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RESEARCH ARTICLE

EFFECT OF SUB MAXIMAL EXERCISE ON ENDOTHELIUM-DERIVED NITRIC OXIDE CONCENTRATIONS IN HEALTHY YOUNG SUBJECTS

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ABSTRACT:

Objective: To determine any possible contribution of 10 minutes single session sub maximal exercise on Nitric oxide level in healthy young population.
Design: A descriptive observational study on forty healthy volunteers (20 male & 20 female) of 20-30 years designed to evaluate the level of nitric oxide after 10 minutes cycling.
Intervention: Young populations are allowed to exercise in a bicycle ergo meter for 10 minutes regular exercise under protocol YMCA Sub maximal Cycle Ergo meter Testing against a constant workload.
Main outcome measures: Serum Creatine Phosphokinase and Nitrate-Nitrite ratio (Surrogate marker of nitric oxide) measured. Continuous heart rate monitoring is done from ECG system. Vo_2 max calculated and energy expenditure for physical activity obtained from prediction equation after adjusting age, gender & body mass.
Results: Comparison of Activity Energy Expenditure (AEE) before and after exercise done, (p value<0.0001)(n = 40). Significant amount of work done in this exercise. Increase in the serum creatine phosphokinase level (p value<0.0001). Serum Nitrate-Nitrite ratio (nitric oxide level) showed increase (p value 0.1113) in post exercise level.
Conclusion: As far as energy expenditure concerned, significant muscular activity in both male & female showed insignificant endothelium derived Nitric oxide production. On the basis of the data obtained it is evident that in a single session sub maximal exercise beneficial effect of vasodilator Nitric oxide was not observed in studied young population.
Keywords: sub maximal exercise, activity energy expenditure, nitrate-nitrite ratio.

INTRODUCTION

Exercise is a subset of physical activity that is planned, structured, repetitive, and purposeful in the sense that improvement or maintenance of physical fitness is the objective[1]. Isolated exercise sessions elicit acute, transient cardiovascular and metabolic response. The magnitude of the exercise effect is influenced by characteristics of the exercise intervention, individual variation regarding age, gender and body mass. In the skeletal muscles changes are manifested by reduction in the level of ATP with the simultaneous increase in creatine phosphokinase activity. During exercise, blood flow to tissue is increased by arteriolar dilatation due to local release of nitric oxide (endothelial derived relaxing factor). Nitric oxide (NO) (half life= 0.05 to 1.8 milliseconds) is generated continuously by oxidation of l-arginine by nitric oxide synthase in endothelium and its production is increased by muscle contractions. It regulates contractility of smooth muscle and neuronal transmission of non-adrenergic, non cholinergic nerves. The exercise-induced augmentation of blood flow elicits an increase in shear, thereby providing a possible coupling between exercise and endogenous NO formation. Such a coupling would above all be limited to the exercise period and the immediate post exercise recovery. An effect of repeated exercise leading to an increase in the formation of NO that is maintained also between the exercise sessions might be important in explaining the beneficial effects of physical fitness on cardiovascular health. But recent studies showed improvement in NO vasodilator function after a single session of acute exercise has been less frequently found in healthy subjects[2, 3]. In the light of the above fact the present study attempts to explore the changes of metabolic parameters in healthy volunteers after expose to a single bout of moderate exercise (for 10 minutes) and its status of NO production.

MATERIALS AND METHODS:

TIME LINE: The present study was undertaken in the Department of Biochemistry, Medical College, Kolkata from January 2012-Septembar 2013.

STUDY DESIGN: It was Institution-based descriptive observational study with intervention.

STUDY POPULATION: Forty human volunteers (20 male & 20 female) of 20 to 30 years of age were included in the study from colleagues, staffs of departments and relatives of patients attending the department by proper standard randomization technique.

INCLUSION CRITERIA: Only those who are diagnosed healthy by history, physical examinations and BMI of 18.5 to 25 were included.

EXCLUSION CRITERIA: The followings were excluded from the study:

- Subjects with ischemic heart disease, any h/o previous episodes of angina, hypertension, hypercholesterolemia, diabetes, obesity, genetically determined risks and myopathy.
- Persons with habit of smoking, alcohol consumption,
- Any history of intake of drugs like antiepileptic, ATD, chemotherapy, OCP,
- Any variation in physiological status like pregnancy, stress, excessive exercise was excluded from the study.

Ethical Clearance and Informed Consent: Necessary approval of Institutional Ethics Committee was taken and subjects were enrolled after adequate informed consent was obtained.

Analytical method:

Collection of Sample: Individuals were allowed to exercise in a KAMACHI bicycle ergo meter for 10 minutes (measured by stopwatch) under exercise protocol YMCA Sub maximal Cycle Ergo meter Testing. Software metronome was used and minimum tempo of 40beats/min is taken to monitor the pedaling speed, pedal rate set at 50 r p m. Both male and female volunteers maintained the same speed. Minimum resistance in KAMACHI is 2-4kg. 10 ml venous blood was collected from volunteers at fasting in early morning with proper aseptic technique. Then he or she performed 10 minutes regular exercise in an air conditioned quiet room at temperature 27^0C. Again 10 ml venous blood was collected just after completion of exercise. Work done here was (600 - 800) kg. m / min which was moderate intensity. Continuous heart rate was measured during the whole period of exercise of 10 min regular pedaling protocol by a digital ECG machine with data retrieval system in ASCII format with a sampling rate $100H_Z$. Resting Heart rate (RHR) was monitored at the beginning of the exercise and at the end of 10 min of cycling Maximal Heart rate (MHR) is monitored. VO_2 max calculation from heart rate: Uth-Sorensen-Overgard-Pederson estimation by Heart rate ratio method: Mass specific VO_2 max in ml/ (kg x min) = 15.3 X (MHR/RHR) ; MHR = maximal heart rate, RHR = resting heart rate.

Energy expenditure calculation: Exercise on a bicycle ergo meter leads to an increment of heart rate and the amount of energy expenditure mainly for physical activity i.e the activity energy expenditure can be calculated by formula.Keytel, L.R; Goedecke, J. H. Noakes, T.D. et al. (2005) produced the prediction equation for energy expenditure after adjusting age, gender, weight and heart rate: Activity Energy Expenditure = gender x (-55.0969 + 0.6309 x heart rate + 0.1988 x weight + 0.2017 x age) + (1 - gender) x (-20.4022 + 0.4472 x heart rate - 0.1263 x weight + 0.074 x age) where gender = 1 for males and gender = 0 for females.[4]

Assay of CPK & Nitric Oxide: In pre and post exercise level, serum CPK were estimated in XL -600 ERBA AUTO ANALYZER by Modified IFCC method.[5] Nitrates is assessed by Cadmium Reduction method; Nitrite is determined by Diazotization of sulphalinamide by coupling it to N-napthathyline diamine dihydrochloride (Cortas & Wakid).[6] Since NO has an extremely short half life, it cannot be measured directly. However as NO is rapidly metabolized to nitrite (NO_2) and nitrate (NO_3) in the cell, concentration of these stable anions can be used to measure the amount of NO that was originally present in a sample. NO_2 is converted to nitrous acid (HNO_2) in acidic solution. HNO_2 is diazotized with the sulphanilic acid and sulphalinamide-diazonium complex is formed. This is coupled with amine of NED and a purple colored compound is obtained which can be measured spectrophotometrically at 545 nm.Total nitrite with cadmium and endogenous nitrite is assayed by taking absorbance in UV double beam spectrophotometer. The nitrate by subtracting endogenous nitrite concentration from total nitrite concentration.

Statistical Method: Statistical analysis were done in S.P.S.S version 17. All variables are normally distributed by Kolmogorov-Smirnov goodness-of-fit test. Comparison of means of numerical variables of pre and post exercise levels were done by Student's t test (paired). Mean values of base line characteristics and biochemical variable between males and females were compared by Student's t test (unpaired). All analyses were 2-tailed and *P* value < level of significance, 0.05 was considered to be statistically significant.

RESULT:

Mean age of the male volunteers is 25.75 and of female volunteers is 24.9. The average BMI of the male volunteers is 22.55 and of female volunteers is 23.25 and the difference is statistically not significant. The male volunteers exercised at mean Vo_2 max of the is 29.56 ml/(kgxmin) and of female volunteers at 34.29 ml/(kg x min). There is significant gender difference between male and female volunteers (n=20)(*p* value <0.0001) in Vo_2max [Table 1].Comparison of Activity Energy Expenditure (AEE) before and after exercise in healthy individuals is done by paired t test. The difference is statistically significant. (*p* value<0.0001)(n = 40)[Table 2]. This explains that significant amount of work done in this exercise (YMCA sub maximal exercise protocol). Serum creatine phosphokinase level showed increase (*p* value<0.0001) in case of both male and female volunteers after single exercise session (n = 40). Serum Nitrate-Nitrite ratio (nitric oxide level) showed statistically insignificant increase after 10 minutes of single moderate exercise session in young volunteers. (*p* value 0.1113)(n =40)[Table 2].

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Table 1: All values (n = 20, 20 male & 20 female) are expressed in Mean ± SD

Parameters	Male (mean ± SD)	Female (mean ± SD)	*P* - Value
Age (yrs)	25.75 ± 2.731	24.9 ± 2.69	0.3279
Weight (kg)	65.85 ± 7.22	50.3 ± 4.82	< 0.0001
Height (mt)	1.7 ± 0.07	1.47 ± 0.10	< 0.0001
BMI	22.55 ± 1.87	23.25 ± 1.99	0.2605
VO_2 max [ml/(kg x min)]	29.56± 1.43	34.29 ± 1.01	< 0.0001

Table 2: All values (n = 40, 20 male & 20 female) are expressed in Mean ± SD .There is two groups of variable, pretest level & post test level

Parameters	Pre test (mean ±SD)	Post test (mean ±SD)	*P* - value
Activity Energy Expenditure (AEE)	19.58 ± 3.87	28.95 ± 3.90	<0.0001
Serum CPK	129 ± 12.79	166 ± 19.51	<0.0001
Serum Nitrate-Nitrite ratio	1.58 ± 0.55	1.74 ± 0.54	0.1113

Gender specific sub analysis:

Comparison of mean of all biochemical parameters and Activity energy expenditure in male and female volunteers separately is analyzed by unpaired t test.

The difference between males and females in Activity energy expenditure (AEE) is statistically significant(*P* – value < 0.0001) [Table 3] . Serum CPK (*P* – value < 0.0001) showed significant gender difference. There is increased level in male volunteers [Table 3]. The change of serum Nitrate-Nitrite ratio before and after exercise [impact of exercise] is compared between males and females. The difference between males and females is statistically not significant (*p* value 0.7183). [Table 3]

Table 3: Gender specific sub analysis

Parameter	male	Female	P - value
Pre-exercise AEE (mean ±SD)	22.2 ± 3.42	16.95 ± 2.13	< 0.0001
Post-exercise AEE (mean ±SD)	31.3 ± 3.67	26.6 ± 2.50	
Pre-exercise CPK level (mean ±SD)	140.9 ± 4.14	117.3 ± 5.15	< 0.0001
Post-exercise CPK level (mean ±SD)	184.9 ± 5.91	148 ± 5.44	
Pre-exercise Nitrate-Nitrite ratio (mean ±SD)	1.61 ± 0.50	1.55 ± 0.61	0.7183
Post-exercise Nitrate-Nitrite ratio (mean ±SD)	1.76 ± 0.47	1.72 ± 0.62	

Bivariate correlation analysis was done in both pre and post exercise level. It was done between Nitrate and Nitrite Ratio (Nitric oxide metabolite) CPK and Activity energy expenditure (AEE) in the whole cohort n=40, done by Pearson's correlation coefficient *r* .In Pre and post exercise level good positive correlation is present between Activity Energy Expenditure (AEE) with CPK (Pre exercise r =0.67[scatter diagram 1], post exercise r =0.55[scatter diagram 2]).

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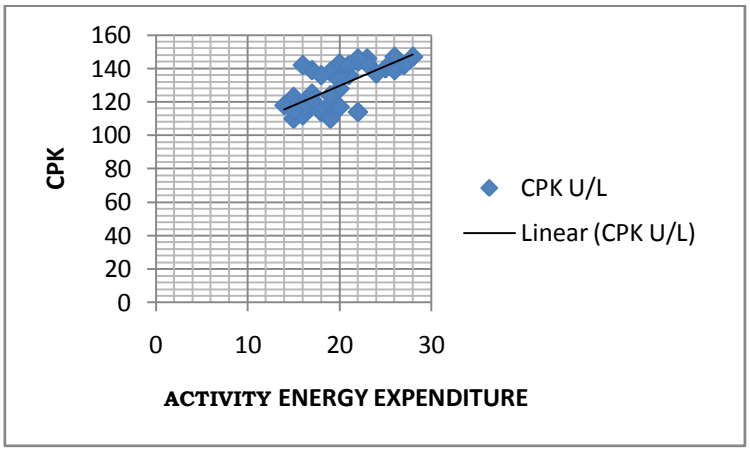

Diagram 1: In Pre exercise level, r =0.67

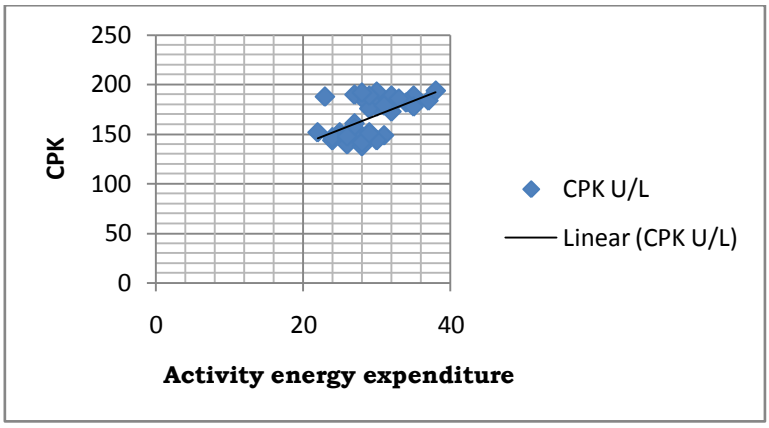

Diagram 2: In post exercise level, r =0.55

DISCUSSION:

It is known that BMR, DIT (diet induced thermo genesis), PA (physical activity energy expenditure) are the components for calculation of net EE (energy expenditure) in an individual, of which PA is the most variable component. The difference of Activity Energy Expenditure before and after exercise is statistically significant among young healthy volunteers having normal BMI. This explains that significant amount of moderate intensity work done in this YMCA sub maximal exercise protocol. Levels of VO_2max indicates the aerobic physical fitness of our volunteers.

Creatine Phosphokinase levels depend on age, gender, race, muscle mass, physical activity and climatic condition. In our study significant increase in CPK concentration serves as an indicator of considerable muscle activity during exercise. In pre & post- exercise level, CPK shows good correlation with AEE and significant increase in male volunteers. It is evident from the studies of Nazmi Sarita et.al CPK level was seen immediately after the exercise in highly trained young individuals and slow return to baseline levels in CK activity[7].

In this study we measured serum Nitrate-Nitrite ratio which is a surrogate marker of vasodilator Nitric oxide. A single 10 minutes session of exercise elicits an acute insignificant elevation of NO production. When gender specific sub analysis is done considering all male and female serum Nitrate-Nitrite ratio values, it showed statistically insignificant gender difference. After exercise training as evident from animal and human studies both urinary and plasma nitrate/nitrite ratio is increased[8,9,10,11] Two hours of cycling in ergometer showed elevated plasma nitrate respectively, in athletes and non athletes, compared with resting nitrate before exercise. [12] A separate study involving Nigerian subjects (19-45), revealed that there was insignificant increase in NO in subjects performing moderate exercise.[13] Kanami Yamamotoa et. al (2007) revealed no statistically significant differences in the means of serum NO level expressed as ratio of serum nitrate/nitrite concentrations in the pre- *vs.* post cycling exercises.[14]

This insignificant rise in the post exercise level of NO in the present instance may have a two- fold origin. The shear stress produced in the vascular lumen stimulates the endothelium to produced NO. It may be such that in the present case the duration of 10 minutes exercise of moderate intensity may not be enough to produce a work load sufficient to induce adequate shear stress in the vessels of our healthy young subjects with a physiological reserve culminating in high coping capabilities. On the other hand, studies undertaking in subjects with *a priori* impaired NO-related vasodilator function produced improvement in conduit or resistance vessel function, whereas studies in healthy normal subjects have been less consistent. That is, depressed

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endothelial function is more amenable to improvement by exercise training than is 'normal' endothelial function in the young and healthy, which might need a higher intensity or volume of exercise training for benefit to be apparent [15]. Presence of confounding effects of unforeseen variables (heterogeneity of ethnicity and religious distribution, dietary habits, physical activity etc) that influence the dependent and independent variables are not included in the present study may contribute to the futile attempt.

CONCLUSION:

The magnitude of the acute single session exercise effect is influenced by characteristics of the exercise intervention, nutritional status and fitness of individual (as assessed by heart rate and VO_2max) regarding age, gender and body mass. In spite of considerable muscular activity, evident from increased CPK level the duration of 10 minutes exercise of moderate intensity may not be enough to produce a work load sufficient to induce adequate shear stress in the vessels of healthy young subjects with a high coping capabilities. Beneficial effect of vasodilator Nitric oxide is not observed in single session exercise. From this study, it can be suggested that the effect of single session exercise on vasodilator response is not mediated by increase nitric oxide release from endothelium.

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