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A MULTI-VITAMIN WITH RELORA® IMPROVES SLEEP LATENCY AND QUALITY BEFORE AN ENDURANCE CYCLING EVENT

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ABSTRACT: ***We examined the effects of one dose of a multi-vitamin with Relora®, a proprietary blend of Magnolia officinalis and Phellodendron amurense bark extracts, designed for nighttime (MVE; containing 200mg Relora), multi-vitamin (MV, 0mg of Relora), or a placebo (P) on subjective sleep quality and sleep quantity the night before an endurance cycling event. Sixty-six recreational athletes (age, 51±11 yr; body fat, 22±8%) completed an 80-164 km sanctioned road cycling event. Prior to the event, subjects reported habitual and previous night's sleep times, latency, and overall sleep quality, and then were randomized and blinded into supplementation groups (MVE, MV, or P). The night before the event, athletes consumed one serving of their supplement and then met with researchers in the morning before the event start. Between groups, there were no differences in athlete demographics, event distance, habitual sleep quality (4.4±1, 4.3±1.3, 4.6±0.9, respectively) or sleep latency (15±14, 24±37, 19±16 min, respectively) (p>0.05). Athletes consuming the MVE fell asleep 28 min faster than P athletes (29±12 vs. 57±59 min, p=0.05). Moreover, the night before the event, athletes not taking the MVE ranked sleep quality worse than habitual sleep (MV: 3.8±1.4, P: 3.5±1.1; p<0.05), while the MVE athletes' sleep rank (3.9±1.1) was not worse. Athletes with an early next day event may experience improved sleep quality and quantity with no perceived effects on readiness with an acute supplementation of the MVE.***

KEY WORDS: Dietary supplement, Exercise, *Magnolia officinalis*, *Phellodendron amurense*

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INTRODUCTION

Disrupted sleep, less than normal sleep, or a shift in sleeping hours are all common situations athletes experience the night before an event with an early start and these sleep disturbances can affect physiological and cognitive functions (Halson, 2014). Sleep is important for almost all biological functions regarding physiological processes (Cirelli et al., 2008), cognitive performance and mood (Belenky et al., 2003), and exercise recovery (Reilly et al., 2007). Acute sleep loss of as little as 80 minutes in one night can result in a reduction of daytime alertness by as much as 32% (Bonnet et al., 1995). However, sleep deprivation of more than a whole day (30+ hours) has very little effect on pacing, cardiorespiratory measures and thermoregulatory function (Oliver et al., 2009) compared to nights of normal sleep, suggesting sleep loss might affect athletes and their performance more psychologically than alter physiologically.

In regards to sleep, animal models have been used most frequently to explore the effects of the extracts of *Magnolia officinalis* bark and its active constituent, honokiol (Chen et al., 2012; Kuribara et al., 1999; Peng et al., 2004; Qiang et al., 2009). The mechanism of action has been compared to the activity of diazepam, a benzodiazepine anxiolytic medication used to treat anxiety disorders for decades (Kuribara et al., 1999), with less negative side effects. Another active ingredient from *Magnolia officinalis* bark, magnolol, acts through the benzodiazepine site of the $GABA_A$ receptor and has been found to exert anti-epileptic and sleep-promoting effects, mediating sleep in both animals and humans (Chen et al., 2012). Berberine, the active constituent of *Phellodendron amurense* bark, has also been found to reduce anxiolytic effects and in combination with *Magnolia officinalis* reduce distress and stress-induced analgesia without causing sedation (Peng

et al., 2004). Together, these constituents have synergistic effects (Qiang et al., 2009) and been added to modern dietary supplements with the intention of inducing relaxation and relieving stress through reducing cortisol levels, as well as mediating evening stress-related eating, and assisting with weight management (Talbott et al., 2013).

Clinically, magnolol administered in as little as five mg/kg has been shown to significantly shorten sleep latency, increase the amount of non-rapid eye movement (non-REM, NREM) and rapid eye movement (REM) sleep for three hours following administration with an increase in the number of NREM and REM sleep episodes (Chen et al., 2012), thus suggesting improvements in quality of sleep. Relora®, a proprietary blend of bark extracts from *Magnolia officinalis* and *Phellodendron amurense*, has anecdotally improved the quality of sleep in athletes after only one night of consumption; however, to date this phenomenon does not appear to have been examined empirically. Likewise, research on acute sleep loss and its effect on athletic contests appear equivocal, specifically in relation to subjective quality of sleep prior to race/competition anticipation in athletes.

We examined athletes the night prior to a long-distance exercise event to explore the acute effects of one dose of a multi-vitamin with Relora on sleep latency issues potentially related to nervousness, uneasiness, and anxiety about the event. These feelings often affect quality of sleep in ultra-endurance cyclists aiming to complete a long distance (e.g. 164 km) event.

We expected the anticipation of the road cycling event, early awakening, and discomfort of sleeping in an unfamiliar bed will evoke changes in sleep pattern. We hypothesized consumption of one serving of the multi-vitamin with 200 mg of **Relora** will improve subjective and objective measures of sleep quality despite the smaller than previously studied quantity. Similarly, we aimed to examine the effects of the multi-vitamin with Relora on subjective levels of energy, readiness, and event outlook compared to other athletes.

METHODS

The 2015 Hotter 'n Hell Hundred (HHH) event was selected for this investigation because of the event's large participation (>13,000 entrants). Cyclists in 80 to 164-km rides at the HHH were recruited via emails to all entrants as well as in person as they visited the event exposition hall and registration site one and two days before the cycling event. Interested athletes attended an informational meeting where they received written and oral descriptions of all procedures, evaluations, time commitment, benefits, and risks before signing the university's Institutional Review Board for Human Studies consent form. Once written informed consent was provided, athletes were considered enrolled. Athletes did not receive any financial compensation for their participation.

Pre-event evaluations

At the event exposition hall and registration site, one or two days before the event, athletes completed a medical history questionnaire. Anthropometric characteristics were then assessed including height through a tape measure attached to the wall, and body mass with a floor scale, accurate to ± 100 g. Body fat was estimated through skinfold calipers measuring thickness at three sites appropriate for sex (men: quadriceps, chest, and abdomen; women: quadriceps, suprailiac, and triceps). Body fat percentage was calculated through formulas appropriate for each sex (Jackson et al., 1978; Jackson et al., 1980).

Athletes completed baseline questionnaires regarding sleep quantity and quality. First, they completed the Pittsburg Sleep

TABLE 1. Micronutrient compositions of the multi-vitamin with Relora and the multi-vitamin (from www.thorne.com). [1]*Magnolia officinalis* bark extract and *Phellodendron amurense* bark extract

Ingredients	Multi-Vitamin with Relora (MVE)	Multi-Vitamin (MV)
Vitamin A	1,000 IU	5,000 IU
Vitamin C	150 mg	250 mg
Vitamin D3	500 IU	2,000 IU
Vitamin E	20 IU	20 IU
Vitamin K	200 mcg	400 mcg
Folate	400 mcg	400 mcg
Biotin	500 mcg	500 mcg
Thiamin	--	50 mg
Riboflavin	--	12 mg
Niacin	--	80 mg
Vitamin B6	--	20 mg
Vitamin B12	--	600 mcg
Pantothenic Acid	--	45 mg
Calcium	50 mg	30 mg
Iodine	75 mcg	75 mcg
Magnesium	107 mg	20 mg
Zinc	20 mg	15 mg
Selenium	100 mcg	200 mcg
Copper	750 mcg	750 mcg
Manganese	1.5 mg	3 mg
Chromium	200 mcg	400 mcg
Molybdenum	50 mcg	100 mcg
Proprietary Blend[1]	200 mg	--
Choline Citrate	100 mg	--
d-Gamma Tocopherol	24 mg	24 mg
Boron	2 mg	2 mg
Lutein	600 mcg	300 mg

Quality Index sleep history for the past month, allowing more detail to researchers for exclusion of athletes in this study, and quantifying habitual sleep and wake times (Buysse et al., 1989). Athletes were excluded from the study if they consumed medications or alcohol to assist falling asleep, or had chronic diagnosed/undiagnosed sleep issues (i.e. restlessness, trouble breathing, waking up multiple times, etc.). Next, athletes reported sleep quantity and quality from the night before, entailing details of sleep-related issues, sleep latency, restlessness, nighttime waking, and morning alertness using a modified version of the Saint Mary's Hospital (SMH) sleep questionnaire (Ellis et al., 1981). Third, athletes were asked to complete a set of visual analog scales (VAS) to assess current mood, readiness, and energy levels, indicating how alert, energetic, sleepy, fatigued, stressed, relaxed, exhausted, and level of clear-headedness they were feeling (Buysse et al., 2007). Athletes verbally reported to researchers their expected finish time for their respective event distance.

Athletes were randomly assigned into one of three groups based on their order of enrollment, and blinded to their supplementation group: a multi-vitamin with Relora (MVE, n=23) (manufactured by Thorne Research, Sandpoint, ID), a multi-vitamin (MV, n=21) (manufactured by Thorne Research, Sandpoint, ID) or an identically-looking cellulose placebo (P, n=22, manufactured by Thorne Research, Sandpoint, ID) (Table 1). Both multi-vitamins were NSF Certified for Sport®, meaning they approved to be free of all banned substances and that actual product contents match what appears on the label. Athletes in the MVE group consumed one serving of a commercially available multi-vitamin that contained 200 mg Relora®, a proprietary blend of a patented extract from *Magnolia officinalis* bark and a proprietary extract from *Phellodendron amurense* bark (NPI, LLC; patent No. 6,582,735; "not less than 1.5% honokiol and 0.1% berberine"). Instructions were given verbally and in writing to take their respective supplement with water or a small amount of food one hour before hopeful time of falling sleep (i.e. if one wanted to be asleep for 10:00 PM, to consume their serving at 9:00 PM) on the night before the cycling event. Before leaving for the evening, athletes scheduled appointments to briefly meet with researchers before the 7:00 AM start on event morning.

Event day

Pre-event: Between 4:30 and 7:00 AM on event morning, athletes met with researchers at their scheduled time at a tent the starting line for pre-event evaluations. At this appointment, athletes were verbally asked if they consumed their supplement one hour before expected sleep time. Next, athletes completed the same SMH questionnaire regarding their last nights' sleep and the same VAS assessments of readiness and energy-related scales based on how they were currently feeling. Following the evaluations athletes proceeded to the starting line.

Post-event: Following the race, athletes reported back to the research tent to complete the same VAS assessments of readiness and energy-related scales based on how they were currently feeling post-race. Actual finish times were collected from the official event results webpage the following week.

Statistical analysis

Seventy-three athletes were enrolled in the study, but seven did not arrive to their scheduled appointment on the morning of the event. Therefore, 66 athletes to cycle 164 km, 100 km or 80 km in the sanctioned road cycling event were analyzed in this study. Statistics were completed using SAS (version 9.4, Cary, NC). All data are presented as mean ± SD unless otherwise stated. Athlete anthropometrics, ride length, and expected/actual finish time were compared by a chi-square for categorical variables (a Fisher's Exact was utilized where expected cell counts were fewer than five) and an ANOVA was used for continuous variables. A Tukey's adjustment was performed for a follow-up, and pairwise comparisons were used for continuous variables where a difference was noted by an ANOVA. The level of significance was set at $p \leq 0.05$.

RESULTS

The three groups of athletes (MVE: n= 23; MV, n= 21; P, n=22) and their characteristics are reported in Table 2. Between groups, there was no significant difference in demographics (age, height, weight or body fat) ($p>0.05$), and event distance was evenly distributed between groups ($p>0.05$).

TABLE 2. Characteristics of athlete participants. MVE= Multi-vitamin with Relora, MV= multi-vitamin, P=placebo.

	Sex (M/F)	Age (yr)	Height (cm)	Weight (kg)	Body fat (%)	Expected finish time (min)	Actual finish time (min)
All (n=66)	54/12	51 ± 11	175 ± 15	85.5 ± 15	22 ± 8	373 ± 97	364 ± 74
MVE (n=23)	22/1	49 ± 11	175 ± 8	86.4 ± 16	19 ± 5	366 ± 97	348 ± 86
MV (n=21)	14/7	51 ± 11	168 ± 23	77.3 ± 12	22 ± 7	359 ± 99	367 ± 66
P (n=22)	18/4	53 ± 11	179 ± 8	89.5 ± 15	25 ± 9	394 ± 95	378 ± 67

Sleep habits

The self-reported times for going to bed and waking up for the days before and the event day are presented in Table 3. On the days leading up to the event, athletes averaged a bedtime between 9:00 and 10:00 PM. However, MVE athlete's bedtime was approximately 20 min later than P athletes ($p<0.05$). The

TABLE 3. Averages of athlete reported bedtimes, wake-up times, and hours slept in the days before and the night before a cycling event. *Duration times are reported in hours:minutes, [1]p-value group over time, [2]p-value between groups

	Days before bedtime	Pre-event bedtime	p-value[1]	Days before wake-up time	Pre-event wake-up time	p-value[1]	Days before sleep duration*	Pre-event sleep duration*	p-value[1]
Multi-Vitamin with Relora	9:52 PM	9:05 PM	0.07	6:23 AM	4:25 AM	0.01	7:40	6:06	0.0001
Multi-vitamin	9:19 PM	9:32 PM	0.08	6:16 AM	4:26 AM	0.01	7:27	6:01	0.0001
Placebo	9:32 PM	9:24 PM	0.17	6:36 AM	4:42 AM	0.01	7:16	5:53	0.0001
p-value[2]	0.03	0.29		0.22	0.78		0.73	0.75	

night before the event, there were no significant differences in bed time between groups (p>0.05), but the MVE athletes trended (p=0.07) towards an earlier than normal bed time and the MV athletes trended (p=0.08) towards a later than normal bedtime. As for wake-up times there were no differences in times for habitual, or the morning of the event. All groups woke up significantly earlier on the pre-event morning than they do normally (p<0.05). As for sleep duration, days prior to the event, all groups of athletes had similar sleep total time, averaging about seven hours and 30 min. On the night before the event all athletes slept significantly less than on prior days, averaging only around six hours of sleep (p<0.001).

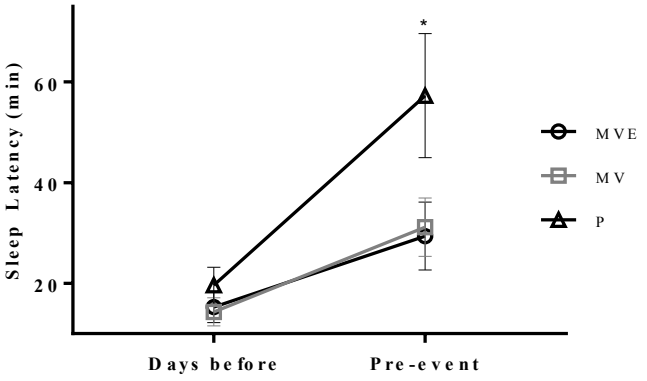
For a night 2-3 days prior to the event, there were no differences between groups for their ranking of perceived sleep quality (MVE: 4.4 ± 1.0, MV: 4.3 ± 1.3, P: 4.6 ± 0.9). However, for the night prior to the event day, the athletes who did not consume the MVE (i.e., MV and P) ranked sleep quality significantly worse than their habitual sleep (MV: 3.8 ± 1.4, P: 3.5 ± 1.1; p<0.05); while, the group who consumed the MVE (3.9 ± 1.1) had no difference in ranking of sleep, despite all athletes having less hours of sleep.

For sleep latency (i.e. the time it takes to fall asleep), there were no significant differences between groups on the days

TABLE 4. Average score ± STD of various readiness variables from athletes a day prior to the cycling event, in the morning pre-event, and immediately post-event. *significance within group over time (p<0.05)

	Days before			Pre-event			Post-event		
	MVE	MV	P	MVE	MV	P	MVE	MV	P
Alert	8.1 ± 1.1	8 ± 1.4	7.6 ± 1.6	7.7 ± 1.7	8.3 ± 1.3	7.4 ± 2.1	7.1 ± 2.3*	7.6 ± 2.5*	6.9 ± 2.5*
Sleepy	2.5 ± 2.2	3 ± 2.7	3.7 ± 2.3	2.6 ± 2.1	2.6 ± 2.2	2.5 ± 2.2	2.6 ± 1.9	2.2 ± 2.7	2.4 ± 2.3
Clearheaded	8.2 ± 1.7	8.7 ± 1.2	7.7 ± 1.5	7.6 ± 1.9	7.8 ± 2.4	7.2 ± 2.1	7 ± 2.7	7.2 ± 2.3	6.1 ± 2.5
Fatigued	2.2 ± 2	3.1 ± 2.5	3.4 ± 2.2	1.9 ± 1.6	2.6 ± 2	2.8 ± 2.3	5.3 ± 2.9*	6.1 ± 2.8*	6 ± 2.4*
Anxious	3.4 ± 2.8	2.3 ± 1.9	2.6 ± 2.3	4.3 ± 2.6	3.9 ± 2.4	3.9 ± 2.6	1.3 ± 0.9*	1.3 ± 1.6*	1.7 ± 2*
Exhausted	1.5 ± 1.4	2.5 ± 2.2	1.8 ± 1.3	1.5 ± 1.3	2.2 ± 1.7	1.9 ± 1.9	5.1 ± 3*	6 ± 2.9*	1.7 ± 2*
Relaxed	7.6 ± 1.7	7.6 ± 2.1	7.3 ± 2.1	6.5 ± 2.4	6.4 ± 2.5	5.4 ± 2.6	6.6 ± 2.8	6.3 ± 2.8	6.8 ± 2.5
Stressed	2.4 ± 2.1	2.1 ± 2.4	2.2 ± 2.2	3.2 ± 2.4	3.4 ± 2.7	3.6 ± 2.8	1.4 ± 1.7	1.7 ± 2.2	2.1 ± 2.3
Energetic	7.7 ± 0.9	7.1 ± 1.6	6.8 ± 2.1	7.2 ± 1.6	7.7 ± 1.4	6.7 ± 2.4	4.7 ± 2.6*	4.3 ± 2.8*	5.3 ± 2.6*
Able to Concentrate	7.7 ± 1.6	8.4 ± 1.4	6.9 ± 2.2	7.8 ± 1.4	8 ± 1.6	7.2 ± 1.6	6.3 ± 2.5	7.1 ± 2.3	6.6 ± 2.3
Overall	8.6 ± 0.7	8.6 ± 1.3	8.5 ± 1.2	9 ± 4.2	8.2 ± 1.3	7.9 ± 1.3	7.8 ± 1.5	7.7 ± 2.3	7.5 ± 2.5

FIGURE 1. Athlete self-reported sleep latency for the day and the night before the cycling event. Mean ± SEM *significant difference between MVE and P athletes



prior to the night before the event (MVE: 15 ± 14, MV: 24 ± 37, P: 19 ± 16 min, p>0.05). However, on the night pre-event, athletes who consumed the MVE fell asleep significantly faster than those who consumed P (29 ± 12 vs. 57 ± 59 min, p=0.05) (Figure 1). The difference between P athletes and MV athletes was not significant.

Readiness

There were no significant differences between groups in any readiness variable at 1-2 days before the event (Table 4). Similarly, all three groups reported similar readiness, including clear-headedness, alertness, and ability to concentrate on the morning of the event, suggesting the MVE and MV did not elicit any perceived mental or energy-related side effects. There were no differences between groups immediately following the event for any measure of readiness; however, there was a main effect of time. Athletes were significantly less alert and more fatigued post-event compared to the day before and pre-event evaluations (p<0.05). Similarly, athletes were more exhausted, less energetic and less anxious post-event than pre-event or the day before the event (p<0.05).

Performance

Final finish times between the groups were not significantly different (p>0.05) (Table 1), and in general athletes completed the event close to their expected finish time. Similarly, there were no statistical differences found between groups for mean difference between expected finish time verses actual finish time.

DISCUSSION

Athletes, including recreational athletes, often experience an undesirable change from their normal sleep patterns the night before an athletic event or competition. This investigation sought to explore the effects of consuming one serving of a multi-vitamin containing 200 mg of Relora®, a proprietary blend of *Magnolia officinalis* bark and *Phellodendron amurense* bark extracts, on the subjective sleep quality and objective sleep quantity the night prior to an endurance cycling event (80-164 km). The night before the endurance cycling event, athletes who supplemented with the Relora containing multi-vitamin fell asleep significantly faster (28 min) and athletes not taking the multi-vitamin with Relora ranked sleep quality significantly worse than their habitual sleep (p<0.05).

Although previous literature is equivocal about the effects of one night of acute sleep loss and its' impact on next-day performance, it is well established that chronic sleep loss can have detrimental effects on physiological elements including exercise performance variables (Belenky et al., 2003; Halson, 2014; Reilly et al., 2007). The ability to fall asleep faster at nighttime through simple multi-vitamin with Relora supplementation in the evening may positively impact athlete's performance overtime.

Relora's bark extracts have been recognized as medicinal and used for centuries in traditional remedies for reducing stress and anxiety in Chinese culture. In past human research, four weeks of supplementation with 500 mg of Relora has been shown to help reduce circulating cortisol levels by 18% (Talbott et al., 2013), a hormone related to physiological stress. In that study, through measures from the Profile of Mood States Survey, subjective reports of overall stress, anger, depression, tension and fatigue were improved by up to 42%, and vigor was increased by 18%. These results suggest that regular supplementation of the combination of bark extracts has the ability to reduce cortisol and perceived daily stress, while improving subjective mood parameters and energy in adults experiencing mental and emotional stress. Relora supplementation at 750 mg for six weeks resulted in lowered evening cortisol levels and increased dehydroandrostenedione (DHEA) levels, along with improvements in psychological measures and perceived stress (Garrison et al., 2006). Similarly, 750 mg daily of the proprietary bark extracts has been shown to reduce mild transitory anxiety (Kalman et al., 2008), which may help to explain the improvements in evening cortisol levels and mechanism of action with improved sleep quality seen in other findings. Research has also suggested Relora to promote mental acuity benefits, and protective effects against eccentric exercise muscle damage with supplementation too (Chiang et al., 2009). Our results with acute supplementation indicate no perceived negative effects from Relora consumption in combination with a multi-vitamin on readiness variables, but the outcomes warrant future investigation of the effects of regular supplementation in athletes on combating the mental and physical stresses and sleep disturbance associated with intense training and competition. Our results are the first to demonstrate the sleep effects of acute, low-dose supplementation with Relora in humans, consistent with research conducted in mice that has identified the active constituents as sleep-promoters without the effects of sedation (Chen et al., 2012).

Athletes experience physical and physiological stress from training and competing. Before a competition or event, it is expected that sleep may be disturbed, and readiness and emotions will reflect a stressed athlete until the competition or event is completed, our results are consistent with this notion. The combination of the present and past results suggests Relora might be an effective, economical, benign means for athletes to combat stress and increase event readiness through perception of good quality sleep.

While the sleep benefits shouldn't be completely attributed to the Relora compound in the multi-vitamin, it is assenting evidence to the support the anecdotal reports of improved sleep patterns by athletes taking these multi-vitamins acutely and regularly. The current study warrants a follow-up study with in-depth sleep analysis studies measuring REM and NREM sleep patterns and ultimately the effects of the Relora containing multi-vitamin on performance and physiological-based outcomes.

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