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Original article

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AUTHOR CONTRIBUTION STATEMENT

AD, SR, CT, and HM conceived and designed research. AD, SR, JB, CT and HM conducted experiments and collected data. AD, SR, QDL and HM analysed data. AD, SR, NA, CT and HM wrote the manuscript. AD, SR, JB, QDL, NA, CT and HM provided critical revisions important for intellectual content of the finished manuscript, approved the final version of the manuscript, and agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. All persons designated as authors qualify for authorship, and all those who qualify for authorship are listed.

CONSENT TO PARTICIPATE

Written informed consent was obtained from all individual included in the study, and from their parents or legal guardians.

CONSENT TO PUBLISH

Participants (and their parents or legal guardians) signed informed consent regarding publishing their data.

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CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

ABSTRACT

Purpose: The aim of the present study was to investigate (i) how glycolytic metabolism assessed by accumulated oxygen deficit (AOD_{gly}) and blood metabolic responses (lactate and pH) resulting from high-intensity exercise change during growth, and (ii) how lean body mass (LBM) influences AOD_{gly} and its relationship with blood markers.

Methods: Thirty-six 11- to 17-year-olds performed a 60-s all-out test on a rowing ergometer. Allometric modelling was used to investigate the influence of LBM and LBM + maturity offset (MO) on AOD_{gly} and its relationship with the extreme post-exercise blood values of lactate ($[La]_{max}$) and pH (pH_{min}) obtained during the recovery period.

Results: AOD_{gly} and $[La]_{max}$ increased while pH_{min} decreased linearly with LBM and MO (r^2= 0.46 to 0.72, $p< 0.001$). Moreover, AOD_{gly} was positively correlated to $[La]_{max}$ (r^2= 0.75, $p< 0.001$) and negatively correlated to pH_{min} (r^2= 0.77, $p< 0.001$). When AOD_{gly} was scaled for LBM, the coefficients of the relationships with blood markers drastically decreased by three to four times ($[La]_{max}$: r^2= 0.24, p= 0.002; pH_{min}: r^2= 0.30, $p< 0.001$). Furthermore, by scaling AOD_{gly} for LBM + MO, the correlation coefficients with blood markers became even lower ($[La]_{max}$: r^2= 0.12, p= 0.037; pH_{min}: r^2= 0.18, p= 0.009). However, MO-related additional changes accounted much less than LBM for the relationships between AOD_{gly} and blood markers.

Conclusion: The results challenge previous reports of maturation-related differences in glycolytic energy turnover and suggest that changes in lean body mass are a more powerful influence than maturity status on glycolytic metabolism during growth.

Keywords: accumulated oxygen deficit, lactate, allometric modelling, maturation, rowing, adolescent

73 **ABBREVIATIONS**

AOD_{gly}	Glycolysis-derived accumulated oxygen deficit
AOD_{tot}	Total accumulated oxygen deficit
APHV	Age at peak height velocity
$[BE]_{min}$	Minimal base excess concentration
BF	Body fat
BM	Body mass
BMI	Body mass index
CA	Chronological age
$[HCO_3^-]_{min}$	Minimal bicarbonate concentration
HR_{max}	Maximal heart rate
LBM	Lean body mass
$[La]_{max}$	Maximal lactate concentration
MO	Maturity offset
MPO	Mean power output
pH_{min}	Minimal pH
$P\dot{V}O_{2max}$	Power at maximal oxygen uptake
$OE_{phos+ox}$	phosphagen- and blood O_2 stores-derived oxygen equivalent
$\dot{V}O_{2max}$	Maximal oxygen uptake

74

INTRODUCTION

Following their finding of lower muscle lactate concentration resulting from maximal exercise in 13.6-year-old boys compared with young men, Eriksson et al. (1971) suggested that glycolytic activity was lower in children than adults. Furthermore, a lower activity level of muscle phosphofructokinase was found in 11- to 13-year-old boys as compared with 17- to 58-year-old adults (Eriksson et al. 1973; Gollnick et al. 1972). Other studies also reported lower glycolytic enzyme activities (lactate dehydrogenase, aldolase, pyruvate kinase) in 3- to 17-year-old children compared with 29- to 54-year-old adults (Berg et al. 1986; Kaczor et al. 2005). In addition, considering the significant positive relationships between testicular volume index and exercise-induced muscle lactate accumulation (Eriksson et al. 1971) and between salivary/serum testosterone concentration and peak blood lactate responses, some authors supported the contention that glycolysis is dependent on maturity status (Falgairette et al. 1991; Fellmann et al. 1988)

However, conclusions from early studies of the potential immaturity of glycolytic activity before puberty must be interpreted cautiously, as these studies were not systematically designed to test the effects of maturation and employed small sample numbers with a lack of continuum throughout the maturation process. In metabolic studies, children were mostly categorised as pre-pubertal and post-pubertal and issues related to the effect of maturation during the circumpubertal period have not been comprehensively addressed (Ratel et al. 2002). Also, early metabolic studies did not integrate dimensional changes into data interpretation from childhood into adolescence. Yet, scientific evidence highlighted the importance of total working muscle mass and muscle power output on lactate production in humans (Jensen-Urstad et al. 1994). In mammals, it has been also shown that activities of enzymes functioning in anaerobic glycogenolysis (glycogen phosphorylase, pyruvate kinase and lactate dehydrogenase) increase with increasing body mass (Emmett and Hochachka 1981). Moreover, while nonoxidative energy supply assessed by the accumulated oxygen deficit (AOD) was found to be positively associated with muscle mass involved during exercise (Bangsbø et al. 1993), it was also found to be correlated to the quantity of lactate accumulated after all-out exercise in adult rowers (Maciejewski et al. 2013) and muscle lactate concentration following maximal exercise in pubertal children (Eriksson et al. 1971). This suggests that there might be a dimensional effect on glycolysis-derived accumulated oxygen deficit (AOD_{gly}), metabolic by-products accumulation and their interrelation throughout growth, but this remains to be proven.

Taken together, the lower blood metabolic disturbances (i.e., lower blood lactate accumulation and higher pH) resulting from high-intensity exercise in young children (Ratel et al. 2002) could be related to their

lower glycolytic energy supply or reduced AOD_{gly} (Carlson and Naughton 1993) owing to their smaller body dimensions, e.g., lean body mass (LBM). Such arguments suggest that with LBM accounted for there might not be significant maturation-related differences in glycolytic energy turnover between pre-pubertal, mid-pubertal and post-pubertal children, contrary to what has been previously reported (Eriksson et al. 1971). However, direct evidence showing the respective influence of LBM and maturity status on the differences in AOD_{gly} and the potentially associated blood metabolic disturbances (i.e., lactate, pH) between pre-, mid- and post-pubertal children is still lacking.

Therefore, the purpose of the present study was to determine whether the increase in glycolytic metabolism during growth, assessed by AOD_{gly} and blood metabolic responses (i.e., increased blood lactate accumulation and decreased pH) is principally influenced by concomitant changes in LBM. We hypothesise that the lower blood lactate accumulation and higher pH resulting from high-intensity exercise in younger children could be explained by a reduced amount of energy released from glycolysis (i.e., AOD_{gly}) owing to a smaller LBM rather than maturity status. A proportional allometric modelling approach will be used to check our assumptions by controlling for the effects of LBM and maturity status.

MATERIALS AND METHODS

Participants

Thirty-six male competitive rowers aged from 11 to 17 years volunteered to participate in the present study. All participants trained three to six times per week (i.e., two to four "on-water" training sessions and one to two physical training sessions) preceding the experiments. None of the participants had a family history of cardiovascular disease or was using any medication. The present study was approved by an institutional ethics review board (Comité d'Éthique pour la Recherche en Sciences et Techniques des Activités Physiques et Sportives – CERSTAPS, n° 2017-29-11-20) and conformed to the standards of use of human participants in research as outlined in the *Sixth Declaration of Helsinki*. The participants were informed of the experimental procedures and gave their written assent before any testing was conducted. In addition, the written informed consent was obtained from the parents or legal guardians of the participants.

Experimental procedure

Volunteers were tested in two experimental sessions separated by at least 48 hours. Participants were instructed not to undertake any strenuous activity during the 24 hours preceding each session. The first session

was dedicated to gathering participants' physical characteristics (anthropometric measurements, body composition and maturity status) and maximal oxygen uptake ($\dot{V}O_{2max}$) assessment. During the second session, the volunteers performed a 60-s all-out test. The two exercise sessions were carried out on a rowing ergometer (Model D, Concept2, Morrisville, VT, USA). The young rowers were fully familiarised with the equipment. The computer of the ergometer continuously delivered the power output and stroke rates (in W and min^{-1}, respectively). The resistance factor was set by the investigators between 100 and 130 according to age and the expertise level of young rowers. The same resistance factor was kept for both tests. Verbal encouragement was systematically provided by the investigators during each exercise session.

Experimental measurements

Session 1: Anthropometric characteristics and body composition. Body mass (BM in kg) was measured using a digital weight scale with a precision of ± 0.01 kg (Seca 899, Seca, Germany). Standing height (in m) was assessed using a stadiometer with a precision of ± 1 mm (Seca 213, Seca, Germany). Sitting height (in m) was also measured with the stadiometer while the participants sat on the floor with their back against a wall. Body mass index (BMI) was subsequently calculated using a standard formula, as follows: mass divided by height squared (in $kg \cdot m^{-2}$). Skinfold thicknesses were measured in triplicate at the triceps and subscapular sites using a Harpenden calliper (British Indicators Ltd, St Albans, UK). The measurements were taken by the same experienced investigator on the right side of the body to reduce variability in the results. Body fat percentage (BF in %) and lean body mass (LBM in kg) were determined using Slaughter's et al. equations (Slaughter et al. 1988). These equations are specific to sex, ethnicity and maturity status, and are recommended for assessing BF and LBM in children 8-18 years of age.

Maturity status. Maturity offset (MO in years) was determined to assess somatic maturity (i.e., years to (from) age at peak height velocity, APHV) by using chronological age (CA), BM, standing height and sitting height. Its calculation was based on sex-specific regression equations according to the method proposed by Mirwald et al. (2002). Children were categorised by their maturity status (pre-, mid- or post-PHV) into discrete bands based on their MO (pre-PHV: < -1, mid-PHV: - 1 to +1, post-PHV: > 1) (Birat et al. 2020).

Maximal oxygen uptake test. Each participant performed a progressive test to exhaustion to determine maximal O_2 uptake ($\dot{V}O_{2max}$ in $L \cdot min^{-1}$). The initial power was set between 40 and 80 W during the first five minutes and

the power was incremented by 10-30 W every three minutes according to age and the expertise level of participants (Maciejewski et al. 2013). Arterialised capillary blood samples (20 μL) were taken from the earlobe at rest and every step to measure the time course of blood lactate concentration. Whole blood lactate concentrations ([La] in $mmol \cdot L^{-1}$) were determined enzymatically using a Biosen C-Line Clinic lactate analyser (EFK Diagnostics GmbH, Barleben, Germany).

Oxygen uptake, carbon dioxide output and ventilation were continuously monitored with a breath-by-breath analyser (Quark CPET, Cosmed, Italy). Heart rate was continuously recorded with a heart rate monitor (HRM-Dual, Garmin, Kansas, USA). The mechanical power output corresponding to $\dot{V}O_{2max}$ ($P\dot{V}O_{2max}$ in W) and the linear relationship between $\dot{V}O_2$ and power output were also assessed. $\dot{V}O_{2max}$ was considered to be reached during the last step when at least two of the following criteria were met: (i) $\dot{V}O_2$ levelling-off, (ii) maximal respiratory exchange ratio (RER_{max}) ≥1.1, (iii) maximal HR (HR_{max}) ≥ 95% of the age-predicted HR_{max} (208.6 – 0.7 · age) (age in years) (Shargal et al. 2015) and (iv) blood lactate concentration higher than 8 $mmol \cdot L^{-1}$.

Session 2: 60-s all-out test. After a standardised 20-min warm-up at about 130-140 $beats \cdot min^{-1}$ and two short sprints (10-s) in the last five minutes, all participants performed a 60-s all-out test. This test was followed by a 10-min sitting recovery. Cardio-respiratory parameters were continuously measured using a breath-by-breath analyser (Quark CPET, Cosmed, Italy). Capillary arterialised blood samples (80 μL) were drawn from the earlobe and collected after warm-up and at 1, 3, 5, and 8 min post-exercise to measure the time course of pH, and lactate ([La] in $mmol \cdot L^{-1}$), bicarbonate ($[HCO_3^-]$ in $mmol \cdot L^{-1}$) and base-excess ([BE] in $mmol \cdot L^{-1}$) concentrations. Blood [La] was determined enzymatically using the same lactate analyser as in the first session while pH, $[HCO_3^-]$ and [BE] were measured by direct potentiometry using an i-STAT® handheld analyser (Abbott Point of Care, Princeton, USA) immediately after collection. The maximal lactate concentration ($[La]_{max}$ in $mmol \cdot L^{-1}$), the minimal pH value (pH_{min}), and the minimal concentrations of bicarbonate ($[HCO_3^-]_{min}$ in $mmol \cdot L^{-1}$) and base-excess ($[BE]_{min}$ in $mmol \cdot L^{-1}$) were identified. Mean power output (MPO in W) was calculated over the entire test. In addition, total accumulated oxygen deficit (AOD_{tot} in L O_2 Eq.) and glycolysis-derived accumulated oxygen deficit (AOD_{gly} in L O_2 Eq.) were individually determined according to the procedures described below.

Measurements and calculations

Total accumulated oxygen deficit. AOD_{tot} was determined by subtracting accumulated O_2 uptake (the measured O_2 uptake integrated over time) from accumulated O_2 demand (the estimated O_2 demand integrated over time). In accordance with Green and Dawson (1996), oxygen demand was extrapolated using the equation of the $\dot{V}O_2$-power output linear regression obtained in session 1 and considering the individual value of $\dot{V}O_{2rest}$ (i.e., $\dot{V}O_2$ measured during three min before the test) for a power output equal to 0 W. Because the present study concerned all-out exercise, O_2 demand was calculated from instantaneous power output (recorded stroke by stroke) rather than mean power output sustained during exercise (i.e., MPO) as initially proposed by Medbø et al. (1988).

Glycolysis-derived AOD. AOD_{gly} was assessed by subtracting phosphagen- and blood O_2 stores-derived oxygen equivalent ($OE_{phos+ox}$) to AOD_{tot}. $OE_{phos+ox}$ was evaluated from the integral of the fast component of $\dot{V}O_2$ recovery kinetic using a bi-exponential 4-parameter model, as previously done (Beneke et al. 2002; di Prampero 1981):

$$y = A_1 \cdot e^{-\frac{t}{\tau_1}} + A_2 \cdot e^{-\frac{t}{\tau_2}} + y_0 \qquad \text{(Eq. 1)}$$

where A_1, A_2 (in $L \cdot min^{-1}$) are the amplitudes of fast and slow components, respectively; τ_1 and τ_2 the corresponding time constants (in min), and y_0 (in $L \cdot min^{-1}$) the asymptotic resting $\dot{V}O_2$ at time $\rightarrow \infty$. Fitted $\dot{V}O_2$ data were then integrated over time from the start of recovery to τ_1 value, and resting $\dot{V}O_2$ value integrated over the same time was subtracted.

Allometric modelling procedures. Allometric approach is used to remove any dimensional effect on parameters, and thereby allow fair comparisons between populations of different body dimensions and compositions. As the large range of LBM in the studied population (30.1 to 78.7 kg) may have influenced the capacity to supply glycolytic nonoxidative energy (i.e., AOD_{gly}), we further investigated the influence of LBM on the relationships between AOD_{gly} and extreme blood metabolic responses (i.e., $[La]_{max}$, pH_{min}, $[HCO_3^-]_{min}$ and $[BE]_{min}$), by analysing these relationships with LBM as scaling factor through an allometric modelling procedure. The allometric relationship obtained between LBM and AOD_{gly} was based on the general allometric equation (Nevill et al. 1992):

$$AOD_{gly} = a_1 \cdot LBM^{b_1} \quad \text{(Eq. 2)}$$

where a_1 is the proportionality coefficient and b_1 the scaling factor associated with LBM. The resultant power function ratio $AOD_{gly} \cdot LBM^{b_1}$ is allegedly free from the confounding influence of LBM. The statistical approach to allometry is to use a simple logarithmic transformation as follows:

$$log\,(AOD_{gly}) = b_1 \cdot log\,(LBM) + log\,(a_1) \quad \text{(Eq. 3)}$$

where b_1 is the slope of the linear regression. This slope is calculated by regression analysis, where b_1 in the regression output is equal to the scaling factor and the inverse log of log a_1 is equivalent to the constant a_1 in the Eq. 2.

If this first methodological approach totally accounts for the effects of LBM on AOD_{gly}, it is important to underline that LBM could also be influenced by individual maturity status. As a result, we also used the multiplicative allometric model proposed by Nevill and Holder (1994) including the maturity indicator as a second factor within an exponential term in addition to the LBM component. This procedure takes into account both LBM and the effect of MO on LBM on AOD_{gly} as follows:

$$AODgly = LBM^{b_2} \cdot exp\,(a_2 + c \cdot MO) \quad \text{(Eq. 4)}$$

where a_2 is the proportionality coefficient, b_2 and c are the scaling factors associated with LBM and MO, respectively.

The statistical approach to allometry is to use a multiple logarithmic transformation (as previously done by Carvalho et al. 2012), as follows:

$$log\left(AOD_{gly}\right) = b_2 \cdot log\,(LBM) + a_2 + c \cdot MO \quad \text{(Eq. 5)}$$

where b_2 and c are the slopes of the multiple linear regression. These slopes are calculated by ordinary multiple regression analysis where b_2 and c are equal to the scaling factors and the inverse log of log a_2 is equivalent to the constant a_2 in Eq. 4.

Statistical analysis

Analyses were performed using OriginPro 2020 software (OriginLab, Massachusetts, USA). Descriptive statistics were expressed as mean ± standard deviation (SD) by maturity groups (pre, mid, post). Differences between maturity groups were analysed using the non-parametric Kruskal-Wallis test. Mann-Whitney test was completed for pairwise comparisons when the Kruskal-Wallis test revealed a significant effect. Linear regression models between MO, LBM, AOD_{gly}, and blood parameters (i.e., $[La]_{max}$, pH_{min}, $[HCO_3^-]_{min}$ and $[BE]_{min}$) were fitted by the least-squares method. The squared Bravais-Pearson correlation coefficients (r^2) of these linear regression models were calculated. The statistical significance level was set at 5% (i.e. $p < 0.05$).

RESULTS

Participants' physical and physiological characteristics

The physical characteristics and aerobic fitness of participants are detailed by maturity groups in Table 1. CA, MO, standing height, BM, BMI, LBM, $\dot{V}O_{2max}$ ($L \cdot min^{-1}$) and $P\dot{V}O_{2max}$ significantly increased while BF significantly decreased from the pre-PHV to post-PHV group ($p < 0.001$).

Table 1 around here

60-s all-out test

Mechanical and physiological parameters obtained from the 60-s all-out test (i.e., MPO, AOD_{tot}, AOD_{gly}, $OE_{phos+ox}$, $[La]_{max}$, pH_{min}, $[HCO_3^-]_{min}$, $[BE]_{min}$) are detailed by maturity groups in Table 2. MPO, AOD_{tot}, AOD_{gly} $OE_{phos+ox}$, and $[La]_{max}$ significantly increased while pH_{min}, $[HCO_3^-]_{min}$ and $[BE]_{min}$ significantly decreased after the 60-s all-out exercise from the pre-PHV to post-PHV group ($p < 0.001$).

Table 2 around here

Allometric modelling exponents

Allometric scaling exponents a_1 and b_1 obtained by the simple procedure (i.e., Eq. 2) were -2.40 and 1.60, respectively. Allometric scaling exponents a_2, b_2 and c obtained from the multiple procedure (i.e., Eq. 4) were -3.96, 1.19 and 0.08, respectively.

Correlations between variables

Relationships between metabolic responses, MO and LBM are displayed in Table 3. AOD_{gly}, $[La]_{max}$, pH_{min}, $[HCO_3^-]_{min}$ and $[BE]_{min}$ were significantly correlated to MO and LBM ($p < 0.001$ for all) (Table 3).

Table 3 around here

In addition, $[La]_{max}$ ($r^2 = 0.75$, $p < 0.001$), pH_{min} ($r^2 = 0.77$, $p < 0.001$), $[HCO_3^-]_{min}$ ($r^2 = 0.69$, $p < 0.001$) and $[BE]_{min}$ ($r^2 = 0.74$, $p < 0.001$) were highly correlated to AOD_{gly} (Figures 1A, 1B, 1C, 1D, respectively). However, when AOD_{gly} was scaled for $LBM^{1.60}$ the determination coefficients of the relationships with blood markers drastically decreased by three to four times ($[La]_{max}$: $r^2 = 0.24$, $p = 0.02$; pH_{min}: $r^2 = 0.30$, $p < 0.001$; $[HCO_3^-]_{min}$: $r^2 = 0.19$, $p = 0.008$; $[BE]_{min}$: $r^2 = 0.18$, $p = 0.009$) (Figures 1E, 1F, 1G, 1H, respectively). Furthermore, by considering the additional effect of MO on LBM, the correlation coefficients between AOD_{gly} and blood markers became even lower ($[La]_{max}$: $r^2 = 0.12$, $p = 0.037$; pH_{min}: $r^2 = 0.18$, $p = 0.009$; $[HCO_3^-]_{min}$: $r^2 = 0.11$, $p = 0.051$; $[BE]_{min}$: $r^2 = 0.10$, $p = 0.061$) (Figures 1I, 1J, 1K, 1L, respectively).

Figure 1 around here

DISCUSSION

The present study aimed to investigate (i) how glycolytic metabolism assessed by accumulated oxygen deficit (AOD_{gly}) and blood metabolic responses (i.e., lactate and pH) changes during growth and (ii) how concomitant changes in lean body mass (LBM) influence AOD_{gly} and its relationship with lactate accumulation and pH reduction. The main results were consistent with our hypotheses since (i) AOD_{gly} and blood metabolic responses concurrently increased with maturity status (MO) and lean body mass (LBM), and were correlated, and (ii) concomitant changes in LBM accounted much more than maturity status for AOD_{gly} and its relationship with lactate accumulation and pH reduction. The results indicate that with LBM accounted for there may not be significant maturation-related differences in glycolytic energy turnover, contrary to what has been previously mentioned.

In the present study, MO and LBM highly accounted for the increment of AOD_{gly} (71% and 61%, respectively (Table 3). This finding is consistent with previous cross-sectional studies showing that (i) total AOD

is significantly lower in children than adolescents and adults during high-intensity exercise (Carlson and Naughton 1993; Naughton et al. 1997) and (ii) the kinetics of O_2 uptake at the onset of high-intensity exercise are faster in children compared with adults (Armon et al. 1991). The results suggest that prepubertal children may adapt their oxidative metabolism faster than their older counterparts to meet the higher energy requirements and, hence, have a lower need for nonoxidative metabolism at the onset of high-intensity exercise. However, the present data indicate for the first time a progressive increase in glycolysis-specifically derived AOD from childhood into adolescence and this is associated with LBM and MO (Table 3).

The blood metabolic responses (i.e., $[La]_{max}$, pH_{min}, $[HCO_3^-]_{min}$, $[BE]_{min}$) were also highly associated with LBM and MO (Table 3). These findings are consistent with previous studies showing lower end-of-exercise blood [La] and higher pH in prepubertal boys than men, either after a Wingate test (Hebestreit et al. 1996) or following repeated cycle sprints (Ratel et al. 2002). However, in the present study, the metabolic stress elicited by high-intensity exercise during rowing was higher than from other forms of exercise in children. Indeed, in previous studies, high-intensity exercises yielded post-exercise peak blood [La] from 7.7 to 8.4 $mmol \cdot L^{-1}$ during cycling (Falgairette et al. 1991) or 7.8 to 11.0 $mmol \cdot L^{-1}$ during running (Paterson et al. 1986) between the ages of 10 and 15 years while data from our population ranged from 10.2 to 15.9 $mmol \cdot L^{-1}$ (Table 2). Similarly, end-of-exercise blood pH values of 7.36-7.37 in 10-year-old boys and 7.28 in 15-year-old male adolescents after repeated sprints have been reported (Kappenstein et al. 2015; Ratel et al. 2004) while values obtained in the present study were 7.24 in pre-pubertal boys and 7.13 in post-pubertal boys. Such discrepancies could be ascribed to differences in body mass engaged during exercise as blood metabolic responses were found to be highly associated with LBM. Even if the body mass is not supported in rowing, it involves a greater muscle mass during exercise [about 85% of the total muscle mass (Mader et al. 1988; Maciejewski et al. 2013)], than other modalities of exercise such as cycling [about 25 to 30% of body mass (Gastin 2001)], or running (Bangsbø et al. 1993). The greater muscle mass involved in rowing could elicit a greater glycolytic energy supply and thereby a higher blood lactate accumulation and pH or $[HCO_3^-]$ reduction (Bangsbø et al. 1993; Saltin 1990). However, direct evidence showing the effect of exercise mode on blood metabolic responses during high-intensity exercise during growth and maturation is still lacking.

Another interesting point is that AOD_{gly} was highly correlated to $[La]_{max}$, pH_{min}, $[HCO_3^-]_{min}$ and $[BE]_{min}$ (Figures 1A, 1B, 1C, 1D). The higher the AOD_{gly}, the greater glycolytic by-products accumulation found during

growth. This finding is novel as no previous studies have analysed the relationships between the (total and glycolysis-derived) accumulated O_2 deficit and blood acid-base disturbance in response to high-intensity exercise during growth. Only Naughton et al. (1997) evaluated total AOD and plasma markers of anaerobic metabolism (lactate, pH, ammonium) during treadmill runs at 120-130% of $\dot{V}O_{2max}$ in 14-year-old trained male and female adolescents but these authors did not establish any relationship between the parameters. In adults, previous studies have provided contradictory data on these relationships; some authors failed to find significant correlations between $[La]_{max}$ and total AOD in elite athletes (Bangsbø et al. 1993) while others found an association between the quantity of lactate accumulated and total AOD in well-trained rowers (Maciejewski et al. 2013). This discrepancy could be related to methodological issues given that $[La]_{max}$ reflects the balance between production and removal of lactate at the time of measurement while the quantity of lactate accumulated is an estimate of total production considering the quantity of lactate removed from the body. Although in the present study the quantity of lactate accumulated was not assessed and $[La]_{max}$ was measured, AOD_{gly} highly accounted for the variance of blood lactate concentration (i.e., 75%). This was also the case for the other blood metabolic responses (i.e., 77%, 69% and 74% for pH_{min}, $[HCO_3^-]_{min}$ and $[BE]_{min}$, respectively).

In the present study, the allometric exponent calculated to reduce the effect of LBM on AOD_{gly} was positive (1.60), supporting the notion that AOD_{gly} increased with LBM. However, this allometric exponent was clearly higher than the coefficients usually calculated between body mass and $\dot{V}O_{2max}$ (e.g., 0.75) (Armstrong and Welsman 2019; Nevill et al. 1992) or between LBM and $\dot{V}O_{2max}$ (i.e., 1.13) in the present study (Table 1). This allometric exponent is consistent with the high body size allometric exponents reported during high-intensity exercises, as for instance in adolescent rowers during a 30-s all-out test (i.e., 1.24) (Maciejewski et al. 2016). Consequently, body size exponents could be higher in anaerobic vs aerobic exercise conditions, and anaerobic metabolic responses could be greater in heavier than lighter individuals, as it is the case for AOD_{gly} in the present study. This could be ascribed to additional anaerobic energy required to move (lean) body mass during all-out exercise compared to aerobic exercise, particularly in heavier individuals.

The results of the present study suggest that LBM is the main influence on the AOD_{gly} and its relationship with blood parameters. When the effect of LBM was considered in the relationships between AOD_{gly} and $[La]_{max}$, pH_{min}, $[HCO_3^-]_{min}$ and $[BE]_{min}$, the coefficients of determination drastically decreased by three to four times with respect to those obtained from absolute AOD_{gly} values (Figures 1E, 1F, 1G, 1H). The additional effect of MO on these relationships accounted for much less than LBM, as the coefficients of

determination decreased to a lesser extent (Figures 1I, 1J, 1K, 1L). Taken together, this indicates that there would be an associated-cumulative effect of maturity status in addition to the significant influence of LBM on AOD_{gly} and its relationship with blood metabolic responses. However, LBM could be considered as the main influence on these metabolic changes.

Several considerations should be mentioned in this study. The number of years the rowers have been training prior to the study was different between younger and older participants. However, this difference should not affect the conclusions of the present study since aerobic fitness evaluated from $\dot{V}O_{2max}$ normalised for $LBM^{1.13}$ was not significantly different between the maturity status groups (Table 1). Moreover, although validation studies of maturity offset indicated several limitations for the prediction of biological maturation (Fransen et al. 2018), the level of accuracy of this method was found to be sufficient to assign a maturational classification with a standard error of 0.5 years between the ages of 8 and 16 years (Mirwald et al. 2002). If we had used the Tanner staging criteria based on pubic hair and testicular volume, we could not have analysed the effect of maturation on AOD_{gly} and its relationship with blood markers from the allometric method, as these criteria are categorical variables while maturity offset is a continuous discrete variable. Another point of consideration is that girls were not evaluated. Sex-related differences on AOD_{gly} and blood responses could be expected during puberty, as body composition (whole-body lean vs fat mass) changes significantly over this period between males and females (Van Praagh and Dore 2002), but this remains to be proven.

In conclusion, AOD_{gly} and blood metabolic responses in boys linearly increased with maturity status and lean body mass. Lactate accumulation and pH reduction resulting from high-intensity exercise were found to be highly associated with the amount of energy released from glycolysis. Changes in lean body mass during growth accounted much more than maturity status for glycolysis-derived accumulated oxygen deficit and its relationship with lactate accumulation and pH reduction. The results challenge previous reports of maturation-related differences in glycolytic energy turnover and suggest that changes in lean body mass are a more powerful influence than maturity status on glycolytic metabolism during growth.

In the occupational, exercise or sporting context, the results of the present study may prove relevant since it has often been considered that anaerobic glycolysis is immature in pre-pubertal children (Eriksson et al. 1971) and it is, therefore, useless to propose physical activities soliciting the glycolytic process to improve

anaerobic capacity before puberty (Ratel and Martin 2012). This work could lead physical educators, teachers, students and coaches who have to train young people to reconsider these conclusions, as dimensional changes could mainly account for glycolytic activity during growth. However, this work needs to be pursued in girls, since body composition (whole-body lean vs fat mass) changes significantly during growth between boys and girls (Tanner et al. 1981), and lean body mass could act differently on glycolytic metabolism between both sexes.

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Table 1: Physical characteristics and aerobic fitness of the different maturity groups (n = 36) (mean ± SD).

	Pre-PHV (n = 8)	Mid-PHV (n = 11)	Post-PHV (n = 17)
Chronological age (years)	12.4 ± 0.9	13.7 ± 0.7 **	16.2 ± 0.9 *** $$$
MO (years)	-1.7 ± 0.6	0.0 ± 0.5 ***	2.2 ± 0.6 *** $$$
Standing height (m)	1.55 ± 0.09	1.70 ± 0.05 **	1.80 ± 0.07 *** $$$
BM (kg)	43.6 ± 6.2	60.7 ± 3.3 ***	70.5 ± 6.8 *** $$$
BMI ($kg \cdot m^{-2}$)	18.0 ± 1.3	21.0 ± 1.1 ***	21.7 ± 1.6 ***
BF (%)	15.1 ± 4.0	10.6 ± 5.3	8.6 ± 3.1 ***
LBM (kg)	37.0 ± 5.0	54.4 ± 4.9 ***	64.4 ± 6.0 *** $$$
$\dot{V}O_{2max}$ ($L \cdot min^{-1}$)	2.36 ± 0.43	3.47 ± 0.55 ***	4.54 ± 0.28 *** $$$
$\dot{V}O_{2max}$ ($mL \cdot min^{-1} \cdot LBM^{-1.13}$)	40 ± 5	38 ± 5	41 ± 4
$P\dot{V}O_{2max}$ (W)	126 ± 24	204 ± 40 **	272 ± 25 *** $$$
HR_{max} (bpm)	203 ± 9	206 ± 7	200 ± 6

**: $p < 0.01$; ***: $p < 0.001$ from Pre ; $$$: $p < 0.001$ from Mid.

MO: maturity offset; BM: body mass; BMI: body mass index; BF: body fat percentage; LBM: lean body mass; $\dot{V}O_{2max}$ ($mL \cdot min^{-1} \cdot kg^{-1.13}$ LBM): $\dot{V}O_{2max}$ normalized for $LBM^{1.13}$, allometric exponent was calculated from the following equation: $\log(\dot{V}O_{2max}) = b_1 \cdot \log(LBM) + \log(a_1)$ (see section *Allometric modelling procedures* for further explanations); $P\dot{V}O_{2max}$ power corresponding to $\dot{V}O_{2max}$; HR_{max}: maximal heart rate.

524 **Table 2:** Mechanical and physiological parameters obtained from the 60-s all-out test by maturity group (n = 36)

525 (mean ± SD).

526

	Pre-PHV (n = 8)	Mid-PHV (n = 11)	Post-PHV (n = 17)
MPO (W)	198 ± 37	343 ± 60 ***	515 ± 45 *** $$$
AOD_{tot} (L O_2 Eq.)	2.08 ± 0.45	3.39 ± 0.60 ***	5.23 ± 0.68 *** $$$
AOD_{gly} (L O_2 Eq.)	1.29 ± 0.33	2.10 ± 0.49 ***	3.39 ± 0.73 *** $$$
$OE_{phos+ox}$ (L)	0.79 ± 0.18	1.29 ± 0.44 **	1.84 ± 0.38 *** $$$
AOD_{gly} (%AOD_{tot})	61.7 ± 6.1	61.9 ± 11.4	64.4 ± 7.5
$OE_{phos+ox}$ (%AOD_{tot})	38.3 ± 6.1	38.1 ± 11.4	35.6 ± 7.5
$[La]_{max}$ (mmol·L^{-1})	10.2 ± 1.5	12.3 ± 1.1 **	15.9 ± 1.8 *** $$$
pH_{min} (-)	7.24 ± 0.03	7.21 ± 0.03 **	7.13 ± 0.04 *** $$$
$[HCO_3^-]_{min}$ (mmol·L^{-1})	13.5 ± 1.3	11.3 ± 1.3 **	8.7 ± 1.3 *** $$$
$[BE]_{min}$ (mmol·L^{-1})	-13.3 ± 2.0	-16.6 ± 1.7 **	-20.3 ± 1.9 *** $$$

527 **: $p < 0.01$; ***: $p < 0.001$ from Pre ; $^{\$\$\$}$: $p < 0.001$ from Mid.

528 MPO: mean power output calculated over the entire test; AOD_{tot}: total accumulated oxygen deficit; AOD_{gly}:

529 glycolysis-derived accumulated oxygen deficit; $OE_{phos+ox}$: phosphagen- and blood O_2 stores-derived oxygen

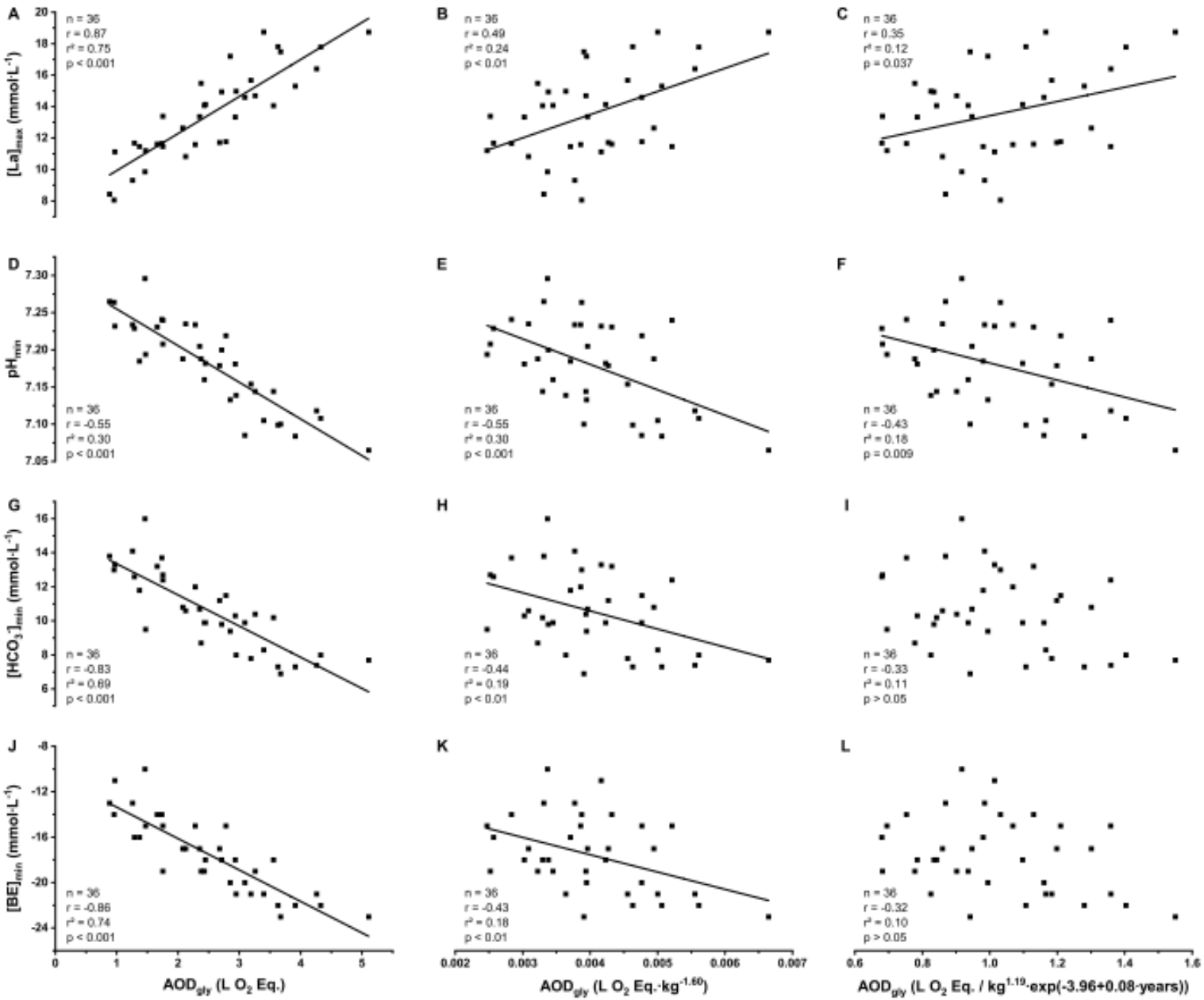
530 equivalent; $[La]_{max}$: maximal blood lactate concentration; pH_{min}: minimal blood pH; $[HCO_3^-]_{min}$: minimal blood

531 bicarbonate concentration; $[BE]_{min}$: minimal blood base excess concentration.

Table 3: Relationships between metabolic responses, maturity offset and lean body mass (n = 36).

	LBM (kg)	AOD_{gly} (L O_2 Eq.)	$[La]_{max}$ ($mmol \cdot L^{-1}$)	pH_{min} (-)	$[HCO_3^-]_{min}$ ($mmol \cdot L^{-1}$)	$[BE]_{min}$ ($mmol \cdot L^{-1}$)
MO (years)	r = 0.91 p < 0.001	r = 0.84 p < 0.001	r = 0.85 p < 0.001	r = -0.78 p < 0.001	r = -0.78 p < 0.001	r = -0.82 p < 0.001
LBM (kg)	-	r = 0.78 p < 0.001	r = 0.78 p < 0.001	r = -0.68 p < 0.001	r = -0.77 p < 0.001	r = -0.79 p < 0.001

MO: maturity offset; LBM: lean body mass; AOD_{gly}: glycolysis-derived accumulated oxygen deficit; $[La]_{max}$: maximal blood lactate concentration; pH_{min}: minimal blood pH; $[HCO_3^-]_{min}$: minimal blood bicarbonate concentration; $[BE]_{min}$: minimal blood base excess concentration.


A
n = 36
r = 0.87
r² = 0.75
p < 0.001
[La]max (mmol·L-1)
B
n = 36
r = 0.49
r² = 0.24
p < 0.01
C
n = 36
r = 0.35
r² = 0.12
p = 0.037
D
n = 36
r = -0.55
r² = 0.30
p < 0.001
pHmin
E
n = 36
r = -0.55
r² = 0.30
p < 0.001
F
n = 36
r = -0.43
r² = 0.18
p = 0.009
G
n = 36
r = -0.83
r² = 0.69
p < 0.001
[HCO3-]min (mmol·L-1)
H
n = 36
r = -0.44
r² = 0.19
p < 0.01
I
n = 36
r = -0.33
r² = 0.11
p > 0.05
J
n = 36
r = -0.86
r² = 0.74
p < 0.001
[BE]min (mmol·L-1)
AODgly (L O2 Eq.)
K
n = 36
r = -0.43
r² = 0.18
p < 0.01
AODgly (L O2 Eq.·kg-1.60)
L
n = 36
r = -0.32
r² = 0.10
p > 0.05
AODgly (L O2 Eq. / kg1.19·exp(-3.96+0.08·years))

536

FIGURE LEGEND

Figure 1: Correlations between blood markers and glycolysis-derived accumulated oxygen deficit (AOD_{gly}) expressed in absolute value (Panels A to D), scaled for $LBM^{1.60}$ (Panels E to H) and scaled for LBM + MO (Panels I to L); n = 36.

$[La]_{max}$: maximal blood lactate concentration; pH_{min}: minimal blood pH; $[HCO_3^-]_{min}$: minimal blood bicarbonate concentration; $[BE]_{min}$: minimal blood base excess concentration; LBM: lean body mass; MO: maturity offset.