Does Hemoglobin Mass Increase from Age 16 to 21 and 28 in Elite Endurance Athletes?

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ABSTRACT

STEINER, T., and J. P. WEHRLIN. Does Hemoglobin Mass Increase from Age 16 to 21 and 28 in Elite Endurance Athletes? *Med. Sci. Sports Exerc.*, Vol. 43, No. 9, pp. 1735–1743, 2011. **Purpose**: It is unclear if hemoglobin mass (Hb_{mass}) and red cell volume (RCV) increase in endurance athletes with several years of endurance training from adolescence to adulthood. The aim of this study, therefore, was to determine with a controlled cross-sectional approach whether endurance athletes at the ages of 16, 21, and 28 yr are characterized by different Hb_{mass}, RCV, plasma volume (PV), and blood volume (BV). **Methods**: BV parameters (CO rebreathing), $\dot{V}O_{2max}$ and other blood, iron, training, and anthropometric parameters were measured in three endurance athlete groups AG16 (n = 14), AG21 (n = 14), and AG28 (n = 16) as well as in three age-matched control groups (<2 h endurance training per week): CG16 (n = 16), CG21 (n = 15), and CG28 (n = 16). **Results**: In AG16, body weight–related Hb_{mass} (12.4 ± 0.7 $g \cdot kg^{-1}$), RCV, BV, and $\dot{V}O_{2max}$ (66.1 ± 3.8 $mL \cdot kg^{-1} \cdot min^{-1}$) were lower ($P < 0.001$) than those in AG21 (14.2 ± 1.1 $g \cdot kg^{-1}$, 72.9 ± 3.6 $mL \cdot kg^{-1} \cdot min^{-1}$) and AG28 (14.6 ± 1.1 $g \cdot kg^{-1}$, 73.4 ± 6.0 $mL \cdot kg^{-1} \cdot min^{-1}$). Results for these parameters did not differ between AG21 and AG28 and among the control groups. $\dot{V}O_{2max}$, PV, and BV were higher for AG16 than for CG16 (12.0 ± 1.0 $g \cdot kg^{-1}$, 58.9 ± 5.0 $mL \cdot kg^{-1} \cdot min^{-1}$) but not Hb_{mass} and RCV. **Conclusions**: Our results suggest that endurance training has major effects on Hb_{mass} and RCV from ages 16 to 21 yr, although there is no further increase from ages 21 to 28 yr in top endurance athletes. On the basis of our findings, an early detection of the aptitude for endurance sports at age 16 yr, solely based on levels of Hb_{mass}, does not seem to be possible. **Key Words:** BLOOD VOLUME, CO REBREATHING, ADOLESCENTS, TRAINING, AEROBIC CAPACITY

Performance in endurance sports depends on the capacity to deliver oxygen to active muscle tissue and the ability of the muscles to use oxygen. In endurance athletes, the oxygen supply to active muscle is regarded as the main limiting factor of maximal oxygen uptake ($\dot{V}O_{2max}$) (23,40). Because oxygen is transported primarily by hemoglobin, the total amount of hemoglobin (Hb_{mass}) determines, in large part, the oxygen transport capacity of the blood (12,35). For the past 50 yr, it has been well known (and supported by more recent investigations) that adult top endurance athletes are characterized by considerably higher levels of body weight–related Hb_{mass} compared with either nonendurance athletes or untrained persons (4,15,21).

However, it is unclear whether this higher Hb_{mass} is an effect of several years of endurance training, due to genetic predisposition, or a combination of both. Studies using the Evans blue dye method have measured increased red cell volume (RCV) after 3 (32) and 12 wk (41) of endurance training. An increase of ~6% of Hb_{mass} measured with the carbon monoxide (CO) rebreathing technique was reported after 9 months of endurance training (34) in relatively untrained subjects. In contrast, it has been shown that Hb_{mass} is stable in adult endurance athletes (11,12,29) and in adolescent athletes (7) independent of training volume and phase. The finding of unaltered Hb_{mass} after prolonged normoxic endurance training is supported by studies that have measured RCV with radioactive isotope methodologies (14,37). Recently, Schmidt and Prommer (34,35) have concluded that, on the basis of all available data, endurance training under normoxic conditions has only small effects on Hb_{mass} and RCV in elite athletes. Therefore, the high-Hb_{mass} and the high-endurance performance of elite athletes are more likely to originate from a specific genetic predisposition. Given that a high Hb_{mass} and consequently, a high $\dot{V}O_{2max}$ are rather hereditary than trainable, measuring Hb_{mass} could be used as a talent identification (7).

Unfortunately, there is a paucity of knowledge regarding the level and the changes of Hb_{mass} and blood volume (BV) parameters during adolescence in endurance athletes. Therefore, the question arises, if, or to what extent, Hb_{mass} and RCV increase with endurance training from adolescence to adulthood in endurance athletes. To our knowledge, there have been no studies to date comparing Hb_{mass} measured with the CO rebreathing method (33) between adolescents at

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the age of 16 yr, under 23 yr (U23) athletes, and adult elite endurance athletes.

The purposes of the present study, therefore, were to compare absolute and relative levels of Hb_{mass} and RCV in adolescent and adult top endurance athletes at different age categories (junior, U23, and elite) with age-matched controls to compare plasma volume (PV) and BV between the aforementioned groups, and, finally, to compare aerobic capacity ($\dot{V}O_{2max}$) between the groups.

METHODS

Subjects. In total, 45 male endurance athletes of different age groups (junior, U23, and elite) and 47 age-matched, healthy, non–endurance-trained and nonsmoking male controls participated in this study. According to their age (that averaged 16, 21, and 28 yr) and training status, the subjects were divided into six subgroups (athletes: AG16, AG21, and AG28; controls: CG16, CG21, and CG28). Group characteristics and averaged self-reported training volumes are reported in Table 1. Subjects neither conducted an altitude training at least 3 months before testing nor donated blood during this period. AG21 and AG28 were recruited from the Swiss National Teams for cross-country skiing (XC skiing) and triathlon. Inclusion criterion for AG16 was a national top 15 overall ranking in either XC skiing or triathlon in the season 2007/2008 because no national team exists at this age. For uniformity of the control groups' training status, a maximum of either 2 h of endurance training per week or 3 h of team sports were set as upper limits for all control subjects, disregarding school sport lessons for AG16 and CG16. One athlete from AG21 was injured and was unable to complete the exercise test. Results for one subject from AG16 were excluded because of the high likelihood of iron deficiency according to the age-specific interpretive standards of Valberg et al. (39).

The age-matched athletes and controls were, on average, of the same height and weight. The athletes trained significantly more than the controls ($P < 0.001$), whereas control groups did not differ ($P = 0.22$) in training volume. Athletes from AG21 and AG28 reported considerably higher ($P < 0.001$) training volumes than athletes from AG16 (Table 1).

All experimental procedures were approved by the Regional Ethics Committee in Berne, Switzerland, and the study was carried out according to the recommendations of the Helsinki Declaration. Written informed consent of the subjects and the parents (for subjects of AG16 and CG16) was obtained before any testing.

Study design. All subjects completed the same series of measurements in the following order: 1) venous blood sampling, 2) anthropometric measurements, 3) an exercise test ($\dot{V}O_{2max}$), and 4) CO rebreathing method to measure Hb_{mass} and BV. Athletes performed the tests at the beginning of the off-season period (XC skiers in June and triathletes in November), whereas controls were all measured between May and July. Before any testing, subjects were required to complete a questionnaire for the assessment of actual training load. Subjects were asked not to perform strenuous exercise within 24 h of the measurements. All tests were carried out in Magglingen (Switzerland) at an altitude of 950 m.

Determination of Hb_{mass} and BV parameters. Hb_{mass} was measured using a slightly modified version of the CO rebreathing procedure (27) described by Schmidt and Prommer (33). Briefly, after the subjects had spent 5 min in the sitting position, three capillary blood samples (35 μL) were taken from an earlobe and analyzed immediately for HbCO (ABL 800flex; Radiometer A/S, Copenhagen, Denmark). Subsequently, the subjects inhaled a bolus of chemically pure CO (Multigas SA, Domdidier, Switzerland) corresponding to 1.2 $mL \cdot kg^{-1}$ for the athletes and 1.0 $mL \cdot kg^{-1}$ for the controls. The gas was always administered via the same 100-mL plastic syringe (Omnifix®; B.Braun, Melsungen, Germany) connected to a specific glass spirometer (Blood Tec GbR, Bayreuth, Germany) with a 3.5-L anesthetic bag filled with oxygen. After inhaling the CO and the oxygen, subjects held their breath for 10 s before they began rebreathing in the closed circuit for 1 min 50 s. A CO gas analyzer (CO Single Gas Detector; BW Technologies, Calgary, Canada) was used during the rebreathing procedure to check for possible CO leakage at the mouthpiece, noseclip, and spirometer. At 6 and 8 min after inhalation of the CO, two final blood samples were taken from an earlobe, and the average was used as the post-HbCO value. To account for the CO exhaled from rebreathing termination to the midway point

TABLE 1. Subject characteristics and self-reported training volumes.

	n	Age (yr)	Height (cm)	Weight (kg)	Body Fat (%)	LBM (kg)	Training ($h \cdot wk^{-1}$)	Endurance Training ($h \cdot wk^{-1}$)
Athlete groups								
AG16	14	16.0 ± 0.6	174.3 ± 6.7*	61.0 ± 8.9**	5.8 ± 2.8	57.3 ± 7.2**	8.6 ± 1.7**‡‡	7.4 ± 1.7**‡‡
AG21	14	21.3 ± 0.9	179.8 ± 4.8	74.4 ± 4.8	6.7 ± 1.5‡	69.4 ± 4.3‡	16.3 ± 2.5‡‡	14.1 ± 3.4‡‡
AG28	16	27.4 ± 2.9	180.4 ± 5.4	72.6 ± 6.2	6.3 ± 1.6‡‡	68.0 ± 5.1	18.7 ± 4.6‡‡	16.1 ± 4.9‡‡
Control groups								
CG16	16	15.9 ± 0.3	175.3 ± 8.1†	62.1 ± 7.4††	7.7 ± 3.0	57.1 ± 5.3††	1.8 ± 1.5	0.6 ± 0.6
CG21	15	21.3 ± 1.2	179.1 ± 5.0	72.4 ± 6.4	9.4 ± 3.9	65.4 ± 5.0	2.7 ± 1.5	1.1 ± 0.5
CG28	16	27.9 ± 3.8	181.2 ± 6.5	76.1 ± 5.5	10.8 ± 4.6	67.7 ± 3.8	2.7 ± 1.9	0.7 ± 0.8

Values are presented as mean ± SD.
* Significantly lower than in older athletes (* $P < 0.05$, ** $P < 0.01$).
† Significantly lower than in older controls († $P < 0.05$, †† $P < 0.01$).
‡ Value significantly different from age-matched controls (‡ $P < 0.05$, ‡‡ $P < 0.001$).
LBM, lean body mass; Training, training per week disregarding school sport lessons for AG16 and CG16.

between the final two blood samples, the differences between the end-tidal CO concentrations measured with a CO gas detector with parts per million sensitivity (Dräger PAC 7000; Dräger Safety; Lübeck, Germany) before and after the rebreathing procedure were multiplied by the estimated alveolar ventilation of 5.25 $L \cdot min^{-1}$ (42). The volume of CO that had not been absorbed by the body was quantified by measuring the CO concentration in the anesthetic bag with the same CO gas detector to measure the end-tidal CO concentration by connecting a tube to the glass spirometer. The measured CO concentration was then multiplied by both the bag volume and the estimated subject's residual volume (25).

Total Hb_{mass} was calculated as described previously (27), using a slightly different correction for loss of CO to myoglobin (0.3%·min^{-1} of administered CO) as recommended by Prommer and Schmidt (28).

RCV, BV, and PV were finally calculated as followed (see also Burge and Skinner [5] and Heinicke et al. [15]).

$$RCV = Hb_{mass}/MCHC \times 100$$

$$BV = RCV \times (100/Hct)$$

$$PV = BV - RCV$$

where MCHC = mean corpuscular hemoglobin concentration and Hct = hematocrit corrected to whole-body Hct by the body/venous hematocrit ratio of 0.91 (6). For the calculations of RCV, PV, and BV, venous hemoglobin concentration (Hb) as well as venous Hct were used.

The typical error (18) for Hb_{mass} in our laboratory assessed with 17 duplicate measures (24- to 48-h time lag between the tests) using the aforementioned CO rebreathing protocol 2 months before the measurements of the present study was 1.4%.

Measurement of aerobic capacity ($\dot{V}O_{2max}$ test). All subjects performed a graded exercise test to determine $\dot{V}O_{2max}$. For CG16, CG21, CG28, AG16, and the triathletes from AG21 and AG28, these tests were conducted on a treadmill (Model Venus; h/p/Cosmos Sports & Medical GmbH, Traunstein, Germany). After a 5-min warm-up jog, control subjects began running at 7 $km \cdot h^{-1}$, and subjects from the athlete groups ran at 9 $km \cdot h^{-1}$. The speed was increased by 1 $km \cdot h^{-1}$ every minute for the first 3 min of the test and thereafter by 0.5 $km \cdot h^{-1}$ every 30 s until exhaustion. The treadmill incline was set at 4° throughout the test. Gas exchange was measured breath by breath with an open-circuit system (Oxycon Pro; Erich Jaeger GmbH, Hoechberg, Germany); HR was continuously registered with a Polar HR monitoring system (Polar S610i; Polar Electro Oy, Kempele, Finland). Subjects' RPE was assessed with Borg's RPE Scale (3).

XC skiers from AG21 and AG28 performed the $\dot{V}O_{2max}$ test with roller skis in the diagonal striding technique on a special roller skiing treadmill (ST Innovation GmbH, Leipzig, Germany). Initial speed was set at 3.2 $m \cdot s^{-1}$ and was thereafter increased every 30 s by 0.1 $m \cdot s^{-1}$. The treadmill incline was set at 6° throughout the test. Gas exchange was measured with a mixing chamber system (Oxycon Pro; Erich Jaeger GmbH), and HR was continuously recorded with a Suunto HR monitoring system (Suunto T6; Suunto Oy, Vantaa, Finland). RPE was measured with the same scale as described for the other groups.

Both $\dot{V}O_{2max}$ protocols were designed to induce exhaustion of the subjects after 5–9 min. The effect of the different protocols and the different gas exchange measurement systems on $\dot{V}O_{2max}$ was tested with 10 young XC skiers who performed both protocols at least 5 h apart. There was no difference between the measured mean $\dot{V}O_{2max}$ values ($P = 0.91$), and the typical error between the protocols was 1.44%. The criteria of a plateau in oxygen uptake, a RER value of ≥1.10, and an HR close to the age-predicted maximum were used to determine whether the subjects reached $\dot{V}O_{2max}$ (19).

Venous blood sampling and analysis. Venous blood was sampled first on the subjects' arrival at the institute. After 15 min of rest in the supine position, two blood samples (4 mL for EDTA blood, 5 mL for blood serum) were drawn from the antecubital vein. Hb, Hct, red blood cell distribution width (RDW), and percentage of reticulocytes (Rct) were measured with an automated hematology analyzer (ADVIA 120; Siemens Healthcare Diagnostics GmbH, Eschborn, Germany). The transferrin saturation and soluble transferrin receptor (sTfR) were quantified with a biochemistry analyzer (Olympus AU 2700; Olympus Medical System Corp., Tokyo, Japan). With two different automated immunoassay systems, serum erythropoietin (sEPO) (Immulite 2000; Siemens Healthcare Systems, Erlangen, Germany) and serum ferritin (Ftn) (ADVIA Centaur; Siemens Healthcare Systems) were measured. ON-hes (index for current recombinant human erythropoietin (rHuEPO) misuse), OFF-hre (index for recent rHuEPO misuse), and OFF-hr (index for recent rHuEPO misuse using only Hb and Rct) scores were calculated according to Gore et al. (13). Subsequently, they were compared with the cutoff scores for false-positive rates of 1–10 for blood samples collected at sea level as well as at altitudes >610 m (13).

Anthropometric measurements. Anthropometrical assessment of subjects, including the determination of height, weight, and skinfold measurements at seven sites (chest/pectoral, midaxillary, suprailiac, abdominal, triceps, subscapular, and thigh), was performed by the same experienced investigator. From these data, body density was determined using the procedure proposed by Jackson and Pollock (20). Subsequently, percent body fat (%) and lean body mass (LBM) were both calculated with age-specific equations (17).

Assessment of biological age for AG16 and CG16. Maturity status (and hence, biological age) of subjects from AG16 and CG16 was estimated with a somatic method (2) that compares the present stature with the projected adult stature. The percentage of adult stature attained on the test day provided an indicator of biological maturity status.

Statistics. All statistical tests were conducted using the statistical package R (Version 2.10.0, R Foundation for

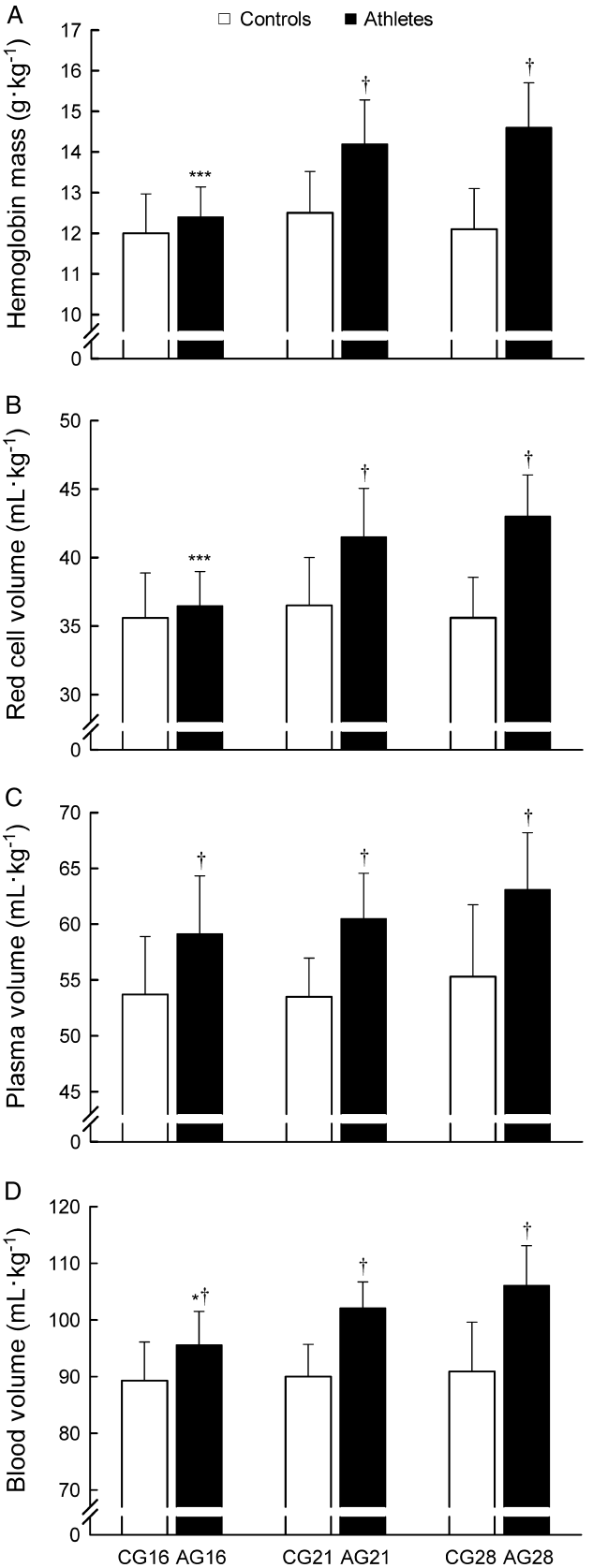

FIGURE 1—Hb_{mass} (A), RCV (B), PV (C), and BV (D) per kilogram body weight in the different athlete and control groups. *Significantly lower than in older athletes (*$P < 0.05$, ***$P < 0.001$). †Significantly higher than in age-matched controls ($P < 0.01$).

Statistical Computing; www.r-project.org). Differences among athlete groups and control groups were assessed with a one-way ANOVA. Tukey HSD *post hoc* tests were used to identify age-specific differences between means. Differences between athletes and control subjects from the specific age groups were analyzed with unpaired *t*-tests. Linear regression was used to test associations between different variables, and correlations were compared using Steiger's *z* test (38). Unless stated otherwise, results are expressed as mean ± SD. Significance was set as $P < 0.05$ for all analyses.

RESULTS

Hb_{mass}. Body weight–related (relative) Hb_{mass} was lower ($P < 0.001$) in AG16 (12.4 ± 0.7 g·kg^{-1}) than in older athletes AG21 (14.2 ± 1.1 g·kg^{-1}) and AG28 (14.6 ± 1.1 g·kg^{-1}). There were no differences ($P = 0.51$) between the older athlete groups (AG21 and AG28) and among the control groups ($P = 0.40$) (Fig. 1A). Hb_{mass} between AG16 and CG16 (12.0 ± 1.0 g·kg^{-1}) did not differ significantly ($P = 0.24$). AG21 had 14% and AG28 had 21% higher relative Hb_{mass} values than the age-matched controls ($P < 0.001$). LBM-related Hb_{mass} (Hb_{mass}/LBM) showed almost the same characteristics as relative Hb_{mass} (Table 2). There was no difference between AG16 and CG16 ($P = 0.641$); athletes from AG16 were significantly lower in Hb_{mass}/LBM than older athletes ($P < 0.001$), and AG21 and AG28 had higher Hb_{mass}/LBM values (+10% and +16%, respectively) than age-matched controls ($P < 0.001$). CG21 had higher Hb_{mass}/LBM values than CG16 ($P = 0.044$). Absolute Hb_{mass} values are reported in Table 2. There was a strong correlation between weight and absolute Hb_{mass} ($r = 0.77$, $P < 0.001$) and LBM and Hb_{mass} ($r = 0.86$, $P < 0.001$) for all 91 subjects.

BV parameters. Athletes from AG16 had lower relative RCV ($P < 0.001$) and BV ($P < 0.05$) than AG21 and AG28, respectively, whereas there were no differences in PV between the three athlete groups ($P = 0.09$) (Figs. 1B–D). Control groups did not differ in relative RCV ($P = 0.66$), PV ($P = 0.56$), and BV ($P = 0.81$). Athletes had higher relative PV (AG16 + 10%, AG21 + 13%, and AG28 + 14%) and BV (AG16 + 7%, AG21 + 13%, and AG28 + 17%) than their age-matched controls ($P < 0.01$). RCV was higher for AG21 and AG28 than for CG21 and CG28 ($P < 0.01$) but not for AG16 in comparison with CG16 ($P = 0.39$). Absolute RCV, PV, and BV for both AG16 and for CG16 were lower in comparisons with either older athletes or controls, respectively ($P < 0.01$). Absolute values for RCV, PV, and BV did not differ between AG16 and CG16.

$\dot{V}O_{2max}$. AG16 (66.1 ± 3.8 mL·kg^{-1}·min^{-1}) had lower ($P < 0.001$) relative $\dot{V}O_{2max}$ values than AG21 (72.9 ± 3.6 mL·kg^{-1}·min^{-1}) and AG28 (73.4 ± 6.0 mL·kg^{-1}·min^{-1}), whereas control groups did not differ ($P = 0.137$). Absolute and relative $\dot{V}O_{2max}$ for athlete groups was higher than in the age-matched controls (Fig. 2 and Table 2). Testing for differences in LBM related $\dot{V}O_{2max}$ ($\dot{V}O_{2max}$ /LBM) between

TABLE 2. Blood volume parameters and $\dot{V}O_{2max}$ for the different athlete and control groups.

	n	Hb_{mass} (g)	Hb_{mass}/LBM (g·kg^{-1})	RCV (mL)	PV (mL)	BV (mL)	$\dot{V}O_{2max}$ (mL·min^{-1})
Athlete groups							
AG16	14	758 ± 124**	13.2 ± 0.87**	2225 ± 361**	3591 ± 512**	5817 ± 815**	4019 ± 547**‡
AG21	14	1055 ± 83‡‡	15.2 ± 1.01‡‡	3084 ± 280‡‡	4508 ± 482‡‡	7592 ± 625‡‡	5432 ± 464‡‡
AG28	16	1059 ± 104‡‡	15.6 ± 1.06‡‡	3118 ± 318‡‡	4575 ± 462‡	7693 ± 720‡‡	5336 ± 556‡‡
Control groups							
CG16	16	745 ± 92††	13.0 ± 0.90§	2204 ± 293††	3323 ± 405††	5526 ± 630††	3641 ± 373††
CG21	15	903 ± 103	13.8 ± 0.90	2642 ± 341	3870 ± 403	6512 ± 684	4160 ± 597
CG28	16	917 ± 81	13.5 ± 0.73	2701 ± 234	4204 ± 525	6905 ± 715	4158 ± 530

Values are presented as mean ± SD.
* Significantly lower than in older athletes (* $P < 0.05$, ** $P < 0.01$).
†† Significantly lower than in older controls ($P < 0.01$).
‡ Significantly different from age-matched controls (‡ $P < 0.05$, ‡‡ $P < 0.001$).
§ Significantly lower than in CG21 ($P < 0.05$).

the groups did not reveal any differences to relative $\dot{V}O_{2max}$. Levels of $\dot{V}O_{2max}$/LBM for the athletes were 70.2 ± 3.5 (AG16), 78.1 ± 3.6 (AG21), and 78.6 ± 6.6 mL·kg^{-1}·min^{-1} (AG28). $\dot{V}O_{2max}$/LBM levels for controls were 63.8 ± 4.4 (CG16), 63.4 ± 6.7 (CG21), and 61.3 ± 6.0 mL·kg^{-1}·min^{-1} (CG28). Absolute $\dot{V}O_{2max}$ for AG16 and CG16 were lower in comparison with either older athletes or controls, respectively ($P < 0.01$). Absolute $\dot{V}O_{2max}$ was highly correlated with absolute Hb_{mass} ($r = 0.86$, $P < 0.001$), whereas the correlations between relative $\dot{V}O_{2max}$ and relative Hb_{mass} ($r = 0.74$, $P < 0.001$) and $\dot{V}O_{2max}$/LBM and Hb_{mass}/LBM ($r = 0.69$, $P < 0.001$) were lower.

Venous blood sampling and analyses. There were no differences in transferrin saturation, mean corpuscular volume, RDW, sEPO, Rct, OFF-hre score, and OFF-hr score between all groups. AG16 had significantly lower Hb, Hct, and Ftn values than older athletes (Table 3). Ftn and sTfR were significantly lower in CG16 than in CG21 and CG28. Two athletes showed higher ON-hes scores than the cutoff score (192.2) for sea level. For OFF-hre and OFF-hr in total, nine subjects (six athletes and three controls) had higher scores than the sea-level cutoff scores (OFF-hre = 99.6; OFF-hr = 104.6). Because four of these six athletes live at altitudes >610 m above sea level, the scores for these athletes were also compared with the cutoffs for blood samples collected >610 m (13). All ON-hes scores were subsequently below the corresponding cutoff score of 209.4, and only two athletes were above the cutoffs of 108.1 (OFF-hre) and 113.7 (OFF-hr). One athlete was an XC skier with a medically certificated high natural Hb level (17.4 g·dL^{-1}), whereas the second athlete from AG16 had a very low Rct value with normal levels of hemoglobin.

Biological age and correlations of Hb_{mass} with body weight and LBM in AG16 and CG16. Biological ages for both AG16 (16.4 ± 0.6 yr) and CG16 (16.2 ± 0.6 yr) were different from chronological ages ($P < 0.05$) but did not differ between the athlete and the control groups ($P = 0.52$). Weight ($r = 0.76$, $P < 0.001$) and LBM ($r = 0.83$, $P < 0.001$) were both strongly correlated to biological age, although the correlations between weight and chronological age ($r = 0.35$, $P = 0.051$), as well as LBM and chronological age ($r = 0.36$, $P = 0.05$), were weak and significantly lower than for biological age ($P < 0.01$).

DISCUSSION

The main findings of the present study are that Hb_{mass} and RCV of adolescent athletes (16 yr) are significantly lower than those in either U23 or adult athletes and—novel and surprising for us—that Hb_{mass} and RCV do not differ between athletes and control subjects at the age of 16 yr.

Hemoglobin mass. We observed approximately 15% lower relative Hb_{mass} and RCV values in 16-yr-old endurance athletes in comparison with older athletes. Whereas comparable data for Hb_{mass} in adolescent athletes at this age do not exist, relative Hb_{mass} of AG21 (14.2 g·kg^{-1}) and AG28 (14.6 g·kg^{-1}) was quite similar compared with cross-sectional studies on adult endurance athletes (4,12,15, 21,22,34). Surprisingly, Hb_{mass} of AG16 did not differ compared with age-matched controls. Differences in Hb_{mass} between adult athletes and controls (15%–20%) were less pronounced than in other investigations (15,21,34). One reason for this could be the fact that control subjects of the present investigation were not totally untrained and had higher Hb_{mass} values than previously reported for untrained subjects (21,34). In addition, Hb_{mass} can vary by approximately 3% in athletes during competitive season, and parts of these variations are related to oscillations in training load (10). Measuring Hb_{mass} and also $\dot{V}O_{2max}$ in the athlete

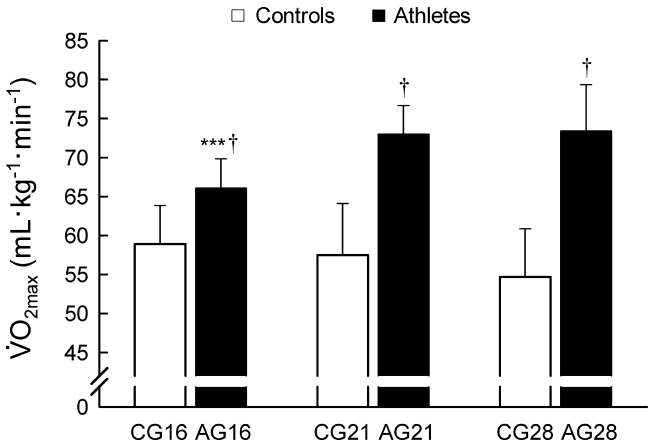

FIGURE 2—Maximal oxygen uptake per kg body weight in the different athlete and control groups. *$\dot{V}O_{2max}$ significantly lower than in older athletes ($P < 0.001$). †$\dot{V}O_{2max}$ significantly higher than in age-matched controls ($P < 0.01$).**

APPLIED SCIENCES

TABLE 3. Blood and iron parameters for the different athlete and control groups.

	Athletes			Controls		
	AG16	AG21	AG28	CG16	CG21	CG28
n	14	14	16	16	15	16
Hb ($g \cdot dL^{-1}$)	14.3 ± 0.8*	15.3 ± 1.1	15.1 ± 0.8	14.8 ± 0.7	15.2 ± 0.6	14.6 ± 0.8
Hct (%)	42.0 ± 2.8*	44.7 ± 3.2	44.6 ± 2.2	43.8 ± 3.0	44.5 ± 2.5	43.1 ± 2.5
Transferrin saturation (%)	31 ± 7	29 ± 12	29 ± 11	25 ± 11	31 ± 12	31 ± 9
Serum ferritin ($ng \cdot mL^{-1}$)	42 ± 20*	75 ± 32	82 ± 35	30 ± 15††	62 ± 34	92 ± 56
MCV (fL)	89 ± 4	90 ± 4	91 ± 3	88 ± 4	88 ± 3	89 ± 3
RDW (%)	12.8 ± 0.4	12.9 ± 0.5	13.0 ± 0.4	13.1 ± 0.4	13.0 ± 0.5	13.0 ± 0.5
sTfR ($mg \cdot L^{-1}$)	1.5 ± 0.2	1.5 ± 0.2	1.5 ± 0.3	1.7 ± 0.4†	1.6 ± 0.3	1.4 ± 0.3
sEPO ($U \cdot L^{-1}$)	10.3 ± 2.4	9.8 ± 3.4	10.4 ± 3.7	9.4 ± 2.6	8.3 ± 2.1	9.3 ± 2.2
Rct (‰)	9.4 ± 3.3	9.9 ± 2.5	11.7 ± 3.5	9.5 ± 2.5	10.7 ± 4.5	10.9 ± 3.3
ON-hes	166 ± 11	174 ± 14	173 ± 8‡	172 ± 9	174 ± 6†	166 ± 10
OFF-hre	79 ± 13	88 ± 14	82 ± 11	84 ± 10	87 ± 11	79 ± 12
OFF-hr	86 ± 15	94 ± 14	87 ± 11	90 ± 10	92 ± 12	85 ± 12

Values are presented as mean ± SD.
* Significantly lower than in older athletes (* $P < 0.05$).
† Significantly different from CG28 († $P < 0.05$, †† $P < 0.001$).
‡ Significantly different from age-matched controls ($P < 0.05$).
MCV, mean corpuscular volume; ON-hes, index for current rHuEPO misuse; OFF-hre, index for recent rHuEPO misuse; OFF-hr, index for recent rHuEPO misuse using only Hb and Rct.

groups during the competitive season instead of off-season probably would have yielded slightly higher values and therefore bigger differences.

Our results indicate that Hb_{mass} increases with endurance training between the ages of 16 and 21 yr, whereas the potential to increase Hb_{mass} with training in normoxia in already highly trained athletes after the U23 category seems limited. Similar findings about the stability of Hb_{mass} in adult endurance athletes independently of training load have been reported in other investigations (11,12,29,34), although endurance training seems to have only some influence on Hb_{mass} for moderately trained subjects (31,34). The same conclusions can be drawn when Hb_{mass} is related to LBM, which best predicts Hb_{mass}. Because athletes had similar percentages of body fat, differences (%) among the athlete groups were identical for Hb_{mass}/LBM in comparison with relative Hb_{mass}. Differences in Hb_{mass}/LBM between adult athletes and age-related controls were even lower (10%–16%) because of a higher body fat content of the control subjects. Owing to marginally lower values of Hb_{mass}/LBM in CG16 than in CG21 as well as CG28, it can be hypothesized that other factors than endurance training could slightly influence the evolution of Hb_{mass} between 16 and 21 yr.

Endurance training before and during puberty seems to have minor influence on Hb_{mass} levels in athletes. This is supported by observations of Eastwood et al. (7), who reported no change in Hb_{mass} in 11- to 15-yr-old cyclists after 12 months of training but an increase in $\dot{V}O_{2max}$. Eriksson (8) also reported a large increase in $\dot{V}O_{2max}$ (14% when corrected for growth) in 11- to 13-yr-old untrained boys after 16 wk of training, whereas Hb_{mass} did not increase more than could be explained by growth. An early detection of the aptitude for endurance sports solely based on Hb_{mass} levels at the age of 16 yr does not seem possible. On one hand, Hb_{mass} does not discriminate between athletes and nonathletes, and on the other hand, nothing is known about the (eventually, quite individual) evolution of Hb_{mass} until the age of 20 yr. However, our results do not oppose the assumption that genetic predisposition could play an important role for a high Hb_{mass} in elite athletes (34) because ranges for Hb_{mass} were very widespread for AG16 (10.9–13.8 $g \cdot kg^{-1}$) as well as for CG16 (10.4–14.1 $g \cdot kg^{-1}$), with a nonathlete having the highest Hb_{mass} of the 16-yr-old subjects. To assess whether the higher Hb_{mass} of some subjects at this age is a prerequisite to reach high Hb_{mass} levels in adulthood, more data about the evolution of Hb_{mass} between 16 and 21 yr are needed.

On the basis of our results, it can be hypothesized that a training-induced relevant erythropoietic stimulation does not occur before late stages of puberty in boys. The limited increase in the rate of erythrocyte production before puberty may be triggered by the up-regulation of androgens during puberty and, hence, the up-regulation of erythropoiesis years after the onset of testosterone secretion (16). The combination of elevated androgen levels and frequently increased training volumes and intensities in junior athletes at the age of 16 may be the reason that the training period between 16 and 21 yr is important for the evolution of high Hb_{mass} in male athletes.

RCV, PV, and BV. Because RCV is directly calculated from Hb_{mass}, all differences and interpretations for Hb_{mass} discussed in the preceding paragraph can be transferred to RCV. The mean PV did not differ among athlete groups and ranged from 59 to 63 $mL \cdot kg^{-1}$. Values were quite similar to those reported by Heinicke et al. (15), as well as Schmidt and Prommer (34), for elite endurance athletes. In contrast to Hb_{mass} and RCV, adolescent athletes were characterized by a significantly higher PV than nonendurance controls. BV levels for AG21 (102.1 $mL \cdot kg^{-1}$) and AG28 (106.1 $mL \cdot kg^{-1}$) were on a level comparable to other investigations as well (15,34). BV for AG16 was significantly lower than in the older athletes, which was mainly due to a lower RCV. These results suggest that the main adaptation to endurance training before the age of 16 yr is an increase in PV (and hence, BV), whereas levels of Hb_{mass} and RCV are only slightly affected.

Aerobic capacity ($\dot{V}O_{2max}$). All athlete groups had significantly higher $\dot{V}O_{2max}$ values than the age-matched controls. However, levels for $\dot{V}O_{2max}$ of the control groups were higher than those reported for untrained subjects (34). Close correlations between $\dot{V}O_{2max}$ and absolute Hb_{mass} (r = 0.87), relative $\dot{V}O_{2max}$ and Hb_{mass} (r = 0.74), and $\dot{V}O_{2max}$/LBM and Hb_{mass}/LBM (r = 0.69) were observed, showing the close relationship between these parameters. Correlations were comparable to other publications that reported correlations between either absolute or relative Hb_{mass} with $\dot{V}O_{2max}$ (12,15,24,35) or BV with $\dot{V}O_{2max}$ (31).

The higher $\dot{V}O_{2max}$ can be explained by the higher BV and Hb_{mass} for AG21 and AG28 than for control subjects, leading to a higher oxygen transport capacity in their blood than in that of control subjects. To which degree other factors such as pulmonary diffusing capacity, capillarization of the muscles, or mitochondrial density (1) had an influence on the elevated $\dot{V}O_{2max}$ value in endurance athletes is unclear because we did not conduct any of these measures. It has been reported that up to ~65% of $\dot{V}O_{2max}$ are inherited, after adjusting for anthropometric characteristics and sports participation (9). Owing to the close relationship of $\dot{V}O_{2max}$ and Hb_{mass}, a large part of these unspecified genetic factors may be related to Hb_{mass} (and BV parameters). This can be illustrated with individually high $\dot{V}O_{2max}$ values (up to 68 $mL \cdot kg^{-1} \cdot min^{-1}$) of control subjects of AG16 with no history of training. These high $\dot{V}O_{2max}$ levels were always associated with naturally high Hb_{mass} levels as already reported by Martino et al. (24).

Blood and iron parameters. We found lower Hb and Hct values in AG16 than in AG21 and AG28. During adolescence, a maturity and growth-related increase of Hb can be observed that levels off at the age of 20 (26). Therefore, the lower Hb and Hct values of AG16 can be attributed to adolescence and the training associated increase of PV with stable RCV (Figs. 1B, C).

Mean serum ferritin levels of AG16 (39 $ng \cdot mL^{-1}$) and CG16 (30 $ng \cdot mL^{-1}$) were significantly lower than those in older subjects of the comparable groups. Nevertheless, these lower values are in line with other investigations measuring Ftn in adolescents (16,26,39). Body growth and the concomitant expansion of the red cell mass place increased demands on the amount of absorbed iron. For this reason, body iron balance in males is most crucial from 16 to 17 yr (26). Using the age-specific interpretive standards of Valberg et al. (39) for the probability of iron deficiency and consequential limited erythropoiesis revealed that only one athlete from AG16 was classified as iron deficient. This subject's results were therefore excluded from analysis.

Individual values for markers of altered erythropoiesis (Hct, sTfR, and EPO) were all in the 95% reference range that was reported by Sharpe et al. (36). Combined scores (ON-hes, OFF-hre, and OFF-hr) did not differ significantly between athletes and control subjects. About the same number of subjects from either the athlete groups or the control groups (after having corrected the cutoffs for four athletes living >610 m above sea level) was above the cutoff scores for false-positive rates of 1 to 10 (13) for OFF-hre and OFF-hr. These results lead us to assume that our results were not confounded by either the recent or the current misuse of rHuEPO. Furthermore, OFF-hr would have been effective at detecting chronic transfusions of autologous blood 1–4 wk after the infusion (27). However, acute transfusions cannot be detected with the OFF-hr score.

Influence of biological age. Biological age, weight, and LBM did not differ between AG16 and CG16, thus differences between these groups are not biased by different stages of development. Weight and LBM were highly correlated with biological age, although there was only a weak correlation of both parameters with chronological age. For this reason, weight and LBM are proxy indicators of maturation in the 16-yr-old subjects, which has also been reported by Raes et al. (30), who concluded that LBM has the advantage of correcting for differences between the pubertal stages in children and adolescents. Because percent body fat did not differ between AG16 and CG16, we assumed that it was appropriate to use body weight–related Hb_{mass} values in adolescents without special maturation indices for the comparisons.

Limitations of the study. The aim of the present study was to investigate whether Hb_{mass} and RCV increase with endurance training from adolescence to adulthood in top endurance athletes. Of course, the best study design to answer this question would be a longitudinal approach. However, we presume, in the absence of longitudinal data, that our controlled, cross-sectional approach provides novel estimates of change in Hb_{mass} and RCV from adolescent to adult athletes, given subjects' selection characteristics (all athletes with similar age-specific performance level, all controls with similar activity levels). However, a possible selection bias cannot be excluded, particularly in the AG16 group. Athletes from AG16 had to be selected based on a seasonal, overall ranking in XC or triathlon. At this age, the lack of a strong physiological predisposition can be partly compensated by high technical skills, a higher training volume, and training history. Consequently, it is possible that athletes with rather low Hb_{mass}, and therefore, a limited aerobic capacity potential, were included. In the U23 (AG21) or elite (AG28) categories, a masking of low Hb_{mass} levels is no longer possible. Therefore, only athletes with a high aerobic capacity level and, hence, high Hb_{mass}, are successful in competitions and members of a national team. Whether athletes from AG16 will reach a similar level of Hb_{mass} as AG21 and AG28 at a comparable age, and whether Hb_{mass} actually develops in these athletes with training and maturation cannot be answered yet but shall be further examined.

CONCLUSIONS

Our results indicate that the age period from 16 to 21 yr seems to be a particularly sensitive phase for male endurance

athletes to elevate Hb_{mass} and RCV with endurance training, whereas in adult athletes, a training-induced increase of these parameters is almost unlikely. Endurance training seems to have only minor effects on Hb_{mass} and RCV until the age of 16 yr in endurance athletes in comparison with nonathletes, whereas training seems to influence and increase PV, BV, and hence $\dot{V}O_{2max}$. An early detection of the aptitude for endurance sports solely based on levels of Hb_{mass} at age 16 yr does not seem possible. However, high Hb_{mass} levels seem to be highly genetically predisposed at this age, but their transferability to adulthood still needs be examined. Our findings must be confirmed with a longitudinal approach to assess possible intraindividual differences of Hb_{mass} adaptations.

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