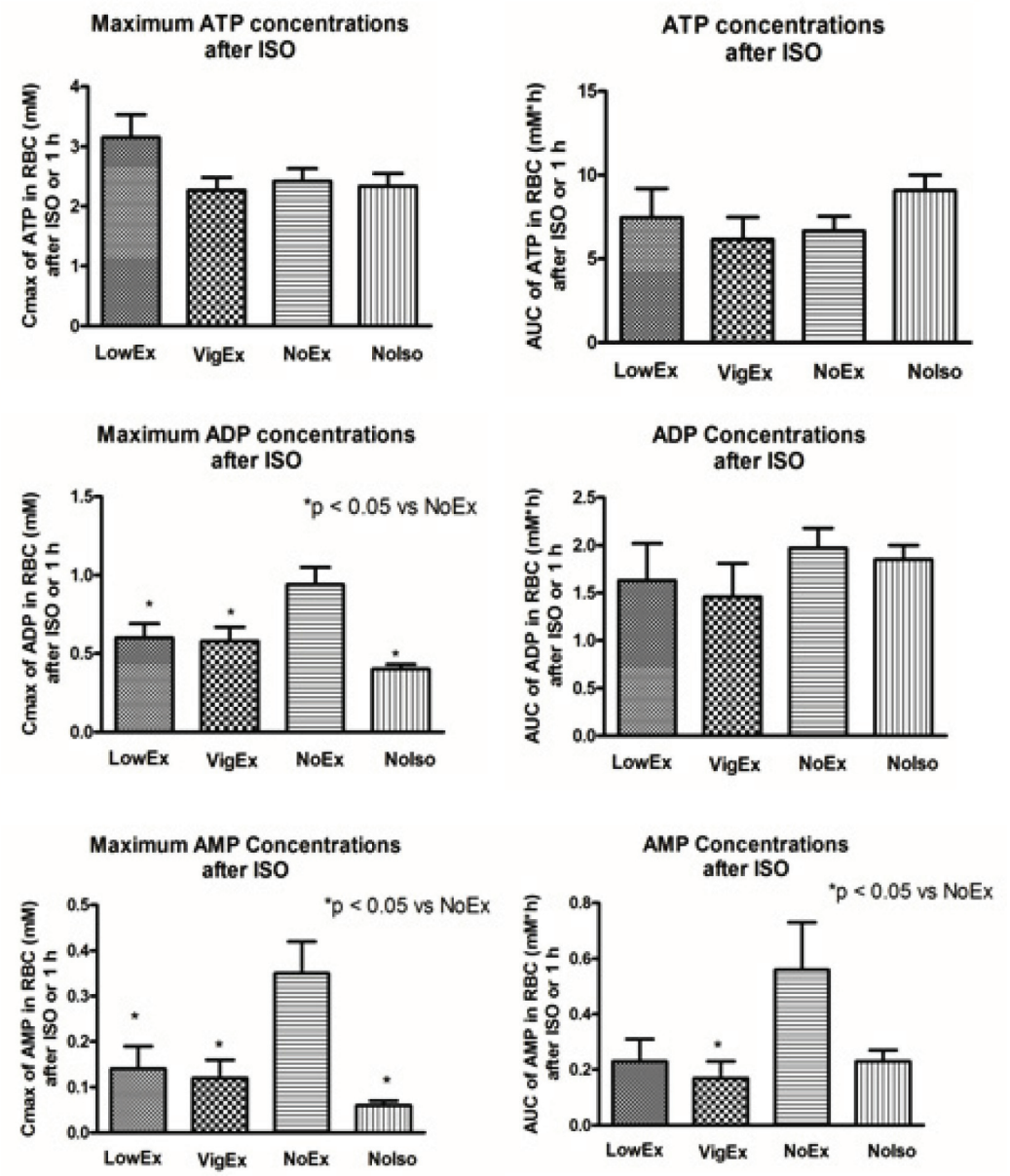

Fig. (5). Effect of exercise preconditioning on adenine nucleotide concentrations in red blood cells (RBC) in response to isoproterenol injection (30 mg/kg) in rats. Each column represents mean ± SEM.

Table 4. Correlation between RBC concentrations of adenine nucleotides in rats after isoproterenol (Iso) or 1 hr.

Biomarkers/Treatment	LowEx Group (n = 7)	VigEx Group (n = 8)	NoEx Group (n = 10)	NoIso Group (n = 11)
ATP vs AMP r	-0.291 ± 0.498[a]	-0.122 ± 0.663	-0.438 ± 0.423*	0.049 ± 0.327
ATP vs AMP β	-0.052 ± 0.103	-0.079 ± 0.140	-0.166 ± 0.204*	-0.001 ± 0.015
ATP vs ADP r	0.107 ± 0.621	0.116 ± 0.642	-0.218 ± 0.559*	0.441 ± 0.436
ATP vs ADP β	-0.023 ± 0.240	-0.100 ± 0.326	-0.260 ± 0.521	0.097 ± 0.118
ADP vs AMP r	0.734 ± 0.332*	0.807 ± 0.278*	0.732 ± 0.314*	0.152 ± 0.532
ADP vs AMP β	0.231 ± 0.239	0.218 ± 0.169*	0.314 ± 0.260*	0.033 ± 0.074

[a]Mean ± SD
*p < 0.05 vs NoIso Group

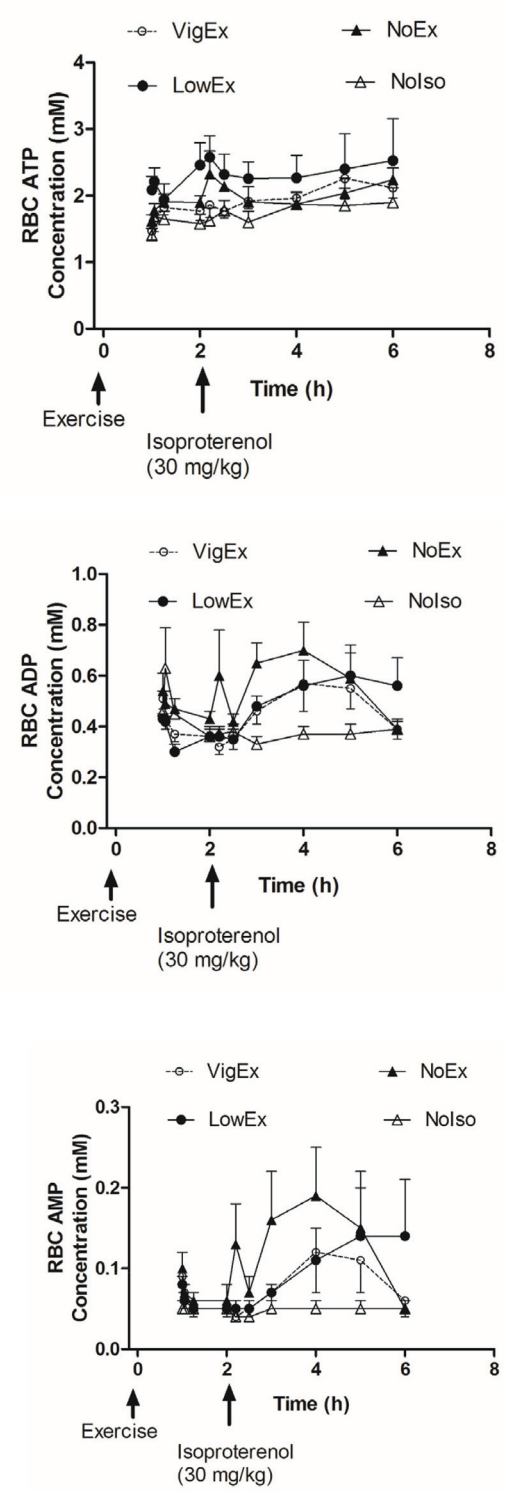

Fig. (6). Concentrations of adenine nucleotides in red blood cells (RBC) in response to isoproterenol injection (30 mg/kg) in rats. Each data point represents mean ± SEM.

concentrations towards the end of the experiment, there were no other notable differences in the cardiovascular protective effect between the two exercise groups. Clinically, it has been suggested that exercise intensity, not frequency or duration, is the most important variable for cardioprotection [40]. Thus further study using varying levels of exercise and longer follow up after isoproterenol may provide more mechanistic insights into the relationship between exercise intensity and ATP metabolism in the RBC in its role in cardiovascular protection.

As reported previously, a single dose of isoproterenol (30 mg/kg) injected subcutaneously can cause significant mortality and profound hemodynamic changes under a similar experimental condition [37]. The high mortality was in part attributed to the serial blood samples taken for measurement of ATP metabolism in the RBC. For this reason, the same number and volume of blood samples was taken from each rat in each group for comparison. As all the rats survived in the NoIso group, the effect of blood sampling was considered minimal without the isoproterenol injection. The current study also showed similar mortality (50%) and an immediate fall of blood pressure (both SBP and DBP) by about 60 mmHg (38 – 102 mmHg for SBP and 18 – 98 mmHg for DBP) which was associated with a rapid increase in HR by about 70 bpm (18 – 178 bpm) after isoproterenol (Fig. **4** and Table **2**). There was a rebound of blood pressure shortly after, but not the HR as it continued to increase until the end of the experiment (Fig. **4**). In addition, a significant increase of RBC ATP concentrations was found in the rats with exercise (LowEx and VigEx) and also in those without receiving isoproterenol (NoIso) ($p < 0.05$ paired t-test), but not in the NoEx group (Table **3**). We have previously observed an increase in RBC ATP concentrations in rats even when they were kept in a restrainer and the increase was greater in SHR than in SDR [36]. The mechanism for the increase ATP concentrations in the RBC in the NoIso group is not currently known. On the other hand, there was a significant increase of RBC AMP concentrations in the NoEx group, but not in the other groups (LowEx, VigEx or NoIso) (Table **3** and Fig. **5**). Similarly, the AUC ratios of AMP to ATP and also AMP to ADP concentrations in RBC were significantly higher in the NoEx group than the other groups (Table **3**) suggesting a greater catabolism of ATP and ADP to AMP in the RBC occurred in the rats received isosproterenol without the exercise preconditioning. This was further supported by the correlation and regression analyses that exercise pre-conditioning (both LowEx and VigEx groups) attenuated breakdown of ATP in the RBC (Table **4**). As has been shown previously, breakdown of ATP in the RBC was a strong indicator for serious cardiovascular events including mortality [37]. Thus the results support our working hypothesis that exercise preconditioning may reduce mortality, attenuates the rebound in blood pressure, and the breakdown of ATP in the RBC induced by isoproterenol.

However, it should be noted that manifestation of the post exercise effect is multi-factorial and there are other mechanisms besides an increase of ATP concentration in RBC that contribute to post-exercise hypotension. Exercise activates nitric oxide synthase activity in the endothelium and the renin angiotensin system [41, 42]. Blockade of angiotensin II receptor has been shown also to augment the

effect of exercise in reducing post-myocardial infarct ventricular remodeling in rats [43]. In contrast, blocking nitric oxide synthase activity attenuates post-exercise hypotension [44]. Recently, exercise has been shown to increase uridine concentrations in systemic blood which may be associated with the post-exercise effect [45]. There are probably other additional mechanisms which contribute to the health benefits associated with cardiovascular exercise [46, 47], and many of these mechanisms may work in tandem to maintain cardiovascular homeostasis in the body.

Although the clinical significance of ATP metabolism in the RBC is not clear, we hypothesize that it may be a measure of energy utilization within the cardiovascular system *in vivo*. *In vitro* studies have shown that ATP is released from human RBC and myocardium in response to a brief period of hypoxia and is subsequently broken down to ADP and AMP [48, 49]. While there is no direct evidence to indicate a similar response to ischemia or exercise occurs *in vivo*, the idea of RBC being an oxygen sensor as suggested by other workers is a plausible explanation and warrants further investigation [50, 51]. It is known that RBC are capable of releasing increased amounts of ATP as oxygen content falls and its haemoglobin becomes desaturated [48]. It was hypothesized that RBC may sense tissue oxygen requirements when they travel through the microcirculation, releasing vasodilatory compounds such as ATP that enhance blood flow in hypoxic tissues [51]. The released ATP would help to increase blood supply to the tissue and preserve an optimum, tissue-specific balance between oxygen supply and demand, thereby modulating the concentrations of tissue ATP within the cardiovascular system. Such a mechanism would eliminate the requirement for a diverse network of sensing sites throughout the vasculature, and should provide a more efficient means of appropriately matching oxygen supply with demand, and allow an immediate switch to alternative energy sources under hypoxia condition [52]. Thus we advocate the potential cardiovascular protective effects of exercise are mainly attributed to the post exercise effect, which is contributed at least partly by an increase of RBC concentrations of ATP. The protection may be measured by a reduction in mortality, attenuating breakdown of ATP in the RBC and rebound of blood pressure in response to sympathetic activation and cardiovascular injury induced by isoproterenol. However it should be cautious owing to the small number of rats used in the study (n = 7 – 11 in each group) and the large variation of the adenine nucleotide concentrations in the RBC, the exercise effect on some of the parameters such as reduction in cardiovascular mortality and breakdown of ATP to ADP (Table **3**) was not statistically significant. Additional study using larger number of rats in each group and including measure of baseline hemodynamic variables and adenine nucleotide concentrations before exercise is warranted. If our working hypothesis is validated, ATP metabolism in the RBC may be used as a drug target for anti-ischemia agents [29], and as surrogate biomarker for management of cardiovascular diseases, which would be an exciting topic with important therapeutic implications for further studies [26, 53].

CONCLUSION

The current study has demonstrated exercise pre-conditioning reduces rebound in blood pressure and breakdown of ATP in the RBC in acute MI induced by isoproterenol. The mechanisms may be attributed to post exercise hypotension secondary at least in part to increased ATP concentrations in RBC which may be affected by intensity of the exercise. Further study probing the potential ATP metabolism in RBC as a drug target for cardiovascular protection is warranted.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflict of interest.

ACKNOWLEDGEMENTS

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