Landgraff, H. E. W., Hallén, J. (2020). Longitudinal Training-related Hematological Changes in Boys and Girls from Ages 12 to 15 yr. *Medicine & Science in Sports & Exercise, 52*(9), 1940-1947. http://dx.doi.org/10.1249/MSS.0000000000002338

Longitudinal Training-related Hematological Changes in Boys and Girls from Age 12 to 15

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The authors declare that they have no conflict of interest. This study was not funded. The authors herewith state that the results of the present study do not constitute endorsement by the American College of Sports Medicine and are presented clearly, honestly, and without fabrication, falsification, or inappropriate data manipulation.

Abstract

Purpose: Natural growth and maturation cause hemoglobin mass (Hbmass) and blood volume (BV) to increase during childhood and adolescence. Whether endurance training during the same period can cause further increases in these variables is not clear. Also, body composition develops differently in girls and boys during puberty, and the effect of these differences on hematological variables has not previously been studied.

Methods: Forty-two endurance athletes (End-group; 24 boys) and 34 athletes from other sports (nonEnd-group; 23 boys) were tested at age 12, 13 and 15 for Hbmass, BV, plasma volume (PV), red cell volume (RCV), hematological variables and anthropometrics.

Results: At age 12, Hbmass and BV showed no difference between sexes or training groups in absolute values or relative to fat-free mass (FFM). Relative to FFM, Hbmass and BV averaged 11.7 (0.8) $g \cdot kg^{-1}$ and 95 (6.8) $ml \cdot kg^{-1}$. Increases in FFM from age 12 – 15 primarily determined the increased Hbmass and BV for both sexes with no differences between training groups. At age 15, Hbmass relative to FFM was higher in boys than girls (13.1 (0.8) $g \cdot kg^{-1}$ and 12.1 (0.9) $g \cdot kg^{-1}$; $p<0.001$) while BV relative to FFM was not significantly different between sexes or training groups at any ages (averaged 100 (6.7) $ml \cdot kg^{-1}$ at age 15). Relative to FFM, PV was higher in the End-group at all ages and RCV was, on average, lower and increased less compared with nonEnd-group.

Conclusion: Our results indicate that increases in Hbmass during puberty are mainly associated with increased FFM and independent of sex or volume of endurance training.
However, the FFM-relative PV was higher and FFM-relative RCV was lower in the End-group compared to the nonEnd-group.

Key Words: Hemoglobin mass, Blood volumes, Longitudinal, Adolescents, Growth

INTRODUCTION

Hemoglobin mass (Hbmass) and blood volume (BV) increase with age during childhood and adolescence because of natural growth and maturation. However, whether endurance training during the same period results in further increases in these variables is not clear. Hbmass and BV are strongly related to maximal oxygen uptake and performance in endurance sport. Cross-sectional studies on adults show that highly trained endurance athletes have up to 40% higher levels of Hbmass and BV than non-endurance trained athletes and untrained individuals (1-3). Whether the higher levels are due to endurance training or genetic predisposition is not entirely clear, and the mechanisms behind the higher levels of Hbmass are not yet fully understood (4, 5).

Only a few longitudinal studies have looked at the effect of endurance training on the development on Hbmass in adolescent boys and girls (6-9). Eastwood et al. (7), Steiner et al. (9) and Ulrich et al. (8) concluded that there was no effect on Hbmass related to body mass with endurance training, while Prommer et al. (6) suggested that endurance training may have an effect beyond what could be expected from growth and maturation. The authors found that the close relationship between Hbmass and fat-free mass (FFM) in adults also applies to children. Some of these studies have methodological challenges, including mixed sex groups (6, 7) and large age spans at inclusion, such as 11-15 years (7), and 7.6-11.5 years (6). Since girls and boys have different physiological features during puberty, this may influence the results. Only one study had separate groups for girls (8) but the age at inclusion was 15 years. They concluded that changes in FFM were the main factors in increasing Hbmass over time, but that training volume had an additional effect.

Hence, the purpose of the present study was to investigate the influence of growth and endurance training on Hbmass, BV and blood profile in active boys and girls around the ages of puberty and peak height velocity (PHV). We used a longitudinal design from age 12 to 15 and recruited children from cross-country ski clubs and children from school classes engaged in different sport activities. Most children in this age group engage in several hours of physical activity per week in different sport activities. Cross-country skiers at age 13 – 15 also include many hours of systematic endurance training. We compared boys and girls during this period, and groups with high and low volumes of endurance training.

MATERIAL AND METHODS

Study design

Using a repeated-measure design, we assessed anthropometrics, Hbmass, hematological variables, sexual maturity, predicted age at PHV and amount of training in 76 healthy children over a period of three years. All tests were performed in one day and the participants were tested three times (ages 12.1 (0.4), 13.4 (0.3), and 15.3 (0.3) years). Written parental consent was obtained prior to any testing. All experimental procedures were approved by the Norwegian Regional Committee for Medical Research Ethics and conformed to the standards set by the Declaration of Helsinki.

Participants

Forty-two endurance athletes (End-group; 24 boys; 93% cross-country skiers) and 34 athletes from other sports (nonEnd-group; 23 boys; 95% team sports) were tested at age 12, 13 and 15 for Hbmass and anthropometrics. Every year, the participants completed a questionnaire to assess

types of sports participation and the amount of weekly training hours. Participants were also interviewed (age 15) in order to get a more detailed picture about weekly training content.

Both training groups participated in organized sport on average from 6.5 (2.2) hours per week at age 12 to 9.3 (3.5) hours per week at age 15. On average over the years, the End-groups trained 1.7 hours more per week than the nonEnd-groups. In the nonEnd-groups most of the training was with focus on technical and tactical skill development, while in the End-groups, the training became gradually more focused on endurance training over the years. At age 14-15, the End-groups performed typical endurance training (continuous or interval workouts using running, skiing or biking) 6.5 (1.6) and 8.0 (1.8) hours per week in girls and boys, respectively. The nonEnd-groups included typical endurance training 1.2 (0.9) and 0.9 (0.7) hours per week in girls and boys, respectively.

Anthropometry

All measurements were conducted with the participants wearing shorts, t-shirt and no shoes. Stature and sitting height were measured to the nearest 0.5 cm using a stadiometer (Seca, Hamburg, Germany) and body mass (BM) to the nearest 0.1 kg using a digital scale (Seca, Hamburg, Germany). Sitting height was used to predict years from PHV (10). Body composition was assessed by bioelectrical impedance analysis (InBody, 720, Biospace Co, Ltd, Seoul, Korea). In 7 out of 76 participants, one out of the three measurements of body composition were missing due to technical error. On average, %FM changed in a near linear manner from age 12 – 15, with similar change per year, within each group (table 1). Based on this, the third missing

%FM value was calculated by interpolation or extrapolation from the two valid assessment. FFM was than calculated based on BM and %FM.

Sexual maturity

All participants underwent a brief health check by a medical doctor. In girls, breast development was assessed according to Tanner (11) and they were asked about menarche. In boys, blood samples were analysed for testosterone. Chronological age was calculated as the difference between date of birth and date of testing.

Venous blood sample

Emla cream (AstraZeneca 55, Lidocain 25 mg/g, Prilocain 25 mg/g) was used as a topical anaesthetic before venepuncture to reduce pain and distress for the participants. Blood samples were drawn from an antecubital vein into 4 mL EDTA glass tubes (EDTA glass, BD vacutainer K2E 7,2 mg) and 5 mL serum gel tubes (VACUETTE® TUBE 5 ml Z Serum Separator Clot Activator). The EDTA coated tubes were sent to a medical laboratory, (Fürst, Oslo, Norway) the following morning and analysed for hemoglobin concentration ([Hb]), red blood cell count (RBCC), hematocrit (Hct) and mean red cell volume (MCV) (Sysmex XN-9000 Automation, Sysmex Corporation, Kobe, Japan). Mean corpuscular hemoglobin concentration (MCHC) was calculated by dividing [Hb] by Hct. The serum tubes were left to rest for at least 30 min before centrifuging at 3500 G for 10 minutes at 4°C. The serum was then transferred to Eppendorf tubes and frozen. All samples were stored at -80°C until analysis. When all the samples had been collected, the serum tubes were sent to a medical laboratory, (Fürst, Oslo, Norway) and analysed

for serum ferritin (Advia Chemistry XPT, Siemens Medical Solutions Diagnostics, Japan) and testosterone (Advia Centaur XPT, Siemens Healthcare Diagnostic Inc., USA).

Assessment of total hemoglobin mass and blood volume parameters

Total Hbmass was assessed using the optimised carbon monoxide rebreathing method as described in detail by Prommer and Schmidt (12). In brief, a bolus of carbon monoxide (CO) was inhaled and rebreathed for 2 min through a closed circuit consisting of a glass spirometer (Blood Tec Gbr, Bayreuth, Germany) and a 3-litre anaesthetic bag containing 100% oxygen (O_2). The administered amount of CO was individually calculated to 1.0 and 0.8 $mL \cdot kg^{-1}$ of body mass for boys and girls, respectively. During CO rebreathing, CO leakage was checked at the mouthpiece, nose clip and spirometer using a portable CO-gas analyser (Dräger-Pac 7000, Dräger Safety AG Co, Lübeck, Germany). Arterialised capillary blood samples (125µl) were taken from a pre-warmed fingertip before and at 6 and 8 min after commencing the rebreathing and immediately analysed twice for percent carboxyhemoglobin (%HbCO) using a diode array spectrophotometer (ABL80 FLEX CO-OX, Radiometer, Copenhagen, Denmark). From the difference in %HbCO before and after the CO application, the Hbmass was calculated as described by Schmidt and Prommer (13). Blood volume (BV), plasma volume (PV) and red cell volume (RCV) were calculated from Hbmass using venous hemoglobin concentration [Hb] and venous hematocrit (Hct) according to Burge and Skinner (14) and Heinicke et al (1).

Before any actual measurements, all participants were familiarized with the rebreathing procedure, with air applied instead of CO, until they fully mastered the technique.

Statistical analyses

A three-way mixed ANOVA was run to examine the effects of sex, training group (group) and age on the different variables. Data are mean (standard deviation) unless otherwise stated. A Shapiro-Wilk test ($p > 0.05$) was used to test whether the variables for the different groups and time points were normally distributed. Testosterone at age 12 and 13 was not normally distributed. For unpaired comparisons, the Student's t-test was run when data were normally distributed, and a Mann-Whitney U Test was used when data were not normally distributed. GraphPad Prism 8.2.1 (GraphPad Software Inc., La Jolla, CA) and Microsoft Excel 2013 were used for statistical analyses.

RESULTS

Participant characteristics

The participants' anthropometrics are given in Table 1. There were no differences in height and FFM between training groups at any age. End-boys were lighter and had lower percent fat mass (%FM) than the nonEnd-boys at age 12 and 13, but not at age 15. End-girls had lower %FM than the nonEnd-girls at age 12 and 15. At the start of the study, menarche had not occurred in any of the girls. Based on breast development, the Tanner stage was 1 and 2 in 25 out of 29 girls and 4 were at Tanner stage 3. At age 15 years, 100% of the nonEnd-girls and 72% of the End-girls had begun menstruation. Forty out of 47 boys had S-Testosterone levels below 3.5 $nmol \cdot L^{-1}$ (100 $ng \cdot dL^{-1}$) at age 12. Levels of S-Testosterone were higher in nonEnd-boys than in End-boys at age 12 (2.6 (2.9) vs 0.7 (1.2) $nmol \cdot L^{-1}$; $p=0.005$) at age 13 (6.4 (5.9) vs 2.2 (3.0) $nmol \cdot L^{-1}$; $p=0.004$) and age 15 (13.2 (5.4) vs 7.3 (4.1) $nmol \cdot L^{-1}$; $p<0.001$). The ferritin levels were on average over the three years 31 (14), 29 (15), 34 (11) and 43 (20) µg/L in End-girls, nonEnd-girls, End-boys

and nonEnd-boys, respectively and girls had lower levels than boys (p=0.044). On average for all groups, ferritin levels decreased from age 12 to 15 by 2.4 (6.3) µg/L per year (p=0.003). However, evaluated by groups, only End-girls had a significant reduction of 3.8 (4.5) µg/L per year (p=0.002).

Hbmass and blood volumes

Hbmass and blood volumes (PV, RCV and BV) increased in absolute values with age for all groups (Figure 1-4, A; $p<0.001$ for all). There was no significant 3-way interaction between age, sex and group for any of these variables. There was a simple 2-way interaction between age and sex for all the variables, but only PV interacted with training groups (for p-values, see Figures). For all these variables, the increase with age was larger in boys than in girls and PV increased more in the End-groups compared with nonEnd-groups. There were strong FFM correlations with changes in Hbmass and blood volumes, from age 12 to 15 ($0.78<r<0.85$; $p<0.001$ for all).

There was no significant 3-way interaction between age, sex and group for Hbmass or blood volumes relative to BM. There was a simple 2-way interaction between age and sex for all these variables (Figure 1-4, B). Hbmass and blood volumes relative to BM increased with age in boys (p=0.001 for all), but not in girls (p=0.086 – 0.335). For girls, Hbmass, RCV, PV and BV relative to BM decreased from age 13 to 15 ($p<0.02$ for all).

There was no significant 3-way interaction between age, sex and group for Hbmass or blood volumes relative to FFM. There was a simple 2-way interaction between age and sex for Hbmass and RCV, but not for PV and BV (Figure 1-4, C). Hbmass relative to FFM increased for boys

(0.43 (0.28) $g \cdot kg^{-1}$; $p<0.001$) and for nonEnd-girls (0.23 (0.38) $g \cdot kg^{-1}$; $p=0.037$) but not for End-girls (0.08 (0.32) $g \cdot kg^{-1}$; $p=0.508$). RCV and BV relative to FFM also increased for boys (1.4 (0.95) and 1.7 (2.3) $ml \cdot kg^{-1}$ per year, respectively; $p<0.001$ for both), but not for girls ($p> 0.05$). For RCV relative to FFM, there was also a simple 2-way interaction between age and training groups. At age 15, nonEnd-groups had higher RCV than the End-group both for girls ($p=0.014$) and for boys ($p=0.005$). PV did not change significantly in any of the groups ($0.186<p<0.852$) but was higher in the End-groups at all ages.

Blood parameters: [Hb] and Hct, MCV, MCHC and RBCC

There was no significant 3-way interaction between age, sex and group for any of the blood parameters. There was a simple 2-way interaction between age and sex for [Hb], Hct and RBCC (Figure 5). This interaction was mainly caused by differences in the age effect of sex in the End-groups, with End-boys having an increase for all these variables, with End-girls either having similar values in all three years (Hb and Hct) or showing a decrease (RBCC; $p=0.030$). For boys, these variables increased on average by 1.6-2.0% per year ($p<0.001$ for all), while there were no significant changes in girls (except the decrease for RBCC in End-girls). At age 15, End-girls had 7-8% lower values for these variables than End-boys ($p<0.001$ for all), with no significant differences in the nonEnd-groups.

There was a simple 2-way interaction between age and group for all measured blood variables except [Hb] (Figure 4). This means that, contrary to what we generally found for blood volumes, the age effects on blood parameters were dependent on training group. MCV increased by 0.3% per year for the End-groups ($p<0.001$) and 0.8% per year in the nonEnd-groups ($p<0.001$), while

MCHC decreased by 0.8% per year in nonEnd-groups ($p<0.001$), with no change in the End-groups.

DISCUSSION

The main findings from the present study are as follows. 1) The increase in Hbmass and blood volumes with age (age effect) was mainly determined by the increases in FFM in both boys and girls. 2) For Hbmass, there was an additional increase in boys, but this increase was less clear in girls. 3) Relative to FFM, BV was not significantly different between age, sex and training groups. 4) PV was higher and RCV was lower in the End-groups compared with the nonEnd-groups. 5) Contrary to the age effects on Hbmass and blood volumes, the age effect on hematological variables measured in blood samples mainly interacted with the volume of endurance training and not with sex.

Hbmass

Cross-sectional studies, in adults, show that highly trained endurance athletes have up to 40% higher Hbmass and BV than non-endurance trained athletes and untrained individuals (1-3). As training studies in adults have only found minor (untrained) or no (previously trained) effects of training on Hbmass, Schmidt and Prommer (3) concluded that training only has a small effect and that genetic predisposition should be considered to be responsible for the high Hbmass in elite ahletes. Later, Prommer et al. (6) hypothesized that erythropoiesis may be influenced by training at a very young age and that this could explain the differences in Hbmass between adult endurance athletes and sedentary participants. In a longitudinal study of young children, they found support for this hypothesis. Although the increase in Hbmass with age was mainly

associated with an increase in FFM, endurance training exerted an additional effect (6). However, the authors also pointed out that they did not include a control group in the longitudinal study, and it is therefore difficult to determine whether the additional effect is due to endurance training or genetic predisposition.

In the present study, we found no additional influence of high volumes of endurance training. In boys, the increase was similar in the group with a high volume of endurance training compared with the group with a low volume of endurance training. In girls, we found a possible negative effect of high volumes of endurance training on Hbmass. Only a few additional studies have looked at the influence of endurance training on Hbmass during adolescence in a longitudinal design (7-9, 15). In these studies, the authors concluded that the increase in Hbmass was related to growth only (body mass) with no additional effect of training. However, we cannot directly compare our results with these studies due to their different designs, which involved male participants only, mixed sex groups and different age groups. In the study by Ulrich et al (8), the participants were older (15 – 17 years) while the participants in the investigation by Prommer et al. (6) were younger (8 – 13 years). Eastwood et al. (7) used participants with similar ages to ours, but had a mixed sex group, and the participants were included in the study at different ages. Hence, as far as we know, our study is the only one to have followed the same participants from age 12 to age 15, with separate groups for girls and boys and where most of the participants went through puberty.

Recently, Steiner et al. (9) showed, in a longitudinal boys' study, that Hbmass normalized to FFM increased from age 16 to age 19 and that the increase was independent of the volume of

endurance training. Our study extends these findings by showing that Hbmass normalized to FFM also increased from age 12 to 15 in boys and that in this age range, the increase was again independent of the volume of endurance training. The rate of increase in our study was 0.43 (0.28) $g \cdot kg^{-1}$ FFM per year. Steiner et al. (9) found a total increase over 3 years of 0.74 $g \cdot kg^{-1}$ FFM. However, their tabulated data show that the increase leveled off after age 17.5. Our calculations from their data indicate a rate of increase from age 16 – 17.5 of approximately 0.5 (0.3) $g \cdot kg^{-1}$ FFM per year, close to our rate of increase from age 12 – 15. Taken together, the findings of Steiner et al. (9) and the present study suggest that in boys, during puberty, Hbmass increases mainly as a function of increased FFM, but there is also an additional effect, independent of both FFM and volume of endurance training.

While Hbmass relative to BM increased in boys with age, there were no significant changes in girls. The %FM increased in girls, but not in boys over these years and when Hbmass was normalized to FFM, the difference between boys and girls was reduced. Hence, the sex effect was mainly due to the increase in %FM in girls. As for boys, Hbmass relative to FFM increased in the nonEnd-girls, but with a rate half that of the boys (0.23 (0.29) $g \cdot kg^{-1} \cdot year^{-1}$; p=0.037). However, in the End-girls, there was no increase (0.08 (0.36) $g \cdot kg^{-1} \cdot year^{-1}$, p=0.6). We cannot say whether endurance-training volume per se could have had a direct negative effect on erythropoiesis, or alternatively, that the effect was due to endurance training in combination with malnutrition. End-girls had significantly lower %FM than nonEnd-girls (on average over all years, 14.4% vs 18.4%; $p<0.03$). Also, only End-girls had a significant reduction in ferritin levels over the three years and half of the End-girls had ferritin levels lower than 20 µg/L (range for these 7 – 19 µg/L) at age 15. RBCC was lower in End-girls than nonEnd-girls at age 15

(p=0.004). Furthermore, End-girls had a reduction in RBCC from age 13 to 15 while the other groups experienced an increase or no change at the same age. Together, the changes in RBCC and MCV resulted in no change in Hct for End-girls, while the other groups experienced an increase in Hct from age 12 – 15. Altogether, our findings indicate that high volumes of endurance training in combination with malnutrition may have negative effect on erythropoiesis in girls. If this is the case, then coaches and parents should be made aware of the need to regularly monitor iron status in growing children, and particularly girls, who undertake high volumes of endurance training.

Our data support the conclusion of Prommer et al. (6), that the increase in absolute Hbmass during these ages was mainly determined by the increase in FFM in both boys and girls. Our results showed that there was an additional increase, independent of FFM and endurance-training volume, and which was most evident in boys. This may be a direct effect of testosterone on erythropoiesis (16). However, based on some indices in our results, we speculated that the direct effect of testosterone on erythropoiesis was minor and that the main effect of testosterone was via the hormone's effect on muscle mass. Since the increase in Hbmass was tightly associated with the increase in FFM, it is difficult to discriminate between a direct effect on erythropoietin or erythropoiesis and an indirect effect via testosterone's effects on muscle mass. In the present cohort of boys, FFM increased on average by 19 kg from age 12 to 15. At age 12, Hbmass per kg FFM was on average 11.7 $g \cdot kg^{-1}$. If this value stayed constant over these years, Hbmass would have increased by 222 g (19 kg $\cdot$ 11.7 $g \cdot kg^{-1}$). From age 12 to age 15, the total increase for the boys was, on average, 295 g. Hence, an average 73 g increase in Hbmass was independent of the increase in FFM. This increase could theoretically have been a direct effect of testosterone on

erythropoiesis. However, it seems that Hbmass increased at a constant rate from age 12-17 (present data and data from ref. 9), while testosterone in boys was low at age 12 and 13, and much higher at age 15. Furthermore, Hbmass relative to FFM seems to level off at age 17-18 (9), when testosterone is at its highest level. Also, for the girls, there was an increase in Hbmass, mostly proportional to the increase in FFM. It is not likely that this increase was determined by testosterone since the hormone level was very low in these girls. For the nonEnd-girls, an increase, on average, of 23 g was independent of the increase in FFM ($p=0.037$), and again probably not determined by testosterone. Hence, from these numbers it is difficult to argue that testosterone has a significant direct effect on erythropoiesis during puberty.

To check whether Hbmass was somehow affected by maturation, we calculated simple correlations between years from PHV and absolute Hbmass values, separately for each year and each sex. For both girls and boys, there were strong correlations between years from PHV and HBmass in absolute values for all 3 years ($0.64<r<0.085$; $p<0.001$). However, for FFM-relative values, there were no correlations with years from PHV. This supports FFM as being the main factor determining Hbmass. However, the fact that there was an additional increase, independent of FFM (i.e. FFM-dependent Hbmass increased), indicates that maturational factors independent of growth exist. If this factor is testosterone, other hormones or other factors related to maturation, it needs further investigation.

Blood volumes and hematological parameters

Absolute values for BV, PV and RCV showed similar patterns to the absolute values for Hbmass. BM-relative blood volumes and Hbmass values showed similar patterns, increasing

with age in the boys, but not changing in the girls. The effect of sex and the interaction of sex and age on blood volumes relative to BM were partly due to body composition, since they disappeared or decreased when the volumes were normalized to FFM. For BV relative to FFM, there were no sex or group differences at any ages. There was a small increase in BV normalized to FFM with age for boys both in the present study and in the study by Steiner et al. (9). Furthermore, BV normalized to FFM at age 16 in Steiner et al.'s (9) investigation, produced results similar to the values in the present study at age 15; approximately 100 $ml \cdot kg^{-1}$ FFM.

Contrary to what was the case for BV and Hbmass, a group effect was evident for PV and RCV relative to FFM, with higher values in the End-groups for PV ($p=0.015$) and lower values for RCV ($p=0.009$). One explanation for the lower RCV in the End-groups is that the End-groups had lower RBCC (per liter of blood). This is in line with the findings of Boyadjiev and Taralov (17) , who compared red blood cell variables in highly trained male and female pubescents athletes from different sport disciplines (age 14.0 (0.06) years) and untrained controls (age 14.6 (0.09) years). They found that the highly trained group had lower RBCC, Hct and [Hb] than the untrained group. In the present study, there was also a group effect on the change in MCV, which increased more with age in the nonEnd-groups compared with the End-groups. There were also group effects on MCHC (Hb-concentration in the red blood cells) as well as MCH (Hb-content of the red blood cells). Taken together, these findings indicate that the higher volume of endurance training induced lower RBCC and lower MCV, resulting in a lower RCV. However, a higher PV compensated for this possible training effect, resulting in the same BV between the training groups.

In adults, long-term endurance training leads to RCV expansion (18). However, prior to the long-term (~ 4-8 weeks) increase in RCV, endurance training induces an expansion of PV, both acutely, following a single training bout, as well as a short-term training effect (~ 1-2 weeks) (5). It is suggested that the mechanism behind the training induced increase in RCV is an erythropoietic feedback to normalize Hct after the acute training induced reduction in Hct due to the PV expansion (5). Importantly, in the current study, the Hct was lower in the End-group already at age 12 and stayed lower to age 15, more so in girls than boys. In boys, the age-related increase in Hct was not different between training groups, due to similar age effect on RBCC in the two groups. This is in line with the changes found in a lager reference cohort (19). Also, in line with this reference group, MCV increased with age in the nonEnd-group (both sexes). However, in the present study, MCV did not increased with age in the End-group (both sexes). Hence, it may be that endurance training blunts the normal increase in MCV during puberty, and that this may be one mechanism behind the reduced Hct. In nonEnd-girls, RBCC did not change with age, again in line with the reference cohort (19), while the RBCC in the End-girls decreased. Since the age effect on RBCC was similar in the two groups of boys, but not the two groups of girls, and endurance training seems to affect MCV in both sexes, it may be hypothesized that the reduction of RBCC in the End-girls is due to low ferritin levels while the lack of increase in MCV is more sensitive to the endurance training. These two mechanisms together may result in the very low Hct and [Hb] in the End-girls relative to nonEnd-girls.

The higher PV relative to FFM in the End-groups compared with the nonEnd-groups, and the lack of change with age, are in line with Steiner et al. (9), and the values for both End-boys and nonEnd-boys are very similar in the two studies, regardless of the age difference. Based on the

two studies, it seems that FFM-relative PV does not change from age 12 to 19, but tends to be higher in endurance-trained populations. In addition, we found that PV relative to FFM was the same for boys and girls both in the End- and nonEnd-groups, respectively. The two studies together indicate that during adolescence, Hbmass is dependent on sex and independent of the volume of endurance training, RCV is dependent on both sex and volume of endurance training while PV is independent of sex and dependent on the volume of endurance training.

One of the limitations of the current study is the relatively small number of participants, especially nonEnd girls. Also, some of the variables seem to be influenced by nutrition. Hence, identified differences and no differences between End- and nonEnd-groups must be regarded with caution. However, our results stand in agreement with other related studies.

In conclusion, our results indicate that in both sexes, increases in Hbmass and blood volumes during puberty were mainly associated with increases in FFM. For Hbmass, there was an additional increase of 0.4-0.5 $g \cdot kg^{-1}$ FFM per year for boys. In girls, this additional increase in Hbmass was less clear and may be influenced by malnutrition, since there was some association between changes in Hbmass and low ferritin levels. BV normalized to FFM was similar between sexes and training groups at all ages between 12 and 15. On average, RCV was lower and PV was higher in children undertaking high volumes of endurance training compared with those with low volumes of endurance training. Endurance training may affect the red blood cells by decreasing MCV and increasing MCH.

Acknowledgments

We thank Hege Nymo Østgaard, Stian Hallén, Anette Aaeng and Carlo Capelli for laboratory assistance.

Conflict of interest

The authors declare that they have no conflict of interest. This study was not funded. The authors herewith state that the results of the present study do not constitute endorsement by the American College of Sports Medicine and are presented clearly, honestly, and without fabrication, falsification, or inappropriate data manipulation.

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Figure legends

Figure 1: A) Hbmass (hemoglobin mass) in absolute values, B) Hbmass relative to BM (body mass) and C) Hbmass relative to FFM (fat free mass) in girls and boys undertaking high (End-girls and End-boys) and low (nonEnd-girls and nonEnd-boys) volumes of endurance training at age 12, 13 and 15.

Figure 2: A) RCV (red cell volume) in absolute values, B) RCV relative to BM (body mass) and C) RCV relative to FFM (fat free mass) at age 12, 13 and 15 (for groups see Fig 1).

Figure 3: A) PV (plasma volume) in absolute values, B) PV relative to BM (body mass) and c) PV relative to FFM (fat free mass) at age 12, 13 and 15 (for groups see Fig 1).

Figure 4: A) BV (blood volume) in absolute values, B) BV relative to BM (body mass) and C) BV relative to FFM (fat free mass) at age 12, 13 and 15 (for groups see Fig 1).

Figure 5: Hb (hemoglobin concentration), Hct (hematocrit), MCHC (mean corpuscular hemoglobin concentration), MCV (mean corpuscular volume), RBCC (red blood cell count) and MCH (mean corpuscular hemoglobin) at age 12, 13 and 15 (for groups see Fig 1).

Figure 1

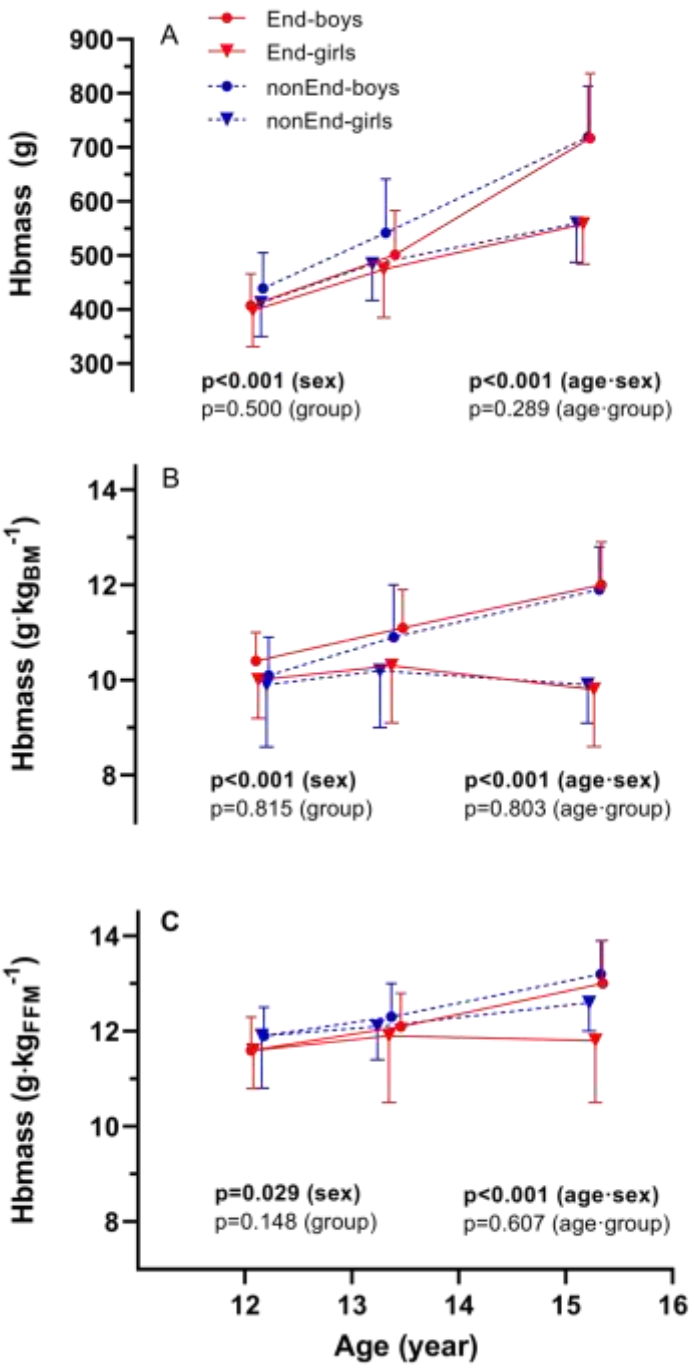
A
End-boys
End-girls
nonEnd-boys
nonEnd-girls
Hbmass (g)
900
800
700
600
500
400
300
p<0.001 (sex)
p=0.500 (group)
p<0.001 (age·sex)
p=0.289 (age·group)
B
Hbmass (g·kg$_{BM}^{-1}$)
14
12
10
8
p<0.001 (sex)
p=0.815 (group)
p<0.001 (age·sex)
p=0.803 (age·group)
C
Hbmass (g·kg$_{FFM}^{-1}$)
14
12
10
8
p=0.029 (sex)
p=0.148 (group)
p<0.001 (age·sex)
p=0.607 (age·group)
12
13
14
15
16
Age (year)

Figure 2

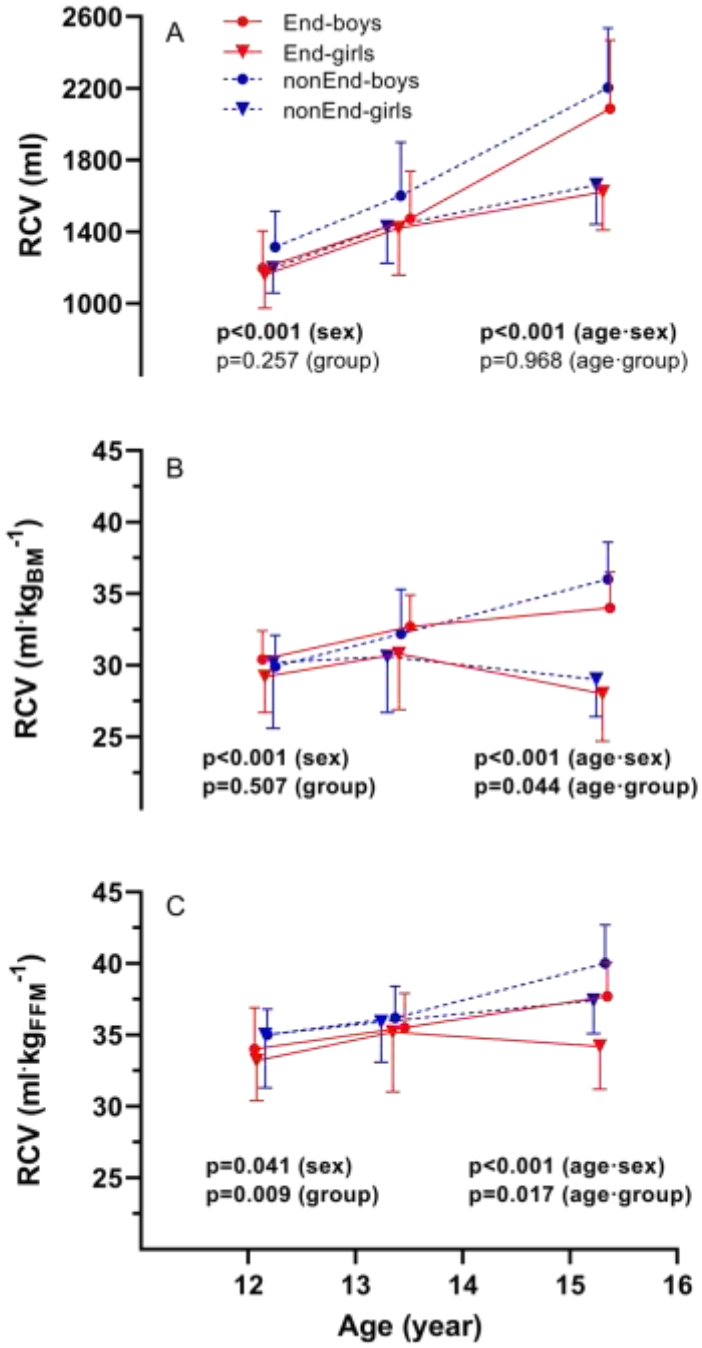
A
End-boys
End-girls
nonEnd-boys
nonEnd-girls
RCV (ml)
2600
2200
1800
1400
1000
p<0.001 (sex)
p=0.257 (group)
p<0.001 (age·sex)
p=0.968 (age·group)
B
RCV (ml·kg$_{BM}^{-1}$)
45
40
35
30
25
p<0.001 (sex)
p=0.507 (group)
p<0.001 (age·sex)
p=0.044 (age·group)
C
RCV (ml·kg$_{FFM}^{-1}$)
45
40
35
30
25
p=0.041 (sex)
p=0.009 (group)
p<0.001 (age·sex)
p=0.017 (age·group)
12
13
14
15
16
Age (year)

Figure 3

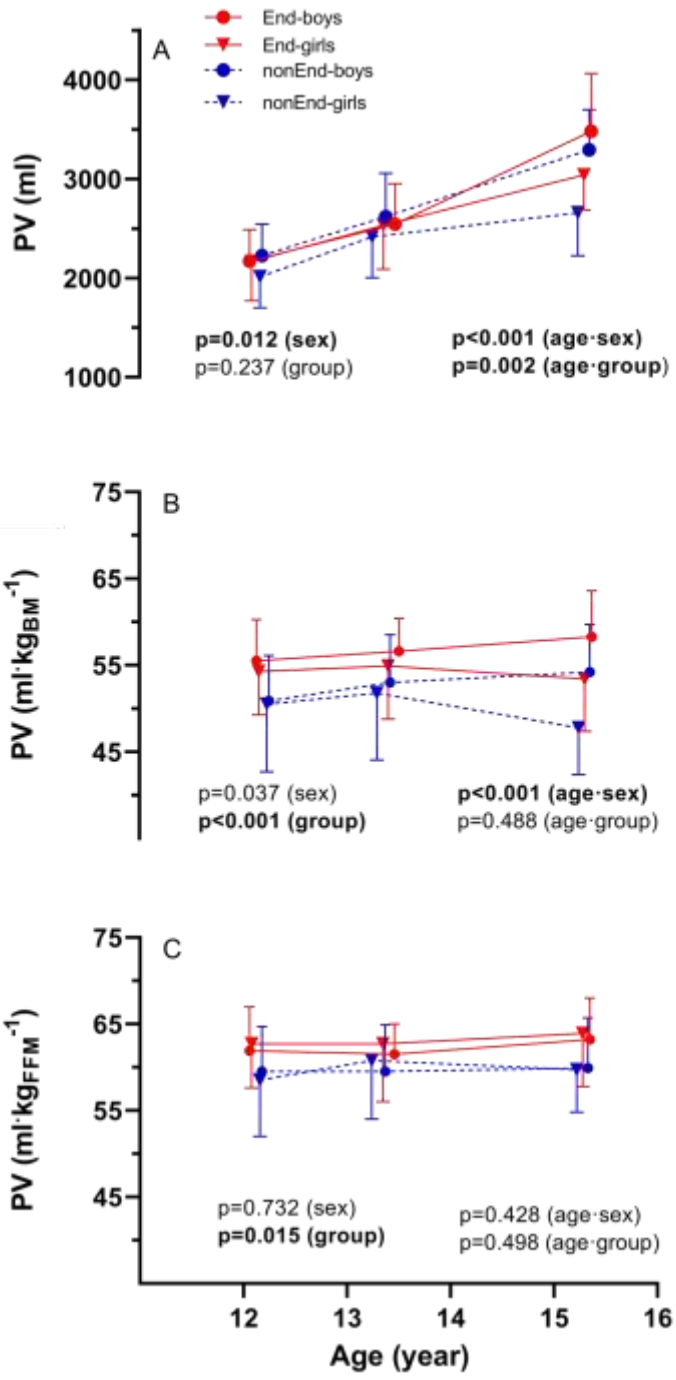
End-boys
End-girls
nonEnd-boys
nonEnd-girls
A
PV (ml)
4000
3000
2000
1000
p=0.012 (sex)
p=0.237 (group)
p<0.001 (age·sex)
p=0.002 (age·group)
B
PV (ml·kgBM-1)
75
65
55
45
p=0.037 (sex)
p<0.001 (group)
p<0.001 (age·sex)
p=0.488 (age·group)
C
PV (ml·kgFFM-1)
75
65
55
45
p=0.732 (sex)
p=0.015 (group)
p=0.428 (age·sex)
p=0.498 (age·group)
12
13
14
15
16
Age (year)

Figure 4

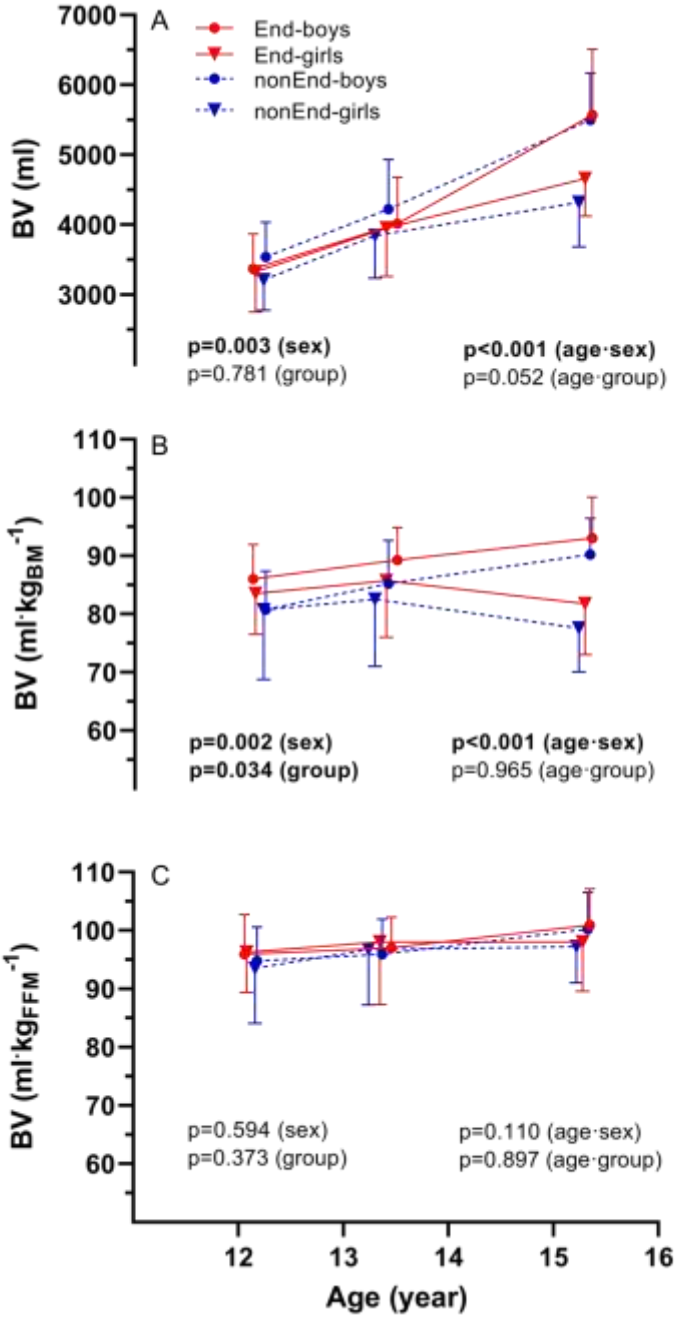
A
End-boys
End-girls
nonEnd-boys
nonEnd-girls
BV (ml)
7000
6000
5000
4000
3000
p=0.003 (sex)
p=0.781 (group)
p<0.001 (age·sex)
p=0.052 (age·group)
B
BV (ml·kgBM-1)
110
100
90
80
70
60
p=0.002 (sex)
p=0.034 (group)
p<0.001 (age·sex)
p=0.965 (age·group)
C
BV (ml·kgFFM-1)
110
100
90
80
70
60
p=0.594 (sex)
p=0.373 (group)
p=0.110 (age·sex)
p=0.897 (age·group)
12
13
14
15
16
Age (year)

Figure 5

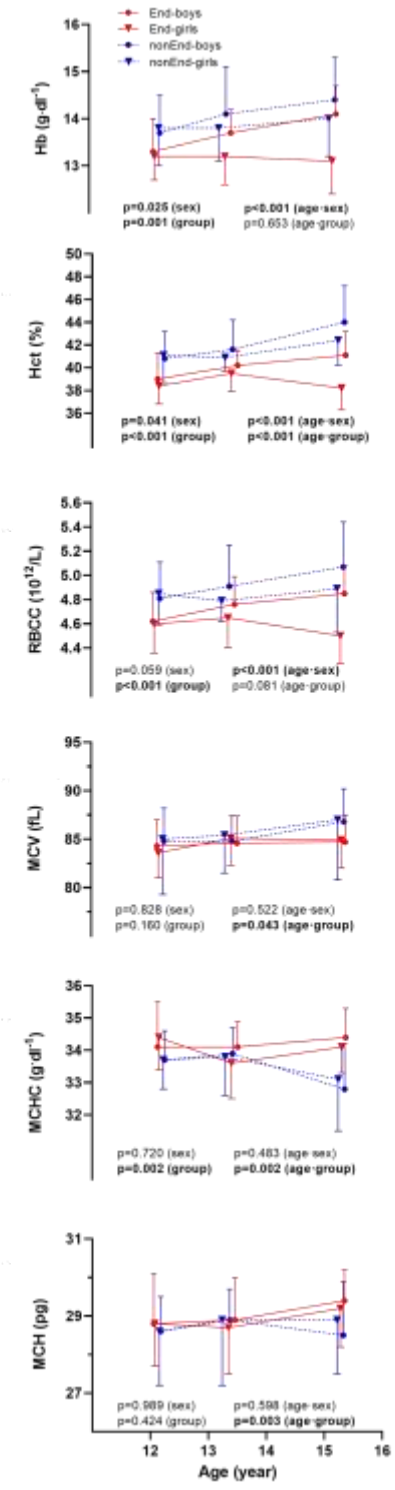
End-boys
End-girls
nonEnd-boys
nonEnd-girls
Hb (g·dl⁻¹)
p=0.026 (sex)
p=0.001 (group)
p<0.001 (age·sex)
p=0.653 (age·group)
Hct (%)
p=0.041 (sex)
p<0.001 (group)
p<0.001 (age·sex)
p<0.001 (age·group)
RBCC (10¹²/L)
p=0.059 (sex)
p<0.001 (group)
p<0.001 (age·sex)
p=0.061 (age·group)
MCV (fL)
p=0.828 (sex)
p=0.160 (group)
p=0.522 (age·sex)
p=0.043 (age·group)
MCHC (g·dl⁻¹)
p=0.720 (sex)
p=0.002 (group)
p=0.483 (age·sex)
p=0.002 (age·group)
MCH (pg)
p=0.989 (sex)
p=0.424 (group)
p=0.598 (age·sex)
p=0.003 (age·group)
Age (year)

Table 1: Subject characteristics at age 12, 13 and 15.

	End-girls (n=18)			**nonEnd-girls (n=11)**		
	Test 1	Test 2	Test 3	Test 1	Test 2	Test 3
Age (year)	12.1 (0.4)	13.3 (0.3)	15.3 (0.3)	12.2 (0.4)	13.2 (0.3)	15.2 (0.3)
Height (cm)	153 (6.1)	161 (7.0)	168 (6.4)#	152 (5.4)	160 (4.2)	165 (5.1)#
Body mass (kg)	39.7 (5.6)	46.0 (6.8)	57.2 (7.3)	42.2 (8.8)	48.4 (9.3)	56.4 (7.4)
FFM (kg)	34.5 (4.9)	39.8 (5.5)	47.4 (5.0)#	34.9 (5.4)	40.0 (4.7)	44.3 (5.0)#
Fat mass (%)	12.0 (3.3)#*	13.8 (2.9)#	16.6 (4.7)#*	16.9 (7.4)	17.2 (6.9)#	21.0 (6.0)#

	End-boys (n=24)			**nonEnd-boys (n=23)**		
	Test 1	Test 2	Test 3	Test 1	Test 2	Test 3
Age (year)	12.1 (0.4)	13.5 (0.3)	15.4 (0.3)	12.3 (0.4)	13.4 (0.3)	15.4 (0.3)
Height (cm)	152 (7.3)	161 (9.2)	175 (10.3)	154 (8.2)	163 (9.3)	176 (8.3)
Body mass (kg)	39.3 (5.9)*	45.1 (7.1)*	60.0 (10.2)	43.5 (6.9)	49.6 (7.4)	60.8 (8.2)
FFM (kg)	35.1 (4.0)	41.3 (5.9)	55.2 (8.7)	37.0 (5.0)	43.9 (6.4)	54.6 (6.1)
Fat mass (%)	9.3 (4.1)*	9.4 (3.1)*	8.2 (3.2)	15.2 (6.6)	12.4 (5.8)	10.1 (3.4)

Values are mean (SD). End, endurance; nonEnd, non-endurance, FFM, fat free mass

** denotes significant difference between training groups $p<0.05$, # denotes significant sex difference within the same training group $p<0.05$*