

Effects of *Ganoderma lucidum* and ‘Essence of Chicken’ on Physical Fatigue Recovery and Exercise Performance Improvement

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Abstract

A fast-paced lifestyle, pressure from the environment and a heavy work load often cause extreme tiredness in modern life. Different kinds of nutritional supplements in the form of functional foods or traditional Chinese medicine, such as ‘essence of chicken’ and *Ganoderma lucidum* have been claimed to benefit physical performance and promote health. Previous studies have revealed that ‘essence of chicken’ or *G. lucidum* have a wide spectrum of biological activities. In this study, we combined these two ingredients together (designated as CEG) to evaluate their synergistic effects on physiological adaption on exercise fatigue and physical activities. The ICR strain mice were allocated as 0, 833, 1666, and 4165 mg/kg dose groups and administrated by oral gavage consecutively for 4 weeks. Physical activities including grip strength and aerobic endurance were evaluated. Various fatigue-associated biochemical variables such as lactate, BUN or CK were also evaluated. The levels of liver and muscle glycogen were measured as an indicator of energy storage at the end of the experiment. Safety assessments for supplementation were also evaluated. CEG supplementation significantly increased the endurance and grip strength and demonstrated beneficial effects on lactate production and clearance rate after an acute exercise challenge. The CEG supplementation significantly mitigated the BUN and CK indexes after extended exercise and elevated the glycogen content in the liver and muscle tissues. According to body composition, biochemical and histopathological data, daily administration of CEG for over 28 days (subacute toxicity) also demonstrated reasonable safety results for supplementation. Combined *G. lucidum* and ‘essence of chicken’ can significantly increase the exercise performance and improve fatigue recovery. It may also provide a viable alternative nutritional supplement for health promotion.

Key Words: clearance rate, essence of chicken, exercise fatigue, physical activities, safety

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Introduction

Physiological fatigue relevant to exercise physiology occurs through two different mechanisms, a central mechanism involving proximal motor neurons (mainly in the brain) and a peripheral mechanism within the motor units (i.e., motor neurons, peripheral nerves, motor endplates, and muscle fibers) (1). The central fatigue hypothesis suggests that an increased ratio of central serotonin to dopamine is associated with feelings of tiredness and lethargy, accelerating the onset of fatigue (25). In contrast to the central fatigue, the processes in the motor neurons, neuromuscular junctions, sarcolemmal membrane, excitation-contraction coupling, accumulation of metabolites, or depletion of fuel are involved in peripheral fatigue (20). Peripheral and central fatigue may happen separately or together, depending on the specific situation or condition. Fatigue can be also further categorized in other ways, such as secondary, and chronic fatigue. Secondary fatigue is caused by an underlying medical condition and may last one month or longer, but less than six months. If the fatigue syndrome lasts longer than 6 months, it can be defined as chronic fatigue (5).

Ganoderma lucidum, called Lingzhi in Mandarin, is a well-known traditional medicinal fungus that has been shown to have many physiological effects. For hundreds of years, this mushroom has been used as a folk medicine and applied for the prevention and treatment of various human diseases, such as hepatitis, hypertension, chronic bronchitis, bronchial asthma, and cancer (3). In evidence-based reports, *G. lucidum* demonstrated hepatoprotective effects on a CCL4-induced liver injury model resulting in significant decrease in the hepatic indexes (13). In addition, exhaustive exercise-induced muscular oxidative stress could be attenuated by supplementation with *G. lucidum* polysaccharides (47). The immune-suppression caused by long-term and high intensity exercise could be mitigated by *G. lucidum* supplementation via non-specific and specific immune response improvement (31) and it could also significantly improve aerobic endurance, lower body flexibility, and velocity (10). Furthermore, *G. lucidum* could improve chemotherapy-induced fatigue via regulation of inflammatory responses, oxidative stress and reduction of nephrotoxicity (28) and also showed beneficial effects on quality of life and immune markers pertaining to cancer-related fatigue (46).

'Essence of chicken' is a liquid nutritional supplement made by cooking whole chickens by high-temperature extraction, centrifugation, vacuum concentration, and sterilization. It contains some

special nutrients such as imidazole dipeptide (carnosine and anserine), taurine, polypeptides, minerals, trace elements, and multiple amino acids (23). Mental fatigue often occurs in populations who sit in meetings, read, or take exams for long periods and daily intake of 'essence of chicken' may be effective for recovery (43). The imidazole dipeptide, are natural antioxidants that have been reported to inhibit tissue damage and suppress the reduction in performance level induced by mental fatigue (4). In addition, 'essence of chicken' has been proved to reduce hyperglycemia and the L-Carnosine component helps to regulate blood sugar levels (36, 44). Chicken essence (CE) supplementation also has been found to have other beneficial effects on mood (45), cognition (22), circadian clock resetting process (41), hypertension (32) and exercise adaption (18).

The food and nutrition industry has grown rapidly in recent years and many different functional or medical food combinations are being tried to obtain better health benefits. In the current study, we investigated the effects of a *G. lucidum* and CE combination (CEG) for on exercise physiological adaption using a slightly modified animal platform. We also evaluated the safety of this supplementation using a subacute toxicity test to understand the potential risk with consecutive and dose-effect supplementation.

Materials and Methods

Materials

The supplementation contained CE and extracts of *G. lucidum* fruiting body. The CE and extracts of *G. lucidum*, designated as CEG, were provided by Tian Yuan Xiang Co. Limited (Tainan City, Taiwan). The dried fruiting bodies of *G. lucidum* were extracted by sequential procedures, first with alcohol at room temperature (12 h) and then water, and combined these extractions for lyophilization. The CEG included the 90% CE and 10% *G. lucidum* extracts as proportion for current study. The CEG was freeze dried for comparable and compatible dosage conversion for animal experimental designs and the averaged lyophilized powder weight was about 4.1 grams for human recommended CEG daily serving (60 mL). The calorie count was about 15.6 Kcal/serving as analyzed by SGS Taiwan, Ltd.

Animals and Experimental Designs

Forty male ICR mice (6 weeks old) from AAALAC certified by BioLASCO Taiwan (Yi-Lan, Taiwan) were used in this study. Mice were acclimatized and allowed food *ad libitum* for 2 weeks

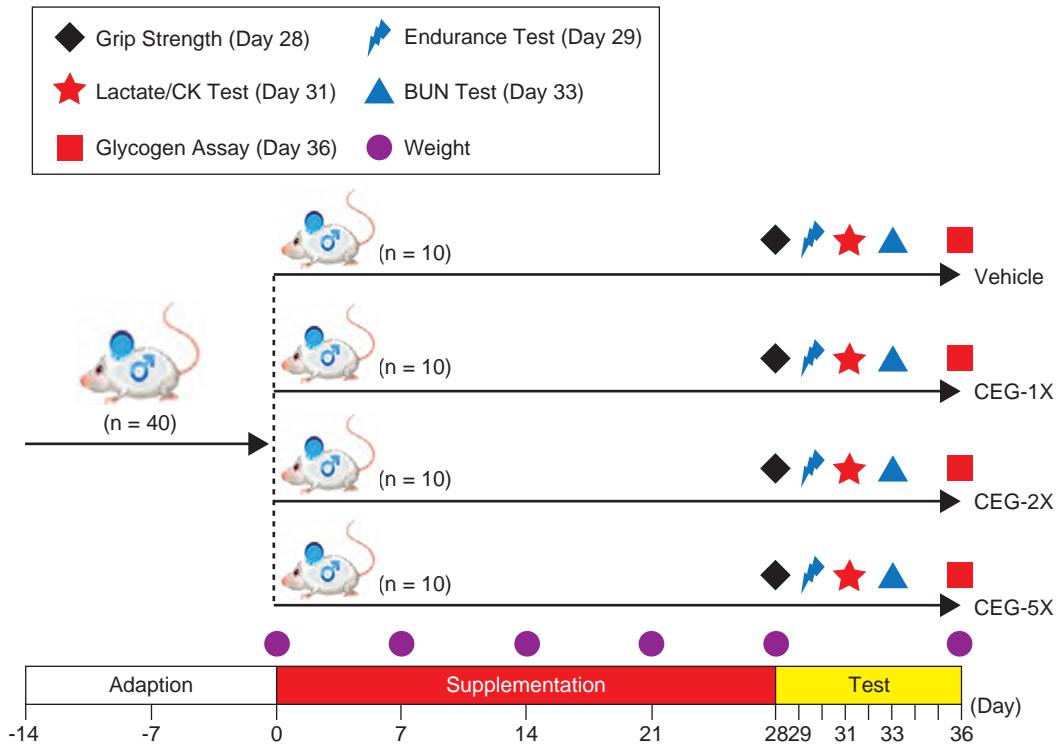


Fig. 1. Experimental designs for the effects of CEG on exercise adaptation. The animals were randomly assigned to the indicated 4 groups (Vehicle, CEG-1X, CEG-2X, and CEG-5X) and consecutively supplemented the CEG until the end of the experiments. The physical capacities and related biochemistries were assessed within test duration.

prior to experiments. All animals were given a standard laboratory diet # 5001 (PMI Nutrition International, MO, USA) and distilled water ad libitum, and maintained in constant photoperiod, temperature, and humidity (12-h light/12-h dark cycle, $24 \pm 2^\circ\text{C}$, and 55%–65%, respectively). Routine cleaning was conducted twice per week. The Institutional Animal Care and Use Committee (IACUC) of National Taiwan Sport University approved all animal experiments in this study, and the study conformed to the guidelines of protocol IACUC-10513 approved by the IACUC ethics committee.

The recommended CEG dosage was 4.1 g per day (lyophilized powder), which is equivalent to CEG at 60 mL/serving/day (18). The mouse CE dose (845 mg/kg) we used was converted from a human equivalent dose (HED) based on body surface area as CEG-1X by the formula from the US Food and Drug Administration. The detailed formula was as described in a previous study (18) and we designed the different dependence-dosages (vehicle, CEG-1X, CEG-2X, and CEG-5X groups).

The detailed experimental procedure is illustrated in Fig. 1. All animals were given an acclimation period of two weeks to adapt to the environment and diet. The weight, dietary, and social

behaviors were monitored during the supplementation period and the daily supplementation began at the regular time with fresh CEG sample preparations. Treatment dosages of 0, 833, 1666, and 4165 mg/kg/day were designated as vehicle, CEG-1X, CEG-2X, and CEG-5X and were administered by oral gavage with a volume 10 ml/kg BW. Physical fitness was evaluated by grip strength and aerobic endurance capacities and the exercise-related biochemistry was immediately assessed after a fixed exercise time/intensity.

Exercise Endurance Performance Test

Exercise performance was based on the survival motives to assess the aerobic endurance capacities. The animals were loaded with a weight equivalent to 5% individual body weight and forced to swim in a tank until exhaustion. The persistent time from beginning to exhaustion was recorded as endurance index. The detailed procedures and protocol were described in our previous article (35).

Forelimb Grip Strength

A low-force testing system (Model-RX-5, Ai-

koh Engineering, Nagoya, Japan) for forelimb grip strength was used to measure the grip strength. The details have been described previously (8).

Determination of Fatigue-Associated Biochemical Variables

Based on our previous reports (15), the effect of CEG supplementation on fatigue-associated biochemical indexes was slightly modified to accurately demonstrate the physiological status. The blood was sampled by submandibular collection for further biochemical analysis. For the lactate metabolites profile, the blood sampling time points were pre-exercise, immediately after 10 min acute exercise, and then after 20 min rest. The other indexes, such as blood urine nitrogen (BUN) and creatine kinase (CK), were immediately assessed at the 60 min rest time point after 90 min extended exercise. The blood samples were centrifuged at $1000 \times g$ and 4°C for 15 min after complete clotting for serum separation and determined by an autoanalyzer (Hitachi 7060, Hitachi, Tokyo, Japan).

Clinical Biochemical Profiles

CEG supplementation was administrated continuously until animal sacrifice. All mice were euthanized by 95% CO_2 asphyxiation one hour after the last treatment and immediately sampled blood by cardiac puncture. Serum was separated by centrifugation and clinical biochemical variables, including aspartate aminotransferase (AST), alanine transaminase (ALT), ammonia (NH_3), CK, glucose (GLU), BUN, creatinine (CREA), uric acid (UA), total cholesterol (TC), triglycerides (TG), albumin (ALB), and total protein (TP) were measured by use of an autoanalyzer (Hitachi 7060).

Body Composition and Glycogen Content Analysis

After sacrifice, the important visceral organs, including liver, kidney, heart, lung, muscle, epididymal fat pad (EFP), and brown adipose tissue (BAT), were accurately excised and weighed. Then, the organs were saved into 10% formalin for further histopathology. Part of muscle was kept in liquid nitrogen for glycogen content analysis as described previously (9).

Histopathology

The visceral organs preserved in 10% formalin were trimmed and embedded in paraffin for tissue sections with $4 \mu\text{m}$ thickness slices. Tissue sections were further stained with hematoxylin and

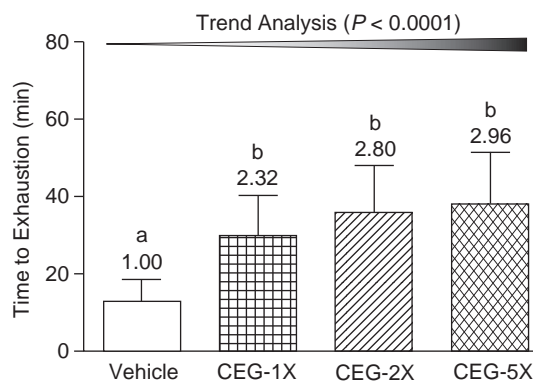


Fig. 2. Effect of 4-week CEG supplementation on exhaustive swimming time. Data are mean \pm SD for $n = 10$ mice per group. Columns with different superscript letters (a, b) are significantly different at $P < 0.05$. Numbers above the columns are the fold increase from vehicle.

eosin (H&E) staining and examined under a light microscope equipped with a CCD camera (BX-51, Olympus, Tokyo, Japan) by a veterinary pathologist.

Statistical Analysis

The data were represented as mean \pm standard deviation (SD). The statistical difference among groups in physical activities, biochemistry, body composition, dietary and glycogen content were analyzed by one-way analysis of variance (ANOVA) and the Cochran-Armitage test for dose-effect trend analysis with use of SAS v. 9.0 (SAS Inst., Cary, NC, USA). A mixed design two-way ANOVA (supplementation \times time) was also applied to the supplementation effects on lactate metabolite profiles and growth curve within repeated time points. Data were considered statistically significant when the probability of a type I error was less than 0.05.

Results

Effects of CEG Supplementation on Endurance Capacity

The endurance capacity was measured by exhaustive swimming test and it showed a significant difference among groups ($F(3,36) = 11.19$, $P < 0.001$). The CEG supplementation groups (CEG-1X, CEG-2X, and CEG-5X) were significantly higher than the vehicle group showing a 2.32-, 2.8-, and 2.96-fold increase and demonstrated the significant dose-dependent effects ($P < 0.001$) in trend analysis (Fig. 2).

Table 1. The effects of CEG on lactate metabolite profiles during acute exercise challenge

Time point	Vehicle	CEG-1X	CEG-2X	CEG-5X
	Lactate (mmol/L)			
Before swimming (A)	2.6 ± 0.3 ^a	2.6 ± 0.5 ^a	2.6 ± 0.4 ^a	2.6 ± 0.3 ^a
After swimming (B)	9.0 ± 1.2 ^b	7.1 ± 0.3 ^a	6.9 ± 0.5 ^a	6.7 ± 0.6 ^a
After a 20-min rest (C)	8.5 ± 1.3 ^c	6.1 ± 0.4 ^b	5.6 ± 0.5 ^{ab}	5.3 ± 0.8 ^a
	Rate of lactate production and clearance			
Production rate = B/A	3.57 ± 0.55 ^b	2.82 ± 0.44 ^a	2.73 ± 0.52 ^a	2.63 ± 0.43 ^a
Clearance rate = (B-C)/B	0.06 ± 0.03 ^a	0.15 ± 0.02 ^b	0.19 ± 0.06 ^{bc}	0.21 ± 0.08 ^c

The lactate metabolites were assessed for the 4 groups allocated as vehicle, CEG-1X, CEG-2X, and CEG-5X at three time points. Lactate production rate was calculated as, after exercise divided by before exercise (B/A) and the lactate difference between after exercise and after rest divided by after rest was defined as the clearance rate. Values in the same row with different superscript letters (a, b, c) differ significantly, $P < 0.05$.

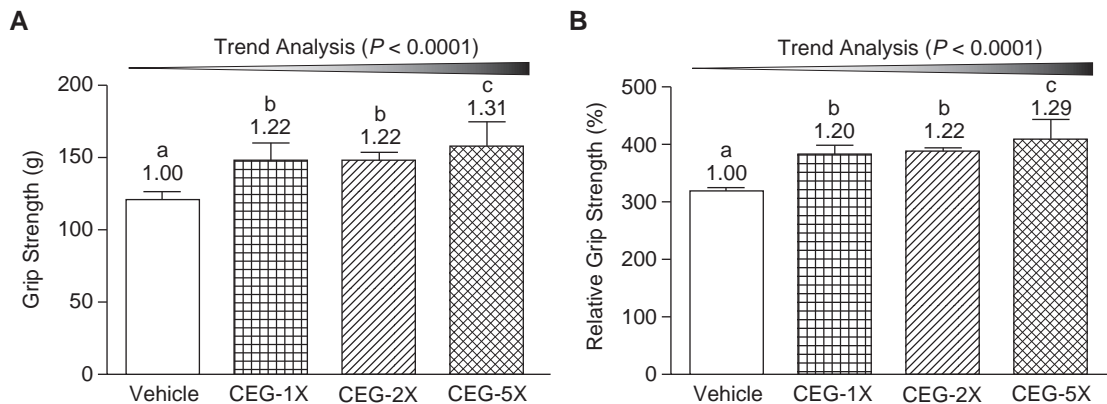


Fig. 3. Effect of 4-week CEG supplementation on absolute forelimb grip strength (A) and forelimb grip strength (%) relative to body weight (B). Data are mean ± SD for $n = 10$ mice per group. Columns with different superscript letters (a, b, c) are significantly different at $P < 0.05$. Numbers above the columns are the fold increase from vehicle.

The Effects of CEG Supplementation on Grip Strength

The grip strength showed significant differences among groups in absolute strength ($F(3,36) = 20.87$, $P < 0.001$) and relative strength adjusted by individual weight ($F(3,36) = 43.11$, $P < 0.001$). The CEG supplementation groups (CEG-1X, CEG-2X, and CEG-5X) were significantly higher than vehicle group ($P < 0.0001$) with regard to absolute strength (1.22-, 1.22-, and 1.31-fold, respectively) and relative strength (1.20-, 1.22-, and 1.28-fold, respectively). Both grip strength measures also showed significant CEG dose-dependent effects ($P < 0.001$) in trend analysis (Fig. 3).

The Effects of CEG Supplementation on Exercise-related Biochemical Indexes after Exercise Challenge

The lactate metabolite was assessed pre-

exercise, immediate post-exercise, and after rest (3 time points) with the different CEG treatments (Table 1). It showed significance difference in supplementation main effect ($F(3,36) = 23.75$, $P < 0.001$) and time main effect ($F(2,72) = 966.9$, $P < 0.001$). The interaction effects was also significant ($F(6,72) = 21.82$, $P < 0.001$). Further analysis of the 3 time points showed a significant difference in the post-exercise ($F(3,36) = 19.74$, $P < 0.001$) and after rest ($F(3,36) = 30.84$, $P < 0.001$) time points. The vehicle group was significantly higher than other CEG treatment groups at time points of post-exercise and after rest by one-way ANOVA. The self-comparison indexes, such as lactate production rate and clearance rate, also demonstrated significant beneficial effects with CEG supplementation ($P < 0.05$).

The other metabolic indicator, BUN, demonstrated significance after extended exercise ($F(3,36)$

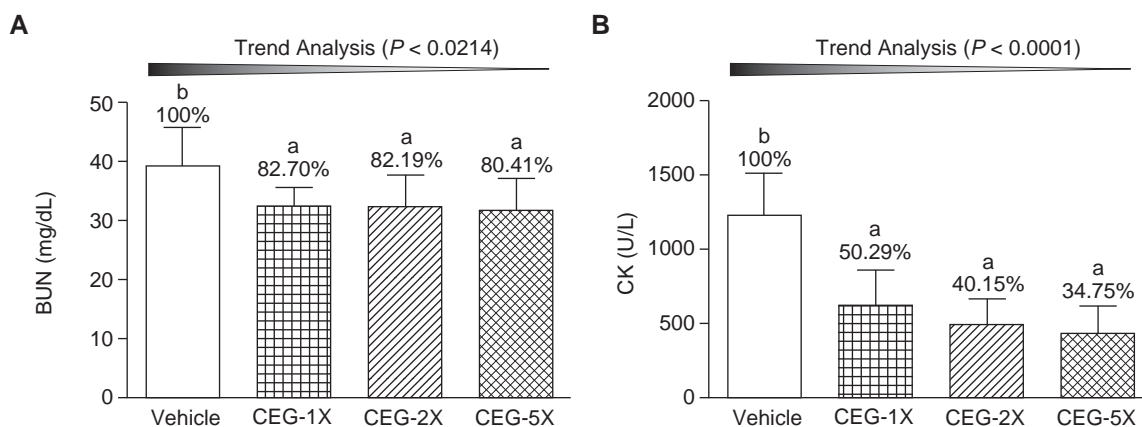


Fig. 4. Effect of 4-week CEG supplementation on the serum BUN (A) and CK (B) levels after extended exercise challenge. The indicated 4 groups underwent 90 min swimming exercise and blood was sampled after 60 min rest. Data are mean \pm SD for $n = 10$ mice per group and the columns with different superscript letters (a, b) are significantly different at $P < 0.05$. Numbers above the columns are the fold-decrease from vehicle.

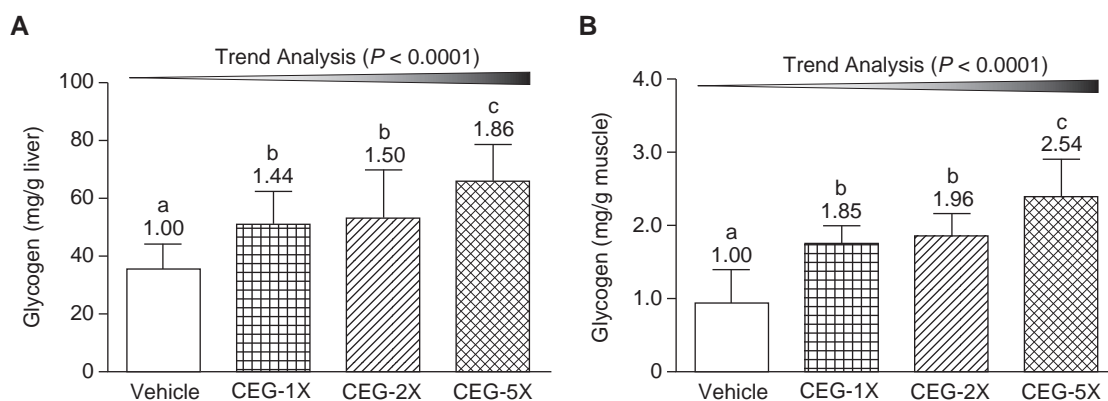


Fig. 5. Effect of 4-week CEG supplementation on hepatic (A) and muscle (B) glycogen level. Data are mean \pm SD for $n = 10$ mice per group. Bars with different superscript letters (a, b, c) are significantly different at $P < 0.05$.

= 4.62, $P = 0.008$) (Fig. 4A). CEG supplementation significantly decreased the exercise-induced BUN elevation by 18-20% ($P < 0.05$) showing a dose-dependent trend ($P = 0.002$). The other important injury index, CK, showed significant difference among groups ($F(3,36) = 25.74$, $P < 0.001$) (Fig. 4B). The CEG supplementation could mitigate the CK increase by 49.3, 39.2, and 65%, respectively, and showed a dose-dependent trend ($P < 0.0001$).

The Effects of CEG Supplementation on Glycogen Content

Glycogen is mainly stored in the liver and muscle tissue for the purpose of energy homeostasis. CEG supplementation was shown to affect the glycogen content in the liver (Fig. 5A; $F(3,36) =$

9.51, $P < 0.0001$). Hepatic glycogen significantly increased 1.44-, 1.50-, and 1.86-fold, respectively, as compared to the vehicle group. Muscular glycogen also showed a significant difference among the groups ($F(3,36) = 22.75$, $P < 0.0001$). The muscular glycogen significantly increased 1.85-, 1.96-, and 2.54-fold, respectively, as compared to the vehicle group (Fig. 5B). Both the hepatic and muscular glycogen showed significant dose-dependent trend ($P < 0.0001$).

Subacute Oral Toxicity Evaluation of CEG Supplementation

Subacute toxicity evaluation following OECD Guideline 407 was performed not only for physiological exercise adaption but also to assess the

Table 2. Growth curve and dietary profiles during experiments

Time point	Vehicle	CEG-1X	CEG-2X	CEG-5X
Initial BW (g)	35.1 ± 0.8 ^a	35.0 ± 1.0 ^a	35.0 ± 1.8 ^a	35.1 ± 0.7 ^a
1st wk BW (g)	35.9 ± 0.9 ^a	35.9 ± 1.5 ^a	35.9 ± 1.5 ^a	35.9 ± 1.1 ^a
2nd wk BW (g)	36.6 ± 1.0 ^a	36.8 ± 1.5 ^a	36.9 ± 1.4 ^a	36.9 ± 1.0 ^a
3rd wk BW (g)	37.5 ± 1.2 ^a	37.7 ± 1.5 ^a	37.7 ± 1.9 ^a	37.9 ± 1.0 ^a
4th wk BW (g)	38.0 ± 1.6 ^a	38.5 ± 1.7 ^a	38.2 ± 1.9 ^a	38.6 ± 1.1 ^a
Final BW (g)	39.3 ± 1.0 ^a	39.8 ± 1.7 ^a	39.9 ± 1.9 ^a	39.6 ± 0.9 ^a
Water intake (mL/mouse/day)	8.8 ± 2.3 ^a	8.8 ± 2.4 ^a	8.8 ± 1.7 ^a	8.9 ± 2.4 ^a
Chow 5001 (g/mouse/day)	6.9 ± 0.8 ^a	6.9 ± 0.7 ^a	6.9 ± 0.7 ^a	6.9 ± 0.8 ^a
*CEG supplement Diet (g/mouse/day)	0.0	0.033	0.067	0.167

The weight and diet were measured regularly for the 4 groups allocated as vehicle, CEG-1X, CEG-2X, and CEG-5X. All the data are represented as mean ± SD and analyzed by one-way ANOVA. Values in the same row with different superscript letters (a, b) differ significantly, $P < 0.05$. (*) CEG supplement was calculated as animal averaged weight as 40 grams. BW, body weight.

Table 3. Effects of CEG on body compositions

Characteristic	Vehicle	CEG-1X	CEG-2X	CEG-5X
Liver (g)	2.38 ± 0.15	2.4 ± 0.17	2.43 ± 0.13	2.45 ± 0.23
Muscle (g)	0.36 ± 0.02 ^a	0.37 ± 0.03 ^a	0.39 ± 0.02 ^b	0.39 ± 0.02 ^b
Kidney (g)	0.62 ± 0.03 ^a	0.62 ± 0.04 ^a	0.65 ± 0.04 ^{a, b}	0.66 ± 0.03 ^b
Heart (g)	0.24 ± 0.03 ^a	0.28 ± 0.02 ^b	0.28 ± 0.03 ^b	0.29 ± 0.03 ^b
Lung (g)	0.24 ± 0.01 ^a	0.25 ± 0.01 ^{a, b}	0.26 ± 0.04 ^{a, b}	0.27 ± 0.04 ^b
EFP (g)	0.38 ± 0.08 ^a	0.33 ± 0.10 ^{a, b}	0.31 ± 0.05 ^{a, b}	0.30 ± 0.09 ^b
BAT (g)	0.12 ± 0.02 ^a	0.15 ± 0.01 ^b	0.15 ± 0.02 ^b	0.16 ± 0.02 ^b
Relative liver weight (%)	6.05 ± 0.25	6.02 ± 0.17	6.09 ± 0.12	6.17 ± 0.46
Relative muscle weight (%)	0.90 ± 0.04 ^a	0.92 ± 0.04 ^a	0.99 ± 0.02 ^b	0.99 ± 0.02 ^b
Relative kidney weight (%)	1.58 ± 0.05 ^a	1.55 ± 0.05 ^a	1.63 ± 0.04 ^b	1.66 ± 0.06 ^b
Relative heart weight (%)	0.61 ± 0.06 ^a	0.7 ± 0.04 ^b	0.69 ± 0.05 ^b	0.72 ± 0.06 ^b
Relative lung weight (%)	0.60 ± 0.02 ^a	0.62 ± 0.02 ^{a, b}	0.64 ± 0.08 ^{a, b}	0.68 ± 0.09 ^b
Relative EFP weight (%)	0.96 ± 0.19 ^b	0.83 ± 0.22 ^{a, b}	0.77 ± 0.09 ^a	0.75 ± 0.22 ^a
Relative BAT weight (%)	2.01 ± 0.26 ^a	2.44 ± 0.17 ^b	2.39 ± 0.34 ^b	2.5 ± 0.21 ^b

Data are mean ± SD for n = 10 mice in each group. Data in the same row with different letters (a, b) differ significantly at $P < 0.05$ by one-way ANOVA; EFP: epididymal fat pad; BAT: brown adipose tissue.

safety of the supplementation. Several indexes including behavior, diet, growth curve, organ weight, biochemical assessments and histopathology were evaluated for the subacute toxic effects of CEG supplementation. Behavior was monitored with daily CEG administration and no abnormal behavior was seen among the groups. As shown in Table 2, the treatment main effect did not show a significant difference ($F(3,36) = 0.07$, $P = 0.976$) but the time main effect showed significant difference ($F(3.33,199.9) = 725.5$, $P < 0.0001$) which indicated time-dependent growth. However, the interaction effect (supplement × time) was not signifi-

cantly different ($F(10,199.9) = 1.26$, $P = 0.261$) and further analysis at different time points by one way ANOVA showed no significant difference among groups. Diet and water intake also did not significantly differ among groups with dose-dependent CEG supplementation ($F(3,120) = 0.005$, $P = 0.999$; $F(3,120) = 0.026$, $P = 0.994$).

Body composition may also reflect the effect of supplementation on different organs. As shown in Table 3, the muscle weight was significantly different among groups ($F(3,36)=6.96$, $P = 0.001$) and the CEG supplementation groups (CEG-2X and CEG-5X) were significantly higher than ve-

Table 4. The effects of CEG on clinical biochemical analysis at the end of the experiment

Parameter	Vehicle	CEG-1X	CEG-2X	CEG-5X
AST (U/L)	89 ± 15 ^b	80 ± 12 ^{a, b}	78 ± 14 ^{a, b}	77 ± 13 ^a
ALT (U/L)	68 ± 12 ^b	67 ± 10 ^b	55 ± 11 ^a	52 ± 10 ^a
NH ₃ (μmol/L)	199 ± 25 ^b	176 ± 9 ^{b, c}	159 ± 40 ^{a, c}	147 ± 27 ^a
CK (U/L)	319 ± 106	258 ± 137	243 ± 156	216 ± 93
GLU (mg/dL)	148 ± 8 ^c	137 ± 11 ^{a, b}	143 ± 10 ^{b, c}	130 ± 8 ^a
CREA (mg/dL)	0.21 ± 0.04 ^a	0.23 ± 0.04 ^a	0.34 ± 0.09 ^b	0.38 ± 0.07 ^b
BUN (mg/dL)	22.2 ± 2.4	22.0 ± 1.7	22.5 ± 1.5	22.1 ± 1.8
UA (mg/dL)	1.6 ± 0.3 ^b	1.1 ± 0.2 ^a	1.0 ± 0.2 ^a	1.0 ± 0.2 ^a
TC (mg/dL)	163 ± 9 ^c	142 ± 21 ^b	125 ± 20 ^a	118 ± 15 ^a
TG (mg/dL)	182 ± 21 ^b	179 ± 19 ^b	158 ± 25 ^a	155 ± 18 ^a
ALB (g/dL)	2.6 ± 0.1 ^a	2.9 ± 0.1 ^b	2.9 ± 0.1 ^b	3.0 ± 0.2 ^b
TP (g/dL)	4.9 ± 0.2 ^a	5.1 ± 0.1 ^{a, b}	5.1 ± 0.2 ^b	5.1 ± 0.2 ^b

Data are mean ± SD for n = 10 mice in each group. Values in the same row with different superscript letters (a, b, c) differ significantly, $P < 0.05$, by one-way ANOVA; AST, aspartate aminotransferase; ALT, alanine transaminase; NH₃, Ammonia; CK, Creatine kinase; GLU, glucose; CREA, Creatinine; BUN, blood urea nitrogen; UA, uric acid; TC, total cholesterol; TG, triacylglycerol; ALB, albumin; TP, total protein.

hicle group ($P < 0.05$). The EFP and BAT weight were significantly different among groups ($F(3,36) = 1.77$, $P = 0.045$ and $F(3,36) = 5.42$, $P = 0.003$, respectively). The EFP of the CEG-5X group was significantly lower than vehicle ($P < 0.05$) and the BAT was significantly higher in CEG groups than vehicle group ($P < 0.05$). The relative organ weight adjusted by individual weight showed similar statistical results which also demonstrated that the CEG supplementation could increase the lean mass and BAT and decrease the EFP weight in the current study.

Clinical biochemistry was also used to evaluate the effects of supplementation on physiological status. As shown in Table 4, the indexes were relevant to the hepatic function, blood lipid, renal function, injury and metabolites index applied to the current biochemical assessment. The indices of hepatic function, AST, and ALT, were significantly different among the groups ($F(3,36) = 1.93$, $P = 0.042$ and $F(3,36) = 5.88$, $P = 0.002$, respectively) and the higher CEG doses (CEG-5X) could significantly decrease the AST and ALT levels as compared to the vehicle group ($P < 0.05$). The blood lipid indexes, TC and TG, were significantly different ($F(3,36) = 14.02$, $P < 0.0001$ and $F(3,36) = 4.4$, $P = 0.01$, respectively) and the CEG supplementation could significantly decrease those indexes as compared to vehicle ($P < 0.05$). For the renal function index, the CREA showed a significant difference ($F(3,36) = 16.41$, $P < 0.0001$) and there was not a significant difference in the BUN index ($F(3,36) = 0.132$, $P =$

0.94). The CEG higher dose (CEG-2X and CEG-5X) administration could significantly increase the CREA levels as compared to vehicle group ($P < 0.05$). As shown in Fig. 6, the pathological results did not show abnormalities attributed to CEG supplementation as observed by a clinical veterinarian.

Discussion

In the current study, we used combined *G. lucidum* and 'essence of chicken' (CEG) supplementation to investigate whether it could improve the exercise fatigue recovery and performance. The physical activities, endurance capacities and grip strength significantly increased with CEG supplementation without training intervention. The biochemical variables including the lactate, BUN, and CK indexes showed beneficial effects with CEG supplementation after intervention of exercise challenges. Furthermore, we found that CEG supplementation could not only increase the physiological adaption but also improve the exercise performance possibly *via* glycogen content increase in the liver and muscle. The subacute oral toxicity of CEG was also evaluated for safety and the results demonstrated beneficial effects on the body composition and biochemistry in a reasonable range.

The treadmill and swimming methods have been widely applied to assess exercise endurance capacity (7, 17). However, the treadmill is generally equipped with an electric shock stimulator to enforce continued exercise. The shock apparatus can

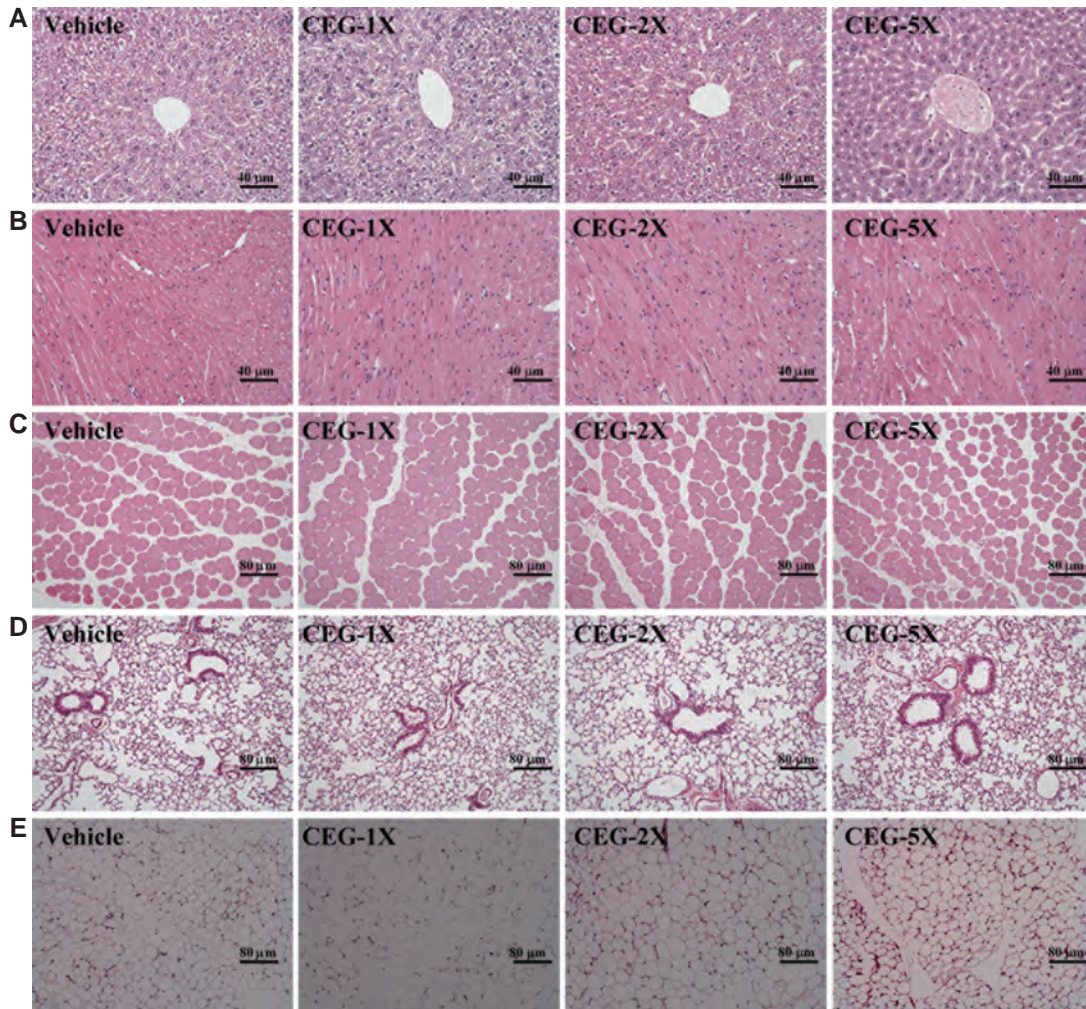


Fig. 6. Effect of CEG supplementation on histomorphologic features of the liver (A), heart (B), soleus muscle (C), lung (D) and adipocyte tissue (E) in mice. Specimens were photographed under a light microscope. (H&E stain, magnification: 200 \times ; bar, 40 or 80 μ m).

interfere with quantitation of running endurance, as well as confound measurements of post-exercise serum hormone and cytokine levels (11). The swimming test (21) could ensure the higher differential capacity with stronger survival instinct as compared to the treadmill methods (16). Therefore, in the current study we chose to assess endurance with CEG supplementation using the swimming method. CEG supplementation in the current study showed increased endurance capacity as compared to a previous CE treatment study (18). Grip strength can potentiate initial motor activity and be related to neuromuscular admittance (26). In a previous report, grip strength was found to be strongly associated with gender and weight in a young population (29). CEG may have positive effects on motor control for better strength performance according to

the current results (Fig. 3).

With regard to exercise-related biochemistry, lactate, was positively correlated with exercise duration and intensity, resulting in the release of hydrogen ions. Increased hydrogen ions cause acidity, which affect physiological metabolism and calcium release to muscular contractions (6), and eventually contribute to the sensation of fatigue. Lactate can also be metabolized as energy and it can be accumulated when the production rate exceeds the clearance rate. In the current study, CEG could decrease the production rate and increase the clearance rate significantly (Table 1). Sufficient energy is critical to physiological maintenance and the energy molecule (ATP, ADP, and AMP) is under homeostasis under normal conditions. ATP is consumed by exercise demand and ADP is converted to AMP for

ATP replenishment. AMP can be further metabolized to IMP and ammonia by deaminase and the ammonia could be metabolized as BUN *via* the urea cycle. Therefore, BUN can be considered to be a biomarker for ATP metabolism (14). In a previous study, CE supplementation improved the lactate and ammonia levels in the recovery phase after exhaustive exercise (24). CEG could keep the efficient energy supplement so the BUN index could be ameliorated with dose-dependence in current study (Fig. 4A).

Oxidative stress, such as reactive oxygen species and free radicals, induced by prolonged/exhaustive exercise can injure the cell membrane permeability and lactic dehydrogenase (LDH) and CK can leak out into the blood and act as damage markers (19, 33). The anti-oxidation capacity of CE active peptides and the total triterpene fraction isolated from *G. lucidum* have been reported (34, 40). In our study, CEG could significantly decrease the CK levels caused by exercise-induced oxidation (Fig. 4B). Glycogen is an important fuel during exercise from the point view of exercise physiology (39) and is considered as a carbohydrate energy store for ATP production. Intermyo-fibrillar, intramyofibrillar, and subsarcolemmal glycogen, about 75% of total body glycogen, is stored in muscular cells (27). The positive correlation between exercise endurance and glycogen content was revealed in the 1960s (2). In our previous study on CE supplementation, glycogen was significantly increased about 70% and 30% in liver and muscle, respectively (18). The muscular glycogen content with CEG dose-dependent supplementation was also significantly elevated more than 80%, which was more than with CE treatment only. *G. lucidum* has been reported to up-regulate glycogen synthesis-related genes (GS2 and GYG1) (38) and acted synergistically with CE to contribute to the increased glycogen content. The efficient glycogen elevation with CEG treatment may explain the higher endurance capacities than those found previously with CE.

G. lucidum has been reported to have potential toxicity and side effects in previous reports, including dizziness, dry nose and throat, headache, and skin irritation (12). Therefore, the subacute oral toxicity of CEG supplementation was assessed. CEG supplementation was not observed to cause abnormality in social behavior, appearance, or diet. With respect to body composition, our previous report showed that CE treatment did not result in a significant difference, but in the current study CEG supplementation caused a significant difference in muscle, fat, and BAT tissues. CE combined with green tea showed a positive clinical effect by reduc-

ing body fat (37) and *G. lucidum* has been reported to up-regulate lipid metabolism-related genes (38). Fat composition and lipid biochemistry (TG and TC) was significantly improved possibly *via* the combination of the effects of CE and *G. lucidum*. Interestingly, brown adipocyte tissue (BAT) showed a significant increase with CEG supplementation which was different as compared to our previous CE treatment only study (18). Exercise might activate and recruit BAT through the activation of the sympathetic nervous system, heart and skeletal muscle (30). The possible effects of CEG on BAT activation relevant to exercise physiology will be worth further investigation. The effect of CE treatment on biochemistry was reported in our previous study (21). However, we found the effect of CEG on several indexes were different than in the previous CE study. The liver indexes, AST and ALT, showed beneficial effects after supplementation *G. lucidum* (42). It is rather remarkable that consecutive CEG supplementation resulted in significantly higher CREA index with a dose-dependence trend, which is consistent with a previous study. The CEG supplementation may therefore pose a potential risk for specific populations of patients, such as those with nephropathy.

Conclusions

CE is widely acceptable as a nutritional supplement. From the perspective of dietary therapy and nourishment, combinations of different functional or medical food are being investigated and developed for better efficacy. However, evidence-based study is still needed to investigate the efficacy and possible toxicity. In this study, we demonstrated that *G. lucidum* combined with CE could synergistically improve the exercise fatigue recovery and exercise performance within a healthy population of mice.

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Competing Interests

The authors declare no conflict of interest.

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