# Athletic, muscular and hormonal evaluation in CrossFit<sup>®</sup> athletes using the "Elevation Training Mask"

# Diego Fernández-Lázaro<sup>1,2</sup>, Juan Mielgo-Ayuso<sup>3</sup>, Darío Fernández-Zoppino<sup>3</sup>, Silvia Novo<sup>1</sup>, María Paz Lázaro-Asensio<sup>4,5</sup>, Nerea Sánchez-Serano<sup>1,6</sup>, César I. Fernández-Lázaro<sup>1,7</sup>

<sup>1</sup>Departamento de Biología Celular, Histología y Farmacología. Facultad de Ciencias de la Salud. Universidad de Valladolid. Soria. <sup>2</sup>Grupo de Investigación de Neurobiología. Facultad de Medicina. Universidad de Valladolid. Valladolid. <sup>3</sup>Departamento de Ciencias de la Salud. Facultad de Ciencias de la Salud. Universidad de Burgos. <sup>4</sup>Departamento de Fisioterapia. Facultad de Ciencias de la Salud. Universidad de Valladolid. Soria. <sup>5</sup>Centro de Salud "La Milagrosa". Salud Castilla y León (SACyL). Soria. <sup>6</sup>Unidad de Microbiología Clínica. Hospital Sta. Bárbara de Soria. Soria. <sup>7</sup>Departamento de Medicina Preventiva y Salud Pública. Facultad de Medicina. Universidad de Navarra. Pamplona.

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#### Summary

**Introduction:** The possibility of performing intense workouts without falling into states of chronic fatigue stimulates the use of devices that improve muscular and hormonal functionality in athletes. The Elevation Training Mask (Training Mask LLC) (ETM) allows the application of hypoxia during exercise. The ETM is integrated into training routines increasing the physical stimulus to improve performance.

**Objective:** We evaluated the impact of ETM on Workouts of the Day (WODs), muscular and hormonal behavior in Crossfit® athletes.

**Material and method:** Prospective cohort study. During 12 weeks 20 Crossfit<sup>®</sup> athletes trained 60 minutes 3 days a week were randomly divided into 2 groups, control group (CG) (n=10) and ETM group (EG) (n=10) applying an additional progressive simulated altitude between 914 and 2743 meters. WODs (press, squat, deadlift, total CF and grace), macular markers: lactate dehydrogenase (LDH); creatine kinase (CK); myoglobin (Mb) and hormones: testosterone (T); cortisol (C), were evaluated at 2 time points of the study: day 1 (T1) and day 84 (T2).

#### Key words:

Elevation Training Mask. Hypoxia. Sport Performance. Muscle. Hormones. Crossfit<sup>®</sup>. **Results:** All WODs and parameters LDH, CK, Mb, T and C showed no significant difference (p>0.05) in the time group interaction. In EG, a substantially lower percentage change ( $\Delta$ ) between T1 and T2 was observed in Mb (-16.01±25.82%), CK (6.16±26.05%) and C (-0.18±4.01%) than in CG (Mb: -094±4.39%; CK: 17.98±27.19%; C: 4.56±3.44%). The  $\Delta$  T1-T2 in the WODs were similar. **Conclusion:** After 12 weeks of training under simulated hypoxia conditions with ETM there are no improvements in athletic performance assessed by WODs. However, the greater tendency to decrease Mb, CK and C, after using ETM, could stimulate recovery and indicate a lower muscle catabolism of the Crossfit<sup>®</sup> athlete in the long term.

## Evaluación deportiva, muscular y hormonal en deportistas de CrossFit<sup>®</sup> que emplean la "Elevation Training Mask"

#### Resumen

Introducción: La posibilidad de realizar entrenamientos intensos sin caer en estados de fatiga crónica, estimula el uso de dispositivos que mejoren la funcionalidad muscular y hormonal en deportistas. La *Elevation Training Mask* (Training Mask LLC) (ETM) permite la aplicación de hipoxia durante el ejercicio. La ETM se integra en las rutinas de entrenamiento incrementando el estímulo físico para mejorar el rendimiento.

**Objetivo:** Evaluamos el impacto de la ETM sobre los entrenamientos del día o Workouts of the Day (WODs), el comportamiento muscular y hormonal en deportistas de Crossfit<sup>®</sup>.

**Material y método:** Estudio de cohorte prospectivo. Durante 12 semanas 20 practicantes de Crossfit® entrenaban 60 minutos 3 días a la semana fueron divididos aleatoriamente en 2 grupos, grupo control (GC) (n=10) y grupo ETM (GE) (n=10) aplicando una altitud simulada adicional progresiva entre 914 y 2743 metros. Los WODs (press, squat, deadlift, CF total y grace), marcadores maculares: lactato deshidrogenasa (LDH); creatina quinasa (CK); mioglobina (Mb) y hormonas: testosterona (T); cortisol (C), se evaluaron en 2 momentos del estudio: día 1 (T1) y día 84 (T2).

**Resultados:** Todos los WODs y los parámetros LDH, CK, Mb, TT y C no mostraron ninguna diferencia significativa (p>0,05) en la interacción grupo tiempo. En el GE se observó un porcentaje de cambio ( $\Delta$ ) entre T1 y T2 sustancialmente menor en Mb (-16,01±25,82%), CK (6,16±26,05%) y C (-0,18±4,01%) que en GC (Mb:-0,94±4,395; CK: 17,98±27,19%; C: 4,56±3,44%). Los  $\Delta$ T1-T2 en los WODs fueron similares.

#### Palabras clave:

Elevation Training Mask. Hipoxia. Rendimiento deportivo. Músculo. Hormonas. Crossfit<sup>®</sup>. **Conclusión:** Tras 12 semanas de entrenamiento en condiciones simuladas de hipoxia con ETM no existen mejoras del rendimiento deportivo evaluadas mediante los WODs. Sin embargo, la mayor tendencia a disminuir de Mb, CK y C, tras usar la ETM, podrían estimular la recuperación e indicar un menor catabolismo muscular del atleta de Crossfit<sup>®</sup> a largo plazo.

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**Correspondence:** Diego Fernández Lázaro E-mail: diego.fernandez.lazaro@uva.es

# Introduction

The demands of competition-level sport push us to seek out systems which improve results. Hypoxic stimulation in athletes has been used as a method to improve athletic performance since the 1960s<sup>1</sup>. Hypoxic training (HT) induces modifications in several body systems, including the central nervous, cardiorespiratory, hormonal and muscular systems, which do not occur in normoxic conditions or, if they do, do so to a lesser degree<sup>2</sup>.

In recent years, the simulation of altitude training in sports medicine has worked to generate beneficial adaptations for both the health and the athletic performance of individuals<sup>3</sup>. These methods remove the drawbacks of travelling and the cost involved, and do not reduce the intensity of training as a result of staying at altitude for prolonged periods<sup>1</sup>. Consequently, the use of various altitude simulation methods to induce a normobaric hypoxic stimulus or minimise the amount of air that a subject is allowed to consume has become popular<sup>4,5</sup>. These methods are presented as ergogenic strategies for athletes to increase training-induced adaptations<sup>6</sup>. Devices have recently arrived on the market for professional and recreational athletes, and the general public to induce/ simulate hypoxic conditions which are easy to acquire and economical<sup>7</sup> compared to the hypobaric chambers or portable devices for exposure to hypoxia available, such as the Altitrainer<sup>®</sup> or GO<sub>2</sub> Altitude<sup>®</sup> hypoxicator.

One of these is the *"Elevation Training Mask"* (ETM), a new instrument for use during workouts which the manufacturer describes as an exercise device with breathing resistance and adjustable capacity. The ETM aims to simulate training at altitude (914 to 5486 metres) by restricting the oxygen ( $O_2$ ) supply, creating this condition when breathing through a system of flow valves designed to limit the amount of air that enters the mask<sup>8</sup>. The altitude simulation of the ETM does not generate a hypobaric situation (reduced partial pressure of  $O_2$ ) but leads to mild arterial hypoxemia as a consequence of the reduced respiratory rate caused by the respiratory restriction produced by the resistance caps and the flow-valve system (Figure 1). Hypoxemia could also be intensified by inhaling carbon dioxide ( $CO_2$ ) and the subsequent shift of the  $O_2$  dissociation curve<sup>9</sup>.

HT is known to produce pronounced changes in lactate concentrations in athletes when compared with normoxic training<sup>10</sup>. However, no significant differences were observed in lactate concentration between the control groups and the group that used the ETM during continuous exercise<sup>11</sup>. Fernandez-Lázaro *et al.*<sup>1</sup> described that a hypoxia stimulation programme in combination with training was able to stimulate improvements in the haematological profile which were related to better results in athletic performance assessment tests. During training with ETM, the haematological profile of the athletes does not change<sup>12,13</sup>. This would justify and corroborate that the ETM does not imitate or simulate altitude situations. Additionally, the restriction of the airflow caused by the valves on the ETM increases the work of the respiratory muscles, which could stimulate improvements in endurance performance through respiratory

# Figure 1. *Elevation Training Mask* 2.0 resistance caps and valve system



muscle training (RMT)<sup>14</sup> although the use of the ETM during a highintensity training programme lasting several weeks has been shown not to increase lung function<sup>7,12,13</sup>. Therefore, although the physiological changes associated with the ETM are not induced<sup>7,11-13</sup>, improvements have been reported on specific performance markers when compared with identical training without the EMT<sup>7,12,13</sup>.

CrossFit® (CF) is a popular new exercise method involving functional movements performed at high intensity. Training consists of functional movements which make up *Workouts of the day* (WODs). In these sessions, all the WODs are performed quickly and repetitively at maximum intensity with little or no recovery time in between<sup>15,16</sup>.

We do not know at present if the ETM could compromise the ability to train at the high intensities that CF demands. The lack of evidence regarding the mechanisms of the ETM on athletic performance calls for considerable research to develop protocols which optimise the balance between efficacy and safety concerning the biological effects of using the mask, fundamentally hormonal safety and muscle response, which have not yet been studied. For these reasons, we set out to evaluate the influence of using the ETM in combination with high-intensity training regimes in subjects who did CF in terms of WOD performance, hormonal response, testosterone (T) and cortisol (C), and the enzymes of muscle activity (damage and inflammation) produced by a training programme under these conditions.

## Materials and method

A prospective cohort study was conducted. Twenty male volunteer CF practitioners took part in a non-placebo controlled, randomised study which evaluated the effect of the ETM 2.0 (Training Mask LLC, Cadillac, Michigan) on athletic performance, muscle response and hormonal behaviour during a 12-week training period. The protocol followed the recommendations of the Declaration of Helsinki, and the study was reviewed and approved by the Ethics Committee for research involving medicinal products in the East Valladolid area (PI 19-1361).

#### **Physical examination**

All the subjects signed informed consent forms. The participants were studied by means of a cardiopulmonary and electrocardiographic examination, and completed a medical questionnaire before joining the study. None of the CF athletes smoked, drank alcohol or took drugs or illegal substances capable of altering their muscular or hormonal response, or their sports performance. No injuries were suffered before or during the test, as these were ruled out by medical records and the clinical examination. All the subjects followed the same diet during the study, supervised by a nutritionist.

#### **Subjects**

The participants were allocated to two groups using a random sampling method. The study group (SG), which used the ETM, included a total of 10 male CF athletes (n=10) (38.4±3.8 years old; body mass index 24.6±2.7 kg/m<sup>2</sup>; 51.5±6.5 mL-kg<sup>-1</sup> -min<sup>-1</sup>) and the control group (CG), which did not use the ETM, consisted of 10 male CF athletes (n=10) (36.7±5.3 years old; body mass index 22.9±3.1 kg/m<sup>2</sup>; 53.1±7.3 mL-kg<sup>-1</sup> -min<sup>-1</sup>). All the study subjects (n=20) had at least one year's experience doing CF. No participant had been recently exposed to altitude, hypoxia or acclimatisation, bar the fact that they lived in Salamanca (802 metres) and Soria (1063 metres) in Spain.

#### Training

The workouts during the 12 weeks of the study consisted of 3 weekly sessions on alternate days. Each session lasted one hour and was divided into a specific warm-up, a strength and/or skill component, programmed strength or metabolic conditioning training lasting from 10 to 30 minutes, and a cooldown and/or mobility work. Each workout was supervised by a certified Level 1 CF Trainer. All the subjects performed the same physical activity routines to ensure that they did the same training during the study.

#### **Dietary assessment**

To calculate and record the nutrient composition and energy intake of the food and drink that the athletes consumed, the methodology used in some of our previous studies was followed<sup>17,18</sup>.

#### Uso de la Elevation Training Mask

The ETM was employed in the 36 training sessions over the 12-week study period. The additional altitude simulation was 914 metres in the first week and 1829 metres in the second in order to accustom the participants to the airflow restriction and acclimatise them to altitude simulation. In the remaining weeks of the study, the simulated altitude was 2743 metres above the altitude at which training was taking place

#### Blood collection and testing

Antecubital venous blood samples were taken from the CF athletes on the first day of study (T1) without prior use of the ETM and after 12 weeks of training with the ETM (T2). For the collection, extraction and transport of the blood samples of the athletes, the methodology of the studies conducted by Fernández-Lázaro *et al.*<sup>1,17</sup> was used.

The athletes' lactate dehydrogenase (LDH), creatine kinase (CK) and myoglobin (Mb) blood serum concentrations were measured by enzymatic chemiluminescence<sup>17</sup>. Total T and C were determined by enzyme-linked immunosorbent assays<sup>19</sup>.

Percentage changes in plasma volume (%  $\Delta$ PV) were calculated using the Van Beaumont formula. The values of the analytical markers were adjusted for the changes in plasma volume, using the following formula: Corrected value = Uncorrected value x ((100 + %  $\Delta$ PV) / 100)<sup>17</sup>.

#### Athletic performance assessment

The performance of the subjects was evaluated in different WODs which respected CF methods considered internationally standard<sup>15</sup>. The exercises performed were: back squat, shoulder press and deadlift, CF Total and Grace.

#### Determination of perceived exertion

Before their blood was drawn, the participants were asked to score their perceived muscle discomfort at each point in time (T1 and T2), using the Borg CR-10 scale validated for rating perceived exertion (RPE)<sup>20,21</sup>.

#### Statistical analysis

Processing was randomly assigned using the Random Sequence Generator. Statistical analysis was performed using the IBM Statistical Package (SPSS Version 22) and Graphpad Prism (Graphpad Software Version 6.01, San Diego, CA). The data are expressed as the mean  $\pm$  standard deviation (SD). The differences in the parameters were evaluated using Scheffe's method to identify significant differences between T1 and T2 independently. Differences were considered significant when p<0.05. A repeated measures ANOVA was used to examine the existence of an interaction effect of training with the ETM (time by group) on all the parameters assessed. The percentage changes of the variables studied in each group between the baseline tests (T1) and post-ETM tests (T2) were calculated as  $\Delta$  (%): [(T2 – T1) / T1]  $\times$  100). The differences between groups in terms of the changes  $\Delta$ (%) were evaluated by means of a parametric or non-parametric test for independent samples after the normality of the data had been confirmed with the Shapiro-Wilk test.

# Results

#### **Dietary intake**

There were no significant differences (p > 0.05) between the study groups (CG and SG) for total caloric, vitamin and mineral intake (Table 1).

#### Muscle markers

Table 2 shows the muscle behaviour markers (LDH, CK and Mb) at two points in the study, T1 and T2. No statistically significant differences (p>0.05%) existed in the two groups (CG and SG) for the muscle parameters analysed, except LDH in SG, in which an increase with a statistically significant difference (p<0.05) between the two points in the study was observed (T1: 167.55±21.30 U/L vs T2: 189.80±27.69 U/L) (Figure 2). None of the muscle markers analysed showed a significant difference in values (p>0.05) in the group by time interaction.

Table 3 shows the percentage changes of the muscle parameters at the end of the study. There were no significant differences (p>0.05) between LDH, CK and Mb. However, in the SG a greater increase in LDH (12.75±15.01%) and a significant downward trend in Mb (-16.01±25.82%) were both observed, as was a smaller increase in CK (6.16±26.05%) in the SG compared with the CG (CK: 17.98±27.19%).

#### Hormonal behaviour

No significant differences (p>0.05) were found in T and C hormones between the two groups (CG and SG) over the 12 weeks of the study.

#### Table 1. Energy and micronutrient intake. Daily mean in the study group (SG) and control group (CG ) of CrossFit® athletes during the 12 weeks of study.

Group	Group Study (SG)	Group Control (CG)	Ρ	Recommended daily*
Energy (kcal/kg)	38.3±5.8	39.7±5.2	0.273	
Ca (mg)	1036±214	1082±193	0.345	1000
Mg (mg)	542±99	551±95	0.863	320
P (mg)	2123±66	2076±84	0.583	700
Fe (mg)	21.1±4.6	23.5±5.7	0.801	10
Zn (mg)	13.7±0.8	14.7±0.8	0.699	8
Vitamin A (µg)	1859±1180	2002±775	0.659	689
Vitamin E (mg)	17.0±2.5	17.3±1.6	0.466	15
Thiamine (mg)	2.62±0.20	2.80±0.62	0.526	1.1
Riboflavin (mg)	2.76±0.23	2.75±0.28	0.693	1.1
Niacin (mg)	40.0±7.1	38.2±3.9	0.815	14
Vitamin B6 (mg)	4.11±0.73	4.36±0.94	0.831	1.3
Folic acid (mg)	634±171	636±169	0.885	400
Vitamin B12 (µg)	9.12±3.91	9.35±3.11	0.877	2.4
Vitamin C (µg)	347±138	356±119	0.733	700

Table 2. Biochemical markers of muscle behaviour and hormonal response in the CrossFit® athletes in the control group (CG) and the study group (SG) with the Elevation Training Mask at two points in the study: T1, at the beginning of the study, and T2, after 12 weeks.

	T1	T2	<i>P</i> (T x G)	
LDH (U/L) [1				
CG	200.00±46.49	195.71±33.70	NC	
SG	167.55±21.30	189.80±27.69*	NS	
Creatine kina	nse (U/L) [38 – 190 U/L]	l		
CG	437.56±467.80	500.22±510.25	NG	
SG	301.20±237.51	315.70±232.48	NS	
Myoglobin (ng/ml) [28 – 72 ng/ml]				
CG	32.67±17.38	33.33±21.17	NC	
SG	38.00±26.25	26.11±5.55	NS	
Total testosterone (ng/ml) [2.49 – 8.36 ng/ml]				
CG	6.19±0.87	6.52±0.91	NS	
SG	6.19±1.03	6.39±1.06	N.S	
Cortisol (ug/dl) [6.0 – 18.4 ug/dl]				
CG	17.79±3.69	18.32±3.88	NG	
SG	17.80±2.55	17.53±3.70	NS	

Data expressed as mean ± standard deviation.

Significant differences during the study period, calculated using Scheffe's method.

P (T x G): 2-factor ANOVA (time by group). \*: Significant difference between T1 and T2 (p<0.05).

NS: Not significant.

Reference values in square brackets.

Table 3. Percentage change in the biochemical markers of muscle behaviour and hormonal response in the control group (CG) and the study group (SG) with the Elevation Training Mask during the 12 weeks of training.

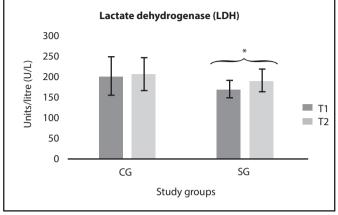
	Δ (T1-T2)	Р	
LDH (%)			
CG	-0.18±28.19	0.620	
SG	12.75±15.01	0.620	
Creatine kinase (%)			
CG	17.98±81.59	0.296	
SG	6.16±26.05	0.290	
Myoglobin (%)			
CG	-0.94±4.39	0.200	
SG	-16.01±25.82	0.289	
Total testosterone (%)			
CG	5.79±0.57	0.762	
SG	3.60±0.52		
Cortisol (%)			
CG	4.56±3.44	0.640	
SG	-0.18±4.01	0.649	

Data expressed as mean ± standard deviation.

 $\Delta$  (T1-T2) = ((T2-T1) /T1) \*100.

P: Statistical differences between groups

Figure 2. Lactate dehydrogenase (LDH) in the CrossFit<sup>®</sup> athletes in the control group (CG) and the study group (SG) with the *Elevation Training Mask* at two points in the study: T1, at the beginning of the study, and T2, after 12 weeks.



\*: Significant difference between T1 and T2 (p<0.05) Control group (CG); Study group (SG)

Table 4. Performance tests, "Workouts of the day" (WODs), completed by the CrossFit<sup>®</sup> athletes in the control group (CG) and the study group (SG) with the *Elevation Training Mask* at two points in the study: T1, at the beginning of the study, and T2, after 12 weeks.

	T1	T2	P (T x G)	
Press (kg)				
CG	70.83±10.69	76.67±10.80 *	NS	
SG	69.17±11.58	80.00±11.40 *	CNI	
Squat (kg)				
CG	142.50±28.94	149.17±23.11	NC	
SG	128.33±15.71	131.67±11.25	NS	
Deadlift (kg)				
CG	160.83±19.34	165.83±18.28	NC	
SG	162.50±9.87	170.00±10.49 *	NS	
CF total (kg)				
CG	374.17±47.48	391.67±37.24 *	NS	
SG	360.00±35.36	381.67±30.93 *	CNI	
Grace (segund	los)			
CG	30.17±5.08	24.33±4.46*	NC	
SG	28.00±6.23	22.67±5.92*	NS	

Data expressed as mean ± standard deviation.

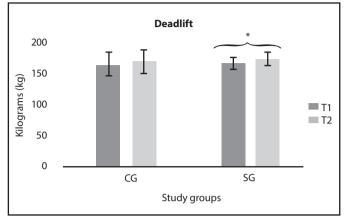
Significant differences during the study period, calculated using Scheffé's method  $P(T \times G)$ : 2-factor ANOVA (time by group)

\*: Significant difference between T1 and T2 (p<0.05).

NS: Not significant

Neither were there any significant differences (p>0.05) in the group by time interaction (Table 2). The CG had a higher percentage of change for T ( $5.79\pm0.57\%$ ) and the SG showed a negative percentage change (- $0.18\pm4.01\%$ ) for C (Table 3).

Figure 3. Deadlift in the CrossFit<sup>®</sup> athletes in the control group (CG) and the study group (SG) with the *Elevation Training Mask* at two points in the study: T1, at the beginning of the study, and T2, after 12 weeks.



\*: Significant difference between T1 and T2 (p<0.05)

Control group (CG); Study group (SG)

Table 5. Percentage change in the performance tests, "Workouts of the day" (WODs), in the control group (CG) and the study group (SG) with the *Elevation Training Mask* during the 12 weeks of training.

	Δ (T1-T2)	Р	
Press (%)			
CG	8.52±3.76	0.614	
SG	10.70±3.76	0.014	
Squat (%)			
CG	5.70±9.83	0.126	
SG	4.89±6.06	0.126	
Deadlift (%)			
CG	3.32±6.33	0.639	
SG	3.78±4.18	0.059	
CF total (%)			
CG	5.07±16.05	0.624	
SG	5.43±9.83	0.024	
Grace (%)			
CG	-24.73±2.48	0.524	
SG	-23.46±1.63	0.524	

Data expressed as mean  $\pm$  standard deviation.

 $\Delta$  (T1-T2) = ((T2-T1) /T1) \*100

P: Differences between groups

#### Sports performance

Table 4 shows the results of the performance tests at T1 and T2. A statistically significant increase in total kilograms was observed both in the CG (T1:  $374.17\pm47.48$  kg vs T2:  $391.67\pm37.24$  kg) and the SG (T1:

Table 6. Determination of exertion perceived, BORG CR-10, by the CrossFit<sup>®</sup> athletes in the control group (CG) and the study group (SG) with the *Elevation Training Mask* at two points in the study: T1, at the beginning of the study, and T2, after 12 weeks.

Test	Group	Time		P (T x G)
		T1	T2	
BORG CR-10	CG	5.23±3.13	5.32±3.24	NS
	SG	5.70±1.29	5.86±1.1	

Data expressed as mean  $\pm$  standard deviation. Significant differences during the study period, calculated using Scheffé's method.

P (T x G): 2-factor ANOVA (time by group)

\*: Significant difference between T1 and T2 (p<0.05)

NS: Not significant; Control Group: SG; Study Group: SG

 $360.00\pm35.36$  kg vs T2:  $381.67\pm30.93$  kg) in CF total. A statistically significant reduction (p<0.05) was also measured in the seconds needed to complete Grace in both the CG (T1:  $30.17\pm5.08$  s vs T2:  $24.33\pm4.46$  s) and the SG (T1:  $28.00\pm6.23$  s vs T2:  $22.67\pm5.92$  s). A significant improvement (p<0.05) in the deadlift was only observed in the SG (T1:  $162.50\pm9.87$  vs T2:  $170.00\pm10.49$ ) (Figure 3). None of the WODs analysed showed a significant difference (p>0.05) in the group by time interaction.

The percentage changes (Table 5) were similar for the CG and SG in CF Total (CG:  $5.07\pm16.05\%$  vs SG:  $5.43\pm9.83\%$ ) and Grace (CG:  $-24.73\pm2.48\%$  vs SG:  $-23.46\pm1.63\%$ ).

#### Determination of perceived exertion

Table 6 shows the RPE in the CG. The Borg CR10 scale shows that there were no significant differences (p>0.05) between T1 and T2 in either group (CG or SG). Neither were there any significant differences (p>0.05) in the group by time interaction.

## Discussion

The purpose of this study was to investigate the effect (36 training sessions) of the ETM on CF performance using WODs, examining muscle behaviour and hormonal variation in recreational CF athletes. To our knowledge, this is the first study of its kind. The study used a training methodology designed to cause a high degree of fatigue to permit biochemical and hormonal assessment, and to be employed as a programme focused on improving WOD performance. According to the results of our study, use of the ETM did not affect the participants' overall training programme. Nor were any side effects from the use of the ETM which led participants to abandon the study reported. Therefore, the use of the ETM during CF training was well tolerated, as confirmed by the absence of significant differences in RPE between the CG and SG, in agreement with the study conducted by Granados *et al.* <sup>9</sup> and contrary to Jagim *et al.*<sup>6</sup> in weightlifters. Our results may be the consequence of acclimatising to the ETM in the first two weeks of the study.

As for athletic performance, evaluated through WODs, the main finding after 12 weeks of training with the ETM was that there were no differences in sports performance between the two situations (CG and SG), although we did observe that the use of the ETM led to a significant increase in deadlift performance and a greater percentage of improvement in CF Total, which is the sum of the three WODs. These results are consistent with those reported by other authors<sup>7,12,13</sup>. Although the results between groups (CG and SG) were not significant, there was a greater increase in  $VO_{2000}^{-7,12,13}$  and anaerobic power output in a standardised cycle ergometer test<sup>12</sup> in the SG wearing the ETM compared with the control group, which did not wear the mask. One hypothesis as to why the use of the ETM may improve performance is that HT optimises muscle response through an increase in certain hormones, such as T<sup>22</sup>, and the accumulation of metabolites which serve as components in the signalling of the key anabolic pathways that stimulate the recruitment of muscle fibres, thereby contributing to hypertrophy and increased muscle strength<sup>2,23,24</sup>. This hypoxic environment, which would permit HT, could be achieved by performing high-intensity exercise with the ETM and would potentially provide benefits similar to altitude training<sup>25</sup>, such as increases in performance variables<sup>26-28</sup>. Furthermore, the restriction of respiratory flow caused by the ETM permits RMT, which would enhance performance in CF WODs due to a possible delay in the triggering of the respiratory muscle metaboreflex and increased respiratory performance<sup>3</sup>.

Jagim et al.<sup>6</sup> reported that maximum speed in the execution of standardised movements such as back squat and bench press in weightlifters was lower with the ETM, although there were no significant differences between groups. Likewise, we observed in the Grace WOD (30 movements/time) a lower speed and intensity of execution in the SG, without significant differences between the two conditions of the study. Although we did not analyse blood lactate concentration values, one study<sup>6</sup> found that less lactate was collected at the end of weightlifting exercises with ETM compared with exercises without ETM. This could be explained by differences in fast muscle fibre recruitment patterns during exercise in the CG and SG<sup>6</sup>. Reduced recruitment of fast muscle fibres suggests an earlier onset of muscle fatigue<sup>29</sup>, affecting the potential to reach maximum execution speed during the Grace WOD with the ETM. The ETM devices also induced respiratory acidosis by increasing CO<sub>2</sub> respiration, as occurs with RMT instruments<sup>30</sup>. This restriction of O<sub>2</sub> may lead to adaptations related to greater buffering capacity, which would lower blood lactate<sup>11</sup>. Therefore, the potential to reach maximum intensity during the Grace WOD could also be compromised with the ETM by the decrease in blood lactate as a consequence of the buffering effect<sup>6</sup>.

The intense isometric and eccentric weight exercises used in CF workouts have a positive influence on body composition and physical fitness but also entail a high risk of skeletal muscle damage<sup>16</sup>. This situation triggers early fatigue, additional oxidative stress, a reduced capacity to carry out exercise, a greater perception of exertion and

uncertain movements<sup>31</sup>. Individual monitoring by determining muscle biomarkers could define the training load and minimise these risks<sup>32</sup>. High circulating levels of enzymes such as LDH, CK, and Mb are indicative of increased exercise-induced muscle damage (EIMD), which negatively affects athletes because it reduces exercise performance and can also put their health at risk<sup>32</sup>.

The findings of our study show that CK increased 10 percentage points less in the SG (6.16±26.05%) than the CG (17.98±81.59%), and the concentration of Mb decreased after 12 weeks of ETM (-16.01±25.82%) while remaining practically constant without the mask (-0.94±4.39%). These results seem to indicate that ETM training could modulate and prevent the muscle damage produced by CF training associated with reductions in CK and Mb compared with the CG. The hypoxic environment generated by CF exercise in combination with the ETM<sup>25</sup> could be responsible for the decrease in Mb, in the way that Villa et al.<sup>33</sup> and Fernández-Lázaro et al.<sup>2</sup> described in situations of hypoxia and physical activity. Since exercise and hypoxia are known to control the mitochondrial function and act positively on EIMD through the hypoxia-inducible factor (HIF), which plays an essential role in activating a molecular signalling cascade after exposure to hypoxia<sup>34,35</sup>, the ETM could modulate the expression of HIF and thus attenuate the histopathological muscle damage caused by CF.

C is released from the adrenal cortex in response to psychophysical stress, and significant increases in C have been reported after endurance exercise which alters training adaptations through the direct catabolic effect<sup>32</sup>. Hypoxia has been reported to affect function in the hypothalamic-pituitary-adrenal axis and increase plasma adrenocorticotropic hormone (ACTH) levels. Hypoxia also stimulates the expression of the steroidogenic acute regulatory protein, increases the secretion of glucocorticoids, such as C<sup>22</sup>, and decreases T<sup>36</sup>. Throughout our study, C levels in the SG remained constant (-0.18±4.01%) but increased in the CG (4.56±3.44%), which could suggest that it is adequate in CF training with ETM. That is, the stabilisation of C levels, in addition to the slight increase in T (3.60±0.52%), would permit the activation of protein synthesis, the antiglucocorticoid effect and the secretion of insulin-like growth factor 1 (IGF -1) and growth hormone (GH). This physiological situation would have an influence on muscle satellite cells which could contribute in part to generating greater muscle strength, less muscle damage and better recovery from training<sup>2,23,37</sup>. Therefore, the enhanced catabolic/anabolic ratio created in ETM conditions would contribute to significant improvements in WODs in the long term.

Certain limitations in this study need to be taken into account. One important limitation was that no dummy mask or placebo was used in the CG. Second, this study was only conducted at a resistance altitude of 2743 metres (after the first two weeks of acclimatisation to 914 and 1890 metres, respectively) even though the manufacturer makes several altitude resistances available (914 m to 5486 m). Applying different altitudes, or resistances, during exercise could lead to different results. Another limitation in our study was the small sample size. Including a

larger number of subjects would provide a greater basis for eliminating error due to individual differences.

In conclusion, after 12 weeks of training with the ETM device, no improvements were observed in athletic performance, evaluated using WODs, compared to the control group. However, there were significant improvements in CF Total and Grace between T1 and T2 in the SG. Furthermore, the greater percentage trend in the decrease of Mb and C, together with the smaller increase in CK after ETM use could stimulate recovery and point towards a lower muscle catabolism in CrossFit® athletes in the long term. It is important to note that the results of this study suggest that the use of the ETM during training does not hinder CF practitioners from achieving the desired workloads or training volume. The ETM does not seem to negatively affect subjective perceptions, such as RPE, nor does it pose a risk of adverse events, as none were reported after the 12 weeks of the study. Such events should be considered before using the ETM device in training programmes in the future studies needed to determine whether this modest hypoxic condition or the increased work that the respiratory muscles are required to do is responsible for potential improvements in athletic performance.

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#### **Conflict of interest**

The authors declare that they are not subject to any type of conflict of interest.

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