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Four Weeks of *Hericium erinaceus* Supplementation Does Not Impact Markers of Metabolic Flexibility or Cognition

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ABSTRACT

International Journal of Exercise Science 15(2): 1366-1380, 2022. *Hericium erinaceus* (HE), also known as Lion's Mane mushroom, has been found to enhance cognition and metabolic flexibility in various animal models. To date however, only four studies exist in humans and none have evaluated the effects of HE on markers of metabolic flexibility or cognitive performance. A single-blind, placebo controlled, parallel-longitudinal study was used to determine the effects of HE on markers of metabolic flexibility and cognition. Twenty-four participants completed a graded exercise test on a cycle ergometer to analyze substrate oxidation rates and markers of cardiorespiratory fitness. Additionally, two dual-task challenges consisting of a Stroop Word Challenge interspersed with a Mental Arithmetic Challenge were performed, pre-post the graded exercise test, to evaluate markers of cognition in a pre-post fatigued state. Participants were stratified into two groups, receiving either 10 g of HE per day or placebo for 4-weeks in the form of two muffins identical in taste and appearance. Repeated-measures analysis of variance were conducted to evaluate potential interactions or main effects. Although group differences were noted at baseline, there were no significant interactions or main effects observed from HE ingestion for any dependent variable (all $p > 0.05$). Our data suggest that ingesting 10 g of HE per day for 4-weeks had no impact on metabolic flexibility and cognition in a college-age cohort. Due to the limited research on HE supplementation, future research is needed to establish an effective supplement dose and duration for potential physiological changes to be observed in humans.

KEY WORDS: Dietary supplementation, mushrooms, metabolism

INTRODUCTION

Fatigue is considered a multidimensional sensation, likely resulting from repeated physiological and psychological stressors, although no universal definition exists. In sport, various mechanisms have been detailed which might cause neural, cognitive, or muscular fatigue including inadequate motor command by the motor cortex (8), modulation of brain neurotransmitters (24), metabolite build-up (25), and depletion of endogenous carbohydrate stores (24). Moreover, it is common in sport for athlete's to be exposed to multiple stressors (i.e., dual-task challenges; DTC) simultaneously, such as concurrent psychological and physiological

stressors, which are known to elicit a greater stress and fatigue response compared to that noted from a single stressor alone (12, 29). Provided these findings, athletes have turned their attention to ingesting various dietary supplements prior to competition to improve cognitive and physical performance and potentially mitigate some aspects of fatigue.

One area of interest for athletes and sport nutritionists alike are the purported anti-fatigue properties offered by the regular consumption of edible mushrooms (9). Mushrooms of a wide variety have been ingested by athletes worldwide due to their bioactive constituents of polysaccharides, proteins, vitamins, and minerals. In the animal model, mushroom ingestion has been shown to inhibit blood lactate accumulation, increase glycogen storage in the liver and muscle, and improve the integrity of the mitochondria (16). In humans, current practices for mitigating fatigue are aimed primarily at delaying skeletal and liver glycogen depletion during competition either through exogenous carbohydrate consumption or through dietary manipulations to enhance fatty acid oxidation rates (i.e., low-carbohydrate diets; 17). Regardless of which dietary technique the athlete adopts, both practices arguably rely on a high-degree of metabolic flexibility and a robust mitochondrial environment (10). Interestingly, the regular consumption of mushrooms in the animal model has been found to improve the mitochondrial environment (e.g., increased biogenesis via an increase in peroxisome proliferator-activated receptor gamma coactivator 1-alpha) and should hypothetically, improve markers of metabolic flexibility and assist in fatigue management (14).

Hericium erinaceus (HE), also known as Lion's Mane, is a popular medicinal mushroom that has been shown in a mouse model to improve markers of metabolic flexibility by shifting the cell's reliance towards fatty acids (13) and reducing the oxidation of endogenous carbohydrate stores during exercise (16). Similar mushrooms (e.g., *Cordyceps sinensis*) have shown a strong capacity to modulate mitochondrial function via their upregulation of mitochondrial biogenesis and enzymes associated with long chain fatty acid β-oxidation (9). Although the metabolic effects of HE are still unclear, the neurological and cognitive aspects of HE are frequently highlighted within the literature (15, 19, 20). For example, HE has continuously demonstrated properties to stimulate nerve growth factor and brain-derived neurotropic factor production, both which are known to increase cognitive functioning through its neurotrophic properties (5, 19). Interestingly, only four studies to date have examined the psychological, gut microbiota, and neuroprotective effects of HE in humans and findings are mixed (19, 20, 27, 30). While data are limited in both animals and humans, discrepancies in findings likely reflect the incorporation of an absolute versus a relative HE dose (grams vs. mg/kg), the dose incorporated for HE consumption (~50 mg/kg – 4 g/day), and the duration of HE consumption (2-16 weeks), Additionally, no studies have yet examined HE consumption on markers of metabolic flexibility or cognitive performance in a human cohort. Therefore, the primary purpose of this study was to examine the effects of a 4-week HE supplementation period on markers of metabolic flexibility (i.e., changes to substrate oxidation rates) with a secondary aim to examine potential changes to markers of cognition, pre-post a fatiguing graded exercise test in a college-age cohort.

METHODS

Participants

A total of twenty-four (*N* = 24; HE: 4 males, 8 females; PLA: 6 males, 6 females; height: 173.5 ± 8.6 cm; body mass: 73.3 ± 15. 4 kg; body fat: 20.3 ± 8.1%; age: 22 ± 2.9 years) apparently healthy, college-age adults participated in this study (Table 1). In brief, participants were allowed to participate in this study if they: (a) met the American College of Sports Medicine qualifications for aerobic activity per week (i.e., 150 min of moderate-intensity or 60 min of vigorous-intensity; American College of Sports Medicine, 2021), (b) had not consumed HE in the previous two weeks in any form, and (c) were currently weight stable (± 2.5 kg) for at least one month prior to the start of the study. Following inclusion criteria, each participant then completed a medical health questionnaire, a physical activity readiness questionnaire, and gave written informed consent. Approval from the University of North Alabama's Institutional Review Board (IRB #: 21-22-088) was obtained prior to recruitment and all participants were informed that they may terminate participation at any time. All procedures in the present study conformed to the standards set by the Declaration of Helsinki and this research was conducted in accordance with the ethical standards of the International Journal of Exercise Science (21).

Table 1. Descriptive characteristics (M = 10; F = 14)

Group	Age (years)	Height (cm)	Weight (kg)		Body Fat %	
			PRE	POST	PRE	POST
PLA	21.8 ± 2.7	173.8 ± 9.1	74.0 ± 13.0	73.4 ± 12.4	19.3 ± 7.3	18.7 ± 7.7
HE	22.3 ± 3.2	173.3 ± 8.5	72.6 ± 18.1	72.7 ± 18.6	21.4 ± 8.9	20.2 ± 9.3

Data are presented as mean ± SD

Protocol

A placebo (PLA) controlled, single-blinded, parallel-longitudinal design was employed to examine the effects of ingesting 10 g/day HE supplement for 4-weeks on markers of metabolic flexibility and cognitive function following a fatiguing graded-exercise test (GXT). Since potential changes to markers of metabolic flexibility were a primary dependent variable, participants were stratified into PLA or the HE group following visit 2 based on absolute fat oxidation rates ($g \cdot min^{-1}$) across the GXT. Each participant was then provided 2 muffins (1 muffin: carbohydrates = 14 g, protein = 3 g, fat = 30 g, total caloric load = ~340 kcals) to consume daily, one in the morning and one at night, for a total of 4-weeks. For the HE group, an additional 10 g of HE (5 g per muffin) were included in the muffins. Pilot testing prior to the initiation of the present study determined that the PLA and HE muffins were identical in taste and appearance. All muffins were baked by the lead investigator prior to distribution to the participants every week. HE was provided by Nammex Organic Mushroom Extracts (Gibsons, BC) and Nammex provided guidance for the chosen dose (10 g/day) and duration (4-weeks) incorporated in the present study. Third party testing validated that the present HE sample included a 1 : 1 ratio of dried mushroom : extract supplement containing a 33.69% volume of β-(1,3) (1,6)-glucans.

Participants completed a total of three visits comprised of a familiarization (Visit 1), and two experimental trials (Visits 2 and 3). During visit 1, preliminary data of age, height (Detecto, Webb City, MO, USA), body mass (BWB-800, Tanita Inc. Tokyo, Japan), and body composition via three-site skinfold for respective sex (male: chest, abdominal, and thigh; female: tricep, suprailiac, and thigh; Lange Skinfold Calipers, Cambridge Scientific Industries, Inc., Cambridge, Maryland) were collected. All visits consisted of a DTC, pre-post-GXT on a Velotron cycling ergometer (RacerMate, Seattle, WA, USA). However, only variables collected during visit 2 (baseline) and visit 3 (post-supplement) were included in statistical analysis.

Prior to visit 2, participants provided the principle investigator with their previous 24-h dietary food log and were reminded to replicate this diet in the 24-h period before returning for visit 3. Moreover, participants provided the primary investigator with a 3-day (Thursday, Friday, Saturday) food log to examine possible dietary changes pre-post intervention during visits 2 and 3, which might impact markers of body composition or metabolic flexibility. Statistical analyses showed no significant differences in macronutrients or overall caloric load pre-post intervention for either group ($p > 0.05$; see Table 2). Participants were also asked to avoid alcohol and caffeine consumption 24-h prior to each experimental session, and were instructed to arrive 4-h fasted prior to testing. All testing was carried out at approximately the same time of day (± 1 h within participants).

Table 2. Macronutrient breakdown pre-post intervention (means ± SD)

		Kcal	PRO (g)	CHO (g)	FAT (g)
PLA					
	PRE	1,569 ± 594	80 ± 36	175 ± 54	61 ± 26
	POST	1,598 ± 734	86 ± 50	165 ± 48	66 ± 38
HE					
	PRE	1,417 ± 322	77 ± 33	131 ± 16	65 ± 14
	POST	1,495 ± 571	81 ± 37	124 ± 36	75 ± 31

PLA = placebo; HE = *Hericium erinaceus;* PRO = protein; CHO = carbohydrate.

Provided the evidence demonstrating a DTC as a greater stressor and inducer of fatigue than a physical or mental stressor alone (12, 29), we chose to adopt a DTC for the present investigation to see if HE had any impact on markers of cognitive performance while participants simultaneously completed a physical challenge. Prior to the start of and following the GXT, a computer screen (MacBook Pro, 13-inch, 2020, Apple California) was placed 2 m in front of the participants at eye level for the completion of the DTC. The DTC consisted of three consecutive 15-s segments composed of two visual-verbal modified Stroop Color Word (SCW; 11) interposed by a mental arithmetic task (MAT). Participants simultaneously completed a Y-Balance Test (YBT) during the DTC (27). The cognitive component of this protocol (i.e., SCW and MAT) was adopted from previous investigations (1, 17, 28) and modified to be performed during the YBT. At the initiation of the SCW protocol, 27 words in various colors (e.g., red, blue, black, green, etc.) would appear simultaneously on the computer screen. The participants were asked to ignore the conflicting color text and as quickly as possible, verbally identify the font color in which each word was presented. Following the completion of the first SCW, participants then

completed the MAT which consisted of addition and subtraction problems using single and double-digit numbers, such as "11 minus 1" or "3 plus 10". The MAT included a total of 10 arithmetic problems. To control for a potential learning effect, participants were never exposed to repeating SCW patterns or identical arithmetic problems during the experimental sessions. Further, the variables recorded and used for analysis include the total number of correct and incorrect responses for both the SCW and MAT, as well as the number of unanswered questions during the DTC.

The YBT was conducted on the participant's dominant limb and consisted of a single leg reach in the anterior, posterolateral, and posteromedial directions, respectively. Participants were instructed to place their hands on their hips and tap the distal portion of the foot to the furthest possible point without placing weight on the extended foot for assistance with balance. Prior to the start of the DTC, participants completed three rounds of the YBT without interference from the SCW to gather a baseline reach distance for the trial. The baseline measurement was then taken as the furthest of the three reach distances (cm). During each trial, participants were instructed to reach as far as possible and complete as many rotations as possible before they finished the SCW and MAT assessment.

Following completion of the first DTC, participants then completed a GXT on a cycle ergometer. Participants first had a heart rate (HR) monitor (T31 Transmitter, Polar Electro, Kempele, Finland) fixed across their chest and then donned metabolic headgear for the collection of cardiorespiratory measures using a metabolic cart (Parvo Medics TrueOne 2400, Sandy, UT, USA) to record oxygen consumption (VO_2), carbon dioxide production (VCO_2), and respiratory exchange ratio (RER). Additionally, rating of perceived exertion (RPE) was gathered in the final 30-s of each stage using the modified cycling Borg 0-10 scale (4). The present GXT was adopted as a valid protocol for measuring an individual's substrate oxidation rates and modified by the present investigative team to start at a lighter intensity since the current study did not require participants to be well-trained (2).

Following connection to the metabolic cart, participants were asked to sit quietly for 5 min to gather resting cardiorespiratory measures. After the resting period, participants were instructed to straddle the cycle ergometer and begin pedaling at 50 W for men and 35 W for females for 5 min (Stage 1). Resistance then increased by 25 W for males and 15 W for females every 3 min until a total of 5 stages had been completed. Following each participant completing 5 stages, resistance then increased by 15 W every minute, until volitional exhaustion was achieved to obtain a VO_{2peak}. Participants were assumed by the investigative team to be in a fatigued state following termination of the VO_{2peak}, as evident by a final VO_{2peak} RPE of 9 ± 1 for both visits 2 and 3.

All cardiorespiratory variables (VO_2, VCO_2, and RER) were averaged from steady-state expired gas and used to calculate substrate oxidation rates. The first 4 min of each stage were excluded and the last 60 s were averaged in two, 30 s cycles from breath-by-breath data. HR, RPE and blood lactate concentrations were also collected during the final 30 s of each stage. Stoichiometric

equations were used to evaluate fat and carbohydrate oxidation rates during the GXT and ignored rates of protein oxidation (6).

$$\text{Fat oxidation } (g \cdot min^{-1}) = 1.67\ VO_2\ (L \cdot min^{-1}) - 1.67\ VCO_2\ (L \cdot min^{-1})$$

$$\text{Carbohydrate oxidation } (g \cdot min^{-1}) = 4.55\ VO_2\ (L \cdot min^{-1}) - 3.21\ VCO_2\ (L \cdot min^{-1})$$

During the experimental sessions, blood lactate concentrations (5 µl) were assessed via a finger stick at a lateral side of the nondominant index finger using a 26-gauge Dynarex (1.8 mm lancet; Orangeburg, NY) self-withdrawing safety lancet and a lactate meter (Nova Biomedical Corporation, Waltham, MA, USA) at the end of each stage during the GXT.

Statistical Analysis

Data are presented at means ± SD. An alpha level of $p \leq 0.05$ was determined *a priori* to be considered statistically significant. Data were tested for normality using the Shapiro-Wilk's test prior to proceeding with the parametric tests as described. Sphericity was evaluated using Mauchly's test. A 2-way (group [PLA vs. HE] × condition [PRE vs. POST]) repeated-measures analysis of variance (ANOVA) was used to assess changes in BM, BF%, as well as VO_{2peak}. A mixed model (group [PLA vs. HE] × stage [1- 5] × condition [PRE vs. POST]) repeated measures ANOVA was conducted to assess VO_2, VCO_2, fat oxidation, carbohydrate oxidation, and blood lactate levels. Regarding cognitive data, DTC (SCW and MAT: correct and incorrect responses, unanswered questions) and YBT data (number of rotations) were analyzed using a 3-way ANOVA (group [PLA vs. HE] × condition [PRE vs. POST] × test [Pre-GXT vs. Post-GXT]). A mixed model (group [PLA vs. HE] × condition [PRE vs. POST]) ANOVA was conducted to evaluate reach distance measures (cm). Note the term "group" refers to HE vs. PLA and "condition" refers to pre- or post-supplementation. When significance occurred, partial eta square (η^2_p: .01 = small effect; .09 = moderate effect; and .25 = large effect) were calculated and reported to provide effect sizes for an interpretation of meaningful differences (7). All data were analyzed using SPSS (Version 28; IBM, Chicago, IL).

RESULTS

Of the twenty-four participants in the present study, there were no significant interactions, main effects or condition effects for BF% or BM ($p > 0.05$; see Table 1).

Fat oxidation found no significant group × stage × condition, group × condition, group × stage interactions, or main effects ($p > 0.05$). There was a main effect for stage ($F = 3.7$, $p < 0.006$, $\eta_p^2 = 0.07$). There were significant decreases to fat oxidation rates across each stage. Mean fat oxidation data are shown in Figure 1.

In terms of carbohydrate oxidation, no significant group × stage × condition, group × condition, or group × stage interactions were noted ($p > 0.05$). There was a main effect for group ($F = 6.6$, $p < 0.011$, $\eta_p^2 = 0.03$) and stage ($F = 55.2$, $p < 0.001$, $\eta_p^2 = 0.51$). There were significantly higher rates

of carbohydrate oxidation in the PLA group compared to the HE group ($p < 0.01$) and a significant increase was observed in carbohydrate oxidation rates across the entire GXT for both groups ($p < 0.001$). Mean carbohydrate oxidation data are shown in Figure 2.

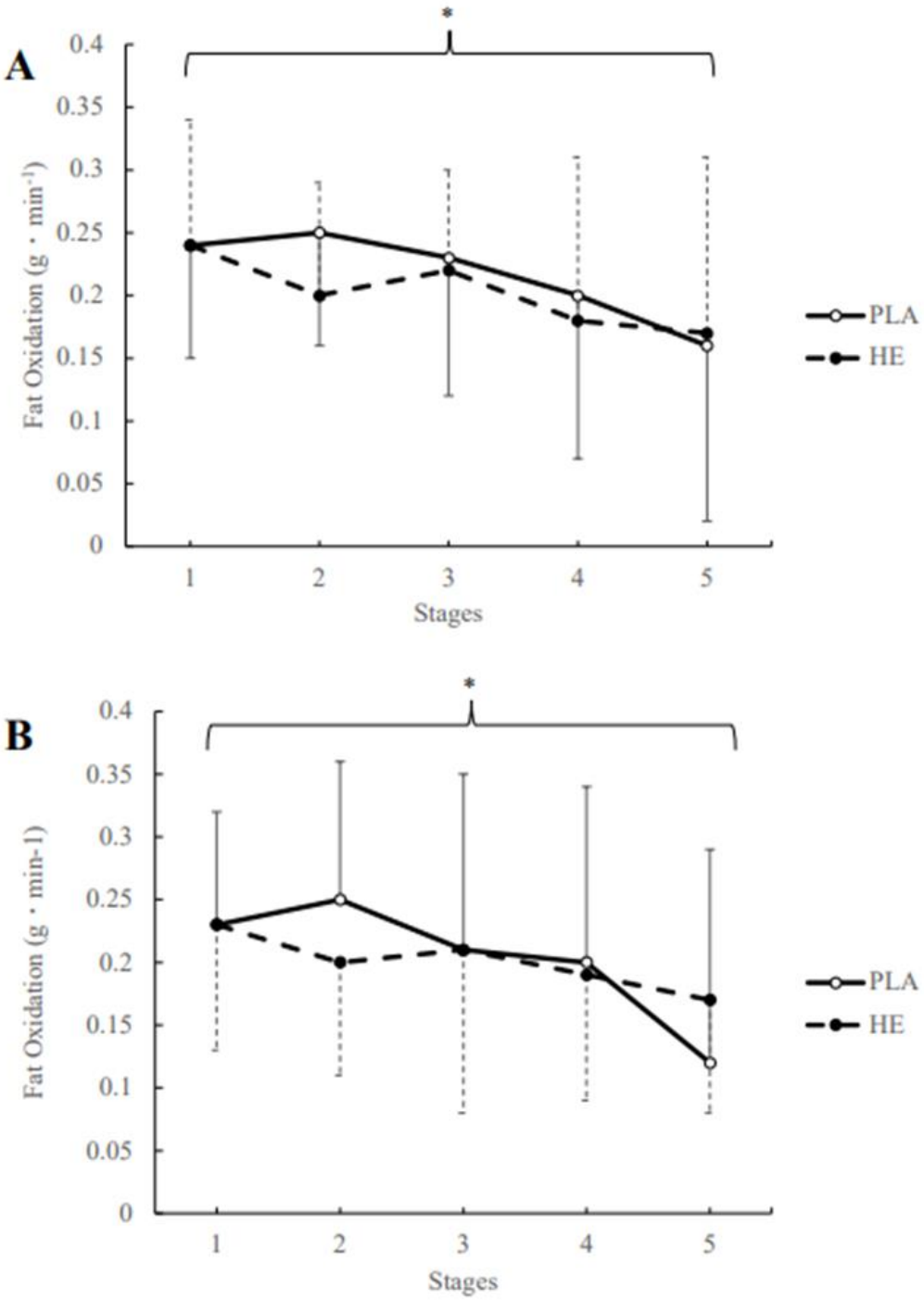

Figure 1. Fat oxidation rates. (A) Pre-supplementation period; (B) Post-supplementation period. PLA = placebo; HE = *Hericium erinaceus*. * Denotes significant main effect for time ($p < 0.01$)

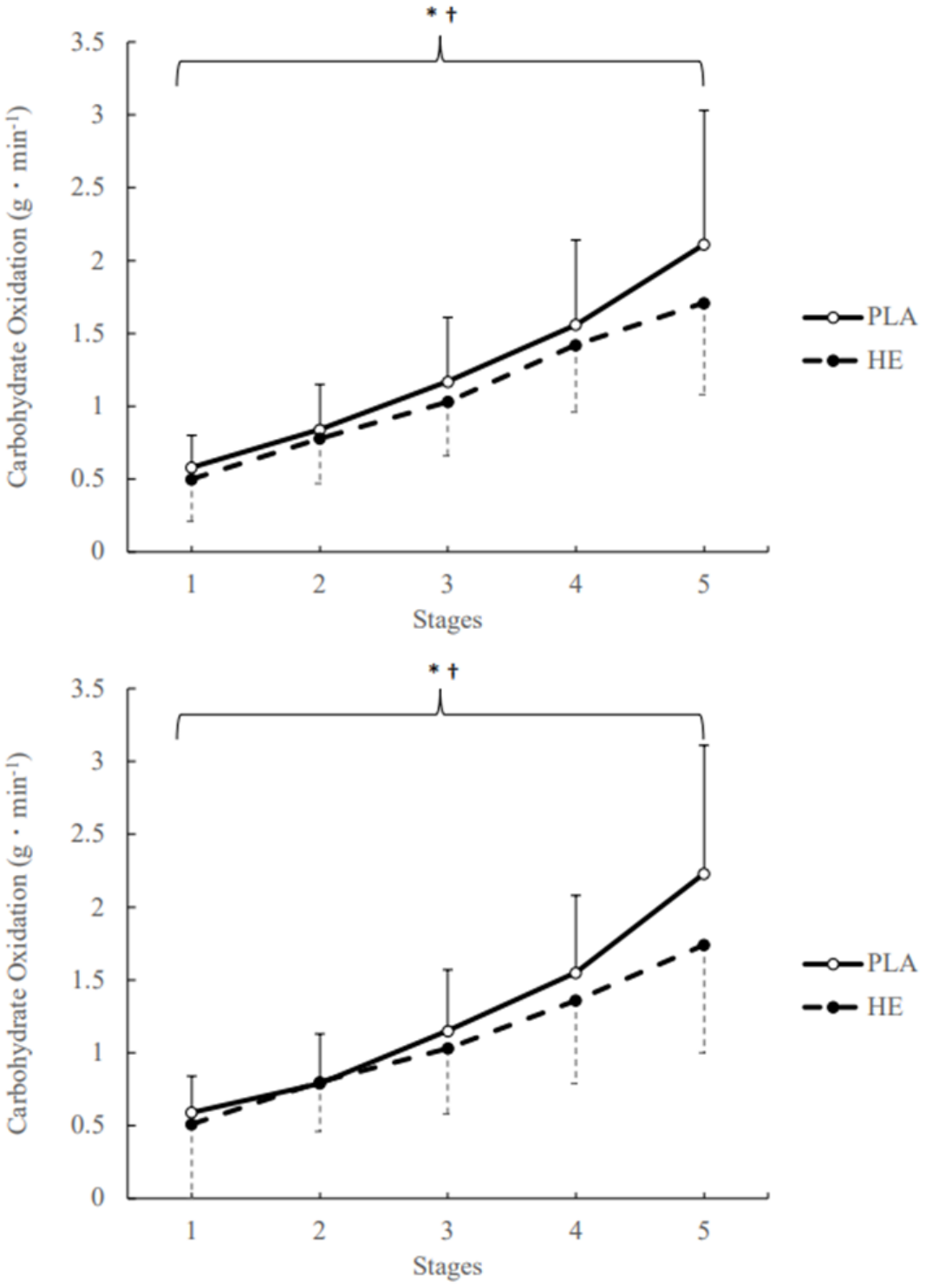

Figure 2. Carbohydrate oxidation rates. (A) Pre-supplementation period; (B) Post-supplementation period. * Denotes significant main effect for time ($p < 0.01$). † Denotes significant differences for group ($p = 0.01$). PLA = placebo; HE = *Hericium erinaceus*

In terms of VO_{2peak}, no significant interactions or main effects were found ($p > 0.05$). No significant group × stage × condition, group × condition, or group × stage interactions were noted for mean VO_2 ($p > 0.05$). However, there was a main effect for group ($F = 7.1$, $p < 0.008$, $\eta_p^2 = 0.03$) and stage ($F = 46.2$, $p < 0.001$, $\eta_p^2 = 0.47$). Overall, the PLA group demonstrated a significantly higher VO_2 across the GXT compared to the HE group and there was a significant increase in mean VO_2 across each stage of the GXT for both groups.

No significant group × stage × condition, group × condition, or group × stage interactions were noted for VCO_2 ($p > 0.05$). There was a main effect for group ($F = 7.8$, $p < 0.006$, $\eta_p^2 = 0.04$) and stage ($F = 54.2$, $p < 0.001$, $\eta_p^2 = 0.51$). There were significantly higher VCO_2 values in the PLA group compared to the HE group and a significant increase in VCO_2 across each stage of the GXT for both groups.

RER found no significant group × stage × condition, group × condition, or group × stage interactions ($p > 0.05$). However, there was a main effect for stage ($F = 23.5$, $p < 0.001$, $\eta_p^2 = 0.31$). There was a significant increase in mean RER across each stage of the GXT for both groups.

Additionally, no significant group × stage × condition, group × condition, or group × stage interactions were noted for HR ($p > 0.05$). Main effects were observed for group ($F = 7.9$, $p < 0.01$, $\eta_p^2 = 0.04$) and stage ($F = 69.9$, $p < 0.001$, $\eta_p^2 = 0.57$). There were significantly higher mean HR levels in the PLA group compared to the HE group, as well as a significant increase in mean HR across each stage of the GXT for both groups.

No significant group × stage × condition, group × condition, or group × stage interactions were noted for RPE ($p > 0.05$). However, there was a main effect for group ($F = 9.8$, $p < 0.01$, $\eta_p^2 = 0.05$) and stage ($F = 142.7$, $p < 0.001$, $\eta_p^2 = 0.73$). Overall, RPE for the PLA group was significantly higher when compared to the HE group and a significant increase in mean RPE across each stage of the GXT was observed for both groups.

There were no significant group × stage × condition, group × condition, or group × stage interactions observed for blood lactate concentration ($p > 0.05$). However, there was a main effect found for group ($F = 19.7$, $p < 0.001$, $\eta_p^2 = 0.09$) and stage ($F = 32.5$, $p < 0.001$, $\eta_p^2 = 0.39$). Higher blood lactate concentration levels were apparent in the PLA group compared to the HE group and there was a significant increase to blood lactate concentration levels across each stage of the GXT for both groups. All cardiorespiratory measures can be viewed in Table 3.

Table 3. Cardiorespiratory data before and after the intervention during the graded exercise test.

	Stage 1	Stage 2	Stage 3	Stage 4	Stage 5	
Trial 1						
VO_2 (L/min)						
*PLA**	0.92 ± 0.23	1.13 ± 0.26	1.34 ± 0.35	1.58 ± 0.40	1.84 ± 0.49	†
HE	0.87 ± 0.20	1.01 ± 0.24	1.22 ± 0.27	1.43 ± 0.36	1.65 ± 0.41	
VCO_2 (L/min)						
*PLA**	0.78 ± 0.19	0.98 ± 0.24	1.20 ± 0.32	1.46 ± 0.39	1.80 ± 0.52	†
HE	0.72 ± 0.19	0.89 ± 0.22	1.09 ± 0.26	1.32 ± 0.38	1.54 ± 0.41	
RER						
PLA	0.85 ± 0.05	0.87 ± 0.05	0.89 ± 0.04	0.92 ± 0.05	0.94 ± 0.06	†
HE	0.83 ± 0.05	0.87 ± 0.05	0.91 ± 0.04	0.92 ± 0.04	0.93 ± 0.06	
BLA (mmol/L)						
*PLA**	2.0 ± 0.4	1.9 ± 0.7	2.1 ± 0.9	3.0 ± 1.5	4.0 ± 1.4	†
HE	1.5 ± 0.4	1.6 ± 0.6	1.5 ± 0.5	2.1 ± 1.1	3.1 ± 1.7	
HR (bpm)						
*PLA**	106 ± 11	116 ± 13	131 ± 16	144 ± 17	159 ± 17	†
HE	107 ± 13	113 ± 13	124 ± 15	136 ± 14	147 ± 15	
RPE (0-10)						
*PLA**	2 ± 1	3 ± 1	4 ± 1	6 ± 1	7 ± 2	†
HE	2 ± 1	3 ± 1	4 ± 1	5 ± 1	6 ± 2	
Trial 2						
VO_2 (L/min)						
*PLA**	0.90 ± 0.16	1.09 ± 0.25	1.29 ± 0.30	1.56 ± 0.38	1.85 ± 0.49	†
HE	0.85 ± 0.19	1.01 ± 0.30	1.18 ± 0.34	1.42 ± 0.42	1.64 ± 0.47	
VCO_2 (L/min)						
*PLA**	0.77 ± 0.15	0.95 ± 0.22	1.16 ± 0.28	1.44 ± 0.36	1.81 ± 0.51	†
HE	0.72 ± 0.17	0.88 ± 0.27	1.06 ± 0.31	1.30 ± 0.41	1.54 ± 0.49	
RER						
PLA	0.85 ± 0.06	0.86 ± 0.06	0.90 ± 0.05	0.91 ± 0.04	0.96 ± 0.06	†
HE	0.83 ± 0.06	0.87 ± 0.06	0.88 ± 0.06	0.90 ± 0.05	0.92 ± 0.04	
BLA (mmol/L)						
*PLA**	1.4 ± 0.3	1.4 ± 0.5	1.8 ± 0.5	2.7 ± 1.0	4.1 ± 1.6	†
HE	1.3 ± 0.5	1.2 ± 0.5	1.5 ± 0.6	2.1 ± 1.3	2.6 ± 1.1	
HR (bpm)						
*PLA**	105 ± 13	114 ± 15	128 ± 17	142 ± 22	157 ± 21	†
HE	103 ± 10	112 ± 12	123 ± 14	133 ± 13	146 ± 15	
RPE (0-10)						
*PLA**	1 ± 1	3 ± 1	4 ± 1	6 ± 1	7 ± 1	†
HE	2 ± 1	2 ± 1	4 ± 1	5 ± 1	6 ± 1	

PLA = placebo; HE = *Hericium erinaceus*; BLA = blood lactate concentrations. Data are represented as means ± SD. *Denotes significant difference between groups ($p < 0.05$). †Denotes significant differences across each stage ($p < 0.05$).

No significant group × timepoint × condition, group × condition, or group × timepoint were noted for SCW tests or unanswered questions ($p > 0.05$). There were no significant main effects for group, test, or condition for either variable ($p > 0.05$). SCW data are shown in Table 4.

Table 4. Stroop and mental arithmetic task tests.

Variables	Trial 1		Trial 2	
SCW (n/27)	PLA	HE	PLA	HE
Correct Answers				
PRE	19 ± 5	20 ± 5	21 ± 4	22 ± 5
POST	19 ± 5	21 ± 5	22 ± 4	23 ± 5
Unanswered				
PRE	7 ± 5	6 ± 5	6 ± 4	5 ± 5
POST	6 ± 5	5 ± 5	5 ± 4	4 ± 5
MAT (n/10)				
Correct Answers				
PRE	8 ± 3	9 ± 1	8 ± 2	9 ± 1
POST	8 ± 2	8 ± 2	8 ± 3	9 ± 2

SCW = Stroop color word; MAT= mental arithmetic task; PLA = placebo; HE = *Hericium erinaceus*. Data are represented as means ± SD.

With respect to reach distance for anterior, posterior lateral, and posterior mediolateral directions, no significant group × condition interactions were found ($p > 0.05$). There were no significant main effects observed for any variable. Further, no significant group × timepoint × condition, group × condition, or group × timepoint were noted for rotations completed ($p > 0.05$). However, there was a main effect for group ($F = 5.5$, $p < 0.022$, $\eta_p^2 = 0.06$). The PLA group initially completed fewer rotations than the HE group. Y-Balance assessment data are shown in Table 5.

Table 5. Y-Balance challenge

Variables	Trial 1		Trial 2	
	PLA	HE	PLA	HE
Rotations Completed				
PRE	10 ± 3	11 ± 4	10 ± 3	12 ± 5
POST	11 ± 3	13 ± 5	11 ± 2	13 ± 4
Reach Distance (cm)				
ANT	66 ± 10	67 ± 7	66 ± 9	67 ± 7
PL	83 ± 15	88 ± 7	88 ± 19	92 ± 9
PM	77 ± 17	85 ± 7	81 ± 16	87 ± 10

ANT = anterior reach; PL = posterolateral reach; PM = posteromedial lateral reach; PLA = placebo; HE = *Hericium erinaceus*. Data represented as means ± SD.

DISCUSSION

The primary aim of this study was to examine the effects of 4-weeks of HE supplementation at 10 g/day on markers of metabolic flexibility during a GXT and cognition using a DTC in college-age adults. First, there was no indication HE resulted in enhanced metabolic flexibility during the GXT and secondly, DTC markers were not improved during pre-post a fatiguing GXT (final-

GXT, RPE 9 ± 1). It is critical to note that although no significant differences were observed between both groups for multiple outcomes, in many cases this simply reflects the groups were not initially stratified by those outcomes (e.g., cardiorespiratory fitness).

Prior to data collection, our team hypothesized that previously established findings from animal models and mushroom consumption might reveal similar metabolic changes in a human cohort. Previous work has found that supplementation with *Cordyceps sinensis* significantly elevated proteins involved in mitochondrial synthesis and long chain fatty acid β-oxidation in mice (14, 21). Theoretically, this should increase some aspects of metabolic health (e.g., reduced BF% or elevated fat oxidation rates) and possibly reduced peripheral fatigue by lowering rates of carbohydrate oxidation while simultaneously reducing circulating lactate concentrations during exercise (16, 26). However, most data regarding HE consumption and metabolic benefits are derived from animal models. Our study is one of four studies that has directly assessed the potential benefits of HE ingestion in a human model. However, we did not find any changes to the aforementioned markers (see Figure 1). Though we were unable to directly assess mitochondrial function or oxidative enzymes as done in previous animal models, our indirect measures from the GXT suggest that 4-weeks of HE supplementation at 10 g/day was insufficient at inducing metabolic changes. Due to the lack of human data, direct comparisons are difficult. It is quite possible that a greater dose of HE or a longer duration of HE ingestion is required to reveal metabolic changes, if any. However, a greater dose than 10 g/day would have unmasked the HE treatment, as pilot data demonstrated inconsistency in taste with a greater dosage and additionally, our duration matched those of a previous HE study conducted in humans where beneficial cognitive effects were observed (19).

Regarding cognitive benefits, previous studies have reported that supplementation with HE can stimulate the synthesis of neural growth factor within the hippocampus and has been shown to upregulate brain-derived neurotropic factor (26). Again, though these findings were reported strictly in animal models, the evidence from HE consumption has shown that the increased expression of nerve growth factor and brain derived neurotropic factor can stimulate improvements in learning and recognition memory (5). Within our study, the DTC aimed to evaluate these effects through recognition and delegation of mentally demanding tasks. The SCW and MAT test, when paired with the YBT, evaluated the participants' ability to differentiate between conflicting stimuli within a given time. Our findings indicated that HE ingestion did not significantly influence any cognitive task (see Tables 4 and 5), and further, we found no evidence that participants focused on the SCW, MAT, or YBT tasks independently.

At present, there are only four studies examining the effects of HE in humans focused on aspects of cognition such as depression and anxiety (20) or the effect of HE on the progression of Alzheimer's disease (15, 19, 27). Our study is unique in that it tested HE supplementation on markers of cognitive performance as opposed to markers of cognitive decline (e.g., dementia). While our study adds to the minimal data on HE supplementation in humans, there are some limitations which should be addressed. While 10 g/day for 4-weeks of HE consumption may indeed have resulted in no significant metabolic or cognitive effects, it is also possible that the

groups were simply underpowered to reveal significant differences as a power analysis was not conducted prior to the study, thus possibly reflecting a Type II error. Additionally, the study is also limited as the dosage and duration we implemented for the present study may not have been sufficient for metabolic or cognitive changes to manifest themselves, if any, in our chosen testing procedures. Lastly, the DTC following the GXT was assumed to be completed in a fatigued state. This assumption was due to the participants post-GXT RPE (~9) and the volitional termination for VO_{2peak} assessment. However, RPE merely represents perceived effort and does not serve as a valid method for solely collecting measures of fatigue. Though this would not have impacted our metabolic data, it may have resulted in a Type II error for our post-GXT DTC data, as the participants may not have been in a fatigued state. Therefore, readers should interpret the post-GXT DTC data with caution. Future studies can correct for this limitation by collecting measures of fatigue pre-post implemented protocol, and demonstrating a reduction in the chosen measure prior to proceeding with their cognitive testing.

In conclusion, the ingestion of 10 g/day of HE for 4-weeks did not elicit any statistically significant changes to markers of metabolic flexibility or cognition in a college-age cohort. Provided that this is only the fifth study to evaluate the effects of HE in humans, and the first to examine metabolic changes or markers of cognitive performance, the outcomes and duration of HE ingestion are still not well understood. Until a standard HE supplementation duration or dosage is established, evaluating changes among studies will remain difficult; therefore, future studies which aim to establish a minimal effective dose or which attempt to elicit mechanistic changes from HE ingestion in humans are warranted.

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