

The Possible Physiological Role of Vascular Endothelial Growth Factor Receptor – 1 (VEGFR-1) in Adrenaline-Induced Myocardial Infarction in Rats with and Without Exercise

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Abstract: Background: We investigated the effect of post-myocardial infarction (MI) exercise on oxidative and angiogenic mediators in the heart of rats with adrenaline-induced MI. **Methodology:** Forty adult male rats that were divided into the following four groups: group I, sedentary control group; group II, exercised control group; group III, sedentary infarct group and group IV, exercised infarct group. MI was established by subcutaneous injection of adrenaline (2 mg/kg body weight) in two subsequent doses, 24 hours apart for 2 consecutive days. Sedentary and exercised control groups received subcutaneous saline. The animals in groups II and IV started swimming immediately after induction of MI for about 15 minutes daily for 5 days/week for 4 weeks. However, rats in groups I and III remained sedentary throughout the experiment period. After 4 weeks, blood and heart tissues were collected for the assay of cardiac enzyme markers lactate dehydrogenase (LDH), and creatine kinase (CK), vascular endothelial growth factor receptor-1(VEGFR-1), malondialdehyde and antioxidant concentrations. **Results:** MI showed increased levels of LDH, CK, and malondialdehyde in association with decreased antioxidant concentrations. However, post-MI exercise attenuated the effects of MI on oxidative stress markers and increased antioxidant activity in cardiac tissue. In addition, cardiac VEGFR-1 was elevated significantly in the sedentary infarct rats with more increase in the exercised infarct group. **Conclusion:** post-MI exercise training could reverse the adverse effects of MI by reducing the extent of myocardial damage, attenuating the oxidative stress, increasing VEGFR-1 expressions, and thereby increasing angiogenesis.

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1. Introduction

Cardiovascular diseases (CVDs) have a high prevalence in developing and developed countries and myocardial infarction accounts for majority of deaths and disabilities [1]. After myocardial infarction (MI), specific growth factors promote cardiac angiogenesis by specific receptors in the endothelium of the cardiac microvasculature, leading to improvement of blood supply to the infarction zone and its periphery [2]. Thus, it seems that the body already possesses an "in-house" rescue system to increase blood flow in ischemic circumstances. Stimulation of this system, termed neovascularization, could be a promising new direction in treating CVD [3]

Angiogenesis is a process by which new capillaries are formed from pre-existing blood vessels in physiological or pathological contexts [4]. The process of angiogenesis in the myocardium is not limited to the developmental periods of life, but may occur when the heart is challenged during ischemia [5]. It is important for the body to initiate the angiogenic process in order to create new circulation in ischemic lesions. Cardiac ischemic injury usually leads to compensatory neovascularization to meet local metabolic demands of the heart [2].

The vascular endothelial growth factor (VEGF) system and its receptors (VEGFRs) are important for the physiological regulation of angiogenesis. Thus, they are considered prime molecular targets for pro-angiogenic and anti-angiogenic therapies [4]. Hypoxia serves as a powerful stimulus for the up-regulation of VEGFs and their receptors that facilitate angiogenesis especially after myocardial ischemia [6,7].

The VEGF family includes VEGF-A, -B, -C, -D, -E and PlGF (placental growth factor) [8]. VEGF-A (VEGF) is the master regulator of angiogenesis during growth and development, as well as in disease states [9]. VEGF-mediated angiogenic response was dependent on both vascular endothelial growth factor receptor – 1 (VEGFR-1) signaling and NO production [10].

VEGFs are predominantly produced by endothelial, hematopoietic, and stromal cells in response to hypoxia [11]. They stimulate proliferation, migration, and tube formation of endothelial cells (ECs) through interacting with receptor tyrosine kinases, VEGF receptor-1, -2, and -3 (VEGFR-1, -2, -3) [12].

VEGFR-1 (or fms-like tyrosine kinase or Flt-1) is widely expressed in normal and pathologic tissue

and contributes to the pathogenesis of many neoplastic and inflammatory diseases [13].

VEGFR-1 is composed of seven extracellular immunoglobulin-like (Ig-like) domain, a single transmembrane region and an intracellular tyrosine kinase domain that is interrupted by a kinase-insert domain [14]. A naturally occurring soluble form of VEGFR-1 (sVEGFR-1), consisting of the extracellular part of VEGFR-1, is produced from endothelial cells [15].

The major pool of endothelial VEGFR1 is largely resident within the Golgi apparatus but translocates to the plasma membrane by ligand or agonist stimulation via a calcium-regulated process. Translocation of VEGFR1 from the Golgi to cytoplasmic vesicles and to the plasma membrane required elevation of cytosolic calcium ion levels [16].

In addition to its expression on endothelial cells [2], VEGFR1 is also expressed on a wide range of non-endothelial cells, including vascular smooth muscle cells, monocytes/macrophages, retina, brain, osteoblasts, pericytes, placental trophoblasts, renal mesangial cells and also in some hematopoietic stem cells. VEGFR-1 expression is up-regulated during angiogenesis by hypoxia [17].

VEGFR-1 is required for endothelial-cell survival as VEGFR1 loss is associated with decreased vascular sprout formation, migration, and vascular branching [18]. Recently, much focus has been paid to the endothelial-immune cell cross-talk in regulating angiogenesis [19]. VEGFR-1 mediated angiogenesis has been shown to be dependent on monocytes [20]. It is found that VEGFR1 is a positive regulator of monocyte and macrophage migration as VEGFR-1 mediates collateral growth in ischemic disease by mobilizing leukocytes and recruiting them to the pericollateral space [21].

Cardiovascular risk factors are raised by sedentary life style and ameliorated by physical fitness in the general population [22]. Regular exercise is rapidly gaining widespread advocacy in the prevention and treatment of many CVD [23].

Post-MI exercise training studies have revealed several positive effects on the improvement of cardiac functions in patients with MI [24]. However, the mechanism of exercise-induced benefit in MI remains poorly defined. With this view, the current study was designed to investigate the effect of post-MI swim exercise training on oxidative and angiogenic mediators in the heart of rats with adrenaline-induced MI. We hypothesized that early exercise training after MI had myocardial salvage and able to reverse the MI-induced abnormalities, thereby attenuating deleterious cardiac remodeling and preserving post-MI cardiac function.

2. Material and Method

This study was conducted on 40 albino adult rats weighing between 190–200g. They were allowed to live at room temperature, fed *ad libitum* on standard laboratory rat chow and had free access to tap water. After the rats were fed for one week to allow adaptation to the new environment, the rats were randomly divided into the following four groups each with 10 animals according to the treatment and exercise conditions: **Group I:** Sedentary control group, **Group II:** Exercised control group, **Group III:** Sedentary infarct group and **Group IV:** Exercised infarct group.

The rat model of myocardial infarction was established by subcutaneous injection of adrenaline (2 mg/kg body weight) in two subsequent doses, 24 hours apart for 2 consecutive days [25]. Adrenaline was purchased from local market. The sedentary and exercised control groups received saline (1 ml) subcutaneously once daily for two consecutive days (24 hours apart).

The exercised infarct rats started exercising immediately post-MI while the sedentary control and sedentary infarct rats remained sedentary throughout the experiment period. Rats were exercised by swimming in a tank of water at 35–37°C with a surface area of 2,830 cm² and a depth of 60 cm. The rats swam for about 15 minutes daily for 5 days / week for 4 weeks. The determination of exercise duration was based on the previous studies [26]. This exercise regimen was well tolerated by rats with MI. There were no mortalities during the four weeks of exercise training.

Biochemical assessment of myocardial infarction induced by adrenaline was done by measuring the levels of marker enzymes lactate dehydrogenase (LDH), and creatine kinase (CK) in sera of rats twenty four hours after the second injection of adrenaline. Blood samples were collected from the retro-orbital plexus under light ether anesthesia.

At the end of the experiment (after 4 weeks), all rats were sacrificed under light anesthesia with ether. Blood samples were collected for the assay of myocardial infarction marker enzymes LDH and CK. Animal heart tissues were dissected out, immediately washed in ice-cold saline and a homogenate was prepared in 0.1 M Tris-HCl buffer (pH 7.4). The homogenate was centrifuged and supernatant was used for the assay of vascular endothelial growth factor receptor-1 (VEGFR – 1), malondialdehyde and antioxidant concentrations. Samples were stored at -80°C until analysis.

Estimation of Vascular Endothelial Growth Factor Receptor-1(VEGFR- 1) in cardiac tissue homogenate [27]:

VEGFR – 1 concentration was measured using the quantitative sandwich enzyme immunoassay technique. The optical density of each sample was determined using a microplate reader set to 450 nm and the concentration of VEGFR was calculated based on standards and expressed in pg/mg of total protein content.

Estimation of total antioxidant concentrations in cardiac tissue homogenate [28] :

Total antioxidant capacity level was measured using colorimetric method. The antioxidative capacity is performed by the reaction of antioxidants in the sample with defined amount of exogenously provide hydrogen peroxide (H₂O₂). The antioxidants in the sample eliminate a certain amount of the provided hydrogen peroxide. The residual H₂O₂ is determined colorimetrically by enzymatic reaction of the colored product and blank absorbances. It was read immediately against distilled water at 505 nm. The total antioxidant concentration was calculated by the following

equation: $m\ M/L = AB-AS \times 3.33$

Estimation of malondialdehyde (MDA) concentrations

in cardiac tissue homogenate [29] :

Malondialdehyde (MDA) levels were measured using colorimetric method. Thiobarbituric acid (TBA) reacts with MDA in acidic medium at temperature of 95 degree for 30 min to form thiobarbituric acid reactive product. The absorbance of the resultant pink product was measured at 534nm, and the concentration in sample was expressed as nmol/ml .

Estimation of serum Creatine kinase(CK) and Lactate dehydrogenase (LDH) [30,31]

Statistical analysis:

Data were expressed as mean \pm SEM. Statistical significance was assessed using analysis of variance (ANOVA) with P-value of ≤ 0.05 was considered as significant.

3. Results

Serum creatine kinase(CK) and serum lactate dehydrogenase (LDH) : Table I

Myocardial infarction (MI) was proved by measuring serum levels of CK and LDH twenty four

hours after the second injection of adrenaline. Both were significantly elevated in the adrenaline -treated rats reflecting heart injury.

At the end of the four weeks, a significant increase in the serum levels of CK and LDH was detected in sedentary infarct group as compared to sedentary control one. A finding that is partially but significantly corrected in the exercised MI group. (F=503.334, $p < 0.05$) and (F=370.542, $p < 0.05$) respectively.

However, there was no significant difference in serum CK and LDH concentrations between the sedentary and exercised non-infarct rats.

Oxidant/antioxidant system markers malondialdehyde (MDA) and antioxidant levels in cardiac tissue homogenate: Table II

The data on the levels of oxidant/antioxidant system markers detected in the heart tissue homogenate of the studied groups were shown in table II. It was shown that MI induced oxidative stress with increased levels of MDA when compared to the control non-infarct group. In addition, MI decreased the activity of total antioxidants significantly when compared to the control non-infarct rats. (F=57.093, $p < 0.05$) and (F=209.564, $p < 0.05$), respectively.

On the other hand, the results showed that swimming exercise attenuated the MI- induced oxidative stress by lowering the levels of MDA when compared to the sedentary infarct group. In addition, swimming exercise in infarct rats increased the levels total antioxidants significantly when compared to the sedentary infarct animals. However, the results obtained showed that swimming exercise attenuated the effects of MI on oxidative stress markers and increased antioxidant activity in cardiac tissue homogenate yet; it did not reach the control non-infarct value.

Vascular Endothelial Growth Factor Receptor – 1 (VEGFR-1) in cardiac tissue homogenate: Table II

Table II showed the levels of cardiac VEGFR-1 that were elevated significantly in the sedentary infarct rats when compared to the non-infarct control ones, with more increase in the exercised infarct group. (F=403.452, $p < 0.05$)

Table 1: Serum cardiac enzyme markers creatine kinase (CK) and lactate dehydrogenase (LDH) in the studied groups at the end of the experimental period:

Variables	Group I (Sedentary control) (N=10)	Group II (Exercised control) (N=10)	Group III (Sedentary infarct) (N=10)	Group IV (Exercised infarct) (N=10)	F-value	P-value
CK (U/L)	84.4 ± 8.6 ^c	88.2 ± 5.4 ^b	183.5 ± 6.7 ^{ac}	167.0 ± 8.1 ^{ab}	503.334*	.000
LDH (U/L)	193 ± 9.9 ^c	194.1 ± 8.8 ^b	617 ± 33.3 ^{ac}	503.8 ± 61.5 ^{ab}	370.542*	.000

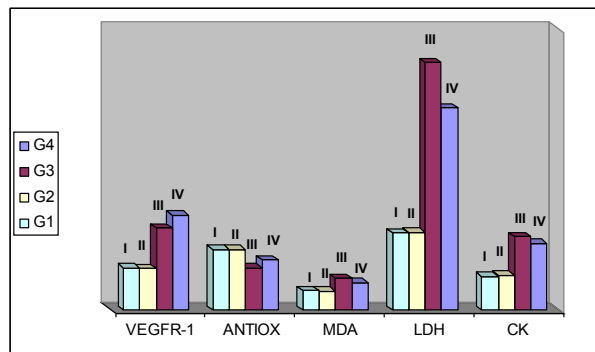
N: number of rats in each group , $P \leq 0.05$: Significant * : Highly significant
 a: Significance between group III and IV. b: Significance between group II and IV.
 c : Significance between group I and III

Table II: Levels of malondaldehyde (MDA) , total antioxidants and vascular growth factor receptor –1 (VEGFR –1) in heart tissue homogenates in the studied groups at the end of the experimental period:

Variables	Group I (Sedentary control) (N=10)	Group II (Exercised control) (N=10)	Group III (Sedentary infarct) (N=10)	Group IV (Exercised infarct) (N=10)	F-value	P-value
VEGFR - 1 (Pg/mg protein)	106.746 ± 11.4 ^c	104.988 ± 13.0 ^b	205.088 ± 9.4 ^{ac}	237.646 ± 8.4 ^{ab}	403.452*	.000
MDA (n mol/L)	48.844 ± 5.3 ^c	47.929 ± 4.9 ^b	79.664 ± 7.5 ^{ac}	69.272 ± 8.0 ^{ab}	57.093*	.000
Total antioxidants (m mol/L)	1.523 ± 2.6 ^c	1.514 ± 2.2 ^b	1.053 ± 1.8 ^{ac}	1.250 ± 9.1 ^{ab}	209.564*	.000

N: number of rats in each group
 * : Highly significant
 b: Significance between group II and IV

$P \leq 0.05$: Significant
 a: Significance between group III and IV
 c : Significance between group I and III

**Figure 1:** Comparison between the four studied groups regarding different studied parameters at the end of the experimental period

CK : Creatine kinase
 LDH: Lactate dehydrogenase
 MDA: Malondialdehyde
 ANTIOX: Total antioxidants
 VEGFR-1: vascular endothelial growth factor receptor – 1
 I: Group I
 II: Group II
 III: Group III
 IV: Group IV

4. Discussion

The purpose of this investigation was to assess the potential protective role of swim exercise training (ET) in rats with adrenaline- induced myocardial infarction (MI) as the exercise-induced adaptations within the heart that provide the protection were still in doubt . This was performed with reference to cardiac enzyme markers, oxidative stress markers and cardiac VEGFR-1 expression .

We explored this phenomenon in a rat model of MI. The administration of adrenaline (2 mg/kg in two subsequent doses subcutaneously, 24 hours apart) had been found to cause infarct-like necrosis of the heart muscle. Adrenaline-induced MI serves as a well-standardized model because the pathophysiological changes following adrenaline administration are comparable to those taking place in human MI [32, 33].

In the present work, the occurrence of MI had been evidenced by the significant increase in the circulating levels of cardiac enzyme markers, creatine kinase (CK) and lactate dehydrogenase (LDH) in adrenaline-injected rats compared to control non-injected ones. This was found to be consistent with previous reports [34].

It is demonstrated that blood concentrations of cardiac enzyme markers are normally very low as a result of physiological wear and tear of the cell [35].

Cardiac enzymes are often used as sensitive markers of MI as when the myocardium is injured, these enzymes can be released from the cells to blood. Therefore, the blood levels of these enzymes reflect the extent of myocardial injury [36].

In this study, the cardioprotective potential of ET was evident by the observation that swim exercise could significantly decrease the blood levels of cardiac enzyme markers in infarct rats. Concerning the issue of the association between cardiac enzyme markers and ET, Nivethetha *et al.* [34] has been demonstrated that cardioprotective effect of ET was probably related to its ability to strengthen the myocardial membrane by its membrane-stabilizing action and reduced the extent of myocardial damage thereby restricted the leakage of these enzymes from the myocardium.

Our findings reported a significant increase in the levels of cardiac malondialdehyde (MDA) together with a reduction in the cardiac antioxidant concentrations in the infarct rats when compared with the non-infarct ones. Therefore, we postulated that this might reflect heightened oxidative stress and consumption of antioxidants which probably had been reported to be causally involved in MI. Indeed, our results confirmed previous studies that reported a similar increase in the markers of oxidative stress following induction of MI [37]. These studies demonstrated that oxidative stress and generation of its markers were strongly implicated in a number of cardiovascular disorders including MI. These oxidative insults induced by MI appeared to play a major role in the pathogenesis of heart damage

There is growing recognition that oxidative stress plays role in the pathogenesis of myocardial repair/remodeling following MI, and antioxidants can be an option for treatment of this disorder [38].

In the ischemic myocardium, reactive oxygen species (ROS) are generated and directly injure the cell membrane causing cell death. However, ROS also stimulate signal transduction to elaborate inflammatory cytokines in the ischemic region and surrounding myocardium as a host reaction. Moreover, calcium overload induced by extensive ROS generation causes necrosis through enhanced permeability of the mitochondrial membrane [39].

In endothelial cells (ECs), many reports observed that low levels of ROS function as signaling molecules to mediate various biological responses such as angiogenesis [40].

Nicotinamide adenine dinucleotide phosphate (NADPH) oxidase was activated by ischemia and considered to be a major source for cardiac ROS production in the infarcted myocardium [41]. Even though NADPH oxidase is a major source of ROS, it has been reported that oxidative stress in the infarcted

heart can be derived from other sources. ROS can also be produced intracellularly through electron leakage from the mitochondria during oxidative phosphorylation and through the activation of several cellular enzymes, including xanthine oxidase and nitric oxide synthase [42].

More benefits of ET had been proved in the present work. A significant increase in the cardiac antioxidants together with a reduction in the cardiac MDA concentrations were observed in the infarct exercised group when compared to the infarct sedentary one. This observation gave the speculation that ET could have a role in alleviating oxidative stress in these animals. This beneficial effect of post-MI exercise training was also shown by other reports

Many studies hypothesized that exercise-induced increase in endogenous antioxidants might be an important change contributing to cardioprotection, especially in high-risk populations [22]. Other investigators found that ET decreased NADPH oxidase activity and consequently diminished ROS production [44]. These might be important mechanisms by which ET could mediate its beneficial effects in MI condition.

Among the interesting findings in this study were that cardiac vascular endothelial growth factor receptor -1 (VEGFR-1) levels were elevated in infarct animals. In agreement with our results, other previous studies reported a similar increase in cardiac VEGFR-1 concentrations in MI [45,46].

After MI, specific growth factors promote cardiac angiogenesis, leading to a therapeutic effect as revascularization is urgently needed in this condition [47]. Vascular endothelial growth factor (VEGF) and its receptor VEGFR-1 are closely associated in human tissues and quantifying their mRNAs might be helpful in evaluating angiogenesis [48].

It is reported that the VEGF/ VEGFR-1 signaling is important in myocardial protection as mice deficient in VEGFR-1 are more susceptible to heart ischemia/reperfusion injury [49]. Interestingly, Vöö *et al.* [50] have been found that the VEGFR-1-mediated response was rapid (2 days following MI).

In support of these assumptions, it has been demonstrated that MI leads to an upregulation of VEGF and VEGFR-1 as cardiac myofibroblasts isolated from the site of infarction were found to express high levels of these growth factors [51].

ROS are critically important for VEGF signaling as VEGF stimulates ROS production via activation of NADPH oxidase in endothelial cells. This ROS production induces myocardial angiogenesis [12,52].

Zhao *et al.* [38] suggested that following MI, angiogenesis is mostly active in the infarcted

myocardium, which is coincident with enhanced ROS.

Cardiac VEGFR-1 was specifically up-regulated in hypoxia and on exposure to oxidative stress. Cardiomyocyte VEGFR-1 activation by VEGF induces compensatory hypertrophy and preserves cardiac function after myocardial infarction [53]. These observations provided strong support to the interrelationship between myocardial ischemia, VEGFR-1, and increased oxidative stress.

Following MI, VEGF recruits circulating blood monocytes to the infarcted myocardium via stimulation of VEGFR-1 [54] as VEGFR1 is expressed exclusively by monocytes. These monocytes are differentiated into macrophages that remove necrotic cardiac myocytes; secrete cytokines, and growth factors that restore cardiac vascularization at a microvascular level [45]. It is found that the migrating monocytes into the infarcted myocardium produce large amounts of ROS [55].

Considering the effect of exercise on cardiac VEGFR-1, the present work showed a significant increase in cardiac VEGFR-1 levels in rats with post-MI swim exercise training. Accordingly, this was another possible beneficial effect for post-MI exercise training on myocardial function as these higher levels of VEGFR-1 with exercise could be predictor of enhanced angiogenesis with exercise. These findings were in accordance with previous studies that found that upregulation of VEGFR-1 levels in the infarcted mice heart in response to exercise resulted in decreased infarct size and improved angiogenesis [56].

Exercise training (ET) is associated with adaptations in the coronary microvasculature including the formation of new capillaries. ET does not stimulate growth of coronary collateral vessels in the normal heart. However, if exercise produces cardiac ischemia, there is evidence that collateral growth can be enhanced [57].

As regard the effect of swim-exercise training, experimental studies have shown that this type of exercise increased the capillary density of the heart [58]. Some investigators revealed that rats exposed to low to moderate exercise after MI offered beneficial effect while higher exercise intensity had detrimental effects in rats [59].

Several mechanisms have been conducted to explain the angiogenic stimulatory effect of exercise training (ET). The activation of VEGF dependent angiogenic pathways represents a crucial molecular mechanism by which exercise triggers angiogenesis [60]. Others have shown that metabolites stimulate angiogenesis [61]. Interestingly; exercise activates 5'-AMP-activated kinase which has been reported to stimulate VEGF-A gene expression [62]. Moreover,

ET could enhance the vasodilatory response to VEGF. In addition, ET in a mouse model was found to increase endothelial progenitor cells (EPCs) and enhanced angiogenesis [63]. This adaptation to exercise was dependent on nitric oxide, cyclooxygenase and tyrosine kinase receptor activity [64].

Gavin *et al.* [65] found that inhibition of nitric oxide synthase attenuated the exercise-induced increase in VEGFR-1 mRNA by approximately 50%. These findings suggest that exercise alters VEGFR-1 gene expression and NO is an important signal in this process. These findings provide direct evidence of exercise-induced angiogenesis and myocardial salvage in the ischemic condition.

We concluded that early exercise training especially swimming exercise after MI was likely playing an important role in reversing the adverse effects of MI by reducing the extent of myocardial damage, attenuating the oxidative stress, increasing VEGFR-1 expressions, and thereby increasing angiogenesis. These improvements, in turn preserve post-MI cardiac function. Indeed, exercise training could provide future modalities in the prevention or treatment of ischemic cardiac diseases especially in those patients who are not suitable for currently available treatment options, such as endovascular intervention or surgical reconstruction. Therefore, biological revascularization via exercise training has emerged as a new therapeutic option and a positive clinically relevant option in post-MI rehabilitation. In addition, the present article attested the importance to the therapeutic values of antioxidants.

Conflict of interest:

Based on our results, it was conceivable to speculate that post-MI swim exercise training for four weeks could not alleviate cardiac damage and oxidative stress completely but at least has disease-modifying effects. This might be due to short duration of swim exercise training. So, we advise to investigate the effect of longer duration of exercise either by prolongation of exercise session or the whole exercise period.

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References

1. Saravanan G, Ponnurugan P, Sathiyavathi M, Vadivukkarasi S, and Sengottuvelu S (2011): Cardioprotective activity of *Amaranthus viridis* Linn: Effect on serum marker enzymes, cardiac troponin and antioxidant

- system in experimental myocardial infarcted rats. *Int J Cardiol* [Epub ahead of print].
2. Wang XH, Zhang GP, Jin HM, and Chen SF(2007): Dynamic changes in the expression of growth factor receptors in the myocardium microvascular endothelium after murine myocardial infarction. *Chin Med J (Engl)*;120(6):485-90.
 3. Carmeliet P(2000): Mechanisms of angiogenesis and arteriogenesis. *Nat Med*; 6:389-95.
 4. Wu FT, Stefanini MO, Mac Gabhann F, Kontos CD, Annex BH, and Popel AS(2010): A system biology perspective on sVEGFR1: its biological function, pathogenic role and therapeutic use. *J Cell Mol Med*;14(3):528-52.
 5. Ushio-Fukai M, and Urao N(2009): Novel role of NADPH oxidase in angiogenesis and stem/progenitor cell function. *Antioxid Redox Signal*;11(10):2517-33.
 6. Zhao T, Zhao W, Chen Y, Ahokas RA, and Sun Y(2010): Vascular endothelial growth factor (VEGF)-A: Role on cardiac angiogenesis following myocardial infarction. *Microvasc Res*; 80(2):188-94.
 7. Lohela M, Bry M, Tammela T, and Alitalo K(2009): VEGFs and receptors involved in angiogenesis versus lymphangiogenesis. *Curr Opin Cell Biol*; 21(2):154-65.
 8. Grünewald FS, Prota AE, and Giese A(2010): Ballmer-Hofer K Structure-function analysis of VEGF receptor activation and the role of coreceptors in angiogenic signaling. *Biochim Biophys Acta* ;1804(3):567-80.
 9. Nieves BJ, D'Amore PA, and Bryan BA(2009): The function of vascular endothelial growth factor. *Biofactors*;35(4):332-7.
 10. Lin MI, and Sessa WC(2006): Vascular endothelial growth factor signaling to endothelial nitric oxide synthase: more than a FLeeTing moment. *Circ Res*;99(7):666-8.
 11. Stutfeld E, and Ballmer-Hofer K(2009): Structure and function of VEGF receptors. *IUBMB Life*; 61(9):915-22.
 12. Ushio-Fukai M, and Nakamura Y (2008): Reactive oxygen species and angiogenesis: NADPH oxidase as target for cancer therapy. *Cancer Lett*;266(1):37-52
 13. Schwartz JD, Rowinsky EK, Youssoufian H, Pytowski B, and Wu Y (2010): Vascular endothelial growth factor receptor-1 in human cancer: concise review and rationale for development of IMC-18F1 (Human antibody targeting vascular endothelial growth factor receptor-1). *Cancer* ; 116 (4 Suppl):1027-32.
 14. Tammela T, Enholm B, Alitalo K, and Paavonen K(2005): The biology of vascular endothelial growth factors. *Cardiovasc Res* 65(3):550-63.
 15. Kishuku M, Nishioka Y, Abe S, Kishi J, Ogino H, Aono Y, Azuma M, Kinoshita K, Batmunkh R, Makino H, Ranjan P, Minakuchi K, and Sone S(2009): Expression of Soluble Vascular Endothelial Growth Factor Receptor-1 in Human Monocyte-Derived Mature Dendritic Cells Contributes to Their Antiangiogenic Property. *J Immunol*;183(12):8176-85.
 16. Mittar S, Ulyatt C, Howell GJ, Bruns AF, Zachary I, Walker JH, and Ponnambalam S(2009): VEGFR1 receptor tyrosine kinase localization to the Golgi apparatus is calcium-dependent. *Exp Cell Res*;315(5):877-89.
 17. Beck H, Raab S, Copanaki E, Heil M, Scholz A, Shibuya M, Deller T, Machein M, and Plate KH (2010): VEGFR-1 Signaling Regulates the Homing of Bone Marrow-Derived Cells in a Mouse Stroke Model. *J Neuropathol Exp Neurol*;69(2):168-75.
 18. Zhang Z, Neiva KG, Lingen MW, Ellis LM, and Nör JE(2010): VEGF-dependent tumor angiogenesis requires inverse and reciprocal regulation of VEGFR1 and VEGFR2. *Cell Death Differ*;17(3):499-512.
 19. Noonan DM, De Lerma Barbaro A, Vannini N, Mortara L, and Albini A(2008): Inflammation, inflammatory cells and angiogenesis: decisions and indecisions. *Cancer Metastasis Rev*;27(1):31-40.
 20. Murakami M, Zheng Y, Hirashima M, Suda T, Morita Y, Oeohara J, Ema H, Fong GH, and Shibuya M(2008): VEGFR1 tyrosine kinase signaling promotes lymphangiogenesis as well as angiogenesis indirectly via macrophage recruitment. *Arterioscler Thromb Vasc Bio*;28(4):658-64.
 21. Clayton JA, Chalothorn D, and Faber JE(2008): Vascular endothelial growth factor-A specifies formation of native collaterals and regulates collateral growth in ischemia. *Circ Res*;103(9):1027-36.
 22. Bai Y, Sigala W, Adams GR, and Vaziri ND(2009): Effect of exercise on cardiac tissue oxidative and inflammatory mediators in chronic kidney disease. *Am J Nephro*; 29(3):213-21.
 23. Yung LM, Laher I, Yao X, Chen ZY, Huang Y, and Leung FP (2009): Exercise, vascular wall and cardiovascular diseases: an update (part 2). *Sports Med*;39(1):45-63.
 24. Xu X, Wan W, Powers AS, Li J, Ji LL, Lao S, Wilson B, Erikson JM, and Zhang JQ (2008): Effects of exercise training on cardiac function and myocardial remodeling in post myocardial infarction rats. *J Mol Cell Cardiol* ;44(1):114-22.
 25. Khan I. Effect of antioxidant vitamins E and C on adrenaline induced myocardial damage in rats. M.Phil. Thesis. University of Dhaka. 1996.
 26. Iemitsu M, Maeda S, Jesmin S, Otsuki T, and Miyauchi T(2006): Exercise training improves aging-induced downregulation of VEGF angiogenic signaling cascade in hearts. *Am J Physiol Heart Circ Physiol*;291(3):H1290-8.
 27. Neufeld G, Cohen T, Gengrinovitch S and Pottorak Z (1999): Vascular endothelial growth factor and its receptors. *FASEB J*;13:9-22.
 28. Kampa M, Nistikaki A, Tsaoussis V, Maliarakis N, Notas G, Castanas E (2002): A new automated method for the determination of the Total Antioxidant Capacity (TAC) of human plasma, based on the crocin bleaching assay. *BMC Clin Pathol*;2(1):3.
 29. Satohk (1978): Serum lipid peroxide in cerebrovascular disorders determined by a new colorimetric method. *Clinica Chimica Acta*;90:7-43.
 30. Szasz G, Gruber W, and Bemt E (1976): Creatine kinase in serum: Determination of optimum reaction conditions. *Clin Chem*;22:650-656.
 31. Moss DW, Henderson AR: Clinical enzymology. In Titez Textbook of Clinical Chemistry. 3rd edition. Edited by Brutis CA, Ashwood ER. Philadelphia: W.B. Saunders Company; 1999:617-721.
 32. Parvin R, and Akhter N. (2008): Protective effect of tomato against adrenaline-induced myocardial infarction in rats. *Bangladesh Med Res Counc Bull*;34(3):104-8.
 33. Sood JD, Empson M, Fitzharris P, and Stewart J(2007): Too much of a good thing, is it bad? Adrenaline on trial. *N Z Med J*;120(1252):U2494.
 34. Nivethetha M, Jayasri J, and Brindha P(2009): Effects of Muntingia calabura L. on isoproterenol-induced myocardial infarction. *Singapore Med J*;50(3):300-2.
 35. Brancaccio P, Maffulli N, Buonauro R, and Limongelli FM(2008): Serum enzyme monitoring in sports medicine. *Clin Sports Med* ; 27 (1): 1-18.
 36. Guo J(2006): Electrocardiogram of acute coronary syndrome. *Journal of Clinical Electrocardiology*;15:128-137.
 37. Zhou SX, Zhou Y, Lei J, and Zhang YL(2008): Effects of oxidative stress on ventricular remodeling after myocardial infarction in rats. *Nan Fang Yi Ke Da Xue Xue Bao*;28(11):2030-4.
 38. Zhao W, Zhao D, Yan R, and Sun Y (2009): Cardiac oxidative stress and remodeling following infarction: role of NADPH oxidase. *Cardiovasc Pathol*; 18(3): 156-166.

39. Hori M, and Nishida K (2009): Oxidative stress and left ventricular remodelling after myocardial infarction. *Cardiovasc Res*;81(3):457-64.
40. Sardina JL, López-Ruano G, Sánchez-Sánchez B, Llanillo M, and Hernández-Hernández A(2012): Reactive oxygen species: Are they important for haematopoiesis? *Crit Rev Oncol Hematol*;81(3):257-74.
41. Frey RS, Ushio-Fukai M, and Malik A (2009): NADPH Oxidase-Dependent Signaling in Endothelial Cells: Role in Physiology and Pathophysiology. *Antioxid Redox Signal*;11(4):791-810.
42. Chen Y, and Gibson SB(2008): Is mitochondrial generation of reactive oxygen species a trigger for autophagy? *Autophagy*;4(2):246-8.
43. Leung FP, Yung LM, Laher I, Yao X, Chen ZY, and Huang Y (2008): Exercise, vascular wall and cardiovascular diseases: an update (Part 1). *Sports Med*;38(12):1009-24.
44. Zanchi NE, Bechara LR, Tanaka LY, Debbas V, Bartholomeu T, and Ramires PR(2008): Moderate exercise training decreases aortic superoxide production in myocardial infarcted rats. *Eur J Appl Physiol*; 104(6):1045-52.
45. Shantsila E, and Lip GY(2009): Monocytes in acute coronary syndromes. *Arterioscler Thromb Vasc Biol*;29(10):1433-8.
46. Siddiqui AJ, Fischer H, Widegren U, Grinnemo KH, Hao X, Månsson-Broberg A, Sylvén C, and Gustafsson T(2010): Depressed expression of angiogenic growth factors in the subacute phase of myocardial ischemia: a mechanism behind the remodeling plateau? *Coron Artery Dis* ; 21(2):65-71.
47. Lähteenvuo JE, Lähteenvuo MT, Kivelä A, Rosenlew C, Falkevall A, Klar J, Heikura T, Rissanen TT, Vähäkangas E, Korpisalo P, Enholm B, Carmeliet P, Alitalo K, Eriksson U, and Ylä-Herttua S(2009): Vascular endothelial growth factor-B induces myocardium-specific angiogenesis and arteriogenesis via vascular endothelial growth factor receptor-1- and neuropilin receptor-1-dependent mechanisms. *Circulation*;119(6):845-56.
48. Uchida N, Nagai K, Sakurada Y, and Shiota K(2008): Distribution of VEGF and flt-1 in the normal dog tissues. *J Vet Med Sci* ;70(11):1273-6.
49. Thirunavukkarasu M, Juhasz B, Zhan L, Menon VP, Tosaki A, Otani H, and Maulik N(2007): VEGFR1 (Flt-1+/-) gene knockout leads to the disruption of VEGF-mediated signaling through the nitric oxide/heme oxygenase pathway in ischemic preconditioned myocardium. *Free Radic Biol Med* ;42(10):1487-95.
50. Vöö S, Eggermann J, Dunaeva M, Ramakers-van Oosterhoud C, and Waltenberger J (2008): Enhanced functional response of CD133+ circulating progenitor cells in patients early after acute myocardial infarction. *Eur Heart J*;29(2):241-50.
51. Chintalgattu V, Nair DM, and Katwa LC(2003): Cardiac myofibroblasts: a novel source of vascular endothelial growth factor (VEGF) and its receptors Flt-1 and KDR. *J Mol Cell Cardiol*;35(3):277-86.
52. Ushio-Fukai M, and Alexander RW. Reactive oxygen species as mediators of angiogenesis signaling: role of NAD(P)H oxidase *Mol Cell Biochem* 2004;264(1-2):85-97.
53. Zentilin L, Puligadda U, Lionetti V, Zacchigna S, Collesi C, Pattarini L, Ruozi G, Camporesi S, Sinagra G, Pepe M, Recchia FA, and Giacca M (2010): Cardiomyocyte VEGFR-1 activation by VEGF-B induces compensatory hypertrophy and preserves cardiac function after myocardial infarction. *FASEB J* ;24(5):1467-78.
54. Tchaikovski V, Fellbrich G, and Waltenberger J(2008): The molecular basis of VEGFR-1 signal transduction pathways in primary human monocytes. *Arterioscler Thromb Vasc Biol*;28(2):322-8.
55. Lambert JM, Lopez EF, and Lindsey ML(2008): Macrophage roles following myocardial infarction. *Int J Cardiol*;130(2):147-58.
56. Wu G, Rana JS, Wykrzykowska J, Du Z, Ke Q, Kang P, Li J, and Laham RJ (2009): Exercise-induced expression of VEGF and salvation of myocardium in the early stage of myocardial infarction. *Am J Physiol Heart Circ Physiol*;296(2):H389-95.
57. Duncker DJ, and Bache RJ(2008): Regulation of coronary blood flow during exercise. *Physiol Rev*; 88(3):1009-86.
58. Batista EC, Batista EC, Ramalho JD, Reis FC, Barros CC, Moraes MR, Pesquero JL, Bacurau RF, Pesquero JB, and Araújo RC(2008): Swimming training exacerbates pathological cardiac hypertrophy in kinin B2 receptor-deficient mice. *Int Immunopharmacol*;8(2):271-5.
59. Wisloff U, Loennechen JP, Currie S, Smith GL, and Ellingsen O. (2002): Aerobic exercise reduces cardiomyocyte hypertrophy and increases contractility, Ca²⁺-sensitivity and SERCA-2 in rat after myocardial infarction. *Cardiovasc Res*;54(1):162-174.
60. Leosco D, Rengo G, Iaccarino G, Golino L, Marchese M, Fortunato F, Zincarelli C, Sanzari E, Ciccarelli M, Galasso G, Altobelli GG, Conti V, Matrone G, Cimini V, Ferrara N, Filippelli A, Koch WJ, and Rengo F(2008): Exercise promotes angiogenesis and improves beta-adrenergic receptor signalling in the post-ischaemic failing rat heart. *Cardiovasc Res*;78(2):385-94.
61. Murray B, Wilson DJ(2001): A study of metabolites as intermediate effectors in angiogenesis. *Angiogenesis*; 4: 71–77.
62. Winder WW, Hardie DG, Mustard KJ, Greenwood LJ, Paxton BE, Park SH, Rubink DS, and Taylor EB(2003): Long-term regulation of AMP-activated protein kinase and acetyl-CoA carboxylase in skeletal muscle. *Biochem Soc Trans*; 31: 182–185.
63. Laufs U, Werner N, Link A, Endres M, Wassmann S, Jurgens K, Miche E, Bohm M, and Nickenig G (2004): Physical training increases endothelial progenitor cells, inhibits neointima formation, and enhances angiogenesis. *Circulation* ; 109: 220–226.
64. Fogarty JA, Muller-Delp JM, Delp MD, Mattox ML, Laughlin MH, and Parker JL(2004): Exercise training enhances vasodilation responses to vascular endothelial growth factor in porcine coronary arterioles exposed to chronic coronary occlusion. *Circulation*; 109: 664–670.
65. Gavin TP, and Wagner PD(2002): Attenuation of the exercise-induced increase in skeletal muscle Flt-1 mRNA by nitric oxide synthase inhibition. *Acta Physiol Scand*; 175(3):201-9.