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The sensitivity of the alternative maximal accumulated oxygen deficit method to discriminate training status

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ABSTRACT

The purpose of the study was to investigate the sensitivity of an alternative maximal accumulated oxygen deficit ($MAOD_{ALT}$) method to discriminate the "*anaerobic*" capacity while comparing: least trained (LT) participants (n = 12), moderately trained (MT) participants (n = 12), endurance trained (ET) participants (n = 16), and rugby (RG) players (n = 11). Participants underwent a graded exercise test on a treadmill and a supramaximal effort for assessing $MAOD_{ALT}$. $MAOD_{ALT}$ was calculated as the sum of oxygen equivalents from the phosphagen and glycolytic metabolic pathways. $MAOD_{ALT}$ was significantly higher ($P < 0.05$) in RG (64.4 ± 12.1 mL · kg^{-1}) than in ET (56.8 ± 5.4 mL · kg^{-1}; effect size [ES] = 0.77; +13.5%), MT (53.8 ± 5.3 mL · kg^{-1}; ES = 1.08; +19.8%), and LT (49.9 ± 4.5 mL · kg^{-1}; ES = 1.50; +36.4%). In addition, the magnitude-based inference analysis revealed that $MAOD_{ALT}$ was *likely* (LT vs. MT), *very likely* (MT vs. RG, and ET vs. RG) and *most likely* (LT vs. ET, and LT vs. RG) different between all groups, except for MT and ET, which presented an *unclear* difference. In conclusion, $MAOD_{ALT}$ was sensitive enough to distinguish the "*anaerobic*" capacity in individuals with different training status, especially for RG players compared with LT participants and MT participants.

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KEYWORDS
"Anaerobic" capacity; blood lactate response; excessive post-exercise oxygen consumption; physical conditioning

Introduction

The blood lactate response (i.e., post-exercise minus resting values) and fast phase of the excess post-exercise oxygen consumption (i.e., $EPOC_{FAST}$) have been jointly used to estimate the "*anaerobic*" capacity in a single exhaustive supramaximal effort, resulting in the so-called alternative maximal accumulated oxygen deficit ($MAOD_{ALT}$) (Bertuzzi, Kiss, Damasceno, Oliveira, & Lima-Silva, 2015; Bertuzzi et al., 2010; Brisola, Miyagi, da Silva, & Zagatto, 2015; Zagatto, Bertuzzi, Miyagi, Padulo, & Papoti, 2016; Zagatto & Gobatto, 2012). This method seems to have good practical application due to its methodological simplicity compared to the conventional maximal accumulated oxygen deficit (MAOD), a time-consuming protocol requiring several exercise bouts to be performed (i.e., ~10 submaximal efforts and a supramaximal effort) (Noordhof, de Koning, & Foster, 2010), while $MAOD_{ALT}$ requires only two sessions (i.e., an incremental test and a supramaximal effort). Besides the assessment of "*anaerobic*" capacity (i.e., the maximal amount of energy that can be resynthesised by non-mitochondrial energy systems), $MAOD_{ALT}$ can also be used to estimate the energy contributions of the phosphagen (E_{PCr}) and glycolytic ($E_{[La]}$) metabolic pathways (Bertuzzi et al., 2015).

$MAOD_{ALT}$ is not statistically different from MAOD (Bertuzzi et al., 2010; Zagatto & Gobatto, 2012; Zagatto, Bertuzzi, et al., 2016), and recently Zagatto, Bertuzzi, et al. (2016) reported that the best intensity to perform the supramaximal effort was 115% of the intensity associated with maximal oxygen uptake ($i\dot{V}O_{2max}$), which leads to a greater $MAOD_{ALT}$ outcome, as well as good reliability (ICC = 0.87). Furthermore, $MAOD_{ALT}$ was not altered after an acute high dose of taurine (Milioni et al., 2016) or caffeine (de Poli, Miyagi, Nakamura, & Zagatto, 2016), demonstrating that $MAOD_{ALT}$ is not affected by these ergogenic aids that improve performance. However, despite all the potential advantages of $MAOD_{ALT}$, it is still unclear if $MAOD_{ALT}$ can distinguish the "*anaerobic*" capacity between groups with different training levels.

Gastin and Lawson (1994) noted higher MAOD values for sprint trained (60 mL · kg^{-1}) than untrained (50 mL · kg^{-1}) or endurance trained (ET) (52 mL · kg^{-1}) individuals. In addition, Brisola et al. (2015) showed that acute sodium bicarbonate ingestion improved $MAOD_{ALT}$ and the $E_{[La]}$, evidencing that $MAOD_{ALT}$ could be a sensitive enough measure to detect physiological adaptations in non-mitochondrial metabolism, resembling the specific training-related effects. Thus, the purpose of the present study was to investigate the sensitivity of $MAOD_{ALT}$ to distinguish the "*anaerobic*" capacity in groups

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with different levels of training. Given that the MAOD method is the most accepted test to assess the "*anaerobic*" capacity, we hypothesised that $MAOD_{ALT}$ would be capable of distinguishing between distinct "*anaerobic*" performance levels. Therefore, in the current study the $MAOD_{ALT}$ values determined in least trained (LT) individuals, moderately trained (MT) individuals, ET runners, and rugby (RG) players who have a known high conditioning of "*anaerobic*" capacity, [i.e., MAOD = ~ 99 mL · kg^{-1}; (Moore & Murphy, 2003)] were compared.

Methods

Participants

About 51 healthy men were recruited for this study and were allocated to the LT group (n = 12), MT group (n = 12), recreational endurance runners (n = 16), and RG sevens group (n = 11). Those in LT engaged in ≤149 min · wk^{-1} of physical activity such as soccer, futsal, walking, and jogging; those in MT engaged in ≥150 min · wk^{-1} of physical activity around 3 times per week in activities such as soccer, futsal, jogging, and others, but were not athletes. The endurance runners systematically performed endurance training with a physical trainer and maintained a training volume of ~200 min · wk^{-1} for 37.1 ± 11.6 km. Members of the national RG seven players trained at least 5 times per week, 845 ± 122 min · wk^{-1} and regularly trained in RG for 11.8 ± 6.3 years. Participants characteristics are summarised in Table 1.

All participants were familiarised with the experimental procedures (i.e., maximal running on treadmill) and equipment (i.e., they performed exercise using the gas analyser at least twice to mimic the exercise procedure) and were instructed to eat the same light meal at least 2 h before the tests, to arrive euhydrated, and to perform no heavy training sessions in the 48 h before the test. The participants were instructed to avoid alcohol or caffeine ingestion during their participation in the study. All procedures were approved by the University's Institutional Review Board for Human Subjects (Human Research Ethics Committee – process number 645.784/2014) and were conducted according to the Declaration of Helsinki. Athletes were informed about the experimental procedures and risks, and provided written informed consent prior to the start of the study.

Experimental procedures

The experimental tests were conducted over a period of 10 days, with the participants performing 3 visits to the laboratory. On the first visit body composition was assessed using dual-energy X-ray absorptiometry (Hologic QDR, Discovery, Bedford, USA); on the second visit a graded exercise test (GXT) was performed, followed by the supramaximal effort on the third visit. The exercise sessions were separated by a minimum interval of 48 h.

The GXT and supramaximal effort tests were performed on a motorised treadmill (ATL, Inbramed, Inbrasport, Porto Alegre, RS, Brazil) with a fixed inclination of 1%. To eliminate any influence of circadian variation, each participant completed all the trials at the same time period of a day in controlled environmental conditions (temperature of 20.3 ± 1.5°C and relative humidity of 50.2 ± 4.3%). During the GXT and the supramaximal test, participants were verbally encouraged to perform maximally and wore a chest harness with the rope attached to the ceiling to ensure maximal effort without fall risk.

Prior to each testing effort, a warm-up lasting 5 min at 8 km · h^{-1} was performed and the tests started 5 min after the end of the warm-up.

Physiological measurements

The respiratory data were collected breath-by-breath during all tests using a Cosmed Quark CPET gas analyzer system (Quark CPET, Cosmed, Rome, Italy) coupled with a heart rate transmitter belt (Wireless HR 138 Monitor, Cosmed, Rome, Italy). Before the start of each test, the gas analyser was calibrated with a high-precision gas mixture and ambient air, whereas the volume transducer was calibrated before each test and verified after each test using a 3-L calibration syringe (Hans-Rudolph, Shawnee, Kans., USA), in accordance with the manufacturer's instructions. The breath-by-breath oxygen uptake ($\dot{V}O_2$) data were smoothed using a rolling 5-s average and interpolated to provide 1-s values for modelling $\dot{V}O_2$ response (OriginPro 8.0; Origin Lab Corporation, Microcal, MA, USA).

To measure blood lactate concentration ([La]), blood samples were drawn from an earlobe (25 µL) at rest before the GXT and supramaximal effort, and after the supramaximal effort. Blood samples were stored at −20°C in tubes containing 50 µL of sodium fluoride (1%) and later analysed using an electrochemical lactate analyser (Yellow Springs Instruments model 2300, Ohio, USA) (measurement error of ±2%).

Graded exercise test (GXT) to assess maximal oxygen uptake ($\dot{V}O_{2max}$) and the associated exercise intensity $i\dot{V}O_{2max}$

The GXT started at 8 km · h^{-1} (after warm-up) and the velocity increased by 1.5 km · h^{-1} every 2-min until voluntary exhaustion (Brisola et al., 2015; Zagatto, Bertuzzi, et al., 2016). The

Table 1. GXT variables for least trained (LT), moderately trained (MT), endurance trained runners (ET), and rugby players (RG).

Variables	LT	MT	ET	RG	$F_{(3;50)}$	P-value
Age (years)	25 ± 8(20–30)	27 ± 5(24–31)	27 ± 6(24–30)	24 ± 4(22–27)	0.72	0.544
Height (cm)	173.4 ± 4.4(170.6–176.2)	175.4 ± 7.2(170.8–180.0)	177.0 ± 6.3(173.6–180.3)	179.9 ± 8.6(174.1–185.7)	1.91	0.141
Body mass (kg)	69.6 ± 9.1 (63.8–75.3)	77.2 ± 11.0 (70.2–84.2)	72.1 ± 7.5 (68.1–76.1)	90.2 ± 12.0 (82.7–97.7)	10.64	0.0001
Fat mass (%)	16.1 ± 3.3 (14.0–18.3)	18.1 ± 4.8 (15.1–21.2)	13.9 ± 5.7 (10.9–16.9)	14.5 ± 4.8 (11.3–17.7) †‡&	2.02	0.124

P < 0.05 least trained group (LT); ‡ P < 0.05 moderately trained group (MA); & P < 0.05 endurance trained runner group (ET). Values are mean ± SD (95%CI)

Borg scale (6–20) was used to assess the rating of perceived exertion (RPE) at the end of each stage and after exhaustion. $\dot{V}O_2$ was measured during the entire test and the highest $\dot{V}O_2$ average (i.e., $\dot{V}O_2$ average over the final 30 s of each stage) was assumed as $\dot{V}O_{2max}$, considering the verification of a plateau in $\dot{V}O_2$ (variation in $\dot{V}O_2 < 2.1$ mL · kg^{-1} · min^{-1} between the final and penultimate stage of exercise). Secondary criteria were: (1) maximal HR (HRmax) ≥ 90% of predicted HRmax (Tanaka, Monahan, & Seals, 2001); (2) respiratory exchange ratio (RER) ≥ 1.10, and (3) peak lactate ≥ 8.0 mmol · L^{-1}. The minimal exercise intensity at which the participant reached $\dot{V}O_{2max}$ was considered as i$\dot{V}O_{2max}$ (Billat, Blondel, & Berthoin, 1999). If the final stage had not been completed, the i$\dot{V}O_{2max}$ was determined using the method proposed by Kuipers, Verstappen, Keizer, Geurten, and van (1985) [i$\dot{V}O_{2max}$= running speed of the final complete stage + (velocity increment after each stage × time sustained during the incomplete stage /total time of stage)].

The respiratory compensation point (RCP) was considered as the exercise intensity at which an increase in both ventilatory equivalents of O_2 ($\dot{V}E/\dot{V}O_2$) and ventilatory equivalents of carbon dioxide production (CO_2) ($\dot{V}E/\dot{V}CO_2$) over workload occurred (Zagatto, Miranda, & Gobatto, 2011; Zagatto, Leite, Papoti, & Beneke, 2016).

Supramaximal exhaustive effort and assessment of $MAOD_{ALT}$

Prior to the supramaximal exhaustive effort, $\dot{V}O_2$ was measured throughout 10 min of seated rest for $\dot{V}O_2$ baseline determination ($\dot{V}O_{2baseline}$; considered as the $\dot{V}O_2$ average over the last 2 min of rest). The supramaximal effort was performed at 115% of i$\dot{V}O_{2max}$ as described by Zagatto, Bertuzzi, et al. (2016). Time-to-exhaustion of the supramaximal effort was recorded. Respiratory data were collected for another 7 min after the end of the supramaximal effort for determination of the fast component of excess post-exercise oxygen consumption ($EPOC_{FAST}$), along with blood sampling from the earlobe in the 3rd, 5th, and 7th minutes to determine [La]. The highest $\dot{V}O_2$ reached at exhaustion was also determined ($\dot{V}O_{2EX}$), as the $\dot{V}O_2$ average over the final 20 s of exercise.

$MAOD_{ALT}$ was calculated as the sum of the oxygen equivalents of the phosphagen metabolic pathway (E_{PCr}) and glycolytic metabolic pathway ($E_{[La]}$) (Bertuzzi et al., 2010; Brisola et al., 2015; de Poli et al., 2016; Zagatto & Gobatto, 2012; Zagatto, Bertuzzi, et al., 2016) (test and retest ICC = 0.87; data from our laboratory (Zagatto, Bertuzzi, et al., 2016)).

$E_{[La]}$ was estimated by subtracting resting [La] from peak post-exercise blood lactate concentration (Δ[La]), considering a value of 1 mmol · L^{-1} to be equivalent to 3 mL O_2·kg^{-1} body mass (di Prampero, 1981; di Prampero & Ferretti, 1999). The E_{PCr} was considered to be the $EPOC_{FAST}$ (di Prampero & Ferretti, 1999; Margaria, Edwards, & Dill, 1933; Zagatto, Leite, et al., in press), which was estimated by multiplication of the amplitude and time constant of the fast component of a bi-exponential model (Equation (1)) using OriginPro 8.0 software (OriginLab Corporation, Microcal, Massachusetts, USA) (Equation (2)) (di Prampero & Ferretti, 1999; Margaria et al., 1933; Zagatto, Bertuzzi, et al., 2016).

$$\dot{V}O_{2(t)} = \dot{V}O_{2baseline} + A_1[e^{-(t-\delta)/\tau_1}] + A_2[e^{-(t-\delta)/\tau_2}] \quad (1)$$

$$E_{PCr} = A1 \times \tau 1, \quad (2)$$

where $\dot{V}O_{2(t)}$ is the oxygen uptake at time t, $\dot{V}O_{2baseline}$ is the oxygen uptake at baseline, A is the amplitude, δ is the time delay, and τ is the time constant. 1 and 2 represent the fast and slow components, respectively.

Statistical analysis

The data are presented as mean ± standard deviation (SD) and confidence interval of 95% (CI 95%). Data normality was initially verified by the Shapiro–Wilk test, allowing the use of parametric statistical analysis. For analysis of the values from the supramaximal effort outcomes and $MAOD_{ALT}$ among groups, a one-way repeated measures analysis of variance was used for comparisons. In addition, Mauchly's sphericity test was applied to the data and sphericity was assumed to be violated when the "F" test was significant. In case of sphericity violation, the Greenhouse-Geisser Epsilon correction was used. Analyses were completed using the "Bonferroni" *post hoc* test. In all cases, a significance level of 5% was assumed.

As the MAOD difference between trained participants was expected to be small (Heugas, Brisswalter, & Vallier, 1997), magnitude-based inference analysis was also used. The raw outcomes were log-transformed prior to the analysis to reduce non-uniformity of error (Hopkins, Marshall, Batterham, & Hanin, 2009). Magnitude-based inference was used to determine the practical significance and smallest worthwhile changes (non-clinical inference), using the method described by Batterham and Hopkins (2006). The threshold values for Cohen's d statistical power were considered as >0.2 (small), >0.5 (moderate), and >0.8 (large) (Cohen, 1988). A Cohen's unit of 0.2 was used to determine the smallest worthwhile value of change, and changes ≥75% likely to exceed the smallest important ES were considered meaningful. Using a Microsoft Excel® spreadsheet designed for sports science research (Batterham & Cox, 2006), mean effects, and the 95% confidence limits were estimated to establish the percentage likelihood of each experimental condition having a positive/trivial/negative effect on $MAOD_{ALT}$. Thus, the chances of benefit were qualitatively evaluated as follows: 0.5–5% = *very unlikely*; 5–25% = *unlikely*; 25–75% = *possibly*; 75–95% = *likely*; 95–99.5% = *very likely*; and >99.5% = *most likely* (Hopkins et al., 2009). When the positive and negative values were both >5%, the inference was classified as *unclear*.

Results

The age, height, and percentage of fat mass were similar among the groups, but the body weight in the RG was higher ($F_{(3.50)}$ = 10.64; P = 0.0001) compared with LT, MT, and ET (Table 1).

The physiological responses during the GXT are shown in Table 2. The $\dot{V}O_{2max}$, i$\dot{V}O_{2max}$, and RCP were statistically different between the groups ($P < 0.001$). The respiratory exchange

Table 2. GXT variables for least trained (LT), moderately trained (MT), endurance trained runners (ET), and rugby players (RG).

Variables	LT	MT	ET	RG	$F_{(3;50)}$	*P*-value	Statistical power (%)
$\dot{V}O_{2max}$ (mL · kg^{-1} · min^{-1})	48.6 ± 4.5(45.8–51.1)	50.0 ± 5.1(46.8–53.2)	55.9 ± 4.9(52.9–57.6)†‡	48.2 ± 3.3(46.0–50.4)$^{\&}$	8.79	0.0001	100.0
$\dot{V}O_{2max}$ (km · h^{-1})	13.2 ± 1.2(12.4–14.0)	14.5 ± 0.6(14.1–14.9)†	16.6 ± 1.4(15.8–17.3)†‡	14.9 ± 1.0(14.3–15.6)$^{\dagger\&}$	23.19	0.00001	100.0
RCP (km · h^{-1})	10.5 ± 0.8(10.0–11.0)	11.8 ± 0.6(11.4–12.2)†	13.9 ± 0.9(13.5–14.4)†‡	12.2 ± 1.1(11.5–12.9)$^{\dagger\&}$	39.20	0.00001	100.0

$^{\dagger}P < 0.05$ least trained group (LT); ‡ $P < 0.05$ moderately trained group (MA); and $P < 0.05$ endurance trained runner group (ET). Values are mean ± SD (95%CI)
$\dot{V}O_{2max}$: maximal oxygen uptake; i$\dot{V}O_{2max}$: intensity associated with maximal oxygen uptake; RCP: respiratory compensation point;

ratio (LT = 1.16 ± 0.08, MT = 1.16 ± 0.05, ET = 1.17 ± 0.05, and RG = 1.18 ± 0.05, P = 0.843), RPE (17.1 ± 1.8, 18.2 ± 1.8, 19.2 ± 1.0, and 18.8 ± 1.3, respectively; P = 0.115) and peak [La] (10.2 ± 2.0, 10.4 ± 1.8, 11.0 ± 2.1, and 12.0 ± 4.0 mmol · L^{-1}, respectively; P = 0.353) did not differ.

Table 3 shows the supramaximal effort values of time-to-exhaustion, $\dot{V}O_2$ reached at exhaustion point ($\dot{V}O_{2EX}$), resting [La], peak [La], Δ[La], and the $EPOC_{FAST}$ outputs. Significant differences were observed only for time-to-exhaustion (P = 0.015) and $\dot{V}O_{2EX}$ (P = 0.013), with the ET group presenting lower time-to-exhaustion compared to the other groups. However, ET presented the highest $\dot{V}O_{2EX}$ during the supramaximal effort (P = 0.004). The RG presented higher peak and Δ[La] than the LT, whereas the τ1 (i.e., time constant of the first exponential) in the RG was higher than in all the other groups. However, the A_1 ($\dot{V}O_2$ amplitude) in the RG was lower than in the ET (Table 3).

In addition, Figure 1 shows the oxygen equivalents from the $E_{[La]}$ (Figure 1(a,b)), E_{PCr} (Figure 1(c,d), and the $MAOD_{ALT}$ values (Figure 1(e,f) for all groups. For the values presented relative to body mass (i.e., mL · kg^{-1}), the RG showed higher $E_{[La]}$, E_{PCr}, and $MAOD_{ALT}$ than the LT (34.5, 45.5, and 36.4%, respectively), MT (17.9%, 32.5%, and 19.8%, respectively) and ET (16.5, 40.6, and 13.5%, respectively), except for $E_{[La]}$ where the RG was statistically higher only in relation to the LT. For the absolute values (i.e., L) the outcomes were similar.

The magnitude-based inference analysis showed meaningful differences for $MAOD_{ALT}$ (both expressed in L and mL · kg^{-1}), presenting moderate to large ESs, and that $MAOD_{ALT}$ was effective in distinguishing individuals with different physical training backgrounds (Table 4) (Probability of difference: ≥92% between LT and MT; ≥99% between LT and ET; 100% between LT and RG; ≥98% between MT and RG; and ≥91% between ET and RG). Only the comparison between the MA and ET showed an *unclear* difference (ES = −0.01; 31% of positive; 41% of trivial, and 28% of negative). The magnitude-based inference analysis also showed that E_{PCr} and $E_{[La]}$ were effective for distinguishing individuals with different training status (Table 4); however, the magnitude-based inference analysis showed an *unclear* outcome for comparisons between LT and ET, and between MT and ET for both parameters.

Discussion

The purpose of the present study was to investigate the sensitivity of $MAOD_{ALT}$ to distinguish the "*anaerobic*" capacity in groups with different levels of training. As hypothesised, $MAOD_{ALT}$ was capable of discriminating between groups that are expected to display distinct levels of "*anaerobic*" capacities.

Team sports players are expected to present very high "*anaerobic*" pathway activity (Moore & Murphy, 2003) to allow them to cope with match demands, which comprise high-intensity intermittent efforts and repeated sprints. For instance, one of the highest values of MAOD recorded in the literature was obtained in elite RG union players (99.4 mL · kg^{-1}) (Moore & Murphy, 2003). To our knowledge, there are no previous studies quantifying the MAOD in RG sevens players. However, their performance levels in sprint, repeated-sprint, Yo-Yo Intermittent Recovery Test level 1, and $\dot{V}O_{2max}$ can be considered high and comparable to other team sports players. For this reason, elite RG sevens players are expected to display superior $MAOD_{ALT}$ levels. In fact, our RG presented higher $MAOD_{ALT}$ than the ET (13.5%), MT (19.8%), and LT (36.4%). These differences favouring the RG were reflected in both the E_{PCr} and $E_{[La]}$, demonstrating that RG sevens players develop both the phosphagen and glycolytic pathways in their selection and training routines.

These findings were reinforced by magnitude-based inference analysis, with $MAOD_{ALT}$ presenting meaningful differences between LT vs. MT (*likely meaningful*), MT vs. RG *(very likely meaningful)* ET vs. RG *(very likely meaningful*), LT vs. ET *(most likely meaningful)*, and LT vs. RG *(most likely meaningful)* (Table 4). The E_{PCr} and $E_{[La]}$ assessed in the RG were also higher than the other groups and presented *very likely* to *most likely meaningful* effects compared to the other groups. However, the *unclear* inference for comparisons between LT and ET, and between MT and ET could be attributed to infrequent high-intensity stimuli generally performed by these groups during exercise.

Concerning the sensitivity of MAOD to training effects, Heugas et al. (1997) reported that conventional MAOD was decreased after 3 months of intense aerobic training in elite sprint runners. In addition, Gastin and Lawson (1994) reported that ET cyclists presented similar conventional MAOD compared to untrained participants (52 vs. 50 mL · kg^{-1}, respectively), which were consistent with our findings [i.e., no significant difference (P > 0.05)] among LT, MT, and ET individuals). However, as expected, both groups were inferior to sprint trained cyclists (when MAOD was expressed in absolute values) (Gastin & Lawson, 1994). This is also consistent with our findings since RG players (the most "anaerobically" trained group) presented higher $MAOD_{ALT}$ that the other groups.

As aforementioned, the $MAOD_{ALT}$ is assessed from sums of the glycolytic and phosphagen metabolic pathways, which are estimated after a supramaximal effort until exhaustion (i.e., 115% $\dot{V}O_{2EX}$) which lasted between 102 and 218 s. According to Medbo et al. (1988), this effort is long enough for a severe depletion in intramuscular stores of phosphocreatine and

Table 3. Supramaximal trial values for least trained (LT), moderately trained (MT), endurance trained runners (ET), and rugby players (RG).

Variable	LT	MT	ET	RG	$F_{(3;50)}$	*P*-value	Statistical power (%)
tlim (s)	163.3 ± 52.4(130.0–196.6)	154.8 ± 38.9(130.1–179.6)	119.2 ± 32.8(101.7–136.7)	176.2 ± 62.5(134.2–218.1)[&]	3.86	0.015	100
$\dot{V}O_{2EX}$ (mL · kg^{-1} · min^{-1})	47.1 ± 3.9(44.7–49.6)	48.8 ± 6.7(44.5–53.1)	53.6 ± 6.0(50.4–56.8)[†]	46.3 ± 4.2(43.4–49.1)[&]	5.10	0.004	100
Resting [La] (mmol · L^{-1})	1.1 ± 0.4(1.0–1.3)	1.2 ± 0.5(0.8–1.5)	1.1 ± 0.3(0.9–1.3)	1.0 ± 0.4(0.7–1.2)	0.545	0.654	76.7
Peak [La] (mmol · L^{-1})	10.7 ± 1.8(9.6–11.8)	11.7 ± 1.6(10.7–12.8)	12.0 ± 2.0(10.9–13.0)	13.4 ± 3.6(11.0–15.9)[†]	2.71	0.045	100
Δ[La] (mmol · L^{-1})	9.6 ± 1.8(8.5–10.8)	10.5 ± 1.4(9.7–11.4)	10.9 ± 2.0(9.8–11.9)	12.5 ± 3.7(10.0–15.0)[†]	2.92	0.044	100
$EPOC_{FAST}$							
A1 (mL · kg^{-1} · min^{-1})	20.1 ± 1.7(19.0–21.2)	20.7 ± 2.7(18.9–22.4)	22.2 ± 2.8(20.7–23.7)	19.1 ± 2.1(17.7–20.7)[&]	3.84	0.015	100
τ1 (min)	1.03 ± 0.16(0.93–1.13)	1.07 ± 0.11(1.00–1.14)	1.00 ± 0.12(0.94–1.07)	1.42 ± 0.17(1.31–1.43)[† ‡&]	22.3	0.0001	100

[†]$P < 0.05$ least trained group; [‡] $P < 0.05$ moderately trained group; and $P < 0.05$ endurance trained runner group. Values are mean±SD (95%CI).
Tlim: Time to exhaustion; $\dot{V}O_{2EX}$: peak oxygen uptake; Resting [La]: resting blood lactate value; Peak [La]: peak blood lactate value; Δ[La]: difference between peak and resting lactate values; A1: Amplitude of fast phase of EPOC; τ1: time constant of fast phase of EPOC.

glycogen to occur. Therefore, the sum of oxygen equivalents from both non-mitochondrial pathways seems to correspond to the intramuscular "*anaerobic*" stores.

The assumptions accepted to estimate $MAOD_{ALT}$ have two important limitations. The first-one concerns the acceptance of the $EPOC_{FAST}$ to estimate the oxygen equivalent from the phosphagen pathway. Despite decades of the assumption that the initial seconds of EPOC are the oxygen above baseline values necessary to replenish the phosphocreatine (PCr) depleted during high-intensity exercise, other studies have reported that $EPOC_{FAST}$ does not seem to correspond exclusively to replenishment of PCr, also seeming to be influenced by the participation of the glycolytic pathway. However, Korzeniewski and Zoladz (2013) reported an inverse relationship between the $\dot{V}O_2$ work-to-rest kinetics and the phosphocreatine work-to-rest kinetics in computer simulations, supporting the hypothesis that in fact the $EPOC_{FAST}$ corresponds to oxygen demand for the replenishment of PCr. It is relevant to point out that in our study the athletes with greater $\dot{V}O_{2max}$ values were the endurance athletes, whereas the RG were the group that had the greater magnitude of the phosphagen pathway (Table 4). Based on the fact that VO_2 amplitude was not altered between the groups, it seems that a likely determinant factor for greater "*anaerobic*" capacity was a higher τ1. The greater τ1 found in the RG (1.42 ± 0.17 min) indicates that these players take longer to replenish PCr, probably due to the fact that these players have higher values of phosphocreatine (i.e., higher phosphagen pathway capacity).

The second limitation, and more relevant, is based on the fact that glycolytic pathway activity was estimated from blood lactate responses considering the difference between peak and resting values. In this procedure, we assumed the oxygen equivalent of 3.0 mL · kg^{-1} to each 1 mmol · L^{-1} of Δ [La] (di Prampero & Ferretti, 1999). Despite several studies having used this procedure to estimate the glycolytic metabolism pathway (Bertuzzi, Franchini, Kokubun, & Kiss, 2007; Bertuzzi et al., 2010, 2015), this relationship was fitted using linear regression (di Prampero & Ferretti, 1999) instead of a stoichiometric relationship. This means that this method is unable to establish a chemical equation that represents, at least in experimental conditions, the exact equivalence to its respective oxygen equivalent for each 1 mmol · L^{-1} of blood lactate accumulated. Bangsbo et al. (1990) reported after a one-legged, dynamic knee-extensor exercise until exhaustion (≈3.2 min), that the muscle lactate concentration increased from 2 to 28.1 mmol · (kg wet wt)$^{-1}$, and the concomitant net lactate release was 14.8 mmol · (kg wet wt)$^{-1}$ (1/3 of the total net lactate production) and during recovery just 70% of the accumulated lactate was released to the blood. Additionally, the glycolytic metabolic pathway estimate from blood samples may not correspond exactly to the non-mitochondrial energy dispensed, however this measurement seems to be strongly related (di Prampero & Ferretti, 1999; Margaria et al., 1933). In fact, this means that an athlete with higher blood lactate response in a maximal effort has greater muscular "*anaerobic*" capacity and/or power (Fujitsuka, Yamamoto, Ohkuwa, Saito, & Miyamura, 1982).

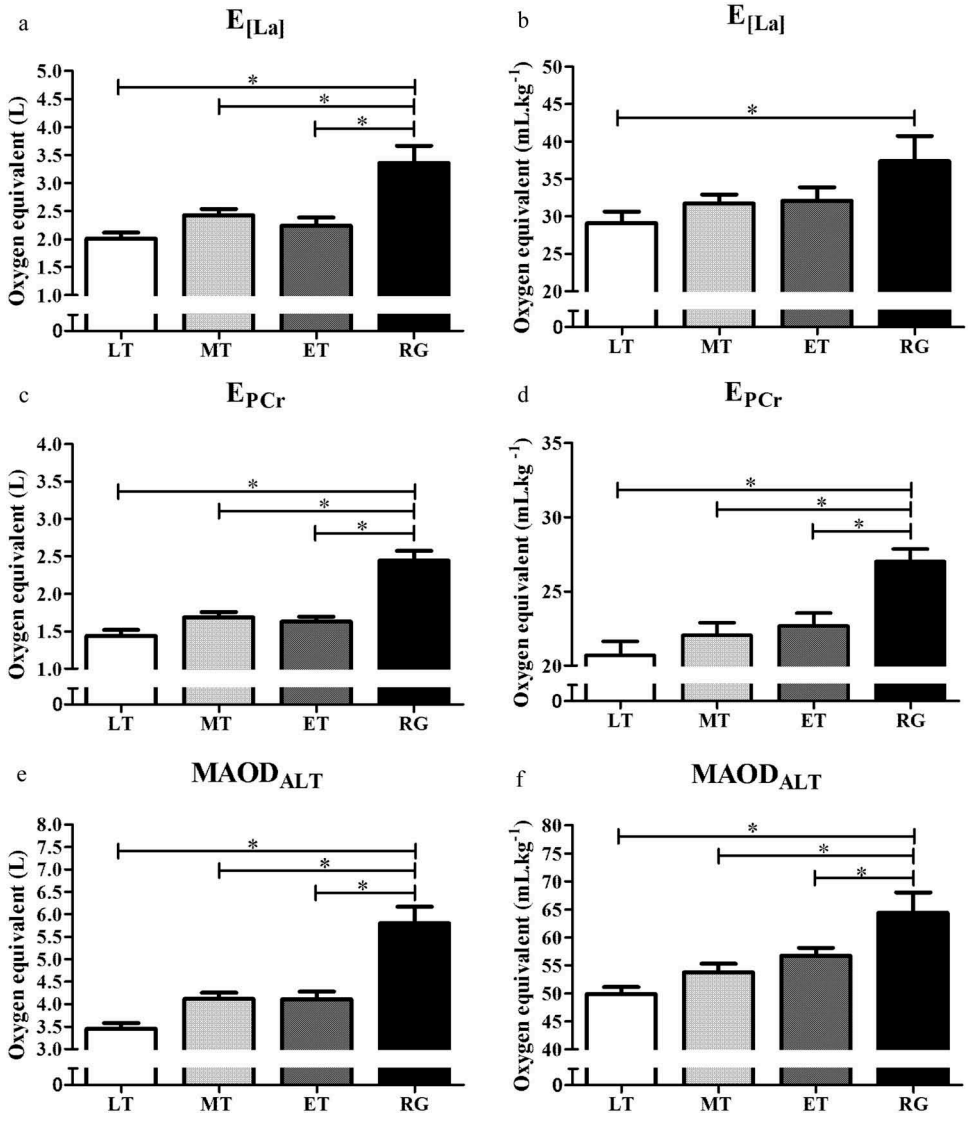

Figure 1. Means and standard deviation of oxygen equivalents from $E_{[La]}$ (Figure 1(a,b)) and E_{PCr} (Figure 1(c,d)), and the $MAOD_{ALT}$ values (Figure 1(e,f) for least trained (LT), moderately trained (MT),endurance trained runners (ET), and rugby sevens players (RG).Note:*$P < 0.05$ as compared to RG.

Despite the aforementioned limitation concerning the contribution of the glycolytic pathway, it was reported that athletes with greater performance in short-distance events and with high non-mitochondrial activity (i.e., 100, 200, 400, and 800 m sprints) have higher blood lactate values after high-intensity effort (Fujitsuka et al., 1982; Hautier et al., 1994; Lacour, Bouvat, & Barthelemy, 1990). In this way, we understand that athletes with higher lactatemia are the athletes with greater glycolytic pathway capacity. This hypothesis is supported by significant differences found in blood lactate in the RG compared to the other groups (Table 4).

Despite our findings, until now, these findings do not allow the assumption that this procedure is able to determine the intramuscular "*anaerobic*" capacity; but we are reporting the sensitivity of $MAOD_{ALT}$ to different levels of training status. These findings suggest that the $MAOD_{ALT}$ could be an attractive index to evaluate the "status of *anaerobic* conditioning" of athletes. It is important to note that the present study provides preliminary normative values of $MAOD_{ALT}$ to classify the "*anaerobic*" capacity of participants with different training backgrounds. Of note, previous studies have demonstrated values of 55.8 mL · kg^{-1} of $MAOD_{ALT}$ in recreationally trained runners (Milioni et al., 2016) and ~52 mL · kg^{-1} of $MAOD_{ALT}$ in MT males (Brisola et al., 2015; Zagatto, Bertuzzi, et al., 2016), which are similar to the values presented herein (53.8 mL · kg^{-1} of $MAOD_{ALT}$ in the MT group). However, in the current study the different levels of training status were classified by training minutes per week instead of a physiological index such as $\dot{V}O_{2max}$, however training minutes per week was used because $\dot{V}O_{2max}$ is not a good parameter to classify "anaerobic" fitness.

Therefore, we can conclude that $MAOD_{ALT}$ assessed in treadmill running can be considered a sensitive enough procedure to distinguish the "*anaerobic*" capacity in individuals with different training levels. However, future studies should determine the $MAOD_{ALT}$ values attained by sprint-trained athletes (e.g., 400-m dash), in order to define the values that can be attained at the extremes of human performance. Due to the single session needed to assess $MAOD_{ALT}$, it has high

Table 4. Magnitude-based inference analysis of results from comparisons between groups.

	Oxygen equivalents in litres			Oxygen equivalents in millilitres		
Comparators	Effect size	Probability of being positive, trivial and negative, %	Magnitude-based inference	Effect size	Probability of being positive, trivial and negative, %	Magnitude-based inference
E_{PCr}						
LT vs. MT	0.91 (0.09 to 1.73)	96/4/1	*Very likely positive*	0.42 (−0.40 to 1.24)	71/23/7	*Unclear*
LT vs. ET	0.67 (−0.10 to 1.44)	89/10/1	*Likely positive*	0.57 (−0.19 to 1.33)	84/14/2	*Likely positive*
LT vs. RG	2.62 (1.77 to 3.47)	100/0/0	*Most likely positive*	1.91 (1.09 to 2.73)	100/0/0	*Most likely positive*
MT vs. ET	−0.22 (−0.98 to 0.54)	13/35/52	*Unclear*	0.19 (−0.57 to 0.94)	49/36/15	*Unclear*
MT vs. RG	2.06 (1.20 to 2.91)	100/0/0	*Most likely positive*	1.54 (0.72 to 2.36)	100/0/0	*Most Likely Positive*
ET vs. RG	2.15 (1.33 to 2.98)	100/0/0	*Most likely positive*	1.23 (0.48 to 1.98)	100/0/0	*Most likely positive*
$E_{[La]}$						
LT vs. MT	1.06 (−0.28 to 1.88)	98/2/0	*Very likely positive*	1.06 (0.24 to 1.88)	98/2/0	*Very likely positive*
LT vs. ET	0.46 (−0.28 to 1.21)	76/20/4	*Unclear*	0.46 (−0.28 to 1.21)	76/20/4	*Unclear*
LT vs. RG	1.65 (0.78 to 2.52)	100/0/0	*Most likely positive*	1.65 (0.78 to 2.52)	100/0/0	*Most likely positive*
MT vs. ET	−0.38 (−1.12 to 0.37)	6/25/68	*Unclear*	−0.38 (−1.12 to 0.37)	6/25/68	*Unclear*
MT vs. RG	1.14 (0.27 to 2.01)	98/2/0	*Very likely*	1.14 (0.27 to 2.01)	98/2/0	*Very likely positive*
ET vs. RG	1.29 (0.45 to 2.12)	99/1/0	*Very likely*	1.29 (0.45 to 2.12)	99/1/0	*Very likely positive*
$MAOD_{ALT}$						
LT vs. MT	1.42 (0.60 to 2.24)	100/0/0/	*Most likely positive*	0.76 (−0.05 to 1.58)	92/7/1	*Likely positive*
LT vs. ET	1.13 (0.39 to 1.87)	99/1/0	*Very likely positive*	1.34 (0.59 to 2.09)	100/0/0	*Most likely positive*
LT vs. RG	2.39 (1.52 to 3.25)	100/0/0	*Most likely positive*	1.50 (0.63 to 2.37)	100/0/0	*Most likely positive*
MT vs. ET	0.54 (−0.22 to 1.30)	81/16/3	*unclear*	−0.01 (−0.76 to 0.73)	28/41/31	*unclear*
MT vs. RG	1.70 (0.87 to 2.56)	100/0/0	*Most likely positive*	1.08 (0.21 to 1.94)	98/2/0	*Very likely positive*
ET vs. RG	1.61 (0.78 to 2.45)	100/0/0	*Most likely positive*	0.77 (0.08 to 1.62)	91/7/1	*Very likely positive*

least trained (LT), moderately trained (MT), endurance trained runners (ET), and rugby players (RG).
E_{PCr}: energy contribution (i.e., oxygen equivalents) from the phosphagen metabolic pathway; $E_{[La]}$: energy contribution (i.e., oxygen equivalents) from the glycolysis metabolic pathway; $MAOD_{ALT}$: Alternative maximal accumulated oxygen deficit method. The quantitative chances were assessed qualitatively as follow: 0.5–5% = very unlikely; 5–25% = unlikely; 25–75% = possibly; 75–95% = likely; 95–99.5% = very likely; and >99.5% = most likely

potential for practical application to assess "*anaerobic*" capacity. In addition, the $MAOD_{ALT}$ can distinguish the energy contribution from the phosphagen and glycolytic pathways, an assessment which is not possible when using the conventional MAOD.

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The author and co-authors reviewed the final stages of the manuscript.

All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

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References

Bangsbo, J., Gollnick, P. D., Graham, T. E., Juel, C., Kiens, B., Mizuno, M., & Saltin, B. (1990). Anaerobic energy production and O2 deficit-debt relationship during exhaustive exercise in humans. *The Journal of Physiology*, *422*, 539–559. doi:10.1113/jphysiol.1990.sp018000

Batterham, A. M., & Cox, A. J. (2006). Spreadsheets for analysis of controlled trials, with adjustment for a subject characteristic. *Sportscience*, *10*, 46–51.

Batterham, A. M., & Hopkins, W. G. (2006). Making meaningful inferences about magnitudes. *International Journal of Sports Physiology and Performance*, *1*(1), 50–57. doi:10.1123/ijspp.1.1.50

Bertuzzi, R., Kiss, M. A., Damasceno, M., Oliveira, R. S., & Lima-Silva, A. E. (2015). Association between anaerobic components of the maximal accumulated oxygen deficit and 30-second Wingate test. *Brazilian Journal of Medical and Biological Research*, *48*(3), 261–266. doi:10.1590/1414-431X20144043

Bertuzzi, R. C., Franchini, E., Kokubun, E., & Kiss, M. A. (2007). Energy system contributions in indoor rock climbing. *European Journal of Applied Physiology*, *101*(3), 293–300. doi:10.1007/s00421-007-0501-0

Bertuzzi, R. C. M., Franchini, E., Ugrinowitsch, C., Kokubun, E., Lima-Silva, A. E., Pires, F. O., … Kiss, M. (2010). Predicting MAOD using only a

supramaximal exhaustive test. *International Journal of Sports Medicine*, *31*(7), 477–481. doi:10.1055/s-0030-1253375

Billat, V. L., Blondel, N., & Berthoin, S. (1999). Determination of the velocity associated with the longest time to exhaustion at maximal oxygen uptake. *European Journal of Applied Physiology and Occupational Physiology*, *80*(2), 159–161. doi:10.1007/s004210050573

Brisola, G. P., Miyagi, W. E., da Silva, H., & Zagatto, A. M. (2015). Sodium bicarbonate supplementation improved MAOD but is not correlated with 200 and 400m running performances: A double blind, crossover and placebo controlled study. *Applied Physiology, Nutrition and Metabolism*, *40*(9), 931–937. doi:10.1139/apnm-2015-0036

Cohen, J. (1988). *Statistical power analysis for the behavioral sciences*. Hillsdale, NJ: L. Erlbaum Associates.

de Poli, R., Miyagi, W. E., Nakamura, F. Y., & Zagatto, A. M. (2016). Caffeine improved time to exhaustion, but did not change alternative maximal accumulated oxygen deficit estimated during a single supramaximal running bout. *International Journal of Sport Nutrition and Exercise Metabolism*. Advance online publication. doi:10.1123/ijsnem.2016-0038

di Prampero, P. E. (1981). Energetics of muscular exercise. *Reviews of Physiology, Biochemistry and Pharmacology*, *89*, 143–222.

di Prampero, P. E., & Ferretti, G. (1999). The energetics of anaerobic muscle metabolism: A reappraisal of older and recent concepts. *Respiration Physiology*, *118*(2–3), 103–115. doi:10.1016/S0034-5687(99)00083-3

Fujitsuka, N., Yamamoto, T., Ohkuwa, T., Saito, M., & Miyamura, M. (1982). Peak blood lactate after short periods of maximal treadmill running. *European Journal of Applied Physiology and Occupational Physiology*, *48*(3), 289–296. doi:10.1007/BF00430218

Gastin, P. B., & Lawson, D. L. (1994). Influence of training status on maximal accumulated oxygen deficit during all-out cycle exercise. *European Journal of Applied Physiology and Occupational Physiology*, *69*(4), 321–330. doi:10.1007/BF00392038

Hautier, C. A., Wouassi, D., Arsac, L. M., Bitanga, E., Thiriet, P., & Lacour, J. R. (1994). Relationships between postcompetition blood lactate concentration and average running velocity over 100-m and 200-m races. *European Journal of Applied Physiology and Occupational Physiology*, *68*(6), 508–513. doi:10.1007/BF00599521

Heugas, A. M., Brisswalter, J., & Vallier, J. M. (1997). Effect of a three month training period on the maximal oxygen deficiency in high level performance sprinters. *Canadian Journal of Applied Physiology*, *22*(2), 171–181.

Hopkins, W. G., Marshall, S. W., Batterham, A. M., & Hanin, J. (2009). Progressive statistics for studies in sports medicine and exercise science. *Medicine and Science in Sports and Exercise*, *41*(1), 3–13. doi:10.1249/MSS.0b013e31818cb278

Korzeniewski, B., & Zoladz, J. A. (2013). Slow VO2 off-kinetics in skeletal muscle is associated with fast PCr off-kinetics–and inversely. *Journal of Applied Physiology*, *115*(5), 605–612. doi:10.1152/japplphysiol.00469.2013

Kuipers, H., Verstappen, F. T., Keizer, H. A., Geurten, P., & van, K. G. (1985). Variability of aerobic performance in the laboratory and its physiologic correlates. *International Journal of Sports Medicine*, *6*(4), 197–201. doi:10.1055/s-2008-1025839

Lacour, J. R., Bouvat, E., & Barthelemy, J. C. (1990). Post-competition blood lactate concentrations as indicators of anaerobic energy expenditure during 400-m and 800-m races. *European Journal of Applied Physiology and Occupational Physiology*, *61*(3–4), 172–176. doi:10.1007/BF00357594

Margaria, R., Edwards, H. T., & Dill, D. B. (1933). The possible mechanisms of contracting and paying the oxygen debt and the role of lactic acid in muscular contraction. *The American Journal of Physiology*, *106*(3), 689–715.

Medbo, J. I., Mohn, A. C., Tabata, I., Bahr, R., Vaage, O., & Sejersted, O. M. (1988). Anaerobic capacity determined by maximal accumulated O2 deficit. *Journal of Applied Physiology*, *64*(1), 50–60.

Milioni, F., Malta, E. S., Rocha, L. G. S. A., Mesquita, C. A. A., Freitas, E. C., & Zagatto, A. M. (2016). Acute administration of high doses of taurine does not substantially improve high-intensity running performance and the effect on maximal accumulated oxygen deficit is unclear. *Applied Physiology, Nutrition and Metabolism*, *41*(5), 498–503. doi:10.1139/apnm-2015-0435

Moore, A., & Murphy, A. (2003). Development of an anaerobic capacity test for field sport athletes. *Journal of Science and Medicine in Sport*, *6*(3), 275–284. doi:10.1016/S1440-2440(03)80021-X

Noordhof, D. A., de Koning, J. J., & Foster, C. (2010). The maximal accumulated oxygen deficit method: A valid and reliable measure of anaerobic capacity? *Sports Medicine*, *40*(4), 285–302. doi:10.2165/11530390-000000000-00000

Tanaka, H., Monahan, K. D., & Seals, D. R. (2001). Age-predicted maximal heart rate revisited. *Journal of the American College of Cardiology*, *37*(1), 153–156. doi:10.1016/S0735-1097(00)01054-8

Zagatto, A., Miranda, M. F., & Gobatto, C. A. (2011). Critical power concept adapted for the specific table tennis test: Comparisons between exhaustion criteria, mathematical modeling, and correlation with gas exchange parameters. *International Journal of Sports Medicine*, *32*(7), 503–510. doi:10.1055/s-0030-1270470

Zagatto, A. M., Leite, J.V., Papoti, M., & Beneke, R. (2016). Energetics of table tennis and table tennis specific exercise testing. *International Journal of Sports Physiology and Performance*, *11*(8), 1012-1017. doi:10.1123/ijspp.2015-0746

Zagatto, A. M., Bertuzzi, R., Miyagi, W. E., Padulo, J., & Papoti, M. (2016). MAOD determined in a single supramaximal test: A study on the reliability and effects of supramaximal intensities. *International Journal of Sports Medicine*, *37*(09), 700–707. doi:10.1055/s-0042-104413

Zagatto, A. M., & Gobatto, C. A. (2012). Relationship between anaerobic parameters provided from MAOD and critical power model in specific table tennis test. *International Journal of Sports Medicine*, *33*(8), 613–620. doi:10.1055/s-0032-1304648