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Caffeine increases motor output entropy and performance in 4 km cycling time trial

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Abstract

Caffeine improves cycling time trial performance through enhanced motor output and muscle recruitment. However, it is unknown if caffeine further increases power output entropy. To investigate the effects of caffeine effects on cycling time trial performance and motor output entropy (MOEn), nine cyclists (VO_{2MAX} of 55 ± 6.1 $mL{\cdot}kg^{-1}min^{-1}$) performed a 4 km cycling time trial (TT_{4km}) after caffeine and placebo ingestion in a counterbalanced order. Power output data were sampled at a 2 Hz frequency, thereafter entropy was estimated on a sliding-window fashion to generate a power output time series. A number of mixed models compared performance and motor output entropy between caffeine and placebo every 25% of the total TT_{4km} distance. Caffeine ingestion improved power output by 8% ($p = 0.003$) and increased MOEn by 7% ($p = 0.018$). Cyclists adopted a U-shaped pacing strategy after caffeine ingestion. MOEn mirrored power output responses as an inverted U-shape MOEn during the time trial. Accordingly, a strong inverse correlation was observed between MOEn and power output responses over the last 25% of the TT_{4km} ($p < 0.001$), regardless of the ingestion, likely reflecting the end spurt during this period ($p = 0.016$). Caffeine ingestion improved TT_{4km} performance and motor output responses likely due to a greater power output entropy.

Introduction

According to the dynamic system theory, the variability presented by a given physiological system, a concept that is known as complexity, may reflect its flexibility to face natural perturbations [1,2]. For example, the neuromuscular system is characterized by regular fluctuations in electrophysiological responses (i.e. complexity) which enable the central nervous system (CNS) to adapt to environment-induced perturbations [3]. Assuming that every single body motion is a dynamic acceleration-deceleration interplay [4], the level of complexity in motor

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output responses may indicate the CNS ability to face a physical task-induced perturbation. Studies have reported an association between motor output complexity and fatigue, as a reduced knee extensor torque entropy has been observed as a fatiguing single-joint isometric exercise progresses [5,6]. In this regard, it has been hypothesized that a "loss of complexity" is likely present in fatiguing exercises, so that variations in neuromuscular complexity such as in motor output entropy (MOEn), may indicate the neuromuscular system ability to face the exercise-induced fatigue [7].

Studies investigating the MOEn-fatigue relationship have used highly controlled isometric muscle tasks as an exercise mode [5,6]. Despite providing a well-controlled intensity and power output response, this exercise mode has a low ecological validity as it reflects an unnatural form of exercise. Consequently, isometric muscle task-derived results cannot provide enough to understand the MOEn in more usual forms of exercise. For example, exercises such as a cycling time trial may be insightful to understand the MOEn-fatigue relationship in strenuous whole-body self-paced exercises, as this exercise mode requires a more complex, moment-to-moment regulation when regulating pacing and exercise performance [8–15]. For example, power output fluctuations during a cycling time trial may indicate the CNS ability to deal with the central-peripheral fatigue interplay during a more natural form of exercise [8,15], thereby offering insights on the role of the neuromuscular complexity in exercise regulation and pacing strategy.

Whether both central and peripheral fatigue increase as a cycling time trial progresses, one may argue that the neuromuscular complexity decreases as a function of the trial distance [16]. Consequently, a likely U-shape pacing strategy during a short cycling trial [17] may indicate a reduction in MOEn, what could be related to the trial fatigue status. Importantly, a shorter cycling time trial may be preferable to emphasize the CNS complexity when regulating the motor output during exercise, given that the magnitude of neural drive required to complete a short time trial such as 4km (TT_{4km}) is greater than the neural drive necessary to complete longer ones (e.g. 40km). In this sense, a higher power output could suggest an enhanced motor unit firing synchronization during exercise, as the electromyography (EMG) entropy is lower in higher (i.e. 330 W) than lower (i.e. 150 W) power output values [18]. Therefore, considering that trained cyclists produce a higher mean power output in short (TT_{4km}) than in long cycling time trials (i.e. 40km) [16], analysis of MOEn in TT_{4km} could elucidate the MOEn-fatigue interplay in a high ecological validity exercise.

Some ergogenic aids could add valuable information to the neuromuscular complexity-cycling paradigm, as some ergogenics have the ability to change neuromuscular properties. For example, caffeine may be an interesting approach to investigate the MOEn-fatigue interplay, indicating if fluctuations in power output responses during cycling time trials may be related to changes in neuromuscular response complexity. It has been suggested that caffeine increases MOEn through amplification of the synaptic inputs to α-motor neurons [19]. Caffeine increases the monoamines synthesis and turnover [20], thereby amplifying the synaptic input and motoneuronal gain [21,22] as indicated by a steeper H-reflex curve and greater self-sustained motor unit firing frequency [22,23]. Consequently, assuming that a higher motor neuron gain is associated with a greater muscle force variability as suggested elsewhere [24], one may hypothesize that caffeine increases MOEn through increased neuromuscular complexity. Assuming this hypothesis is right, one may also expect that caffeine may further attenuate the fatigue-induced reduction in MOEn as the trial progresses, thereby likely improving power output and performance [25].

Therefore, the present study aimed to characterize MOEn in a TT_{4km} and verify if caffeine ingestion increases power output complexity and performance in this trial. We hypothesized

that caffeine would attenuate a fatigue-induced reduction in power output complexity, improving power output and performance during TT_{4km}.

Methods

Participants and experimental design

Nine endurance-trained male cyclists (32.0 ± 7.5 years, body mass of 74.9 ± 8.6 kg, height of 1.73 ± 5.2 m, VO_{2MAX} of 55.0 ± 6.1 $mL \cdot kg^{-1} \cdot min^{-1}$,) having a minimum 3 years training experience competing at regional competitions, classified as performance level 3 [26] and experienced in cycling time trials, volunteered to participate in this study. They were non-smokers and had no neuromuscular or cardiopulmonary disorder that could affect the study outcomes. Most cyclists (n = 7) were low-to-moderate consumers of caffeine (50–250 mg of caffeine per day) and two were classified as non-consumers ($\leq$50 mg of caffeine per day), according to classification used elsewhere [27,28]. The experimental procedures were previously approved by the Research Ethics Committee of the University of São Paulo (#0023.0.342.000–10) and explained to participants before the informed consent form signature.

After a preliminary visit to obtain anthropometric measures and assess the VO_{2MAX} through a maximal incremental cycling exercise performed with a 80 rpm pedal cadence (25 $W \cdot min^{-1}$ increases until exhaustion), cyclists attended to 3 sessions in a counterbalanced order; 1) a baseline 4 km cycling time trial (TT_{4km}); 2) a TT_{4km} after caffeine ingestion; 3) a TT_{4km} after placebo ingestion. All visits were interspersed by a ~7 days interval. The cyclists were encouraged to maintain the training schedule (intensity and volume) throughout the study period and avoid vigorous exercise, alcohol, and stimulant or caffeine beverages for the last 24 h before the sessions. Briefly, we chose a TT_{4km} as a strenuous whole-body self-paced exercise and assumed that endurance-trained cyclists complete this trial having a mean power output higher than 300 W [15,16], therefore potentiating a likely reduction in MOEn [18]. In contrast, caffeine ingestion may increase MOEn and TT_{4km} performance.

Caffeine and placebo ingestion

Caffeine and placebo capsules (6 $mg \cdot kg^{-1}$ of body mass) were ingested ~ 60 min before the TT_{4km} commencement. Caffeine and sucrose-based (i.e. placebo) substances were formulated in opaque capsules of equal size, color and taste to prevent that participants rightly guessed the treatment. Importantly, instead of a double-blind, randomized placebo-controlled clinical trial, we used a placebo-deceived design, as some have argued that the use of double-blind designs is a possible source of bias in clinical trials [29,30]. To ensure that eventual differences between caffeine and placebo were solely due to caffeine pharmacological effects, cyclists were led to believe they ingested caffeine in both sessions and the study was investigating the reproducibility of caffeine effects on TT_{4km} performance. They were informed about the presence of a placebo condition at the study completion, as reported elsewhere [31]. Informal and anecdotal communication revealed that participants were blinded about the presence of a true placebo pill.

Instruments, measures, and analysis

All cyclists performed the TT_{4km} on the same road bike (Giant®, Thousand Oaks, CA, USA) attached to a cycle-simulator calibrated before every test (Racer Mate®, Computrainer, Seattle, WA, EUA), individually fitted with crank, pedals and saddle. This equipment provided power output measures (W) at a 2Hz sampling rate. The validity and reliability of this system have been previously reported [32,33]. Cyclists performed a standard 7 min warm-up,

consisting of a 5 min self-paced (gear and cadence freely adjusted) and a 2 min controlled-pace cycling (fixed gear at 100 W and 80 rpm pedal cadence). When they were still cycling at the end of the controlled-pace warmup, they immediately started the TT_{4km}. The cyclists were oriented to rate their perceived exertion (RPE) at each 0.5 km, according to the 6–20 Borg's scale [34], so that the mean RPE during the TT_{4km} was calculated. A researcher unaware of the substance ingested encouraged the cyclists to complete the distance as fast as possible, while distance feedback was available to cyclists to pace themselves.

Entropy calculation

The entropy could be interpreted as a non-linear analysis that provides a measure of the complexity of a system [35]. Based on the information theory, entropy is a measure that reflects the level of uncertainty of a dataset or time series. Entropy can be obtained as the probability (p_k) of each possible event multiplied by log of the inverse probability of each event ($\log\left(\frac{1}{p_k}\right)$) [36] as described in Eq 1.

$$H = \sum_{i=1}^{N} p_k \log\left(\frac{1}{p_k}\right) \quad (1)$$

However, the prior knowledge of the probability (p_k) for the occurrence of all events is impossible in stochastic processes, therefore, adequate methodologies such as the sample entropy (SampEn) have been suggested [37]. The SampEn (Eq 2) fits the approximate entropy [38] to generate less time series length-dependence and self-matching-reduced bias (Eq 2).

$$SampEn(m, r, N) = -\ln\left(\frac{A_{m+1}(r)}{A_m(r)}\right) \quad (2)$$

Where m is the length of sequences to be compared, r is the tolerance for accepting matches and N is the length of the time series. In the present study, the input parameters were set as $r = 0.2$, $m = 2$, $N = 120$. In the SampEn algorithm, r is multiplied by the standard deviation (SD) of N, providing a matching threshold and allowing comparisons among sequences of m points. Readers are referred to a seminal work by Richman et al. [37] for a comprehensive SampEn demonstration.

Data analysis and statistics

In this study MOEn was estimated applying SampEn algorithm in the mechanical power output signal obtained during TT_{4km}. A custom code (Matlab v.2013a, The Mathworks, EUA) was used to estimate MOEn over time, by applying a sliding-window over 120 samples epochs having 10 samples overlap. Thereafter, absolute power output data, as well as MOEn vectors, were expressed at each 25% of the total TT_{4km} distance (i.e. 25%, 50%, 75% and 100% of the trial).

Data were reported as mean (± SD) and 95% confidence limits (CI 95%). Power output and MOEn obtained at each 25% of the cycling trial were compared through a number of mixed models, having substance (caffeine and placebo) and distance (25%, 50%, 75% and 100% of the TT_{4km}) as fixed factors, and cyclists as the random factor. The Pearson correlation coefficient was calculated between mean values of power output and MOEn for each 25% of the TT_{4km}, as we expected that MOEn would decrease if cyclists significantly increased the power output. Significant results were accepted as $p < 0.05$ (SPSS software, version 17.0, SPSS Inc., Chicago, IL, USA).

Results

Ingestion of caffeine resulted in a 8% increase in mean power output ($p = 0.003$, $F = 9.69$) when compared to placebo, as mean power output was 331.4 ± 53 W (CI 95% [306.5–356.3]) in caffeine vs 306.2 ± 40 W (CI 95% [281.3–331.1]) in placebo (Fig 1A). This improved power output in caffeine was reflected in ~1.8% shorter times ($p > 0.05$) in caffeine (350.0 ± 14.6 s) than placebo (357.0 ± 13.2 s). Additionally, cyclists presented comparable mean RPE during the TT_{4km} in both supplementations with caffeine (16 ± 0.62 a.u.) and placebo (16 ± 0.63 a.u.).

Cyclists adopted a U-shaped pacing strategy (Fig 2) so that a distance main effect was detected ($p = 0.002$, $F = 5.70$), and power output decreased by 14% from 25% to 75% of the TT_{4km} (-41.6 ± 11.4 w; CI 95% [-10.3, -72.9], $p = 0.004$), but increased by 11% from 75% to 100% of the TT_{4km} (35.9 ± 11.4 w; CI 95% [4.6, 67.2], $p = 0.016$). No substance by distance interaction effects were found in power output responses ($p = 0.178$, $F = 1.697$).

We observed a substance main effect on MOEn results, as MOEn was 7% greater in caffeine than placebo ($p = 0.018$, $F = 5.983$; CI 95% [0.019, 0.190]) (Fig 1B). We observed a distance main effect in MOEn ($p < 0.001$, $F = 10{,}118$; CI 95% [0.032, 0.339]), so that MOEn increased by ~ 20% from 25% to 50% of the trial (0.284 ± 0.060 A.U.; CI 95% [0.120, -0.449], $p < 0.001$), but remained unchanged between 50% and 75% (- 0.005 ± 0.06 A.U.; CI 95% [- 0.170, 0.160], $p < 0.05$) and between 75% and 100% (- 0.115 ± 0.060 A.U.; CI 95% [- 0.280, 0.050], $p = 0.368$). No substance by distance interaction effect was found in MOEn ($p = 0.337$, $F = 1.151$). Fig 3 depicts MOEn responses during the cycling trial, and Table 1 shows individual power output and MOEn responses over the TT_{4km} in both supplementations.

Correlations analysis revealed that MOEn was inversely correlated with power output in the first 25% ($r = - 0.82$; $p < 0.001$) of placebo condition, but not in caffeine. Negative correlations were also founded between 25% and 50% of the TT_{4km} in caffeine ($r = - 0.76$; $p = 0.03$), but not in placebo, perhaps as a result of the steady power distribution in placebo during this part of the trial. Furthermore, MOEn was inversely correlated with power output in the last 25% of the TT_{4km} in both caffeine ($r = - 0.92$, $p < 0.001$) and placebo trials ($r = - 0.83$, $p < 0.001$), being coincident with a ~11% increase in power output at the end of the trial, regardless of the supplementation. Table 2 shows all correlation coefficients between MOEn and power output.

Discussion

This study aimed to characterize the MOEn during a TT_{4km} and investigate if caffeine could change the MOEn-fatigue interplay during this strenuous, whole-body short cycling exercise. Our results showed a progressive reduction in motor output complexity as the TT_{4km} progressed, however caffeine increased TT_{4km} performance through an altered MOEn-fatigue interplay. These results may support the notion that caffeine increases power output responses and attenuates the fatigue-induced reduction in MOEn during TT_{4km}.

This is the first study characterizing the MOEn during a natural exercise mode with high ecological validity such as a strenuous, whole-body short cycling time trial. In the present study, cyclists used a U-shaped pacing strategy to complete the TT_{4km}, as they yielded an end spurt in the last 25% of the trial, after an increased power output in the initial 25% and unaltered power output in the intermediate 50%. In contrast, there was a progressive reduction in MOEn in the last 25% of the TT_{4km}, regardless of the ingested substance, thereby supporting the fatigue-induced loss of entropy hypothesis as suggested in single-joint isometric exercises [6,39]. Briefly, MOEn responses could involve changes in neuromuscular complexity such as in CNS areas such as cortical, subcortical and spinal areas, as well as in motor neuron conduction to skeletal muscles. In this regard, the 20% increase in SampEn during the first half of the

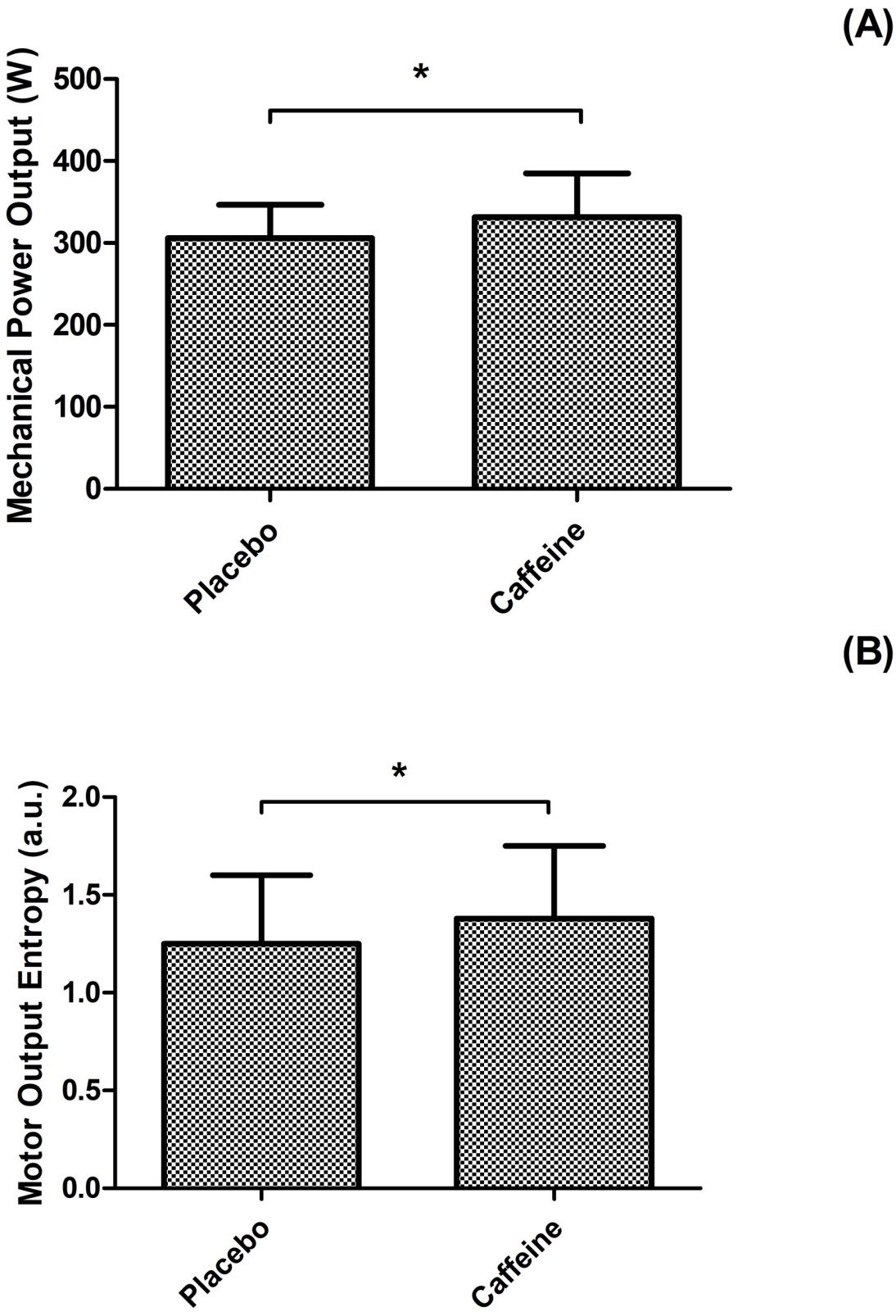

Fig 1. Cycling time trial performance and entropy. Mechanical power output (panel A) and motor output entropy (panel B) in placebo and caffeine trials. * indicates supplementation main effect in power output ($p = 0.003$, $F = 9.69$) and motor output entropy ($p = 0.018$, $F = 5.983$).

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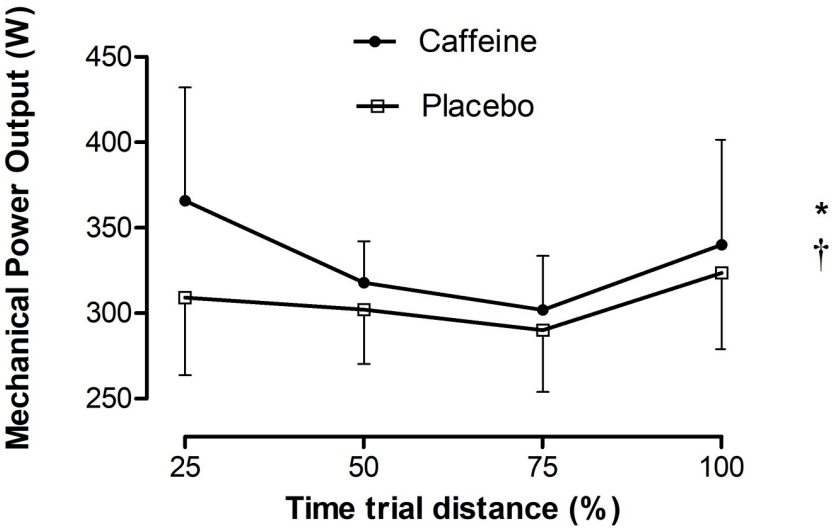

Fig 2. Pacing strategy. Mechanical power output relative to a percentage of the TT_{4km} distance. * indicates distance main effect (p = 0.002, F = 5.70) and † indicates supplementation main effect (p = 0.003, F = 9.69).

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TT_{4km} was likely due to an enhanced exercise-induced perturbation, given that most relevant increases in psychophysiological responses take place in this part of the trial [15]. However, despite the increasing exercise-induced perturbation, neuromuscular fatigue was likely low over this half of the trial and probably allowed an increased MOEn when regulating the motor output during this part [40].

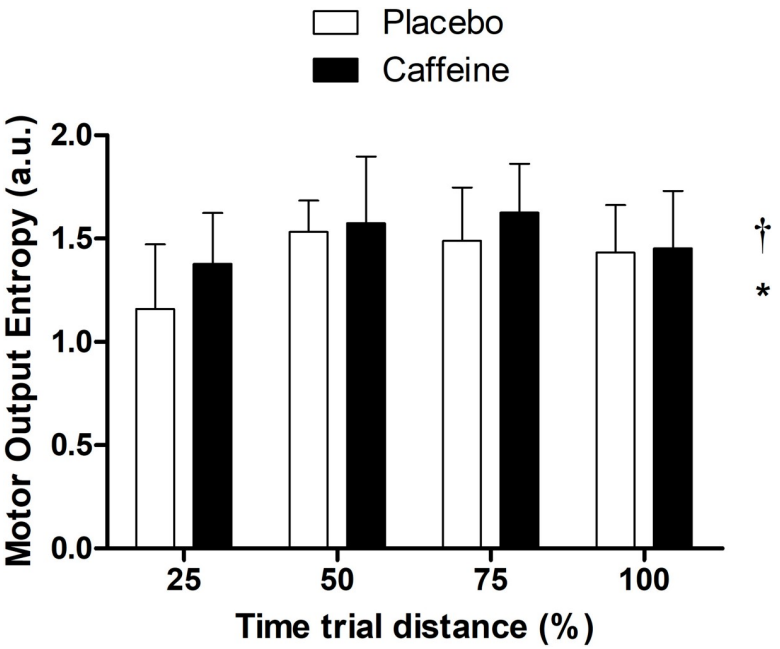

Fig 3. Motor output entropy during the cycling time trial. Motor output entropy was expressed relative to a percentage of the TT_{4km} distance. * indicates distance main effect ($p < 0.001$, F = 10.11) and † indicates supplementation main effect (p = 0.018, F = 5.98).

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Table 1. Individual power output (PO) and motor output entropy (MOEn) responses were reported as a percentage of the total cycling time trial distance.

		Time trial distance (%)							
		25		50		75		100	
Cyclists	Caffeine	PO	MOEn	PO	MOEn	PO	MOEn	PO	MOEn
1		342.34	1.19	290.04	1.31	270.39	1.56	288.26	1.31
2		315.38	1.19	344.43	1.1	301.1	1.64	324.77	1.37
3		336.24	1.62	322.99	1.95	309.11	1.84	308.23	1.68
4		305.98	1.39	243.81	2.08	234.07	2.03	238.69	1.96
5		379.99	1.11	299.51	1.45	274.32	1.31	262.33	1.39
6		322.42	1.37	339.16	1.45	366.02	1.32	386.87	1.49
7		376.9	1.13	311.7	1.46	299.67	1.53	302.76	1.03
8		341.9	1.78	300.49	1.88	280.63	1.78	246.09	1.64
9		344.6	1.62	326.72	1.48	335.18	1.61	364.72	1.35
	Placebo	PO	MOEn	PO	MOEn	PO	MOEn	PO	MOEn
1		308.22	1.21	291.75	1.48	267.53	1.32	268.5	1.63
2		298.72	1.24	339.59	1.44	319.98	0.91	342.24	1.03
3		349.09	1.19	283.82	1.7	268.22	1.67	256.75	1.61
4		253.83	1.28	235.22	1.75	224.6	1.69	240.1	1.68
5		365.88	0.58	297.12	1.38	275.27	1.59	291.38	1.36
6		364.19	0.9	370.11	1.38	359.13	1.51	364.02	1.38
7		369.75	0.99	295.63	1.57	281.31	1.5	287.63	1.55
8		311.61	1.7	308	1.7	310.87	1.77	320.73	1.54
9		365.57	1.35	311.37	1.39	314.31	1.45	370.08	1.12

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In the present study, we observed that cyclists attacked the first 25% of the TT_{4km} more aggressively when they ingested caffeine rather than placebo, somehow influencing the significant inverse correlation between power output and MOEn observed only with caffeine for this part of the trial. One may argue that neuromuscular fatigue is low during this initial part of the trial, thus likely allowing an adequate response of the neuromuscular system to the exercise-imposed perturbation through an increased motor unit firing variability. Moreover, the power output reduction observed from 25% to 50% of the caffeine TT_{4km} resulted in an inverse correlation between MOEn and power output during this part of the trial. In contrast, such a correlation between power output and MOEn was not observed in placebo TT_{4km} during these parts. In particular, the lowest MOEn and power output values were observed from 50% to 75% of the trials, so that no correlation between MOEn and power output was observed during this part, regardless of the ingested substance. Importantly, MOEn was inversely correlated with power output during the last 25% of the TT_{4km}, regardless the ingested substance. This is a part of the cycling trial usually characterized by a sharp increase in power output (i.e. end spurt), so that one may hypothesize that the loss of MOEn during this latter part of the TT_{4km}

Table 2. Pearson's correlation coefficient between power outptut (PO) and motor output entropy (MOEn) over the 4km cycling time trial (TT4km) expressed as a percentage of the trial distance, in both caffeine and placebo supplementations.

$\%TT_{4km}$	Caffeine	p-value	Placebo	p-value
0–25	-0.25	0.33	-0.82	< 0.001
25–50	-0.76	0.03	-0.36	0.15
50–75	-0.36	0.16	-0.30	0.25
75–100	-0.92	< 0.001	-0.83	< 0.001

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was possibly related to a higher motor unit firing frequency, as neuromuscular fatigue is higher in the second half of a cycling trial [40].

A short cycling time trial having an end spurt may be a challenging scenario for the neuromuscular system, as this may represent fewer chances to vary muscle recruitment during pedaling mainly at the final stages of the trial [18], thereby reducing the mechanical power output variability (i.e. power output bandwidth) and MOEn. This hypothesis is based on a previous study that reported a different neuromuscular strategy as indicated by EMG analysis when contrasting fixed-load cycling at 150 W vs 300 W [18]. The authors of that study concluded that the lower EMG entropy observed during higher cycling power output was likely due to a higher synchronism of motor units firing.

The present study hypothesized that caffeine may increase MOEn by increasing motoneuronal gain and changing the input-output relationship in the motor pathway, thereby resulting in a greater variability in motor output. Although caffeine effects on skeletal muscles cannot be ruled out [41], the most convincing caffeine mechanism involves its action on neuronal A_1 adenosine receptors, as improvements in exercise performance after caffeine ingestion have been associated with increases in spinal and supraspinal excitability [42,43]. Accordingly, the 7% increase observed in MOEn during the TT_{4km} after caffeine ingestion may be related to the caffeine's action on neuronal tissue. Considering the 8% increase in mean power output in caffeine, one may argue that the higher power output observed in this condition was also related to a higher synchronism of motor units firing [18].

Analysis of movement variability have been used in different research fields [1,2,44], so that such analysis have been recently incorporated in neuromuscular fatigue studies [5,6]. In an exercise performance scenario, nonlinear measures such as MOEn may be a useful mean to estimate exercise-induced neuromuscular fatigue and its repercussion on motor control and performance responses [5]. Therefore, such a nonlinear measure could be helpful to improve the understanding of exercise performance and fatigue in different fields of sports sciences.

Limitations and methodological considerations

The increased motoneuronal gain suggestion should be interpreted with caution, as no specific measures were performed to indicate motoneuronal gain. Insights to a motoneuronal gain mechanism could be obtained with advanced EMG techniques, such as the motor unit decomposition algorithms from electrode matrices-derived signal [45]. However, this technique is still restricted to low-intensity isometric contractions so that the dynamic whole-body exercise used in the present study limited the use of these measures to provide motoneuronal gain mechanisms insights after caffeine ingestion. Future studies comparing recruitment and derecruitment frequencies of pairs of motor units could shed-light on caffeine effects on motoneuronal gain during voluntary contractions [46].

The present study is descriptive rather than mechanistic, and its design and methods may not elucidate if losses in power output entropy during cycling time trial were due to central or peripheral fatigue factors. In this sense, the power output was sampled at a 2 Hz frequency, a sampling rate that may not detect all variability in power output data, given the possible aliasing effect resulted from sampling the data in different pedal positions at each revolution. Another limitation was the absence of EMG responses, a measure that could have assessed the neuromuscular system and power output entropy, simultaneously.

Furthermore, we disregarded eventual subgroup comparisons based on the habitual caffeine consumption effects on performance, given that a recent well-designed study [28] and an important sports nutrients position stand challenged [27] the myth that habituation to caffeine consumption affects the caffeine's potential as an ergogenic aid. However, considering that

habitual caffeine consumption may change physiological responses to caffeine supplementation such as heart rate and ventilation, future studies may want to investigate potential habitual caffeine consumption effects on MOEn and EMG during cycling time trial.

Conclusion

Results of the present study showed a progressive reduction in MOEn during the TT_{4km}, thus revealing a progressive loss of motor output complexity as the trial progressed, mainly during the last 25% of the TT_{4km}. However, caffeine ingestion improved TT_{4km} performance and MOEn. These results reinforce a likely fatigue-induced loss of complexity hypothesis.

Supporting information

S1 Raw data.
(XLSX)

Author Contributions

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Data curation: Flávio Oliveira Pires.

Formal analysis: Bruno Ferreira Viana, Gabriel S. Trajano, Flávio Oliveira Pires.

Funding acquisition: Carlos Ugrinowitsch, Flávio Oliveira Pires.

Investigation: Flávio Oliveira Pires.

Methodology: Bruno Ferreira Viana, Gabriel S. Trajano, Carlos Ugrinowitsch, Flávio Oliveira Pires.

Project administration: Carlos Ugrinowitsch, Flávio Oliveira Pires.

Resources: Flávio Oliveira Pires.

Software: Bruno Ferreira Viana, Flávio Oliveira Pires.

Supervision: Flávio Oliveira Pires.

Validation: Flávio Oliveira Pires.

Visualization: Bruno Ferreira Viana, Gabriel S. Trajano, Flávio Oliveira Pires.

Writing – original draft: Bruno Ferreira Viana, Gabriel S. Trajano, Carlos Ugrinowitsch, Flávio Oliveira Pires.

Writing – review & editing: Bruno Ferreira Viana, Gabriel S. Trajano, Carlos Ugrinowitsch, Flávio Oliveira Pires.

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