



## Hematological, Hormonal and Fitness Indices in Youth Swimmers: Gender-Related Comparisons

by

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*This study objective was to evaluate gender differences in hematological, hormonal and fitness variables among youth swimmers and to explore relationships between erythrocyte indices and aerobic and anaerobic capacity. 137 girls and 171 boys participated in the study and were divided into three groups based on their training experience. Blood samples were obtained to determine red blood cell counts, hemoglobin concentration, hematocrit, and plasma erythropoietin and testosterone levels.  $VO_{2max}$  was assessed using a submaximal cycle protocol. 76 girls and 102 boys also undertook a Wingate test to determine their peak anaerobic power. Boys had higher ( $p < 0.05$ ) means than girls for all hematological variables except for erythropoietin and these variables demonstrated an increase with training in boys. The average  $VO_{2max}$  in  $l \cdot min^{-1}$  and peak anaerobic power in watts were also higher in boys ( $2.91 \pm 0.08$  and  $547 \pm 28$ , respectively) than girls ( $2.25 \pm 0.07$  and  $450 \pm 26$ , respectively). Modest but significant ( $p < 0.05$ ) correlations were found between  $VO_{2max}$  and red blood cell counts ( $r = 0.252$ ), hemoglobin concentration ( $r = 0.345$ ), or hematocrit ( $r = 0.345$ ) and between peak anaerobic power and red blood cell counts ( $r = 0.304$ ), hemoglobin concentration ( $r = 0.319$ ) or hematocrit ( $r = 0.351$ ). This study revealed relatively lower yet age- and gender-appropriate hematological, hormonal and fitness indices in youth swimmers. The gender-related differences in erythrocyte indices seem unrelated to erythropoietin and may be explained by the higher testosterone levels seen in boys. Given their correlation to both aerobic and anaerobic capacity, erythrocyte indices may be used as part of talent identification for sports.*

**Key words:** erythrocytes, testosterone, aerobic capacity, anaerobic power, training.

### Introduction

The primary role of red blood cells (RBCs) is to transport respiratory gases. Oxygen ( $O_2$ ) brought into the pulmonary circulation is bound by hemoglobin (Hb) via oxygenation. The resulting oxyhemoglobin ( $Hb-O_2$ ) is then circulated by the cardiovascular system to the periphery where  $O_2$  is released from  $Hb-O_2$  and diffuses into tissue cells. The physiological significance of  $O_2$  transport by Hb is well-illustrated by anemia where decreased Hb also decreases sports performance despite a compensatory increase in cardiac output (Eichner, 1992), and by a strong positive correlation between aerobic performance and total Hb (Schmidt and Prommer, 2008). In fact, Hb has

been regarded as a key limiting factor to maximum  $O_2$  uptake ( $VO_{2max}$ ), which predicts endurance performance. RBCs also fulfill a variety of other functions such as buffering changes in blood pH by transport of  $CO_2$  and by binding of  $H^+$  to hemoglobin.

It has long been found that endurance athletes as compared to untrained counterparts have hematological abnormalities and decreased Hb levels near to or below the lower limit of the normal range (Coates et al., 2016, Rietjens et al., 2002). Previous studies also demonstrated a decrease in RBCs, Hb, and mean corpuscular volume (MCV) following an acute bout of intense exercise (Cordova and Escanero, 1992; Yalcin et

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al., 2003). Explanations for this deformation in erythrocytes include expanded plasma volume, increased intravascular hemolysis, gastrointestinal blood loss, and insufficient dietary intake of iron. Most observations have been carried out based on middle- and long-distance running in which oxidative energy transformation is critical to successful performance.

Much less is known regarding hematological profiles in children. In particular, there is a paucity of literature regarding gender-differences in erythrocyte variables and how they may be related to fitness of young athletes. Not only is the research in this area limited, but the existing evidence is also inconsistent. Boyadjiev and Taralov (2000) observed gender differences in RBCs, Hb, and hematocrit (Hct) with boys demonstrating higher values than girls. The same research group also found lower RBC and Hb levels in pubescent swimmers and rowers as compared to their control counterparts, which suggests that erythrocyte indices may not necessarily explain gains in fitness as children train. In contrary, Eastwood et al. (2009) revealed a positive correlation between  $VO_{2max}$  and a total quantity of Hb ( $Hb_{mass}$ ) in adolescent cyclists, indicating that measuring variables related to erythrocytes can serve as an indicator of aerobic capacity. In this latter study, no gender comparisons were made due to a small sample size.

Given a direct correlation between  $VO_2$  and swimming velocity,  $VO_{2max}$  is viewed as one of the most relevant measure of functional capacity among swimmers. It is considered that swimmers with higher  $VO_{2max}$  can sustain a large training volume, swim faster and more economically, and recover faster (Pyne and Sharp, 2014; Toussaint and Hollander, 1994). In this context, it is imperative that monitoring responses of erythrocyte indices be carried out among swimmers. Most swimming events last <2 minutes. Thus, training for swimming must also emphasize developing anaerobic capacity. Children generally have limited anaerobic power due to their under-developed muscle mass, neuromuscular coordination, and glycolytic ability (Bar-Or, 1987). Inbar and Bar-Or (1986) and Van Praagh (2000) reported no appreciable gender gaps in anaerobic power in prepubescent

children. However, it is unclear if this is the case in youth athletes. In addition, no data has been found that connect erythrocyte indices with anaerobic performance.

The aim of this study was to evaluate gender differences in hematological and fitness variables among youth swimmers and to explore relationships between erythrocyte indices and aerobic as well as anaerobic capacity. By stratifying study participants based on their training experience, we were also able to assess gender differences in hematological response to training. We hypothesized that 1) boys would demonstrate higher values than girls in erythrocyte indices and aerobic and anaerobic capacity and 2) erythrocyte indices would be correlated to both aerobic and anaerobic capacity in youth swimmers.

## Methods

### *Participants*

308 youth swimmers including 137 girls and 171 boys aged 8 to 16 volunteered to participate in this study. They typically trained six days a week with each training session consisting of two hours in water and a half hour on land. Both children and their parents were informed of the purpose and procedure of the study. While written consent was obtained from their parents, each child also gave their ascent to participate. The research protocol was approved by the Ethics Committee of the Fudan University.

To assess gender differences in hematological response to training, participants within each gender were further divided into three groups according to their training experience. The level of training was measured by their qualifying time in swimming events. The following are the criteria used in forming the three groups:

- Advanced - those who qualified to compete at national-level competitions.
- Intermediate - those who qualified to compete at provincial-level competitions,
- Beginner - those who competed at various school- and district-level competitions.

The gender- and training-specific physical and physiological characteristics of the participants are presented in Table 1.

### *Experimental procedure*

Participants reported to the laboratory at ~8 am after an overnight fast and at least 24 hours

after their last training session. Body mass and height were measured using an electronic weight scale and a wall-mounted stadiometer. This was followed by measuring participants' skeletal age using a portable digital radiography system (CXDI-50G, Canon, Japan). After 10-min of sitting, blood samples were taken from antecubital veins. Participants then consumed a regular breakfast and 2 hours later returned to the laboratory for a submaximal cycle ergometry test that determined their  $\text{VO}_{2\text{max}}$ . A subset of 178 participants including 76 girls and 102 boys returned to the laboratory in the afternoon to undergo a Wingate anaerobic power test. Prior to each test, participants were reviewed with the protocol carefully and were told to report any discomfort, pain, or unusual fatigue should they occur so that the test could be terminated immediately. Research technicians were trained and were provided with a manual of testing procedures and standardized data forms to insure a high degree of accuracy and consistency in data collection.

#### *Blood assays*

Blood samples were collected by EDTA and heparin vacutainers using a HENSO vacuum blood collection system (Hensor Medical Co, China). Samples collected in tubes containing EDTA were used to determine RBCs, Hb, Hct, and MCV with a hematology analyzer (Beckman Coulter AcT 5diff Autoloader, Beckman Inc., USA) that uses the Coulter AcT 5diff diluent and has coefficients of variation (CV) of  $< 2.0\%$ ,  $< 1.0$ , and  $< 2.0$  for RBCs, Hb, and Hct, respectively. Samples collected in tubes containing heparin were used to determine plasma levels of erythropoietin (EPO) and TST using an immunoassay analyzer (Access 2, Beckman Inc., USA), which has a CV of  $< 10\%$  for both variables.

#### *Aerobic capacity test*

Aerobic capacity tests were conducted using the Åstrand-Ryhming cycle protocol (Åstrand and Ryhming, 1954). This protocol has proven reliable across a wider age range including adolescents (Åstrand, 1960). Test equipment included a stationary cycle ergometer (Monark Ergomedic 839E, Varberg, Sweden) and a Polar chest-strap telemetry system (Polar A300, Woodbury, NY, USA). Both seat height and handlebars were adjusted to ensure an optimal riding position. A heart rate (HR) chest strap was placed at the level near the participant's xiphoid process of the

sternum and tighten snugly to insure proper functioning of the HR monitor. A 10-min warm-up was performed before the test.

The protocol calls for an initial workload of 50 watts (W) and a workload progression of 50 W every six minutes until the HR reaches the target range between 120 and 170  $\text{beats}\cdot\text{min}^{-1}$  (Åstrand and Ryhming, 1954). As this study used youth participants, the protocol was modified so that the workload was increased by 25 W every three minutes (Cai et al., 2010). The HR was recorded at the end of the 2<sup>nd</sup> and 3<sup>rd</sup> minute of each 3-min stage. If the difference between the two HRs exceeded 5 beats, the work bout would be extended for another minute. The highest workload achieved along with the average HR determined during the last stage were used to predict  $\text{VO}_{2\text{max}}$  using a nomogram. The pedal rate was maintained at 50  $\text{revs}\cdot\text{min}^{-1}$  throughout the test and guided by a metronome.

#### *Anaerobic power test*

Anaerobic power tests were conducted using a Wingate 30-second all-out protocol on a cycle ergometer (Monark Ergomedic 894E, Varberg, Sweden). This protocol has proven valid and reliable in assessing anaerobic capacity in children (Bar-Or, 1987). Both seat height and handlebars were adjusted to ensure an optimal riding position and participants were given a 5-min warm up followed by two to three 5-second sprints. After the weight basket being loaded with the appropriate amount of weights (i.e.,  $75 \text{ g}\cdot\text{kg}^{-1}$  body mass), the participant began to accelerate without a load. Upon reaching the maximum speed, the investigator dropped the weight basket and started timing simultaneously. The participant was encouraged to pedal as fast as possible for 30 seconds. Peak power output ( $\text{WAnT}_{\text{peak}}$ ) was recorded and normalized to the participants' body mass.

#### *Statistical analysis*

One-way analysis of variance (ANOVA) was used to compare gender differences in hematological and hormonal indices and aerobic and anaerobic capacities. Two-way (gender  $\times$  training) ANOVA was used to analyze the interactive effect of gender and training on hematological variables. Given the differences in sample size, the homogeneity of variance across different groups was examined using the Levene's test. A significant F ratio was followed by post-

hoc comparisons using Bonferroni's adjustments. The Pearson product-moment correlation coefficients between erythrocyte indices and aerobic and anaerobic capacities were tested using the 2-tailed option. A probability level of 0.05 was established to denote statistical significance.

## Results

Gender- and training-specific means and standard deviations for RBCs, Hb, Hct, MCV, EPO, and TST were displayed and compared with the corresponding reference ranges based on standard laboratory tests for adult males and females (Beckman Inc., USA). As shown in Table 2, gender- and training-specific means for all variables fell within their respective reference ranges, although most of those associated with the intermediate and beginner groups were closer to the lower end of the range.

A significant main effect of gender was found on RBCs ( $F = 39.249, p < 0.001$ ), Hb ( $F = 44.133, p < 0.001$ ), Hct ( $F = 45.440, p < 0.001$ ), TST ( $F = 127.652, p < 0.001$ ), while no gender differences were noted for MCV and EPO (Table 3). For variables in which gender differences were detected, boys demonstrated higher values than girls. A significant main effect of gender was also noted for  $VO_{2max}$  in  $l \cdot min^{-1}$  ( $F = 34.788, p < 0.001$ ) and  $VO_{2max}$  in  $ml \cdot kg^{-1} \cdot min^{-1}$  ( $F = 5.870, p < 0.05$ ) (Table 3). These variables were all greater in boys than

girls. A subset of 178 participants including 76 girls and 102 boys undertook a Wingate anaerobic power test. Boys were also greater than girls in absolute  $WAnT_{peak}$  in watts ( $F = 6.478, p < 0.01$ ), while no gender differences were noted for relative  $WAnT_{peak}$  in  $W \cdot kg^{-1}$  (Table 3).

Our two-way ANOVA revealed a significant interaction between gender and training for RBCs ( $F = 3.94, p < 0.05$ ), Hb ( $F = 8.917, p < 0.001$ ), Hct ( $F = 8.484, p < 0.001$ ), and TST ( $F = 30.178, p < 0.001$ ), but not for EPO. There were significant ( $p < 0.05$ ) training-induced increases ( $p < 0.05$ ) in RBCs, Hb, and Hct in boys, but not in girls (Figure 1). The differences in TST followed the same response pattern as those of RBCs, Hb, and Hct, while neither gender- nor training-related difference were observed for EPO (Figure 2).

$VO_{2max}$  was correlated significantly to RBCs ( $r = 0.252, p < 0.001$ ), Hb ( $r = 0.345, p < 0.001$ ), Hct ( $r = 0.345, p < 0.001$ ), MCV ( $r = 0.111, p < 0.05$ ), and TST ( $r = 0.452, p < 0.001$ ), while no significant correlation was found between  $VO_{2max}$  and EPO (Table 4).  $WAnT_{peak}$  was also correlated significantly to RBCs ( $r = 0.304, p < 0.001$ ), Hb ( $r = 0.319, p < 0.001$ ), Hct ( $r = 0.351, p < 0.001$ ), and TST ( $r = 0.399, p < 0.001$ ), while no significant correlation was found between  $WAnT_{peak}$  and MCV or EPO.

**Table 1**

*Physical characteristics of participants*

Gender	Training Experience	Age (yr)	Skeletal Age (yr)	Years of Training (yr)	Height (cm)	Body Mass (kg)
Girls (n = 37)	Beginner (n = 93)	10.2 ± 1.8	10.3 ± 1.8	4.5 ± 1.9	146.8 ± 10.5	39.9 ± 9.1
	Intermediate (n = 32)	12.2 ± 1.3	11.8 ± 2.5	6.4 ± 1.8	159.8 ± 6.0	47.3 ± 7.8
	Advanced (n = 12)	13.4 ± 1.1	13.3 ± 1.3	8.7 ± 1.6	166.9 ± 4.7	56.6 ± 6.1
Boys (n = 171)	Beginner (n = 126)	10.5 ± 1.9	11.0 ± 1.9	4.7 ± 2.1	149.9 ± 12.0	43.8 ± 10.2
	Intermediate (n = 37)	12.9 ± 1.3	13.2 ± 1.5	7.3 ± 1.7	165.9 ± 10.1	54.8 ± 13.8
	Advanced (n = 8)	14.2 ± 1.3	14.3 ± 1.4	7.1 ± 1.9	175.6 ± 3.6	61.7 ± 5.6

*Note: Data are means ± SD.*

Table 2

*Comparisons of hematological and hormonal variables with respective reference ranges of normal adults*

Variables	Gender	Training Experience	Means $\pm$ SD	Reference Range*
RBC ( $\times 10^{12} \text{ l}^{-1}$ )	Girls	Beginner	4.66 $\pm$ 0.28	4.2-5.4
		Intermediate	4.64 $\pm$ 0.28	
		Advanced	4.70 $\pm$ 0.30	
	Boys	Beginner	4.82 $\pm$ 0.31	4.7-6.1
		Intermediate	4.96 $\pm$ 0.31	
		Advanced	5.21 $\pm$ 0.27	
Hb (g·dl <sup>-1</sup> )	Girls	Beginner	13.44 $\pm$ 0.72	11-15
		Intermediate	13.57 $\pm$ 0.81	
		Advanced	13.68 $\pm$ 0.77	
	Boys	Beginner	13.74 $\pm$ 0.88	12-17
		Intermediate	14.41 $\pm$ 0.96	
		Advanced	15.46 $\pm$ 0.72	
Hct (%)	Girls	Beginner	40.30 $\pm$ 2.10	34.9-44.5
		Intermediate	41.01 $\pm$ 2.38	
		Advanced	41.35 $\pm$ 2.34	
	Boys	Beginner	41.27 $\pm$ 2.45	38.8-50
		Intermediate	43.39 $\pm$ 2.81	
		Advanced	46.51 $\pm$ 1.99	
MCV (fl)	Girls	Beginner	87.74 $\pm$ 3.69	79-101
		Intermediate	88.36 $\pm$ 3.49	
		Advanced	88.00 $\pm$ 1.84	
	Boys	Beginner	85.55 $\pm$ 3.52	79-101
		Intermediate	87.49 $\pm$ 3.44	
		Advanced	89.38 $\pm$ 2.92	
EPO (mIU·ml <sup>-1</sup> )	Girls	Beginner	8.39 $\pm$ 3.97	2.6-18.5
		Intermediate	8.40 $\pm$ 3.92	
		Advanced	9.27 $\pm$ 4.50	
	Boys	Beginner	7.77 $\pm$ 2.95	2.6-18.5
		Intermediate	8.80 $\pm$ 3.73	
		Advanced	7.42 $\pm$ 1.54	
TST (ng·dl <sup>-1</sup> )	Girls	Beginner	32.10 $\pm$ 32.81	10-100
		Intermediate	49.32 $\pm$ 17.31	
		Advanced	53.69 $\pm$ 22.77	
	Boys	Beginner	91.41 $\pm$ 146.94	270-1000
		Intermediate	289.43 $\pm$ 183.73	
		Advanced	468.09 $\pm$ 166.89	

*Note: RBC = Red blood counts; Hb = Hemoglobin; Hct = Hematocrit; MCV = Mean corpuscular volume; EPO = Erythropoietin; TST = Testosterone. \*Values were plasma concentrations based on standard laboratory tests provided by Bechman Inc.*

**Table 3***Comparisons of hematological, hormonal and fitness variables between boys and girls*

Variables	Gender	N	Mean	SE	F ratio	p
RBC ( $\times 10^{12} \cdot l^{-1}$ )	Girls	137	4.668	0.035	39.249	0.000**
	Boys	171	4.996	0.039		
Hb (g·dl <sup>-1</sup> )	Girls	137	13.551	0.098	44.133	0.000**
	Boys	171	14.535	0.111		
Hct (%)	Girls	137	40.861	0.281	45.440	0.000**
	Boys	171	43.715	0.317		
MCV (fl)	Girls	137	87.574	0.414	0.018	0.895
	Boys	171	87.491	0.467		
EPO (mIU·ml <sup>-1</sup> )	Girls	137	8.601	0.414	0.862	0.354
	Boys	171	8.021	0.467		
TST (ng·dl <sup>-1</sup> )	Girls	137	44.716	14.002	127.652	0.000**
	Boys	171	283.394	15.818		
VO <sub>2max</sub> (l·min <sup>-1</sup> )	Girls	137	2.25	0.07	34.788	0.000**
	Boys	171	2.91	0.08		
VO <sub>2max</sub> (ml·kg <sup>-1</sup> ·min <sup>-1</sup> )	Girls	137	47.53	1.85	5.87	0.016*
	Boys	171	54.27	2.08		
WAnT <sub>peak</sub> (W)	Girls	75	449.53	25.65	6.569	0.011*
	Boys	102	546.95	28.05		
WAnT <sub>peak</sub> (W/kg)	Girls	75	8.96	50.12	2.106	0.149
	Boys	102	9.97	54.81		

Note: RBC = Red blood counts; Hb = Hemoglobin; Hct = Hematocrit; MCV = Mean corpuscular volume; EPO = Erythropoietin; TST = Testosterone.

\* $p < 0.05$ ; \*\* $p < 0.01$ .

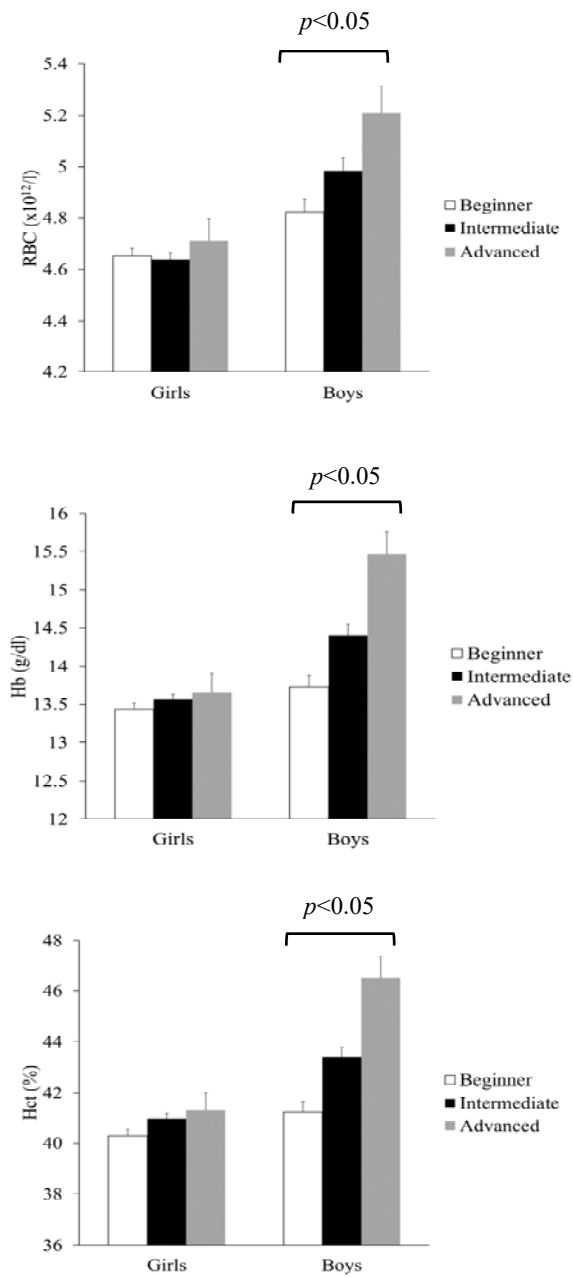
**Table 4**

*Correlation coefficients between hematological variables and aerobic and anaerobic power in boys and girls combined*

	RBC ( $\times 10^{12}/L$ )	Hb (g/dL)	Hct (%)	MCV (fl)	EPO (mIU/ml)	TST (ng/dl)
VO <sub>2max</sub> (l·min <sup>-1</sup> ) (N = 308)	0.252**	0.345**	0.345**	0.111*	-0.047	0.452**
WAnT <sub>peak</sub> (W) (N = 178)	0.304**	0.319**	0.351**	0.066	0.052	0.399*

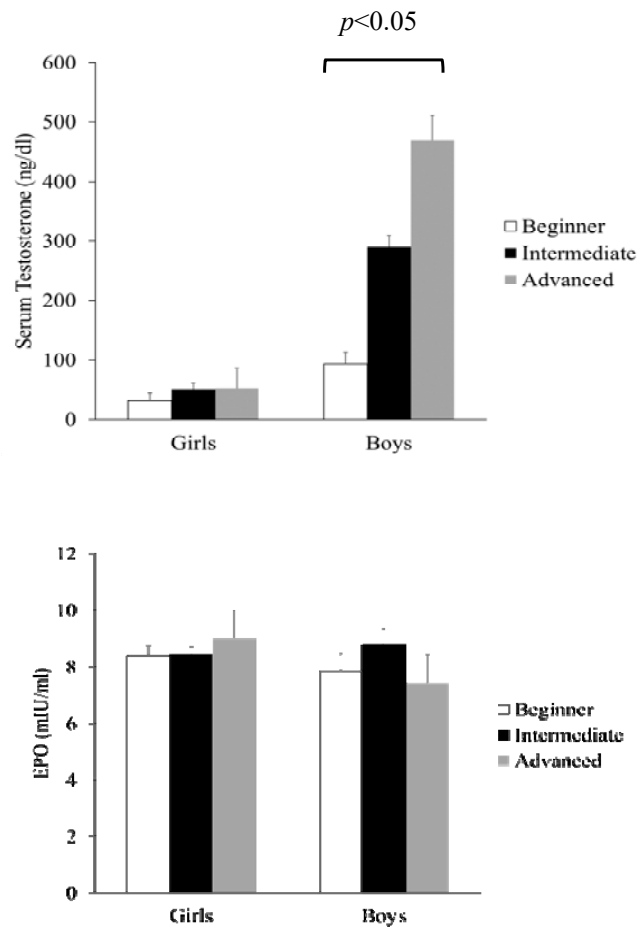
Note: RBC = Red blood counts; Hb = Hemoglobin; Hct = Hematocrit; MCV = Mean corpuscular volume; EPO = Erythropoietin; TST = Testosterone, VO<sub>2max</sub> = Maximum oxygen uptake; WAnT<sub>peak</sub> = Peak anaerobic power.

\* $p < 0.05$ ; \*\* $p < 0.01$ .



**Figure 1**

Comparisons of red blood cell counts (RBCs), hemoglobin concentrations (Hb), and hematocrits (Hct) between boys and girls stratified by their training level. Values are means  $\pm$  SE. Note: There were significant ( $p < 0.05$ ) increases in RBCs, Hb, and Hct in boys, but not in girls.



**Figure 2**

*Comparisons of serum EPO and testosterone concentrations between boys and girls stratified by their training level.*

*Values are means  $\pm$  SE. Note: There was a significant ( $p < 0.05$ ) increase in TST in boys, but not in girls.*



## Discussion

Although the gender-specific means of RBCs, Hb, Hct, MCV, EPO and TST were mostly within the adult-based reference ranges, values were closer to the lower end of their respective spectrums and this is especially the case for the least experienced group (Table 2). However, the results of red blood cell indices were comparable to those of studies that used athletes of similar age. For example, Boyadjiev and Taralov (2000) showed that mean RBCs, Hb, Hct, and MCV of highly trained boys and girls of various sports aged between 13 and 15 were  $4.61 \times 10^{12} \cdot l^{-1}$ , 13.3 g·dl<sup>-1</sup>, 39%, and 85 fl, respectively, while our corresponding results were  $4.66 \times 10^{12} \cdot l^{-1}$ , 13.5 g·dl<sup>-1</sup>, 41%, and 87 fl in girls and  $4.88 \times 10^{12} \cdot l^{-1}$ , 14.0 g·dl<sup>-1</sup>, 42%, and 86 fl in boys. Koch and Rocker (1977) also reported Hb of 13.4 g·dl<sup>-1</sup> from a group of trained boys aged between 14 and 15. Our results also matched well with the normative values developed for healthy untrained children. For example, the healthy ranges of Hb and Hct for children aged from 8 to 14 were recommended to be 13.5-14 g·dl<sup>-1</sup> and 40-43%, respectively (Osiki, 1993), and our corresponding values were 13.5 g·dl<sup>-1</sup> and 41% in girls and 14.0 g·dl<sup>-1</sup> and 42% in boys. Our results, however, were lower than those of adult athletes. For example, the mean RBCs, Hb, Hct, and MCV determined from highly trained adults of various sports were found to be  $5.0\text{-}5.4 \times 10^{12} \cdot l^{-1}$ , 15.4-16.0 g·dl<sup>-1</sup>, 45.8-47.4%, and 85.5-91.3 fl, respectively (Biancotti et al., 1992; Borges et al., 2012). These values, except for MCV, were closer to the upper end of their respective reference ranges and clearly higher than what we found in this youth cohort.

Endurance training, particularly running, has been reported to increase the rate of red blood cell destruction and thus to reduce RBCs, Hb, and Hct in adult athletes. Limited evidence exists as to whether this "anemic" condition could also concern youth athletes. Boyadjiev and Taralov (2000) found lower RBCs, Hb, and Hct in pubescent athletes pursuing swimming and rowing compared to their control counterparts. They also demonstrated a positive correlation between training experience and the degree of reduction in RBCs and Hb. These training-induced decreases in erythrocyte indices, however, were not observed by Eastwood et al. (2009) who used cyclists of similar age and by

Mayr et al. (2006) who used elite speed skaters aged between 14 and 18. The condition of sports anemia can be caused by factors such as training volume and intensity, condition of athletes, and dietary intake of iron, protein, vitamin C, vitamin B<sub>12</sub>, or folic acid (Eichner, 1992). Thus, it is difficult to discern why such discrepancy existed between the studies. Our data revealed relatively normal red blood cell indices in youth swimmers. However, these children trained regularly and many participants had values near the lower end of the reference range. Therefore, to ensure the safety and well-being of athletes, a regular hematological screening for young athletes should be implemented.

RBCs, Hb, and Hct were 7-8% higher in boys than girls (Table 3). These findings are in close agreement with those of Boyadjiev and Taralov (2000) who observed gender gaps of similar magnitude in these variables. Using relatively older athletes, Ulrich et al. (2011) also showed 20% higher weight-adjusted Hbmass in adolescent male than female athletes. Our two-way ANOVA further indicated an interaction between gender and training. As shown in Figure 1, training-induced increases in RBC, Hb, and Hct were much greater in boys than in girls. Although these erythrocyte indices did not seem to be negatively affected by training that these children have endured in both genders, a lack of appreciable increases in erythrocyte indices seen in girls warrants caution. It remains to be determined if this gender difference persists as children continue their training. Interestingly, responses of plasma testosterone were in close resemblance to those of RBCs, Hb, and Hct (Figure 2). This may be a testament to the potential linkage between testosterone and biosynthesis of erythrocytes. Beside its well-known effects on protein synthesis and tissue growth, testosterone has been found to have strong stimulating effect on erythropoiesis especially in pubescent populations (Hero et al., 2005).

Plasma EPO did not seem to be affected by either genders (Table 3) or training (Figure 2). In addition, no significant correlation was found between VO<sub>2max</sub> and EPO (i.e.,  $r = 0.047$ ) (Table 4). We are not aware of any published data concerning gender- and training-related differences in EPO in youth, but studies using

both trained and untrained adults have shown that the mean EPO recorded over a 24-h period was not correlated to training status or  $VO_{2max}$  (Klausen et al., 1993). Although the major function of EPO is to stimulate erythropoiesis, the production of EPO is thought to be mainly induced by exposure to a hypoxic environment (Mairbäurl, 2013), which is not the case in the present study as our participants had all been trained at sea level. Of interest is that EPO and testosterone responded differently and there was virtually no correlation (i.e.,  $r = 0.005$ ) between these two variables. It has long been thought that testosterone stimulates erythropoiesis via its stimulatory effect on EPO release (Shahani et al., 2009). However, this notion does not seem to corroborate our current findings. The discordance between the two hormones was also illustrated in a recent study where a transdermal testosterone treatment for 3 years significantly increased hemoglobin, but not EPO levels (Maggio et al., 2013).

Both absolute ( $l \cdot min^{-1}$ ) and relative ( $ml \cdot kg^{-1} \cdot min^{-1}$ )  $VO_{2max}$  were higher in boys than girls, although the difference in relative  $VO_{2max}$  was much smaller (Table 3). Our  $VO_{2max}$  values were similar to those reported in untrained children of similar age. For example, Kemper et al. (2013) observed an average of  $VO_{2max}$  of 2.3-2.5 for girls and 2.6-2.8  $l \cdot min^{-1}$  for boys aged 12-13, whereas our values were 2.3 and 2.9  $l \cdot min^{-1}$ , respectively. As for anaerobic capacity, gender differences in absolute (i.e., watts)  $WAnT_{peak}$  also existed with boys demonstrating higher values than girls (Table 3). This gender difference was, however, largely abolished when  $WAnT_{peak}$  was normalized to body mass (i.e.,  $W \cdot kg^{-1}$ ) (Table 3). Gender differences were also reported by Bencke et al. (2002) who used youth swimmers of similar age range. Their  $WAnT_{peak}$  results, however, were somewhat lower than ours. For example, in their study the average  $WAnT_{peak}$  were 339 W for girls and 355 W for boys, whereas our values were 449 W and 547 W, respectively. This difference may be explained in part by the fact that the brake resistance that we chose was slightly higher, i.e., 75 vs. 68-70  $g \cdot kg^{-1}$ , although other factors such as the condition of athletes cannot be ruled out.  $WAnT_{peak}$  values for normal children aged from 7 to 14 have been reported to range from 250 to 500 W with no appreciable gender gaps before

puberty (Inbar and Bar-Or, 1986; Van Praagh, 2000). The gender gap in  $WAnT_{peak}$  found presently corroborates our findings of TST, which suggests that strength gains may respond to training more favorably in boys than girls.

In the present study, modest but significant positive correlations (i.e.,  $r \approx 0.25-0.35$ ) were observed between  $VO_{2max}$  and red blood cell variables including RBCs, Hb, and Hct (Table 4). Given that the primary role of red blood cells is to transport  $O_2$  to the working tissue, these findings make sense physiologically. Our findings are consistent with those of Eastwood et al. (2009) who also revealed a positive correlation between  $VO_{2max}$  and  $Hb_{mass}$  in adolescent cyclists. Interestingly, significant correlations of similar magnitude ( $r \approx 0.30-0.35$ ) were also found between  $WAnT_{peak}$  and the erythrocyte indices. This correlation may be explained by TST that can cause parallel gains in both erythrocytes and muscle strength. As mentioned earlier, TST stimulates erythropoiesis, while also enhances protein synthesis. From a practical perspective, our findings provided additional support for using erythrocyte indices, i.e., Hb or  $Hb_{mass}$ , as part of talent identification for sports. It has been shown that endurance athletes have higher  $Hb_{mass}$  than non-endurance or untrained counterparts and their Hb contents are relatively stable upon reaching puberty (Eastwood et al., 2009; Prommer et al., 2008; Ulrich et al., 2011).

A potential limitation of this study is that we did not assess children's maturity and grouping was made based on their training experience. As such, how many children were pre-pubertal or pubertal within each group remains unknown. As both puberty and menstruation can affect blood-borne variables especially those that are growth-related such as TST, our results should be interpreted with caution.

In conclusion, this study revealed relatively lower yet age- and gender-appropriate erythrocyte indices and aerobic and anaerobic capacity in youth swimmers with boys demonstrating higher values than girls in most of these variables. There were significant training-induced increases in erythrocyte indices in boys, but not in girls. The gender differences in erythrocyte indices seem unrelated to EPO and may be explained by the higher plasma

testosterone levels seen in boys. Given their correlation to both aerobic and anaerobic capacity,

erythrocyte indices may be used as part of talent identification for sports.

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