OXYGEN UPTAKE, ACID-BASE BALANCE AND HORMONAL RESPONSES IN MAXIMAL 300 – 400 M RUNNING IN CHILD, ADOLESCENT AND ADULT ATHLETES

Nikke Vilmi

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Department of Biology of Physical Activity
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Supervisors: Antti Mero and Keijo Häkkinen

ABSTRACT

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The 400 m sprint demands both aerobic and anaerobic energy systems. During the race the acid-base balance of the body is disturbed and that has effects on the oxygen uptake. The purpose of this study was to investigate the oxygen uptake and acid-base balance during and after the maximal anaerobic running test in child (300 m), adolescent (350 m) and adult (400 m) athletes. Furthermore, the aim was also to determine aerobic and anaerobic energy contributions during the test for the different age groups.

Eight adult male 400 m runners (age 21 ± 2 years), eight male adolescent athletes (age 15 ± 1 years) and eight male child athletes (age 13 ± 1 years) volunteered for the study. Participants performed two running tests on the same 200 m indoor track. The first test was a maximal running test (MRT) and the second test was a VO_{2max} running test. In both running tests oxygen uptake (VO_2) was recorded with a portable gas exchange analyser. In MRT adult athletes ran 400 m, adolescent athletes 350 m and child athletes 300 m. VO_{2max} running test included three different aerobic running speeds. Adults and adolescents ran three times 800 m and children ran three times 600 m. After these three runs the speed was increased by 0.3 m/s every 200 m until exhaustion. Blood samples to analyse lactate (La^-) and pH were taken in the morning, before and after warm-up, just before and 3, 6, 9, 12, 15, 30 and 60 minutes after MRT and before and 3, 6, 9, 12, 15 and 30 minutes after VO_{2max} running test. Countermovement jumps (CMJ) were performed before and after MRT. Energy system contributions were estimated using accumulated oxygen deficit (AOD) method.

Maximal oxygen uptake (VO_{2max}) as the greatest 30 s average during the VO_{2max} running test was 60.4 ± 6.0 ml/kg/min for adults, 58.7 ± 6.3 ml/kg/min for adolescents and 55.6 ± 5.1 ml/kg/min for children. Performance times in MRT were 52.1 ± 2.1 s in adults, 53.3 ± 2.3 s in adolescents and 53.6 ± 5.7 s in children. In CMJ the jumping height decreased after MRT in every group, but significantly only in adolescents ($P < 0.01$). VO_{2max} during MRT using 30 s averages was 50.3 ± 3.9 ml/kg/min for children, 55.8 ± 4.7 ml/kg/min for adolescents and 57.7 ± 3.0 for adults. Difference in oxygen uptake between children and adults was significant ($P < 0.05$). These results were 91 ± 11 %, 96 ± 7 % and 96 ± 9 % from VO_{2max} measured in VO_{2max} running test. Peak oxygen uptake (VO_{2peak}) during MRT using 5 s averages was significantly 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, $P < 0.01$) during MRT. After MRT La^- was 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, $P < 0.01$). Blood pH was lowest in adults (6.97 ± 0.06) compared to adolescents (7.14 ± 0.07, $P < 0.05$) and to children (7.18 ± 0.03, $P < 0.001$). The estimated anaerobic energy percentage during MRT calculated using AOD method was greatest in adults (53 ± 5 %) compared to adolescents (44 ± 7 %, $P < 0.05$) and to children (45 ± 5 %, $P < 0.05$).

The present data demonstrated that all age groups could achieve over 90 % of their real maximal oxygen uptake during maximal anaerobic 52 - 54 s run and adult male athletes used mainly anaerobic energy and achieve greater acidosis than adolescents and children who used mainly aerobic energy. This study suggests that training for pre-pubertal children should focus on skill, speed and endurance and for post-pubertal the focus should transfer more to anaerobic training.

Keywords: Long sprint running, 400 m run, oxygen uptake, acid-base balance, hormonal responses, pH

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1 INTRODUCTION

The 400 m run is the longest sprint event in athletics (track and field). It has belonged to the athletics program at the Summer Olympics since 1896. For elite level male runners it takes about 44 s to 46 s to run 400 m. The world record is currently 43.18 s and it was made by Michael Johnson in the World Championships in Sevilla 1999.

The 400 m sprint is a unique race distance because of the need of great running speed (average speed 9.26 m/s in Johnson's world record run) and the need of endurance to keep up that running speed during the whole race. Because of these two needs, all three energy systems are important for optimal result in the 400 m race. Newsholme et al. (1992) in their classic work evaluated that in maximal 400 m running energy contribution to adenosine triphosphate (ATP) is as follows: phosphocreatine (PCr) 12.5%, anaerobic glycolysis 62.5% and aerobic energy production 25%. All three energy systems are working all the time during running, but the emphasis is different. In the beginning of the run the first energy system involves mainly the splitting of the high-energy phosphagen, phosphocreatine, which together with the stored ATP in the cell provides the immediate energy in the initial stages of 400 m race. The second energy system involves the anaerobic breakdown of muscle glycogen and the third energy system, aerobic (oxidative) metabolism, involves the combustion of mainly carbohydrates and probably little fats in the presence of oxygen. Anaerobic energy production is dominant during the first half and the aerobic energy production during the second half of the 400 m race (Spencer and Gastin 2001). Because of the fast metabolic reactions during the 400 m 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 (e.g. Hanon and Gajer 2009). The total anaerobic energy production (anaerobic glycolysis and PCr together) is the greatest energy system during a 400 m race and has been reported to be 57 - 65 % of the total energetic needs during a race (Duffield et al. 2005; Spencer and Gastin 2001; Zouhal et al. 2010) and might be even as great as 75 % in top level athletes (Newsholme et al. 1992.)

Previous results have indicated that the peak oxygen uptake reached during a 400 m running race or in 400 m treadmill running is between 80 and 95 % of VO_{2max} (Duffield et al. 2005; Hanon et al. 2010; James et al. 2007; Nummela and Rusko 1995; Spencer and Gastin 2001). There have also been reports that oxygen uptake decreases during the last 100 m of the 400 m race (Hanon et al. 2010) and the similar decrease has been reported during other maximal exhaustive running exercises realized on constant pace in treadmill running (Nummela and Rusko 1995; Perrey et al. 2002) or in field conditions (Hanon et al. 2007; Thomas et al. 2005).

After the 400 m race blood lactate concentration could rise to over 20 mmol/l (Lacour et al. 1990) and after the simulated 400 m race blood pH could drop to 7.00 (Hanon et al. 2010). 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 (Slowinska-Lisowska and Majda 2002).

Children have similar maximal aerobic capacity as compared to adults. 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. (Zwiren 1989.)

Previous studies have been focused on adult subjects and to the best knowledge of the author, this is the first study to investigate maturity-related differences in oxygen uptake and acid-base balance in maximal anaerobic running performance. Therefore, the purpose of this study was to investigate oxygen uptake, acid-base balance and hormonal responses during 400 m running in adult male athletes and to compare the results to those of adolescent and child athletes running shorter distances (300 m or 350 m) so that duration of the run was similar (about 50 s).

2 AEROBIC AND ANAEROBIC ENERGY METABOLISM IN EXERCISE

The immediate source of energy for muscle contractions comes from the hydrolysis of adenosine triphosphate (ATP). Each kilogram of skeletal muscle contains 3 to 8 mmol of ATP (McArdle 2007, 166). As ATP exists in very low concentrations in the muscle, and regulatory mechanisms appear to prevent its complete degradation, there are chemical pathways to regenerate ATP. The anaerobic pathways are capable of regenerating ATP at high rates. However, the amount of energy that can be released in a single bout of intense exercise is limited. In contrast, the aerobic system has an enormous capacity yet is somewhat hampered in its ability to deliver energy fast. (Gastin 2001.)

The anaerobic energy system can be devided into alactic and lactic components, referring to to the processes involved in the splitting of the stored phosphogens, ATP and phosphocreatine (PCr), and the nonaerobic breakdown of carbohydrate to lactic acid through glycolysis. (Gastin 2001.) When ATP concentration starts to decrease, the first process to produce more ATP involves the splitting of the high-energy phosphagen. PCr together with the stored ATP in the cell provides the immediate energy in the initial stages of intense or explosive exercise (Gastin 2001). Muscle contains 4 to 5 times more PCr than ATP (McArdle 2007, 166).

The second process involves the nonaerobic breakdown of carbohydrate, mainly in the form of muscle glycogen, to pyruvic acid and then lactic acid through glycolysis (Gastin 2001). In the anaerobic glycolysis the maximal energy transfer rate is about 45 % that of the high-energy phosphates. In a way, anaerobic glycolysis buys time. It allows ATP to form rapidly by substrate-level phosphorylation, even though outstrip the muscle's capacity to resynthesise ATP aerobically. (McArdle 2007, 166).

Aerobic energy metabolism refers to energy-generating catabolic reactions in which oxygen serves as the final electron acceptor in the respiratory chain and combines with hydrogen from water. Oxygen presence at the "end of the line" largely determines the

capacity for aerobic ATP production and sustainability of high-intensity endurance exercise. (McArdle 2007, 144.)

2.1 Energy system contributions in exercise

Aerobic energy release from the combustion of carbohydrates and fats is readily quantified, as there is a direct relationship between the oxygen uptake (VO_2) measured at the mouth and a whole-body aerobic production of ATP (Åstrand 1981). For each litre of oxygen [at standard temperature, pressure and density (STPD)] utilised in the respiratory chain, approximately 20 kJ is yielded. Determination of the metabolic respiratory quotient (RQ) to quantify the proportion of carbohydrate and fat broken down provides an exact measurement of the aerobic energy yield. (Gastin 2001.)

On the other hand, methods to quantify anaerobic energy release are less precise. As anaerobic ATP production is an intracellular process with little reliance on central processes, a universally accepted method does not exist. (Gastin 2001.)

The peak blood lactate concentration is often used as a measure for evaluation of anaerobic energy release during exercise (Jacobs 1986). Although lactate in the blood may provide an indication of the extent of glycolysis, it cannot be used to quantify muscle lactate production nor does it provide any indication of the energy derived from the stored phosphagens, ATP and PCr (Gastin 2001). The peak blood lactate concentration cannot be recommended as a basis for comparison of anaerobic capacity in different subjects (Medbø 1987).

The amount of oxygen taken up in excess of the resting value during the recovery period has been referred to as the oxygen debt (Gastin 2001). The classical oxygen hypothesis predicted that the volume of oxygen consumed after exercise was linked to the metabolism of lactate during the post-exercise recovery period (Hill and Lupton 1923). However, the use of oxygen debt as a measure of anaerobic energy release has been discredited by several authors (Hermansen 1969; Vandewalle et al. 1987; Saltin 1990).

Ergometric assessments of mechanical work are frequently used as noninvasive, indirect and performance-based measures of power and capacity of the three energy systems. Contribution of the energy systems is dependent on the intensity and duration of the work effort, such that tests generally attempt to select a duration that maximizes the contribution of one particular energy system while minimizing the contribution of the others. In practice, the energy systems are overlapped at any given time during exercise and it is impossible to measure just one energy system's power or capacity. (Gastin 2001.)

The use of the needle biopsy technique has enabled the direct measurement of the decrease in muscle ATP and PCr, as well as accumulation of metabolites like pyruvate and lactate, thereby allowing an assessment of the anaerobic energy production of the biopsied muscle. The muscle biopsy technique provides the measurement of concentrations and not amounts. Having determined changes in ATP, PCr and lactate concentrations, the total anaerobic energy release during whole-body exercise is calculated by estimating the active muscle mass involved in exercise. (Gastin 2001.) Main problems with this method are inaccurate estimation of the active muscle mass (Gastin 2001), low representativeness of the biopsy sample (Blomstrand and Ekblom 1982), and a possible underestimation of the anaerobic energy release because of changes occurring between the cessation of exercise and the attainment of the biopsy sample (Bangsbo 1998; Gastin 1994).

The concept of accumulated oxygen deficit (AOD) was first introduced by Krogh and Lindhard (1920) and has been used since as a means to determine anaerobic energy production during both sub- and supramaximal exercise. During supramaximal exercise, the appropriateness of its use relies on the validity of the assumption that supramaximal energy demand can be determined from the relationship between submaximal work intensity and oxygen consumption (Gastin 2001). 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 (Bangsbo 1998). Several investigators have reported decreasing efficiencies with increasing power output (Gaesser and Brooks 1975; Gladden and Welch 1978; Luhtanen et al. 1987). However, the linear extrapolation of submaximal work may

inherently compensate for this changing efficiency, as it is similar to the calculation of delta efficiency in that it reflects each additional increment in work (Gastin 1994; Gastin et al. 1995). In so doing, it follows changes at any point along the regression of energy release and exercise intensity (Gladden and Welch 1978). Ramsbottom et al. (1994) suggested based on their results that the AOD is a unique and reproducible physiological characteristic, which is strongly correlated with sprint capacity. Since the AOD 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 the accumulated oxygen deficit (Medbø et al. 1988).

To summarize the various methods estimating anaerobic energy metabolism during exercise we can say that from the methods listed above the muscle biopsy technique and the accumulated oxygen deficit method provide the best possible insights into anaerobic energy production during intense exercise (Gastin 2001).

Gastin (2001) collected data from over 30 studies and based on those studies he has estimated energy contributions on different durations of intense exercises lasting from ten seconds to four minutes (Table 1). Earlier Newsholme et al. (1992) collected data from various studies and did estimations of the percentages of the contributions of different fuels to ATP generation in various running events (Table 2.)

TABLE 1. Estimates of anaerobic and aerobic energy contribution during selected periods of maximal exercise (Gastin 2001).

Duration of exhaustive exercise (s)	Anaerobic %	Aerobic %
0 -10	94	6
0 -15	88	12
0 - 20	82	18
0 - 30	73	27
0 - 45	63	37
0 - 60	55	45
0 - 75	49	51
0 - 90	44	56
0 - 120	37	63
0 - 180	27	73
0 - 240	21	79

TABLE 2. Estimate of the percent contribution of different fuels to ATP generation in various running events (Newsholme et al. 1992).

	Percent contribution to ATP generation				
	Phospho-	Glycogen		Blood	Triacyl-
Event	creatine	Anaerobic	Aerobic	glucose	glycerol
100 M	50	50	-	-	-
200 M	25	65	10	-	-
400 M	12.5	62.5	25	-	-
800 M	6	50	44	-	-
1500 M	[a]	25	75	-	-
5000 M	[a]	12.5	87.5	-	-
10 000 M	[a]	3	87	-	-
Marathon	-	-	75	5	20
80 KM	-	-	35	5	60
24 H Race	-	-	10	2	88

[a] In such events phosphocreatine is used for the first few seconds and, if it has been resynthesized during the race, in the sprint to the finish.

Not just the duration of intense exercise but also the training background of the athlete can affect energy contributions. Gastin and Lawson (1994) tested sprint trained and endurance trained cyclists in 90 s of all-out cycle exercise (Figure 1). They reported that the sprint trained group had significantly greater peak power output. Although the final VO_2 during the test and the separately measured VO_{2max} in the sprint group were lower than in the endurance group, VO_2 kinetics were faster in the sprint trained athletes. In both groups final power output is almost directly attributable to the rate of aerobic energy supply, providing clear support for the existence of an anaerobic capacity. In a study by Nummela and Rusko (1995) it was also reported that sprint trained subjects had greater anaerobic contribution, oxygen deficit, running speed, peak lactate and oxygen demand but lower oxygen uptake compared to endurance trained athletes in 50 s supramaximal run on treadmill. The difference in energy production between the sprint and endurance trained athletes occurred only during the second half of the supramaximal exercise when sprinters used more the anaerobic and endurance athletes aerobic pathways for energy production (Nummela and Rusko 1995).

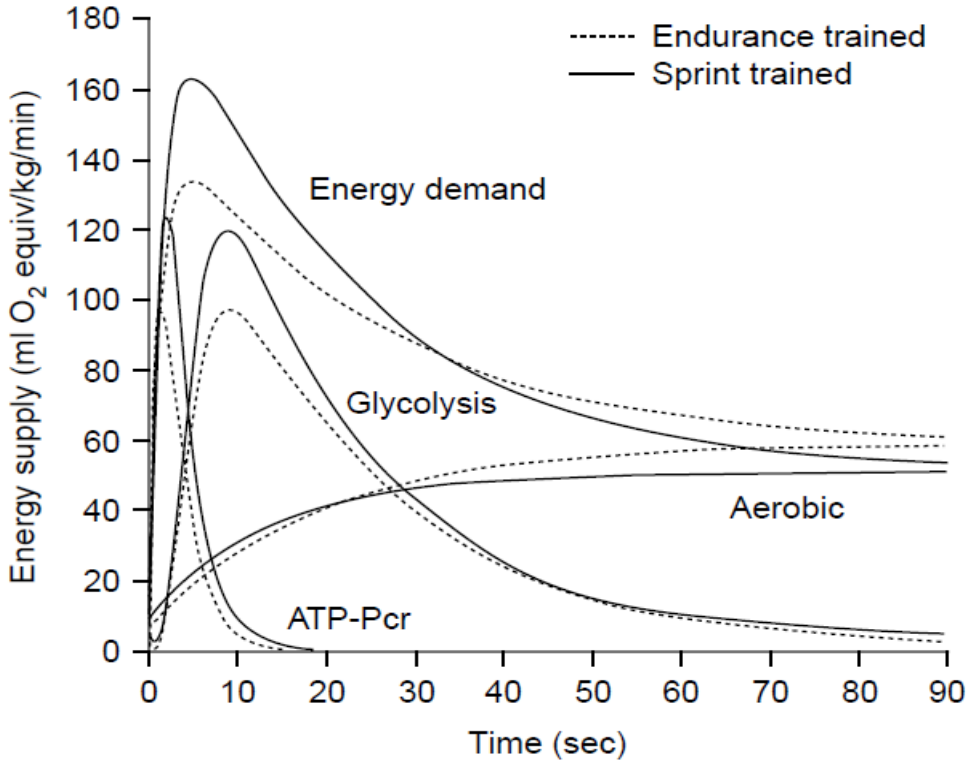

FIGURE 1. Relative contribution of the three energy systems to the total energy supply during 90 seconds of all-out cycle exercise. Participants were 6 male sprint-trained cyclists (mean VO_{2max} 58 ml/kg/min) and 8 endurance-trained triathletes (mean VO_{2max} 65 ml/kg/min). Data from Gastin and Lawson (1994).

2.2 Energy metabolism in children

Absolute aerobic capacity as well as absolute and relative anaerobic capacities have been observed to increase with growth. Aerobic capacity, however, when expressed relative to body weight, remains the same or even decreases with age. Limited evidence suggests that training during prepubescence does not increase maximal oxygen uptake values beyond that attributed to growth. However, increases in anaerobic metabolic systems and in Wingate Anaerobic Test (WAT) scores with training, beyond that expected from growth, have been observed. (Zwiren 1989.)

Children are characterized by a weaker anaerobic capacity and similar aerobic fitness compared with adults (Rowland 2007). Decreased 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 (Berthoin et al. 2003; Eriksson et al. 1971). On the contrary, children are characterized by better muscle oxidative metabolism, represented by faster oxygen uptake at the onset of exercise (Fawkner and Armstrong 2003). There is an almost linear increase in boys' VO_{2max} (l/min) in relation to chronological age between ages 8 and 16 (Armstrong et al. 2011).

The blood lactate concentration after maximal short-term, high-intensity exercise has been frequently described to be lower in children than adolescent and adults. However, such cases should be considered with care because the blood lactate concentration does not reflect only an increase in lactate in the muscle compartment but also a number of other processes modulating the transport of lactate into and its elimination out of the blood compartment. In a study made by Beneke et al. (2005) they compared blood lactate kinetics at 30 s Wingate test in children (12.0 ± 0.6 years), adolescent (16.3 ± 0.7 years) and adults (27.2 ± 4.5 years). Maximal blood lactate concentration and corresponding time were lower ($P < 0.05$) in children (10.2 ± 1.3 mmol/l and 4.1 ± 0.4 min) than in adolescent (12.7 ± 1.0 mmol/l and 5.5 ± 0.7 min) and adults (13.7 ± 1.4 mmol/l and 5.7 ± 1.1 min). The difference in the kinetics of the blood lactate concentration between children, adolescent 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. The extravascular increase of lactate seems to have a maximum during the third decade of life. Nevertheless, the latter does not support the hypothesis of a reduced muscular glycolytic rate, but it is consistent with lower relative muscle mass, greater relative total lactate water space and favourable conditions for the aerobic metabolic system in children than adolescent and adults, respectively.

In a study made by Mero (1988) he investigated blood lactate production in anaerobic exercise in 19 trained and 6 untrained prepubescent boys. Peak blood lactate concentration after 60 s maximal bicycle ergometer test was 13.1 ± 2.6 mmol/l in the trained group and 12.8 ± 2.3 mmol/l in the untrained group. Following the 15 s maximal bicycle ergometer test blood lactate concentration in all subjects was 60.6 % of the values measured after 60 s test.

3 ACID-BASE BALANCE IN HUMAN BODY

Acid-base balance means the condition of the body where hydrogen ions' intake and production are in balance with the hydrogen ion removal. Normally in the human body there is a balance between acids and bases. Acid is a substance that can emit a hydrogen ion (H^+). Base, on the other hand, is a substance that can receive a hydrogen ion. Hydrogen ion is a single free proton that is left when a hydrogen atom has emitted its only electron.

The concentration of free hydrogen ions determines how acidic the solution is. Because hydrogen ion concentration is normally low, and changes in concentration can be quite high, it is customary to express hydrogen ion concentration on a logarithm scale, using pH units. pH is related to the actual hydrogen ion concentration by the following formula: $pH = -\log [H^+]$. When the solution is neutral, its pH value is seven. When the pH value is below seven, the solution is acidic; and when the pH value is greater than seven, the solution is basic.

For normal functions of the human body, acid-base balance is a significant factor. Hydrogen ion concentration has great influence, for instance, on enzyme activity. In arterial blood the normal pH is 7.4. When pH is below 7.4 the term for that condition is acidosis, and if pH is greater than 7.4 the term for that condition is alkalosis. The lower limit of pH at which a person can live more than a few hours is about 6.8, and the upper limit is about 8.0. Acidosis and alkalosis can be divided into respiratory and metabolic acidosis and alkalosis by their origin. Acidosis and alkalosis are usually just temporary conditions but they can also turn to chronic conditions. In veins and extracellular matrix pH is usually slightly lower, about 7.35, because of greater concentration of carbon dioxide. In cells, pH is also lower than in arterial blood because of the acids that are produced in cells during normal metabolism. (Guyton and Hall 2006, 383–384.)

There are three independent variables, which affect hydrogen ion concentration in the human body: strong ion difference (SID), total concentration of the weak acids (A_{tot}) and partial pressure of carbon dioxide (pCO_2). The strong ion difference is the

difference between the sums of concentrations of strong cations and strong anions. A simplified formula for this can be written as: SID = $(Na^+ + K^+) – (Cl^- + La^-)$. When SID increases hydrogen ion concentration decreases, and therefore pH increases. In practice A_{tot} means albumin and phosphate concentrations because these two are the main weak acids. (Kellum 2000.) $A_{tot} = A^- + AH$ where AH is associated form of weak electrolytes and A^- is dissociated form. The formula describes the total effect of weak acids. (Heigenhauser 1995.) Rise in the partial pressure of carbon dioxide also raises the hydrogen ion concentration. Changes in these independent variables cause also changes in dependent variables, which are hydrogen ion concentration and bicarbonate ion concentration. (Kellum 2000.)

3.1 Chemical buffer systems

There are three primary systems that regulate hydrogen ion concentration in the body fluids to maintain the acid-base balance: (1) the chemical acid-base buffer systems of the body fluids, (2) the respiratory center and (3) the kidneys. The fastest of these systems is the chemical acid-base buffer system, which starts reacting within a fraction of a second after the first change in hydrogen ion concentration. Chemical buffers cannot eliminate hydrogen ions from the body, but only keep them bound until the balance can be re-established. Hydrogen ions' final removal from the body is done by the other systems. (Guyton and Hall 2006, 384.)

Chemical buffer is a substance that can bind a proton and later emit it. The general form of the buffering reaction is: Buffer + H^+ ↔ H Buffer. When free hydrogen ion concentration increases, the reaction is forced to the right, and when free hydrogen ion concentration decreases, reaction is forced to the left. In this way changes in pH are minimized. (Guyton and Hall 2006, 385.)

Chemical buffer systems always consist of weak acid and salt of the same acid. Like, for example, carbonic acid H_2CO_3 (weak acid) and sodium bicarbonate $NaHCO_3$ (bicarbonate salt). The most important buffers in human body are bicarbonate, phosphate and protein buffers. In blood the most important buffer is haemoglobin,

which is a protein buffer. $H^+ + Hb^- \leftrightarrow HHb$. The phosphate buffer system plays a major role in buffering renal tubular fluids and intracellular fluids where concentration of phosphate is high. Bicarbonate buffer is an important buffer especially in extra cellular fluids and its function is linked to respiratory systems functions as acid-base balance regulator. (McArdle et al. 2007, 308–310.) About 60–70 % of the chemical acid-base buffering is taking place in cells and rest 30–40 % in extracellular fluids. This is mainly explained by the fact that in intracellular fluids there are more proteins than in extracellular fluids. Also phosphate ion concentration is greater in intracellular fluids than in extracellular fluids. Furthermore, because intracellular pH is lower than extracellular pH phosphate buffer works better in intracellular fluid. (Guyton and Hall 2006, 387–388.)

3.2 Physiological buffer systems

After the body's chemical buffers function, the second line of defence, the respiratory system, also acts within a few minutes to eliminate carbon dioxide and, therefore, carbonic acid from the body. An increase in hydrogen ion concentration in extracellular fluids and plasma stimulates the respiratory centre. Breathing removes carbon dioxide from extracellular fluids. Increased ventilation decreases carbon dioxide concentration and, therefore, also the partial pressure on carbon dioxide (pCO_2), which is one of the independent variables that regulate acid-base balance. Removal of carbon dioxide is linked to acid-base balance also with bicarbonate buffer through the following formula:

$HCO_3^- + H^+ \leftrightarrow H_2CO_3 \leftrightarrow CO_2 + H_2O$

When hydrogen ion concentration increases the reaction is forced to the right when hydrogen ion and bicarbonate ion combine into carbonic acid and again carbonic acid dissociate into carbon dioxide and water. Carbon dioxide is removed from the body through lungs and reaction can continue moving to the right. So, an increase in ventilation decreases partial pressure of carbon dioxide and hydrogen ion concentration and therefore increases pH. Respiratory buffering can be up to two times more effective than all chemical buffers combined (McArdle et al. 2007, 310–311).

The first two lines of defence keep the hydrogen ion concentration from changing too much until the more slowly responding third line of defence, the kidneys, can eliminate the excess acid or base from the body. From all acids that are formed in the body only carbonic acid is removed by the respiratory system. Other routes must remove all other acids. The main route to remove acids is to remove them in urine so kidneys have an important role regulating the acid-base balance in the long run. (McArdle et al. 2007, 311.) The kidneys control acid-base balance by excreting either acidic or basic urine. That is done by controlling how much hydrogen or bicarbonate ions are excreted into the urine. Usually the kidneys reabsorb much more bicarbonates than hydrogen ions from the urine, which is important because otherwise hydrogen ions would accumulate faster than bicarbonates can buffer them. Normally about 90 % of the bicarbonate ions in urine are reabsorbed to the blood stream. This equals to about 4320 mEq of bicarbonate in 24 hours. In the kidneys reabsorption of bicarbonate ions is linked to secretion of hydrogen ions. For every bicarbonate ion that is reabsorbed there must be secretion of a hydrogen ion so the kidneys must excrete about 4400 mEq of hydrogen ions in 24 hours. In urine most of the hydrogen ions are buffered and those that are not buffered by bicarbonate, but some other buffers, leave free bicarbonates that can be also reabsorbed back to the blood stream. This explains the small difference in bicarbonate reabsorption and hydrogen secretion. In acidosis bicarbonate ions reabsorption is boosted and in alkalosis it is reduced. Bicarbonate ions reabsorption and hydrogen ions secretion are regulated by partial pressure of carbon dioxide, hydrogen ion concentration, bicarbonate ion concentration, angiotensin II, aldosterone and volume of extracellular fluids. (Guyton and Hall 2006, 390–397.)

3.3 Anaerobic energy metabolism and acid-base balance

All energy that cells need comes from adenosine triphosphate (ATP). ATP stores in the cells last only a few seconds in high intensity physical exercise. In the first seconds of anaerobic physical exercise energy is produced from the instant energy resources, which are ATP and phosphocreatine (PCr). PCr can emit its phosphate group and that group can be added to an adenosine diphosphate (ADP) to form ATP. These energy resources last only about six seconds. In longer anaerobic physical exercise ATP is produced

mainly via anaerobic glycolysis. (McArdle 2007, 138-141, 231-233.) In anaerobic glycolysis' source material can be either glucose or glycogen and in the end product is pyruvate.

$$glucose + 2\,ADP + 2\,P_i + 2\,NAD^+$$
$$\rightarrow 2\,pyruvate + 2\,ATP + 2\,NADH + 2\,H_2O + \mathbf{2}\,\mathbf{H}^+$$
$$glycogen_n + 3\,ADP + 3\,P_i + 2\,NAD^+$$
$$\rightarrow glycogen_{n-1} + 2\,pyruvate + 3\,ATP + 2\,NADH + 2\,H_2O + \mathbf{H}^+$$

If the need of energy is high and aerobic energy metabolism is not producing ATP fast enough, pyruvate is turned to lactate. When we combine these two reactions we get the following reaction:

$$glucose + 2\,ADP + 2\,P_i \rightarrow 2\,lactate + 2\,ATP + 2\,H_2O$$
$$glycogen_n + 3\,ADP + 3\,P_i + \mathbf{H}^+ \rightarrow glycogen_{n-1} + 2\,lactate + 3\,ATP + 2\,H_2O$$

When produced ATP molecules are dissociated, energy is released for the cell functions.

$$ATP + H_2O \leftrightarrow ADP + P_i + \mathbf{H}^+$$

Phosphate ion that is released can buffer the hydrogen ion that is also released, but usually phosphate is needed to form new ATP and the hydrogen ion stays free. That's why especially this hydrogen ion is the main reason for the acidosis in the human body during exercise. When ATPs hydrolysis and anaerobic glycolysis are combined, total reaction can be written in a short form as follows:

$$glucose \rightarrow 2\,lactate + \mathbf{2}\,\mathbf{H}^+$$
$$glycogen \rightarrow 2\,lactate + \mathbf{H}^+$$

Released lactate ion has its roles as being raw material for making glucose and it also facilitates hydrogen ion removal from muscle to extracellular fluids (the blood stream)

via H^+ + $lactate^-$ symporters. (Robergs et al. 2004.) Bloods lactate concentration is a usable marker for exercise intensity and adaptation (Cairns 2006).

A decrease in pH have been consired to be the main factor in fatique during anaerobic exercise. A decrease in pH effects on energy metabolism mechanisms and muscle contraction. When pH decreases below 6.9 it inhibits phosphofructokinase, which is an important enzyme in glycolysis, and when pH decreases below 6.4 glycolysis is totally stopped. (Willmore and Costill 2004, 152.) This view has also received some critisism with to the observation that many studies in isolated muscle have been done in unnaturally cold temperatures and, on the other hand, strength and power recovers faster than pH after exercise (Westblad et al. 2002). Westblad et al. (2002) have presented that phosphate ion that has been released from the PCr would be even a greater factor than acidosis in causing fatigue. This theory has been showed to be false because the phosphate ion realeased from PCr combines immediately with ADP molecule forming ATP. Phosphate ions in cells are from hydrolysis of ATP when physical exercise is so intense that there is no balance between breakdown and syntetisation of ATP and both phosphate and hydrogen ion are releashed into the body. (Robergs ym. 2004.) In any case, it is certain that hydrogen ion is not the only factor that causes fatigue in intensive exercise (Cairns 2006).

3.4 Acid-base balance in children

Acid-base balance in children is linked to their lower anaerobic capacity compared to adults. As stated in the previous chapter anaerobic glycolysis and ATP breakdown together produce free hydrogen ions and as children have lower glycolytic enzymes concentration and activity than adults they do not produce that much free hydrogen ions as adults during high intensity exercise. In a study by Klimek et al. (1998) untrained pre- and post-pubertal boys (12 and 16 years old) were tested in a 30 s Wingate test and post-pubertal boys exhibited a better tolerance of disturbances of acid-base balance as reflected by a significantly greater increases in lactate and hydrogen ion concentration and a greater decrement of base excess. Leithauser et al. (2007) compared acid-base status after Wingate maximal anaerobic tests in children (12 year old boys) and

adolescents (16 years old boys) and 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. Differences in maximum blood lactate (children 10.2 ± 1.3 mmol/l and adolescent 12.7 ± 1.0 mmol/l) and minimum pH (children 7.22 ± 0.03 and adolescent 7.15 ± 0.04) cause corresponding differences in the metabolic but not in selected measures of the respiratory compensation (Leithauser et al. 2007).

4 ACUTE TESTOSTERONE AND CORTISOL RESPONSES IN EXERCISE

Testosterone is secreted by the interstitial cells of Leydig in the testes, but only when they are stimulated by luteinizing hormone (LH) from the anterior pituitary gland. Testosterone secreted by the testes in response to LH has the reciprocal effect of inhibiting anterior pituitary secretion of LH. (Guyton and Hall 2006, 1007.)

Testosterone has an anabolic, tissue-building role and it contributes the male-female differences in muscle mass and strength that emerge at the onset of puberty. Plasma testosterone concentration commonly serves as a physiologic marker of the anabolic status. In addition to its direct affects on muscle tissue synthesis, testosterone indirectly affects a muscle fiber's protein content by promoting growth-hormone release. Testosterone's effect on the cell nucleus remains controversial. More than likely, a transport protein (sex hormone-binding globulin, SHBG) delivers testosterone to target tissues, after which testosterone associates with a membrane-bound or cytosolic receptor. It subsequently migrates to the cell nucleus where it interacts with nuclear receptors to initiate protein synthesis. (McArdle 2007, 437.)

No testosterone is produced during childhood until about the ages of 10 to 13 years. Then testosterone production increases rapidly especially in boys under the stimulus of anterior pituitary gonadotropic hormones at the onset of puberty and lasts throughout most of the remainder of life (Guyton and Hall 2006, 1004). Plasma testosterone concentration in females, although only one tenth that in males, increases with exercise (McArdle 2007, 437). Acute increase in salivary testosterone concentration has been observed on post-pubertal boys after anaerobic exerecise (Thomas et al. 2009). It is shown in many studies that on adult males blood testosterone concentration peaks right after intensive exercise and decreases back to pre-exercise values within an hour (McArdle 2007, 438).

Cortisol is the major glucocorticoid hormone of the adrenal cortex and it affects glucose, protein and free fatty acid metabolism. Cortisol is secreted with a strong

diurnal rhythm; secretion normally peaks in the morning and diminishes at night. Cortisol secretion increases with stress; thus it is sometimes called the stress hormone. Even though considered a catabolic hormone, cortisol's important effect counters hypoglycemia and is thus essential for life. (McArdle 2007, 434.)

Cortisol turnover, the difference between its production and removal, provides a convenient means to study cortisol response to exercise. Considerable variability exists in cortisol turnover with exercise, depending on intensity and duration, fitness level, nutritional status, end even circadian rhythm. Most research indicates that cortisol output increases with exercise intensity; this accelerates lipolysis, ketogenesis, and proteolysis. Even during moderate exercise, plasma cortisol concentration rises with prolonged duraton. Cortisol levels also can remain elevated for up to two hours following exercise, suggesting that cortisol plays a role in tissue recovery and repair. (McArdle 2007, 435.)

Studies for acute changes in testosterone and cortisol concentrations after anaerobic exercises have mainly shown increase in both of these hormones. Stokes et al. (2013) studied eight healthy non-obese young adults (18–25 years) on 30 seconds cycle ergometer sprint and measured testosterone and cortisol concentrations before and after the sprint. This sprint exercise significantly increased testosterone ($P = 0.001$) and cortisol ($P = 0.004$). In other study made by Zeinali et al. (2012) they tested track and field athletes (speed runners) in repeated running with increasing time (each of the first three repetitions lasted 15 s, whereas each of the second three repetitions took 20 s, one repetition was for 30 s and another repetition lasted for 40 s and was performed at the most speed and the most distance). Immediately after the exercise there was significant increase compared to pre-test values in cortisol (408 ± 162 nmol/l vs. 686 ± 251 nmol/l, $P < 0.05$) but not in testosterone (29.08 ± 5.92 vs. 31.90 ± 9.42, $P > 0.05$). There are also studies made with a protocol where subjects run 7 times 35 m with 25 s of light running between. Results have shown that in most subjects there has been acute increase in testosterone and cortisol concentration in handball players (Živanović et al. 2011) and football players (Palić et al. 2012) after the tests using this protocol.

5 PHYSIOLOGY OF A 400 M RUN

The 400 m race takes about 44 to 46 s in elite male runners and about 47 to 50 s in national level male runners. The optimal performance in a 400 m running consists of a fast start followed by an approximate 15 % decrease in velocity, relative to peak velocity, during the last 100 m of the race (Hirvonen et al. 1992; Hanon and Gajer 2009; Hanon et al. 2010). The decrease in speed is larger in elite runners compared to lower level runners and the decrease in speed is mainly due the decrease in stride length not the stride frequency (Gajer et al. 2007). The large anaerobic contribution and subsequent accumulation of metabolites (Kindermann et al. 1977) may contribute to the decrease in velocity observed in during the final 100 m (Hanon and Gajer 2009; Hanon et al. 2010).

During the acceleration phase of the 400 m sprint most of the ATP is resynthesized through the breakdown of PCr and the role of glycolysis is small. Between 100 m and 200 m, the contribution of high-energy phosphate is reduced and the role of glycolysis is increased. After 200 m, fatigue is observed and the running speed starts to decrease, although PCr is not depleted and lactate concentration is not at maximum level. At the end of the 400 m sprint, PCr stores are depleted and lactate concentration attains an individual maximum. (Hirvonen et al. 1992.)

After the 400 m race very high concentration of blood lactate (from 18.5 to 20.1 mmol/l) has been reported in elite level male runners (Lacour et al. 1990; Slowinska-Lisowska and Majda 2002). After the simulated 400 m races low blood pH values (from 7.07 to 7.00) (Medbø and Sejersted 1985; Hanon et al. 2010), high blood lactate concentrations (from 13.5 to 22.0 mmol/l) (Ohkuwa et al. 1984, Medbø and Sejersted 1985, Nummela et al. 1992, Nummela et al. 1995, Hill 1999, Reis et al. 2004, Duffield et al. 2005, Hanon et al. 2010, Zouhal et al. 2010; Saraslanidis et al. 2011, Bret et al. 2013), high muscle lactate concentration (17.3 mmol/kg) (Hirvonen et al. 1992) low blood bicarbonate concentrations (from 4.9 to 7.1 mmol/l) (Medbø and Sejersted 1985; Hanon et al. 2010) and low blood pCO_2 (3.33 kPa) (Medbø and Sejersted 1985) have

been reported. While similar data have not been reported for a competitive 400 m race, it is likely that similar changes occur.

There are not much studies about hormonal changes in 400 m race but in the study by Slowinska-Lisowska and Majda (2002) they noticed that immediately after the 400 m run in the group of top class sportsmen increase luteinizing hormone (LH) (from 4.37 ± 2.17 to 8.13 ± 2.31 U/l) and follicle-stimulating hormone (FSH) (from 3.05 ± 1.91 to 4.75 ± 1.83 U/l) was determined as well as decrease in total (from 26.17 ± 5.84 to 23.13 ± 5.94 nmol/l) and free testosterone (from 74.60 ± 9.22 to 55.36 ± 11.26 pmol/l) concentration. In the group of athletes with lower training level increase in FSH (from 2.98 ± 2.10 to 3.71 ± 2.45 U/l) and total (from 25.36 ± 6.28 to 32.24 ± 6.62 nmol/l) and free testosterone (from 81.82 ± 24.33 to 130.70 ± 45.59 pmol/l) concentration was noticed. In both groups after the 24 h restitution, the examined parameters, except LH levels in the group of top class sportsmen, showed concentrations similar to those before the run. (Slowinska-Lisowska and Majda 2002.)

5.1 Energy system contributions during a 400 m run

There has been great variation in the results of studies trying to determine the energy system contributions during the 400 m run (Table 3). Even recent studies that have used the same methods to calculate energy system contributions and have had similar level athletes as subjects have got over 15 % difference on anaerobic and aerobic contributions (Duffield et al. 2005; Reis et al. 2004). If we take the average or the median from the results of these selected studies we get the result of about 66 % of anaerobic and 34 % aerobic metabolism during 400 m run. Even if we select only the studies that have used the most reliable methods, that is, accumulated oxygen deficit for calculating energy system contributions and measurements done on a real track (and not on a treadmill), we get about the same results as average. On the other hand, as mentioned earlier even in this selected group of studies the deviation in the results is huge so the real energy system contributions stay questionable. It is also important to remember that almost all studies have been done in simulated 400 m races and not in actual race situations and that might underestimate the anaerobic part of the energy

system contributions. It is also speculated that faster runners have greater total energy cost in 400 m sprint and that it comes mainly from the anaerobic half of the energy contributions and, therefore, they also have greater anaerobic percentage compared to slower runners on the 400 m sprint (Arcelli et al. 2008). The anaerobic energy contribution can also be devided into alactic and lactic components, referring to to the processes involved in the splitting of the stored phosphogens, ATP and phosphocreatine (PCr), and the nonaerobic breakdown of carbohydrate to lactic acid through glycolysis (Gastin 2001.) It has been speculated that alactic component would be about 12.5 % and lactic component about 62.5 % of the total energy during a 400 m race in elite level athletes (Newsholme 1992) but more research is needed to comfirm that.

TABLE 3. Anaerobic and aerobic energy system contributions in 400 m run in various studies.

Study	Measurement	Distance (m)	Time (s)	Anaerobic %	Aerobic %
Weyand et al. 1993	Treadmill (AOD)	400	?	36	64
Medbø and Sejersted 1985	Treadmill (AOD)	335	57	56	44
Spencer and Gastin 2001	Treadmill (AOD)	400	49.3	57	43
Duffield et al. 2005	Track (AOD)	400	52.2	59	41
Nummela and Rusko 1995	Treadmill (AOD)	370	49.5	63	37
Spencer et al. 1996	-	400	?	63	37
Zouhal et al. 2010	Track (AOD)	400	55.0	63	37
Duffield et al. 2005	Track (La and PCr)	400	52.2	65	35
Hill 1999	Treadmill and race (La)	400	49.3	68	32
Peronnet and Thibault 1989	Mathematical model	400	44	70	30
Lacour et al. 1990	Race (La)	400	?	72	28
Ward-Smith 1985	Mathematical model	400	44.9	72	28
Newsholme et al. 1992	-	400	?	75	25
Reis et al. 2004	Track (AOD)	400	53.9	76	24
Foss and Keteyian 1998	-	400	45	82	18
van Ingen Schneau et al. 1991	Mathematical model	400	44.4	83	17

During the 400 m sprint the aerobic system contribution percentage follows closely oxygen uptake curve (Figure 3.) and reaches 50 % in about half way of the 400 m sprint (Spencer and Gastin 2001).

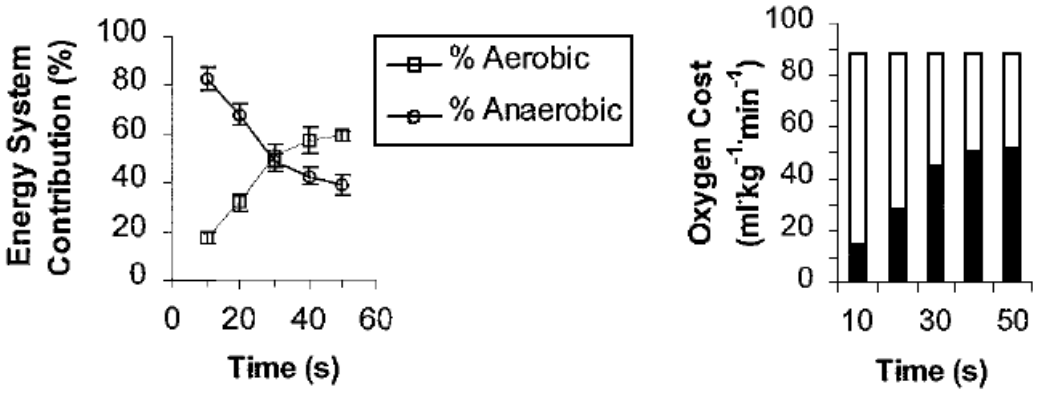

FIGURE 3. Energy system contributions (on the left) and oxygen deficit and oxygen uptake (on the right) in 10 s time intervals for the 400 m run (Spencer and Gastin 2001).

5.2 Oxygen consumption during a 400 m run

A recent study by Hanon et al. (2010) indicates that the peak oxygen uptake reached during a 400 m simulated race is about 94 % of VO_{2max}, although lower values have been reported earlier (from 52 to 93.1 %) (Nummela and Rusko 1995; Spencer and Gastin 2001; Reis et al. 2004; Duffield et al. 2005; James et al. 2007). However, these studies did not exactly replicate the competition race conditions and the sampling windows for VO_2 kinetics have been varied between studies. In absolute values these are about 49 - 55 ml/kg/min (Hanon et al. 2010; Duffield et al. 2005; James et al. 2007).

The decrease in velocity during the final 100 m of 400 m race has been reported to occur concomitantly with a decrease in VO_2 (Figure 4.). As there is similar decrease in both speed and VO_2 the oxygen uptake presented as ml/kg/m does not decrease during the last 100 m but stays flat (Hanon et al. 2010). One reason for the drop in the oxygen uptake might be that acidosis inhibits oxidative phosphorylation and can limit ATP supply in exercising muscle to below the mitochondrial capacity (Jubrias et al. 2003).

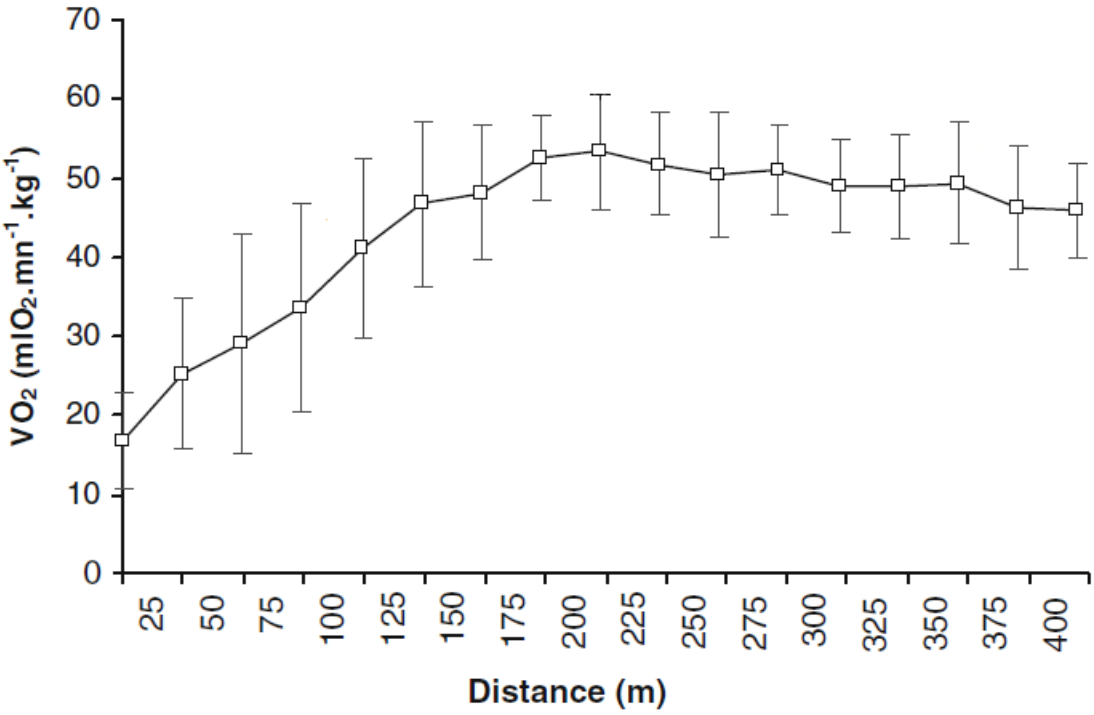

FIGURE 4. Oxygen uptake (ml/kg/min) during 400 m run (Hanon et al. 2010).

5.3 Fatigue in a 400 m run

Fatigue is the transient decrease in performance capacity of the muscles, usually seen as a failure to maintain or develop expected force or power output. Both central and peripheral mechanisms have been postulated as causes of muscle fatigue. Any one of the many links in the long chain from the voluntary motor centres in the brain to the contractile apparatus in the single muscle fibers may contribute. (Asmussen 1979.) While evidence does point to the central nervous system as a possible site of fatigue, most research implicates changes in the periphery as the major limiting factor (McLester 1997). Maximal muscle activation increases during 400 m run, especially during the last 150 m. This indicates that neural input is maximal during the last 100 m as runner is trying to maintain the running speed but despite of maximal effort fatigue in muscle level forces running speed to decrease. (Mero et al. 1987, 198-199.) Nummela et al. (1992) reported in their 400 m study that the decrease in speed in the last 100 m of the 400 m run with an increase in acidity and the EMG:force ratio confirms that in trained athletes fatigue in the 400 m sprint is mainly related to the processes within skeletal muscle and not to the central nervous system. Running velocity and stride

length decrease and contact time increase during the 400 m sprint (Nummela et al. 1992).

Because of the fast metabolic reactions during the 400 m race, the acid-base balance of the body is disturbed by increase of free hydrogen ions (H^+). This increase in hydrogen ion concentration causes acidosis in the body and it is the main reasons of fatigue during the 400 m race (Hanon and Gajer 2009).

At high intensity, short duration exercise like 400 m run, the accumulation of inorganic phosphate (P_i) due the use of the immediate (phosphagen) energy system would occur even prior to ionic concentration changes, and, therefore, may be one contributor to fatigue. High concentration of inorganic phosphates during exercise prevents the powerstroke and causes a reversal of the crossbridges from the high-force to the low-force states, resulting in a lower force production capacity. This accumulation of crossbridges in the low-force states, along with oscillation at the proper frequency, could possibly lead to activation of a parallel cycle specialized to oscillatory work. This cycle would allow force production to continue, but at lower level and greater cost. In addition, any contributors to fatigue are exacerbated by high hydrogen ion concentration. (McLester 1997.)

6 PURPOSE OF THE STUDY AND HYPOTHESES

Prior studies have shown that adult male athletes are able to attain a high percentage of their VO_{2max} during the 400 m sprint and that there can be a significant decrease in VO_2 during the last 100 m (Hanon et al. 2010). Furthermore, 400 m sprint causes great decrease in blood pH (Medbø and Sejersted 1985; Hanon et al. 2010), which is the main factor while determining acute whole body acid-base balance. Little is known about the differences in oxygen uptake, acid-base balance and energy system contributions between child, adolescent and adult athletes during (and after) maximal anaerobic 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 the maximal anaerobic running test in child, adolescent and adult athletes. Furthermore, the aim was also to determine aerobic and anaerobic energy contributions during the test for the different age groups.

1. Research problem: Which percentage of VO_{2max} can be achieved during a 50 s maximal running test?

Adult athletes can achieve over 90 % of their VO_{2max} during a 400 m run (Hanon et al. 2010).

1. Hypothesis: All groups achieve over 90 % of their VO_{2max} during a 50 s maximal running test.

2. Research problem: Does age affect anaerobic percentage of the energy system contribution during a 50 s maximal running test?

Maximal blood lactate concentration after 30 s maximal exercise has been reported to be lower in children than in adolescents and adults (Beneke at al. 2005).

2. Hypothesis: Adults use more anaerobic energy sources than adolescents and adolescents more than children during a 50 s maximal running test.

3. Research problem: Does age affect acidosis after a 50 s maximal running test?

Children have been reported to have smaller acidosis compared to adolescents after anaerobic exercises (Klimek et al. 1998; Leithauser et al. 2007)

3. Hypothesis: Adults have lower pH than adolescents and adolescents lower than children after a 50 s maximal running test.

7 METHODS

Subjects. Eight adult male 400 m runners (age 21 ± 2 years, height 1.82 ± 0.07 m and body mass 73.2 ± 7.0 kg), eight male adolescent athletes (age 15 ± 1 years, height 1.80 ± 0.03 m and body mass 63.9 ± 4.2 kg) and eight male child athletes (age 13 ± 1 years, height 1.61 ± 0.10 m and body mass 47.9 ± 7.1 kg) volunteered for the study. 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 this experimental study, which was approved by the local University Ethics Committee.

Experimental protocol. The study was undertaken between the end of the competitive indoor season and the beginning of the outdoor season (May-June 2011). Participants performed two running tests on the same 200 m indoor track, separated by at least one week. The first test (1) was maximal running test (MRT), which means simulated race performed according to the normal competition pacing strategy. The second (2) test was 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 the airflow volume and gas analysers was performed according to the manufacturer's instructions before each test for each subject.

Maximal running test (MRT). The present protocol is presented in figure 5. The subjects 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 own breakfast. Subjects started their warm-up one hour after the first blood sample was taken. Participants were instructed to do similar warm-up as they were used to do normally before their competitions. Warm-up lasted one hour and included at least one 80 - 100 m run with a competition speed at the end of warm-up. At the end of the warm-

up participants performed countermovement jumps. After warm-up other neuromuscular tests were performed. These measurements lasted less than 15 minutes and after these subjects were allowed to do 5 - 10 minutes warm-up before MRT.

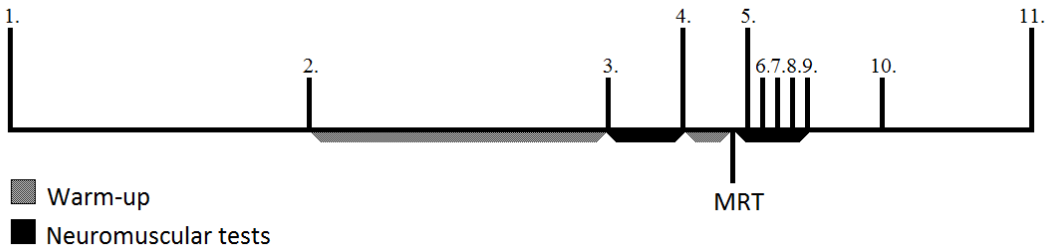

FIGURE 5. The research protocol of maximal running test. Numbers are blood samples. Longer poles mean blood sample from both arterial blood and a fingertip. Shorter poles mean blood samples from a fingertip only. MRT = Maximal Running Test.

Subjects were familiarized with the portable gas exchange analyser and breathing mask during the second warm-up phase. Adult athletes ran 400 m, adolescent athletes 350 m and 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 stage to run the test as fast as possible. Split times were recorded by hand stopwatch every 100 m using 0.1 s accuracy. After the run subjects were transported to do the same neuromuscular tests as before the run. These tests lasted again less than 15 minutes. After these tests subjects performed countermovement jumps, and after that they were allowed to move a little bit and start their own cool-down after 30 minutes of MRT.

VO_{2max} running test. Subjects 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 minutes. The test included three different aerobic running speeds followed by a 30 s recovery. Adults ran three times 800 m at speeds of 3.0 m/s, 3.4 m/s and 3.8 m/s, adolescents ran three times 800 m at speeds of 2.6 m/s, 3.0 m/s and 3.4 m/s and 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 5 m intervals inside of the running track and turning on and off in a correct speed). After these three runs each subject 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 subject was not able to run at the speed by the "light pacemaker". Lap times were recorded by hand stopwatch using the 0.1 s accuracy.

Blood samples. Blood samples were taken from both a fingertip and from antecubital vein. During the day when MRT was performed blood samples from a fingertip were taken 8 - 9 AM after 10 h of fasting, and before and after warm-up, before MRT and 3, 6, 9, 12, 15, 30 and 60 minutes after MRT. Each blood sample from the fingertip contained a 20 µl 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 µl), which was used to analyse blood pH, bicarbonate concentration (HCO_3^-), base excess (BE), partial pressure of carbon dioxide (pCO_2), partial pressure of oxygen (pO_2), sodium ion concentration (Na^+) and potassium ion concentration (K^+) (GEM Premier 3000, Instrumentation Laboratory, Lexington, MA, USA). During the day when MRT was performed blood samples (3.5 ml) were taken from an antecubital vein in the morning, before MRT and 3 and 60 minutes after MRT. From antecubital vein blood samples testosterone, cortisol and sex hormone-binding globulin concentrations (SHBG) were analysed (Immulite 1000 Immunoassay System, Siemens, Germany). From these samples we also analysed total protein concentration and calculated total concentration of the weak acids (A_{tot}). We used the formula: [Atot] (mEq/l) = 2.43 x [total protein] (g/dl), which is the most widely used method to assign a value for A_{tot} of human plasma (Constable 2001). In addition, we analysed chloride ion concentration (Cl^-) and together with sodium ion concentration (Na^+) and potassium ion concentration (K^+) from fingertip blood samples we calculated strong ion difference (SID) using the formula: [SID] (mEq/l) = $(Na^+ + K^+) - (Cl^- + La^-)$ (Lindinger 1995).

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

Neuromuscular tests. The pre-fatigue measurements included the H/M-ratio, the supramaximal passive twitch, isometric MVC effort, and superimposed twitch during isometric MVC contraction. After MRT subjects did the same neuromuscular tests as

before MRT, except that it was started with two supramaximal stimuli for measuring the maximal M-wave. During measurements subjects remained relaxed and passive except for a few seconds of maximal voluntary contractions from calf muscles. Neuromuscular variables are reported more precisely in another report (Äyrämö 2013).

Jumping ability. Counter movement jump (CMJ) was performed on a contact mat and flight time was recorded to analyse height of jump with a formula: $h = gt^2/8$ (Komi & Bosco 1978). From 3 to 6 jumps were performed both pre and post MRT. Best pre CMJ and best post CMJ were used for analysis. Percentage changes between pre and post CMJs were used for further analysis.

Data analysis. Breath-by-breath gas exchange values were averaged with 5 s intervals to obtain $VO_{2peak.}$ In order to compare with the literature, the greatest 30 s VO_{2max} was also calculated from 5 s values. Accumulated oxygen deficit (AOD) was calculated as a difference between the theoretical oxygen demand and oxygen uptake during MRT (Krogh and Lindhard 1920). 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 VO_2 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 MRT and the oxygen uptake value was used as the oxygen demand. As a comparison we also calculated anaerobic energy system contribution using blood lactate levels to estimate anaerobic energy used during MRT. One mmol/l increase in lactate concentration above the resting values (values before MRT) was assumed to be equal to 3.0 ml/kg of oxygen uptake (diPrampero 1981). The energy cost of running (C_r) (ml/kg/m) was calculated by dividing the theoretical oxygen demand by the running distance in MRT.

Statistical analysis. Results are 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 groups. All statistical analyses were conducted using SPSS software (Version 18). The level of significance was set at $P < 0.05$.

8 RESULTS

8.1 VO_{2max} running test

Maximal oxygen uptake as the greatest 30 s average was 60.4 ± 6.0 ml/kg/min for adults, 58.7 ± 6.3 ml/kg/min for adolescents and 55.6 ± 5.1 ml/kg/min for children (P > 0.05). Blood lactate levels after and oxygen uptake during aerobic running speeds are shown in Table 4. Other variables are shown in Table 5.

TABLE 4. Mean ± SD values for blood lactate (La^-) after and oxygen uptake during the last full minute of aerobic running speeds at VO_{2max} running test.

Speed (m/s)	Blood lactate (mmol/l)			Oxygen uptake (ml/kg/min)		
	Children	Adolescents	Adults	Children	Adolescents	Adults
2.2	2.0 ± 0.5	-	-	35.9 ± 2.3	-	-
2.6	2.4 ± 1.1	2.3 ± 0.8	-	40.8 ± 2.4 [a]	36.8 ± 3.3	-
3.0	3.1 ± 1.1	2.7 ± 1.2	2.3 ± 0.3	45.6 ± 2.3 [aa,bb]	41.2 ± 3.0	40.6 ± 2.3
3.4	-	3.5 ± 1.5	2.2 ± 0.8	-	46.0 ± 2.4	46.1 ± 3.0
3.8	-	-	3.7 ± 0.9	-	-	50.8 ± 3.0

Significantly different between children and adolescents ; [a] P < 0.05, [aa] P < 0.01.
Significantly different between children and adults ; [bb] P < 0.01.

TABLE 5. Mean ± SD values for maximal blood lactate (La^-) and maximal pH after the VO_{2max} running test, time for increase running speed part of the test and running speed of the last 200m of the test.

Group	La^- (mmol/l)	pH	Time (s)	Running speed (m/s)
Children	7.3 ± 2.0 [a,bb]	7.29 ± 0.05 [bb]	329 ± 63	4.36 ± 0.35 [aa,bb]
Adolescents	10.3 ± 2.7	7.23 ± 0.08	349 ± 53	5.11 ± 0.42 [c]
Adults	12.4 ± 1.8	7.15 ± 0.06	310 ± 29	5.59 ± 0.44

Significantly different between children and adolescents ; [a] P < 0.05, [aa] P < 0.01.
Significantly different between children and adults ; [bb] P < 0.01.
Significantly different between adolescents and adults ; [c] P < 0.05.

8.2 Maximal running test (MRT)

Running performance, Cr and jumping ability. Performance times, running speeds and mean energy cost of running are shown in Table 6. The performance time of the adults was 95 ± 3 % of their personal best in 400 m. A 100 m split times for the adults were 12.6 ± 0.4 s for the first, 12.2 ± 0.5 s for the second, 13.4 ± 0.7 s for the third and 13.9 ± 0.7 s for the last 100 m. In all groups the running speed decreased significantly between each 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. Height in CMJ decreased after MRT in every group. In children the decrease was 8.5 ± 11.2 % ($P = 0.079$), in adolescents 7.5 ± 5.6 % ($P < 0.01$) and in adults 6.5 ± 7.2 % ($P = 0.064$).

TABLE 6. Average running speed differed significantly ($P < 0.001$) between the groups.

Group	Distance (m)	Time (s)	Speed (m/s)	Mean energy cost of running (Cr) (ml/kg/m)
Children	300	53.6 ± 5.7	5.65 ± 0.54	0.228 ± 0.012 [a]
Adolescents	350	53.3 ± 2.3	6.57 ± 0.27	0.209 ± 0.022
Adults	400	52.1 ± 2.1	7.68 ± 0.30	0.218 ± 0.021

Significantly different between children and adolescents ; [a] $P < 0.05$.

Oxygen uptake. Maximal oxygen uptake (VO_{2max}) during MRT using 30 s averages was 50.3 ± 3.9 ml/kg/min for children, 55.8 ± 4.7 ml/kg/min for adolescents and 57.7 ± 3.0 ml/kg/min for adults. Difference in oxygen uptake between children and adults were statistically significant ($P < 0.05$). These results were 91 ± 11 %, 96 ± 7 % and 96 ± 9 % from VO_{2max} measured in VO_{2max} running test. Peak oxygen uptake (VO_{2peak}) during MRT using 5 s averages was significantly 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, $P < 0.01$) during MRT. These results were 96 ± 11 %, 103 ± 9 % and 101 ± 10 % from VO_{2max} measured in VO_{2max} running test. Table 7 displays VO_2 in 5 s intervals. In adults the decrease in VO_2 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 VO_2 in other groups during the run.

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

TABLE 7. Mean ± SD values oxygen uptake (VO_2) in MRT.

	VO_2 (ml/kg/min)		
Group	Children	Adolescents	Adults
-5 – 0 s	13.3 ± 2.9	13.1 ± 3.1	13.2 ± 4.6
0 - 5 s	18.3 ± 6.0	15.4 ± 9.3	21.6 ± 6.5
5 – 10 s	25.1 ± 5.4	28.3 ± 10.6	27.8 ± 5.1
10 - 15 s	30.8 ± 4.2	32.2 ± 8.7	34.5 ± 7.3
15 - 20 s	44.2 ± 7.6	45.0 ± 8.9	48.5 ± 7.4
20 - 25 s	48.3 ± 5.8 [b]	52.6 ± 5.6	56.0 ± 4.3
25 - 30 s	49.9 ± 4.8 [b]	54.5 ± 6.1	58.4 ± 3.0
30 - 35 s	49.2 ± 6.4 [a,bb]	56.2 ± 5.6	59.7 ± 4.0
35 - 40 s	50.4 ± 3.9 [a,b]	56.4 ± 5.5	58.3 ± 3.0
40 - 45 s	50.2 ± 4.2 [b]	54.3 ± 9.1	58.0 ± 3.3
45 - 50 s	50.3 ± 4.0 [a,b]	56.3 ± 4.6	55.3 ± 4.4
50 - 55 s	47.8 ± 6.5	54.5 ± 5.6	51.7 ± 4.4
55 - 60 s	46.3 ± 3.6	49.6 ± 7.4	48.1 ± 4.2

Significantly different between children and adolescents ; [a] $P < 0.05$.

Significantly different between children and adults ; [b] $P < 0.05$, [bb] $P < 0.01$.

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

significantly greater percentage (53 ± 2 %) than adolescents (45 ± 7 %, $P < 0.05$) and children (39 ± 4 %, $P < 0.01$).

8.3 Blood samples in MRT

Blood lactate and pH. La^- recovered back to pre-values at one hour after MRT in adolescents and children ($P > 0.05$) but not in adults (Table 8). Blood pH recovered back to pre-values at half an hour after MRT in children ($P > 0.05$), one hour after MRT in adolescents ($P > 0.05$) but not in adults.

TABLE 8. Mean ± SD values for blood lactate (La^-) and blood pH in MRT.

	La^- (mmol/l)			pH		
Group	Children	Adolescents	Adults	Children	Adolescents	Adults
Morning	1.2 ± 0.4	1.3 ± 0.4	1.3 ± 1.0	7.39 ± 0.01	7.39 ± 0.01	7.40 ± 0.02
Pre WU	1.6 ± 0.3	1.7 ± 0.5	1.8 ± 0.3	7.41 ± 0.01	7.40 ± 0.01	7.40 ± 0.02
Post WU	2.1 ± 1.0 ^{a}	3.4 ± 2.4	2.6 ± 1.0	7.41 ± 0.02	7.38 ± 0.04	7.40 ± 0.02
Pre	2.0 ± 0.8	2.0 ± 1.2	1.8 ± 0.4	7.40 ± 0.02	7.41 ± 0.01	7.41 ± 0.02
Post 3	9.9 ± 1.4 ^{bb}	12.2 ± 3.4	14.7 ± 2.5	7.18 ± 0.03 ^{bb}	7.15 ± 0.06 ^{cc}	7.05 ± 0.06
Post 6	10.0 ± 0.8 ^{bb}	13.0 ± 4.0	16.1 ± 2.3	7.19 ± 0.03 ^{bbb}	7.14 ± 0.08 ^{cc}	6.98 ± 0.06
Post 9	9.5 ± 0.9 ^{bb}	12.3 ± 3.5 ^{c}	16.9 ± 1.8	7.23 ± 0.03 ^{bbb}	7.16 ± 0.08 ^{cc}	6.99 ± 0.06
Post 12	8.6 ± 1.0 ^{a,bb}	12.2 ± 3.2 ^{cc}	16.5 ± 1.9	7.26 ± 0.03 ^{bbb}	7.19 ± 0.09 ^{cc}	7.01 ± 0.07
Post 15	8.5 ± 1.6 ^{bbb}	10.8 ± 2.9 ^{cc}	15.7 ± 2.0	7.29 ± 0.03 ^{bb}	7.23 ± 0.08 ^{cc}	7.05 ± 0.08
Post 30	3.4 ± 0.5 ^{a,bbb}	5.3 ± 2.0 ^{c}	8.6 ± 2.3	7.40 ± 0.02 ^{a,bbb}	7.36 ± 0.03 ^{cc}	7.30 ± 0.04
Post 60	1.8 ± 0.5	2.1 ± 0.7	2.4 ± 0.4	7.39 ± 0.03	7.40 ± 0.02	7.39 ± 0.02
Post Max	10.2 ± 1.1 ^{bb}	13.3 ± 3.7 ^{c}	17.4 ± 1.8	7.18 ± 0.03 ^{bbb}	7.14 ± 0.07 ^{cc}	6.97 ± 0.06
Tmax	5 ± 2 ^{bb}	6 ± 3	9 ± 3	4 ± 1 ^{a,bb}	5 ± 2	7 ± 2

Post Max = individual maximal (or minimal in pH) value regardless from which sample it was measured, Tmax = time in minutes from end of MRT to individual maximal (or minimal in pH) value, Pre WU = before warm-up, Post WU = after warm-up, Pre = before MRT, Post n = n minutes after MRT.

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} $P < 0.01$.

Blood bicarbonate and base excess. HCO_3^- recovered back to pre-values at one hour after MRT in adolescents and children (P > 0.05) but not in adults (Table 9). BE recovered back to pre-values at one hour after MRT in adolescents (P > 0.05) but not in children and adults.

TABLE 9. Mean ± SD values for blood bicarbonate (HCO_3^-) and blood base excess (BE) in MRT.

	HCO_3^- (mmol/l)			BE (mEq/l)		
Group	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 ^{b}	13.6 ± 2.9 ^{c}	9.9 ± 3.1	-14.3 ± 1.5 ^{b}	-15.0 ± 4.0 ^{c}	-19.6 ± 4.3
Post 6	13.7 ± 1.3 ^{bb}	12.2 ± 3.1 ^{cc}	7.1 ± 2.5	-15.3 ± 1.9 ^{bb}	-17.1 ± 4.0 ^{cc}	-23.2 ± 3.3
Post 9	14.6 ± 1.2 ^{bb}	12.7 ± 3.4 ^{cc}	6.7 ± 2.0	-14.3 ± 1.5 ^{bb}	-16.6 ± 4.4 ^{cc}	-24.0 ± 2.5
Post 12	16.0 ± 1.3 ^{bb}	13.8 ± 3.6 ^{cc}	7.2 ± 2.3	-12.5 ± 1.8 ^{bb}	-15.1 ± 4.7 ^{cc}	-23.3 ± 2.9
Post 15	17.4 ± 1.6 ^{bb}	15.4 ± 3.8 ^{cc}	8.4 ± 2.6	-10.6 ± 2.2 ^{bb}	-13.2 ± 5.2 ^{cc}	-22.1 ± 3.3
Post 30	23.1 ± 1.7 ^{bb}	21.9 ± 2.0 ^{c}	18.1 ± 2.5	-2.7 ± 2.4 ^{bb}	-4.2 ± 2.9 ^{c}	-9.7 ± 3.6
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
Post Min	13.6 ± 1.4 ^{bb}	12.2 ± 3.2 ^{cc}	6.6 ± 2.0	-15.4 ± 1.9 ^{bb}	-17.2 ± 4.1 ^{cc}	-24.2 ± 2.5
Tmin	4 ± 2 ^{a,b}	6 ± 1	7 ± 2	5 ± 2 ^{b}	7 ± 1	9 ± 3

Post Min = individual minimal value regardless from which sample it was measured, Tmin = time in minutes from end of MRT to individual minimal value, Pre WU = before warm-up, Post WU = after warm-up, Pre = before MRT, Post n = n minutes after MRT.

Significantly different between children and adolescents ; ^{a} P < 0.05.

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

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

Blood partial pressures of carbon dioxide and oxygen. In pCO_2 there were no differences between the values before and three minutes after MRT at any of the groups (Table 10). Three minutes after MRT values were significantly lower than before and recovered back to pre-values at one hour after MRT in adolescents and adults (P > 0.05)

but not in children. pO_2 did not recover back to pre-values within an hour at any of the groups.

TABLE 10. Mean ± SD values for blood's partial pressure of carbon dioxide (pCO_2) and blood's partial pressure of oxygen (pO_2) in MRT.

	pCO_2 (kPa)			pO_2 (kPa)		
Group	Children	Adolescents	Adults	Children	Adolescents	Adults
Morning	5.5 ± 0.3	5.8 ± 0.4	5.7 ± 0.5	9.1 ± 1.2	10.2 ± 1.2	10.0 ± 0.9
Pre WU	5.4 ± 0.3 ^{aa,b}	5.8 ± 0.2	5.8 ± 0.4	9.6 ± 1.3	9.3 ± 1.0	9.6 ± 0.6
Post WU	5.2 ± 0.4	5.2 ± 0.3	5.5 ± 0.5	10.2 ± 2.0	9.7 ± 1.1	9.6 ± 0.6
Pre	5.4 ± 0.4	5.4 ± 0.2 ^{c}	5.8 ± 0.3	9.2 ± 1.1	9.2 ± 0.7	8.5 ± 0.8
Post 3	5.0 ± 0.5	5.2 ± 0.6	5.2 ± 0.8	10.7 ± 1.3	11.0 ± 1.0	11.6 ± 1.4
Post 6	4.6 ± 0.4	4.5 ± 0.4	4.8 ± 0.8	11.0 ± 1.7	11.9 ± 1.4	12.2 ± 0.8
Post 9	4.3 ± 0.3	4.4 ± 0.3	4.2 ± 0.6	12.3 ± 1.4	12.6 ± 1.1	12.9 ± 0.9
Post 12	4.4 ± 0.3	4.5 ± 0.4	4.3 ± 0.7	11.3 ± 1.4	11.5 ± 1.2 ^{c}	12.5 ± 0.6
Post 15	4.5 ± 0.2	4.4 ± 0.4	4.0 ± 0.5	10.5 ± 0.9 ^{a,bb}	11.8 ± 0.8 ^{c}	12.8 ± 0.5
Post 30	4.8 ± 0.3	5.0 ± 0.4 ^{c}	4.4 ± 0.5	10.4 ± 0.9	9.9 ± 0.8	10.4 ± 0.8
Post 60	5.2 ± 0.4	5.5 ± 0.2	5.5 ± 0.3	10.0 ± 1.5	10.4 ± 1.1	9.7 ± 1.0

Pre WU = before warm-up, Post WU = after warm-up, Pre = before MRT, Post n = n minutes after MRT.

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

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

Significantly different between adolescents and adults ; ^{c} $P < 0.05$.

Strong ion difference and total concentration of the weak acids. In children SID values three minutes after MRT differed from those measured before ($P < 0.001$) and one hour after ($P < 0.01$) (Table 11). In adolescents SID values three minutes after MRT differed from those measured before ($P < 0.001$) and one hour after ($P < 0.05$). In adults SID values three minutes after MRT differed from those measured before ($P < 0.001$) and one hour after ($P < 0.001$). The decrease from SID values before MRT to values three minutes after is significantly smaller in children compared to adolescents (6.8 ± 2.3 vs. 10.1 ± 3.8 mEq/l, $P < 0.05$) and to adults (6.8 ± 2.3 vs. 13.2 ± 2.3 mEq/l, $P < 0.01$). In children A_{tot} values three minutes after MRT differed from those measured before ($P < 0.001$) and one hour after ($P < 0.01$). In adolescents A_{tot} values three minutes after MRT

differed from those measured before ($P < 0.001$) and one hour after ($P < 0.001$. In adults A_{tot} values three minutes after MRT differed from those measured before ($P < 0.001$) and one hour after ($P < 0.001$). The increase from A_{tot} values before MRT to values three minutes after was greater in adults compared to children (2.4 ± 0.5 vs. 1.6 ± 0.6 mEq/l, $P < 0.01$) and to adolescents (2.4 ± 0.5 vs. 1.8 ± 0.5 mEq/l, $P < 0.05$).

TABLE 11. Mean ± SD values for strong ion difference (SID) and total concentration of the weak acids (A_{tot}) in MRT.

	SID (mEq/l)			A_{tot} (mEq/l)		
Group	Children	Adolescents	Adults	Children	Adolescents	Adults
Morning	35.8 ± 4.5	41.7 ± 3.1 [c]	38.6 ± 3.7	16.5 ± 0.6	17.3 ± 0.7	17.2 ± 1.0
Pre	38.6 ± 4.9	40.4 ± 2.3	41.2 ± 4.7	16.5 ± 0.8 [a,b]	17.5 ± 0.8	16.8 ± 0.9
Post 3	31.9 ± 5.7	30.3 ± 4.5	27.7 ± 5.0	18.1 ± 0.7 [aa,bb]	19.3 ± 0.7	19.3 ± 1.0
Post 60	35.4 ± 5.7	38.4 ± 3.8	38.3 ± 4.6	16.5 ± 1.0	17.0 ± 0.6	17.1 ± 0.7

Pre = before MRT, Post n = n minutes after MRT.

Significantly different between children and adolescents ; [a] $P < 0.05$, [aa] $P < 0.01$.

Significantly different between children and adults ; [b] $P < 0.05$, [bb] $P < 0.01$.

Significantly different between adolescents and adults ; [c] $P < 0.05$.

Blood testosterone and cortisol. In children testosterone concentration three minutes after MRT differed from those measured before ($P < 0.05$) (Table 12). In adolescents testosterone concentration one hour after MRT differed from those measured before ($P < 0.01$) and three minutes after ($P < 0.01$). In adults testosterone concentration three minutes after MRT differed from those measured before ($P < 0.05$) and one hour after ($P < 0.01$). The change in testosterone concentration from the values measured before MRT to the values measured three minutes after differed in adults compared to children (2.35 ± 2.15 vs. -0.33 ± 0.39 nmol/l, $P < 0.05$) and to adolescents (2.35 ± 2.15 vs. -0.06 ± 2.39 nmol/l, $P < 0.05$). Cortisol concentration three minutes after MRT differed from those measured before in children ($P < 0.01$) and adults ($P < 0.05$). The increase from cortisol concentration values before MRT to values three minutes after differed between groups (children 102 ± 76 nmol/l vs. adolescents 12 ± 46 nmol/l, $P < 0.05$, children 102 ± 76 nmol/l vs. adults 115 ± 93 nmol/l, $P < 0.05$ and adolescents 12 ± 46 nmol/l vs. adults 115 ± 93 nmol/l, $P < 0.05$).

TABLE 12. Mean ± SD testosterone and cortisol in MRT.

	Testosterone (nmol/l)			Cortisol (nmol/l)		
Group	Children	Adolescents	Adults	Children	Adolescents	Adults
Morning	5.1 ± 5.1 ^{aa,bbb}	18.7 ± 4.4	17.0 ± 3.2	404 ± 103 ^{a,b}	500 ± 97	526 ± 72
Pre	3.1 ± 3.5 ^{aa,bb}	15.0 ± 4.1	13.6 ± 3.3	243 ± 112 ^{bb}	276 ± 56 ^{cc}	466 ± 139
Post 3	2.8 ± 3.1 ^{aa,bbb}	14.9 ± 3.5	16.0 ± 3.6	346 ± 161 ^{bb}	289 ± 57 ^{cc}	581 ± 137
Post 60	2.2 ± 2.8 ^{aa,bb}	11.3 ± 3.6	12.6 ± 4.2	312 ± 98 ^{bb}	289 ± 74 ^{cc}	535 ± 117

Pre = before MRT, Post n = n minutes after MRT.

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

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

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

Blood sex-hormone binding globulin and testosterone/cortisol –ratio. In children SHBG values three minutes after MRT differed from those measured before ($P < 0.05$) and one hour after ($P < 0.01$) (Table 13). In adolescents SHBG values three minutes after MRT differed from those measured one hour after ($P < 0.05$). In adults SHBG values three minutes after MRT differed from those measured before ($P < 0.01$) and one hour after ($P < 0.01$). In children the testosterone/cortisol-ratio values three minutes after MRT differed from those measured before ($P < 0.05$). In adolescents the testosterone/cortisol -ratio values one hour after MRT differed from those measured before ($P < 0.05$) and three minutes after ($P < 0.01$). In adults the testosterone/cortisol -ratio values three minutes after MRT differed from those measured one hour after the run ($P < 0.05$).

TABLE 13. Mean ± SD values for sex-hormone binding globulin (SHBG) and testosterone/cortisol -ratio in MRT.

	SHBG (nmol/l)			Testosterone/Cortisol -ratio		
Group	Children	Adolescents	Adults	Children	Adolescents	Adults
Morning	55.2 ± 21.6 [b]	38.7 ± 12.5	33.4 ± 8.0	13.2 ± 13.2 [aa,bb]	38.8 ± 13.3	33.2 ± 6.6
Pre	58.9 ± 22.6 [b]	37.4 ± 8.9	32.2 ± 8.5	16.8 ± 18.4 [aa,bb]	57.9 ± 26.4 [c]	32.2 ± 15.5
Post 3	63.0 ± 24.7	41.1 ± 10.9	38.4 ± 9.4	12.6 ± 15.0 [aa,bb]	54.8 ± 21.0 [cc]	29.6 ± 11.7
Post 60	58.7 ± 22.4 [a,b]	35.8 ± 8.7	33.0 ± 9.7	11.2 ± 16.6 [a,bb]	41.2 ± 14.6 [c]	24.7 ± 9.5

Pre = before MRT, Post n = n minutes after MRT.

Significantly different between children and adolescents ; [a] $P < 0.05$, [aa] $P < 0.01$.

Significantly different between children and adults ; [b] $P < 0.05$, [bb] $P < 0.01$.

Significantly different between adolescents and adults ; [c] $P < 0.05$, [cc] $P < 0.01$.

9 DISCUSSION

Main findings. All three age groups were able to reach a high 5 s peak oxygen uptake (VO_{2peak} 96 - 103 %) and also a high 30 s maximal oxygen uptake (VO_{2max} 91 – 96 %) of their real VO_{2max} during the last 30 s in 52 - 54 s sprint running. Furthermore, in adult male athletes the role of anaerobic energy production was greater than in child and adolescent athletes and, therefore, they also achieved greater acidosis.

Running performance and fatigue. Each of the three age groups experienced significant fatigue during MRT. This was observed through the consistent slowdown of running speed after the acceleration phase. In all groups the running speed decreased significantly between each successive 100 m intervals. The running speed decreased significantly from the fastest to the last 100 m interval by 12.2 %, 9.8 %, and 12.2 % in children, adolescents and adults, respectively. 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 (Hirvonen et al. 1992). 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 (Hanon and Gajer 2009). 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 subjects in each age group were able to use the aggressive pacing strategy that is characteristics to the competitive runs in speed endurance events, and, hence, induced fatigue. Also CMJ in every group worsened after the runs and showed strong fatigue in leg muscles.

Oxygen uptake. In the VO_{2max} running test maximal oxygen uptake, as the greatest 30 s average, was 60.4 ml/kg/min for adults, which is close to VO_{2max} measured in elite level 400 m runners (Slowinska-Lisowska and Majda 2002). The present study indicated that

during 400 m simulated race well-trained adult male athletes were able to reach high peak oxygen uptake (VO_{2peak}) before the decrease in VO_2. This VO_{2peak} value (101 % of their VO_{2max} measured in VO_{2max} running test) was greater than observed in previous field studies where subjects reached 52 % (Reis et al. 2004), 82 % (Duffield et al. 2005), 89 % (Spencer and Gastin 2001) and 94 % (Hanon et al. 2010) of their VO_{2max} during a 400 m race. Previous studies have indicated that VO_2 during maximal exercise can be increased by employing an all-out start (Bishop et al. 2002; Gardner et al. 2003; Gastin et al. 1995), or a competition-start strategy (Hanon et al. 2008; Thomas et al. 2005). This may be attributable to the greater phosphocreatine (PCr) breakdown at the onset of exercise when using a very fast start procedure (Hirvonen et al. 1992). 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 (Duffield et al. 2005; Spencer and Gastin 2001) 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. (2010). Moreover, previous authors (Reis et al. 2004) have most likely used one minute moving average even though they reported using 10 s intervals or there have been some other errors in data collection, as 52 % does not sound reliable. In comparison, VO_{2max} in adults during MRT 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 (Hanon et al. 2010) and on treadmill (Nummela and Rusko 1995). The decreases in VO_2 and in velocity could be related to acidosis-induced inhibition of oxidative phosphorylation in

contracting muscle (Jubrias et al. 2003) and consequently, with the large decrease in ATP and PCr observed at the end of a 400 m race (Hirvonen et al. 1992).

Acid-base balance. In previous studies in adults, the post-fatigue levels of blood lactate concentration (from 13.5 to 22.0 mmol/l) (Ohkuwa et al. 1984; Medbø and Sejersted 1985; Nummela et al. 1992; Nummela et al. 1995; Hill 1999; Reis et al. 2004; Duffield et al. 2005; Hanon et al. 2010; Zouhal et al. 2010; Saraslanidis et al. 2011; Bret et al. 2013), blood pH (from 7.07 to 7.00) (Medbø and Sejersted 1985; Hanon et al. 2010) and blood bicarbonate concentrations (from 4.9 to 7.1 mmol/l) (Medbø and Sejersted 1985; Hanon et al. 2010) 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 (Kindermann and Keul 1977). The greatest single values in the current study were blood lactate concentration of 20.2 mmol/l and 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 (Kazcor et al. 2005) and faster elimination of glycolytic by-products in children (Beneke et al. 2005). The large anaerobic contribution and subsequent accumulation of metabolites especially hydrogen ions (low pH) (Kindermann et al. 1977) may contribute to the decrease in velocity observed in during the final 100 m (Hanon and Gajer 2009; Hanon et al. 2010).

The difference in main acid-base balance variables (La^-, pH, HCO_3^- and BE) was greater between adults and adolescents than children and adolescents. This also 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 30 s Wingate test (Beneke et al. 2005). 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 (Leithauser et al. 2007). The difference in the kinetics of the blood lactate concentration between children, adolescent 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.

The extravascular increase of lactate seems to have a maximum during the third decade of life (Beneke et al. 2005).

Energy system contributions. In the current study the anaerobic energy system contribution in adults was 53 % when calculated with the accumulated oxygen deficit (AOD) method. This anaerobic percentage was slightly smaller to that observed in other recent studies in track where percentages were 59 % (Duffield et al. 2005), 63 % (Zouhal et al. 2010), 76 % (Reis et al. 2005) and on treadmill where percentage was 57 % (Spencer and Gastin 2001). There could be many explanations why there are differences in energy system contributions between the results of this study and majority of other 400 m running studies. One of the reasons is the difference in 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 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 the AOD (Medbø et al. 1988). 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 (Bangsbo 1998). 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 it was and 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 same the anaerobic percentage as with the AOD method.

It should be noted that in this study and most of the other studies anaerobic energy production is not divided to lactic and alactic components. Alactic component PCr breakdown are estimated to be 12.5 % of the total energy used during a 400 m run

(Newsholme et al. 1992). The latter researchers evaluated that in world elite level 400m 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 (Arcelli et al. 2008). We could speculate that Newsholme's values might be true in elite level male 400 m runners (43 – 45 s) but on slower 400 m runners (around 50 s) the total ATP might be produced as follows: PCr 10 %, anaerobic glycolysis 50 % and oxygen uptake 40 %.

Even though the time used for MRT was similar in all three groups, the amount of work varied because of the different running distance and running speed for each group. 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 (Berthoin et al. 2003; Eriksson et al. 1971).

In addition to the divide into energy system distributions, performance could be divided into metabolic energy and neuromuscular energy. Metabolic energy is production of ATP and neuromuscular energy is neural activation of the muscles and force produced by the muscles including energy stored in elastic components during stretch-shortening cycle. It is impossible to calculate and compare these energies but they are both very important for running performance together with running economy (running technique).

Hormonal responses. In adults testosterone response was similar to the one that has been reported earlier by Slowinska-Lisowska and Majda (2002) 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 (2002) testosterone response in 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 hard to reach any conclusions about them. The greatest testosterone concentration in children was lower than the lowest 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. (2012) 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 showed 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.

Conclusions. These data demonstrate that in MRT lasting 52 - 54 s all age groups using normal pacing strategy can achieve a high 5 s peak value (96 – 103 %) and a high 30 s average value (91 – 96 %) of their individual maximal oxygen uptake. 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 running speed, 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 males comes mainly from speed and the ability of the males to use more anaerobic energy production.

Practical applications. This study suggests 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. Nevertheless, when planning training programs for young athletes, the aspects of mental and mechanical load on the immature body should not be left out of consideration. 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.

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