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Brief Report

Effects of Acute Mistletoe (*Viscum album* L.) Ingestion on Aerobic Exercise Performance

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Abstract: Mistletoe (*Viscum album* L.; VA) has been traditionally used in folk medicine to combat fatigue and stress. Evidence has shown that chronic consumption of VA results in an enhancement of oxidative metabolism and exercise performance. However, no studies have investigated how acute VA consumption influences performance. The purpose of this brief report was to investigate the effects of acute VA ingestion on rowing exercise performance. Physically active females were recruited for this study. In a crossover, counterbalanced design, participants completed two trials each with a different treatment: (1) VA (2000 mg) and (2) placebo (PL; gluten-free cornstarch; 2000 mg). A total of 30 min prior to exercise, participants consumed their treatment. The participants were familiarized with the rowing ergometer and warmed up for 5 min at 50% of age-predicted heart rate max. Immediately following the warm-up, the participants completed a 2000 m rowing time trial. Blood lactate (La) was obtained with a lactate meter via finger prick before and after exercise. Power output, trial time, heart rate, rating of perceived exertion (RPE), and La were analyzed. The findings revealed no significant differences for the relative power output ($p = 0.936$), trial time ($p = 0.842$) or heart rate ($p = 0.762$). Rating of perceived exertion was lower with VA ingestion ($p = 0.027$). La was significantly higher post-exercise regardless of treatment ($p < 0.001$). However, post-exercise La was lower with VA ingestion ($p = 0.032$). Findings do not support VA as an ergogenic aid but suggest ingestion may alter metabolism resulting in less La formation and subjective fatigue.

Keywords: rowing; lactate; power output; ergogenic aid; mistletoe plant

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1. Introduction

Mistletoe (*Viscum album* L.; VA) is a hemiparasitic plant belonging to the *Santalaceae* family that is native to various European and Asian regions, although various subspecies of VA exist and may be present in other areas. VA has been used for centuries as a purported natural remedy for pain, backaches, fatigue, and stress [1]. However, much of the original folklore surrounding VA has since been tempered. More recently, VA has been empirically studied showing immunomodulatory, vasodilatory, and adaptive metabolic actions [2–4]. Because of these actions, VA has been investigated as a means to increase physical capacity and endurance [4,5]. However, research describing the possible ergogenic effects of VA in humans is sparse, leaving the possible benefits unclear.

Currently, VA is widely available and commercially sold for dietary enrichment in the form of whole plant and extracts. The characterization of VA has been previously reported to include a number of phytochemicals including lectins, polysaccharides, alkaloids, terpenoids, glycosides, amines, peptides, polyphenols, flavonoids, phytosterols, and amino acids [6,7]. While multiple studies have deemed that VA is safe for human consumption in controlled doses [8,9], the physiological effects of VA remain to be fully delineated, especially regarding exercise. In vitro studies have shown VA increases cellular oxygen consumption rate in rat myoblasts [4]. Supporting this, peroxisome proliferator-activated

receptor gamma coactivator 1-alpha (PGC-1α) and AMP-activated protein kinase (AMPK), which are pivotal regulators of mitochondrial metabolism and energy homeostasis, have been shown to be potently induced with VA [4,10]. In animal models, VA administration has been shown to enhance longevity and exercise capacity [4,11,12]. Lee et al. reported VA increased the lifespan and exerted anti-aging effects in *C. elegans* and *D. melanogaster* models [11]. Increases in longevity were concomitant with increased *Sir2* (homologous to human *SIRT1*) gene expression which largely regulates energy homeostasis and cellular stress responses. VA has also been shown to increase forced running time and skeletal muscle function in mice [2,4,12]. While not fully clear, increased physical capacity may be mediated by increases in the availability of plasma-free fatty acids (FFA) and mitochondrial adaptations [4,12]. Overall, studies to date using cellular and animal models have described mechanisms that may result in a shift toward an oxidative phenotype. However, less is known about the effects of VA in human physiology leaving a demand to identify if the aforementioned physiological mechanisms translate to sport and exercise.

To date, only a small number of studies investigating VA and exercise performance exist in humans with almost exclusive use of chronic dosing protocols [13,14]. Lim et al. reported increased knee strength and balance in elderly males and females with 12 weeks of VA supplementation [13]. Furthermore, Ha et al. reported that 8 weeks of twice-daily VA supplementation enhanced endurance rowing capacity and lowered inflammatory markers in young males [14]. While intriguing, chronic inter-day recurring dosing strategies may not be practical and/or desirable to some athletes and recreational exercisers. Since long-term nutritional adherence has been reported as being poor in athletes and the general population [15,16], identifying possible acute dietary interventions as a means of improving exercise performance is imperative. It is currently unknown if acute VA ingestion induces any benefits towards exercise performance. Furthermore, studies on VA supplementation in females are scarce and relatively non-existent in the exercise literature [17,18]. Thus, the purpose of the current brief report was to elucidate the acute effects of VA ingestion on endurance rowing performance and responses to exercise in females. Given the previous reports on adaptogenic actions of VA, we hypothesized that acute VA ingestion would enhance rowing performance but would attenuate increases in physical stress markers (i.e., HR, RPE, blood lactate).

2. Materials and Methods

2.1. Study Design

A convenience sample of physically active females (n = 12) was recruited. In a crossover, counterbalanced design, all the participants completed two rowing time trial visits each with a different randomized treatment: (1) Mistletoe (VA; *Viscum album* L.) or (2) placebo (PL; gluten-free cornstarch). The participants ingested a single dose of the corresponding treatment followed by the completion of a 2000 m rowing time trial. Trial time, relative power output, HR, and RPE were taken every minute during the exercise and averaged for analysis. Blood La was measured prior to exercise (Pre) and immediately after exercise (Post). The trials were separated by 48 h. Prior to any data collection, verbal and written informed consent was obtained from each participant. All experimental procedures were conducted in accordance with the Declaration of Helsinki and approved by the Samford University Institutional Review Board (EXPD-HP-22-S-12; January 2022).

2.2. Participants

Healthy, physically active females (age = 21.0 year ± 0.8; height = 164.4 cm ± 6.3; body mass = 71.9 kg ± 15.7) volunteered to participate in this study. Physically active was defined as accruing 150 min/week of moderate-intensity physical activity [19]. A Physical Activity Questionnaire (PARQ) was completed by each participant prior to testing to screen for exercise safety [19,20]. To qualify for testing, participants had to be free of injury in the past 6 months, free of chronic disease (i.e., cardiovascular, metabolic), and not currently supplementing with VA or other herbal supplements. Before each visit, the

participants were asked to refrain from vigorous activity 24 h prior and from consuming caffeine, nicotine, and alcohol 12 h prior [19,21]. Prior to each visit, the participants were asked to maintain identical normal sleep and dietary routines.

2.3. VA Supplement and Placebo

For the experimental treatment, a single 2000 mg dose (2 capsules × 1000 mg) of either PL (gluten-free cornstarch) or VA (Fluxias GmbH, Württemberg, Germany) was admitted 30 min prior to exercise [13]. For the PL treatment, gluten-free cornstarch was encapsulated and matched in appearance to the VA treatment [22]. For the VA treatment, organic whole dried cut 100% *Viscum album* L. leaves were used (Naturegrail, Fluxias GmbH, Württemberg, Germany). VA has been well characterized previously to include a number of phytochemicals including lectins, polysaccharides, alkaloids, terpenoids, proteins, amines, peptides, polyphenols, flavonoids, phytosterols, and amino acids [6]. The country of plant origin was Poland and was processed in Germany. The host of the harvested VA was *Populus nigra* (black poplar tree). To ensure homogeneity of the VA treatment, the whole leaves were ground to a fine powder where no distinguishable leaves were present. The powder was then weighed and encapsulated in an identical manner as the PL. The VA was stored under dry, cool, dark conditions to maintain product integrity according to the manufacturer's recommendations. The capsules and opaque bags were made in such a way to where the treatments were unidentifiable [22]. To maintain data integrity, all the treatments were distributed in a double-blinded manner, whereby an independent researcher organized each treatment. To ensure compliance, empty bags were returned by the participants and recorded. No participants reported any side effects from supplementation throughout the investigation. The participants were unaware of any experimental hypotheses throughout the testing.

2.4. Procedures

Following corresponding treatment ingestion, height and weight were recorded. The participants were outfitted with a chest strap HR monitor (Polar Electro, Lake Success, NY, USA). Blood La was then measured prior to exercise (Pre) as previously described by our lab [23,24]. Briefly, the distal end of the third finger was cleaned with 70% isopropanol. Bleeding was then induced with a 28 G 1.0 mm depth disposable lancet (McKesson Corp, Irving, TX, USA). The first drop of blood that was yielded was discarded/wiped away and the second drop was analyzed using a portable lactate meter (Lactate Plus Meter, Nova Biomedical, Waltham, MA, USA). Following the La reading, the participants completed a 5 min rowing warm-up at 50% of their age-predicted HR_{max} on a rowing ergometer (Concept2, Morristown, VT, USA). The participants then completed a 2000 m rowing time trial where they were instructed to finish as quickly as possible. No time, distance, or feedback about their progress was presented during the trial. Every minute, rate of perceived exertion (RPE) (1–10 scale), heart rate (HR), time, and power output were collected. Immediately following the commencement of exercise, blood La measurements were repeated as previously described.

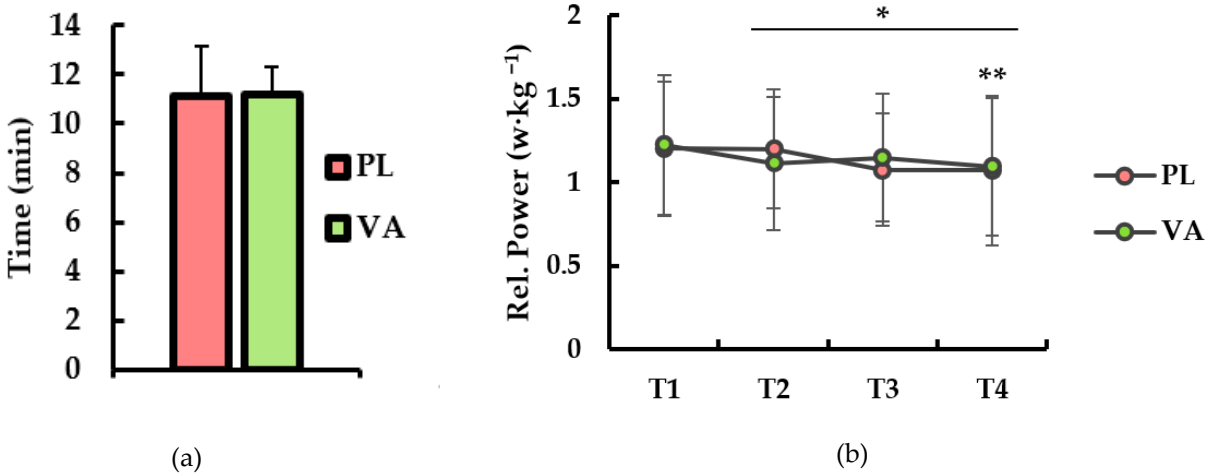
2.5. Data Analysis

All data were analyzed using Jamovi software (v.0.9; Sydney, Australia). A Shapiro–Wilk test was used to test the normality of all the data. No comparisons violated normality (p = 0.067–0.984). Statistical analysis for the trial time was conducted using a paired-samples t-test. For the relative power output, HR and RPE data points were grouped into 4 time quartiles (T1, T2, T3, T4) and analyzed using a [2 × 4] treatment × time repeated measures ANOVA with a Bonferroni–Holm post hoc test in cases of significance. For blood La, a [2 × 2] treatment × time repeated measures ANOVA was utilized with a Bonferroni–Holm post hoc test. The effect size estimates between the means were calculated between the treatments using Cohen's d effect sizes (d) and interpreted as: 0.2—small; 0.5—moderate; and 0.8—large [25,26]. Estimates of the effect size for the main effects were

calculated using eta squared (η^2) and interpreted as: 0.01—small; 0.06—medium; and ≥0.14—large [25,26]. Significance was set at $p \leq 0.05$ a priori. All the data are presented as the mean ± standard deviation (SD).

3. Results

The trial time (min) and relative power output (RPO; watts·kg^{-1}) data are shown in (Figure 1). For the trial time (Figure 1a), there were no significant differences between the treatments ($p = 0.842$; d = 0.11). For RPO (Figure 1b), there was no main effect for treatment ($p = 0.936$; $\eta^2 < 0.001$) but a significant main effect for time ($p = 0.017$; $\eta^2 = 0.019$) existed. No interaction between treatment × time was observed ($p = 0.498$; $\eta^2 = 0.002$) for RPO. For time, RPO during T1 was significantly higher than T2 ($p = 0.047$; d= 0.34), T3 ($p = 0.032$; d = 0.44), and T4 ($p = 0.022$; d = 0.32). RPO was also significantly higher during T2 than T4 ($p = 0.023$; d = 0.41).



(a) (b)

Figure 1. Comparison of (**a**) the total trial time (min) and (**b**) relative power output (w·kg^{-1}) over each time quartile (T1, T2, T3, T4) of the rowing exercise between placebo (PL; red) and *Viscum album* (VA; green) treatments. The data are presented as the mean ± SD. * indicates significantly different than T1 ($p < 0.05$). ** indicates significantly different from T2 ($p < 0.05$).

HR (bpm), RPE (1–10 scale), and blood La (mmol·L^{-1}) are shown in Figure 2. For HR (Figure 2a), there was not a main effect for treatment ($p = 0.762$; $\eta^2 = 0.001$), but a significant main effect was found for time ($p < 0.001$; $\eta^2 = 0.194$). No interaction between treatment × time was observed ($p = 0.623$; $\eta^2 = 0.002$). During T1, HR was significantly lower than T2 ($p < 0.001$; d = 1.90), T3 ($p = 0.004$; d = 1.47), and T4 ($p = 0.004$; d = 1.18). For T2, HR was significantly higher than both T3 ($p = 0.046$; d = 0.58) and T4 ($p = 0.045$; d = 0.61). HR was also significantly higher during T4 than T3 ($p = 0.045$; d = 0.49).

Analysis of RPE (Figure 2b) revealed a main effect for treatment ($p = 0.027$; $\eta^2 = 0.028$) and time ($p < 0.001$; $\eta^2 = 0.518$). There was also an interaction between treatment × time ($p < 0.001$; $\eta^2 = 0.035$). During T1, RPE was significantly lower than T2 ($p < 0.001$; d = 1.99), T3 ($p < 0.001$; d = 2.42) and T4 ($p < 0.001$; d = 4.83). For T2, RPE was significantly higher than both T3 ($p < 0.001$; d = 1.43) and T4 ($p < 0.001$; d = 4.83). RPE was also significantly higher during T4 than T3 ($p < 0.001$; d = 1.47). Regarding the main effect for treatment, RPE was significantly higher with PL vs. VA ($p < 0.001$; d = 0.65). At T4, RPE was significantly lower with VA treatment compared to PL ($p = 0.005$; d = 1.48).

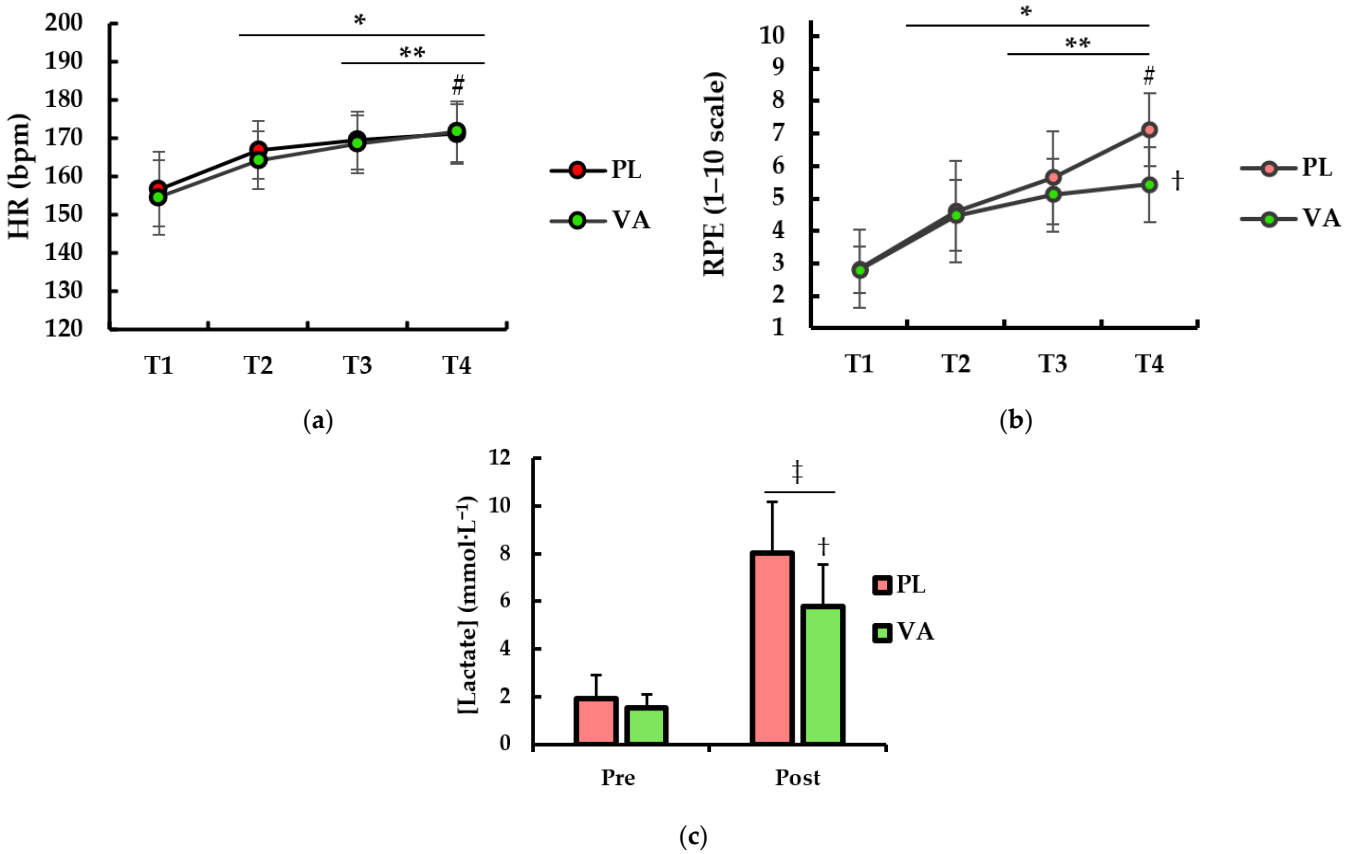


Figure 2. Comparison of (**a**) heart rate (bpm), (**b**) RPE (1–10 scale) [over each time quartile (T1, T2, T3, T4) of the rowing exercise], and (**c**) pre- and post-exercise blood lactate concentrations (mmol·L^{-1}) between placebo (PL; red) and *Viscum album* (VA; green) treatments. The data are presented the mean ± SD. * indicates significantly different than T1 ($p < 0.05$). ** indicates significantly different from T2 ($p < 0.05$). # indicates significantly different from T3 ($p < 0.05$). † indicates significantly different from PL ($p < 0.05$). ‡ indicates significantly different from pre ($p < 0.05$).

For La (Figure 2c), there was a main effect for treatment ($p = 0.022$; $\eta^2 = 0.041$) and time ($p < 0.001$; $\eta^2 = 0.639$). An interaction between treatment × time also existed ($p = 0.022$; $\eta^2 = 0.021$). Specifically, La was significantly lower during the VA treatment compared to PL ($p = 0.022$; $\eta^2 = 0.041$) and post La levels were significantly higher than the pre time ($p < 0.001$; $\eta^2 = 0.639$). Lastly, the post La levels were significantly lower with the VA treatment ($p = 0.032$; d = 0.84) compared to the PL.

4. Discussion

Although VA has been used for centuries in folk medicine, the nutraceutical benefits have only recently been empirically investigated. Initial studies in animals and humans support the use of chronic VA supplementation as a means to improve aerobic exercise performance and possibly enhance oxidative capacity [2,4,14]. However, little to no information exists as to whether an acute dose of VA, which may be more practical for some individuals, imparts a performance enhancement. Current findings from this brief report suggest that VA does not enhance endurance rowing performance or alter HR and RPE. However, acute VA ingestion resulted in pronounced decreases in blood La post-exercise compared to the PL. While precise mechanisms remain largely speculative, alterations in La may suggest alterations in metabolism and potentially support the use of VA chronically.

A lack of alterations in performance with VA is in direct opposition to previous reports although no changes in HR are supported [13,14]. In contrast to current findings, Ha et al. used an identical exercise bout but showed improved rowing efficiency (i.e., individuals

took fewer strokes to complete the rowing trial in a similar time) following 8 weeks of twice-daily VA supplementation [14]. Differences in the findings are likely in part due to the differences in the supplementation protocols. Ha et al. used a water-extracted VA tincture and chronic dosing regimen. It is plausible that the use of the whole dried plant with a capsule may not have resulted in comparable bioavailability to that of a tincture delivery system. Indeed, liquid extracts of herbal supplements have been implicated in better absorption in some applications [27]. Furthermore, the beneficial effects may not have been fully realized due to the differences in regimen duration. The plethora of cellular signaling induction which has been proposed from chronic VA supplementation likely does not fully translate to an acute dose. Indeed, increases in the gene expression of PGC-1α and AMPK, which are key signaling molecules in metabolic adaptation, following VA administration have been documented in the hours following introduction [1,12]. Since the exercise in the current investigation was initiated 30 min after ingestion, the benefits from adaptive gene expression may not have been fully realized in that short of a time frame. It should be noted that the optimal dosage amount, duration, and delivery system for VA in exercise contexts remain unknown. Indeed, there is a large amount of heterogeneity between studies regarding dosage and form of delivery (i.e., subcutaneous, intravenous, oral) which obscures the ability to provide proper dietary enrichment recommendations. Although VA is tolerated well in moderate to low doses, these factors are important for consideration in practical applications as some lectins in VA have cytotoxic properties and can result in illness when it is taken in larger quantities [28]. However, no adverse effects were noted with the current oral dosage and previous oral supplementation protocols have shown similar findings with chronic supplementation [14]. Future dose-response exercise studies with VA will be imperative in developing ideal dietary enrichment guidelines for performance enhancement and safety.

Interestingly, a single acute dose of VA resulted in pronounced reductions in the post-exercise blood La levels. Although it has been previously shown that VA ingestion may alter blood lactate and substrate utilization using animal models [4], the current findings are the first that we are aware of to describe this phenomenon in humans. Lee et al. showed that chronic VA administration resulted in greater plasma-free fatty acid (FFA) availability and utilization in mice [2]. While speculative, the acute decreases in post-exercise La may be due to a greater reliance on fat metabolism during exercise, thereby decreasing glycolytic energy utilization and La formation. This would support previously reported mitochondrial adaptations and glycogen sparing with chronic VA supplementation [2,4]. Furthermore, the lower La levels that were currently observed may be related to changes in La clearance. VA has been shown to have vasodilatory effects and result in smooth muscle relaxation [29]. Thus, it is plausible that the acute VA dose may have exacerbated skeletal muscle hyperemia thereby aiding in La clearance. However, it should be cautioned that fat utilization and La clearance were not measured currently and should be included in future human investigations. RPE was also lower with VA treatment in the last quarter of the rowing exercise bout. Indeed, RPE has been shown to be strongly associated with blood La levels during incremental exercise [30,31]. Higher levels of dissociation (i.e., lower RPE) have been documented to allow for greater endurance and exercise volume [32,33]. Thus, the collective findings of lower La and RPE may provide further insight into previous reports of long-term adaptations and fatigue attenuation with chronic VA consumption.

Although the present data reveal novel information on acute VA dosing and exercise, there were several limitations. Admittedly, the sample size was small and homogenous, although few comparable studies exist. Larger and more diverse samples will be needed to bolster findings, and this brief report should serve as a foundation for more in-depth studies. Also, only a single variety of VA and host species was used for supplementation. Previous evidence has shown that different subspecies of VA or host species can influence the supplement character and efficacy [6,34]. The current findings may not fully translate to other types of VA. Lastly, direct biochemical characterization of the VA was not conducted although the chemical content of VA has been well described [6]. Responses to VA in the

current study cannot be directly attributed to any single or joint effect of molecules. This supports the need for future studies using standardized chemical extracts to allow for more comparable comparisons.

5. Conclusions

The current results show that acute VA ingestion does not improve aerobic performance but results in lower post-exercise blood La which may indicate alterations in substrate metabolism. Furthermore, lower RPE towards the end of the time trial coincided with lower La. While not confirmed, increased fat utilization and lower reliance on glycolysis may underpin the current findings. While promising, more in-depth study is needed with both physiological and psychological assessment to identify the mechanisms that are responsible for adaptive responses to exercise.

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