Received: 29 January 2021 | Accepted: 28 April 2021

DOI: 10.1113/EP089433

Experimental Physiology WILEY

RESEARCH PAPER

Quantification of testosterone-dependent erythropoiesis during male puberty

Erica Mancera-Soto[1,2] | Diana Marcela Ramos-Caballero[3] | Jose Magalhaes[4] | Sandra Chaves Gomez[5] | Walter F. J. Schmidt[2] | Edgar Cristancho-Mejía[3]

[1] Departamento del Movimiento Corporal Humano, Facultad de Medicina, Universidad Nacional de Colombia, Bogotá, Colombia

[2] Department of Sports Medicine and Sports Physiology, University of Bayreuth, Bayreuth, Germany

[3] Departamento de Biología, Facultad de Ciencias, Universidad Nacional de Colombia, Bogotá, Colombia

[4] Laboratory of Metabolism and Exercise (LaMetEx) Research Center in Physical Activity, Health and Leisure (CIAFEL), Faculty of Sport (FADEUP), University of Porto, Porto, Portugal

[5] Laboratorio de Control al Dopaje, Ministerio del Deporte de Colombia, Bogotá, Colombia

Correspondence
Walter Schmidt, Department of Sports Medicine/Sports Physiology, University of Bayreuth, Universitaetsstrasse 30, 95440 Bayreuth, Germany.
Email: walter.schmidt@uni-bayreuth.de

Funding information
National Program of Projects for the strengthening of research, National University of Colombia

Edited by: Andrew Sheel

[Correction added on 17 June 2021, after first online publication: Projekt DEAL funding statement has been added.]

Walter F. J. Schmidt and Edgar Cristancho-Mejía are joint senior authors.

Abstract

The amount of haemoglobin during puberty is related to endurance performance in adulthood. During male puberty, testosterone stimulates erythropoiesis and could therefore be used as a marker for later endurance performance. This cross-sectional study aimed to determine the relationship between serum testosterone concentration and haemoglobin mass (Hbmass) in both male and female children and adolescents and to evaluate the possible influences of altitude and training. Three-hundred and thirteen differentially trained boys and girls aged from 9 to 18 years and living at altitudes of 1000 and 2600 m above sea level entered the study. The stage of sexual maturation was determined according to the classification of Tanner. Testosterone was measured by ELISA. Hbmass was determined by CO-rebreathing. Haemoglobin concentration did not change during maturation in girls and was 11% higher during puberty in boys, while Hbmass was elevated by 33% in Tanner stage V compared to stage II in girls (498 ± 77 vs. 373 ± 88 g) and by 95% in boys (832 ± 143 vs. 428 ± 95 g). This difference can most likely be attributed to indirect testosterone influences through an increase in lean body mass (LBM) and to direct testosterone effects on erythropoiesis, which increase the Hbmass by ~65 g per 1 ng/ml. Altitude and training statuses were not associated with testosterone, but with an increase in Hbmass (altitude by 1.1 g/kg LBM, training by 0.8 g/kg LBM). Changes in Hbmass are closely related to testosterone levels during male puberty. Further studies will show whether testosterone and Hbmass during childhood and adolescence can be used as diagnostic tools for endurance talents.

KEYWORDS
altitude, blood volume, haemoglobin mass, lean body mass, sexual maturation, talent diagnostic, Tanner, training

1 | INTRODUCTION

Maximum oxygen uptake as one limiting factor of endurance capacity in adulthood depends to a decisive extent on the total amount of haemoglobin mass (Hbmass; e.g., Schmidt & Prommer, 2010). This amount is already predetermined in adolescence and only slightly modified later (Landgraff & Hallén, 2020; Steiner et al., 2019). So far, however, it is not known which factors are responsible for

the individual development of Hbmass in adolescence. During male puberty, testosterone plays a crucial role in the growth of various tissues, and is reflected in a higher lean body mass (LBM) in men than in women. It is a well-known fact that testosterone also has a dose-dependent stimulatory effect on erythropoiesis (Coviello, et al., 2008), although the underlying mechanisms are not yet fully understood. During male puberty, the onset of testicular testosterone production is closely related to a subsequent increase in haemoglobin concentration ([Hb]), and the different testosterone concentrations are also assumed to be the main reason for the higher [Hb] in men than in women (Handelsman et al., 2018; Hero et al., 2005).

In general, [Hb] depends on Hbmass or red cell volume (RCV) and on the amount of plasma volume (PV), which are regulated by different hormonal mechanisms, i.e. mainly by erythropoietin and by the volume regulating hormones antidiuretic hormone, aldosterone and atrial naturetic peptide. In the short term, physiological changes in [Hb] are due exclusively to changes in PV; in the long term, changes in both Hbmass or RCV and PV must be considered (Prommer et al., 2018; Schmidt & Prommer, 2010).

During childhood, Hbmass, blood volume (BV) and PV similarly increase in both girls and boys during development, a change which is closely related to the increase in LBM (Prommer et al., 2018; Raes et al., 2006). Thereafter, [Hb] remains constant in girls and female adolescents until adulthood, while it increases in boys at the onset of puberty, a change which is closely related to the increasing level of blood testosterone (Hero et al., 2005). Because [Hb] also depends on the magnitude of PV it is not yet possible to quantify the absolute amount of haemoglobin or red cell mass which is produced under the influence of elevated testosterone levels during puberty.

In contrast to [Hb], Hbmass remains unaffected by changes in plasma volume and its measurement could quantify more precisely the degree of erythropoiesis during adolescence. Available data on Hbmass show a similar continuous increase in boys and girls until the age of ~12 years, which in boys is followed by an exponential increase in the years afterward (Åstrand, 1952; Karlberg & Lind, 1955; Prommer et al., 2018; von Döbeln & Eriksson, 1972). In girls, however, just a continuous increase can be observed before and during puberty without any further change after that age.

To date, there exist no simultaneous measurements of Hbmass and serum testosterone concentrations in children and adolescents which could more precisely quantify the effects of increasing testosterone levels on erythropoiesis during male puberty. One reason for lacking data concerns health issues when applying the so-called gold standard method to children using radioactive tracer substances. With the optimized CO-rebreathing technique, there is now a simple and non-invasive method available to conduct such investigations (Gore et al., 2005; Schmidt & Prommer, 2005). The first aim of this study was, therefore, to determine simultaneously Hbmass and serum testosterone levels to estimate more precisely the relationship between testosterone and erythropoiesis in children and adolescents (Zachmann et al., 1974). Since both testosterone and Hbmass might be influenced by confounding environmental factors, a second aim was to investigate the possible relationship between training status or chronic altitude exposure with the pubertal testosterone concentration and with Hbmass.

New Findings

- **What is the central question of this study?**
  To what extent does testosterone influence haemoglobin formation during male puberty?
- **What is the main finding and its importance?**
  In boys, testosterone might be responsible for about 65% of the increase in haemoglobin mass during puberty. The underlying mechanisms are assumed to be twofold: (i) indirectly, mediated by the increase in lean body mass, and (ii) directly by immediate testosterone effects on erythropoiesis. Thereby, an increase in testosterone of 1 ng/ml is associated with an increase in haemoglobin mass of ~65 g. These processes are likely to determine endurance performance in adulthood.

2 | METHODS

2.1 | Ethical approval

Ethical approval was granted by the ethics committee of the National University of Colombia at Bogota (reference: ID 06/2015). The study conformed to the standards set by the *Declaration of Helsinki*, except for registration in a database. Written informed consent was obtained from all children and their parents. The subjects volunteered to participate in the study and were free to withdraw at any time without any need to provide a reason.

2.2 | Subjects

In total, 313 healthy and differentially trained children and adolescents (girls $n = 94$, boys $n = 219$; untrained group $n = 123$, trained group $n = 190$; training volume 15.0 ± 5.9 h/week) aged from 9 to 18 years participated in the study. All of the trained children and adolescents reported they practiced endurance sports, i.e. medium- and long-distance running, cycling, speed skating, race walking and triathlon. The pre-condition for participation in the trained group was a training history of at least 2 years, a training volume of at least 6 h per week (see Table 1) and a training frequency of at least 3 times per week. The participants in the untrained group did not practice any sports, except school sports. The subjects were recruited in the Colombian regions

TABLE 1 Anthropometric data of the tests subjects classified according to their stage of maturation

	Tanner I	Tanner II	Tanner III	Tanner IV	Tanner V	ANOVA *P* (Tanner, Sex, Interaction)
n						
Boys	21	49	44	80	25	
Girls	18	19	27	18	12	
Age (years)						
Boys	9.7 ±1.0	11.2 ± 1.2^{+++}; *1.3*	13.8 ± 1.7^{+++}; *1.8*	15.7 ± 1.5^{+++}; *1.2*	16.3 ± 1.5; *0.4*	≤0.000
Girls	10.2 ± 1.7	10.4 ± 1.0; *0.1*	13.8 ± 2.2^{+++}; *1.9*	15.3 ± 1.9; *0.7*	15.9 ± 1.6; *0.3*	n.s.
d_{Cohen}	*0.4*	*0.7*	*0.0*	*0.3*	*0.3*	n.s.
Body mass (kg)						
Boys	31.8 ± 4.7	38.9 ± 9.0^{++}; *0.9*	47.8 ± 8.2^{+++}; *1.0*	56.8 ± 7.4^{+++}; *1.2*	58.1 ± 7.4; *0.2*	≤0.000
Girls	30.1 ± 5.2	34.6 ± 7.1; *0.7*	46.0 ± 7.8^{+++}; *1.3*	52.8 ± 9.6; *0.8*	52.5 ± 7.3; *0*	≤0.001
d_{Cohen}	*0.3*	*0.5*	*0.2*	*0.5*	*; *0.8*	n.s.
Height (cm)						
Boys	134.7 ± 7.3	145.2 ± 8.7^{+++}; *1.3*	159.2 ± 8.7^{+++}; *1.6*	168.1 ± 6.0^{+++}; *1.3*	169.9 ± 8.1; *0.3*	≤0.000
Girls	134.8 ± 5.9	140.8 ± 9.1; *0.8*	154.3 ± 5.8^{+++}; *1.8*	157.7 ± 8.2; *0.5*	160.6 ± 6.8; *0.4*	≤0.000
d_{Cohen}	*0.0*	*0.5*	**; *0.6*	**; *1.6*	**; *1.2*	≤0.01
BMI (kg/m^2)						
Boys	17.5 ± 2.1	18.3 ± 2.8; *0.3*	18.7 ± 1.8; *0.2*	20.0 ± 2.1^{+}; *0.7*	20.1 ± 1.5; *0.1*	≤0.000
Girls	16.5 ±2.1	17.4 ± 2.6; *0.4*	19.2 ± 2.5; *0.7*	21.2 ± 2.9; *0.7*	20.3 ± 2.2; *0.3*	n.s.
d_{Cohen}	*0.5*	*0.4*	*0.2*	*0.5*	*0.1*	n.s.
Body fat (%)						
Boys	16.4 ± 7.0	17.5 ± 7.0; *0.2*	13.9 ± 5.5; *0.6*	13.2 ± 4.8; *0.1*	11.6 ± 3.2; *0.4*	n.s.
Girls	16.4 ± 3.5	18.5 ± 6.2; *0.4*	18.0 ± 4.0; *0.1*	21.2 ± 7.3; *0.6*	24.9 ± 4.6; *0.5*	≤0.000
d_{Cohen}	*0.0*	*0.1*	**; *0.8*	***; *1.5*	***; *3.6*	≤0.001
LBM (kg)						
Boys	26.4 ± 3.5	31.9 ± 6.6^{+}; *0.9*	41.1 ± 6.9^{+++}; *1.4*	49.2 ± 6.2^{+++}; *1.3*	51.4 ± 6.9; *0.3*	≤0.000
Girls	25.0 ± 3.6	28.1 ± 5.6; *0.6*	37.6 ± 5.8^{+++}; *1.6*	41.2 ± 5.5; *0.6*	39.2 ± 4.5; *0.4*	≤0.000
d_{Cohen}	*0.4*	*; *0.6*	*; *0.5*	***; *1.3*	***; *2.0*	≤0.001
Training volume (h/week)						
Boys	11.5 ± 4.0	9.9 ± 3.7	14.5 ± 4.9	16.4 ± 5.7	19.9 ± 9.0	≤0.000
Girls	11.9 ± 4.1	12.6 ± 1.1	15.7 ± 4.7	12.5 ± 3.3	19.0 ± 4.5	n.s.
						n.s.

Tanner, stage of maturation; LBM, lean body mass; training volume, hours/week in the trained group only. Effect sizes (Cohen's *d*) for the sex-specific change from the previous Tanner level are shown in italic next to the respective values; those for the differences between the sexes are indicated below the respective parameters. Significant differences from the previous stage of maturation: $^{+}P < 0.05$, $^{++}P < 0.01$, $^{+++}P < 0.001$; significant differences between girls and boys of identical stage of maturation: $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$. No statistical analyses were performed for the parameter 'training volume'. The column on the right presents the results of the 2-way ANOVA with the between-subject's factors Tanner, sex, and interaction of both variables; n.s., not significant.

around Bogota (2600 m – 3000 m above sea level, $n = 163$) and Cali (~1000 m above sea level; $n = 150$). They had lived at their respective altitude for at least 5 years and had not changed their altitudes for more than 1 week during the past year. None of the females indicated the use of hormonal contraceptives and none used iron, folic acid or any supplements which could affect the parameters determined in this study. The anthropometric data of the test subjects are presented in Table 1.

2.3 | Study design

The study was conducted in the laboratories of the Universidad Nacional de Colombia in Bogotá and of the Unidad Central del Valle in Tuluá, both in Colombia. After subjects' arrival, a medical examination was performed followed by an anthropometrical evaluation. Hbmass was determined using the optimized CO-rebreathing method. Two cubital venous blood samples, i.e. 4 ml heparinized blood and

8 ml for serum analyses, were taken for the determination of basic haematological parameters as well as testosterone, ferritin, erythropoietin and C-reactive protein.

2.4 | Medical examination and anthropometrical evaluation

Health status was checked by medical examination including electrocardiogram under resting conditions. The status of biological maturation was evaluated by using the method of co-evaluation according to Tanner (1962), and then the children were classified into stages from I to V according to their external primary and secondary sexual characteristics.

For the anthropometrical evaluation, the five-component method was applied and data for body mass, height and body composition were obtained (Kerr, 1988; Stewart & Marfell-Jones, 2011). Skinfold caliper measurements were performed by the same scientist using triceps, subscapular, supra-iliac, abdominal, tight and medial calf. Percentage body fat and absolute lean body mass were estimated using a correction for the age and sex of the participants (Slaughter et al., 1988). The typical error was 2.5% (Prommer et al., 2018).

2.5 | Sample transport and storage

The whole procedure, including blood sampling, sample transport, sample storage and sample analyses, was performed under standardized conditions oriented to current guidelines of the World Anti-Doping Agency (WADA, 2019). The blood samples were taken after leaving the subject for at least 15 min in a sitting position. Within 24 h, the samples were transported under monitored cool conditions to the WADA-related Institute of Doping Analysis in Bogotá, Colombia.

2.6 | Blood analytical procedures

The Sysmex XT2000i haematological analyser (Sysmex, Norderstedt, Germany) was used for the determination of [Hb], haematocrit (Hct), reticulocyte number, immature reticulocyte fraction (IRF), red cell indices and other routine haematological parameters.

2.7 | Serum parameters

In serum, the following parameters were determined: testosterone (Elisa NovaTec DNOV0002; NovaTec Immunodiagnostica GmbH, Dietzenbach, Germany; intra-assay CV $\leq$ 5.8%, sensitivity 0.07 ng/ml); erythropoietin (EPO; Elisa Novus NBP2-31082; Novus Biologicals, Centennial, CO, USA; overall intra-assay CV 3.4%, sensitivity 0.17mU/ml); ferritin (Elisa NovaTec DNOV002; NovaTec Immunodiagnostica GmbH; intra-assay CV < 7%, sensitivity 0.10 ng/ml), and C-reactive protein (CRP; Quantikine ELISA kits, R&D Systems, Bio-Techne, Wiesbaden, Germany; minimum and maximum detectable concentrations are 0.1 mg/l and 10 mg/l).

2.8 | Haemoglobin mass determination

Haemoglobin mass was determined by using the optimized CO-rebreathing method as described by Schmidt and Prommer (2005) and modified by Prommer and Schmidt (2007). Briefly, a bolus of 99.97% carbon monoxide (CO; 0.7–1.2 ml CO per kg body mass) was administered to subjects and rebreathed along with 2–3 litres of 100% O_2 for 2 min. Because Hbmass might be influenced by training status (Prommer et al., 2018) the CO bolus was adjusted to a dose of 0.7 ml/kg (untrained) and 0.8 ml/kg (trained) for all children until the age of 14 years. Above 14 years old, girls received 0.8 ml/kg (untrained) and 1.0 ml/kg (trained), and boys 1.0 ml/kg untrained) and 1.2 ml/kg (trained). To compensate for the lower barometric pressure at altitude (ambient pressure at 2600 m approx. 600 mmHg), the doses were extended by the factor 1.25.

Arterialized capillary blood samples were taken from a hyperemized earlobe before and 7 min after the rebreathing procedure and analysed in sextuplicate using an OSM3 haemoximeter (Radiometer, Denmark). End tidal [CO] was assessed before and 2 min after the rebreathing procedure by a portable CO detector (Draeger Pac7000, Lübeck, Germany). After familiarization with the equipment and the breathing procedure, the children performed the method without any problems. Possible leakage, especially around the mouth and nose, was permanently controlled with a CO detector (see above). At the two laboratories, the identical equipment was used by the same staff. The typical error of this method obtained in our laboratory is 2.2% and is in accordance with the typical error published by (Gore et al., 2005).

2.9 | Statistics

For statistical analysis the software SPSS Statistics version 25 was used (IBM Corp., Armonk, NY, USA). Data mostly refer to the stage of biological maturation and are presented as means and standard deviation for the respective stages according to Tanner (1962).

To compare the mean values of the different Tanner stage groups, a two-way ANOVA with two between-subjects factors, i.e., sex and Tanner stage, was applied. For *post hoc* tests, Student's unpaired *t*-test was used to check differences between boys and girls.

In addition to the two-way ANOVA, we also performed a one-way ANOVA (Tanner stage only) for boys and girls separately followed by a Bonferroni *post hoc* test which more clearly indicated the sex-specific development. To exclude the possible influences of different group sizes, effect sizes (Cohen's *d*) were calculated with pooled standard deviations to judge the differences between the individual Tanner groups (Lakens, 2013; Sullivan & Feinn, 2012).

Simple bivariate linear regression analyses were performed for [Hb], Hbmass and LBM as dependent variables and testosterone as the independent variable. A further bivariate linear regression analysis

was done for Hbmass as the dependent and LBM as the independent variable.

Multi-factorial analyses of variance were conducted to determine possible influences of environmental and individual variables on the dependent variables EPO, testosterone in the boys' group only, and normalized Hbmass (g/kg LBM). The independent variables for testing the dependent variable EPO were sex, altitude, training status and Tanner as fixed factors and testosterone and ferritin as co-variates. After drawing a directed acyclic graph to evaluate possible interactions between these variables (Westreich & Greenland, 2013), additional to the main effects, the interaction of sex, testosterone and Tanner was included in the model. For testosterone as the dependent variable, the independent variables were altitude, training status, Tanner stage, percentage fat and LBM. For normalized Hbmass (g/kg LBM) as dependent variable, the independent variables were sex, altitude, training status, testosterone and EPO. As described above, and also before conducting these analyses, possible interactions between the independent variables were checked after drawing a directed acyclic graph. To estimate the regression coefficients for the relationship between normalized Hbmass (g/kg LBM) and these independent variables, a multiple linear regression analysis was performed.

3 | RESULTS

The anthropometrical characteristics show the well-known time course during the development with increasing fat mass in the girls and a higher increase in LBM in the boys during puberty (Table 1).

[Hb] increased in the boys from 14.6 ± 0.8 g/dl at the age of 9 years to 16.0 ± 1.1 g/dl at the age of 18 years, while in the girls no difference was observed between the different age groups (Figure 1a). In contrast, Hbmass increased in both groups (Figure 1b); it was, however, much more pronounced in the boys (from 365 ± 54 to 883 ± 136 g) than in the girls (from 297 ± 58 to 534 ± 128 g).

For the haematological data for the state of biological maturation (Table 2), significant increases of absolute Hbmass and the blood volumes (RCV, BV, PV) can be seen from Tanner stage I to stage III in the girls and to stage IV in the boys. At stage I and II no significant differences in [Hb], Hct, Hbmass and the blood volumes were detected between the sexes while from stages III to V all haematological values were considerably higher in the boys.

When Hbmass, RCV, BV and PV were normalized to LBM, no change occurred in girls during maturation, while Hbmass and RCV increased in boys from stage II. In the boys, too, the PV did not change in the course of maturation and did not differ from the girls' values with the exception of stage III. The BV only slightly increased from Tanner II to Tanner III.

Ferritin showed an opposite course in boys and girls; it was at the same level until stage III, then tended to increase in boys and to decrease in girls. Erythropoietin concentration was similar in both boys and girls and did not change during maturation (Table 2). Testosterone was mainly below the detection limit in both sexes up to stage II. Thereafter, it increased significantly only in boys as far as stage IV and then tended to increase further at stage V (Table 2).

3.1 | Regression analyses

When [Hb] and Hbmass of the male groups were related as dependent variables to serum testosterone as the independent variable (Figure 2a, b), we found significant relationships for both analyses, which was closer for Hbmass ($r = 0.761$) than for [Hb] ($r = 0.534$; $P < 0.001$). For LBM as the dependent variable vs. testosterone (Figure 2c) there existed a similar close relationship ($r = 0.748$) as for Hbmass vs. testosterone ($r = 0.761$). For Hbmass as the dependent vs. LBM as the independent variable (Figure 2d), we found close correlations for boys ($r = 0.949$) as well as for girls ($r = 0.892$).

The multi-factorial ANOVA with EPO as the dependent variable yielded no significant relationship to any of the independent variables except to ferritin. As expected, testosterone was significantly ($P < 0.001$) influenced by the Tanner stage, but not by altitude and training status. Hbmass normalized to LBM as the dependent variable was significantly influenced by testosterone, sex, altitude and training status (in all cases $P < 0.001$), but not by EPO or any interaction of these variables. The results of a multiple regression analysis with normalized Hbmass (g/kg LBM) as the dependent and the significant independent variables are shown in Table 3.

4 | DISCUSSION

The most important result from this study is the quantification of the relationship between the male-specific erythropoiesis and increasing serum testosterone levels during puberty. Before puberty, i.e. during Tanner stages I and II, Hbmass develops similarly in both girls and boys. Thereafter, only a moderate increase occurs in girls to stage III, while in boys a steep augmentation occurs to stage IV. This results finally in approx. 65% higher Hbmass in post-pubescent boys than in the mature girls.

4.1 | Testosterone

As expected, there was no significant change in testosterone concentration in the course of ageing or sexual development in the female groups. The changes found in the boys for the different levels of maturity agree with the literature values (Khairullah et al., 2014; Konforte et al., 2013).

In our study, the increase in testosterone concentration only correlates with the degree of sexual maturation. The training status and the altitude of the place of residence have no significant influence, which is in accordance with data from Gonzales (2013) and D'Andrea et al. (2020) describing acute stimulatory influences of both parameters, which, however, do not exerct any chronic effect.

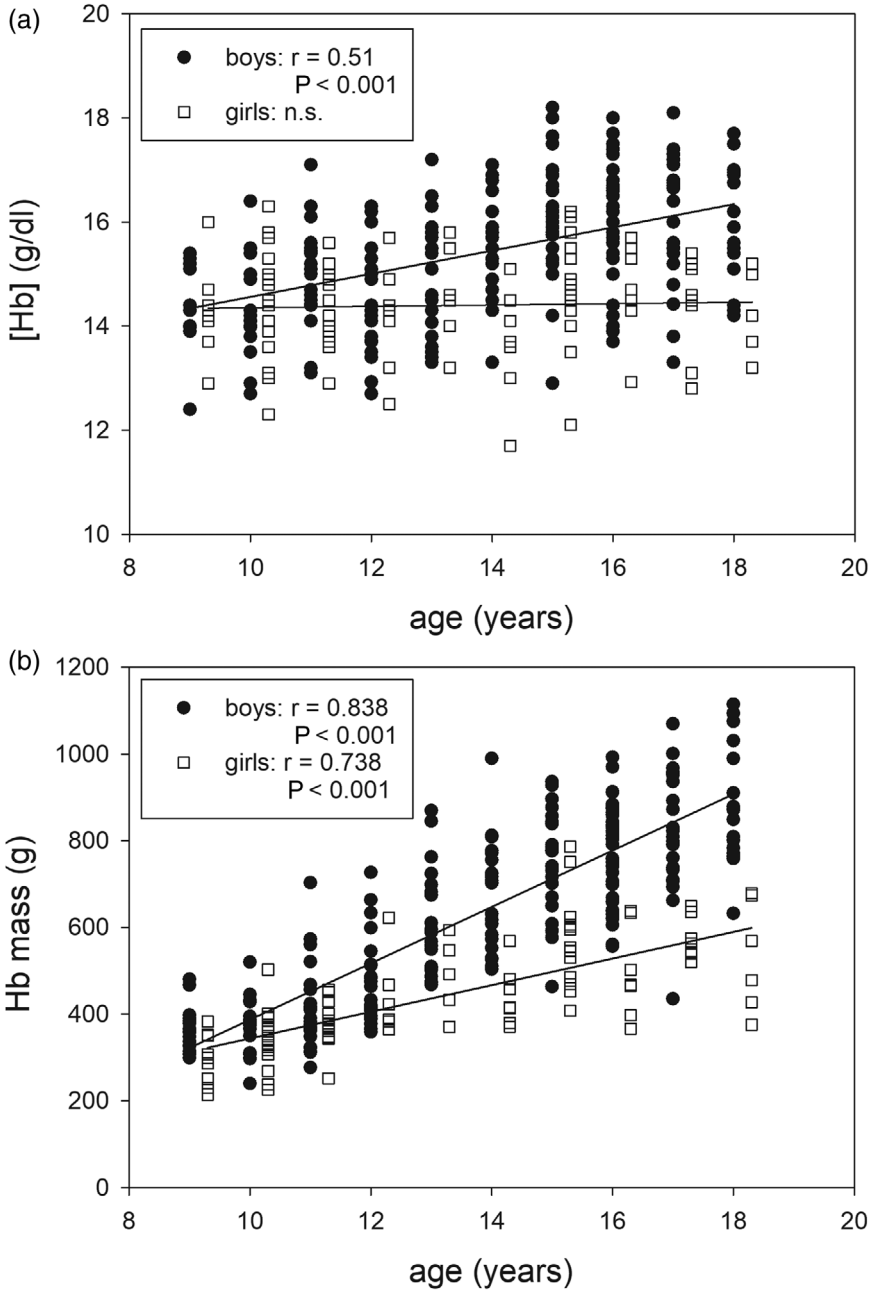

FIGURE 1 Development of haemoglobin concentration ([Hb], a) and haemoglobin mass (Hbmass, b) from the ages of 9 to 18 years. The regression lines for [Hb] vs. age (a) are $y = 0.23x + 12.3$ in boys; n.s.: not significant in girls; and for Hbmass vs. age (b) $y = 65.1x - 263.6$ in boys and $y = 30.7x + 46.0$ in girls

4.2 | EPO

The serum EPO concentration showed no difference between the sexes and remained unchanged over the maturation process. This behaviour agrees well with data from Eckardt et al. (1990) and Yanamandra et al. (2019), who did not find any change in the EPO concentration in boys during puberty. The lack of influence of altitude and training status also agrees with the literature as neither of these exerts chronic stimulatory effects on EPO (Cristancho et al., 2016; Gunga et al., 2007; Jelkmann, 2011; Schmidt et al., 1999, 2002). As is discussed in detail below, it is assumed that the testosterone effects on erythropoiesis are caused in part by increased EPO stimulation (Bachman et al., 2014; Maggio et al., 2013). However, no dependencies between serum EPO and testosterone concentrations could be detected here. The only significant relationship of EPO was with the ferritin concentration, which in 15 of the test persons was below 10 ng/ml, thus indicating the dependence of the [EPO] on the iron availability (Yanamandra et al., 2019).

4.3 | Haemoglobin concentration

As expected, [Hb] in the females remains constant from the ages of 9 to 18 years. In boys, we found an increase in [Hb] with increasing testosterone levels from 14.6 g/dl in stage II to 16.2 g/dl in stage V, which corresponds to the data of the literature. Hero et al. (2005) described a first increase in [Hb] from 13.6 to 14.6 years by 0.3 g/dl and a further development by 1.5 g/dl at the age of 16.6 years. During

TABLE 2 Haematological and hormonal data, classified according to the stage of maturation

	Tanner I	Tanner II	Tanner III	Tanner IV	Tanner V	ANOVA *P*(Tanner, Sex, Interaction)
n						
Boys	21	49	44	80	25	
Girls	18	19	27	18	12	
[Hb] (g/dl)						
Boys	14.6 ± 1.0	14.6 ± 0.9; *0,0*	15.3 ± 1.2^{+}; *0.7*	16.0 ± 1.1^{++}; *0.6*	16.2 ± 1.0; *0.2*	≤0.000
Girls	14.5 ± 1.0	14.2 ± 0.9; *0.2*	14.5 ± 0.9; *0.2*	14.5 ± 1.2; *0.0*	14.3 ± 1.1; *0.1*	≤0.000
d_{Cohen}	*0.10*	*0.4*	**; *0.7*	***; *1.3*	***; *1.8*	≤0.000
Hct (%)						
Boys	42.1 ± 2.6	42.0 ± 2.3; *0.0*	44.2 ± 2.8^{++}; *0.8*	46.5 ± 2.8^{+++}; *0.8*	47.4 ± 2.4; *0.3*	≤0.000
Girls	42.1 ± 2.6	41.5 ± 2.2; *0.2*	42.6 ± 2.4; *0.4*	42.8 ± 3.1; *1.1*	42.4 ± 2.8; *0.1*	≤0.000
d_{Cohen}	*0.0*	*0.2*	**; *0.6*	***; *1.3*	***; *2.0*	≤0.000
Hbmass (g)						
Boys	365 ± 60	428 ± 95; *0.7*	636 ± 148^{+++}; *1.7*	774 ± 141^{+++}; *0.9*	832 ± 143; *0.4*	≤0.000
Girls	330 ± 58	373 ± 88; *0.5*	482 ± 111^{++}; *1.1*	543 ± 117; *1.0*	498 ± 77; *0.4*	≤0.000
d_{Cohen}	*0.6*	*; *0.6*	***; *1.1*	***; *1.7*	***; *2.6*	≤0.000
Hbmass (g/kg LBM)						
Boys	13.8 ± 1.4	13.5 ± 1.7; *0.2*	15.4 ± 1.6^{+++}; *1.1*	15.7 ± 1.5; *0.2*	16.1 ± 1.2; *0.3*	≤0.000
Girls	13.2 ± 1.5	13.2 ± 1.6: *0.0*	12.8 ± 1.9; *0.2*	13.1 ± 1.6: *0.2*	12.7 ± 0.9; *0.3*	≤0.001
d_{Cohen}	*0.4*	*0.2*	***; *1.5*	***; *1.7*	***; *3.0*	≤0.000
RCV (ml)						
Boys	1052 ± 187	1235 ± 277; *0.7*	1846 ± 432^{+++}; *1.7*	2259 ± 414^{+++}; *1.0*	2434 ± 444; *0.4*	≤0.000
Girls	958 ± 163	1087 ± 249; *0.6*	1419 ± 316^{++}; *1.1*	1605 ± 333; *0.6*	1479 ± 240; *0.4*	≤0.000
d_{Cohen}	*0.5*	*; *0.5*	***; *1.1*	***; *1.6*	***; *2.4*	≤0.000
RCV (ml/kg LBM)						
Boys	39.8 ± 4.4	38.9 ± 4.8; *0.2*	44.6 ± 4.7^{+++}; *1.0*	45.7 ± 4.3; *0.2*	47.1 ± 3.7; *0.3*	≤0.000
Girls	38.3 ± 4.5	38.7 ± 4.5; *0.1*	37.6 ± 5.4; *0.2*	38.7 ± 4.2; *0.2*	37.5 ± 2.8; *0.3*	≤0.000
d_{Cohen}	*0.3*	*0.0*	***; *1.4*	***; *1.6*	***; *2.4*	≤0.000
BV (ml)						
Boys	2750 ± 469	3233 ± 706; *0.7*	4572 ± 967^{+++}; *1.6*	5329 ± 896^{+++}; *0.8*	5652 ± 1064; *0.3*	≤0.000
Girls	2502 ± 410	2878 ± 627; *0.7*	3656 ± 744^{++}; *1.1*	4115 ± 759; *0.6*	3847 ± 648; *0.3*	≤0.000
d_{Cohen}	*0.5*	*0.5*	***; *1.0*	***; *1.4*	***; *1.9*	≤0.000
BV (ml/kg LBM)						
Boys	104.1 ± 10.8	101.7 ± 10.9; *0.2*	110.8 ± 11.0^{+++}; *0.8*	108.1 ± 9.1; *0.3*	109.5 ± 9.6; *0.3*	≤0.000
Girls	100.2 ± 11.9	102.4 ± 10.5; *0.2*	97.0 ± 11.6; *0.5*	99.5 ± 8.1; *0.2*	97.7 ± 9.6; *0.2*	n.s.
d_{Cohen}	*0.3*	*0.1*	***; *1.2*	***; *1.0*	**; *1.2*	≤0.01
PV (ml)						
Boys	1697 ± 298	1999 ± 443; *0.7*	2726 ± 564^{+++}; *1.4*	3070 ± 522^{++}; *0.6*	3218 ± 650; *0.3*	≤0.000
Girls	1545 ± 265	1791 ± 390; *0.8*	2237 ± 446^{++}; *1.1*	2510 ± 460; *0.6*	2367 ± 434; *0.3*	≤0.000
d_{Cohen}	*0.5*	*0.5*	***; *0.9*	***; *1.1*	***; *1.4*	≤0.01
PV (ml/kg LBM)						
Boys	64.2 ± 7.6	62.8 ± 6.9; *0.2*	66.3 ± 7.7; *0.5*	62.4 ± 6.5; *0.6*	62.3 ± 6.9; *0.0*	n.s.
Girls	62.0 ± 8.7	63.8 ± 7.0; *0.2*	59.3 ± 6.9; *0.6*	60.8 ± 5.8; *0.2*	60.2 ± 7.6; *0.1*	≤0.01
d_{Cohen}	*0.3*	*0.2*	**; *0.9*	*0.2*	*0.3*	≤0.05

(Continues)

TABLE 2 (Continued)

	Tanner I	Tanner II	Tanner III	Tanner IV	Tanner V	ANOVA *P*(Tanner, Sex, Interaction)
Ferritin (ng/ml)						
Boys	42.1 ± 31.2	36.1 ± 15.9	41.8 ± 24.1	55.0 ± 33.5	78.7 ± 71.1	n.s.
Girls	25.6 ± 11.4	33.1 ± 18.8	45.0 ± 23.0	30.0 ±21.4	25.0 ± 21.7	≤0.000
d_{Cohen}	*0.7*	*0.2*	*0.5*	**; *0.8*	*; *0.9*	≤0.01
EPO (mU/ml)						
Boys	8.0 ± 3.6	10.4 ± 4.3; *0.6*	10.9 ± 5.0; *0.1*	8.9 ± 4.4; *0.4*	7.6 ± 2.4; *0.3*	n.s.
Girls	8.3 ± 3.7	9.4 ± 5.3; *0.2*	9.6 ± 4.7; *0.0*	11.2 ± 6.0; *0.3*	9.0 ± 4.5; *0.4*	n.s.
d_{Cohen}	*0.1*	*0.2*	*0.3*	*0.5*	*0.4*	n.s.
Testosterone (ng/ml)						
Boys	0.2 ± 0.5	0.5 ± 0.9; *0.4*	2.7 ± 2.0^{+++}; *1.4*	4.0 ± 2.0^{+++}; *0.7*	4.7 ± 2.4; *0.3*	≤0.000
Girls	0.0 ± 0.0	0.0 ± 0.0; *0.0*	0.2 ± 0.4; *0.6*	0.2 ± 0.3; *0.0*	0.1 ± 0.1; *0.4*	≤0.000
d_{Cohen}	*0.5*	*0.6*	***; *1.5*	***; *2.1*	***; *2.3*	≤0.000

Effect sizes (Cohen's *d*) for the sex-specific change from the previous Tanner level are shown in italic next to the respective values; those for the differences between the sexes are indicated below the respective parameters. Significant differences from the previous stage of maturation: $^{+}P < 0.05$, $^{++}P < 0.01$, $^{+++}P < 0.001$; significant differences between girls and boys of identical stage of maturation: $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$. The column on the right presents the results of the 2-way ANOVA with the between-subject's factors Tanner, sex and interaction of both variables; n.s., not significant. BV, blood volume; [EPO], serum erythropoietin; [Hb], haemoglobin concentration; Hbmass, haemoglobin mass; Hct, haematocrit; LBM, lean body mass; PV, plasma volume; RCV, red cell volume.

TABLE 3 Influence of sex, testosterone, altitude and training status on Hbmass (g/kg LBM)

	Regression coefficient	*β* coefficient	*P*
Constant	11.2		<0.000
Sex (boys/girls)	0.88	0.21	<0.001
Testosterone (ng/ml)	0.38	0.46	<0.001
Altitude (2600 m/1000 m)	1.06	0.27	<0.001
Physical activity (high – low)	0.77	0.19	<0.001

this time, serum testosterone increased by 4.9 ng/ml, which is similar to our results (+4.2 ng/ml). Similar effects are also observed in boys with delayed puberty receiving a testosterone treatment and increasing their [Hb] concentration by 1.6 g/dl (Hero et al., 2005). Krabbe et al. (1978) related [Hb] to serum testosterone level and found a good relationship of $r = 0.7$, which is closer than that demonstrated here ($r = 0.54$). This may be due to the delay of the testosterone-mediated effects on erythropoiesis which is assumed to be 4–5 months (Thomsen et al., 1986). The cross-sectional design of our study, therefore, may slightly reduce the relationship between testosterone and [Hb] during puberty.

4.4 | Absolute haemoglobin mass

To date, Hbmass and/or red cell volume (RCV) has not been determined simultaneously with serum testosterone levels to evaluate the relationship between what is probably the most important influencing factor during puberty on erythropoiesis. In an approximation, Hero et al. (2005) calculated RCV using haematocrit and body mass and found a continuous increase by approx. 350 ml from the age of 11.7 years to 16.6 years in healthy boys. In delayed puberty, a similar increase was calculated after a 12-month treatment with testosterone (Hero et al., 2005). In our study, RCV rose during the same age (stage II to V), by 1140 ml, indicating that erythropoiesis was by far underestimated when RCV was calculated as mentioned above. Our data, therefore, show more accurately the real effect of puberty on erythropoiesis than changes in [Hb] and calculated RCV can. In our study, the difference in [Hb] between stages II and V was 11% (from 14.6 to 16.2 g/dl) while the difference in Hbmass accounted for ~95% (from 428 to 832 g). Similar differences in [Hb] and Hbmass are observed between mature girls and boys (Tanner stage V) being approx. 15% and 65%, respectively. In other words, Hbmass and also the blood volumes of mature girls are only slightly above those values at the beginning of puberty.

The reason for the discrepancy in judging erythropoiesis by [Hb] or by Hbmass is the increasing plasma volume during growth and maturation. In girls, there occurs a parallel increase in Hbmass and PV resulting in an unchanged [Hb] during maturation, while in boys the percentage increase in Hbmass is considerably higher than the

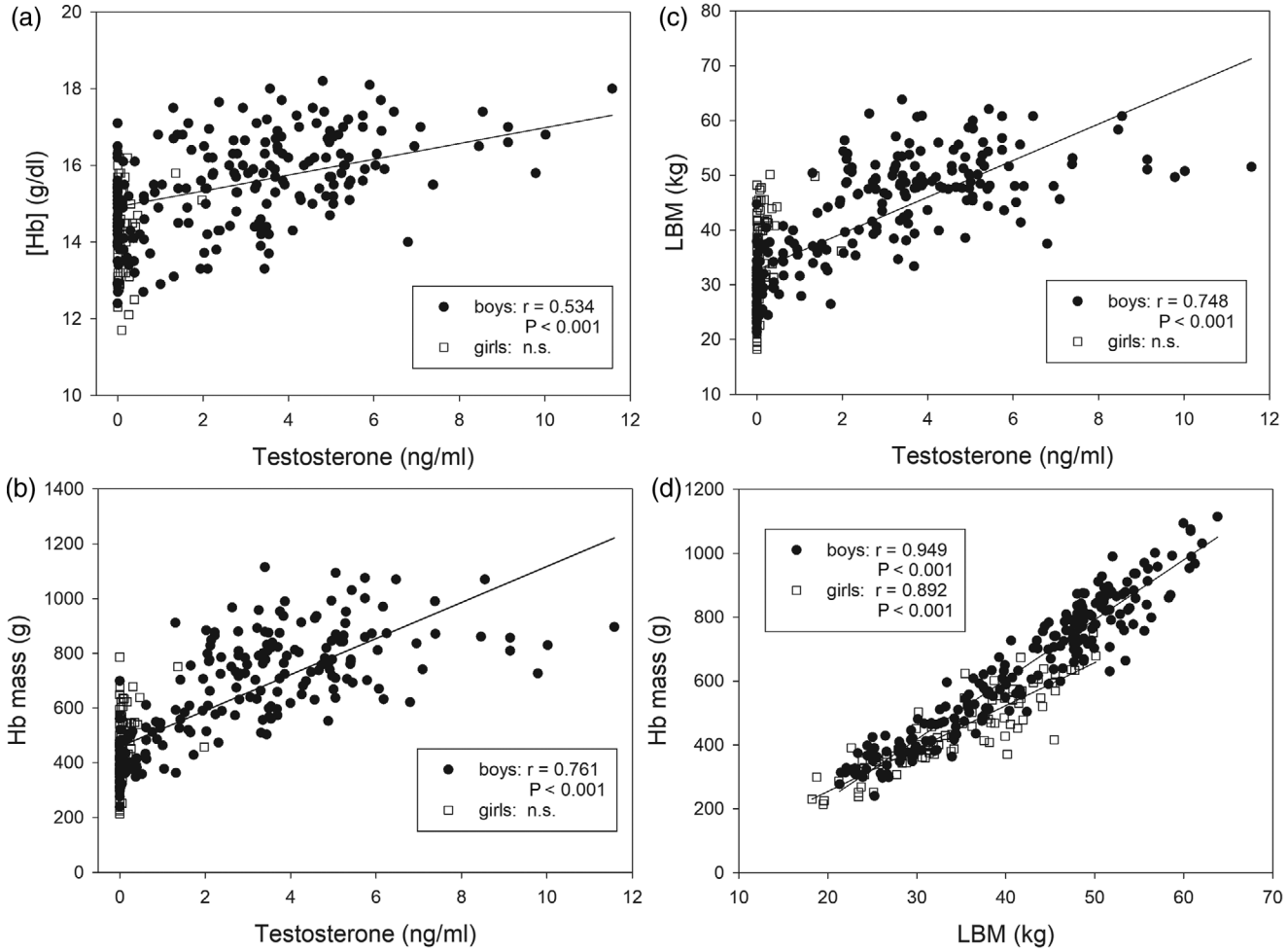

FIGURE 2 Relationship between serum testosterone concentration (independent variable) with the dependent variables haemoglobin concentration ([Hb], a), haemoglobin mass (Hbmass, b), and lean body mass (LBM, c) as well as the relationship between LBM (independent variable) and Hbmass (d). The regression lines were calculated for the male subjects in (a–c) (a: $y = 0.27x + 14.7$; b: $y = 65.9x + 458.7$; c: $y = 3.3x + 32.9$) and in (d) for both sexes (boys: $y = 18.6x - 142.3$; girls: $y = 13.5x - 15.3$); n.s.: not significant

percentage increase in PV (Hbmass ~+95% vs. PV ~+60% from stages II to V).

4.5 | Haemoglobin mass and testosterone

In both girls and boys a close relationship exists between Hbmass and LBM (Figure 2d). The augmentation of both parameters in girls during the whole maturation process and in boys to stage II occurs independently of testosterone and is probably regulated mainly by the (human growth hormone–insulin-like growth factor) hGH - IGF-1 axis (Vihervuori et al., 1996). One reason for the slowdown of erythropoiesis in girls during later maturation might be related to the appearance of female hormones generally completing the growth process and slightly counteracting erythropoiesis (Murphy, 2014; Peschle et al., 1973).

A close relationship of testosterone and Hbmass in boys first becomes obvious in stage III and, as can be observed in Figure 2b, there is a sensitive phase up to the plasma concentration of approx. 6 ng/ml. When calculating the slope of the regression line for this concentration interval, the increase in testosterone by 1 ng/ml corresponds to an increase in Hbmass by ~65 g. Above 6 ng/ml the efficiency slows down and higher concentrations are less related to erythropoiesis. A very similar behaviour was described previously by Hero et al. (2005) comparing testosterone and [Hb] and showing a similar narrow range for testosterone efficiency up to 5.7 ng/ml.

The erythropoietic stimulation via testosterone is likely to take place in two different ways. On the one hand, testosterone increases the LBM, which, as it was before puberty, is associated with a proportional increase in Hbmass. In addition, testosterone stimulates erythropoiesis in a special way that is independent of LBM, as becomes obvious from the normalized Hbmass (g/kg LBM), which increases from Tanner III resulting in a close relationship between normalized Hbmass and serum testosterone concentration (Table 3). Several direct testosterone effects on erythropoiesis are discussed in the literature. Bachman et al. (2014) showed a significant increase in EPO concentration lasting several months during testosterone administration in the elderly, which, however, and according to our

data, could often not be confirmed in healthy younger people (Bachman et al., 2014; Maggio et al., 2013; Shahani et al., 2009). Other possible effects are increasing bone marrow activity via androgenic receptors in erythroblasts (Claustres & Sultan, 1988) and testosterone-mediated increased iron incorporation into the erythrocyte precursor cells (Coviello et al., 2008) as well as changes in renal microcirculation (Murphy, 2014), which may stimulate the erythropoietic activity.

Interestingly from the blood compartments, only the Hbmass or RCV is directly influenced by testosterone. The PV, related to LBM, does not differ between boys and girls and shows almost no changes during the maturation process. Due to the constant PV and the increase in Hbmass/RCV in the boys, BV also slightly increases during puberty.

In addition, there are, however, some environmental factors, i.e. altitude and training status, which have affected Hbmass independently of testosterone in our study. As shown in Table 3, moderate altitude is associated with an increase in Hbmass by 1.1 g/kg LBM, corresponding to ~8%, which is in accordance with data obtained from differentially trained male and female adults living at similar altitudes as the subjects in this study (Böning et al., 2004; Schmidt et al., 2002). To the best of our knowledge, these are the first data on altitude adaptation of Hbmass in children and adolescents, and show that adaptation to hypoxia also takes place at a young age. The higher training status is associated with an elevated Hbmass by 0.8 g/kg LBM (~6%), which is almost identical to the values from Prommer et al. (2018) for children between 9.9 and 12.4 years. It also shows that physical training in childhood and adolescence, similar to adulthood, has only a relatively minor influence on erythropoiesis (Schmidt & Prommer, 2008). In addition, it cannot be ruled out that the higher Hbmass in the trained group is possibly due to selection processes, as it represents a more advantageous basis for better endurance performance.

4.6 | Practical impact

The data obtained here can also have a direct practical impact for clinical medicine and sports. Since the aerobic performance correlates closely with the Hbmass (a change of 1 g changes the $\dot{V}_{O_2max}$ by 4 ml/min) (Schmidt & Prommer, 2010), it becomes obvious that boys suffering from hypogonadism are massively limited in performance during daily life (Zitzmann & Nieschlag, 2003). Increasing the Hbmass through testosterone application will therefore very probably increase everyday performance. On the other hand, athletes with endogenously high testosterone concentrations in puberty should have an advantage. According to Steiner et al. (2019), at the end of puberty there are already large individual differences in the levels of Hbmass among young athletes, which only slightly change in the following years. Only those athletes presenting the highest Hbmass values at the age of 16 years are able to perform at highest national and international level during adulthood (Wehrlin & Steiner, 2021). Since the Hbmass, therefore, correlates very closely with the performance and competition success (Zelenkova et al., 2019), the testosterone-dependent erythropoiesis can be assumed to have an important influence on talent in endurance sports.

Unfortunately, testosterone also plays a negative role in endurance sports. Before EPO was abused as a doping agent in almost all endurance disciplines, testosterone was used for the same purpose and probably still is (Vorona & Nieschlag, 2018). Interestingly, athletics is currently debating whether women with endogenously high testosterone levels should be excluded from international competitions (Handelsman et al., 2018). A maximum value of 5 nmol/l was set as the upper limit, which corresponds to a concentration of 1.4 ng/ml. Our data clearly show that exceeding this value would increase the Hbmass by at least 70 g corresponding to an increase in $\dot{V}_{O_2max}$ by at least 280 ml/min, leading to a remarkable advantage for the athlete.

4.7 | Limitations

The correlations between testosterone and haemoglobin do not prove cause and effect, but only associations between the two variables. Cause and effect can only be determined by testosterone application, which should not be done in healthy children due to ethical concerns. Due to the change in [Hb] in hypogonadal children after testosterone treatment (Hero et al., 2005), however, it is very likely that general conclusions can also be drawn about the testosterone effect on the Hbmass level in children.

The LBM was not directly measured here, but estimated by skinfold measurements and, based on this, determination of the percentage of body fat took place. Although this method correlates sufficiently with the gold standard methods (dual-energy X-ray absorptiometry (Loftin et al., 2007), three component method (Aguirre et al., 2015)), the results should be interpreted with caution. Because of their ease of use and robustness, however, skinfold measurements have often been used successfully for the determination of fat mass and LBM in comparable groups of children and adolescents in Colombia (Spurr et al., 1992).

5 | CONCLUSIONS

In conclusion, we have defined the relationship between testosterone and erythropoiesis in male puberty. Hbmass increases from the beginning of puberty until complete maturation by 33% in girls, and by 95% in boys. This is much greater than hitherto assumed from the increase in [Hb], which only amounts to 11% in boys. In the most sensible phase of puberty, the increase in testosterone plasma concentration by each 1 ng/ml is correlated with an increase in Hbmass by ~65 g. Testosterone-mediated effects via increased LBM and specific testosterone effects on erythropoiesis are most likely the causes.

ACKNOWLEDGEMENTS

The authors are grateful to those who assisted with the data collection: Joel Rojas and Duque Lohover. They also would like to thank all the

study participants, parents and coaches for their support. This study was financially supported by funds of the National Program of Projects for the strengthening of research, creation and innovation in post-graduate courses of the National University of Colombia in the editions 2013–2015 and 2016–2018.

Open access funding enabled and organized by Projekt DEAL.

COMPETING INTERESTS

W.F.J.S. is a managing partner of the company 'Blood tec GmbH', but he is unaware of any direct or indirect conflict of interest with the contents of this paper. The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

AUTHOR CONTRIBUTIONS

E.M.S., W.F.J.S. and E.C.M. were involved in the conception and design of the study, the acquisition of data, the analysis and interpretation of the data and the drafting of the manuscript. D.M.R.C., J.M. and S.C.G. were involved in the acquisition of data, analysis and interpretation of data for the work as well as in the critical revision of the manuscript. All authors approved the final version of the manuscript and agreed to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. All persons designated as authors qualify for authorship, and all those who qualify for authorship are listed.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

ORCID

Diana Marcela Ramos-Caballero https://orcid.org/0000-0003-2456-2959

Walter F. J. Schmidt https://orcid.org/0000-0002-9766-5564

REFERENCES

Aguirre, C. A., Salazar, G. D. C., Lopez De Romaña, D. V., Kain, J. A., Corvalán, C. L., & Uauy, R. E. (2015). Evaluation of simple body composition methods: Assessment of validity in prepubertal Chilean children. *European Journal of Clinical Nutrition*, *69*(2), 269–273.

Åstrand, P.-O. (1952). *Experimental studies of physical working capacity in relation to sex and age*. Copenhagen: Ejnar Munksgaard.

Bachman, E., Travison, T. G., Basaria, S., Davda, M. N., Guo, W., Li, M., Westfall, J. C., Bae, H., Gordeuk, V., & Bhasin, S. (2014). Testosterone induces erythrocytosis via increased erythropoietin and suppressed hepcidin: Evidence for a new erythropoietin/hemoglobin set point. *Journals of Gerontology. Series A, Biological Sciences and Medical Sciences*, *69*(6), 725–735.

Böning, D., Cristancho, E., Serrato, M., Reyes, O., Mora, M., Coy, L., & Rojas, J. (2004). Diurnal changes of arterial oxygen saturation and erythropoietin concentration in male and female highlanders. *International Journal of Sports Medicine*, *25*(8), 561–568.

Claustres, M., & Sultan, C. (1988). Androgen and erythropoiesis: Evidence for an androgen receptor in erythroblasts from human bone marrow cultures. *Hormone Research*, *29*(1), 17–22.

Coviello, A., Kaplan, B., Lakshman, K., Chen, T., Singh, A., & Bhasin, S. (2008). Effects of graded doses of testosterone on erythropoiesis in healthy young and older men. *Journal of Clinical Endocrinology & Metabolism*, *93*(3), 914–919.

Cristancho, E., Riveros, A., Sánchez, A., Peñuela, O., & Böning, D. (2016). Diurnal changes of arterial oxygen saturation and erythropoietin concentration in male and female highlanders. *Physiological Reports*, *4*(17), e12901.

D'Andrea, S., Spaggiari, G., Barbonetti, A., & Santi, D. (2020). Endogenous transient doping: Physical exercise acutely increases testosterone levels—results from a meta-analysis. *Journal of Endocrinological Investigation*, *43*(10), 1349–1371.

Eckardt, K.-U., Hartmann, W., Vetter, U., Pohlandt, F., Burghardt, R., & Kurtz, A. (1990). Serum immunoreactive erythropoietin of children in health and disease. *European Journal of Pediatrics*, *149*(7), 459–464.

Gonzales, G. (2013). Serum testosterone levels and excessive erythrocytosis during the process of adaptation to high altitudes. *Asian Journal of Andrology*, *15*(3), 368–374.

Gore, C., Hopkins, W., & Burge, C. (2005). Errors of measurement for blood volume parameters: A meta-analysis. *Journal of Applied Physiology*, *99*(5), 1745–1758.

Gunga, H., Kirsch, K., Roecker, L., Kohlberg, E., Tiedemann, J., Steinach, M., & Schobersberger, W. (2007). Erythropoietin regulations in humans under different environmental and experimental conditions. *Respiratory Physiology & Neurobiology*, *158*(2–3), 287–297.

Handelsman, D., Hirschberg, A., & Bermon, S. (2018). Circulating testosterone as the hormonal basis of sex differences in athletic performance. *Endocrine Reviews*, *39*(5), 803–829.

Hero, M., Wickman, S., Hanhijärvi, R., Siimes, M., & Dunkel, L. (2005). Pubertal upregulation of erythropoiesis in boys is determined primarily by androgen. *Journal of Pediatrics*, *146*(2), 245–252.

Jelkmann, W. (2011). Regulation of erythropoietin production. *Journal of Physiology*, *589*(6), 1251–1258.

Karlberg, P., & Lind, J. (1955). Studies of the total amount of hemoglobin and the blood volume in children. *Acta Paediatrica*, *44*(1), 17–34.

Kerr, D. A. (1988). An anthropometric method for fractionation of skin, adipose, bone, muscle and residual masses in males and females age 6 to 77 years. MSc Thesis, Simon Fraser University.

Khairullah, A., Cousino Klein, L., Ingle, S., May, M., Whetzel, C., Susman, E., & Paus, T. (2014). Testosterone trajectories and reference ranges in a large longitudinal sample of male adolescents. *PLoS One*, *9*(9), e108838.

Konforte, D., Shea, J., Kyriakopoulou, L., Colantonio, D., Cohen, A., Shaw, J., Bailey, D., Chan, M. K., Armbruster, D., & Adeli, K. (2013). Complex biological pattern of fertility hormones in children and adolescents: A study of healthy children from the CALIPER cohort and establishment of pediatric reference intervals. *Clinical Chemistry*, *59*(8), 1215–1227.

Krabbe, S., Christensen, T., Worm, J., Christiansen, C., & Transbøl, I. (1978). Relationship between haemoglobin and serum testosterone in normal children and adolescents and in boys with delayed puberty. *Acta Paediatrica Scandinavica*, *67*(5), 655–658.

Lakens, D. (2013). Calculating and reporting effect sizes to facilitate cumulative science: A practical primer for *t*-tests and ANOVAs. *Frontiers in Psychology*, *4*, 00863.

Landgraff, H., & Hallén, J. (2020). Longitudinal training-related hematological changes in boys and girls from age 12 to 15. *Medicine & Science in Sports & Exercise*, *52*(9), 1940–1947.

Loftin, M., Nichols, J., Going, S., Sothern, M., Schmitz, K. H., Ring, K., Tuuri, G., & Stevens, J. (2007). Comparison of the validity of anthropometric and bioelectric impedance equations to assess body composition in adolescent girls. *International Journal of Body Composition Research*, *5*(1), 1–8.

Maggio, M., Snyder, P., Ceda, G. P., Milaneschi, Y., Luci, M., Cattabiani, C., Masoni, S., Vignali, A., Volpi, R., Lauretani, F., Peachey, H., Valenti, G., Cappola, A. R., Longo, D., & Ferrucci, L. (2013). Is the haematopoietic

effect of testosterone mediated by erythropoietin? The results of a clinical trial in older men. *Andrology*, *1*(1), 24–28.

Murphy, W. G. (2014). The sex difference in haemoglobin levels in adults – Mechanisms, causes, and consequences. *Blood Reviews*, *28*(2), 41–47.

Peschle, C., Rappaport, I. A., Sasso, G. F., Condorelli, M., & Gordon, A. S. (1973). The role of estrogen in the regulation of erythropoietin production. *Endocrinology*, *92*(2), 358–362.

Prommer, N., & Schmidt, W. (2007). Loss of CO from the intravascular bed and its impact on the optimised CO-rebreathing method. *European Journal of Applied Physiology*, *100*(4), 383–391.

Prommer, N., Wachsmuth, N., Thieme, I., Wachsmuth, C., Mancera-Soto, E., Hohmann, A., & Schmidt, W. (2018). Influence of endurance training during childhood on total hemoglobin mass. *Frontiers in Physiology*, *9*, 251.

Raes, A., Van Aken, S., Craen, M., Donckerwolcke, R., & Walle, J. (2006). A reference frame for blood volume in children and adolescents. *BMC Pediatrics*, *6*, 3.

Schmidt, W., Heinicke, K., Rojas, J., Gomez, J. M., Serrato, M., Mora, M., Wolfarth, B., Schmid, A., & Keul, J. (2002). Blood volume and hemoglobin mass in endurance athletes from moderate altitude. *Medicine and Science in Sports and Exercise*, *34*(12), 934–1940.

Schmidt, W., & Prommer, N. (2005). The optimised CO-rebreathing method: A new tool to determine total haemoglobin mass routinely. *European Journal of Applied Physiology*, *95*(5–6), 486–495.

Schmidt, W., & Prommer, N. (2008). Effects of various training modalities on blood volume. *Scandinavian Journal of Medicine & Science in Sports*, *18*(Suppl 1), 57–69.

Schmidt, W., & Prommer, N. (2010). Impact of alterations in total hemoglobin mass on VO2max. *Exercise and Sport Sciences Reviews*, *38*(2), 68–75.

Schmidt, W., Rojas, J., Böning, D., Bernal, H., Garcia, S., & Garcia, O. (1999). Plasma-electrolytes in natives to hypoxia after marathon races at different altitudes. *Medicine and Science in Sports and Exercise*, *31*(10), 1406–1413.

Shahani, S., Braga-Basaria, M., Maggio, M., & Basaria, S. (2009). Androgens and erythropoiesis: Past and present. *Journal of Endocrinological Investigation*, *32*(8), 704–716.

Slaughter, M., Lohman, T., Boileau, R., Horswill, C., Stillman, R., Van Loan, M., & Bemben, D. (1988). Skinfold equations for estimation of body fatness in children and youth. *Human Biology*, *60*(5), 709–723.

Spurr, G. B., Reina, J. C., & Hoffmann, R. G. (1992). Basal metabolic rate of Colombian children 2–16 y of age: Ethnicity and nutritional status. *American Journal of Clinical Nutrition*, *56*(4), 623–629.

Steiner, T., Maier, T., & Wehrlin, J. (2019). Effect of endurance training on hemoglobin mass and VO2max in male adolescent athletes. *Medicine & Science in Sports & Exercise*, *51*(5), 912–919.

Stewart, A., & Marfell-Jones, M. (2011). International standards for anthropometric assessment. International Society for the Advancement of Kinanthropometry.

Sullivan, G. M., & Feinn, R. (2012). Using effect size—or why the *P* value is not enough. *Journal of Graduate Medical Education*, *4*(3), 279–282.

Tanner, J. (1962). *Growth at Adolescence*. Oxford: Blackwell Scientific Publications.

Thomsen, K., Riis, B., Krabbe, S., & Christiansen, C. (1986). Testosterone regulates the haemoglobin concentration in male puberty. *Acta paediatrica Scandinavica*, *75*(5), 793–796.

Vihervuori, E., Virtanen, M., Koistinen, H., Koistinen, R., Seppälä, M., & Siimes, M. A. (1996). Haemoglobin level is linked to growth hormone-dependent proteins in short children. *Blood*, *87*(5), 2075–2081.

von Döbeln, W., & Eriksson, B. (1972). Physical training, maximal oxygen uptake and dimensions of the oxygen transporting and metabolizing organs in boys 11–13 years of age. *Acta Paediatrica*, *61*(5), 653–660.

Vorona, E., & Nieschlag, E. (2018). Adverse effects of doping with anabolic androgenic steroids in competitive athletics, recreational sports and bodybuilding. *Minerva Endocrinologica*, *43*(4), 476–488.

WADA. (2019). *Guidelines – blood sample collection*. Montreal: WADA/AMA. https://www.wada-ama.org/sites/default/files/resources/files/isti.

Wehrlin, J. P., & Steiner, T. (2021). Is hemoglobin mass at age 16 a predictor for national team membership at age 25 in cross-country skiers and triathletes? *Frontiers in Sports and Active Living*, *3*, 580486.

Westreich, D., & Greenland, S. (2013). The table 2 fallacy: Presenting and interpreting confounder and modifier coefficients. *American Journal of Epidemiology*, *177*(4), 292–298.

Yanamandra, U., Senee, H., Yanamadra, S., Das, S. K., Bhattachar, S. A., Das, R., Kumar, S., Malhotra, P., Varma, S., Varma, N., & Nair, V. (2019). Erythropoietin and ferritin response in native highlanders aged 4–19 years from the Leh-Ladakh region of India. *British Journal of Haematology*, *184*(2), 263–268.

Zachmann, M., Prader, A., Kind, H., Häfliger, H., & Budliger, H. (1974). Testicular volume during adolescence. Cross-sectional and longitudinal studies. *Helvetica Paediatrica Acta*, *29*(1), 61–72.

Zelenkova, I. E., Zotkin, S. V., Korneev, p. V., Koprov, S. V., & Grushin, A. A. (2019). Relationship between total hemoglobin mass and competitive performance in endurance athletes. *Journal of Sports Medicine and Physical Fitness*, *59*(3), 352–356.

Zitzmann, M., & Nieschlag, E. (2003). Effects of androgen replacement on metabolism and physical performances in male hypogonadism. *Journal of Endocrinological Investigation*, *26*(9), 886–892.

How to cite this article: Mancera-Soto, E., Ramos-Caballero, D. M., Magalhaes, J., Chaves Gomez, S., Schmidt, W. F.J., & Cristancho-Mejía, E. (2021). Quantification of testosterone-dependent erythropoiesis during male puberty. *Experimental Physiology*, *106*, 1470—1481. https://doi.org/10.1113/EP089433