

## ANTHROPOLOGICAL, HEMATOLOGICAL AND CARDIO-PHYSIOLOGICAL VARIABLES IN PHYSICALLY ACTIVE BOYS AND GIRLS

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**Abstract.** *Physical activity (PA) is stimulatory physiologic stress for the human body and regular PA induces significant changes in many physiological, biochemical and anthropological parameters. The aim of this study is to determine the values and the differences among the cardio-physiological, hematological and anthropological parameters for different age and gender subgroups in healthy physically active children and an adolescent population. An anthropometric evaluation (Matiegka protocol), hematological analysis i.e., red blood cells count (RBC), hemoglobin level, hematocrit, and ergometrical testing (Brus protocol) were made. Regarding mass and height until the age of 12 (U12), girls were heavier and taller than boys. The mean values for relative muscle mass in all the groups of boys and girls of different ages are in the optimal range (>50%) and indicate well developed muscle mass. There are no differences between the same age subgroups for this anthropometric parameter between the boys and girls. All the subgroups of girls of different ages showed higher body fat percent than their male siblings. Among the girls, RBC variables did not show a difference in the age subgroups. Regarding gender, all RBC variables were significantly higher among the male groups, except the U12. Cardio-physiological parameters of heart frequency at rest, exercise time duration and maximal oxygen consumption ( $VO_{2max}$ ), were significantly higher among the boys. In all age-based male subgroups  $VO_{2max}$  was higher among the older boys. Conclusion: This study has shown age related changes in anthropological, hematological and ergometrical parameters in a male and female young active population. The girls showed significantly lower levels of cardio-physiological fitness which can be contributed either to gender and lower volume of PA.*

**Key words:** *Body Composition, Red Blood Cell, Hemoglobin, Hematocrit, Adolescents*

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## INTRODUCTION

The significance of regular physical activity (PA) for the physical, mental and social health of youth is acknowledged in the scientific public, and therefore it is important to promote and support regular PA as a foundation of good health for young generations. Sports medicine aims to achieve the optimum benefit and to avoid health risk from exercise. One of the roles of pre-participation examinations, besides providing optimal health safety and decreasing the risk for health accidents for a person during physical activities is to monitor and follow the changes in the relevant physiological parameters. The health status check includes checking the blood parameters, ergometrical testing and anthropometrical status of young athletes (Cvejić, Pejović, & Ostojić, 2013).

PA is physiologic stress for the human body and regular physical activities induce important changes in many physiological, anthropological and biochemical parameters. Acute and long-term effects of PA influence the hematological, cardiovascular and body composition parameters (Mairbaurl, 2013).

Habitual monitoring of relevant physiological parameters in young athletes during different phases of training and the competition process could help to detect potential iron deficiency, anemia or other health problems, which are important for successful exercise planning and training programming (Anđelković et al., 2014). The permanence of hematological variable status is one of the key determinants of good health and a precondition for optimal exercise performance. Hematological variables in a young population, children and adolescents are exposed to changes during the growth and maturation of the relevant organs (Hero, Wickman, Hanhijarvi, Siimes, & Dunkel, 2005; Price, 2008).

Body composition is also affected by PA. Body composition analysis is important for evaluating nutritional status and growth level in children (Ellis, Shypailo, Abrams, & Wong, 2000). The assessment of body composition in children is basically demanding, and needs to be made constantly at certain periods of time because of the rapid growth-related changes in longitudinal and circumferential dimensions and all body components (Kyle, Earthman, Pichard, & Coss-Bu, 2015). Just as the whole body grows and matures, several physiological, hematological and anthropological parameters have also been shown to change with age. Maximal oxygen uptake ( $VO_{2max}$ ) is measured, arterial oxygen content and oxygen extraction and in addition to the functionality of many organic systems and health and fitness status, it depends on age and gender (da Cunha, Farinatti, & Midgley, 2011). In adolescents as in adults, gender differences in  $VO_{2max}$  have been accredited to several external and internal factors such as body composition, blood hemoglobin concentration and cardiac size and function, and amount of PA (Saghiv, Sherve, Sira, Saghiv, & Goldhammer, 2017). Changes that occur in body dimensions, body composition, hematological and cardio-physiological parameters should be quantitatively determined as they indicate good growth and development in a young person's body.

The aim of this study is to establish the reference values and the differences for the cardio-physiological, hematological and anthropological parameters for different age and gender subgroups in healthy physically active children and an adolescent population.

## METHODS

This study included 608 participants (406 boys and 202 girls; age span 8 to 18 years), attending regular health checks including a laboratory investigation and exercise testing at our department. The investigated population was divided by gender into two groups, and by age into five different age subgroups: U10 (8.0 to 9.99 years); U12 (10 to 11.99 years); U14 (12 to 13.99 years); U16 (14 to 15.99 years) and U18 group (16 to 18.0 years). All of the participants fulfilled a sports medicine history questionnaire for assessing their PA, sports discipline, and a weekly training workload. Written informed consents for participation in the study was obtained from the all participants or their parents. The study was done at the Institute of Medical and Applied Physiology and Anthropology, Faculty of Medicine, UKIM, in Skopje, the Republic of North Macedonia, between September 2016 and March 2017. Some findings concerning the investigated population in this paper have been published before (Plunčević Gligoroska et al., 2019).

### **Anthropometric measurements**

The anthropological procedure by Matiegka was used to assess body composition. The Matiegka protocol uses several anthropometric measurements: standing height, body mass, limb circumferences at certain measuring points (upper arm relaxed and flexed, forearm, thigh, calf), four limb diameters (wrist, elbow, ankle, knee) and seven skinfolds (biceps, triceps, forearm, subscapular, thigh, calf, suprailiac). Participants' height was measured to with a fixed stadiometer (Holtain Ltd., Crymich, U.K.). Their body mass was measured with the SECA beam balance (Seca, Hamburg, Germany) to the nearest 0.1 kg. For skinfold thickness, a Harpenden skinfold caliper (British indicators Ltd., Luton) was used with 0.1 mm accuracy and a Vernier caliper was used to determine the ankle diameters. Elastic tape was used to take circumferences with 0.01mm accuracy. The values of these measurements were used in the Matiegka protocol equations (Cartryse et al., 2002) to assess absolute muscle mass (MM in kg), relative muscle mass (MM in %), bone mass (BM in kg), relative bone mass (BM in %), absolute body fat (BF in kg), body fat percentage (BF in %), lean body mass (LBM in kg), while the body mass index (BMI in kg/m<sup>2</sup>) was also calculated.

### **Ergometrical procedures**

Participants performed normal treadmill exercise testing according to the Bruce sub-maximal protocol and the ACSM guidelines (2000). The Bruce test is an ergometric test consisting of several stages of gradually increasing workloads, which is performed until reaching the previously established sub-maximum heart rate or until a subjective or objective reason has been noted. After the end of the test, the main variable is the duration of time (ET=exercise time) which is inserted in a software formula for calculating VO<sub>2max</sub>, general endurance, specific endurance and speed abilities scores.

### **Hematologic measurements**

A hematologic analysis was done using a automated hematology analyzer ABX Micros 60-OT (ABX hematology, Montpellier, France). Blood samples were collected from capillary vessel using sterile plastic containers with an anticoagulant (EDTA K3). The following hematological parameters were analyzed: Red blood cells count (RBC in

10<sup>9</sup>/dl); hemoglobin level (Hb in g/dl); hematocrit (Hct in %); mean corpuscular volume (MCV in  $\mu\text{m}^3$ ); Mean corpuscular hemoglobin (MCH in pg) and mean corpuscular hemoglobin concentration (MCHC in g/dl).

### Ethics

Institutional ethical approval was received from the Ethics Committee of the Medical Faculty, Ss Cyril and Methodius University of Skopje. Informed consent was obtained from the parents.

### Statistics

For statistical analyses, the software Statistica 7.0 for Windows was used. The data are presented as group means with the respective standard deviation (SD). After checking the normality and homogeneity of variances with Shapiro-Wilk's *W* test and Levene's test respectively, the data were analyzed by a two-way ANOVA/MANOVA. Whenever the ANOVA disclosed significant results, the post-hoc Tukey test was applied. The Spearman correlation coefficient was used to find specific linear associations. Statistically significant differences were considered at the level  $p < 0.05$ .

## RESULTS

The mean values (Mean $\pm$ SD) for age and quantity of training duration for all the subgroups are presented in Table 1.

**Table 1** Training characteristics of the age different groups (Mean $\pm$ SD) of the boys and girls

| Group | N          | Age              | Years of training | Hours per week  |
|-------|------------|------------------|-------------------|-----------------|
|       | boys/girls | boys/girls       | boys/girls        | boys/girls      |
| U10   | 48/12      | 8.19 $\pm$ 1.04  | 1.6 $\pm$ 1.0     | 3.5 $\pm$ 1.59  |
|       |            | 8.23 $\pm$ 1.17  | 1.35 $\pm$ 1.21   | 2.77 $\pm$ 0.83 |
| U12   | 74/47      | 10.54 $\pm$ 0.5  | 3.46 $\pm$ 1.83   | 3.69 $\pm$ 1.27 |
|       |            | 9.53 $\pm$ 1.9   | 2.37 $\pm$ 1.65   | 2.93 $\pm$ 1.03 |
| U14   | 114/54     | 12.63 $\pm$ 0.48 | 4.68 $\pm$ 1.78   | 4.62 $\pm$ 2.18 |
|       |            | 12.52 $\pm$ 0.5  | 2.82 $\pm$ 1.61   | 3.07 $\pm$ 1.59 |
| U16   | 88/49      | 14.42 $\pm$ 0.5  | 5.67 $\pm$ 2.08   | 5.82 $\pm$ 3.36 |
|       |            | 14.47 $\pm$ 0.5  | 4.37 $\pm$ 2.47   | 4.26 $\pm$ 1.35 |
| U18   | 82/40      | 16.73 $\pm$ 0.74 | 7.04 $\pm$ 2.9    | 6.35 $\pm$ 3.33 |
|       |            | 16.98 $\pm$ 0.77 | 5.28 $\pm$ 3.54   | 4.26 $\pm$ 2.38 |
| Total | 406/202    |                  |                   |                 |

*Legend:* U10-under 10 years; U12-under 12 years; U14-under 16 years; U18-under 18 years

The boys showed greater frequency of training session and training history (duration in years) than the girls in all the subgroups.

**Table 2** Descriptive statistics and age and gender differences for anthropological parameters for the subgroups of boys and girls of different ages (Mean±SD)

| Parameter | U10                        |                             | U12                         |                             | U14                         |                             | U16                         |                             | U18                         |                             |
|-----------|----------------------------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|
|           | male                       | female                      | male                        | female                      | male                        | female                      | male                        | female                      | male                        | female                      |
| Mass (kg) | 38.88 ± 15.86 <sup>a</sup> | 39.91 ± 9.89 <sup>a*</sup>  | 54.35 ± 15.85 <sup>b</sup>  | 62.13 ± 13.25 <sup>b</sup>  | 72.73 ± 12.17 <sup>c*</sup> | 34.46 ± 7.70 <sup>a</sup>   | 46.70 ± 10.94 <sup>b*</sup> | 53.25 ± 9.68 <sup>c</sup>   | 56.15 ± 8.39 <sup>c</sup>   | 61.04 ± 9.41 <sup>d*</sup>  |
|           | Height (cm)                | 132.95 ± 19.35 <sup>a</sup> | 146.29 ± 8.59 <sup>b*</sup> | 162.08 ± 10.48 <sup>c</sup> | 173.22 ± 7.91 <sup>d*</sup> | 179.67 ± 7.76 <sup>d*</sup> | 138.65 ± 9.72 <sup>a</sup>  | 152.18 ± 9.75 <sup>b*</sup> | 160.22 ± 5.95 <sup>c</sup>  | 162.36 ± 6.33 <sup>c*</sup> |
| MM (kg)   | 16.45 ± 4.24 <sup>a</sup>  | 20.87 ± 5.13 <sup>b*</sup>  | 28.72 ± 7.62 <sup>c</sup>   | 33.77 ± 7.23 <sup>d</sup>   | 39.66 ± 7.22 <sup>e*</sup>  | 18.30 ± 3.97 <sup>a</sup>   | 24.38 ± 5.87 <sup>a*</sup>  | 29.37 ± 9.67 <sup>b</sup>   | 30.49 ± 4.95 <sup>b</sup>   | 33.35 ± 3.34 <sup>b*</sup>  |
|           | MM (%)                     | 50.24 ± 3.41 <sup>a</sup>   | 51.81 ± 2.95 <sup>b</sup>   | 52.77 ± 2.77 <sup>bc</sup>  | 53.56 ± 2.33 <sup>cd</sup>  | 54.32 ± 3.06 <sup>d</sup>   | 52.12 ± 1.22                | 52.22 ± 3.38                | 52.78 ± 3.46                | 54.11 ± 2.25                |
| BM (kg)   | 6.64 ± 1.26 <sup>a</sup>   | 7.99 ± 1.6 <sup>bd</sup>    | 9.51 ± 2.75 <sup>c</sup>    | 9.15 ± 1.23 <sup>d*</sup>   | 9.89 ± 1.23 <sup>e*</sup>   | 6.45 ± 1.33 <sup>a</sup>    | 7.74 ± 1.47 <sup>a</sup>    | 10.14 ± 1.95 <sup>a</sup>   | 11.75 ± 2.46 <sup>b*</sup>  | 12.82 ± 1.60 <sup>b*</sup>  |
|           | BM (%)                     | 19.93 ± 1.85                | 19.92 ± 1.86 <sup>*</sup>   | 19.08 ± 2.28 <sup>*</sup>   | 18.81 ± 2.31 <sup>*</sup>   | 17.84 ± 1.76 <sup>*</sup>   | 19.09 ± 1.43 <sup>a</sup>   | 17.64 ± 1.77 <sup>ab*</sup> | 17.33 ± 2.07 <sup>bc*</sup> | 16.29 ± 1.09 <sup>d*</sup>  |
| BF (kg)   | 5.07 ± 2.05 <sup>a</sup>   | 6.57 ± 2.76 <sup>a*</sup>   | 9.10 ± 4.05 <sup>b</sup>    | 9.36 ± 2.93 <sup>b</sup>    | 11.33 ± 3.41 <sup>c</sup>   | 6.08 ± 1.92 <sup>a</sup>    | 8.24 ± 3.41 <sup>ab*</sup>  | 9.65 ± 3.49 <sup>bc</sup>   | 10.16 ± 3.16 <sup>bc</sup>  | 11.37 ± 3.51 <sup>c</sup>   |
|           | BF (%)                     | 15.39 ± 3.45                | 16.05 ± 3.02 <sup>*</sup>   | 16.10 ± 3.71 <sup>*</sup>   | 15.54 ± 5.64 <sup>*</sup>   | 16.79 ± 14.78 <sup>*</sup>  | 16.99 ± 2.55                | 17.45 ± 3.35 <sup>*</sup>   | 17.46 ± 3.19 <sup>*</sup>   | 17.53 ± 2.38 <sup>*</sup>   |
| LBM (kg)  | 26.99 ± 4.90 <sup>a</sup>  | 33.24 ± 6.82 <sup>b*</sup>  | 44.96 ± 9.80 <sup>c</sup>   | 52.83 ± 9.57 <sup>d*</sup>  | 59.62 ± 11.62 <sup>e*</sup> | 29.35 ± 5.91 <sup>a</sup>   | 37.76 ± 8.21 <sup>b*</sup>  | 44.02 ± 6.49 <sup>c</sup>   | 45.85 ± 5.72 <sup>c*</sup>  | 49.39 ± 7.10 <sup>d*</sup>  |
|           | BMI (kg/m <sup>2</sup> )   | 17.52 ± 2.93 <sup>a</sup>   | 18.73 ± 3.00 <sup>a</sup>   | 20.39 ± 3.57 <sup>bc</sup>  | 20.82 ± 3.09 <sup>c</sup>   | 22.95 ± 5.60 <sup>d</sup>   | 17.92 ± 2.32 <sup>a</sup>   | 19.71 ± 2.93 <sup>ab</sup>  | 20.78 ± 3.32 <sup>b</sup>   | 21.05 ± 2.47 <sup>b</sup>   |

Legend: <sup>1</sup>values are expressed as mean (standard deviation); For every metric, different superscript letters (<sup>a, b, c, d</sup>) represents differences between the different age groups for each gender (read horizontal) and stars (\*) represent differences between genders within each age group; MM-absolute muscle mass in kg; MM%-relative muscle mass; BM-bone mass in kg; BM%-relative bone mass; BF-absolute body fat in kilograms; BF%-body fat percentage; LBM-lean body mass in kilograms; BMI-body mass index in kg/m<sup>2</sup>.

Table 2 displays descriptive statistics for anthropological parameters and body composition for subgroups of boys and girls of different ages. A high statistically significant difference was found for the main characteristics of the participants: age, height and mass. With an increase in the age of the participants, the mass also increased in both groups, the male and female. The boys had approximate mass in groups U10 and U12. Among the girls, groups U14 and U16 had approximate mass. A significant difference between females and males was noted in the U12 groups where girls were heavier than the boys (46.7 kg vs 39.91 kg) and in the U18 groups where the boys were heavier (72.73 kg vs 61.04 kg). Regarding height until the age of 12, the girls were taller than the boys. In the U14 group they have similar height, and after that boys older than 14 were taller than their female peers.

Absolute muscle mass (MM in kg) and relative muscle mass (MM in %) increased with significant differences between all the subgroups among the boys. Relative MM among the girls showed constant values for all the age groups. Regarding gender, relative MM was insignificantly different between the boys and girls.

Absolute bone mass (BM in kg) increased distinctively with age in all the male subgroups. Girls older than 14 had a higher BM than U10, U12 and U14 group. Although absolute bone mass increased with age, relative bone mass (BM in %) decreased with age, from 19.93% in the youngest to the 17.84% in the oldest group of boys, but without statistical significance. Among the girls the decrease of relative BM was significant, from 19.09 % in the youngest to the 16.29%, both in the U16 and U18 group. In all the age subgroups, except U10, boys had significantly higher relative BM than their female peers.

The amount of body fat (BF in kg) gradually increased with age, in boys and girls. Body fat percent (BF in %) was insignificantly different regarding age in all the subgroups in boys and girls. In terms of gender, the girls showed significantly higher relative BF than the boys, except group U10.

Lean body mass (LBM in kg), just like its subcomponents (MM in kg+BM in kg), showed a significantly distinctive difference between the age groups in both genders. In group U10 and U12 the girls showed higher LBM than the boys, and after the age of 14, in groups U16 and U18 the advantage for this parameter was in favor of the boys. The body mass index (BMI in kg/m<sup>2</sup>) mean values were significantly different between the different age groups for both genders. Among the boys BMI increased from U10=17.52 to U18=22.95. Among the girls BMI rose too, from U10=17.92 to U18=21.92. There were no significant differences in the BMI between boys and girls.

**Table 3** Descriptive statistics and age and gender differences for hematological parameters for different age subgroups of the boys and girls (Mean±SD)

| Parameter                    | U10                       |                           | U12                       |              | U14                        |                           | U16                        |                           | U18                        |                           |
|------------------------------|---------------------------|---------------------------|---------------------------|--------------|----------------------------|---------------------------|----------------------------|---------------------------|----------------------------|---------------------------|
|                              | male                      | female                    | male                      | female       | male                       | female                    | male                       | female                    | male                       | female                    |
| RBC<br>(10 <sup>9</sup> /dl) | 4.99 ± 1.06 <sup>a</sup>  | 4.86 ± 0.33 <sup>a</sup>  | 4.86 ± 0.33 <sup>a</sup>  | 4.78 ± 0.42  | 5.04 ± 0.40 <sup>a*</sup>  | 4.87 ± 0.37 <sup>*</sup>  | 5.26 ± 0.41 <sup>b*</sup>  | 4.78 ± 0.43 <sup>*</sup>  | 5.29 ± 0.36 <sup>b*</sup>  | 4.63 ± 0.30 <sup>*</sup>  |
| Hb<br>(g/dl)                 | 12.87 ± 0.84 <sup>a</sup> | 13.07 ± 0.96 <sup>a</sup> | 13.07 ± 0.96 <sup>a</sup> | 12.86 ± 0.96 | 13.74 ± 1.15 <sup>b*</sup> | 13.14 ± 0.96 <sup>*</sup> | 14.40 ± 1.19 <sup>c*</sup> | 12.74 ± 0.98 <sup>*</sup> | 14.90 ± 1.02 <sup>d*</sup> | 12.79 ± 1.13 <sup>*</sup> |
| Hct<br>(%)                   | 40.62 ± 2.74              | 40.84 ± 4.89              | 40.84 ± 4.89              | 41.16 ± 3.32 | 43.20 ± 3.37 <sup>*</sup>  | 41.35 ± 3.57 <sup>*</sup> | 45.93 ± 3.59 <sup>*</sup>  | 41.36 ± 3.32 <sup>*</sup> | 46.86 ± 5.78 <sup>*</sup>  | 39.68 ± 6.67 <sup>*</sup> |
| MCV<br>(µm <sup>3</sup> )    | 84.12 ± 3.28 <sup>a</sup> | 85.24 ± 3.54 <sup>a</sup> | 85.24 ± 3.54 <sup>a</sup> | 86.45 ± 6.20 | 85.88 ± 4.49 <sup>ab</sup> | 84.91 ± 6.38              | 87.27 ± 5.40 <sup>b</sup>  | 86.51 ± 5.79              | 89.90 ± 3.98 <sup>c*</sup> | 87.75 ± 5.90 <sup>*</sup> |
| MCH<br>(pg)                  | 26.70 ± 1.40 <sup>a</sup> | 27.09 ± 1.82 <sup>a</sup> | 27.09 ± 1.82 <sup>a</sup> | 26.90 ± 2.68 | 27.34 ± 2.06 <sup>a</sup>  | 27.09 ± 2.10              | 27.37 ± 2.32 <sup>b</sup>  | 26.81 ± 2.60              | 28.24 ± 1.93 <sup>b</sup>  | 27.67 ± 2.51              |
| MCHC<br>(g/dl)               | 31.71 ± 1.25              | 31.42 ± 2.90              | 31.42 ± 2.90              | 31.16 ± 2.69 | 31.83 ± 1.66               | 31.92 ± 2.69              | 31.45 ± 1.58 <sup>*</sup>  | 30.42 ± 3.87 <sup>*</sup> | 31.45 ± 1.49               | 31.50 ± 1.80              |

Legend: <sup>1</sup>values are expressed as mean (standard deviation); For every metric, different superscript letters (<sup>a, b, c, d</sup>) represent differences between the different age groups for each gender (read horizontal) and stars (\*) represent differences between genders within each age group; RBC-red blood cells count in 10<sup>9</sup>/dl; Hb-hemoglobin level in g/dl; Hct-hematocrit in %; MCV-mean corpuscular volume in µm<sup>3</sup>; MCH-mean corpuscular hemoglobin in pg; MCHC-mean corpuscular hemoglobin concentration in g/dl.

Differences between mean values of the RBC count between the boys and girls are statistically insignificant for the U10 and U12 group. The RBC count is similar in boys younger than 14, and significantly higher in boys older than 14 (U16 and U18). Among the girls there was no difference in the RBC count. Hemoglobin concentration values

were insignificantly different for the U10 and U12 groups of boys (12.87 g/dl vs 13.07 g/dl), while between older groups there were significant differences in favor of the older boys (13.74 g/dl vs 14.4 g/dl vs 14.9 g/dl). Hemoglobin values (Hb in g/dl) among the girls did not show statistically significant differences between different age groups (12.83 g/dl vs 12.86 g/dl vs 13.14 g/dl vs 12.74 g/dl vs 12.79 g/dl). Hematocrit (Hct in %) did not show age differences either among the boys or the girls. Gender differences between mean Hct values are presented in boys and girls older than 12. Hematological indexes (MCV and MCH) show a tendency to rise with the increased age of the participants, and are statistically different between boys over the age of 14. Among the girls MCV did not differ between the age subgroups. Only in older groups under 18 was there a gender difference for MCV. MCHC was insignificantly different between the boys and girls regarding age and gender, with the exception of the gender difference in the U16 group.

**Table 4** Descriptive statistics and age and gender differences for cardio-physiological parameters obtained from Brus ergometry for different age subgroups of the boys and girls

| Parameter                  | U10                        |                            | U12                        |                            | U14                        |                | U16           |                | U18           |                |
|----------------------------|----------------------------|----------------------------|----------------------------|----------------------------|----------------------------|----------------|---------------|----------------|---------------|----------------|
|                            | male                       | female                     | male                       | female                     | male                       | female         | male          | female         | male          | female         |
| HRR (bpm)                  | 84.53 ± 7.56*              | 89.42 ± 17.1               | 85.73 ± 11.0*              | 84.67 ± 13.8               | 84.40 ± 16.0*              | 105.00 ± 4.89* | 94.59 ± 13.39 | 98.95 ± 15.01* | 89.86 ± 18.71 | 95.52 ± 10.70* |
| ET (minutes)               | 9.00 ± 1.97*               | 10.76 ± 2.01               | 11.46 ± 2.19*              | 11.57 ± 2.23*              | 13.29 ± 2.51*              | 5.89 ± 3.02*   | 8.37 ± 2.15   | 7.32 ± 2.00*   | 7.81 ± 2.37*  | 7.59 ± 1.45*   |
| VO <sub>2max</sub> (ml/kg) | 35.53 ± 6.19 <sup>a*</sup> | 35.72 ± 6.79 <sup>a*</sup> | 38.32 ± 7.34 <sup>a*</sup> | 40.85 ± 7.16 <sup>b*</sup> | 41.89 ± 7.01 <sup>b*</sup> | 25.40 ± 8.76*  | 31.67 ± 6.54* | 28.63 ± 5.69*  | 29.74 ± 8.21* | 28.40 ± 7.01*  |

*Legend:* <sup>1</sup>values are expressed as mean (standard deviation); For every metric, different superscript letters (<sup>a, b, c, d</sup>) represent differences between the different age groups for each gender (read horizontal) and stars (\*) represent differences between genders within each age group; HRR-heart rate at rest in bpm; ET-exercise time in minutes; VO<sub>2max</sub>-maximal oxygen consumption in ml/kg.

For heart rate at rest (HRR in bpm) gender differences were determined in the U10, U14, and U18 groups. In all the different age groups, HRR was higher among the girls (U10, U14, U16 and U18,  $p < 0.005$ ). Exercise time (ET in minutes), which is the time that every participant needed to achieve submaximal heart rate during the incremental treadmill test, was insignificantly different within the same gender, but ET was significantly different between the genders. Maximal oxygen consumption (VO<sub>2max</sub> in ml/kg) was significantly higher in boys older than 14 compared to the younger boys, while among the girls there was no significant difference for this parameter. VO<sub>2max</sub> was significantly higher for the boys compared to the girls in all the different age subgroups.

## DISCUSSION

From this study the reference values for anthropological, physiological and hematological parameters for young healthy populations were obtained and analyzed. This study exposed that significant age and sex specific differences were observed among some different age subgroups; this indicates which period of growth and maturation those parameters are age differentiated and when they have reached constant values.

### **Anthropological parameters and body mass components regarding age and gender**

**Mass and height:** The biggest rise in mass and height in the boys was noticed between the U12 and U14 group. Among the girls, the biggest difference in mass and height was between the U10 and U12 groups. The girls were considerably heavier and taller than the boys in the U12 group. The boys were significantly heavier and taller than the girls in the U18 group. All the remaining different age groups did not show a difference for height and mass between the boys and girls. In the different age subgroups, with a difference of two years between each, height increased significantly among the boys and the girls in all the subsequent groups. The boys groups U16 and U18 and the girls groups U14 and U16 were insignificantly different.

**Body mass composition** analyzed by the Matiegka protocol is separated into three components: the muscle, bone and fat component. All components can be expressed as absolute measurements in kilograms or as relative components (percent part of body mass). For comparison reasons we could get more relevant information if they are expressed as percent of whole body mass. An analysis of body composition shows that absolute muscle mass (MM in kg) among the boys increased distinctly with age with each older group. The boys showed a substantial increase in muscle mass after the age of 12. Relative muscle mass (MM in %) among the boys showed a trend of increase with the age, with a certain stable phase in group U14. Relative MM among the girls was insignificantly higher with age. The mean values for relative MM in all different age groups among the boys and girls were within the optimal range (>50%) and indicated well developed muscle mass. There were no differences between the same age subgroups for this anthropometric parameter between the boys and girls. The boys had a significantly higher amount of muscles (MM in kg) only in the oldest group, U18.

High bone mass could indicate the capacity of the person to grow in height, and our findings are consistent with this opinion because the participants in the phase of intensive growth showed the highest bone mass. The mean value of relative bone mass (BM in %) was highest in the youngest groups in both genders, boys and girls (19.93% vs 19.09%). Although the relative BM among the boys was highest in the U10 group, it was almost the same with all the other age subgroups and insignificantly lower in the oldest group (17.84%). Among the girls, bone mass significantly decreased in the older groups. The boys and girls have an approximate relative BM only in group U10, and after that, the girls have a smaller relative BM than the boys.

The amount of absolute body fat (BF in kg) gradually increased with the age of the groups, and body fat percent (BF in %) was insignificantly different between the different age groups of both genders. Mean values of body fat percent for both genders were at about the optimal value for healthy young people (15-20%). Relative BF was significantly higher among the girls in all the groups except group U10.

Lean body mass (LBM in kg) which represents a sum of non-fat body components, muscles and bones, was the most dynamic parameter and increased significantly with the increase in age among the boys and girls. The girls had a higher LBM than the boys until the age of 12; in groups U14 both genders have an approximate LBM, then after the age of 14, the values of this parameter were in favor of the boys. The mean values for the body mass index (BMI in kg/m<sup>2</sup>) regarding gender, were insignificantly different, ranging from 17.52 to 22.95 among the boys and from 17.92 to 21.92 among the girls.



Reference values for body fat indicators for children are reported from studies which included different techniques (skinfolts, anthropometry, BIA, DEXA, etc.), which made direct comparison of data complicated. From the study of Plachta Danielzik et al. (2015), 24 children from Germany, aged 2 to 18, measured by BIA methodology (Tanita), derived reference data for anthropometric parameters which showed median values for relative BF for U10=18.7%, U12=19.4%; U14=17.7%; U16=17.8% for boys, and U10=21.2%, U12=20.6%; U14=24.6%; U16=27% for girls. Compared to the German results, our data showed lower relative BF for all age subgroups. The lower values in our participants could be due to different methodology or the fact that our study population was composed of physically active children and adolescent.

BMI which is acknowledged as an indicator of nutrition status differs in reference values for children and adolescents compared to adults. In a population younger than 18, reference curves and percentiles for BMI, FMI, LBM, height and mass are frequently used (Weber, Moore, Leonard, & Zemel, 2013). Kids with a BMI among the 85<sup>th</sup> and 95<sup>th</sup> percentiles are classified as overweight and those with a BMI in the 95<sup>th</sup> percentile as obese (Krebs et al., 2000). The yearly increase in BMI from the middle of childhood onward is mainly due to increasing lean body mass rather than increasing fat mass (Wells, 2000; Maynard et al., 2001).

Studies of the children populations tend to use percentage body fat (BF%) as better parameters of obesity and to illustrate deficiencies in BMI as a substitute of adiposity in the young population (Flegal et al., 2010). The use of relative BF as the gold standard of adiposity is also an incomplete solution that does not consider height, body proportions, and LBM (Wells, 2001). Assessment of body composition in adolescent school children in Manipur, India, using BIA methodology, in boys and girls aged from 12 to 19, divided at a one-year difference, found relative BF as follows: for boys between 12.9% for youngest, and 16.8% for 17 years old. For girls: the youngest, 12 years had BF 20.1%, and the oldest, 19 years=27.2% (Rajkumari, Akoijam, Akoijam, & Longjam, 2012). The increase in body mass, standing height, hip and waist perimeters, fat mass and fat percent which were noted among the boys could be due to the intensive physical development during the first phase of adolescence (Oner et al., 2004). According to some studies, among girls a relative BF increase is seen only up to age of 16 because at that age girls have a tendency to be more careful regarding their food intake (Lloyd, Chinchilli, Egli, Rollings, & Kulin, 1998).

The general scientific attitude is that the anthropological differences which occurred between boys and girls during adolescence might be the result of sex hormones effects. In puberty, an increase in lean body components (muscle and bones) among boys was recorded, while girls showed an increase of body fat mass (Demerath et al., 2006). Until the age of 9 growth curves are similar between boys and girls. The highest values for body fat for boys are reached about the age of 11 while for girls they continue to increase throughout adolescence. Median BF% at the age of 18 is 17.0% and 27.8% for boys and girls, respectively (Laurson, Eisenmann, & Welk, 2011).

### **Red blood cell variables age and gender differences**

It is well known, thoroughly investigated and documented that RBC variables depend on gender and change significantly with age (Taylor et al., 1997; Murphy, 2014; Mandala et al., 2017). Our aim was to confirm this on a current sample of our population. The results of our study showed that gender differences for hematological variables RBC and Hct occur in participants older than 12, in favor of the boys. Regarding age, RBC and Hb

had higher values among the older groups of boys, while among the girls these parameters were not age dependent, they showed constant values in all age different groups. Hematological parameters (MCV, MCH and MCHC) were also insignificantly different in different age groups among the girls. Among the boys MCV and MCH showed significantly higher values in boys older than 14.

Gender differences in hemoglobin levels have been reported after the onset of adulthood in male mammals, even in the birds as they move away from the younger stages of development. Females also increase their hemoglobin level, but not to the same degree compared to males. Women have approximately 12% lower Hb concentration than men, which is a physiological phenomenon which is presented in many species of adult mammals, too. These gender related differences in the Hb level are probably the result of modulation of the erythropoietic response and differences in microcirculation in males and females (Murphy, 2014). The higher RBC counts and Hb levels in men compared to women result from the stimulation of erythropoiesis by androgens and its inhibition by estrogens. Observations of physiological studies lead to the conclusion that adult women uphold their hemoglobin at a lower concentration than adult men (Murphy, 2014). Other studies also found that the concentrations of Hb and Hct have similar trends during certain periods of development. Median Hb concentration is low in neonates and young children and it rises in males between the ages of 5-10, to achieve the highest levels (14.40 g/dL) at the age 15-20. Boys in the age group of 5-10 had significantly higher Hb (13.05 g/dL) and Hct (42.5%) compared to girls of the same age, 10.4 g/dL and 32.6% (Mandala et al., 2017). Several studies conducted among children in Caucasian (Taylor et al., 1997) and African populations (Menard et al., 2003; Onwurah et al., 2018), noted similar Hb and Hct levels despite of gender until the age of 10. Other studies on Hb values regarding age and gender (Yandamuri & Yandamuri, 2013) noted that boys had higher Hb values compared to girls from the age of 12 onward. Some other observations are that the age of 14 is the borderline when RBC variables (RBC count, hemoglobin level, and hematocrit) become typically higher in males compared to females. At the end of adolescence, between the ages of 17 and 20, the RBC count increases to  $4.9 \times 10^6/\mu\text{l}$  in adolescent males and  $4.7 \times 10^6/\mu\text{l}$  in adolescent females. The average normal RBC count in adult males is  $5.4 \times 10^6/\mu\text{l}$ , (between  $4.5-5.9 \times 10^6/\mu\text{l}$ ) and  $4.8 \times 10^6/\mu\text{l}$  in women (between  $4.1-5.1 \times 10^6/\mu\text{l}$ ) (Yandamuri & Yandamuri, 2013). Similar finding as in our study, that girls demonstrated no significant changes in hemoglobin in hematocrit as they progress through puberty, are reported by Willows, Grimston, Smith, & Hanley (1995).

### **Cardio-physiological parameters - age and gender differences**

Cardio-physiological parameters analyzed in this study were parameters which originated from the Bruce ergometry test. We analyzed the resting heart frequency (HRR in bpm), at the preparatory phase for the ergometry, before the treadmill starts to move. The girls in all the age subgroups had a visibly higher heart rate than their male peers. In group U10, U14 and U18 group the difference was significant, around 10 beats higher. The boys showed significantly better exercise time (ET in minutes) than the girls in all different age groups. The longer time for reaching individual submaximal heart rates could be due to a lower resting heart rate in boys, longer training history and better endurance. Estimated values of  $\text{VO}_{2\text{max}}$  are devised from ET, which is why  $\text{VO}_{2\text{max}}$  is also significantly higher in boys in every age group. The only cardio-physiological parameter in

our study that showed age dependency was  $VO_{2max}$  in the group of boys. Among the girls  $VO_{2max}$  was not different regarding age.

Studies of the differences between adolescent males and females, (mean age  $15.0 \pm 1.1$  vs  $14.9 \pm 1.1$  years; body fat percent: 15.5% vs 20.2%) indicated the following cardio physiologic parameters: HRR (bpm)=73.7 vs 78.1;  $VO_{2max}$  (ml/kg/min)=51.2 vs 48.4 (Saghiv et al., 2017). Contrary to adults, findings in pre-pubescent children demonstrate that the exclusive restraining factor of  $VO_{2max}$  that distinguished boys from girls could be a poorer systolic volume ( $SV_{max}$ ), and this gender difference vanished when normalized for LBM. Thus, the gender dissimilarity in heart size and cardiac function during exercise should be interpreted as only one significant aspect of the lower LBM in girls (Vinet et al., 2003). It is not completely explained how gender affects overall cardiac dimensions and amount of  $VO_{2max}$  in boys and girls. It is not clear whether heart size differences between adolescent girls and adolescent boys are affected by general body dimensions (especially of lean body mass), or whether there are other essential functional differences (Saghiv et al., 2017).

#### CONCLUSIONS

This study has shown that age and gender made a difference in anthropometrical, hematological and cardio-physiological parameters in healthy active young participants. Until the age of 12, girls are significantly heavier and taller and have higher LBM than boys. In groups older than 14, boys had an advantage in terms of mass, height and LBM. Relative muscular mass (MM in %) is well developed in both group and insignificantly different regarding gender. Relative bone mass (BM in %) in boys decreased insignificantly with age, while among the girls relative BM decreased significantly with constant values in U16 and U18, which could be an indicator of the end of growth. Body fat percent is in optimal ranges for healthy and physically active pubescent and adolescent participants (between 15 and 18 %). Relative BF is of similar values for all the different age groups, among the boys and girls. Girls older than 12 have higher relative BF than their male peers. RBC variables among the girls showed no age dependency, while among the boys they are significantly higher in subsequent older groups. Gender differences for hematological variables are found in groups older than the age of 12. The analyzed cardio-physiological parameters did not show age dependency, except  $VO_{2max}$  in boys, which is higher in groups older than 14. The boys showed significantly higher  $VO_{2max}$  than the girls. In conclusion, this study presents the first reference physiological data for body composition, hematological and ergometrical results in children and adolescents conducted from a large sample of a healthy and physically active young population from the Republic of North Macedonia.

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## ANTROPOLOŠKE, HEMATOLOŠKE I KARDIO-FIZIOLOŠKE VARIJABLE FIZIČKI AKTIVNIH DEČAKA I DEVOJČICA

Fizička aktivnost (FA) predstavlja stimulativni fiziološki stres za ljudsko telo koja izaziva značajne promene u mnogim fiziološkim, biohemijskim i antropološkim parametrima. Cilj ovog istraživanja je da se utvrde vrednosti i razlike kardio-fizioloških, hematoloških i antropoloških parametara različitih starosnih i polnih podgrupa zdrave fizički aktivne dece i adolescenata. Sprovedena je antropometrijska procena (Matiegka protokol), hematološka analiza, tj. broj eritrocita (RBC), nivo hemoglobina, hematokrit i ergometrijsko testiranje (Brus protokol). Što se tiče mase i visine tela do 12. godine (U12), devojčice su bile teže i više od dečaka. Srednje vrednosti relativne mišićne mase u svim uzrasnim kategorijama dečaka i devojčica su u optimalnom rasponu (>50%) i ukazuju na dobro razvijenu mišićnu masu. Ne postoje razlike između podgrupa istog uzrasta za navedeni antropometrijski parametar između dečaka i devojčica. U svim uzrasnim kategorijama devojčicama je utvrđen veći procenat telesne masnoće u odnosu na njihove vršnjake. RBC varijable devojčica nisu se razlikovale u uzrasnim kategorijama. Što se tiče pola, vrednosti svih varijabli RBC bile su značajno veće u muškim grupama, osim u U12. Kardio-fiziološki parametri broj otkucaja srca u mirovanju, vreme vežbanja i maksimalna potrošnja kiseonika ( $VO_{2max}$ ), bili su značajno veći kod dečaka.  $VO_{2max}$  je bio veći kod starijih dečaka u odnosu na mlađe dečake. Zaključak: Ovo istraživanje je pokazalo promene u antropološkim, hematološkim i ergometrijskim parametrima mlade i aktivne populacije muškog i ženskog pola. Devojčicama je utvrđen značajno niži nivo kardio-fiziološke kondicije, čemu mogu doprineti pol i manji obim FA.

Ključne reči: Sastav tela, crvena krvna zrnca, hemoglobin, hematokrit, adolescenti