# Inclusion of blood lactate O<sub>2</sub> equivalent in the VO<sub>2</sub>-intensity regression at level and 10.5% grade running

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Victor Machado REIS\* Diogo Roberto OLIVEIRA\* André Luiz CARNEIRO\*\* Hélder Miguel FERNANDES\* Christopher SCOTT\*\*\* \*Research Center in Sports Sciences, Health Sciences & Human Development, Vila Real, Portugal. \*\*Centro de Ciências Biológicas e da Saúde, Universidade Estadual de Montes Claros, Montes Claros, MG, Brasil. \*\*\*University of

Southern Maine, Gorham, ME, USA.

## **Abstract**

This study aimed to analyze the influence of adding blood lactate ([La $^-$ ]) energy equivalent (Eq) to the VO $_2$  measurements during running exercise at different grades (0% and 10.5%) in order to estimate energy cost of running. Thirteen male highly-trained middle- and long-distance runners (age 28.1  $\pm$  4.2 years; stature 1.75  $\pm$  0.07 m; body mass 65.2  $\pm$  4.9 kg; and maximal oxygen uptake 70.3  $\pm$  4.9 ml·kg $^-$ 1 min $^-$ 1) volunteered after medical approval and performed two treadmill tests (0% and 10.5% grade) which included several bouts at a constant speed. Individual VO $_2$ -speed regressions were determined for each subject and alternative regressions were established by adding an energy equivalent of 3 ml O $_2$  Eq·kg $^-$ 1 ·mM [La $^-$ ] to the mean VO $_2$  values. No significant interaction between [La $^-$ ] O $_2$  equivalent inclusion and grade running was found. Results of within-subjects ANOVAs indicated a significant effect of [La $^-$ ] inclusion in the regression slope and in the estimated energy cost of running at both level and grade running. However, the obtained effect sizes suggest that this effect is considerably higher at level compared with grade running. These findings indicate that the inclusion of [La $^-$ ] measurements in VO $_2$ -intensity regression estimates at sub maximal running should be considered when testing highly trained runners on the treadmill.

KEY WORDS: Horizontal vs. inclined running; Energy cost; Error of estimate.

### Introduction

The energy cost of running  $(C_R)$  has been vastly assessed by the relationship between sub maximal oxygen uptake  $(VO_2)$  and running speed, both in level and in grade treadmill running 1-3. This relationship is usually established with sub maximal  $VO_2$  measurements at various exercise intensities and it assumes that  $VO_2$  represents the overall  $C_R$ . Complying with this rationale, the vast majority of the studies that used the  $VO_2$ -intensity relationship to assess  $C_R$  have not assessed the potential of blood lactate ([La<sup>-</sup>]) energy equivalents (Eq) in their calculations, though an anaerobic energy cost component appears likely at heavy to severe exercise intensities.

The pioneering studies by Margaria et al.<sup>4-5</sup>, later followed by Cerretelli et al.<sup>6</sup>, and subsequently completed by those of Di Prampero<sup>7</sup> allowed the

establishment of a quantitative energy equivalent for post-exercise lactate accumulation in the blood that could be used to quantify the energy yielded by the anaerobic lactic source during running or swimming exercise (between 2.7 and 3.3 ml VO<sub>2</sub>·kg<sup>-1</sup>·mM). DI Prampero<sup>7</sup> clearly stated that this equivalent does not represent an energy equivalent of lactate formation. Rather, the equivalent represents an amount of energy that could be attributed to the lactic metabolism when the rate of lactate formation greatly surpasses that of its removal<sup>7-8</sup>. Because the studies by Margaria used only linear models, when estimating energy expenditure between subjects the anaerobic energy cost equivalent rises with work at the same rate (slope) as oxygen uptake, providing a generalized description of a linear and proportionate

increase in energy costs to work rates. However, it also is apparent that within subjects heavy to severe exercise intensities - such as during an incremental test to exhaustion - eventually lead to increases in total (aerobic + anaerobic) energy costs that are not proportional to increases in work<sup>9</sup>.

It is well established that grade running demands a high energy turnover<sup>10</sup> possibly due to a worsening running economy<sup>11</sup> and imposes a different mechanical pattern<sup>12</sup>, as compared with level running. The original studies by Margaria et al.<sup>4-5</sup> involved both level and grade treadmill running. In addition, in his review paper on the matter, DI Prampero<sup>7</sup> also mentioned both running conditions and provided possible explanations for

the different mechanical efficiency between level and grade treadmill running.

Given that [La] accumulation is expected to rise more with incremental inclined grade as opposed to running on the level, one could expect a greater influence of [La] as part of the overall energy cost estimate during running at higher grades. To date no previous research has analyzed the differentiated effects of [La] energy equivalent inclusion on VO<sub>2</sub>-intensity regression estimates when running at different treadmill grade conditions. With this in mind, the aim of the present study was to investigate the influence of adding [La] energy equivalent to the VO<sub>2</sub> measurements during running exercise at different grades (0% and 10.5%).

#### Method

#### **Participants**

Thirteen male subjects volunteered after medical approval and gave informed consent to participate in the present study. The mean  $(\pm s)$  age, stature, body mass and maximal oxygen uptake of the subjects were, respectively,  $28.1 \pm 4.2$  years,  $1.75 \pm 0.07$  m, 65.2± 4.9 kg, and 70.3 ± 4.9 ml·kg<sup>-1</sup>·min<sup>-1</sup>. All subjects were apparently healthy trained runners who were involved in systematic endurance training programs (from 6 to 9 weekly endurance running sessions). The present study was approved by the Institutional Ethics Committee and written informed consent was signed by each and every participant in the study. During one week prior to the experiment the subjects were not engaged in high-intensity training sessions and limited their exercise to a single daily low-intensity running session. In addition the subjects were instructed not to use any ergogenic supplement and to report any drug that they might have been using.

# **Experimental procedures** and measurements

All exercise was conducted on a laboratory in the morning (i.e. 09.00-12.00 h), with a controlled temperature (20-23 °C) and relative humidity (35-40%). The subjects completed a level treadmill running test and a 10.5% treadmill running test on a Master ATL motorized treadmill (Inbrasport, Porto Alegre, Brazil). The time interval between the two tests was 96 hours. During the testing period

the subjects limited their training program to a daily low intensity 20 min running session. The testing was performed in a counter-balanced order, where 6 subjects were drafted to start with the level running and the remaining 6 were assigned to start with the 10.5% grade running. Each test included 6 min bouts at a constant speed; six 6 min bouts for level running; five 6 min bouts for inclined running. The starting running speed was 3 m·s<sup>-1</sup> for level running and 2 m·s<sup>-1</sup> for grade running. The speed increases in each subsequent bout were 0.5 m·s<sup>-1</sup> for level running and 0.3 m·s<sup>-1</sup> for grade running. The starting running speeds for each subject, as well as speed increases, were based on laboratory pre-testing of 4 subjects randomly selected. The pre-testing occurred 2 weeks prior to the start of the experimental sessions. The recovery between bouts was individual and based on VO, measurements. The subjects started each bout when their VO presented two consecutive 20 s average values within 2 ml·kg<sup>-1</sup>·min<sup>-1</sup> from the individual's resting VO<sub>2</sub> <sup>13</sup>.

Through all testing expired gases were collected and analyzed with a K4b2 gas analyzer (Cosmed, Rome, Italy). Collected data, including the VO<sub>2</sub> were averaged to 20 s intervals. Before each test, a reference air calibration of the device was performed using a gas sample with a 16% O<sub>2</sub> concentration and a 5% CO<sub>2</sub> concentration. The flow meter was also calibrated before each testing with a 3000 ml syringe. For both level and inclined running oxygen uptake attained a steady state that did not differ more than 2 ml·kg<sup>-1</sup>·min<sup>-1</sup> by the end of each 6 min run. Before

(resting measurement) and immediately after the conclusion of each bout, capillary blood samples were collected to determine the [La<sup>-</sup>] concentration with a YSI 1500 Sport lactate analyzer (YSI Inc., Yellow Springs, USA). Before testing a calibration of the YSI was performed with several YSI 1530 Standard Lactate Solutions (2, 4, 8 and 10 mM·L<sup>-1</sup>).

The  $\mathrm{VO}_2$ -speed regression was developed for each individual using the steady-state  $\mathrm{VO}_2$  values during the last min of each exercise bout for each of the two tests. An individual resting  $\mathrm{VO}_2$  measurement (zero speed  $\mathrm{VO}_2$ ) was also included in the regressions, i.e., the standard method <sup>14</sup>. Oxygen uptake measurements from bouts that lasted less than 6 min were not included in the regression.

The energy cost of running  $(C_R)$  was determined from the slope of the  $VO_2$ -speed regression fit line. For each subject, an alternative regression model was established, where an energy equivalent of 3 ml

O<sub>2</sub> Eq·kg<sup>-1</sup>·mM [La<sup>-</sup>] was added to the mean VO<sub>2</sub> (alternative method) values. The latter calculation used the net [La<sup>-</sup>] (post-exercise [La<sup>-</sup>] minus resting [La<sup>-</sup>]).

#### Statistical analysis

Data was analyzed with SPSS 17.0 (SPSS Science, Chicago, USA) software. Linear regression was used on all appropriate data. The scatter around the regression line was used as measures of the fitness of the regression lines. A 2 (without or with the inclusion of [La<sup>-</sup>]  $O_2$  equivalent) x 2 (level vs. 10.5% grade running) repeated measures analysis of variance (ANOVA) was performed for each regression estimates (slope, *y*-intercept, scatter around the regression line) and for  $C_R$ . Partial eta-squared values  $(\eta_p^{-2})$  were reported as measures of effect size, with values higher than 0.01, 0.06 and 0.14 representing small, medium, and large effects, respectively<sup>15</sup>.

#### Results

Mean resting [La] was  $\approx 1.7 \text{ mM} \cdot \text{L}^{-1}$ . The mean [La] in the first four stages in the level running and in the first three stages of the graded running were up to  $4 \text{ mM} \cdot \text{L}^{-1}$  and the anaerobic lactic contribution in these bouts of exercise was lower than 1 ml  $O_2 \cdot \text{kg}^{-1}$  in most cases. In the last two bouts at both treadmill inclinations, [La] was higher (between 5 and 9 mM·L¹) and the anaerobic lactic contribution varied between 1.5 and 4 ml  $O_2 \cdot \text{kg}^{-1}$  (see TABLES 1 and 2).

FIGURE 1 depicts the various regressions obtained when using mean data with the standard and the alternative procedures in both tests (level and grade running).

Descriptive results for the mean parameters extracted from the regression (slope, *y*-intercept and the scatter around the regression line), as well as  $C_R$ , are presented in TABLE 3.

TABLE 1 - Mean (± sd) of oxygen uptake (VO<sub>2</sub>) and blood lactate ([La<sup>-</sup>]) and O<sub>2</sub> energy equivalent due to net lactate (VO<sub>2</sub>LA) at the various running speeds in the level running test.

Speed	VO <sub>2</sub>	[La <sup>-</sup> ]	VO <sub>2</sub> LA
$(m \cdot s^{-1})$	$(ml \cdot kg^{-1} \cdot min^{-1})$	$(mM \cdot L^{-1})$	(ml·kg <sup>-1</sup> ·min <sup>-1</sup> )
3.0	38.16 ± 3.62	2.55 ± 0.49	0.43
3.5	$42.31 \pm 3.41$	$2.67 \pm 0.57$	0.49
4.0	$46.63 \pm 5.53$	$2.98 \pm 0.69$	0.64
4.5	$53.09 \pm 5.81$	$3.89 \pm 1.24$	1.10
5.0	57.94 ± 6.21	$5.71 \pm 1.73$	2.01
5.5	$65.79 \pm 6.34$	$8.78 \pm 1.79$	3.54

TABLE 2 - Mean (± sd) of oxygen uptake (VO<sub>2</sub>), blood lactate ([La<sup>-</sup>]) and O<sub>2</sub> energy equivalent due to net lactate (VO<sub>2</sub>LA) at the various running speeds in the 10.5% grade running test.

Speed	$VO_2$	[La <sup>-</sup> ]	VO <sub>2</sub> LA
$(m \cdot s^{-1})$	$(ml \cdot kg^{-1} \cdot min^{-1})$	$(mM \cdot L^{-1})$	$(ml \cdot kg^{-1} \cdot min^{-1})$
2.0	44.80 ± 4.56	$3.06 \pm 0.80$	0.83
2.3	$50.02 \pm 4.57$	$3.41 \pm 1.26$	0.86
2.6	$53.96 \pm 3.82$	$4.07 \pm 1.48$	1.19
2.9	59.15 ± 5.05	$5.40 \pm 2.05$	1.85
3.2	64.53 ± 5.53	$7.89 \pm 2.93$	3.10

LVO2: level standard regression; LVO2+BL: level alternative regression; GVO2: 10.5% grade standard regression; GVO2+BL: 10.5% grade alternative regression.

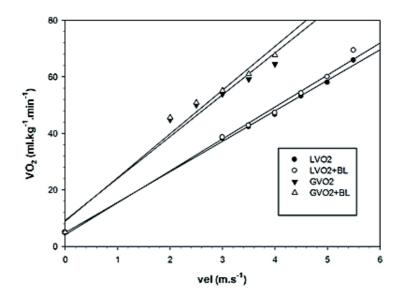


FIGURE 1 - Regression lines computed for running at different grades and with different calculation methods.

TABLE 3 -Mean  $(\pm sd)$  and main effects of the slope, the y-intercept, the scatter around the regression line and  $C_R$  at different grades and with or without the blood lactate ([La-]) energy equivalent (Eq) inclusion.

	Without [La-] Eq	With [La-] Eq	F	p
	$(Mean \pm sd)$	$(Mean \pm sd)$		
Level running				
Slope	$10.95 \pm 0.69$	$11.76 \pm 0.83$	15.42	0.002
<i>y</i> -intercept	$4.08 \pm 0.80$	$3.76 \pm 1.00$	1.05	0.327
Scatter	$1.62 \pm 0.83$	$2.58 \pm 1.09$	5.98	0.031
$C_R (ml \cdot kg^{-1} \cdot m^{-1})$	$0.18 \pm 0.12$	$0.20 \pm 0.01$	15.29	0.002
10.5% grade running				
Slope	$18.23 \pm 0.57$	$19.54 \pm 2.31$	4.93	0.046
<i>y</i> -intercept	$5.41 \pm 1.38$	$5.00 \pm 1.04$	0.75	0.404
Scatter	$1.51 \pm 0.73$	$2.16 \pm 0.95$	3.18	0.100
$C_R (ml \cdot kg^{-1} \cdot m^{-1})$	$0.30 \pm 0.01$	$0.33 \pm 0.04$	4.59	0.053

No significant interaction (p > 0.05) between [La<sup>-</sup>]  $O_2$  equivalent inclusion and grade running was found in the repeated measures ANOVAs. To assess the effect of [La<sup>-</sup>] inclusion in the regression estimates and CR, we conducted separate within-subjects ANOVAs on the data from the level and the 10.5% grade running. For level running, results indicated a significant increase in the slope (F<sub>1,12</sub> = 15.42, p = 0.002,  $\eta_p^2$  = 0.56), the scatter (F<sub>1,12</sub> = 5.98, p = 0.031,  $\eta_p^2$  = 0.33) and in the CR (F<sub>1,12</sub> = 15.29, p = 0.002,

 $\eta_p^{~2}$  = 0.56), but not in the y-intercept estimate ( $F_{1,12}$  = 1.05, p = 0.327,  $\eta_p^{~2}$  = 0.08). Regarding the 10.5% grade running, the same trend of increasing effects was found in the slope and CR, however, with lower effect sizes than found for level running, namely: slope ( $F_{1,12}$  = 4.93, p = 0.046,  $\eta_p^{~2}$  = 0.29) and CR ( $F_{1,12}$  = 4.59, p = 0.053,  $\eta_p^{~2}$  = 0.28). A nonsignificant effect was found in the scatter ( $F_{1,12}$  = 3.18, p = 0.100,  $\eta_p^{~2}$  = 0.20) and the y-intercept estimate ( $F_{1,12}$  = 0.75, p = 0.404,  $\eta_p^{~2}$  = 0.06) at grade running.

# **Discussion**

The present study investigated if the inclusion of [La-] measurements in level vs. grade running significantly affects the VO<sub>2</sub>-intensity regression estimates in the same way. For both level and inclined conditions a slight but significant increase in the energy cost slope was found (FIGURE 1, TABLE 3). Since the [La-] accumulation is expected to rise more with incremental inclined grade running, we hypothesized that the [La-] energy equivalent when added to the VO<sub>2</sub> measurements during running exercise would greatly influence the 10.5% grade running, when compared with level running. It has been shown that grade running demands a high energy turnover<sup>10</sup>, possibly due to a poor running economy<sup>11</sup>. Inclined running imposes a different mechanical pattern<sup>12</sup>, as compared with level running and the former pattern relies more on concentric muscle contractions. Based on these facts, a more pronounced lactate accumulation is expected when running at a steeper grade.

In fact our data with highly-trained subjects indicated the opposite tendency; i.e. VO<sub>2</sub>-intensity regression seemed to be more affected with the inclusion of [La<sup>-</sup>] measurements in the level running condition. Despite the larger energy cost of running with the inclusion of [La<sup>-</sup>] measurements in both exercise conditions, the error of its estimate only increased in the case of level running. As for 10.5% grade running the error of the regression decreased (though not significantly) when the [La<sup>-</sup>] measurements were included in the calculations.

It is of interest that the estimates in the present study were less affected by the [La<sup>-</sup>] measurements at 10.5% running. In our opinion the fact that the subjects in the present study were highly-trained runners that often perform inclined running, both in training and in competitions, may help explain these

results. Previously, Pringle et al.2 have observed, in untrained runners, that the VO, slow component was higher for uphill when compared to level treadmill running. However, Reis et al.3 confirmed that, for endurance-trained subjects, the slow component is not greatly affected by the treadmill grade. In the present study the slow component (as quantified by the difference between end- VO, and the VO, at the 3rd min of exercise) was below 200 ml·min<sup>-1</sup> in every bout (sample mean). However, in the last bout of the level running there were two subjects which presented values above 300 ml·min<sup>-1</sup>. Hence there could be a possible underestimation of energy cost without the BL adding during level running. However, possible deviations from linearity due to a slow component would be the same for each pair of regressions (with and without BL).

The issue of the treadmill inclination may affect differently untrained subjects as opposed to highly trained runners. If this line of reasoning is correct, the results of the present study may not be applicable to other subject cohorts, such as less-trained or untrained subjects. Similarly, the study design itself is limiting when it is considered that the total energy costs are a product of both aerobic and anaerobic energy system interactions that may differ at a given work rate/power output<sup>16</sup>: the regression lines between level and graded conditions were not parallel (FIGURE 1). Indeed, from a relative perspective the [La<sup>-</sup>] accumulation across the two testing protocols (see TABLES 1 and 2) did not seem to be much affected by the treadmill grade, yet both the slope and  $C_{\scriptscriptstyle R}$  grew with an increasing difference between level and inclined conditions (TABLE 3).

It is concluded that the inclusion of [La] equivalent on  $VO_2$ -intensity estimates, in highly trained runners, slightly but significantly influences the mean

parameters associated to the energy expenditure (slope and the  $C_R$ ) at 10.5% grade running, albeit at a smaller magnitude when compared to level running. This evidence demonstrates that graded exercise testing in highly trained athletes is a useful and feasible form of cardiorespiratory assessment, and the possible inclusion of [La-] measurements in  $VO_2$ -intensity regression estimates at sub maximal running should be considered when testing these subjects. Nevertheless, it is possible that this recommendation may not be applicable to sedentary individuals or less trained athletes,

with more studies required to confirm or refute these findings.

Limitations of the current study were the homogeneity of the recruited sample and the potential for differences in aerobic and anaerobic metabolic interactions or responses between the two protocols. Therefore, we recommend testing subjects with different aerobic abilities and training backgrounds, both at level and inclined grades. In addition, different treadmill grades are encouraged to be used in order to provide a more complete and comprehensive knowledge on this issue.

#### Resumo

Inclusão do equivalente energético do lactato na regressão  $VO_2$ -intensidade em corrida horizontal e inclinada (10,5%)

O estudo teve por objetivo analisar o efeito da adição do equivalente energético do lactato sanguíneo com a medida de  $VO_2$  durante a corrida em esteira horizontal (0%) e inclinada (10,5%), como forma de estimativa do custo energético da corrida. Treze corredores de meia e longa distância (idade 28,1  $\pm$  4,2 anos; estatura 1,75  $\pm$  0,07 m; massa corporal 65,2  $\pm$  4,9 kg;  $VO_2$ max 70,3  $\pm$  4,9 ml·kg<sup>-1</sup>·min<sup>-1</sup>) cumpriram dois testes em esteira rolante (0% e 10,5%) que incluíram vários estágios em intensidade constante. Foram calculadas para cada atleta as regressões  $VO_2$ -velocidade, bem como regressões alternativas com a adição de um equivalente energético de 3 ml  $O_2$  Eq·kg<sup>-1</sup>·mM [La-] às medições de  $VO_2$ . Não se verificou interação significativa entre a adição do equivalente do lactato e a inclinação da esteira. A ANOVA indicou um efeito significativo da adição do equivalente do lactato na inclinação da reta de regressão e na estimativa do custo energético. Os tamanhos do efeito obtidos indicam que este efeito é mais forte na corrida horizontal. Estes resultados sugerem que em testes laboratoriais com corredores treinados se deverá considerar a adição dos valores de  $VO_2$  com os equivalentes energéticos do lactato.

Palavras-chave: Corrida horizontal vs. inclinada; Custo energético; Erro de estimativa.

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ENDEREÇO Victor Machado Reis Universidade de Trás-os-Montes & Alto Douro Quinta de Prados, 5001-801 Vila Real - PORTUGAL e-mail: victormachadoreis@gmail.com

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