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Effect of Thirty Days Supplementation of Standardized Lingzhi Extract on Aerobic, Anaerobic and Strength Parameters in Trained Athletes

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The mushroom fungus lingzhi (Ganoderma lucidum) has been used for centuries as a traditional health tonic in the Far East. One of its reputed effects, albeit still unproven, is the ability to improve human physical performance. The purpose of this study was to assess the potential ergogenic effects of thirty days of standardized lingzhi extract supplementation on exercise performance. The study adopted a randomized, double-blind, placebo-controlled experimental design. Nineteen players from the men's national field-hockey team were randomly divided into the experimental (LIN, n = 10) and control (CON, n = 9) groups. All players completed tests for percentage body fat, maximal oxygen uptake, 30 s anaerobic Wingate cycling, and hand-grip strength, at pre- and post-thirty days of supplementation. Throughout the 30 days, the LIN group consumed 440 mg lingzhi extract (dose equivalent to 1.5 g dry fruiting body) daily while the CON group was given an equivalent placebo. All players underwent a similar centralized training programme during the intervention period. There were no significant differences in the physical and performance parameters between the two groups, either at the pre- and post-supplementation stages. In conclusion, chronic daily supplementation days of 440 mg lingzhi extract for thirty-days did not enhance training related gains in the aerobic, anaerobic and strength parameters in well-trained field-hockey players.

key words: *herbal extract, ergogenic, Chinese medicine, traditional medicine, Asia*

Introduction

The mushroom fungus known as *lingzhi* by the Chinese, and *reishi* by the Japanese, enjoys a legendary and somewhat mystical status in traditional Chinese

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medicine as a superior health tonic, and has been said to “lighten the spirit and prolong youthfulness” (Jong & Birmingham, 1992; Wong, 1996). There are six known sub-species of *lingzhi,* with the most widely used variety being the “red” *lingzhi*, or Ganoderma *Lucidum*. There are more than 150 chemical compounds found in lingzhi that have apparent medicinal value. These included polysaccharides, furanes, ribosides, peptides and triterpenes (Watchel-Galor et al., 2003; Wong, 1996). There are several types of *Ganoderma Lucidum* preparations currently available on the market. Preparation methods include grinding, drying, pulverizing or using hot water or alcohol extracts of the substrate, which may be the fruiting bodies, mycelium, and/or the mushroom primordial (Chang & Buswell, 1999). Dosages which have anecdotally been recommended range from 1.5 g to 9.0 g dry fruiting body daily, depending on preparation and intended use (Hsu, 1985; Liu & Bau, 1980; Matsumotto, 1979). Chang (1994), in a review that attempted to establish a standard and a rationale for *Ganoderma* dosages, used stimulation of B-glucan receptor activity on white blood cells by standardized extract of *Ganoderma* dry fruiting body as a measure of the biological activity of *Ganoderma lucidum*. He reported that such biological activity could potentially occur with extract from approximately 300 mg of *Ganoderma* dry fruiting body, although the dose dependant biological activity could encompass a relatively very large range (Chang, 1994).

Among the most clinically potent of the chemical constituents of *lingzhi* are the Polysaccharide - linked - Peptides (PSP’s) which are purported to help to modulate the immune system, and prevent or treat cancer (Teeguarden, 1998; Willard, 1990). The triterpenes, which cause the bitter taste of the fungi, are also thought to be “physiologically activating” (Willard, 1990). Other purported effects of *Ganoderma* include that of working as an anti-oxidant against free radicals, an anti-aging effect, reduction of triglyceride levels, anti-inflammatory properties, and even helping with insomnia and stress (Chen & Miles, 1996; Watchel-Galor et al., 2003; Watchel-Galor et al., 2004b; Willard, 1990).

Of particular interest to athletes would be the claim that *lingzhi* helps “oxygenate the blood to alleviate height sickness” (Willard, 1990). In one animal study, hot water extracts of *Ganoderma Lucidum* (GL-57 strain) were administered to three groups of mice in different dosages, and their survival times in the absence of oxygen were compared against that of a control group (Yang & Wang, 1994). There were significant improvements in survival times of the mice that were administered

Ganoderma. Furthermore, this effect appeared to be dose-dependent, as those administered with the higher dosages survived longer. In the following investigation of the same study, three groups of mice were again administered different dosages of *Ganoderma*, and were made to swim continuously (Yang & Wang, 1994). The rodents swimming times were used as a measure of endurance capacity. Again, there was a dose-related improvement in survival time of the mice. These experimental observations on animals raise the possibility that human physical performance may also be enhanced by consuming *Ganoderma*. For example, a previously published study in Chinese scientific literature had reported that regular consumption of lingzhi significantly elevated the levels of 2,3 - diphosphoglyceric acid (2,3-DPG) in both human erythrocytes (in-vitro), as well as rat erythrocytes in vivo (Chen, 1983). 2,3-DPG functions by binding loosely with subunits of the hemoglobin molecule, making it reduce its affinity for oxygen. At the lung level, 2,3-DPG will not significantly affect oxygen binding because of the high oxygen saturation (Brooks, Fahey & White, 1996) but in peripheral tissues, it may be plausible that the extra 2,3-DPG would result in more oxygen being made available during exercise (Dempsey et al., 1971; McArdle, Katch & Katch, 2004). Thus it can be postulated that chronic ingestion of *lingzhi* would lead to higher 2,3-DPG levels which in turn will facilitate or enhance the release of more oxygen (i.e. oxygen delivery) to the working muscles; and this could then be translated to an improvement in predominantly aerobic or endurance-type of performance. In addition, it is also not known whether *lingzhi* supplementation could enhance other aspects of human physical performance such as strength and anaerobic capacity, and such possibilities have not been explored, even in the previous research on animals.

Thus the purpose of this study was to examine the effects of thirty days of chronic supplementation of standardized *lingzhi* extract on several exercise performance tests that assess the aerobic, anaerobic and strength attributes in well-trained athletes who were engaged in training.

Methods

Subjects

The entire squad of twenty-two players from the Singapore National men's field-hockey team was recruited as subjects. Players have represented the country between 2 to 10 years and all were healthy and were not consuming any supplements prior to and during the period of the study. Taking into account the positions played, the players were randomly divided, equally into the experimental (LIN) and control (CON) groups. But injuries sustained during the intervention period, allowed only 19 players (LIN, n = 10 and CON, n = 9) to complete all the requirements of the investigation. Written informed consent from the team's manager, coach and the players were obtained at the start of the study, which was approved by the institutional human subjects review committee.

Lingzhi Preparation

A local company (Prima Research and Technologies, Singapore) produces a hot water and alcohol extract of the fruiting bodies of *Ganoderma Lucidum*, which are evaporated to dryness and made up into capsules of a standardized dosage. This method of preparation extracts the polysaccharides and triterpenes, which are the biologically active components of the mushroom. For the purpose of this study, standardized capsules containing 220 mg extract per capsule derived from 750 mg dried fruiting body were used.

Experimental Procedures

The present study adopted a randomized, double-blind, placebo-controlled experimental trial design. Only one of the investigators had the knowledge of the substance given to each player. The players, coaches, and the sports trainer carrying out the distribution of the supplementation capsules as well as other investigators involved in the performance tests were blinded to this information. The LIN group received *lingzhi* extract 220 mg capsules, while the CON group received identical looking placebo capsules; both types of capsules were provided by the manufacturer (Prima Research and Technologies, Singapore). Unmarked bottles containing either the *lingzhi* or placebo capsules were written with the specific player's name and individually issued to the player. Players were instructed to take two capsules (440 mg *lingzhi* extract, equivalent to 1.5 g dry fruiting body or placebo) from their own bottle every morning for the next thirty days. At the time when the present study was conducted, there was no "ideal" recommended daily amount of *lingzhi* in humans published in the literature. Thus the amount of *lingzhi* given to the present LIN group was based solely on the known common usage of this herbal supplement as well as the recommended dose as advised by the manufacturer (Prima Research and Technologies, Singapore), at the start of the study.

During the period of the study, the entire team was in a thirty-day intensive training camp as part of their pre-competition preparation phase. Accordingly, all players underwent an identical training schedule of six to eight sessions a week, for ~60 to 90 min per session, which included combinations of aerobic and speed fitness training, individual skills, team tactics and drills, open-field play, and six friendly matches. Their diet, although not controlled, was wholly catered and thus, we assume to be similar within the team during the study. The team's sport trainer ensured that each player took their assigned capsules and supervised their consumption at the breakfast table each day. The sport trainer reported 100% compliance.

Table 1. *Physical characteristics of the Control (CON, n = 9) and Lingzhi (LIN, n = 10) groups*

	CON	LIN
Age (yrs)	24.0 ± 4.7	23.6 ± 4.8
Stature (cm)	172 ± 8	170 ± 8
Body mass (kg)	63.9 ± 7.5	61.3 ± 7.7
Body fat (%)	13.8 ± 3.2	14.5 ± 4.4

Table 2. *Body mass, percentage body fat and exercise performance tests results at the pre- and post-supplementation in the Control (CON, n = 9) and Lingzhi (LIN, n = 10) groups*

	Pre-supplementation		Post-supplementation	
	CON	LIN	CON	LIN
Body mass (kg)	63.9 ± 7.5	61.3 ± 7.7	64.1 ± 7.4	61.1 ± 7.3
Body fat (%)	13.8 ± 3.2	14.5 ± 4.4	13.2 ± 2.9	13.4 ± 4.4
Aerobic test				
VO_{2max} ($L \cdot min^{-1}$)	3.74 ± 0.42	3.45 ± 0.57	3.85 ± 0.24	3.67 ± 0.51
VO_{2max} ($ml \cdot kg^{-1} \cdot min^{-1}$)	58.9 ± 5.7	56.4 ± 6.8	60.5 ± 5.6	0.4 ± 8.3
Anaerobic Wingate 30 s test				
Peak power (W)	792 ± 86	739 ± 136	830 ± 78	784 ± 79
Mean power(W)	571 ± 53	541 ± 64	597 ± 53	560 ± 60
Fatigue index(%)	49 ± 7	45 ± 10	46 ± 4	46 ± 7
Peak blood [La] ($mmol \cdot L^{-1}$)	11.9 ± 1.9	11.3 ± 1.0	11.4 ± 0.8	11.4 ± 1.6
Strength test				
Hand grip(N)	406 ± 46	383 ± 47	416 ± 43	413 ± 32

VO_{2max} = maximal oxygen uptake; [La] = lactate concentration

Players underwent the following measurements and assessments at baseline (pre-supplementation) and at the end of the thirty days (post-supplementation): body mass, estimated percentage body fat, maximal aerobic power, Wingate 30s anaerobic cycle and isometric handgrip strength tests. All players were familiarized with the test procedures because these tests were already part of their routine fitness test battery. Players were tested between three to five days prior to and after their return from the training camp. Because of the exhaustive nature of some of the tests, the tests were conducted in two different days for each player, separated by at least 24 hr. All tests were conducted in the morning between 0800 to 1100 hr under similar laboratory conditions.

Percentage Body Fat

Body mass was measured using an electronic scale (*708 Seca, Hamburg, Germany*). Percentage body fat was estimated using a previous method which involves skinfolds, girth and skeletal diameter measurements, and is particularly suited to active adult males (Zuti & Golding, 1973). All measurements were taken by the same investigator at pre- and post-supplementation. The investigator was a Level II ISAK (International Society for the Advancement of Kinanthropometry) anthropometrist.

Maximal Oxygen Uptake (VO_{2max})

The VO_{2max} test was to measure the players' aerobic fitness parameter. The test was conducted using a continuous progressive incremental running protocol on a treadmill (*Marquette model 1900, Milwaukee, WI, USA*). After a standardized warm-up, the test commenced with a treadmill velocity of between 10 and 12 $km \cdot h^{-1}$, at 0% grade. For the first 5 minutes, treadmill elevation was increased by 2% at the end of each minute. Subsequently, this was increased by 1% every minute until the player achieved volitional exhaustion. Treadmill speed and elevation was kept similar for both the pre- and post-supplementation testing for each player. Expired gas was analyzed and the mean values of the output variables were recorded every 20 s using open-circuit spirometry (*Sensormedics 2900Z, Yorba Linda, CA, USA*). The oxygen and carbon dioxide gas analyzers were calibrated prior to each run with known concentrations of standard gases and the flowmeter was calibrated using a 3-l syringe. The individual's VO_{2max} was defined as the highest 20 s of oxygen value if any three of the following criteria as advocated by Davies (1995) were met: i) RER >1.05, ii) HR at termination >95% of age-predicted maximum HR, iii) leveling of oxygen uptake with increasing intensity, and, iv) volitional exhaustion. Heart rate data were monitored continuously via a short-range telemetry (*Sport-tester, Polar Electro Oy, Kempele, Finland*).

Wingate 30s Anaerobic Cycling (WG) Test

The WG test was used to assess the players' ability to sustain high-intensity work for a short duration, i.e. anaerobic power capability (Inbar, Bar-Or & Skinner, 1996). The test employed a 30 s maximal effort bout on a friction-braked cycle ergometer with a weighted-basket loading system (*834E Monark, Verberg, Sweden*) equipped with computerized data acquisition software (*Monark version 1.0*). The test load or resistance level was set at 0.75 $kp \cdot kgBW^{-1}$, based on the body mass obtained at pre-supplementation (Inbar et al., 1996). The same resistance level was retained for the individual's post-supplementation WG test. A standardized warm-up was followed with two run-up practices of 2-3 s during which the actual test load was imposed to accustom players with the resistance. For the actual test, the players started slowly and then on command would begin peddling as fast as they could against an unloaded ergometer. Within the next 1-2 s the weighted-basket was immediately loaded onto the flywheel. Players were then verbally encouraged to maintain their maximum pedal rate throughout the test. The data collected was not corrected for inertia of the flywheel. However, measurement was not initiated until players had attained maximal unresisted acceleration of the flywheel in accordance with the previous methodology (Inbar et al., 1996). Post-exercise blood lactate concentration ([La]) level was taken via finger-prick at the 2nd and 4th min marks, with the highest value reported as peak blood [La]. Blood La was measured with a portable lactate meter (*Accusport, Boehringer Manneheim, Germany*). Performance indices were averaged every 5 s, in absolute (W) and relative (i.e. based on the individual's body mass in kg, W·kgBW-1):

i. Peak Power is the highest power obtained during the first 5 s,
ii. Mean Power is the average power exerted throughout the 30 s, and
iii. Fatigue Index is the percentage decrement between highest and lowest power output during the test

Isometric Hand Grip Strength Test

The isometric hand grip strength was to assess the players' arm strength. The test was conducted using an electronic dynamometer (*T.K.K. 5101 Takei, Tokyo, Japan*) with a standardized protocol (Heyward, 2002). Briefly, the dynamometer grip was first individually-adjusted. The player stood erect with his arm by the side away from the body and the elbow flexed slightly. The player squeezed the hand dynamometer as hard as possible using a maximal effort and minimal extraneous body movements. Each player was allowed two trials for the dominant hand, with at least 60 s rest in-between trials. The highest value was taken as the isometric grip strength. The results obtained from the Takei dynamometer was in kilogram force, but these were converted to Newton (N), in the study.

Statistical Analysis

All analyses were conducted using SPSS for Windows v. 11.0 software (*SPSS Inc., Chicago, IL*) and data were presented as mean ± SD. Data were analyzed using two-way analysis of variance (ANOVA) with repeated measures on the second factor. The t-tests for dependent and independent samples were used for follow-up analysis, if significant interactions were detected. An alpha level of 0.05 was used as the standard for significance.

Results

There were no significant differences between the two groups in their age, height, body mass and percentage body fat at the start of the study (all $P > 0.05$; Table 1). Table 2 shows the players' performance tests results at pre- and post-supplementation time points. These data generally indicate that the present players' physical and fitness attributes compare favorably with previous published work on well-trained hockey players (Reilly & Borrie, 1992).

When the two groups' data were pooled, there were significant declines in percentage body fat and increases in the team's VO_{2max}, peak power, mean power and grip strength from pre- to post-supplementation (all $P < 0.05$). These data indicate that the 30 days of training camp had a positive effect on the players' physical and fitness attributes. However, when statistical analysis was conducted on each group (i.e. LIN and CON separately) independently, the analysis showed that there were no significant differences in all the physical and exercise performance tests' variables between the two groups, either at pre- or post-supplementation time points (all $P > 0.05$). Additionally, we ran a statistical analysis on the magnitude of change made (i.e. the improvement gains) in all the physical and exercise tests between the two groups. Again, the analysis showed that there were no significant differences in the magnitude of changes and/or gains made in the physical and exercise performance tests between the two groups (all $P > 0.05$).

Discussion

For athletes involved in sporting competition, the issue of whether *lingzhi* contains any performance-enhancing substances banned by the International Olympic Committee Medical Commission (2003) must seriously be considered. As a prelude to this study therefore, specimens of the *lingzhi* capsules used in the present study were sent to an independent forensic laboratory for scientific analysis. No stimulants, narcotics, anabolic agents, beta-blockers or diuretics were found (Department of Scientific Services, Report no. 97014269).

Previous studies on *lingzhi* have mainly focused on effects of this herbal supplement on immune-modulation in humans (Lin, 2005), and the present study, to our knowledge, is the first study to investigate the possibility of ergogenic effects of regular *lingzhi* ingestion on exercise performance in humans. The pooled mean exercise performance data showed that both groups combined had higher mean results from the pre- compared to post-30 days of training intervention indicating that the players have generally improved their overall fitness attributes. However, for the purpose of the present study's aim, we observed no significant differences in all of the post-supplementation tests results as well as no significant differences in the magnitude of changes made as a result of the 30 days intervention between the two groups. These latter data did not provide any evidence to indicate that the regular consumption of *lingzhi* has had additional benefit for the players' exercise performance measures. Thus the major finding of this study was that thirty days of *lingzhi* supplementation provided no additive physiological benefits on aerobic, anaerobic and strength parameters in well-trained field-hockey players, above that which could normally be obtained with training.

One of the more compelling postulated ergogenic effects of *lingzhi* consumption is on enhancing aerobic fitness (Chen, 1983), and hence it is prudent that the effect of lingzhi supplementation on this physical attribute is discussed in greater detail here. Aerobic fitness has been established as a key performance parameter in intermittent sports and is usually indicated by the player's VO_{2max} value (Helgerud et al., 2001; Jenkins, 1993). VO_{2max} is the most widely accepted "gold standard" of aerobic fitness and represents the maximal rate of energy supplied aerobically (Basset & Howley, 2000). It was postulated that the supplementation of *lingzhi* would lead to elevated levels of 2,3-DPG which could help to release more oxygen to the working peripheral muscles, and this in turn could results in an enhanced VO_{2max} value during the treadmill test (Brooks et al., 1996; Chen, 1983, McArdle et al., 2004). Interestingly, the VO_{2max} test data did show that the LIN group had a higher magnitude of improvement in mean VO_{2max} compared to the CON group (4.0 vs. 1.6 $ml \cdot kg^{-1} \cdot min^{-1}$, $P > 0.05$). However this difference between groups did not achieve statistical significance and hence our initially postulation that *lingzhi* supplementation may be ergogenic for the aerobic fitness attribute in humans is not tenable in the present study. For practical reasons, we were not able to draw blood from our players and thus it was not possible to determine the direct effects of *lingzhi* supplementation on players' 2,3-DPG levels; and this represents a limitation of the present study.

The present study findings indirectly support a previous study on long-term lingzhi supplementation on healthy humans (Watchel-Galor et al., 2004a). In the well-controlled study, the investigators assessed the effects of four weeks of *lingzhi* supplementation (1.44 g per day) on a range of biomarkers of antioxidant status, cardiovascular heart disease risk, immune status and inflammation. They reported no evidence for the benefits of long-term *lingzhi* supplementation in all of these measures (Watchel-Galor et al., 2004a). In addition, the study also showed that long-term supplementation was not associated with any toxic effect on the subjects' liver and kidney blood markers, nor on any hematological variables (Watchel-Galor et al., 2004a). Taking this study as well as the present study's data together, it can be suggested that *lingzhi* supplementation of up to 1.5 g per day for 30 days does not seem to have any positive response or effects in many of the physiological and metabolic markers in healthy humans. Consequently, a higher dosage is recommended for future studies on the efficacy of long-term *lingzhi* supplementation. Such a proposal will always lead to the concern that there would be the dangers of toxicity or even side-effects. It is, however, reassuring that Ganoderma products have been repeatedly reported to be lacking in significant toxicity and side effects (Chang & Buswell, 1994). Also, lethal doses of Ganoderma polysaccharide in rats had been reported as greater than 5 g·kg body mass^{-1}. This far exceeds the dosages normally given to humans (Chang, 1994). The dosages of *lingzhi* used in the present study as well as that of Watchel-Galor et al. (2004a) were clearly within the lower end of the spectrum of effective dosages covered in Chang's (1994) review. It is however important to highlight to the reader that the present study showed that the improvements made in several of the exercise performance tests (i.e. peak power and grip strength results) in the LIN group were consistently higher than that of the CON group, albeit not statistically significant. This suggest that it remains possible that using a relatively higher dosage levels and/or for the longer-term > 4 weeks, may induce a greater magnitude of changes in humans physical attributes; and this is a fertile area for further research. Nonetheless based on the above observations (Chang, 1994; Chang & Buswell, 1994; Watchel-Galor et al., 2004a; 2004b), it is recommended that a higher dose of between > 2.0 to 4.0 g·kg body mass^{-1} be used in future studies investigating the ergogenic effects of *lingzhi* on human physical performance.

Several possible reasons can be put forth for the lack of significance difference in the magnitude of the gains observed at post-supplementation between the LIN and CON groups. The limited number of players in each group as well as the large variation in the individuals' tests scores could have lowered the statistical power of the study and this could be considered as a major limitation of the present study. This view is supported by the fact that when the two groups' data were pooled, statistically significant differences were observed in all the tests results when comparing pre- and post-supplementation. Another plausible reason is that the tests used in the present study were not sufficiently sensitive to detect the proposed, but probably very small, changes in exercise performance as a result of *lingzhi* ingestion. For example, it has been alternatively argued that oxygen utilization rather than oxygen delivery perse, is the predominant limiting factor in VO_{2max} (Basset & Howley, 2000; Bergh et al., 2000; Green & Patla, 1992; Noakes, 1997), which implies that the postulated increase in levels of 2,3-DPG due to *lingzhi* supplementation would consequently have only a very limited effect, if any at all, on enhancing VO_{2max}.

In conclusion, the present study's data indicated that thirty days of regular administration of 440 mg of *lingzhi* extract daily had no ergogenic effects on aerobic, anaerobic and strength parameters when combined with a regular training program in trained athletes. It is not known whether an increase in dosage would result in a greater magnitude of effects; this being a potential area for further research. Further basic research is also needed to study the effects of *lingzhi* at the cellular level, particularly in humans.

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