HIGH INTENSITY EXERCISE INDUCED ALTERATION OF HAEMATOLOGICAL PROFILE IN SEDENTARY PREPUBERTAL AND POSTPUBERTAL BOYS: A COMPARATIVE STUDY

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The purpose of the study was to investigate the influence of high intensity incremental running exercise induced perturbations of haematological profile in sedentary prepubertal (n=32, age = 10.21 ± 0.14 years) and postpubertal (n=32, age = 15.58 ± 0.10 years) boys. Blood samples were collected before and immediately after exercise to assess hematological parameters like RBC count, WBC count, haematocrit, haemoglobin concentration, absolute and relative count of lymphocyte, monocyte, neutrophil, eosinophil and basophil. The baseline RBC count, hemoglobin (Hb) concentration and haematocrit increased significantly as a function of age with attainment of puberty while total resting WBC count, absolute and relative lymphocyte and monocyte count declined steadily with increase in age. Blood RBC count, Hb concentration and haematocrit did not change significantly after exercise in both the groups. However, high intensity exercise stress resulted in significant increase in total leukocyte count in both the groups. The postpubertal boys reported significantly higher leucocytosis as compared to the prepubertal boys. The magnitude of lymphocytosis and neutrophilia was also significantly higher in postpubertal boys as evident from significantly higher percentage increase in absolute lymphocyte and neutrophil count. Significant positive correlation of age with percentage increase in absolute lymphocyte and neutrophil count indicated that the rate of mobilization of lymphocyte and neutrophil in response to exercise increased with advancement of age. However, the extent of monocyte trafficking into circulation following exercise was significantly higher in prepubertal boys and the extent of exercise induced monocytosis decreased with age. The relative proportion of lymphocyte in total leukocyte count was significantly higher in prepubertal boys while relative neutrophil count in total white blood cells remained significantly higher in postpubertal boys both before and after exercise. Despite of having higher absolute and relative neutrophil count than the prepubertal boys, the relative neutrophil count in postpubertal boys decreased in response to exercise stress possibly on account of comparatively higher rate of lymphocytosis as opposed to magnitude of neutrophilia after exercise in this group. Although exercise resulted in significant eosinophlia and basophilia in both the groups, pubertal transition seems to have no influence on exercise induced trafficking of these two variables. Therefore, the present investigation depicted that the postpubertal boys might be comparatively more prone to exercise induced perturbations in blood cell count than the prepubertal boys on account of higher rate of leucocytosis mediated by significantly higher lymphocytosis and neutrophila.

Increased total leukocyte count especially neutrophil count is widely used to indicate immune system perturbation in relation to activation of inflammatory process and such

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changes in immune cell count has been shown to be associated with development of various pathological conditions like cardiovascular disease, type-2 diabetes and renal disease (Mochizuki *et al*., 2012; Manabe *et al*., 2011). Moreover, leukocyte count and neutrophil level have been shown to be positively associated with cardiovascular disease risk factor (Oda and Kawai 2010). Regular moderate exercise improved immune system while high intensity exercise or overtraining might cause adverse perturbations in immune system (Nieman 1994). Acute high intensity exercise increased WBC count in circulation due to increased trafficking of blood cells into circulation (Edwards *et al.,* 2007; Timmons *et al.,* 2006). While some of the previous studies reported that the extent of perturbation in Leukocyte count depends on intensity, duration and individual fitness level, some other studies reported no significant influence of exercise trial on leukocyte subset counts (Da Silva *et al*., 2015; Edwards *et al*., 2007; Kendall *et al*., 1990; Nieman *et al*., 2007). Da Silva *et al*., (2015) reported that high intensity exercise led to significant leucocytosis mediated mostly by transitory lymphocytosis and monocytosis, followed by a delayed neutrophilia in young adults. Although several studies have examined the impact of high intensity exercise in blood immune cell perturbation in adult population (Da Silva *et al*., 2015; Kendall *et al*., 1990), very few investigations examined the impact of high intensity exercise on paediatric and adolescent population. Christensen and Hill (1987) previously reported that concentration of circulating neutrophil, eosinophil and lymphocyte increased following exercise in teenage athletes. One of the classical immune response to exercise stress in adult population has been a sustained neutrophilia during one to five hours following exercise (Pedersen and Hoffman-Goetz 2000). Some of the paediatric studies have reported similar neutrophilia immediately after acute exercise in both prepubertal (Timmons *et al.,* 2004) and postpubertal boys (Timmons 2006).

Impact of pubertal attainment on exercise induced perturbations in blood cell count has not been adequately investigated, especially among sedentary subjects. One of the previous studies (Timmons *et al*., 2006) examined the influence of exercise stress on immune function by comparing the extent of perturbation in cellular immune function among young and older boys and girls belonging to the age group of 12-14 years following cycling exercise. However, the study did not specifically divide the subjects into pre- and post-pubertal group and some of the subjects in young group were in the early pubertal state. However, influence of puberty on exercise induced changes in haematological parameters can be better assessed by directly comparing such changes following exercise among prepubertal boys with those who have already attained puberty. Moreover, to our knowledge no previous study has directly examined and compared the exercise induced alteration in blood cell count between sedentary prepubertal and postpubertal boys following high intensity incremental running exercise. Moreover, literature on exercise induced perturbations of haematological profile is scanty in Indian context and unavailable in Indian paediatric population.

The present investigation was therefore aimed to examine the impact of high intensity incremental treadmill running on baseline as well as exercise induced changes in total RBC count, WBC count, haematocrit, hemoglobin concentration, absolute and relative count of lymphocyte, monocyte, neutrophil, eosinophil and basophil among sedentary prepubertal and postpubertal boys.

MATERIALS AND METHODS

Subjects:

Sedentary school going prepubertal (n = 32, age = 10.21 ± 0.14 years) and postpubertal (n = 32, age =15.58 ± 0.10 years) boys were randomly taken for the study. The participants neither took part in any physical conditioning programme nor associated with any professional sport. Subjects with history of any major disease(s) or undergoing any medication were excluded from the study. Attainment of puberty was assessed by Tanner's staging criterion (Tanner, 1962). The pre-pubertal boys were in Tanner's stage I while the postpubertal boys belonged to Tanner's stage III to IV. The subjects did not participate in any physical exercise from two weeks prior to the experimental trial. Written informed consent was obtained from all the subjects and their parents. The study was approved by the Human Ethics Committee of the Department of Physiology, University of Calcutta.

Familiarisation Trial:

The familiarisation trial was conducted 3 weeks before the experimental trial not only to familiarise the subjects with the experimental protocol but also to select the speed and inclination of the treadmill at which the subjects attained their 80% of age-predicted maximum heart rate (HRmax) as calculated from standard equation (Pal *et al.*, 2017). Physical examination of the subjects was performed by recording their pre-exercise heart rate, blood pressure and electrocardiograph. The trial involved a progressively incremental treadmill (Viasys, Germany) running by increasing the speed (*2* $km.h^{-1)}$ and inclination (1%) alternatively after each 3 minutes until 80% of HRmax was reached (Pal *et al.*, 2017).

Experimental Protocol:

Subjects reported at the laboratory at 8 AM after an overnight fast of 12 hrs. They were asked to take rest for 30 minutes on an easy chair. A heart rate monitor was secured on the subject's chest to monitor the resting, exercising and recovery heart rates. The pre-exercise heart rate and blood pressure were recorded after the resting period.

Subjects performed warm up exercise at a speed of 3 $km.h^{-1}$ at 0% elevation for 5 minutes followed by progressive incremental treadmill running with change in speed (by 2 $km.h^{-1}$) and elevation (by 1%) alternatively after each 3 minutes to reach the specific speed and grade that elicited 80% of HRmax during the pre-experimental trial. The subjects continued to exercise at that specified speed and inclination until onset of fatigue that was indicated by volitional exhaustion.

Blood samples were collected from antecubital vein just before commencement of the exercise trial (T1) and immediately after cessation of exercise (T2) for estimation of different haematological parameters.

Estimation of haematocrit Parameters:

RBC Count, haematocrit, haemoglobin (Hb) concentration, total leucocyte count and differential leucocyte count were estimated using Sysmex XT 400i automated hematology analyser.

Statistical Analysis:

Statistical package for social sciences (SPSS, v 20.0; Chicago, IL) was used for the statistical analysis of the data. Data have been presented as mean ± SE. Paired t-tests were performed separately in each group to locate significant difference between the mean values of blood cell count and haematological parameters measured before and after the exercise trial. Independent sample t-tests were performed to locate any significant difference in mean values of the parameters between two groups and also to find out significant inter-group difference in the mean value of percentage change in each parameter following exercise. Pearson's product-moment correlations were performed to assess the strength and direction of association between values of the haematological parameters and age of the subjects. The level of significance was set at $p<0.05$.

RESULTS

Physical characteristics, body mass index (BMI) pre-exercise heart rate, blood pressure, running time to exhaustion and distance covered by subjects during exercise trial have been presented in Table 1. Postpubertal boys had significantly higher BMI ($p<0.001$), body height ($p<0.001$), body weight ($p<0.001$), pre-exercise heart rate ($p<0.05$), systolic and diastolic blood pressure ($p<0.001$) as compared to their prepubertal counterparts. However, there were no significant inter-group differences in mean running time to exhaustion and average distance covered during treadmill running (Table 1).

Table 1: Physical characteristics, pre-exercise heart rate, blood pressure, mean running time and distance covered by the subjects.

Groups	Body height (cm)	Body weight (kg)	BMI ($kg.m^{-2}$)	Pre-exercise Heart Rate ($beats.min^{-1}$)	Blood Pressure (mm of Hg)		Running time (min)	Distance covered (km)
					Systolic	Diastolic		
Prepubertal Boys (n=32)	130.77 ± 0.92	22.11 ± 0.43	12.94 ±0.25	68.66 ± 0.69	95.84 ± 1.03	59.56 ± 0.93	51.58 ± 0.69	7.18 ± 0.12
Postpubertal Boys (n=32)	158.50 ±0.73**	50.81 ±0.63**	20.25 ±0.27**	70.69 ± 0.77*	111.56 ± 0.89**	70.63 ± 0.50**	52.79 ± 1.62	7.61 ± 0.29

Values were expressed as mean ± SE, *$p<0.05$, **$p<0.001$

The pre-exercise and the post-exercise values as well as percentage change in haematological parameters have been presented in Table 2. Pre-exercise and post-exercise RBC Count, hematocrit and Hb concentration were significantly higher in postpubertal boys ($p<0.001$). No significant post-exercise perturbations and intergroup variation in percentage change were observed for these parameters after exercise. Absolute leukocyte count increased significantly in both the groups following exercise ($p<0.001$). Although, baseline total leukocyte count was significantly higher in prepubertal boys ($p<0.01$), postpubertal boys reported significantly higher post-exercise value ($p<0.001$) and percentage increase ($p<0.001$) in WBC count (Table 2).

Table-2: Changes in haematological parameters following exercise.

Variable	Group	Absolute Count		Percentage Change
		Before Exercise	After Exercise	
RBC (X 10^{12}/L)	Pre	4.52 ± 0.01	4.57 ± 0.02	1.02 ± 0.55
	Post	4.95 ± 0.05**	4.99 ± 0.06**	0.76 ± 0.53
WBC (/ cumm)	Pre	8332 ± 154*	10211 ± 217 #	22.43 ± 0.74
	Post	7854 ± 34	11654 ± 96#**	48.51 ± 1.44**
Hematocrit (%)	Pre	37.06 ± 0.17	37.11 ± 0.14	0.14 ± 0.12
	Post	40.37 ± 0.37**	40.48 ± 0.37**	0.28 ± 0.19
Hb (g/dl)	Pre	12.35 ± 0.05	12.37 ± 0.04	0.15 ± 0.12
	Post	13.45 ± 0.12**	13.49 ± 0.12**	0.28 ± 0.20

Values are expressed as Means ± SE. Pre = Prepubertal, Post = Postpubertal, #p<0.001 when compared with the pre-exercise value within the group, *p<0.01 between the groups at respective time points, **p<0.001 between the groups at respective time points.

The absolute and relative count of leukocyte subtypes has been presented in Table 3. Prepubertal boys had significantly higher pre-exercise absolute lymphocyte and monocyte count (p<0.001) (Table 3). The baseline relative neutrophil count was significantly higher in postpubertal boys (p<0.001), while pre-exercise relative lymphocyte and monocyte count were significantly higher in prepubertal boys (p<0.001) (Table 3).

Table-3: Changes in absolute and relative leucocyte count following exercise.

Variable	Group	Absolute Count		Percentage Change in Absolute Count	Relative Count (%)		Percentage Change in Relative Count
		Before Exercise	After Exercise		Before Exercise	After Exercise	
Lymphocyte (/ cumm)	Pre	2797 ±56***	3448 ±84###	22.97 ±0.61	33.72 ±0.59***	33.87 ±0.57##**	0.47 ±0.14
	Post	2237 ±32	3684 ±76###*	64.45 ±1.85***	28.54 ±0.51	31.60 ±0.60###	10.70 ±0.36***
Monocyte (/ cumm)	Pre	309 ±8***	450 ±14###***	45.17 ±2.10***	3.71 ±0.06***	4.38 ±0.06###***	18.65 ±1.72***
	Post	240 ±8	258 ±10##	7.19 ±2.54	3.05 ±0.09	2.20 ±0.08###	-27.89 ±1.53
Neutrophil (/ cumm)	Pre	4660 ±107	5721 ±130###	22.88 ±0.86	55.88 ±0.61	56.05 ±0.56###	0.35 ±0.15***
	Post	4829 ±65	7125 ±77###***	47.86 ±1.32***	61.42 ±0.57***	61.19 ±0.63##***	-0.41 ±0.13

Variable	Group	Absolute Count		Percentage Change in Absolute Count	Relative Count (%)		Percentage Change in Relative Count
		Before Exercise	After Exercise		Before Exercise	After Exercise	
EosinophilL (/cumm)	Pre	550 ±32	572 ±33###	4.26 ±0.76	6.52 ±0.34	5.52 ±0.28###*	-14.83 ±0.39
	Post	533 ±18	569 ±23#	6.63 ±2.36	6.80 ±0.23	4.85 ±0.16###	-28.36 ±1.12***
Basophil (/cumm)	Pre	15 ±0.30	17 ±0.44###	17.98 ±3.53	0.177 ±0.004	0.172 ±0.007	-3.62 ±2.82
	Post	15 ±0.20	19 ±0.21###**	27.25 ±1.05*	0.186 ±0.003	0.159 ±0.001###	-14.00 ±1.24**

Values are expressed as Means ± SE. Pre = Prepubertal, Post = Postpubertal. Negative values indicate decrease.
Significant change within group from pre-exercise value: p < 0.001.
*** Significant Difference between group at respective time points: p < 0.001.
Significant change within group from pre-exercise value: p < 0.01.
** Significant Difference between group at respective time points: p < 0.01.
# Significant change within group from pre-exercise value: p < 0.05.
* Significant Difference between group at respective time points: p < 0.05.

There were significant increases in post-exercise absolute counts of lymphocyte (p<0.001), neutrophil (p<0.001), basophil (p<0.001), monocyte and eosinophil in both the groups following exercise. Post-exercise absolute lymphocyte (p<0.05), neutrophil (p<0.001) and basophil count (p<0.01) was significantly higher in postpubertal boys. While postpubertal boys reported significantly higher percentage increase in absolute lymphocyte (p<0.001), neutrophil (p<0.001) and basophil count (p<0.05), prepubertal boys had significantly higher post-exercise value and percentage increase in absolute monocyte count (p<0.001) (Table 3).

Relative count of lymphocyte increased significantly in both the groups with significantly higher percentage increase in postpubertal boys (p<0.001). In postpubertal boys relative neutrophil (p<0.01) and monocyte count (p<0.001) decreased significantly following exercise while these two parameters increased significantly in prepubertal boys (p<0.001). Relative proportion of eosinophil and basophil decreased significantly in prepubertal boys after exercise while only relative basophil count decreased significantly in postpubertal group. Prepubertal boys reported significantly higher post-exercise relative lymphocyte (p<0.01), monocyte (p<0.001) and eosinophil (p<0.05) count, whereas postpubertal boys had significantly higher post-exercise relative neutrophil count. Percentage increase in relative lymphocyte count was significantly higher in postpubertal boys (p<0.001) while percentage change in monocyte and neutrophil count was significantly higher in prepubertal boys (p<0.001). Magnitude of percentage decrease in eosinophil and basophil was significantly higher in pospubertal boys (Table 3).

Baseline RBC count had significant positive correlation with age (p<0.001) while WBC count ($p<0.01$), absolute lymphocyte ($p<0.001$) and monocyte count ($p<0.001$) showed significant negative correlation with age. Post-exercise RBC ($p<0.001$), WBC ($p<0.001$), absolute neutrophil ($p<0.001$) and basophil count ($p<0.01$) also had significant positive correlation with age. Percentage change in WBC ($p<0.001$), absolute lymphocyte ($p<0.001$), neutrophil ($p<0.001$) and basophil count ($p<0.001$) also showed significant positive correlation with age. However post-exercise monocyte count and percentage change had significant negative correlation with age ($p<0.001$). Both Pre-exercise and post-exercise relative lymphocyte and monocyte count showed significant negative correlation with age while neutrophil count had significant positive correlation (Table 4).

Table-4: Values of correlation coefficient between age and different haematological parameters before and after the exercise trial.

	Values of correlation coefficient (r) with age						
	RBC	WBC	Lymphocyte	Monocyte	Neutrophil	Eosinophil	Basophil
Pre-exercise absolute count	0.652***	-0.375**	-0.754***	-0.618***	0.164	-0.079	-0.005
Post-exercise absolute count	0.602**	0.573***	0.214	-0.807***	0.738***	-0.033	0.343**
Percentage change in absolute count of	0.037	0.868***	0.914***	-0.816***	0.866***	0.11	0.308*
Pre-exercise relative count	-	-	-0.644***	-0.577***	0.649***	0.067	0.236
Post-exercise relative count	-	-	-0.366**	-0.921***	0.616***	-0.266*	-0.17

*p<0.05, **p<0.01, ***p<0.001.

DISCUSSION:

Exercise leads to transient perturbation in blood cell count especially lymphocyte and neutrophil which reflects stress induced alteration of immune function and inflammatory response to exercise. The present investigation examined the influence of pubertal transition and age on exercise induced perturbations in blood cell count and haematological parameters among sedentary boys after challenging them with novel high intensity incremental treadmill running.

Significantly higher pre-exercise and post-exercise erythrocyte (RBC) count in postpubertal boys ($p<0.001$) along with significant positive correlation of age with both pre-exercise ($r = 0.652$, $p<0.001$) and post-exercise RBC count ($r = 0.602$, $p<0.01$) suggested that postpubertal boys have higher RBC count both before and after exercise and RBC count increased with advancement of age. Similarly, the present study also depicted that Hb concentration and haematocrit also increased with advancement in age. This finding may be due to the fact that androgens like testosterone during puberty up-regulated the rate of erythropoiesis as well as increased formation of Hb (Hero *et al.,* 2005). However, the present investigation depicted that acute exercise had no immediate impact on RBC count, Hb concentration and haematocrit as evident from insignificant changes in respective post-exercise values from the pre-exercise levels. Similar insignificant percentage change in RBC count, Hb concentration and hematocrit in both the groups following exercise together with lack of any significant correlation between age and post-exercise percentage change in these parameters also suggested that pubertal attainment did not have any differential impact on exercise induced perturbations in these haematological parameters.

The present study also depicted that the baseline total leukocyte count (WBC) was significantly higher in prepubertal boys as compared to their postpubertal counterparts ($p<0.01$). Moreover, there was significant negative correlation between age and pre-exercise value of total leukocyte count ($r = -0.375$, $p<0.01$) which suggested that unlike RBC count, baseline leukocyte count decreased with advancement of age. This finding of the current investigation corroborated with previous studies which indicated that total leukocyte count decreased consistently throughout childhood (Erkeller-Yuksel *et al.* 1992; Shahabuddin 1998 *et al*., Shearer *et al.,* 2003). Exercise resulted in profuse leukocytosis in both the groups as indicated by significant increase in total leukocyte count in both the groups following exercise ($p<0.001$). Exposure to acute stress such as high intensity physical exercise had been shown to induce leukocytosis, the extent of which is dependent on duration and intensity of exercise (Edwards *et al.,* 2007; Timmons *et al.,* 2006; Da Silva Neves *et al.,* 2015). Studies suggested that such exercise induced leukocytosis was mostly caused by release of lymphocyte, monocytes and neutrophil (Da Silva *et al.,* 2015, Timmons 2004; 2006) into the circulation in response to exercise stress. High intensity exercise leads to an intensity dependent increase in sympathetic activity resulting in increased release of catecholamines like epinephrine and norepinephrine which are responsible for increased trafficking of lymphocyte from spleen (Kruger 2008, Natale *et al.,* 2003; Benschop *et al.,* 1996). On the other hand, neutrophils are mobilised into the peripheral circulation in response to exercise mostly from the bone marrow and other marginated pools like lungs leading to profuse leukocytosis (Timmons 2005). One of the important findings of the present study was that the extent of leukocytosis was significantly higher in postpubertal boys as evident from significantly higher post-exercise value ($p<0.001$) and more than double percentage increase in the postpubertal boys ($p<0.001$) despite of having lower baseline total leukocyte count as compared to their prepubertal counterparts. The magnitude of leukocytosis increased as a function of age which is indicted by significant positive correlation of age with percentage increase in WBC count ($r =0.868$, $p<0.001$).

Examination of WBC sub-types suggested that baseline absolute and relative lymphocyte count was significantly higher in prepubertal boys as evident from significantly higher baseline absolute and relative lymphocyte count ($p<0.001$). Moreover, significant negative correlation of age with both pre-exercise absolute ($r = -0.754$, $p<0.001$) and relative lymphocyte count ($r = -0.644$, $p<0.001$) also suggested that basal level lymphocyte count as well as their relative proportion in circulation was significantly higher before puberty and the same decreased significantly with advancement of age and pubertal transition. Previous studies also suggested that total peripheral lymphocyte pool and absolute lymphocyte count gradually decreased during childhood (Erkeller-Yuksel *et al.,* 1992; Shahabuddin 1998 *et al.,* Shearer *et al.,* 2003; Timmons 2005). Present investigation clearly suggested that not only the absolute count but also the relative proportion of lymphocyte in circulation decreased gradually with advancement of age. Exercise stress led to immediate lymphocytosis in both the age groups. The immediate increase in lymphocyte count after exercise might be attributed to the increase in sympathetic activity following exercise stress which resulted in an intensity dependent increase in catecholamines like epinephrine and norepinephrine (Kruger 2008). These hormones are responsible for increased trafficking of lymphocyte following exercise from lymphoid organs like spleen (Natale *et al.,* 2003). However, the post-exercise value ($p<0.05$) and the magnitude of increase in absolute lymphocyte count as measured by percentage increase in this parameter was significantly higher in postpubertal boys ($p<0.001$) despite of the fact that the postpubertal boys had significantly lower pre-exercise absolute lymphocyte count as opposed to postpubertal boys ($p<0.001$). Postpubertal boys recorded more than three times higher percentage increase in lymphocyte count as compared to their prepubertal counterpart and the extent of percentage increase in absolute lymphocyte count had significant positive correlation with age ($r = 0.914$, $p<0.001$). This indicated that postpubertal boys are relatively more prone to exercise induced purturbations in blood lymphocyte count. Although the percentage increase in relative lymphocyte count was significantly higher in postpubertal boys ($p<0.001$) (10.70% increase in postpubertal boys as opposed to 0.47% in prepubertal boys), the post-exercise value of relative lymphocyte count was still higher in prepubertal boys ($p<0.01$). This suggested that despite of robust post-exercise increase in both absolute and relative lymphocyte count in postpubertal boys, the relative proportion of lymphocyte in total WBC count (i.e., total leukocyte in circulation) still remained significantly higher in prepubertal boys. This notion is further supported by the fact that there was significant negative correlation of age with both pre-exercise ($r = -0.644$, $p<0.001$) and post-exercise relative lymphocyte count ($r = 0.366$, $p<0.01$).

Exercise stress also led to significant monocytosis in both the groups. However, prepubertal boys had significantly higher absolute and relative monocyte count both before and after exercise ($p<0.001$). Significant negative correlation of age with baseline absolute ($r = -0.618$, $p<0.01$) and relative monocyte count ($r = -0.577$, $p<0.001$) indicated that the resting monocyte level decreased as a function of age. Significantly higher percentage increase in absolute monocyte count following exercise in prepubertal boys ($p<0.001$) together with significant negative correlation of age with post-exercise percentage increase ($r = -0.816$, $p<0.01$) indicated that the rate and extent of monocyte trafficking into circulation following exercise was relatively higher in prepubertal boys as compared to their postpubertal counterpart. Although relative proportion of monocyte in total WBC count increased in prepubertal boys by 18.65%, this parameter decreased by 27.89% in postpubertal boys despite of a significant increase in absolute count following exercise. Therefore, it is evident that in postpubertal boys the trafficking of other blood cells like lymphocyte into circulation was possibly much higher as compared to monocyte following exercise which might have resulted in decreased relative proportion of monocyte in circulation.

Exercise also caused significant neutrophilia in both the groups following the exercise trial as evident from significantly higher post-exercise absolute neutrophil count in both the age groups ($p<0.001$). Previous studies confirmed similar immediate neutrophilia in response to acute exercise in both prepubertal (Timmons *et al.,* 2004) and postpubertal boys (Timmons *et al.,* 2006). It is also apparent from the present study that extent of mobilization of neutrophil into circulation in response to exercise stress was significantly higher in postpubertal boys as indicated by significantly higher post exercise value and percentage increase in absolute neutrophil count ($p<0.001$). Examination of relative neutrophil count also suggested that postpubertal boys had significantly higher relative neutrophil count both before and after exercise ($p<0.001$). Age of the subjects had significant positive correlation with post-exercise absolute ($r = 0.738$, $p<0.001$) and relative neutrophil count ($r = 0.616$, $p<0.001$) as well as percentage increase in absolute count ($r = 0.866$, $p<0.001$) which also indicated that the absolute and relative proportion of neutrophil in total leukocyte count is always higher in post-pubertal boys. Although adequate experimental evidences regarding exercise induced neutrophilia is lacking in the literature for this age group, one of the previous studies reported similar result that the adolescents are likely to have higher rate of exercise induced neutrophilia as compared to children (Timmons *et al.,* 2006). It has been previously reported that exercise induced increase in cortisol level may have a direct role in post-exercise neutrophilia and individuals with higher post exercise cortisol level are likely to have higher exercise induced neutrophilia (Timmons *et al.,* 2004, 2006). Research also indicated that Interleukin-6 (IL-6) may be another potential mediator of exercise induced mobilization of neutrophil into circulation and exercise induced release of IL-6 from working muscle might also be responsible for elevated secretion of cortisol during acute high intensity exercise (Steensberg *et al.,* 2003). Previous studies also showed statistical association between post-exercise IL-6 level and neutrophil counts following exercise (Yamada *et al.,* 2002; Timmons *et al.,* 2005; Suzuki *et al.,* 1999). Infusion of IL-6 also resulted in increase of systemic level of neutrophils in circulation in rabbit (Suwa *et al.,* 2000). Previous research indicated that children might experience substantially lower exercise induced IL-6 response as compared to adults (Timmons *et al.,* 2006) and such response is directly related to muscle mass recruited during exercise (Febbraio and Pedersen, 2002). Hence a smaller muscle mass in prepubertal boys might have accounted for relatively lower exercise induced neutrophilia on account of lower IL-6 response as compared to postpubertal boys. However, further studies are required to establish this fact. Post-exercise elevation in neutrophil count has also been attributed to the inflammatory response to exercise induced muscle damage wherein neutrophils are recruited to the damaged muscle fibres to remove the dead tissues (Stupka 2000, Suzuki *et al.,* 1999).Therefore, total muscle mass of the subjects might be an important determinant of the extent of post-exercise neutrophilia. Timmons *et al.,* (2006) previously indicated that post-exercise neutrophilia might be significantly correlated with fat-free mass in healthy children and adolescents Research also indicated that adolescent boys experienced relatively higher exercise induced muscle damage as compared to prepubertal boys (Marginson *et al.,* 2005; Chen *et al.,* 2014). Therefore, higher muscle mass in postpubertal boys together with higher IL-6 response and higher extent of muscle damage might have accounted for higher recruitment of neutrophils in response to exercise in postpubertal boys.

One of the important findings of the present study is that although absolute and relative neutrophil count was consistently higher in postpubertal boys as compared to their prepubertal counterparts, the relative neutrophil count actually decreased significantly in postpubertal boys after exercise ($p<0.01$) while it increased in prepubertal boys from basal level ($p<0.001$) in response to exercise stress. The relative proportion of neutrophil in total leukocyte count decreased in postpubertal boys following exercise possibly on account of comparatively higher rate of mobilization of lymphocyte (lymphocytosis) as opposed to release of neutrophil (neutrophilia) in circulation after exercise. This factor might have accounted for slight decline in relative proportion of neutrophil in total leukocyte count following exercise in postpubertal boys despite of having higher value of absolute and relative count as compared to prepubertal boys. This finding is further supported by the fact that postpubertal boys reported 10.70% increase in relative lymphocyte count following exercise while relative neutrophil count reduced by 0.41 % after exercise. This indicated that the rate of exercise induced lymphocytosis is relatively higher in postpubertal boys as compared to magnitude of neutrophilia.

Absolute eosinophil and basophil count also increased significantly in both the groups but no intergroup differences could be noted in exercise induced trafficking of eosinophil and basophil as indicated by similar post exercise increase in both the groups. However, there was significant decrease in relative count of these two variables following exercise despite of increase in absolute count with postpubertal boys reporting significantly higher percentage decrease in relative count as compared to the prepubertal group. Since the relative rate of increase in lymphocyte and neutrophil count following exercise was much higher in both the groups as compared to exercise induced trafficking of eosinophil and basophil into circulation, there was a decrease in relative proportion of these two parameters in total WBC count.

CONCLUSION

Pubertal transition was accompanied by substantial up regulation of baseline RBC count and Hb concentration along with increase in heamatocrit value. However, neither acute high intensity exercise nor pubertal maturation seemed to have any significant influence on these variables. Pubertal maturation led to progressive decline in basal WBC, lymphocyte and monocyte count. Exercise stress caused substantial perturbation in leukocyte count in both the groups mostly on account of exercise induced lymphocytosis, monocytosis and neutrophilia. However, postpubertal boys were relatively more prone to exercise induced perturbation in leukocyte count which appeared to increase as a function of age. High intensity exercise resulted in decrease in relative neutrophil count in postpubertal boys due to comparatively higher rate of exercise induced lymphocytosis as opposed to neutrophilia in this group. Although exercise resulted in significant eosinophlia and basophilia in both the groups, pubertal transition appeared to have no influence on exercise induced trafficking of these two parameters.

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