



Oxygen Uptake, Acid-Base Balance and Anaerobic Energy System Contribution in Maximal 300 – 400 M Running in Child, Adolescent and Adult Athletes

Nikke Vilmi^{1*}, Sami Äyrämö¹, Ari Nummela², Teemu Pullinen¹, Vesa Linnamo¹, Keijo Häkkinen¹ and Antti A Mero¹

Abstract

Objective: The purpose of this study was to investigate oxygen uptake, acid-base balance and energy system contributions during and after short maximal running in adult (n=8), adolescent (n=8) and child (n=8) male athletes.

Methods: The tests included a maximal time trial of 400 m, 350 m and 300 m for different age groups respectively and a VO_{2max} running test on a 200 m indoor track. Capillary blood samples to analyse pH and lactate were taken before and after the time trial. Energy system contributions were estimated using the accumulated oxygen deficit (AOD) method.

Results: Peak oxygen uptake (VO_{2peak}) during the time trial was the lowest in children (53.1 ± 4.6 ml/kg/min) compared to adolescents (59.9 ± 3.7 ml/kg/min, $P < 0.01$) and to adults (60.7 ± 2.4 ml/kg/min, $P < 0.01$). After the time trial minimal blood pH was the lowest in adults (6.97 ± 0.06) compared to adolescents (7.14 ± 0.07 , $P < 0.05$) and children (7.18 ± 0.03 , $P < 0.001$) and maximal blood lactate was the greatest in adults (17.4 ± 1.8 mmol/l) compared to adolescents (13.3 ± 3.7 mmol/l, $P < 0.05$) and children (10.2 ± 1.1 mmol/l, $P < 0.01$). The estimated anaerobic energy percentage during the time trial was the greatest in adults ($53 \pm 5\%$) compared to adolescents ($44 \pm 7\%$, $P < 0.05$) and children ($45 \pm 5\%$, $P < 0.05$).

Conclusion: The present data demonstrated that adult and adolescent male athletes achieved greater oxygen uptake than child athletes during maximal 52–54 s running and adult athletes used mainly anaerobic energy and achieved greater acidosis than adolescents and children, who used mainly aerobic energy.

Keywords

Long sprint running; 400 m run; pH; Anaerobic performance; Aerobic performance; Oxygen uptake; Acid-base balance; Energy system contributions

Introduction

Because of the fast metabolic reactions during the 400 m running race, the acid-base balance of the body is disturbed by the increase of free hydrogen ions (H^+). This increase in hydrogen ion concentration causes acidosis in the body and it is the main reason of fatigue during the 400 m race [1]. The total anaerobic energy production (anaerobic glycolysis and phosphocreatine [PCr] together) is the dominant energy system during a 400 m race and has been reported to be 57 - 65% of the total energetic needs during a race [2-4] and might be even as great as 75% in top level athletes [5]. Anaerobic energy production is dominant during the first half and the aerobic energy production during the second half of the 400 m running race [3].

Previous results have indicated that the peak oxygen uptake (VO_{2peak}) reached during a 400 m track or treadmill run is between 80 and 95% of maximal oxygen uptake (VO_{2max}) [2,3,6-8]. There has been report that oxygen uptake decreases during the last 100 m of the 400 m race [6] and the similar decrease has been reported during other maximal exhaustive running exercises realized on constant pace in treadmill running [8,9] or in field conditions [10,11]. The decrease in VO_2 (and in running velocity) could be related to acidosis-induced inhibition of oxidative phosphorylation in contracting muscles [12] and consequently, with the large decrease in ATP observed at the end of a 400 m race [13].

After the 400 m race blood lactate concentration could rise to over 20 mmol/l [14] and after the simulated 400 m race blood pH could drop to 7.00 [6] in adults. In the top class runners immediately after the 400 m run the total and free testosterone concentrations have decreased from pre-run values, whereas, in athletes with lower training level there has been an increase in the total and free testosterone concentrations [15].

VO_{2peak} is strongly correlated with body mass and this is conventionally controlled for by simply dividing VO_{2peak} (ml/min) by body mass (kg) and expressing it as the simple ratio ml/min/kg. When VO_{2peak} is expressed in this manner boys' VO_{2peak} remain remarkably consistent from 6 to 18 years [16]. However, children have been found to have lower anaerobic capacity. Maximal oxygen uptake relative to body weight is observed not to change or even to decrease, while there is a growth related increase in anaerobic performance in adolescence mainly due to hormonal and strength development [17].

Previous studies have been focused on adult participants and to the best knowledge of the present authors; this is the first study to investigate maturity-related differences in oxygen uptake and acid-base balance in maximal running performance. Thus, the main purpose of this study was to investigate the oxygen uptake response and acid-base balance in the blood during and after maximal running in child, adolescent and adult athletes. Furthermore, the aim was also to determine aerobic and anaerobic energy contributions during maximal running for the different age groups. Understanding of maturity-related differences would help to determine how to optimise training through athletic career from child to adult in 400 m running. We hypothesize that adult athletes use more anaerobic energy sources than adolescent athletes and adolescent athletes more than child athletes during the maximal time trial.

*Corresponding author: Nikke Vilmi, Department of Biology of Physical Activity, University of Jyväskylä, PL 35, 40014, Finland, Tel: +358-50-5769053; E-mail: nikke.vilmi@jyu.fi

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Methods

Participants

Twenty four male volunteers participated in the study. Based on age, blood testosterone concentration levels and the previous performances in the running events they were divided into three groups (Table 1) children (n=8; 11-13 years), adolescents (n=8; 14-16 years), and adults (n=8; 18-24 years). The adult runners were competing at a national level and their personal best in 400 m was 49.49 ± 1.84 s. Adolescent and child athletes were actively training and competing in running events. Participants gave voluntary written consent to participate in the present study, which was approved by the Jyväskylä University Ethical Committee.

Experimental protocol

The study was undertaken between the end of the competitive indoor season and the beginning of the outdoor season (May–June). Participants performed two running tests on the same 200 m indoor track, separated by at least one week. The first test was a maximal time trial, which means a simulated race performed according to the normal competition pacing strategy. The second test was a VO_{2max} running test.

In both running tests oxygen uptake (VO_2), minute ventilation (VE) and breathing frequency (BF) were recorded continuously breath-by-breath with a portable gas exchange analyser (Jager Oxygen Mobile, Viasys Healthcare, Germany) and analysed with LABmanager 5.2.01 program. Calibration of both airflow volume and gas analysers was performed according to the manufacturer's instructions before each test for each participant.

Maximal time trial

The participants were advised to rest or exercise lightly the day before the test day and have a fasting period of ten hours before the blood sampling in the morning of the test day. The test day started with taking blood samples at 8–9 AM. After that subjects ate their breakfast. Participants started warm-up one hour after the first blood sample was taken. The participants were instructed to do a similar warm-up as they were used to do normally before their competitions. Participants drank 0.5 l of water during the warm-up. The first warm-up lasted one hour and included at least one 80 – 100 m run with a competition speed at the end of the warm-up. In order to simulate real competition conditions participants finished their first warm-up phase 20-30 min before the start of time trial and participants were allowed to do 5 – 10 minutes warm-up just before it.

Participants were familiarized with the portable gas exchange analyser and breathing mask during the second warm-up phase. The adult athletes ran 400 m, the adolescent athletes 350 m and the child athletes 300 m. This experimental design was performed in order to have the same running time for each age group. Each run started

from a standing position, and the participants were instructed to use their optimal pacing strategy to run the test as fast as possible. Split times were recorded by stopwatch every 100 m with the 0.1 s accuracy. For adolescents the first split time was at 50 m from the start so every group got last three 100 m split times similarly and adults got additional fourth 100 m split time in the start of the time trial. After the run participants were allowed to sit or walk around and start cooling down 30 minutes after the time trial.

VO_{2max} running test

The participants were advised to rest or exercise lightly the day before the test day. For this test the warm-up was shorter and lasted 15-30 min. The test included three different aerobic running speeds followed by a 30 s recovery. The adults ran three times 800 m at speeds of 3.0 m/s, 3.4 m/s and 3.8 m/s, the adolescents ran three times 800 m at speeds of 2.6 m/s, 3.0 m/s and 3.4 m/s and the children ran three times 600 m at speeds of 2.2 m/s, 2.6 m/s and 3.0 m/s. Running speed was controlled by using "light pacemaker" (lights in 4 m intervals inside of the running track and turning on and off in a correct speed). After these three runs each participant continued running at the same speed as his last aerobic run and the speed was increased by 0.3 m/s every 200 m. The test ended when the participant was not able to run at the speed by the "light pacemaker". Lap times were recorded by stopwatch using the 0.1 s accuracy. Total running distance during the test varied from 2600 m to 4200 m lasting 18-22 min.

Blood samples

Blood samples were taken from both a fingertip and from an antecubital vein. During the day when time trial was performed blood samples from a fingertip were taken 8-9 AM after 10 h of fasting, before and after the warm-up, before and 3, 6, 9, 12, 15, 30 and 60 min after the time trial. Each blood sample from the fingertip contained a 20 μ l sample, which was used to analyse blood lactate concentration (La^-) using Biosen Lactate analyser (Biosen C-Sport analyser, EKF Industrie, Elektronik GmbH, Barleben, Germany) and Li-heparinized whole blood samples (200 μ l), which was used to analyse blood pH, bicarbonate concentration (HCO_3^-) and base excess (BE) (GEM Premier 3000, Instrumentation Laboratory, Lexington, MA, USA) immediately after they were taken. During the day when the time trial was performed blood samples (3.5 ml) were taken from an antecubital vein in the morning, before and 3 and 60 min after the time trial. From the antecubital vein blood samples testosterone, cortisol and sex hormone-binding globulin concentrations (SHBG) were analysed (Immulite 1000 Immunoassay System, Siemens, Germany).

During the VO_{2max} running test blood samples were taken only from the fingertip. Blood samples were taken before the test and 3, 6, 9, 12, 15 and 30 min after the test to analyse La^- , pH, HCO_3^- and BE. Blood samples were also taken after each of the three aerobic 600/800 m runs in the beginning of the test for La^- analysis.

Table 1: Subject characteristics and running performance in the maximal anaerobic time trial (300 – 400 m).

Group	Age (yr)	Height (cm)	Weight (kg)	Running time (s)	Average speed (m/s)	Mean energy cost of running (Cr) (ml/kg/min)
Children	11.9 \pm 1.4 ^{aa}	161.4 \pm 9.6 ^{aa}	47.9 \pm 7.1 ^{aa}	53.6 \pm 5.7	5.65 \pm 0.54 ^{aaa}	0.228 \pm 0.012 ^a
Adolescents	14.9 \pm 1.1 ^{cc}	179.6 \pm 2.8	63.9 \pm 4.2 ^c	53.5 \pm 2.3	6.57 \pm 0.27 ^{ccc}	0.209 \pm 0.022
Adults	21.3 \pm 3.3 ^{bbb}	182.0 \pm 6.7 ^{bbb}	73.2 \pm 7.0 ^{bbb}	52.1 \pm 2.1	7.68 \pm 0.30 ^{bbb}	0.218 \pm 0.021

Significantly different between children and adolescents; ^aP<0.05, ^{aa}P<0.01, ^{aaa}P<0.001.

Significantly different between adults and children; ^{bbb}P<0.001.

Significantly different between adolescents and adults; ^cP<0.05, ^{cc}P<0.01, ^{ccc}P<0.001.

VO_{2peak}, accumulated oxygen deficit (AOD), anaerobic energy contribution, and energy cost of running (C_r) analysis

Breath-by-breath gas exchange values were averaged with 5 s intervals to obtain VO_{2peak} and the greatest 30 s average (VO_{2end}) was also calculated from 5 s values. The allometric exponent was determined from the logarithmic equation $\text{Log } y = \text{Log } a + b \text{ Log } x$, where x is the body mass (kg), y is the VO_{2peak} (l/min), a is VO_{2peak} (l/min/kg) and b is the allometric exponent. Accumulated oxygen deficit (AOD) was calculated as a difference between the theoretical oxygen demand and oxygen uptake during the time trial [18]. The method is based on estimation of the O₂ demand by extrapolating the linear relationship between running speed and O₂ uptake at submaximal intensities [19]. Oxygen uptake was the actual value measured by a portable gas exchange analyser and the theoretical oxygen demand was calculated using submaximal oxygen uptake values from the VO_{2max} running test. Average VO₂ from the last full minute of each 600/800 m run were used to calculate individual linear relationship between running speed and oxygen uptake. This linear line was then extrapolated using running speed from the time trial and the oxygen uptake value was used as the oxygen demand. As a comparison we also calculated anaerobic energy system contribution using maximal blood lactate levels to estimate anaerobic energy used during the time trial. One mmol/l increase in lactate concentration above the resting values (values before the time trial) was assumed to be equal to 3.0 ml/kg of oxygen uptake [20]. The energy cost of running (C_r) (ml/kg/m) was calculated by dividing the theoretical oxygen demand by the running distance in the time trial.

Statistical analysis

The results were reported as mean ± SD. Statistical differences in time within groups were evaluated by paired sample t-test. Because of the small group size non-parametric independent Kruskal-Wallis test was used for evaluating statistical differences between the groups. Relationships between variables were analysed by a Pearson's correlation coefficient. All statistical analyses were conducted using SPSS software (Version 18). The level of significance was set at P<0.05.

Results

VO_{2max} running test

Submaximal values for oxygen uptake and blood lactate during the constant speed phases are presented in Table 2. The same table shows maximal values during and after increased running speed phase for oxygen uptake, running time and speed and blood pH and lactate.

Maximal time trial

Running performance and energy cost of running: Performance times, running speeds and mean energy cost of running are shown in Table 1. The performance time of the adults was 95 ± 3% of their personal best in 400 m. Split times are shown in Table 3. In all groups the running speed decreased between last three successive 100 m intervals (P<0.05). The running speed decreased from the fastest to the last 100 m interval by 12.2 ± 6.5% (P<0.01), 9.8 ± 5.1% (P<0.001), and 12.2 ± 3.1% (P<0.001) in children, adolescents and adults, respectively.

Oxygen uptake: The calculated allometric exponent was 1.00 for these participants but also theoretical exponent's 2/3 and 3/4 were used in analyses. Oxygen uptake values are presented in Table 4. VO_{2end} results were 91 ± 11%, 96 ± 7% and 96 ± 9% from VO_{2max} measured in the VO_{2max} running test in children, adolescents and adults,

respectively. VO_{2peak} results were 96 ± 11%, 103 ± 9% and 101 ± 10% from VO_{2max} measured in the VO_{2max} running test. Figure 1 displays VO₂ in 5 s intervals. In adults the decrease in VO₂ was significant from 30 – 35 s (59.7 ± 4.0 ml/kg/min) to 45 – 50 s (55.3 ± 4.4 ml/kg/min) (P<0.001). There were no significant decreases in VO₂ in other groups during the run.

Ventilation (VE) and breathing frequency (BF): Both VE and BF peaked in adults between 40 s and 45 s being 163 ± 19 l/min and 71 ± 3 breaths per minute, in adolescents between 35 s and 40 s being 139 ± 19 l/min and 66 ± 9 breaths per minute and in children between 40 s and 45 s being 102 ± 20 l/min and 67 ± 8 breaths per minute. In VE children had the lowest values from 20 s after the start to the end of the time trial compared to both adolescents (P<0.05) and adults (P<0.01). In BF there were no statistically significant differences between groups at any point of the time trial.

Anaerobic energy production: Estimated anaerobic contribution using the accumulated oxygen deficit method was the greatest in adults (53 ± 5%) compared to both adolescents (44 ± 7%, P<0.05) and children (45 ± 5%, P<0.05). For adults the estimated anaerobic energy production contribution were 75 ± 6% for the first, 55 ± 5% for the second, 40 ± 6% for the third and 41 ± 5% for the last 100 m. Using blood lactate levels to estimate anaerobic energy production contribution adults had the greater percentage (53 ± 2%) than adolescents (45 ± 7%, P<0.05) and children (39 ± 4%, P<0.01)

Blood lactate (La⁻), pH, bicarbonate (HCO₃⁻), and base excess (BE): After the time trial maximal blood lactate was the greatest in adults (17.4 ± 1.8 mmol/l) compared to adolescents (13.3 ± 3.7 mmol/l, P<0.05) and children (10.2 ± 1.1 mmol/l, P<0.01) and minimal blood pH was the lowest in adults (6.97 ± 0.06) compared to adolescents (7.14 ± 0.07, P<0.05) and children (7.18 ± 0.03, P<0.001). La⁻ recovered back to pre-values at one hour after the time trial in adolescents (from 2.0 ± 1.2 to 2.1 ± 0.7 mmol/l, P>0.05) and children (from 2.0 ± 0.8 to 1.8 ± 0.5 mmol/l, P>0.05), but not in adults (from 1.8 ± 0.4 to 2.4 ± 0.4 mmol/l, P<0.05) (Figure 2a). Blood pH recovered back to pre-values at half an hour after the time trial in children (from 7.40 ± 0.02 to 7.40 ± 0.02, P>0.05), one hour after the time trial in adolescents (from 7.41 ± 0.01 to 7.40 ± 0.02, P>0.05), but not in adults (from 7.41 ± 0.02 to 7.39 ± 0.02, P<0.01) (Figure 2b). HCO₃⁻ recovered back to pre-values at one hour after the time trial in adolescents and children (P>0.05), but not in adults (Table 5). BE recovered back to pre-values at one hour after the time trial in adolescents (P>0.05) but not in children and adults.

Blood testosterone, cortisol, sex-hormone binding globulin and testosterone/cortisol –ratio: The blood testosterone concentration was smaller in children than in adolescents (P<0.01) and adults (P<0.001). Other hormonal results and significant differences are presented in Table 6.

Relationships between running performance and oxygen uptake and metabolic variables. Table 7 shows that running performance as average running speed values was positively correlated with oxygen uptake and La⁻ and negatively correlated with pH, HCO₃⁻ and BE measured after the time trial.

Discussion

Main findings

Adult and adolescent male athletes achieved greater oxygen uptake (ml/kg/min) during 52-54 s sprint running than child athletes. Adult male athletes achieved greater disturbance in their acid-base variables after the sprint running than adolescent and child

Table 2: Mean ± SD values for blood lactate (La), oxygen uptake (VO₂), blood pH (pH), time for increase running speed part of the test (Time) and running speed of the last 200 m of the test (Speed) during VO_{2max} running test session.

Running speed		Children	Adolescents	Adults
2.2 m/s	La ⁻ (mmol/l)	2.0 ± 0.5		
	VO ₂ (ml/kg/min)	35.9 ± 2.3		
2.6 m/s	La ⁻ (mmol/l)	2.4 ± 1.1	2.3 ± 0.8	
	VO ₂ (ml/kg/min)	40.8 ± 2.4 ^a	36.8 ± 3.3	
3.0 m/s	La ⁻ (mmol/l)	3.1 ± 1.1	2.7 ± 1.2	2.3 ± 0.3
	VO ₂ (ml/kg/min)	45.6 ± 2.3 ^{aa}	41.2 ± 3.0	40.6 ± 2.3 ^{bb}
3.4 m/s	La ⁻ (mmol/l)		3.5 ± 1.5	2.2 ± 0.8
	VO ₂ (ml/kg/min)		46.0 ± 2.4	46.1 ± 3.0
3.8 m/s	La ⁻ (mmol/l)			3.7 ± 0.9
	VO ₂ (ml/kg/min)			50.8 ± 3.0
Increased running speed until exhaustion	La ⁻ (mmol/l)	7.3 ± 2.0 ^a	10.3 ± 2.7	12.4 ± 1.8 ^{bb}
	pH	7.29 ± 0.05	7.23 ± 0.08	7.15 ± 0.06 ^{bb}
	VO _{2max} (ml/kg/min)	55.6 ± 5.1	58.7 ± 6.3	60.4 ± 6.0 ^b
	Time (s)	329 ± 63	349 ± 53	310 ± 29
	Speed (m/s)	4.36 ± 0.35 ^{aa}	5.11 ± 0.42 ^c	5.59 ± 0.44 ^{bb}

Significantly different between children and adolescents; ^aP<0.05, ^{aa}P<0.01.

Significantly different between adults and children; ^bP<0.05, ^{bb}P<0.01.

Significantly different between adolescents and adults; ^cP<0.05.

Table 3: Split times in the maximal time trial (300 - 400 m).

Group	400 – 300 m from the finish line (s)	300 – 200 m from the finish line (s)	200 – 100 m from the finish line (s)	100 – 0 m from the finish line (s)
Children		16.7 ± 1.6 ^{aa}	18.0 ± 12.3 ^{aa}	18.9 ± 2.1 ^{aa}
Adolescents		14.4 ± 0.7 ^{cc}	15.4 ± 0.9 ^{cc}	16.0 ± 0.9 ^{cc}
Adults	12.6 ± 0.4	12.2 ± 0.5 ^{bb}	13.4 ± 0.7 ^{bb}	13.9 ± 0.9 ^{bb}

Significantly different between children and adolescents; ^{aa}P<0.01.

Significantly different between adults and children; ^{bb}P<0.01.

Significantly different between adolescents and adults; ^{cc}P<0.01.

Table 4: Oxygen uptake during the maximal time trial.

Group	VO _{2end} (ml · min ⁻¹ · kg ⁻¹)	VO _{2peak} (l · min ⁻¹)	VO _{2peak} (ml · min ⁻¹ · kg ⁻¹)	VO _{2peak} (ml · min ⁻¹ · kg ^{-2/3})	VO _{2peak} (ml · min ⁻¹ · kg ^{-3/4})
Children	50.3 ± 3.9 ^a	2.6 ± 0.5 ^{aa}	53.1 ± 4.5 ^{aa}	192.7 ± 23.2 ^{aa}	139.5 ± 15.5 ^{aa}
Adolescents	55.8 ± 4.7	3.8 ± 0.4 ^c	59.9 ± 4.1	239.4 ± 18.2 ^c	169.3 ± 12.5 ^c
Adults	57.7 ± 3.0 ^{bb}	4.4 ± 0.4 ^{bb}	60.7 ± 2.4 ^{bb}	252.6 ± 12.1 ^{bb}	176.9 ± 7.7 ^{bb}

Significantly different between children and adolescents; ^aP<0.05, ^{aa}P<0.01.

Significantly different between adults and children; ^{bb}P<0.01.

Significantly different between adolescents and adults; ^cP<0.05.

athletes. Furthermore, in adult male athletes the role of anaerobic energy production was greater than in children and adolescents and, therefore, they also achieved greater acidosis.

Running speed

Each of the three age groups experienced significant fatigue during the time trial. This was observed through the consistent slowdown of running speed after the acceleration phase. Deceleration of 12.2% observed in adults was slightly lower than 15.5% that was reported in a study where 100 m segmentation was used for split timing in experimental runs on an indoor track at the end of the competitive season [13]. On the other hand, 12.2% is exactly the same as in Michael Johnson's World Record run. In an outdoor racing situation the decrease in running speed has been analysed for 400 m distance using 50 m intervals, which showed deceleration of 13.9, 14.4, and 23.0% for the regional, national, and world-class athletes, respectively [1]. However, it should be noticed that shortening of the measurement segment tends to increase the variation in the estimates of the average running speed, which, thereby, leads to a

greater difference between the most extreme values of running speed. Hence, the changes in running speed observed in this study indicate that the participants in each age group were able to use the aggressive pacing strategy that is characteristics to the competitive runs in speed endurance events (like 400 m running), and, hence, induced fatigue.

Oxygen uptake

Difference in oxygen uptake was greater between children and adolescent than between adolescent and adults. One explanatory factor is that differences in ventilation were also greater between children and adolescents than between adolescents and adults while there were no differences in breathing frequency between any groups. Results indicate that the main energy system increase in anaerobic running performance before and during puberty comes from the increase in aerobic metabolism.

In the VO_{2max} running test maximal oxygen uptake was close to VO_{2max} measured in elite level 400 m runners [15]. The present study indicated that during 400 m simulated race well-trained adult

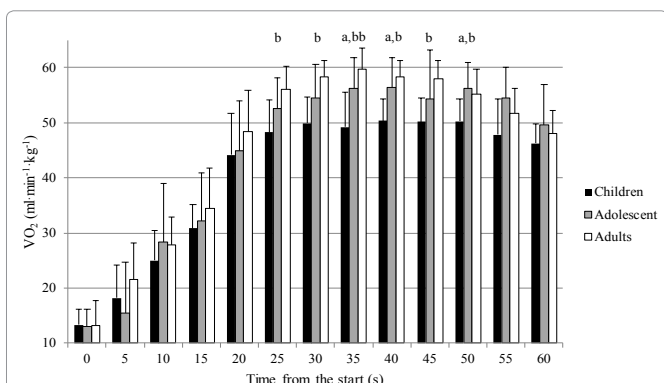


Figure 1: Oxygen uptake during the time trial. Significantly different between children and adolescents; ^a P < 0.05. Significantly different between children and adults; ^b P < 0.05, ^{bb} P < 0.01.

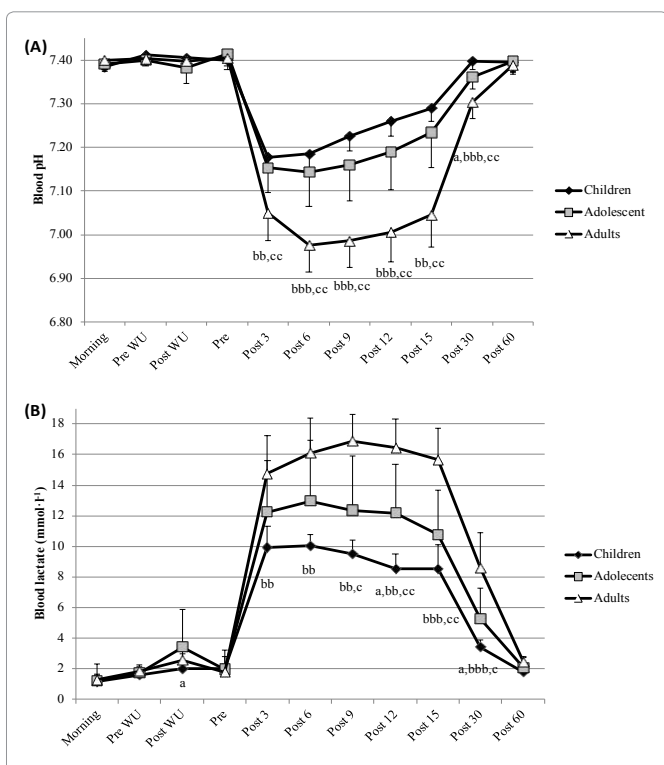


Figure 2(A) Blood pH and **(B)** blood lactate during the time trial. Significantly different between children and adolescents; ^a P < 0.05. Significantly different between children and adults; ^{bb} P < 0.01, ^{bbb} P < 0.001. Significantly different between adolescents and adults; ^c P < 0.05, ^{cc} P < 0.01. Pre WU=before warm-up, Post WU=after warm-up, Pre=before time trial, Post n=n minutes after time trial.

male athletes were able to reach high peak oxygen uptake before the decrease in VO_2 . This VO_{2peak} value during a 400 m race was greater than observed in previous field studies where participants reached 82% [2], 89% [3] and 94% [6]. Previous studies have indicated that VO_2 during a short maximal sprint can be increased by employing an all-out start [21-23], or a competition-start strategy [11,24]. This may be attributable to the greater PCr breakdown at the onset of exercise when using a very fast start procedure [13]. Greater relative VO_{2peak} with respect to values observed in some previous 400 m studies may also be due to the differences in the VO_2 sampling window.

For example, previous authors [2,3] aiming to determine the energy system contributions used longer sampling windows than was used in the present study and in the study by Hanon et al. [6]. In comparison, VO_{2end} in adults during the time trial using 30 s averages was 96% from VO_{2max} measured in the VO_{2max} running test. As the velocity and, therefore, the O_2 uptake are never in a steady state during a 400 m running race, the use of a large sampling window will tend to smooth and decrease peak values.

In the current study adults and adolescents were able to reach VO_{2peak} that was over 100% of their VO_{2max} measured in VO_{2max} running test (101 and 103% respectively). The main reason was that we did not analyse 5 s peaks in the VO_{2max} running test. Another reason might be that they did not reach their true VO_{2max} during the VO_{2max} running test and reasons for that might be an inexperience and unpleasantness of the breathing mask in the longer running test. In the present study, a VO_2 decrease was observed in adults during the final 100 m, confirming previous results obtained during 400 m running on track [6] and on treadmill [8]. The decreases in VO_2 and in velocity could be related to acidosis-induced inhibition of oxidative phosphorylation in contracting muscle [12] and consequently, with the large decrease in ATP and PCr observed at the end of a 400 m race [13].

Accumulated oxygen deficit (AOD)

In the current study the anaerobic percentage was slightly smaller to that observed in other recent studies in track running, where percentages were 59% [2] and 63% [4]. The same has been observed on treadmill running, where percentage was 57% [3]. One factor behind the differences is measured oxygen uptake, which was greater in our study than any other published study. Failures to measure real oxygen uptake throughout the run with short enough sampling window could overestimate the anaerobic energy contribution percentage, when using the AOD method. Since the AOD method is defined as the difference between the accumulated oxygen demand and the accumulated oxygen uptake, an error in the measured oxygen uptake will also affect the calculation of AOD [19]. On the other hand, the quantification of anaerobic energy release using the AOD method might be underestimated during very short, intense exercise, where average power outputs are well above maximal aerobic power, as the efficiency relationship used to predict energy demand may not remain linear [25]. Furthermore, errors in calculating the linear curve between running speed and oxygen demand in aerobic running speeds can affect the final results. In the current study there remains a question of whether the third aerobic running speed was too fast, when determining the relationship between running speed and oxygen demand. If anaerobic energy production was involved more than during previous running speeds, it might cause underestimation in anaerobic energy contribution percentage in the final results. In comparison, using blood lactate levels to estimate anaerobic energy production contribution, in the current study adults had the same anaerobic percentage as with the AOD method. It has also been speculated that faster runners have greater total energy cost in the 400 m sprint and that it comes mainly from the anaerobic part of the energy contributions and, therefore, they also have greater anaerobic percentage compared to slower runners in the 400 m sprint [26].

Acid-base variables

In previous studies in adults, the post-fatigue levels of blood lactate concentration (from 13.5 to 22.0 mmol/l) [2,4,6,8, 27-34], blood pH (from 7.12 to 7.00) [6,27,29], blood bicarbonate concentrations (from

Table 5: Mean ± SD values for blood bicarbonate (HCO₃⁻) and base excess (BE) during the maximal anaerobic time trial.

	HCO ₃ ⁻ (mmol/l)			BE (mEq/l)		
	Children	Adolescents	Adults	Children	Adolescents	Adults
Morning	24.4 ± 1.1	25.6 ± 1.1	25.8 ± 1.8	-0.4 ± 1.7	1.3 ± 1.7	1.5 ± 2.6
Pre WU	25.6 ± 1.3	26.1 ± 1.0	26.3 ± 0.9	1.0 ± 1.9	2.1 ± 1.4	2.2 ± 1.4
Post WU	24.7 ± 1.7	23.5 ± 2.5	25.2 ± 1.9	-0.3 ± 2.7	-1.8 ± 3.6	0.6 ± 3.0
Pre	25.1 ± 1.6	25.9 ± 1.1	26.5 ± 1.5	0.4 ± 2.4	1.6 ± 1.6	2.6 ± 2.1
Post 3	14.1 ± 1.5	13.6 ± 2.9 ^c	9.9 ± 3.1 ^b	-14.3 ± 1.5	-15.0 ± 4.0 ^c	-19.6 ± 4.3 ^b
Post 6	13.7 ± 1.3	12.2 ± 3.1 ^{cc}	7.1 ± 2.5 ^{bb}	-15.3 ± 1.9	-17.1 ± 4.0 ^{cc}	-23.2 ± 3.3 ^{bb}
Post 9	14.6 ± 1.2	12.7 ± 3.4 ^{cc}	6.7 ± 2.0 ^{bb}	-14.3 ± 1.5	-16.6 ± 4.4 ^{cc}	-24.0 ± 2.5 ^{bb}
Post 12	16.0 ± 1.3	13.8 ± 3.6 ^{cc}	7.2 ± 2.3 ^{bb}	-12.5 ± 1.8	-15.1 ± 4.7 ^{cc}	-23.3 ± 2.9 ^{bb}
Post 15	17.4 ± 1.6	15.4 ± 3.8 ^{cc}	8.4 ± 2.6 ^{bb}	-10.6 ± 2.2	-13.2 ± 5.2 ^{cc}	-22.1 ± 3.3 ^{bb}
Post 30	23.1 ± 1.7	21.9 ± 2.0 ^c	18.1 ± 2.5 ^{bb}	-2.7 ± 2.4	-4.2 ± 2.9 ^c	-9.7 ± 3.6 ^{bb}
Post 60	24.2 ± 1.2	25.1 ± 1.3	24.9 ± 1.5	-1.0 ± 1.7	0.4 ± 1.9	0.2 ± 2.2
Max	13.6 ± 1.4	12.2 ± 3.2 ^{cc}	6.6 ± 2.0 ^{bb}	-15.4 ± 1.9	-17.2 ± 4.1 ^{cc}	-24.2 ± 2.5 ^{bb}
Time (min)	4 ± 2 ^a	6 ± 1	7 ± 2 ^b	5 ± 2	7 ± 1	9 ± 3 ^b

Pre WU=before warm-up, Post WU=after warm-up, Pre=before time trial, Post n=n minutes after time trial, Max=maximal values at any time point, Time=time after time trial to reach individual maximal value.

Significantly different between children and adolescents; ^aP<0.05, ^{aa}P<0.01.

Significantly different between adults and children; ^bP<0.05, ^{bb}P<0.01.

Significantly different between adolescents and adults; ^cP<0.05.

Table 6: Mean ± SD values for blood testosterone, cortisol, sex-hormone binding globulin (SHBG) and testosterone-cortisol –ratio (T/C) during maximal anaerobic time trial.

		Testosterone (nmol/l)	Cortisol (nmol/l)	SHBG (nmol/l)	T/C
Morning	Children	5.1 ± 5.1 ^{aa}	404 ± 103 ^a	55.2 ± 21.6	13.2 ± 13.2 ^{aa}
	Adolescents	18.7 ± 4.4	500 ± 97	38.7 ± 12.5	38.8 ± 13.3
	Adults	17.0 ± 3.2 ^{bbb}	526 ± 72 ^b	33.4 ± 8.0 ^b	33.2 ± 6.6 ^{bb}
Pre	Children	3.1 ± 3.5 ^{aa}	243 ± 112	58.9 ± 22.6 ^b	16.8 ± 18.4 ^{aa}
	Adolescents	15.0 ± 4.1	276 ± 56 ^{cc}	37.4 ± 8.9	57.9 ± 26.4 ^c
	Adults	13.6 ± 3.3 ^{bb}	466 ± 139 ^{bb}	32.2 ± 8.5	32.2 ± 15.5 ^{bb}
Post 3	Children	2.8 ± 3.1 ^{aa}	346 ± 161	63.0 ± 24.7	12.6 ± 15.0 ^{aa}
	Adolescents	14.9 ± 3.5	289 ± 57 ^{cc}	41.1 ± 10.9	54.8 ± 21.0 ^{cc}
	Adults	16.0 ± 3.6 ^{bbb}	581 ± 137 ^{bb}	38.4 ± 9.4	29.6 ± 11.7 ^{bb}
Post 60	Children	2.2 ± 2.8 ^{aa}	312 ± 98	58.7 ± 22.4 ^a	11.2 ± 16.6 ^a
	Adolescents	11.3 ± 3.6	289 ± 74 ^{cc}	35.8 ± 8.7	41.2 ± 14.6 ^c
	Adults	12.6 ± 4.2 ^{bb}	535 ± 117 ^{bb}	33.0 ± 9.7 ^b	24.7 ± 9.5 ^{bb}

Pre=before time trial, Post n=n minutes after time trial.

Significantly different between children and adolescents; ^aP<0.05, ^{aa}P<0.01.

Significantly different between adults and children; ^bP<0.05, ^{bb}P<0.01, ^{bbb}P<0.001.

Significantly different between adolescents and adults; ^cP<0.05, ^{cc}P<0.01.

4.9 to 9.7 mmol/l) [6,27,29] and base excess values (-22,4 mEq/l) [27] have been comparable with the present study, which also indicate that the runners were able to perform the test run in an all-out manner. Following a 400 m race of 45.5 s, an international-calibre runner had a lactate concentration of 25.0 mmol/l with a pH of 6.92 [35]. The greatest single values in the current study were in blood lactate concentration of 20.2 mmol/l and in pH of 6.87. The smaller accumulation and earlier peaking of blood lactate and pH in children and adolescents compared to adults can be explained by several maturity-related factors, such as lower glycolytic enzyme activity [36] and faster elimination of glycolytic by-products in children [37]. The large anaerobic contribution and subsequent accumulation of metabolites especially hydrogen ions (low pH) [27] may contribute to the decrease in velocity observed during the final 100 m [1,6].

The differences in main acid-base balance variables (La⁻, pH, HCO₃⁻ and BE) were greater between adults and adolescents than children and adolescents. This indicates that the main energy system

increase in anaerobic running performance after puberty comes from the increase in anaerobic metabolism. The post exercise blood lactate concentration in children and adolescents in this current study were similar to the results previously found after a 30 s Wingate test [37]. It has been reported that changes in blood lactate and acid-base status are smaller and faster in children than in adolescents and that the differences in the extreme values seem to be smaller than those observed at given time points [38]. The difference in the kinetics of the blood lactate concentration between children, adolescents and adults appears to reflect a lower extravascular increase in lactate generated by the maximal short-term exercise combined with faster elimination of the blood lactate concentration in children. The extravascular increase of lactate seems to have a maximum during the third decade of life [37].

Alactic component

It should be noted that in this study and in most of the other

Table 7: Pearson correlation coefficients (r) between average running speed and oxygen uptake (VO_{2peak} and VO_{2end}) and metabolic parameters (La^- , pH, HCO_3^- and BE) as greatest values measured after the time trial.

	VO_{2peak}	VO_{2end}	pH	La^-	HCO_3^-	BE
All groups	0.70 ***	0.70 ***	-0.87 ***	0.87 ***	-0.83 ***	-0.82 ***
Children	0.61	0.62	-0.45	0.73 *	-0.18	-0.10
Adolescents	0.49	0.51	-0.81 *	0.95 ***	-0.91 **	-0.92 **
Adults	0.31	0.29	-0.66	0.53	-0.85 *	-0.87 *

* P<0.05, ** P<0.01, *** P<0.001

studies anaerobic energy production is not divided to lactic and alactic components. The breakdown of the alactic component, PCr, is estimated to be 12.5% of the total energy used during a 400 m runs [5]. The latter researchers evaluated that in world elite level 400 m running the total ATP is produced as follows: PCr 12.5%, anaerobic glycolysis 62.5% and oxygen uptake 25%. It has also been speculated that faster runners have greater total energy cost in the 400 m sprint and that it comes mainly from the anaerobic part of the energy contributions and, therefore, they also have greater anaerobic percentage compared to slower runners in the 400 m sprint [26]. We could speculate that Newsholme's values might be true in elite level male 400 m runners (43-45 s), but in the slower 400 m runners (around 50 s) there is more variation. Some runners may have high anaerobic and some others may have high aerobic energy production. In average the total ATP might be produced as follows: PCr 10%, anaerobic glycolysis 50% and oxygen uptake 40%. But in every case, the training background affects the energy contributions.

Amount of work

Even though the time used for the time trial was similar in all three groups, the amount of work varied because of the different running distance and running speed for each group. Also Cr differed between children and adolescents which is probably due the fact that children usually don't have as good running technique as more experienced runners. The weaker anaerobic performance of children has been related to lower glycolytic enzymes concentration and activity, lower glycogen concentration, probably associated to the reported lower post exercise lactate concentration after exhaustive exercise [39,40]. In addition, performance can be divided as discussed into metabolic energy production, but also to neuromuscular contribution. In the latter, there are included the force produced by the muscles including energy stored in elastic components during stretch-shortening cycle. Improved temporal sequencing of muscle activation, especially improved fast twitch fibre recruitment and increased speed of impulse transmission along the motor axon may contribute to greater sprint performance [41]. It is complicated to calculate and compare these contributions, but they are both very important for running performance together with running economy (running technique).

Hormone concentrations

In adults the testosterone response was similar as reported earlier by Slowinska-Lisowska and Majda [15] in a similar group of 400 m athletes. They were able to run 400 m in 48.6 s in the study and, therefore, it is the closest point of reference to the adult group in the current study. Both studies demonstrated acute increases in testosterone concentration immediately after the 400 m run. On the other hand, in the study by Slowinska-Lisowska and Majda [15] testosterone response in a group of elite of 400 m runners (400 m 45.9 s in the study) was opposite. The authors speculated that the observed hormonal changes in the master class athletes induced by the years-long anaerobic training might provide evidence for the reduction of functional reserves of the gonads, when compared to the

group of less trained sportsmen, in whom the endocrine response was quite opposite. In adolescents, there were no similar responses and in children concentrations are so small that it is difficult to present any conclusions about them. The greatest individual testosterone concentration in children was lower than the lowest individual testosterone concentration in adolescents and, therefore, we can conclude that there was a clear difference in maturity between these two groups in the current study.

For cortisol responses there are no data available in 400 m run before this current study, but the acute responses were similar to those reported earlier by Zeinali et al. [42] after repeated running with increasing time (3 times 15 s, 3 times 20 s, once 30 s and once 40 s with maximal effort) in sprint runners. Both studies show acute increases in cortisol concentration immediately after exercise in adults. Again, in adolescents there were no similar strong responses (only very small increase), but in children responses were similar to adults.

Practical applications

In practise, based on the results of present study it can be suggested that coaches coaching pre-pubertal children should focus on skill training to improve their running speed and technique, and thus, their running economy and basic endurance training to improve their oxygen uptake. Later on, in and after puberty, the focus of training energy systems should transfer more to improve anaerobic energy production, although the overall main focus is in speed and strength training. In coaching, it should be remembered that the 400 m running performance is a combination of speed, skill, strength, speed endurance, endurance and psychological factors. However, energy system contributions could vary greatly between persons with different age, gender, training background, genetics and performance level.

One limitation of the present research was small sample size. Additionally the participants were not competing at the same level as adults were at national level and adolescents and children were not. Also the training programs are not the same (quantity and quality) between the age groups.

Conclusion

These data demonstrate that in the time trial lasting 52-54 s anaerobic energy contribution and also the acidosis are greater in adults than in adolescents or in children. The difference in running performance between pre-pubertal and pubertal boys comes probably from higher running speed and better running economy of the pubertal boys and their ability to use more oxygen for energy production. The difference in running performance between pubertal boys and adult men comes mainly from higher running speed and greater usage of anaerobic energy production in adults.

The present data demonstrated that adult and adolescent male athletes achieve greater oxygen uptake than child athletes during maximal 52-54 s running and adult athletes used mainly anaerobic

energy and achieved greater acidosis than adolescents and children, who used mainly aerobic energy.

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Author Affiliation

Top

¹Department of Biology of Physical Activity, University of Jyväskylä, Jyväskylä, Finland

²Research Institute for Olympic Sports, Jyväskylä, Finland