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Navigating Anti-Fatigue Effect: Aqueous Extract of *Cistanche tubulosa* plus QH *in Vitro* and *in Vivo* Study

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Abstract

The effects of coenzyme Q10 (CoQ10) on exercise-induced fatigue remain unclear, as previous studies have reported inconsistent results. To address fatigue associated with physiological stress, the combined use of *Cistanche tubulosa* aqueous extract (CTE) and ubiquinol (QH) has increasingly been reported to provide antioxidant benefits and support mitochondrial energy production, including adenosine triphosphate (ATP) synthesis; however, the underlying mechanisms remain to be fully elucidated. This study aimed to evaluate the potential synergistic effects of QH in combination with CTE on fatigue reduction and performance enhancement under physiological challenge. The CTE100 plus QH group demonstrated significant improvements in forelimb grip strength and exhaustive swimming time compared with QH alone, indicating enhanced physical performance. In the mechanistic assessment of anti-fatigue activity, the CTE100 plus QH group showed reduced serum levels of lactate, ammonia, and creatine kinase (CK) after acute exercise, as well as increased concentrations of free fatty acids (FFA). Although these differences were not statistically significant relative to the QH-only group, the results demonstrated a consistent downward trend in acute fatigue-related biomarkers. To further investigate the metabolic regulatory ef-

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fects of CTE plus QH, a TNF-α-induced C2C12 cell injury model was employed. Cellular metabolism was assessed by measuring mitochondrial oxygen consumption rate (OCR) using the Seahorse XF24 extracellular flux analyzer. The results showed that the combination of CTE and QH enhanced ATP production and maximal respiratory capacity compared with QH alone. Overall, these findings suggest that the anti-fatigue effects observed in animal studies may be partially attributable to the ability of CTE to enhance mitochondrial respiratory oxygen consumption.

Subject Areas

Food Science, Technology

Keywords

Ubiquinol, *Cistanche tubulosa*, Anti-Fatigue, Exercise Performance, C2C12, OCR

1. Introduction

During exercise, the blood and oxygen supply and circulation, as well as normal cellular biochemical metabolic pathways, play a crucial role in bodily activity. Particularly in aerobic forms of exercise, the demand for oxygen by bodily tissues is significantly higher than at rest, with oxygen consumption during activity potentially increasing by 10 to 15 times. However, vigorous or eccentric exercise can lead to damage to muscle cells, resulting in inflammation and the generation of harmful substances, particularly an excess of free radicals, including reactive oxygen species (ROS) and reactive nitrogen species (RNS). When these substances accumulate excessively and disrupt the body's natural balance, if the body's antioxidant system fails to counteract the attack of free radicals, oxidative stress can rise, causing damage to cellular tissues and affecting exercise performance [1] [2]. Therefore, the continuous efforts and focus on the field of sports science have been on how to mitigate the fatigue and decline in exercise performance caused by inflammation and oxidative damage induced by exercise through the supplementation of antioxidant substances or supplements. It is hoped that by supplementing with food factors contained in the diet, the oxidative balance in the body during exercise can be maintained or balanced, thereby preventing or reducing the occurrence of tissue damage and achieving improvements in exercise performance [1]-[3].

Fatigue refers to a loss of normal levels of activity or exercise performance, while fatigue sensation is a subjective feeling, usually associated with tissue damage and energy shortage [4]. Recent studies suggest that in today's busy lifestyles and stress often leads to states of fatigue, which may endanger health [5]. Fatigue or the sensation of fatigue occurs after enduring challenges from exercise or other types of stress, and the duration of recovery may vary depending on different chal-

lenge patterns and interventions with natural supplements. Therefore, after intervention with health foods, if it can: 1) accelerate the recovery of exercise performance or enhance the ability to withstand exercise challenges; 2) reduce physiological stress levels during recovery; 3) accelerate the recovery of tissue damage; 4) accelerate the recovery of muscle tissue energy storage or increase the amount of energy stored in muscle tissue; or 5) expedite the elimination of fatigue and pain sensation, it indicates that the health food has a considerable degree of anti-fatigue effect.

Coenzyme Q10 (also known as Ubiquinone or CoQ10) is a lipid-soluble nutrient that resembles a vitamin and is naturally synthesized within the body, serving as an endogenous antioxidant [6]. In the mitochondria, CoQ10 is a crucial component of the mitochondrial respiratory chain, utilized in the synthesis of adenosine triphosphate (ATP) [6] [7]. Its two primary functions are energy production and antioxidation. CoQ10 participates in aerobic cellular respiration and acts as a cofactor in the mitochondrial electron transport chain, thus playing a vital role in ATP production. CoQ10 is primarily synthesized and concentrated in tissues and organs with high metabolic rates or energy demands, such as the heart, kidneys, liver, and muscles [8]. There is increasing emphasis on the application of CoQ10 as an antioxidant for the prevention and treatment of various functional diseases like fatigue.

CoQ10 has two main drawbacks. Firstly, it is a relatively large hydrophilic molecule, leading to slow and limited absorption [9]. Secondly, ubiquinol (QH) is the reduced active antioxidant form of CoQ10 [10]. Although CoQ10 typically undergoes enzymatic reduction to ubiquinol (QH) after dietary intake, research suggests that this process becomes increasingly challenging with age [11] [12]. Our previous study showed QH has anti-fatigue effects and promotes exercise performance.

The dried stem of *Cistanche tubulosa* (Schrenk) R. Wight, Rou Cong Rong, is widely harvested in the Xhinjang, China desert. It is an important traditional Chinese medicine that belongs to tonic Chinese medicine indexed in the China Pharmacopedia and used for thousands of years for the treatment of physical weakness, kidney deficiency, infertility, forgetfulness, impotence and senile constipation [13] [14]. *C. tubulosa* aqueous extract (CTE) capsules have been approved as a botanical drug for vascular dementia in China. According to a clinical trial of 18 patients diagnosed with mild to moderate AD, CTE capsules gave patients a more stable memory condition compared to acetyl cholinesterase inhibitors [15]. In our previous study, the CTE decreased Amyloid β peptide (Aβ) deposition and improved memory loss in Alzheimer's disease-like rats [16]. Recent research indicates that CTE possesses significant antioxidant activity *in vitro* and exerts anti-fatigue effects as well [17] [18].

This study aims to investigate the anti-fatigue activity of CTE combined with QH using a mouse weight-bearing swimming model, including assessments of endurance, creatine kinase levels, blood lactate concentration, blood ammonia lev-

els, and blood glucose levels. Furthermore, TNFα-induced C2C12 cell injury and Seahorse XF24 extracellular flux analysis were employed to evaluate CTE plus QH-mediated metabolic regulation.

2. Materials and Methods

Cistanche tubulosa Aqueous Extract (CTE) and Ubiquinol (QH)

The stem of *C. tubulosa* were cut into one cubic centimeter cubes and extracted by refluxing with water and then filtered to collect filtrate. The filtrate was concentrated. Following the addition of ethanol to the concentrate, the supernatant is collected. The filtrate was eluted and collected and subsequently subjected to resin separation using macro-porous absorption resin; then spray-dried to obtain aqueous extract (CTE) at 10% yield. This extract is produced by Sinphar Tian-Li Pharmaceutical Co, Ltd, Hangzhou, Sinphar Group, China. The mixture of CTE plus QH and QH were provided by Sinphar Pharmaceutical Co, Ltd, Taiwan.

Animals

This study utilized forty 8-week-old male Institute of Cancer Research (ICR) mice bred under specific pathogen-free (SPF) conditions. All mice were provided ad libitum access to a standard commercial chow diet (No. 5001, PMI Nutrition International, Brentwood, MO) and distilled water. Mice were housed under standard laboratory conditions with a 12-hour light-dark cycle, maintained at a constant ambient temperature of 22 ± 2˚C, and relative humidity ranging from 50% to 60%. Bedding and water bottles were changed and cleaned twice weekly. All experimental procedures were conducted following the approved protocol by the Institutional Animal Care and Use Committee (IACUC) ethics committee of the National Taiwan Sport University, adhering to the guidelines of protocol IACUC-10516. After a one-week acclimatization, the mice were randomly grouped according to different doses and used for experiments. Thirty mice were used in this experiment and randomly divided into five groups of 4 rats each for orally given different complexes containing *C. tubulosa* aqueous extract (CTE) plus ubiquinol (QH) or ubiquinol (QH) for 14 days. The groups received the following treatments: Control Group; QH Group (6.15 mg/kg) (human dose: 30 mg QH); CTE50 + QH Group ((10.25 mg CTE + 6.15 mg QH) /kg) (human dose: 50 mg CTE + 30 mg QH); CTE100 + QH Group ((20.5 mg CTE + 6.15 mg QH)/kg) (human dose: 100 mg CTE + 30 mg QH). The daily dose for each mouse was derived by converting the human daily intake of one capsule. After 2 weeks of administration, endurance exercise protocols and blood analysis were conducted to assess the anti-fatigue effects.

Exhaustive swimming exercise

Endurance performance serves as a vital measure in assessing anti-fatigue effects, gauged through an exhaustive swimming test. On the 14th day of the study, mice received oral administration followed by a 30-minute period before being placed individually in a cylindrical swimming pool. The pool, 65 cm in height with a 20 cm radius, contained fresh water maintained at 28˚C ± 1˚C, with a depth of

approximately 40 cm. Each mouse carried a lead block attached to its tail, weighing around 5% of its body weight. Endurance performance was quantified by the swimming duration, measured from the onset until exhaustion. Exhaustion was determined by the presence of uncoordinated movements and the inability to reach the surface within a 7-second timeframe [19]. The exhaustive swimming duration served as a metric for exercise endurance.

Forelimb grip strength

The forelimb grip strength was assessed using a grip strength meter (Model-RX-5; Aikoh Engineering, Nagoya, Japan). This device features a metal bar (2 mm in diameter, 7.5 cm long) onto which the mouse grasps, with the tensile force being captured by a digital force transducer. The methodology for this assessment was detailed in our prior research [20]. Following the administration of different formulas, the forelimb grip strength test was conducted. Each mouse underwent 10 consecutive trials, with the highest recorded value from each trial being noted using the attached force gauge. The mean maximal force measured in grams via this low-force system represented the forelimb grip strength.

The 15-min Free Swimming Test with Serum Biochemical Measurements

The blood sampling time points were pre-exercise, after 15 min of the swimming exercise, and following 20 min of rest. Serum samples were collected for analysis of serum lactate levels, serum glucose, creatine kinase (CK), ammonia (NH3) and free fatty acids (FFA) after centrifugation at 1000× g for 15 min at 4 ˚C and were analyzed by an autoanalyzer (Hitachi 7060, Hitachi, Tokyo, Japan).

Cell culture

C2C12 cells (purchased from ATCC) were counted and seeded into Seahorse XF24 cell culture plates at a density of 1×10^4 cells each well. The C2C12 cells were cultured in H-DMEM (purchased from Sigma) supplemented with 10% FBS (purchased from BI) and incubated until they reached 80% confluence. Afterward, the medium was replaced with fresh H-DMEM, and 0.4 μL (500-fold dilution) of 15 different test samples (**Table 1**) was added. Six hours later, the cells were switched to H-DMEM + 2% HS differentiation medium containing 10 ng/mL TNF-α (1000-fold dilution), and 0.4 μL of different test samples were added. The cells were cultured in differentiation medium, and the medium was replaced the next day with the test samples added. After 4 days of culture, the cells showed differentiation.

Energy metabolism Measurements

Tumor necrosis factor-α (TNFα) inhibits the survival, proliferation, and differentiation of myoblasts and serves as a cellular model for studying the protective effects of drugs and nutrients on muscle function [21]. The TNFα-induced myoblast cell injury model is a valuable tool for cellular-level anti-fatigue research [21]. In this study, we employed this model to investigate the cellular metabolic regulatory effects of different ratios of CTE plus QH.

Cellular metabolism was assessed using mitochondrial oxygen consumption

Table 1. 15 different ratios of CTE and QH test samples.

Sample	CTE (mg)	QH (mg)	ratio
1	50	300	1:0.17
2	50	150	1:0.33
3	50	75	1:0.67
4	30	30	1:1
5	50	30	1:1.6
6	100	30	1:3.3
7	200	30	1:6.6
8	300	30	1:10
9	0	30	
10	0	75	
11	0	150	
12	0	300	
13	50	0	
14	100	0	
15	200	0	

rate (OCR) analysis, measured with the Seahorse XF24 Extracellular Flux Analyzer. The Seahorse XF24 Extracellular Flux Analyzer is widely used for assessing energy metabolism in cells by measuring key metabolic parameters [22]. These measurements are based on real-time monitoring of cellular oxygen consumption rate (OCR), including baseline respiration, ATP- associated oxygen consumption, and maximal respiration, which provides insights into mitochondrial respiration. Using the XF24 analyzer, the dynamic flux of metabolic processes can be measured in a high-throughput manner, offering a detailed understanding of cellular energy production, metabolism, and stress responses.

Statistical analysis

Data were expressed as the mean ± SEM. All the statistical analyses were performed using SAS 9.4 (SAS Inst., Cary, NC, USA) based on one-way analysis of variance (ANOVA). The Cochran-Armitage test was used for the dose-effect trend analysis. The level of statistical significance was set at $P < 0.05$.

3. Results

Effects of Mixture of *C. tubulosa* Aqueous extract (CTE) plus Ubiquinol (QH) and QH on Body Weight

During the experiment, the initial and final weights of each group of animals are shown in Figure 1. As depicted in Figure 1(a), before the start of the trial, the initial weights of the control group (Con) and the three experimental groups QH, CTE50 + QH, and CTE100 + QH were 31.5 ± 0.3, 31.3 ± 0.5, 32.3 ± 0.5, and 31.5

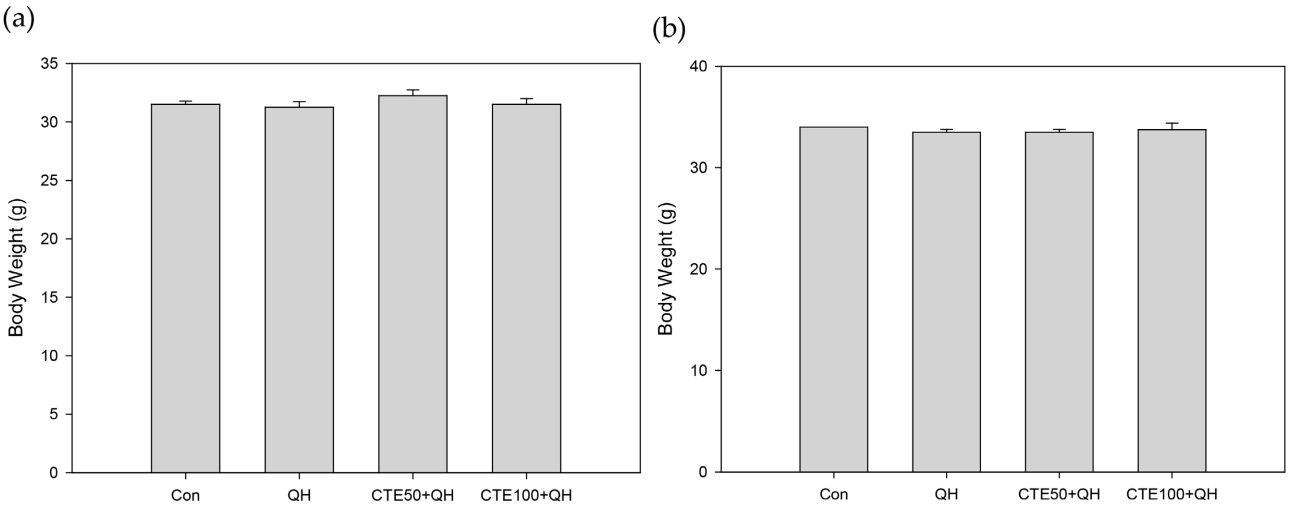

Figure 1. The change in initial weight (a) and the weights of each group after feeding different mixtures of CTE plus QH and QH for 14 days (b) in the experiment. Data are the mean ± SEM for n = 4 mice in each group.

± 0.5 (g), respectively, with no significant differences observed among groups. After 14 days of feeding with different QH formulations (**Figure 1(b)**), the final weights of the control group (Con) and the three experimental groups QH, CTE50 + QH, and CTE100 + QH were 34.0, 33.5 ± 0.3, 33.5 ± 0.3, and 33.8 ± 0.6 (g), respectively, with no significant differences observed among groups. Throughout the experimental period, the weights of the three groups of animals steadily increased, indicating that the supplementation with the three different mixture of CTE plus QH and QH for 14 days did not cause any adverse effects on animal growth.

Effects of Mixture of *C. tubulosa* Aqueous extract (CTE) plus Ubiquinol (QH) and QH on Endurance Capacity in the Exhaustive Swimming Test

In the results section of the exhaustive swimming performance under load, as shown in **Figure 2**, the exhaustive times (5% body weight load) for the Control, QH, CTE50 + QH, CTE100 + QH groups were 4.2 ± 0.3, 7.1 ± 0.5, 9.1 ± 2.3, and 10.1 ± 1.4 minutes, respectively. Compared to the Control group, the QH and CTE100 + QH groups both showed significant improvements. Although there is no statistically significant difference between CTE50 + QH group and the Control group, there is an increasing trend. Furthermore, CTE100 + QH showed a significant increase of 1.42-fold compared to the QH group. Therefore, supplementation with the CTE significantly enhances exhaustive swimming performance under load.

Effects of Mixture of *C. tubulosa* Aqueous extract (CTE) plus Ubiquinol (QH) and QH on Grip Strength

To assess and compare the potential muscle strength-enhancing effects of different complex of CTE plus QH and QH, after supplementation over 14 days, grip strength tests were conducted on four groups of mice, and their respective performances were recorded. As shown in **Figure 3(a)**, the grip strength values for the Control, QH, CTE50 + QH, CTE100 + QH groups were 137.5 ± 0.6, 160.3 ±

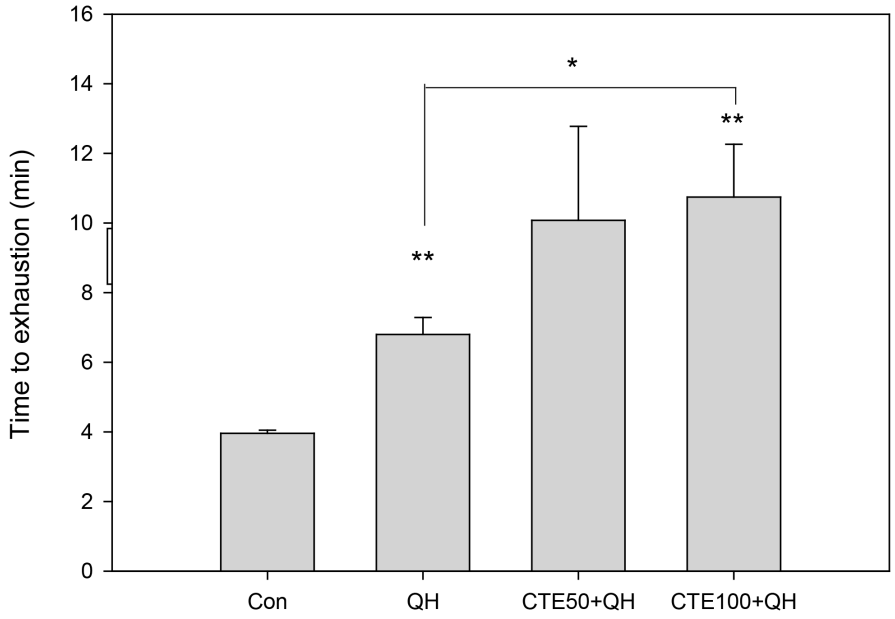

Figure 2. Effect of 14 days of different mixtures of CTE plus QH and QH on exhaustive swimming test. Data are the mean ± SEM for n = 4 mice in each group. $^{**}P < 0.01$ compared with Control group. $^{*}P < 0.05$ compared between CTE100 + QH group and QH group.

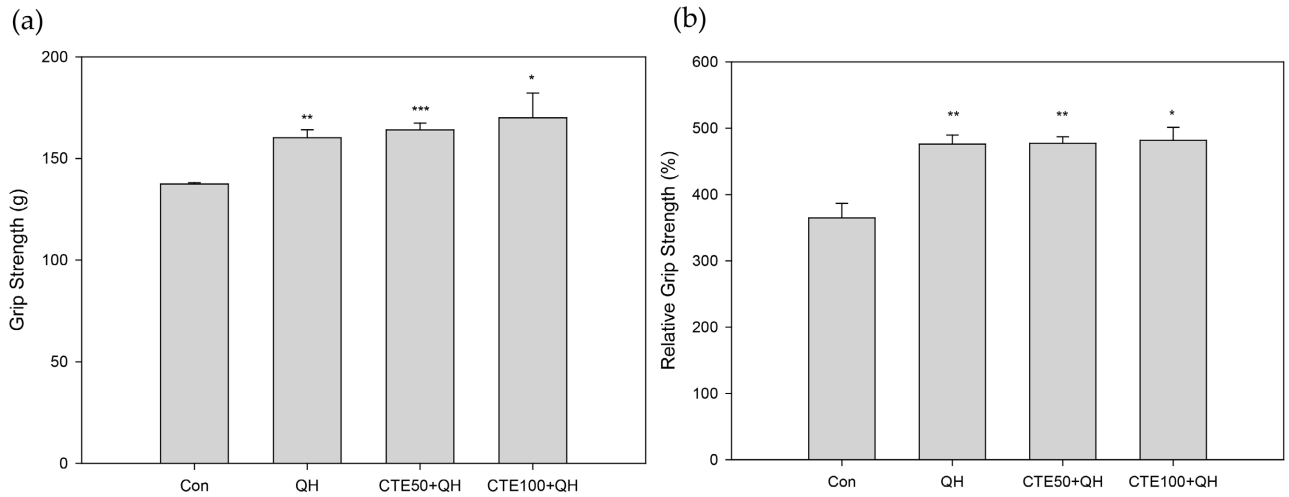

Figure 3. Effect of 14 days of different complex of CTE plus QH and QH on (a) forelimb grip strength; (b) relative grip strength (%) Data are the .mean ± SEM for n = 4 mice in each group. $^{*}P < 0.05$, $^{**}P < 0.01$, and $^{***}P < 0.001$ compared with Control group.

3.9, 164.0 ± 3.4, and 170.0 ± 12.2 grams (g). Compared to the Control group, supplementation with the QH, CTE50 + QH, CTE100 + QH groups significantly increased grip strength, respectively.

Since grip strength performance may be influenced by individual differences in body weight, this study measured absolute grip strength and also calculated the relative grip strength (%) to mitigate the impact of body weight. As depicted in **Figure 3(b)**, the relative grip strength (%) for the Control, QH, CTE50 + QH, CTE100 + QH groups were 364.5 ± 22.1, 476.0 ± 13.5, 477.0 ± 10.1, and 481.8 ± 19.4 (%), respectively. Compared to the Control group, supplementation with the

QH, CTE50 + QH, CTE100 + QH groups significantly enhanced relative grip strength.

Effects of Mixture of *C. tubulosa* Aqueous extract (CTE) plus Ubiquinol (QH) and QH on Lactate, ammonia (NH_3), free fatty acids (FFA), serum glucose, and creatine kinase (CK) after a 15-min Swimming Test

As shown in Figure 4(a), following a single 15-minute swimming test, the blood lactate concentrations for the Control, QH, CTE50 + QH, CTE100 + QH groups were 10.0 ± 1.0, 7.0 ± 0.3, 6.7 ± 0.5, and 6.3 ± 0.4 mmol/L, respectively. Compared to the Control group, supplementation with the QH, CTE50 + QH, CTE100 + QH groups significantly reduced blood lactate concentrations respectively. This indicates that supplementation with these three different formulas effectively lowered blood lactate concentrations following exercise. Although there is no statistically significant difference between the QH, CTE50 + QH, CTE100 + QH groups, there is a decreasing trend.

As shown in Figure 4(b), following a single 15-minute swimming test, the blood am-monia concentrations for the Control, QH, CTE50 + QH, CTE100 + QH groups were 218.5 ± 18.6, 134.8 ± 11.1, 130.0 ± 4.9, and 122.5 ± 5.6 μmol/L, respectively. Compared to the Con-trol group, supplementation with the QH, CTE50 + QH, CTE100 + QH groups significantly reduced blood ammonia concentrations respectively. This indicates that supplementation with these four different formulas effectively lowered blood ammonia concentrations following exercise. Although there is no statistically significant difference between the QH, CTE50 + QH, CTE100 + QH groups, there is a decreasing trend.

As shown in Figure 4(c), following a single 15-minute swimming challenge, the concentrations of free fatty acids in the blood for the Control, QH, CTE50 + QH, CTE100 + QH groups were 11.1 ± 1.0, 13.2 ± 0.4, 13.1 ± 0.1, and 13.8 ± 0.1 μM, respectively. Compared to the Control group, only supplementation with CTE100 + QH group significantly increased blood free fatty acid concentration.

As shown in Figure 4(d), following a single 15-minute swimming challenge, the blood glucose concentrations for the Control, QH, CTE50 + QH, CTE100 + QH groups were 132.3 ± 5.0, 173 ± 6.6, 163 ± 6.0, 176 ± 18, and 185.8 ± 6.0 mg/dL, respectively. Compared to the Control group, supplementation with the QH, CTE50 + QH, CTE100 + QH groups significantly increased blood glucose concentrations, respectively. There was no significant difference observed between the QH, CTE50 + QH, CTE100 + QH groups and there is an increasing trend.

As shown in Figure 4(e), following a single 15-minute swimming challenge, the CK (creatine kinase) activity for the Control, QH, CTE50 + QH, CTE100 + QH groups were 1954 ± 270.9, 843.8 ± 76.3, 864,5 ± 85.9, and 757.3 ± 170.1 U/L, respectively. Compared to the Control group, supplementation with the QH, CTE50 + QH, CTE100 + QH groups significantly decreased CK activity, respectively. There were no significant differences in CK activity among the QH, CTE50 + QH, CTE100 + QH groups and there is a decreasing trend.

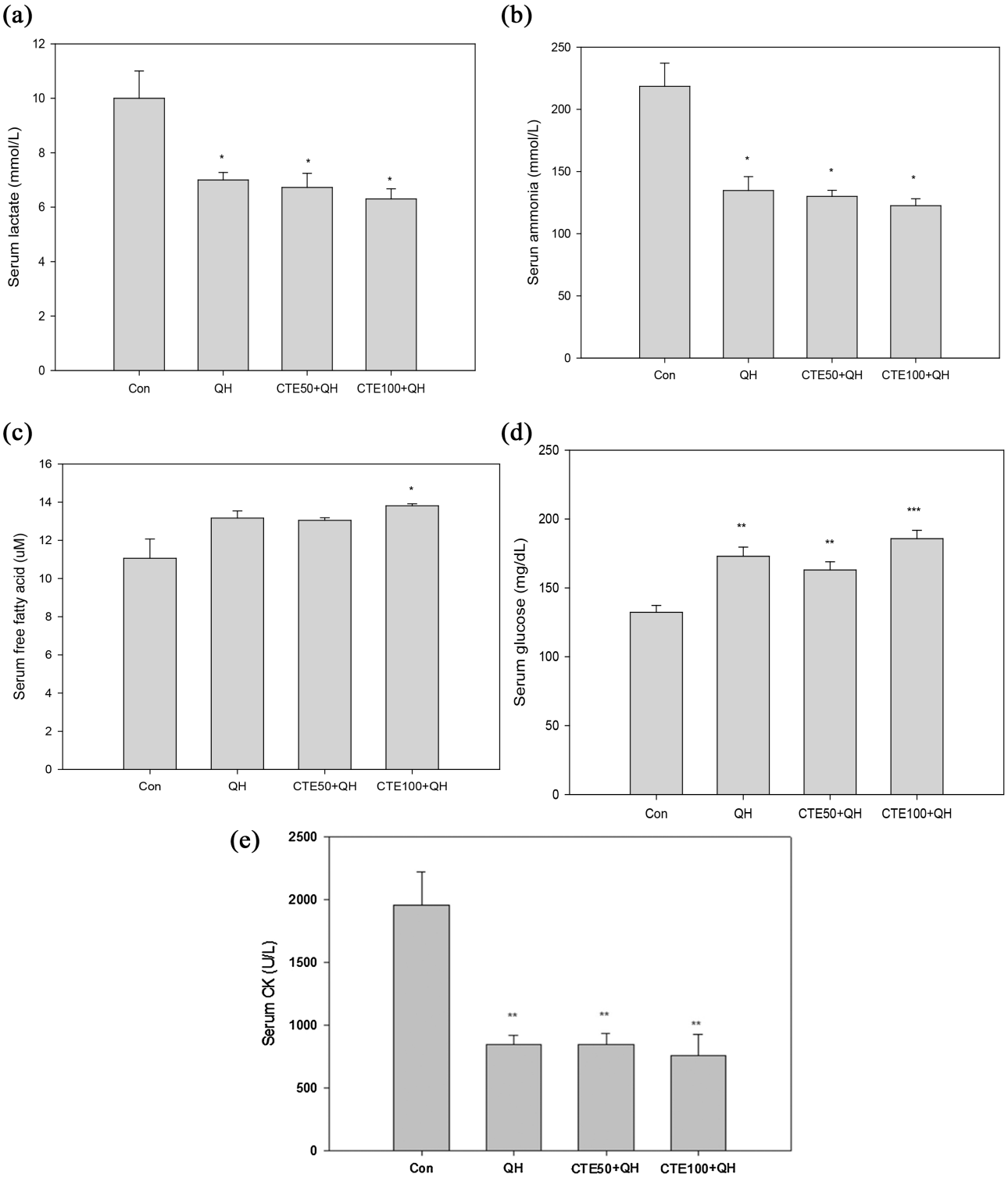

Figure 4. Effects of different mixtures of CTE plus QH and QH on (a) Lactate, (b) ammonia (NH_3), (c) free fatty acids (FFA), (d) serum glucose, and (e) creatine kinase (CK) after a 15-min swimming test. Data are the .mean ± SEM for n = 4 mice in each group. *$P < 0.05$, **$P < 0.01$, and ***$P < 0.001$ compared with Control group.

OCR analysis

Mitochondrial function is crucial for energy production and endurance capac-

ity [23]. In this study, we utilized OCR analysis with a TNF-α-induced C2C12 cell injury model to evaluate mitochondrial respiratory function [24] [25]. As shown in Figure 5, among the tested samples, sample 7 from significantly increased ATP production and maximal res-piratory oxygen consumption, while extract No. 8 enhanced maximal respiratory oxygen consumption. In contrast, extracts 9, 10, and 11 showed no significant effects. Notably, samples 14 and 15 significantly increased ATP production and maximal respiratory oxygen consumption. These OCR results suggest that samples 7, 14, and 15 can significantly improve mitochondrial respiratory capacity.

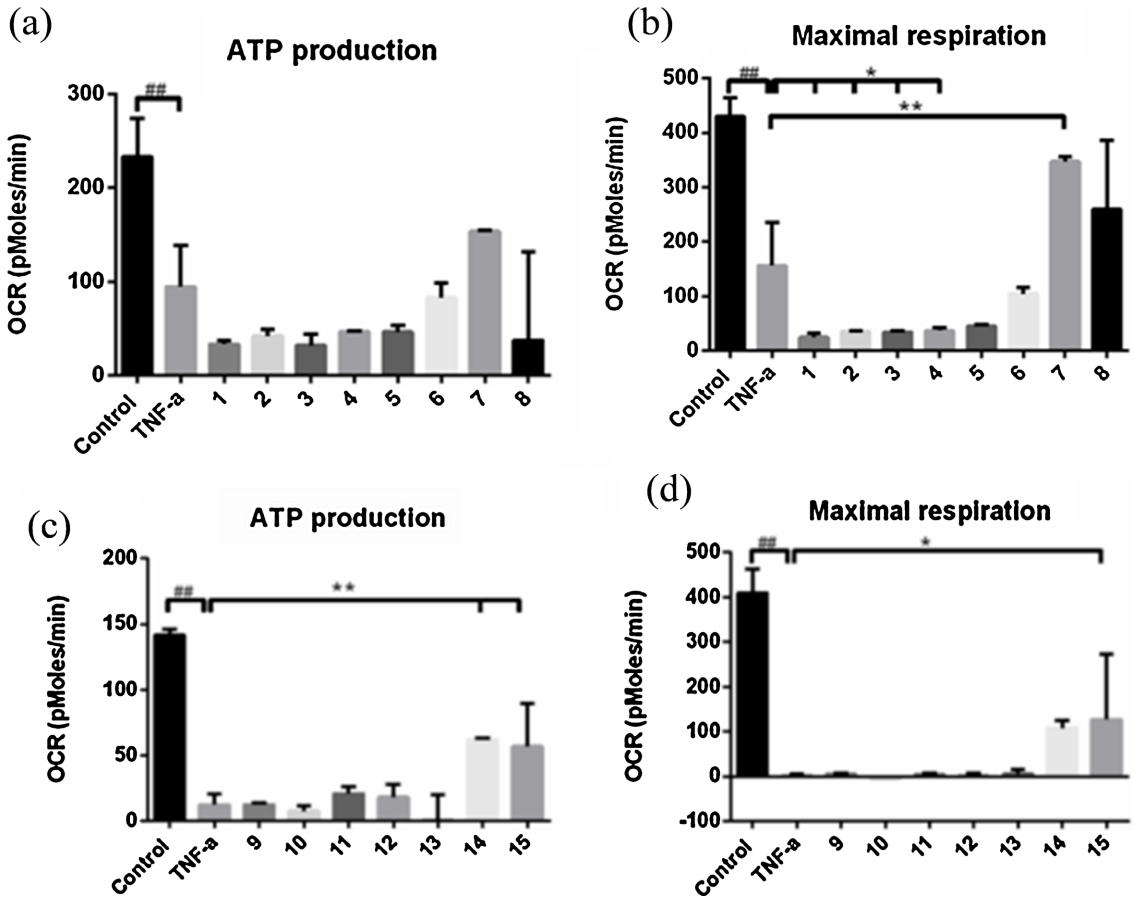

Figure 5. Effects of different mixtures of CTE plus QH (sample 1-15) on C2C12 cell line induced by TNF-α by OCR analysis. Data are the mean ± SD for n = 4. In all comparisons, the level of significance was set at $^{*}P < 0.05$, $^{**}P < 0.01$, and $^{\#\#}P < 0.01$.

4. Discussion

Ubiquinol (QH) plays a pivotal role in cellular energy production, exhibiting both energizing and activating physiological effects [26]. In our previous study, we observed a positive correlation between muscle strength and grip strength, and supplementation with QH was shown to enhance grip strength [27]. The swimming exhaustion test is widely used as an experimental model to evaluate physical fatigue. In earlier work, we demonstrated that QH supplementation significantly

prolonged endurance time in mice, highlighting its anti-fatigue potential [27].

Fatigue is a multifactorial physiological response resulting from prolonged physical exertion and is closely related to metabolic disturbances, mitochondrial dysfunction, and the accumulation of exercise-induced biochemical markers. In the present study, 14-day supplementation with a combination of *Cistanche tubulosa* aqueous extract (CTE100) and QH markedly improved endurance performance, as reflected by an increased time to exhaustion in the weighted swimming test. This enhancement suggests that the CTE100 + QH combination may offer greater fatigue-delaying potential compared to QH alone.

To further clarify the anti-fatigue mechanisms, biochemical markers associated with fatigue were analyzed. The CTE100 + QH group exhibited lower serum lactate, ammonia, and creatine kinase (CK) levels following acute exercise, indicating improved metabolic efficiency and reduced muscle damage. Although free fatty acid (FFA) levels were elevated, they did not differ significantly from those in the QH group, suggesting that the anti-fatigue effects of CTE100 + QH may not be driven solely by enhanced lipid metabolism. Instead, additional mechanisms—such as improved mitochondrial function—may be involved. It is important to note that some differences, while trending toward improvement, did not reach statistical significance, possibly due to limited statistical power.

Mitochondrial function is fundamental to energy production and endurance capacity [23]. In this study, mitochondrial respiratory function was assessed using an oxygen consumption rate (OCR) analysis in a TNF-α-induced C2C12 cell injury model [28]. The results demonstrated that supplementation with CTE100 + QH significantly enhanced ATP production-linked oxygen consumption and maximal respiratory capacity compared with QH alone. These findings indicate that CTE100 contributes to improved mitochondrial bioenergetics, thereby supporting its anti-fatigue effects. Enhanced mitochondrial respiration may facilitate more efficient ATP generation, reduce oxidative stress, and sustain muscle performance during prolonged exercise.

Nonetheless, this study has certain limitations. First, the relatively small sample size (n = 4 per group) used for ANOVA may have reduced statistical power, particularly for outcomes showing nonsignificant trends. Second, the OCR analyses conducted in cultured cell lines may not fully represent mitochondrial function *in vivo*, as they cannot capture total mitochondrial efficiency or capacity under physiological conditions.

Overall, the findings suggest that the anti-fatigue effects of CTE100 + QH observed in animal models may be closely related to its ability to enhance mitochondrial respiratory function. Future studies should further investigate the molecular mechanisms underlying these effects and explore the potential application of CTE100 + QH supplementation in human clinical trials.

5. Conclusions

The results of this study demonstrate that 14-day supplementation with a *Cistanche*

tubulosa aqueous extract (CTE100) and ubiquinol (QH) mixture significantly increased time to exhaustion in the weighted swimming test compared with QH alone. The CTE100 + QH group also exhibited reduced serum lactate, ammonia, and creatine kinase (CK) levels following acute exercise, alongside an increase in free fatty acid (FFA) concentrations, although the latter did not differ significantly from the QH group.

OCR analysis using a TNF-α-induced C2C12 cell injury model further showed that CTE100 + QH supplementation enhanced ATP production-linked oxygen consumption and maximal respiratory capacity relative to QH alone. These findings suggest that the anti-fatigue effects observed in animal studies may be attributable, at least in part, to the ability of CTE to enhance mitochondrial respiratory function.

Credit Authorship Contribution Statement

Conceptualization, M.-H.S.; methodology, C.-C.H. and J.-G.L.; formal analysis, A.-L.Y.; investigation, C.-J.W.; writing—original draft preparation, C.-L.C, C.-T. C, and Y.-T.W.; writing—review and editing, C.-J.W., F.-W.H., M.-H.S, and. J.-G.L. All authors have read and agreed to the published version of this manuscript.

Statement of Accordance with ARRIVE Guidelines

This study has been reported in accordance with the ARRIVE guidelines.

Ethical Approval

This study was approved by the Institutional Animal Care and Use Committee (IACUC) ethics committee of the National Taiwan Sport University.

Institutional Review Board Statement

All experimental procedures were conducted following the approved protocol by the Institutional Animal Care and Use Committee (IACUC) ethics committee of the National Taiwan Sport University, adhering to the guidelines of protocol IACUC-10402.

Acknowledgments

We are grateful to Sinphar Pharmaceutical group for providing the commercial natural product (*C. tubulosa* extract).

Data Availability

The data presented in this study are available on request from the corresponding authors. The data are not publicly available.

Conflicts of Interest

The authors declare no conflicts of interest.

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