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Anaerobic Contributions Are Influenced by Active Muscle Mass and The Applied Methodology in Well-Controlled Muscle Group

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ABSTRACT

International Journal of Exercise Science 15(7): 599-615, 2022. The anaerobic metabolism determination is complex and the applied methodologies present limitations. Thus, the purpose of this study was to investigate the effects of different calculations (MAOD vs. AOD) on the anaerobic contribution using the dynamic knee extension. Twenty-four male were recruited [Mean (SD); age 27 (1) years, body mass 90 (3) kg, height 181 (2) cm]. This study was divided into two independent experiments ($EXP_{1:}$ one-legged; EXP_2: two-legged). In both experiments, it was performed a graded exercise test to determine maximal power (MP-GXT); 2-4 submaximal efforts (VO_2-intensity relationship); and an exhaustive effort. The theoretical energy demand for the exhaustive effort (TED_{ex}) was constructed from the submaximal efforts. Therefore, MAOD was assumed as the difference between the TED_{ex} and the accumulated VO_2 (AVO_2). In contrast, the energy demand for AOD was calculated as the product between VO_2 at the end of exercise and time to exhaustion (TED_{aod}). Thus, AOD was assumed as the difference between TED_{aod} and AVO_2. Bayesian paired *t*-test was used to compare the differences between the applied methods. Also, correlations between the anaerobic indices and performance were verified. In EXP_1, AOD was higher than MAOD [1855 (741) vs. 434 (245); BF_{10} = 2925; ES = 2.5]. In contrast, in EXP_2, MAOD was higher than AOD [2832 (959) vs. 1636 (549); BF_{10} = 3.33; ES = 1.4]. Also, AOD was correlated to performance (*r* = .59; BF_{10} = 4.38). We concluded that MAOD and AOD are a distinct phenomenon and must be utilized according to the exercise model.

KEY WORDS: High-intensity effort, anaerobic estimation, dynamic knee extension, performance, oxygen deficit, anaerobic capacity, time to exhaustion

INTRODUCTION

The anaerobic energy supply [i.e. high energy phosphates (anaerobic alactic) and glycolytic pathway (anaerobic lactic)] provides energy for the resynthesis of the adenosine triphosphate at a faster rate than the oxygen-dependent pathway (22); however, their relative contribution decreases with exercise duration (39). Therefore, this metabolism is determinant in high-intensity, short-duration events, such as track and field (i.e. 100 to 400 meters running) (39) and swimming events (e.g. 50 to 400 meters) (13). Since its associate with performance in these events, an accurate anaerobic determination can be valid for training evaluation, monitoring, and prescription. Contrary to the aerobic metabolism which could be instantaneously measured by oxygen consumption (VO_2) data, the quantification of the anaerobic contribution is complex and claims for further investigations (4).

Investigations about anaerobic sources have been made through an invasive process (e.g. muscle biopsy) and the anaerobic production of ATP is estimated by their metabolites (4). Also, indirect estimations of the anaerobic contribution can be estimated by the oxygen deficit, proposed by Krogh and Lindhard (28). The oxygen deficit is attributed to the delayed response of the VO_2 at the onset of the exercise until a steady state is reached (12) or predicted (36). This "deficit" was later termed accumulated oxygen deficit (AOD) and is calculated as the difference between the energy cost for a given intensity and the accumulated oxygen uptake (AVO_2) (6, 29, 36, 37, 40).

The maximal attainable AOD was termed maximal accumulated oxygen deficit (MAOD) and has been attributed to the individual anaerobic capacity, which represents the highest anaerobic energy storage (29, 37). The MAOD was determined in a supramaximal exhaustive test lasting 2-5 minutes, where it reached its highest values (i.e. anaerobic fuels depletion) (29). Despite its criticism (4, 35), this methodology was sensitive to discriminate training status (38), anaerobic training (30), and hypoxia (21, 29).

Different from AOD, the energy demand for a supramaximal effort is estimated by the extrapolation of a linear relationship between a submaximal VO_2 data and exercise intensity (VO_2-intensity relationship) (29). Therefore, their feasibility was questionable since it demands several laboratory visits (8). Also, the assumptions regarding MAOD may not be valid in whole-body exercises (e.g. running or cycling) (2-4), since it was influenced by active muscle mass (5, 43).

Therefore, investigations regarding the anaerobic contribution (e.g. AOD and MAOD) could be improved utilizing a well-known muscle group. The ergometer for dynamic knee extension (DKE) promotes an exercise model in which the main motor agent during the effort is the Quadriceps Femoris (1). Thus, the metabolic responses reflect the sustained contractions of this muscle group (27), and the energy cost estimation could be improved. Bangsbo et al. (4) utilized the DKE model and found that the oxygen deficit method is associated with the anaerobic energy production (e.g. lactate, adenosine triphosphate, and creatine phosphate) in the biopsied muscle; however, oxygen deficit seems valid only when used in small muscle groups (2). Also, these authors only applied the MAOD method and AOD estimations remain to be investigated.

Considering the need for a better tool for the anaerobic estimations and the feasibility of the AOD in contrast to the MAOD, we investigated the differences between these methodologies for the determination of the anaerobic status in both one-legged and two-legged DKE, in two distinct experiments. Thus, the purpose of this present study was twofold. Firstly, we aim to investigate the effect of active muscle mass in the anaerobic contribution in both methodologies. Secondly, we aim to determine which anaerobic parameter most correlate with high-intensity exhaustive performance in different modes of exercise. We hypothesize that MAOD would be higher than AOD and could be strongly correlated with performance in both experiments.

Table 1. Abbreviations.

Abbreviation	Definition
AVO_2	accumulated oxygen deficit
BF_{10}	bayes factor
DKE	dynamic knee extension
ES	effect size
EXP_1	experiment 1 with one-legged exercise
EXP_2	experiment 2 with two-legged exercise
GXT	graded exercise test
HR	hear rate
MP-GXT	maximal power obtained in graded exercise test
r	person's product-moment correlation coefficient
RER	respiratory exchange ratio
RPE	rate of perceived exertion
TED_{ex}	theoretical energy demand extrapoled with force data
TED_{aod}	theoretical energy demand from VO_2 kinetics
t_{lim}	time-to-exhaustion
VE	minute ventilation
VO_2	oxygen consumption
VO_{2exh}	oxygen consumption at exhaustion
VO_{2peak}	peak values of oxygen consumption in GXT
95% CI	95% intervals for confidence interval
95% CR	95% intervals for credible interval

METHODS

Participants

A power analysis conducted with G*Power (v. 3.1.9.7, University of Dusseldorf, Germany) (18) determined that at least 6 participants were needed for a power of .80, with an effect size of 1.2 (data from previous pilot study) and α = .05. Thus, twenty-four healthy males were voluntarily recruited [Mean (SD); age 27 (1) years, body mass 90 (3) kg, height 181 (2) cm]. This study was divided into two independent experiments, with different participants and time of data collection. The physical characteristics of the subjects included in each experiment were summarized (Table 2). All subjects were healthy and had previous experience with this model of exercise, with anterior participation in other experiments. They were all habitually physically active, enrolled in a wide range of modalities (e.g., triathlon, cycling, resistance training, team sports), at a moderate level. They were advised to maintain their usual exercise, sleep, and diet habits throughout the study. Participants were informed about procedures and their potential risks, given written informed consent, previously approved by the Human Research Ethics Committee. All procedures were conducted in accordance with the Declaration of Helsinki. This research was carried out fully in accordance with the ethical standards of the International Journal of Exercise Science (34).

Table 2. Physical characteristics of the participants in both studies.

	Experiment 1	Experiment 2
n	17	7
Age, y	25 (3)	28 (4)
Height, cm	180 (5)	183 (8)
Body mass, kg	84 (16)	94 (11)

Protocol

Experiments 1 (EXP_1) and 2 (EXP_2) follow the same general procedures, except for the quantity of active muscle mass involved and few methodological adjustments. The EXP_1 was a one-legged exercise, in which the chosen leg was determined randomly (i.e. contralateral leg remain passive during all effort). The EXP_2 was a two-legged exercise, where both legs were active simultaneously. In both studies, the experimental design lasted at most ten days and each test was followed by at least a 24 h recovery. The evaluation followed this order: 1) graded exercise test to volitional exhaustion (GXT) to determine its maximal power (MP-GXT); 2) 2-4 submaximal constant efforts with intensities ranging from 20-80% of MP-GXT to determine VO_2-intensity relationship; 3) a high-intensity exhaustive effort resulting in a two to three minutes fatigue, approximately. The exhaustion during GXT and in the exhaustive efforts were characterized when the subjects were unable to sustain the target cadence for at least 10 s, despite strong verbal encouragements.

All efforts were conducted in a prototype for a dynamic knee extension (DKE) ergometer (1). This prototype developed in our laboratory allows a movement pattern in which the anterior thigh muscles (Quadriceps Femoris) are the primary motor agent (27). In our DKE ergometer, both one-legged (EXP_1) and two-legged exercise (EXP_2) was feasible by simplistic adjustments in the metal rod and connections. To minimize any possible learning effect during the experimental protocol, we conducted several random efforts, with diverse intensity (i.e. submaximal and exhaustive efforts) and duration (i.e. 2-15 minutes). This familiarization protocol was completed at least 24 h before the beginning of the main study.

For details about construction, force characterization, electromyographic responses, and applicability of the DKE ergometer, see Kalva-Filho et al. (27). Briefly, in this apparatus, the pedal of a mechanically braked cycle ergometer (Monark 828; Monark Exercise AB, Vansbro, Sweden) is replaced by a metal bar, connected with the participant's ankles by a stainless-steel boot. Load cells were positioned in a semicircle (i.e., two-legged exercise) or in a prolongation of the metal bar (i.e. one-legged exercise), allowing continuous force monitoring. Also, participants remain seated with the hip and torso supported in a seat by a belt, with his back to the cycle ergometer. This DKE allowed smooth movement at each knee extension, returning to the initial phase (i.e., knee flexion) passively due to inertia. Thus, all significant metabolic responses (e.g. oxygen consumption) were provided from the Quadriceps muscles isolated, with small or insignificant contribution from the hamstrings or stabilizers muscles (1, 27).

The power determination in the DKE can be determined similarly as in conventional cycle ergometers [see Kalva-Filho et al. (27) for details]. These assumptions were used in the GXT and for the prescription of submaximal and exhaustive efforts. However, these were only mean values for the external power of the ergometer, not reflecting the actual exertion being produced by the active legs (19). To determine the power values using the force signal, we developed specific mathematical routines for this proposal in the Matlab® environment (Matlab® R2018b, MathWorks® Inc., MA, USA). Thus, force data analysis was performed after all the data collection.

Force and ventilatory data were continuously monitored during all efforts. Voltage (mV) was obtained through a load cell equipped with strain gauges (250 kg capacity; CSR-1T, MK Controle, São Paulo, Brazil) connected to a signal acquisition board (NI-USB 6009, National Instruments®) and a signal amplifier (Output 0 to 10 VDC; CSR-1T, MK Controle, São Paulo, Brazil). Calibrations were routinely performed with the superposition of known weights (8 weights ranged from 0 kg to 10.2 kg), allowing the conversion of voltage data to force values (N). Acquisition frequency was set at 1000 Hz and the signal was posterior smoothed in a digital Butterworth filter of order 5, with a bandpass cutoff frequency of 0.3 and 5 Hz. All procedures for signal treatment were performed in Matlab® environment (Matlab® R2018b, MathWorks® Inc., MA, USA).

The ventilatory data was acquired by an oronasal mask (7450 Series Silicone V2™, Hans Rudolph Inc., USA) connected to a metabolic analyzer (Quark CPET or K4b2, Cosmed, Italy), providing breath-by-breath data. To enhance the physiological responses and to standardize the gap between each data point, data were interpolated to each second. Before all efforts, participants remained calmly seated for at least five minutes and the VO_2 baseline was computed. Calibrations were made according to the specifications of the manufacturer. The ambient air was used, along with a gas containing 16% O_2 and 5% of CO_2 (White Martins, Osasco, SP, Brazil). The spirometer was calibrated through a 3 L syringe (Hans Rudolph Inc., USA).

After a five-minute (EXP_1) or seven-minute (EXP_2) warm-up at 13 W, a progressive effort was performed with 13 $W \cdot min^{-1}$ increments. MP-GXT was attributed to the power of the last complete stage or adjusted according to the Kuipers formula (Equation 1) when exhaustion occurred during the stages.

Equation 1: $MP\text{-}GXT\ (W) = PC + (T \cdot 60^{-1} \cdot 13)$

Where PC is the power of the last complete stage (W), T is the duration of the incomplete stage (s), 60 is the duration in seconds of each stage, 13 is the power increment of each stage (W).

Heart rate (HR) was monitored by a chest strap (H7, Polar Electro Oy, Kempele, Finland) integrated through an interface of the gas analyzer. The final 30 s of ventilatory data and HR were the average of each stage. The rate of perceived exertion (RPE) was monitored by 10 points scale (20). The highest values for HR, minute ventilation (VE), and RPE were considered. Also, the highest 30 s mean of VO_2 was termed VO_{2peak}.

The submaximal and exhaustive efforts were preceded by the same warm-up as in the GXT. In both experiments, 2-4 submaximal constant efforts with the duration of five (EXP_1) or seven (EXP_2) minutes were applied. These intensities ranged from 20-85% of MP-GXT and were conducted to determine the VO_2-intensity relationship equation. The real VO_2 for each effort was attributed to the highest 30 s means. In addition, subjects were submitted to an exhaustive effort lasting 2-3 minutes. This intensity was approximately 100% and 110% of MP-GXT in EXP_1 and EXP_2, respectively. Also. this effort was used to determine t_{lim} and AVO_2. The highest 5 s VO_2 averages were considered for this intensity (VO_{2exh}). The peak values in force data correspond to each full knee extension moment (Kalva-Filho et al, 2020). Thus, all peaks in the force sampling were averaged (Figure 1). Also, t_{lim} was the time from the first and last valid peak.

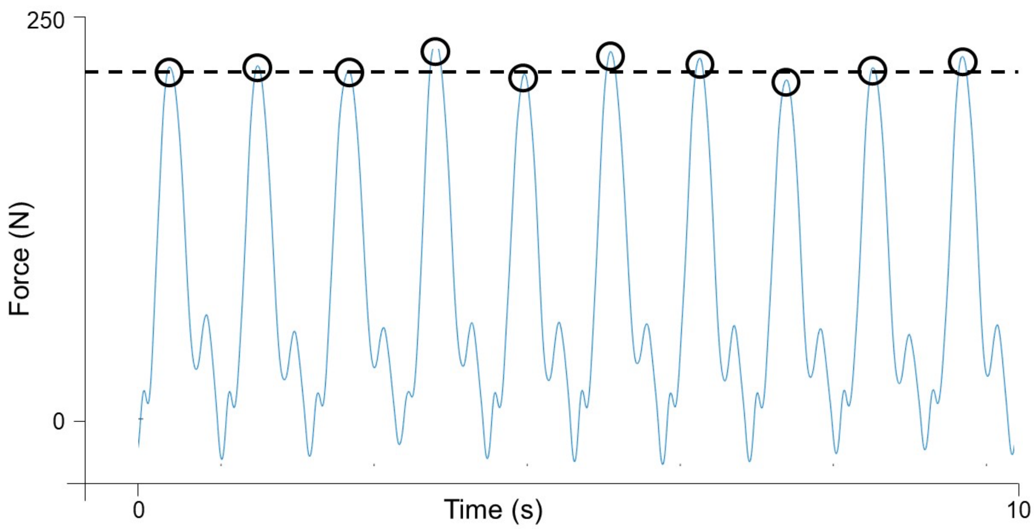

Figure 1. Illustrative representation of the force characteristics during dynamic knee extension. In this figure, 10 consecutive extensions were selected. Dashed line = theoretical average force; Circles = peak value of each knee extension.

As described above, the VO_2-intensity relationship was constructed individually through a linear regression fitting with the mean force's value and the VO_2 of the submaximal efforts. The y-intercept was fixed individually at their respective basal oxygen consumption (i.e. 5 minutes average from baseline data) (14, 29). This equation was used to estimate the theoretical energy demand for the exhaustive effort (TED_{ex}).

The intensity of the exhaustive effort remained constant (i.e., no alteration in the mechanically braked system); however, the force presents considerable variation along with the exercise. Therefore, the TED_{ex} cannot be assumed as linear as in other modalities (e.g. traditional cycle ergometer or treadmill). Thus, the VO_2-intensity equation was applied at each peak in the force signal, along with the entire effort. This was an attempt to enhance the characteristics of energy

cost in this model of exercise. Therefore, the MAOD was assumed as the difference between the total TED_{ex} (i.e. integral of TED_{ex} and t_{lim}) and the AVO_2 (Figure 2). This strategy has already been applied in tethered swimming (26).
In contrast, AOD determination does not involve the force values and the energy demand was calculated as the product between VO_{2exh} and t_{lim} (TED_{aod}). Thus, AOD was assumed as the difference between TED_{aod} and AVO_2.

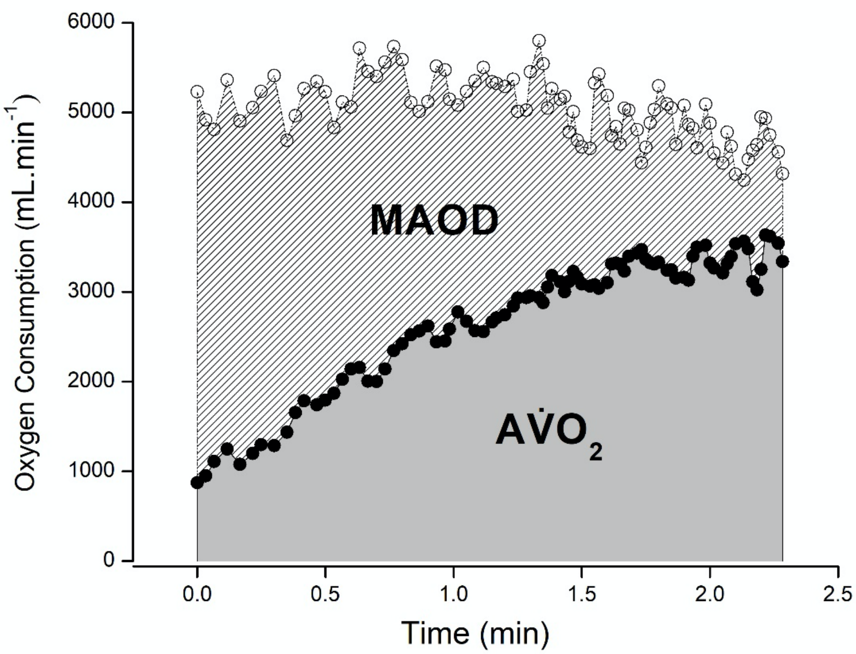

Figure 2. Representative illustration of MAOD calculation. The MAOD was assumed as the difference between the total TED_{ex} (TED_{ex} extrapolated at each knee extension) and the AVO_2.

Statistical Analysis
Statistical analysis was performed using JASP statistical software (v. 0.12.2, Amsterdam, Netherlands). In both experiments, data normality was tested by Shapiro-Wilk allowing the use of parametric procedures and presentation of data through Mean ± SD. Considering the limitations of the conventional null hypothesis of significance testing (NHST) (24), the NHST was replaced by the Bayesian inference (i.e. Bayes factor hypothesis testing) (41, 42). A Bayesian paired *t*-test was used to compare the probabilities of the differences between the dependent variables. Comparisons are based on the default prior [Cauchy scale; r = 1 / sqrt(2)]. Evidence for alternative hypothesis (H1) was set at Bayes factor (BF_{10}) > 3 and evidence for the null hypothesis (H0) was set at $BF_{10} < 1/3$. Qualitative outcomes were used to indicate the strength of the probabilistic inference. The evidence for H1 was classified as follows: $1 < BF_{10} < 3$ = "anecdotal"; $3 < BF_{10} < 10$ = "moderate"; $10 < BF_{10} < 30$ = "strong"; $30 < BF_{10} < 100$ = "very strong"; $BF_{10} > 100$ = "extreme".

In addition, the Bayesian Pearson's product-moment correlation coefficient (*r*) was used to test the relationship between the anaerobic indices (i.e. MAOD and AOD) and the performance (i.e.

t_{lim}). The credible intervals (CR) for *r* were also calculated. The correlations were classified as: trivial < 0.1; small 0.1-0.3; moderate 0.3-0.5; large 0.5-0.7; very large 0.7-0.9; and nearly perfect > 0.9 (25).

Furthermore, the magnitude of these differences (i.e. standardize mean differences; Cohen's *d*) was calculated as a measure of the effect size (ES) (17). A small sample bias adjustment was applied to each ES. Also, the respective confidence interval (CI) was calculated as a variation around the ES. These calculations were made in an Excel® spreadsheet (Microsoft 365®, MA, USA) with the proposed equations (33). Following the adaptation for sports sciences made by Hopkins (25), the ES was interpreted as: trivial < 0.2; small 0.2-0.6; moderate 0.6-1.2; large 1.2-2.0; very large 2.0-4.0; and nearly perfect > 4.0 (7).

RESULTS

As the EXP_1 and EXP_2 were conducted with different subjects and moments, the specific results of each experiment are presented separately below. One-legged dynamic knee extension (EXP_1) (n = 16): The physiological responses obtained after GXT are summarized (Table 3). In this experiment, the VO_2-intensity relationship was constructed by three submaximal trials. The submaximal forces ranged from 24 (10), 53 (11), and 70 (15) % of the exhaustive force. Figure 3 shows the average values from VO_2 and force obtained in the submaximal effort and the linear relationship constructed. The parameters of the linear regression fitting obtained are demonstrated (Table 4). Also, the results obtained in the exhaustive efforts are shown (Table 5). The AOD was significantly higher than MAOD, with an "extreme" evidence for H_1 (BF_{10} = 2925) and "very large" effect (ES = 2.5; 95% CI = 1.6-3.4) (Figure 4). We found "trivial" to "large" correlations between t_{lim} and the anaerobic parameters (Table 6).

Table 3. Physiological variables obtained after graded exercise test (GXT) in one-legged exercise.

	Mean	SD	Range
Duration (s)	289	69	190-495
MP-GXT (W)	53	15	34-100
VO_{2peak} ($mL \cdot min^{-1}$)	1500	246	1017-1937
VE ($L \cdot min^{-1}$)	83	27	31-157
RER (a.u.)	1.30	.28	.97-2.02
RPE (a.u.)	8.8	1.5	5-10
HR ($beats \cdot min^{-1}$)	135	16	114-168

Note. MP-GXT = maximal power obtained in graded exercise test; VO_{2peak} = highest 30 s VO_2 averages; VE = minute ventilation; RER = respiratory exchange ratio; RPE = rate of perceived exhaustion; HR_{peak} = highest 30 s averages

Table 4. Mean parameters of the linear regression fitting obtained by the individual VO_2-intensity relationship in one-legged exercise.

	Mean	SD	Range
Slope ($mL \cdot min^{-1} \cdot N^{-1}$)	6.32	3.57	2.19-12.87
y-intercept ($mL \cdot min^{-1}$)	467	54	377-550
r^2	.97	.04	.85-1.00

Note. Slope = angular coefficient (β); y-intercept = linear coefficient (α); r^2 = coefficient of determination

Table 5. Variables obtained after the exhaustive effort in one-legged exercise.

	Mean	SD	Range
t_{lim} (s)	168	51	102-271
Mean Force (N)	227	104	95-406
VO_{2exh} (mL · min^{-1})	1936	363	1292-2750
TED_{ex} (mL)	3971	1275	2346-6939
TED_{aod} (mL)	5392	1787	2809-9175
AVO_2 (mL)	3537	1238	1929-6579
AOD (mL)	1855	741	723-3144
MAOD (mL)	434	245	68-937

Note. t_{lim} = time-to-exhaustion; VO_{2exh} = highest 5 s VO_2 averages; TED_{ex} = integral of the theoretical energy demand constructed by the VO2-intensity relationship; TED_{aod} = integral of the theoretical energy demand estimated by the product between VO_{2exh} and t_{lim}; AVO_2 = integral of the VO_2 and t_{lim}; AOD = accumulated oxygen deficit; MAOD = maximal accumulated oxygen deficit

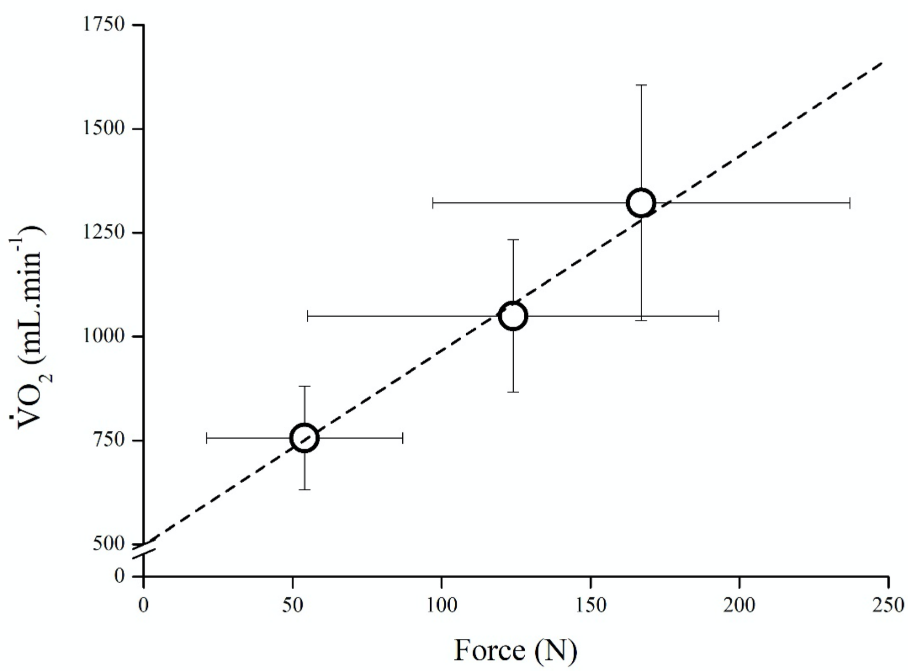

Figure 3. Average values from VO_2 and force obtained in the submaximal effort and the linear relationship constructed in EXP_1.

Two-legged dynamic knee extension (EXP_2) (n = 7): The parameters of the GXT are presented (Table 7). Four submaximal efforts were utilized for this VO_2-intensity equation (Figure 5). The percentual of the exhaustive force was 51 (12), 63 (13), 69 (10), and 86 (24). The fitting parameters of the equation are demonstrated (Table 8). Table 9 shows all physiological responses after the exhaustive bout. Conversely to the first experiment, the MAOD was significantly higher than AOD, with an "moderate" evidence for H_1 (BF_{10} = 3.33) and "large" effect (ES = 1.4; 95% CI =.2-2.6) (Figure 6). Performance and the anaerobic methodologies were "moderate" to "large" correlated (Table 10).

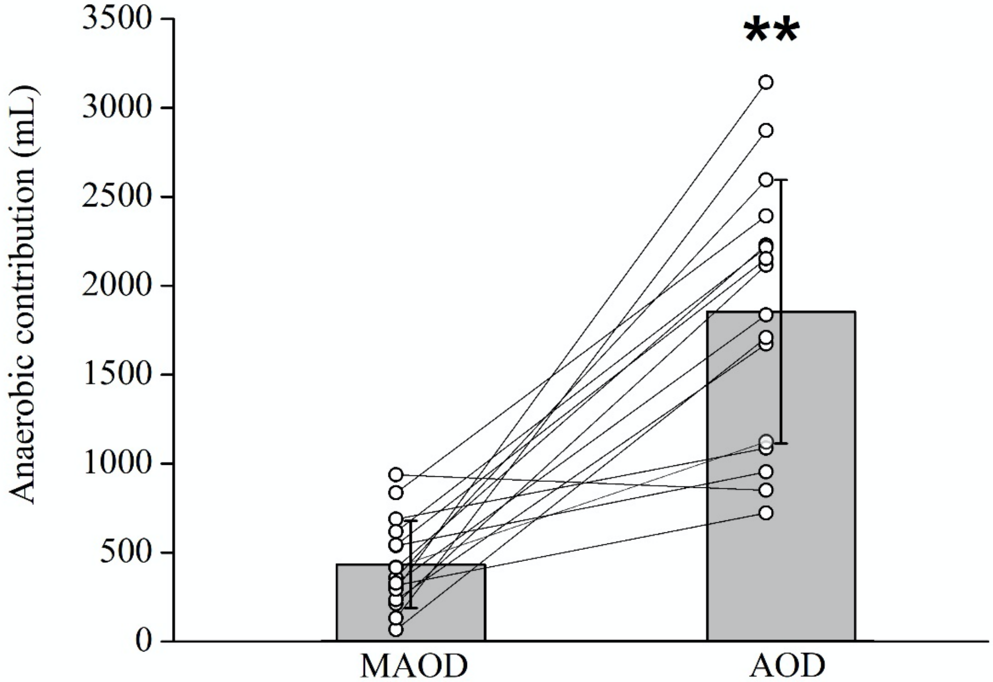

Figure 4. Absolute (bars) and individual (dots) values from MAOD and AOD in one-legged dynamic knee extension. **BF_{10} > 100.

Table 6. Bayesian Pearson's product-moment for time-to-exhaustion and anaerobic indices in one-legged exercise.

	r	95% CR	Classification	BF_{10}	Probability
t_{lim} x MAOD	.16	.57 to -.33	"trivial"	.36	"anecdotal"
t_{lim} x AOD	.59	.81 to .10	"large"	4.38*	"moderate"

Note. r = Bayesian Pearson's product-moment; 95% CR = credible intervals; BF_{10} = Bayes factor; t_{lim} = time-to-exhaustion; AOD = accumulated oxygen deficit; MAOD = maximal accumulated oxygen deficit

Table 7. Physiological variables obtained after graded exercise test (GXT) in two-legged exercise.

	Mean	SD	Range
Duration (s)	485 (104)	104	360-660
MP-GXT (W)	93 (14)	14	14-93
VO_{2peak} (mL · min^{-1})	1853 (514)	514	1508-2852
VE (L · min^{-1})	97 (17)	17	71-121
RER (a.u.)	1.22 (.17)	.17	.98-1.41
RPE (a.u.)	9.6 (.5)	.5	9-10
HR (beats · min^{-1})	147 (19)	19	129-172

Note. MP-GXT = maximal power obtained in the graded exercise test; VO_{2peak} = highest 30 s VO_2 averages; VE = minute ventilation; RER = respiratory exchange ratio; RPE = rate of perceived exhaustion; HR_{peak} = highest 30 s averages

Table 8. Mean parameters of the linear regression fitting obtained by the individual VO_2-intensity relationship in two-legged exercise.

	Mean	SD	Range
Slope ($mL \cdot min^{-1} \cdot N^{-1}$)	15.57	.89	14.40-16.76
y-intercept ($mL \cdot min^{-1}$)	469	79	378-568
r^2	.99	.01	.97-.99

Note. Slope = angular coefficient (β); *y*-intercept = linear coefficient (α); r^2 = coefficient of determination

Table 9. Variables obtained after the exhaustive effort in two-legged exercise.

	Mean	SD	Range
t_{lim} (s)	132	28	98-171
Mean Force (N)	167	19	139-191
VO_{2exh} ($mL \cdot min^{-1}$)	2867	569	2164-3600
TED_{ex} (mL)	7505	122	5641-9134
TED_{aod} (mL)	6256	1611	4508-8535
AVO_2 (mL)	4619	1152	3051-6182
AOD (mL)	1636	549	899-2354
MAOD (mL)	2832	959	1726-4345

Note. t_{lim} = time-to-exhaustion; VO_{2exh} = highest 5 s VO_2 averages; TED_{ex} = integral of the theoretical energy demand constructed by the VO2-intensity relationship; TED_{aod} = integral of the theoretical energy demand estimated by the product between VO_{2exh} and t_{lim} = AVO_2: integral of the VO_2 and t_{lim}; AOD = accumulated oxygen deficit; MAOD = maximal accumulated oxygen deficit

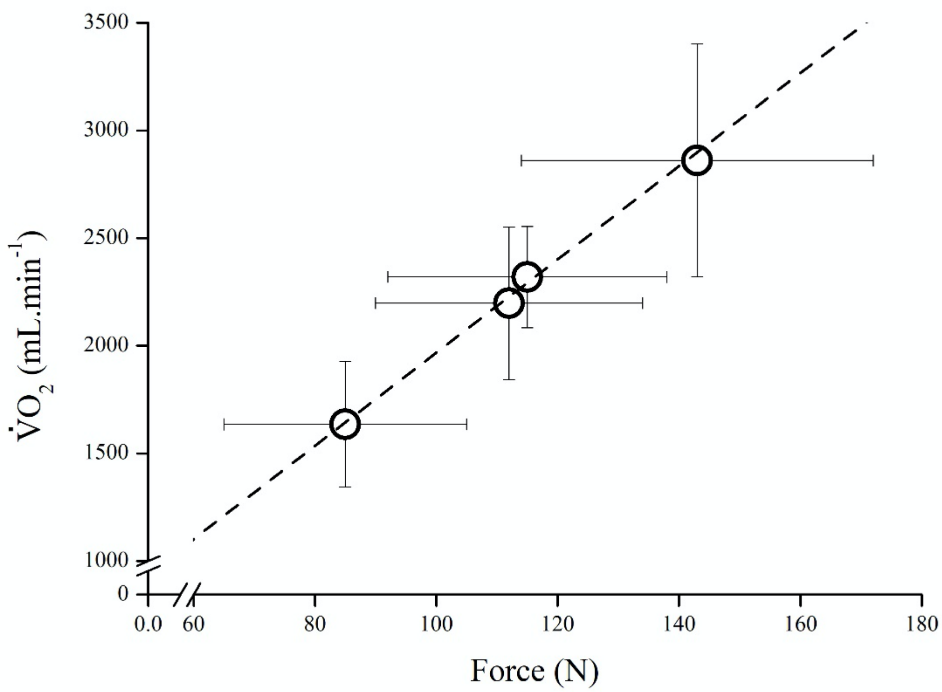

Figure 5. Average values from VO_2 and force obtained in the submaximal effort and the linear relationship constructed in EXP_2.

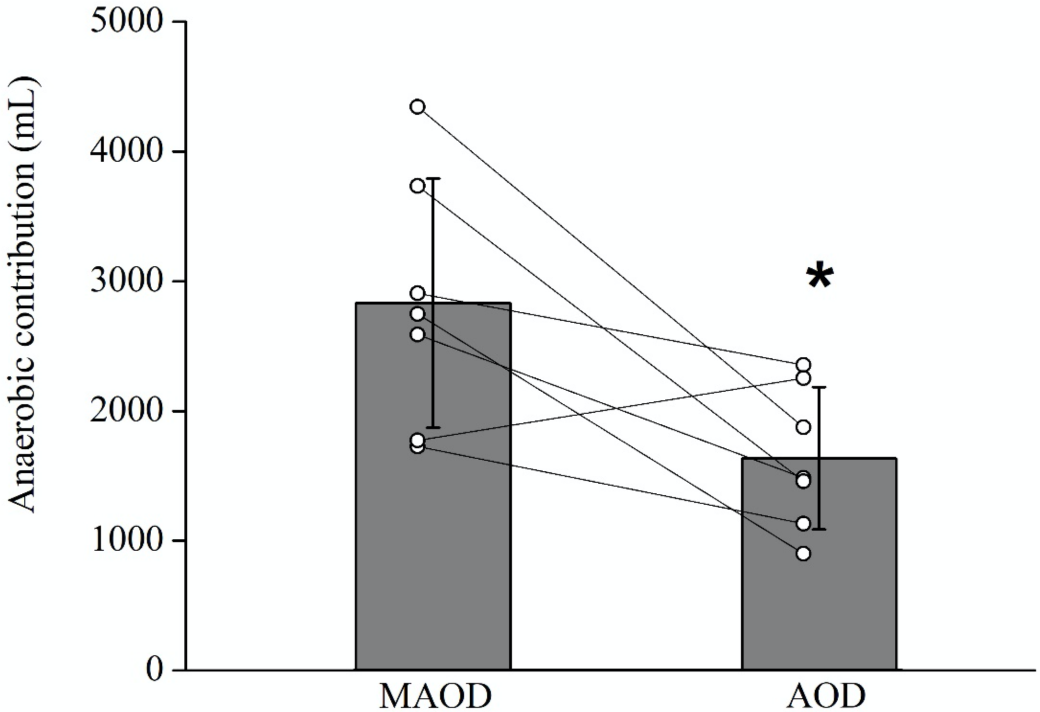

Figure 6. Absolute (bars) and individual (dots) values from MAOD and AOD in one-legged dynamic knee extension. *BF_{10} > 3.

Table 10. Bayesian Pearson's product-moment for time-to-exhaustion and anaerobic indices in two-legged exercise.

	r	95% CR	Classification	BF_{10}	Probability
t_{lim} x MAOD	.44	.82 to -.38	"moderate"	.70	"anecdotal"
t_{lim} x AOD	.63	.89 to -.21	"large"	1.24	"anecdotal"

Note. *r* = Bayesian Pearson's product-moment; 95% CR = credible intervals; BF_{10} = Bayes factor; t_{lim} = time-to-exhaustion; AOD = accumulated oxygen deficit; MAOD = maximal accumulated oxygen deficit

DISCUSSION

The main aim of this study was to investigate two methodologies usually applied in the determination of the anaerobic status (i.e. MAOD and AOD) in a knee extensor exercise. In this model, the majority of the metabolic responses (e.g. oxygen consumption and lactacidemic variation) and the anaerobically attributable ATP produced (4) are provided by an isolated muscle group (1). Thus, the non-active musculature does not confound the metabolic demands and the accuracy of these estimations could be improved (3). Therefore, we aimed to verify if these methodologies differ with the change in active muscle mass (i.e. one or two-legged knee extension). Partially contributing to our hypotheses, the main findings of the present study were that the chosen methodology is influenced by the quantity of muscle mass; however, the methodologies present discrepant findings. We also aim to verify the relationship between these anaerobic parameters and the performance. According to our second hypothesis, a "large" correlation was found between AOD and t_{lim} in EXP_1 (r = .59; 95% CR = .81 to .10; BF_{10} = 4.38 "moderate").

In the first experiment, conducted in a one-legged dynamic knee extension model, AOD was significantly higher than MAOD. This finding was contrary to our hypothesis and reveal the inconsistency of the MAOD, at least in our settings. To the best of our knowledge, only one study investigates MAOD in isolated muscle group (4). Similarly, Bangsbo et al. (4) obtained MAOD values close to 460 mL in a sample of eight physically active participants. Despite this congruence, the MAOD should represent the maximal values of anaerobic contribution (3) and therefore cannot be lower than AOD. Thus, the MAOD does not actually reflect the anaerobic capacity in this model of exercise. Differently, in the two-legged model, MAOD was higher than AOD. This was the first study that proposes to investigate anaerobic sources in two-legged exercise. Therefore, there is no available data to direct comparison; however, these findings support the use of MAOD instead of AOD. Despite this, the correlation was only "moderate" with "anecdotal" probabilistic inference.

The MAOD was investigated in several other modalities including different levels of fitness (e.g. sprinters vs. endurance runners) (38), age (younger vs. older athletes) (14), and environmental conditions (e.g. hypoxia and normoxia) (21, 29). Compared to other sports, the present study shows elevated values of MAOD in EXP_2 than untrained cyclists (2600 mL) (44); young swimmers (2900 mL) (26); young soccer players (3200 mL) (14); cycling (3990 mL) (32); or running (4700 mL) (45). The lack of accessory muscles involved in this type of exercise could decrease the metabolites removal or lactate turnover for the active muscle (9). Therefore, higher depletion of anaerobic sources and MAOD in DKE could be expected.

In the present study, it was elucidated that MAOD and AOD are distinct phenomena and the methodology must be chosen according to the exercise model. The inconsistency of the MAOD could be attributed to the regression fitting utilized for the VO_2-intensity relationship. There is plenty of discussion about the construction of the TED_{ex}, including the number of submaximal efforts required, the duration, and the intensity of them (35). Medbø et al. (29) stated that are necessary at least 10 submaximal bouts with 10 minutes duration; however, there is a wide range of efforts and durations utilized in the literature [e.g. 2 to 10 efforts; 2 to 15 minutes duration (35)].

The current investigation utilized 2-4 submaximal efforts, which could be pointed to as a limitation. To deal with the lower number of efforts, it was proposed the use of intensities that are in the extremities (i.e. the lowest and the highest values of the equation) (11) and the fixation of y-intercept (29, 35). Here, we adopt these strategies to increase the viability of the experimental design, and it was used intensities ranged from 24 to 86% of the force obtained in the exhaustive effort and the y-intercept was fixed at the individual VO_2 baseline. This number of submaximal has already been applied with acceptable reliability (16).

As mentioned above, the duration of the submaximal intensities could also influence the MAOD (2, 10, 35), especially at higher submaximal intensities (i.e. above the anaerobic threshold) (35). Therefore, the chosen duration in this study (i.e. 5 minutes in EXP_1; 7 minutes in EXP_2) agreed with the current literature; however, the VO_2 kinetics and their slow component must be further explored in this model of exercise, as it could influence the VO_2-intensity relationship and therefore, the MAOD (23, 36, 37). Despite this, high levels of linearity were founded (r^2 = .97 and .99, EXP_1 and EXP_2, respectively).

Besides the VO_2-intensity relationship, the lack of significant correlations between MAOD and t_{lim} in both experiments (r < .44; BF_{10} < .70) could be attributed to the force characterization during the exhaustive bout. In this investigation, the TED_{ex} was extrapolated each knee extension, which amplified the real signal and could be pointed as a methodological advantage. However, it was observed in previous pilot experiments conducted in our laboratory that force tends to fall at the end of these high-intensity efforts, but the VO_2 remains elevated (unpublished data). We speculate that this response in the DKE ergometer may have underestimated the MAOD, especially in EXP_1. Thus, AOD should be preferred in one-legged exercise since its estimation only depends on VO_2 data and a "large" correlation was found.

It was stated that exhaustion must occur between 2 and 3 minutes to guarantee the full depletion of the anaerobic sources (3, 29, 31). In the present study, the exhaustion falls in this range and the t_{lim} was 168 (51) and 132 (28) for EXP_1 and EXP_2, respectively. This is another congruent findings with the study conducted by Bangsbo et al. (4), who shows a t_{lim} of 3.2 minutes and similar MAOD values.

Through a critical analysis of this study, we pointed out as the main limitation the use of separated experiments for MAOD and AOD calculation in one and two-legged exercises. In fact, different participants were recruited, which did not allow for direct comparisons. Also, the sample was physically active, and these results could not be extrapolated to athletes, especially those involved in high-intensity modalities. These athletes may exhibit improved tolerance in this type of exercise (15) and greater anaerobic storages (38), modifying MAOD and AOD calculations. Also, besides the great methodological advantage with the use of the DKE approach for exercise physiology experiments, the findings found in this study should be extrapolated to other modalities with caution. Therefore, the time-efficient advantages of the AOD utilization must be further investigated in other sports contexts which present different movement patterns and muscle activation than in DKE.

In conclusion, MAOD and AOD are distinct phenomena and must be utilized according to the exercise model. Also, the AOD presents a large correlation with the performance during intense exhaustive exercise for a single muscle group (EXP_1). This conclusion was limited to the specific population investigated (physically active health males) and exercise model (i.e., DKE). Therefore, these methodologies must be further investigated in this type of exercise, including the utilization of biopsy technique for the anaerobic ATP production, which could improve the study of its validity, since the active muscle mass is known. Also, the reliability and sensitivity of AOD to anaerobic training or environmental stress (e.g. hypoxia) should be further tested. These investigations could improve the understanding of the anaerobic metabolic process for further analysis in whole-body exercise.

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