Effects of Postexercise Supplementation of Chicken Essence on the Elimination of Exercise-Induced Plasma Lactate and Ammonia

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Abstract

We investigated the effects of chicken essence (CE) supplementation on exercise-induced changes of lactate and ammonia during recovery. In this randomized, double blind, crossover study, twelve healthy subjects performed a single bout of exercise to exhaustion, and then consumed either a placebo or CE within 5-min of the exercise cessation. Blood samples were collected before exercise, at exhaustion (0 minute), and 20, 40, 60, and 120 minutes, respectively during the recovery period. There were no differences in plasma glucose, creatine kinase, or heart rate responses between treatments. The exercise exhaustion significantly increased the levels of lactate and ammonia, and both measured values gradually declined during the recovery period. Ammonia levels at 40, 60, and 120 min. of the recovery period were observed lower significantly in the CE group, as compared to those in the placebo group. Additionally, lactate concentrations at 60 and 120 min were lower in the CE group, as compared to those in the placebo group. In conclusion, the main finding of this study was that CE supplementation after exercise reduces plasma lactate and ammonia levels. The results indicated that CE supplementation after an exhaustive exercise could enhance physiological recovery in humans.

Key Words: chicken essence, recovery, amino acid, fatigue, exercise

Introduction

Chicken essense (CE) is a commonly used nutritional supplement, mainly due to its protein or amino acid-enriched ingredients (5, 26). It increases metabolic rate (10), enhances iron restoration (30), reduces mental fatigue (19), attenuates the chronic development of cardiac hypertrophy and artheriosclerosis in rats (24), and increases colostrum levels of lactoferrin, epidermal growth factor and transforming growth factor-β2 in lactating women (6). However, no information related to the use of CE for postexercise recovery has been revealed.

An acute bout of exercise induces alteration in protein metabolism with a decrease in whole-body protein synthesis and an increase in whole-body protein and amino acid catabolism (9, 20). Under this condition, the working cell forms ammonia during the metabolism of amino acids, especially glutamine and glutamate. However, the toxic effect of ammonia toward living cells has also been widely recognized (16). Although, CE supplementation provides a sub-

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strate for ammonia production during metabolism towards a catabolic state, no study has been performed to assess the effects of CE ingestion on ammonia metabolism after postexercise recovery. The main purpose of the current study was to examine whether postexercise supplementation of CE affects the plasma level of ammonia during recovery following a single bout of exercise to exhaustion.

Materials and Methods

Subjects

Twelve healthy females, with a mean age (±SE) of 21.6±0.6 yrs, weight 54.0±2.2 kg, height of 160.6 ±1.2 cm, and maximum oxygen consumption ($\dot{V}O_{2max}$) of 39.5±0.9 ml/kg/min, participated in the experiment. The study conformed to the ethical guidelines of the National College of Physical Education and Sports (Taoyuan, Taiwan) and was approved by the Human Research Ethics Committee. All volunteers signed informed consent forms. In a pre-study interview, information on routine use of vitamins and other nutritional supplements was obtained for each participant. Volunteers found to be taking regular medication were excluded in the study.

Two weeks prior to the tests, subjects were required to cease vitamin and supplement intake. Subjects were instructed to avoid exercise or strenuous physical activity for 3 days prior to the tests. In the twenty-four hours preceding the study, subjects recorded all food and drink intake and this dietary pattern was duplicated in the second part of the cross over study. $\dot{V}O_{2max}$ of each subject was determined one week before the exhaustive exercise tests were conducted.

Experimental Design

In this randomized, double blind, crossover study, 12 subjects were randomly allocated into two treatment groups, placebo (PL) and chicken essence (CE). The CE group received a 140 ml solution of chicken essence (provided by Cerebos Taiwan, Ltd., Chang-Hua, Taiwan). The composition of the CE as determined by high performance liquid chromatography was shown in Table 1. The PL group received a 140 ml 0.5% gelatin with 0.3% caramel solution, which has similar appearance and flavor to CE and has been previously used (at a different concentration) by Nagai *et al*. (19).

All tests were conducted under laboratory conditions at 26.88±0.23°C and a relative humidity of 47.95±1.48 %. On arrival at the laboratory on the morning of the test day, the fasted subjects (for 8 hr prior to tests) were instructed to consume 300 ml of water. After resting for 20 min, baseline blood samples (pre-exercise) were taken.

Table 1. Composition of the chicken essence

Amino acids	% (w/v)	Peptides	μg/ml
Glutamic acid	0.935	carnosine	1238.5
Glycine	0.878		
Aspartic acid	0.519		
Alanine	0.519		
Histidine	0.460		
Arginine	0.435		
Lysine	0.331		
Leucine	0.324		

Exhaustive Exercise Test

Individual subjects were required to run on a treadmill at an initial speed corresponding to 80% of their individual $\dot{V}O_{2max}$ for 30 min. After 30 min, the intensity (incline) was incrementally increased by 1% every minute until exhaustion was reached. The heart rate and rating of perceived exertion (RPE) scale were recorded throughout. Exhaustion was considered when the subject's heart rate reached a maximum level (220-age) and the RPE scale scored at least 18 (4). On completion of the exercise test the subjects were seated and consumed either 140 mL of chilled PL or CE (10°C) within 5-min of exercise cessation.

Following a 7-day washout period, the subjects from the PL group were switched into the CE group, and vice versa. The exhaustive exercise test was then repeated.

Sample Collection and Analysis

Fingertip blood samples (200 μl) were collected from each subject before exercise, exhaustion, and 20, 40, 60 and 120 min during the recovery period. Plasma from the samples was isolated, and plasma glucose, lactate, ammonia, and creatine kinase activity were determined at the appropriate wavelength, using a DT-60 analyzer (Johnson & Johnson, Rochester, NY, USA).

Statistical Analysis

Data from group CE and group PL were expressed as mean ± standard error (SE). The repeated-measures analysis of variance test followed by Tukey's post hoc test was used to determine the significant differences between values taken at the various time points throughout the test. Differences were considered significant when $P < 0.05$.

Table 2. Heart rate and biochemical parameters, at exhaustion and during the recovery phase[a, b]

Parameter	Group	Pre-Ex.	Exhaustion	Recovery phase			
				20 min	40 min	60 min	120 min
Heart rate (beats/min)	CE	63.3±2.2	190.9±1.9	103.7±2.9[#]	96.8±2.7[#]	94.1±2.8[#]	–[c]
	PL	63.3±2.2	188.5±2.5	102.5±3.7[#]	97.1±3.2[#]	92.7±2.8[#]	–
Glucose (mg/dl)	CE	98.9±2.4	135.0±8.4	116.0±6.6[#]	93.6±3.4[#]	89.0±3.4[#]	–
	PL	93.9±3.2	127.7±5.2	106.9±4.2[#]	91.1±4.3[#]	88.6±3.6[#]	–
Lactate (mmol/l)	CE	1.6±0.1	10.1±0.6	6.0±0.3[#]	3.6±0.2[#]	2.6±0.2[#]	2.2±0.1[#]
	PL	1.6±0.1	8.7±0.6	5.0±0.4[#]	3.5±0.3[#]	2.8±0.3[#]	2.4±0.2[#]
NH_3 (μmol/l)	CE	40.9±9.0	95.7±12.3	58.4±9.9[#]	47.0±10.2[#]	41.3±8.3[#]	31.7±7.9[#]
	PL	35.9±6.6	94.0±5.9	70.3±9.8[#]	56.5±8.1[#]	53.1±6.6[#]	49.5±7.8[#]
CK[d] (U/l)	CE	71.8±20.3	99.5±23.2	88.2±21.2[#]	83.7±20.9[#]	79.2±19.0[#]	75.3±16.8[#]
	PL	66.5±15.0	97.2±17.7	83.5±17.0[#]	79.5±14.0[#]	80.7±13.7[#]	78.0±13.4

[a] CE: chicken essence supplement; PL: placebo supplement.
[b] Values are expressed as mean ± SE (n = 12).
[c] Ellipses indicate parameter not measured at this point.
[d] CK: creatine kinase.
[#] Indicates significant difference to values at exhaustion ($P < 0.05$).

The changes in the biochemical parameters during the recovery period were expressed as a percentage relative to the value at exhaustion. Decreased percentage (%) = [(data during recovery period - data at exhaustion)/ data at exhaustion] × 100% (11). A paired *t*-test was performed to compare the mean values between the placebo (group PL) and chicken essence (group CE) supplemented groups. Differences were considered significant when $P < 0.05$.

Results

The biochemical and heart rate measurements obtained from the subjects are listed in Table 2. During recovery from exhaustive exercise, the heart rate and plasma glucose concentration decreased significantly at 20, 40 and 60 mins ($P < 0.05$, Table 2) in both CE and PL groups. However, there was no significant difference in the recovery rates for heart rate and plasma glucose between the CE and PL group.

Plasma lactate levels decreased significantly at all recovery time points in both CE and PL groups ($P < 0.05$, Table 2). The CE group exhibited a greater reduction in plasma lactate than the PL group, with a 72.7 % decrease at 60 min, as compared to 66.6% in the PL group, and a 76.9% decrease at 120 min, as compared to 71.2 % in the PL group (Fig. 1). The plasma lactate differences at 60 and 120 minutes between CE and PL groups were significant ($P < 0.05$) and hence indicated that CE supplementation may reduce lactate accumulation.

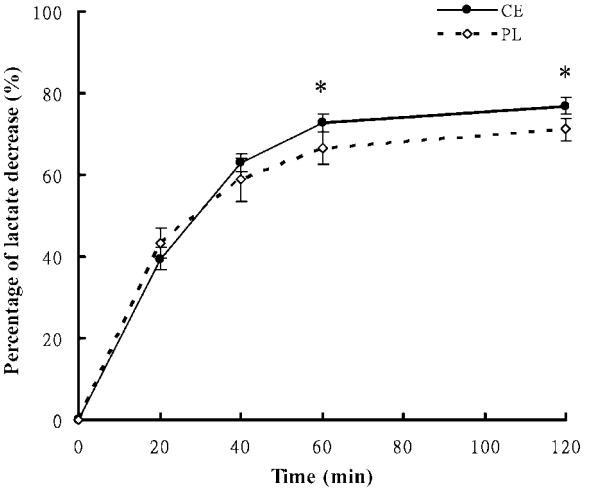

Fig. 1. Percentage of plasma lactate decrease relative to the value observed at exhaustion in group CE (– ● –) and group PL (-- ◊ --). Each point represents the mean ± SE (n = 12). *Indicates significant difference between PL and CE groups ($P < 0.05$).

Plasma ammonia levels reduced significantly during recovery at all measured time points ($P < 0.05$, Table 2) in both CE and PL groups. The CE group showed a greater clearance of plasma ammonia, as

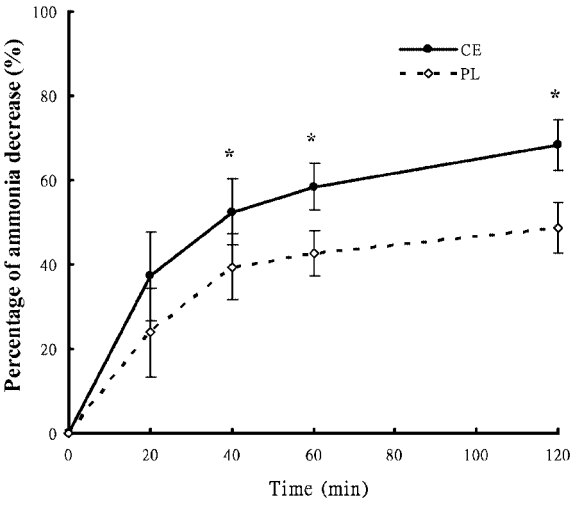

Fig. 2. Percentage of ammonia decrease relative to the value observed at exhaustion in group CE (– ● –) and group PL (-- ◊ --). Each point represents the mean ± SE (n = 12). *Indicates significant difference between PL and CE groups ($P < 0.05$).

compared to the PL group. This was significant at 40, 60 and 120 minutes ($P < 0.05$, Fig. 2). In the CE group, the percentage of plasma ammonia reduction at 40, 60, and 120 min was 52.4%, 58.4% and 68.4% respectively, as compared to 39.4%, 42.7% and 48.6% in the PL group.

Plasma creatine kinase activity increased in response to exercise. On cessation of exercise, creatine kinase levels decreased significantly ($P < 0.05$, Table 2) in both the CE and PL groups. There was no significant difference in the recovery rate for creatine kinase activity between the CE and PL groups.

Discussion

To our knowledge, this is the first human clinical trial investigating the effects of CE supplementation on plasma ammonia level during recovery from a single bout of exercise to exhaustion. We also examined its effects on creatine kinase, plasma glucose, and lactate levels. We found that the plasma lactate and ammonia accumulation of these subjects were reduced after a single bout of exercise to exhaustion. Furthermore, this reduction was significantly greater in the CE supplementation group than those of the placebo group.

In our study, the total creatine kinase activity significantly increased in both groups after exhaustive exercise. We ascribe this to plasma creatine kinase increasing in both groups of the subjects due to exercise-induced skeletal muscle damage. Mechanical disruption in the muscle is one of the basic mechanisms to explain exercise initiated muscle damage. Mair *et al.* (15) demonstrated a transient rise in the serum concentrations of muscle proteins such as creatine kinase, an indicator of muscle damage due to sarcolemma disruption, and subsequent creatine kinase leakage into the blood (15). However, CE did not appear to affect CK measures in this study.

Our study showed that CE can increase the rate of lactate removal during recovery from exercise. The accumulation of blood lactate is seen as a response to anaerobic and increasing-intensity exercise and is frequently and reliably measured as a marker for physical fatigue (12, 21). There are several implications of lactate accumulation and the parallel rise in H^+ concentrations in muscle. A high concentration of H^+ will result in decreased phosphocreatine (21). In addition to phosphocreatine depletion, the cumulative effects of ADP increase and a low pH environment will be the inhibition of local muscle contraction process (12, 22), and an interference with energy supply (22), all of which contribute to muscle fatigue. Carnosine (β-alanyl-L-histidine) is one of the active peptide components present in CE. It has an intracellular pH buffering capacity by virtue of its imidazole carrying histidine residue (1). Carnosine is proposed to function as a pH buffer (hydrogen ion carriers) to neutralize lactic acid produced in skeletal muscle (25). Thus, carnosine may mediate the attenuating effect of CE on plasma lactate levels. Our study revealed that CE can increase the rate of lactate removal during recovery from exercise and hence may play a role in reducing muscle fatigue. Further investigation into whether the increased lactate clearance is associated with an improved tolerance or a delay in muscle fatigue is needed.

The CE group showed a greater clearance of plasma ammonia, as compared to the PL group. This was significant at 40, 60 and 120 minutes. During intense or prolonged exercise, there is an augmented production of ammonia and inosine monophosphate in the exercised muscle that could contribute to the establishment of physical fatigue. Ammonia produced during exercise rapidly diffuses into the bloodstream, where elevated levels interfere with GABA- (gamma-aminobutyric acid) mediated actions in the brain, including synaptic neurophysiological control (3). The accumulation of ammonia is linked to physical fatigue and has been implicated in the development of mental fatigue (2). In order to prevent ammonia accumulation, the urea cycle in the liver eliminates ammonia in the form of urea and the skeletal muscle buffers the increase of ammonia *via* deamination and transamination reactions (30). The amino acid glutamate plays an important role in this metabolic pathway. The intrahepatic concentration of glutamate modulates the rate of ammonia detoxification to urea (16). Glutamate is also the precursor of

the inhibitory neurotransmitter GABA, a potential mediator of hyperammonemic neurotoxicity (14). During intense exercise, the muscle and plasma glutamate concentrations are markedly decreasd due to the significant increase in amino acid catabolism. As a result, detoxification of ammonia by glutamine synthetase may be limited due to a shortage of glutamate. CE contains high levels of glutamate, which may accelerate the removal rate of exercise-induced ammonia during the recovery phase. The results of our study were similar to those of Mourtzakis and Graham in which glutamate supplementation reduced exercise-induced accumulation of plasma ammonia (18).

Furthermore, the amino acid aspartate is a necessary substrate for the urea cycle. Urea synthesis is primarily derived from ammonia and aspartate generated through transamination reactions (16). Arginine is also an intermediate product in the urea cycle. CE contains both aspartate and arginine which have been demonstrated to reduce exercise-induced increases in plasma lactate and ammonia in human subjects (7, 23).

Compared to the PL group, CE supplementation significantly reduced the levels of exercise induced lactate and ammonia observed during the recovery period. HPLC has confirmed the presence of amino acids in CE, several of which have been previously investigated and shown to reduce exercise-induced accumulation of plasma lactate and/or ammonia (7, 18, 23, 25). Therefore, in response to CE supplementation in this study, the amino acids present in CE may be responsible, or contribute to the enhanced reduction of post-exercise lactate and ammonia levels.

Adequate dietary supplements or nutritional ergogenic aids are an important means of optimizing exercise performance and warding off fatigue. Exercise produces changes in the protein balance with a decrease in whole-body protein synthesis and an increase in whole-body protein breakdown and amino acid catabolism (20). Fielding and Parkington (8) indicated that individuals engaging in strenuous activity should consume a meal rich in amino acids and carbohydrates soon after the exercise bout. Studies have shown that the combined ingestion of both carbohydrate and protein improves net protein balance and stimulates glycogen synthesis during the post-exercise recovery phase (13, 29). Oral ingestion of essential amino acids also results in a change from net muscle protein degradation to net muscle protein synthesis in response to resistance exercise (27). As CE is abundant in amino acids, it may provide a natural food source for amino acid supplementation during the post-exercise recovery phase.

In conclusion, the results of this study have demonstrated the role of CE in increasing the elimination of lactate and ammonia during recovery from a single bout of exercise to exhaustion. CE used as a nutritional supplement may aid in recovery from intense physical exercise.

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References

1. Abe, H. Role of histidine-related compounds as intracellular proton buffering constituents in vertebrate muscle. *Biochemistry* 65: 757-765, 2000.
2. Banister, E.W. and Cameron, B.J. Exercise-induced hyperammonemia: peripheral and central effects. *Int. J. Sports Med*. 11 Suppl 2: S129-S142, 1990.
3. Banister, E.W., Rajendra, W. and Mutch, B.J. Ammonia as an indicator of exercise stress: implication of recent findings to sports medicine. *Sports Med.* 2: 34, 1983.
4. Borg, G. and Ottodon, D. The perception of exertion in physical work., London: The Macmillan Press Ltd. 1986
5. Chan, K.-H. and Hsu, M.-C. The supplementation usage pattern of elite athletes in Taiwan regional games from 1994 to 1997. *Research Award Special Issue, Sport Science in Higher Education*, 149-159, 1998.
6. Chao, J.C.-J., Tseng, H.-P., Chang, C.W., Chien, Y.-Y., Au, H.K., Chen, J.-R. and Chen, C.-F. Chicken extract affects colostrums protein composition in lactating women. *J. Nutr. Biochem.* 15: 37-44, 2004.
7. Eto, B., Press, G. and Le Moel, G. Effects of an ingested glutamate arginine salt on ammonemia during and after long lasting cycling. *Arch. Int. Physiol. Biochim. Biophys*. 102: 161-162, 1994.
8. Fielding, R.A. and Parkington, J. What are the dietary protein requirements of physically active individuals? New evidence on the effects of exercise on protein utilization during post-exercise recovery. *Nutr. Clin. Care*. 5: 191-196, 2002.
9. Gaitanos, G.C., Williams, C., Boobis, L.H. and Brooks, S. Human muscle metabolism during intermittent maximal exercise. *J. Appl. Physio*l. 75: 712-719, 1993.
10. Geisser, C., Boroumand-Naini, M. and Tomassen, C. Large acute thermic response to chicken essence in humans. *Nutr. Rep. Int.* 39: 547-556, 1989.
11. Hopkins, W.G., Hawley, J.A. and Burke, L.M. Design and analysis of research on sport performance enhancement. *Med. Sci. Sports Exerc*. 31: 472-485, 1999.
12. Hultman, E. and Spriet, L.L. Biochemistry of muscle fatigue. Soderlund K. *Biomed. Biochim. Acta.* 45: S97-106, 1986.
13. Ivy, J.L., Goforth, H.W. Jr., Damon, B.M., McCauley, T.R., Parsons, E.C. and Price, T.B. Early postexercise muscle glycogen recovery is enhanced with a carbohydrate-protein supplement. *J. Appl. Physiol*. 93: 1337-1344, 2002.
14. Kelly, A. and Stanley, C.A. Disorders of glutamate metabolism. *Ment. Retard. Dev. Disabil. Res. Rev*. 7: 287-295, 2001.
15. Mair, J., Mayr, M., Muller, E., Koller, A., Haid, C., Artner-Dworzak, E., Calzolari, C., Larue, C. and Puschendorf, B. Rapid adaptation to eccentric exercise-induced muscle damage. *Int. J. Sports Med*. 16: 352-356, 1995.
16. Malcolm, W. Glutamine and glutamate metabolism across the liver sinusoid. *J. Nutr.* 130: 983s-987s, 2000.
17. Martinelle, K. and Haggstrom, L. Mechanisms of ammonia and ammonium ion toxicity in animal cells: transport across cell

membranes. *J. Biotech*. 30: 339-350, 1993.
18. Mourtzakis, M. and Graham, T.E. Glutamate ingestion and its effects at rest and during exercise in humans. *J. Appl. Physiol*. 93: 1251-1259, 2002.
19. Nagai, H., Harada, M., Nakagawa, M., Tanaka, T., Gunadi, B., Setiabudi, M.L.J., Uktolesja, J.L.A. and Miyata, Y. Effects of chicken extract on the recovery from fatigue caused by mental workload. *Appl. Human Sci*. 15: 281-286, 1996.
20. Rennie, M.J., Edwards, R.H., Krywawych, S., Davies, C.T., Halliday, D., Waterlow, J.C. and Millward, D.J. Effect of exercise on protein turnover in man. *Clin. Sci (Lond)*. 61: 627-639, 1981.
21. Sahlin, K. Muscle fatigue and lactic acid accumulation. *Acta. Physiol. Scand. Suppl*. 556: 83-91, 1986.
22. Sahlin, K., Tonkonogi, M. and Soderlund, K. Energy supply and muscle fatigue in humans. *Acta Physiol. Scand*. 162: 261-266, 1998.
23. Schaefer, A., Piquard, F., Geny, B., Doutreleau, S., Lampert, E., Mettauer, B. and Lonsdorfer, J. L-arginine reduces exercise-induced increase in plasma lactate and ammonia. *Int. J. Sports Med*. 23: 403-407, 2002.
24. Sim, M.-K. Cardiovascular actions of chicken-meat extract in normo- and hypertensive rats. *Brit. J. Nutr*. 86: 97-103, 2001.
25. Skulachev, V.P. Carnosine and anserine as specialized pH buffers-hydrogen ion carriers. *Biokhim* 57: 1311-1316, 1992.
26. Slater, G., Tan, B. and Teh, K.C. Dietary supplementation practice of Singaporean atheletes. *Int. J. Sport Nutr. Exerc. Metab.* 13: 320-332, 2003.
27. Tipton, K.D., Ferrando, A.A., Phillips, S.M., Doyle, D. Jr. and Wolfe, R.R. Postexercise net protein synthesis in human muscle from orally administered amino acids. *Am. J. Physiol*., 276: E628-E634, 1999.
28. van Hall, G., van der Vusse, G.J., Soderlund, K. and Wagenmakers, A.J. Deamination of amino acids as a source for ammonia production in human skeletal muscle during prolonged exercise. *J. Physiol.* 489: 251-261, 1995.
29. van Loon, L.J.C., Saris, W.H.S., Kruijshoop, M. and Wagenmarkers, A.J.M. Maximizing postexercise muscle glycogen synthesis: carbohydrate supplementation and the application of amino acid or protein hydrolysate mixtures. *Am. J. Clin. Nutr*. 72: 106-111, 2000.
30. Williams, A.T. and Schey, S.A. Use a traditional blood remedy. A study on regular blood donors. *Int. J. Food Sci. Nutr*. 44: 17-20, 1993.